AN INNOVATIVE APPROACH TO THE CONTROL OF SEQUENCING BATCH REACTORS USED FOR NITRIFICATION AND DENITRIFICATION

by

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To Helena and Gabriela.

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ABBREVIATIONS

A/D	Analog-to-Digital
ATP	Adenosine Triphosphate
BOD	Biochemical Oxygen Demand
BOD_5	5-day Biochemical Oxygen Demand
CFS	Continuous Flow Systems
COD	Chemical Oxygen Demand
DA&C	Data Aquisition and Control
DO	Dissolved Oxygen
EBP	Enhanced Biological Phosphorus
F/M	Food-to-Microorganism Ratio
FA	Free Ammonia
FNA	Free Nitrous Acid
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
N(%)	Nitrification Efficiency
N_{org}	Organic Nitrogen
NO_X-N	Oxidized Nitrogen
Phb	Poly-2-hydroxybutyrate
SBR	Sequencing Batch Reactor
SHE	Standard Hydrogen Eletrode
SOTR	Specific Oxygen Transfer Rate
SRP	Soluble Residual Product
SS	Suspended Solids
SVI	Sludge Volume Index
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Nitrogen
VSS	Volatile Suspended Solids
$e_{(subscript)}$	Effluent
$i_{(subscript)}$	Influent

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Ever since human beings developed from a hunter-gatherer to a more settled way of life, the pressure on the environment has been mounting dayly. Specifically in terms of fresh water pollution, two events of the most far-reaching significance have increased the pressure still further. One was the industrial revolution in Europe; the other was the invention of the modern waterborne sewage system and, in particular, of the water closet. As a direct result of those events, rivers near to human settlements became fouled by industrial waste and by bacteriological pollution. Since rivers were the primary source of water for human consumption almost everywhere, pollution suddenly became a vital public problem. Over the years, attention was subsequently given to the treatment of sewage prior to its disposal to the rivers. However, the development of suitable sewage treatment processes was slow and certainly unable to keep pace with man's capacity of fouling the nest.

Nowadays, with the advent of a so called 'green consciousness', people are becoming more and more aware of the water pollution problems. In response, the official bodies responsible for the regulations in this field, are providing tougher guidelines for wastewater treatment and disposal. The need for innovative technologies in the wastewater field, in order to meet these new standards is compelling.

Nitrogen compounds are among those substances requiring better and more economical ways of treatment. Untreated sewage contains nitrogen mostly in the form of organic and/or ammonia nitrogen. Conventional biological wastewater treatment

processes do little in terms of nitrogen removal. Therefore, the wastewater leaving the treatment will have about the same concentration of nitrogen as initially present in the influent, although predominantly in the form of ammonia or, maybe, an oxidized form. The disposal of such effluent can be undesirable for several reasons. Nitrogen in the form of free ammonia is toxic to fish and other aquatic organisms. As the ammonium-ion it may deplete the dissolved oxygen in receiving water bodies. Nitrogen in all forms can be used as nutrient to aquatic plants and consequently contribute to eutrophication. Finally, the oxidized nitrogen forms, nitrite and nitrate are potential public health hazard in water consumed by infants due to the problem of methaemoglobinaemia.

Biological nitrification and denitrification are among those processes capable of dealing with the nitrogen problem. If effluent requirements allow the presence of nitrogen oxidized forms, the nitrification process can be used by itself in order to alleviate ammonia toxicity and oxygen demand in the receiving water body. On the other hand, if nitrogen removal is required, both nitrification and denitrification are used together. Nitrification employs oxic autotrophs to oxidized ammonia to nitrite and nitrate. Subsequent denitrification utilizes facultative heterotrophs to reduce nitrite and/or nitrate to nitrogen gas under anoxic conditions. In combination with the basic goal of organic carbon removal, these biological processes require rather diverse environmental conditions and considerable operating attention in order to maximize their effect.

The sequencing batch reactor (SBR) is one of the available technologies capable of providing those variations in environmental conditions required for the nitrification and/or denitrification process. The principle behind the SBR technology is based on the well known activated sludge process. When comparing SBRs and conventional activated sludge systems, it is possible to say that the former does in time what the later does in space. The SBR is a fill-and-draw process housed only in one tank, where the two basic activated sludge process steps, reaction and separation take place in different times. Because of that, there is no need for the structures required by the conventional activated sludge process, secondary clarifier

and sludge recirculation. As a fill-and-draw process. it can be operated in such a fashion that only effluent meeting the required standards shall be discharged. Therefore, in principle, SBRs can be more efficient than the conventional activated sludge process.

As it stands at the moment, SBRs have to comply with a fixed cycle established in advance, in function of the operational goals. This schedule is chosen through studies using bench or pilot scale models. In order to get this cycle implemented some kind of automation has to be used. Fortunately, today's technology can supply the necessary tools to do that. Computers, time switches and solenoid valves among others have already been in use for some time. A SBR working in this way, can be said to be automated, but not a real time automation, as the system outputs are still initiated by a prior selected timed cycle.

SBRs as well as other systems are monitored by means of conventional laboratory determinations performed on a regular basis on spot samples or daily average samples, including organic matter, solids and nitrogen and phosphorus species. This traditional way of process monitoring does not take full advantage of the SBR potential. Besides that, the inherent delay in the processing of a laboratory analysis limits its use to a historical record, making immediate corrective action difficult. On-line process monitoring is the logical alternative. Unfortunatelly, the sensors available today to monitor the nitrification and/or denitrification process, such as ammonia and nitrate probes, are expensive, short lasting and far from reliable. In this context, the use of inferential measurements may be of value. For example, as shown later, the use of redox potential measurements as a potential monitoring tool for the denitrification process has been reported in the literature (Peddie *et al.* , 1990 and Charpentier *et al.* , 1989).

The operation of a SBR for biological nitrification and/or denitrification, controlled by on-line measurements, may effect substantial improvements in treatment efficiency and savings in costs. However, before such a stage can be reached, more research is necessary both in terms of SBR operation and inferential monitoring tools.

1.2 Objectives of this study

As can be inferred from the above considerations the main aim of this study was to investigate potential monitoring tools for controlling SBRs operated for nitrification and denitrification. pH, redox and DO measurements were chosen as possible inferential monitoring tools.

More specific objectives were:

a) Construct a bench-scale SBR capable of being operated under the changing environmental conditions needed for nitrification and/or denitrification, on a reliable and regular basis.

b) Assemble a data acquisition and control (DA & C) system to monitor pH, redox and DO measurements while controlling the SBR operation.

c) Operate the SBR system for nitrification and/or denitrification

d) Monitor the performance of the reactor for each of the above processes and try to optimize the corresponding SBR cycle.

e) Measure pH, redox and DO as potential monitoring tools for process control and determine the effect of system changes upon then.

f) Select the most appropriate monitoring tool from pH, redox and DO for the nitrification and/or denitrification control.

g) Investigate the evolution of SBR protozoan and metazoan populations as nitrification and/or denitrification progresses, and evaluate their potential use in process monitoring and control.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Historically, biological wastewater treatment has developed from empirical methods to highly sophisticated techniques and technologies. In the course of this century, the understanding of the processes occurring in biological wastewater treatment plants has improved significantly. In this field, an extremely interdisciplinary one, a knowledge of chemistry, biochemistry and microbiology has been traditionally combined with a knowledge of civil, sanitary and mechanical engineering. Lately, even some knowledge in computer sciences and electric/electronic engineering has become necessary. Biological nutrient removal is one of the areas which has profited most upon this interdisciplinary knowledge.

The removal of carbon and nutrients from sewage, can be accomplished in properly designed, operated and controlled biological wastewater treatment systems. However, the biochemical reactions involved are rather diverse in nature and require particular environmental conditions which are, in some instances, incompatible. Because of that, some kind of segregation should be provided. In the case of the activated sludge process, the separation in different zones or reactors is one solution employed. The Sequencing Batch Reactor (SBR) performs this separation in time rather than in space. As such, the reactor would operate on the basis of a chronological sequencing of environments, as opposed to the spatial distribution of segmented reactors and zones.

In order to properly operate and control such a system, an almost instantaneous knowledge of the process dynamics is necessary. The provision for

on-line monitoring of pH, Redox and Dissolved Oxygen (DO), can supply this kind of information. Also, some insights can be gained from the microscopic examination of the microfauna present in the activated sludge.

This chapter will provide referenced background information pertinent to the different aspects of this study. Firstly, the basic biochemistry and microbiology of the activated sludge will be summarized, with a special attention to the most important facets of the SBR operation, which relate to this study, namely: carbon removal, nitrification, and denitrification. Secondly, an overview of the SBR treatment system will be presented. After that, the emphasis will shift to process monitoring and control. At this point, a literature survey on using pH, Redox and DO will be introduced.

2.2 The Activated Sludge Process

In a series of three communications to the Journal of the Society of Chemical Industry, Ardern and Lockett (1914a; 1914b; 1915) laid down the basic principles behind of the activated sludge process. They reported:

> "It was shown that the accumulated deposit, designated "activated sludge", resulting from the complete oxidation by prolonged aeration of successive quantities of sewage, had the property of increasing enormously the purification effected by simple aeration of sewage."

They had seeded in fertile soil. Today, this is one of the most widely employed wastewater treatment technologies. Since Ardern and Lockett's time, the original process has suffered a considerable number of modifications. However, they are all similar in essence. The basis of the process is still an active flocculant microbial mass growing at expense of the influent sewage, but the prerequisite of continuous aerobic conditions no longer holds. The advent of biological nutrient removal in activated sludge systems, provoked the introduction of anoxic and anaerobic periods during the course of the treatment. In this way, the term activated

sludge, gained a broader meaning. Therefore, activated sludge may be redefined as the flocculant microbial mass which is produced when sewage is exposed to a sequence of different environmental conditions. This sequence will be established in function of the treatment objectives.

Hänel (1988) describes the activated sludge process as a biotechnology application, emphasising the principal differences regarding common applied microbiology processes. He describes the latter as a process in which a special nutrient medium is processed by a pure culture of microorganisms to produce an specific end-product, e.g., alcohol, cheese, yogurt, etc. The process is microbiological in nature, and it employs only one specie of microorganism, producing as a waste the non-utilized diluted substrate, which has become unsuitable for the process. In the case of the activated sludge process, a diluted substrate medium, the sewage, is processed by a mixed culture of microorganisms in order to produce more biomass (which is at the same time, the end-product in this analogy), and the process waste. The activated sludge utilises a diverse community of microorganisms, and the process is not only microbiological, but also chemical and physical in nature. There is no direct analogy between the activated sludge process and any industrial microbiological application, in terms of the true end-product of the former, the treated effluent.

The essential component of the activated sludge is the bioflocculated microbial aggregates, known as floc. This structure is formed by: living organisms, dead cells, undigested large organic fragments trapped in the floc, and an inorganic fraction composed, for example, of grit. The latter representing from 10 to 50 percent, in weight, of the activated sludge floc (Eikelboom and van Buijsen, 1983). The community of microorganisms living in, on, or among the activated sludge floc, is subject to all kinds of ecological interactions, such as, competition, parasitism, predation, inhibition, and stimulation. This community includes: bacteria, fungi, protozoa, algae, and some metazoa. The bacteria are by far the most numerous and important group present in the activated sludge. They can be classified in terms of their morphology, in terms of the complexity of their cellular structure, on the basis

of their optimum temperature for growth and metabolic activity, based on their nutritional requirements, and others. However, from the point of view of the activated sludge process, the most important classification is the one regarding their nutritional requirements, in other words, according to the carbon source used in their metabolic pathways. In this way, they can be classified according to the following key (Mckane and Kandel, 1986):

a) Chemoheterotrophs: Microorganisms that use organic carbon both, as carbon and as energy sources. Usually, they use the same compounds for both. Examples: Most bacteria, protozoa and fungi.

b) Chemoautotrophs: Microorganisms that use carbon dioxide as carbon source and inorganic compounds as energy source. Examples: Sulphur, iron and ammonia-oxidising bacteria, and several types of methane-producing bacteria.

c) Chemomixotrophs: Microorganisms that depending on the environmental conditions, can be either chemoheterotrophs or chemoautotrophs. Example: Some sulphur bacteria capable of heterotrophic desulphatation, under anaerobic conditions, and autotrophic oxidation of reduced sulphur forms, under oxic conditions.

In the activated sludge process, the microorganisms utilise the substrate present in the influent sewage for, energy generation, maintenance and cell synthesis. Among other factors, the environmental or cultivation conditions, will determinate in which way this substrate is going to be used, and the predominant microbial population. The terms oxic, anoxic and anaerobic, will be employed to describe these conditions. When the biological process takes place in the presence of physically dissolved oxygen, the conditions are said to be oxic. Anoxic conditions are achieved in the absence , or very low concentrations, of free dissolved oxygen, but in the the presence of molecular oxygen, e.g., nitrate and nitrite anions. Finally, anaerobic conditions occur in the absence of both, free dissolved and molecular oxygen. These

environmental conditions are artificially engineered in order to achieve the desired treatment goals of, nitrification, denitrification, phosphorus removal, etc.

Inside the activated sludge reactor, depending on the environmental conditions above described, different biochemical reactions may occur. Wanner(1991) presented a very good summary of this (Table 2.1). It is necessary to recognize that in practice, the metabolic reactions can be even more diverse than those shown in that table. For example, in recent studies on enhanced biological phosphorus removal, it has been argued about the probable existence of organisms capable of outcompeting, in anaerobic conditions, the polyphosphate accumulating (Poly-P) bacteria for organic substrate (Cech and Hartman, 1990; Cech *et al.*, 1991). They maintained that the metabolic mechanisms which are employed by these bacteria to utilise organic substrate in anaerobic conditions, is still unknown. Before any of these reactions can occur, the high-molecular organic compounds present in the sewage, have to be broken down to low-molecular compounds by hydrolysis. It has been demonstrated that hydrolysis may occur under any of the three possible environmental conditions, although the rate of hydrolysis is higher under oxic conditions than under anoxic or anaerobic ones (Ekama and Marais, 1986). In order to gain a better understanding of how these reactions occur, it is wise to study them from an environmental conditions viewpoint.

a) Oxic conditions: The organic substrate is removed by oxidative and synthesis reactions. During oxidation, electrons are transferred from the electron donors to the free dissolved oxygen, the electron acceptor. In oxic heterotrophic respiration, organic carbon is the electron donor. In the case of nitrification, electrons are transferred from reduced forms of nitrogen. Yet other electron donors are reduced forms of sulphur, during their oxic oxidation to sulphates. All these processes produce energy, which is used for cell maintenance and synthesis. The latter requires a carbon source, which is either organic carbon for heterotrophs, or carbon dioxide for autotrophs, such as, nitrifiers. Some heterotrophic bacteria employ storage products which have been synthesised

Metabolic activity	Required carbon form	Electron donor	Reaction providing energy	Electron acceptor	Products	Growth forms in activated sludge
Oxic heterotrophic	Organic carbon	Organic carbon	Oxic oxidation of organic carbon	O_2	$CO_2 + H_2O$	Floc forming and filamentous bacteria
Anaerobic fermentation microbes	Organic carbon	Organic carbon	Fermentation of organic carbon	Organic carbon	Low molecular compounds and $CH_4 + CO_2$	Floc forming bacteria (?)
Denitrifiers	Organic carbon	Organic carbon	Anoxic oxidation of organic carbon	NO_3^- and NO_2^-	N_2, CO_2, H_2O and OH^-	Floc forming and, rarely, filamentous bacteria
Nitrifiers	Inorganic carbon	NH_4^+ and NO_2^-	Oxic oxidation of NH_4^+ and NO_2^+	O_2	NO_3^-, NO_2^-, H^+ and H_2O	Attached on the floc
Sulphate reducing microbes	Organic carbon	Organic carbon	Anaerobic oxidation of organic carbon	SO_4^{2-}	S^0 and S^{2-}	Floc forming bacteria
Sulphur and Sulphide oxidising microbes	Inorganic carbon	S^0 and S^{2-}	Oxic oxidation of S^0 and S^{2-}	O_2	SO_4^{2-}	Floc forming bacteria (?) and, often, filamentous
Polyphosphate accumulating (Poly-P) bacteria	Organic carbon (storage products)	Organic carbon (storage products)	Oxic oxidation of storage products and phosphate uptake	O_2	$CO_2 + H_2O$, and polymerised phosphorus	Cluster of individual cells
Polyphosphate accumulating (Poly-P) bacteria	Organic carbon (storage products)	Organic carbon (storage products)	Anaerobic formation of storage products, and phosphate release	Organic carbon (low molecular compounds)	Storage products and PO_4^{2-}	Cluster of individual cells

Table 2.1: Common Activated Sludge Reactions (Wanner, 1991) Modified

under anaerobic conditions as carbon and energy source. During this process, the excess of energy produced is accumulated in polyphosphates, with consequent phosphate uptake. Besides the above processes, when the available substrate is exhausted the intracellular material starts to be used as carbon source in a process called endogenous respiration.

b) Anoxic conditions: Under these conditions, essentially the same metabolic processes take place as under the oxic ones. The difference is that instead of using free dissolved oxygen as the electron acceptor, this time, molecular oxygen is employed. Therefore, during heterotrophic denitrification, electrons are transferred from organic carbon to nitrate or nitrite, causing their ultimate reduction to nitrogen gas. It has been shown that even nitrifiers can use oxidised nitrogen forms as electron acceptors (Bock *et al.*, 1988). Lotter and Murphy (1985), also argued for a possible formation of polyphosphate, with the consequent utilization of storage products, under these conditions. In this case, some phosphate uptake may occur.

c) Anaerobic conditions: During anaerobic desulphatation and fermentation, energy is produced through the transfer of electrons from organic compounds to sulphate sulphur and other organic compounds, respectively. In this way, both electron acceptors are reduced. Another important process that may take place under these conditions, is enhanced phosphate release. It occurs when polyphosphates are depolymerised, releasing energy. This energy can be used for the synthesis of storage products from low-molecular weight compounds, the latter being intermediary products from the anaerobic fermentation process.

The objective of placing this summary of possible metabolic processes at the beginning of this literature review, is to provide a theoretical framework, which will facilitate the development of the remaining topics. In the following sections, some of these processes will be dealt with in more detail.

2.3 Carbon Removal

2.3.1 Background

The removal of organic matter is the principal goal of sewage treatment, and also it was the most important achievement of Arden and Lockett's activated sludge process. If the sewage was discharged untreated to a water body, the organic matter contained in it would cause the oxygen dissolved in the water, to be depleted, which in the worst case scenario could lead to septic conditions prevailing. In a sewage of medium strength, about 75% of the suspended solids (SS) and 40% of the volatile suspended solids (VSS) are of organic nature (Tchobanoglous and Burton, 1991). This organic matter normally comprises a combination of carbon, hydrogen, oxygen, nitrogen, sulphur and others atoms of a lesser importance. These atoms are combined in complex molecules like proteins, carbohydrates, fats and oils. Besides these, some synthetic organic compounds may be present, such as, surfactants, organic priority pollutants and agricultural pesticides.

From a close look at Table 2.1, it is possible to infer that organic carbon can be utilised in all 3 environmental conditions. Under oxic conditions, the heterotrophs will use it as carbon and energy sources during the oxic oxidation of the organic matter and the phosphorus polymerization. In this case, the organic matter degradation follows the glycolysis/ pyruvic acid/ krebs cycle metabolic pathways (Hänel, 1988; Horan, 1990; Mckane and Kandel, 1986). This scheme is highly efficient in terms of energy production, yielding 38 ATP(Adenosine Triphosphate) molecules per mol of glucose oxidized. That is the principal reason why this is the preferred scheme over other possible pathways, in the presence of free dissolved oxygen. During denitrification, under anoxic conditions, the organic matter is employed also for bacteria catabolism and anabolism. The metabolic pathways are the same as for oxic oxidation, but this time less energy is generated (Barnes and Bliss, 1983). As a result of a shorter pyruvic acid decarboxylation cycle, and because the resulting product of the former does not enter the Krebs cycle at the beginning,

this time, only 26 ATP's are produced per mol of glucose utilised (Grandie and Lim, 1980). Finally, under anaerobic conditions, in the fermentative process, again the organic matter is utilised for energy and synthesis with a resulting energy gain of only 2 ATP's per mol of glucose employed (Anderson, 1980; Barnes and Bliss, 1983; Hänel, 1988; Horan, 1990; Mckane and Kandel, 1986). The ATP molecules formed came from the glycolysis pathway, with no energy generated during the fermentative pathway, and with the resulting end products still being very energetic. Therefore, this is a very inefficient process, and will only occur when the environmental conditions are not suitable for the previous ones to occur.

For the sake of completeness, yet another process can occur; the formation and consumption of storage products. Storage products are defined as "those cellular compounds which undergo rapid synthesis in the presence of a soluble exogenous substrate and rapid degradation upon exhaustion of the external food supply" (Walters *et al.*, 1968). The two most common carbon and energy storage compounds are glycogen, a glucose polymer, and poly-2-hydroxybutyrate (phb), a polymer of hydroxybutyric acid. Relatively little is known regarding the metabolic pathways utilised during the synthesis and degradation of these compounds. Glycogen is a glucose polimer, which provides cells with a reserve of carbohydrate. Its degradation is accomplished through the glycolysis pathway. Therefore, the end-products will be dependent upon the prevailing environmental conditions. The glycolysis pathway is also used for its synthesis, this time in the reverse way. Carbon dioxide, and intermediary products of the Krebs cycle, e.g., succinate and malate, are used as substrates for the synthesis of glycogen, when there is a surplus of exogenous organic material and ATP is not needed by the cell (Allamong and Mertens, 1976). Dawes and Ribbons (1964) mentioned that some products of the fermentative pathway, such as, acetate and butyrate, are the principal substrates for the synthesis of phb. They also reported crotonic acid as a possible alternative substrate, which would bypass the need for acetoacetate. In terms of phb degradation, they claimed that it first goes to acetoacetate, before entering the Krebs cycle. The importance of these storage products, as they relate to the denitrification process, will be discussed later in this chapter.

From the above paragraphs it is clear that the organic matter can be used in a multitude of different ways. Even so, the bulk of organic carbon removal will still be performed by the heterotrophs under oxic conditions. In the following equations, using glucose to represent the organic matter, the oxic oxidation process is outlined (Mckane and Kandel, 1986).

$$C_6H_{12}O_6 + O_2 \xrightarrow{\text{Heterotrophs}} 6CO_2 + 6H_2O + \text{Energy} \qquad 2.1$$

(Glucose)

$$ADP + P_i + \text{Energy} \xrightarrow{\text{Synthesis}} ATP \qquad 2.2$$

$$ATP \xrightarrow{\text{Hydrolysis}} ADP + P_i + \text{Energy} \qquad 2.3$$

$$\text{COHNS} + \text{Energy} \xrightarrow{\text{Heterotrophs}} C_5H_7NO_2 + \text{other end-products} \qquad 2.4$$

(Krebs Cycle Products) (New Cells)

Equations 2.1 through 2.4 show the coupling of catabolism and anabolism through ATP. In equation 2.1, a highly reduced and energy-rich compound, glucose, is oxidized to an energy-poor and highly oxidized compound, carbon dioxide. Most of the energy liberated during the process is lost as heat, but part is transferred to the chemical bonds of ATP (equation 2.2), during a process called phosphorylation. In this way, the energy is stored until it is needed by the cell. By the process of hydrolysis (equation 2.3), the ATP releases its energy in order that it can be used during the synthesis of new cells (see equation 2.4). Besides these reactions, yet

another process can occur, that of endogenous respiration. Endogenous metabolism may be defined as "the total metabolic reactions that occur inside the living cell when it is held in the absence of compounds or elements which may serve as specific exogenous substrate" (Dawes and Ribbons, 1964). The following equation shows the stoichiometry of the process as proposed by Tchobanoglous and Burton (1991).

$$\underset{\text{(bacterial cells)}}{C_5H_7NO_2} + 5O_2 \xrightarrow{\text{bacteria}} 5CO_2 + 2H_2O + NH_3 + \text{energy} \qquad 2.5$$

2.3.2 Kinetics of Microbial Growth and Substrate Utilization

In order to understand the kinetics of the oxic oxidation of the organic matter, it is necessary to appreciate the dynamics of bacterial growth and substrate utilization. Figure 2.1 shows, for a batch reactor, the schematic microbial growth and substrate utilization curves, against time, for a pure culture of microorganisms growing on a single substrate. In addition, a third curve is showed, representing the specific growth rate, also against time. The latter is the first derivative of the growth curve, divided by the bacterial density. The following phases can be spotted:

a) Lag phase: This initial time is required for the bacteria to adapt to their new environment, with the bacterial cell presenting a long generation time and a zero specific growth rate. By generation time is understood "the time required for each bacterial fission, anything from some days to 20 minutes" (Tchobanoglous and Burton, 1991). The number of microorganisms remains constant, but some substrate uptake may occur, with an increase in the size and the mass of bacteria (Gray, 1990).

b) Exponential or log phase: During this phase there is a rapid increase in the number and mass of microorganisms, with the cells dividing at a rate

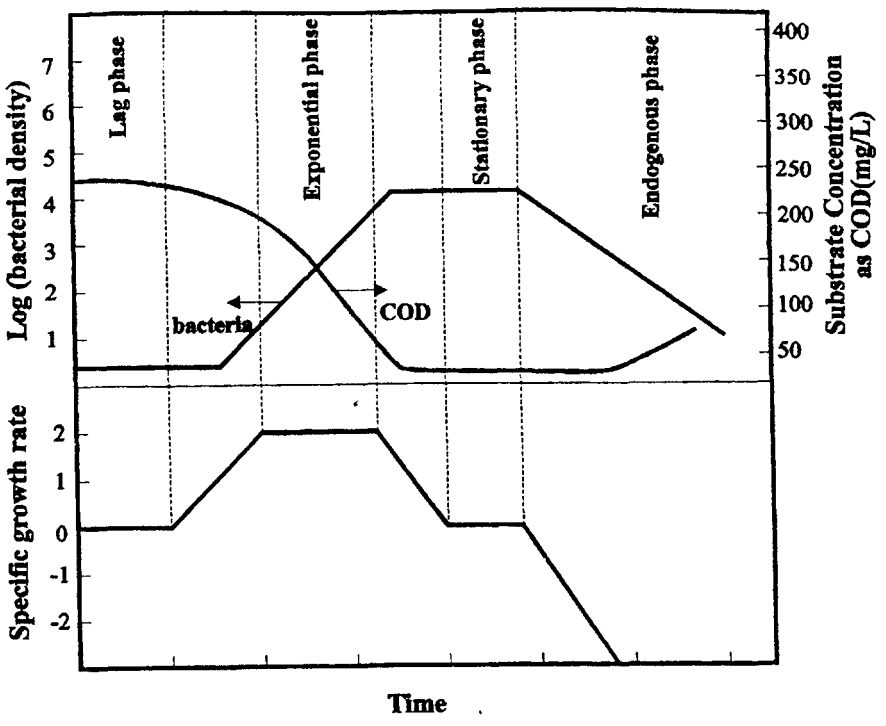


Figure 2.1: Bacterial Growth and Substrate Uptake Curves (Gray, 1990)

determined by their generation times and their ability to process the organic matter. The growth is exponential, the generation time is minimum and the specific growth rate is maximum and constant. This phase will last until the substrate becomes limiting. When this happen, together with other factors, like the accumulation of toxic metabolites and the environmental conditions deterioration, e.g., pH, the specific growth rate decreases and the number or mass of microorganisms will reach their maximum (Barnes and Bliss, 1983). In this phase, the substrate uptake rate is maximum. Because of that, this can be the chosen phase for the activated sludge operation, since the principal treatment aim is to remove substrate as quickly as possible (Horan, 1990). Even so, this phase presents some setbacks. As can be seen from figure 2.1, there is still some room for further substrate removal. Besides that, the accelerated bacterial growth will mean large volumes of surplus sludge to

handle. Also, it has been shown that high rate activated sludge suffers from poor settleability (Gray, 1990).

c) Stationary phase: The bacterial population reaches a stationary stage. This happens because the growing of new cells is being matched by the dead of old ones, and the substrate has been exhausted. The maximum number or mass of microorganisms is reached. The substrate concentration reaches it minimum. From the above considerations is easy to inferred that this would be the ideal phase to operate activated sludge systems. However, activated sludge systems are known by their inherent operational fluctuations. Therefore, to operate a system inside this narrow range will be an almost impossible task to perform, and, at end of the day, they finish by being operated in a intermediary phase, between the log and the stationary ones. This is the case of conventional activated sludge systems (Gray, 1990). By the end of this period, the environmental conditions have deteriorated further, no longer being able to sustain any growth.

d) Endogenous phase: As the substrate concentration has reached its minimum, the bacteria starts to use their own energy reserves, decreasing the active biomass. The bacteria is dyeing at a pace greater than their production rate. Therefore, the number or mass of microorganisms starts to decrease, with the specific growth rate becoming negative, and decreasing at a faster rate. After a while, the substrate concentration can start to increase from its background level, due to the release to the medium of soluble residual products(SRP), which are related to the hydrolysis of non-viable cellular material (Orhon *et al.*, 1989). The extended aeration activated sludge system works in this range (Gray, 1990).

The kinetics of activated sludge are easier to express during the exponential or log phase. During this phase, the bacterial growth is exponential (first-order reaction) and can be mathematically represented by,

$$\frac{dX}{dt} = \mu X \qquad 2.6$$

where X is the microorganism concentration (mg/L) and μ is the specific growth rate (d^{-1}), in other words, the reaction rate. A variety of different expressions for μ have been proposed. Lopes (1988) presented a very good review on the subject, but, by large, the most employed expression is the one proposed by Monod (1949). This author, using data from batch experiments on a pure culture of *E. coli* growing on glucose, found that several mathematically different formulations could be made to fit the data, but that it was both convenient and logical to adopt a hyperbolic equation of the form:

$$\mu = \mu_{max} \frac{S}{K_S + S} \qquad 2.7$$

where μ_{max} is the maximum specific growth rate at a saturation concentration of the growth limiting substrate (d^{-1}), S is the substrate concentration (mg/L), and K_S is the half-saturation constant (mg/L), which is the concentration of the limiting substrate at which the specific growth rate equals one-half of the maximum specific growth rate. Equation 2.7 can be simplified for extreme situations:

When $S << K_S$, e.g., for some heterotrophs:

$$\mu = \mu_{max} \qquad 2.8$$

When $S >> K_S$, e.g., for some autotrophs:

$$\mu = \frac{\mu_{max}}{K_S} S \qquad 2.9$$

By substituting μ in Equation 2.6, by the right side of Equation 2.7, the growth rate can be expressed as follows:

$$\frac{dX}{dt} = \mu_{max} \frac{SX}{K_S + S} \qquad 2.10$$

It has also been showed that, during the log phase, the bacterial growth is proportional to the substrate uptake by a proportionality factor Y, the yield coefficient (mg cells / mg substrate):

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \qquad 2.11$$

An equivalent expression for the substrate utilization rate is obtained by combining Equations 2.11 and 2.6, in terms of substrate concentration.

$$\frac{dS}{dt} = -\frac{\mu_{max}}{Y} \left(\frac{SX}{K_S + S} \right) \qquad 2.12$$

During the development of the above equations, the yield coefficient (Y) has been considered constant. In the real world of activated sludge, a multiple substrate is going to be employed by a mixed culture of microorganisms, which most of the time will be beyond the log phase, maybe in the stationary or, even in the endogenous phases. In this situation Y will vary. To account for that, two new terms have been introduced. First of all, Y has been replaced by Y_T, the true growth yield, which represents the maximum theoretical cell yield when maintenance energy requirement is zero. The second term, is the endogenous decay coefficient, k_d (d^{-1}). This term includes all the processes responsible for loss of bacterial mass, such as, predation, death, cell lysis and storage products utilization. Benefield and Randal (1980), referred to in Barnes and Bliss (1983) and in Gray (1990), proposed the following expression relating specific growth rate, substrate utilization, the true yield coefficient and endogenous decay.

$$\frac{dS}{dt} = -\frac{\mu}{Y_T}X - k_d X \qquad 2.13$$

In order to employ the above equations for simulation and design of the activated sludge process, it is necessary to determine the kinetic parameters: μ_{max}, K_S, Y_T and k_d. This parameters should be determined experimentally, using bench or pilot scale methods (Grandie and Lim, 1980). In addition to these conventional methods, it is also possible to use parameter estimation techniques. For example, Anderson(1978) proposed a method based on the maximum likelihood principle for the determination of model parameters, when all measured variables are subject to error. However, due to the difficulties and costs involved in the experimental methods employed to obtain the data for the parameter determination, most wastewater engineers and designers prefer to rely on data presented in the literature. Table 2.2 lists a range of reported values of the kinetics parameters for mixed culture aerobic systems (Alleman, 1978; Barnes and Bliss, 1983; Grandie and Lim, 1980; Gray, 1990; Horan, 1990; Tchobanoglous and Burton, 1991). As it would be expected, the values of these coefficients are subject to great variations, which again emphasises the need for these coefficients to be determined from experimental data.

Kinetic Parameter	Units	Values
μ_{max}	d^{-1}	2.3 to 13.2
K_S	mg/L as COD mg/L as BOD_5	10.0 to 70.0 25.0 to 120.0
Y_T	mg MLVSS/mg COD mg MLVSS/mg BOD_5	0.25 to 0.5 0.4 to 0.8
k_d	d^{-1}	0.025 to 0.1

Table 2.2: Kinetic parameters for mixed culture aerobic systems.

The above kinetic equations do not exhaust today's knowledge on the subject. Further elaborations have been often proposed, increasing considerably the model

complexity. For example, Andrews and Tien (1977) proposed a model that reflects the importance of external substrate tension on the substrate storage mechanism. As such, the rate of storage is proportional to the exogenous carbon concentration, in addition to the existing cell mass and the present level of storage as compared to some maximum value. Another example is the modelling of soluble residual products, SRP (Orhon *et al.*, 1989). These authors related SRP formation to the hydrolysis of non-viable cellular material. It established a theoretical, as well as practical, way of explaining the organic matter accumulation during the endogenous phase. Here it is necessary to emphasise that the merit of any model formulation in accurately represent the process or processes being studied, will depend on the way in which the proposed model provides the necessary operational tools to predict and control system behaviour under different conditions. The success of this approach is related to the level of understanding of the biochemical reactions involved. Unfortunately, these reactions are too complex to be accurately represented in any model. Therefore, any model formulation is obliged to remain empirical despite all possible refinements. In this respect, the simplest approach would provide the most workable model as long as it defines the performance of the system being studied, within acceptable accuracy. If this accuracy is not good enough, additional mechanistic concepts will be considered for a more valid modelling approach.

2.3.3 Environmental Factors Affecting Carbon Removal

Diverse environmental factors can affect the oxic heterotrophic organic carbon oxidation process, including, temperature, dissolved oxygen concentration, pH, presence of toxic substances, redox and presence of nutrients and trace elements. From these environmental factors, only the temperature is out of control of the activated sludge operator. Below, some of these factors will be briefly discussed.

a) Temperature: This affects the bacterial growth, and consequently substrate uptake, by influencing the rates of enzymatically catalyzed reactions, and by altering the rate of diffusion of substrate to the cell (Grandie and Lim, 1980). Activated sludge systems usually work between 12 and 25°C, which put them

inside the mesophillic range (Gray, 1990). Barnes and Bliss (1983) mention that when the temperature goes above 35°C, the microorganisms undergo a rapid decrease in metabolic activity, probably caused by enzyme inactivation, and that at 45°C biological activity is halted. On the other hand, heterotrophic activity has been observed at temperatures as low as 5°C. The Van't Hoff's law establishes that inside the of 5 to 35°C, the biological activity doubles with each 10°C rise in temperature. Specifically in terms of the effect of temperature on the rate of reaction, the Arrhenius' formula, below, has been largely employed (Barnes and Bliss, 1983; Grandie and Lim, 1980; Gray, 1990; Hänel, 1988; Horan, 1990; Tchobanoglous and Burton, 1991).

$$K_T = K_{20}\theta^{T-20} \qquad 2.14$$

In this formula, T is temperature (°C), K is the reaction rate constant at temperature T, K_{20} is the reaction rate constant at 20°C (d^{-1}), and θ is the temperature coefficient.

b) Dissolved Oxygen (DO) Concentration: A DO concentration between 1 and 2 mg/L is sufficient for active oxic heterotrophic microbial activity (Gray, 1990). In practical terms, it has been recommended that the DO concentration inside the aeration tank, should be maintained at 10% of the saturation, during all times, and when nitrification is not important (IWPC, 1987).

c) pH: For the majority of the heterotrophs, the optimum pH for carbonaceous oxidation is in a range of 2 to 3 pH units centred around pH 7. Gray(1990) suggests that this range is from pH 6.5 to 8.5. Besides that, he states that above pH 9, the microbial activity is inhibited, and that below pH 6.5 fungi will predominate over bacteria in the competition for the substrate.

d) Nutrient and Trace Elements Concentration: It has long been recognized how important is the presence of nitrogen and phosphorus for optimum bacterial growth. Normally, both are present in excess in domestic sewage. However, some industrial sewages are deficient in N and P. In these cases, it is

important to supplement the sewage with these nutrients. It has been widely accepted that for optimum growth, the relative quantities of organic carbon, expressed as BOD_5, N and P should be 100:5:1 (Grandie and Lim, 1980). Apart from N and P, bacterial growth is also dependent on the presence of trace elements, like sulphur, iron, calcium, magnesium, potassium, manganese, copper, zinc and molybdenum.

Pollutant	Concentration (mg/L)
Aluminum	15 - 26
Ammonia	480
Arsenic	0.1
Borate (Boron)	0.05 - 100
Cadmium	10 - 100
Calcium	2,500
Chromium (hexavalent)	1 - 10
Chromium (trivalent)	50
Copper	1.0
Cyanide	0.1 - 5
Iron	1,000
Manganese	10
Mercury	0.1 - 5.0
Nickel	1.0 - 2.5
Silver	5
Zinc	0.8 - 10
Phenol	200

Table 2.3: Threshold Concentrations of Pollutants Inhibitory to the Activated Sludge Process (Tchobanoglous, 1991)Modified

e) Presence of Toxic Substances: Heavy metals and other substances, including some organic compounds, may have an toxic or inhibitory effect upon the activated sludge community of microorganisms, if they are present in concentrations which exceeds threshold limits. Some of the compounds that have an inhibitory effect upon organic carbon removal, on activated sludge systems, together with their threshold limits, are presented in Table 2.3 (Tchobanoglous and Burton, 1991).

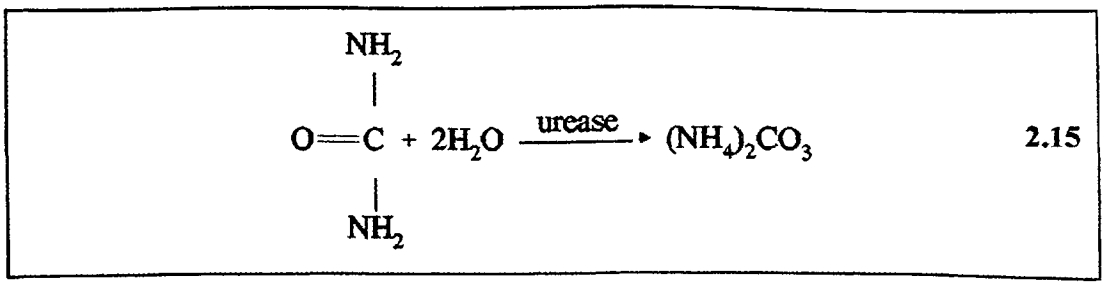
f) Redox: Wanner (1991) suggests that the oxic oxidation of the organic matter is accomplished under a redox between +50 and +250 mV, using the standard hydrogen electrode (SHE) as reference. In other words, this range characterizes the oxic conditions.

2.4 Nitrification

As defined by Grandie and Lim (1980), nitrification is the biological conversion of ammonia nitrogen to nitrate nitrogen, under oxic conditions. The original discovery of this process is attributed to Schloesin and Muntz, as early as 1877 (Alleman, 1978).

2.4.1 Background

The nitrogen contained in domestic sewage is either organically bound as protein and nucleic acids, as urea or as the ammonium anion. In the sewerage system, most of the organically bound nitrogen is rapidly deaminated and urea is hydrolysed by the enzyme urease to release ammonia (see equation 2.15). This process is known as ammonification.



$$\begin{array}{l} NH_2 \\ | \\ O{=}C \\ | \\ NH_2 \end{array} + 2H_2O \xrightarrow{\text{urease}} (NH_4)_2CO_3 \qquad 2.15$$

Ammonia is assimilated by heterotrophs during the oxic oxidation of the organic carbon, but domestic sewage has nitrogen in excess of the heterotrophs needs. Therefore, the excess is going to be released as ammonium ion (NH_4^+), which in its turn, will be oxidized by chemoautotrophs to nitrate via nitrite, during the nitrification process.

Already by 1891, Warington (cited in Alleman (1978)), indicated that nitrification occurred in two distinct steps, each promoted by a separate group of bacteria. Both groups are composed of chemoautotrophic bacteria, generically known as nitrifiers. Unlike heterotrophs, these bacteria have separate metabolic routes for carbon incorporation and energy production. Carbon incorporation is achieved through the Calvin cycle, using carbon dioxide as the sole source of carbon. These bacteria, like the heterotrophs, also uses the Krebs pathway for the generation of intermediate products necessary for the synthesis of new cells, but in a different manner. In this case the Krebs pathway does not produce energy, due to a slight alteration which prevents the pathway from working in a cyclical way. The lack of the enzyme responsible for the oxidation of the α-ketoglutaric acid causes the destruction of the cyclic operation (Grandie and Lim, 1980). Their energy requirements are met by the oxidation of ammonia and nitrite, under oxic conditions. Therefore, they obtain energy through the nitrification process. The overall stoichiometric reactions, involved in the energy production process, can be written as follows (Sharma and Ahlert, 1977):

$$NH_4^+ + 1.5O_2 \xrightarrow{Nitrosomonas} NO_2^- + 2H^+ + H_2O + (240 - 342\ kJ/mol) \qquad 2.16$$

$$NO_2^- + 0.5O_2 \xrightarrow{Nitrobacter} NO_3^- + (63 - 99\ kJ/mol) \qquad 2.17$$

As already mentioned, the nitrification occurs in two separate steps. Equation 2.16 represents the so called nitritification step, where the ammonium ion is oxidized to nitrite by the genera *Nitrosomonas*. The pathway by which *Nitrosomonas* oxidizes ammonia to nitrite is still largely speculative, but it is believed to occur in three separate steps, each consisting of one or two electron changes, from oxidation state -3 to +3 (Sharma and Ahlert, 1977). These authors also suggest that the energy is generated from the oxidation of the hydroxylamine (NH_2OH) and that ATP is produced by oxidative phosphorylation, when the electrons pass through the electron transport chain.

$$\underset{(-3)}{NH_4^+} + 0.5O_2 \longrightarrow \underset{(-1)}{NH_2OH} + H^+ \longrightarrow \underset{(+1)}{?} \longrightarrow \underset{(+3)}{NO_2^-} \qquad 2.18$$

In the subsequent nitratification step (Equation 2.17), nitrite is further oxidized to nitrate, this time by the *Nitrobacter* genera. This oxidation involves a single electron pair transfer. Again the electrons pass to an electron transport chain, where ATP is generated. It has been shown that the oxygen incorporated in the nitrate does not come from molecular oxygen as in the nitritification, but from water. Strictly speaking, there are a few other genera, which have been reported in the literature, capable of either ammonia or nitrite oxidation, e.g., *Nitrosococcus*, *Nitrocystis*, and *Nitrospira* (Gray, 1990). Nevertheless, for convenience, the terms *Nitrosomonas* and *Nitrobacter* will be used throughout this text, as all-embracing designations for, respectively, ammonia and nitrite oxidizers.

The nitrifiers are strictly aerobic when growing on their respective substrates, but anoxic or anaerobic conditions, even for long periods of time, are not lethal to them (IWPC, 1987). Besides that, it has been shown that under these conditions, *Nitrobacter* is capable of becoming a nitrate reducer (Kiesow, 1964 cited in Sharma and Ahlert, 1977), and that *Nitrosomonas europaea* can reduce nitrite, in the presence of hydroxylamine (Hooper, 1968 referred to in Alleman, 1978).

Based upon experimentally obtained yield coefficients and oxygen consumptions, the following overall equation for nitrifier synthesis and nitrification has been proposed (EPA, 1975 cited in Sharma and Ahlert(1977):

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \longrightarrow 0.021C_5H_7NO_2 + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \qquad 2.19$$

Some interesting observations can be inferred from this equation:

a) There is a significant oxygen requirement during the nitrification process, amounting to approximately 4.2 g O_2/ g NH_4^+-N removed or 4.3 g O_2/ g NO_3^--N formed. This consumption is slightly smaller than the theoretical nitrogenous oxygen demand (NOD) calculated from equations 2.16 and 2.17. There, the overall theoretical NOD is 4.57 g O_2/ (g NH_4^+-N removed or oxidized). A good review on reported disagreements between theoretical and actual NOD has been presented by Sharma and Ahlert (1977). They suggest different possible explanations, for instance that the oxygen consumption may depend on the partial pressure of carbon dioxide, and the fact that during nitratification step some of the oxygen used comes from the water molecules.

b) The amount of bacterial cells synthesized in the process is represented by $C_5H_7NO_2$ in equation 2.19. The experimentally determined cell yield (Y) used in this equation is 0.17 g cells/ g NH_4^+-N removed, which is very low

compared to that of heterotrophs. This indicates how poor contenders the nitrifiers are in the competition against the heterotrophs.

c) There is a high consumption of alkalinity, associated with the oxidation of ammonia and removal of bicarbonate, amounting to 8.63 g HCO_3^-/ g NH_4^+-N removed or, approximately 7.1 g of alkalinity (as $CaCO_3$)/ g NH_4^+-N removed.

2.4.2 Kinetics of Nitrification

The nitrification process has been traditionally modeled using the Monod relationship, since 1965, when Knowles *et al.* demonstrated its soundness. Barnes and Bliss (1983) stated that the nitrification process complies with the Monod model assumptions even better than the oxic heterotrophic oxidation one. Regarding the nitrification process, the need for a pure culture is approached by the specific set of nitrifiers, and the assumption of a single growth limiting substrate is met by these bacteria specific energy substrate, ammonium and nitrite.

The Monod model was already discussed in some detail under the section 2.3.2 . Therefore, now only the pertinent equations will be presented.

$$\mu_n = \mu_{max,n} \frac{S_n}{K_{S,n} + S_n} \qquad 2.20$$

$$\frac{dX_n}{dt} = \mu_{max,n} \frac{S_n X_n}{K_{S,n} + S_n} \qquad 2.21$$

$$\frac{dS_n}{dt} = - \frac{\mu_{max,n}}{Y_n} \left(\frac{S_n X_n}{K_{S,n} + S_n} \right) \qquad 2.22$$

$$\frac{dS_n}{dt} = - \frac{\mu_n}{Y_n} X_n - k_{d,s} X_n \qquad 2.23$$

This set of equations can be used to model either the nitratification, the nitritification, or both steps. Care should be taken to use the right kinetic parameters, energy substrate and concentration of microorganisms, depending on which process is being modeled. For example, in the case of nitratification, $\mu_{max,n}$, $K_{S,n}$, Y_n and $k_{d,n}$ are the kinetics parameters associated with growth, substrate uptake and endogenous decay for the genera Nitrosomonas. S_n represents ammonium nitrogen (NH_4^+-N), and X_n is the *Nitrosomonas* concentration. Similar definitions can be worked out for nitritification.

Table 2.4 presents some typical values of Monod parameters for nitrifying bacteria (Alleman, 1978; Barnes and Bliss, 1983; Grandie and Lim, 1980; Gray, 1990; Horan, 1990; Sharma and Ahlert, 1977; Tchobanoglous and Burton, 1991). The first thing to notice in this table, is that μ_{max} for nitrifiers is at least an order of magnitude smaller than that for heterotrophs. This once more highlights the idea that nitrification will only occur in reactors in which the specific growth rate is quite low. It is also worth observing that the μ_{max} for *Nitrosomonas* is somewhat greater than that for *Nitrobacter*; K_S is also quite low for both genera. This depicts the case where the monod expression is simplified to the relationship presented in equation 2.8, which characterizes a zero order reaction with respect to the substrate (Alleman, 1978; Grandie and Lim, 1980). The nitrifiers yield is also much smaller than for heterotrophs. The explanation can be ascertained from the nominal energy availability in the oxidation of ammonium and nitrite (Alleman, 1978). Finally, is still worth noticing that the *Nitrosomonas* yield is generally greater than that of *Nitrobacter*, which reflects the relationship between the energy produced, for both genera, during the nitritification and nitratification steps.

2.4.3 Environmental Factors Affecting Nitrification

From the above considerations about nitrification, it is easy to infer that environmental factors will have a more influential effect on this process than for oxic heterotrophic oxidation. Therefore, in this section, this subject will be looked at in

Parameter	Basis	*Nitrosomonas*	*Nitrobacter*
μ_{max}	d^{-1}	0.3 to 2.2	0.14 to 1.44
K_S	NH_4^+-N mg/L NO_2^--N mg/L	0.06 to 5.6	0.06 to 8.4
Y	NH_4^+-N mg VSS/mg NO_2^--N mg VSS/mg	0.03 to 0.13	0.02 to 0.08
k_d	d^{-1}	0.03 to 0.12	0.03 to 0.13

Table 2.4: Typical values of Monod parameters for nitrifiers

some detail.

a) Temperature: Both, nitrifiers growth rate and half-saturation coefficient are intensely affected by changes in temperature. The temperature effect on the specific growth rate has been proposed to follow an Arrhenius' equation (Barnes and Bliss, 1983; Grandie and Lim, 1980; Gray, 1990; Sedlak, 1991; Sharma and Ahlert, 1977; Tchobanoglous and Burton, 1991) namely:

$$\mu_{max,T} = \mu_{max,15^\circ C} e^{K(T - 15)} \qquad 2.24$$

where, $\mu_{max,T}$ is the specific growth rate at any temperature, and $\mu_{max,15^\circ C}$ is the rate at 15°C. The latter has been reported to be 0.47 d^{-1}. K is the temperature constant, expressed in $(°C)^{-1}$. For *Nitrosomonas*, K has been found to be in the range of 0.095 to 0.12 $(°C)^{-1}$, while in the case of *Nitrobacter*, the range is between 0.056 and 0.069 $(°C)^{-1}$. The following equations have been proposed to represent the effect of the temperature upon the half-saturation coefficient (Barnes and Bliss, 1983):

For *Nitrosomonas*:

$$K_{S,T} = 0.405\, e^{0.118(T - 15)} \qquad 2.25$$

For *Nitrobacter*:

$$K_{S,T} = 0.625\, e^{0.146(T - 15)} \qquad 2.26$$

Finally, Sharma and Ahlert (1977) presented a review on the subject. They concluded that the overall optimum temperature for nitrification is in the range of 28 to 36°C. In this review, the minimum temperature at which some nitrifier growth could still be observed was 5°C.

b) Dissolved Oxygen (DO) Concentration: Oxygen is utilized in the oxidation reactions carried out by the nitrifiers. In activated sludge systems, the oxygen concentration is frequently present at a level which limits nitrifiers growth to less than the maximum value. In this case, the oxygen concentration has to be considered as a limiting substrate in addition to the energy substrate, ammonium nitrogen. Barnes and Bliss (1983), presented the Monod equation combining the effects of ammonium nitrogen and dissolved oxygen on the growth of *Nitrosomonas*,

$$\mu_n = \mu_{max,n}\left[\frac{(NH_4^+ - N)}{K_{S,n} + (NH_4^+ - N)}\right]\left[\frac{DO}{K_O + DO}\right] \qquad 2.27$$

where K_O, the half-saturation coefficient for oxygen, has been reported to vary from 0.15 to 2.0 mg/L, with 1.3 mg/L being a typical value. Results from a number of studies on the effect of DO concentration upon the nitrification process have been summarized by Sharma and Ahlert (1977). The optimum DO concentration for nitrifiers was found to be in the range of 2 to 3 mg/L. They concluded that below 1 mg/L, the DO concentration starts to be rate limiting, and that the critical value below which nitrification does not happen was 0.2 mg/L. In principle, there is no problems with an upper limit. No adverse effects were verified at DO concentrations up to 60 mg/L.

c) pH: As can be seen from equation 2.19, alkalinity is depleted during nitrification. As a consequence the pH may go down to values as low as 5.0 or even lower, depending on the sewage original alkalinity (IWPC, 1987). It may cause nitrification to come to a halt, because of the inhibition caused by the low pH value. Besides affecting the nitrifiers, this low pH will also affect the heterotrophs (see section 2.3.3). The effect on the nitrifiers specific growth rate has been suggested to follow equation 2.28 over the pH range of 7.2 to 8.0 (Barnes and Bliss, 1983).

$$\mu_{n,pH} = \mu_{max,n}\,[1 - 0.83(7.2 - pH)] \qquad 2.28$$

An alternative expression has been proposed by Benefield and Randal (1980), also reviewed by Barnes and Bliss (1983),

$$\mu_{n,pH} = \frac{\mu_{max,n,pH_{opt}}}{1 + 0.04\left[10^{(pH_{opt} - pH)} - 1\right]} \qquad 2.29$$

where, pH_{opt} is in the range of 8.0 to 8.4 . In reality, the optimum range of pH values for the overall nitrification process, has been reported to be much wider than that. Sharma and Ahlert(1977) summarized a range of 7.0 to 9.0, from the works reviewed by these authors. Alleman (1984) suggested two separate ranges, one for *Nitrosomonas*, 7.9 to 8.2, and other for *Nitrobacter*, 7.2 to 7.6 . This suggests that *Nitrobacter* favours a somewhat less basic environment than *Nitrosomonas*. Some criticism have been made about the way these ranges were calculated (Barnes and Bliss, 1983; Gray, 1990). These authors maintained that the majority of these studies were short-term, and that the possibility of acclimatization was not taken in consideration. They claimed that when acclimatization is allowed, there have been reports of nitrifiers growing at near maximum rates at pH 6.5 or even lower. The acclimatization process may take several weeks. For example, a pH shift from 7 to 6 required 10 days of acclimatization before nitrification returned to its former rate. However, there are limits beyond which no acclimatization will help. Sharma

and Ahlert (1977) reported pH 11 and pH 4, as the maximum and minimum limits, outside which no nitrification would be possible: pH also affects the chemical equilibrium between ionized and non-ionized forms of ammonia and nitrite. As it will be seen in the next item, this has a profound effect upon nitrification due to possible inhibitory effects upon the nitrifier genera.

d) Presence of Energy Substrate and Products: Both, *Nitrosomonas* and *Nitrobacter*, are sensitive to the presence of ammonia and nitrite. Normally, reported critical concentrations are high, and most of then disagree with each other. Anthonisen, 1974, cited in Sharma and Ahlert (1977), put forward a mechanism by which pH affects the inhibition of the nitrification process, caused by ammonia and nitrite. The degree of inhibition will depend on the ammonia-ammonium and the nitrite-nitrous acid equilibria, which is pH function. He postulated that, depending on the pH of both intracellular and extracellular medium, free ammonia (FA) or free nitrous acid (FNA) may penetrate the cell, causing inhibition. Their ionized forms, ammonium and nitrite ions, on the contrary, are not capable of doing so, and therefore they remain in the extracellular medium. Based on that, other authors have suggested that the true energy substrate for *Nitrosomonas* and *Nitrobacter*, are respectively FA and FNA, rather than their ionized forms. In his original work, Anthonisen prepared an operational chart for evaluating nitrifying systems relating pH, ionized and un-ionized energy substrate presence and inhibition ranges (see Figure 2.2). Based on chemical equilibrium theory, the following formula can be used to calculate the ammonium ion percentage as function of the pH.

$$NH_4^+ (\%) = \frac{100}{1 + K_a \times 10^{pH}} \qquad 2.30$$

In this equation, K_a represents the ionization constant, which is function of the temperature. For example, for 15°C K_a is 2.73×10^{-10} and for 20°C, K_a is 3.98×10^{-10}(Barnes and Bliss, 1983). From a close examination of Figure 2.2

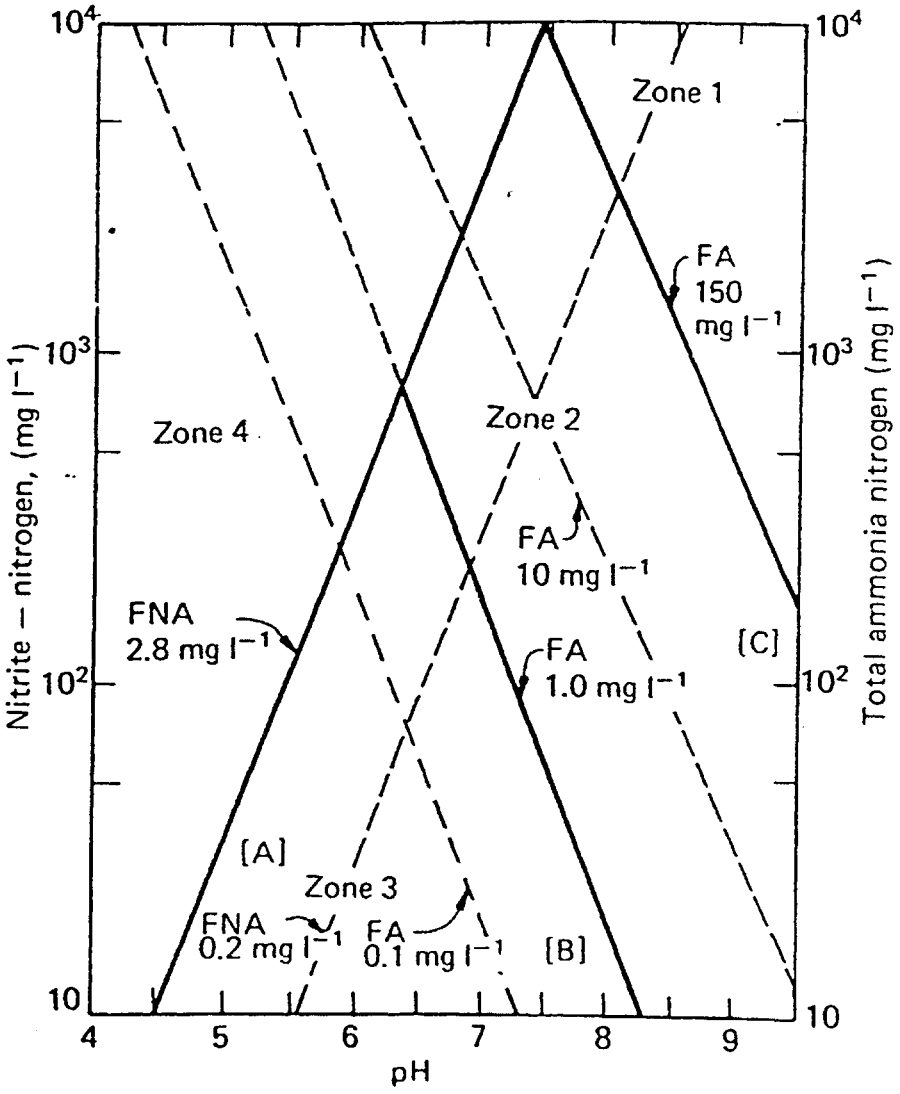


Figure 2.2: Inhibition of nitrifying bacteria by free ammonia (FA) and free nitrous acid (FNA)
(Anthonisen, 1974) Modified by Barnes and Bliss (1983)

it is possible to conclude that inhibition of *Nitrosomonas* by FA is likely in the range of 10 to 150 mg/L, and that inhibition of *Nitrobacter* by FA is probable at a lower value, from 0.1 to 1.0 mg/L. *Nitrobacter* can be further inhibited by FNA in the range of 0.2 to 2.8 mg/L. If only *Nitrobacter* is inhibited, nitrification may come to a halt at the nitrite stage, and nitrite will accumulate as a result. Contrary to the common view, it is clear that *Nitrobacter* is the weaker link in the nitrification chain. Recognizing that, and its potentialities regarding the denitrification process (see next section), Alleman (1984) presented a comprehensive review on the factors that could cause *Nitrobacter* inhibition with the consequent nitrite accumulation. He listed the following stress conditions capable of favour nitrite accumulation:

- Reduced temperature;
- Limiting O_2 or CO_2 presence;
- Elevated pH;
- Free ammonia presence associated with nitrification startups, acute process spikes, and batch or plug-flow type reactors;
- Excess or acute solids wastage;
- Acute process loadings;
- Cryptic nitrate reduction.

Finally, because of the pH effect on the energy substrate specie present, ionized or non-ionized, care should be taken when using equations 2.28 and 2.29 .

e) Redox: It has been suggested, in the literature, an optimum redox range for nitrification of 100 to 300 mV, using the standard hydrogen electrode (SHE) as reference (Wanner, 1991).

f) Others: It has been argued that due to the autotrophic nature of nitrifiers, they would be somehow inhibited by the presence of organic matter. This has been proved to be false, both for pure and mixed cultures. Indeed, nitrification can proceed at maximum rates in the presence of organic matter, provided that other environmental conditions are adequate, e.g., pH, DO, temperature, etc

(Grandie and Lim, 1980). On the other hand, there are some organic compounds that are inhibitory to nitrifiers and not to heterotrophs. For example, it has been found that some metal chelating compounds containing sulphur, such as thiourea, thioacetamide and dithio-oxamide, may inhibit nitrifiers because many of these bacteria enzymes require metal for activation (Sharma and Ahlert, 1977). This author also reported some works, which suggest that light may inhibit the activity of nitrifiers. It seems that nitrification proceeds more rapidly in cultures placed in the dark. Finally, as it would be expected, the sludge age is of paramount importance in nitrification process. A sludge age of at least 3 to 4 days, appears to be required in most suspended-growth systems, to achieve a high degree of nitrification. The optimum sludge age can be calculated as the inverse of the specific growth rate for the nitrifiers, $\mu_{max,n}$, and applying a safety factor to it (Sedlak, 1991).

2.5 Denitrification

The first to notice bacterial nitrate reduction were Gayon and Dupetit, in 1886. They observed the disappearance of nitrate and nitrite, with simultaneous production of nitrogen and nitrous oxide gases, when they were studying the application of nitrified sewage effluent to sand columns (Alleman, 1978). Despite this early discovery, knowledge in this field is still very limited.

2.5.1 Background

In biological processes, nitrate may be reduced in two different ways, each one requiring the use of a separate enzymatic system (Grandie and Lim, 1980). They are: assimilatory nitrate reduction and dissimilatory nitrate reduction. During assimilatory nitrate reduction, the enzyme assimilatory nitrate reductase catalyzes the transformation of nitrate to ammonia by following the inverse metabolic route of nitrification, the ammonia resultant being used for the synthesis of new cell structures.

$$\underset{(+5)}{NO_3^-} \rightharpoonup \underset{(+3)}{NO_2^-} \rightharpoonup \underset{(+1)}{?} \rightharpoonup \underset{(-1)}{NH_2OH} \rightharpoonup \underset{(-3)}{NH_4^+} \qquad 2.31$$

This process only works when nitrate is the sole form of nitrogen present. It can happen in the presence of dissolved oxygen and it is not coupled to the electron transport chain. The other one, dissimilatory nitrate reduction, is also known as anaerobic or nitrate respiration, or as denitrification.

Denitrification can be defined as a biological process that occurs under low, or zero, dissolved oxygen concentrations, where oxidized forms of nitrogen, nitrate and nitrite, are converted to more reduced forms, such as molecular nitrogen (N_2), nitrous oxide (N_2O) and nitric oxide (NO). This process is carried out by facultative heterotrophic bacteria, which are usually found in large numbers in biological wastewater treatment plants, including: *Denitrobacillum, Micrococcus denitrificans, Spirillum, Bacillus, Pseudomonas, Alcaligenes,* and *Achromobacter,* among others (Grandie and Lim, 1980; Hänel, 1988; Horan, 1990). These same bacteria are capable of performing organic carbon oxidation under oxic conditions. Also, the metabolic pathways utilized for oxic and anaerobic respiration are very similar, and the only major differences are in the enzymes catalyzing the terminal electron transfer and their sites in the electron transport chain (Grandie and Lim, 1980; Sedlak, 1991). Payne (1973), cited in Alleman (1978), presented the following denitrification pathway.

$$\underset{(+5)}{NO_3^-} \rightharpoonup \underset{(+3)}{NO_2^-} \rightharpoonup \underset{(+2)}{NO_{(gas)}} \rightharpoonup \underset{(+1)}{N_2O_{(gas)}} \rightharpoonup \underset{(0)}{N_{2\,(gas)}} \qquad 2.32$$

Grandie, (1980) explains this multistep reaction sequence as folows. The first step involves the reduction of nitrate to nitrite by the enzyme dissimilatory nitrate reductase, utilizing electrons from cytochrome b of the electron transport chain. Delineation of the pathway involved beyond the nitrite form is not well defined, but some works have suggested that before the formation of nitrous oxide (N_2O) and molecular nitrogen (N_2), nitric oxide (NO) is formed as an intermediate by the

activity of cytochrome oxidase, which transfers electrons from cytochrome$_c$ to nitrite. As a consequence, cytochrome$_a$ is bypassed in the electron transport chain, and therefore only two moles of ATP are formed, compared with the three possible under oxic respiration. The true denitrification end product will depend on diverse factors, e.g., environmental parameters, bacteria involved, etc.

From the above comments, it can be easily inferred that denitrification will proceed only if a source of electron donor is available. After all, the electrons used to transform nitrate and nitrite to their reduced gaseous forms, have to come from somewhere. Normally, organic compounds are used as electron donors. Before going any further, it is necessary to understand that bacteria do not use the denitrification process just to reduce nitrogen, but as a coupled reaction for the simultaneous oxidation of organic carbon substrates. Historically, different sources of electron donors have been tried, which can be grouped as: wastewater, endogenous and storage products, and supplemental (Alleman, 1978).

There has been some evidence that points toward the possibility of nitrite accumulation caused by either a shortage of electron donor or lack of time for denitrification to be completed. Koné and Brehrens (1981), reviewed in Hänel (1988), reported an intermediate nitrite accumulation as high as 700 mg/l during denitrification, with acetate as the electron donor.

Influent raw or settled wastewater may be used as a reliable and inexpensive source of organic carbon. Barnes and Bliss (1983) commented that as sewage contains other materials besides organic matter, such as organic nitrogen, ammonia and pathogenic organisms, among others, care should be taken in choosing the appropriate sequence of nitrification/ denitrification, in order to make sure that they are effectively removed, before leaving treatment.

Endogenous carbon is yet another source of electron donor. It has been suggested that the carbon present inside the bacterial cell would meet the needs of denitrification (Alleman, 1978). This author concentrated his attention on the use of

cell storage products, such as PHB and glycogen, to provide organic carbon for denitrification. He obtained 92% nitrogen removal, using a sequencing batch reactor (SBR) operated for nitrification/ denitrification, employing storage products as electron donors. When the cell reserves had been exhausted, denitrification proceeded at the expense of cell integrity. This author also showed that some release of ammonia ocurred during the anoxic denitrification period. This can be explained by the destruction of nitrogen-containing cell material or by some inefficiency in the enzymatic nitrogen-reducing pathway.

By far the most widely used electron donor source is the supplemental one. By supplemental carbon source it is understood any chemical or mixture of chemicals capable of meeting the denitrifying cell demand, in terms of electron donor. Barnes and Bliss (1983) listed the following axioms that, ideally, any supplemental carbon source should meet: readily available at the site, non-inhibitory to the biological community, in an inexpensive reproducible form and at an appropriate concentration. Methanol, acetic acid, acetone and citric acid are among those more frequently mentioned in the literature. The use of any of these organic chemicals will add to the treatment plant running costs. On this basis, methanol has been seen as the most favourable (Sedlak, 1991). Besides these, some industrial and agricultural wastes, have been mentioned as a possible alternatives (Hänel, 1988; Horan, 1990). Hänel (1988) also suggested the use of some source of hydrogen as an electron donor, such as methane and molecular hydrogen, together with the cell reserves, would meet the denitrification requirements.

McCarty *et al.* (1969) proposed the following stoichiometry for the reactions involved in the denitrification process, using methanol as carbon source (IWPC, 1987).

Overall nitrate removal:

$$NO_3^- + 1.08CH_3OH + H^+ \longrightarrow 0.065C_5H_7NO_2 + 0.47N_2 + 0.76CO_2 + 2.44H_2O \qquad 2.33$$

Overall nitrite removal:

$$NO_2^- + 0.67CH_3OH + H^+ \longrightarrow 0.04C_5H_7NO_2 + 0.48N_2 + 0.47CO_2 + 1.7H_2O \quad (2.34)$$

Overall deoxygenation reaction:

$$O_2 + 0.93CH_3OH + 0.056NO_3^+ + 0.056H^+ \longrightarrow 0.056C_5H_7NO_2 + 0.65CO_2 + 1.69H_2O \quad (2.35)$$

Equation 2.33 represents the nitrate reduction to nitrogen gas. In this reaction, 1 mg/L nitrate-nitrogen requires 2.47 mg/L of methanol to produce 0.525 mg/L of new cells. A nitrified sewage is likely to contain some nitrite. In this case, denitrification can proceed as shown in equation 2.34 , requiring 1.53 mg/L of methanol and producing 0.322 mg/L of new cells, for each mg/L of nitrite reduced. If at the beginning of the denitrification, the sewage still contains some dissolved oxygen, it will be used to oxidize a portion of the methanol added, before the reduction of nitrate or nitrite would take place. This reaction is shown in equation 2.35 . It is worth noticing that some nitrate will be reduced and that for each mg/L of dissolved oxygen, 0.93 mg/L of methanol are used, 0.025 mg/L of nitrate are reduced, and 0.2 mg/L of new cells are formed. From these equations, formulas for the calculation of the methanol needed and of the biomass produced, to achieve full denitrification were derived as follows,

$$C_{CH_3OH} = 2.47(NO_3^- - N) + 1.53(NO_2^- - N) + 0.87(DO) \quad (2.37)$$

$$C_{Biomass} = 0.53(NO_3^- - N) + 0.32(NO_2^- - N) + 0.19(DO) \quad (2.38)$$

where, the nitrate, nitrite and DO concentrations, are the amounts present at the beginning of the denitrification. The terms in these formulas are self explanatory.

Looking at equation 2.35 and to the stoichiometric quantities related to it, one could speculate if it would not be more sensible to start the denitrification process at the nitrite level. The advantages of such a shortcut (see Figure 2.3) are evident; less carbon source required and less biomass produced. In order to make this proposition feasible, it would be necessary to stop the nitrification process before the nitrite oxidation could take place, in this way allowing nitrite to accumulate. From the point of view of nitrification, it would be beneficial as well, because less electron acceptor (DO) would be necessary. Indeed, some research has been done on this matter. Among them it is worth mentioning the works of Pantea-kiser (1989), Turk and Mavinic (1989a; 1989b), and Alleman (1984). These authors have claimed that it is possible to sustain denitrification in these conditions. However, it seems that they reached a deadlock regarding the nitrification process. In their experiments, they were able to selectively inhibit *Nitrobacter*, therefore allowing the nitrite to accumulate. The problem was that this situation could not be maintained for more than 4 months at the most, due to the acclimatization of *Nitrobacter* to all inhibitory compounds and procedures used up to then. Yet, this is an interesting and very promising research line.

From equations 2.34 and 2.35, it can be seen that denitrification will consume protons, consequently increasing the sewage alkalinity. This can be particularly important for sewages with low alkalinity and poorly buffered, when they undergo a sequenced nitrification/ denitrification process. It has been calculated that denitrification replaces approximately 50% of the alkalinity lost during nitrification. As it has been shown in section 2.4.1, nitrification will reduce alkalinity by approximately 7.1 mg $CaCO_3$/ mg N oxidized, while denitrification will produce 3.6 mg $CaCO_3$/ mg N reduced (Wilson, 1981).

An endogenous respiration equation, using nitrate instead of dissolved oxygen, has been also suggested (Sedlak, 1991).

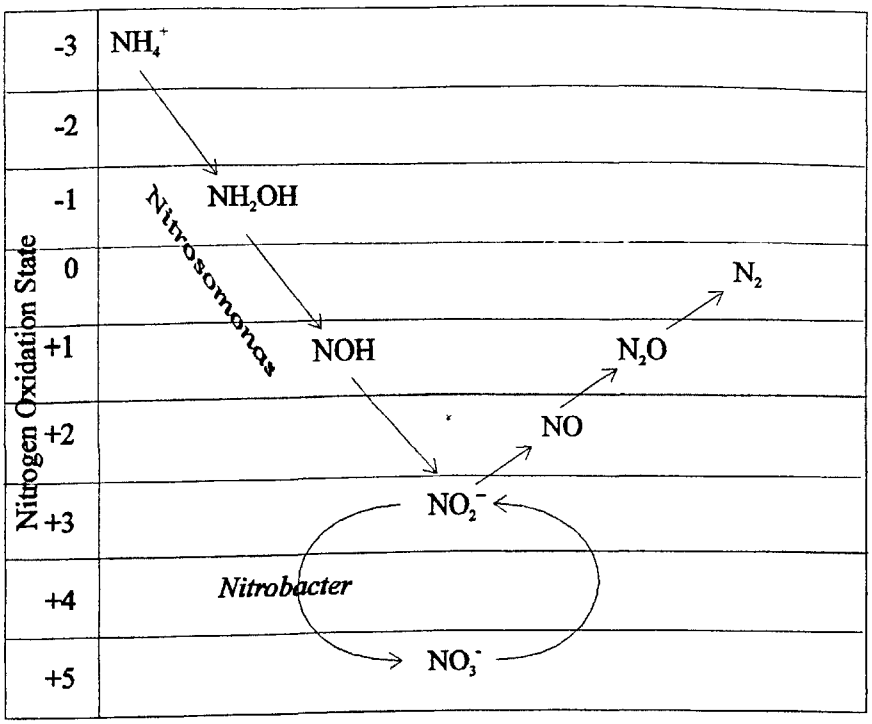


Figure 2.3: Nitrogen Removal Shortcut (Alleman, 1984) Modified

$$C_5H_7NO_2 + 4.6NO_3^- \longrightarrow 5CO_2 + 2.8N_2 + 4.6OH^- + 1.2H_2O \qquad 2.38$$

2.5.2 Kinetics of Denitrification

The concept of a single-rate limiting substrate was utilized to model both, oxic organic carbon oxidation and nitrification processes. In their cases, this kind of approach was justified, because they met the requirements of this kind of model, i.e, the existence of only one limiting substrate at any time. These limiting substrates were organic matter for oxic oxidation of the organic matter, and ammonia or nitrite for nitrification. Denitrification presents a somehow more complex mechanism, involving the concept of a dual limiting substrate., in this case the nitrate or nitrite

and the electron donor source (Alleman, 1978; Grandie and Lim, 1980). These authors suggested the use of the Monod relationship applied to the dual limiting substrate assumption, as follows:

$$\mu_d = \mu_{max,d}\left(\frac{S}{K_S + S}\right)\left(\frac{N}{K_N + N}\right) \qquad 2.39$$

where, S and N represent organic matter and nitrate nitrogen concentration (mg/L), respectively, μ_n and $\mu_{max,n}$ are related to the denitrifiers growth rate (d^{-1}) and, K_S and K_N are the half saturation coefficients (mg/L).

Focht and Chang (1975) pointed out the complexity involved in understanding and interpreting the dual limiting substrate model, let alone the lack of understanding of the involved metabolic pathways (Alleman, 1978). Because of that, a more simplistic approach has been favoured by the majority of researchers in this field. This approach involves the use of the traditional one limiting substrate Monod kinetics. As reviewed in Horan (1990), Hänel (1988), and Sedlak (1991), denitrification is thought to occur in the presence of a carbon source substrate in excess of the bacterial needs. Therefore, the only limiting substrate is nitrate, and the rate of nitrate uptake can be expressed as:

$$\frac{dN}{dt} = \mu_{max,d}\frac{N}{K_N + N}X \qquad 2.40$$

where, X is the concentration of denitrifiers (mg MLVSS/L). However, K_N values are generally very low compared with N, which characterizes the condition where the rate of nitrate uptake is zero order with relation to the nitrate concentration. Therefore, equation 2.40 can be simplified as follows.

$$\frac{dN}{dt} = \mu_{max,n}X \qquad 2.41$$

Table 2.5 presents a range of values of the kinetic coefficients for the denitrification process, as reported in the literature.

Parameter	Basis	NO_3^--N Substrate	Carbon Source Substrate
μ_{max}	d^{-1}	0.24 to 0.9	3.1 to 8.0
K_S	mg(NO_3^--N)/L mg(COD)/L	0.06 to 0.2	10 to 72.5
Y	mg(VSS)/mg (NO_3^--N) mg(VSS)/mg (COD)	0.4 to 0.9	0.18
k_d	d^{-1}	0.04 to 0.08	0.048

Table 2.5: Kinetic Constants for Denitrification. (Alleman, 1978; Barnes and Bliss, 1983; Grandie and Lim, 1980; Horan, 1990; Tchobanoglous and Burton, 1991)

Finally, it seems that the rate of denitrification is influenced by the sludge age and by the organic load. Barnes and Bliss (1983) mentioned that an increase in sludge age from 3 to 15 days, decreased the denitrification rate by a factor of 2 to 3. Systems with a low organic loading have been reported to present also,low denitrification rates (Hänel, 1988). The explanation presented by these authors is related to the fact that a more heavily loaded sludge will attain a higher concentration of active biomass. Therefore, a low denitrification rate would be expected for lightly loaded systems.

2.5.3 Environmental Parameters Affecting Denitrification

As denitrification is carried out by heterotrophs, it is understandable that this is a much more robust process than nitrification. Therefore, denitrification will be less affected by the environmental factors than nitrification. These factors are:

a) Temperature: Temperature will affect the performance of denitrification systems, as it would for any other biological process. The optimum temperature range reported in the literature is from 25 to 35°C, being considered that even at temperatures as low as 0°C, and as high as 50°C, some denitrification can still occur (Barnes and Bliss, 1983). In terms of the temperature effect upon the maximum specific growth rate for denitrifiers, the following equation has been proposed (Sedlak, 1991):

$$\mu_{max,T} = \mu_{max,20^\circ C} K^{(T - 20^\circ C)} \qquad 2.42$$

where, K varies from 1.03 to 1.1 (dimensionless), and $\mu_{max,T}$ and $\mu_{max,20°C}$ are the maximum specific growth rate at the temperature T and at 20°C, respectively.

b) Dissolved oxygen (DO) concentration: The ideal condition for denitrification is zero DO, but there are some evidence suggesting that denitrification can still occur if some DO is present. Grandie, (1980) presented a very good review regarding the DO effects upon denitrification. He mentioned that the biosynthesis of denitrifying enzymes is inversely related to DO. Yet it is known that the dissimilatory nitrate reductase can be formed under low DO concentrations, although no nitrite reductase can be formed in this condition. From his review, it is possible to infer that denitrification would occur at limited rates in the presence of DO, provided that a period of anoxic conditions had occurred before, during which enzymes would be formed. Alleman (1978) acknowledged the above arguments, emphasizing that the principal effect is related to the presence of a preferred electron acceptor, the DO. A maximum DO concentration of 0.2 mg/L has been suggested to describe the low DO condition, and that above 1.0 mg/L, denitrification can be ignored (Sedlak, 1991).

c) pH: As already mentioned, denitrification will increase the pH of the sewage (see equations (2,34) and (2,35)). It has been reported that at pH above 7.3, the release of nitrous oxide, as the end product of denitrification,increased

(Grandie and Lim, 1980). A possible explanation for the phenomena would be related to a selective pH effect on the biomass. An optimum pH range of 6.5 to 7.5 has been recommended by different authors. Focht and Chang (1975) claimed that a slightly alkaline pH, would be beneficial for the process, favouring faster denitrification rates and a more complete reduction to gas.

d) Presence of inhibitory compounds: Denitrification can be inhibited by much the same compounds that inhibit heterotrophs in general. As already mentioned, DO is the principal inhibitor for denitrification.

e) Presence of different electron donor sources: The kind of carbon source will have a profound twofold effect on denitrification. First of all, the substrate uptake rate will vary in function of the chosen electron donor source. The other effect is related to the denitrification rate. As observed by Hänel (1988) the denitrification rate when using methanol can be up to ten times greater than when using wastewater or endogenous respiration products. Table 2.6 presents observed denitrification rate and substrate demand ranges for methanol, wastewater and endogenous respiration products (Sedlak, 1991).

Electron Donor Substrate	**Denitrification Rates $g(NO_3^-N)/g(MLVSS).d$**	**Substrate Demand $g(BOD_5)/g(NO_3^-N)$**
Raw Sewage	0.03 to 0.11	1.5 to 6.0
Endogenous Products	0.017 to 0.048	2.0 to 3.5
Methanol	0.12 to 0.9	1.9

Table 2.6: Denitrification: Rates and Substrate demands (Hänel, 1988 and Sedlak, 1991)

f) Redox: A narrow range of redox characterizes the anoxic conditions necessary for denitrification. Wanner (1991) suggests the range from -50 to +50 mV (SHE) for that.

2.6 The Sequencing Batch Reactor

2.6.1 Introduction

The processes described above, namely carbon removal, nitrification and denitrification, are normally accomplished by using segmented activated sludge reactors. While such a plant would likely meet whatever effluent goals the designer was faced with, the complexity of operation, and the costs involved would not make it the best alternative available. Essentially the problem calls for a single stage reactor, whose routine operation provides considerable variation in environmental conditions and does so in a cyclical way. A "fill-and-draw" reactor would comply with these requirements. This kind of reactor, given its temporal control over operating modes, could provide for a diversity of environmental conditions in a repetitive way designed for carbon and nitrogen removal. Basically, during the react phases, this reactor would operate in a manner similar to the spatial flow through the diverse zones of a true plug flow system. This "fill-and-draw" reactor has been renamed as Sequencing Batch Reactor, SBR for short (Irvine and Davis, 1971). The new name has been proposed in recognition of the fact that additional cycles would have to be added to the basic "fill-and-draw" scheme. In fact, the terminology seems appropriate for the description of a reactor whose sequential and cyclic operation revolves around a batch type kinetics.

2.6.2 Process Description

The SBR can be described as a time based activated sludge system. In this way it can be said that the SBR provides in time what the conventional activated sludge process provides in space. Each tank in a SBR system is filled with wastewater during a discrete period of time. When the working volume has been

completed, the flow is diverted to another SBR, in the case of a multiple SBR system, or to a storage tank, for single SBR configurations. After treatment, the mixed liquor is allowed to settle for a predetermined amount of time and, then, the clarified supernatant is decanted.

As a periodic system, the SBR works complying with a cycle. Irvine(1979), proposed a standardized nomenclature to describe the five basic operational steps of the SBR cycle (see Figure 2.4). They were called: Fill, React, Settle, Draw and Idle. Each one of them has a specific function and, although they are quite descriptive, they may be somehow misleading. For example, Fill clearly means that the tank is being filled. However, it is erroneous to assume that no treatment occurs during this phase. Before Fill starts, there is an active and sizeable biomass inside the reactor, which will interact with the incoming sewage if mixing and/or aeration is provided. As a result, the bulk of the treatment may be completed before Fill ends, unless some action is taken to slow down the reactions. When the inflow stops, the React phase starts. Now, aeration and/or mixing should be supplied, according to the operational goals, in order to meet the required effluent quality. A short burst of air may be supplied at the end of this period, to avoid denitrification occurring during the next phase. In the Settle phase, clarification occurs under almost perfect quiescent conditions, mostly because there is no incoming flow to disturb the process. Care should be exercised not to prolong this period more than necessary, in order to avoid settled sludge being provoked to float as a result of an inconvenient denitrification process. In the next phase, the Draw period, the clarified effluent is discharged. After Draw, the remaining biomass is allowed to rest until the beginning of the next cycle. This phase is called Idle. Yet another operation may take place, normally during the React or Idle phases. It is the wastage of surplus biomass, the frequency being determined by the net solids increase in the system each day and the capacity of the mixing and aeration equipment.

Although the SBR works in a batch fashion, it can be used to treat either continuous or discontinuous influents. In the first case, a system with two or more

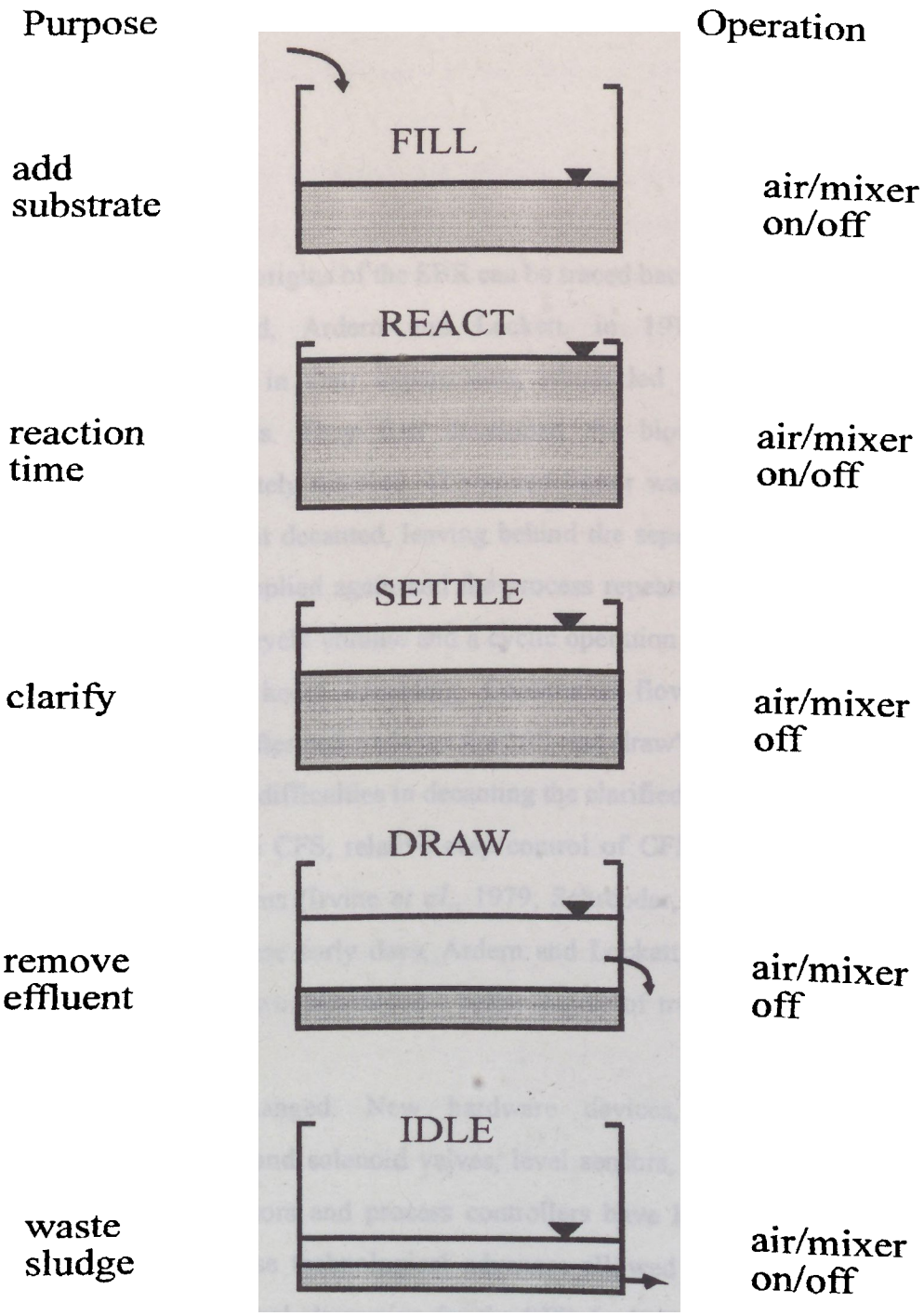


Figure 2.4: Typical SBR Cycle

SBRs would cope with this kind of flow, the criteria being that one reactor must have completed the Draw phase before to another completing Fill. In the case of discontinuous flows, like those that occur in the food-processing industry or in small towns, a system with one SBR and a storage tank would be enough (Irvine and Bush, 1979).

2.6.3 Historical

Surprisingly, the origins of the SBR can be traced back to the activated sludge discovery itself. Indeed, Ardern and Lockett, in 1914, used bench scale "fill-and-draw" reactors in their experiments, which led to the investigation of activated sludge process. They first developed the biomass by aerating raw wastewater until completely nitrified. The mixed liquor was then allowed to settle and, the clear supernatant decanted, leaving behind the separated suspended solids. Raw wastewater was supplied again and the process repeated. Good treatment was obtained using a 20% recycle volume and a cyclic operation of 4 hours aeration, 1.5 hours settling and 0.5 hours decanting. Continuous flow systems (CFS) were developed shortly thereafter and replaced the "fill-and-draw" treatment scheme. The reasons for that included difficulties in decanting the clarified effluent, lower effluent SS concentrations of the CFS, relative easy control of CFS and diffuser clogging problems in batch systems (Irvine *et al.*, 1979; Schroeder, 1982). It is interesting noticing that even in these early days, Ardern and Lockett, 1914, mentioning that "fill-and-draw" systems would achieve a better degree of treatment than the CFS.

Times have changed. New hardware devices, such as motorized, pneumatically operated and solenoid valves, level sensors, flow meters, automatic timers and microprocessors and process controllers have been developed and are routinely available. These technological advances allowed for the "fill-and-draw" reactor revival, as an actual alternative for the CFS. In 1971, Irvine started working with bench scale units at the University of Notre Dame, Indiana, USA (Irvine and Davis, 1971). As already mentioned, he proposed the SBR term to differentiate it from the conventional full-time aerated "fill-and-draw" process. Since then, much

work have been done by Irvine and co-authors in investigating the use of SBRs for the treatment of domestic and industrial sewage treatment and nutrient removal. Today, there are researchers working with this process all around the world.

2.6.4 Advantages and Disadvantages of the SBR compared with the CFS

Probably, the most attractive and obvious advantage is related to the capital savings that can be achieved through the use of a SBR scheme, as opposed to a CFS one. The conventional activated sludge process, at least, consists of one aeration tank, one sedimentation tank and a sludge recirculation system, while only one tank is necessary for SBRs (Irvine and Bush, 1979). Besides that, simulation studies conducted by Irvine and Richter (1976) have suggested that a several fold reduction in total reactor volume required may be possible, when compared with CFS.

Most of the advantages of SBR systems are attributed to the very flexible nature of the operating parameters. A large choice of system operation may be brought about for a fixed reactor volume, just by changing the Fill/React ratios and aeration modes. However, these very advantages can also be handicaps, needing a much more complex control strategy. Additionally, the flexibility in operation, also complicates the basic mechanistic understanding of the process (Orhon *et al.*, 1986).

Some more specific advantages and disadvantages, mentioned in the specialized literature, are listed below.

a) During the Fill phase, the SBR can be used as an equalization basin, consequently being able to tolerate more easily peak flows and shock loads (Silverstein and Schroeder, 1983).

b) Because effluent discharge is periodic, within limits, it can be held until it meets the required quality. Also, because of that mixed liquor solids can not be washed out by hydraulic surges (Arora *et al.*, 1985).

c) During early design life, when flow is significantly lower than the full design capacity, liquid level sensors can be set at a lower level in order that only a fraction of the SBR tank capacity will be used (Arora *et al.*, 1985).

d) As already mentioned, during the Settle phase, sedimentation will occur under almost perfect quiescent conditions, with short-circuiting, the common plague of continuous flow settling tanks, almost non-existent (Arora *et al.*, 1985).e) Each tank in the system can be operated in a way that either plug-flow (short Fill periods) or completely mixed (extended Fill period) behaviour would be encouraged without extra costs (Irvine and Bush, 1979).

f) Because the DO concentration is zero or near zero during the anoxic Fill period, the initial oxygen driving gradient at the start of the React phase will be greater than under normal conditions. This would provoke somehow higher overall oxygen transfer efficiency with the same aeration equipment (Arora *et al.*, 1985).

g) Filamentous growth can be easily controlled by the introduction of anoxic Fill period (Chiesa and Irvine, 1985; Irvine and Bush, 1979).

h) Batch systems are known to be better than the CFS for treating industrial wastewater, because the former are not designed to be operated under steady-state conditions. Therefore, the SBR will cope much better than CFS, with the influent variations in flow and strength (Dennis and Irvine, 1979).

i) There are some indications that SBRs have a kinetic advantage over CFSs. It has been showed that the microorganism ribonucleic acid contents are three or four times greater in SBRs (Irvine *et al.*, 1985).

j) Ketchum and Liao (1979) suggested that SBRs may have a lower sludge yield than CFSs.

k) As a research tool, SBRs are much more useful than continuous systems, because the former allow the researcher to observe the reaction time course (Corei *et al.*, 1990).

l) Irvine and Richter (1976) put forward a possible SBR setback. In systems employing an anoxic Fill routine, the oxygen uptake rate at the beginning of the React phase, can be very high. Indeed, they concluded that, in some cases, the oxygen demand would be greater than the maximum capacity of the aeration system.

2.6.5 Process Characteristics

Batch systems are inherently non-steady-state processes, therefore any possible relationship between growth rate and sludge age, as it is defined for CFS under steady-state conditions, is totally invalid. The emphasis should be placed on process stoichiometry and kinetics rather than on sludge age. For example, wasting 10% of the reactor working volume each day, results in a calculated 10-day sludge age, but this reveals nothing about the actual growth rates experienced (Hoepker and Schroeder, 1979; Irvine and Richter, 1976).

Effluent turbidity is of paramount importance for SBRs, therefore it should be a critical and operating constraint (Schroeder, 1982). Irvine and Richter (1976) suggested that the settleability of the SBR is influenced by the Fill/React ratio. In another work, Dennis and Irvine, 1979, mentioned that there is strong circumstantial evidence that the importance of Fill/React ratio can be mitigated by introducing an anoxic period during Fill.

The fundamental characteristic of the SBR design is its innate flexibility of cyclic phasing. Depending on the treatment objectives, there are a profusion of possible operating modes. Below, some examples of these operating strategies will be given.

Carbon removal, as discussed earlier in this chapter, will occur in all 3 environmental conditions. When aeration starts, carbon will be taken in at a rapid rate, reaching its background levels within minutes. In the case of anaerobic or anoxic conditions, the organic carbon is going to be removed, first, at a rapid rate and then, it will slow down until aeration starts (McCartney and Oleszkiewicz, 1990). These authors attributed this carbon removal to biosorption and the presence of terminal electron acceptors, like O_2 and NO_3^-. They defined biosorption as the rapid transport of organic molecules onto the starved cell, because of the sudden increase in substrate concentration outside the cells. The following operating mode to achieve carbon and suspended solids removal, has been proposed (Arora *et al.*, 1985).

a) Fill: Anaerobic or anoxic period which will induce elevated organic carbon build up. In the final part of this period, mixing may be supplied in order to promote organism contact with the incoming sewage to start substrate degradation.

b) React: Oxic stirred period to promote oxic heterotrophic organic carbon degradation. A period as short as 15 minutes may be enough.

c) Settle: Quiescent settling.

d) Draw: Effluent decanting.

As for carbon removal, nitrification was also reviewed earlier in this chapter. Specifically in terms of SBR applications, Irvine and Bush, 1979, recommended 1 mg/L as a minimum DO concentration for nitrification. Also, they suggested a low average specific growth rate, to ensure optimum conditions for the nitrifiers. Below, a typical SBR operation mode, optimized for nitrification is shown (Wun-Jern and Droste, 1989).

a) Fill: Anaerobic or anoxic period, without mixing, in order to save energy.

b) React: Oxic stirred period to promote oxic organic carbon oxidation and nitrification. In this case, longer aeration periods, 4 hours or more, are normally required.

c) Settle: Quiescent conditions.

d) Draw: Effluent decanting.

In order that the denitrification process occurs, the right environmental conditions should be implemented. As a stand alone process, denitrification has been comprehensively studied in section 2.5 . Most wastewaters contain very little oxidized nitrogen, including those from domestic origin. In this cases, denitrification has to be looked at as complementary process to nitrification. Nitrification/ denitrification, herein referred to as nitrogen removal, can be achieved by using alternating periods of high and low DO concentration (Manning and Irvine, 1985). As already mentioned, there are 3 possible sources of electron donor for denitrification: endogenous and exogenous carbon, and supplemental carbon. Because this work will just deal with the exogenous carbon alternative, i.e., the carbon contained in the incoming sewage, the example of an operational mode, presented below, is specific for this source of organic carbon (Alleman, 1978).

a) Fill: A period with cyclic repetition of oxic and anoxic conditions.

b) React: Repetitive oxic and anoxic periods continued until substrate has been sufficiently depleted. After that, a final period of aeration to cause the displacement of gaseous nitrogen entrained in the floc structure, is recommended.

C) Settle: Quiescent settling.

d) Draw: Effluent discharging.

In this operation mode, emphasis is given to the availability of incoming organic matter to drive the denitrification reaction. During the first period, Fill, continued repetition of short oxic/anoxic periods will provide for nitrogen oxidation and denitrification, using influent substrate carbon, respectively. After Fill, the repetition of oxic and anoxic periods would likely be continued until both carbon and nitrogen are reduced.

2.6.6 Nitrification and/or Denitrification in SBRs

Alleman and Irvine (1980b) mentioned that nitrification and denitrification were part of a mechanism which was not fully determined. As a complimentary reaction to nitrification, the process of denitrification could be conducted by variety of heterotrophic and, in some instances, autotrophic bacteria. When they conducted the studies on a bench-scale SBR, using a synthetic, high strength waste stream, a capacity for nitrification and denitrification, with a constant total nitrogen removal of over 92%, was observed. Yet, in another paper using the data from the above experiment, they developed a model for nitrogen removal, including equations capable of representing the storage product production and utilization (Alleman and Irvine, 1980a).

Silverstein and Schroeder (1983), studying bench-scale SBRs, reported a good performance with respect to standard wastewater criteria, for removal of organic carbon, nitrogen and suspended solids. They still noted that the optimization of aeration and subsequent anoxic mixing will depend on nitrification and denitrification requirements.

Irvine *et al.* (1983), achieved approximately 90% nitrogen removal in a high loaded full-scale SBR. In particular, the results demonstrated that an operating strategy which encouraged some denitrification to occur during Fill, would remove a large percentage of the incoming nitrogen.

Alleman (1984) presented a comprehensive review in factors that can induce elevated nitrite concentration in activated sludge systems, with particular emphasis on SBRs.

Palis and Irvine (1985), conducted an experiment to demonstrate how denitrification can be achieved in a low loaded SBR. They mentioned that nitrification can be easily achieved and essentially completed in both continuously aerated and intermittently aerated systems. They also said that the SBR which used an alternating aeration sequence during Fill would be preferable to a continuously aerated one. Finally, they commented that the reactor with a continuous aeration presented a better sludge settling than the intermittent one.

Abufayed and Schroeder (1986), studied the performance of SBR in terms of denitrification, using primary sludge as carbon source. They emphasized that primary sludge is an excellent source of organic carbon for denitrification in SBRs. They still noted a steady pH increase which remained between 6.56 and 8.63, and this showed the need for chemical addition for pH control. In addition a short aeration period was necessary to purge the gases released as a result of denitrification reactions and this also prevented potential sludge bulking problems. However, they also showed that this kind of operation may cause high SVIs.

Oleszkiewicz and Berquist (1988), studied the performance of SBR/de-nitrification systems at low temperatures. The range of temperatures analyzed was from 2 to 15°C. They concluded that the SBR is a process well able to cope with low temperatures and still keeping its nitrogen removal capacity.

Murthy *et al.* (1988), employed SBR technology to treat an industrial waste containing glyphosate, an active ingredient of an herbicide. They concluded that operating strategies which enrich for the nitrifiers and denitrifiers, also enrich for glyphosate degrading ability. Addition of ammonia to the feed increased the relative fraction of nitrifiers in the biomass.

Hosomi *et al.* (1989), studied the treatment of landfill leachates containing nitrogen and refractory organic compounds, using SBRs. They reported a drop in pH value due to the nitrification process, to pH value as low as 5.2 . Besides that, they used methanol as electron donor for denitrification.

Jones *et al.* (1990b), proposed a 3-stage SBR system for carbon removal, nitrification and denitrification. The system included one SBR, one trickling filter and a secondary clarifier. One of the assumptions was that in a first stage, the SBR would be used only for carbon removal, under high load conditions, fostering sludge carbon storage. Nitrification would occur in the fixed-film reactor and, this highly nitrified effluent, after solids separation in the secondary clarifier, would return to the SBR. There, during the third stage, denitrification would arise using the stored substrate from the first stage as carbon source. They obtained good carbon removal, 95% of nitrification and an almost complete denitrification. Subsequently, in another publication about the same experiment, they concluded that Fill, during the carbon storage stage, should be unmixed to help sludge settling and to provide for a high initial substrate concentration, at the start of aeration. They also indicated the possibility of improving denitrification by as much as 30%, by allowing the nitrified effluent from stage 2, to contain some nitrite (Jones *et al.*, 1990a).

McCartney and Oleszkiewicz (1990), studied the effects of F/M ratio and low temperature in SBRs operated for carbon and nutrient removal. Settleability was not affected by the decreasing temperature, however at low F/M (0.5 g COD/g VSS.d), deflocculation and a consequent increase in effluent solids were observed. They also reported that nitrification was more sensitive than carbon removal to the decreasing temperature, and it did not exceed 10% at 4°C.

Osada *et al.* (1991), employed a SBR to treat swine wastewater, very strong in terms of TOC, nitrogen and phosphorus. They noticed that denitrification occurred only after TOC was reduced to its background levels. They suggested that TOC is removed from the sewage by adsorption on the floc or as storage product, and that this storage products are going to be used later in the denitrification process. They

also reported a decrease in phosphorus removal caused by the oxidized nitrogen present during the anoxic phase.

Brenner *et al.* (1992), used SBRs to test alternative operational strategies for the treatment of sewage containing phenolic compounds. They reported problems related to the low alkalinity of the sewage and nitrification. This caused a sharp drop in pH values.

2.7 pH, Redox and DO Measurements

The implementation of any SBR system will require some kind of automation. The discontinuous operation of valves, blowers, mixers, etc., in this and any other intermittent process, is too time-consuming for human operators to successfully manage on a routine shift basis. Besides that, the labor costs involved, and the consequences and costs related to a possible human error should also be taken in consideration. Computer technology can provide a timely solution for the automation of such systems (Alleman *et al.*, 1989). Some time ago this approach would have involved the use of dedicated main-frame type computer systems, which are expensive, complex and difficult to maintain. Nowadays inexpensive, small personal computer systems can solve the problem. Normally, once such a system has been implemented there will be enough unused capacity left, which can be employed for biological process monitoring and/or control. As already mentioned in chapter 1, one of the main objectives of this work is to evaluate and compare the use of pH, redox and DO as monitoring tools for controlling the biological nitrification and/or denitrification processes in SBRs. This item will provide theoretical background information about these parameters, as well as a quick review regarding their application in biological nutrient removal process monitoring and control.

2.7.1 Theoretical Background Information

a) pH:

Sorenson (1909), cited in Sawyer (1978), proposed the term pH to express the hydrogen-ion activity in a given solution, as its negative logarithm,

$$pH = -\log a_{H^+} \quad \text{or} \quad pH = \log \frac{1}{a_{H^+}} \qquad 2.43$$

where, a_{H+} represents the hydrogen-ion activity in solution. The term pH has been used universally to express the intensity of the acid or alkaline conditions of a solution. The pH scale, is usually in the range of 0 to 14, with 7 representing neutrality, pH values less than 7 representing acid conditions and, ph values greater than 7, alkaline conditions.

pH is a very important parameter, finding application in almost every corner of the environmental engineering practice. Specifically in terms of the biological wastewater treatment field, pH must be controlled within a range suitable to the organisms involved. In order to measure the pH value of a solution, a high impedance voltmeter and amplifier, a measuring electrode, and a reference electrode, are needed. Normally, this two electrodes are coupled together as a combination probe (Liptak, 1974).

The pH electrode is built based on the potentiometric principle. Potentiometric electrodes develop a voltage generated by the activity of a particular ion, in the case, H^+. Their individual potential cannot be determined. The measurable quantity is the difference between the measuring electrode and the inerte reference electrode (Ingold, 1980). Different types of electrodes can be found. By large, the most accepted is the glass electrode. When a pH glass electrode, comes in contact with an aqueous

solution, a layer of gel develops on the pH-sensitive glass membrane. Another gel layer is formed on the inside of the glass membrane, which is in contact with a specific buffer solution. The H^+ ions either diffuse out or into the gel layer, depending on the pH value of the measured solution. When this solution is alkaline, the hydrogen ions will diffuse out, whereby a negative charge is established on the outer side of the gel layer. Since the internal buffer has a constant pH value, the potential at the inner surface of the membrane is also constant. The total membrane potential, is the difference between the inner and the outer charges (Meier *et al.*, 1989).

The following equation, derivated from the Nernst relatioship, is used to convert voltage to pH values (Liptak, 1974; Meier *et al.*, 1989; Sawyer and McCarty, 1978):

$$pH_{sol} = pH_{buffer} + \frac{E_{meter} - E_0}{E_N} \qquad 2.44$$

where, pH_{sol} is the pH value of the measured solution, pH_{buffer} is the pH value of the internal buffer, E_{meter} is the electrode potential, E_0 is the zero potential and E_N is the Nernst coefficient (approximately 60 mV/pH unit at 25°C).

As the activity of the hydrogen ions is temperature dependent, the temperature will also influenciate the pH values. Most of today's pH measuring systems are temperature compensated, therefore overcoming this problem. The pH electrode can be subjected to errors induced by very acid and alkaline conditions or due to the presence of organic compounds, but only at very high concentrations (Meier *et al.*, 1989).

b) Redox

Reduction and oxidation reactions are always coupled together and complementary to each other, in order that one reaction releases just as many

electrons as the other consumes. Therefore, a pair of reactions always takes place in the process. These simultaneous and complementary reactions are known as redox reactions. Qualitative definitions like "acid", "neutral", or "alkaline" have been replaced by a more precise numerical values through the use of pH measurements. The same can be done with other qualitative statements, related to the redox reactions, "oxidizing" and "reducing" powers, by using redox potential values. As in the case of pH values, which represent the hydrogen-ion activity of the measuring solution, the redox potential is determined by the electron activity. Yet another way of defining redox, is considering it as "a measure of the ease with which a solution either absorbs or releases electrons" (Bühler and Galster, 1979).

The redox potential is also measured by a potentiometric electrode. However, two major differences can be spotted when comparing redox and pH probes. Firstly, redox uses a metal electrode as opposed to the pH glass one. Secondly pH electrodes measure the activity of an specific ion, H^+, while redox probe measurements have no specificity, i.e., it indicates neither the presence nor the absence of a particular ion. They indicate the activity ratio of oxidizing species to that of reducing species (Sawyer and McCarty, 1978). As a potentiometric electrode, practically no current flows through the sample during the determination, therefore mitigating the effects of electrolysis and probe polarization. When the electrode is immersed in a solution, electrons either flow from the electrode to the redox system and vice versa. The separation of charges causes a potential to be formed on the metal surface, which opposes any further migration of electrons. When equilibrium is achieved, the electrochemical force (potential), and the chemical forces (oxidizing and reducing forces) balance each other out (Bühler and Galster, 1979).

Liptak (1974) presented the following equation to represent the redox potential.

$$E_{meter} = E_0 + \frac{E_N}{n} \log \frac{[Oxid]}{[Red]} \qquad 2.45$$

where, E_{meter} is the redox measured, E_0 is a constant which depends on the choice of the reference electrode, E_N is the Nernst constant, [Oxid] and [Red] are the activity of the oxidized and reduced forms of the ions being measured, and n represents the number of electrons involved.

As for pH, the redox measurement system is also made of three components: the high impedance voltmeter, the indicator electrode and the reference electrode. These two electrodes are normally combined together in a combination electrode. Most of the redox probes marked today, have an indicator electrode made of platinum or gold (Sawyer and McCarty, 1978). Reference electrodes, made of different materials, will produce different redox values for the same solution. In order to obtain valid information about any individual redox pair, a standard reference electrode to which all measurements can be referred to is required. The universal reference electrode is known as Standard Hydrogen Electrode (SHE). Usually, measurements taken with other reference electrodes, are converted to the SHE as reference electrode, through the following expression (Bühler and Galster, 1979):

$$E_{SHE} = E_{meter} + E_{ref} \qquad 2.46$$

where, E_{SHE} is the redox potential against the SHE, E_{meter} is the redox potential against the reference electrode and E_{ref} is the standard potential of the reference electrode. For example, E_{ref} is approximately 200 mV for the silver/silver chloride reference electrode at 25°C.

Redox potential applications in biological wastewater treatment have been subjected to some scepticism. It has been said that meaningful measurement of the redox potential is not possible in complex wastewater systems, which are generally not in equilibrium and which contain many oxidants and reductants (Sawyer and McCarty, 1978). Nevertheless, these same authors support the view that the use of redox measurements in such systems has sometimes been useful in an empirical way,

to indicate whether a system is aerobic and oxidizing or anaerobic and reducing. As it will be seen later, this view has somehow changed in the last 4 or 5 years.

The redox potential can be either pH dependent or pH independent, depending on the reactions involved. If the hydrogen ion is involved in these reactions, the redox will be pH dependent, varying with the number of H^+ taking part in the reactions. Also, the redox probe is knowned for its sluggish response to changes in the solution oxidation state, when free dissolved oxygen is present. It has been proposed that the free oxygen is adsorbed on the platinum surface. The oxide layer so produced, conducts electrons, therefore it does not affect the redox sensitivity of the electrode. However, it does act as an oxidation reserve, which tends to maintain the electrode potential at high levels, even when the solution redox comes down (Bühler and Galster, 1979).

c) DO:

Mostly, DO concentrations have been ascertained through the use of DO electrodes. They are amperometric or polarograph electrodes, rather different than the previous ones. As such, activity evaluation is based on a current measurement, and not on a potential (Ingold, 1980).

DO electrodes consist of a cathode and an anode conductively connected by an electrolyte. A suitable polarization voltage, applied between the anode and the cathode, selectively reduces the oxygen at the cathode, resulting in an electric current, which in proportional to the partial pressure of oxygen. The cathode can be made of gold or platinum and the anode, of silver. The oxygen enters the system through the permeable membrane that covers the cathode (Liptak, 1974).

The relatioship between the measured current and the oxygen partial pressure is based on the Fick's law, and can be expressed as follows (Ingold, 1980):

$$i = k.D.a.A.\frac{pO_2}{X} \qquad 2.47$$

where, i is the electrode current, k is a constant, D is the coefficient of oxygen diffusion in the membrane, a is the solubility of the oxygen in the membrane material, A is the cathode area and X is the thickness of the gas-permeable membrane.

The importance of knowing the DO concentration in activated sludge applications cannot be overestimated. As already seen in this chapter, oxic heterotrophics and nitrifiers use free dissolved oxygen in their metabolism. On the other hand, as a control parameter its importance is even bigger. DO regulation schemes have been already in use for some time.

DO determinations are temperature dependent, and the temperature has a two fold effect on DO measuring systems. Firstly, temperature changes will cause mechanical and electrical changes within the electrode and secondly, the solubility of oxygen in water changes with the temperature. In modern DO probes, temperature compensation has been provided to minimize this problem. Besides that, DO measurements are also affected by the atmospheric pressure and by the solution salinity. These effects have to be taken in account when working with DO concentration, instead of DO saturation percentage (Sawyer and McCarty, 1978). The viscosity and flow rate of the solution in contact with the DO electrode, are also important. The reason for this is that DO probes consume oxygen, which is continuously extracted from the solution. Therefore, the liquid that comes in contact with the probe has to be continuously exchanged in order to avoid false DO concentration readings (Ingold, 1980).

2.7.2 Using pH, Redox and DO for process monitoring and control

Redox Potential (Ag/AgCl)	Treatment Conditions	Carbon Removal	Nitrogen Removal	Phosphorus Removal
+100 mV	Presence of DO Aerobic zone Aerobic respiration	Oxidative pathway $\rightarrow CO_2 + H_2O$	Nitrification $NH_4^+ \rightarrow NO_3^-$	Uptake
0 mV	Presence of NO_3^- Absence of DO Anoxic zone Anaerobic respiration	Oxidative pathway $\rightarrow CO_2 + H_2O$	Denitrification $NO_3^- \rightarrow N_2$	P is trapped in the sewage
-300 mV	Absence of DO and NO_3^-	Fermentative pathway $\rightarrow$ volatile acids	Enhanced Reduction	Release
-500 mV	Anaerobic zone Fermentation	Fermentative pathway $\rightarrow$ methane	$\rightarrow NH_4^+$	P is released into the interstitial liquid

Table 2.7: Redox Potential and C, N and P removal Charpentier *et al.* (1987)

The history of the utilization of electrochemical tools to monitor wastewater treatment processes goes back to the beginning of the century. This kind of tool has been treated with suspicion, because of its inherent lack of reliability and reproducibility. Times have changed, and today new and somewhat more reliable electrodes have been developed. Together with that, the computer age advent allowed that data could be gathered and analyzed with easy. Putting these factors together, with the relatively recent need for improved wastewater treatment systems, with nutrient removal capability, it is easy to understand why this subject has only recently started to mature. Even so, the use of electrodes is not yet a straight forward thing. To quote from Grune and Chueh (1958), cited in Koch and Oldham (1985): " ... a tool is no better than its user and intelligent application requires a profound understanding of the underlying principles and the circumstances under which measurements are made". The failure in recognizing this basic principle, for example in terms of redox measurements, resulted in much useless data being published (Ciaccio, 1973). Because of that, in this text, only the more recent developments on the subject will be reported.

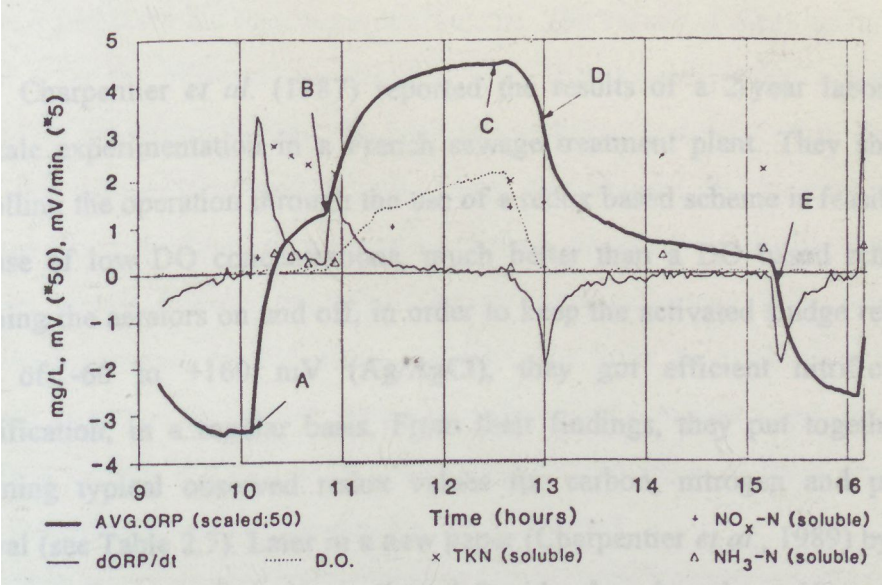


Figure 2.5: Digestor Redox Curve
Peddie *et al.* (1990), Modified

Koch and Oldham (1985) presented a comprehensive literature review on the use of redox probes in the biological wastewater treatment field. Besides that, they have undertaken an extensive 3-year project to investigate the use of redox potential as an operational tool for biological nutrient removal processes. Some intersting remarks were made regarding practical aspects of the redox measurement. For example, they reported that in batch systems, or when time is a process variable, the redox probe response time must somehow be taken into account, indicating the already mentioned sluggish response problem. From some batch experiments under non-oxic conditions, where redox was continuously monitored, they reported the discovery of two interesting features on the redox curve. Firstly, they observed a distinct "knee" in that curve, when nitrates were reduced to zero. This point also coincided with the onset of phosphorus release. The redox levels where this feature took place were not always the same. Most of the time, it fell inside the range of -40 to -140 mV (Ag/AgCl). Secondly, another distinct "knee" was observed in the redox curve. This time they found that it coincided with the disappearance of DO. The range where it occurred was between +100 and +200 mV (Ag/AgCl). They claimed

that this second breakpoint delineated the point at which aerobic respiratory activity ceased and anaerobic respiration began.

Charpentier *et al.* (1987) reported the results of a 2-year laboratory and full-scale experimentation in a French sewage treatment plant. They showed that controlling the operation through the use of a redox based scheme is feasible and, in the case of low DO concentrations, much better than a DO based scheme. By switching the aerators on and off, in order to keep the activated sludge redox in the range of -60 to +160 mV (Ag/AgCl), they got efficient nitrification and denitrification, in a regular basis. From their findings, they put together a table containing typical observed redox values for carbon, nitrogen and phosphorus removal (see Table 2.7). Later in a new paper (Charpentier *et al.*, 1989) by the same authors, the above results were confirmed. Besides that, they showed very interesting new observations from a pilot-scale study on nitrogen removal. They reported a maximum nitrification efficiency for redox values greater than +250 mV(SHE). From track analysis studies on this pilot-scale experiment, they were able to identify two interesting features of the redox curve. The first one, observed after the aeration was switched on, was a inflection point in the ascending part of the redox curve. They correlated it with the nitrate levels stabilization, in the absence of ammonia. The DO curve, at the same time, showed a sharp increase in DO levels. The second observed inflexion point was the same which was observed by Koch and Oldham(1985). It occurred on the descending part of the redox curve, after aeration has been switched off. They also related it with the disappearance of DO. Additionally, they observed an increase in phosphate levels, which they related with the well known phosphate release that may occur under true anaerobic conditions.

Heduit and Thevenot(1989) carried out tests, both in laboratory and full-scale treatment plants, to investigate the relationship between redox potential and DO concentration, in the activated sludge process. From these experiments, they found that the two parameters complied with the following logarithm relationship:

$$E_h = a + b \log [O_2] \qquad 2.48$$

where, a and b are the regression coefficients. The values of these coefficients have varied a lot for different experimental sets. They proposed that a and b mainly depend on the sludge loading, the aeration conditions and the sludge concentration. They also mentioned an earlier work, where they showed that for pilot-scale plants subjected to cyclic feed and aeration conditions, the redox reached at the end of the aeration periods, could be related to the nitrification efficiency. Some work was also done to investigate the pH effect on redox. In order to establish a relationship between these two parameters, they varied the pH of a treated water, saturated with dissolved oxygen at 21°C, while monitoring pH and redox at fixed time intervals. They following linear relationship has been obtained.

$$E_h = 0.721 - 0.046\ pH \qquad 2.49$$

where E_h was expressed in Volts.

Peddie *et al.* (1990) continuously monitored redox and DO within sludge digesters undergoing alternating oxic and non-oxic conditions. Besides this continuous monitoring, a time series of chemical analysis was performed. A curve showing the general form of the redox tracking is showed in Figure 2.5 . They identified 5 points of interest, which they were able to relate to the other measurements. The point labelled "A" in this curve, corresponds to the onset of aeration. The "B" point, coined "elbow" seems to be the same inflexion point observed by Charpentier *et al.* (1989), although here the explanation is somewhat different. In this work, they associated it with the transition from the effects on redox of combined oxygen in the form of nitrates, to the effects of free dissolved oxygen. They also claimed that, in one case, this point coincided with the ammonia disapearance. Point "C", the "plateau", was reckoned to indicate a fully oxidized state or, at least, the state where the rate of oxidation equals the rate of aeration. The "D" point, named "oxygen knee", coincided with the depletion of measurable DO levels. The authors claimed it indicates when the denitrification process starts. This feature has also been reported by Koch and Oldham (1985). Finally, the "E" point was

related to the complete disappearance of nitrates, indicating the transition from anoxic to anaerobic condition, which agreed with the observations of Koch and Oldham (1985) and Charpentier *et al.* (1989). Besides this curve, they also obtained linear relationships between redox and the logarithm of DO, and between redox and the logarithm of nitrate concentration.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

In order to investigate variations in pH, redox and DO during biological nitrification and/or denitrification in SBRs, a three fold development program has being undertaken. First of all it was necessary to design and assemble a SBR bench-scale rig capable of being operated on a reliable and regular basis, under the changing environmental conditions needed for nitrification and/or denitrification. Secondly, it was also essential to develop a data acquisition and control (DA & C) system. This system was used to gather pH, redox, DO and temperature data (DA), while controlling the time based SBR schedule (C). Finally, once the above steps had been completed, it was possible to start the series of experiments planned. Separate experiments were performed for nitrification and nitrification/denitrification. Besides these, yet another experiment was carried out regarding nitrification and enhanced biological phosphorus (EBP) removal. The reasons why this experiment was performed will be explained later in section 3.5 .

The above steps can be grouped into four discrete phases. In phase 1, all the activities connected with the SBR rig and the data acquisition and control system development have been put together. Phases 2, 3 and 4 comprised the main experiments, respectively, nitrification, nitrification/denitrification and nitrification/EBP removal.

During all these experiments, the reactors were fed only with real sewage. It was decided not to use a synthetic feed to avoid the inherent experimental bias associated with this. Parameters like pH and redox, are known to be heavily affected

by variations in the medium composition. This decision caused some serious logistic problems, like the wastewater collection, transport and storage. In order to overcome them in a manageable way, it was necessary to constrain the collection frequency. Wastewater was collected and brought to the laboratory twice a week and on arrival was autoclaved at 121°C, for one hour, to attenuate changes in composition during storage. This sewage, mainly of domestic origin, was collected from Pudsey Sewage Treatment Works, a small activated sludge plant situated in the town of Pudsey, west of Leeds, between Leeds and Bradford. The plant is operated by Yorkshire Water, North Central Division, and serves a population of approximately 10,000 people. Sewage was collected after the primary sedimentation tank, just before entering the aeration tank. Its composition varied a great deal in terms of organic carbon and nitrogen concentration during this research, from very low to medium strength.

All experiments were carried out at ambient temperature.

3.2 Phase 1: System Development and Initial Tests

The emphasis during this phase was on the design and development of a reliable SBR system. The entire experimental set-up comprised the SBR rig and the data acquisition and control (DA & C) system.

The SBR rig consisted of two equal and completely independent configurations. Each of them included, a reactor, an influent storage tank, auxiliary equipment, and plastic connections and tubes.

Each reactor was constructed from plexiglass cylinders with three lateral holes provided to hold the pH, redox and DO probes (Figure 3.1). In all experiments, the SBRs were operated with a minimum volume prior to Fill of 2 litres, and a maximum working volume of 6 litres, hence 4 litres were fed to the reactor, each cycle.

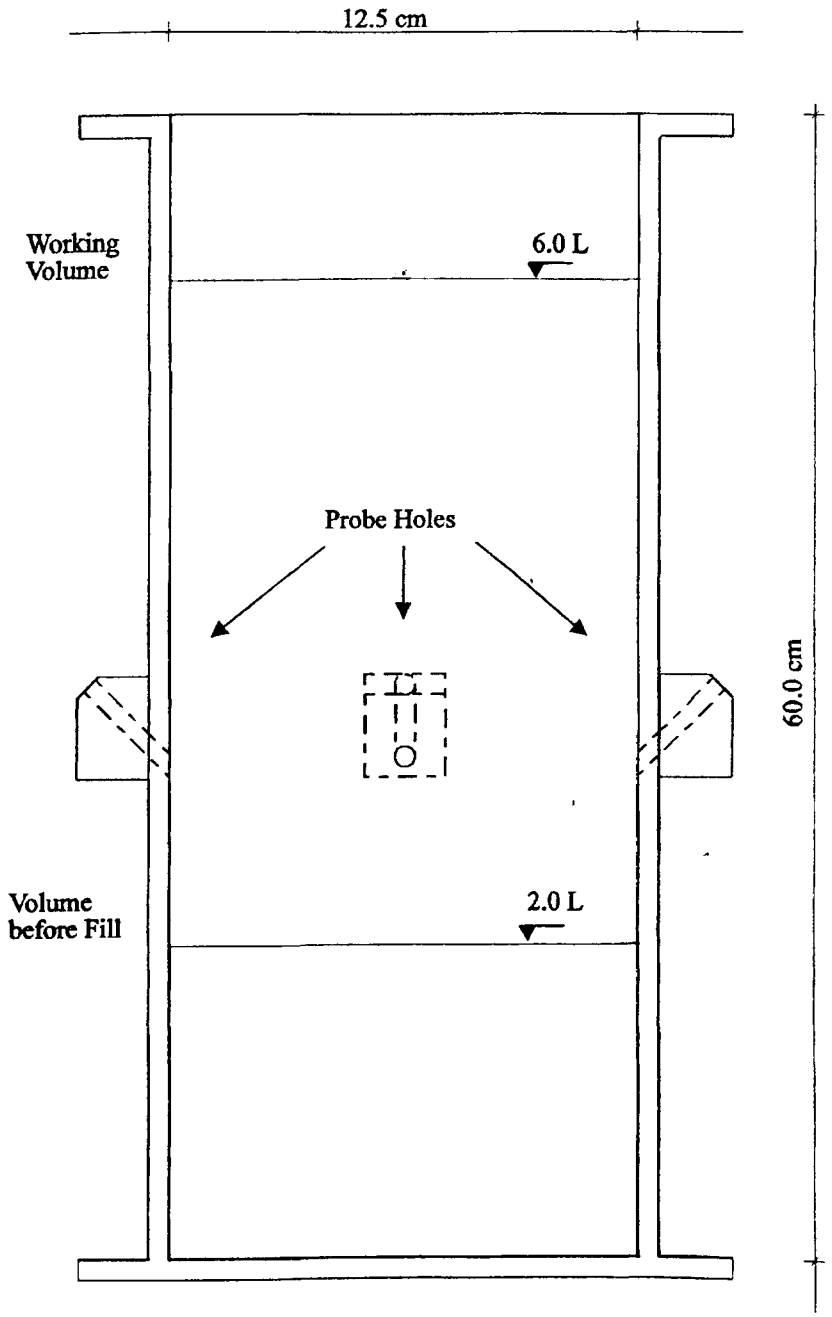


Figure 3.1: Schematic diagram of the SBR system

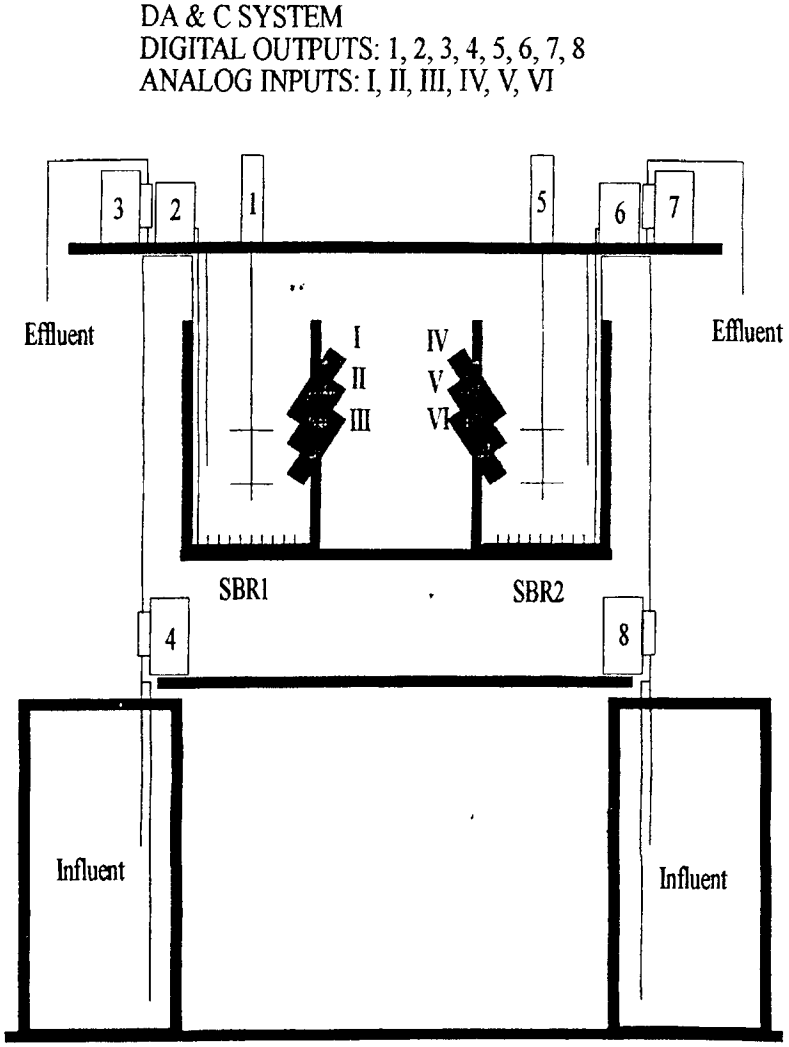
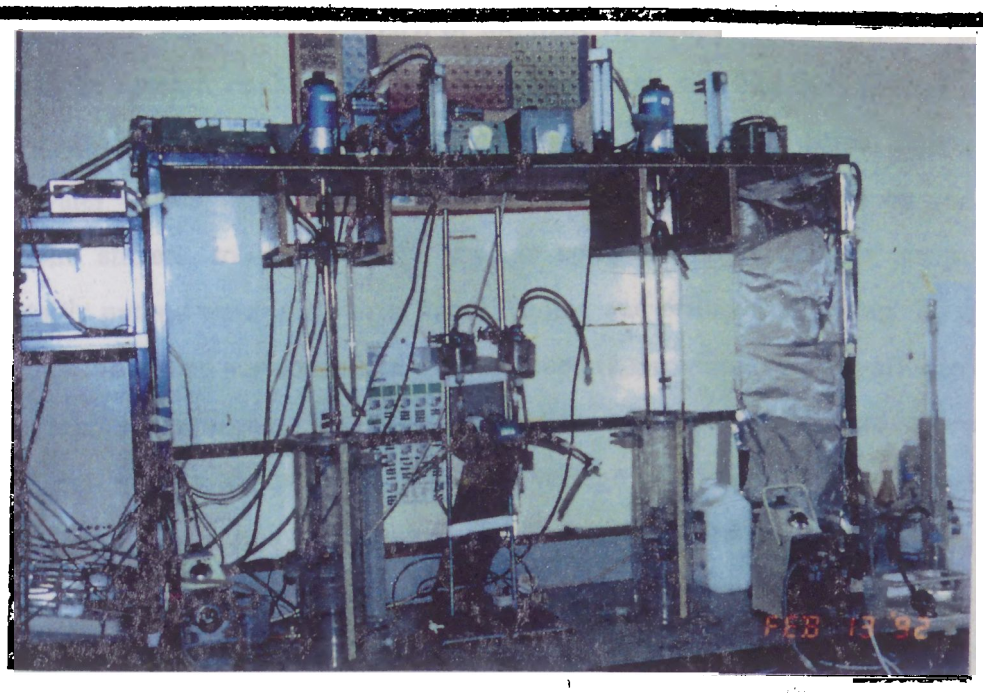


Figure 3.2: SBR Rig



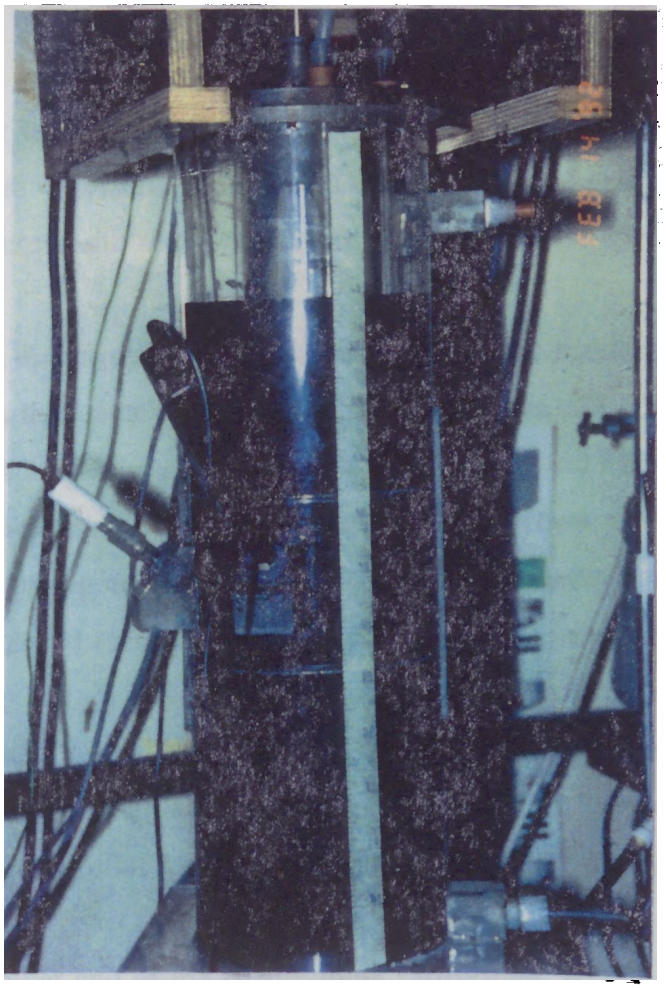


Figure 3.3: SBR Rig and Reactor Pictures

Figure 3.2 presents a sketch of the SBR rig and, figure 3.3 shows the SBR rig and the reactor. Each one of the influent storage tanks had the capacity to store the wastewater necessary for 2 SBR cycles, approximately 8 litres. The influent wastewater in the storage tanks was kept under constant agitation by means of magnetic stirrers, to maintain the sewage composition as uniform as possible. Peristaltic pumps were used for influent feeding and effluent decanting. To provide for non-oxic mixed periods, a low mixing speed (approximately 40 rpm) was one of the system prerequisites. This was accomplished by using rather crude, variable-speed, pad stirrers, coupled together with voltage regulators. Finally, the aeration system comprised aeration pumps connected to aeration stones placed at the bottom of the reactors. A rough air flow rate control was provided by means of air flow rotameters. Throughout these experiments, the air flow rate was maintained at, approximately, 1 litre per minute.

In order to accomplish the main goals of this research, it was necessary to develop a DA & C system. This system comprised three main sub-systems: analog input, digital output and control program. (figure 3.4).

The DA & C system can be better understood by making an analogy with the human body. In this context, the heart of the system was a digital personal computer (PC), an Elonex 286 PC, equipped with an analog input and a digital output interface boards. Both boards were manufactured by Burr-Brown Corporation. The PCI-20089W-1, the analog interface board, performs the necessary analog to digital (A/D) conversions required to make analog input data, in this case a voltage, compatible with the digital PC. It has an input multiplexer that can select from between 8 different channels. Signal scaling is provided by a programmable gain amplifier. Gains of 1, 10 and 100 are available under software control. The 12-bit A/D converter was configured for the input range of 0 to 10 volts full scale.

At the other end of the analog input sub-system, are the probes. Carrying on with the proposed analogy, they represent the sensitive organs of the system.

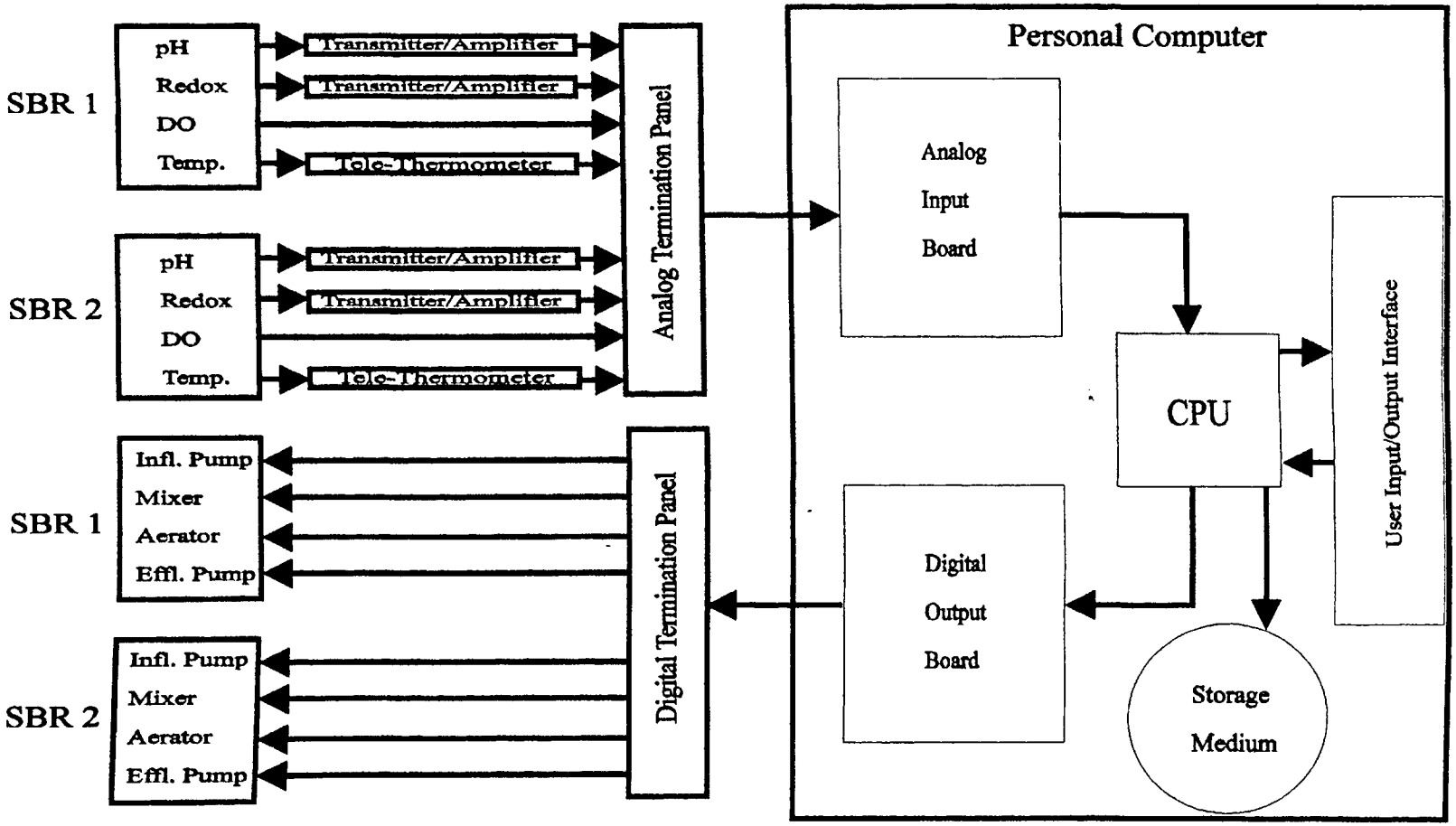


Figure 3.4: DA & C System

The pH was measured by pH combination electrodes (Hanna Instruments), comprising a glass and a gelled reference electrodes. The probe was connected to a transmitter/amplifier from Anglicon, which had its output range modified for this project. After that, the amplified signal was transmitted to the analog interface board through the analog termination panel.

The redox probes were Asean Brown Boveri (ABB), wire platinum electrode, using as reference element Ag/AgCl. As with pH, their signal was amplified (modified Anglicon transmitter/amplifier) before going to the analog interface board.

DO was measured by means of "Clark" type polarographic oxygen electrodes, coupled with oxygen meters, in a portable, battery operated, DO measurement system manufactured by Jenway. Their signal was sent directly to the analog interface board without need of amplification.

Finally, in order to monitor temperature, Yellow Springs Instrument (YSI) 400 flexible temperature probes were used. They were connected to a YSI Tele-thermometer (model 44 TD). Its output signal, already amplified inside the Tele-thermometer, was transmitted directly to the analog interface board through the termination panel. Table 3.1 shows how the signal coming out of the probes evolves until it reaches its final value, inside the PC.

The actions performed by the digital output sub-system may be compared with the actions taken by the human body, such as, walking, speaking and lifting an arm. The PCI-20087W-1 digital output interface board is capable of switching on and off up to 40 appliances. In this work, only 8 digital output channels have been used for switching appliances. As seen in figures 3.2 and 3.3, they were: influent and effluent pumps, aeration pumps and stirrers. Contrary to the analog input sub-system, where the termination panel was used only for connection purposes, in the digital output sub-system, it had a very important function. It provided digital isolation to the system. When driving heavier loads than the board is rated for, digital isolation can provide the necessary protection. In this work, optical digital isolation was employed.

Parameter	Probe (mV)	Transmitter/ Amplifier[1] (V)	Gain[2]	Final Values
pH	-407 to 407	0 to 10	1	0 to 14 (pH units)
redox	-800 to 800	0 to 10	1	-800 to 800 (mV)
DO	-	0 to 0.1	100	0 to 10 (mg/L)
Temp.	-	0 to 0.1	100	0 to 50 (°C)

1. For DO and Temperature, the DO meter and the Tele-thermometer, respectively, performed the transmitter/amplifier functions.
2. Set by software.

Table 3.1: Probes Signal Evolution

It provides high voltage separation between the load and the DA & C system, without using mechanical relays.

As a last comparison in the proposed analogy, the brains of the system would be represented by the driving software. DA & C systems are made operational by a software. Together with the interface boards, Burr-Brown also supplied some function sub-routine libraries, written in QuickBasic. Based on these sub-routines, a program also coded in QuickBasic, was developed to command the diverse DA & C functions. Figure 3.5 presents in a very concise way, the program main modules.

The control program was developed as a menu-driven software. As such, all the program functions can be reached through the use of menus. The program continuously display on the computer screen, information about the digital output channels status, and the analog input channels readings. The program functions can be grouped in three main categories: system maintenance, automatic operation and manual operation. The system maintenance functions or modules, allow changes in the configuration of any channel, either digital or analog. For example, the module "analog initial settings" can be used to change an analog channel gain. Besides that,

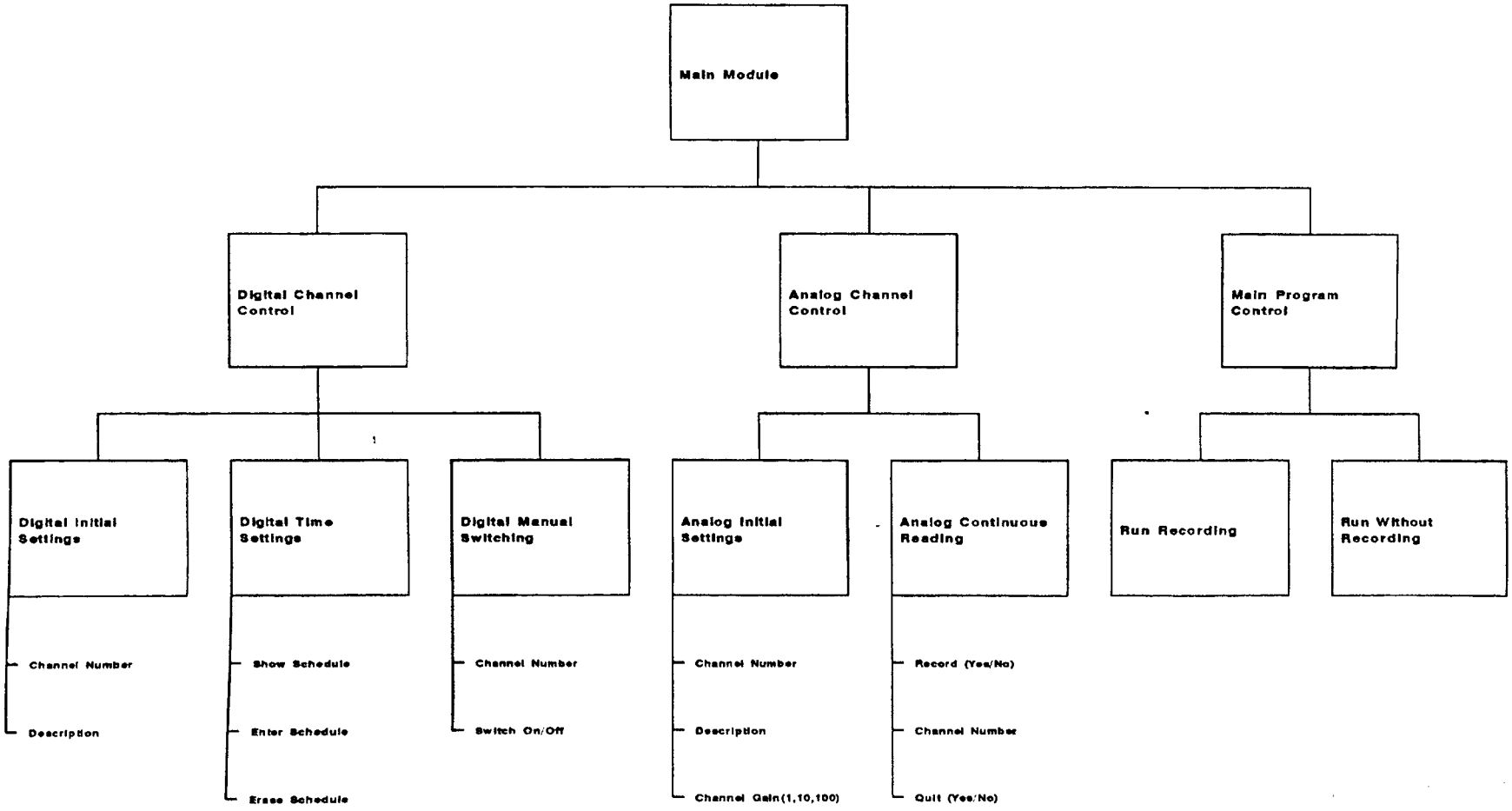


Figure 3.5: DA & C Software

the maintenance of the SBR time-based schedule is also attained by using this group of functions. For example, the module "enter schedule" allows entry of the specific time when the SBR 2 effluent and aerator pumps should be switched on.

The functions relating to the automatic operation represent the main part of the program. They are responsible for the automatic running of the SBR rig, without human interference. They were designed to perform the following activities:

a) Continuously read the analog inputs (DO, pH, redox and temperature), convert them to volts and, then, to the respective unit;

b) Store the read data in ASCII format, in a file on disk, with each entry stamped by the time;

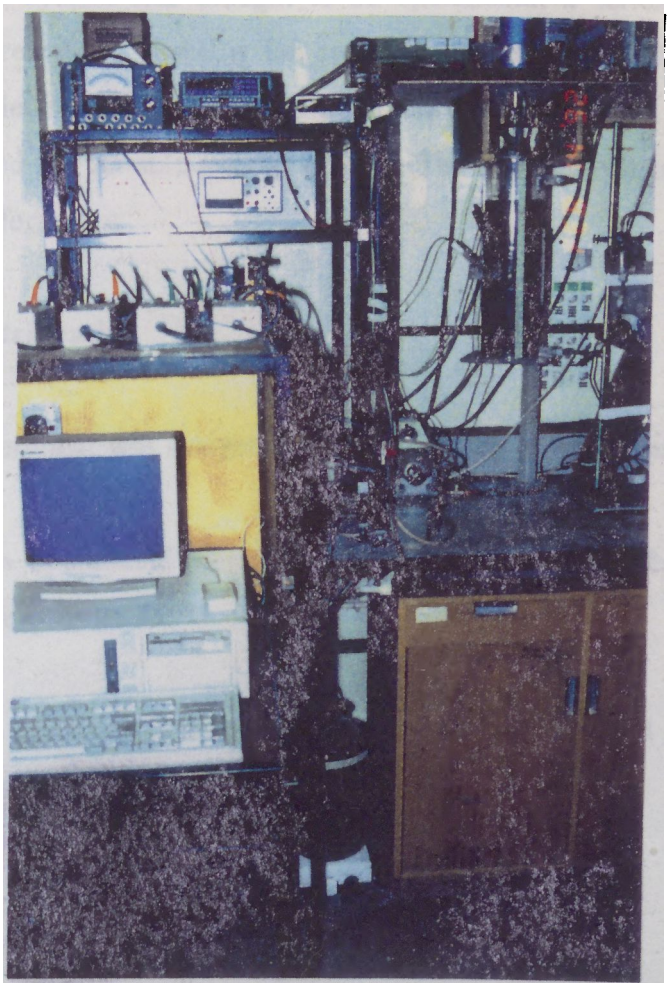
c) Based on the SBR schedule, also stored on disk, switch the appliances on or off as required;

d) Continuously update the screen information based on the above steps;

e) Monitor the user interface (keyboard) for any requests.

Finally, the functions grouped under manual operation, were designed to allow digital switching of appliances and analog channel readings outside the automatic operation environment. For example, the "digital manual switching" module would be used in case of some urgent situation needing prompt action, and the "analog continuous reading" module was used throughout this work for probe calibration.

Figure 3.6 presents two views of the system, the first one showing the computer and part of the SBR rig, and the second one, showing a close-up of the transmitter/amplifiers assembling.



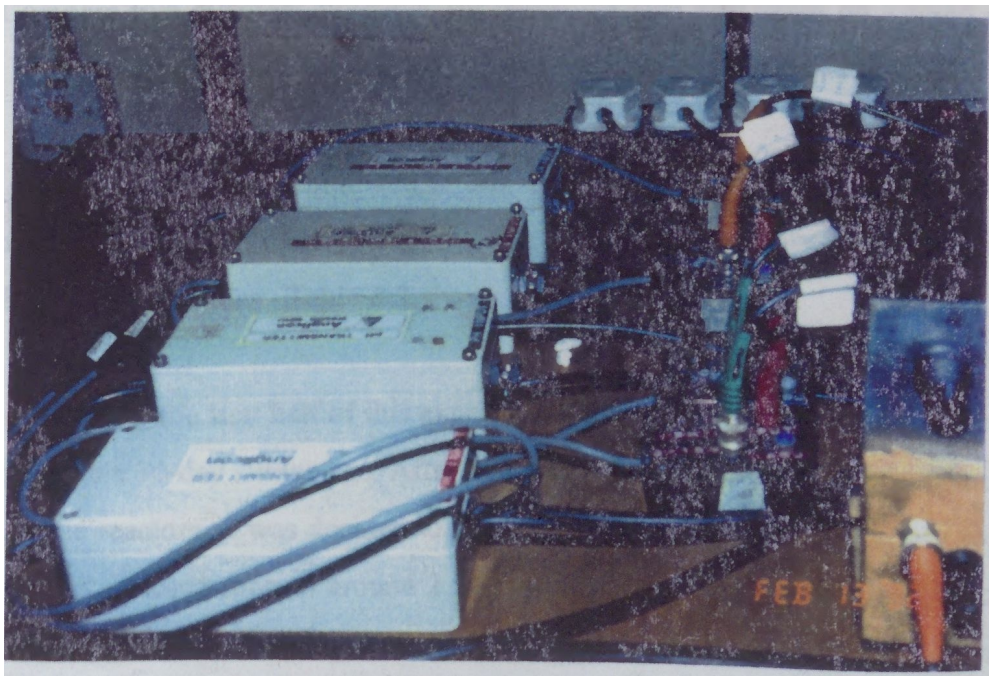


Figure 3.6: SBR System and Transmitter/Amplifiers Assembling Pictures

Once the system was thought to be operational, a series of short-lasting experiments were run to check it for eventual problems. In order to do so, the reactors were filled some times with tap water and other times with activated sludge, and then operated for variable periods of time. The main emphasis was on the DA & C system performance.

3.3 Phase 2: Nitrification

This experiment was designed with the following objectives in mind:

a) Implement an operational strategy capable of promoting carbon removal and nitrification in SBRs;

b) Monitor these processes in terms of both conventional chemical analysis and pH, redox and DO measurements;

c) Compare the performance of both reactors for reproducibility;

d) Compare two different strategies for solids wastage one based on a fixed sludge age and the other one on mantaining a fixed MLSS concentration.

Both SBRs were employed in this experiment, being operated in exactly the same way during the entire first month of functioning. Their operation comprised 2 12-hour cycles per day. Table 3.2 shows their cycle schedule.

During the first half of this experiment, sludge was wasted on a regular basis, to promote a 30-day sludge age. After that, because of excessive solids accumulation inside the reactors, it was decided to waste the amount of sludge necessary to lower the MLSS concentration to around 2,500 mg/L (full reactor volume) and, from then on, to reduce the sludge age of one of the reactors (SBR 1) to 20 days. The other reactor (SBR 2) stopped being operated in terms of sludge age, in favour of a fixed MLSS concentration strategy, in this experiment 2,500 mg/L.

Period	Duration (hours)	Sequence (hours)	Description
Fill	2:00	1:00 nM/nA 1:00 M/ A	Anoxic/Anaerobic Anoxic/Stir only
React	6:00	M/ A	Oxic period
Settle	1:00	-	Quiescent conditions
Draw	0:30	-	Effluent decanting
Idle	2:30	-	Anaerobic period

M - Mixed nM - Unmixed
A - Aerated nA - Unaerated

Table 3.2: Phase 2 - SBR Cycle

Besides the routine analyses described later in this chapter, track studies were performed on SBR 1 and 2. By track study it is meant a study where the reactor contents were sampled and analyzed at specific times during the entire cycle span.

3.4 Phase 3: Nitrification/Denitrification

This experiments main objectives were:

a) Implement an operational strategy capable of promoting carbon removal, nitrification and denitrification. The latter being expected to take place without the need for a supplemental electron donor source, by using the organic carbon contained in the influent wastewater and from the endogenous respiration products;

b) Monitor the process in terms of conventional chemical analyses and pH, redox and DO measurements;

c) Observe protozoan and metazoan communities in the activated sludge.

Only one reactor was employed. It was operated based on a routine of one cycle per day. Table 3.3 shows the initial SBR cycle implemented.

Period	Duration (hours)	Sequence (hours)	Description
Fill	8:00	3:00 nM/nA 1:30 M/ A 1:00 M/nA 1:00 M/ A 1:30 M/nA	Substrate accumulation Oxic period Anoxic period Oxic period Anoxic period
React	8:00	1:00 M/ A 6:45 M/nA 0:15 M/ A	Oxic period Anoxic/anaerobic period Oxic period
Settle	1:00	-	Quiescent conditions
Draw	0:30	-	Effluent decanting
Idle	6:00	-	Anaerobic period

M - Mixed nM - Unmixed
A - Aerated nA - Unaerated

Table 3.3: Phase 3 - Initial SBR Cycle

This cycle remained the same for almost the entire experiment duration. After 3 months of operation, it was noticed that the total time provided for oxic conditions was not sufficient to bring nitrification to completion. In order to try to overcome this problem, a series of different cycle schedules have been tried. Table 3.4 presents a summary of these cycle alterations

Also at the same time that the cycle changes started, a sharp drop in pH values was observed during oxic periods. The conclusion reached was that the influent initial alkalinity was not sufficient to counterpoise its consumption during nitrification. Accordingly, it was decided to increase the influent wastewater alkalinity by adding calcium hydroxide. The amount of lime to be added to each new wastewater batch was calculated as function of the sewage initial alkalinity and Total

Cycle number	86	87	88	89 - end
Fill	3:00 nM/nA 1:45 M/ A 0:45 M/nA 1:00 M/ A 1:30 M/nA	2:35 nM/nA 1:55 M/ A 1:00 M/nA 1:30 M/ A 1:00 M/nA	2:30 nM/nA 2:00 M/ A 1:00 M/nA 1:30 M/ A 1:00 M/nA	1:30 M/ A 1:30 M/nA 1:30 M/ A 1:15 M/nA 1:15 M/ A 1:00 M/nA
React	2:00 M/ A 5:45 M/nA 0:15 M/ A	1:30 M/ A 6:15 M/nA 0:15 M/ A	1:30 M/ A 6:15 M/nA 0:15 M/ A	1:30 M/ A 6:15 M/nA 0:15 M/ A
Settle	1:00	1:00	1:00	1:00
Draw	0:30	0:30	0:30	0:30
Idle	6:00	6:00	6:00	6:00

M - Mixed nM - Unmixed
A - Aerated nA - Unaerated

Table 3.4: Phase 3 - SBR Cycle Alterations

Kjeldhal Nitrogen (TKN). The objective was to keep a minimum alkalinity of 100 mg/L (as $CaCO_3$) at all times during the SBR cycle. It was considered that both nitrification and denitrification would proceed to completion. In section 2.5.1, it was shown that denitrification replaces almost 50% of the alkalinity lost during nitrification. With that in mind and, considering that nitrification consumes 7.1 mg (as $CaCO_3$) per milligram of nitrogen oxidized, it is relatively straight forward to calculate the amount of lime needed.

Throughout the experiment duration, solids were wasted to keep a MLSS of 2,500 mg/L (full reactor volume). This phase was characterized by a very low sludge yield. As a direct result, the wastage procedure was effected only a few times.

In addition to the analyses performed for all phases of this work, total alkalinity was also determined, from the moment when the pH problem was realized onwards. Also, as the experimental objectives suggests, the sludge microscopic composition was observed, on a regular basis, throughout the experimental period.

A track study was carried out during the 78th cycle. In this study, total alkalinity was included in the monitored parameters list.

3.5 Phase 4: Nitrification/EBP Removal

This experiment was carried out due to the desire to complete the nitrification study, mainly in terms of protozoal microscopic examination, which was not possible to perform during phase 2. Also, from the phase 2 results, the possibility of accomplishing enhanced biological phosphorus (EBP) removal and nitrification concomitantly was realised. The experimental main objectives are listed below.

a) Implement an operational strategy capable of promoting nitrification together, if possible, with EBP removal;

b) Monitor both processes in terms of conventional chemical analyses and pH, redox and DO measurements;

c) Observe protozoan and metazoan population development during the experiment duration;

d) Confirm the results obtained in phase 2.

This experiment was performed concomitantly with phase 3. In consequence, the influent wastewater employed in both phases was the same.

Two 12-hour cycles per day were performed during this phase. Table 3.5 shows the initial cycle schedule implemented.

This cycle was observed for more than two months of operation. At the end of this period, a sharp drop in the reactor pH, reaching values as low as 6.5 gave rise to changing the original cycle. As with phase 3, the pH drop was caused by the

Period	Duration (hours)	Sequence (hours)	Description
Fill	3:00	1:30 nM/nA 1:30 M/nA	Substrate accumulation Anoxic/anaerobic period
React	6:00	M/ A	Oxic period
Settle	1:00	-	Quiescent conditions
Draw	0:30	-	Effluent decanting
Idle	1:30	-	Anaerobic period

M - Mixed nM - Unmixed
A - Aerated nA - Unaerated

Table 3.5: Phase 4 - Initial SBR Cycle

combination of low to medium influent wastewater alkalinity and, high influent TKN concentration. The new schedule, implemented on cycle 139, differed from the original by the fact that the first hour of the React period was transformed from aerated and mixed, to mixed only. The change was implemented hoping that it would induce some denitrification to occur. After a while, it was decided that the denitrification occurring in new period, if any, was not sufficient to counterbalance the nitrification effect on the reactor alkalinity. As a consequence, it was decided to add lime to the effluent wastewater. The calculation of the amount of lime to be added, was performed in much the same way as it was done for phase 3. The only difference was that, this time no denitrification effect was considered. A further cycle modification was implemented on the 169th cycle, in order to try the improve conditions for EBP removal. Table 3.6 presents the two commented cycle alterations.

During most of this phase, solids were wasted to keep the MLSS concentration around 2,500 mg/L (full reactor volume) on a regular basis. After the pH problems, the wastage became irregular and a somewhat greater MLSS concentration was allowed to occur (up to 3,500 mg/L).

In this experiment, as many as 3 track studies were performed. Alkalinity was monitored in the last two of them. Also, as can be implied from this phase objectives,

Cycle number	139	169
Fill	1:30 nM/nA 1:30 M/nA	1:00 nM/nA
React	1:00 M/nA 5:00 M/ A	2:00 M/nA 6:00 M/ A
Settle	1:00	1:00
Draw	0:30	0:30
Idle	1:30	1:30

M - Mixed nM - Unmixed
A - Aerated nA - Unaerated

Table 3.6: Phase 4 - SBR Cycle Alterations

the sludge microfauna composition was followed throughout the entire phase.

3.6 System Maintenance

In order to reduce the effects of poor laboratory procedures on the reliability of the results as far as possible, some precautions were observed throughout all phases.

Reactor walls, aeration stones, and stirrer pads were brushed once a week to attenuate the effects of solids attachment. Also, the influent storage tanks were washed thoroughly before each new wastewater batch. Tubes and connectors were washed and others replaced, in between experiments.

pH, redox and DO electrodes required continuous close attention. After each monitoring session, they were removed from their bays and rinsed with distilled water. To avoid the need for calibration and repolarization before using them again, they were stored overnight connected to the system. The DO electrodes had their membrane replaced normally, once a fortnight. Besides that, they required daily calibration. On the other hand, pH and redox electrodes needed recalibration once each 4 or 5 days. Finally, the electrolyte solution chamber of the redox probes had

to be refilled every 3 or 4 weeks. This precaution was not necessary for the pH electrodes because they use a gel electrolyte instead of a liquid one.

3.7 Collection Schedule and Analytical Methods

As already mentioned earlier in this chapter, Pudsey settled wastewater batches were brought to the laboratory once every 3 or 4 days. This sewage, after being sterilized in an autoclave, was fed to the reactors. For every new sewage batch, at least two SBR cycles were monitored (normally, the first and last cycles fed on it). Influent and effluent samples were collected and analyzed for COD, SS, nitrogen species and orthophosphate. Also, for the same cycles, during the last 10 minutes of the React period, reactor samples were collected for MLSS and MLVSS determination. Thirty minutes after the Settle period had initiated, the sludge blanket level was measured. This measurement was used for SVI calculations. Besides that, pH, redox, DO and temperature were continuously monitored, during the entire cycle duration.

At least once for each experiment, the reactor contents were sampled at discrete time intervals, during an entire cycle span (track studies). Those samples were analyzed for MLSS, MLVSS and filtered COD, nitrogen species and orthophosphate. Besides that, the cycle was continuously monitored for pH, redox, DO and temperature.

The analyses performed during all experiments have been conducted, as far as possible, in a diligent and meticulous mode, with strict attention given to reagent quality, equipment calibration and clean glassware. Besides that, all analyses have been performed in duplicate.

Table 3.7 shows the analytical methods used for parameter determinations. The parameters pH, redox, DO and temperature were measured by means of electrodes, as described in session 3.2 .

Parameter	Method[1]	Description
SS	2540 D	Total SS dried at 103 - 105°C
VSS	2540 E	VSS ignited at 550°C
COD	5220 C	Closed reflux, Titrimetric method
TKN	4500-N_{org} C	Semi-micro-Kjeldhal method
NH_3^+-N	4500-NH_3 B and E	Preliminary distillation and Titrimetric methods
NO_3^--N NO_2^--N Orthophosphate	4110 B	Ion chromatography with chemical suppression of eluant conductivity
Alkalinity	2320 B	Titration Method

[1] (APHA; AWWA and WPCF, 1989)

Table 3.7: Analytical Methods

A Olympus BH-2 phase contrast microscope was employed for the activated sludge microscopic observation. A drop of activated sludge was placed in a glass slide and a cover glass placed over the drop, taken care to avoid air bubbles entrapment. This wet mount was then observed under 100X and 400X magnification. Some identification keys provided in the literature were used for recognition of the microfauna observed (Martin, 1968; Eikelboom and van Buijsen, 1983; Finlay, *et al.*, 1988).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter will present and discuss the results of the experimental phases described in the last chapter. Following the same structural organization employed there, each of the experimental phases will be presented in succession. After that, a pH, redox and DO measurements general overview comprising all the experiments together will be introduced.

The formulae used for calculating nitrification and denitrification efficiencies, as well as their respective rates, have been placed in Appendix A. Also, the experimental data not included in this chapter, can be found in separate appendices at the end of this thesis.

4.2 Phase 1: System Development and Initial Tests

4.2.1 General Remarks

The system configuration presented in chapter 3 turned out to be reliable, accurate and flexible. However, that configuration was the result of a long, and sometimes frustrating, development process.

Initially, it was necessary to overcome those problems common to any laboratory bench-scale experiment, such as: reactor tubing and connections leaking;

calibration of reactor influent storage tanks and pumps; and clogging problems. To complicate things further, the implementation of "fill-and-draw" intermittent reactors, such as SBRs, requires the development of a reliable, time-based, control system. As explained in chapter 3, this was accomplished through the digital output subsystem. It proved to be the most reliable part of the entire system. During almost two years of unattended operation, it never failed.

The analog subsystem developed was a completely different story, being subjected to interferences of one kind or other. However these problems did not emerge during the system development. Only when the initial tests started, did they emerge.This will be cover in the next section.

4.2.2 Initial Tests

Before the series of planned experiments could begin, it was necessary to test and establish a suitable, reliable method of performing pH, redox and DO measurements. A review of the literature on the subject had revealed many anomalies and inconsistencies evident in previous investigations (section 2.7.2).

Although it was never the intention of this study to carry out these measurements using discrete samples, sometimes it was necessary to proceed in such a fashion, mostly during the initial tests. It proved to be totally unsuitable, undependable and misleading in terms of the results obtained. Due to the dynamic nature of the biological process and possibly other undetermined factors, the measurements were subject to sharp and rapid changes. Things were even worse in the case of anoxic or anaerobic samples, due to the effect of air entrapment. These observations call into question about the validity of often reported discrete pH, DO, redox and other electrode-based measurements.

In the initial design of the SBR system, the probes were intended to be housed in a specially built chamber. The chamber was connected to a recirculation

stream outside the reactor. This was undertaken in order to mitigate the effect of air bubbles, mostly on the DO probes. At first, it seemed to be a very promising approach, however, in the end it had to be rejected, due to the pressure inflicted by the recirculation pump inside the probes chamber. This pressure besides not being constant due to reactor level variations, also caused problems such as cavitation and air entrapment.

The final design approach, as presented in figure 3.1, provided for *in situ* measurement. It proved to be the most dependable and reliable method, just stopping short of being the perfect solution because of the antecipated air bubble problem with regards to DO measurements. The obvious solution would be to provide an internal chamber for probe housing. However, that was dismissed on the basis that eventual improvements in the DO measurement conditions, would not counterbalance one side effect that such approach would provoke. In a small reactor already packed with tubes, stirrer pads and aeration stones, the provision of an internal probe chamber would certainly cause more attached bacteria growth, besides making reactor maintenance more difficult.

At this stage, when it appear that all problems have been resolved, electrical generated interference or noise problems occurred. The possibility of magnetic interferences, such as those induced by the switching of high current loads in near-by signal wiring, or capacity coupling, as for example caused by AC power cables running close to signal wires, had been accounted for. Only shielded cables were employed for the analog input subsystem wiring. However, the possibility of a third interference transfer way, namely conduction, was overlooked. In this case the interference is produced by ground loops. It was this last type of noise transfer mechanism which caused the interference problems mentioned above. To solve them, it was necessary to rewire the analog termination panel, in order to set the analog interface board to work under a different grounding and connection technique, from a single-ended to a differential configuration. Differential connections would avoid any ground current from flowing in the signal wires. Besides that, it was also necessary to electrically isolate both reactors. For example, the stirrer shaft inside the

reactors had to be isolated from the stirrers themselves, to avoid external ground loops.

Although very annoying and time consuming, the above problems had a positive side. In the process of solving them, other small problems occurred, allowing prompt corrective action to be taken. Besides that, their occurrence also allowed a better understanding of the DA & C system, which proved to be helpful during the remaining experimental phases.

Just before starting the planned experiments, the reactors aeration systems were calibrated. That was effected by regulating the air flow by means of a rotameter, as described in the last chapter. The specific oxygen transfer rate (SOTR) in clean water, was used as calibration parameter. SOTR for both reactors were determined through the standard method prescribed in ASCE, 1984. After calibration, both reactors were characterized by SOTR of approximately 12 mg O_2 /min (K_La=0.23 min^{-1}), at an air flow of 1 L/min.

4.3 Nitrification

4.3.1 Initial Remarks

After a long system development and initial tests phase, it was possible to proceed with the experimental phases. The phase 2 main objective was to operate a SBR in a fashion conducive to the nitrification process, and monitor it in terms of both conventional wastewater treatment analysis and electrochemical measurements. Both SBR systems were used. Secondary objectives were to compare the SBRs for reproducibility and later, to access the effect of two different wastage strategies, a fixed sludge age or a fixed mixed liquor suspended solids concentration.

As will be seen later the results for both reactors were very similar. Because of that, it was decided to present and discuss the results of only one of the reactors, SBR 2. Until cycle 40, this reactor was operated with a 30-day sludge age. After that, the fixed mixed liquor concentration strategy was implemented. Data referent to the SBR 1, as well as some additional data about SBR 2, is placed at the end of this thesis, in appendix B.

4.3.2 SBR Operation

During the entire phase 2 period, the timed schedule presented in table 3.2 was respected. Besides Settle, Draw and Idle periods the schedule also comprised a 2-hour Fill (one hour stir only and one hour aerated) and a 6-hour aerated react period.

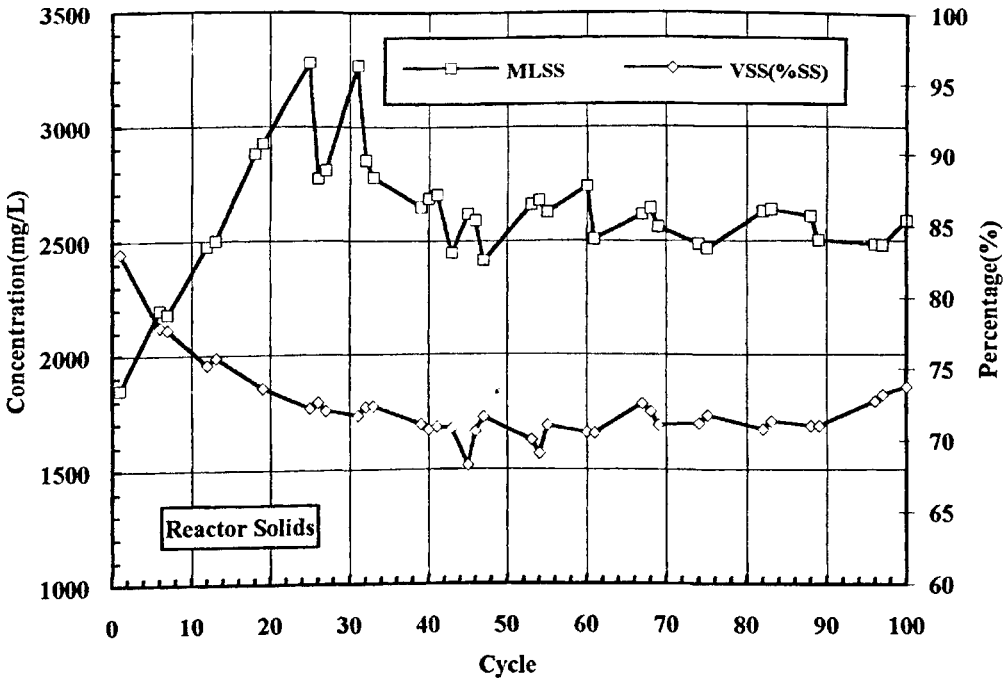
Table 4.1 presents the average composition of the influent wastewater fed to the reactors, which varyed from very weak to medium strength in terms of organic matter and suspended solids, and from weak to strong in terms of reduced forms of nitrogen. COD/TKN ratios varied throughout the experiment, being characterized by high values. On some occasions, values as high as 19 mg COD/mg TKN were observed.

Using influent COD and MLVSS, food to microorganism ratio (F/M) were calculated in the range of 0.03 to 0.09 day^{-1} with an average value of 0.06 day^{-1}. These values fell well inside the reported ranges for extended aeration systems.

Figures 4.1 to 4.5 present reactor and effluent chronological data for SBR 2.

Parameter (mg/L)	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Total COD	408.0	129.8	184.0	710.7
Soluble COD	198.2	62.1	80.0	294.4
Suspended Solids	138.9	50.6	61.5	276.5
Total TKN	40.6	18.4	17.6	75.6
Soluble TKN	37.8	15.6	14.0	72.6
Ammonia-N	18.3	10.5	4.6	37.5
Soluble Oxidized-N	0.3	0.3	0.1	1.3
Soluble PO4-P	3.1	0.8	2.1	4.4

Table 4.1: Average Influent Wastewater Composition for Phase 2, SBR 1 and SBR 2



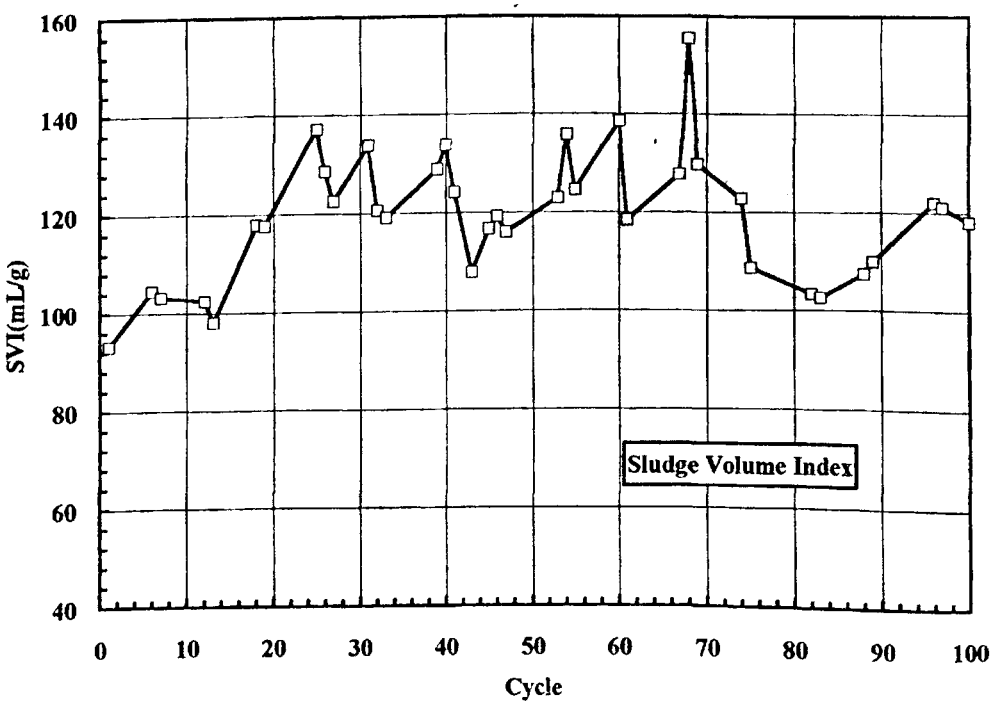


Figure 4.1: Reactor Solids and SVI for Phase 2, SBR 2

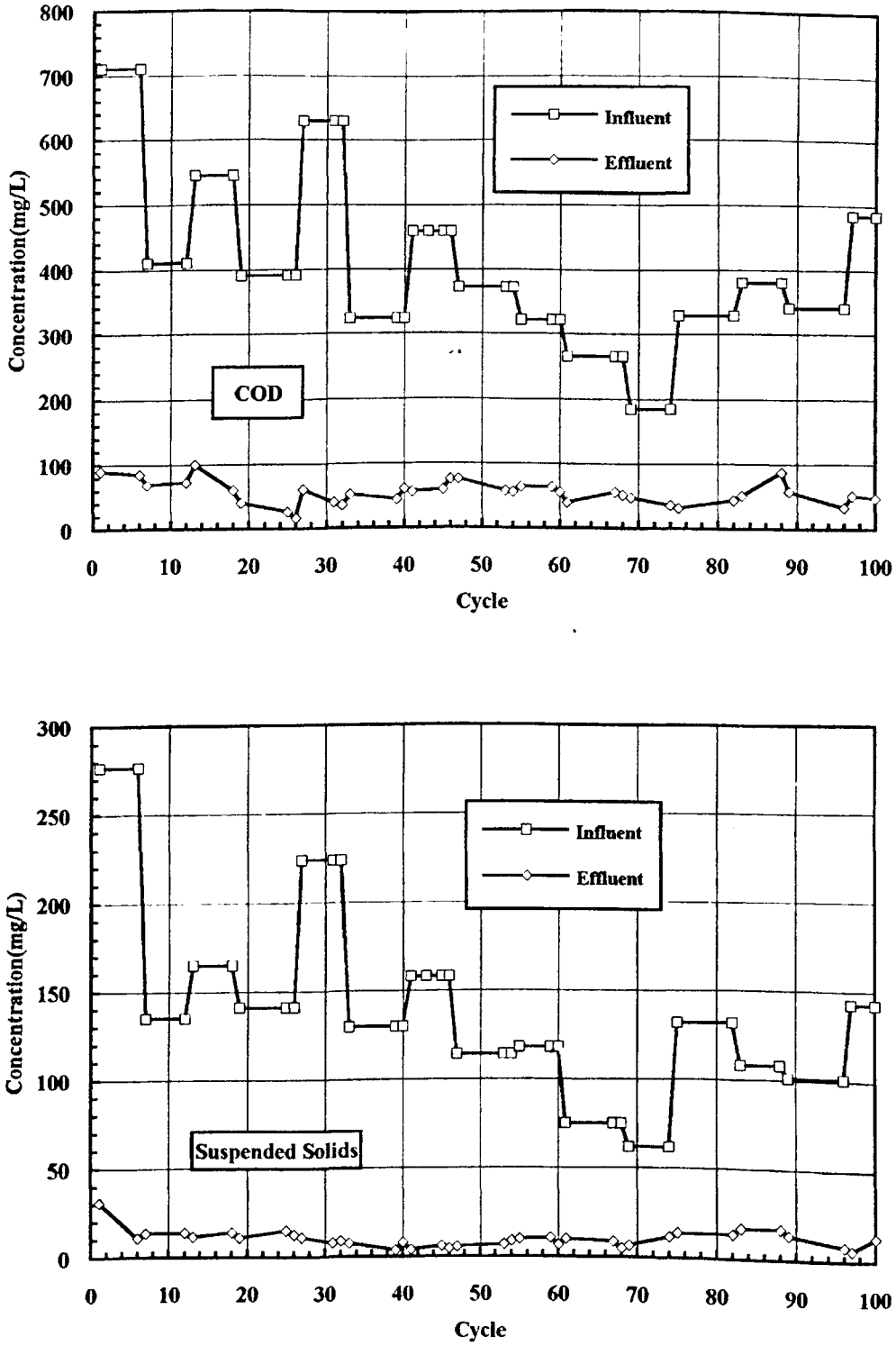
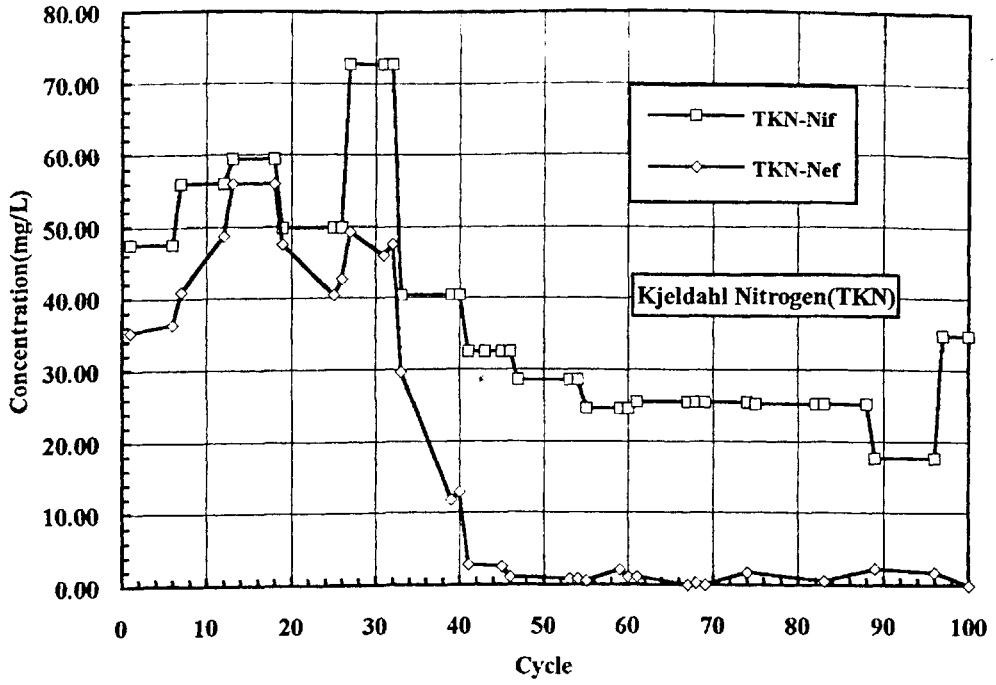


Figure 4.2: COD and SS for Phase 2, SBR 2



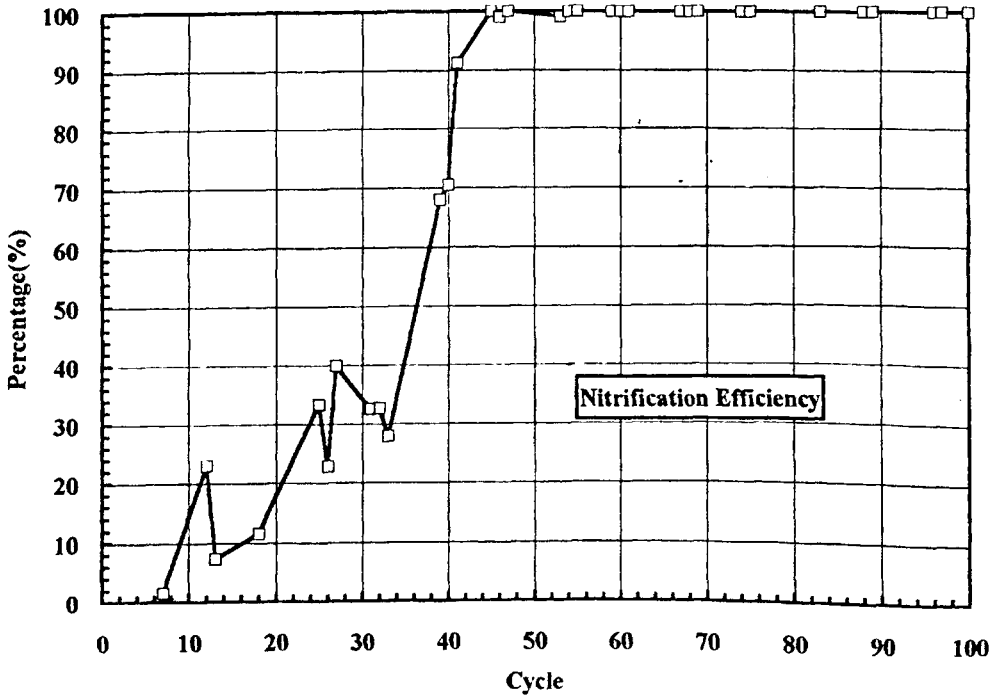


Figure 4.3: Kjeldahl Nitrogen and Nitrification Efficiency for Phase 2, SBR 2

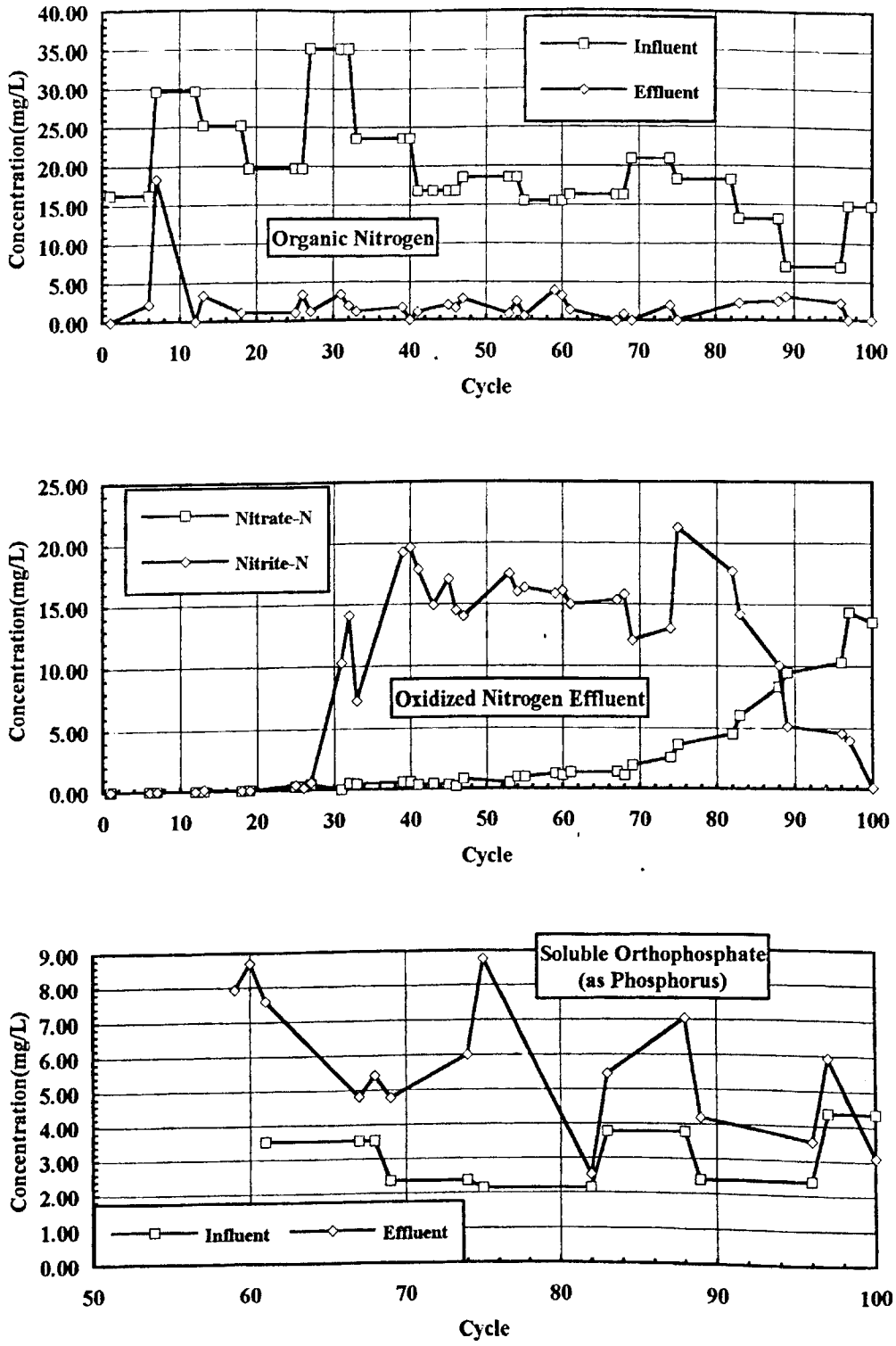


Figure 4.4: Organic Nitrogen, Oxidized Nitrogen and Soluble Orthophosphate for Phase 2, SBR 2

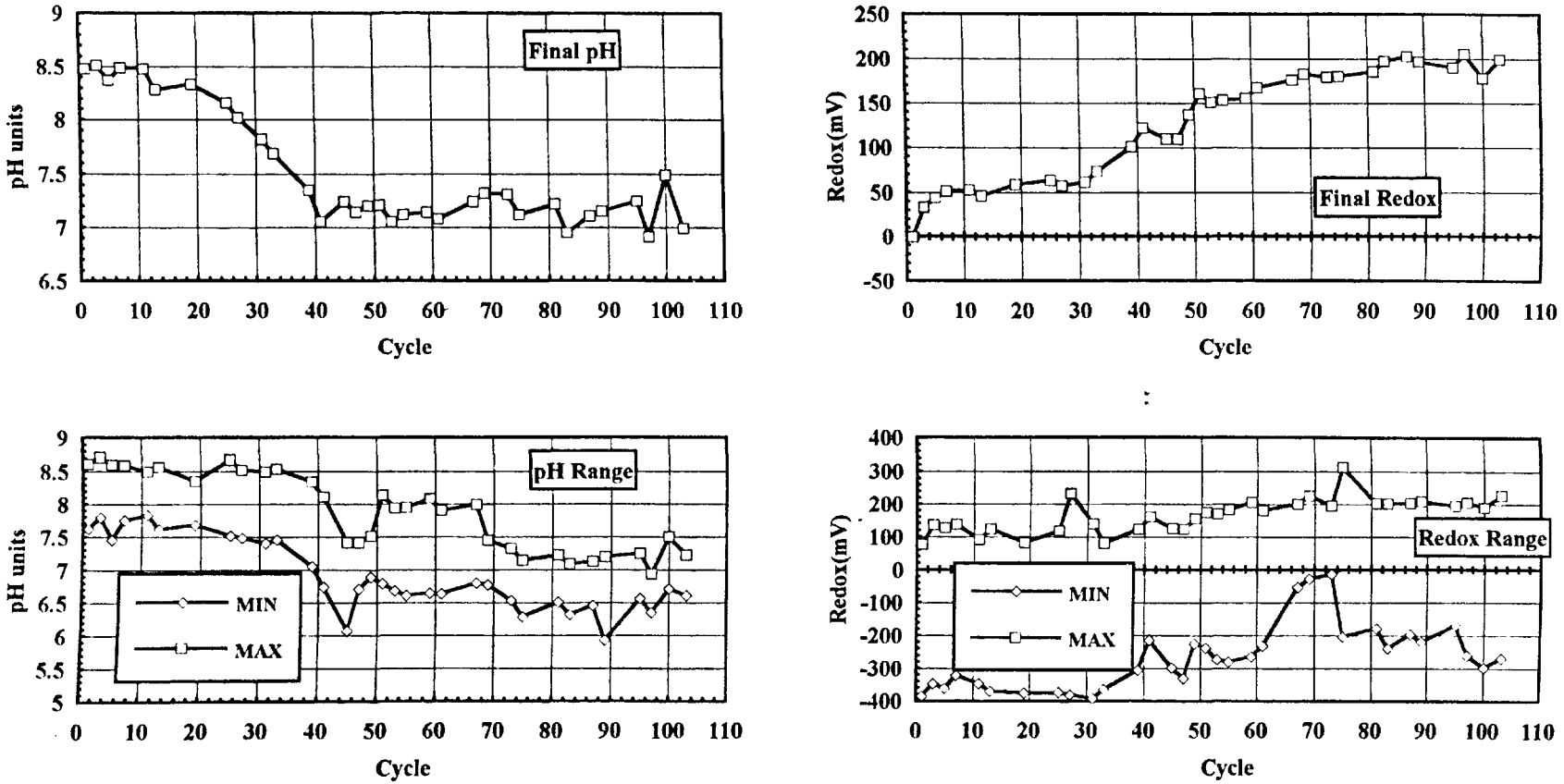


Figure 4.5: pH and Redox for Phase 2, SBR 2

Phase 2 can be divided in two separate periods, start up and "pseudo" steady-state. The start up period goes from the beginning of the experiment until cycle 40. It is a period in which the bacterial population, both in terms of size and composition, is adjusting itself to the new dynamics. The "pseudo" steady-state period goes from the 40th cycle until the experiment conclusion. It is characterized by a roughly constant effluent, but not a constant bacterial growth. Indeed, even during the period of a single cycle, the biomass is in constant change, mostly due to the batch nature of the process.

During the start up period the MLSS concentration increased from 1,850 to 3,280 mg/L (figure 4.1). At this point, the sludge blanket interface, just before the beginning of the Draw period, started to rise above the influent intake point, causing uncontrolled wastage at the time of effluent decanting. As already mentioned, in order to correct the problem, both SBRs had their solids contents lowered to approximately 2,500 mg/L. Besides that, reactor 1 had its sludge age lowered to 20 days. SBR 2, in turn, had its wastage strategy changed by the introduction of a MLSS concentration inside SBR 2, varying between 2,412 and 2,734 mg/L, with a mean value of 2,578 mg/L.

It is important to notice that the initial sludge age was set to 30 days as a result of previous experiments not reported here. Using Pudsey wastewater, tentative approaches to controlling the SBR process through sludge ages from 10 to 15 days had failed, mainly because of excessive wastage. The conclusion was that for the SBR configuration employed, the influent wastewater was too weak to maintain a sizeable bacterial population inside the reactor. In the present case, the reverse phenomena occurred. During the start up period, influent COD concentrations reached values as high as 700 mg/L. SVIs averaging around 121 mL/g show that the problem was not poor settleability, confirming the above observations. On the other hand, the new wastage control approach chosen for SBR 2, based on a MLSS threshold of 2,500 mg/L, turned out to be effective and it was implemented with ease.

Figure 4.2 shows the reactor performance in terms of COD and SS. The treatment achieved consistently low effluent COD and SS concentrations throughout the entire phase. Effluent COD averaged 55.4 mg/L, varying in the range of 32 to 88 mg/L. In terms of SS, the experiment was characterized by a clarified effluent devoid of pin-floc or transient settling abnormalities.

As explained in appendix A, the nitrification efficiency was accessed through Kjeldahl nitrogen and organic nitrogen parameters. The reason for that is obvious. TKN comprises ammonia and organic nitrogen. As the wastwater enters the treatment process, most of the organic nitrogen is transformed to ammonia by the ammonification process. Therefore, depending on the amount of organic nitrogen initially present, the actual quantity of ammonia available for the nitrifiers would be a great deal larger than the initial influent ammonia content.

The start-up period was characterized by high effluent TKN and very low, near zero, effluent oxidized nitrogen. By the end of this period, a sharp drop in effluent TKN concentration concomitantly with a sharp increase in oxidized nitrogen concentrations, marked the onset of the nitrification process. From there on, the treatment achieved complete nitrification on a regular basis (figures 4.3 and 4.4).

Daily specific nitrification rates, calculated as outlined in appendix A, averaged 30.2 g/kg.day, varying from 13.6 to 44.7 g/kg.day. Similar rates have been reported in the literature. Olezkiewicz and Berquist (1988) in a review on the subject reported values of specific nitrification rates varying from 24 to 38 g/kg.day. From their own work on the effects of temperatures in SBR systems operated for nitrification, they reported somewhat greater values, varying within the range of 49 to 90 g/kg.day, for different temperatures.

The nitrification process was marked by a strong nitrite build up. For most of the time, nitrite was the predominant form of oxidized nitrogen in the effluent, reaching values as high as 21 mg/L. It was not until around cycle 60 that the effluent nitrate concentration started to rise. By the end of this phase, the levels of nitrite in

the effluent dropped to zero, while nitrate became the predominant form. Several authors have reported similar occurrences. As reviewed in section 2.4.3, Alleman (1984) listed different stress conditions capable of favouring nitrite accumulation. The most likely in this case, is the presence of free ammonia. Indeed, the circumstance of free ammonia stress on the nitrification process has been associated with nitrification start-ups in batch or plug flow reactors, among others.

All redox data presented in this thesis are expressed with reference to silver/silver chloride (Ag/AgCl) electrode, unless otherwise specifically indicated. Each point in the curves presented in figure 4.5 represents a special point of interest in the daily pH and redox curve. For example,in the case of the final pH curve, each point represents the final pH value reached at the end of the React period for that cycle. Some interesting features have been noticed (figure 4.5).

First of all, the maximum pH values reached during the cycles support the hypothesis of free ammonia presence. At pH levels of 8, an ammonia concentration of 10 mg/l would present a free ammonia concentration of 0.5 mg/l, well in excess of the 0.1 mg/l inhibitory level suggested by Barnes ***et al.*** (1983). The maximum pH curve, may also indicate the reason why near the end of the experiment, ***Nitrobacter*** inhibition appears to have ceased. By then, the maximum pH levels dropped from an average pH of 9, to a less dangerous pH averaging 7.3. For the same ammonia concentration of 10 mg/L, this time only 0.08 mg/L of free ammonia would be present, which is below the inhibitory range. Besides that, some acclimatization effect may have played its part.

Other interesting points can be made by a careful examination of this curves, for example:

a) Even though the final pH during the "pseudo" steady-state period, was kept more or less constant around pH 7.16, a close examination of the minimum pH curve indicates that pH values as low as 5.93 occurred somewhere in the middle of the cycle.

b) The Final pH curve shows that after a while, during the start-up period, pH values dropped steadily from 8.5 to around 7.0 until around the 40th cycle, when the nitrification process, for the first time achieved complete ammonia oxidation. From then on, the final pH fluctuated around 7.0.

c) Finally, the Final redox curve evolved more or less in the reverse fashion to the final pH curve. It seems that complete nitrification was achieved only when the final redox value went above 100 mV. A similar value was reported by Charpentier *et al.* (1989)

4.3.3 Track Study

To conclude phase 2, two separate track studies were run, one for each reactor. Again, in this text, only SBR 2 will be discussed. A table containing SBR 1 track study data has been placed in appendix B.3.

Figures 4.6 through 4.9 present the SBR 2 track study data. In these figures, numbers 1, 2 and 3, inside the boxes, represent the different parts of the cycles, as follows:

1- First hour of the Fill period,without air or mixing;
2- Second hour of the Fill period, air still off, stirrers on;
3- React period, air and mixers on.

Figure 4.6 presents the evolution in time of MLSS and COD. The dilution effect played the major part in the MLSS evolution. The cycle was characterized by a low sludge yield of 0.3 mg MLVSS/mg COD removed. The COD profile shows a sharp rise during the unmixed Fill period, where influent accumulation occurred. After that , during the second part of the Fill period, it remained unchanged. With an influent soluble COD of 205 mg/L, a calculated maximum theoretical COD peak of 133 mg/L would be expected at the end of the Fill period. An actual COD peak of only 88 mg/L suggests the possibility of storage product formation or anaerobic

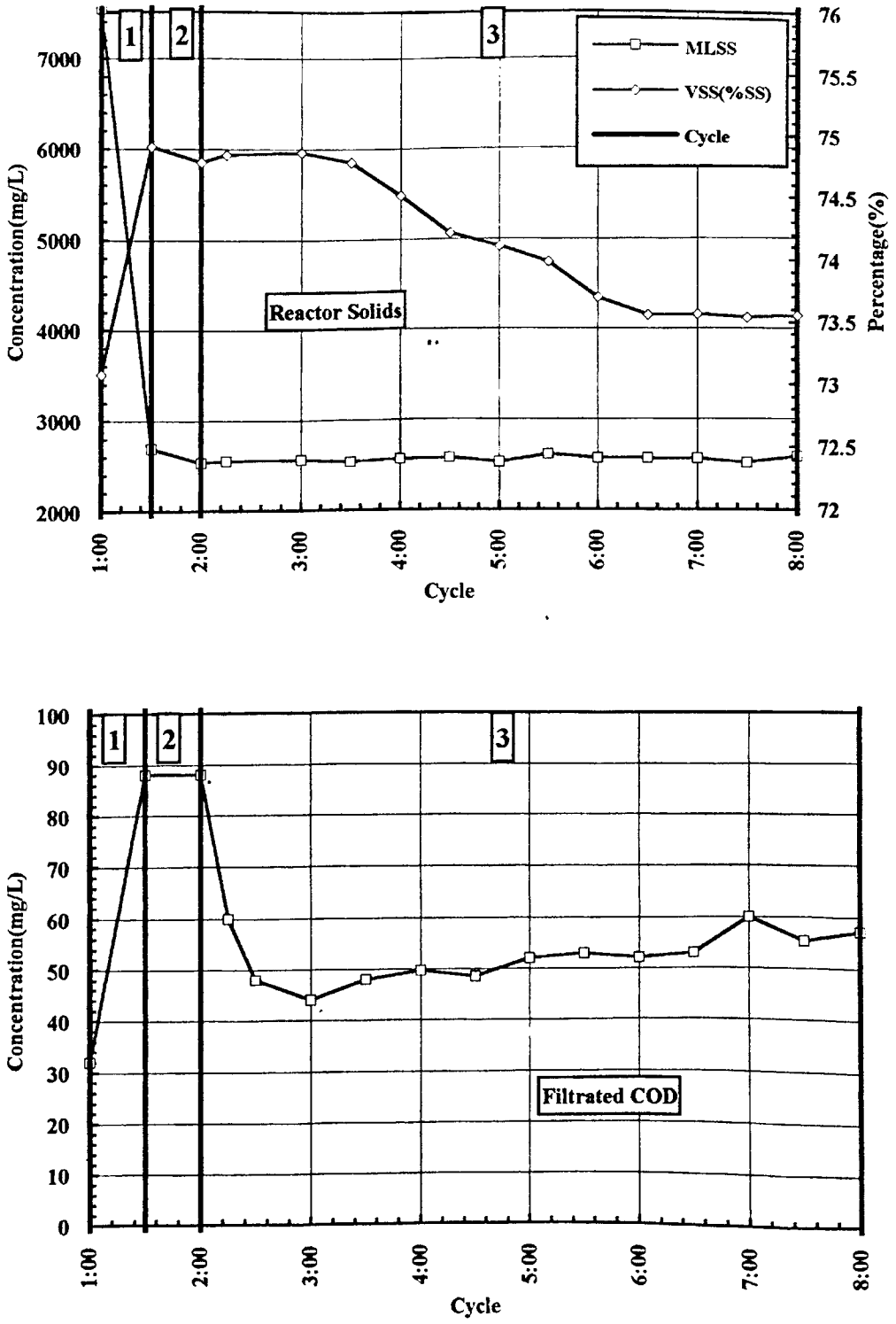


Figure 4.6: Reactor Solids and Soluble COD for Track Study, Phase 2, SBR2

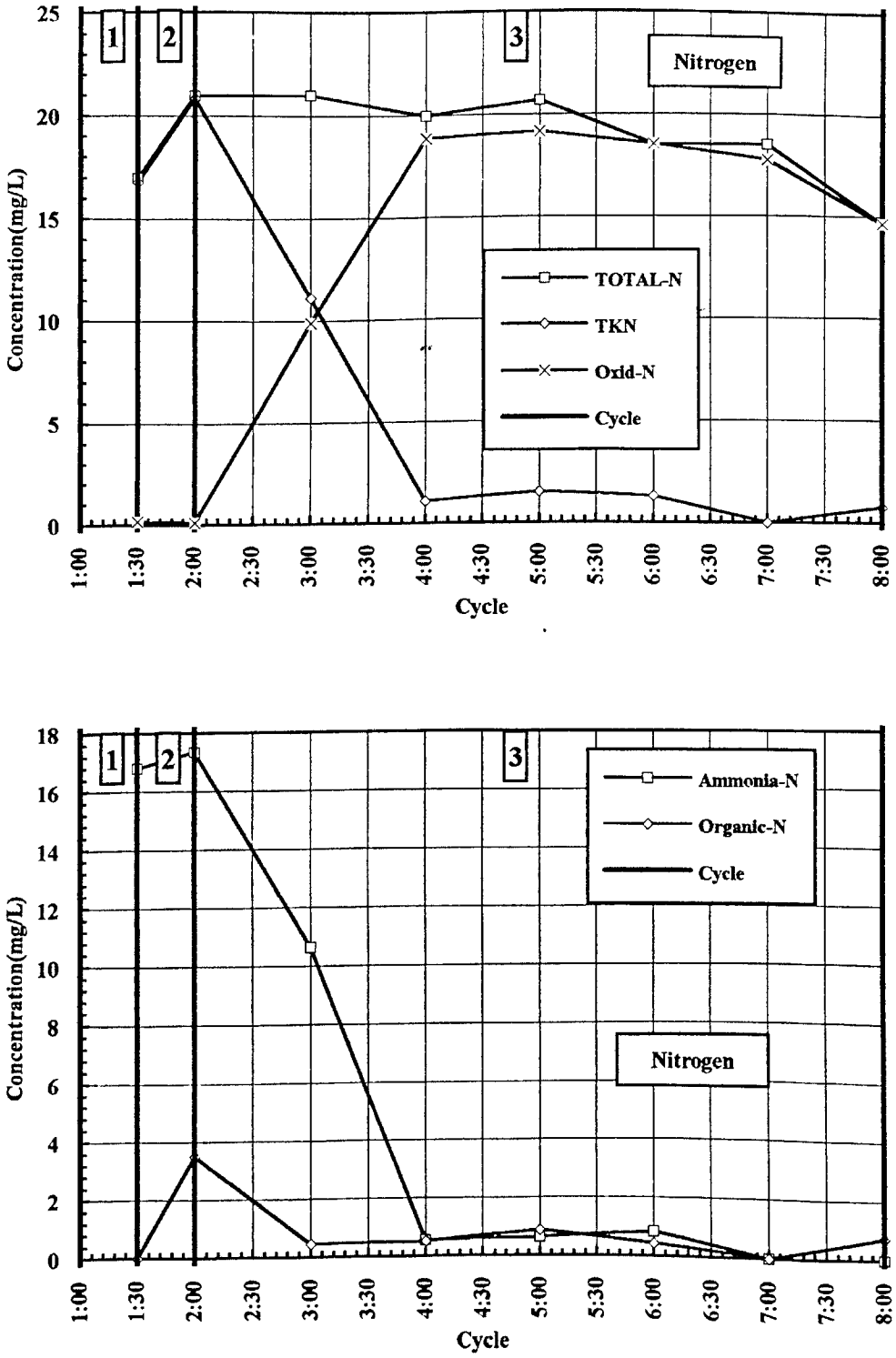


Figure 4.7: Nitrogen Data for Track Study, Phase 2, SBR 2

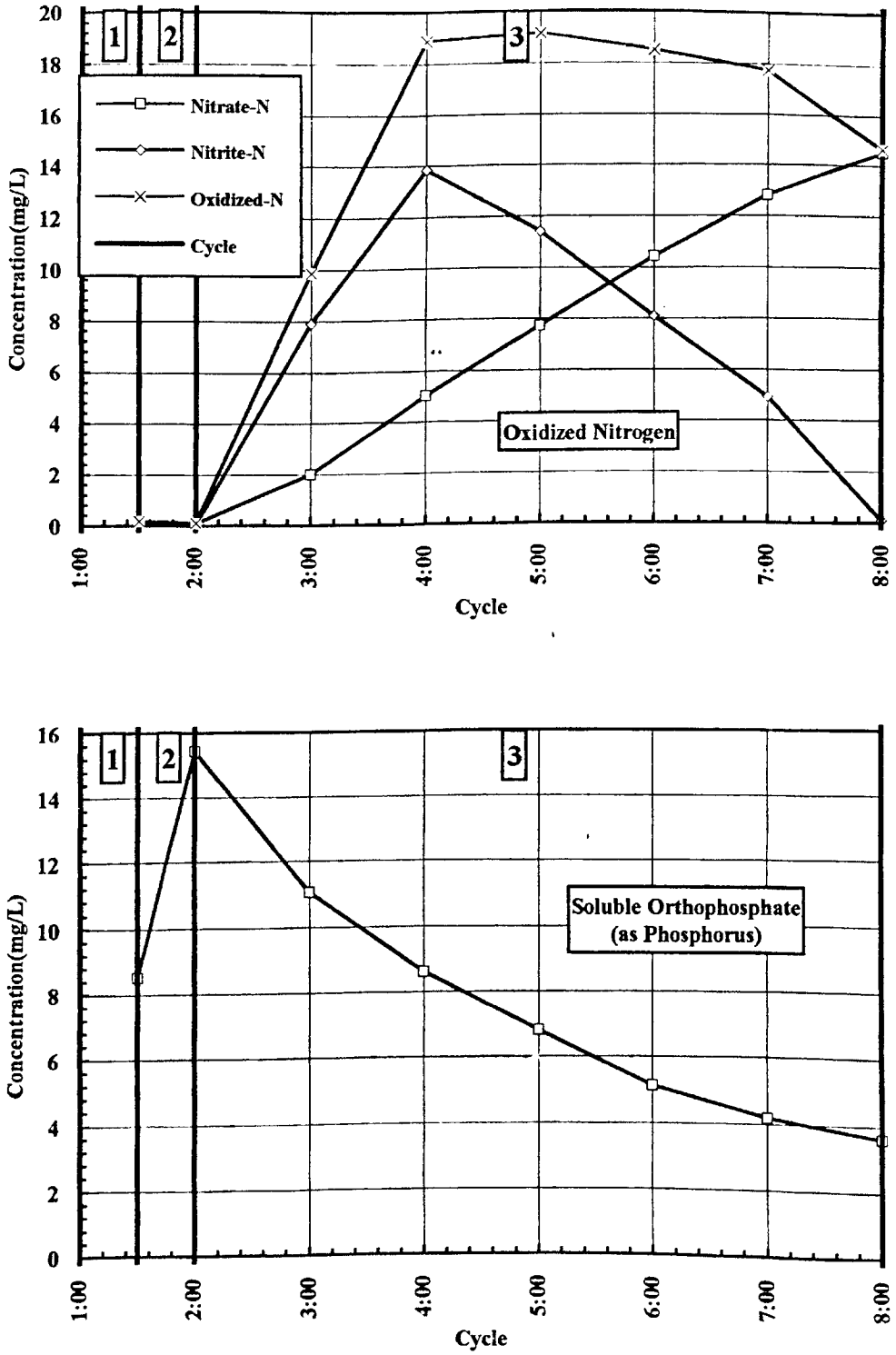


Figure 4.8: Oxidized Nitrogen and Soluble Orthophosphate for Track Study, Phase 2, SBR 2

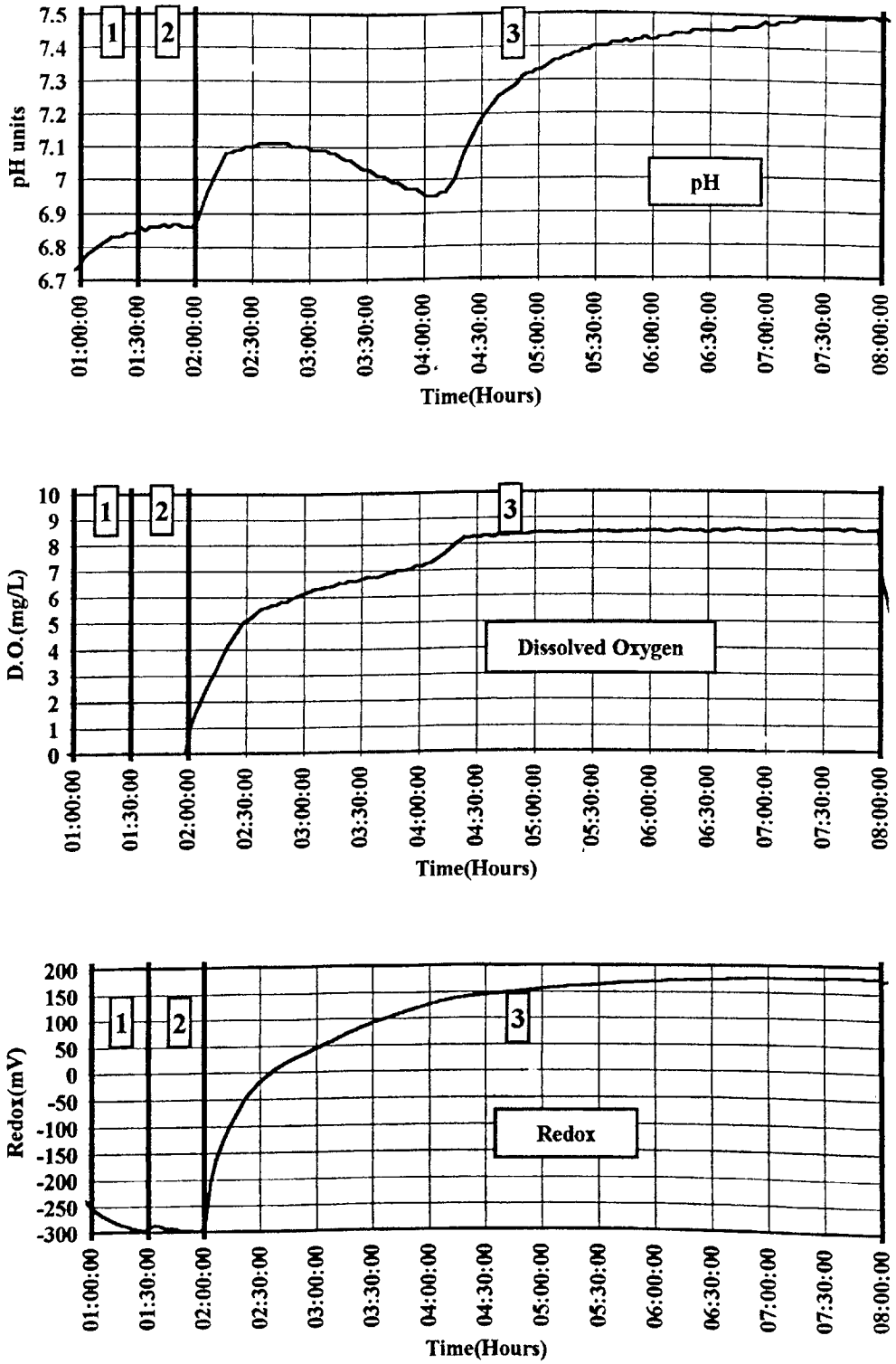


Figure 4.9: Simultaneous pH, DO and Redox Measurements for Track Study, Phase 2, SBR 2

respiration. Yet another possibility is that some oxic oxidation would have occurred, using oxygen supplied through the air-liquid interface. After aeration started, COD levels dropped sharply to a very low value of 44 mg/L, within an hour. From this point onwards, COD concentrations presented a steady, slow rising trend reaching at the end of the React period 57 mg/L. Such COD accumulation phenomena, at the end of SBR React period, have been attributed to the formation of soluble residual products (SRP). Their formation has been related to the hydrolysis of non-viable cellular materials, during endogenous respiration (Orhon *et al.*, 1989).

Ammonia concentrations during the Fill period increased up to a maximum of 17.4 mg/L, by the end of the period. Based on an influent ammonia concentration of 19.9 mg/L, a maximum theoretical concentration of 13 mg/L indicates that fermentative deamination would have occurred. Once aeration started, ammonia dropped sharply reaching background levels at 4:00 o'clock, which indicated the end of the nitrification process (figure 4.7).

The oxidized nitrogen profile shows the presence of neither nitrate nor nitrite, during the Fill period. It is interesting to observe that in the previous cycle, before the track study, two litres of treated sewage containing almost 30 mg/L of oxidized nitrogen were left over. Therefore, high oxidized nitrogen levels would be expected at the beginning of the new cycle. However, the two samples collected half way through the Fill period and just before the onset of the React period presented almost zero oxidized concentrations. From this, it has to be assumed that denitrification occurred throughout the anaerobic Idle period. Indeed, on numerous occasions bubbles have been observed escaping from the sludge blanket, and as a somewhat less often occurrence, the sludge blanket itself floated to the surface of the liquid.

The increase in oxidized nitrogen species, primarily nitrite nitrogen, occurred as soon as the aeration started At the same time, ammonia levels reached their maximum value of 17.4 mg/L, with a coincident pH around 7.0. Based on these data, the correspondent calculated free-ammonia concentration was approximately 0.1 mg/L. This figure is precisely the minimum inhibitory free ammonia level reported

by Barnes and Bliss (1983). Therefore some *Nitrobacter* inhibition may have occurred. As the React period progressed the maximum theoretical free ammonia concentration decreased rapidly, very soon reaching non-inhibitory concentrations. In fact, nitrite concentrations peaked around 14 mg/L at 4 o'clock, and then dropped to non detectable levels at the end of the React period. Nitrate-nitrogen concentrations increased more or less steadily reaching 14.5 mg/L at the end of the period.

During the React period, the steepest slope of the oxidized nitrogen curve was used to determine a maximum theoretical nitrification rate of 9.78 mg NO_X-N/L.h. With an average MLVSS of 1,916 mg/L, the maximum daily specific nitrification rate calculated was 122.5 g/kg.day, which is in the upper part of the range of values reported in the literature. Palis and Irvine (1985), working with a low loaded SBR used for nitrogen removal, reported values in the range of 21.6 to 62.4 g/kg.day. Alleman (1978) presented a value of 170 g/kg.day also obtained from nitrification studies on SBRs. Oleszkiewicz and Berquist (1988) in a study on the temperature effect on the nitrification/denitrification process in SBRs, reported values in the range of 63 to 185 g/kg.day.

Some nitrogen removal seems to have occurred in the last half of the React period (see oxidized nitrogen and total nitrogen curves). At the time, the system was saturated with dissolved oxygen, and the influent degradable organic matter had already been exhausted. Therefore, the reactor conditions were not conducive to denitrification. One explanation for the phenomena would be the presence of anoxic niches inside the reactor, perhaps in the attached form. In this case, denitrification would proceed using endogenous respiration products as an electron donor source. Goronszy (1979) reported a similar case in an intermittent-cycle, extended aeration system. Another explanation that may account for some of the nitrogen removed would be the assimilation process.

Adding this nitrogen removal to the denitrification occurring during the Idle and maybe Fill periods, it was realized that a sizeable nitrogen removal would be expected on a more or less regular basis. Indeed, going back to the chronological

nitrogen data, an average denitrification efficiency of 30.2% was found, varying from 13.6 to 44.7%.

The examination of the soluble orthophosphate profile revealed that enhanced biological phosphorus (EBP) removal occurred in the system. From the influent orthophosphate concentration, a maximum concentration inside the reactor of 5.14 mg/L of soluble orthophosphate was calculated. As can be seen from figure 4.8 (soluble orthophosphate), its concentration reached 15.4 mg/L at the end of the Fill period, and then dropped sharply during the React period to 3.6 mg/L at the end. A close examination of the other parameters, suggested that conditions inside the reactor were right for the EBP removal process. The denitrification occurring during the previous Idle period, removed oxidized nitrogen from the system. The unmixed first part of the Fill period provided for organic substrate accumulation. At the beginning of the mixed only, second part of the fill period, the oxidized nitrogen introduced through the influent, (if any), would be rapidly removed by denitrification. Once anaerobic conditions had been established, phosphates would be released from the flocs with the concomitant storage product synthesis. During the React period, the so called "luxury phosphate uptake" took place. In this process, the organic carbon stored inside the cells is released to the medium, which may help to explain the increase in COD levels towards the end of React.

Figure 4.9 presents pH, DO and redox profiles recorded during the track study. By comparing these profiles with the others,the following observations have been inferred:

a) Unmixed Fill (period 1): This period is characterized by the dilution of the incoming wastewater with the reactor contents. pH showed a more or less steady increase from 6.76 to 6.85 pH units. The DO curve confirmed the absence of DO tension. Redox started at -250 mV and went down further, reaching -300 mV at the end of the period, characterizing fermentative conditions, specifically the presence of volatile acids (Charpentier *et al.* 1987). It should be noted that, under quiescent conditions, the probes readings

may be unrepresentative of the real conditions inside the reactor mostly due to the lack of medium homogeneity..

b) Mixed, non aerated Fill (period 2): All three parameters maintained the trends observed in the previous period. The main factors capable of affecting pH were: influent dilution, volatile acids production through the fermentation process and gas stripping caused by the mixing. As the pH graph had levelled off at around 6.87 pH units, it is conceivable that all the factors evolved have cancelled each other out. DO was zero for the entire period, and redox kept more or less constant at -300 mV.

c) React period: With the onset of aeration, abrupt changes occurred in all three curves. As soon as aeration started, the pH rose steeply up to 7.08, where a clear inflexion point can be observed. This increase was probably caused by three processes: heterotrophic oxic oxidation of the organic matter; ammonification of organic nitrogen compounds and air stripping. After that, the pH increased at a much slower ratee, reaching an maximum of 7.1, just before starting to drop. The most reasonable explanation would be the nitrification process, during which alkalinity is expended by the nitrifiers causing a drop in the pH levels. The meaning of the inflexion point between these two parts of the graph is somewhat uncertain. A possible explanation would be the combination of two processes, a slow down in COD uptake and a concomitant increase in the nitrifier activity. As nitrification proceeded, the pH curve declined until it reached a trough of 6.95, at around 4:10 hours. A quick look at the oxidized nitrogen curve in figure 4.8 reveals that this point marks the decline of the ammonia nitrogen to background levels, indicating the end of the nitratification process. After that, the pH increased sharpely for some time, showing in the end a tendency to level off. The DO curve expressed the DO tension of the system and it also showed the point where nitratification reached completion. It appeared on the DO curve at the same time as in the pH curve and as an inflexion point. Such a feature in the DO curve can be easily explained in terms of oxygen demand. When the

nitrification process comes to a close, the nitrogenous biochemical oxygen demand (NBOD) also ceases. As a result, the reactor oxygen tension will increase. The redox curve resembles the DO curve, at the beginning of aeration the redox rose sharply, at first, slowing down afterwards, and levelling off at the end of the React period at about 180 mV. At first glance, there is no indication of a nitratification end point showing. However, from other recorded cycles, it was realized that an inflexion point occurred at the times at which both pH and DO features appeared. From a detailed observation of the redox curve in figure 4.9 and by calculating its first derivative, it was concluded that an inflexion point had occurred as well. It occurred for a redox value of approximately 150 mV, at around 4:30 hours, showing a small delay compared to the pH and DO values.

As reviewed in section 2.7.2, Charpentier *et al.* (1989) reported an inflexion point in the ascending part of the redox curve which was related to the stabilization of the nitrate content, in the absence of ammonia to be nitrified, and to a rapid increase in the dissolved oxygen content. They worked with a continuous flow activated sludge pilot-scale system. By examining their graphs it is clear that this point corresponds to the nitratification end-point features described above.

The above observations completed the track study analysis. The next logical step was to check all recordings from SBR 1 and SBR 2, for the nitratification end-point features. As would be expected, this analysis revealed their occurrence in all recordings which refer to the cycles where the nitrification process had reached completion, confirming the importance of such a feature as a control parameter.

Figure 4.10 presents the pH, redox and DO profiles for two cycles, one where nitrification did not occurred (left side) and other where it did (right side). They have been provided for comparative purposes.

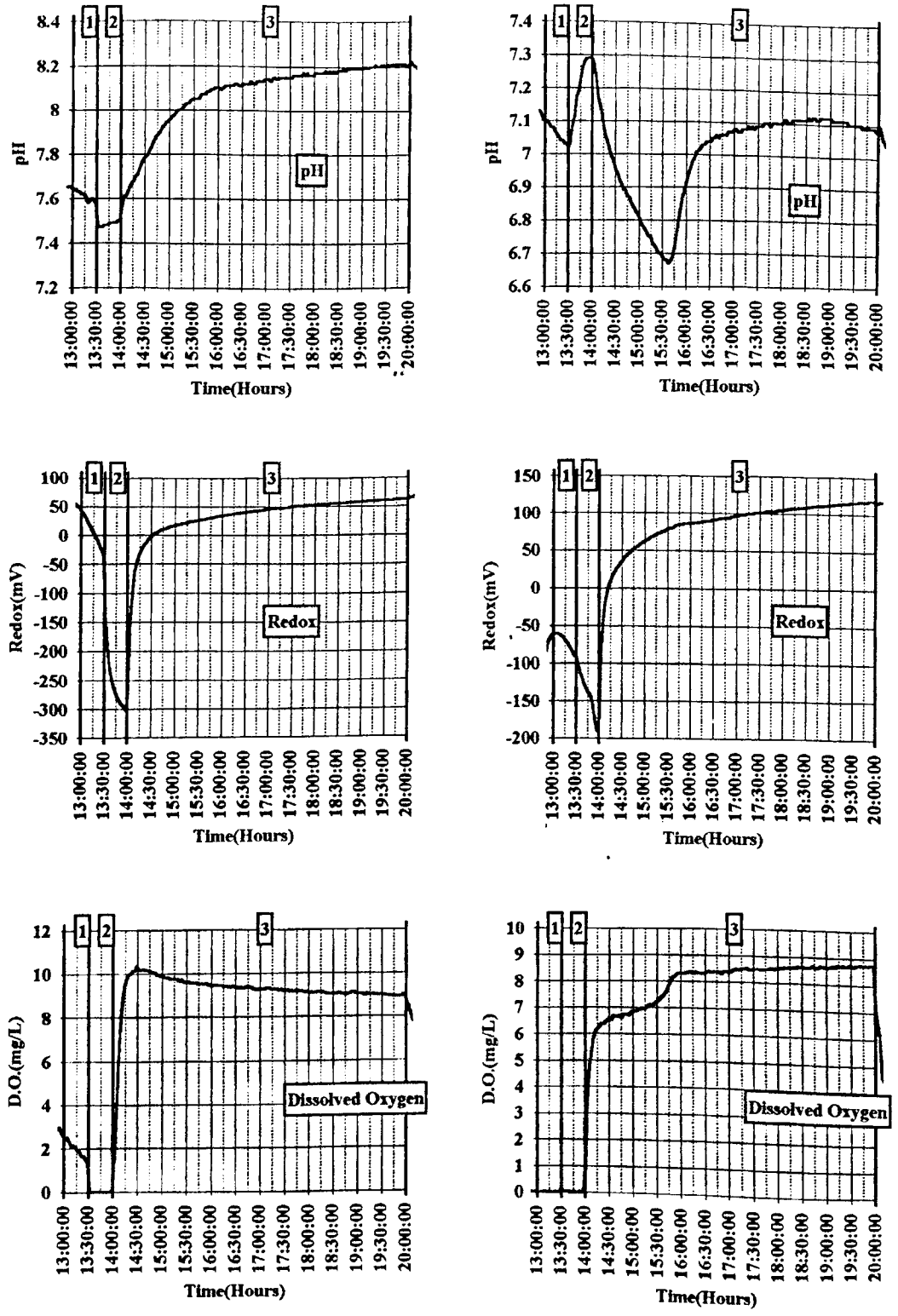


Figure 4.10: Comparative pH, Redox and DO Profiles (Before and After Nitrification) for Phase 2

4.3.4 SBR 1 and SBR 2 Comparison

Figures 4.11 and 4.12 present chronological data for SBR 1 and SBR 2 plotted together in order to compare the reactors performance against each other.

From the beginning of the experiment until cycle 40 they were operated under exactly the same conditions (sludge age 30 days). At cycle 40, the MLSS was reduced to around 2,500 mg/L (full working volume) for both reactors. From then on, SBR 1 was operated under a sludge age of 20 days and SBR 2 had its solids contents fixed to 2,500 mg/L.

The first 40 cycles were used to access both reactors reproducibility. In other words, how well they would repeat the results of each other under equal operational conditions. As already mentioned, there was no significant differences during the period and this was the direct result of careful system calibration before the beginning of the experiment, mostly in terms of aeration capacity and flows rate.

The second part of this phase was used to try to validate the author's ideas about SBR solids wastage strategy. As already mentioned, the authors practical experience in employing a mean cell residence time strategy, was not a successful one, mainly due to the extreme variations in influent strength. For the sludge age range employed (10 to 15 days), two extreme problems occurred. First, during long periods of very dilute influent, a sharp reduction in the reactor solids content was observed, in one case even bringing the system treatment ability to a halt. On the other hand, during periods of medium to strong sewage strength, the problem was high sludge blanket levels after the Settle period. On these occasions, the strategy had to be abandoned in favour of a maximum possible sludge blanket level approach, which in its turn required undue close attention.

At first glance, the results seemed to corroborate the author's view. The MLSS curve in figure 4.11 shows the effect of the two strategies on the reactors solids contents. SBR 1 MLSS profile showed a gradual decline reaching a minimum of

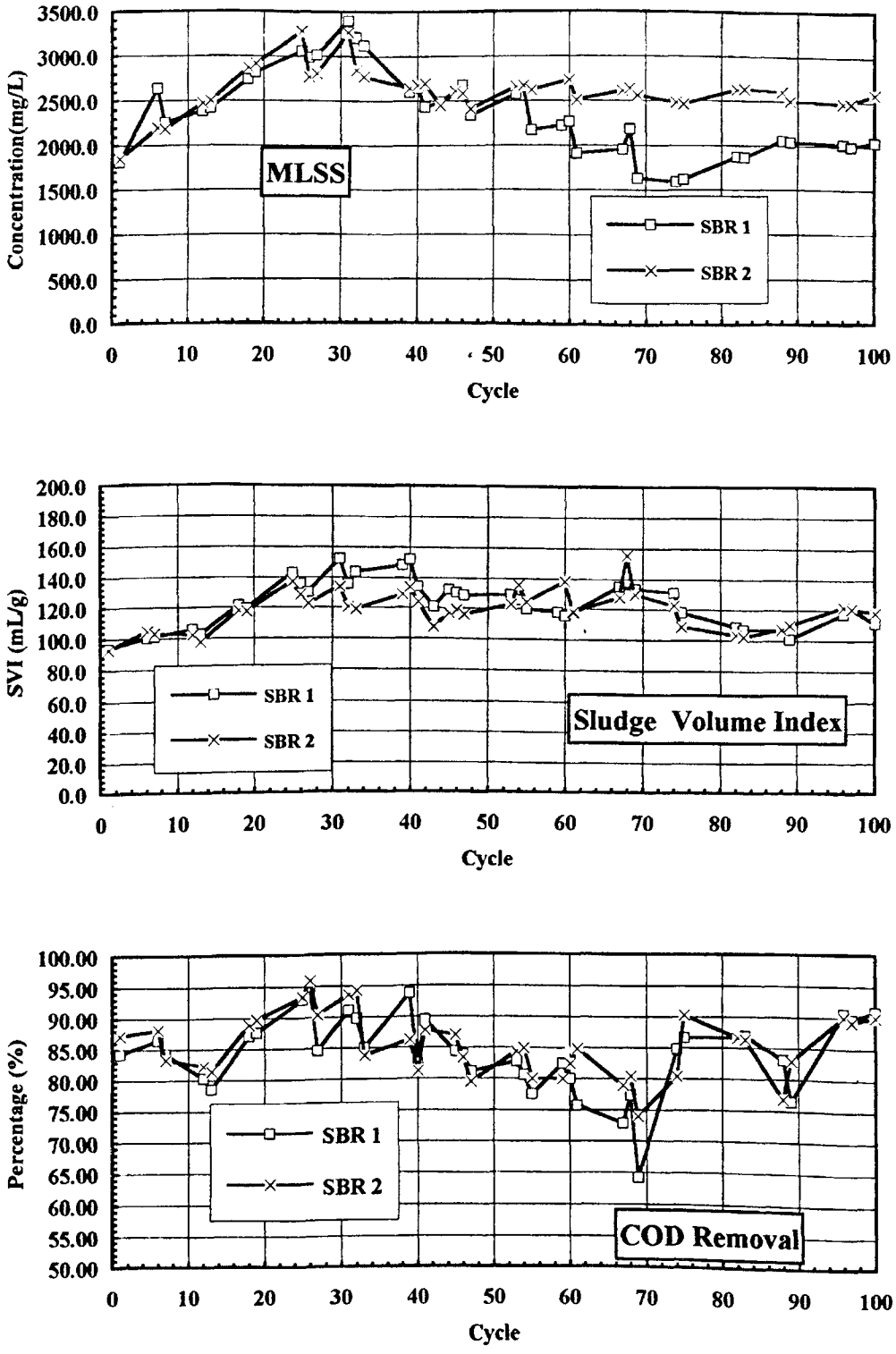
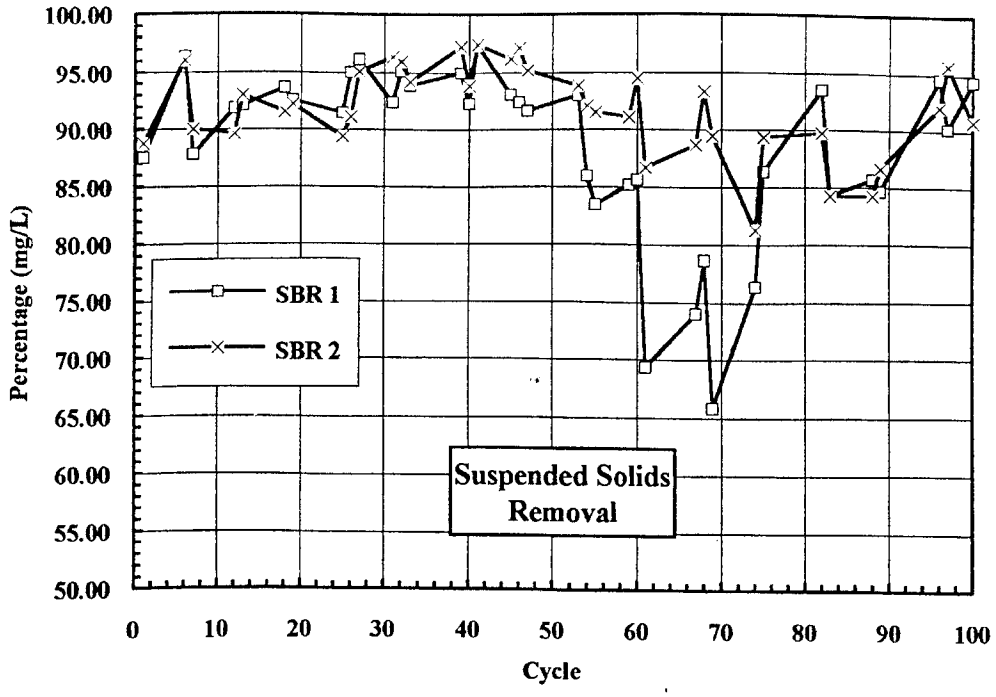


Figure 4.11: MLSS, SVI and COD Removal for Phase 2, SBRs 1 and 2



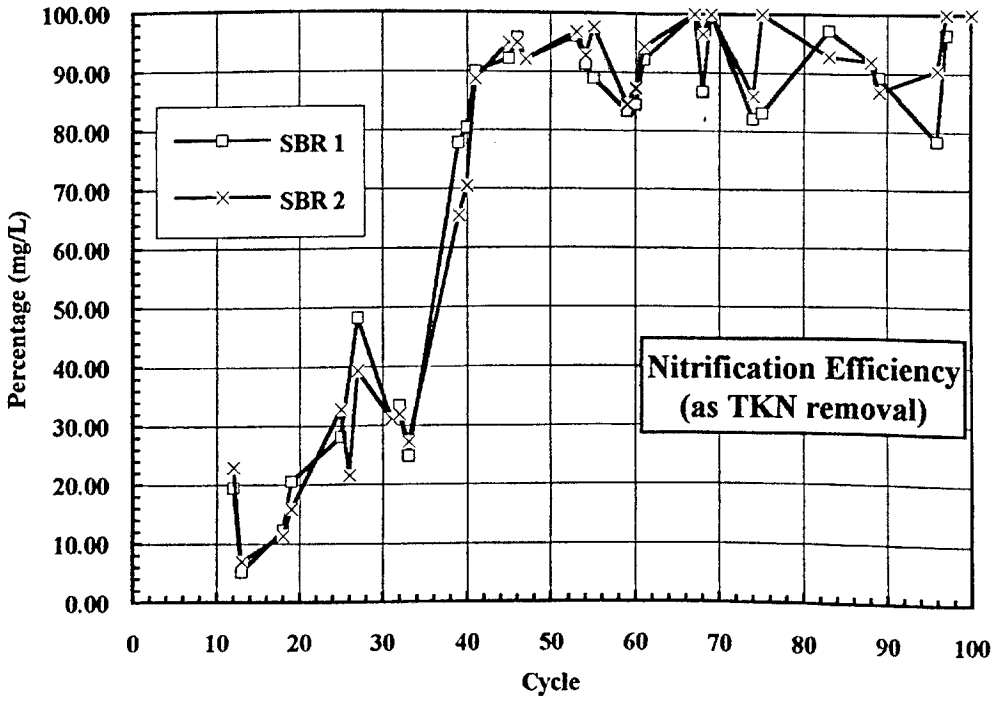


Figure 4.12: SS Removal and Nitrification Efficiency for Phase 2, SBRs 1 and 2

1,592 mg/L at cycle 74. After that, it increased for a while, levelling off in the end, at around 2,000 mg/L. SBR 2 MLSS profile was kept at an average 2,578 mg/L with small variations. The variations in SBR 1 solids contents were caused by the influent COD variations (see figure 4.2).

Parameter	SBR 1			SBR 2		
	Mean	Min	Max	Mean	Min	Max
SVI (mL/g)	125	100	185	121	100	155
COD Removal (%)	82.9	64.1	99.1	84.2	73.9	99.1
SS Removal (%)	86.6	65.9	99.2	91.5	81.3	99.8
TKN Removal (%)	91.3	78.3	99.9	93.3	70.4	99.9

TABLE 4.2: Average Performance Parameter for Phase 2, SBRs 1 and 2

In terms of reactor performance it seems that SBR 2 had a slight edge. Table 4.2 presents some average performance parameters for SBR 1 and SBR 2, from cycle 40 onwards.

SBR 2 apparently out performed SBR 1 in all fronts but the differences were very small. Because of that, an statistical comparative analysis of the data for both SBRs was performed. From a student's test for difference between means, with the assumption of identical standard deviations, it was found that the only significant difference ($\alpha = 0.05$) occurred for SS removal. Therefore, statistically speaking, there was no difference in performance for all other parameters.

From the above considerations, it was inferred that even if the difference in performance was not significantly big enough, except for the SS parameter, the SBR 1 instability in terms of MLSS contents and its potential implications, would turn the balance in favour of SBR 2 strategy.

In fact, other authors have expressed similar opinions on theoretical grounds. Irvine *et al.* (1977) commented that because sludge age, as defined for SBR in terms of stoichiometry and kinetics, varies throughout the Fill and React periods, its value for conventional systems is lost in SBRs. Hoepker and Schroeder (1979) commented that due to the inherent non-steady-state characteristic of SBRs, the relationship between growth rate and sludge age is completely invalid, revealing nothing about the actual growth rates experienced.

4.4 Phase 3: Nitrification/denitrification

4.4.1 Initial Remarks

The strategy employed in the discussion of phase 2 results, intentionally emphasized the evolution in time, of each performance parameter as part of the process being studied. Such an approach was taken for two main reasons. First of all, it was used to provide a detailed description of how each performance parameter is related to the process as a whole and to introduce the structural organization of the graphs employed to present the data. As performance parameters and graphs used in phase 3, and also in phase 4, are the same as those employed in phase 2, it was decided to adopt a new approach for the last two phases. As such, the results will be presented and discussed on a process level rather than on a parameter level. The emphasis will be placed on the process evolution in time, calling on whatever parameter whenever necessary to illustrate the discussion.

The main objective of phase 3 was to implement the nitrification/denitrification process in a SBR and study it using conventional analysis and pH, redox and DO measurements. The operational schedule implemented, intends that the organic matter in the influent wastewater and the products of the endogenous respiration will serve as electron donors for the denitrification process. In addition to the main objective, as a minor study, the reactor protozoan and metazoan communities were observed on a regular basis, to assess the potential of microscopy in plant control.

Data not included in this discussion can be found in appendix C.

4.4.2 SBR Operation

As a result of the reproducibility demonstrated by both system reactors during phase 2, it was decided to carry out the nitrification/denitrification phase using only one reactor.

The time based schedule presented in chapter 3, table 3.3, was observed for most of this phase. As already mentioned, this schedule would provide for the nitrification/denitrification process, by alternating periods of high and low oxygen tension. It was assumed that nitrification would proceed during oxic periods and denitrification during anoxic ones. Yet another assumption was that denitrification would employ the influent wastewater during Fill, and the products of the endogenous metabolism during React, as sources of electron donors.

Table 4.3 presents the average composition of the influent wastewater used during this phase (and also in phase 4). Such wastewater can be characterized as of medium to strong strength in terms of COD, SS and TKN, contrasting with the weaker phase 2 influent. Alkalinity was not determined until the 70^{th} cycle, therefore the values presented in table 4.3 are only representative of the last 25 cycles of this experiment.

Parameter (mg/L)	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Total COD	453.5	90.5	306.3	706.0
Soluble COD	207.2	49.8	104.7	334.0
SS	162.6	39.4	103.0	294.5
Total TKN	45.7	16.7	14.6	77.6
Soluble TKN	39.2	15.2	10.1	65.9
Ammonia-N	22.7	7.8	9.2	39.2
Total Alkalinity (as CACO3)	252.5	49.2	203.3	360.9
Soluble Oxidized-N	0.8	0.1	0.1	3.4
Soluble PO4-P	4.8	1.2	2.2	7.3

Table 4.3: Average Influent Wastewater Composition for Phases 3 and 4

This stronger wastewater composition was caused by a prolonged dry period in the region, effecting a more concentrated influent wastewater arriving at Pudsey works. As a direct consequence, this experiment was characterized for F/M ratios, based on COD and MLVSS, averaging 0.16 day $^{-1}$, varying from 0.11 to 0.21 day^{-1}. Such a range is higher than the recomended values for SBR applications (0.025 to 0.15 day^{-1}), as showed in Tchobanoglous and Burton (1991). Also the average volumetric load of 302 g COD/ m^3.day is just about the maximum SBR load proposed by Hoepker and Schroeder (1979).

Figures 4.13 to 4.20 present the chronological performance parameters observed during phase 3. In order to proceed with the presentation and discussion of phase 3 results, this phase has been separated into 3 distinct periods. The first comprises the first 40 cycles and can be portrayed as the start-up period. The second period goes from cycle 40 to the cycle 85, embracing the remaining 45 periods under the initial time schedule showed in table 3.3. Finally, the last period, includes the cycles up to the very end of this experiment. These cycles were used to experiment with the alternative time based schedules presented in table 3.4. The MLSS was kept approximately at the 2,500 mg/L set point throughout the experiment.

As with phase 2, the start-up period can be characterized as the period of time necessary for the biomass to acclimatize to the new conditions and for the nitrifiers to establish themselves. Bearing in mind that the influent wastewater contained almost no oxidized nitrogen, it is understandable that denitrification was dependent on the oxidized nitrogen supplied through nitrification. Therefore, denitrification was not important during most of this period.

The start-up period proceed initially with high effluent COD and SS concentrations, gradually dropping to values around 50 mg/L for COD and less than 20 mg/L for SS, which proved to be the norm for the remainder of this period. As would be expected, due to the influent strength, it took longer than phase 2 for the effluent COD and SS reach those low values, approximately 20 cycles. The period

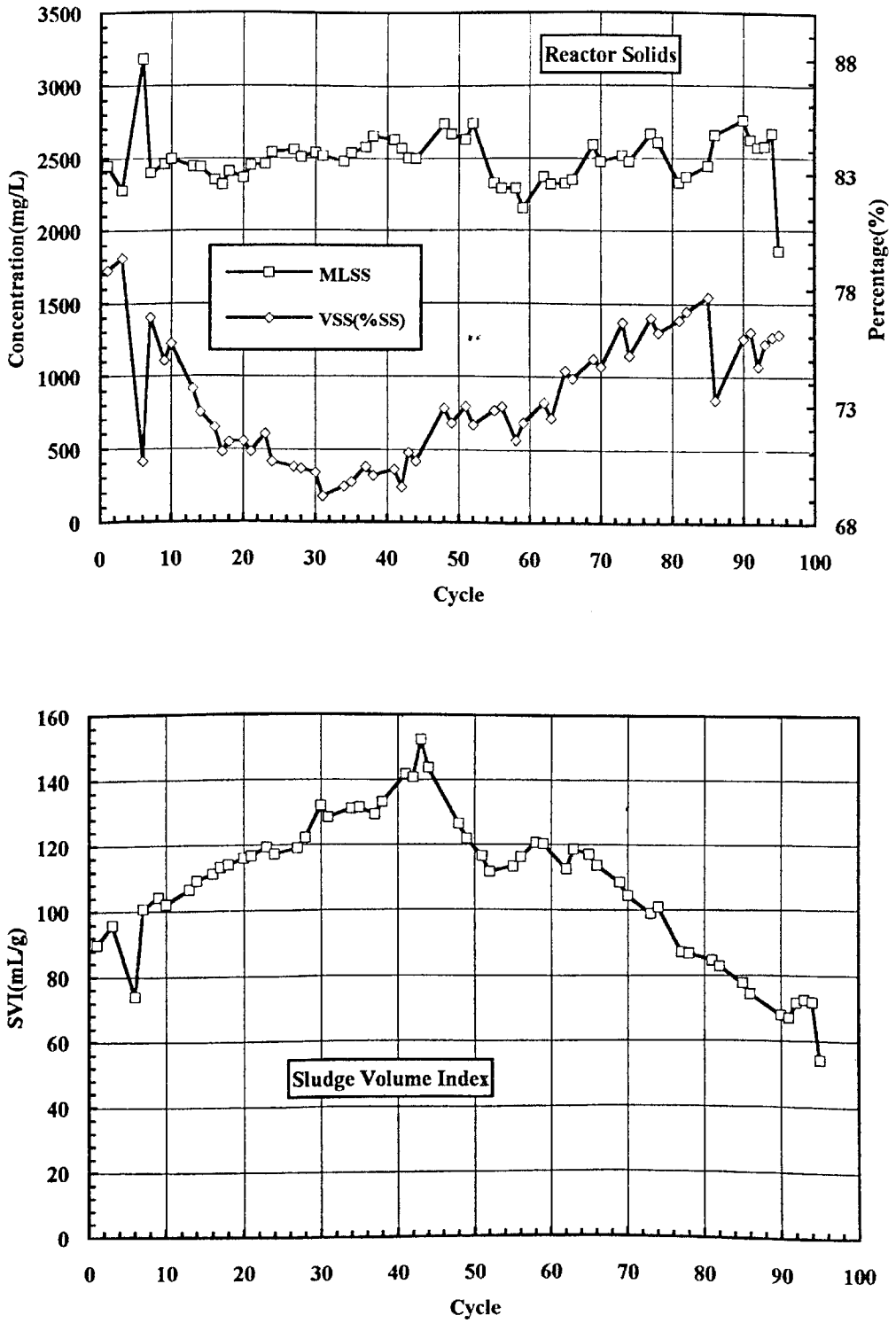
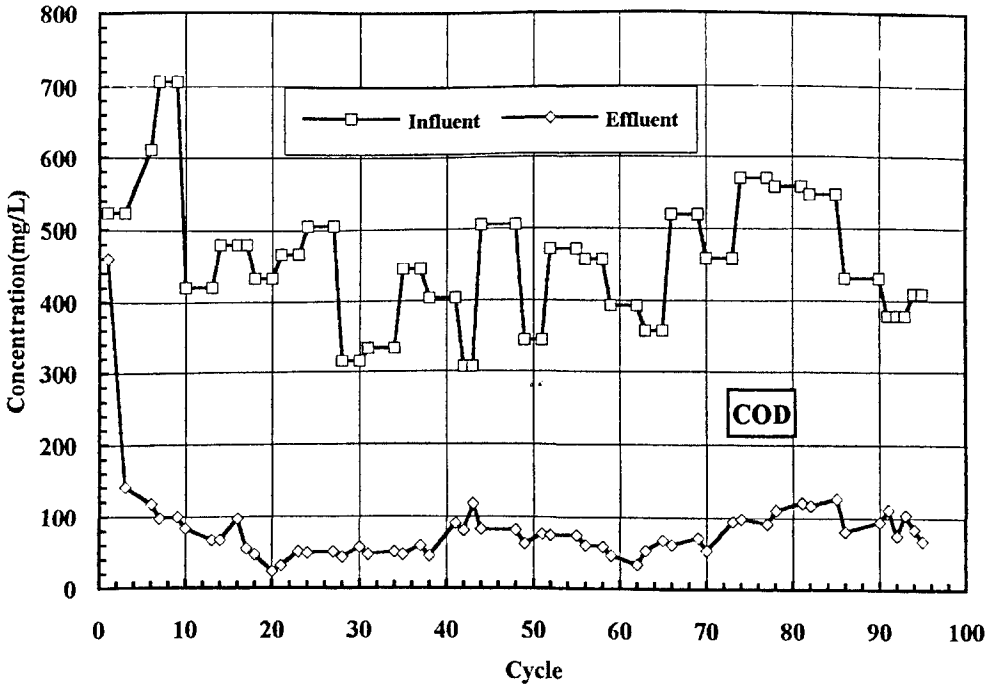


Figure 4.13: Reactor Solids and SVI for Phase 3



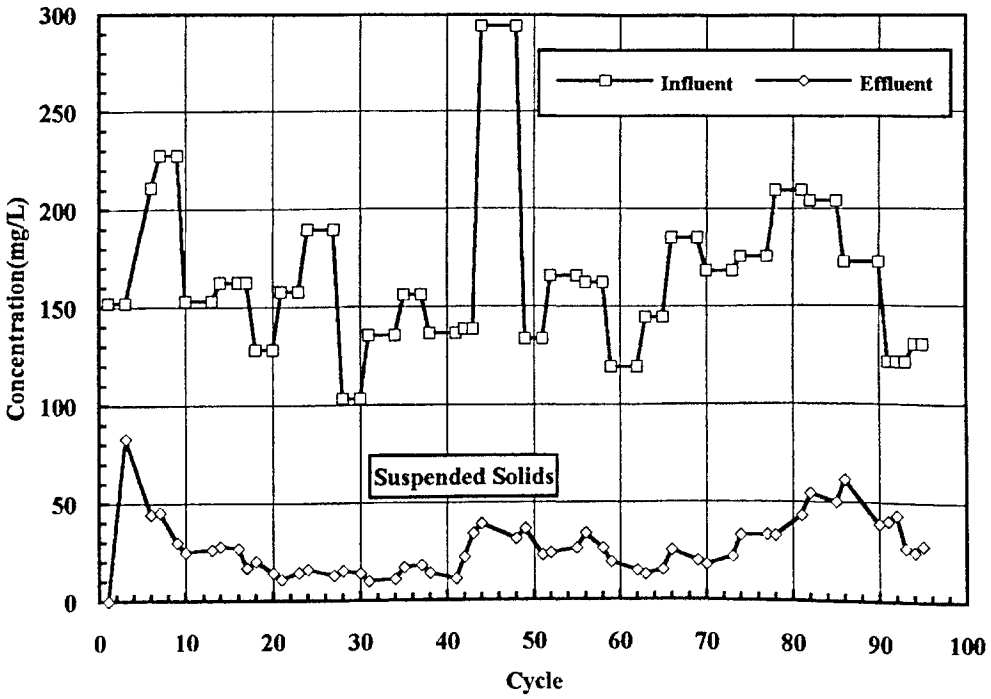
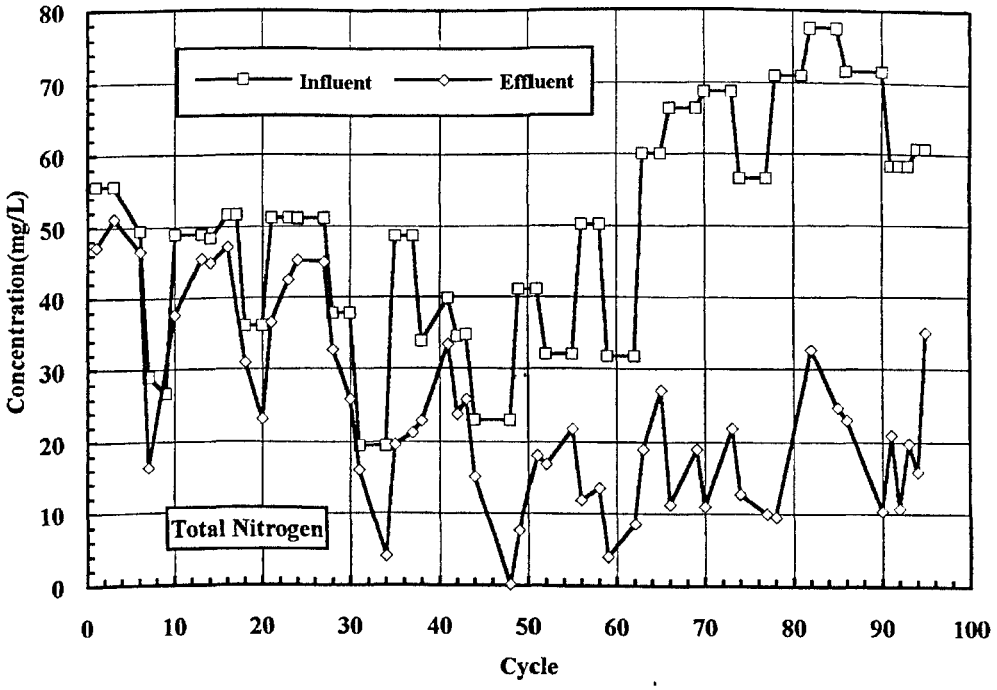


Figure 4.14: COD and SS for Phase 3



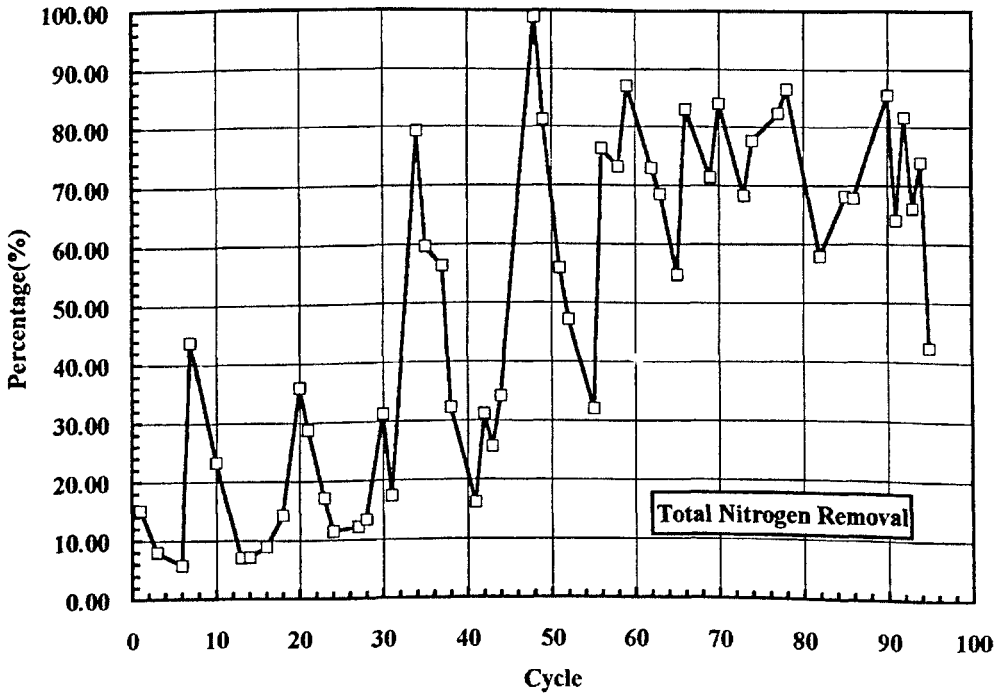


Figure 4.15: Total Nitrogen for Phase 3

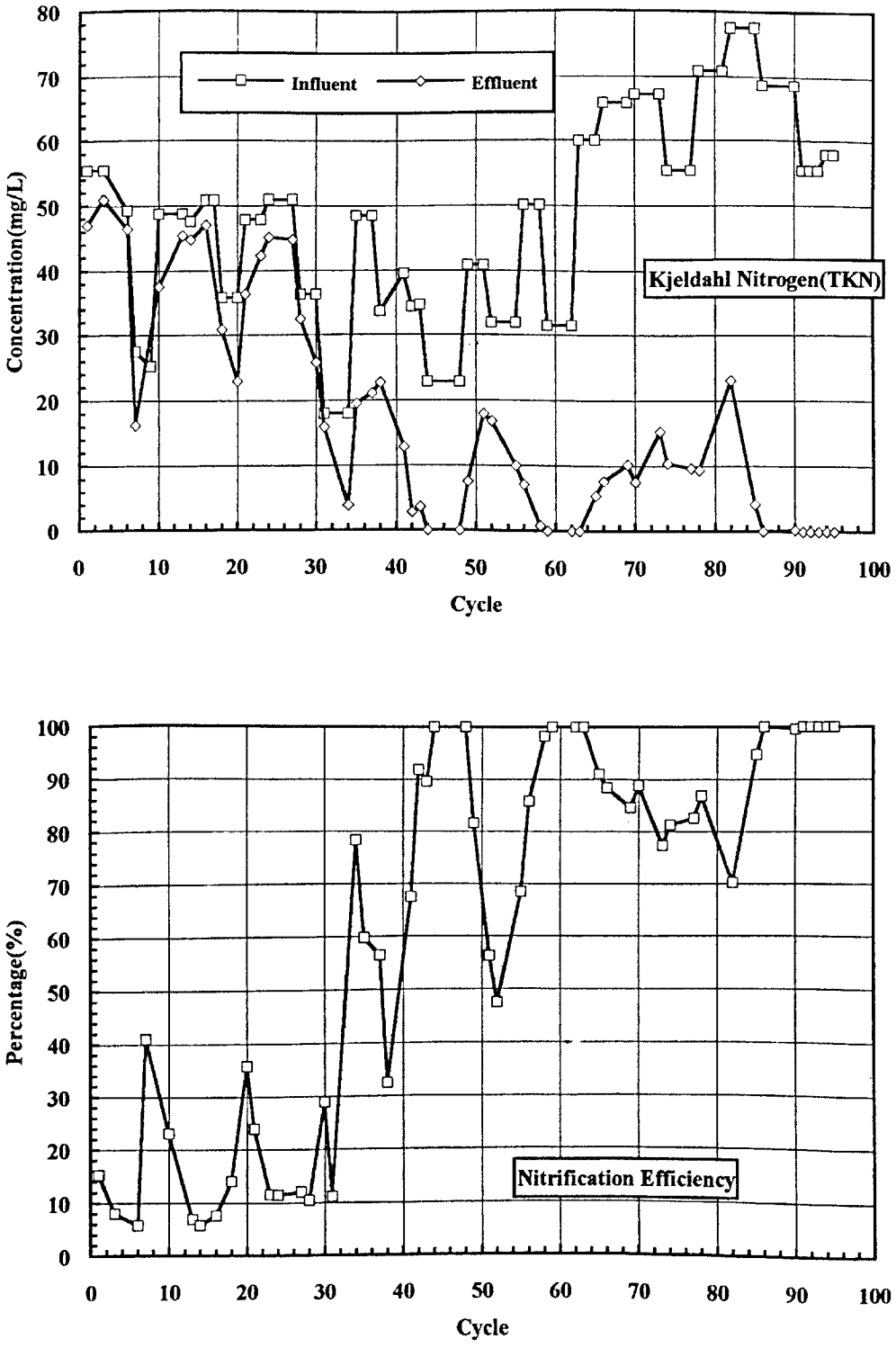


Figure 4.16: Kjeldhal Nitrogen and Nitrification Efficiency for Phase 3

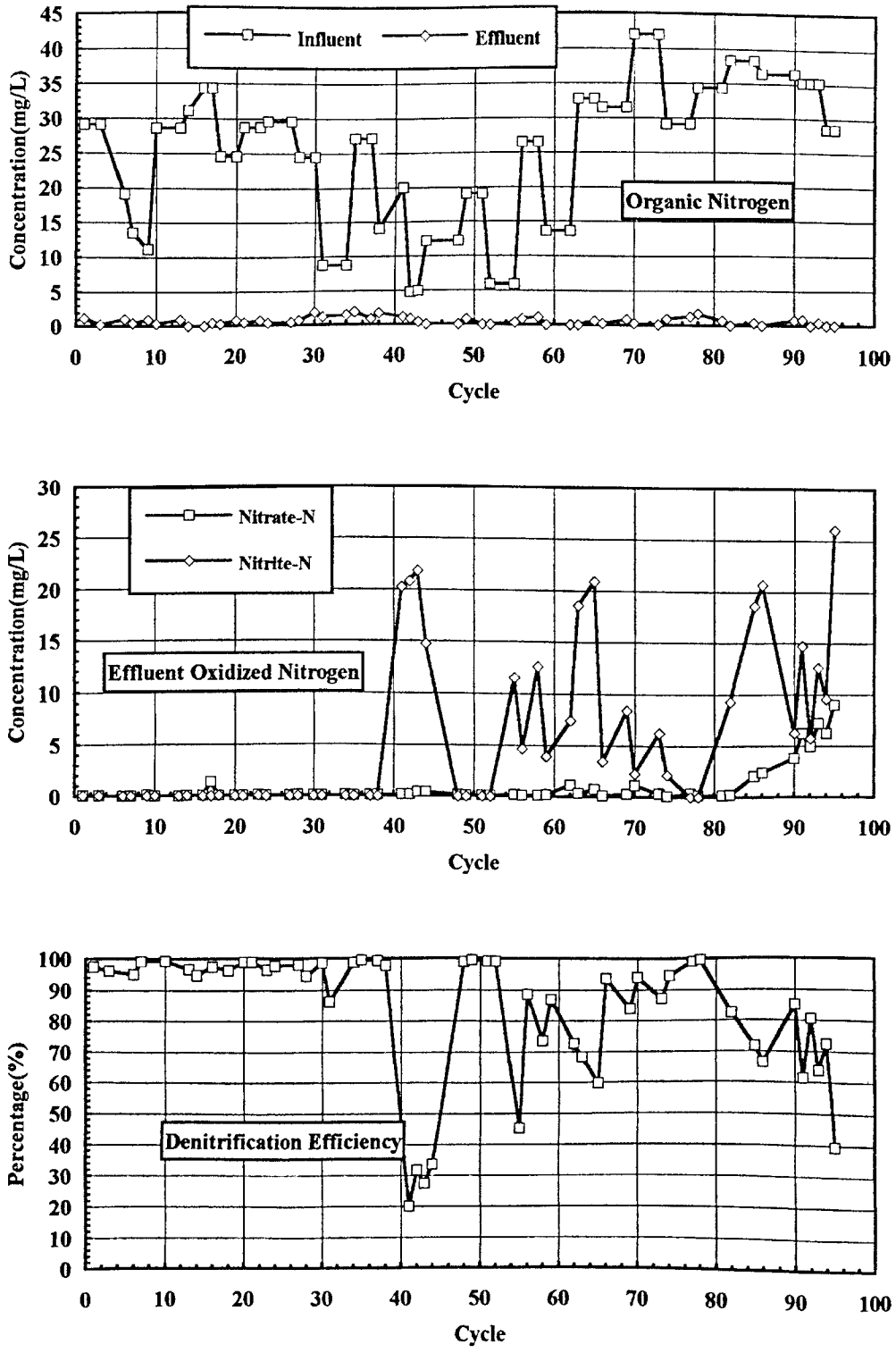


Figure 4.17: Organic Nitrogen, Oxidized Nitrogen and Denitrification Efficiency for Phase 3

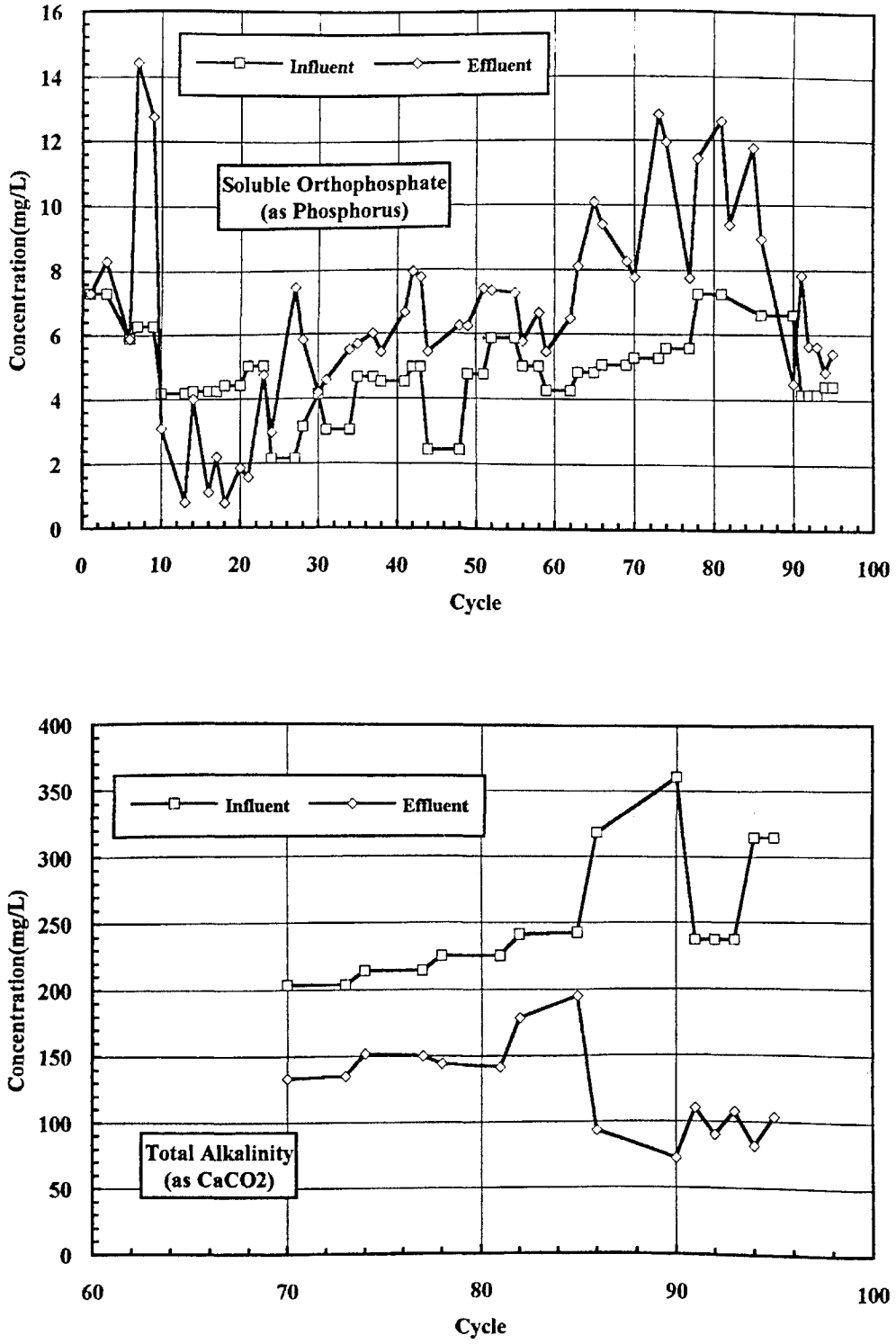


Figure 4.18: Soluble Orthophosphate and Total Alkalinity for Phase 3

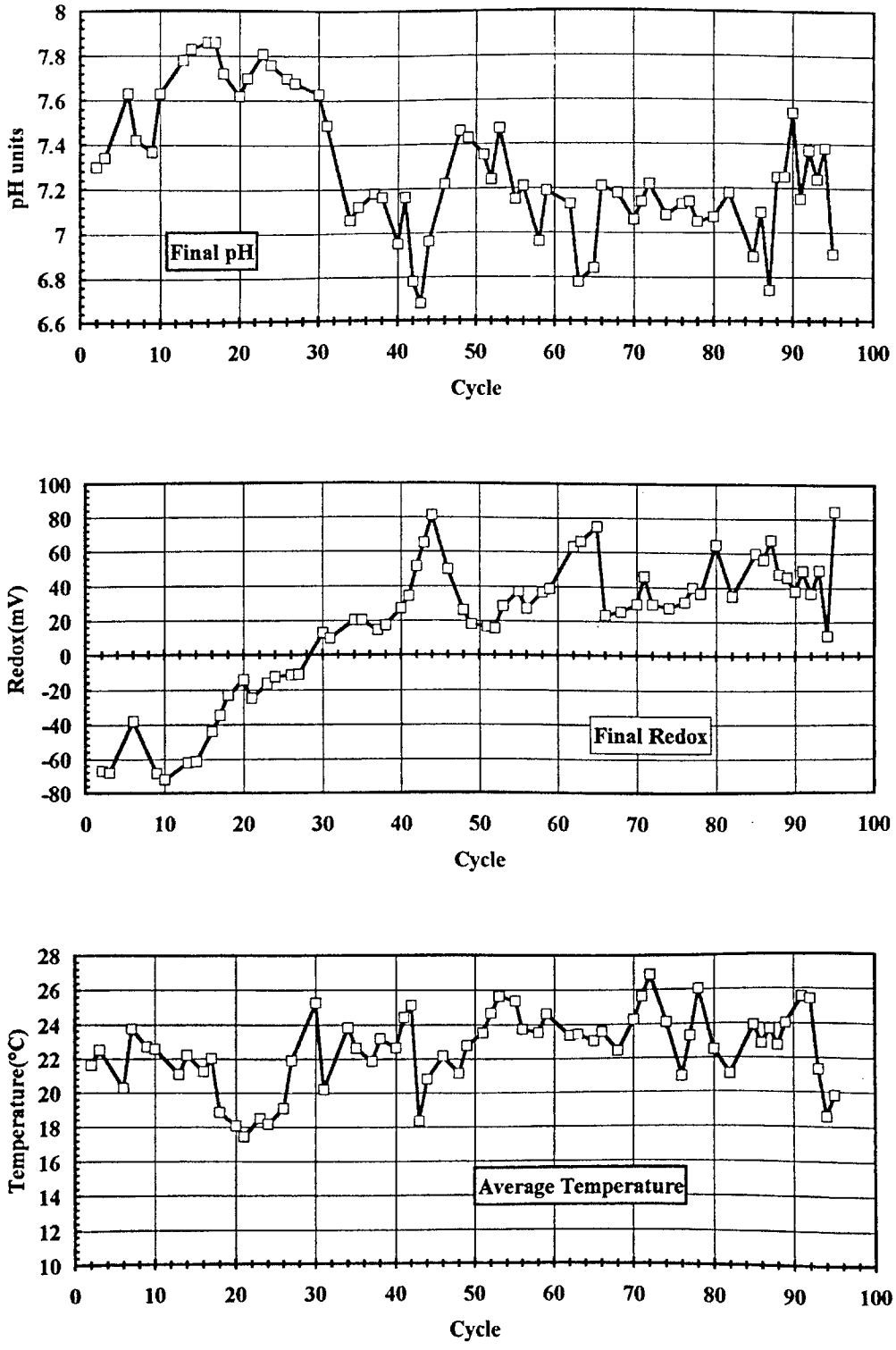


Figure 4.19: Final pH, Final Redox and Average Temperature for Phase 3

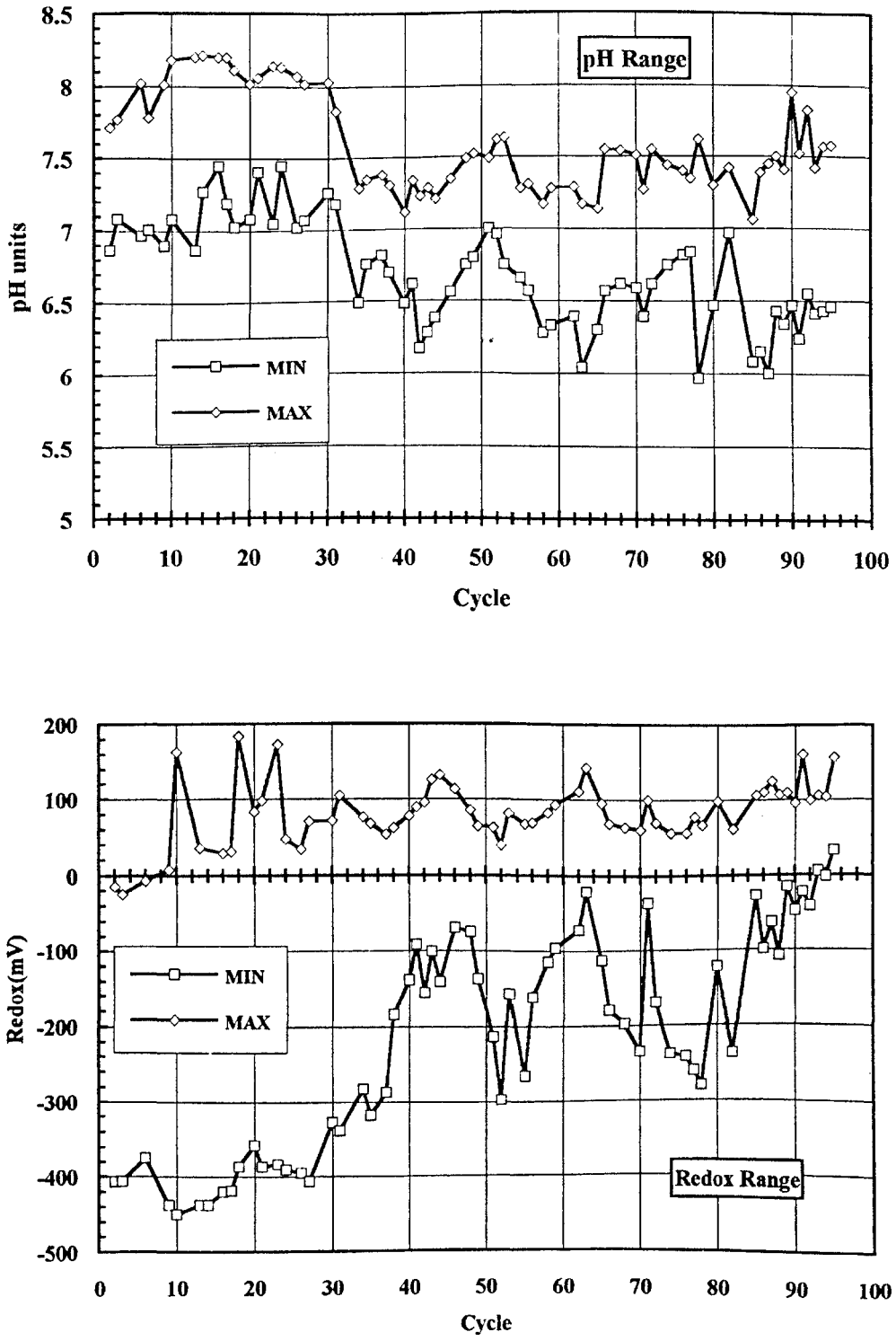


Figure 4.20: pH and Redox Ranges for Phase 3

was also characterized by a steady increase in SVI values from 90 to 140 mL/g.

By the end of the start-up period, the first signs of nitrification appeared. The total nitrogen removal and nitrification efficiency, during the first 30 cycles, most likely represent the ammonia nitrogen uptake by heterotrophic assimilation and perhaps some incipient nitrification (figures 4.15 and 4.16).

In the same graphs big spikes can be seen which correspond to concomitant sharp decreases in influent nitrogen concentrations. Again, the explanation is the same. Smaller influent nitrogen concentrations will result in a bigger percentage of it being assimilated by the heterotrophs. Besides that, some nitrification may have occurred.

The pH and redox (figures 4.19 and 4.20) showed the same tendency presented during phase 2, which again confirms their utility as control parameters for the nitrification process. A sharp drop in pH values around the 30th cycle seems to indicate the beginning of effective nitrification. At the same time, the final redox curve started to show positive values and the minimum redox graph stoped being anaerobic (around -400 mV) rising to a zone more characteristic of anoxic conditions.

One more thing worth noticing during the start-up period was the sizeable removal of orthophosphate between cycles 10 and 20 (figure 4.18). The minimum redox curve seems to confirm the necessary anaerobic conditions for the EBP removal first step, phosphorus release to the medium. Besides that, effluent COD concentrations at that stage were relatively high, indicating the availability of organic matter for the process. After a while however the system lost its EBP removal ability, mostly due to the presence of oxidized nitrogen.

The second period, from cycle 40 to cycle 85, was characterised by instability. At first glance, the graphs seem to be confusing with big fluctuations in almost all monitored parameters, such as effluent COD, SS and nitrogen forms throughout the period. However, after a careful examination of the pH (figures 4.19 and 4.20)

together with the other parameters, a reasonable explanation for such behaviour can be suggested. The effluent wastewater alkalinity was not high enough to prevent nitrification dropping the process pH to inhibitory levels.

As nitrification proceeds, the system alkalinity is depressed, lowering the pH in the process. Depending on the amount of ammonia present, the initial alkalinity and the denitrification intensity, the pH decrease will be more or less acute. In some cases, the minimum pH during a cycle can be reduced to such a extent that it will inhibit the microbial community, including heterotrophic and autotrophic bacteria, protozoa and metazoa. Probably the first to be inhibited will be the nitrifiers, due to their poor competitiveness. The low pH values will also inhibit the heterotrophs to some extent, and this is showed in terms of COD and SS removal, and in terms of denitrification efficiency. A similar process occurred during this period. For example, during cycle 42, the minimum pH value was 6.2, representing a drop of approximately 0.5 pH units from the previous cycle. Around the same cycle, the effluent COD and SS concentration increased from their background values to above 100 mg/L and 40 mg/L, respectively. Also, after the pH shock, the reactor SVI, which had increased steadily for the entire start-up period, showed a sharp drop. Putting these two facts together, it is possible to infer that the fraction of flocculant organisms decreased as a result of the pH shock, causing the advent of a dispersed growth of bacteria. This observation was supported by the microscopic sludge investigation, as it will be seen later. Both nitrification and denitrification efficiencies, also indicated the occurrence of a pH shock. Similar pH shock effects on activated sludge systems were observed by Smith and Wilderer (1986). After a while, the system pH increased, as a result of the reduction in nitrification efficiency, and the reactor efficiency improved somewhat. In consequence, the recovered nitrification process started to lower the pH until a new pH shock was produced, starting all over again. However, as can be seen in figure 4.14, effluent COD and SS concentrations fluctuated for the entire period, varying with the influent strength. George and Gaudy, (1973) observing the response of completely mixed systems to pH shocks, suggested an explanation for this phenomena. They mantain that every time the microbial community is subjected to a pH shock, some kind of natural

selection is effected. As a consequence, this community will be acclimatized to the new prevailing pH conditions. However, the lowered diversity of species in the system can be expected to restrict the range of adaptive response to the variety of environmental changes to which the process is subjected, such as changes in influent concentration and composition, temperature and pH. Therefore, those influent variations which, before the pH shock, did not cause any effect on the effluent COD and SS concentrations, started to cause problems with the new less diversified bacterial community.

The effluent oxidized nitrogen was predominantly nitrite. Again, the maximum pH values and the influent TKN concentrations indicates *Nitrobacter* inhibition due to the presence of free ammonia. Besides that, it is possible that incomplete denitrification, stopping at the nitrite stage, may have played its part.

Nitrification and denitrification efficiencies, calculated as shown in appendix A, varied within the same range, from 46.0 to 99.9%. The overall nitrification/denitrification process effectiveness for the period, can be better assessed through the total nitrogen removal parameter. It varied in the range of 16.1 to 99.0%, averaging 65.4%. Average specific daily rates of nitrification and denitrification were 23.9 (7.6 to 40.9) g/kg.day , and 17.7 (2.5 to 31.1) g/kg.day , respectively. As would be expected from a system subjected to transient inhibition, the nitrification rates are smaller than those of phase 2, but still well inside the ranges reported in the literature. Similar daily specific denitrification rates were reported by Oleszkiewicz and Berquist (1988). From table 2.6, it can be seen that these specific denitrification rates are characteristic of a system combining influent wastewater and endogenous products, as source of electron donors for the denitrification process.

Because of this transient pH inhibition, overall the system did not perform well. Even though the pH problem was identified in the early stages of this period, it was decided not to take action to correct it. As the main objective was to follow the system pH, redox and DO evolution, it was concluded that any action taken at that stage would put this objective at risk. Instead, it was decided to carry on with

the experiment without alterations until a cycle where complete denitrification would occur, in order to perform a track study. After a careful scrutiny of daily results, cycle 78 was chosen for the track study. These results will be dealt with later in section 4.4.3.

After the track study, some time was allowed for the system to recover from the solids loss inflicted by this study analyses. Then, on cycle 86, the first of a series of different time based schedules (see table 3.4) was implemented to increase the time allowed for aeration, in order to improve conditions for nitrification. Also, lime started to be added to the influent wastewater to increase its initial alkalinity. Based on the nitrification efficiency and pH range (figures 4.16 and 4.20), these actions were successful. with nitrification reaching completion and the minimum pH experienced during the cycle, increasing to a less inhibitory level of about 6.5.

In reality, the cycles shown in table 3.4 were not fixed in advance. They were the result, perhaps, of the first application of a pH controlled nitrification process. By following the pH development in time on the computer screen, the nitrification end-point feature, described in phase 2, was used to guarantee complete nitrification. When necessary, aerated periods were prolonged with the consequent denitrification period reduction.

As already mentioned, alkalinity was determined only from cycle 70 onwards. From this data, an average net specific alkalinity uptake rate of 2.9 (0.9 to 5.5) mg $CaCO_3$/mg N removed was calculated for the joint nitrification/denitrification process.

During cycle 94, in the last part of the React period, an excessive accidental sludge wastage made it impossible to continue the experiment.

4.4.3 Track Study

The track study was performed on cycle 78 to observe the pH, redox and DO development when complete denitrification was achieved. Figures 4.21 through 4.24

present the data referent to this study. The numbers 1 to 8 inside boxes, represent the different parts of the cycle, as follows:

1- Fill period: without air or mixing
2- Fill period: mixed and aerated
3- Fill period: mixed only
4- Fill period: mixed and aerated
5- Fill period: mixed only
6- React period: mixed and aerated
7- React period: mixed only
8- React period: mixed and aerated

Figure 4.21 shows reactor solids and soluble COD graphs for this study. As for phase 2, the dilution effect determined the MLSS evolution during the cycle. This time, no yield was observed, probably as a function of the long endogenous period. COD accumulated in the reactor during the unmixed Fill period. After that, during aerated Fill periods, COD was consumed by heterotrophic oxic oxidation and, during anoxic Fill periods by anaerobic respiration. Some storage product synthesis seems to have occurred during the final 45 minutes of the last non-oxic Fill period (period 5), and after 16:00 hours during the non-oxic react period. On these occasions, as can be seen from the soluble orthophosphate curve (figure 4.23), phosphorous was released from the biomass under anaerobic conditions. As for phase 2, the COD increased during the last hours of the cycle, indicating the release of soluble residual products (SRP) to the medium.

As expected, both nitrification and denitrification occurred during this cycle, with the denitrification process using the oxidized nitrogen produced by nitrification during the previous period. Besides those processes, some heterotrophic ammonia assimilation also occurred. For example, a maximum theoretical total nitrogen concentration of 39.0 mg/L, based on the influent concentration of 61.9 mg/L, was calculated for the end of this first aerated Fill period (2). At this point, the actual total nitrogen concentration was 34.3 mg/L, indicating the occurrence of the

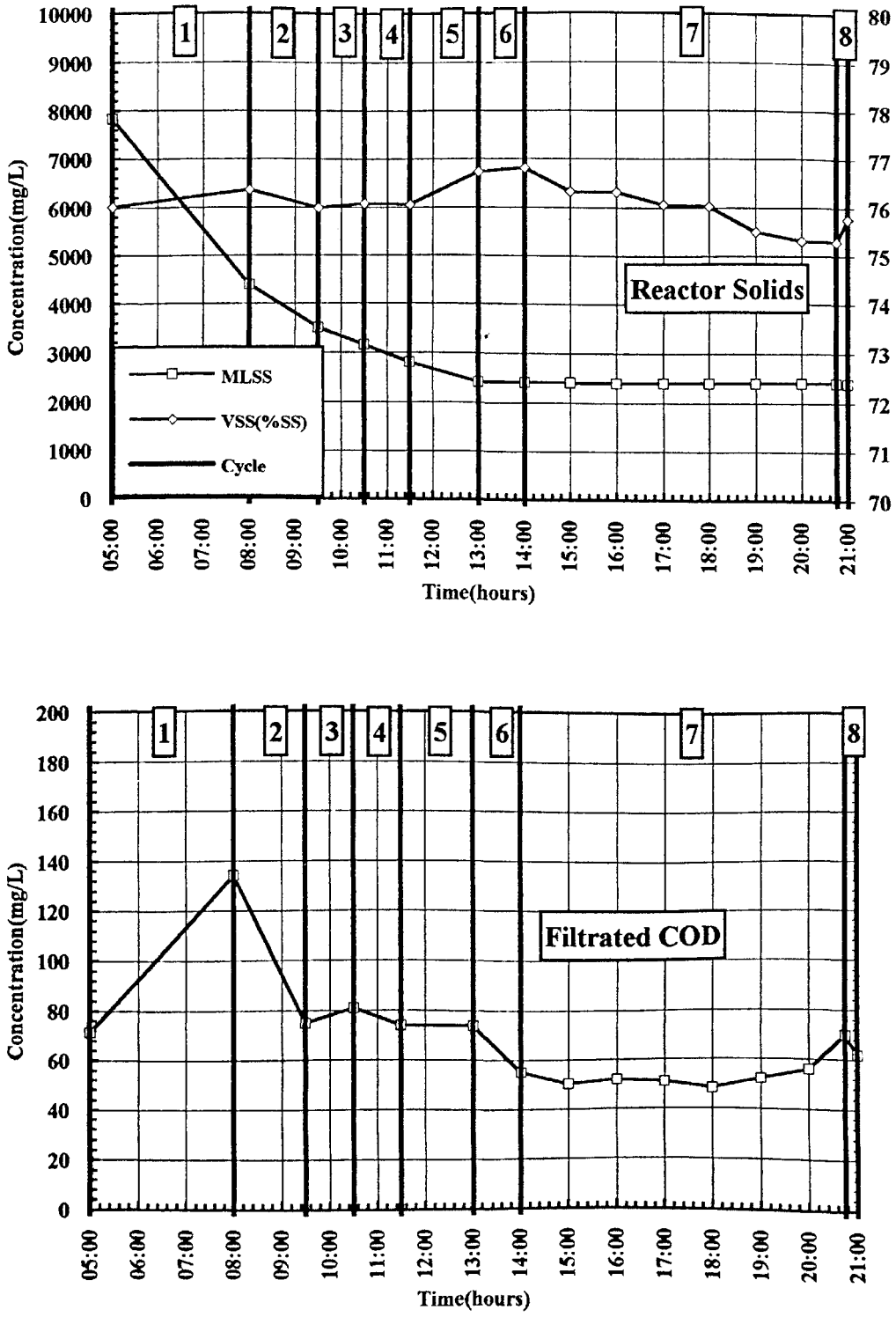


Figure 4.21: Reactor Solids and Soluble COD for Track Study, Phase 3

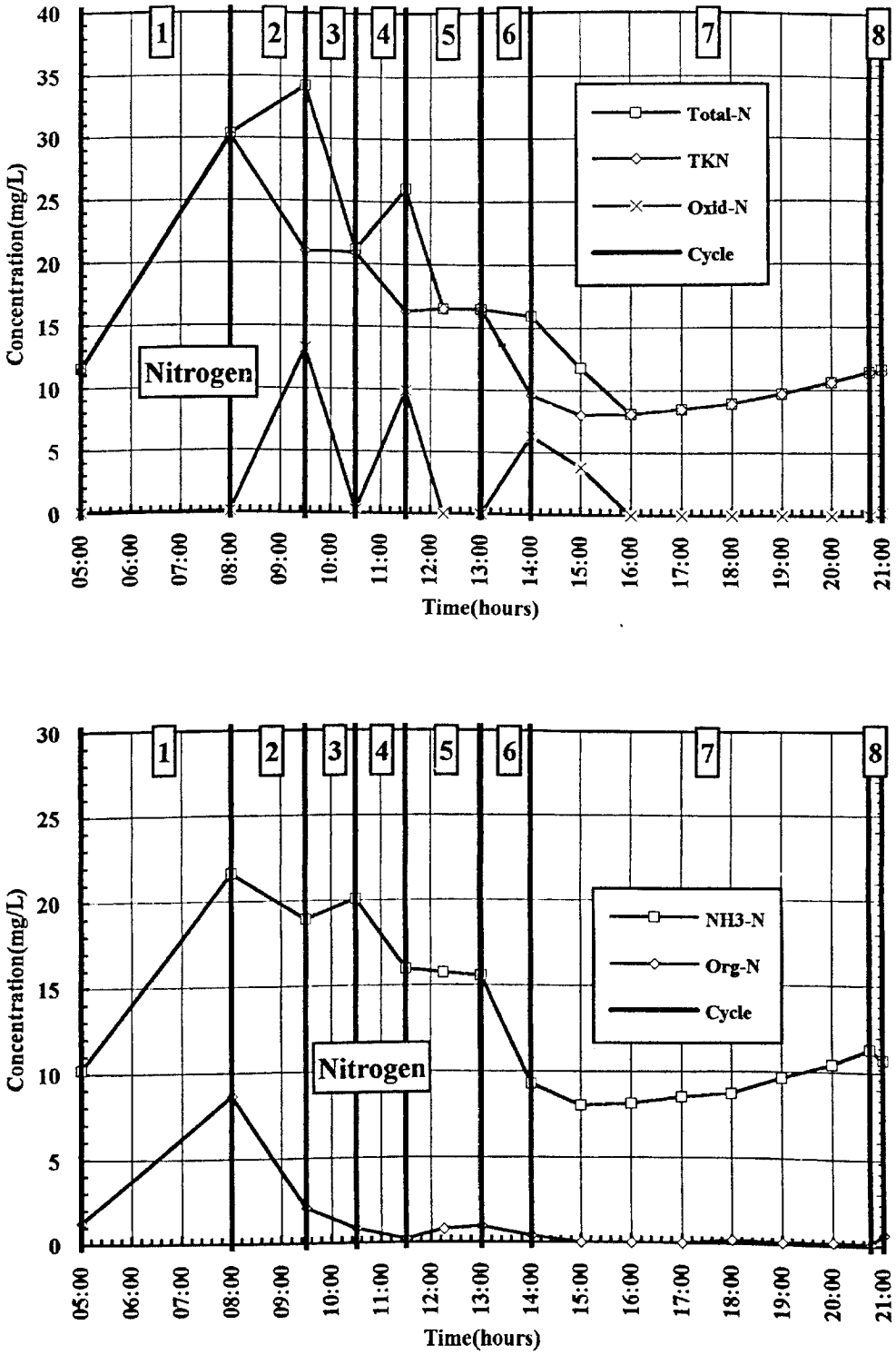


Figure 4.22: Nitrogen Data for Track Study, Phase 3

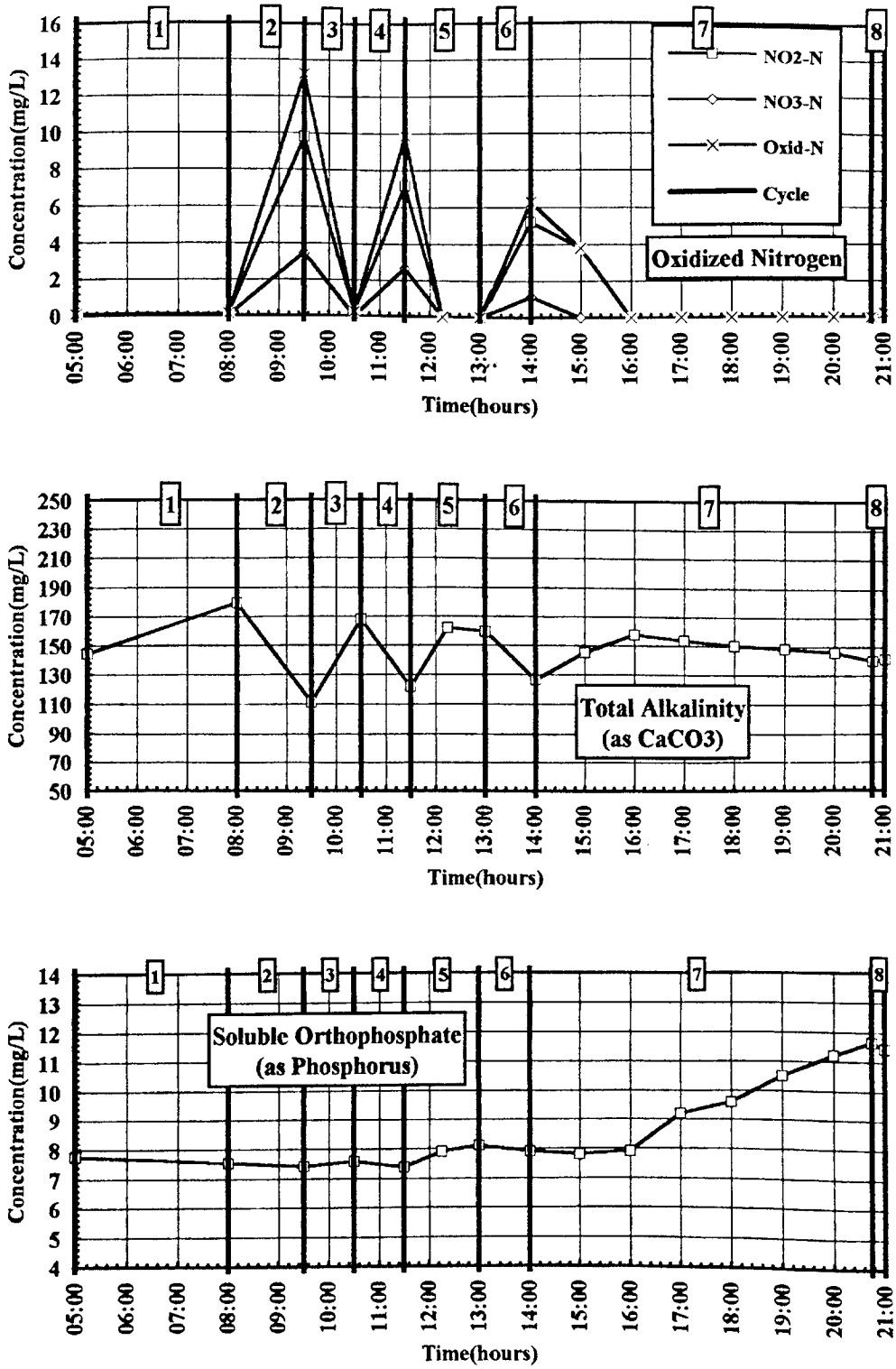


Figure 4.23: Oxidized Nitrogen, Total Alkalinity and Soluble Orthophosphate for Track Study, Phase 3

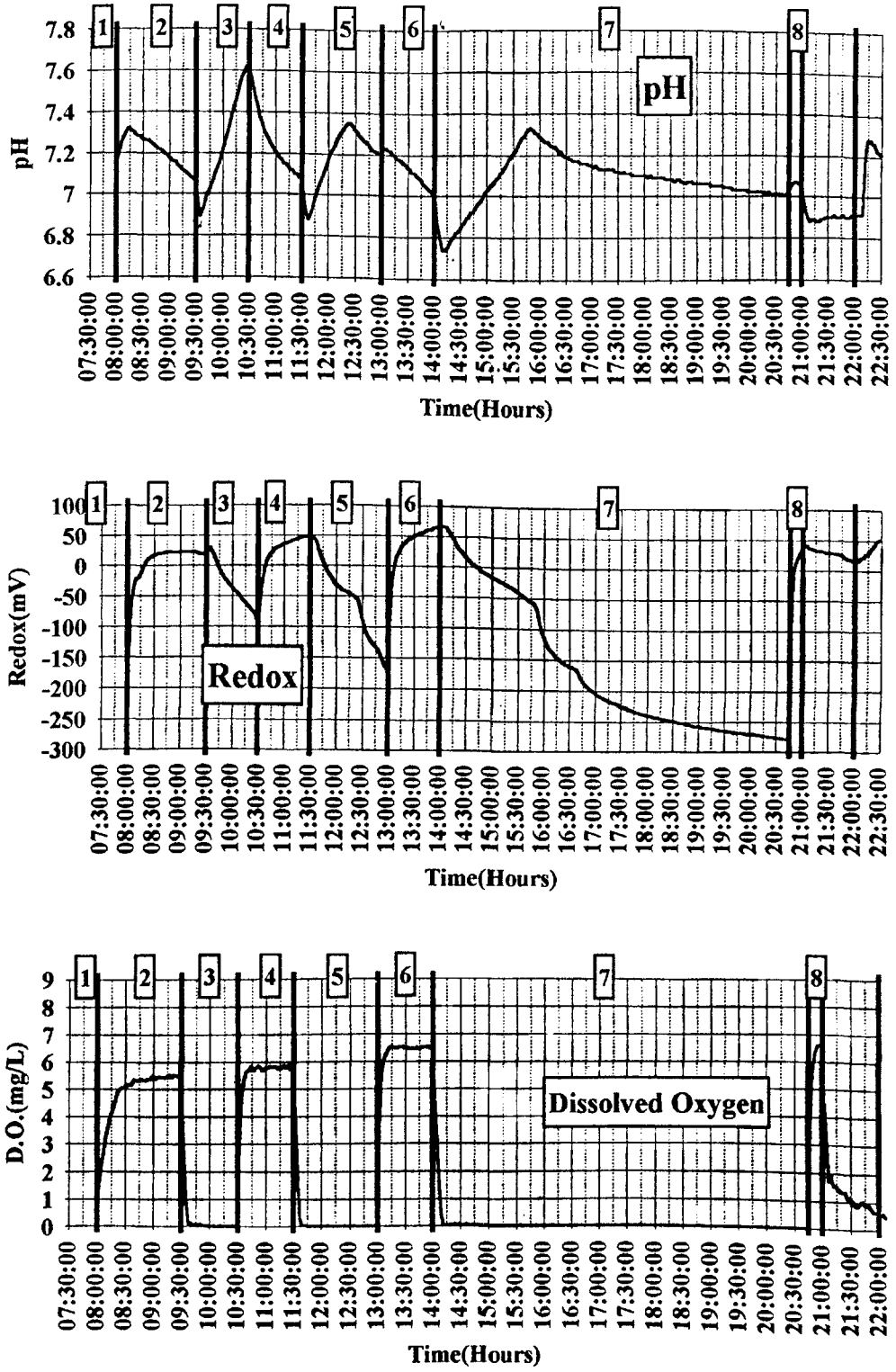


Figure 4.24: Simultaneous pH, Redox and DO Measurements for Track Study, Phase 3

mentioned process.

Nitrite was the predominant oxidized nitrogen specie (figure 4.23) and again the free ammonia levels inside the reactor seems to explain the phenomena. For the pH range of 6.70 to 7.63 experienced during the cycle, free ammonia concentrations as high as 0.35 mg/L may have occurred. Another possible explanation is the interruption of either assimilatory or dissimilatory nitrate reductase at the nitrite stage. Whatever the process responsible for the transient nitrite accumulation, in the end, the denitrification process removed all oxidized nitrogen.

The maximum hourly nitrification rate was 9.6 mg/L.h which occurred between 10:30 and 11:30 hours (period 4), and converting to a daily specific rate resulted in 101.7 g/kg.day. Also, maximum hourly and daily specific denitrification rates of 13.16 mg/L.h and 158.7 g/Kg.day, were determined during the anoxic period 5, between 11:30 and 12:15 hours. Similar nitrification rates were found for phase 2. In terms of denitrification, the observed rates compared well with reported values in the literature. Similar rates have been reported for highly efficient denitrification systems using influent wastewater as the sole organic carbon source (Tchobanoglous and Burton, 1991).

Ammonia levels increased towards the end of the long non-oxic React period, from 8.0 to 10.7 mg/L, probably as a result of either assimilatory reductase in excess of anaerobic needs or the release of constitutive cellular nitrogen.

The total alkalinity (figure 4.23) expresses the cycle of alkalinity consumption during nitrification and its production during denitrification. Using these and the oxidized nitrogen data, a maximum specific alkalinity uptake rate of 5.87 mg alkalinity/mg oxid-N produced, for the nitrification process, was calculated in the last aerated Fill period (6). In terms of denitrification, a maximum of 3.82 mg alkalinity/mg oxid-N reduced was observed in the first non-oxic Fill period (3). Similar values have been reported in the literature for both processes (Sedlak, 1991; Barnes and Bliss, 1983; Gray, 1990; Horan, 1990).

Figure 4.24 presents the results of the simultaneous and continuous monitoring of pH, redox and DO for the track study. As expected no nitratification end-point occurred in this study, due to the incomplete ammonia nitrification. The following observations were inferred from these graphs:

a) pH: Alkalinity and pH graphs are in good agreement with each other and the nitrification process expresses itself in the pH graph as a negative slope during oxic periods, while positive slopes during anoxic periods represent the denitrification process. During the first oxic Fill period (2), a clear peak can be observed at 8:15 hours. This feature already observed in phase 2 track study, was attributed to the combined effect of a slow down in COD uptake simultaneously with an increase in nitrification rates. Two new features can be noticed in the non-oxic periods. The first one, appearing in all three non-oxic periods (3, 5 and 7), can be identified as a minimum point occurring a few minutes after aeration is switched off. These points coincide with the dissolved oxygen disappearance, indicating the passage of oxic to anoxic conditions. The second feature to be observed occurred clearly only in the last two non-oxic periods (5 and 7) and appear as peaks at around 12:15 and 15:30 hours. As can be seen from the oxidized nitrogen curve (figure 4.23) they coincide with the almost complete disappearance of oxidized nitrogen. However, at first glance, no such feature was observed during the first non-oxic period (3), even though the oxidized nitrogen data indicates that denitrification also reached completion in that period. By the evidence presented in the pH profile of other cycles, it was judged that aeration was turned on at the exactly the moment when the peak was reached and the pH curve seems to corroborate this. The negative pH slope after the denitrification end point can be envisaged as the effect of acido- or acetogenesis.

b) Redox: In the case of redox, it is difficult to identify nitrification and denitrification by the redox curve first derivative signal as done for pH. Nitrification turns the medium more and more oxidized, producing a positive

redox slope, but all other aerobic processes also do that. Denitrification and anaerobic processes reduce the medium, causing negative slopes in the redox curve. The redox profile showed features similar to the pH profile. During the first aerated Fill (2), coinciding with the pH peak, a clear inflexion point is seen. Such a feature has been reported as the "elbow" point corresponding to the initial response of the DO probe (Peddie *et al.*, 1990). These authors suggest that this point represents the transition from the effects of combined oxygen in the form of nitrates to the effect of free dissolved oxygen on redox. As there was no oxidized nitrogen species before the beginning of that period, such interpretation cannot be applied in this case. The DO zero features identified in the pH curve also show in the redox profile, in this case as inflexion points. The visual identification of the exact moment where they occurred may be cumbersome. The best strategy is to plot the first derivative of the redox curve, where these inflexion points will appear as maximums or minimums, and thus much easier to identify. They appeared after the sharp fall in redox values caused by the start of aeration. The denitrification end point feature is much easier to identify. It appears as an inflexion point during non-oxic periods. Many authors have reported such a redox feature (see section 2.7.2). As for pH, this feature can be seen in the last two non-oxic periods (5 and 7), but not in the first one. Also in this case, it seems that it was occurring when DO was turned on. There is yet another inflexion point occurring after the denitrification end point (see periods 5 and 7), which seems to have an effect on the pH curve as well. It appears as a change in the slope value, rather than a change in signal. No clear explanation was found for it. It may be possible that such a feature would be connected to one or more existing anaerobic processes, such as acidogenesis, methanogenesis, desulphatation and depolymerization of orthophosphate.

c) DO: There is no role for DO profile during anoxic or anaerobic periods (DO below detection levels). Besides the DO zero point (periods 3, 5 and 7), the only interesting thing to notice is an increase in DO levels from the previous

to the next aerated periods, indicating, as would be expected, that the DO demand decreases with treatment time.

After these new features were identified, all recorded cycles where checked for these and for those features discovered in phase 2. As a result, the interpretations were validated. Figures 4.25 through 4.28 present some selected examples of monitored cycles, showing their evolution as the phase progressed. A close study of these profiles permitted the following observations:

a) Cycle 24 (figure 4.25): This cycle was recorded during the start-up period and there was no evidence for significant, if any, nitrification or denitrification occurring during it. The slopes signal presented in the pH profile are not representative of either nitrification nor denitrification. The DO zero point is the only recognizable feature.

b) Cycle 41 (figure 4.26): Both nitrification and denitrification occurred during this cycle. Neither of them reached completion. In the pH profile the change in the slopes signal is noticeable.

c) Cycle 62 (figure 4.27): Complete nitrification was reached in this cycle, as corroborated by the nitratification end point feature occurring in all oxic periods but the last one. Denitrification occurred to some extent.

d) Cycle 76 (figure 4.28): Complete denitrification occurred as confirmed by the denitrification end point feature. Nitrification was not complete.

Unfortunately, due to the inherent instability of this phase, complete nitratification and denitrification never occurred together. Because of that, no recorded cycle showed both end point features.

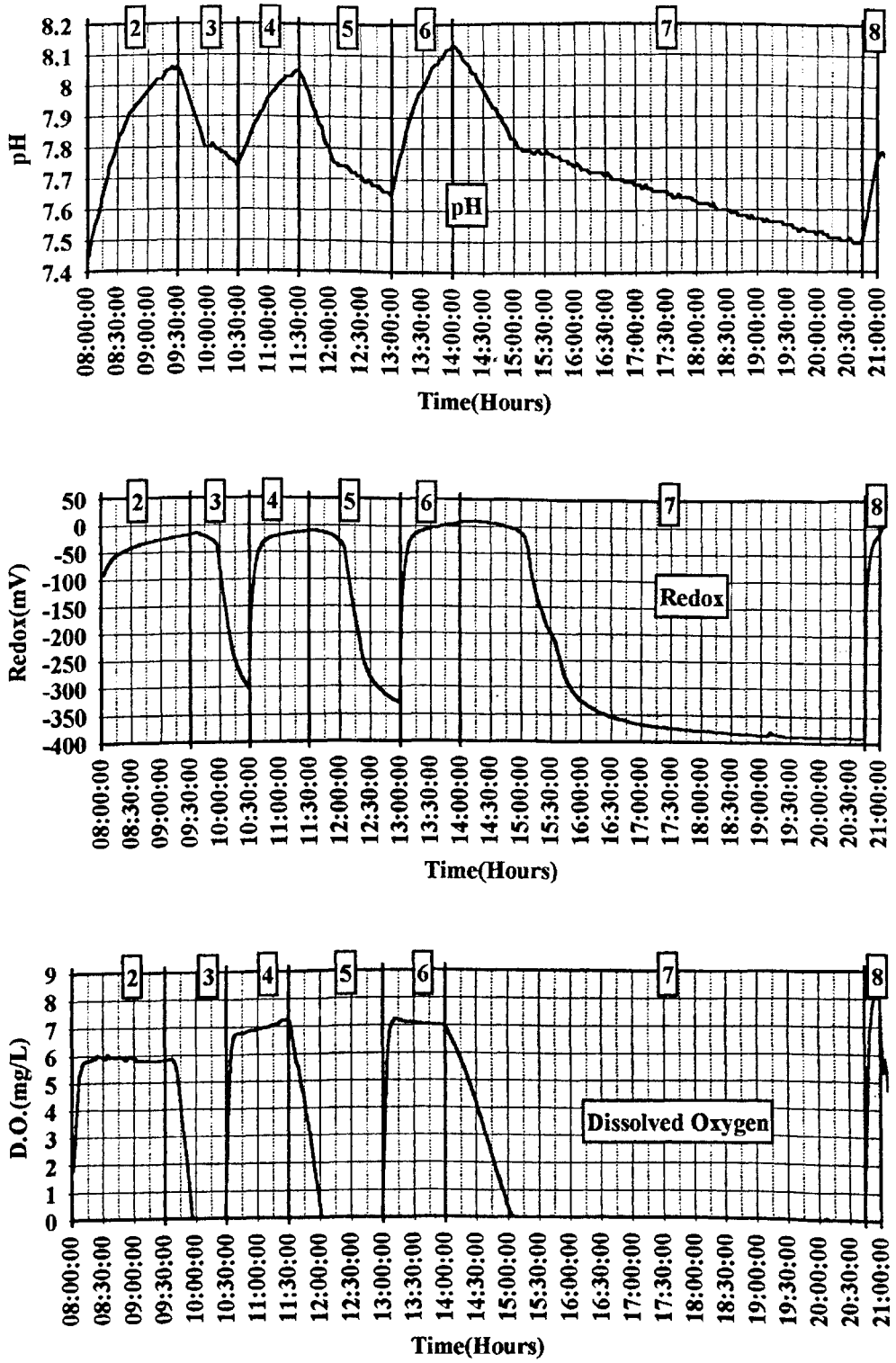


Figure 4.25: pH, Redox and DO Profiles for Cycle 24, Phase 3

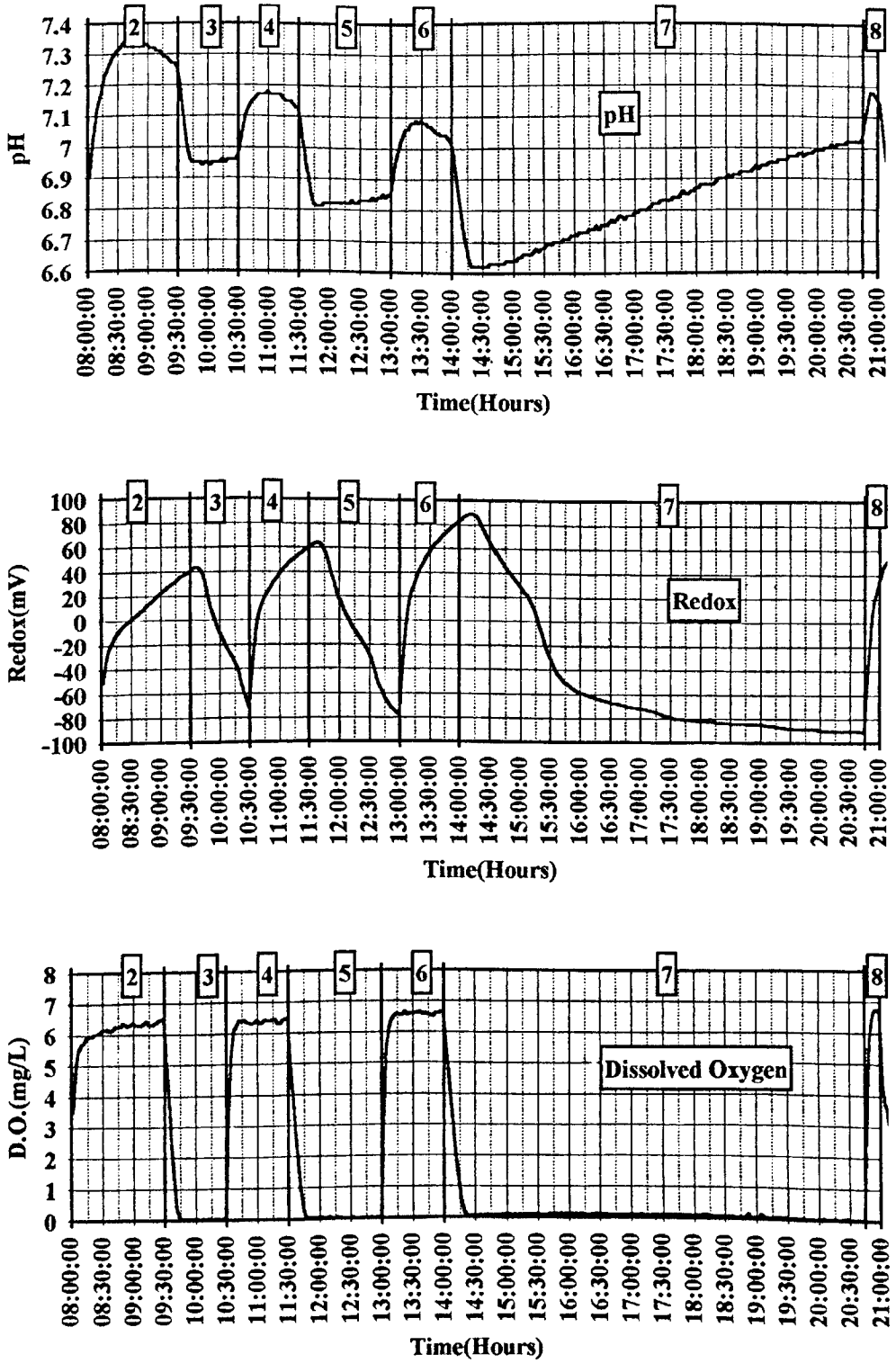


Figure 4.26: pH, Redox and DO Profiles for Cycle 41, Phase 3

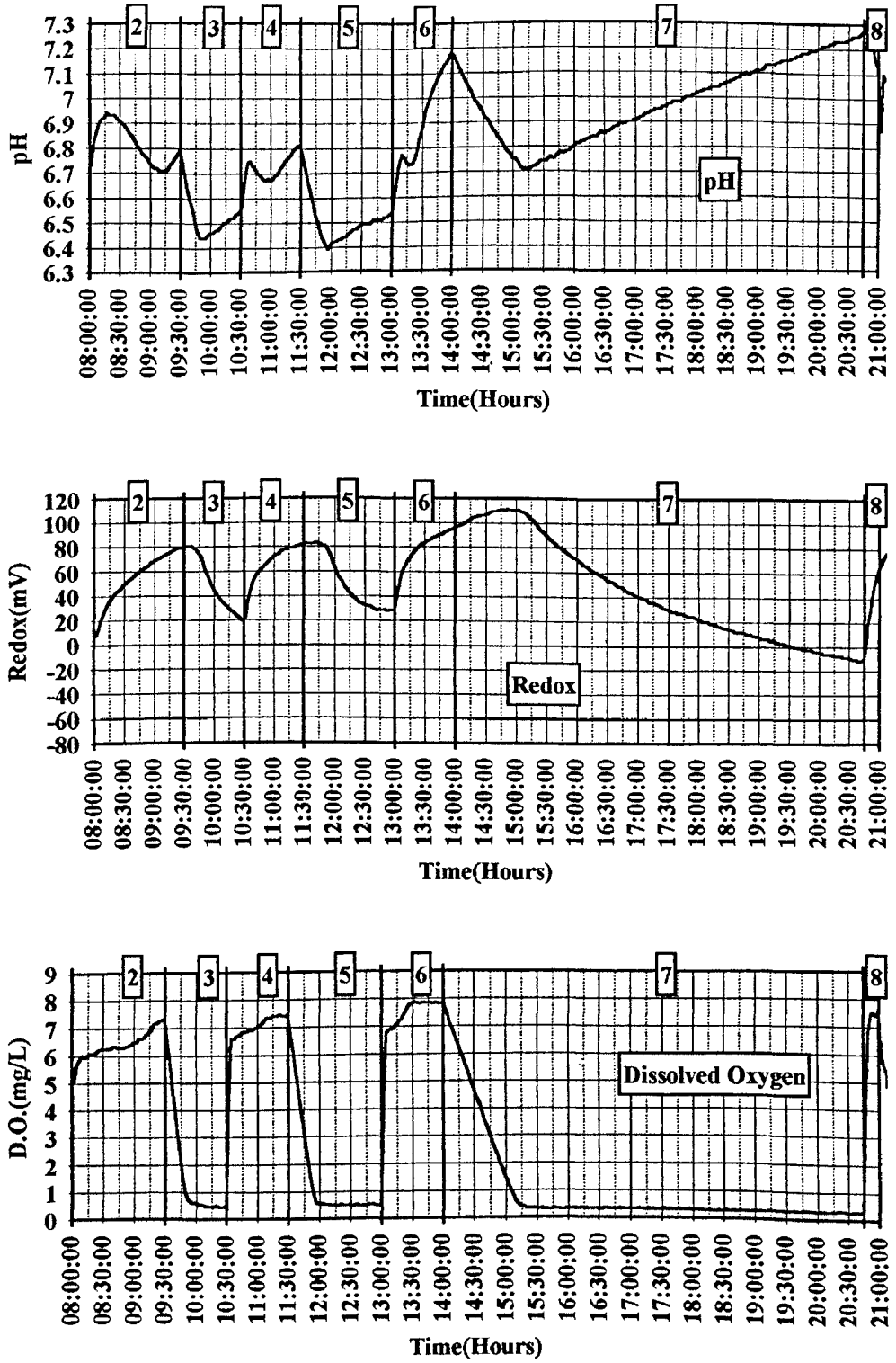


Figure 4.27: pH, Redox and DO Profiles for Cycle 62, Phase 3

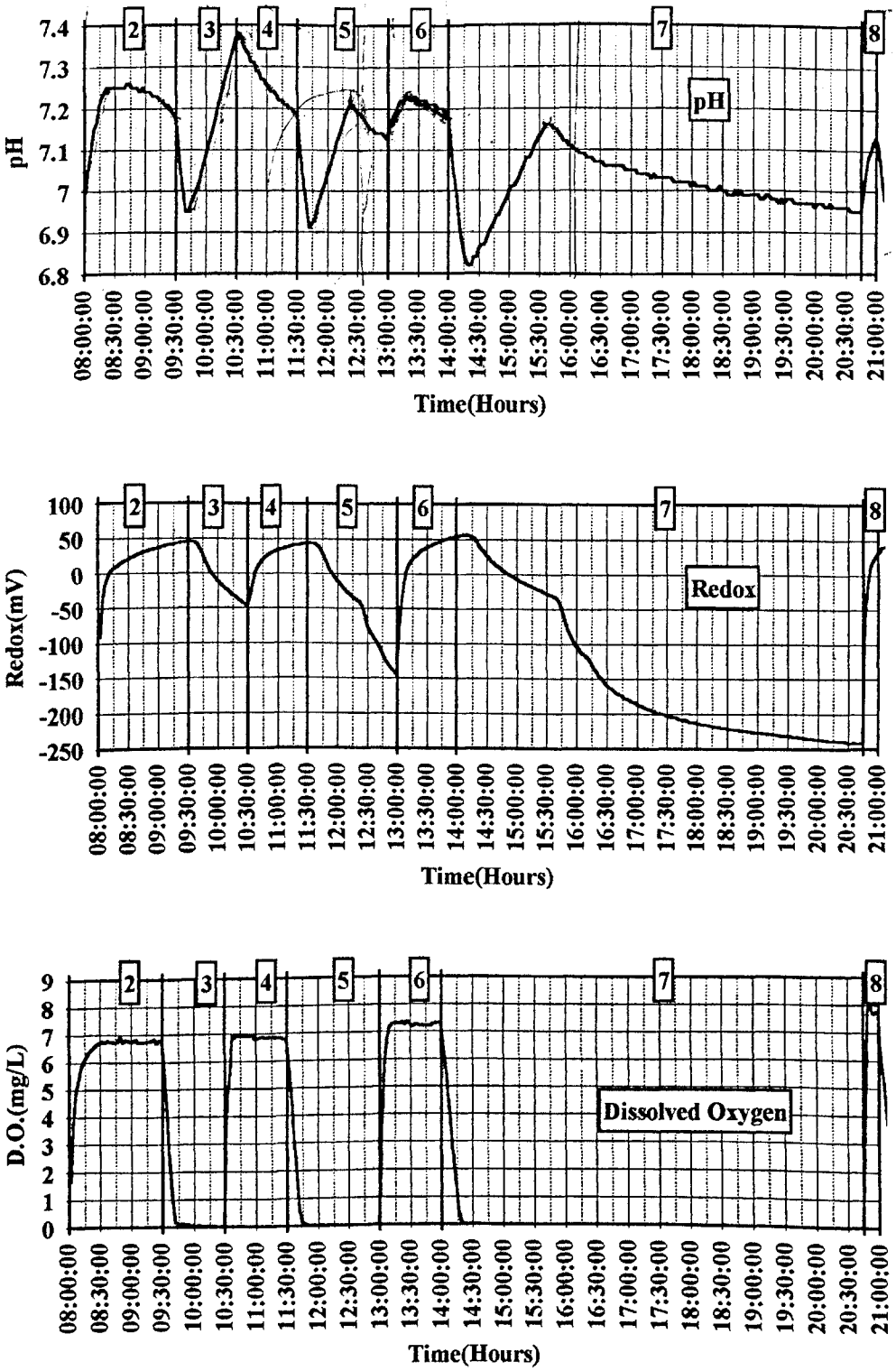


Figure 4.28 pH, Redox and DO Profiles for Cycle 76, Phase 3

4.4.4 Microfauna Study

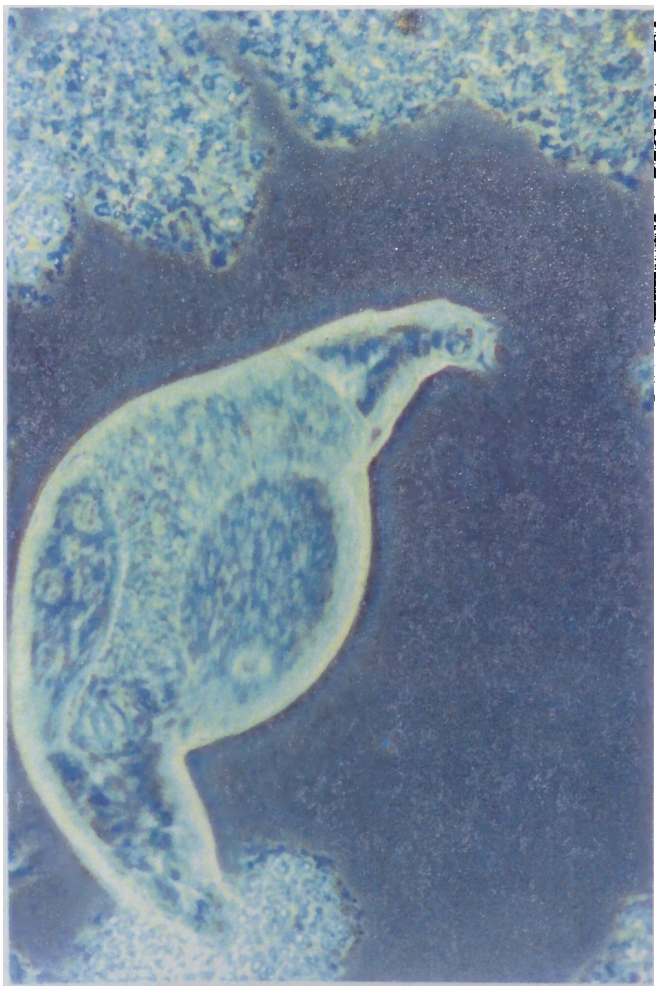
This study was carried out to provide aid for phase 3 chronological data interpretation. It was not designed to be a study on its own. Therefore, only a succinct description of the results and the discussion of the microfauna indicator value for the nitrification/denitrification process will be presented. Similar study was carried out for phase 4.

Using the techniques described in section 3.7 for collection, wet mount preparation and microscopic observation, the SBR microfauna was observed on a regular basis throughout the entire experiment. The SBR mixed liquor was sampled during the last 15 minutes of the React period, and observed in the microscope at least once for each new feed. Organisms identification only was made to the specie level.

Throughout the phase 3 experiment, the sludge was characterized by a brownish-black, somewhat rounded, weak and compact floc. Most of time dispersed growth of bacteria was observed, indicating both medium to high F/M and oxygen limitation during the predominant non-oxic periods. Sometimes *Spirils* were observed which are known to appear in plants where the oxygen tension is low (Eikelboom and Buijsen, 1983).

Figures 4.29 through 4.31 present microphotographs of some observed protozoa and metazoa organisms common for this and phase 4 study.

In order to give a semi quantitative description to the rather qualitative microscopic observations, a relative protozoan and metazoan ranking was used. This ranking comprises 5 scores (0, 5, 10, 30 and 100) which corresponds respectively to the protozoa and metazoa presence as none (0), incidentally (<5), some (5-10), many (>10) and large numbers (>30). With this relative ranking, each microscopic observation proceeded by identifying the different microfauna species, and based on their frequency, a score was given for each of them. After the end of the experiment,



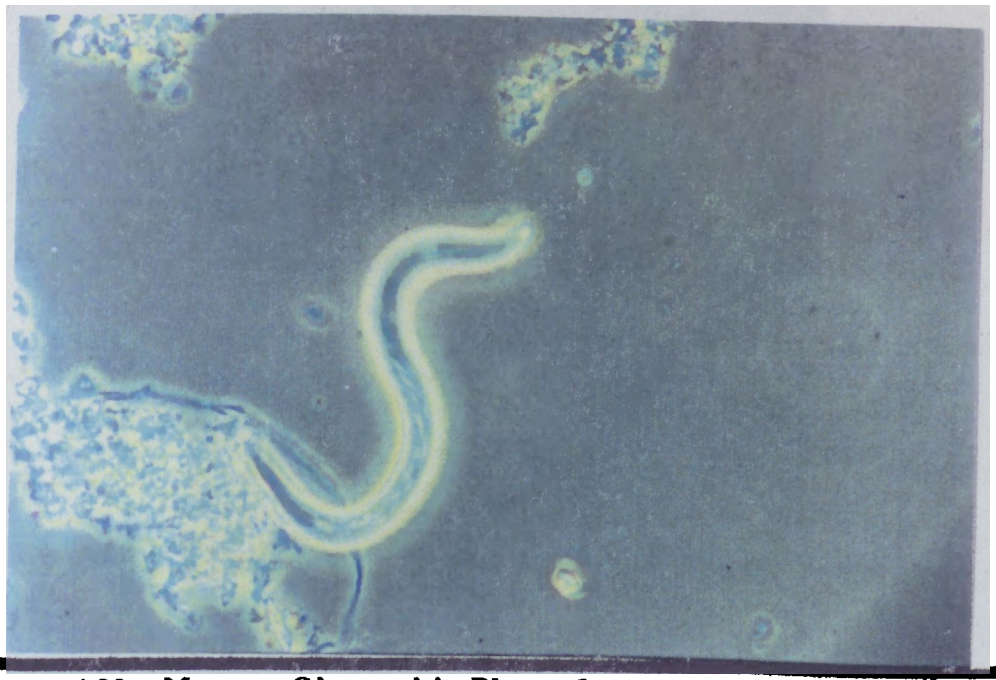


Figure 4.29: Metazoa Observed in Phases 3 and 4
Top: Rotifer 400X Bottom: Nematode 100X

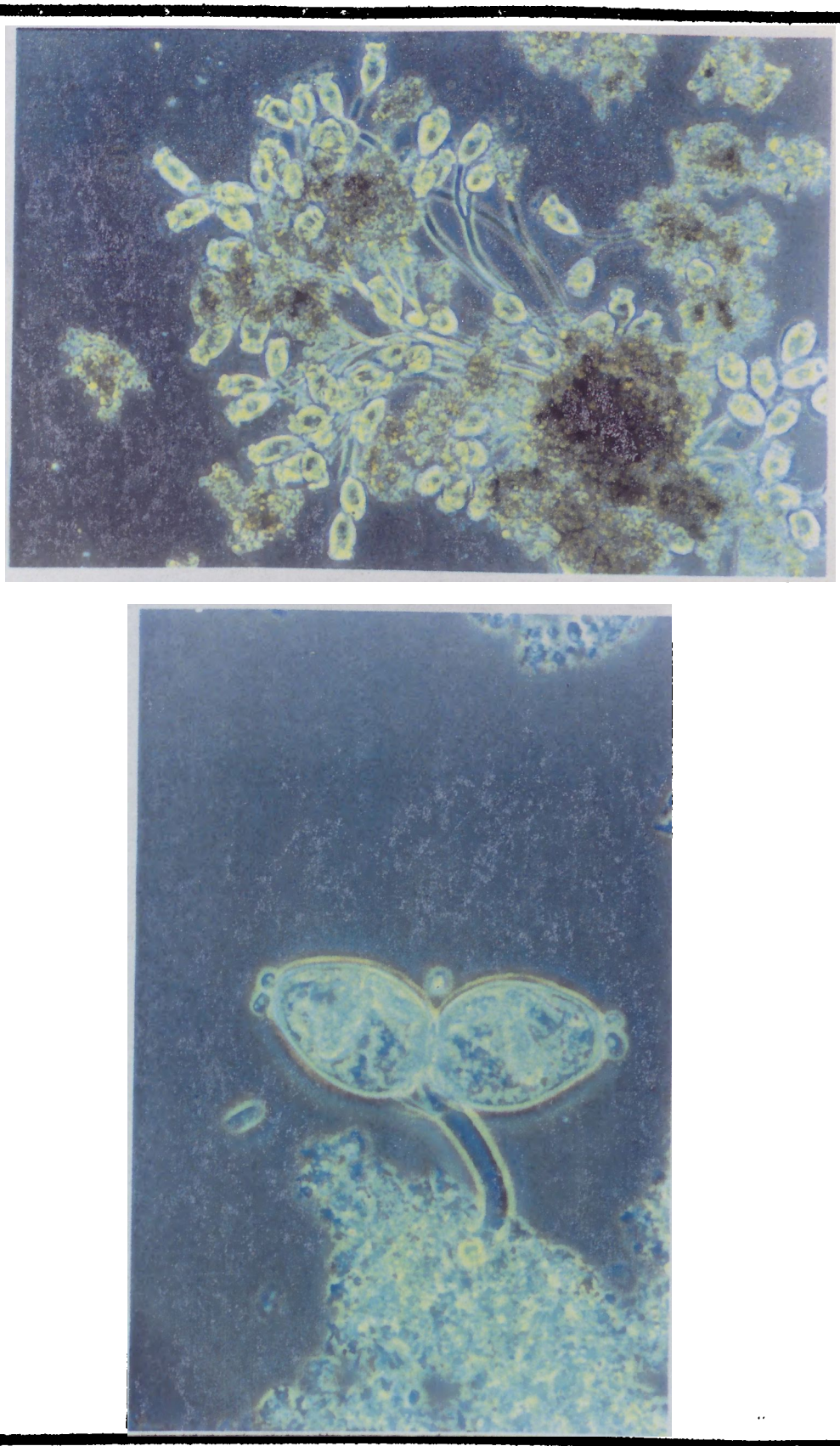


Figure 4.30: Protozoa Observed in Phases 3 and 4
Top: *Epystilis sp.* 100X Bottom: *Opercularia sp.* 400X

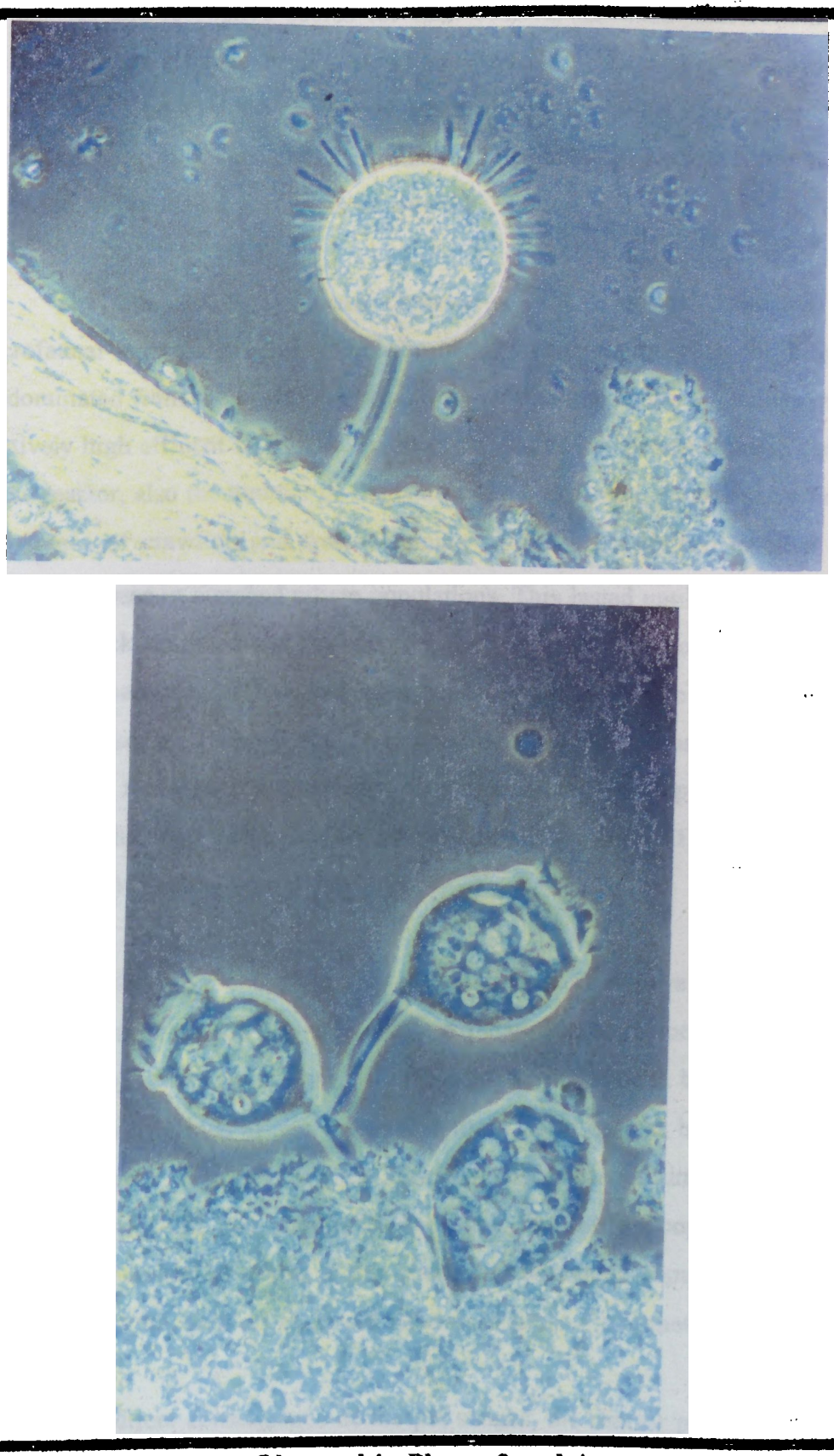


Figure 4.31: Protozoa Observed in Phases 3 and 4
Top: *Podoprya sp.* 400X Bottom: *Carchesium sp.* 100X

the species and respective scores for each observation, were joint conbined in a functional group classification. Table 4.4 presents a list of all the organisms identified to specie level under their respective functional group. Figure 4.32 presents the main results of this study in which the relative distribution of protozoa and metazoa, classified as functional groups, is introduced.

The first 40 cycles were characterized as a normal activated sludge microfaunal succession and establishment during start-up periods. Flagellates predominated from the beginning of phase 3 until around cycle 20, coinciding with relatively high effluent COD and SS. As the number of dispersed bacteria decreased in the reactor, also the number of flagellates dropped sharply. At the same time the appearance of crawling and free-swimming ciliates were noticed together with a relative increase in attached ciliate populations. This lasted until cycle 42 when the first pH shock occurred. As already seen in figure 4.14, the period before this pH shock was characterized by a very good performance in terms of both COD and SS removals. Also, by cycle 40, the first appearance of rotifers was noticed. Such variations in the protozoa population structure, during start-up periods have been reported by different authors, including: McKinney and Gram (1956), Curds (1982), Horan (1991) and Madonni (1991).

The pH shock occurring after cycle 40 seemed to have had a direct effect upon free-swimming and crawling ciliate populations and as soon as the first shock occurred, they disappeared. Small resurgences can be noticed between pH shocks, coinciding with periods of improvement in the effluent quality, but they never really recovered. Again, flagellates predominated for most of the remaining period, showing sharp drops when the effluent quality improved. The rotifera population established itself during the last 30 cycles of this phase. The nematode population development was rather patchy, with no observed relation to process parameters.

As a direct consequence of the pH shocks, the reactor protozoal population had its diversity reduced. By the end of the experiment, practically only flagellates and rotifers were observed. After cycle 86, when alkalinity was added, return of the

Functional groups	Functional Subgroups	Observed Species
Ciliated protozoa	Stalked	*Charchesium sp.* *Vorticella sp.* *Platycola sp.* *Opercularia sp.* *Epystilis sp.* *Podophrya sp.*
	Free-Swimming	*Urotrichia sp.* *Leptopharynx sp.* *Lionotus sp.* *Loxocephalus sp*
	Crawling	*Aspidisca sp* *Trachelophilum sp.*
Flagellated protozoa	-	*Bodo sp.* *Treponoma sp.* *Monosiga sp.* *Pleuromonas sp.*
Metazoa	Rotifers	*Philodina sp.*
	Nematodes[1]	-

1- Observed only as a subgroup

Table 4.4: Protozoa and Metazoa observed species for phase 3 microfauna study

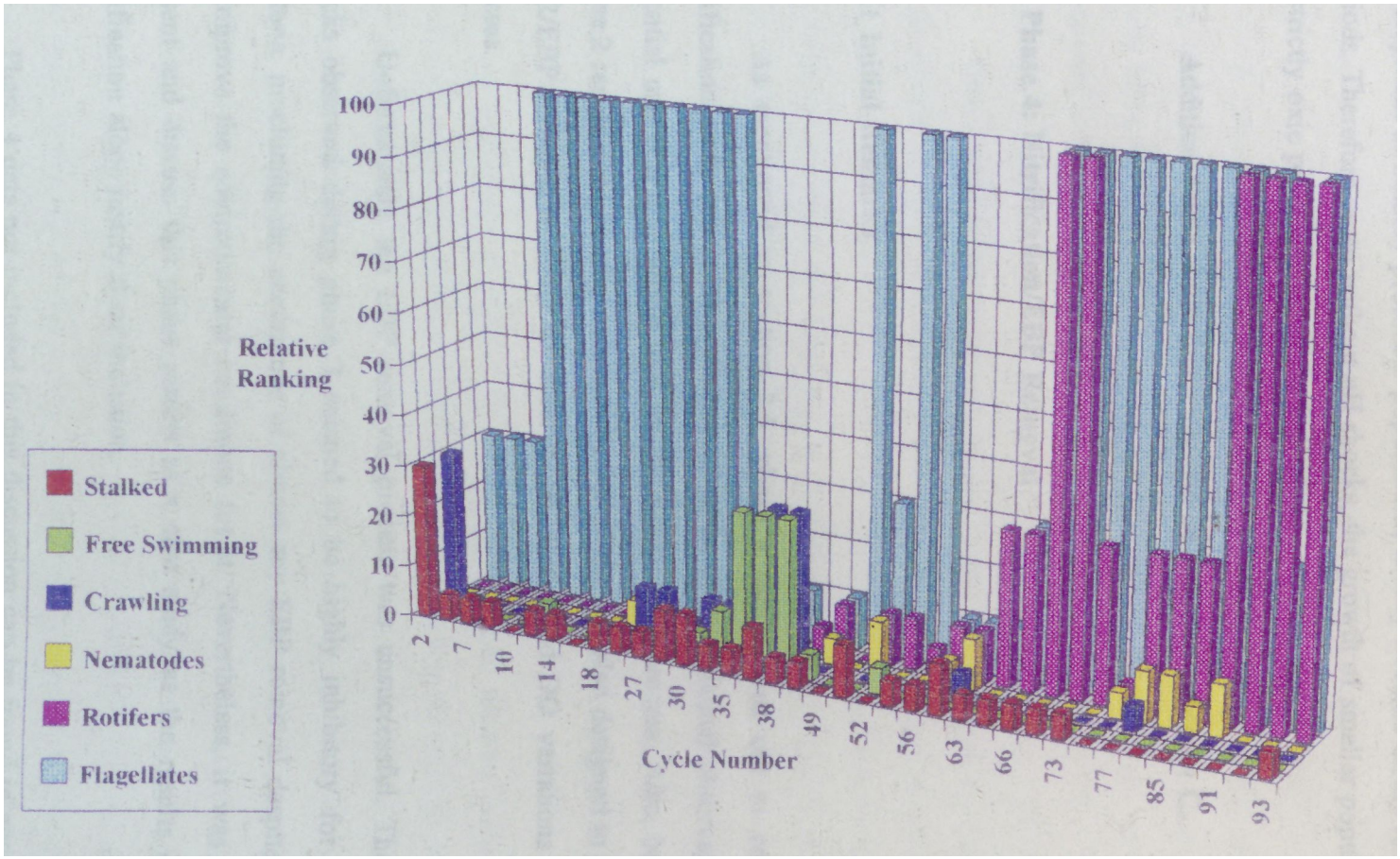


Figure 4.32: Relative Microfauna Distribution for Phase 3

the ciliate population was expected. However, it never occurred. Other authors have indicated that after situations like that, it would take months before the re-establishment of the ciliate population (Brkovic-Popovic and Popovic, 1991).

Overall, the nitrification/denitrification process is not conducive to the development of healthy protozoal populations, mostly because of the long non-oxic periods. Therefore, even without pH shocks, the growth of smaller populations than in strictly oxic processes would be expected.

Additional data about this study can be found in appendix C.

4.5 Phase 4: Nitrification/EBP Removal

4.5.1 Initial Remarks

As mentioned in section 3.5, phase 4 was carried out to complete the nitrification study (phase 2) primarely in terms of microfaunal observation and its potential use as a monitoring tool for the nitrification process. Also, based on the phase 2 results for phosphorus removal, this phase was also designed to provide for SBR/EBP removal in order to observe pH, redox and DO variations during the process.

Unfortunately the EBP removal process was unsuccessful. The same pH shocks observed during phase 3 seemed to be highly inhibitory for the poly-P bacteria, precluding the occurrence of almost any EBP removal despite all efforts to improve the environmental conditions for it. Nevertheless, it was decided to present and discuss this phase results as a case study as the results in terms of nitrification alone justify their inclusion.

Phase 4 data not included in this discussion can be found in appendix D.

4.5.2 SBR Operation

Phase 4 was carried out concomitantly with phase 3 and thus the same influent wastewater was used for both phases (table 4.3), being characterized by a medium to strong composition, in terms of both organic carbon and nitrogen. The average F/M ratio based on COD and MLVSS was 0.13 day^{-1}, varying from 0.08 to 0.19 day^{-1}, which again portrayed this phase as a highly loaded SBR application.

A rather loose solids wastage strategy was employed during this phase. At first, the fixed 2,500 mg/L MLSS concentration threshold was maintained on a regular basis. After a while, when SVIs declined to very low values, no wastage was practised for long periods of time.

Only one SBR was employed, achieving two 12-hour cycles per day. For most of this phase, the SBR was operated under the cycle presented in table 4.3, which comprised: the initial part of the Fill period without mixing or aeration to provide for substrate accumulation; the final part of the Fill period, mixing only, to provide for denitrification followed by enhanced biological phosphorus release; the React oxic period to provide for nitrification and "luxury phosphorus uptake"; the Settle and Draw periods; and a shorter Idle period than the one used in the phase 2 study. Figures 4.33 through 4.38 present the chronological data for this phase.

As with phase 3, phase 4 can be divided in three separate periods: start-up, nitrification under the initial time based schedule (cycles 40 through 138) and the final period where new cycles were tried (table 4.4) and alkalinity was added to the influent wastewater.The start-up period lasted until around cycle 40, as it did for phase 2. This period was characterized by a rapid decline of effluent COD and SS concentrations reaching a background level of about 50 mg/L and 10 mg/L respectively. The first indications of nitrification appeared around cycle 30, and reached completion, for the first time, in cycle 40. Some EBP removal was observed from cycle 10 to cycle 20 (fig. 4.37). As with phase 3, the system appeard to lose its capacity for EBP removal, probably because of incipient nitrification, causing

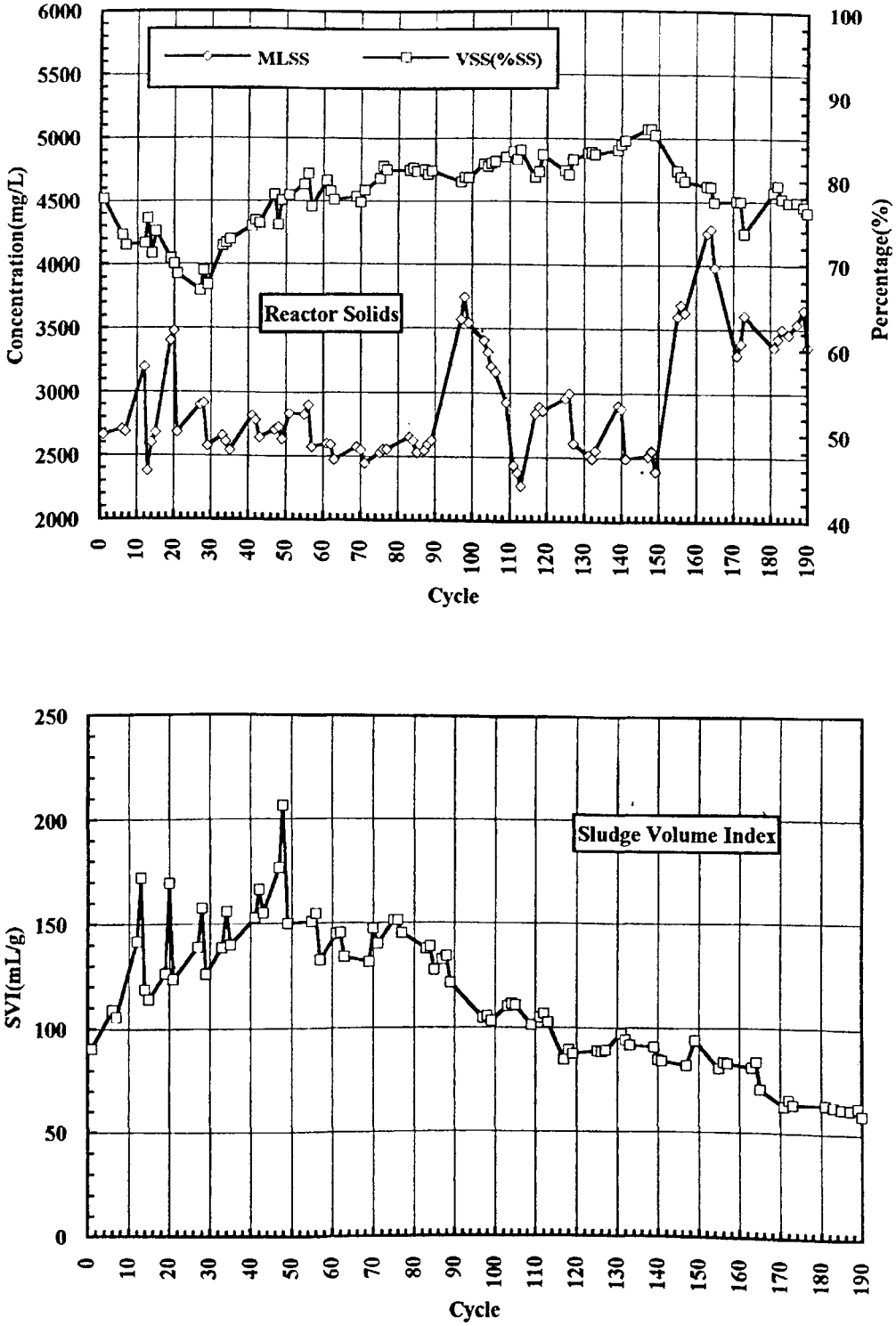


Figure 4.33: Reactor Solids and SVI for Phase 4

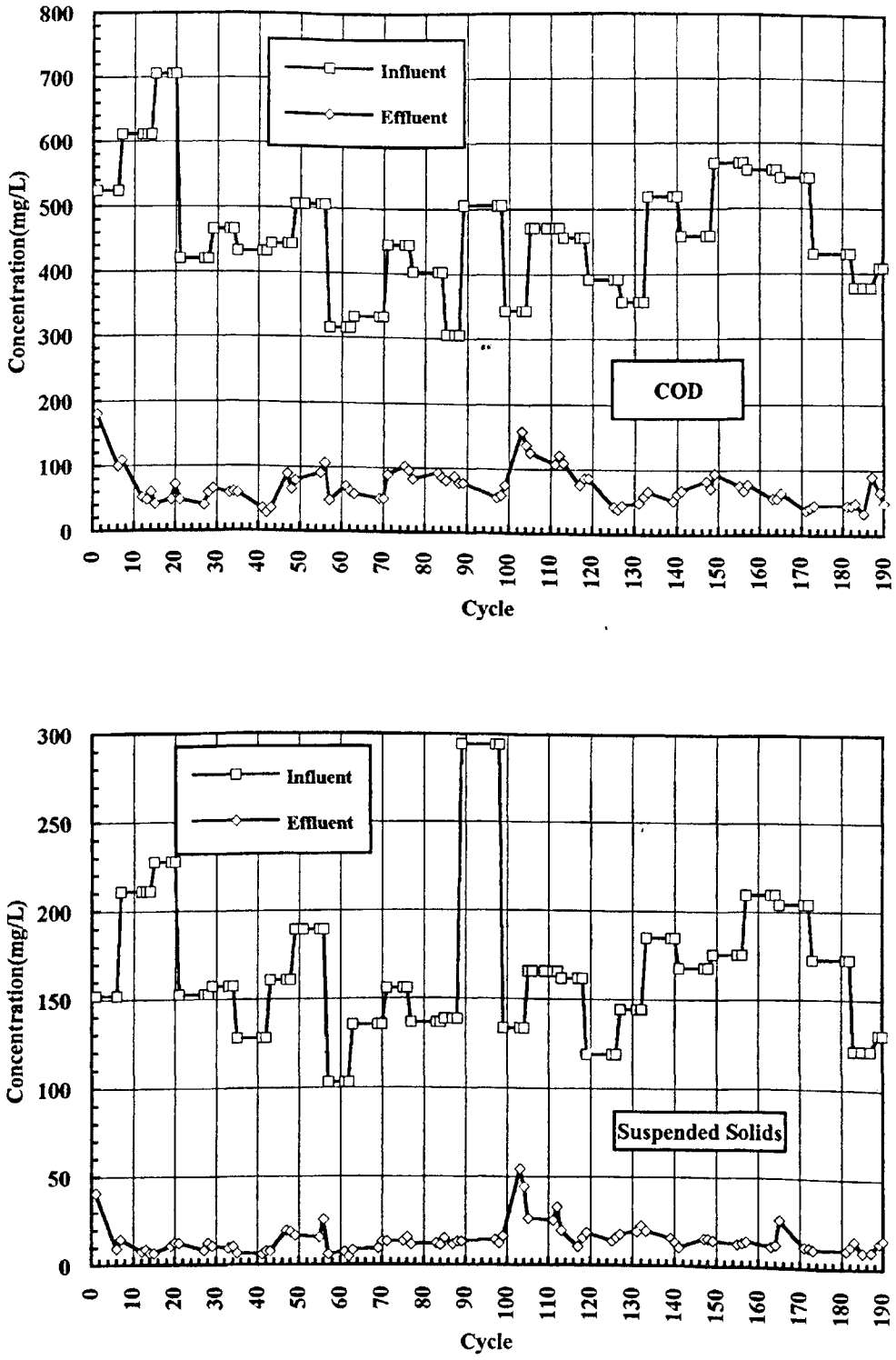


Figure 4.34: COD and SS for Phase 4

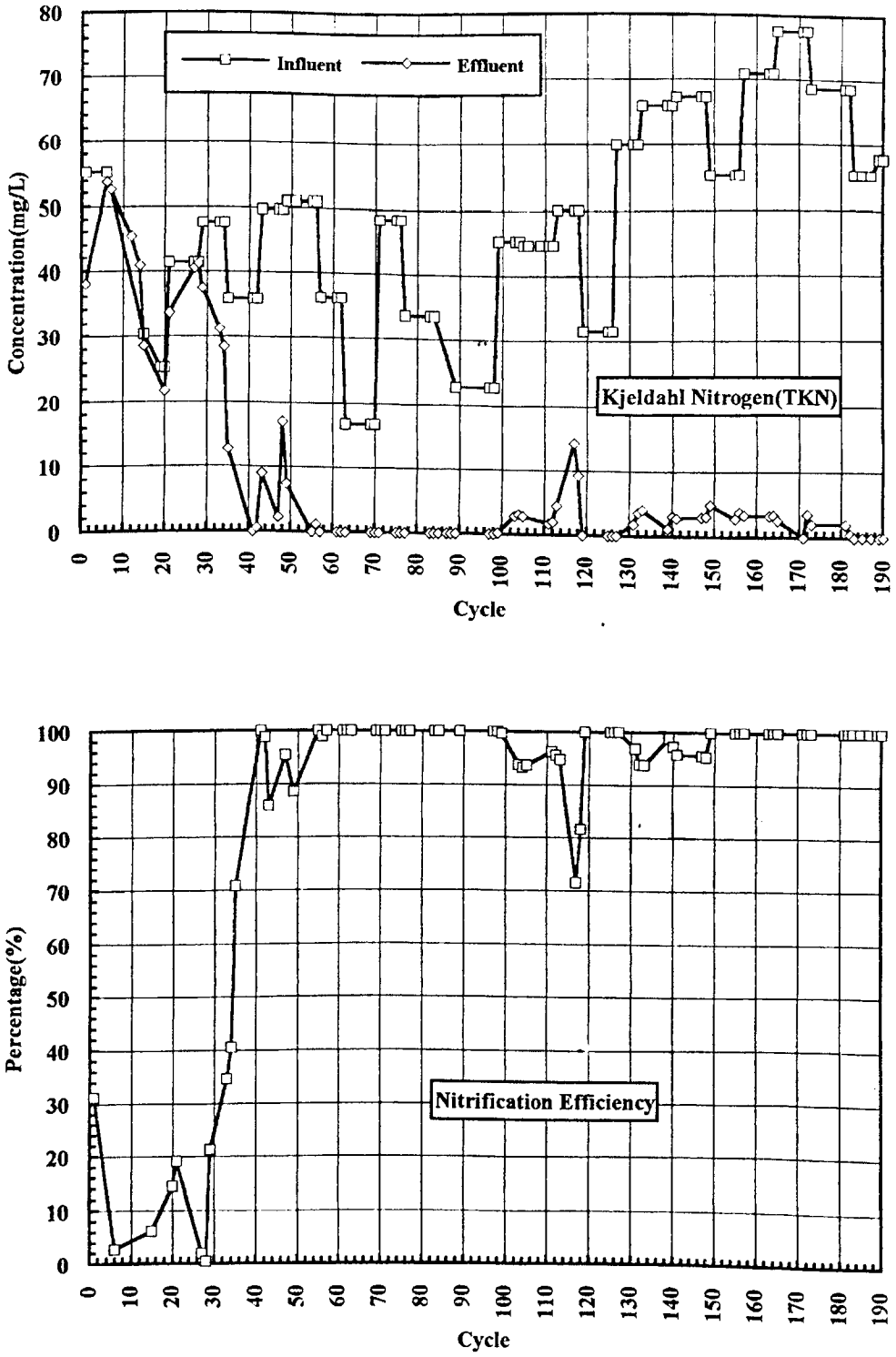


Figure 4.35: Kjeldahl Nitrogen and Nitrification Efficiency for Phase 4

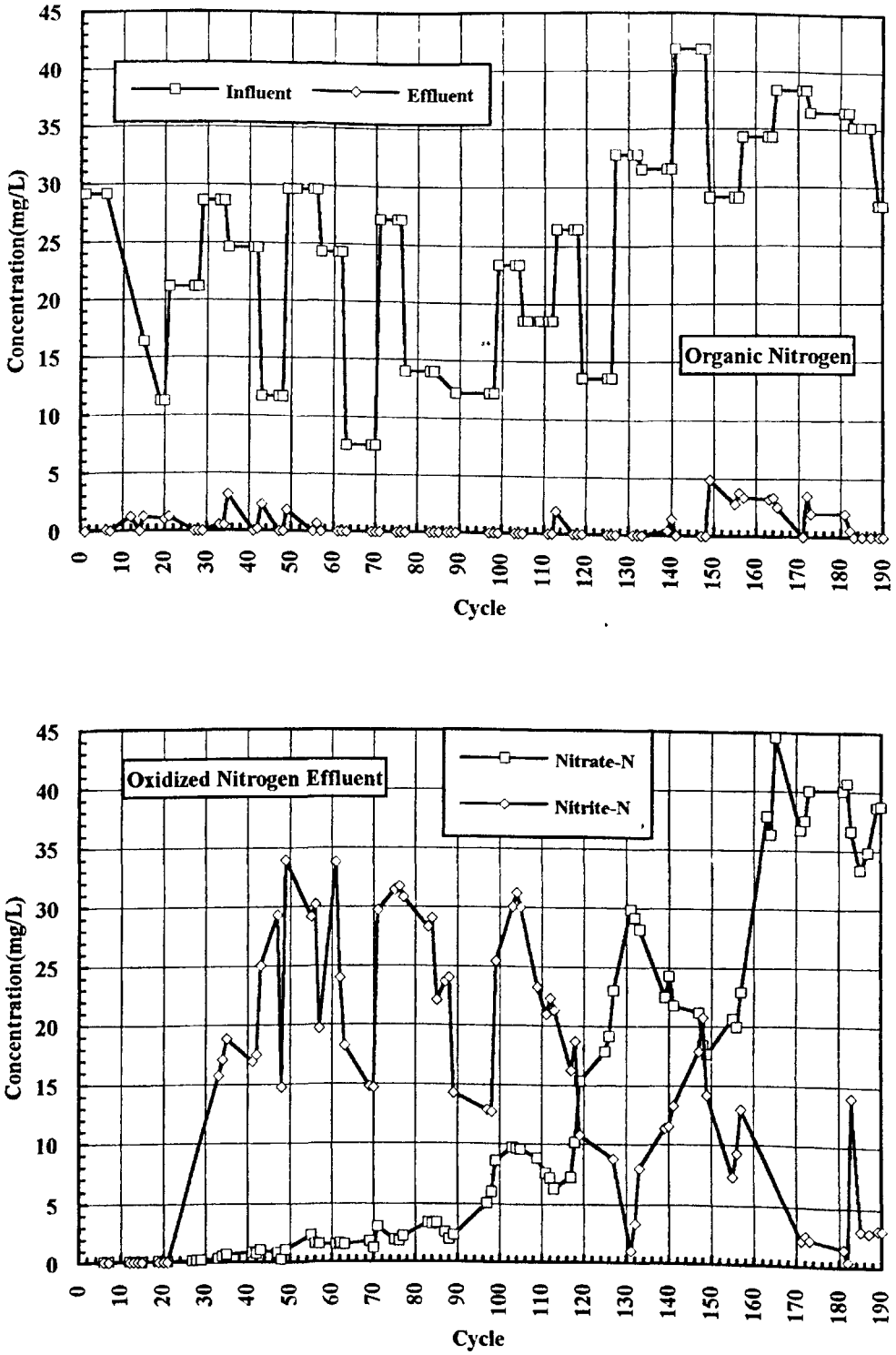
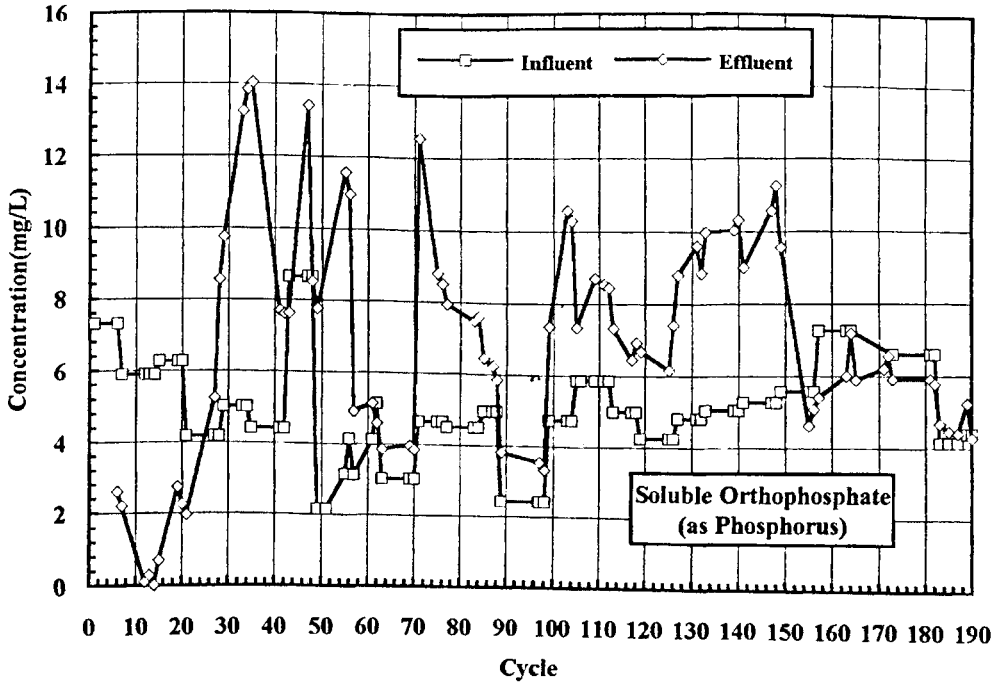


Figure 4.36: Organic Nitrogen and Oxidized Nitrogen for Phase 4



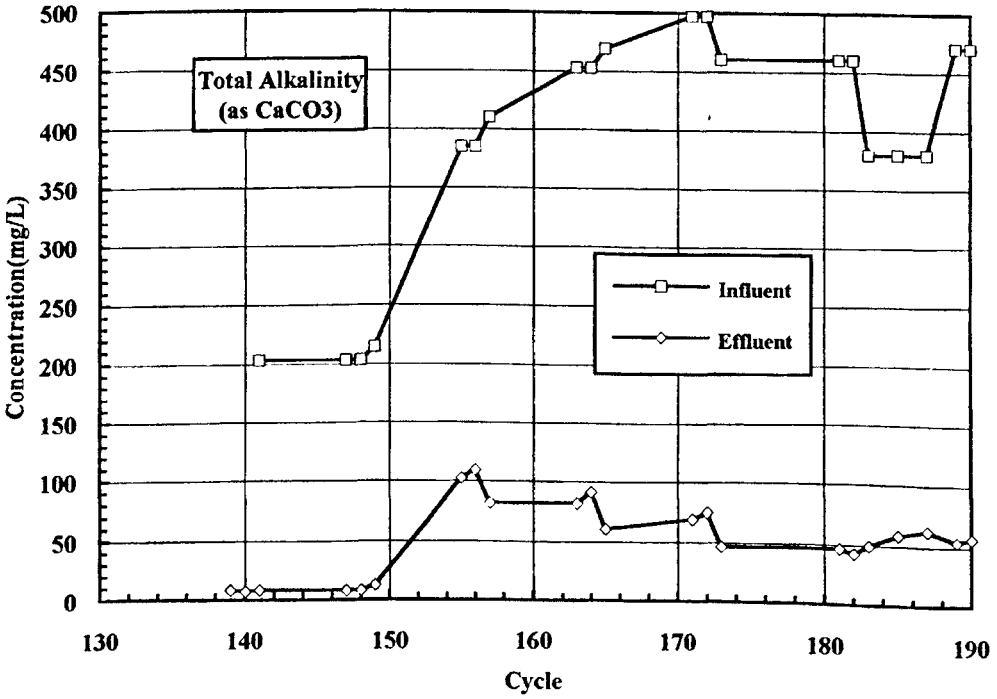
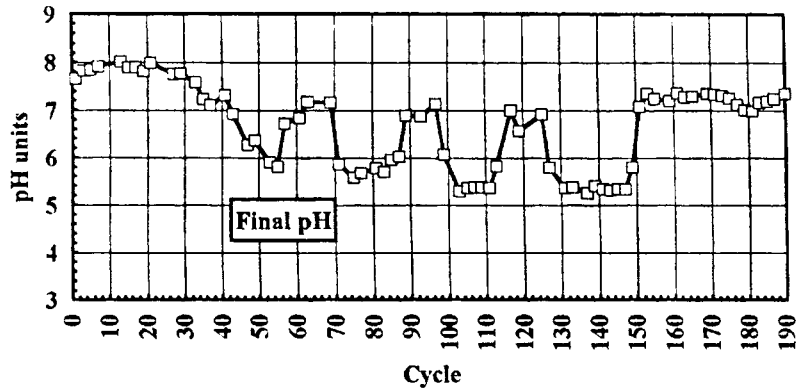
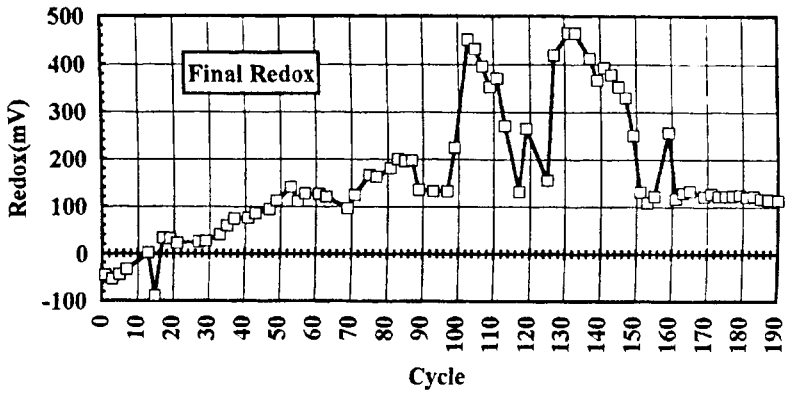
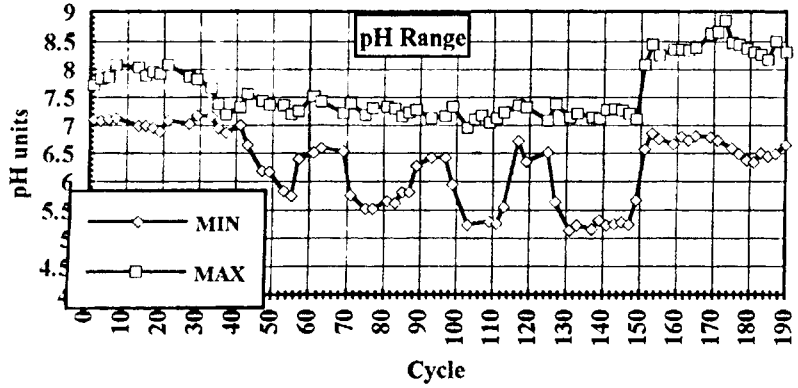


Figure 4.37: Soluble Orthophosphate and Total Alkalinity for Phase 4







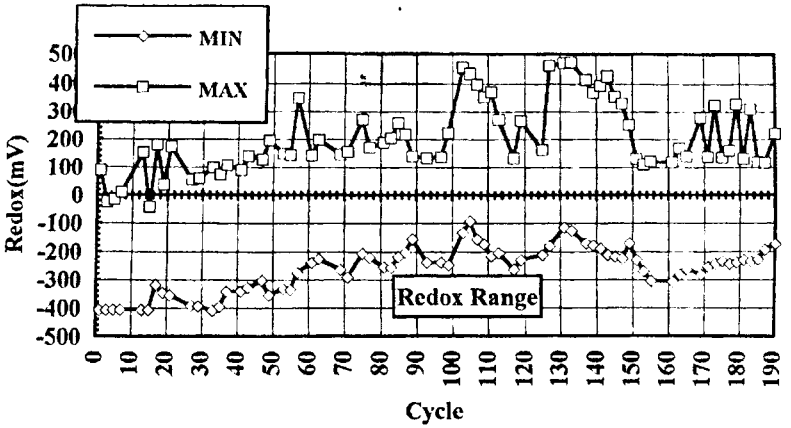


Figure 4.38: pH and Redox for Phase 4

prevailing environment conditions during non-oxic periods to change from anaerobic to anoxic, precluding the EBP removal process. The minimum redox curve in figure 4.38 corroborates this assumption. Again, pH and redox showed their typical evolution for start-up periods, as they did for phases 2 and 3 with a drop in pH value from 8.0 to 7.0, concomitantly with the final redox value reaching 100 mV, indicating the onset of the nitrification process.

It can be seen from the minimum pH graph in figure 4.38 that as soon as nitrification reached completion, pH values dropped from about 7.0 to 5.75. This marked the beginning of a series of pH shocks which were the predominant feature of this period, as it was for phase 3. However, this time the pH shocks were even stronger than in phase 3 mostly due to the lack of an effective denitrification process to counterbalance some of the alkalinity lost during nitrification. pH values as low as 5.14 were reached. Basically, these pH shocks caused high effluent COD and SS concentrations, incomplete nitrification and inhibition of poly-P bacteria. Similar effect of low pH values on EBP removal have been reported by Tracy and Flammino (1985) cited in Sedlak (1991). These authors studied the effect of pH on the specific phosphorus uptake rate. They found that below a pH of 6.5, activity steadily declined and all activity was lost at a pH of 5.2. It is interesting to notice that concomitantly with the pH shocks, a significant phosphorus release occurred (figure 4.37), with effluent orthophosphate containing higher concentrations than in the influent. Even though no explanation for the phenomena was offered, Levin and Shapiro (1965), also cited in Sedlack (1991), reported a similar development. They mentioned that when they held sludge under anaerobic conditions or acidified it, phosphorus release took place. From the above observations it was inferred that a chemical process, rather than a biological one, was responsible for this release of phosphorus under low pH conditions. As for phase 3, periods of low pH levels were intercalated with periods of pH between 6.5 and 7.0. Such periods, were characterized by low effluent COD and SS concentrations, complete nitrification and somewhat less phosphorus being released from the sludge.

For most of this period, nitrite was the predominant oxidized nitrogen form, again indicating *Nitrobacter* inhibition by presence of free-ammonia.

On cycle 138 the first cycle alteration was introduced (table 3.6). The first hour of the React period was transformed from mixed and aerated to mixed only, in order to improve conditions for denitrification. As a result, an increase in pH values due to the alkalinity produced during the denitrification process was expected. After a while, it was evident that the amount of denitrification obtained through this alteration, if any, was not sufficient to make an impact on the system pH. As a last resource, on cycle 150, lime was added to increase pH to a less inhibitory level. Immediately, the minimum pH increased, levelling off at about 6.5. In consequence, effluent COD and SS concentrations, and nitrification efficiency showed noticeable improvements. However, the same cannot be said for EBP removal. On cycle 169, the second cycle alteration was implemented. In order to improve conditions for the EBP removal process, the Fill period was reduced to only one hour without air or mixing. This was done to increase the level of organic matter accumulation inside reactor. In addition, the first 2 hours of this 8-hour long React period was set as a mixed only period to foster denitrification and EBP release. As before, it did not work. On cycle 190, the experiment was terminated.

Apart from some fluctuations, reasonable removal of organic matter, suspended solids and ammonia were achieved during this phase, despite the pH inhibition. Average COD and SS removal were 83.1% and 89.9% respectively. The nitrification efficiency averaged 97.6%, varying from 71.5% to 99.9%. The average daily specific nitrification rate of 40.2 g/kg.day was the highest observed for all 3 phases. Using the alkalinity data provided from cycle 142 onwards, an average specific alkalinity uptake rate of 5.3 (3.0 - 7.2) mg $CaCO_3$/mg TKN removed was calculated.

4.5.3 Track Study

Track studies were performed on cycles 109, 165 and 190. In view of the similarity of the results only the results of cycle 165 are presented. Figures 4.39 through 4.43 present the data referent to this cycle. In these figures, the numbers inside the boxes represent the different periods of the cycle as follows:

1 - First half of the Fill period, mixer and aeration off;
2 - Second half of the Fill period, mixed only;
3 - First hour of the react period, mixed only;
4 - Remainder of React period, mixer and aeration on.

At first glance the phase 4 track study data resembles that of phase 2. However, some important differences can be observed. As for phases 2 and 3, the MLSS graph (figure 4.39) expresses only the influent dilution, as the growth occurring during the period of one cycle is very small compared the magnitude of the MLSS concentration magnitude. Specifically in this case, no growth was observed.

During the second part of Fill and the first hour of React, periods 2 and 3, COD was consumed as indicated by its theoretical maximum value. By the end of period 3, COD would have accumulated up to a maximum of 160 mg/L, instead its value at this point was 97.7 mg/L, suggesting that it was used for either anaerobic respiration or fermentative degradation. Another possibility could be for oxic degradation using oxygen supplied from the liquid-air interface. With the onset of the aeration (period 4), COD was taken up by oxic heterotrophs, quickly at first, and then slowing down towards the end of the React period. This time no COD increase was observed, as occurred for phases 2 and 3. It is interesting to notice that in the phases 2 and 3 track studies, storage product formation was observed, as compared to phase 4. Therefore, it may be inferred that the storage products oxidation during oxic periods is linked to the release of SRP (Orhon *et al.*, 1989).

The Idle period did not seem to have allow complete denitrification to occur (figure 4.40) as it did in phase 2. It is apparent that some oxidized nitrogen was left from the preceding cycle. An Idle period of 1.5 hours was thus not long enough to

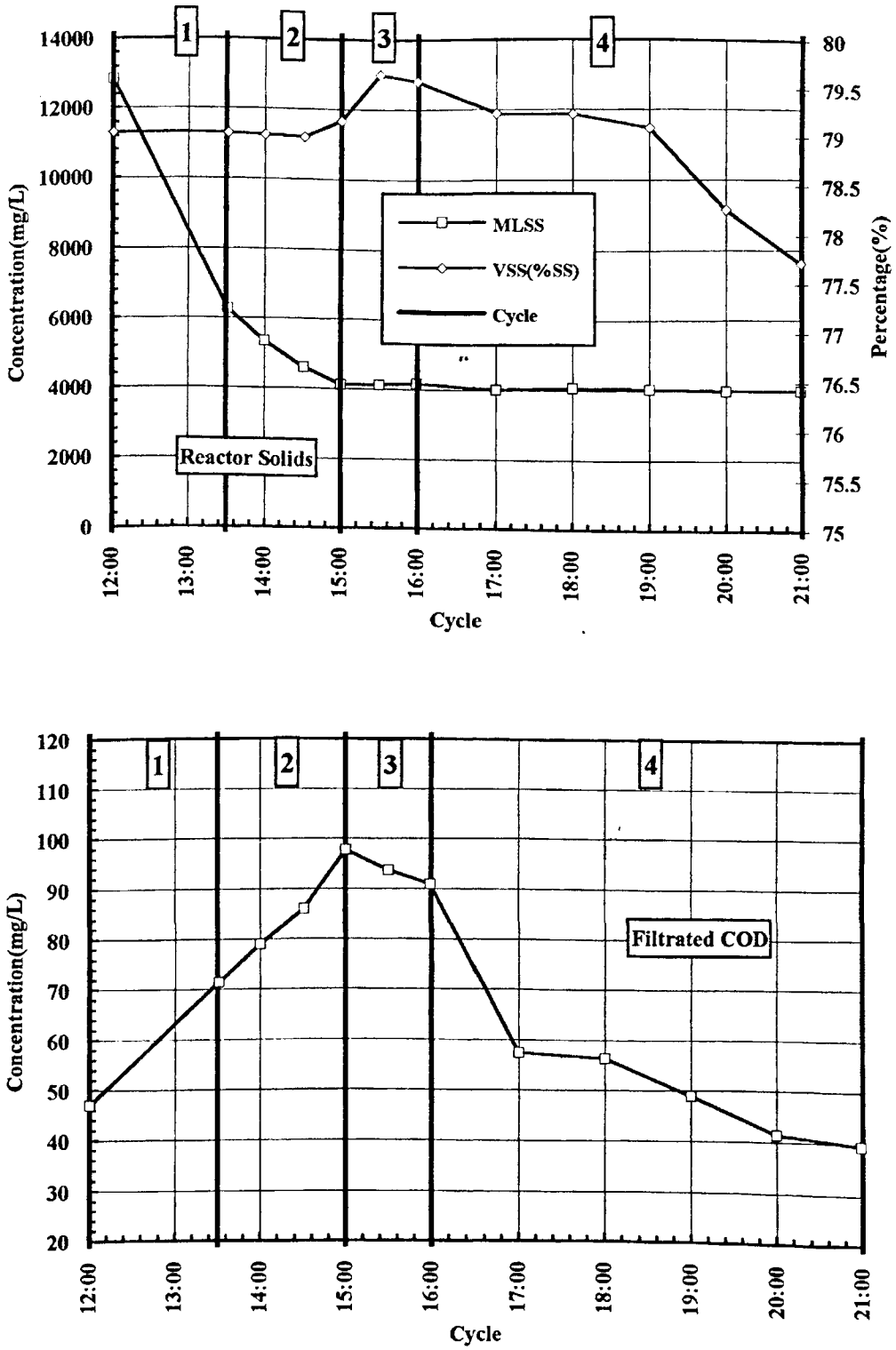


Figure 4.39: Reactor Solids and Filtered COD for Track Study, Phase 4

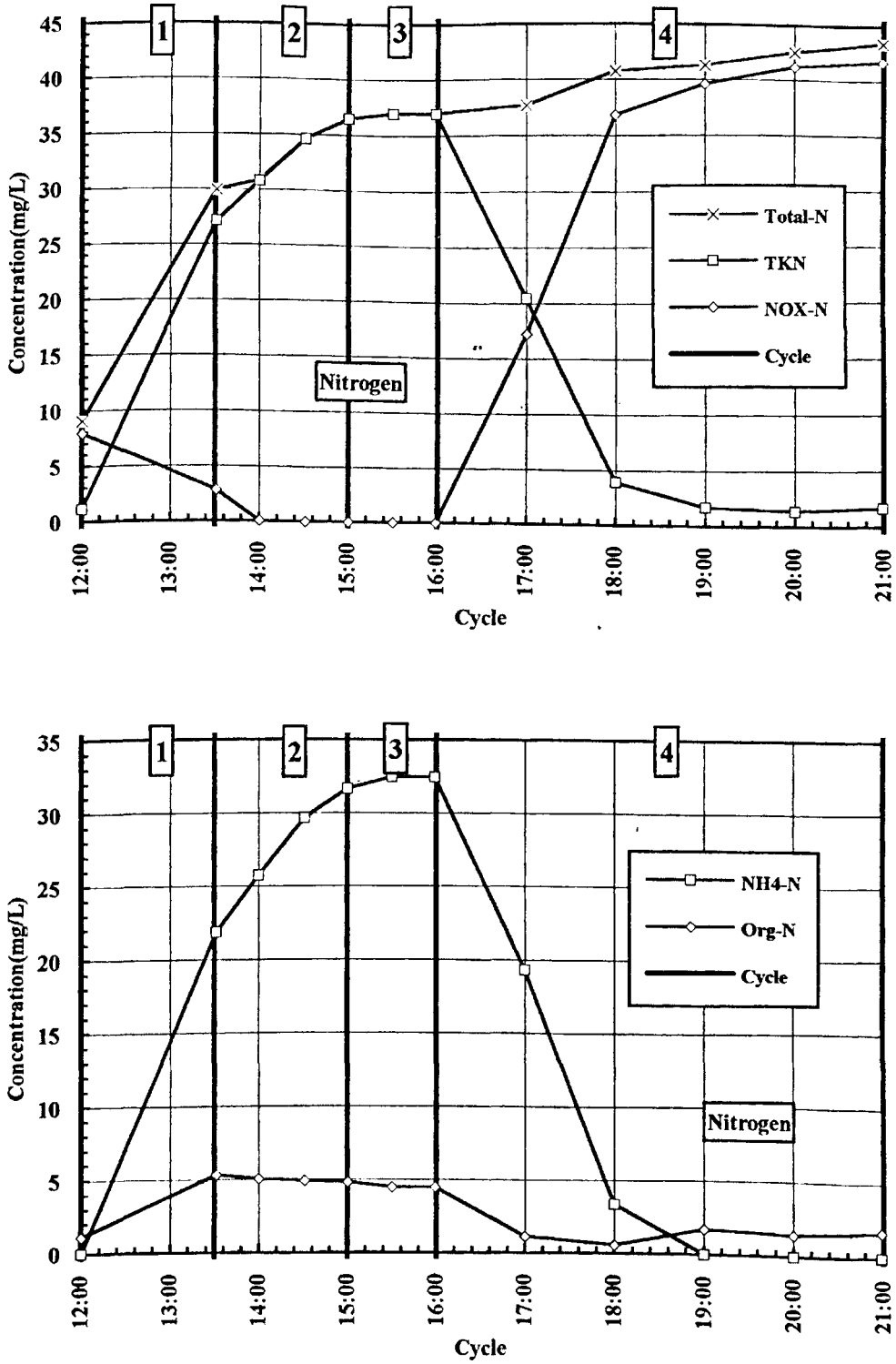


Figure 4.40: Nitrogen Data for Track Study, Phase 4

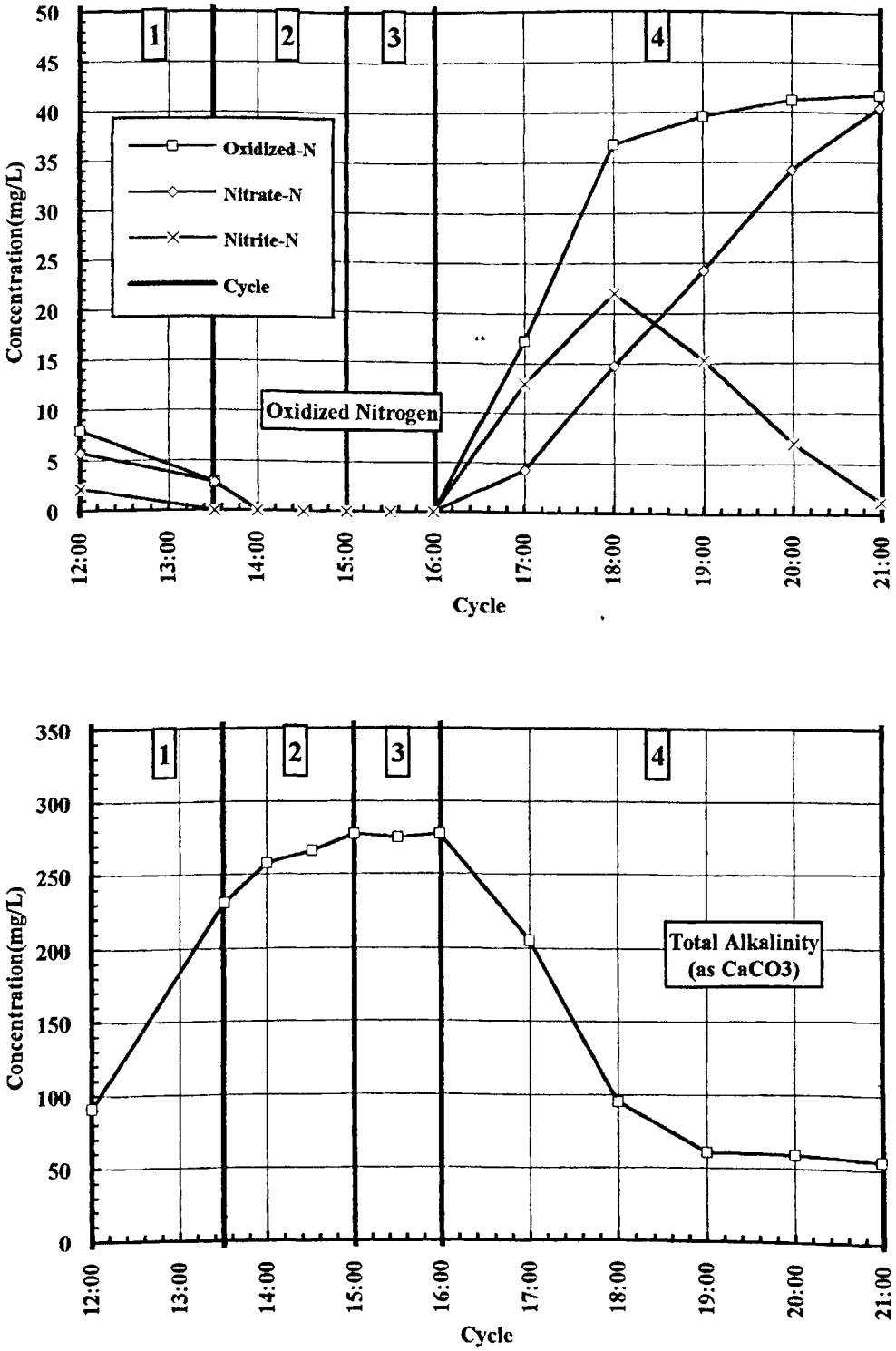


Figure 4.41: Oxidized Nitrogen and Total Alkalinity for Track Study, Phase 4

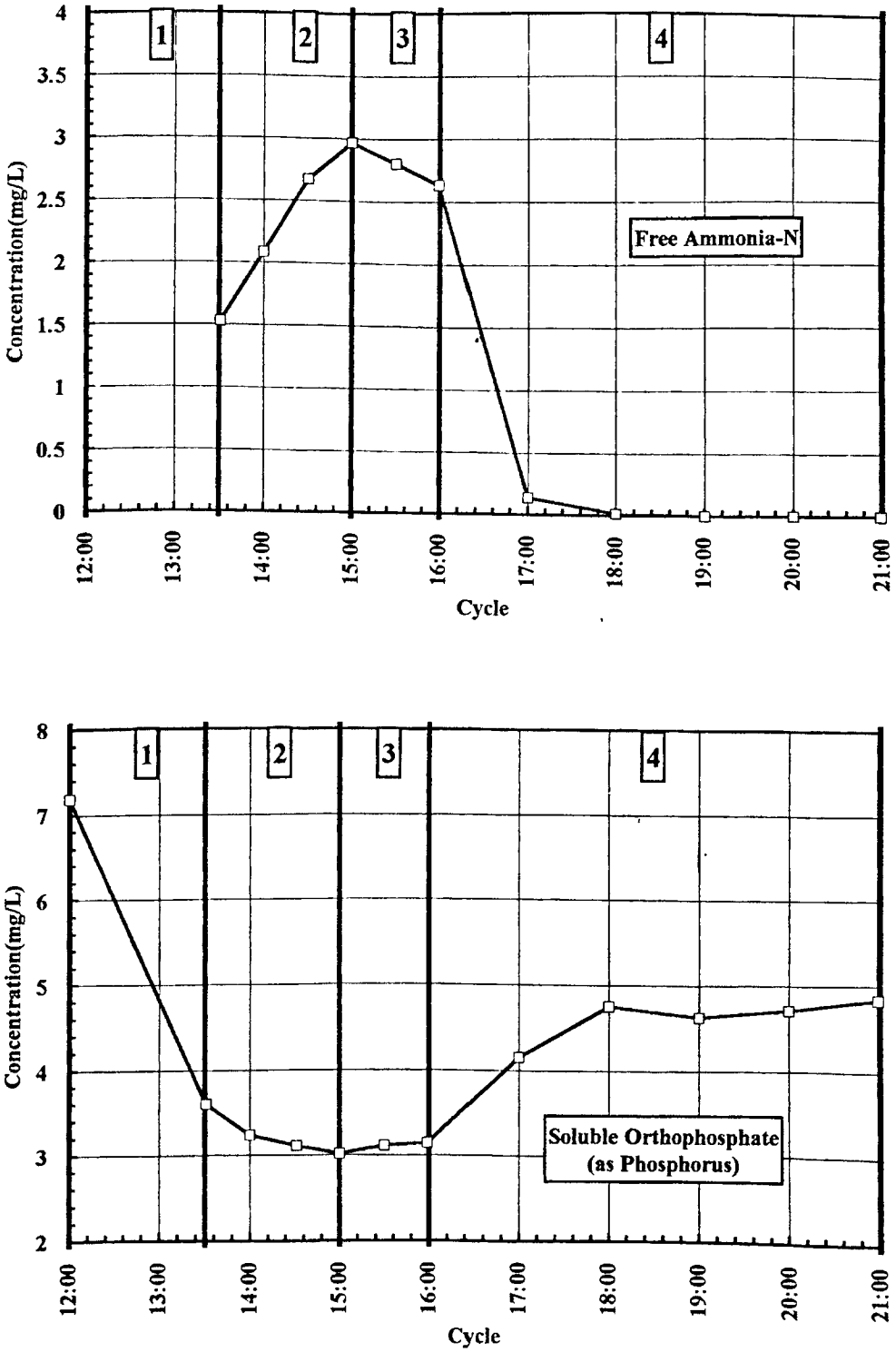


Figure 4.42: Free-Ammonia and Soluble Orthophosphate for Track Study, Phase 4

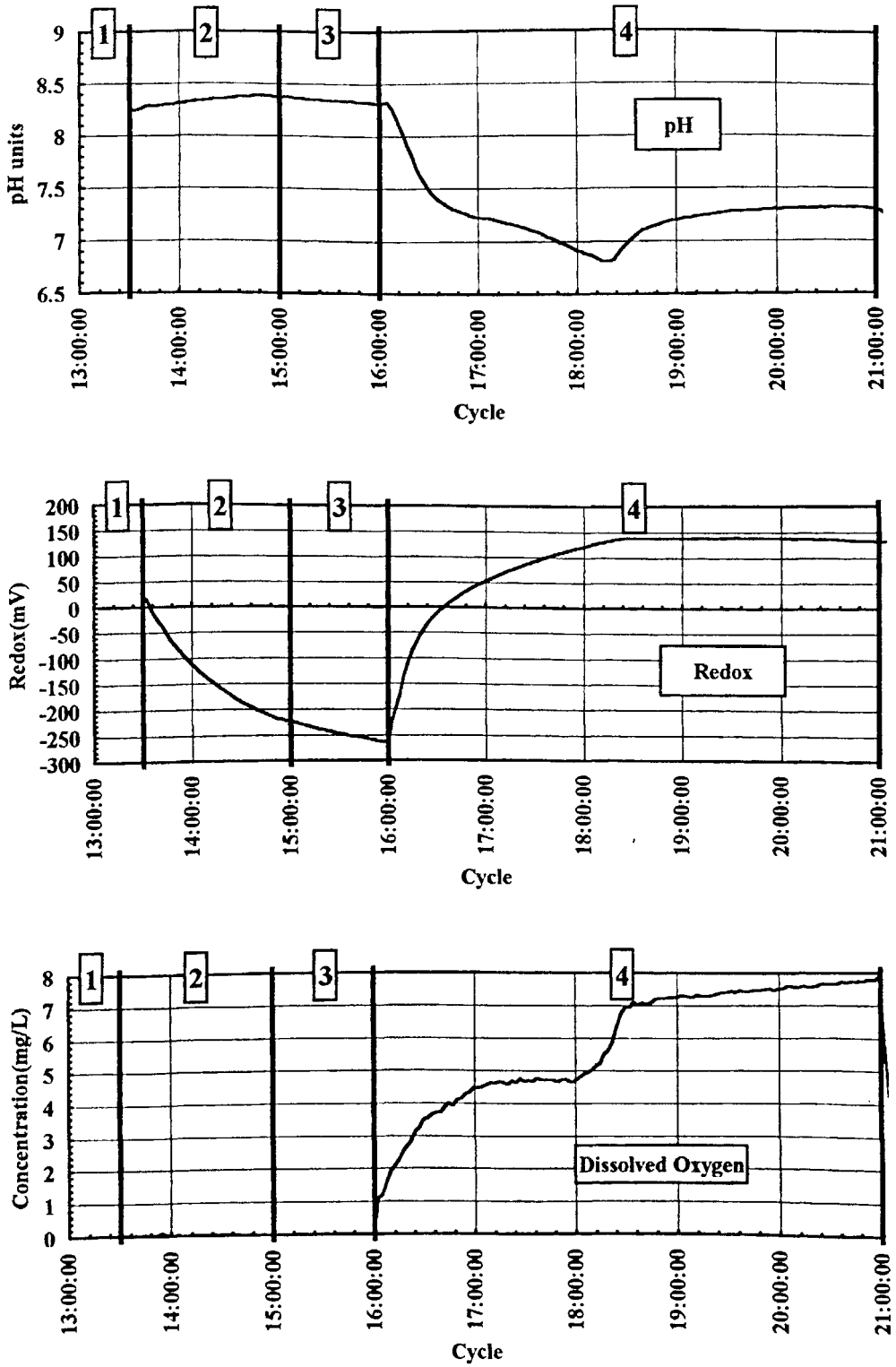


Figure 4.43: Simultaneous pH, Redox and DO Measurements for Track Study, Phase 4

completely reduce the oxidized nitrogen. Nevertheless, this oxidized nitrogen was denitrified during period 2, reaching non detectable values at around 14:00 hours. Also during this period and the first hour of the React period (3), some organic nitrogen was converted to ammonia by fermentative deamination or oxic degradation. When aeration started, the organic nitrogen left from the preceding period was rapidly transformed to ammonia. Concomitantly, the nitrification process started to oxidize ammonia to nitrite and nitrate, reaching completion between 18:00 and 19:00 hours. As with the phase 2 and 3 track studies, nitrite accumulated in the system for a while, before being oxidized to nitrate. Again, free-ammonia was well in excess of its inhibitory level until around 18:00 hours as demonstrated by the free ammonia graph (figure 4.42). Maximum hourly, and maximum daily specific nitrification rates of 19.6 mg NO_x-N/L.h and 146.6 g/Kg.day, respectively, were observed between 17:00 and 18:00 hours. At the same time, an maximum specific alkalinity uptake rate of 5.65 mg $CaCO_3$ consumed/mg NO_x-N produced, was calculated.

No EBP removal was observed during the entire cycle and an increase in soluble orthophosphate during the first two hours of the React period, from 3.1 to 4.8 mg/L (as phosphorus) was probably caused by low pH values.

Figure 4.43 presents pH, redox and DO measurements during the cycle. From these graphs the following observations were inferred.

a) pH: This curve shows both the nitratification and denitrification end-points features. The first one appears as a point of maximum during the second half of the Fill period (2). It is not as sharp as it was for phase 3, probably due to the smaller amounts of oxidized nitrogen denitrified. The second feature to appear, the nitrification end-point, occurred between 18:00 and 19:00 hours during the oxic React period (4). Again, as with phases 2 and 3, an inflexion point appears a few minutes after aeration was turned on (period 4), which seems to be related to a balance between oxic oxidation of organic carbon and nitrification. No explanation was found for the change in slope between 16:00 and 17:00 hours.

b) Redox: Redox failed to show the point where denitrification reached completion (period 2) but it showed the nitratification end-point feature as an inflexion point between 18:00 and 19:00 hours. An inflexion point also appears a few minutes after the onset of aeration concomitantly with the pH inflexion point. Again, it did not seem to be connected with the transition of the effects, on the probes, of nitrate respiration to oxygen respiration, as there was no oxidized nitrogen present at the time that aeration was started.

c) DO: As usual, DO only demonstrated the lack of oxygen tension during the non oxic periods. The nitratification end-point feature appears clearly as a noticeable inflexion point at the same time as it did for pH and redox.

As with all the preceding phases, all recorded cycles were checked against the chronological data, which once again confirmed the reliability of the nitratification and denitrification end-points as control parameters. Figure 4.44 presents pH, redox and DO profiles for two cycles. The profiles on the left are for cycle 21 in which neither complete nitrification nor complete denitrification, during non-oxic period, occurred. On the right, cycle 63 profiles showed both features. It is worth noticing that this time the denitrification end-point feature also appears in the redox profile.

4.5.4 Microfauna Study

This study was performed to help with the interpretation of the chronological data, and to verify the indicator value of microfauna for SBR operational control. The techniques utilized were described in sections 3.7 and 4.4.4 . The same relative protozoan and metazoan ranking system employed in phase 3, was also used in this phase. Table 4.5 shows the protozoa and metazoa species identified throughout this study.

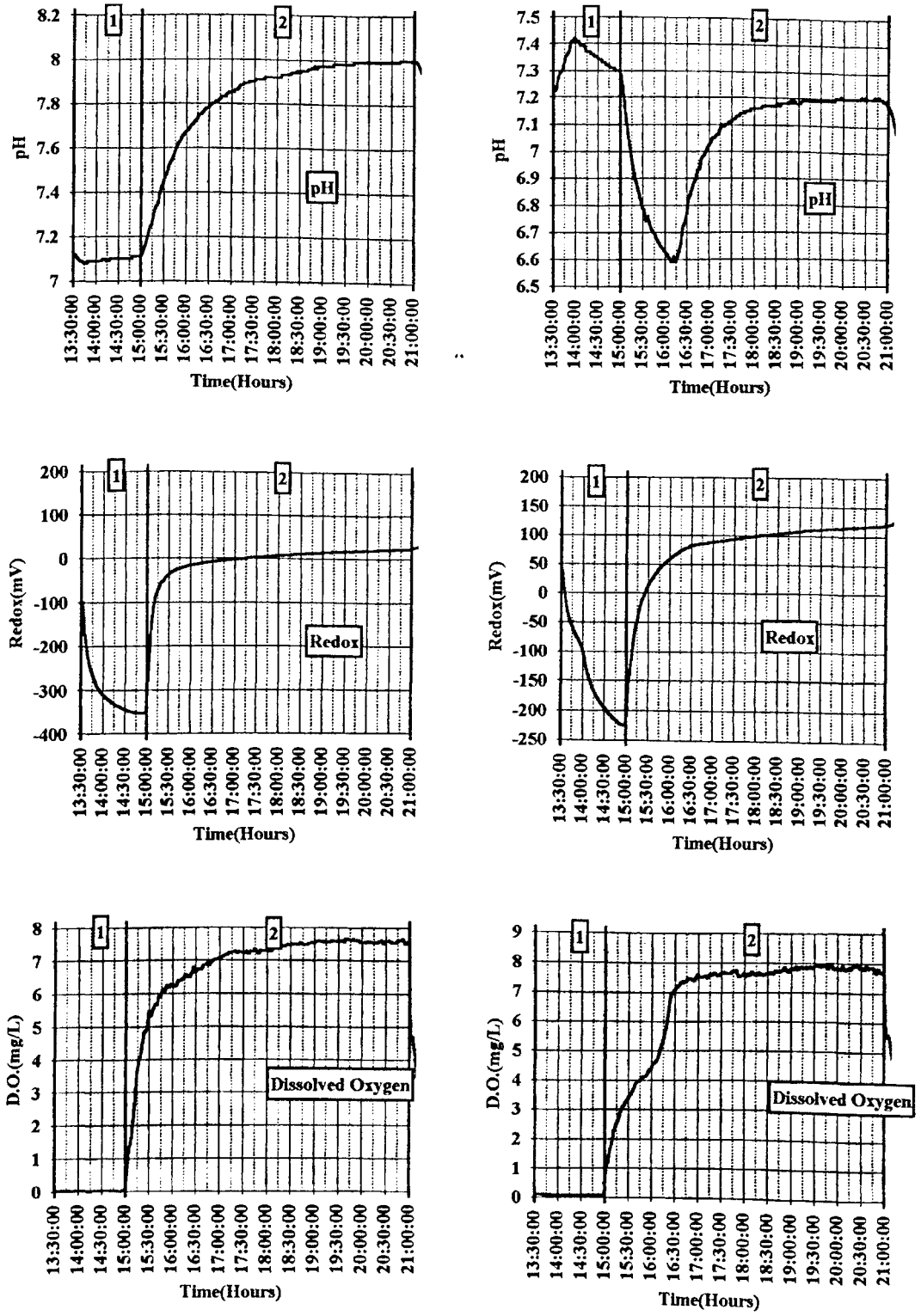


Figure 4.44: Comparative pH, Redox and DO Profiles for Phase 4

Functional groups	Functional Subgroups	Observed Species
Ciliated protozoa	Stalked	*Charchesium sp.* *Vorticella sp.* *Platycola sp.* *Opercularia sp.* *Epystilis sp.* *Podophrya sp.*
	Free-Swimming	*Euplotes sp.* *Tetrahymena sp.* *Lionotus sp.* *Spirostonous sp.* *Colpidium sp.*
	Crawling	*Aspidisca sp* *Trachelophilum sp.*
Flagellated protozoa	-	*Bodo sp.*
	Rotifers	*Rotaria sp.*
	Nematodes[1]	-

1- Observed only as a subgroup

Table 4.5: Protozoa and Metazoa observed species for phase 4 microfauna study

During the start-up period, the sludge had a characteristic brownish-black colour, comprising small rounded flocs. After the onset of complete nitrification it changed for a more yellowish-grey colour and the flocs became somewhat bigger and granular in appearance.

Figure 4.45 presents the relative protozoan and metazoan distribution for this phase and it is apparent that there is a completely different functional group distribution. Even though the phase 4 cycle comprised some non-oxic periods, the predominance of oxic conditions is evident, therefore, healthier conditions for the microfauna development were expected than in phase 3.

During the first 40 cycles, a healthy diversified protozoan population predominated, comprising free swimming, crawling and attached ciliates. As would be expected this period was characterized by efficient COD and SS removal. With the onset of complete nitrification (probably caused by the concomitant pH shock) a drastic change in the microfauna composition occurred: ciliates as a whole almost disappeared and an explosive growth of flagellated protozoa was observed, with the consequent increase in effluent COD and SS concentrations. The flagellated protozoan predominance lasted until around cycle 100. During this period, flagellates were some times observed attached to the flocs through their flagella. It was inferred that such a phenomena occurred as a consequence of the pH shocks. Cech, *et al.*(1991) reported similar behaviour following toxic shock loads. It was speculated that under these conditions a less inhibitory environment occurs around the floc than in the interstitial liquid.

Rotifers appeared in the system around cycle 80, after 40 days of operation, which was approximately the same time as in phase 3 indicating that the rotifer establishment is connected with its generation time. Similar conclusions were reported by Poole (1984). Nematode establishment and distribution could not be linked to any of the parameters measured during this study.

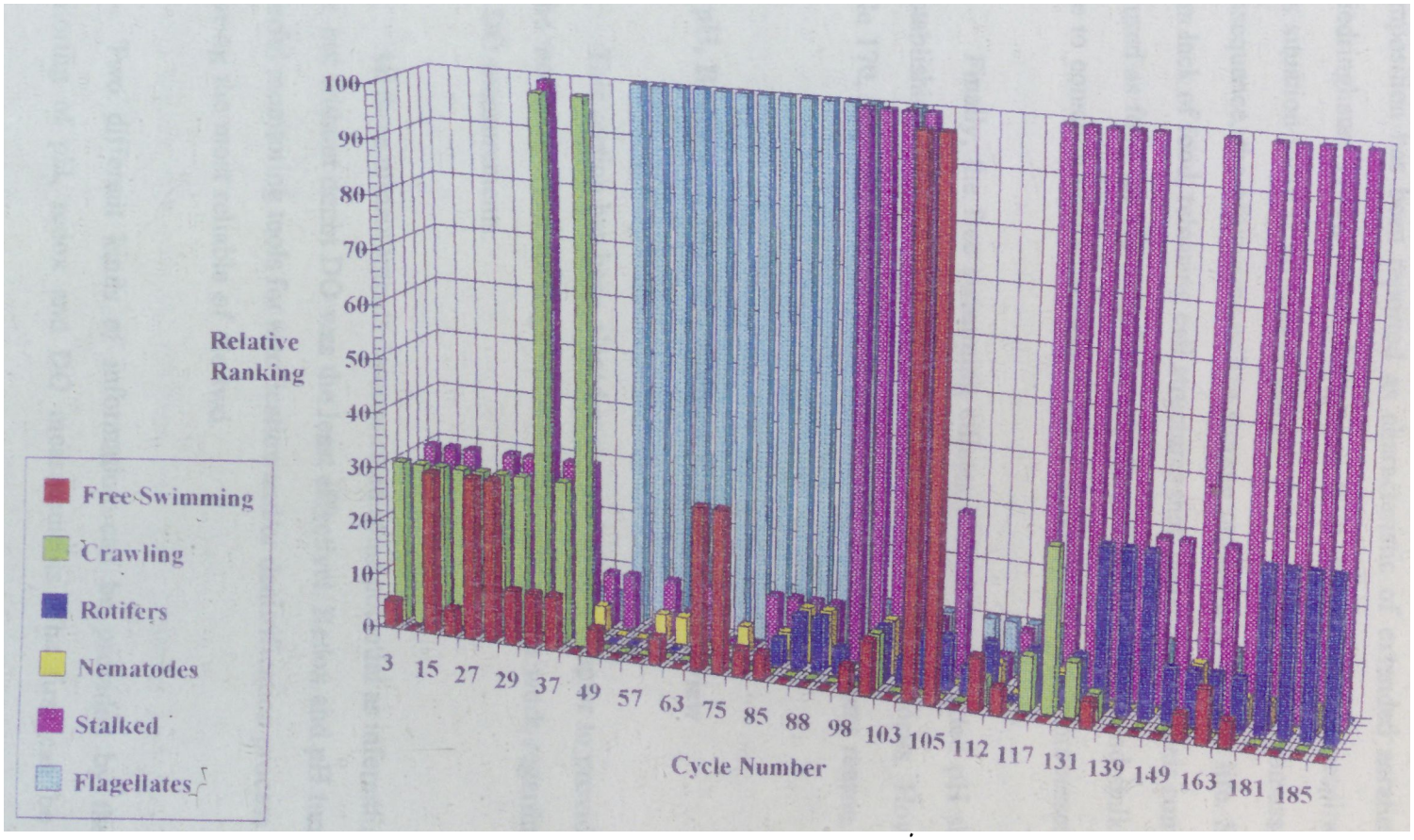


Figure 4.45: Relative Microfauna Distribution for Phase 4

Around cycle 100, attached ciliate protozoa became the predominant functional subgroup and from then on demonstrated an increasing adaptability to repeated pH shocks. With the succession of pH shocks, the microfauna becomes less diversified and from cycle 120 onwards, the sludge population comprised almost exclusively sessile ciliates, rotifers and nematodes. Such a microfaunal population composition has been reported as characteristic of extended aeration applications (Niedringhaus, 1982), where the system is in the endogenous respiration phase. In this situation there is no longer enough food to support the biomass present. As a consequence, the organisms utilize storage products to support life. Some cells die from lack of food, releasing cell structures that, depending on their composition, may be used as food for the remaining microorganisms. Rotifera and stalked ciliates are able to consume whole bacteria in order to sustain their life processes.

Finally, the free swimming ciliates accurately indicated pH shocks, always reestablishing themselves after the attenuation of these shocks. However, around cycle 170, they disappeared from the system without apparent reason.

4.6 pH, Redox and DO Measurements: General Overview

This section has been placed at the end of this chapter to provide a summary of the more important findings obtained throughout this work regarding pH, redox and DO measurements.

All three measurements demonstrated some potential as inferential monitoring tools, but without doubt DO was the least effectivel. Redox and pH turned out to be powerful monitoring tools for nitrification and/or denitrification process control, with pH being the most reliable of the two.

Two different kinds of information can be provided by the continuous monitoring of pH, redox and DO measurements. The first can be regarded as

chronological information, providing insights on a cycle to cycle basis. The other one provides information about temporal process development within a single cycle.

Regarding their use as chronological data for the control of the nitrification and/or denitrification processes, the following remarks summarize some of the findings of this thesis.

a) Both final pH and final redox showed similar development during the nitrification start-up periods for all 3 experimental phases. The final pH characteristically was around pH 8.0 for most of the period, dropping sharply at the onset of nitrification, and then levelling off at around pH 7.2 when complete nitrification was achieved. The final redox parameter by its turn, started the period at very low values (around -400 mV), increasing as time progressed, reaching values above +50 mV when complete nitrification was achieved. Similar redox values were reported by Charpentier, *et al.* (1989) and Peddie, *et al.* (1990).

b) Both minimum pH and minimum redox showed clearly when the nitrification driven pH shocks occurred, as would be expected.

c) Denitrification only occurred when the minimum redox parameter was above -100 mV, which agrees with values reported in the literature (Charpentier, *et al.*, 1989).

d) EBP release occurred during anaerobic periods when the minimum redox parameter was between -350 mV and -450 mV. Again, similar values were found by Charpentier *et al.*(1989).

e) Good correlations between the maximum pH parameter and ammonia or TKN removal were observed, with a correlation coefficient above 0.8

f) Such correlation was also found using the minimum redox parameter instead of pH, also with a correlation coefficient above 0.8.

From the above remarks it is patent that redox had an edge over pH, for the denitrification and EBP removal processes. No clear relationship was found between these processes and chronological pH data. Absolute values above presented should be looked at critically. Those values are valid for the experimental conditions of this work. Changes in influent strength and composition, operational parameters and reactor configuration, among others, may alter these values. A much wiser approach would certainly be to rely on average values established for each specific application.

Figure 4.46 presents, in a schematic manner, the principal findings of this work regarding the use of pH, redox and DO measurements during the track studies performed for all three experimental phases. The points indicated by capital letters represent the pH, redox and DO features that can be used for the nitrification and/or denitrification processes control. Below, a brief description of these features is presented.

Feature A - This point represents the rapid response of pH, redox and DO probes to the onset of aeration.

Feature B - This feature only shows in the pH and redox profiles. As already mentioned, no clear explanation was found for it, but it is conceivable that it represents a decline in the organic carbon uptake rates concomitantly with an increasing in nitrification rates. It is also conceivable that this point shows the actual onset of the nitrification process, however, no clear evidence was found to support it. The explanation proposed by Peddie *et al.* (1990) relating similar observation in the redox profile with the transition of the effects of combined oxygen in the form of nitrates, to the effects of free dissolved oxygen, on the probes, have been ruled out, mostly because no oxidized forms were present in the system prior to that point.

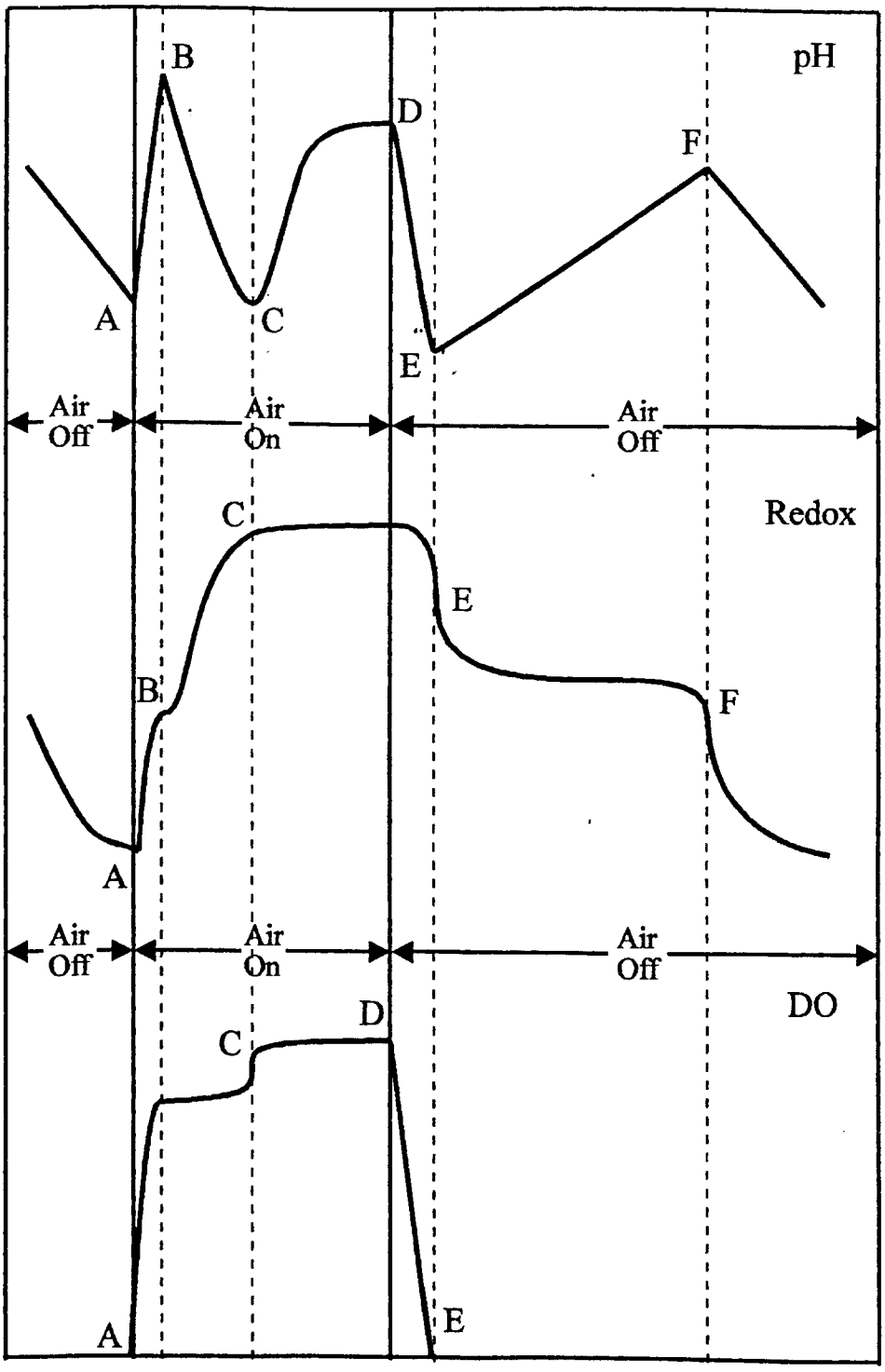


Figure 4.46: Track Studies pH, Redox and DO Measurements Features

Feature C - This feature marks the end of the nitratification process. All three measurements show it in a more or less sharp way, but without doubt the pH feature is the most noticeable. This feature indicates the point where all ammonia has been oxidized to nitrite or nitrate.

Feature D - This shows the point where aeration is turned off. It only appears in the pH and DO profiles.

Feature E - This feature expresses the point where DO concentrations are no longer detectable, indicating the onset of denitrification.

Feature F - Finally, this is the denitrification end-point feature, showing the point where all oxidized nitrogen forms have been denitrified. It also indicates the onset of true anaerobic conditions.

By looking at those 3 profiles it can be inferred that pH is the best alternative for process control. DO measurements did not show as clearly as pH, the nitrification end-point feature. In addition, it is easier to produce a process control algorithm triggered by the pH points of maximum and minimum than it is for the redox inflexion points.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The conclusions below were inferred from all four phases together, unless otherwise indicated.

a) SBR systems can achieve good organic carbon removal, nitrification and denitrification.

b) When operated for carbon removal and nitrification only, under a F/M ratio of 0.06 day^{-1} (phase 2), the system achieved consistently 84.2% COD removal, 91.5% SS removal and 99.9% nitrification efficiency.

c) When operated in a similar manner to item b, but under a F/M ratio of 0.13 day^{-1} (phase 4), 83.1% COD removal, 89.8% SS removal and 97.8% nitrification efficiency were achieved.

d) When operated to achieve the removal of organic carbon and nitrogen, under a F/M ratio of 0.16 day^{-1}, the system achieved averages of 81.1% COD removal, 81.2% SS removal, 87.2% nitrification efficiency and 73.3% denitrification efficiency.

e) Sludge settleability remained good during SBR operation for all operational modes, with an average SVI of 110 mL/g, probably due to quiescent

conditions during the settle period and due to the inherent plug-flow characteristics of SBR systems.

f) Most of the time transient nitrite accumulation was observed in the reactors. This behaviour was connected with free ammonia presence above inhibitory levels.

g) Nitrification (to nitrite) proceeded at an average specific daily rate of 30.2 g/kg.day (phase 2), 23.9 g/kg.day (phase 3) and 40.2 g/kg.day (phase 4), in terms of COD and MLVSS.

h) Denitrification (from nitrite) proceeded at an average daily rate of 17.7 g/kg.day (phase 3)

i) The fixed MLSS strategy for sludge wastage proved to be a better strategy than sludge age, particularly when large variations in influent strength occurred, and, for phase 2 experimental conditions, it produced smaller average effluent SS levels and comparable performance in terms of the other monitored parameters.

j) All inferential monitoring tools utilized (pH, redox and DO) showed some potential as control parameters, but of them pH seems the more promising.

k) pH demonstrated good agreement with alkalinity during the track studies. It was observed to decrease during oxic periods until the completion of nitrification. At this point, pH started to rise (nitrification end point). During non-oxic periods, pH was observed to rise until denitrification reached completion, then it started to decrease (denitrification end point).

l) Redox showed the nitrification end point feature as an almost imperceptible inflexion point. It also showed the denitrification end point as a sharper 'knee', followed by a second inflexion point.

m) DO only showed the nitrification end point by a decrease in oxygen demand.

n) The pH minimum during cycles dropped to values as low as 5.14 for phase 4, effecting transient pH shocks in the system.

o) Ciliated and flagellated protozoa showed good visual correlation with effluent COD and SS. They also responded to pH shocks as an increase in flagellates, concomitant with a decrease in ciliates numbers.

t) Finally, the value of the SBR as a research tool cannot be underestimated. It provided a unique opportunity of following the metabolism of nitrogen through each successive period.

5.2 Recommendations for further research

a) Full scale SBR operation for nitrification and/or denitrification, using pH, redox and DO, is essential in order to investigate other aspects of on-line monitoring, specially in terms of representative measurements.

b) A bench-scale SBR system for EBP removal is necessary to validate the use of pH, redox and DO measurements as controls parameters for this process.

c) The possibility exists to reduce the start-up period for the nitrification process by lowering the system pH to values near 7. It would be useful to verify it.

d) In addition to their use in SBR systems, pH and redox measurements may also prove of value to investigate anaerobic digestion systems.

e) Finally, an investigation of the probes response when monitoring plug-flow and completely mixed activated sludge systems would indicate the usefulness of such a control strategy for nutrient removal activated sludge systems.

REFERENCES

Abllah and Lee. (1991). A Batch Activated Sludge Study of Pinapple Wastewater Using a Bioaugmentation Process. *Water Sci. Tech.* **24**(5): 233-240.

Abufayed and Schroeder. (1986). Performance of SBR/Denitrification with a Primary Sludge Carbon Source. *Jour. Water Poll. Control Fed.* **58**(5): 387-397.

Abufayed and Schroeder. (1986). Kinetics and Stoichiometry of SBR/Denitrification with a Primary Sludge Carbon Source. *Jour. Water Poll. Control Fed.* **58**(5): 398-405.

Akashi, Ikeda, Shibai, Kobayashi and Hirose. (1978). Determination of Redox Potencial Levels Critical for Cell Respiration and Suitable for L-Leucine Production. *Biotech. Bioengng.* **XX**: 27-41.

Al-Shahwani (1988). *Studies on the Contribution of Microfauna to the Performance of Activated Sludge Plants.* Ph.D. Thesis, Leeds University, Leeds, UK.

Al-Shahwani and Horan. (1991). The Use of Protozoa to Indicate Changes in the Performance of Activated Sludge Plants. *Water Res.* **25**(6): 633-638.

Alexander. (1992). Remote Monitoring of Very Small Sewage Works. *Measurement + Control.* **25**: 44-45.

Allamong and Mertens. (1976). *Energy for Life: Photosynthesis and Respiration.* John Wiley & Sons, Inc., New York, USA.

Alleman (1978). *Nitrogen Removal from Wastewater Using Sequencing Batch Reactors.* Ph.D. Thesis, University of Notre Dame, Notre Dame, Indiana, USA.

Alleman. (1984). Elevated Nitrite Ocurrence in Biological Wastewater Treatment Systems. *Water. Sci. Tech.* **17**(2/3): 409-419.

Alleman and Irvine. (1980). Nitrification in the Sequencing Batch Reactor. *Jour. Water Poll. Control Fed.* **52**(11): 2747-2755.

Alleman and Irvine. (1980). Storage-Induced Denitrification Using Sequencing Batch Reactor Operation. *Water Res.* **14**: 1483-1488.

Alleman, Sweeney and Kamber. (1989). Automation of Batch Wastewater Treatment Systems Using Programmable Logic Controllers. *Water. Sci. Tech.* **21**: 1271-1282.

Anderson. (1980). *Bioenergetics of Autotrophs and Heterotrophs.* Series: Studies in Biology. Edward Arnold Publishers Ltd., London, U.K.

Anderson, Abrams and Grens. (1978). Evaluation of Parameters for Nonlinear Thermodynamic Models. *AIChE Jounal.* **24**(1): 20-29.

Andrews and Tien. (1977). New Approach to Bacterial Kinetics in Wastewater. *Jour. Environ. Engng. Division, ASCE.* **103**(EE6): 1057-1074.

Anonymous. (1985). Treatment of Wastewater by Batches Saves Money. *Chem. Engng.* **92**(2): 67.

Antipa. (1977). Use of Commensal Protozoa as Biological Indicators of Water Quality and Pollution. *Trans. Amer. Micros. Soc.* **96**(4): 482-489.

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

Appeldoorn, Kortstee and Zehnder. (1992). Biological Phosphate Removal by Activated Sludge Under Defined Conditions. *Water Res.* **26**(4): 453-460.

Ardern. (1917). A Resume of the Present Position of the Activated Sludge Process of Sewage Purification. *Jour. Soc. Chem. Ind.* **XXXVI**(14): 822-829.

Ardern and Lockett. (1914). Experiments on the Oxidation of Sewage Without the Aid of Filters. *Jour. Soc. Chem. Ind.* **XXXIII**(10): 523-536.

Ardern and Lockett. (1914). The Oxidation of Sewage Without the Aid of Filters. Part II. *Jour. Soc. Chem. Ind.* **XXXIII**(23): 1123-1124.

Ardern and Lockett. (1915). The Oxidation of Sewage Without the Aid of Filters. Part III. *Jour. Soc. Chem. Ind.* **XXXIV**(18): 937-943.

Argaman. (1988). Continuously Fed Intermittently Decanted Activated Sludge: A Rational Basis for Design. *Water Res.* **3**: 303-311.

Arora, Barth and Umphres. (1985). Technology Evaluation of Sequencing Batch Reactors. *Jour. Water Poll. Control Fed.* **57**(8): 867-875.

ASCE. (1984). *A Standard for the Measurement of Oxygen Transfer in Clean Water.* American Society of Civil Engineers, New York.

Azimi and Horan. (1991). The Effects of Diurnal Variations in Flow Rate and Ammonium Load on Nitrification Efficiency in the Activated Sludge Process. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 399-408.

Bahre. (1987). Computer Aided Control of Nitrogen Removal in Small Activated Sludge Wastewater Treatment Plants. *Water Sci. Tech.* **19**: 657-667.

Baines, Hawkes, Hewitt and Jenkins. (1953). Protozoa as Indicators in Activated Sludge Treatment. *Sewage Ind. Wastes.* **25**(9): 1023-1033.

Barker. (1946). The Ecology and Function of Protozoa in Sewage Purification. *Ann. Appl. Biol.* **33**: 314-325.

Barnes and Bliss. (1983). *Biological Control of Nitrogen in Wastewater Treatment.* E. & F. N. Spon Ltd., London, UK.

Batchelor. (1983). Simulation of Single-Sludge Nitrogen Removal. *Jour. Envir. Engng.* **109**(1): 1-16.

Baykal, Orhon and Artan. (1990). Implications of the Task Group Model - II. Response to Intermittent Loading. *Water Res.* **24**(10): 1259-1268.

Bedogni, Falanelli and Pedrazzi. (1991). Evaluation of the Abundance Ratio Between Crawling and Attached Ciliates in the Management of an Activated Sludge Treatment Plant. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 229-233.

Benefield, Kim and Randall. (1989). The Effect of Pentachlorophenol on Enhanced Biological Phosphorus Removal on SBR Systems. *Proc. 44th Purdue Ind. Waste Conf.,* Purdue University, 141-147.

Berry and Casaschi. (1991). Observation of the Microfauna and Evolution of Nitrification and Denitrification Activity of Biofilm on the RBC. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 75-81.

Bock, Wilderer and Freitag. (1988). Growth of *Nitrobacter* in the Absence of Dissolved Oxygen. *Water Res.* 7: 245-250.

Bocken, Braae and Dold. (1989). Dissolved Oxygen Control and Oxygen Utilization Rate Estimation: Extension of Holmberg/Olsson Method. *Water Sci. Tech.* **21**(10/11): 1197-1208.

Bonalberti, Kumer, Milan, Verniani and Garasto. (1991). Control of an Activated Sludge Plant Subjected to a Discontinious Load. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 273-279.

Bottom. (1991). Whitey pH? *Measurement + Control.* **24**: 229-230.

Bowker and Stensel. (1990). *Phosphorus Removal from Wastewater.* Series: Pollution Technology Review, No. 189. Noyes Data Corporation, New Jersey, USA.

Brenner, Chozick and Irvine. (1992). Treatment of a High-Strength, Mixed Phenolic Waste in an SBR. *Wat. Environ. Res.* **64**(2): 128-133.

Briggs. (1983). On-Line Analytical Instrumentation in the Water Industry - Problems and Practical Solutions. Part 1: Problems. *Measurement + Control.* **16**: 94-97.

Briggs. (1983). On-Line Analytical Instrumentation in the Water Industry - Problems and Practical Solutions. Part 2: Potential Solutions. *Measurement + Control.* **16**: 151-155.

Briggs. (1991). Instrumentation and Support System for Monitoring and Control of Source and Receiving Water Quality and Water and Wastewater Treatment Processes. *Measurement + Control.* **24**: 137-141.

Brkovic-Popovic and Popovic. (1991). Relationship Between some Biological and Process Parameters of Activated Sludge. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 235-239.

Bühler and Galster. (1979). *Redox Measurement: Principles and Problems.* Dr. W. Ingold AG, Zürich, Switzerland.

Burbanck and Spoon. (1967). The Use of Sessile Ciliates Collected in Plastic Dishes for Rapid Assessment of Water Pollution. *Jour. Protozool.* **14**(4): 739-744.

Cardello and San. (1988). The Design of Controllers for Batch Bioreactors. *Biotech. Bioengng.* **32**: 519-526.

Carmichael and Rickabaugh. (1989). Treatment of an Organic Contaminated Soil in Activated Sludge Sequencing Batch Reactors. *Proc. 44th Purdue Ind. Waste Conf.,* Purdue University, 161-173.

Cech and Hartman. (1990). Glucose Induced Break Down of Enhanced Biological Phosphate Removal. *Envir. Tech. (Letters).* **11**: 651-656.

Cech, Macek and Wanner. (1991). Limits of Enhanced Biological Phosphate Removal Process. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 437-439.

Charpentier, Florentz and David. (1987). Oxidation-Reduction Potential(ORP) Regulation: A Way to Optimize Pollution Removal and Energy Savings in the Low Load Activated Sludge Process. *Wat. Sci. Tech.* **19**: 645-655.

Charpentier, Godart, Martin and Mogno. (1989). Oxidation-Reduction Potential(ORP) Regulation as a Way to Optimize Aeration and C, N and P Removal: Experimental Basis and Various Full-Scale Examples. *Wat. Sci. Tech.* **21**: 1209-1223.

Chiesa and Irvine. (1985). Growth and Control of Filamentous Microbes in Activated Sludge: An Integrated Hypothesis. *Water Res.* **19**(4): 471-479.

Chin. (1989). Post Treatment of Anaerobic Effluent Using Sequencing Batch Reactors. *Water. Sci. Tech.* **21**: 23-28.

Chin, Ng and Wong. (1987). Treatability Studies of Palm Oil Refinery Wastewaters. *Water. Sci. Tech.* **19**: 23-29.

Chin and Wun-Jern. (1985). Biooxidation and Denitrification Study Using The Sequencing Batch Reactors. In: *Advances in Water Engineering.* Ed. by Tebbutt, T. H. Y., Elsevier Applied Science Publishers Ltd., London, 248-253.

Chozick and Irvine. (1991). Preliminary Studies on the Granular Activated Carbon-Sequencing Batch Biofilm Reactor. *Environ. Progress.* **10**(4): 282-289.

Ciaccio. (1973). *Water and Water Pollution Handbook/Vol. 4.* Marcel Dekker, Inc., New York, New York, USA.

Ciaccio, Cardenas and Jeris. (1973). Automated and Instrumental Methods in Water Analysis. In: *Water and Water Pollution Handbook.* Ed. by Ciaccio, L. L., Marcel Dekker, INC., New York, USA, 1431-1555.

Cingolani, Cossignani and Miliani. (1991). The Role of Microfauna in the Prediction and Control of the Activated Sludge Disfunctions of a Municipal Plant. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 93-96.

Colmenarejo, Morcillo, Parra, Esteban, Bautista, Tellez and Alvarez. (1991). Phosphorus Removal Under Cyclic Anaerobic/Aerobic Conditions. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 447-450.

Corei, Montgomery and Benefield. (1990). Performance Characteristics of an Activated Sludge System When Nitrate Is the Sole Source of Nitrogen. *Proc. 45th Purdue Ind. Waste Conf.*, Purdue University, 359-367.

Corliss. (1986). The Kingdoms of Organisms - From a Microscopist's Point of View. *Trans. Amer. Micros. Soc.* **105**(1): 1-10.

Corliss and Esser. (1974). Comments on the Role of the Cyst in the Life Cycle and Survival of Free-Swimming Protozoa. *Trans. Amer. Micros. Soc.* **93**(4): 578-593.

Couillard, Chartier and Mercier. (1991). Effect of pH and Oxido-Reduction Potential on Biological Solubilization of Metals from Aerobically Digested Sewage Sludge. *Envir. Tech. (Letters).* **12**(12): 1095-1105.

Curds. (1971). Computer Simulations of Microbial Population Dynamics in the Activated-Sludge Process. *Water Res.* **5**: 1049-1066.

Curds. (1982). The Ecology and Role of Protozoa in Aerobic Sewage Treatment Processes. *Ann. Rev. Microbiol.* **36**: 27-46.

Curds and Bazin. (1974). Protozoan Predation in Batch and Continuous Culture. In:

Curds and Cockburn. (1970). Protozoa in Biological Sewage-Treatment Processes - I. A Survey of the Protozoan Fauna of British Percolating Filters and Activated-Sludge Plants. *Water Res.* **4**: 225-236.

Curds and Cockburn. (1970). Protozoa in Biological Sewage-Treatment Processes - II. Protozoa as Indicators in the Activated-Sludge Process. *Water Res.* **4**: 237-249.

Dague and Pidaparti. (1991). Anaerobic Sequencing Batch Reactor Treatment of Swine Wastes. *Proc. 46th Purdue Ind. Waste Conf.*, 751-760.

Dahod. (1982). Redox Potencial as a Better Substitute for Dissolved Oxigen in Fermentation Process Control. *Biotech. Bioengng.* **XXIV**: 2123-2125.

Dawes and Ribbons. (1964). Some Aspects of the Endogenous Metabolism of Bacteria. *Bacteriological Review*. **28**(2): 126-149.

Deininger. (1986). Chapter 4: Operations Control Using Microcomputers. In: *Computerization in the Water and Wastewater Fields*. Ed. by Glysson, E. A., Way, E. J., Force, R. W. and Abbott, W. H., Jr., Lewis Publishers, Chelsea, USA, 35-43.

Dennis and Irvine. (1977). Laboratory Investigation of Fill-and-Draw Biological Reactors for the Treatment of High-Strength Wastes. *Proc. 32nd Purdue Ind. Waste Conf.*, Purdue University, 776-785.

Dennis and Irvine. (1979). Effect of Fill/React Ratio on Sequencing Batch Biological Reactors. *Jour. Water Poll. Control Fed.* **51**(2): 255-263.

Di Pinto, Passino, Ramadori and Tomei. (1990). Modelling of Two Stage Single Sludge System for Nitrogen Removal. *Envir. Tech. (Letters)*. **11**: 509-520.

Drnevich and Stuck. (1975). Error Sources in the Operation of Bench and Pilot Scale Systems Used to Evaluate the Activated Sludge Process. *Proc. 30th Purdue Ind. Waste Conf.*, Purdue University, 561-575.

Duchene. (1991). The Most Significant Protozoa and Metazoa Currently Found in Activated Sludge. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives*, Perugia, Italy, 33-97.

Eikelboom. (1982). Microscopic Sludge Investigation in Relation to Treatment Plant Operation. In: *Bulking of Activated Sludge: Preventative + Remedial Methods (Chapter 3)*. Ed. by Chambers and Tomusen, Ellis Hoawood Ltd,

Eikelboom and van Buijsen. (1983). *Microscopic Sludge Investigation Manual.* 2nd Edition, TNO Research Institute for Environmental Hygiene, Delft, Netherlands.

Ekama and Marais. (1986). The Implication of the IAWPRC Hydrolysis Hypothesis on Low F/M Bulking. *Water Sci. Tech.* **18**: 11-19.

Eroglu, Ozturk, Demir, Akça and Alp. (1991). Sequencing Batch and Hybrid Anaerobic Reactors Treatment of Dairy Wastes. *Proc. 46th Purdue Ind. Waste Conf.*, 413-422.

Esteban, Colmenarejo and Tellez. (1991). Dynamics of Ciliate Communities During Biological Phosphorus Removal Process on Wastewater Treatment. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 71-74.

Esteban, Tellez and Bautista. (1991). Dynamics of Ciliated Protozoa Communities in Activated Sludge Process. *Water Res.* **25**(8): 967-972.

Esteban, Tellez and Bautista. (1991). Effects of Habitat Quality on Ciliated Protozoa Communities in Sewage Treatment Plants. *Envir. Tech. (Letters).* **12**(4): 381-386.

Finlay, Rogerson and Cowling. (1988). *A Beginner's Guide to the Collection, Isolation, Cultivation and Identification of Freshwater Protozoa.* 1st Edition, Culture Collection of Algae and Protozoa, Freshwater Biological Association, Ambleside, U.K.

Ganczarczyk. (1983). *Activated Sludge Process: Theory and Practice.* Series: Pollution Engineering and Technology. Marcel Dekker, Inc., New York, New York, USA.

Gaudy. (1975). The Transient Response to pH and Temperature Shock Loading of Fermentation Systems. *Biotech. Bioengng.* **XVII**: 1051-1064.

Gee, Pfeffer and Suidam. (1990). *Nitrosomonas* and *Nitrobacter* Interactions in Biological Nitrification. *Jour. Envir. Engng.* **116**(1): 4-17.

Geiger, Wheaton, Brinsfield and Alleman. (1985). Biological Treatment of Crab Processing Plant Wastewater. *Jour. Water Poll. Control Fed.* **57**(12): 1128-1133.

George and Gaudy. (1973). Response of Completely Mixed Systems to pH Shock. *Biotech. Bioengng.* **XV**: 933-949.

George and Gaudy. (1973). Transient Response of Continuosly Cultured Heterogeneous Population to Changes in Temperature. *Appl. Microbiol.* **26**(5): 796-803.

Gerder, Mostert and de Villiers. (1987). Interactions Between Phosphate, Nitrate and Organic Substrate in Biological Nutrient Removal Processes. *Water Sci. Tech.* **19**: 183-194.

Gonzales-Martinez, Staud, Wilderer, Hartman and Norouzian. (1987). Alternating Aerobic and Anaerobic Operation of an Activated Sludge Plant. *Jour. Water Poll. Control Fed.* **59**(2): 65-71.

Gordon. (1989). Instrumentation, Control and Automation: The Grampian Way. *Jour. IWEM.* **3**: 459-464.

Gori, Greco, Guarnieri and Minelli. (1991). Microfauna Analysis in Performance Evaluation of the Activated Sludge Depuration Plant in Foligno (Italy). *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 97-99.

Goronszy. (1979). Intermittent Operation of the Extended Aeration Process for Small Systems. *Jour. Water Poll. Control Fed.* **51**(2): 274-287.

Goronszy. (1989). Batch Reactor Treatment of Dairy Wastewaters. *Proc. 44th Purdue Ind. Waste Conf.*, Purdue University, 795-805.

Goronszy. (1991). Coupled Phosphorus and Nitrogen Removal in a Fed-Batch Reactor Without Formal Anoxic Mixing Sequences. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 425-435.

Goronszy and Rigel. (1991). Biological Phosphorus Removal in a Fed-Batch Reactor Without Anoxic Mixing Sequences. *Research Jour. Water Poll. Control Fed.* **63**(3): 248-258.

Goronszy and White. (1989). Biological Nutrient Removal with Sludge Bulking Control in a Batch Activated Sludge System. *Water Sci. Tech.* **21**(12): 1731-1734.

Grandie and Lim. (1980). *Biological Wastewater Treatment: Theory and Applications.* Series: Pollution Engineering and Technology. Marcel Dekker, Inc., New York, New York, USA.

Gray. (1990). *Activated Sludge: Theory and Practice.* Oxford University Press, Oxford, U.K.

Haines. (1992). Automatic Control of Stills for Removal of Ammonia from Coke-Oven Effluents. *Measurement + Control.* **25**: 46-47.

Hänel. (1988). *Biological Treatment of Sewage by the Activated Sludge Process.* Series: Ellis Horwood Series in Water and Wastewater Technology. Ellis Horwood Ltd., Chichester, UK.

Hao and Chang. (1988). Metal Toxicity on Phosphate Removal in Pure Culture and in Activated Sludge Systems. *Jour. Environ. Engng., ASCE.* **114**(1): 38-53.

Hatzinikolaou and Wang. (1991). Quantitative Evaluation of the pH Profile in Organic Acid Fermentations. *Biotech. Bioengng.* **37**: 190-195.

Hayakawa, Tsuji and Hamamoto. (1986). Simultaneous Nitrogen and Phosphate Removal by the Intermittent Cyclic Process. *Water Sci. Tech.* **18**: 319-326.

Heduit and Thevenot. (1989). Relation Between Redox Potential and Oxygen Levels in Activated-Sludge Reactors. *Wat. Sci. Tech.* **21**(8/9): 947-956.

Herzbrun, Hanchak and Irvine. (1984). Treatment of Hazardous Wastes on a Sequencing Batch Reactor. *Proc. 39th Purdue Ind. Waste Conf.*, Purdue University, 385-393.

Herzbrun, Irvine and Malinowski. (1985). Biological Treatment of Hazardous Waste in Sequencing Batch Reactors. *Jour. Water Poll. Control Fed.* **57**(12): 1163-1167.

Hoepker and Schroeder. (1979). The Effect of Loading Rate on Batch-Activated Sludge Effluent Quality. *Jour. Water Poll. Control Fed.* **51**(2): 264-273.

Hoffman and Atlas. (1987). Measurement of the Effects of Cadmium Stress on Protozoa Grazing (Bacterivory) in Activated Sludge by Fluorescence Microscopy. *Appl. Envir. Microbiol.* **53**(10): 2440-2444.

Holmberg, Olsson and Anderson. (1989). Simultaneous DO Control and Respiration Estimation. *Water Sci. Tech.* **21**(10/11): 1185-1195.

Hood. (1948). Measurements and Control of Sewage Treatment Process Efficiency by Oxidation-Reduction Potential. *Sewage Works Jour.* **20**(4): 640-650.

Horan. (1990). *Biological Wastewater Treatment Systems: Theory and Operation.* John Wiley & Sons Ltd., Chichester, UK.

Horan. (1991). The Use of Protozoa as Indicators of Activated Sludge Plant Performance. In: *Microscopic Examination of Activated Sludge for Process Control: Course Notes*. Ed. by Horan, N. J., University of Leeds, Leeds, U.K., 72-91.

Hosomi, Matsusige, Inamori, Sudo, Yamada and Yoshino. (1989). Sequencing Batch Reactor Activated Sludge Processes for the Municipal Landfill Leachate: Removal of Nitrogen and Refractory Organic Compounds. *Water Sci. Tech.* **21**(12): 1651-1654.

Hostettler. (1984). Electrode Electrons, Aqueous Electrons, and Redox Potentials in Natural Waters. *American Journal of Science*. **284**: 734-759.

Hsu. (1986). Treatment of a Petrochemical Wastewater in Sequencing Batch Reactors. *Environmental Progress*. **5**(2): 71-81.

Hu and Strom. (1991). Effect of pH on Fungal Growth and Bulking in Laboratory-Activated Sludges. *Research Jour. Water Poll. Control Fed.* **63**(3): 276-277.

Huang and Drew. (1985). Investigation of the Removal of Organics and Nitrogen in an Oxidation Ditch. *Jour. Water Poll. Control Fed.* **57**(2): 151-156.

Hull, Klimiuk and Janczukowicz. (1990). Comparative Studies of Activated Sludge and Rotating Biological Disk Process. (II- Microfauna). *Proc. 45th Purdue Ind. Waste Conf.*, Purdue University, 339-346.

Ingold. (1980). *Operating Instructions: Oxygen Electrodes*. Zürich, Switzerland.

Inomae, Araki, Awaya, Kusuda and Matsuo. (1987). Nitrogen Removal in an Oxidation Ditch with Intermittent Aeration. *Water Sci. Tech.* **19**: 209-218.

Irvine, Alleman, Miller and Dennis. (1980). Stoichiometry and Kinetics of Biological Waste Treatment. *Jour. Water Poll. Control Fed.* **52**(7): 1997-2006.

Irvine and Bush. (1979). Sequencing Batch Reactors - an Overview. *Jour. Water Poll. Control Fed.* **51**(2): 235-243.

Irvine and Davis. (1971). Use of Sequencing Batch Reactors for Waste Treatment - CPC International, Corpus Christi, Texas. *Proc. 26th Purdue Ind. Waste Conf.*, Purdue University, 450-462.

Irvine, Fox and Richter. (1977). Investigation of Fill and Batch Periods of Sequencing Biological Reactors. *Water Res.* **11**: 713-717.

Irvine, Ketchum, Arora and Barth. (1985). An Organic Loading Study of Full-Scale Sequencing Batch Reactors. *Jour. Water Poll. Control Fed.* **57**(8): 847-853.

Irvine, Ketchum, Breyfogle and Barth. (1983). Municipal Application of Sequencing Batch Treatment. *Jour. Water Poll. Control Fed.* **55**(5): 484-489.

Irvine, Miller and Bhamrah. (1979). Sequencing Batch Treatment of Wastewaters in Rural Areas. *Jour. Water Poll. Control Fed.* **51**(2): 245-254.

Irvine, Murthy, Arora, Copeman and Heidman. (1987). Analysis of Full-Scale SBR Operation at Grundy Center, Iowa. *Jour. Water Poll. Control Fed.* **59**(3): 132-138.

Irvine and Richter. (1976). Computer Simulation and Design of Sequencing Batch Biological Reactors. *Proc. 31st Purdue Ind. Waste Conf.*, Purdue University, 182-192.

Irvine and Richter. (1978). Comparative Evaluation of Sequencing Batch Reactors. *Jour. Environ. Engng., ASCE.* **104**(EE3): 503-514.

Irvine, Richter and Fox. (1975). Batch Treatment for Industrial Wastes. *Proc. 30th Purdue Ind. Waste Conf.*, Purdue University, 841-849.

Irvine and Schaezler. (1971). Kinetic Analysis of Data from Biological Systems. *Jour. San. Engng. Div., ASCE.* **97**(SA4): 409-424.

Irvine and Schertenleib. (1982). The Use of Periodic Biological Reactors in Developing Nations. In: *Management of Industrial Wastewater in Developing Nations.* Pergamon Press, 191-199.

Ishizaki, Shibai and Hirose. (1974). Basic Aspects of Electrode Potential Change in Submerged Fermentation. *Agr. Biol. Chem.* **38**(12): 2399-2406.

IWPC. (1987). *Unit Processes: Activated Sludge.* Series: Manuals of British Practice in Water Pollution Control. Maidstone, Kent, UK.

Jenkins and Mavinic. (1989). Anoxic-Aerobic Digestion of Waste Activated Sludge: Part II - Supertnatant Characteristics, ORP Monitoring Results and Overall Rating System. *Envir. Tech. Letters.* **10**(4): 371-384.

Jing, Benefield and Hill. (1992). Observations Relating to Enhanced Phosphorus Removal in Biological Systems. *Water Res.* **26**(2): 213-223.

Jones, Schroeder and Wilderer. (1990). Denitrification in a Batch Wastewater Treatment System Using Sequestered Organic Substances. *Jour. Water Poll. Control Fed.* **62**(3): 259-267.

Jones, Wilderer and Schroeder. (1990). Operation of a Three-Stage SBR System for Nitrogen Removal from Wastewater. *Jour. Water Poll. Control Fed.* **62**(3): 268-274.

Kennedy, Sanchez, Hamoda and Droste. (1991). Performance of Anaerobic Sludge Blanket Sequencing Batch Reactors. *Research Jour. Water Poll. Control Fed.* **63**(1): 75-83.

Kerrison, McEwen, Phillips and Crook. (1989). The Use of Redox Potential to Control Ferric Sulphate Dosing During Phosphate Removal. *Jour. IWEM.* **3**(4): 397-403.

Ketchum, Irvine and Liao. (1979). First Cost Analysis of Sequencing Batch Biological Reactors. *Jour. Water Poll. Control Fed.* **51**(2): 288-297.

Ketchum, Irvine and Manning. (1987). A Comparison of Biological and Chemical Phosphorus Removals is Continuous and Sequencing Batch Reactors. *Jour. Water Poll. Control Fed.* **59**(1): 13-18.

Ketchum, Liao and Irvine. (1979). Economic Evaluation of Sequencing Batch Reactors. *Proc. 33rd Purdue Ind. Waste Conf.*, Purdue University, 357-376.

Ketchum and Liao. (1979). Tertiary Chemical Treatment for Phosphorus Reduction Using Sequencing Batch Reactors. *Jour. Water Poll. Control Fed.* **51**(2): 298-304.

Kida, Tanemura, Ohno and Sonoda. (1991). Comparison of Performance Among Four Different Processes for the Anaerobic Treatment of Wastewater with a Low Concentration of Organic Matter. *Envir. Tech. (Letters).* **12**(6): 497-502.

Kikkeri and Viraraghavan. (1988). Sequencing Batch Reactors for Wastewater Treatment - A Review. *Asian Environment, Journal of Environmental Science and Technology for Balanced Development.* **10**(1): 27-41.

Kim and Armstrong. (1981). A Comprehensive Study on the Biological Treatabilities of Phenol and Methanol - II. The Effects of Temperature, pH, Salinity and Nutrients. *Water Res.* **15**: 1233-1247.

Kim and Armstrong. (1981). A Comprehensive Study on the Biological Treatabilities of Phenol and Methanol - III Treatment System Design. *Water Res.* **15**: 1249-1257.

Kim, Humenick and Armstrong. (1981). A Comprehensive Study on the Biological Treatabilities of Phenol and Methanol - I. Analysis of Bacterial Kinetics by a Statistical Method. *Water Res.* **15**: 1221-1231.

Kinner and Curds. (1987). Development of Protozoan and Metazoan Communities in Rotating Biological Contactor Biofilms. *Water Res.* **21**(4): 481-490.

Koch and Oldham. (1985). Oxidation-Reduction Potential - A Tool for Monitoring, Control and Optimization of Biological Nutrient Removal Systems. *Wat. Sci. Tech.* **17**: 259-281.

Lallai and Mura. (1989). pH Variation During Phenol Biodegradation in Mixed Cultures of Microorganisms. *Water Res.* **23**(11): 1335-1338.

Lallai, Mura, Miliddi and Mastinu. (1988). Effect of pH on Growth of Mixed Cultures in Batch Reactor. *Biotech. Bioengng.* **31**: 130-134.

Lamb, Westgarth, Rogers and Vernimmen. (1964). A Technique for Evaluating the Biological Treatability of Industrial Wastes. *Jour. Water Poll. Control Fed.* **36**(10): 1263-1284.

Lamberti. (1991). The Role of Metazoa in the Biological Process of Water Purification. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 29-32.

Lee, Small, Lynn and Bovee. (1985). Some Techniques for Collection, Cultivating and Observing Protozoa. :

Lee, Takashima and Speece. (1987). Microcomputer pH Control of Multiple Bioreactors. *Biotech. Bioengng.* **XXX**: 329-330.

Levenspiel. (1972). *Chemical Reaction Engineering.* 2nd Edition, John Wiley & Sons, Inc., New York, New York, USA.

Li and Ganczarczyk. (1990). Structure of Activated Sludge Flocs. *Biotech. Bioengng.* **35**: 57-65.

Lin and Tsang. (1989). Nitrogen Removal in an Intermittently Aerated Completely Mixed Reactor. *Envir. Tech. Letters.* **10**(1): 1-8.

Liptak. (1974). *Environmental Enginners' Handbook. Volume 1: Water Pollution.* Chilton Book Company, Radnor, Pennsylvania, USA.

Lopez (1988). *The Relationship Between Respiration Rate and Microbial Activity and Kinetic Modelling of the Activated Sludge Extended Aeration Process.* Ph.D. Thesis, The University of Leeds, Leeds, U.K.

Lötter and Murthy. (1985). The Identification of Heterotrophic Bacteria in an Activated Sludge Plant with Particular Reference to Polyphosphate Accumulation. *Water SA.* **11**: 179-184.

Macek. (1991). Single-Species Ciliate Cultures Controlling Bacterial Flocs Distribution. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 109-114.

Madoni. (1991). Role of Protozoans and Their Indicator Value in the Activated Sludge Process. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 21-27.

Mallory, Yuk, Liang and Alexander. (1983). Alternative Prey: A Mechanism for Elimination of Bacterial Species by Protozoa. *Appl. Envir. Microbiol.* **46**(5): 1073-1079.

Manning and Irvine. (1985). The Biological Removal of Phosphorus in a Sequencing Batch Reactor. *Jour. Water Poll. Control Fed.* **57**(1): 87-94.

Manning, Irvine, Barth and Chiesa. (1985). The Biological Removal of Phosphorus in a Sequencing Batch Reactor (SBR) - Practical Considerations and Research Observations. *Water Sci. Tech.* **17**: 311-312.

Martin. (1968). *Microfauna of Biological Filters*. Series: University of Newcastle upon Thyne, Department of Civil Engineering, Bulletin 39. Oriel Press Ltd, Newcastle upon Thyne, U. K.

McCartney and Oleszkiewicz. (1990). Carbon and Nutrient Removal in a Sequencing Batch Reactor at Low Temperatures. *Envir. Tech. Letters*. **11**(2): 99-112.

Mckane and Kandel. (1986). *Microbiology: Essentials and Applications*. Series: Life Science Series. McGraw-Hill Book Company, Singapore.

McKinney and Gram. (1956). Protozoa and Activated Sludge. *Sewage Ind. Wastes*. **28**(10): 1219-1231.

Meier, Lohrum and Gareiss. (1989). *Practice and Theory of pH Measurement*. Ingold Messtechnik AG, Urdorf, Switzerland.

Melcer, Bedford, Topnik and Schmidtke. (1987). Conversion of Small Municipal Wastewater Treatment Plants to Sequencing Batch Reactors. *Jour. Water Poll. Control Fed.* **59**(2): 79-85.

Misbahuddin and Farooq. (1991). Biological Treatment of a Petrochemical Wastewater Using Sequencing Batch Reactors. *Envir. Tech. (Letters)*. **12**(2): 131-145.

Monod. (1949). The Growth of Bacteria Cultures. *Ann. Rev. Microbiol.* **3**: 371-394.

Moore and Ruchhoft. (1943). II. Oxidation-Reduction Potentials Developed in Sewage and Sewage-Activated Sludge Mixtures. *Sewage Works Jour.* **15**(5): 880-893.

Murphy, Sutton and Jank. (1977). Dynamic Nature of Nitrifying Biological Suspended Growth Systems. *Prog. Water Tech.* **9**: 279-290.

Murthy, Irvine and Hallas. (1988). Principles of Organism Selection for the Degradation of Glyphosate in Sequencing Batch Reactor. *Proc. 43rd Purdue Ind. Waste Conf.*, Purdue University, 267-274.

Niedringhaus. (1982). Keeping Track of the Bugs: An Operator's Guide to Understanding the Activated Sludge Process. *Jour. Water Poll. Control Fed. (Highlights).* **19**(10): 7-11.

Nielsen and Thompson. (1988). Operating Experiences at a Large Continuously Fed, Intermittently Decanted, Activated Sludge Plant. *Jour. Water Poll. Control Fed.* **60**(2): 199-205.

Nussberger. (1953). Application of Oxidation-Reduction Potentials to the Control of Sewage Treatment Processes. *Sewage Ind. Wastes.* **25**(9): 1003-1014.

Okada and Sudo. (1985). Simultaneous Removal of Phosphorus and Nitrogen by Sequencing Batch Reactor Activated Sludge Process. *Water Sci. Tech.* **17**: 315-316.

Okada and Sudo. (1986). Performance of Sequencing Batch Reactor Activated Sludge Processes for Simultaneous Removal of Nitrogen, Phosphorus and BOD as Applied to Small Community Sewage Treatment. *Water Sci. Tech.* **18**: 363-370.

Oleszkiewicz and Berquist. (1988). Low Temperature Nitrogen Removal in Sequencing Batch Reactors. *Water Res.* **22**(9): 1163-1171.

Oleszkiewicz, Janeczko and Trebacz. (1991). Removal of Simple Phenolics in Aerobic and Denitrifying Sequencing Batch Reactors. *Envir. Tech. (Letters)*. **12**(11): 1017-1026.

Oleszkiewicz, Mateja and Hutchison. (1990). Treatment of Food Industry Wastewater in Sequencing Batch Reactors. *Envir. Tech. Letters*. **11**(6): 499-508.

Oosterlee. (1991). Process Automation of the Hague Sewage-Treatment Plant. *Jour. IWEM*. **5**: 134-140.

Orhon, Artan and Cimsit. (1989). The Concept of Soluble Residual Product Formation in the Modelling of Activated Sludge. *Water. Sci. Tech.* **21**: 339-350.

Orhon, Cimsit and Tunay. (1986). Substrate Removal Mechanism for Sequencing Batch Reactors. *Water Sci. Tech.* **18**: 21-33.

Osada, Haga and Harada. (1991). Removal of Nitrogen and Phosphorus from Swine Wastewater by the Activated Sludge Units with Intermittent Aeration Process. *Water Res.* **25**(11): 1377-1388.

Palis and Irvine. (1985). Nitrogen Removal in a Low-Loaded Single Tank Sequencing Batch Reactor. *Jour. Water Poll. Control Fed.* **57**(1): 82-86.

Pantea-kiser, Wukasch and Alleman. (1989). The Effect of Inhibitory Compounds on Biological Nitrification. *Proc. 44th Purdue Ind. Waste Conf.*, Purdue University, 465-474.

Peddie, Mavinic and Jenkins. (1990). Use of ORP for Monitoring and Control of Aerobic Sludge Digestion. *Jour. Environ. Engng.* **116**(3): 461-471.

Pisano, O'Shaughnessy, LaMarre, Gray, Pederson and Sykes. (1989). Toxic Organic Shock Loading of Rotating Biological Contactors and Sequencing Batch Reactors. *Proc. 44th Purdue Ind. Waste Conf.*, Purdue University, 125-140.

Poole. (1984). A Study of the Relationship Between the Mixed Liquor Fauna and Plant Performance for a Variety of Activated Sludge Sewage Treatment Works. *Water Res.* **18**(3): 281-287.

Ramadori, Rozzi and Tandoi. (1980). An Automated System for Monitoring the Kinetics of Biological Oxidation of Ammonia. *Water Res.* **14**: 1555-1557.

Ranzani, Romano and Lorenzi. (1991). Relationship Between Some Process Parameters and Microfauna Variations in a Two Millions Equivalent Inhabitants Plant. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 241-246.

Rea. (1977). Single-Cell Activated Sludge Using Fill-and-Draw for a Combined Industrial/Domestic Waste Treatment Plant. *Proc. 32nd Purdue Ind. Waste Conf.*, Purdue University, 611-616.

Robinson. (1990). Operating Experiences of Instrumentation, Control and Automation at Holdenhurst Sewage-Treatment Works, Bournemouth. *Jour. IWEM.* **4**(6): 559-569.

Rohlich. (1944). Oxidation-Reduction Potential Measurements in Activated Sludge and Activated Sludge-Sewage Mixtures. *Sewage Works Jour.* **16**(3): 540-557.

Rohlich. (1948). Measurements and Control of Sewage Treatment Process by Oxidation-Reduction Potential - A Discussion. *Sewage Works Jour.* **20**(4): 650-653.

Sanchez, Kennedy, Hamoda and Droste. (1989). A Study of Anaerobic Sequencing Batch Reactors for the Treatment of Soluble Wastes. *Proc. 44th Purdue Ind. Waste Conf.*, Purdue University, 235-246.

Sartory. (1976). The Peritrich Ciliates as Biological Indicators for Activated Sludge. *Microscopy.* **33**: 85-89.

Satchwell. (1991). Microscopic Examination of Activated Sludge. In: *Microscopic Examination of Activated Sludge for Process Control: Course Notes.* Ed. by Horan, N. J., Leeds University, Leeds, U.K., 92-97.

Sawyer and McCarty. (1978). *Chemistry for Environmental Engineering.* Series: McGraw-Hill Series in Water Resources and Environmental Engineering. 3rd Edition, McGraw-Hill Book Company, Singapore.

Schroeder. (1982). Design of Sequencing Batch Reactor Activated Sludge Process. *Civil Engineering for Practicing and Design Engineers.* **2**: 33-44.

Sedlak. (1991). *Phosphorus and Nitrogen Removal from Municipal Wastewater: Principles and Practice.* 2nd Edition Edition, Lewis Publishers, Inc., New York, USA.

Sekine, Sato, Furuya and Sunahara. (1988). Supervision and Control of the Activated Sludge Process Utilising the Respiration Rate Activity. *Envir. Tech. Letters.* **9**: 1317-1326.

Sharma and Ahlert. (1977). Nitrification and Nitrogen Removal. *Water Res.* **11**: 897-925.

Shibai, Ishizaki, Kobayashi and Hirose. (1974). Simultaneous Measurement of Dissolved Oxygen and Oxidation-Reduction Potentials in the Aerobic Culture. *Agr. Biol. Chem.* **38**(12): 2407-2411.

Shin and Jun. (1992). Development of Excess Phosphorus Removal Characteristics in a Sequencing Batch Reactor. *Water Sci. Tech.* **25**(4/5): 433-440.

Silverstein and Schroeder. (1983). Performance of SBR Activated Sludge Process with Nitrification/Denitrification. *Jour. Water Poll. Control Fed.* **55**(4): 377-384.

Smith and Wilderer. (1986). Treatment of Hazardous Landfill Leachate Using Sequencing Batch Reactors. *Proc. 41th Purdue Ind. Waste Conf.*, Purdue University, 272-282.

Society of Protozoologists. (1980). A Newly Revised Classification of the Protozoa. *Jour. Protozool.* **27**(1): 37-58.

Speirs and Hill. (1987). Field Verification of On-Line Instrumentation at a Municipal Wastewater Treatment Plant. *Water Sci. Tech.* **19**: 669-680.

Sudo, Inamori, Kuniyasu and Ouchiyama. (1989). Predation and Deodorization of Musty Odor-Producing Filamentous Algae by the Protozoa. *Water Sci. Tech.* **21**(12): 1743-1746.

Sürücü and Çetim. (1990). Effects of Temperature, pH and D.O. Concentration on Settleability of Activated Sludge. *Envir. Tech.(Letters).* **11**(3): 205-212.

Suzuki and Yoon. (1989). Kinetics of Phosphorus Release and Uptake by Micro-Organisms Under Cyclic Anaerobic/Aerobic Conditions - Experimental Study. *Water Sci. Tech.* **21**(12): 1717-1720.

Sydenham. (1971). A Re-Assessment of the Relative Importance of Ciliates, Rhizopods and Rotatorians in the Ecology of Activated Sludge. *Hydrobiologia.* **38**(3/4): 553-563.

Takahashi. (1986). The Operation and Maintenance of Small Scale Wastewater Treatment Plants. *Water Sci. Tech.* **18**: 313-318.

Tate and Eckenfelder. (1986). Application of the Batch Activated Sludge Process. *Proc. 41th Purdue Ind. Waste Conf.*, Purdue University, 209-218.

Tchobanoglous and Burton. (1991). *Wastewater Engineering: Treatment, Disposal and Reuse/Metcalf & Eddy, Inc.* Series: McGraw-Hill Series in Water Resources and Environmental Engineering. 3rd Edition, McGraw-Hill, Inc., New York, New York, USA.

Tellez and Esteban. (1991). The Density of Ciliate Populations in the Prediction of the Activated-Sludge Performance. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 49-52.

Tetreault, Rusten, Benedict and Kreissl. (1987). Assessment of Phased Isolation Ditch Technologies. *Jour. Water Poll. Control Fed.* **59**(9): 833-840.

Thabaraj and Gaudy. (1969). Effect of Dissolved Oxygen Concentration on the Metabolic Response of Completely Mixed Activated Sludge. *Jour. Water Poll. Control Fed.* **41**(8): R322-R334.

Tringham. (1992). Implications of the Environmental Protection Act 1990. *Measurement + Control.* **25**: 41-43.

Turk and Mavinic. (1989). Mantaining Nitrite Build-Up in a System Acclimated to Free Ammonia. *Water Res.* **23**(11): 1383-1388.

Turk and Mavinic. (1989). Stability of Nitrite Build-Up on an Activated Sludge System. *Jour. Water Poll. Control Fed.* **61**(8): 1440-1448.

van der Geest and Witvoet. (1977). Nitrification and Denitrification in Carrousel Systems. *Prog. Water Tech.* **8**(4/5): 653-660.

Verhagen and Laanbroek. (1992). Effects of Grazing by Flagellates on Competition for Ammonium Between Nitrifying and Heterotrophic Bacteria in Chemostats. *Appl. Envir. Microbiol.* **58**(6): 1962-1969.

Vlekke, Comeau and Oldham. (1988). Biological Phosphate Removal from Wastewater with Oxygen or Nitrate in Sequencing Batch Reactors. *Envir. Tech. Letters.* **9**: 791-796.

Volk. (1991). Examples for Plant Operation Improvement by Using Long-Term Microscopic Examinations. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 39-47.

Walters, Engelbrecht and Speece. (1968). Microbial Substrate Storage in Activated Sludge. *Jour. Sanit. Engng. Division, ASCE.* **94**(SA2): 257-269.

Wanner. (1991). Use of the Routine Biological Analyses to Check the Performance of Sewage Treatment Plants. *Proc. International Symposium Biological Approach to Sewage Treatment Process: Current Status and Perspectives,* Perugia, Italy, 199-210.

Wanner and Grau. (1988). Filamentous Bulking in Nutrient Removal Activated Sludge Systems. *Water Sci. Tech.* **20**(4/5): 1-8.

Weber and Matsumoto. (1987). Feasibility of Intermittent Biological Treatment for Hazardous Wastes. *Environmental Progress.* **6**(3): 166-171.

Wilson. (1981). *Design Calculations in Wastewater Treatment.* E. & F. N. Spon, London, UK.

Wilson and Wharfe. (1989). Application of Microscopic Examination of Activated Sludge to Operational Control. *Water Sci. Tech.* **21**(12): 1647-1650.

Wong and Choi. (1989). Pilot Scale Aerobic Sequencing Batch Reactor for Pig Waste Treatment. *Jour. IWEM.* **3**: 75-81.

Woombs and Laybourn-Parry. (1987). Seasonal Species Composition, Density and Role of Nematodes in Activated-Sludge Effluent Treatment Works. *Water Res.* **21**(4): 459-467.

Wun-Jern and Droste. (1989). Sequencing Batch Reactor(SBR) Treatment of Wastewaters. *Environmental Sanitation Reviews.* **28**: 1-55.

Ying, Bonk, Lloyd and Sojka. (1986). Biological Treatment of a Landfill Leachate in Sequencing Batch Reactors. *Environmental Progress.* **5**(1): 41-50.

Ying, Bonk and Sojka. (1987). Treatment of a Landfill Leachate in Powdered Activated Carbon Enhanced Sequencing Batch Bioreactors. *Environmental Progress.* **6**(1): 1-8.

Young. (1986). Treatment of Combined Municipal/Packing House Wastewater Using an Innovative Continuously Fed-Intermittently Operated Activated Sludge Process: A Design Rationale. *Proc. 41th Purdue Ind. Waste Conf.,* Purdue University, 115-123.

Zoetemeyer, van den Heuvel and Cohen. (1982). pH Influence on Acidogenic Dissimilation of Glucose in an Anaerobic Digestor. *Water Res.* **16**: 303-311.

APPENDIX A

NITRIFICATION AND DENITRIFICATION PERFORMANCE PARAMETERS CALCULATION

Efficiencies and specific rates for nitrification and denitrification were calculated based in the following simplified nitrogen mass balance.

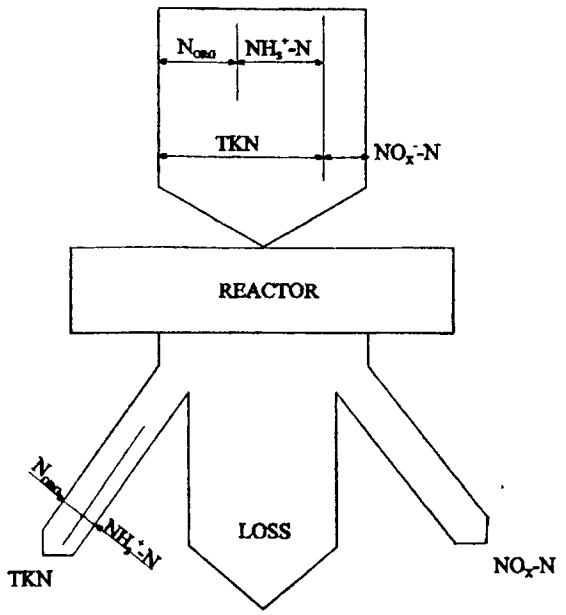


Figure A.1: Nitrogen Mass Balance (Oleszkiewicz and Berquist, 1988) Modified

The nitrification efficiency was calculated in terms of total TKN and organic nitrogen concentrations:

$$N\ (\%) = \frac{(TKN_i - TKN_e)}{(TKN_i - Norg_e)} \times 100 \qquad \text{(A.1)}$$

where, the subscripts i and e refers to influent and effluent respectively.

The specific nitrification rate was calculated on a daily basis as a function of the influent and effluent total TKN, and the total MLVSS mass.

$$K_N\ (g/Kg.day) = \frac{(TKN_i - TKN_e)_{MASS}}{MLVSS_{MASS} \times day} \qquad (A.2)$$

However, this rate should be looked at as per length of aeration time, rather than per day, as nitrification can only proceed during aerated periods.

Hourly maximum nitrification rates were determined from track study data. These rates were calculated as the steepest slope of the oxidized nitrogen concentration time curve during the aeration period, in mg/L.h. The corresponding maximum specific nitrification rates were calculated by dividing the above rate, by the average MLVSS concentration for the period and expressing it as g/kg.day as if this rate prevailed for 24 hours per day.

The development of the expressions for denitrification efficiencies and specific rates was also based on the nitrogen balance presented in figure A.1.

$$DN\ (\%) = \frac{(TKN_i - TKN_e) - [NO_X\text{-}N_e] + [NO_X\text{-}N_i]}{(TKN_i - TKN_e) + [NO_X\text{-}N_i]} \times 100 \qquad (A.3)$$

$$K_{DN}\ (g/Kg.day) = \frac{(TKN_i - TKN_e)_{MASS} - [NO_X\text{-}N_e]_{MASS} + [NO_X\text{-}N_i]_{MASS}}{MLVSS_{MASS} \times day} \qquad (A.4)$$

In the case of denitrification track studies, the hourly maximum denitrification rate was determined in the same fashion as for the nitrification rates. The only difference was that the slope was measured in the anoxic period.

APPENDIX B

PHASE 2: NITRIFICATION

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
MLSS	mg/L	2196.5	359.9	1592.0	2776.0
MLVSS	mg/L	1615.4	246.8	1140.0	2036.0
MLVSS	% MLSS	74.0	1.6	71.6	76.8
SVI	mL/g	125.0	17.2	100.8	185.3
F/M	1/day	0.07	0.01	0.05	0.10
Effl. COD (t)	mg/l	58.6	14.0	28.0	80.0
COD (t) Rem.	%	82.9	7.2	64.1	99.1
Effl. COD (f)	mg/L	43.6	15.4	8.0	66.0
COD (f) Rem.	%	74.4	12.3	45.0	99.2
Effl. SS	mg/L	13.9	5.1	4.0	23.0
SS Rem.	%	86.6	8.7	65.9	99.2
Effl. TKN (t)	mg/L	2.8	1.9	n/d	11.2
TKN (t) Rem.	%	91.3	6.8	78.3	99.9
Effl. TKN (f)	mg/L	1.2	2.4	n/d	11.2
TKN (f) Rem.	%	96.3	6.1	72.2	99.9
Effl. Ammonia-N	mg/L	0.8	2.7	n/d	9.5
Effl. Nitrate-N (f)	mg/L	3.5	4.9	0.3	16.9
Effl. Nitrite-N (f)	mg/L	13.1	5.6	0.1	20.6
Effl. N (f)	mg/L	17.8	2.9	13.9	25.4
N (f) Rem.	%	35.5	10.1	12.7	59.1
Effl. Orthophosphate (f)	mg/L	5.1	1.9	2.0	8.5
Nitrif. Efficiency	%	99.9	-	99.9	99.9
Specif. Nitrif. Rate	g/Kg.day	32.3	8.3	20.1	51.4
Denitrif. Efficiency	%	37.8	12.2	20.0	61.0
Specif. Denitrif. Rate	g/Kg.day	7.3	3.9	2.5	16.8

(t) - total (f) - filtrated n/d - not detected

Table B.1: Reactor Performance Parameters for Phase 2, SBR 1

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Minimum pH	pH units	6.87	0.37	6.07	7.57
Maximum pH	pH units	7.78	0.51	7.05	8.63
pH Range	pH units	0.91	0.36	0.48	1.95
Average pH	pH units	7.32	0.37	6.83	8.01
Last pH	pH units	7.47	0.41	7.01	8.21
Minimum Redox	mV	-240.6	101.7	-383.0	-10.3
Maximum Redox	mV	139.2	53.5	80.3	380.2
Redox Range	mV	379.8	82.3	170.7	477.9
Average Redox	mV	47.8	39.0	-57.0	114.3
Last Redox	mV	105.3	31.7	17.5	139.9
Last DO	mg/L	8.5	0.7	7.6	9.9

Table B.2: pH, Redox and DO Data for Phase 2, SBR 1

Time (Hours)	MLSS (mg/L)	MLVSS (mg/L)	COD (mg/L)	TKN (mg/L)	NH3-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	PO4-P (mg/L)
01:30	2264.0	1744.0	140.0	19.6	16.8	0.2	n/d	8.7
02:00	2072.0	1628.0	124.0	20.7	17.4	0.2	n/d	14.4
02:15	2048.0	1612.0	84.0	-	-	-	-	-
02:30	2080.0	1624.0	52.0	-	-	-	-	-
03:00	2068.0	1504.0	52.0	9.5	9.5	2.2	9.1	11.1
03:30	2056.0	1588.0	76.0	-	-	-	-	-
04:00	2064.0	1600.0	76.0	1.1	0.6	5.5	13.5	9.1
04:30	2096.0	1572.0	64.0	-	-	-	-	-
04:56	2072.0	1608.0	68.0	1.8	1.0	8.6	10.6	7.6
05:30	2140.0	1652.0	64.0	-	-	-	-	-
06:00	2080.0	1500.0	64.0	1.8	0.6	11.4	6.6	6.3
06:30	2072.0	1584.0	64.0	-	-	-	-	-
07:00	2024.0	1560.0	-	0.8	0.5	13.6	3.3	5.2
07:30	2016.0	1504.0	40.0	-	-	-	-	-
08:00	2048.0	1572.0	44.0	n/d	n/d	15.0	0.1	5.3

n/d - not detected

Table B.3: Track Study Data for Phase 2, SBR 1

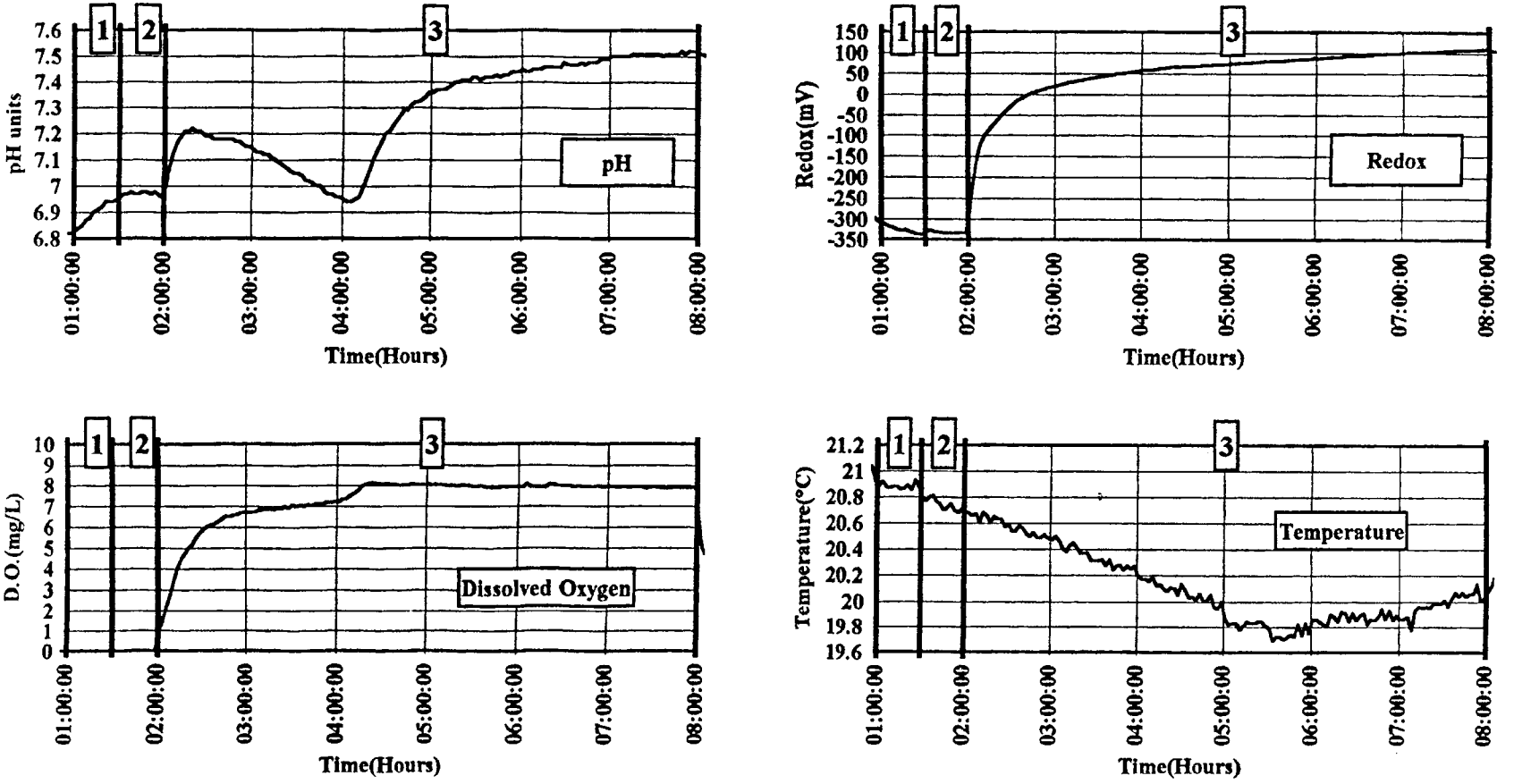


Figure B.1: Simultaneous ph, Redox, DO and Temperature Measurements for Track Study, Phase 2, SBR 1

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
MLSS	mg/L	2578.6	89.1	2412.0	2734.0
MLVSS	mg/L	1835.0	59.7	1728.0	1930.0
MLVSS	% MLSS	71.2	1.2	68.3	73.7
SVI	mL/g	121.0	12.4	102.5	155.5
F/M	1/day	0.06	0.02	0.03	0.09
Effl. COD (t)	mg/l	55.4	13.5	32.0	88.0
COD (t) Rem.	%	84.2	5.5	73.9	99.1
Effl. COD (f)	mg/L	43.0	12.6	20.0	72.0
COD (f) Rem.	%	74.5	10.9	51.9	99.2
Effl. SS	mg/L	9.3	3.9	4.0	17.0
SS Rem.	%	91.5	4.6	81.3	99.9
Effl. TKN (t)	mg/L	2.3	2.8	n/d	13.4
TKN (t) Rem.	%	93.3	7.0	85.0	99.9
Effl. TKN (f)	mg/L	1.8	2.9	n/d	12.9
TKN (f) Rem.	%	95.4	6.9	68.1	99.9
Effl. Ammonia-N	mg/L	0.8	2.9	n/d	12.9
Effl. Nitrate-N (f)	mg/L	3.6	4.4	0.2	14.4
Effl. Nitrite-N (f)	mg/L	13.7	5.3	0.1	21.3
Effl. N (f)	mg/L	18.6	4.0	13.7	33.1
N (f) Rem.	%	32.0	15.2	2.0	60.8
Effl. Orthophosphate (f)	mg/L	5.7	2.0	2.5	8.8
Nitrif. Efficiency	%	99.9	-	99.9	99.9
Specif. Nitrif. Rate	g/Kg.day	30.2	9.0	13.6	44.7
Denitrif. Efficiency	%	39.9	13.6	19.4	66.4
Specif. Denitrif. Rate	g/Kg.day	7.7	3.9	2.2	14.1

(t) - total (f) - filtrated n/d - not detected

Table B.4: Reactor Performance Parameters for Phase 2, SBR 2

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Minimum pH	pH units	6.92	0.54	5.93	7.83
Maximum pH	pH units	7.89	0.59	6.93	8.71
pH Range	pH units	0.97	0.28	0.58	1.43
Average pH	pH units	7.39	0.51	6.77	8.31
Last pH	pH units	7.52	0.55	6.91	8.51
Minimum Redox	mV	-266.9	100.4	-392.1	-12.9
Maximum Redox	mV	166.8	52.3	77.1	312.2
Redox Range	mV	433.7	80.2	207.5	611.7
Average Redox	mV	57.9	60.8	-63.2	146.3
Last Redox	mV	126.5	62.5	-0.8	205.5
Last DO	mg/L	8.2	0.8	6.4	10.2

Table B.5: pH, Redox and DO Data for Phase 2, SBR 2

Time (Hours)	MLSS (mg/L)	MLVSS (mg/L)	COD (mg/L)	TKN (mg/L)	NH3-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	PO4-P (mg/L)
01:00	7540.0	5512.0	32.0	n/d	n/d	-	-	-
01:30	2696.0	2020.0	88.0	16.8	16.8	0.1	0.1	8.5
02:00	2540.0	1900.0	88.0	20.9	17.4	0.1	n/d	15.4
02:15	2554.1	1912.0	60.0	-	-	-	-	-
02:30	-	-	48.0	-	-	-	-	-
03:00	2564.0	1920.0	44.0	11.1	10.6	2.0	7.9	11.1
03:30	2540.0	1900.0	48.0	-	-	-	-	-
04:00	2560.0	1908.0	49.7	1.1	0.6	5.0	13.8	8.6
04:30	2576.0	1912.0	48.5	-	-	-	-	-
05:00	2528.0	1873.8	52.0	1.5	0.7	7.7	11.4	6.8
05:30	2616.0	1935.7	52.9	-	-	-	-	-
06:00	2568.0	1892.8	52.0	1.3	0.8	10.4	8.1	5.1
06:30	2572.0	1892.0	52.9	-	-	-	-	-
07:00	2564.0	1886.2	60.0	n/d	n/d	12.8	4.9	4.2
07:30	2520.0	1853.2	55.3	-	-	-	-	-
08:00	2588.0	1903.6	57.1	0.7	n/d	14.5	0.1	3.6

n/d - not detected

Table B.6: Track Study Data for Phase 2, SBR 2

APPENDIX C

PHASE 3: NITRIFICATION/DENITRIFICATION

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
MLSS	mg/L	2487.8	194.8	1858.0	2760.0
MLVSS	mg/L	1842.0	153.1	1414.0	2096.0
MLVSS	% MLSS	74.1	2.2	69.5	77.7
SVI	mL/g	103.0	25.6	54.1	152.5
F/M	1/day	0.16	0.03	0.11	0.21
Effl. COD (t)	mg/l	82.1	22.9	34.6	125.9
COD (t) Rem.	%	81.1	6.0	61.4	91.2
Effl. COD (f)	mg/L	48.8	12.7	23.1	85.8
COD (f) Rem.	%	73.9	10.6	37.9	87.8
Effl. SS	mg/L	30.8	11.5	13.0	61.5
SS Rem.	%	81.1	6.3	64.2	90.3
Effl. TKN (t)	mg/L	8.5	7.8	n/d	30.2
TKN (t) Rem.	%	80.1	18.2	43.4	99.9
Effl. TKN (f)	mg/L	6.3	7.1	n/d	24.9
TKN (f) Rem.	%	90.4	14.9	43.4	99.9
Effl. Ammonia-N	mg/L	6.2	6.6	n/d	23.1
Effl. Nitrate-N (f)	mg/L	1.6	2.5	0.1	9.1
Effl. Nitrite-N (f)	mg/L	9.8	8.0	n/d	26.0
Effl. N (f)	mg/L	17.3	8.4	0.2	35.1
N (f) Rem.	%	65.4	20.5	16.1	99.0
Effl. Alkalinity (t)	mg/L as CaCO3	119.6	42.6	75.7	194.8
Specif. Net Alkal. Uptake	mg Alkal./mg N rem.	2.9	1.3	0.9	5.5
Effl. Orthophosphate (f)	mg/L	7.8	2.3	4.5	12.8
Specif. Nitrif. Rate	g/Kg.day	23.9	8.4	7.6	40.9
Denitrif. Efficiency	%	73.3	25.1	16.5	99.9
Specif. Denitrif. Rate	g/Kg.day	17.7	8.0	2.5	31.1

(t) - total (f) - filtrated n/d - not detected

Table C.1: Reactor Performance Parameters for Phase 3

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Minimum pH	pH units	6.69	0.37	5.97	7.45
Maximum pH	pH units	7.60	0.34	7.06	8.21
pH Range	pH units	0.94	0.33	0.45	2.66
Average pH	pH units	7.14	0.36	6.52	7.84
Last pH	pH units	7.28	0.30	6.68	7.86
Minimum Redox	mV	-213.5	144.2	-450.0	32.8
Maximum Redox	mV	82.5	42.3	-24.2	184.0
Redox Range	mV	292.4	127.0	99.6	612.9
Average Redox	mV	-54.8	105.5	-284.7	89.9
Last Redox	mV	18.7	40.1	-71.9	84.4
Last DO	mg/L	8.2	1.1	6.2	10.4

Table C.2: pH, Redox and DO Data for Phase 3

Time (Hours)	MLSS (mg/L)	MLVSS (mg/L)	COD (mg/L)	TKN (mg/L)	NH3-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	PO4-P (mg/L)	Total Alkalinity (as CaCO3)
05:00	7830.0	5951.0	71.4	11.5	10.2	n/d	0.1	7.8	144.4
08:00	4396.0	3357.0	134.1	30.2	21.6	n/d	0.1	7.5	179.1
09:30	3510.0	2667.0	75.0	21.0	19.0	3.5	9.8	7.4	111.0
10:30	3160.0	2404.0	81.0	20.9	20.1	n/d	0.3	7.6	167.9
11:30	2798.0	2128.0	74.0	16.2	16.0	2.7	7.2	7.4	121.7
12:15	-	-	-	16.5	15.7	n/d	n/d	7.9	161.8
13:00	2415.0	1853.0	74.0	16.5	15.6	n/d	n/d	8.1	159.6
14:00	2415.0	1855.0	55.0	9.6	9.3	1.1	5.2	7.9	126.6
15:00	2408.0	1838.0	50.0	8.0	8.0	n/d	3.8	7.8	145.2
16:00	2402.0	1833.0	51.8	8.1	8.1	n/d	n/d	7.9	157.2
17:00	2406.0	1830.0	50.8	8.5	8.5	n/d	n/d	9.2	153.6
18:00	2406.0	1829.0	48.0	8.9	8.7	n/d	n/d	9.6	150.0
19:00	2404.0	1815.0	52.0	9.7	9.6	n/d	n/d	10.5	148.2
20:00	2398.0	1806.0	56.0	10.6	10.4	n/d	n/d	11.2	146.0
20:45	2400.0	1807.0	70.0	11.4	11.3	n/d	n/d	11.7	140.4
21:00	2389.0	1810.0	62.0	11.3	10.7	n/d	0.2	11.5	141.6

n/d - not detected

Table C.3: Track Study Data for Phase 3

Cycle	Stalked Ciliates	Free Swimming Ciliates	Crawling Ciliates	Flagellates	Rotifers	Nematodes
2	30	0	30	30	0	0
6	5	0	0	30	0	0
7	5	0	0	30	0	0
9	5	0	0	100	0	0
10	0	0	0	100	0	0
13	5	5	0	100	0	0
14	5	0	0	100	0	0
16	0	0	0	100	0	0
18	5	0	0	100	0	5
24	5	0	10	100	0	0
27	5	0	10	100	0	0
28	10	0	5	100	0	0
30	10	5	10	100	0	5
31	5	10	10	10	0	0
35	5	30	5	10	0	0
37	10	30	30	10	0	0
38	5	30	30	0	5	0
45	5	5	0	10	10	5
49	0	0	0	100	0	0
51	10	0	0	30	10	10
52	0	5	0	100	10	0
55	5	0	0	100	5	0
56	5	0	0	10	10	5
58	10	0	5	10	10	10
63	5	0	0	10	30	0
65	5	0	0	30	30	0
66	5	0	0	100	100	0
69	5	0	0	100	100	0
73	5	0	0	100	30	0
74	0	0	0	100	5	5
77	0	0	5	100	30	10
81	0	0	0	100	30	10
85	0	0	0	100	30	5
90	0	0	0	100	100	10
91	0	0	0	100	100	0
92	0	0	0	30	100	0
93	5	0	0	100	100	0

Table C.4: Relative Distribution of Protozoan and Metazoan Functional Groups, for Phase 3

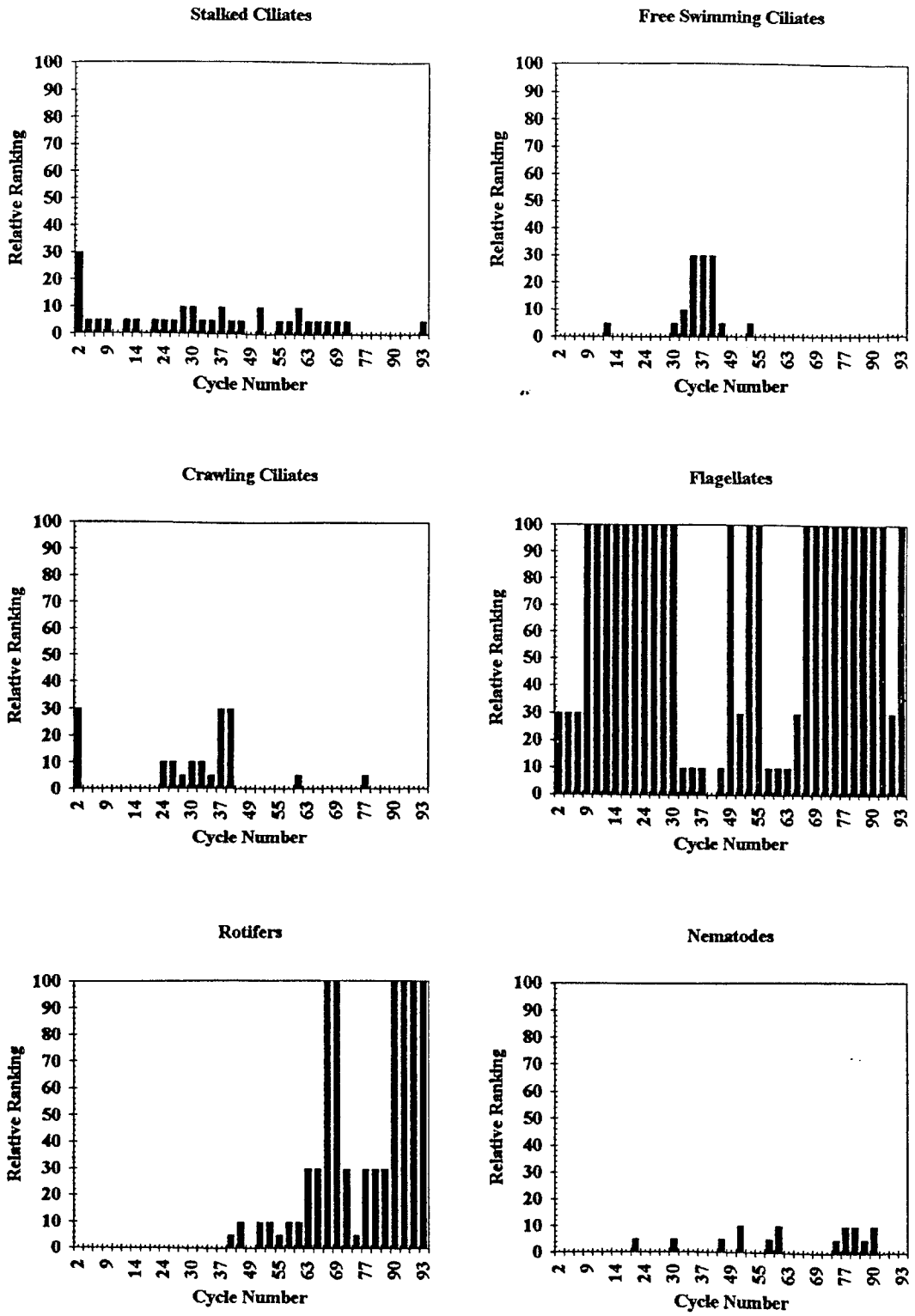


Figure C.1: Functional Groups Relative Scores for Phase 3

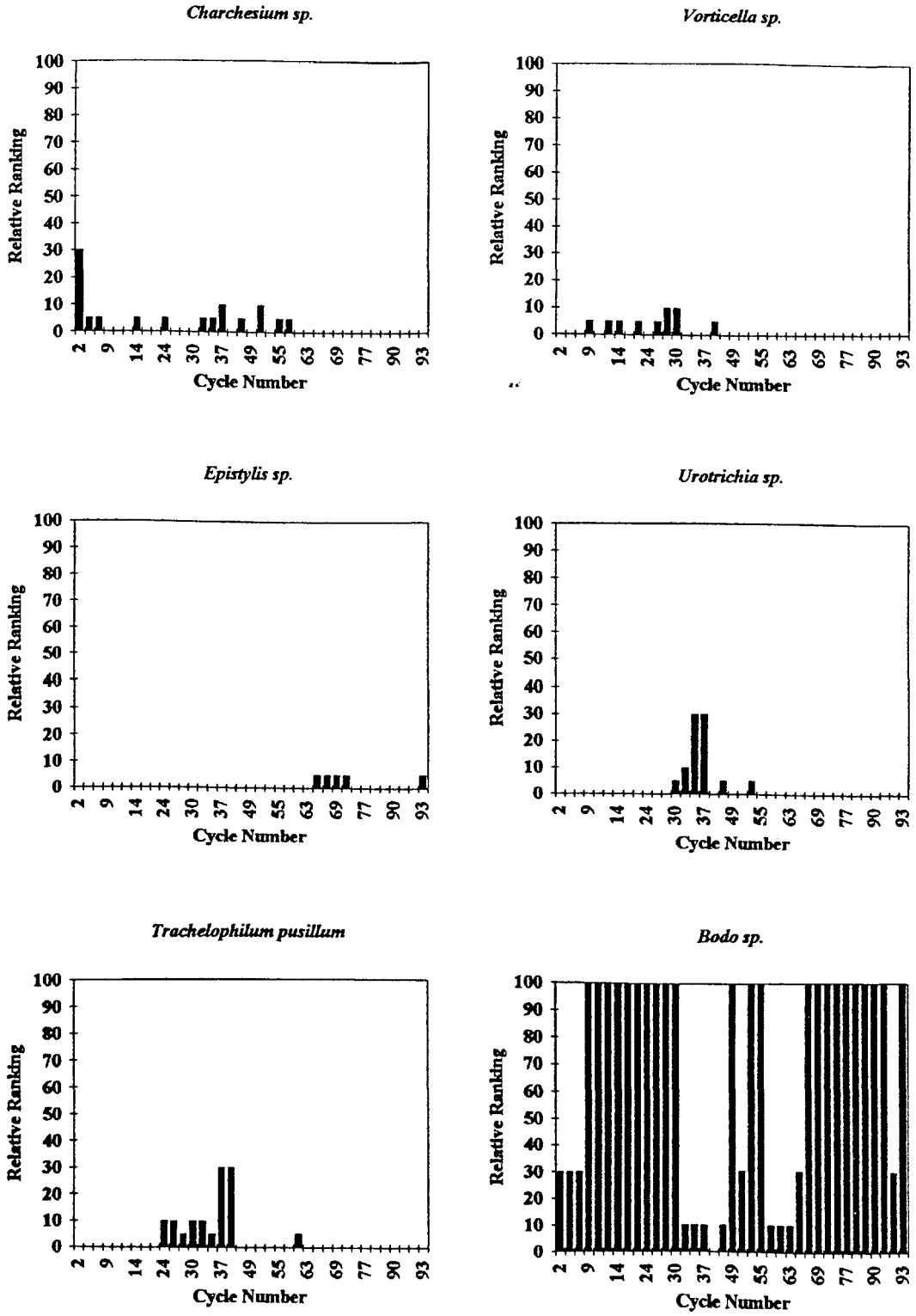


Figure C.2: Most Common Protozoa Species Observed for Phase 3

APPENDIX D

PHASE 4: NITRIFICATION/EBP REMOVAL

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
MLSS	mg/L	2959.7	492.2	2274.0	4286.0
MLVSS	mg/L	2368.2	373.5	1900.0	3398.0
MLVSS	% MLSS	80.2	2.8	73.7	86.1
SVI	mL/g	109.1	34.1	59.1	206.2
F/M	1/day	0.13	0.02	0.08	0.19
Effl. COD (t)	mg/l	71.3	26.4	27.6	158.2
COD (t) Rem.	%	83.1	7.3	54.0	93.6
Effl. COD (f)	mg/L	57.3	19.5	15.7	102.3
COD (f) Rem.	%	75.3	11.2	38.1	93.6
Effl. SS	mg/L	15.8	8.1	5.0	54.5
SS Rem.	%	89.8	6.0	59.2	95.8
Effl. TKN (t)	mg/L	2.1	3.3	n/d	17.1
TKN (t) Rem.	%	96.0	6.6	65.7	99.9
Effl. TKN (f)	mg/L	1.6	3.2	n/d	17.1
TKN (f) Rem.	%	95.8	8.4	50.8	99.9
Effl. Ammonia-N	mg/L	1.5	3.2	n/d	17.1
Effl. Nitrate-N (f)	mg/L	15.2	14.2	0.3	44.6
Effl. Nitrite-N (f)	mg/L	17.3	10.0	0.5	33.9
Effl. N (f)	mg/L	33.2	8.4	16.5	50.7
N (f) Rem.	%	29.7	14.0	2.2	50.5
Effl. Alkalinity (t)	mg/L as CaCO3	52.0	32.0	7.1	109.3
Specif. Alkal. Uptake	mg Alkal./mg TKN rem.	5.3	1.2	3.0	7.2
Effl. Orthophosphate (f)	mg/L	7.3	2.4	3.3	13.4
Nitrif. Efficiency	%	97.6	5.0	71.5	99.9
Specif. Nitrif. Rate	g/Kg.day	40.2	12.0	15.2	61.0
Denitrif. Efficiency	%	30.3	15.6	1.1	54.1
Specif. Denitrif. Rate	g/Kg.day	6.7	4.3	0.1	15.1

(t) - total (f) - filtrated n/d - not detected

Table D.1: Reactor Performance Parameters for Phase 4

Parameter	Units	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Minimum pH	pH units	6.09	0.58	5.14	7.00
Maximum pH	pH units	7.60	0.56	6.96	8.85
pH Range	pH units	1.51	0.45	0.32	2.04
Average pH	pH units	6.75	0.56	5.61	7.59
Last pH	pH units	6.41	0.79	5.25	7.37
Minimum Redox	mV	-227.9	62.6	-353.9	-91.8
Maximum Redox	mV	253.5	121.5	88.7	471.1
Redox Range	mV	481.3	86.7	294.6	641.4
Average Redox	mV	71.5	70.6	-36.8	241.2
Last Redox	mV	231.5	127.7	75.8	465.2
Last DO	mg/L	7.0	0.8	4.1	10.1

Table D.2: pH, Redox and DO Data for Phase 4

Time (Hours)	MLSS (mg/L)	MLVSS (mg/L)	COD (mg/L)	TKN (mg/L)	NH3-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	PO4-P (mg/L)	Total Alkalinity (as CaCO3)
12:00	12849.4	10155.7	47.0	1.1	n/d	5.7	2.2	7.2	91.2
13:31	6268.0	4954.0	71.4	27.2	21.8	5.7	n/d	3.6	230.9
14:00	5338.0	4218.0	79.0	30.8	25.8	5.7	n/d	3.2	257.5
14:31	4596.0	3630.8	86.0	34.6	29.7	n/d	n/d	3.1	266.0
15:00	4128.0	3268.0	97.7	36.4	31.6	n/d	n/d	3.0	277.4
15:30	4126.0	3286.0	93.7	37.0	32.5	n/d	n/d	3.1	275.0
15:59	4162.0	3312.0	90.9	37.0	32.5	n/d	n/d	3.1	277.4
17:00	4028.0	3192.0	57.4	20.4	19.3	4.3	13.0	4.2	205.2
18:00	4066.0	3222.0	56.4	3.9	3.4	14.8	22.0	4.8	95.0
19:00	4048.0	3202.0	48.9	1.7	n/d	24.3	15.3	4.6	60.8
20:00	3994.0	3125.7	41.4	1.4	n/d	34.1	7.0	4.7	58.9
20:59	3986.0	3098.0	39.5	1.7	n/d	40.5	1.3	4.8	55.1

n/d - not detected

Table D.3: Track Study Data for Phase 4

Cycle	Stalked Ciliates	Free Swimming Ciliates	Crawling Ciliates	Flagellates	Rotifers	Nematodes
3	30	5	30	0	0	0
7	30	0	30	0	0	5
15	30	30	30	0	0	0
20	5	5	30	0	0	0
27	30	30	30	0	0	0
28	30	30	30	0	0	0
29	100	10	30	10	0	0
33	30	10	100	5	0	0
37	30	10	30	5	0	0
42	10	0	100	5	0	5
49	10	5	0	100	0	0
55	0	0	0	100	0	0
57	10	0	0	100	0	5
61	5	5	0	100	0	5
63	10	0	0	100	0	0
71	0	30	0	100	0	0
75	0	30	0	100	0	5
77	10	5	0	100	0	0
85	10	5	0	100	5	5
87	10	0	0	100	10	10
88	10	0	0	100	10	10
90	100	0	0	100	5	0
98	100	5	0	10	0	0
99	100	10	10	100	10	10
103	100	0	0	10	5	0
104	10	100	0	10	10	0
105	30	100	0	0	10	0
111	0	0	0	10	5	0
112	5	10	5	10	10	5
113	10	5	0	10	0	0
117	100	0	10	10	10	5
127	100	0	30	0	5	5
131	100	0	10	0	10	0
133	100	5	5	0	30	0
139	100	0	0	0	30	0
147	30	0	0	10	30	0
149	30	0	0	0	10	0
155	100	5	0	0	10	10
163	30	10	0	10	10	0
171	100	5	0	5	10	5
181	100	0	0	0	30	5
183	100	0	0	0	30	0
185	100	0	0	0	30	5
187	100	0	0	0	30	0

Table D.4: Relative Distribution of Protozoan and Metazoan Functional Groups, for Phase 4

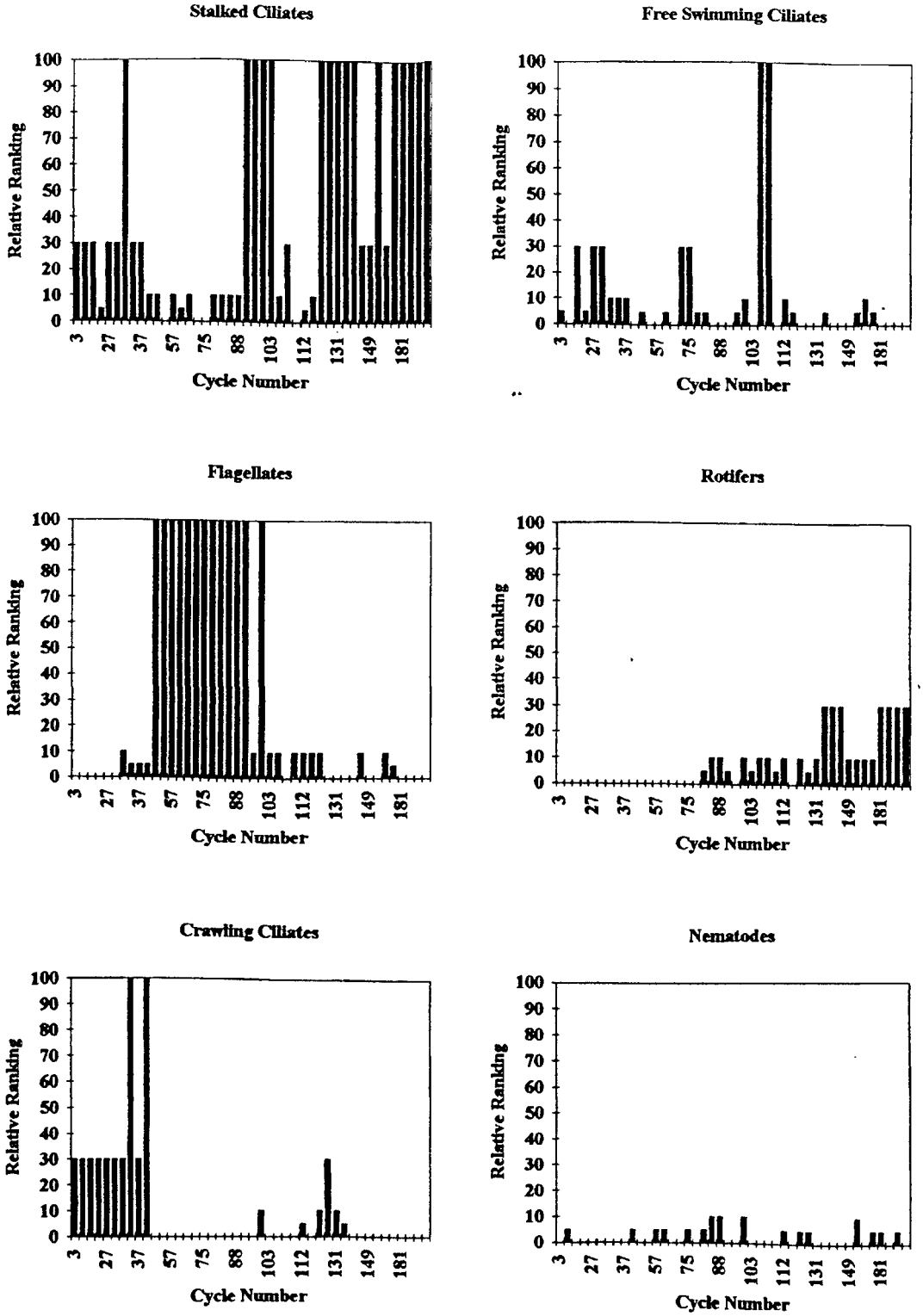


Figure D.1: Functional Groups Relative Scores for Phase 4

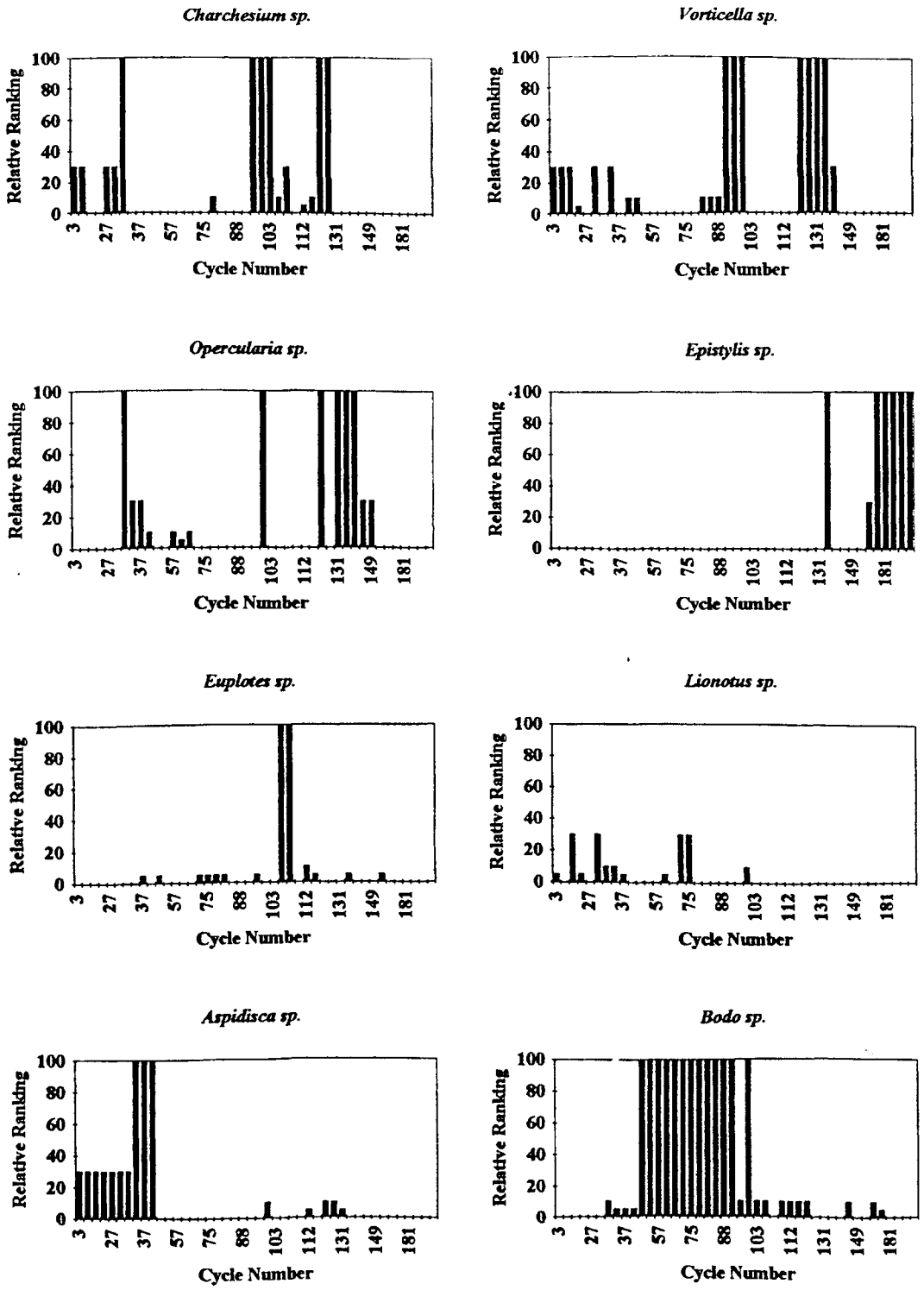


Figure D.2: Most Common Protozoa Species Observed for Phase 4