Investigation of Hypothesized Anaerobic Stabilization Mechanisms in Biological Phosphorus Removal Systems

by

Milind Vishnu Wable

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Civil Engineering

APPROVED:

Clifford W. Randall, Chairman

John T. Novak

William R. Knocke

Robert E. Benoit

James G. Ferry

February, 1992

Blacksburg, Virginia

Investigation of Hypothesized Anaerobic Stabilization Mechanisms in Biological Phosphorus Removal Systems

by

Milind Vishnu Wable
Committee Chairman: Clifford W. Randall
Civil Engineering

(ABSTRACT)

"Anaerobic Stabilization" (AnS) is a phenomenon previously observed in biological nutrient removal (BNR) systems that use anaerobic-aerobic sequencing for phosphorus and/or nitrogen removal. AnS manifests itself in the form of less-than-theoretical oxygen requirements for the extent of organics stabilization observed. The objectives of this study were to develop an improved methodology for the quantitative determination of AnS, verify the occurrence and validate the statistical significance of AnS, identify components of the AnS-related redox balance, and investigate possible explanations for AnS.

A lab-scale continuous-flow A/O[1] system was operated with chemical inhibition of nitrification at a 12-hour nominal HRT, 10-day BSRT (Biological Solids Retention Time), 1Q RAS flow, and varying synthetic feed compositions. Data from this system were used to demonstrate that, by eliminating the need to quantify the clarifier OUR, the Boundary Exchange AnS determination method developed in this study afforded a major advantage over earlier methods. Non-zero AnS was shown to be a statistically significant, reproducible

[1]A/O and A2/O are trademarks of Air Products and Chemicals, Inc., Allentown, PA, U.S.A.

phenomenon. Carbon, oxygen, and sulfur were identified as the three main elements affecting AnS in the A/O system studied.

A second lab-scale A2/O system operating at a 6-hour nominal HRT, 5-day BSRT, 1Q RAS flow, and 2Q RNX flow, and receiving raw municipal wastewater feed spiked with acetate, was used in conjunction with the A/O system to study possible AnS explanations. A combination of processes accounted for varying percentages of observed AnS. Hydrogen production explained 0.1 percent or less, while methane production explained almost 19 percent with formate in the feed but no more than 0.8 percent without it. Aeration-induced stripping of reduced volatiles explained up to 6 percent. Attempts to identify the reduced volatiles revealed traces of ethanol but no n-butanol in the A2/O system. Limitations of the COD test were identified as a possible explanation for AnS that warrants further investigation.

A unified speculative biochemical model consistent with all results of this study and with established theory, and capable of partially explaining observed AnS, is proposed in this study.

ABSTRACT

Acknowledgements

Indispensable contributions to this work were made by several individuals. I consider it an honor, a privilege, and my extreme good fortune that I had Dr. Clifford W. Randall as my major advisor and chair of my advisory committee. He has, however, been much more to me during my program here. Not once can I remember being turned down when I approached him for help. He went out of his way to make sure that I always had what I needed, sharing his personal resources at times. The patient understanding, support, and encouragement he provided will always be remembered and appreciated.

I would also like to thank Drs. John T. Novak, William R. Knocke, Robert E. Benoit, and James G. Ferry for serving on my advisory committee. Dr. Novak made many useful suggestions, especially for the stripping experiments, and took a personal interest in helping with the ethanol/butanol analyses. The thorough scrutiny and constructive criticism provided by Dr. Knocke significantly improved the quality of this document, especially with respect to methods validation. The comments and suggestions made by Dr. Benoit and Dr. Ferry were useful in providing a different perspective on and alternate solution approaches to some of the

issues in this research. Dr. Ferry also helped with information on quantification of dissolved gases. The help and time provided by all members of the committee is much appreciated.

Thanks are due to Drs. Joseph H. Sherrard and Richard G. Krutchkoff for serving as members of the advisory committee during the early part of my program, and to Dr. Daniel Gallagher for his help with statistical simulation.

Dr. Vikram M. Pattarkine, Dr. Samuel A. McClintock, and Donald C. Marickovich were all of great help during the course of this research. All of them made it possible for me to take breaks from running the A/O system. They also provided seed biomass for the A/O system from their pilot-scale unit. Vikram analyzed several total phosphorus samples for me, and Vikram and Don were always ready to help with just about anything.

Rodney Chapin worked hard hauling large quantities of wastewater daily to run the A2/O system and manage its routine maintenance during the data collection period reported here. His help and cooperation were invaluable.

Julie Petruska and Marilyn Grender both provided indispensable help in the lab in countless ways, including set-up of the experimental system, sampling, and sample analysis.

Some useful suggestions concerning the stripping experiments came from Trina Mastran.

Dave Parker of HRSD's Lambert's Point Plant and several of his staff were helpful in providing wastewater and seed biomass.

Thanks are due to Pat Furey for efficiently handling administrative matters and paperwork.

Financial support during a part of this research was provided by George H. Yowell through the Virginia Environmental Endowment. The generous support is greatly appreciated.

I would like to thank my parents and family for their understanding and support of my educational and career plans.

And thanks also to all my peers and friends, in and outside Environmental Engineering, who turned the rigors of a doctoral program into a rewarding and enjoyable experience.

Table of Contents

List of Figures

List of Tables

Glossary of Terms

Acronyms

αKG	α-Keto Glutarate
A/O	Anaerobic/Oxic
A2/O	Anaerobic-Anoxic/Oxic
Ac	Acetic/Acetate
ADP	Adenosine Di-Phosphate
Aer	Aerobic
AMMS	Anion Micro Membrane Suppressor
Ana	Anaerobic
AnS	Anaerobic Stabilization
Anx	Anoxic
APHA	American Public Health Association
ATP	Adenosine Tri-Phosphate
AWWA	American Water Works Association
bio-P	(Used interchangeably with) poly-P
BNR	Biological Nitrogen Removal
BOD	Biochemical Oxygen Demand
BP	Bacto-Peptone[1]
BPR	Biological Phosphorus Removal
BSRT	Biological Solids Retention Time (sludge age)
Bu	Butanol
CoA	Co-Enzyme A
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DW	Distilled Water
EBPR	Enhanced Biological Phosphorus Removal
eeq	Electron EQuivalents
Eff	Effluent
Et	Ethanol
Fd_{ox}	Ferredoxin (oxidized)
Fd_{red}	Ferredoxin (reduced)
FID	Flame Ionization Detector
Fm	Formic/Formate
FOP	Filtrable Ortho-Phosphate
GC	Gas Chromatograph(y)

[1]Difco Laboratories, Detroit, MI, U.S.A.

HRT	Hydraulic Retention Time
Inf	Influent
iV	iso-Valeric/iso-Valerate
Lct	Lactic/Lactate
meq	milli electron equivalents
mgd	million gallons per day
ML	Mixed Liquor
MLSS	Mixed Liquor Suspended Solids
N	Nitrogen
NA	Not Available/Applicable
NAD	Nicotinamide Adenine Dinucleotide (oxidized)
NADH	Nicotinamide Adenine Dinucleotide (reduced)
N_{ox}	Oxidized Nitrogen
OAA	Oxalo Acetic Acid
OUR	Oxygen Uptake/Utilization Rate
P	Phosphorus
PHB	Poly Hydroxy-Butyrate
poly-P	Polyphosphate
Pr	Propionic/Propionate
PVC	Poly Vinyl Chloride
Pyr	Pyruvate
RAS	Return/Recycle Activated Sludge
RNX	Oxidized Nitrogen Recycle (aerobic mixed liquor to anoxic reactor)
rpm	rotations per minute
S	Substrate
S_{bs}	Readily Biodegradable Substrate
SOUR	Specific Oxygen Uptake/Utilization Rate
TCA	Tri Carboxylic Acid
TCD	Thermal Conductivity Detector
TKN	Total Kjeldahl Nitrogen
TSt	Total Stabilization
UCT	University of Cape Town
VFA	Volatile Fatty Acid
VIP	Virginia Initiative Plant
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge
WEF	Water Environment Federation (formerly WPCF)
WPCF	Water Pollution Control Federation (renamed WEF in October, 1991)

Prefixes

F-	Filtrable
n-	normal (isomer)
T-	Total

Greek Alphabetical Symbols

Δox	Oxidizing Capacity Deviation Vector
Δox_{el}	Δox of an Element
Δox_{sp}	Δox of a Species

1 Introduction

The presence of excessive amounts of nutrients (phosphorus and nitrogen) in domestic, agricultural, and industrial wastewaters is one of the major causes of eutrophication in receiving waters (streams, lakes, estuaries). Eutrophication is a serious concern for many of the world's fresh and estuarine water supplies. Once a water-body reaches the eutrophic stage, clean-up and restoration are difficult and expensive. In some cases, reclamation may not be possible at all, and the water source may be permanently lost. Nutrient control (i.e., prevention of excessive nutrient loadings to receiving waters) is thus an extremely attractive alternative. While non-point nutrient sources (such as erosion, agricultural and urban runoff, and acid rain) need elaborate controls closer to the origin, point sources (such as domestic and industrial wastewaters and landfill leachates) are easier to treat. Biological nutrient removal (BNR) systems are a relatively recent development in this area, and offer distinct economic advantages over conventional physico-chemical processes. This study confines itself to Biological Phosphorus Removal (BPR) systems.

1.1 Anaerobic Stabilization Defined

BPR systems require anaerobic-aerobic reactor sequencing, which is essential for achieving the process objective of excess biological phosphorus uptake. The anaerobic zone is crucial to the successful operation of this process, and it is the site of complex interactions between at least two different categories of bacterial populations. The introduction of an anaerobic zone was recently reported to result in less-than-theoretical oxygen requirements for the extent of stabilization observed, even after accounting for other redox processes (nitrification, denitrification, and sulfate utilization/production). The measured oxygen use in the system left a fraction of the measured stabilization unaccounted for. Because this was the result of introducing an anaerobic zone, earlier researchers (Brannan, 1986; Randall *et al.*, 1987) termed this fraction Anaerobic Stabilization (AnS). This study investigated the phenomenon of AnS.

The issues raised by AnS are very fundamental. Reduction and oxidation are coupled, complementary processes. When a species is oxidized, an equivalent amount of another species must be reduced. AnS may be described to be the result of the fact that the identities of all reactants and all products involved in the redox reaction are not known. A logical first step towards understanding AnS, then, was to identify all species involved, without initially addressing details of the biochemical pathway(s). The terminal electron acceptor, or the species reduced to complement organics oxidation, and the product of such reduction were the key unknowns.

1.2 Engineering Applications

AnS reduces the oxygen requirements of wastewater treatment. Systems operating with AnS can provide organic removal treatment equivalent to that of conventional processes, with a significant reduction in required oxygen. This directly translates into savings in aeration power costs. A better understanding of and control over AnS is necessary before such savings can be fully exploited.

A less obvious application of AnS research concerns its significance to BPR itself. AnS and BPR (or, more precisely, anaerobic phosphorus release) are believed to be closely related, and AnS research could provide a better understanding of both. Also, the combination of AnS-related economy and fuller comprehension of BPR could render BNR processes more readily acceptable to decision-makers, who have so far held a skeptical view of them.

Finally, better understanding of AnS could lead to its application to systems other than BNR systems, i.e., it may become possible to induce AnS in environments where it would not naturally occur, with significant advantages.

1.3 Quantitative Determination of AnS

Quantitative determination of AnS is an involved, data-intensive process for several reasons. Although AnS apparently originates in the anaerobic zone, the site where AnS eventually manifests itself is not known. Also, a localized redox balance around the anaerobic zone presents practical operational problems. This necessitates a system-wide balance, which can

be accomplished in various ways. Methods for AnS determination are described in a following chapter. It is important to understand the assumptions made by each method, and recognize its limitations.

1.4 Possible Explanations for AnS

AnS has so far rested on a highly speculative base, unsupported by theory, and commonly dismissed as an artefact. Little effort has been made to address this matter, particularly the possible role played by the gas phase and its potential to provide answers to some very fundamental questions. Analysis of the gaseous phase in equilibrium with the anaerobic mixed liquor and/or the gases stripped in the aerobic reactor could result in a better understanding of AnS. Table 1 lists the range of possible explanations for AnS. Unless unknown species are involved in the redox chemistry underlying AnS, any observed AnS should be traceable either to mixed liquor interactions with the gas phase or to the COD test.

1.4.1 Evolution of Reduced Volatile Substances

One way in which reduced gases could leave the system is through anaerobic respiration. However, most known anaerobic respirers such as methanogens and sulfate reducers (denitrifiers are the exception) are obligate anaerobes. Most of the bacteria with the less common anaerobic respiration pathways such as the fumarate reductase system are also obligate anaerobes. Molecular oxygen is actually fatal to such bacteria. Although the ecology of the floc can provide an *internal* anaerobic environment where obligate anaerobes can grow even under bulk aerobic conditions in a BPR system, the population so supported is not likely

Table 1. Possible Explanations for AnS

1. A reduced[1] volatile substance (such as molecular hydrogen, acetate, or ethanol) is lost to the gas phase. Theoretically, this could occur in any part of the system, not necessarily the anaerobic reactor. Also, the substance could be organic or inorganic. Thus, possible loss pathways are:
   a. Reduced gas production in the anaerobic reactor.
   b. Stripping of reduced volatiles in the aerobic reactor.
2. An additional external oxidant (terminal electron acceptor) enters the system as a dissolved gas. In the aerobic zone, the oxidant would have to be a species other than oxygen, which is accounted for in AnS calculations. These calculations are described in a later chapter. Examples of such oxidants are nitrogen and carbon dioxide, which participate in N- and C-fixation respectively.
3. Limitations of the COD (Chemical Oxygen Demand) test used in AnS calculations:
   a. A fraction of the incoming COD is converted to a reduced species with an oxidation potential low enough to effectively resist oxidation by the dichromate oxidant under the COD test conditions. Examples of such species are pyridine and related compounds (APHA, 1989), and possibly NAD/NADH.
   b. A fraction of the incoming COD is converted to a reduced species with an oxidation potential high enough to make oxidation in the COD test thermodynamically possible, but not high enough to ensure completion of the oxidation reaction in the 2-hour COD test period. This causes underestimation of the COD leaving the system.

[1]The terms "reduced" and "oxidized" are precisely defined and explained in detail in a following chapter.

to form a significant fraction of the overall biomass. Since AnS has been reported in the absence of any significant attached anaerobic growth, anaerobic respiration cannot be wholly responsible for AnS. However, processes that are not strictly classified as anaerobic respiration, but do involve transfer of electrons to an external acceptor other than oxygen or oxidized nitrogen, resulting in a volatile product, can occur in a facultative biomass. Production of molecular hydrogen is a specific example of such a process.

In the absence of electron transfer to an external acceptor, it is conceivable that anaerobic processes result in the formation of one or more reduced volatile intermediates, which are subsequently stripped out of the mixed liquor in the aerobic zone. Acetic acid, ethanol, butanol, and acetone are examples.

1.4.2 Dissolution of Gaseous Oxidant

Dissolution and utilization of oxygen is inherently considered in the calculation of AnS. However, N- or C- fixation considerations may not be inherent to the calculation method. While these processes are unlikely in a non-nitrifying BPR system, they are valid possibilities.

1.4.3 Limitations of the COD Test

In a system as complex as an activated sludge system treating municipal wastewater or synthetic feed with complex organics, the treatment process essentially involves transfer of electrons between several individual donors and acceptors, both organic and inorganic. A comprehensive electron balance, needed to calculate process oxygen requirements or AnS,

would therefore require quantification of each individual species, which is virtually impossible. Recognizing that the primary information needed was the number of electrons regardless of the species that carried them, environmental engineers invented the concept of Chemical Oxygen Demand (COD), which ideally measures the number of electrons contained in all organic and some inorganic species in a given sample, relative to predefined base states. In reality, however, several organic and inorganic species are known to resist oxidation in the COD test, and the number of electrons in these species is not measured. Further, it is conceivable that not all such species are known. Presence of any of these species in a sample results in the COD value providing an inaccurate estimate of the number of electrons in the sample.

COD data are an important input in calculating the contribution of the "reduced organics - oxygen" redox pair to AnS. Possible sources of COD over- or underestimation thus become equally important.

1.5 Factors Affecting AnS

Previous work on AnS (described in detail in a section of the following chapter) indicated that AnS is a function of the nature (composition and concentration) of the substrate. In particular, the relative amounts in the feed of complex organics and fermentation products such as volatile fatty acids (VFAs), and of reduced organics and phosphorus, were found to have strong effects on AnS. Previous work has also established the strong dependence of BPR on

the nature of the substrate, and extensive data are available on the effects of several specific VFAs.

1.6 Objectives

The following specific objectives were set for this study:

1. To develop an improved methodology for the quantitative determination of AnS.
2. To apply this methodology to lab-scale BPR systems and verify the occurrence of AnS.
3. To validate the statistical significance of the experimentally determined AnS values.
4. To identify the various components that contribute to the AnS-related redox balance.
5. To investigate the possible explanations for AnS identified in Table 1.

2 Literature Review

Although BPR systems are a relatively recent development, their potential for greater economy (Lan *et al.*, 1983; Randall, 1984; Randall *et al.*, 1984 and 1985) has attracted considerable research effort in the past few years. A great deal is known today about *what* happens in a BPR system and under what conditions. However, many of the answers to *how* and *why* it happens are speculative hypotheses rather than definite knowledge. It is interesting that the existence of anaerobic stabilization was first suspected because of indirect evidence of reduction in oxygen use during research in which anaerobic stabilization was not of primary concern. Little work has been done on the AnS aspect of the process since then. Phosphorus removal mechanisms, however, are under continued investigation. It is suspected that the two phenomena are closely related.

2.1 Biological Phosphorus Removal

The major obstacle to full-scale commercial use of BPR systems has been the lack of an integrated quantitative model that can predict phosphorus removal performance with reasonable accuracy for a given set of conditions. The models available today (some of which are discussed in a later section of this chapter) are empirical or semi-empirical models with

limited applicability, and cannot guarantee consistent performance. Better models with wider applications are currently in development, and call for a better understanding of the details of the BPR process, and factors that affect it.

2.1.1 Effect of Substrate Composition and Concentration

The dependence of the extent of phosphorus release and uptake on the composition and concentration of the substrate has been the subject of several investigations, and has provided clues to the role of fermenters in BPR systems. Apparently contradictory results have been reported in some cases, however, and few definite conclusions can be drawn. Full-scale studies on the five-stage Bardenpho process (Nicholls *et al.*, 1985) revealed that availability of fermentation products, particularly short-chain volatile fatty acids (VFAs), could "greatly assist" both phosphorus and nitrogen removal. The authors emphasized that VFAs were only a fraction of the quantity referred to as the "readily biodegradable substrate," and the fraction was not always a large one. Readily biodegradable substrate is defined to be that portion of the biodegradable substrate which can easily pass through the bacterial cell membrane without first being transformed, and is represented by the symbol S_{bs}. Procedures for determination of S_{bs} have been described by the Water Research Commission of South Africa (1984). It is commonly believed that, while the VFAs can be directly sequestered by the phosphorus-accumulating biomass, the remainder of the S_{bs} must first be converted to VFAs by the fermenting biomass. This is an important distinction between the two fractions of S_{bs}.

Arvin and Kristensen (1985) quantitatively confirmed the above result. Using batch experiments under anaerobic conditions, they studied the effect of various substrates on phosphorus-rich sludges taken from the aerobic zones of two different BPR plants. Acetate, propionate, butyrate, and lactate caused significant and immediate phosphorus release, at a rate approximately proportional to that of COD uptake. The release/uptake ratio ranged from 0.33 to 0.74 mg P/mg COD. With ethanol and formate, no significant COD uptake was seen, yet substantial phosphorus release was observed in some cases. This resulted in a wide release/uptake ratio range, 0.21 to 1.7 mg P/mg COD. The authors could not find any explanation for this behavior. The behavior of the sludge that received glucose, on the other hand, was as expected. With sludge not acclimated to glucose feed, there was very little COD uptake or phosphorus release (good correlation). With glucose-acclimated sludge, however, rapid COD uptake was observed, while the release of phosphorus showed only a small increase and was considerably delayed relative to COD uptake.

Iwema and Meunier (1985) showed that if acetate was present in the substrate, phosphorus release could occur even under anoxic conditions, with NO_3-N concentrations up to 40 mg/L. This, they argued, was evidence that *phosphorus accumulating bacteria could effectively compete with denitrifying bacteria for acetate, even at low acetate concentrations*. This evidence invalidated earlier assumptions (Wentzel *et al.*, 1985, for instance) that all available acetate is rapidly consumed by the denitrifiers, leaving nothing for the phosphorus-accumulating "poly-P" (polyphosphate) bacteria. According to Iwema and Meunier, the competition created by the presence of nitrate reduces the "specific phosphorus release efficiency" (mg P released/g mixed liquor suspended solids (MLSS)/mg acetate utilized), but

does not totally eliminate it. This efficiency is directly proportional to the acetate concentration, and inversely to the nitrate concentration. When acetate is completely utilized (as in a batch system, for example), P-release stops, and if excess nitrate is still present, subsequent P-uptake occurs. Similar results were reported by Gerber *et al.* (1987). After studying the release-uptake behavior of many different substrates in batch systems, they concluded that "*release of phosphorus from phosphorus-rich sludge is controlled primarily by the nature of the substrate rather than the creation of an anaerobic state.*" Thus, short-chain VFAs such as formate, acetate, and propionate (no data provided for formate and propionate) induce P-release even under anoxic or aerobic conditions, while other readily biodegradable substrates like glucose, ethanol, methanol, and citrate cause P-release under anaerobic conditions only. Further, if fermentation products (VFAs) are available in the presence of nitrate, P-release and P-uptake occur simultaneously. It is interesting that this last statement seems to represent the continuous-flow version of Iwema and Meunier's results describing acetate exhaustion in a batch system.

Taking the anoxic-phosphorus-release result one step further, Hascoet *et al.* (1985) reported that such release was possible in batch systems "if the initial COD was sufficiently high" (greater than 200 mg/L in their case). The COD used in this case comprised albumose, amino acids, peptone, and other constituents of meat extract, but apparently no fermentation products. Thus, according to these results, sufficiently high influent COD (not necessarily composed of fermentation products) is the only requirement for anoxic phosphorus release to occur. If the COD is not high enough, P-uptake occurs instead of P-release. Also, under *anaerobic* conditions, P-release *with* substrate was reported to be significantly higher than that

without, which again underscores the important role played by the organics in the anaerobic zone of a BPR system.

Two different theories have been proposed to explain the release of phosphorus observed under anoxic conditions:

1. N_{ox} (oxidized nitrogen) is masked by the overwhelming excess of COD, and release may partially originate in a portion of the biomass incapable of reducing N_{ox}, such as *Acinetobacter lwoffi* (Lawson and Tonhazy, 1980; Fuhs and Chen, 1975).
2. High concentration of reduced organics results in a low reduction potential, which triggers the P-release.

Fukase *et al.* (1985) reported unusual results from their pilot-scale study of an Anaerobic/Oxic (A/O) system. Biochemical oxygen demand (BOD) loading rates no greater than 2 kg BOD/kg MLSS/day and anaerobic BOD/MLSS ratios no greater than 0.1 kg BOD/kg MLSS were required to obtain good P-removal. This contradicted the general observation that a higher concentration of organics in the influent induced higher anaerobic P-release, and consequently higher excess P-uptake. The authors proposed no explanation for this uncharacteristic behavior.

Heymann (1985) presented a different aspect of the effect of substrate composition on biological phosphorus removal. The idea presented concerned the effect of the nature of the substrate on *aerobic* phosphorus metabolism. According to Heymann, the rate of aerobic

intracellular polyphosphate accumulation is controlled by the prevailing level of biosynthetic activity. An increase in such activity results in a higher adenosine tri-phosphate (ATP) utilization rate, thus leaving a smaller fraction of the total amount of ATP generated for polyphosphate formation. This results in lower P-removal. Presence of biosynthetic precursors in the influent can therefore be expected to reduce overall P-removal, and better removal will result if the substrate has no biosynthetic value. Since biosynthetic precursors usually contain large amounts of nitrogen, in contrast to the very low levels found in high-energy compounds, the influent COD/TKN (total kjeldahl nitrogen) ratio assumes a special significance. This ratio is considered to be an important parameter affecting a given wastewater's treatability with respect to BPR.

Abu-Ghararah (1988) investigated the effects of separate additions (to municipal wastewater influent) of formic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids, plus glucose, on anaerobic phosphorus release, aerobic phosphorus uptake, nitrogen and COD removals, and the specific oxygen utilization rate (SOUR) in a pilot-scale University of Cape Town (UCT) process. He reported that all added substrates, except formic acid and dextrose, caused a significant increase in anaerobic phosphorus release and aerobic phosphorus uptake, and consequently in the overall phosphorus removal efficiency. However, none of the substrates affected TKN or COD removals. These results directly contradict those of Gerber *et al.* (1987), who reported formate-induced P-release even under anoxic or aerobic conditions, although actual data were not presented.

2.1.2 Effect of Oxidized Nitrogen

Researchers and professionals today generally agree that the presence of oxidized nitrogen (nitrate and nitrite, represented by the symbol N_{ox}) in the anaerobic zone of a BPR system (the intended anaerobic zone becomes anoxic, of course, in presence of N_{ox}) adversely affects (reduces) phosphorus release and subsequent excess phosphorus uptake. Again, the exact mechanism responsible for such adverse effects is yet a matter of speculation. This is another example of limited current knowledge about "how" and "why" in spite of the general consensus on "what".

Based on full-scale studies on the five-stage Bardenpho process, Nicholls *et al.* (1985) concluded that "efficient denitrification is a pre-requisite for good phosphorus removal." This statement evidently assumed either significant nitrification or the presence of oxidized nitrogen in the influent. In effect, any N_{ox} entering the anaerobic zone adversely affects phosphorus removal. Hascoet *et al.* (1985) reported similar results in batch experiments. They found that for the same initial COD, presence of N_{ox} lowered the maximum batch P-release, and caused earlier onset of P-uptake.

2.1.3 Role of Phosphorus Release

Regardless of the factors that affect (induce or inhibit) P-release, it is generally agreed today that this phenomenon plays a key role in the process of biological phosphorus removal. Experienced researchers (Marais *et al.*, 1983, for instance) believe that P-release is a pre-requisite for excess P-uptake. Results published by Hascoet *et al.* (1985), however, showed

that while P-uptake was enhanced by preceding P-release, significant anoxic P-uptake and net excess P-removal could occur even in the absence of any preceding P-release. This was a significant finding, since most of the theories proposed to explain the effects of factors like substrate composition and N_{ox} actually relate to P-release, and if excess P-uptake can occur without P-release, the validity and accuracy of these theories is open to question.

2.1.4 Role of Metal Ions

Claims by several researchers that significant chemical precipitation of phosphorus occurs in BPR systems have led to an examination of the possible role played by metal cations. The orthophosphate anion and polyphosphate chains carry negative charges, and electroneutrality considerations suggest that metal cations may be closely associated with these species. Changes in polyphosphate concentration can be expected to cause changes in the metal ion concentrations. Chemical precipitation of orthophosphate with metal ions, however, depends on conditions such as temperature, pH, and concentrations.

Arvin and Kristensen (1985) reported K and Mg release and Ca uptake along with the P-release and substrate uptake observed in anaerobic batch experiments with two different phosphorus-rich sludges. The $\Delta K/\Delta P$ value was about 0.29 mg/mg, and $\Delta Mg/\Delta P$ was about 0.25 mg/mg. The most significant observation reported was the simultaneous chemical precipitation of orthophosphate as it was released into solution by the biomass under anaerobic conditions, claimed to result in 60 percent of the total P in the sludge being chemically bound.

Ca and Mg were the dominant counter-ions ((Ca+Mg)/P molar ratio in the chemical sludge was about 1.1), while P-bound Fe comprised about 0.6 percent of the sludge. The ratio between chemically-bound P and total P in the sludge was relatively constant.

The results reported by Arvin and Kristensen are unique in that the literature contains no other reference to such a high sludge chemical-P content in a BPR process (without chemical addition). A possible reason for this is the questionable reliability of the sludge characterization procedures used in their study, specifically the cold PCA (perchloric acid) extraction procedure. It is strongly suspected that this procedure extracted a significant portion of non-chemically-bound P as well. Also, cold-storage of samples (which lasted several months in this case) before fractionation was later shown to result in erroneous measurements (DeHaas, 1989).

In another batch study (Hascoet *et al.*, 1985), Cu^{2+} (cuprous ion) was found to enhance P-release in the anaerobic phase, but inhibited P-uptake (and, at higher concentrations, caused P-release) in the aerobic phase. The following hypotheses were proposed to explain the observed behavior:

1. Enhancement in anaerobic P-release was the result of Cu^{2+}-induced reduction in pH.
2. Cu^{2+} blocked the transport of orthophosphate across the cell membrane. Thus, polyphosphate was hydrolyzed to satisfy the cell's energy requirements, even under aerobic conditions, just as it is under anaerobic conditions.
3. Cu^{2+} activated polyphosphatase.

4. Cu^{2+} caused cellular lysis.

Kerdachi and Roberts (1985) postulated an additional function for the anaerobic zone in a BPR system: to provide ideal conditions for reduction of influent ferric iron to ferrous iron, and for subsequent precipitation and extracellular adsorption of mixed metallic phosphates, such as the colloidal modification of ferrous calcium phosphate (1/0.4/1 molar), which forms in the pH-range 6 to 7.5. The reduction of ferric iron to ferrous iron was found to require reduction potentials much lower (and consequently anaerobic retention times much longer) than those required for P-release. The authors reported that partial conversion of intracellular polyphosphate to extracellularly adsorbed metallic phosphates was found to be essential for truly enhanced phosphorus removal by a BPR process. In the full-scale BPR system they studied, metabolic phosphate, excess biological uptake, and extracellular precipitation/adsorption each accounted for about one-third of the total phosphorus removed. This supported the observation made by Arvin and Kristensen (1985) that the anaerobically released orthophosphate was simultaneously precipitated, but again the reasoning behind this conclusion was unconvincing. The complexing and extracellular adsorption of phosphate could have been purely incidental, and not really essential for true enhanced P-removal.

Pattarkine (1991) used A/O, UCT, and batch systems to study the role of metal cations, specifically potassium, magnesium, and calcium, in the stoichiometry of EBPR. He concluded that "potassium and magnesium are simultaneously required for efficient EBPR." Neither potassium nor magnesium can induce EBPR in the absence of the other, and (0.43 mol K + 0.37 mol Mg) per mol P in the influent wastewater "may be considered adequate to achieve

maximum EBPR." He also reported that calcium "did not appear to be required for EBPR, but may be released and taken up (co-transported) with P to a limited extent." Calcium did not seem to be involved in biologically mediated chemical precipitation, either, but sustained absence of calcium adversely affected P removal. This was attributed to a general system failure caused by the lack of a required growth element.

2.2 BPR Models

BPR models are conceptual or mathematical representations that attempt to describe the series of steps involved in the BPR process. The representations are based on such experimentally acquired knowledge about the process as the factors that affect it and what the effects are. Over the years, researchers have proposed qualitative as well as quantitative BPR models to describe either part or all of the BPR process. Some of these models are highly informative because they represent a concise summary of the proponent's view and understanding of a particular step or the process as a whole.

Based on work done at the University of Cape Town in South Africa, Siebritz *et al.* (1983) developed an empirical model that provided a formula for biological phosphorus removal as a function of several operating conditions and influent wastewater characteristics. The parameters of the model were evaluated by fitting the proposed empirical equation to experimental data, and the model predictions were reported to agree well with actual results. No physical significance was attached to the parameters, however, and the model had little theoretical basis. Rather, it was based on observations made during several laboratory

experiments. The model and its development were also presented in a monograph published by the Water Research Commission of South Africa (1984).

Wentzel *et al.* (1985) recognized that P-release and P-uptake are essentially separate processes, and must therefore be modeled separately. Accordingly, they proposed a model to quantify anaerobic P-release only, unlike the earlier model by Siebritz *et al.* (1983), which directly quantified the overall net P-uptake. Although the new model had a stronger theoretical base, it was still semi-empirical, because the anaerobic $\Delta P/\Delta COD$ ratio, which was key to the model, was experimentally determined. Several assumptions were involved in the model, which actually evaluated the COD utilization kinetics first and then related these to P-release through the $\Delta P/\Delta COD$ ratio. The authors developed an equation for ΔP across a completely mixed continuous-flow reactor as a function of several operating parameters and waste characteristics, but if the model parameters represent the true, intrinsic kinetics of the system, they could be applied to any process configuration. The mathematical model was based on a biochemical model very similar to those of Siebritz *et al.* (1983) and Comeau *et al.* (1985). Specifically, the model assumed that:

1. Anaerobic P-release is a two-step process involving two different groups of microorganisms.
    a. "Non-poly-P heterotrophs" first convert the readily biodegradable substrate to VFAs (the "conversion" step).
    b. The poly-P organisms then sequester the VFAs into intracellular organic complexes (the "sequestration" step). The energy for sequestration is obtained

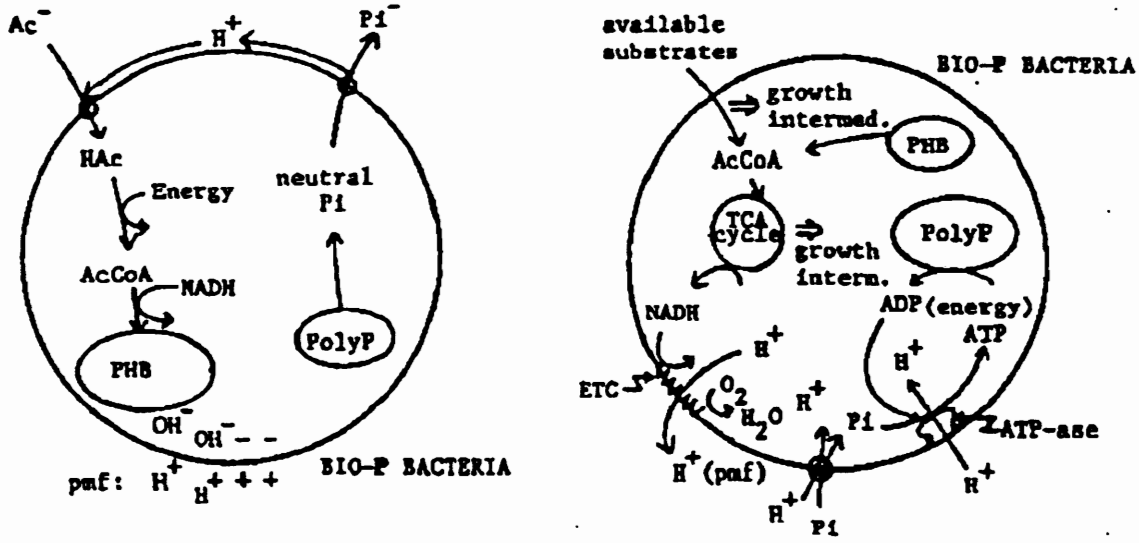
from poly-P hydrolysis, which results in simultaneous P-release. Only VFAs can be sequestered by the poly-P organisms, and $\Delta P/\Delta S_{bs} \approx 0.5$ mg PO_4-P/mg COD.

2. Sequestration is much faster than conversion, so that conversion is the rate limiting step, and the rate of P-release is controlled by the rate of conversion.

The model was verified using several modified UCT (University of Cape Town) processes under various conditions. The authors also presented preliminary experimental evidence indicating that anoxic/aerobic P-uptake was a linear function of anaerobic P-release.

Comeau *et al.* (1985) presented a conceptual BPR model, which is shown in Figure 1. This model was almost identical to the description of the BPR process presented by the Water Research Commission of South Africa (1984). The model divided BPR metabolism into anaerobic and aerobic stages. In the anaerobic stage, the bio-P (used interchangeably with poly-P) bacteria use the energy released by polyphosphate hydrolysis to sequester VFAs such as acetate into intracellular polymeric hydrocarbons such as poly-β-hydroxybutyrate (PHB). This results in P-release and organics uptake. In the aerobic stage, the stored PHB is oxidized along with external substrate, and energy produced in excess of the growth requirements is stored in the form of polyphosphate. This results in excess P-uptake and net P-removal.

Takashi *et al.* (1985) conducted batch experiments with radiolabeled P to investigate the behavior of intracellular polyphosphate in the BPR process. They reported that intracellular polyphosphate was found to exist in two distinct molecular weight fractions: low molecular



(a) Anaerobic stage

(b) Aerobic stage

Figure 1. A Conceptual BPR Model: (a) Anaerobic stage (b) Aerobic Stage. After Comeau *et al.* (1985).

weight polyphosphate (L) and high molecular weight polyphosphate (H). Fractionation for analytical purposes was done using the "STS Method". According to the authors, the two poly-P fractions play significantly different roles in BPR metabolism. Based on mass-balance and radioactivity-balance calculations at predetermined time intervals during the batch experiments, they proposed a biochemical model that explained the metabolic movement of P between the L, H, and soluble (S) fractions during the course of the experiment. This model is shown in Figure 2. According to the model, L is the primary source of energy in the anaerobic stage, while H is the source of phosphorus in the aerobic stage. The accuracy of the numbers on which these conclusions were based is open to question, however, because of an error in the mass- and radioactivity-balance calculations. The authors used mg P/g MLSS and cpm/g MLSS as conservative quantities, which is highly doubtful. While mg P and cpm (over a short time period) should both be conserved, MLSS will no doubt increase during a batch experiment. It is not clear why the authors chose MLSS and not the more commonly used reaction volume as the basis for their calculations.

By gradually weaning UCT and Bardenpho systems from municipal wastewater to a synthetic feed containing acetate as the major carbon- and energy-source, Wentzel *et al.* (1988) developed "enhanced cultures" comprising greater than 90 percent *Acinetobacter* spp., which they used to develop a conceptual mechanistic model (Wentzel *et al.*, 1989a) that explained the "complex interactions between the compounds involved in biological excess P removal and the processes acting on these compounds." The model identified 12 "essential compounds" and 13 "essential processes" involved in biological excess P removal, and formed the basis for a mathematical kinetic model (Wentzel *et al.*, 1989b).

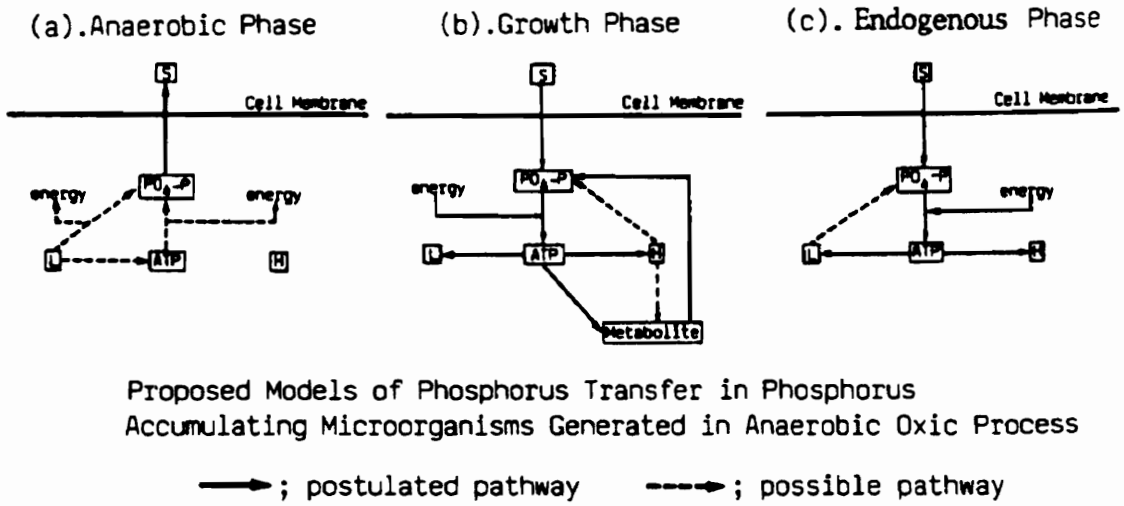


Figure 2. Biochemical Model for the Movement of P between the S, L, and H Polyphosphate Fractions. After Takashi *et al.* (1985).

Jenkins and Tandoi (1991) suggested experimental methods and conditions designed to enable a more realistic duplication of the activated sludge environment and processes in axenic cultures of organisms thought to be responsible for enhanced biological phosphorus removal in activated sludge.

Randall (personal communication, 1991) reported a new mechanism for the anaerobic uptake of acetate, propionate, and lactate in an "anaerobic aerobic" BPR process. The reducing power required for the uptake and sequestration of these acids was shown to come from glycolysis of intracellular carbohydrates.

Dold described a general activated sludge kinetic model capable of modelling the biological processes of carbonaceous energy removal, nitrification, denitrification, and excess phosphorus removal. The new model combined the IAWPRC model for non-poly-P heterotrophic and autotrophic organisms and the model of Wentzel *et al.* (1989b) for poly-P organisms.

2.3 Anaerobic Stabilization in BPR Systems

Anaerobic Stabilization is an aspect of BPR systems not adequately addressed in the literature. This aspect is closely related to the potential of BPR processes for greater economy in terms of reduced aeration requirements, and therefore merits further investigation. This section

presents literature reports of direct and indirect evidence supporting the existence of anaerobic stabilization.

2.3.1 Indirect Evidence

The first indication of the possibility that AnS occurs in a BPR system was observed by Lan *et al.* (1983). They found that introduction of an anaerobic stage ahead of the aerobic stage reduced the specific OUR (oxygen uptake rate) in the aerobic stage, without reducing the substrate removal efficiency. The authors did not concern themselves with the specific implications of this observation at the time, but noted that it might be possible to reduce the aeration requirements of a conventional activated sludge system by introducing an anaerobic stage ahead of the aerobic stage. The concept of AnS was first proposed by Randall *et al.* (1984).

2.3.2 Direct Evidence

Based on experiments on a unit with 2 anaerobic stages and 1 aerobic stage, Ramadori *et al.* (1985) reported that "part of the carbonaceous substrate which disappears during the first anaerobic stage is metabolized." They also reported that Org-N (organic nitrogen) completely vanished in this reactor (1st anaerobic), and a large release of NH_3-N was observed. It is not clear whether the authors considered the disappearance of Org-N and release of NH_3-N to be evidence of the reported substrate metabolization.

The most conclusive AnS evidence to date was presented by Brannan (1986) and Randall *et al.* (1987). They developed an elaborate oxygen-balance equation to quantify AnS, and demonstrated its existence beyond doubt. The elaborate oxygen-balance was considered necessary because the accuracy of the COD test on mixed liquor was believed to be questionable (not high enough to reproducibly detect small differences). Use of a theoretical COD/VSS ratio was deemed more appropriate.

2.4 Factors Affecting Anaerobic Stabilization in BPR Systems

Besides establishing the existence of AnS, Brannan (1986) and Randall *et al.* (1987) also studied the effects of system sludge-age and substrate composition on such stabilization. In lab-scale studies on a continuous-flow BNR system, they found that with fermentable (as opposed to pre-fermented or non-fermentable) substrate, AnS increased when sludge-age was increased. When dextrose (a complex substrate), which initially formed 30 percent of the influent COD, was replaced with the same amount of COD as acetate (fermented substrate), a "washout-type" decline in AnS was observed. It was concluded that "the primary mechanism of AnS is the growth of non-P-storing bacteria (not necessarily the fermenters) in the anaerobic zone." It was interesting that changes in substrate composition had opposite effects on anaerobic P-release and AnS. While P-release was enhanced by a higher concentration of fermentation products (VFAs), AnS was adversely affected. Thus, simultaneous P-release and AnS may require a proper balance between fermented and fermentable substrates.

Pilot-scale studies at the Lambert's Point Plant in Virginia (Randall *et al.*, 1987) yielded some interesting results. AnS was found to be a strong linear function of the influent COD beyond a certain "threshold" influent COD value (≈ 190 mg/L in this case). AnS was zero below the threshold value. Two main conclusions were drawn from these observations:

1. Influent wastewater was already fermented before entering the plant, so that COD was taken up by the poly-P bacteria first.
2. The system was P-limited, so that COD uptake by poly-P bacteria stopped when stored poly-P was exhausted, and excess COD was then available to non-poly-P bacteria. This was the threshold point, beyond which AnS occurred. AnS was thus a function of COD available in excess of that stoichiometrically equivalent to the stored poly-P. This conclusion again indicated that organisms other than poly-P bacteria were responsible for AnS.

Since pre-fermented substrate will not support fermenter growth, non-poly-P organisms other than fermenters were indicated as being responsible for AnS. The authors also noted that the P-removal/COD-removal ratio would vary considerably depending on the extent of pre-fermentation. The results of the pilot-scale studies must be interpreted with caution, however, because no actual AnS data were presented for influent COD values lower than 190 mg/L, nor was it checked if an increase in influent P resulted in a higher threshold influent COD.

2.5 *Anaerobic Metabolism of Amino Acids*

"Amino acid degradation by anaerobic bacteria always involves oxidation and reduction reactions between one or more amino acids or non-nitrogenous compounds derived from amino acids. Electron acceptors used by amino acid-fermenting bacteria include amino acids, α- and β-keto acids, α, β unsaturated acids or their coenzyme A thiolesters, and protons. The ultimate reduction products include a variety of short-chain fatty acids, succinic acid, δ-amino valeric acid, and molecular hydrogen" (Barker, 1981).

Dürre and Andreesen (1983) presented evidence of an energetic advantage for the glycine reductase system in three purinolytic *Clostridia*. Glycine was directly reduced to acetate in all three species.

2.6 *The Case for Anaerobic Reduced Gas Production*

The fact that certain anaerobes can bring about transport of electrons to the hydrogen ion (proton), thus resulting in the formation of hydrogen gas, is well documented in the literature (Cruden *et al.*, 1983, for example). It is well known, for instance, that certain species of the genus *Clostridium* route electrons to protons via the transport protein ferredoxin (Fd), and evolve hydrogen gas as a metabolic product in the process. Martin *et al.* (1983) showed that *Clostridium thermoaceticum* could "vent reductant as H_2" when cultivated heterotrophically on dextrose under carbon monoxide (CO). H_2 production was significantly reduced when CO was replaced by carbon dioxide (CO_2). They speculated that CO inhibited electron flow to acetate, resulting in enhanced H_2 production. Similar results were reported by Kellum and

Drake (1984). They tested hydrogen evolution and the hydrogenase level in growing heterotrophic cultures of *C. thermoaceticum* under five different gas phases, and found that "hydrogenase was maximal from cells cultivated under CO."

Kim *et al.* (1984) reported that the effect of CO on H_2 production by *Clostridium acetobutylicum* was quite the opposite. An increase in the CO partial pressure above the bacterial cultures resulted in lower H_2 production, which was attributed to lower activity of the enzyme hydrogenase.

While the biomass in a BPR system may or may not contain *Clostridia*, their metabolism suggests possibilities. There may exist other bacteria that can use protons as electron acceptors, and some of these may be present in a BPR system. Ferredoxin and/or hydrogenase may be the common factors among such bacteria.

2.7 Experimental Determination of Aerobic OUR

The aerobic OUR is an important input to any AnS calculation method. Proper determination of this quantity is therefore crucial to the accuracy of the calculated AnS value. Section 2710.B (APHA, 1989) describes a simple method for determination of the "oxygen consumption rate" (equivalent to OUR), but warns that "because test conditions are not necessarily identical to conditions at the sampling site, the observed measurement may not be identical with the actual oxygen consumption rate." Mueller and Stensel (1987) described a respirometric technique for the in-situ measurement of OUR, and based on comparison of

results with those obtained using the BOD-bottle method (Section 2710.B, APHA, 1989), concluded that the BOD-bottle method could significantly over- or underestimate the in-situ OUR depending on test conditions. An in-situ OUR measurement technique (such as the one described by McClintock, 1990) must therefore be used to ensure an accurate reading.

3 Theoretical Calculation of Anaerobic Stabilization

The process of quantifying AnS involves an electron balance around the system under consideration. The purpose is to check if all the reduction and oxidation reactions in the system are mutually balanced, and if not, quantify the difference. This may be done in various ways, some of which are described in the following sections. The relative merits and demerits of these methods are also compared.

3.1 Localized Balance around the Anaerobic Zone

This is the least rigorous way of calculating AnS. It is based on the assumption that AnS manifests itself in the anaerobic zone. The balance is restricted to the streams entering and leaving the anaerobic zone only, and the calculations are therefore not very intensive. The assumption made, however, is open to question. AnS values reported in previous work (Brannan, 1986; Randall *et al.*, 1987) were based on a balance around the entire system under investigation, not just the anaerobic zone. Theoretically, therefore, some or all of the AnS observed could have occurred outside the anaerobic zone. Admittedly, this is speculation, but

if true, a balance around the anaerobic zone alone would certainly produce inaccurate AnS values. This is a major drawback of this method.

Also, in this particular study, the suspended solids concentration (and consequently the TCOD) in the RAS, which was one of the streams entering the anaerobic zone, was often very high and varied considerably. The high TCOD values meant amplified dilution error, and the high variability meant that the anaerobic zone, isolated from the rest of the system, was rarely even close to steady-state. In general, therefore, the localized anaerobic balance is of little use. The following methods use a system-wide balance.

3.2 The Unit Process Method

This method does not restrict itself to the anaerobic zone, but accounts separately for each individual redox process occurring in the system. Thus, organics stabilization, nitrification, denitrification, sulfate utilization and production, and oxygen utilization are all calculated separately, converted to oxygen equivalents, and used to figure AnS. Such calculations require detailed information about all redox processes in the system, and must be customized for each BNR process configuration. It must be known whether a given process occurs at all, where it occurs, and to what extent. For example, nitrification may not occur because of chemical inhibition, low temperature, low BSRT, or low influent TKN. Similarly, denitrification will not factor if there is no nitrification, but could occur in either the anoxic or the anaerobic zone, or both, in presence of nitrification. This method is thus highly process-specific, and must be tailored to the system configuration.

A major drawback of this method is the uncertainty in the oxygen uptake rate value for the clarifier. Neither the BOD bottle technique, nor an in-situ procedure would be appropriate for this measurement, since the clarifier is not a completely mixed unit during normal operation. An assumption is therefore required, which is often unrealistic. For example, Brannan (1986) and Randall *et al.* (1987) assumed that the clarifier OUR was equal to that in the final aerobic reactor. This assumption was based on BOD bottle measurements of clarifier and aerobic OURs. Thus, the clarifier OUR, and therefore AnS, can only be approximated at best. Also, oxygen utilization in the tubing connecting the system components is ignored in this method.

The method of choice is a new method that was developed as part of this study. This is a generalized, process-independent method that eliminates the need to make the assumptions and approximations of the earlier methods.

3.3 The Boundary Exchange Method

Rather than calculate the contribution of each redox process, this method focuses on *exchanges* between the system and its surroundings across the system-surroundings boundary. The processes within the system are irrelevant. Regardless of the configuration, the only information needed is the composition and flow-rate of each stream entering and leaving the system as a whole. In most cases, only four streams are involved: the influent (Inf), the effluent (Eff), the waste activated sludge (WAS), and the air (Air).

3.3.1 Base Oxidation State, Oxidizing Capacity, and Electron Equivalents

A "base oxidation state" is assigned to each element known to be potentially subject to reduction or oxidation in the system under consideration. Since an element in its base oxidation state does not factor into AnS calculations, the choice of base oxidation states may be exploited to minimize sampling and analysis requirements. Every other oxidation state of each element is characterized by the magnitude and direction of its deviation from the base state. The deviation vector is represented by the symbol Δox, and, for an element, it is defined to be the actual oxidation state of the element in a compound or species minus its base oxidation state.

$$\Delta ox_{el} = \text{actual oxidation state} - \text{base oxidation state} \quad (1)$$

Δox_{el} is a measure of the "oxidizing capacity" of the element in the actual oxidation state, and is assigned the units "electron equivalents" (eeq) or "milli electron equivalents" (meq = one thousandth of one eeq). Positive deviations represent higher-than-base oxidation states (more oxidized), while negative deviations represent lower-than-base oxidation states (more reduced).

Table 2 lists, for each element of significance, the base oxidation state assigned in this study, other possible oxidation states and the corresponding species or analytically measured quantities, and the contribution of the element to the oxidizing capacity per mole and per gram of each species/quantity. The quantity COD, as analytically measured without corrections, represents the sum of the electron equivalencies of carbon species (except for oxidation-resistant species, such as pyridine), metals, and the oxidation of nitrite to nitrate and sulfide to sulfate. Corrections for the nitrite and sulfide oxidation components must therefore be applied

Table 2. Base Oxidation States and Oxidizing Capacities.

Element	Base oxidation state (examples)	Other oxidation states (species/analytically measured quantities)	$^{*}n\Delta ox_{el}$, eeq/	
			mol	g
			species/quantity	
C	+4 (CO_2)	-4 (CH_4) +2 (CO)	-8 -2	-0.5 -0.071
		Variable (Reduced organics not oxidized in the COD test, such as pyridine)	Species-specific	Species-specific
		Variable (COD = organics oxidized in the COD test + reduced metals + S^{2-} + NO_2^-)	N/A	-0.125
Metals	That of the oxidized species produced at the end of the COD test (Fe^{3+}, Cu^{3+})	Addressed in the COD test (above)		
H	+1 (H^+, H_2O)	0 (H_2)	-2	-1
O	-2 (OH^-, H_2O)	0 (O_2)	+4	+0.125
N	0 (N_2)	-3 (TKN) +3 (NO_2^--N) +5 (NO_3^--N)	-3 +3 +5	-0.214 +0.214 +0.357
S	-2 (H_2S)	+6 (SO_4^{2-})	+8	+0.083

* n = number of moles of element per mole of species or quantity

to COD in order to isolate the carbon and metals components. The oxidizing capacities of oxidation-resistant species like pyridine must also be considered separately.

The oxidizing capacity of a species (Δox_{sp}) is defined to be the sum of the products of the oxidizing capacity of each element in the species and the number of moles (n) of that element present in one mole of the species.

$$\Delta ox_{sp} = \sum_i n_i(\Delta ox_{el})_i \qquad (2)$$

where n_i = number of moles of element i per mole of species

Throughout this document, a species is referred to as "reduced" if $\Delta ox_{sp} < 0$, and "oxidized" if $\Delta ox_{sp} > 0$. It is important to note that the value of Δox_{sp} depends on the base oxidation states chosen, and the descriptors "reduced" and "oxidized" used in this study are based on the base states listed in Table 2.

3.3.2 Electron Balance

With the above convention in place, AnS may be calculated from the following fundamental law: at steady state, the total oxidizing capacity entering the system per unit time must equal the total oxidizing capacity leaving the system per unit time. Thus:

$$AnS = \Delta ox_{Inf} + \Delta ox_{Air} - \Delta ox_{Eff} - \Delta ox_{WAS} \qquad (3)$$

where Δox_{Inf} = oxidizing capacity entering system per unit time with the influent

Δox_{Air} = oxidizing capacity entering system per unit time with the air

Δox_{Eff} = oxidizing capacity leaving system per unit time with the effluent

Δox_{WAS} = oxidizing capacity leaving system per unit time with the waste activated sludge

Each term on the right-hand-side of the above equation represents the oxidizing capacity entering or leaving the system per unit time with a specific process stream. All terms in the equation have the units eeq/time. AnS may be considered to be an outgoing stream representing the portion of the incoming Δox not accounted for by the other two outgoing streams, Eff and WAS. A negative AnS value thus implies that some oxidation half-reactions appear to occur in the system without corresponding reduction half-reactions, while a positive value implies apparent reductions without corresponding oxidations. A balanced system must yield AnS=0.

In this study, the following assumptions were made in deriving the terms on the right-hand-side of the above equation:

1. Oxidation-resistant organics (such as pyridine) are neither reduced nor oxidized in the system, nor is there any interconversion between these and oxidizable organics.
2. No stream other than Inf contains sulfide in concentrations high enough to significantly interfere with the COD test.
3. Phosphorus is neither reduced nor oxidized in the system.
4. No oxygen enters the system through the aerobic effluent stream or the top of the clarifier.

Based on these assumptions, the terms on the right-hand-side of the electron balance equation are calculated by expanding them as shown in Appendix 1. The AnS value obtained from the equation may be readily converted to "oxygen equivalents" if so desired.

By its very nature, this method eliminates the need to track individual redox processes in different zones of the system. It also eliminates the need for assumptions about clarifier OUR values or oxygen utilization in system tubing/piping. By using the generalized units of eeq, this method unifies all individual redox reactions involving various species onto a common base, which makes it easy to quantify the relative contribution of each reaction.

3.4 Total Stabilization (TSt)

From a wastewater treatment process design point of view, it is useful to define a related quantity, Total Stabilization (TSt). TSt represents the electrons routed out of the system via streams other than WAS and Eff, which carry electrons primarily in the form of COD. TSt is defined as follows:

$$TSt = \Delta ox_{Inf} - \Delta ox_{Eff} - \Delta ox_{WAS} \quad (4)$$

Like AnS, TSt has the units eeq/d, and is useful as a basis for evaluating the relative extent of anaerobic stabilization in a system. AnS, expressed as a fraction or percentage of TSt, may be easier to comprehend than the absolute AnS value.

4 Methods and Materials

This chapter describes, in detail, the design and configuration of the experimental systems used in this study, their operation over the study-period, and the procedures followed in collecting and analyzing samples and evaluating the data. Experiments were conducted on continuous flow as well as batch systems. Two types of continuous flow systems (A/O and A2/O) were used. Batch experiments were conducted on mixed liquor samples drawn from the continuous flow systems.

4.1 Continuous Flow Experimental Set-up

4.1.1 The A/O System

Since nitrification and denitrification were not considered essential for achieving the objectives of this study, the A/O process was chosen for its simplicity, and was operated with chemical inhibition of nitrification, which ensured that nitrification and denitrification effects were virtually eliminated. The study could thus focus on phosphorus and AnS.

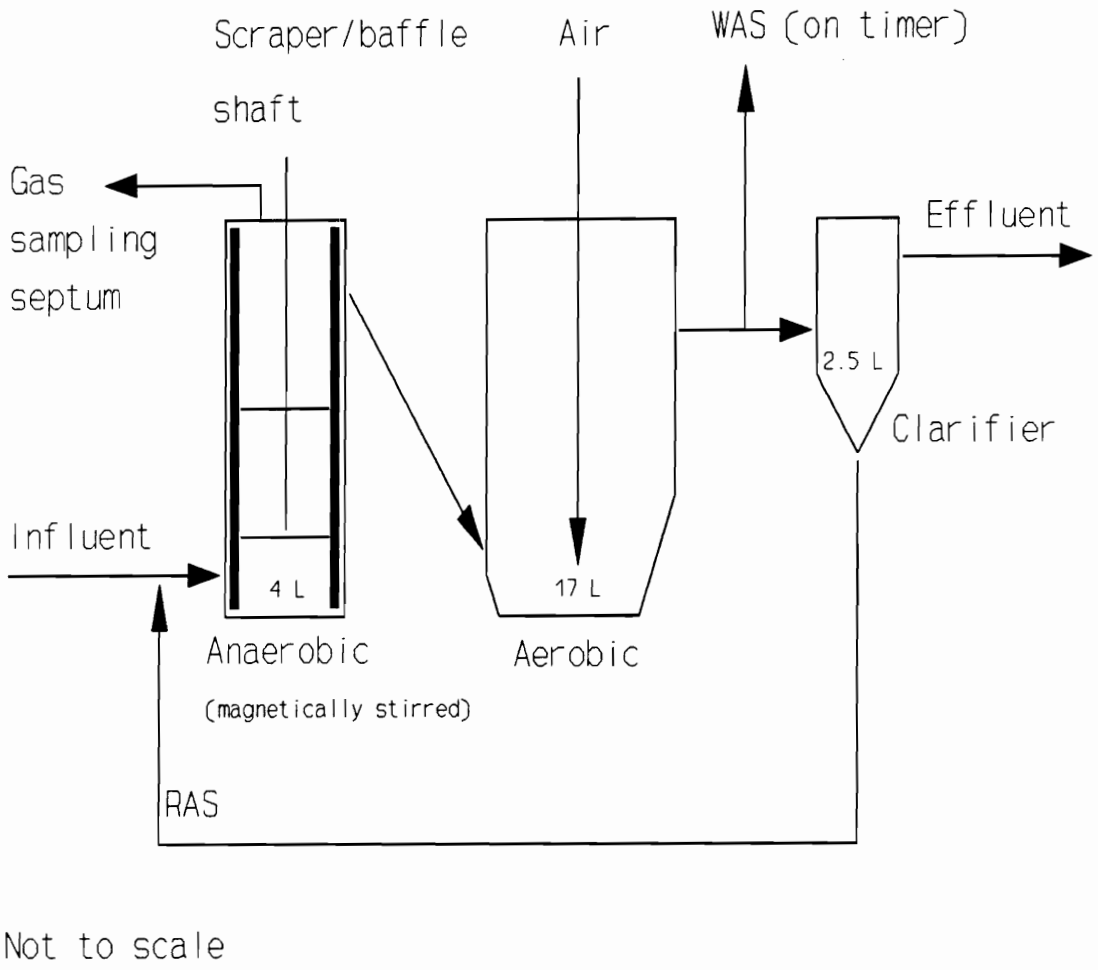


Figure 3. **Schematic of the A/O System Used in this Study.**

A schematic of the A/O system used is shown in Figure 3. Gravity flow was used except for the influent, RAS, and WAS streams, which were driven by Masterflex peristaltic pumps (Cole-Parmer Instrument Company, Chicago, Illinois). The WAS stream could not be operated continuously because of the very low flow-rates required and the obstruction caused by solids at such low flows. The WAS pump was therefore operated six times a day (at four hour intervals) with an electronic timer. The pumping time was adjusted periodically to maintain the target BSRT.

The cylindrical anaerobic reactor was placed on a magnetic stirrer and mixed with a stir-bar. The mixed liquor volume in this reactor was maintained at 4L by a fixed overflow level. A flanged top closure with a rubber gasket and bolts sealed the reactor gas-tight, while a liquid lock on the effluent end created an isolated anaerobic gas space so that the gases evolved could be sampled and analyzed. To prevent anaerobic attached growth and improve mixing, a scraper-baffle was fabricated from aluminum and PVC. An oil seal for the scraper-baffle shaft was housed in the center of the top closure to provide gas-tight operation of the mechanism. Because of the high torque required, the scraper could not be operated continuously, but was manually turned several times at least once every day.

After experimenting with various devices for collecting anaerobic gas, it was found that the pressure in the sealed anaerobic gas space was lower than 1 atmosphere most of the time (a vacuum existed rather than positive pressure). This rendered defunct any device designed to maintain a constant pressure, since such a device cannot remedy a vacuum without diluting

and contaminating the anaerobic gas with ambient air. The gas was therefore sampled through a septum installed directly in the reactor's top closure.

The aerobic reactor was shaped roughly as shown in Figure 3. This reactor was open to the atmosphere at the top (no top closure), and a fixed overflow level maintained the mixed liquor volume at 17L. Compressed air bubbled through a diffuser stone accomplished both mixing and aeration.

The volume of the clarifier contents was 2.5L. A 1 rpm gear-motor drive was used to operate the clarifier scraper mechanism. The entire set-up was placed in a temperature-controlled room for operation at 20°C.

4.1.2 The A2/O System

A schematic of the A2/O system used in this study is shown in Figure 4. Gravity flow was used in this system as well, except for the influent, RAS, and RNX streams, which were driven using four identical Masterflex peristaltic pump-heads on the same drive. The target BSRT was maintained by manually wasting mixed liquor once every day from the aerobic reactor only. The volumes shown in Figure 4 are those of the liquid contained in each unit. Paddle mixers were used in the anaerobic and anoxic reactors, while compressed air bubbled through a diffuser stone accomplished both mixing and aeration in the aerobic reactor. This system was also placed in a temperature controlled room for operation at 20°C.

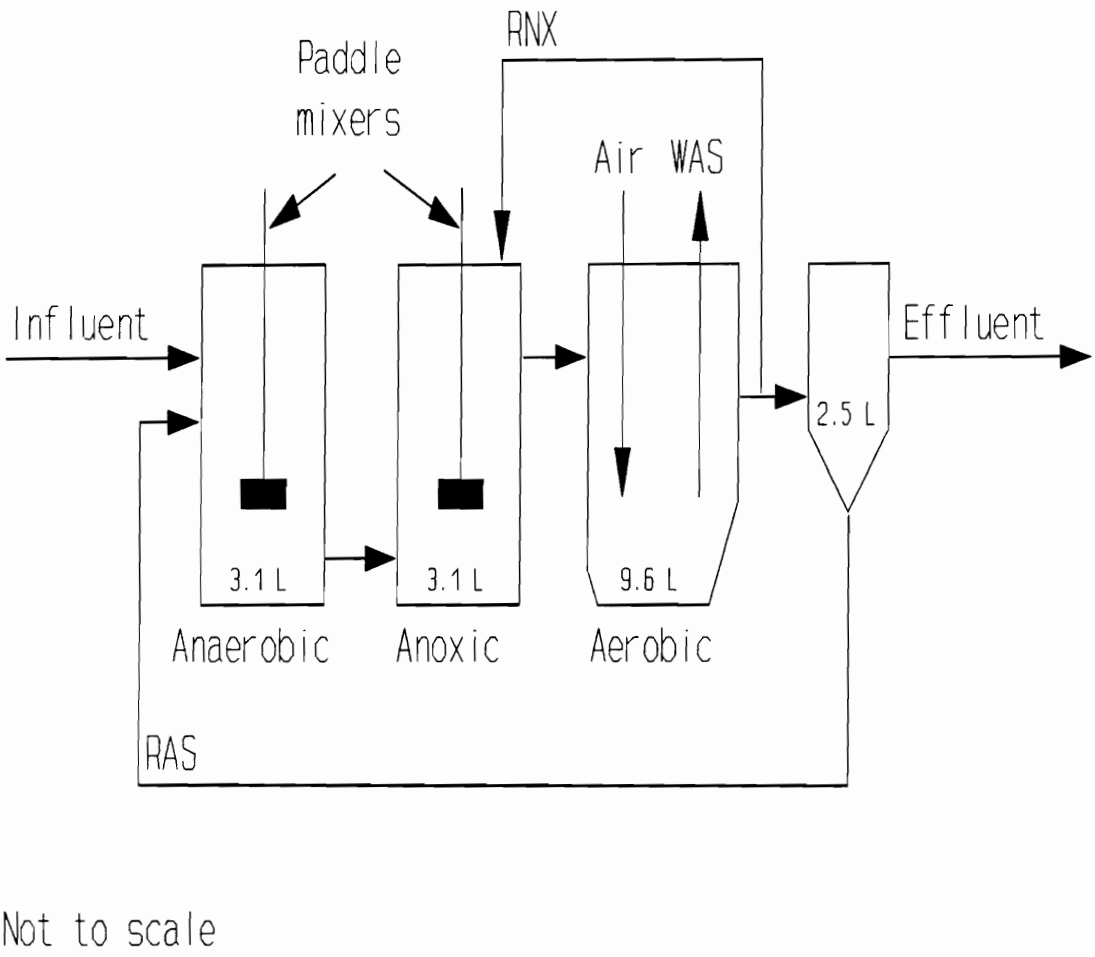


Figure 4. Schematic of the A2/O System Used in this Study.

4.2 Feed Wastewater and Seed Biomass

4.2.1 The A/O System

A synthetic feed was used to enable precise control of and facilitate constancy in the influent composition, and thus meet a basic requirement for steady-state operation. The feed was designed to provide reduced organic carbon (carbon and electron/energy source), nutritional nitrogen and phosphorus, trace nutrients such as various metal ions and anions, and the nitrification inhibitor 2- imidazolidinethione at all times. In addition, varying amounts of excess phosphorus were added at different times. Carbonate was used to keep the feed mildly alkaline. No excess nitrogen was added by design (in an attempt to eliminate nitrification and oxidized nitrogen from the system).

Dextrose was initially used as the only reduced-carbon source, but had to be abandoned because it caused severe bulking sludge problems and led to system failure. Bactopeptone[1] was subsequently substituted as the major reduced-carbon source. This, however, resulted in a substantial excess of reduced nitrogen (TKN) in the feed. It was therefore necessary to chemically inhibit nitrification in order to prevent oxidized nitrogen from interfering with phosphorus removal. 2-Imidazolidinethione ($C_3H_6N_2S$) was chosen as the inhibitor because it had been successfully used before (Lan *et al.*, 1983). One hundred (100) mg/L COD-

[1]Difco Laboratories, Detroit, Michigan

Table 3. A/O Feed Composition.

Compound, M.W.			Element/Quantity	mg Element or Quantity / L	mg Compound / L
From Stock Solution 1 (Organic)					
*Bactopeptone			COD	450	400
2-Imidazolidinethione ($C_3H_6N_2S$), 102			-	-	10.0
**KH_2PO_4, 136	Phases 1-3	10/25/89 - 4/5/90	P	15	65.9
	Phase 4	4/6 - 8/16/90	P	25	109.8
	Phase 5	8/17 - 10/8/90	P	20	87.8
$NaHCO_3$, 84			Na	41	150
From Stock Solution 2 (Inorganic)					
$CaCl_2.2H_2O$, 147			Ca	1.0	3.7
$MgSO_4$, 120			Mg	4.9	24.4
$FeSO_4.7H_2O$, 278			Fe	0.4	2.0
$MnSO_4.H_2O$, 169			Mn	0.7	2.0

*Difco Laboratories, Detroit, MI, U.S.A.
**Phases defined in a later section of this chapter.

equivalent of bactopeptone was replaced, at two different times, with the same COD-equivalent of acetate and formate to study the effects of the substitutions on anaerobic reduced gas production. Table 3 describes the composition of the synthetic A/O feed and the changes in it over the study period.

Forty-two liters of feed were prepared daily from two concentrated stock solutions. It was necessary to maintain two different stock solutions in order to prevent precipitation of sparingly soluble compounds such as calcium phosphate. Stock Solution 1 contained the major C-source (bactopeptone), 2-Imidazolidinethione, monobasic potassium phosphate, and sodium bicarbonate, while Stock Solution 2 contained the inorganic salts calcium chloride, magnesium sulfate, ferrous sulfate, and manganous sulfate. These salts were included in the feed-stock primarily to supply trace minerals, which may not have been present in adequate amounts in the tap water used for final dilution. The required concentrations of these minerals were determined by combining information from various sources (Lan *et al.*, 1983; Wentzel *et al.*, 1988; McClintock, 1986). Three and one-half liters of Stock Solution 1 and 1.4 L of Stock Solution 2 were prepared every week (or whenever the feed composition was changed) using deionized distilled water. Stock Solution 1 was autoclaved at 120°C and 14 psig for 15-30 minutes, then refrigerated to 4°C in order to ensure sterility and prevent biodegradation during storage. Five hundred milliliters of Stock Solution 1 and 200 mL of Stock Solution 2 were combined in a 50 L carboy and diluted to 42 L with tap water, daily. Nitrogen gas was then bubbled through the final feed for about 30 minutes or until the DO was down to about 0.5 mg/L, before putting the feed online.

Seed biomass was obtained from the final aerobic reactor of a pilot-scale UCT system that was being operated on the campus of VPI & SU with municipal wastewater feed.

4.2.2 The A2/O System

Raw municipal wastewater pumped from a sewer manhole in Blacksburg, Virginia and spiked with 400 mg acetate anion (sodium acetate) and about 26 mg P (KH_2PO_4) per liter was used as the feed for this system. The waste was collected and spiked daily.

Seed biomass for this system was obtained from the final aerobic reactor of the pilot-scale UCT system that was being operated on the campus of VPI & SU with municipal wastewater feed, mentioned earlier.

4.3 Operation and Sample Collection

4.3.1 The A/O System

A maintenance schedule was established to ensure smooth, trouble-free operation of the system. Stock feed solutions were prepared weekly (or when composition was changed) and appropriate dilutions were used to prepare the final feed daily. The influent flow was monitored and adjusted daily. Based on measured VSS values and influent flow, the WAS pump timer was reprogrammed after each sampling run to maintain a pre-determined target BSRT. The RAS flow was also measured and adjusted periodically. In order to keep the

A/O anaerobic reactor gas-tight and allow only dissolved gases to enter it, the influent and RAS tubing leading into this unit was kept completely filled with liquid/sludge at all times. Any accumulated gases (particularly nitrogen produced by denitrification in the RAS during uninhibited aerobic nitrification), and the ambient air that entered this tubing when switching feed containers, were purged daily. Various housekeeping tasks such as washing and disinfecting the feed containers and influent tubing, and removing attached growth were also performed at appropriate times. Appendix 2 summarizes the maintenance schedule for the A/O system.

4.3.1.1 Start-Up

The system was started up with bactopeptone as the sole carbon- and energy-source on October 25, 1989. Before this time, the system had been run to failure with dextrose as the only carbon- and energy-source. The problem of bulking sludge was encountered frequently during the first few weeks after start-up, and was remedied in this initial period by adding varying amounts of commercial bleach (5.25% NaOCl) at various times directly to the clarifier. Good settling was achieved in the last week of November, 1989. Precise control of the BSRT was not attempted during the start-up period, but it was maintained at a high value (about 20 days) to discourage growth of filamentous bacteria. The nominal HRT was maintained at 12 hours and the RAS flow-rate at 1Q (Q=influent flow-rate) throughout the study.

4.3.1.2 Operating Phases

To enable meaningful data analysis, the A/O study period was divided into numbered "operating phases", including the start-up phase, which was numbered 1. The phasewise operation of the A/O system is summarized in Table 4.

4.3.1.3 Sample Collection and Sampling Frequency

A complete sampling and analysis run was performed at least once every week. Appendix 3 identifies the parameters that were measured/analyzed for each of the process streams.

All samples were collected in as short a time as possible and analyzed immediately thereafter. No preservation was necessary except for occasional refrigeration of 0.45μ membrane-filtered samples for ion chromatographic determination of anions. Gases were sampled and analyzed no more than one day before or after the liquid/sludge sampling. Samples were collected in a specific sequence, starting at the effluent end of the system and moving towards the influent end, so that the sampling process did not significantly perturb the system until after all samples were collected. DO measurements were made at appropriate times during sampling, in-situ where possible, while pH measurements were made immediately after sampling was completed.

The collected samples were filtered under vacuum, first through a pre-washed and -tared glass-fiber filter (Whatman) and then through a pre-soaked 0.45μ membrane filter (Gelman Sciences, Inc., Ann Arbor, Michigan). The glass-fiber filtration step also accomplished suspended solids measurement. Appropriate volumes of the filtered and unfiltered samples

Table 4. A/O Phasewise Operation Summary.

Phase	Inclusive dates	Target BSRT, days	Target inf FOP, mg/L	Comments/Description
1	Oct 25 - Nov 26, 1989	-	15	Start-up: bulking sludge, frequent clarifier bleaching
2	Nov 27, 1989 - Jan 18, 1990	About 20	15	Acclimation, establishment of nitrification
3	Jan 19 - Apr 5, 1990	10	15	Began chemical inhibition of nitrification, gradual increase in excess BPR
4	Apr 6 - Aug 16, 1990	10	25	Peak BPR at end of phase
5	Aug 17 - Oct 8, 1990	10	20	Higher inf COD/P, recurrence of bulking sludge

were then transferred into various analyses.

4.3.2 The A2/O System

Steady-state data were collected from the A2/O system between December 24, 1991, and January 4, 1992. The system had been operating with the constant feed composition described earlier and under constant operating conditions (target BSRT= 5 days, nominal HRT=6 hours, RAS flow=1Q, RNX flow=2Q) for three BSRTs (about 15 days) before any data were collected. These conditions were maintained throughout the sampling period mentioned above. Maintenance tasks on this system included monitoring and adjustment of the influent flow, calculation of WAS volume needed to maintain the target BSRT, manual wasting, and housekeeping tasks similar to those described for the A/O system.

The same general sample collection procedure was followed as for the A/O system. All parameters required for process control (flow-rates and suspended solids) and to determine AnS (DO, aerobic OUR, COD, TKN, nitrite, nitrate, and sulfate) were measured/analyzed for each sampling run. The anaerobic off-gas and mixed liquor were both analyzed for hydrogen and methane. The phosphorus profile through the system was also analyzed.

This system was mainly used to investigate production and stripping of reduced volatiles as a possible explanation for AnS. Batch aeration experiments and gas chromatographic analyses for ethanol and n-butanol performed on anaerobic and anoxic mixed liquors from this system are described in detail in separate sections in this chapter.

4.4 Analytical Procedures

For liquid/sludge samples, procedures published by the American Public Health Association (APHA, 1989) were followed as closely as possible. Gas-solid chromatography was used for analysis of the gas samples, while gas-liquid chromatography was used to analyze ethanol and butanol in filtered and unfiltered mixed liquor samples.

4.4.1 Suspended Solids

Total suspended solids (TSS) and volatile suspended solids (VSS) analyses followed procedures described in Sections 2540.D and 2540.E (APHA, 1989) respectively.

4.4.2 COD

The dichromate closed reflux titrimetric method described in Section 5220.C (APHA, 1989) was used for determination of COD.

4.4.3 TKN

The semi-micro-kjeldahl method (Section 4500-N_{org}.C, APHA, 1989) was used to digest and distill TKN samples. Borosilicate glass erlenmeyer flasks with ground glass necks were used in the distillation step instead of the micro-kjeldahl distillation apparatus. Before use, the distillation set-up was steamed out as recommended in Section 4500-NH_3.B.4a (APHA, 1989). The ammonia distilled over was received into indicating boric acid, and final ammonia

measurement was performed according to the titrimetric method described in Section 4500-NH_3.E (APHA, 1989).

4.4.4 Anions

Chloride (Cl^-), Nitrite-N (NO_2^--N), Nitrate-N (NO_3^--N), Orthophosphate-P (PO_4^{3-}-P), and Sulfate (SO_4^{2-}) were quantitatively analyzed using ion chromatography. A Dionex 2010i ion chromatograph system (Dionex Corporation, Sunnyvale, California) was used for this purpose. The system consisted of a 50 µl sample loop, a separation column containing a stationary phase with a quaternary ammonium exchange function on a polystyrene/divinylbenzene core, immediately followed by an "Anion Micro Membrane Suppressor" (AMMS), and an electrical conductivity cell detector. A pH 9.5 sodium carbonate - sodium bicarbonate mixture was used as the eluant, while a 0.025 N sulfuric acid solution was used as the AMMS suppressant. The AMMS consisted of a cation exchange column continuously regenerated by the suppressant, separated from the eluant flow by a semi-permeable membrane. The AMMS lowered the background conductivity of the eluant (thereby increasing detector sensitivity to anions of interest) by transporting highly mobile Na^+ cations from the eluant, across the membrane, to the suppressant.

The actual anion determination procedure closely followed that described in Section 4110.B (APHA, 1989). The anion separator column used fell under the "fast-run" category. Samples were filtered through a 0.45 µ membrane filter prior to injection. An eluant flow-rate of 2 mL/min was used. A 3 mL syringe with a male luer-lock connection was washed, first with

deionized distilled water and then with the sample. About 2 mL of sample was withdrawn into the syringe and injected into the sample loop after eliminating air bubbles. This volume was enough to flush the loop several times before filling it with the sample. The machine was then switched to the "inject" mode to start the analysis. Each run was completed in about 8 minutes, and an extra minute was allowed before the next sample was injected. A mixed anion standard was run with every set of samples. Peak areas were used in quantifying the unknown samples.

4.4.5 TP

The persulfate digestion method (Section 4500-P.B.5, APHA, 1989) followed by the ascorbic acid colorimetric method (Section 4500-P.E, APHA, 1989) were used to measure TP. The digestion was performed in an autoclave (30 minutes at 120°C and 14 psig). A Beckman DU®-6 UV-Visible Spectrophotometer (Beckman Instruments, Inc., Irvine, California) was used to perform the colorimetric analysis.

4.4.6 Gases

An HP 5880A Series gas chromatograph (Hewlett-Packard Company, Avondale, Pennsylvania) equipped with a single-filament thermal conductivity detector (TCD) was used for analysis of gas samples. One-eighth inch OD (outer diameter) stainless steel packed columns of varying lengths and containing two different kinds of packings were used for component separation. Identical sample and reference columns were used initially. Both were 2 meters long and were packed with Molecular Sieve 13X (Supelco, Inc., Bellefonte,

Pennsylvania). One of the columns was later replaced with a 10-foot long column packed with 100/120 mesh Carbosieve[1] S-II (Supelco, Inc., Bellefonte, Pennsylvania), primarily in order to improve resolution of early-eluting hydrogen gas.

Two separate sets of analytical conditions were established: one for analyzing hydrogen and methane, and the other for analyzing methane and carbon monoxide. These conditions are listed in Table 5. Samples were withdrawn (through a septum installed at the sampling point) into a 1 mL gas-tight Pressure-Lok[2] syringe (Dynatech Precision Sampling Corporation, Baton Rouge, Louisiana). One of two different sampling techniques was used. In the first method, the syringe was filled to capacity, closed, and transported to the chromatograph. The syringe valve was opened and the sample volume quickly adjusted to the desired value (at 1 atm) just before injection. Alternatively, the exact desired sample volume was withdrawn into the syringe at the "in-situ" sample pressure, and was injected into the machine at that same pressure. A calibration gas of known composition (Scott Specialty Gases, Plumsteadville, Pennsylvania) was injected with each set of samples for each set of analytical conditions. Peak areas (or peak heights in case of poorly resolved peaks) were used in quantifying the unknown samples.

---

[1]Trademark, Supelco, Inc., Bellefonte, Pennsylvania. British Pat. No. 1310422, German Pat. No. 193550. Patent holder - Badische Anilin- & Soda-Fabrik Aktiengesellschaft.

[2]Trademark, Precision Sampling Corp., Baton Rouge, Louisiana.

Table 5. GC Conditions for Analysis of Gases.

Parameter	Hydrogen/Methane Analysis	Methane and Carbon Monoxide Analysis
Carrier and modulator gas	Nitrogen (Grade 4.5)	Helium
Carrier gas flow-rate in each of the two columns, mL/min	30, 20	30, 20
Modulator gas pressure, psig	40, 26	40, 26
Oven temperature	Isothermal: 35°C, 30°C	Programmed: 100°C for 5 mins, increase to 225°C at 25°C/min; or Isothermal: 30°C
Detector temperature	55°C, 50°C	245°C, 50°C
Signal attenuation	2^7 - 2^{10}	2^9 - 2^{10}
Peak threshold	5, 6	6
Peak width	0.04	0.04

4.4.6.1 Extraction of Dissolved Gas

Before chromatographic analysis, vacuum stripping was used to extract gases dissolved in the anaerobic mixed liquor. A mixed liquor sample was drawn into a 20 mL luer-tip syringe. The luer tip was capped with a half-hole septum after carefully expelling all air from the syringe. The mixed liquor volume in the syringe at this point was usually 10 mL. The syringe plunger was then retracted as far as possible and held there, creating a vacuum inside the syringe, which stripped dissolved gas out of the liquid phase. The formation of gas bubbles within the liquid provided visible evidence of stripping. The syringe was gently tapped/shaken several times to dislodge bubbles adhering to the wall, until bubble formation stopped. This happened approximately 3-5 minutes after applying the vacuum. The gas pressure inside the syringe was believed to be low enough to overwhelmingly drive the dissolution equilibrium in the direction of the gas phase. The gas was sampled at the in-situ syringe gas pressure through the half-hole septum covering the luer tip. The volume of stripped gas was noted for calculation purposes.

The validity of this extraction technique was determined by studying both the stripping kinetics and recoveries in a controlled test system. A mixture of hydrogen and methane in nitrogen was equilibrated with distilled water at 20°C for several days in a sealed container equipped with a gas sampling septum. The dissolved-gas concentrations determined by extraction were compared with those calculated by applying Henry's Law to concentrations measured in the gas phase. Also, experiments were performed where extracted gas samples were withdrawn from the 20 mL luer-tip syringe at various time intervals after application of vacuum, and analyzed for hydrogen and methane. The recoveries obtained from the gas-distilled water

equilibrium system are shown in Appendix 5, while the results of the kinetic study are presented in Appendix 6. Distilled water was used in the validation experiments to ensure applicability of Henry's Law. While the actual anaerobic mixed liquor-gas system significantly deviated from Henry's Law (Henry's Law either over- or under-estimated the dissolved gas concentrations at different times), this did not affect the validity of the extraction technique for determination of mixed liquor dissolved gas concentrations.

4.4.7 Ethanol and n-Butanol

A flame ionization detector (FID) installed on the HP 5880A Series gas chromatograph used for gas analysis was used to analyze ethanol and n-butanol. Component separation was achieved using a one-eighth inch OD stainless steel column packed with a 0.2% Carbowax 1500 coating on 80/100 mesh Carbopack C (Supelco, Inc., Bellefonte, Pennsylvania). Grade 4.5 nitrogen was used as the carrier gas at a flow-rate of about 16.7 mL/min. Isothermal runs were used with a 175°C injection temperature, 105°C oven temperature, and 225°C detector temperature. Signal attenuation was set at 2^4, peak threshold at 2, and peak width at 0.04. Injections were made using a 10 μL glass syringe with a fixed needle and a metal wire plunger. The syringe was first rinsed several times with distilled water, then dried with heat and suction. It was then rinsed with the sample several times and overfilled beyond the desired volume (10 μL were used for all injections in this study). All air was expelled from the syringe, the sample volume precisely adjusted, and excess sample lightly wiped from the needle tip just before injection. The syringe was held only at the barrel ends and by the plunger button to avoid thermal expansion and soiled-plunger errors. A mixed aqueous

standard stored at 4°C in a sealed vial with minimal head-space was injected with each set of samples after warming to room temperature. Peak areas were used in quantifying the unknown samples.

4.4.8 OUR

In order to avoid under- or over-estimation that the BOD-bottle method is commonly criticized of, an in-situ technique was used to measure aerobic OUR in this study. Aeration was discontinued and a paddle mixer was switched on to provide just enough agitation to keep the sludge suspended while minimizing dissolution of ambient air. The change in DO was then recorded as a function of time, using a YSI 5739 submersible polarographic membrane probe placed in the center of the aerobic reactor and connected to a YSI Model 57 DO Meter (Yellow Springs Instrument Co., Yellow Springs, Ohio). All flows were continued at their normal rates, except that the aerobic effluent stopped overflowing for a few minutes because of the lowering of the aerobic mixed liquor level caused by the lack of aeration turbulence. For calculation purposes, the time when this flow resumed was set as time zero. Assuming no oxygen entered the mixed liquor from the atmosphere, and knowing the flow-rates and DO values of all streams entering and leaving the aerobic reactor, OUR was calculated from a dynamic non-steady-state oxygen balance around the reactor. The theoretical derivation of the equation used to calculate OUR and a sample calculation are shown in Appendix 4.

4.4.9 DO

All DO values were measured with the same probe - meter combination described in the "OUR" section above. The instrument was polarized and calibrated according to the manufacturer's instructions before use.

4.4.10 pH

All pH measurements were made with a Fisher Accumet Model 610A pH Meter (Fisher Scientific Company, Pittsburgh, Pennsylvania). Manufacturer's instructions were followed in making the measurements.

4.5 Calculation of Clarifier OUR

The clarifier OUR was calculated as follows:

Assuming there is no oxygen exchange between the atmosphere and the clarifier contents, a steady-state oxygen balance around the clarifier requires:

Aer effluent flow * Aer effluent DO = Eff flow * Eff DO + RAS flow * RAS DO + Clarifier volume * Clarifier OUR

All flows are in mL/min, DO concentrations in mg/L, clarifier volume in mL, and OUR in mg/L/min. All quantities in the above equation except the clarifier OUR were known for each sampling run. The clarifier OUR was calculated by substituting appropriate values.

4.6 Statistical Validation of AnS Significance

Statistical simulation of the Boundary Exchange AnS Model was performed using the Latin Hypercube method to sample input distributions, which were all assumed to be triangular. Latin Hypercube sampling was chosen as a superior stratified method designed to more accurately recreate the input distribution in fewer iterations than the traditional completely random Monte Carlo Sampling (detailed descriptions of each method are provided by Palisade Corporation, 1989). The minimum, mean (assumed to be the "most likely" value), and maximum values defining these triangular distributions were all obtained from actual steady-state data collected under the conditions being considered. Separate simulations were performed for each set of conditions, which coincided with a single numbered operating phase. The values of the arguments used to define the triangular distribution for each model input parameter during each operating phase simulated are listed in Appendix 7.

The simulations resulted in an output AnS distribution and summary statistics for the distribution for each phase. The summary statistics included the mean, standard deviation, and cumulative probability distribution tables (including the probability that $AnS < 0$) for the empirical output distribution. These statistics were used to draw inferences about the statistical significance of the numbers produced by the model. The risk analysis and simulation add-in for Lotus Development Corporation's 1-2-3 spreadsheet, @RISK, developed by the Palisade Corporation, Newfield, New York, was used to perform the simulations. Details of the triangular distribution and simulation procedure are provided in the @RISK Users Guide (Palisade Corporation, 1989).

4.7 Aerobic Stripping of Reduced Volatiles: Batch Experiments on the A2/O System

4.7.1 Stripping Experiments

Samples of anaerobic and anoxic mixed liquors were pressure-filtered through a filter disc-60 mL plastic luer-tip syringe combination to eliminate possible losses of reduced volatiles during vacuum filtration. A small volume (60-95 mL) of the filtrate was then aerated for 120 minutes. Filtrate samples were analyzed for COD at several pre-determined intervals (0, 15, 30, 60, and 120 minutes) during aeration. Two traps were used to remove possible contaminants form the air used for aeration. The air was first passed through a column of granular carbon retained between cotton plugs, and then bubbled through distilled water before introduction into the filtrate. The filtration and aeration steps were accomplished using one of two different procedures termed "Open System" and "Closed System" as described below.

4.7.1.1 Open System Stripping

A 500 mL erlenmeyer flask was completely filled with the mixed liquor sample and sealed gas-tight without any air-bubbles with a plastic film stretched across the top. About 15 minutes were allowed for the biomass to settle, and the supernatant was then pipetted off, with minimum turbulence or aeration, into a 100 mL beaker. The settling step was necessary to achieve filtration in the least possible time given the limited capacity of a single filter. This supernatant was then pressure-filtered, first through a glass fiber filter, and then through a

0.2μ or 0.45μ membrane filter. Turbulence and aeration were minimized as much as possible throughout this procedure, as was the time required for filtration. The aeration step was started immediately with about 60 mL of the final filtrate.

4.7.1.2 Closed System Stripping

The set-up used for closed system stripping is shown in Figure 5. A mixed liquor sample was withdrawn directly into the filtration syringe, which was immediately connected to the inlet of the filter disk. The air supply line to the empty erlenmeyer flask was closed at this time, so that any volatiles escaping from the sample would have to pass through the COD trap. The syringe was held vertically for about 15 minutes with the filter disk at the top, allowing biomass to settle. The supernatant and as much of the rest of the sample as possible were then pushed through the filter disk into the flask. The air displaced from the flask was again routed to the COD trap. Only a single-step filtration through a glass fiber filter was used in this case. Two such syringe-fulls of mixed liquor sample provided between 60 and 95 mL filtrate. The filtrate inlet to the flask was then closed and the air line was opened to initiate aeration. Filtrate samples were analyzed for COD at 0, 15, 30, 60, and 120 minutes. In addition, the trap was kept online for the duration of aeration, and the trap COD was determined against an identical blank directly receiving aeration air.

4.7.2 Search for Ethanol and n-Butanol

Ethanol and n-butanol were considered probable fermentation products in a BPR system. Anaerobic and aerobic mixed liquors from the A2/O system were therefore analyzed for these

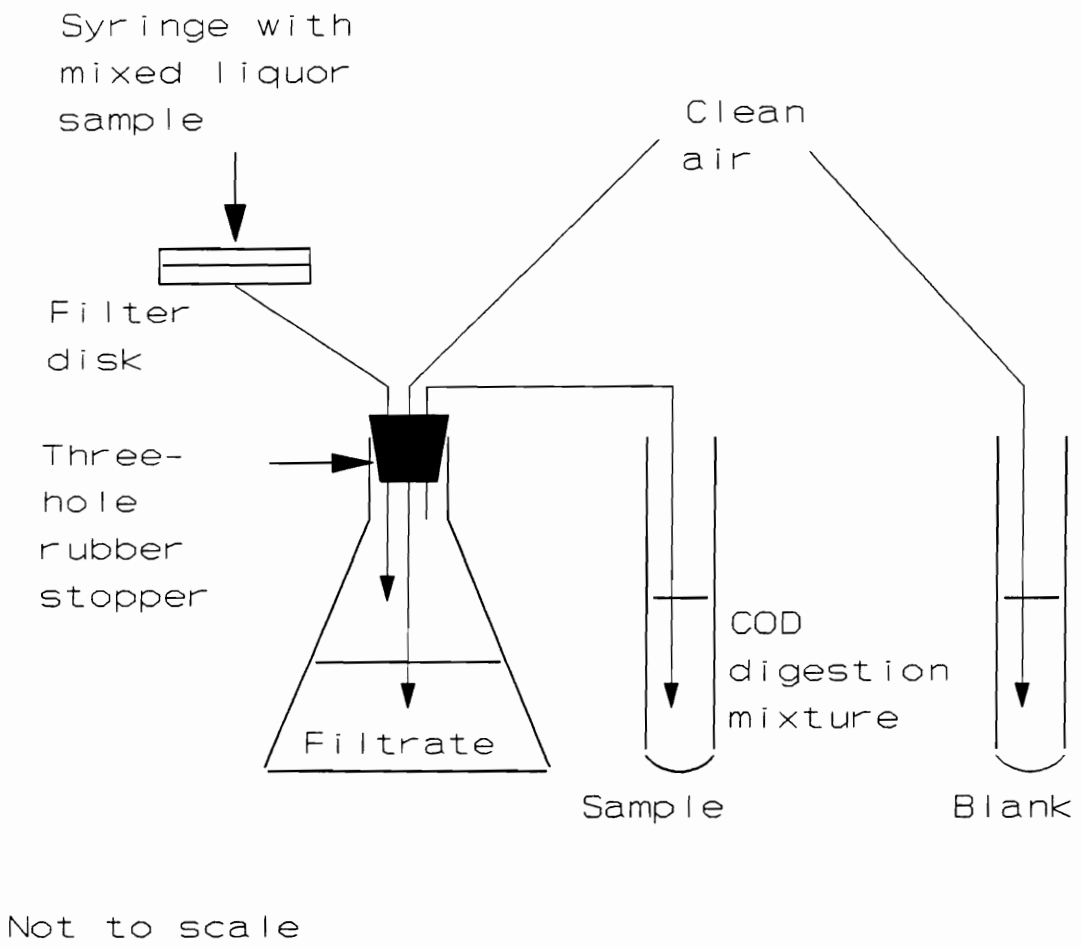


Figure 5. The Closed System Used for Stripping Experiments with Mixed Liquor from the A2/O System.

alcohols in an attempt to identify some of the reduced volatiles possibly being stripped from the system.

Samples were collected in 5mL glass vials. The vials were completely filled and sealed gas tight with plastic film without any air bubbles. About 15 minutes were allowed for the biomass to settle before sampling and analyzing the supernatant with a 10 μL syringe as described earlier.

4.8 Limitations of the COD Test

If it is indeed true that the COD test is subject to limitations, then these must be manifested either as lower-than-theoretical COD values for one or more products in the system or as an increase in calculated COD with increasing digestion time. Experiments were undertaken to investigate both possibilities.

NAD and NADH were chosen as possible oxidation resistant biosynthetic products that might be subject to thermodynamic limitations. The reduced and oxidized forms were purchased from the Sigma Chemical Company, and subjected to the COD determination procedure. The measured COD values were compared with theoretical values for complete oxidation. A similar comparison was performed for dextrose.

To determine whether the digestion time affected the resulting COD values, split samples from each A/O process stream were digested for 2, 12, and 24 hours, and the resulting COD values were compared.

5 Results

Results are presented separately for the A/O and A2/O systems, except where the logical development of an idea is based on selected data from each system. Results of batch experiments are also presented separately.

5.1 A/O Data Screening and Selection

Appendix 8 is a comprehensive tabulation of all raw data obtained for the A/O system over the study period. Data representing reasonable steady-states for various sets of conditions (the previously defined phases) were extracted by subjecting the raw data to a thorough, careful screening. The screening procedure assigned a steady-state code to each data point. These codes are listed in the "Process Control and Operating Conditions" section of Appendix 8. A steady-state code value of zero was given to all data points obtained less than 3 BSRTs after the last change in operating conditions. The phase number was prefixed with a minus sign where the system had not stabilized after 3 BSRTs. The remaining points received positive steady-state codes equal to the phase number, and were considered to represent steady-state conditions. Aside from A2/O and batch data, only steady-state points with AnS values available are shown in the results presented in this chapter.

5.2 A/O Phosphorus Removal

Table 6 shows the changes in influent and effluent filtrable ortho-phosphate (FOP) values over the study period. The table shows that the system removed about 17 mg/L FOP at the end of Phase 4 (around 8/15/90). The sludge contained more than 10 percent phosphorus on a VSS basis at this time. This demonstrated the ability of the A/O system to accomplish phosphorus removal in the presence of 2-imidazolidinethione, the nitrification inhibitor.

5.3 Comparison of AnS Determination Methods: Boundary Exchange Vs Unit Process

Figure 6 compares the oxygen uptake rate in the clarifier with that in the aerobic reactor of the A/O system over the study period. The aerobic OUR in this figure was determined using the direct in-situ measurement technique, while the clarifier OUR was calculated from an oxygen balance around the clarifier, both described earlier. It is clear that the aerobic OUR was always higher than the clarifier OUR, by a factor of 3 on the average. The Unit Process method assumption of equal clarifier and aerobic OURs thus significantly overestimated the clarifier OUR, which resulted in underestimation of the absolute AnS value (the calculated AnS value was higher, or less negative, than the true value). This underestimation and the otherwise excellent correlation between the results of the two methods are evident in the scatterplot in Figure 7.

Table 6. Changes in the A/O System Influent and Effluent FOP Demonstrating Excess BPR.

Date	Phase	BSRT, days	Inf FOP, mg/L	Eff FOP, mg/L	P removal mg/L	Waste sludge P/VSS
02-Apr-90	3	10.1	12.6	0.9	11.7	0.070
07-May-90	4	11.1	22.7	8.5	14.2	0.080
11-Jun-90	4	9.0	22.8	11.5	11.4	0.094
18-Jun-90	4	12.4	23.0	6.1	16.9	0.074
25-Jun-90	4	10.7	21.4	6.2	15.2	0.112
09-Jul-90	4	10.5	21.6	6.1	15.4	0.105
16-Jul-90	4	9.7	22.1	6.0	16.0	0.107
23-Jul-90	4	10.2	22.7	5.7	16.9	0.107
06-Aug-90	4	10.0	23.4	5.7	17.7	0.105
13-Aug-90	4	10.5	23.9	7.2	16.6	0.102
17-Sep-90	5	9.9	17.6	3.1	14.5	0.096
24-Sep-90	5	9.6	17.0	1.7	15.4	0.105
08-Oct-90	5	9.4	17.8	2.8	15.0	0.091

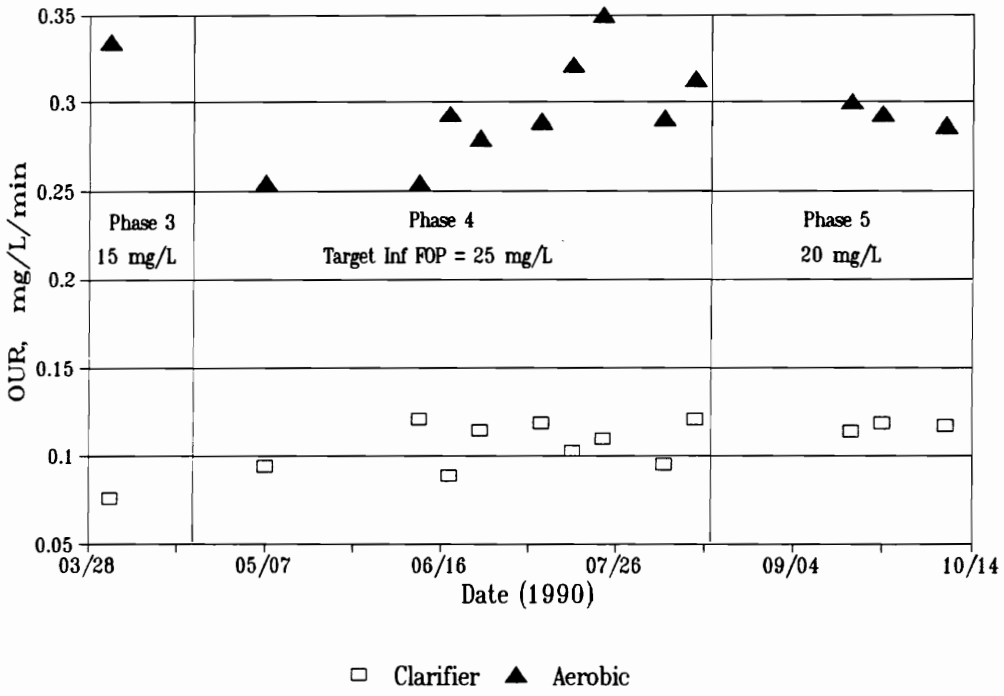


Figure 6. Comparison of A/O Clarifier and Aerobic Oxygen Uptake Rates.

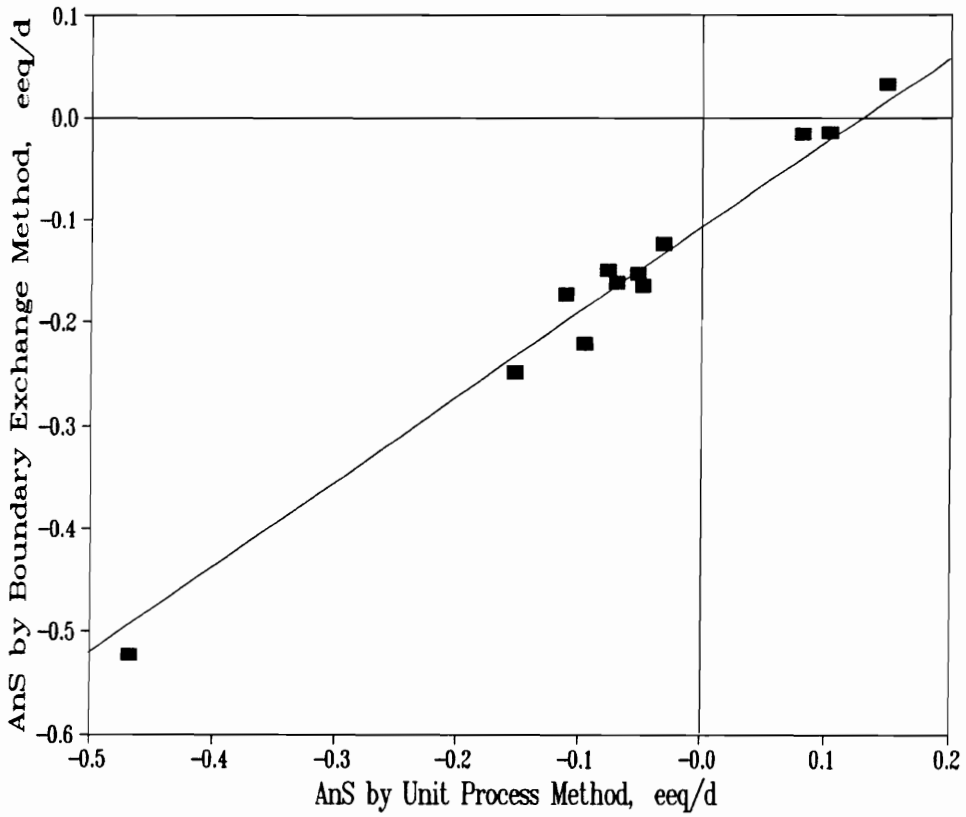


Figure 7. Correlation between AnS Values from the Boundary Exchange and Unit Process Methods Applied to the A/O System.

5.4 Statistical Validation of AnS Significance in the A/O System

Table 7 lists the calculated anaerobic stabilization values in eeq/d and as fractions of the corresponding total stabilization (AnS/TSt). Barring a few exceptions, the AnS values were negative and the AnS/TSt values were positive. AnS was about 20 percent of TSt at the end of Phase 4.

Statistical data (means, standard deviations, probabilities of negative result, and shapes) for AnS distributions obtained through Latin Hypercube simulation of the Boundary Exchange model are shown in Table 8. Only steady-state data sorted by phase were used to define the input distributions.

Table 8 shows that in spite of the high standard deviations, the means were consistently negative. Phase 5 showed AnS significant at a 95% significance level, while the Phase 4 AnS value was significant at the slightly lower 89% level.

5.5 Redox Balance Components

Figure 8 and Figure 9 are graphs of the individual components of the generalized electron balance equation as applied to the A/O system. The graphs show the relative significance of the contribution of each component in the overall balance. Using the same convention and base oxidation states used in the Boundary Exchange method, the individual contributions of DO, COD, TKN, nitrite-N, nitrate-N, and sulfate to the oxidizing capacity of each of the

Table 7. Anaerobic Stabilization in the A/O System.

Date	Phase	BSRT, days	Boundary Exchange AnS, eeq/d	Boundary Exchange AnS/TSt
02-Apr-90	3	10.1	-0.02	0.01
07-May-90	4	11.1	-0.52	0.37
11-Jun-90	4	9.0	-0.17	0.16
18-Jun-90	4	12.4	-0.22	0.18
25-Jun-90	4	10.7	-0.15	0.13
09-Jul-90	4	10.5	-0.15	0.13
16-Jul-90	4	9.7	-0.13	0.11
23-Jul-90	4	10.2	0.03	-0.03
06-Aug-90	4	10.0	-0.02	0.02
13-Aug-90	4	10.5	-0.16	0.13
17-Sep-90	5	9.9	-0.16	0.13
24-Sep-90	5	9.6	-0.12	0.11
08-Oct-90	5	9.4	-0.25	0.20

Table 8. A/O Phasewise Statistical Data for Output AnS Distributions Obtained Using Latin Hypercube Sampling of Assumed Triangular Input Parameter Distributions to Simulate the Boundary Exchange AnS Model.

A/O Steady-state phase*	Target Inf FOP, mg/L	AnS, eeq/d		Probability that AnS < 0	General shape of AnS probability distribution
		Mean	Standard deviation		
4	25	-0.17	0.14	0.886	Unimodal, skewed to left
5	20	-0.18	0.03	> 0.999	Unimodal, skewed to left

*Only steady-state phases with AnS data available were subjected to simulation.

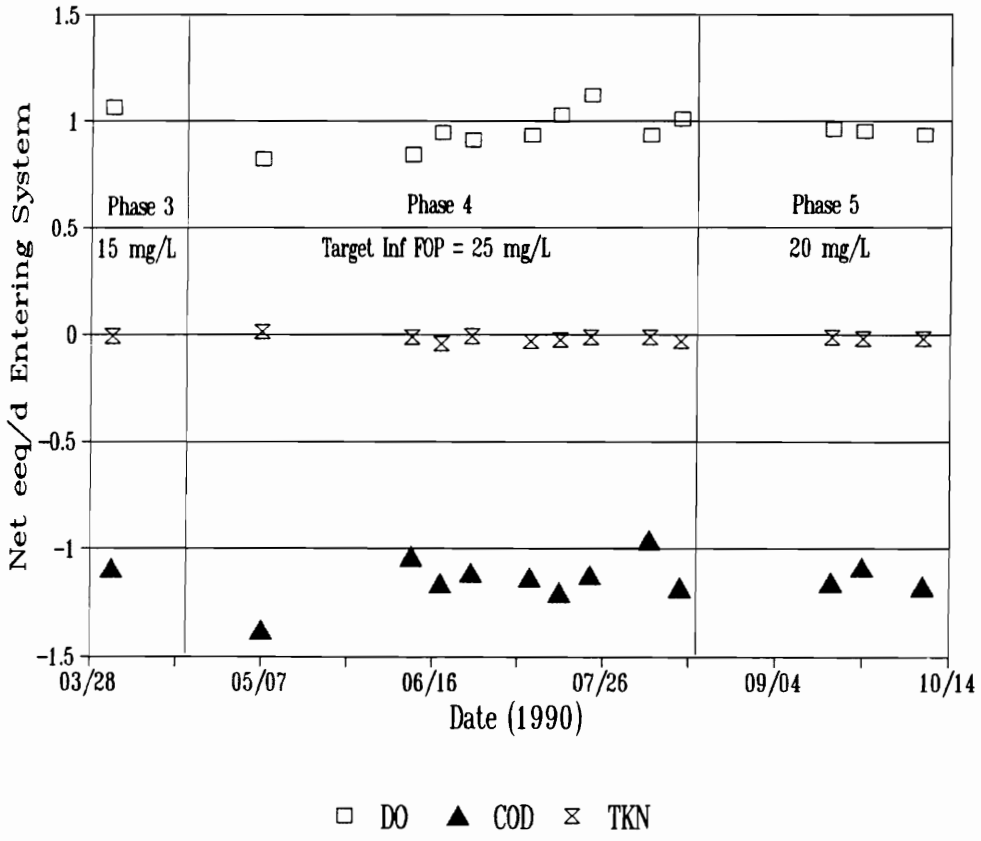


Figure 8. DO, COD, and TKN Contributions to AnS in the A/O System.

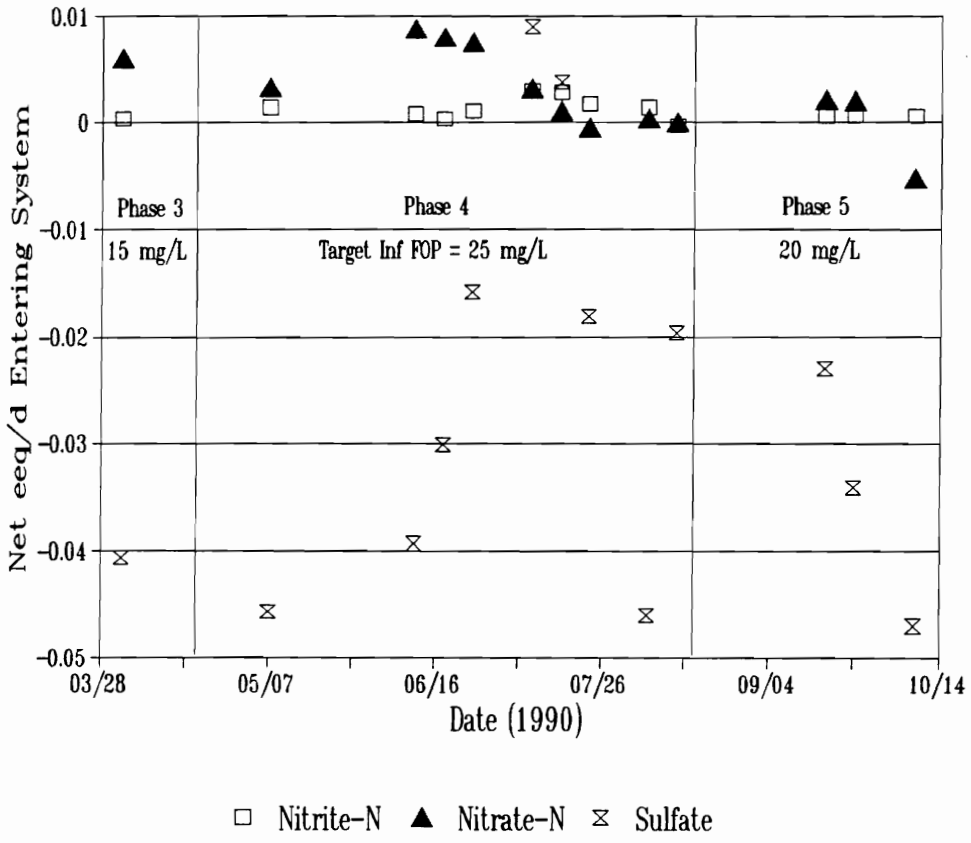


Figure 9. Nitrite, Nitrate, and Sulfate Contributions to AnS in the A/O System.

streams Inf, WAS, Eff, and Air were first calculated separately in the common units of eeq/d. These numbers were then used to calculate the net oxidizing capacity *entering* (Inf+Air-Eff-WAS) the system with each component.

It can be seen that TKN, nitrite-N, and nitrate-N made no significant contribution, as expected with chemical inhibition of nitrification. DO made the only positive contribution, which averaged around +1 eeq/d with little variation. COD was the major negative contributor, averaging approximately -1.2 eeq/d over the study period. The sulfate contribution varied between -0.01 and -0.05 eeq/d. AnS was believed to occur because COD and, to a lesser extent, reduced sulfur together introduced more reducing capacity into the system than the oxidizing capacity provided by oxygen. The contribution made by the reduced sulfur - sulfate redox pair was smaller than that made by either COD or oxygen, but was significantly different from zero. Figure 10 shows the anaerobic reduction of influent sulfate and confirms the small and variable but non-zero contribution of sulfur to the overall redox balance.

5.6 Alternate Expressions of AnS

Figure 11 and Figure 12 show the variation in the A/O AnS expressed as equivalent hydrogen and methane gas production, which may be easier to comprehend than the unfamiliar units of eeq/d. Figure 11 graphs the equivalent production as the concentration of each gas in the anaerobic mixed liquor required to explain all of the observed AnS. Figure 12 expresses the equivalency in the form of the required mass flow rate of each gas out of the system. Hydrogen production is assumed to be the result of proton reduction. Similar hypothetical

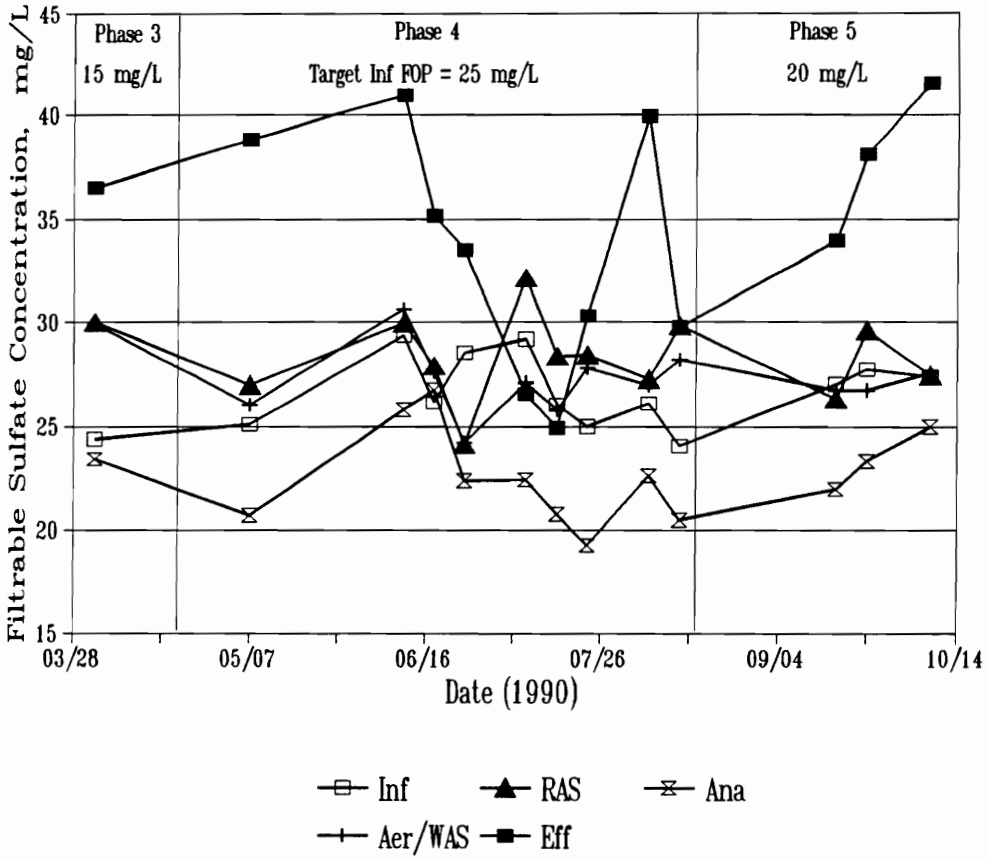


Figure 10. Variation in the Sulfate Concentrations in the A/O Process Streams over the Study Period.

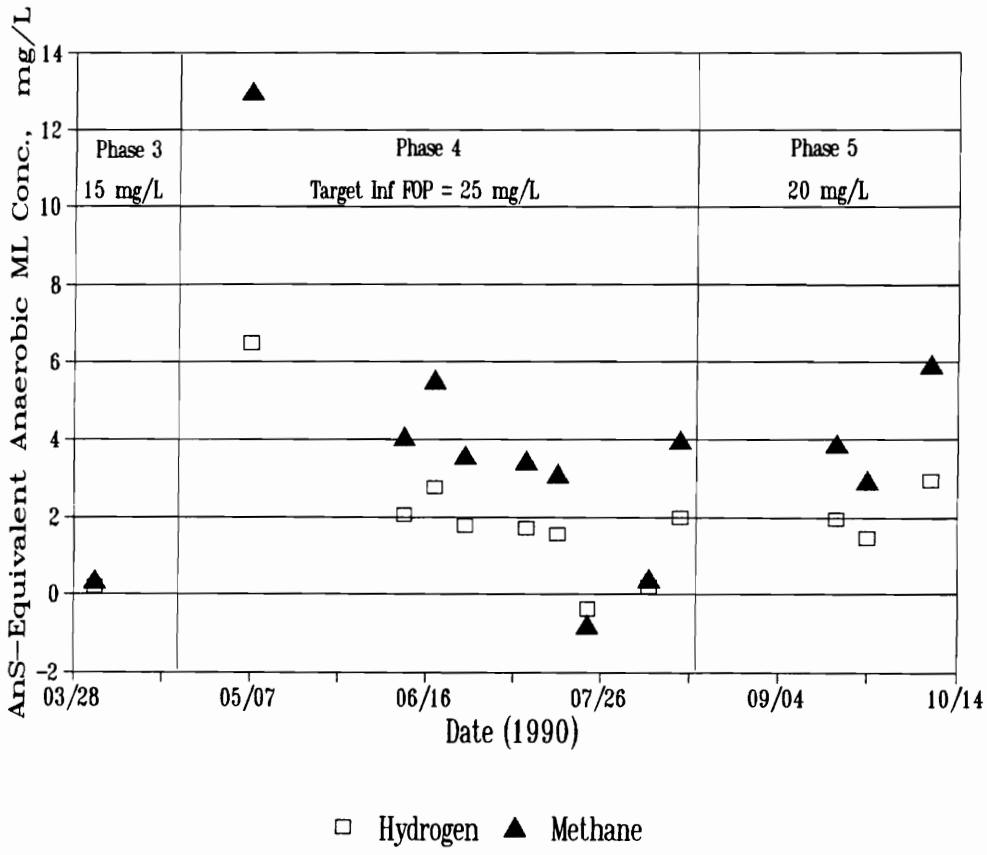


Figure 11. Anaerobic Stabilization in the A/O System Expressed as Equivalent Hydrogen and Methane Concentrations in the Anaerobic Mixed Liquor.

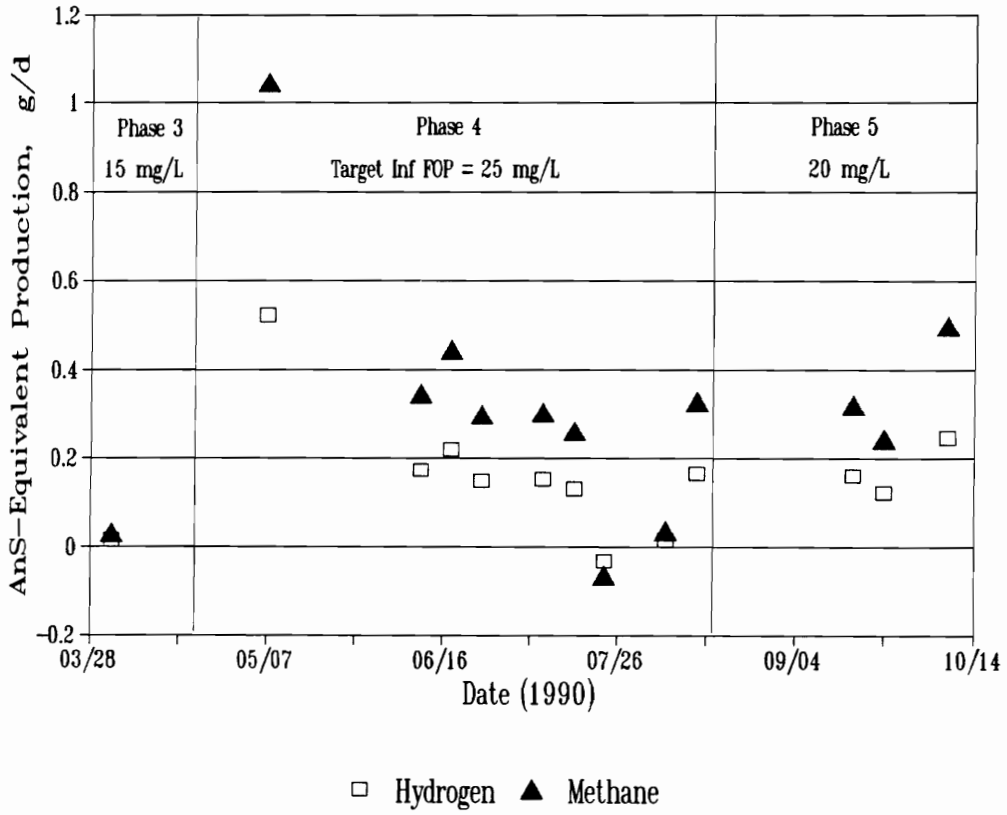


Figure 12. Anaerobic Stabilization in the A/O System Expressed as Equivalent Hydrogen and Methane Mass Flow Rates Leaving the System.

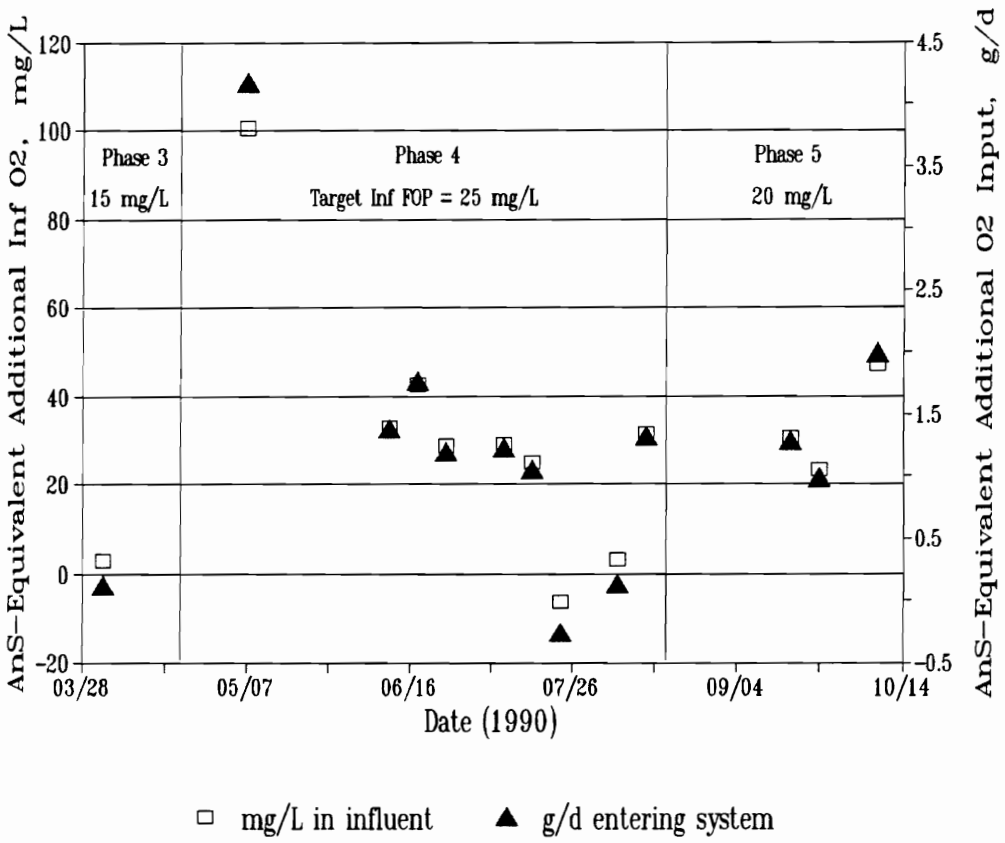


Figure 13. Anaerobic Stabilization in the A/O System Expressed as Equivalent Additional Oxygen Input.

influent oxygen concentration and mass flow-rate values are shown in Figure 13. In the case of oxygen, the values graphed must be in addition to the actually measured dissolved oxygen input to the system, which is already considered in AnS calculations.

5.7 BPR and AnS in the A2/O System

Table 9 shows the AnS and P removal performance of the A2/O system between December 24, 1991 and January 3, 1992. The table shows clear evidence of both significant AnS and P removal during this period. Between 41 and 54 percent of the TSt in the system was due to AnS. At an influent TP of about 30 mg/L, from 16 to 26 mg/L of TP removal was observed across the system, resulting in a waste sludge P content of 6-10 percent based on VSS.

5.8 Explanations for AnS

This section describes the results of experiments conducted to investigate the specific possible AnS explanations listed in Table 1, in fulfillment of Objective 5. Data are presented from both the A/O and A2/O systems.

5.8.1 Anaerobic Reduced Gas Production

The A/O anaerobic off-gas consisted mainly of nitrogen and carbon dioxide, with small amounts of hydrogen (partial pressures ranging from 0 to 0.05 m.atm) and methane (0 to 0.6 m.atm). Nitrogen and carbon dioxide were qualitatively detected, but not quantified because they did not affect the redox balance. Oxygen peaks resulting from unavoidable atmospheric

Table 9. AnS and P Removal Data for the A2/O System.

Date	AnS, eeq/d	AnS/TSt	Influent TP, mg/L	Effluent TP, mg/L	TP removal, mg/L	Waste sludge P/VSS
24-Dec-91	-2.42	0.54	28.3	2.2	26.1	0.063
31-Dec-91	-1.72	0.41	30.6	9.3	21.3	0.087
03-Jan-92	-2.00	0.42	31.9	15.9	16.0	0.101

contamination during sampling and injection (a small but finite amount of ambient air, which could not be expelled even with the plunger completely depressed, was always present in the needle) were not quantified either. The GC analysis focused on quantification of hydrogen and methane, mainly in the anaerobic mixed liquors of the A/O and A2/O systems.

5.8.1.1 Validation of Dissolved Gas Extraction Technique

The fractional recoveries obtained from the vacuum stripping dissolved gas extraction procedure applied to the validation system with a distilled water liquid phase are presented in Appendix 5. Average recoveries of about 86 and 88 percent were obtained for hydrogen and methane respectively. These recoveries compare favorably with those reported by Schauer and Ferry (1980) for a different dissolved hydrogen estimation procedure.

Results of the extraction kinetics study are shown in Appendix 6. The figures show that except for Run 1, only small changes occurred in the hydrogen and methane peak areas for extracted gas samples withdrawn between 2 and 15 minutes after vacuum application. The large decrease between 2 and 5 minutes in Run 1 is believed to be the result of leakage through a bad septum that compromised the seal after the first puncture. It may be concluded from these results that the routinely used sampling time of 3-5 minutes after vacuum application was adequate to reach near-equilibrium conditions.

5.8.1.2 A/O Hydrogen Production

Table 10 shows the variations in the A/O anaerobic gas space hydrogen partial pressure, the anaerobic mixed liquor dissolved hydrogen concentration calculated from Henry's Law, and

Table 10. A/O Anaerobic Hydrogen Gas Production over the Study Period.

Phase	Date 1990	Ana gas hydrogen partial pressure, m.atm	Henry's Law-hydrogen conc. in Ana ML, ug/L	Henry's Law Ana hydrogen production, meq/d
3	04/02	0	0	0
4	06/11	0	0	0
4	06/18	0	0	0
4	06/25	0	0	0
4	07/09	0	0	0
4	07/16	0	0	0
4	07/23	0	0	0
4	08/06	0	0	0
4	08/13	0	0	0
5	09/17	0	0	0
5	09/24	0.05	0.07	-0.01
5	10/08	0	0	0

Table 11. A/O Phasewise Percentages of AnS Explained by Hydrogen Gas Production/Evolution.

Phase	AnS, eeq/d	Measured H2 production, meq/d	Henry's Law H2 production, meq/d	* Percent AnS explained
3	-0.02		0.00	0.00
4	-0.17		0.00	0.00
5	-0.18		0.00	0.00
Ac	-0.20		-0.04	0.02
Fm	-0.23	0.00	-0.03	0.01

* Actually measured values used when available and non-zero, else used Henry's Law.

the oxidizing capacity of the hydrogen produced. There was an isolated instance of small but non-zero hydrogen production. The phasewise percentages of AnS explained by hydrogen production are shown in Table 11. The phases "Ac" and "Fm" represent the presence in the influent of acetate and formate, respectively.

5.8.1.3 A/O Methane Production

Table 12 and Table 13 present similar results for anaerobic methane production. A relatively large AnS fraction (over 18 percent) was explained by methane production when formic acid was present in the feed.

5.8.1.4 A/O Combined Reduced Gas Production

The combined hydrogen and methane production and its contribution to AnS are shown in Table 14. It is noteworthy that a significantly higher fraction of AnS could be explained by methane production resulting from formic acid in the influent than by either hydrogen or methane production with acetate.

5.8.1.5 Hydrogen and Methane Production in the A2/O System

The actually measured anaerobic mixed liquor dissolved hydrogen and methane concentrations in the A2/O system are compared with those theoretically required to explain calculated AnS in Table 15. No hydrogen or methane peaks were detected in the actual samples. Tests performed in this study indicated that the procedure used for mixed liquor hydrogen and methane analysis had detection limits of no greater than 0.001 mg/L for hydrogen and 1.0 mg/L for methane. Past experience (Ferry, 1992) supports these high sensitivities.

Table 12. A/O Anaerobic Methane Gas Production over the Study Period.

Phase	Date 1990	Ana gas methane partial pressure, m.atm	Henry's Law-methane conc. in Ana ML, ug/L	Henry's Law Ana methane production, meq/d
3	04/02	0	0	0
4	06/11	0	0	0
4	07/23	0	0	0
5	09/24	0.6	14.6	-0.6
5	10/08	0	0	0

Table 13. A/O Phasewise Percentages of AnS Explained by Methane Gas Production/Evolution.

Phase	AnS, eeq/d	Measured CH4 production, meq/d	Henry's Law CH4 production, meq/d	* Percent AnS explained
3	-0.02		0.0	0.0
4	-0.17		0.0	0.0
5	-0.18		-0.3	0.2
Ac	-0.20		-1.6	0.8
Fm	-0.23	-43.5	-67.3	18.8

* Actually measured values used when available and non-zero, else used Henry's Law.

Table 14. A/O Phasewise Percentages of AnS Explained by the Combined Anaerobic Production of Hydrogen and Methane.

Phase	AnS, eeq/d	Combined measured H2 and CH4 production, meq/d	Combined Henry's Law H2 and CH4 production, meq/d	* Percent AnS explained
3	-0.02		0.0	0.0
4	-0.17		0.0	0.0
5	-0.18		-0.3	0.2
Ac	-0.20		-1.7	0.8
Fm	-0.23	-43.5	-67.3	18.8

* Actually measured values used when available and non-zero, else used Henry's Law.

Table 15. Comparison of Actual and Theoretical (Required to Explain AnS) Dissolved Hydrogen and Methane Concentrations in the Anaerobic Mixed Liquor of the A2/O System.

AnS, eeq/d	Theoretical hydrogen conc., mg/L	Actual hydrogen conc., mg/L	Theoretical methane conc., mg/L	Actual methane conc., mg/L
-1.7 to -2.4	13.9 to 19.1	0.0	27.2 to 38.1	0.0

5.8.2 Aeration-Induced Stripping of Reduced Volatiles: Batch Experiments with the A2/O System

5.8.2.1 Decrease in Anaerobic/Anoxic FCOD

Table 16 and Figure 14 show results of stripping experiments conducted on pressure-filtered anaerobic and anoxic mixed liquor samples from the A2/O system. It is clear that there was no significant change in the sample COD over the aeration period of two hours. No significant COD accumulation was recorded in the traps either. A maximum COD decrease of 7 mg/L (from 93 to 86) was recorded on January 3, 1992, which explained about 6 percent of the -2.0 eeq/d AnS value calculated for that day.

5.8.2.2 Ethanol/n-Butanol Production

Table 17 shows the results of attempts to quantify dissolved ethanol and n-butanol concentrations in the A2/O anaerobic and anoxic mixed liquors. The table shows that, compared to the theoretical concentrations, no significant ethanol or n-butanol production was seen in the system.

5.8.3 Dissolution of External Oxidant

The possibility of dissolution of an external gaseous oxidant into the A/O system was minimized by design. Since the anaerobic reactor was completely sealed, such dissolution could occur only through the feed, aerobic aeration, or the clarifier. Further, the Boundary

Table 16. Changes in the COD of Pressure-Filtered Anaerobic and Anoxic Mixed Liquor Samples from the A2/O System as a Function of Aeration Time with Two Different Filtration Systems.

Date-->	12/28/91	12/30/91	01/01/92	01/02/92	01/03/92
Sampling location-->	Anoxic	Anoxic	Anoxic	Anaerobic	Anaerobic
Filtration system type-->	Open	Open	Open	Closed	Closed
Filter type-->	Membrane	Membrane	Membrane	Glass fiber	Glass fiber
Filter pore size-->	0.2u	0.2u	0.45u	NA	NA
Approx. initial filtrate volume, mL-->	60	55	60	60	95
0 min COD, mg/L	26	30	25	104	93
15 min COD, mg/L	NA	NA	NA	108	93
30 min COD, mg/L	32	32	26	109	94
60 min COD, mg/L	32	33	27	109	89
120 min COD, mg/L	33	31	26	111	86
Trap COD, mg	NA	NA	NA	0.057	0.137

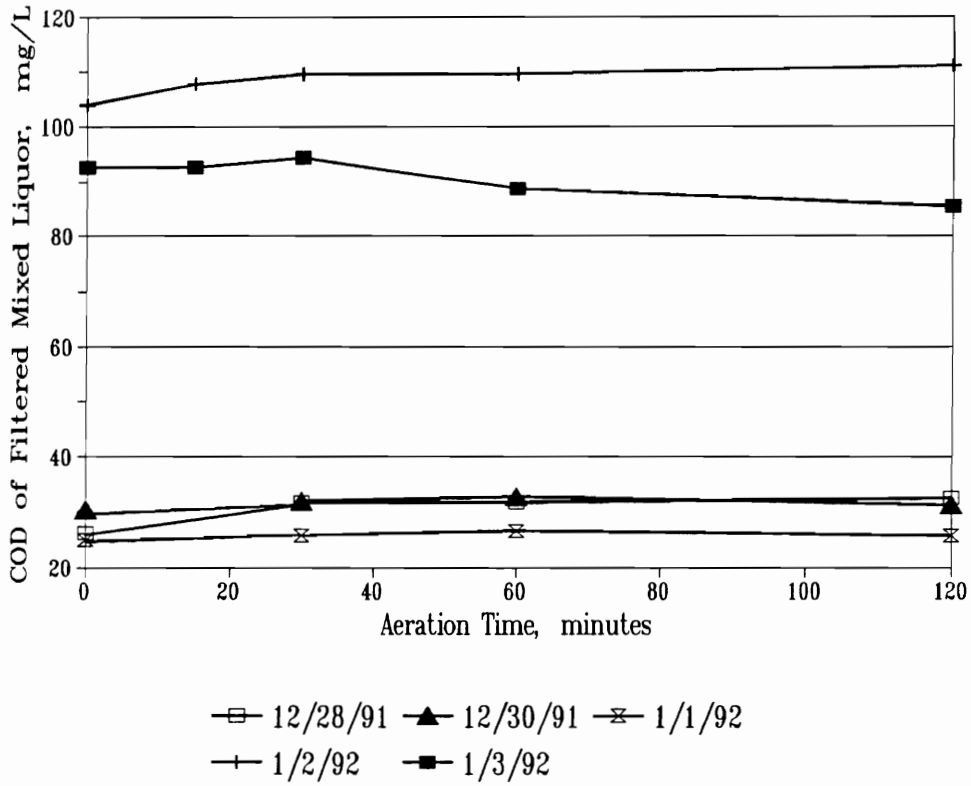


Figure 14. Changes in the COD of Pressure-Filtered Anaerobic and Anoxic Mixed Liquor Samples from the A2/O System as a Function of Aeration Time with Two Different Filtration Systems.

Table 17. Comparison of Actual and Theoretical (Required to Explain Observed AnS) Dissolved Ethanol and n-Butanol Concentrations in the Anaerobic and Anoxic Mixed Liquors of the A2/O System.

AnS, eeq/d	Theoretical Ana ML ethanol conc., mg/L	Actual Ana ML ethanol conc., mg/L	Theoretical Anx ML ethanol conc., mg/L	Actual Anx ML ethanol conc., mg/L	Theoretical Ana ML butanol conc., mg/L	Actual Ana ML butanol conc., mg/L	Theoretical Anx ML butanol conc., mg/L	Actual Anx ML butanol conc., mg/L
-1.7 to -2.4	53.1 to 73.1	0.2 to 0.4	26.5 to 36.5	0.00 to 0.12	42.7 to 58.8	0.0	21.4 to 29.4	0.0

Note: All actual values were for unfiltered samples, except one, where the samples were pressure-filtered through a 0.45μ membrane filter.

Exchange Method automatically accounts for the dissolution not only of oxygen, but also of molecular nitrogen gas and carbon dioxide, if any. Dissolution of an external oxidant was therefore not considered a realistic possibility in this study.

Leakage of oxygen into anaerobic systems is known to be a persistent problem, even with elaborate precautions. This was a potential concern in the A/O system, especially because of the vacuum in the anaerobic reactor. To account for AnS = -0.2 eeq/d (approximate average over the study period), however, about 5.7 L/d of atmospheric air would have to leak into the system. Such a high leakage rate was considered highly unlikely. Oxygen leakage was therefore not considered to be a significant problem in this study.

5.8.4 Limitations of the COD Test

5.8.4.1 Oxidizing Capacities of NAD, NADH, and Dextrose

The results of COD tests on NAD, NADH, and dextrose are presented in Table 18. The numbers in the table represent COD expressed as eeq/mol of each compound. Theoretically, the difference between the oxidizing capacities of the oxidized and reduced forms of NAD should be -2 eeq/mol. Actual measurements, however, yielded an average difference of -17 eeq/mol, significantly different from the theoretical value. Also, the experimentally determined individual NAD and NADH oxidizing capacities were only about 50 percent of the corresponding theoretical values. The experimentally determined oxidizing capacities of

Table 18. Comparison of Theoretical and Experimental Oxidizing Capacities of NAD, NADH, and Dextrose.

Date	eeq/mol compound NAD	eeq/mol compound NADH	Difference NADH-NAD	eeq/mol compound Dextrose
Theoretical	-128	-130	-2	-24
Experimental				
08/03/89				-24
08/20/89				-22
08/23/89				-26
03/23/90	-53	-67	-14	
06/08/90	-50	-69	-19	
02/13/91	-52	-69	-18	
Experimental average	-52	-68	-17	-24

dextrose, on the other hand, closely matched the theoretical value of -24 eeq/d.

5.8.4.2 Digestion Time Effects

The effects of three different COD digestion times (2, 12, and 24 hours) on the resulting TCOD values are shown in Table 19 for samples drawn from the A/O system. Because of the larger time difference, the 2-24 comparison was considered more reliable and was performed more often, resulting in a larger number of samples. Since limitations cannot cause a lower COD value with longer digestion, negative values in Table 19 were considered to represent the absence of such limitations. Average increases of about 3 and 2 percent were recorded, respectively, for influent and effluent samples in the 2-24 comparison.

5.8.5 Combined Effect of All AnS Explanations Studied

Table 20 summarizes the results of all experiments performed in this study to investigate possible AnS explanations.

5.9 Sulfur Imbalance in the A/O System During VFA Addition to Influent

As a matter of interest to researchers planning further AnS investigations, it is important to mention here the observed effect of influent VFAs on the sulfur balance across the A/O system. Unusually high effluent sulfate concentrations (but "normal" aerobic sulfate concentrations) were recorded when the influent contained a VFA. This effect was observed with acetic, formic, iso-valeric, and propionic acids. Elemental sulfur could not be balanced

Table 19. Streamwise Effects of COD Digestion Times of 2, 12, and 24 Hours on Measured TCOD Values of Samples from the A/O System.

Stream	Average fractional COD increase caused by increasing digestion time from 2h to 12h	Average fractional COD increase caused by increasing digestion time from 2h to 24h	Average fractional COD increase caused by increasing digestion time from 12h to 24h	Sample size for 2-12 average	Sample size for 2-24 average	Sample size for 12-24 average
Inf	0.009	0.028		2	6	0
RAS	0.005	-0.016	-0.027	6	7	2
Ana	0.012	-0.017	0.078	6	8	2
Aer	-0.002	-0.009		7	7	0
Eff	0.064	0.021		4	4	0

Table 20. Summary of Results for All Possible AnS Explanations Studied.

Explanation	Approximate percent AnS explained based on A/O system results	Approximate percent AnS explained based on A2/O system results
Hydrogen production	<1	0
Methane production	<1*	0
Stripping of reduced volatiles, Ethanol/n-butanol production	-	≤6
Oxygen leakage into system	Probably insignificant	
Limitations of the COD test	Possibly significant based on preliminary results	
Combination of all explanations	<2*	≤6
Unexplained AnS	>98*	≥94

*Except during formate addition to the A/O influent.

across the system under these conditions (calculated output was significantly higher than calculated input). No explanation was found for the high effluent sulfate values, nor was it clear why the increase occurred in the clarifier. The data collected under these conditions were not considered reliable and are not presented in this document.

6 Discussion

The results of this study indicate that several of the possible explanations for AnS identified in Table 1 were partial contributors to the AnS observed. Limitations of the COD test could not be fully quantified or conclusively shown to contribute to AnS in this study, but were indicated as a possibility. These results and those relating to the other objectives of this study are interpreted and discussed in greater detail in this chapter.

6.1 Advantages of the Boundary Exchange AnS Determination Method

The following advantages of the Boundary Exchange Method over the Unit Process Method were established in this study:

1. Eliminated the need to know the OUR in the clarifier, and hence the inaccuracy involved in approximating it.
2. Addressed any oxygen utilization or other redox reactions occurring in the tubing connecting the system components.

3. Provided the flexibility to minimize sampling and analysis requirements with a suitable choice of base oxidation states.
4. Inherently accounted for the effects of any possible external oxidant dissolution, such as carbon- or nitrogen-fixation.
5. Provided a more structured, logical framework to quantify components of the overall redox process, applicable to any system at steady-state regardless of the process configuration or operating conditions.

Elimination of the need to quantify the clarifier OUR was certainly the most significant benefit. The assumption of equal aerobic and clarifier OURs was shown to result in significant overestimation of the clarifier OUR and underestimation of the absolute AnS value. Aside from this, the AnS values provided by both methods correlated well. The Boundary Exchange Method thus yielded more accurate results with major procedural advantages.

The Boundary Exchange Method was used in conjunction with a newly developed in-situ aerobic OUR determination technique. The BOD bottle method of measuring OUR is believed to be accurate only under specific conditions (APHA, 1989; Mueller and Stensel, 1987). Mueller and Stensel (1987) described a closed respirometric technique for the in-situ measurement of OUR that required a complex set-up and elaborate instrumentation and control. McClintock (1990) used a simpler in-situ procedure where all flows and aeration were simply discontinued (which resulted in conditions not much different from the BOD bottle method), and no mechanical mixing was used. The dynamic non-steady-state oxygen

balance used in this study allowed a much closer approximation of true in-situ conditions with only an additional paddle mixer and a DO meter.

6.2 *Existence of AnS*

Notwithstanding increasing evidence in favor (Randall *et al.*, 1984 and 1985; Brannan, 1986; Randall *et al.*, 1987 and 1991; Wable and Randall, 1992), the existence of AnS is not widely recognized or supported. Bordacs and Tracy (1988) attributed the oxygen savings observed in an "anaerobic-aerobic" process to the "retention of storage products such as PHB." This explanation is related to the possible COD test limitations mentioned in this study. Retention of storage products in the waste sludge cannot explain AnS (and the related oxygen savings) as defined and calculated in this study if the COD test can accurately quantify the oxidizing capacity of such products without any limitation. If, however, a compound such as PHB is present in the waste sludge, and it resists complete oxidation during COD digestion, this limitation will manifest itself as AnS.

The results of this study strengthened the case for AnS and supported the possibility that limitations of the COD test are responsible for AnS, at least in part. The predominance of negative AnS values obtained under several operating conditions qualitatively supported the existence of AnS in the A/O system. Conclusive evidence, however, came from the statistical data obtained from rigorous simulation of the Boundary Exchange Model under actual operating conditions. For the A2/O system, the AnS values themselves established the occurrence of AnS beyond doubt.

6.3 *Redox Balance Components*

As expected in a non-nitrifying system, neither TKN nor oxidized nitrogen (nitrite and nitrate) played a prominent role in the redox reactions occurring in the A/O system. Nitrogen was therefore not significant in AnS calculation. The oxygen/water and COD/carbon dioxide, and, to a lesser extent, the sulfate/sulfide redox pairs were the most active contributors. This information served as the basis for the inference that AnS must originate, at least in part, in the electron exchanges between these pairs and/or the intermediate electron carriers that facilitate such exchanges. Unlike in the study reported by Brannan (1986), sulfur was found to play a significant role in the overall electron balance in this study. Figure 9 and Figure 10 clearly showed that the sulfur contribution was relatively small and variable, but definitely non-zero.

6.4 *Explanations for AnS*

6.4.1 Anaerobic Reduced Gas Production

No hydrogen or methane peaks were detected for the A2/O system. In the A/O system, hydrogen and methane production was positively demonstrated. Each explained percentages of AnS no larger than 0.1 for every steady-state phase, except when formate was present in the influent. With formate in the influent, methane production was high enough to explain more than 18 percent of observed AnS.

According to Claus (1988), "all methanogens are obligate anaerobes and the entire family *Methanobacteriaceae* is very sensitive to both oxygen and nitrate." The production of methane in the A/O anaerobic mixed liquor, however, clearly demonstrated methanogenic activity and the occurrence of anaerobic respiration, which was significantly enhanced in the presence of formate in the feed, in spite of the biomass cycling through an aerobic zone. Moreover, no enhancement in methane production was observed with acetate as the influent VFA, even though "acetic acid is the major substrate oxidized by the methanogens in most environments" (Claus, 1988). It would therefore seem that only very specific species of methanogens existed in the system. Formic acid was the only VFA they could use as an energy source. When this was not present in the influent, they were forced to rely on the hydrogen and/or formate produced by other bacteria from fermentation of complex organics. This resulted in an insignificant methane production in the absence of formate.

Another possible explanation is that acetate-degrading methanogens were present in the system, but did not produce significant methane from acetate because of slow growth kinetics. It is also possible that acetate-degrading methanogens could not effectively compete for acetate with the poly-P bacteria, and therefore did not have enough substrate for active methanogenesis.

6.4.2 Aeration-Induced Stripping of Reduced Volatiles

Among all the experiments performed in this study to quantify the loss of reduced volatiles by stripping, no more than 6 percent of AnS could be explained by such a loss. This is not

surprising given the current understanding of anaerobic bacterial metabolism, which suggests the formation of only a limited number of reduced metabolic products that can be considered volatile and be lost by aeration-induced stripping. Amino-acid fermenting bacteria (particularly relevant with bactopeptone as the substrate), for example, produce "a variety of short-chain fatty acids (VFAs), succinic acid, δ-amino valeric acid, and molecular hydrogen" (Barker, 1981). The list of strippable species is further shortened by the fact that most of the VFAs are almost completely deprotonated (ionized) in the BPR system operating pH range.

When electrons are not being routed to hydrogen or methane, ethanol and, to a lesser extent, butanol and/or acetone are likely products of anaerobic metabolism (Ferry, 1991). The A2/O system in this study, however, produced no detectable butanol and only small amounts of ethanol (explained less than one percent of AnS). No acetone peaks were detected either (if present, would be detected under the same analytical conditions).

6.4.3 Limitations of the COD Test

Use of COD data in AnS calculations pre-supposes that all organics, reduced metals, sulfide, and nitrite present in the sample will be completely oxidized to their highest possible oxidation states in the 2-hour digestion period. Because it attempts to consolidate such a wide variety of reduced species (organic acids, alcohols, aldehydes, ketones, amines, and other aliphatic, cyclic, aromatic, and polymeric organics), the COD test is liable to yield grossly underestimated values in some cases. Anaerobic metabolism is known to route electrons into the formation of a variety of complex species such as PHB (as in BPR systems), glycogen

(Cruden *et al.*, 1983), succinate (the fumarate reductase system), NAD, ATP, and nucleic acids. If any of these species yields a less-than-theoretical COD value and is a metabolic product in a system, that is a source of AnS.

The COD tests on NAD and NADH confirmed the hypothesis that both compounds are subject to limitations. The experimental COD values and oxidizing capacities for both NAD and NADH were found to be only about half the theoretical values. Thus, any production of NAD/NADH could explain at least part of the AnS. Such production obviously occurs with all bacterial growth, but may be significantly enhanced in BPR systems compared to conventional activated sludge systems because of the metabolic advantage afforded by the ability of the poly-P bacteria to polymerize and store both phosphorus and organics), leading to higher levels of these complex products. The COD tests also revealed that the measured difference between the oxidizing capacities of NAD and NADH was, on the average, about 8 times the theoretical difference. This means that conversion of NADH to NAD without recycling to NADH is another possible source of AnS.

The numbers in Table 18 can be used to calculate the amount of NAD or NADH that must be produced to explain a given AnS value. As an example, the AnS in the A2/O system averaged about -2.0 eeq/d under the conditions used. Since NAD(H) registers only about half its theoretical COD, approximately -65 eeq of AnS is explained per mole of NAD(H) produced. Thus, -2/-65=0.031 moles, or 31 mmoles of NAD(H) must be produced per day to explain all of the AnS mentioned. About ten grams of VSS were produced in the system

per day, which yields a ratio of 3.1 mmol NAD(H)/g VSS in the system at steady state. This is the ratio required to explain AnS based solely on NAD(H) production.

It is important to emphasize that the NAD(H) data were collected and used only as an example to illustrate the concept of COD test limitations. Other oxidation-resistant species may be more important in explaining the contribution of COD test limitations to AnS, while NAD(H) itself may actually play no role in AnS at all.

Three of the A/O process streams, RAS, Ana, and Aer/WAS registered, on the average, TCOD *decreases* when the COD digestion time was increased from 2 to 24 hours as part of the digestion time study. Of these, however, Aer/WAS was the only stream that affected AnS. Inf and Eff, the other streams affecting AnS, showed TCOD increases. On a percentage basis, both the decreases and the increases were small, the largest being 2.8 percent. An average Eff TCOD increase of 2.1 percent, which, by itself, could account for a fraction of AnS, was offset by an average Aer/WAS TCOD decrease of 0.9 percent and an average Inf TCOD increase of 2.8 percent. COD digestion time effects were therefore not considered a likely explanation for AnS.

6.5 Anaerobic Stabilization and Phosphorus Removal

The fact that AnS has so far always been linked with BPR systems raises the issue of whether or not BPR is a pre-requisite for AnS, or AnS for BPR. It is generally believed that AnS is related to BPR, but organisms other than poly-P bacteria are responsible for AnS. Relevant

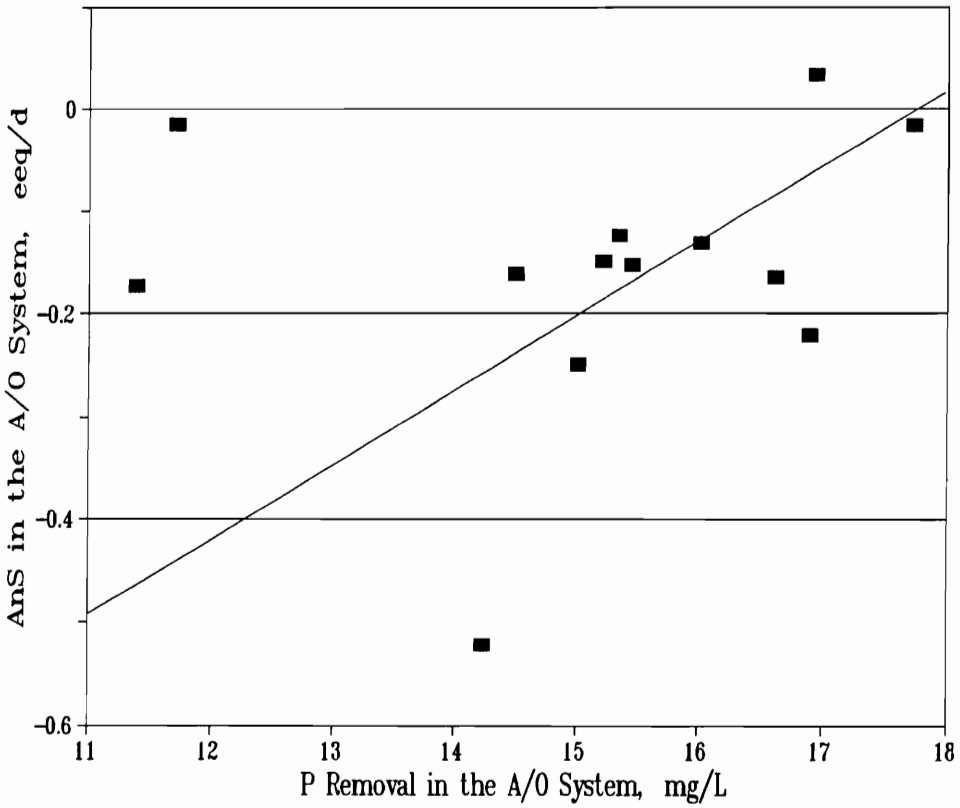


Figure 15. Scatterplot of AnS against P Removal Showing All Steady-State Data for the A/O System.

data for the A/O system from this study are presented in Figure 15. A weak correlation (with significant variability) is discernible in the graph. A general trend of higher absolute AnS values at lower P removals is apparent. While no cause-effect relationship should be inferred from mere correlation, the data do seem to suggest that AnS is not a requirement for P removal, since substantial P removal was achieved at some very low absolute AnS values. Further, substantial P removal (no less than 11 mg/L) was achieved at all times, which precludes any inference about the necessity of P removal for AnS.

6.6 Speculative AnS Model

Based on all the results of this study and established biochemical and physiological theory, a unified speculative model is proposed, consistent with the results and theory, and capable of partially explaining AnS. This model is presented in Figure 16. Part (A) outlines the proposed biochemical pathways that result in formation of reduced volatiles, while Part (B) illustrates the concept of limitations of the COD test and their manifestation as AnS. Although these limitations are manifested as AnS, they are really only an artefact because they cannot be exploited to effect oxygen savings. All processes shown in Part (A) are anaerobic, except for aerobic stripping and possibly the TCA cycle. The interrogative against the TCA cycle indicates that it may or may not occur in the anaerobic zone of a BNR system, depending primarily on the absence or occurrence of respiration. The electron transfer step shown in Part (B) could occur anaerobically with an electron acceptor other than oxygen. This model, however, proposes that oxygen is required to produce oxidation resistant species in amounts large enough to cause significant COD underestimation.

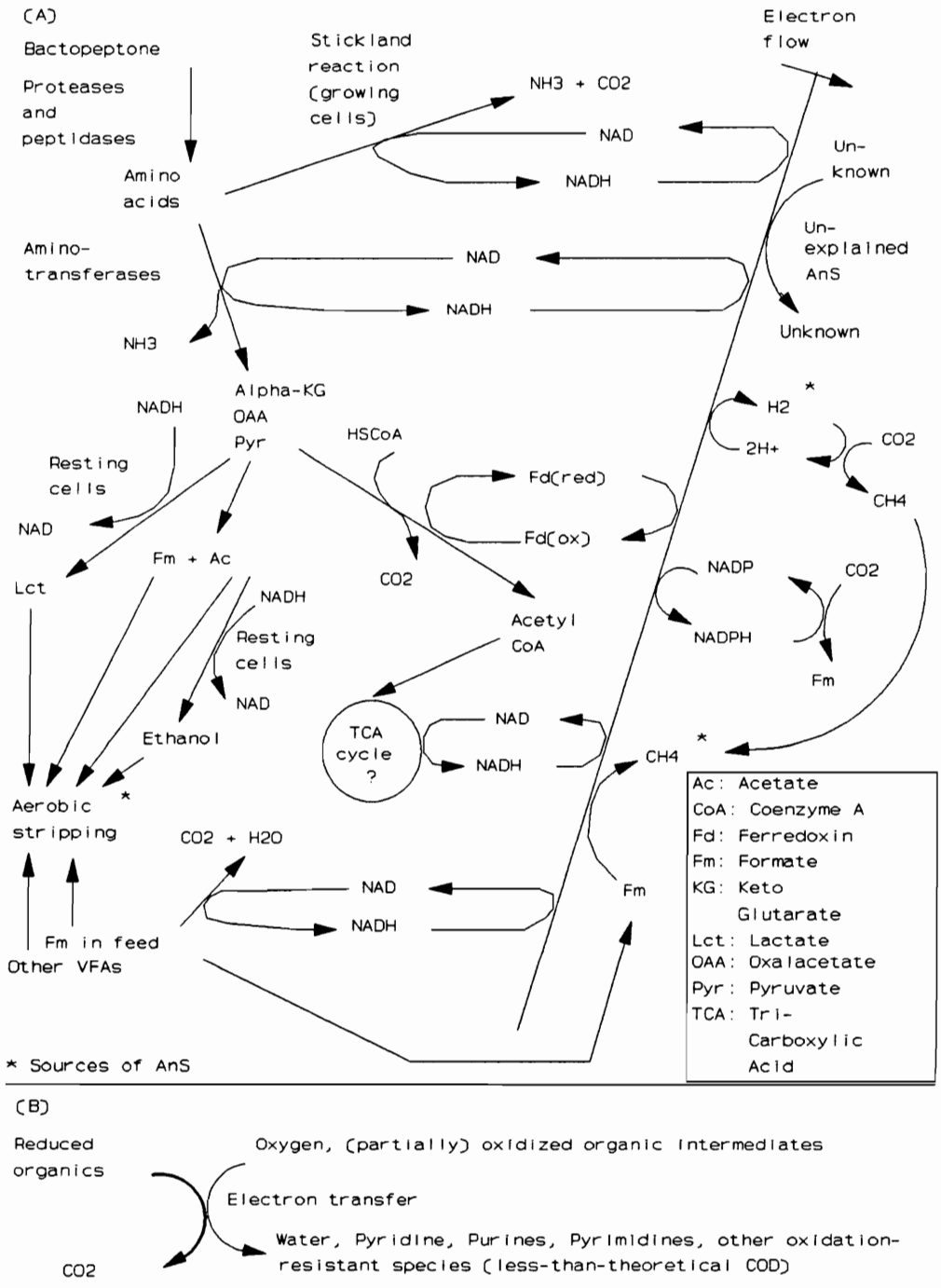


Figure 16. A Speculative Biochemical Model that Partially Explains AnS, Based on the Results Obtained in this Study: (A) Biochemical pathways producing reduced volatiles, and (B) Thermodynamic limitations of the COD test.

Reactions that depend on the metabolic state of the cell are identified in Figure 16 with the descriptors "growing cells" and "resting cells." As a rule, the metabolic state affects reactions that use reducing equivalents (NADH, NADPH) for non-anabolic purposes. Reduction of acetate to ethanol in homolactic bacteria is a good example, where the product (ethanol) of the reaction is actually excreted by the cell as a waste. During active growth, reducing equivalents must be conserved for biosynthesis, and are diverted away from such wasteful reactions. Under resting conditions, however, the low demand on reducing equivalents causes the cell to use the waste production and excretion step as a carrier recycling mechanism (Claus, 1988). In a continuous flow system, both growing and resting cells are present simultaneously. Thus, in theory, all reactions in Figure 16 can occur simultaneously.

Since anaerobic conditions are required for most of the reactions in Figure 16 (A), these reactions cannot occur in all-aerobic conventional activated sludge systems, which is consistent with the zero AnS reported for conventional systems (McClintock, 1990). The presence of the anaerobic zone in BNR systems makes these reactions, and hence AnS, possible. The model divides the anaerobic processes into two main functional categories: reducing equivalents generation, and generation of reduced excretion products resulting from electron transfer to acceptors via carriers. These categories are separated in Figure 16 (A) by the long slant-line across which the electron flow direction is indicated. The model shows the flow of electrons starting from the substrates bactopeptone and VFAs (including the special case of formate), through the various electron carriers (NAD/NADH, Fd, NADP/NADPH), to the products that are the immediate cause of AnS.

The electron transfer process in Figure 16 (B) occurs in all-aerobic conventional systems as well, but, it is proposed, does not cause significant production of oxidation resistant species because of a limited supply of reducing equivalents. In contrast, the intracellular storage products accumulated by the poly-P bacteria dominant in BPR systems provide a relatively abundant supply of reducing equivalents in the aerobic zone. It is speculated that significant production of oxidation resistant species occurs in BPR systems because of a less restricted supply of reducing equivalents. This artefact contributes to the total AnS measured in BPR systems, but is insignificant in conventional systems.

According to results obtained in this study, pathways leading to aerobic stripping seemed to be more active than the pathways leading to hydrogen or methane production, except when formate was present in the influent. The "unknown" pathway also seemed to be active under all conditions studied, resulting in a substantial unexplained AnS fraction. The presence of formate in the influent apparently caused an increase in methanogenic activity at the expense of the activity of the unknown pathway.

7 Conclusions

The following conclusions were drawn from this study:

1. By eliminating the need to quantify the clarifier OUR, the Boundary Exchange AnS determination method affords a major advantage over methods developed and used earlier.
2. Non-zero AnS in BPR systems is a statistically significant, reproducible phenomenon.
3. Carbon and oxygen were the three main elements participating in redox reactions in the non-nitrifying A/O system studied. Sulfur played a less significant role. AnS in this system thus involved one or more of these elements.
4. A combination of processes accounted for varying percentages of the AnS observed:
   a. Hydrogen production explained less than 0.1 percent.
   b. Methane production explained no more than 0.8 percent without formate in the influent, and about 19 percent when formate was present in the feed. The methanogens present in the A/O system could use formate, but not acetate, as the electron source.
   c. Aeration-induced stripping of reduced volatiles explained no more than 6 percent.

d. Limitations of the COD test were shown to have the potential to partially explain AnS.

5. A unified speculative biochemical model consistent with all the results of this study and with established biochemical and physiological theory, and capable of partially explaining observed AnS, is proposed in this study.

8 Areas for Further Research

8.1 Identification of Reduced Volatiles in the Anaerobic/Anoxic Mixed Liquor

The results presented in this study concerning aeration-induced stripping of reduced volatiles are fairly conclusive. However, some of the speculation concerning biochemical pathways leading to this process can be eliminated by analytically confirming the presence or absence of suspected volatiles in the strip-gas and/or in the mixed liquor. The procedures involved in such analyses can be quite complex, and may require special sampling techniques such as "purge and trap" and use of sophisticated instruments like the GC-MS.

8.2 Anaerobic, Anoxic, or Aerobic Formation of Oxidation-Resistant Reduced Substances

This study investigated the limitations associated with the chemical oxidation (in the COD test) of one suspected oxidation resistant reduced metabolic product: NAD(H). Extending this

investigation to other known and suspected recalcitrants could reveal additional sources of artefact-related AnS.

8.3 Tracer Studies with Radiolabeled Substrates

These are of limited value for tracking electrons as must be done in a redox investigation, since electrons cannot be radiolabeled, and can be freely exchanged between labeled and non-labeled species. However, since carbon is one of the major participants in biologically mediated redox reactions, radiolabeled carbon can help indirectly track electron flow in a given system.

References

Abu-Gharara, Z. (1988). The effect of Influent Organic Compounds on the Performance of Biological Nutrient Removal Systems, Ph.D. Dissertation, VPI&SU, Blacksburg, Virginia, U.S.A.

APHA, AWWA, and WPCF (1989). Standard Methods for the Examination of Water and Wastewater, 15th Edition, APHA Washington, D.C., U.S.A.

Arvin, E. and Kristensen, G.H. (1985). Exchange of organics, phosphate, and cations between sludge and water in biological phosphorus and nitrogen removal processes. Wat. Sci. Tech., 17(11/12):147-62.

Barker, H.A. (1981). Amino acid degradation by anaerobic bacteria. Ann. Rev. Biochem., 50:23-40

Bordacs, K. and Tracy, K.D. (1988). Retention of organic storage products in anaerobic-aerobic activated sludge. Paper presented at the 61st Annual Conference of the Water Pollution Control Federation, Dallas, Texas.

Brannan, K.P. (1986). Substrate Stabilization in the Anaerobic Stage of a Biological Phosphorus Removal System, Ph.D. Dissertation, VPI&SU, Blacksburg, Virginia, U.S.A.

Claus, G.W. (1988). Functional Concepts of Microbial Metabolism, Lecture Notes for "Physiology of Microorganisms", Biology 5662, Revision 871130, Winter 1987-88, VPI&SU, Blacksburg, Virginia.

Comeau, Y., Hall, K.J., and Oldham, W.K. (1985). A biochemical model for biological enhanced phosphorus removal. Wat. Sci. Tech, 17(11/12):313-4.

Comeau, Y., Rabinowitz, B., Hall, K.J., and Oldham, W.K. (1987). Phosphate release and uptake in enhanced biological phosphorus removal from wastewater. J. WPCF, 59:707-15.

Cruden, D.L., Durbin, W.E., and Markovetz, A.J. (1983). Utilization of PP_i as an energy source by *Clostridium* sp. Appl. Environ. Microbiol., 46(6):1403-8.

DeHaas, D.W. and Dubery, I.A. (1989). Unreliability of cold-stored samples for assessment of chemical precipitates of phosphate in activated sludge. Water S.A., 15(4):257-60.

Dold, P.L. Incorporation of biological excess phosphorus removal in a general activated sludge model. McMaster University, Hamilton, Ontario.

Dürre, P. and Andreesen, J.R. (1983). Purine and glycine metabolism by purinolytic clostridia. Journal of Bacteriology, 154(1):192-9.

Ferry, J.G. (1991). Personal communication, VPI&SU, Blacksburg, Virginia.

Ferry, J.G. (1992). Personal communication, VPI&SU, Blacksburg, Virginia.

Florentz, M. and Granger, P. (1983). Phosphorus-31 nuclear magnetic resonance of activated sludge: use for the study of the biological removal of phosphates from wastewater. Environ. Technol. Lett., 4:9-14.

Fuhs, G.W. and Chen, M. (1975). Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. Microbial Ecology, 2:119-38.

Fukase, T., Shibata, M., and Miyaji, Y. (1985). Factors affecting biological removal of phosphorus. Wat. Sci. Tech, 17(11/12):187-98.

Gerber, A., Mostert, E.S., Winter, C.T., and de Villiers, R.H. (1987). Interactions between phosphate, nitrate, and organic substrate in biological nutrient removal processes. Wat. Sci. Tech., 19:183-94.

Hao, O.J. and Chang, C.H. (1988). Metal toxicity on phosphate removal in pure culture and in activated sludge systems. J. Environ. Eng. (NY), 114:38-53.

Hascoet, M.C., Florentz, M., and Granger, P. (1985). Biochemical aspects of enhanced biological phosphorus removal from wastewater. Wat. Sci. Tech, 17(11/12):23-41.

Heymann, J.B. (1985). The biochemistry of enhanced phosphorus removal by activated sludge. Wat. Sci. Tech, 17(11/12):203-4.

Iwema, A. and Meunier, A. (1985). Influence of nitrate on acetic acid induced biological phosphorus removal. Wat. Sci. Tech, 17(11/12):289-94.

Jenkins, D. and Tandoi, V. (1991). The applied microbiology of enhanced biological phosphate removal-accomplishments and needs. Water Research, 25(12):1471-8.

Kellum, R. and Drake, H.L. (1984). Effects of cultivation gas phase on hydrogenase of the acetogen *Clostridium thermoaceticum*. J.Bacteriol., 160:466-9.

Kerdachi, D.A. and Roberts, M.R. (1985). Phosphate removal at Pinetown's Umhlatuzana Plant: *Acinetobacter* and ferrous ions in anaerobic zones. Wat. Sci. Tech, 17(11/12):301-2.

Kim, B.H., Bellows, P. Datta, R., and Zeikus, J.G. (1984). Control of carbon and electron flow in *Clostridium acetobutylicum* fermentations: Utilization of carbon monoxide to inhibit hydrogen production and to enhance butanol yields. Appl. Environ. Microbiol., 48:764-70.

Kröger, A. (1977). Phosphorylative electron transport with fumarate and nitrate as terminal hydrogen acceptors. In 27th Symp. Soc. Gen. Microbiol. and Microb. Energetics, ed. B.A. Haddock, W.A. Hamilton, Cambridge Univ. Press, London, pp 61-93.

Lan, J.C., Benefield, L.D., and Randall, C.W. (1983). Phosphorus removal in the activated sludge process. Water Res., 17:1193-200.

Lawson, E.N. and Tonhazy, N.E. (1980). Changes in morphology and phosphate uptake patterns of *Acinetobacter calcoaceticus*. Water S.A., 6(3):105-12.

Marais, G.v.R., Loewenthal, R.E., and Siebritz, I.P. (1983). Review: Observations supporting phosphate removal by biological excess uptake. Wat. Sci. Tech., 15(3/4):15-41.

Martin, D.R., Lundie, L.L., Kellum, R., and Drake, H.L. (1983). Carbon monoxide-dependent evolution of hydrogen by the homoacetate-fermenting bacterium *Clostridium thermoaceticum*. Curr. Microbiol., 8:337-40.

McClintock, S.A. (1986). A Study of Nitrate versus Oxygen Respiration in the Activated Sludge Process, M.S. Thesis, VPI&SU, Blacksburg, Virginia, U.S.A.

McClintock, S.A. (1990). Effects of Temperature and Mean Cell Residence Time on the Performance of High-Rate Biological Nutrient Removal Processes, Ph.D. Dissertation, VPI&SU, Blacksburg, Virginia, U.S.A.

Mueller, J.A. and Stensel, H.D. (1987). Biologically enhanced oxygen transfer in the activated sludge process - fact or folly? Paper presented at the 60th Annual WPCF Conference, Philadelphia.

Nicholls, H.A., Pitman, A.R., and Osborn, D.W. (1985). The readily biodegradable fraction of sewage: its influence on phosphorus removal and measurement. Wat. Sci. Tech, 17(11/12):73-87.

Palisade Corporation (1989). @RISK: Risk analysis and simulation add-in for Lotus 1-2-3. Version 1.5 Users Guide, Palisade Corporation, Newfield, New York.

Pattarkine, V.M. (1991). The Role of Metals in Enhanced Biological Phosphorus Removal from Wastewater, Ph.D. Dissertation, VPI&SU, Blacksburg, Virginia, U.S.A.

Ramadori, R., Tandoi, V., and Misiti A. (1985). Bench scale and pilot scale experiments on biological phosphorus removal. Wat. Sci. Tech, 17(11/12):287-8.

Randall, C.W. (1984). The development of energy efficient activated sludge systems through utilization of nutrient removal processes. Proc. of 16th Mid-Atlantic Ind. Waste Conf., Penn. State Univ., 52-63.

Randall, C.W., Benefield, L.D., and Lan, J.C. (1984). The utilization of biological phosphorus removal processes to reduce the aeration requirements of activated sludge systems. Proc. of the 12th Conf. of the IAWPRC, Post Conf. Seminar on Enhanced Biological Phosphorus Removal from Wastewater, Paris, France, I:229-38.

Randall, C.W., Brannan, K.P., and Benefield, L.D. (1985). The oxygen requirements of biological nutrient removal processes. Proc. of the Internat. Conf. on New Directions and Research in Waste Treatment and Residuals Management, Univ. of British Columbia, Vancouver, B.C., Canada, 487.

Randall, C.W., Brannan, K.P., and Benefield, L.D. (1987). Factors affecting anaerobic stabilization during biological phosphorus removal. Proc. of the IAWPRC International Specialized Conference, Biological Phosphate Removal from Wastewater, Rome, Italy, September 28-30, 1987, 111-22.

Randall, C.W., (Personal communication, 1991). Uptake of organic substrates and accumulation of polyhydroxyalkanoates linked with glycolysis of intracellular carbohydrates under anaerobic conditions in the biological phosphate removal processes.

Randall, C.W., Brannan, K.P., McClintock, S.A., and Pattarkine, V.M. (1991). The case for anaerobic reduction of oxygen requirements in biological phosphorus removal systems. Personal communication from C.W. Randall, VPI&SU, Blacksburg, Virginia.

Schauer, N.L. and Ferry, J.G. (1980). Metabolism of formate in *Methanobacterium formicicum*. Journal of Bacteriology, 142(3):800-7.

Siebritz, I.P., Ekama, G.A., and Marais, G.v.R. (1983). A parametric model for biological excess phosphorus removal. Wat. Sci. Tech, 15(3/4):127-52.

Takashi, M., Kawakami, T., and Matsuo, T. (1985). Behavior of intracellular polyphosphate in the biological phosphate removal process. Wat. Sci. Tech, 17(11/12):11-21.

Wable, M.V. and Randall, C.W. (1992). Investigation of reduction in oxygen requirements of biological phosphorus removal systems. To be presented at the 16th Biennial IAWPRC International Conference, Water Quality '92, Washington, D.C.

Water Research Commission of South Africa (1984). Theory, Design, and Operation of Nutrient Removal Activated Sludge Processes, Publication of the Water Research Commission of South Africa, P.O. Box 824, Pretoria 0001, South Africa.

Wentzel, M.C., Dold, P.L., Ekama, G.A., and Marais, G.v.R. (1985). Kinetics of biological phosphorus release. Wat. Sci. Tech, 17(11/12):57-71.

Wentzel, M.C., Loewenthal, R.E., Ekama, G.A., and Marais, G.v.R. (1988). Enhanced polyphosphate organism cultures in activated sludge systems - Part 1: Enhanced culture development. Water S.A., 14(2):81-92.

Wentzel, M.C., Ekama, G.A., Loewenthal, R.E., Dold, P.L., and Marais, G.v.R. (1989a). Enhanced polyphosphate organism cultures in activated sludge systems. Part II: Experimental behavior. Water S.A., 15(2):71-88.

Wentzel, M.C., Dold, P.L., Ekama, G.A., and Marais, G.v.R. (1989b). Enhanced polyphosphate organism cultures in activated sludge systems. Part III: Kinetic model. Water S.A., 15:89-102.

Appendices

Appendix 1: Expanded Equations Describing Oxidizing Capacities in the Electron Balance Equation

(5)

Δox_{Inf} = $1.44*10^{-3}$ * Inf flow * [(-0.125 * Inf TCOD + 0.143 * Inf NO_2^--N + 0.249 * Inf S^{2-}) + 0.125 * Inf DO - 0.214 * Inf TKN + 0.214 * Inf NO_2^--N + 0.357 * Inf NO_3^--N + 0.083 * Inf SO_4^{2-}]

(6)

Δox_{Air} = $1.44*10^{-3}$ * 0.125 * Aer vol * [Aer OUR + (Aer DO - Pre-Aer DO)/Actual Aer HRT]

(7)

Δox_{Eff} = $1.44*10^{-3}$ * Eff flow * [(-0.125 * Eff TCOD + 0.143 * Eff NO_2^--N) + 0.125 * Eff DO - 0.214 * Eff TKN + 0.214 * Eff NO_2^--N + 0.357 * Eff NO_3^--N + 0.083 * Eff SO_4^{2-}]

(8)

Δox_{WAS} = $1.44*10^{-3}$ * WAS flow * [(-0.125 * WAS TCOD + 0.143 * WAS NO_2^--N) + 0.125 * WAS DO - 0.214 * WAS TKN + 0.214 * WAS NO_2^--N + 0.357 * WAS NO_3^--N + 0.083 * WAS SO_4^{2-}]

All flows are in mL/min, concentrations in mg/L, Aer vol in mL, Aer OUR in mg/L/min, and Actual Aer HRT in min. $1.44*10^{-3}$ is a units-conversion factor, while all other numbers are eeq/g values for the corresponding species, except 0.143, which represents eeq/g N for the oxidation of nitrite to nitrate, and is applied as a correction to eliminate the positive interference it causes in the COD test. Although sulfide contains S in its base oxidation state, it appears in the equation for the influent stream oxidizing capacity as a correction to eliminate the positive interference it causes in the COD test. The resulting value on the left-hand-side of each of the above equations has the units eeq/d.

Appendix 2: A/O System Maintenance Schedule

Table 21. A/O System Maintenance Schedule.

Maintenance Task/Operation	Minimum Frequency
Prepared stock feed solutions	Weekly
Prepared final feed, measured and adjusted influent flow, thoroughly washed feed container	Daily
Reprogrammed WAS timer to maintain target BSRT, measured and adjusted RAS flow	Weekly
Purged accumulated gases from influent and RAS tubing	Daily
Disinfected feed containers and influent tubing with commercial chlorine bleach, thoroughly washed with tap water to prevent bleach from entering system	As required
Removed attached growth from system surfaces (reactor and tubing walls), freed bottlenecks (such as narrow tubing) of obstructions created by solids accumulation	Daily

Appendix 3: A/O Sampling Matrix

Table 22. Sampling Matrix Showing Parameters Measured/Analyzed in Each Process Stream.

Parameter	Inf	RAS	Ana	Aer / WAS	Eff	Ana Gas
Flow-rate	x	x		x (WAS)		
TSS		x	x	x	x	
VSS		x	x	x	x	
TCOD	x	x	x	x	x	
FCOD		x	x	x	x	
TKN	x		x	x	x	
Nitrite-N	x	x	x	x	x	
Nitrate-N	x	x	x	x	x	
Sulfate	x	x	x	x	x	
FOP	x	x	x	x	x	
TP	x	x	x	x	x	
$H_{2(g)}$						x
$CH_{4(g)}$						x
$CO_{(g)}$						x
DO	x	x	x	x	x	
OUR				x		
pH	x		x	x		

Parameter/Stream combinations that were measured/analyzed are marked with an "x".

Appendix 4: Derivation of Aerobic OUR Equation and Sample Calculation

Derivation

A general dynamic non-steady-state oxygen mass balance around the aerobic reactor is described by the following first-order differential equation:

$$V\frac{dC}{dt}=Q_{aer}(C_{in}-C)-V*OUR+V*K_La_f(\beta C^*_{\infty}-C) \quad (9)$$

where: V = volume of the aerobic reactor
C = DO concentration in the reactor at time "t"
t = time
Q_{aer} = flow-rate through the reactor = Inf flow + RAS flow + RNX flow
C_{in} = DO concentration influent to the reactor = Ana/Anx DO
K_La_f = overall oxygen mass transfer coefficient under process conditions
β = ratio of DO saturation concentration in the Aer ML to that in clean water
C^*_{∞} = DO saturation concentration in clean water

Since aeration is discontinued during the OUR test, the mass transfer term in the above equation may be dropped (set equal to zero). Rearrangement and separation of variables then yields the following equation:

$$\int_{C_o}^{C}\frac{dC}{C_{in}-C-\theta_{aer}*OUR}=\int_{0}^{t}\frac{dt}{\theta_{aer}} \quad (10)$$

where: C_o = reactor (Aer) DO concentration at time 0
θ_{aer} = actual aerobic hydraulic retention time (HRT) = V/Q_{aer}

Integration of the equation between the limits shown yields:

$$C=(C_{in}-\theta_{aer}*OUR)+(C_o-C_{in}+\theta_{aer}*OUR)e^{-\frac{t}{\theta_{aer}}} \quad (11)$$

Linear regression of a scatterplot of C on the Y-axis versus $e^{-t/\theta aer}$ on the X-axis gives:

slope = C_o - C_{in} + θ_{aer}*OUR, and
Y-intercept = C_{in} - θ_{aer}*OUR

OUR can thus be calculated using either or both of the following equations:

$$OUR = \frac{slope - C_o + C_{in}}{\theta_{aer}} \quad (12)$$

$$OUR = \frac{C_{in} - Y\ intercept}{\theta_{aer}} \quad (13)$$

Sample Calculation

The data relevant to OUR calculation in the A/O system for the date August 13, 1990 are listed below:

V = 17L and Q_{aer} = 57.6 mL/min, resulting in θ_{aer} = 295 min; C_{in} = 0.2 mg/L; C_o = 4.87 mg/L; and C-t data as follows:

t, min	C, mg/L	e^(-t/THETAaer)
0.00	4.87	1.00
0.50	4.67	1.00
1.00	4.52	1.00
1.50	4.37	0.99
2.00	4.17	0.99
2.50	4.05	0.99
3.00	3.85	0.99
3.50	3.68	0.99
4.00	3.57	0.99
4.50	3.38	0.98
5.00	3.22	0.98
5.50	3.03	0.98
6.00	2.91	0.98
6.50	2.73	0.98
7.00	2.59	0.98
7.50	2.42	0.97

Linear regression yields slope = 96.9 and Y-intercept = -92.1. Upon appropriate substitutions, both the OUR equations yield the same OUR value of 0.31 mg/L/min.

Appendix 5: Recoveries from Dissolved Gas Extraction by Vacuum Stripping

Recoveries of Hydrogen and Methane Obtained Using the Vacuum Stripping Dissolved Gas Extraction Technique on Distilled Water Equilibrated with a Gas of Known Composition

No.	Dissolved hydrogen conc., by extraction, mmols/L	Dissolved hydrogen conc., from Henry's Law, mmols/L	Hydrogen recovery, fraction	Dissolved methane conc., by extraction, mmols/L	Dissolved methane conc., from Henry's Law, mmols/L	Methane recovery, fraction
1	0.188	0.201	0.933	0.434	0.464	0.936
2	0.019	0.025	0.776		0.004	
3	0.552	0.694	0.795		0.002	
4	0.040	0.049	0.804	0.738	1.012	0.729
5	0.059	0.071	0.836	0.192	0.228	0.842
6	0.059	0.057	1.035	0.192	0.191	1.007
Avg.			0.863			0.878

Appendix 6: Kinetics of Dissolved Gas Extraction by Vacuum Stripping

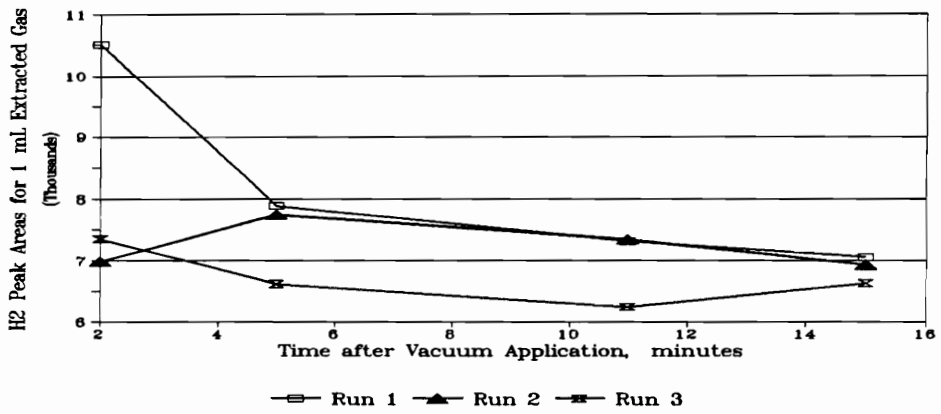


Figure 17. Hydrogen Peak Areas Obtained for 1 mL Extracted Gas Samples at Various Time Intervals after Application of Vacuum to Distilled Water Solvent.

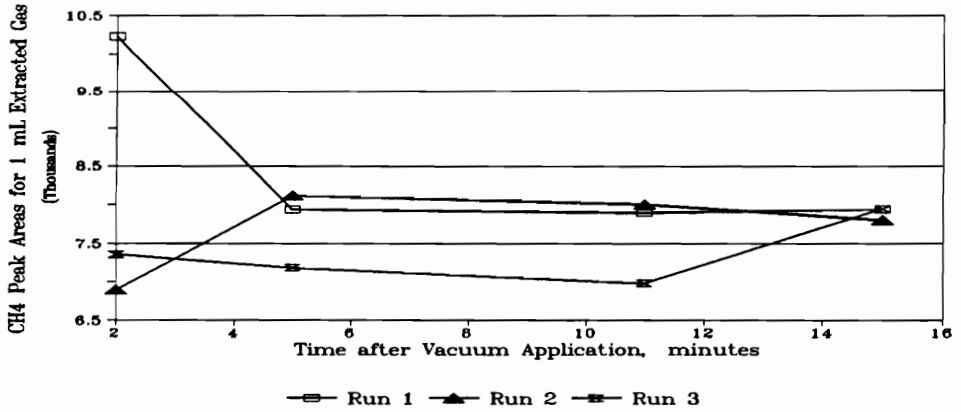


Figure 18. Methane Peak Areas Obtained for 1 mL Extracted Gas Samples at Various Time Intervals after Application of Vacuum to Distilled Water Solvent.

Appendix 7: Input Parameter Triangular Distribution Arguments for Boundary Exchange AnS Model Simulation

Parameter	Phase 4 Minimum	Phase 4 Mean	Phase 4 Maximum	Phase 5 Minimum	Phase 5 Mean	Phase 5 Maximum
WAS flow, mL/d	1300.000	1716.200	1985.000	2035.000	2093.000	2125.000
RAS flow, mL/min	26.500	29.060	32.500	28.200	28.733	29.100
Inf DO, mg/L	0.200	1.220	2.950	0.500	1.033	1.500
RAS DO, mg/L	0.100	0.710	1.500	0.400	0.500	0.700
Ana DO, mg/L	0.200	0.278	0.400	0.400	0.467	0.600
Aer DO, mg/L	6.700	6.875	7.050	6.800	6.817	6.850
Eff DO, mg/L	2.500	3.640	4.300	2.600	2.850	3.050
Aer OUR, mg/L/min	0.255	0.293	0.350	0.287	0.293	0.300
Inf flow, mL/min	28.805	29.041	29.350	28.972	29.062	29.111
Eff flow, mL/min	27.637	27.849	28.218	27.559	27.609	27.635
Inf TCOD, mg/L	427.177	455.958	476.701	456.699	466.682	474.400
RAS TCOD, mg/L	6913.257	9722.226	13411.760	8233.010	8617.319	9078.947
Ana TCOD, mg/L	3378.069	4157.773	4659.794	4140.000	4272.466	4414.239
Aer TCOD, mg/L	3859.247	4382.404	4721.649	4180.000	4251.492	4302.632
Eff TCOD, mg/L	35.074	59.046	97.967	34.175	37.753	40.400
Inf SO4, mg/L	24.060	26.418	29.347	27.013	27.355	27.703
RAS SO4, mg/L	24.143	28.198	32.159	26.333	27.795	29.612
Ana SO4, mg/L	19.247	22.323	26.747	21.964	23.429	25.019
Aer SO4, mg/L	24.240	27.199	30.622	26.724	26.996	27.531
Eff SO4, mg/L	24.907	36.169	61.721	33.983	37.883	41.596
Inf NO3-N, mg/L	0.013	0.282	0.592	0.166	0.198	0.250
RAS NO3-N, mg/L	0.011	0.269	2.510	0.010	0.576	1.705
Ana NO3-N, mg/L	0.000	0.016	0.060	0.010	0.266	0.775
Aer NO3-N, mg/L	0.000	0.362	3.553	0.000	0.054	0.149
Eff NO3-N, mg/L	0.007	0.013	0.026	0.020	0.228	0.554
Inf NO2-N, mg/L	0.000	0.157	0.336	0.078	0.080	0.083
RAS NO2-N, mg/L	0.000	0.005	0.026	0.000	0.000	0.000
Ana NO2-N, mg/L	0.000	0.000	0.000	0.000	0.000	0.000
Aer NO2-N, mg/L	0.007	0.011	0.024	0.013	0.030	0.046
Eff NO2-N, mg/L	0.007	0.014	0.031	0.011	0.012	0.013
Inf TKN, mg/L	56.447	64.384	67.872	63.324	63.618	63.875
Ana TKN, mg/L	NA	NA	NA	396.600	416.826	437.419
Aer TKN, mg/L	392.526	434.799	468.600	403.771	422.526	446.244
Eff TKN, mg/L	43.025	46.589	49.716	42.584	43.172	43.797
Inf Bactopeptone, mg/L	400.000	400.000	400.000	400.000	400.000	400.000

Appendix 8: Continuous Flow A/O System Raw Data

Process Control and Operating Conditions

Phase	Date	Day	* Steady-state code	Target BSRT, days	VFA added	Inf FOP, mg/L	Inf flow, mL/min	RAS flow, mL/min	WAS flow, mL/day	Nominal HRT, hours
1	07-Nov-89	1	0		None					
2	18-Dec-89	42	0		None	15.0	29.1	29.5	920	12.0
2	25-Dec-89	49	0		None	13.7	29.1	36.0	840	12.0
2	01-Jan-90	56	0		None	13.3	29.1	29.0	840	12.0
3	22-Jan-90	77	0		None	13.8	28.7	31.0	986	12.2
3	29-Jan-90	84	0		None	13.5	29.4	28.0	1000	11.9
3	05-Feb-90	91	0	10	None	14.0	29.0	29.0	1535	12.1
3	12-Feb-90	98	0	10	None	13.7	28.7	29.0	1763	12.2
3	19-Feb-90	105	3	10	None	13.6	28.8	29.0	1692	12.2
3	26-Feb-90	112	3	10	None	13.0	28.4	28.0	1633	12.3
3	05-Mar-90	119	-3	10	None	13.0	29.5	30.0	1740	11.9
3	12-Mar-90	126	-3	10	None	11.9	28.7	31.5	1320	12.2
3	19-Mar-90	133	-3	10	None	12.1	28.7	29.5	1650	12.2
3	26-Mar-90	140	-3	10	None	12.7	29.0	28.5	1608	12.1
3	02-Apr-90	147	3	10	None	12.6	28.8	28.0	1730	12.2
4	30-Apr-90	175	0	10	None	23.0	29.3	28.0	1775	11.9
4	07-May-90	182	4	10	None	22.7	28.9	27.0	1520	12.1
4	14-May-90	189	-4	10	None	23.0	28.6	28.0	1675	12.2
4	21-May-90	196	-4	10	None	23.9	28.8	27.0	1670	12.1
4	28-May-90	203	4	10	None	22.3	29.3	29.0	1630	11.9
4	04-Jun-90	210	4	10	None	22.8	29.0	29.0	1408	12.1
4	11-Jun-90	217	4	10	None	22.8	29.2	29.5	1540	12.0
4	18-Jun-90	224	4	10	None	23.0	28.8	26.5	1300	12.2
4	25-Jun-90	231	4	10	None	21.4	28.8	29.5	1695	12.1
4	09-Jul-90	245	4	10	None	21.6	29.0	32.5	1875	12.1
4	16-Jul-90	252	4	10	None	22.1	29.2	29.5	1985	12.0
4	23-Jul-90	259	4	10	None	22.7	29.1	29.5	1902	12.0
4	30-Jul-90	266	-4	10	None	22.8	29.2	29.5	1940	12.0
4	06-Aug-90	273	4	10	None	23.4	29.1	29.1	1915	12.0
4	13-Aug-90	280	4	10	None	23.9	29.1	28.5	1800	12.0
5	20-Aug-90	287	0	10	None	17.9	29.2	29.1	1858	12.0
5	27-Aug-90	294	0	10	None	17.9	29.4	29.0	1922	11.9
5	03-Sep-90	301	0	10	None	17.9	29.0	29.0	1899	12.0
5	10-Sep-90	308	0	10	None	18.1	28.7	28.8	1970	12.2
5	17-Sep-90	315	5	10	None	17.6	29.0	28.2	2035	12.1
5	24-Sep-90	322	5	10	None	17.0	29.1	28.9	2125	12.0
5	01-Oct-90	329	-5	10	None	18.3	29.2	28.9	2032	12.0
5	08-Oct-90	336	5	10	None	17.8	29.1	29.1	2119	12.0

* <0 3 or more BSRTs elapsed under current conditions, but system not stabilized.
=0 Fewer than 3 BSRTs elapsed under current conditions.
>0 Considered steady-state data for current conditions.

DO and pH

Phase	Date	Day	Inf DO, mg/L	RAS DO, mg/L	Ana DO, mg/L	Aer DO, mg/L	Eff DO, mg/L	Aer OUR mg/L/min	Inf pH	Ana pH	Aer pH
1	07-Nov-89	1	3.50	1.10	0.20	7.00					
2	18-Dec-89	42	0.15	0.15	0.20	6.50		0.339		7.73	7.06
2	25-Dec-89	49	1.90	0.50	0.25	6.50		0.379		7.79	7.00
2	01-Jan-90	56	0.20	0.20	0.20	6.30		0.482		7.60	6.80
3	22-Jan-90	77	1.70	1.00	0.10	6.70		0.311		7.39	7.89
3	29-Jan-90	84	0.10	1.00	0.10	6.70		0.334	7.45	7.23	7.71
3	05-Feb-90	91	0.10	0.60	0.10	6.60		0.369	7.70	7.21	7.77
3	12-Feb-90	98	0.10	1.00	0.10	7.00		0.286	7.70	7.34	7.67
3	19-Feb-90	105	0.10	0.20	0.10	6.30		0.428	7.66	7.15	7.70
3	26-Feb-90	112	1.90	2.40	0.40	7.00	5.50	0.340	8.07	7.23	7.68
3	05-Mar-90	119	0.20	0.20	0.20	6.05	2.90	0.253	8.00	7.20	7.90
3	12-Mar-90	126	0.70	3.00	0.40	6.90	4.40	0.284	8.39	7.18	7.92
3	19-Mar-90	133	0.30	0.20	0.20	7.10	3.30	0.325	8.25	7.18	7.80
3	26-Mar-90	140	2.90	1.80	0.60	6.85	3.65	0.311	8.55	7.17	7.89
3	02-Apr-90	147	0.30	2.90	0.65	6.90	4.10	0.334	8.30	7.17	7.85
4	30-Apr-90	175	0.30	0.40	0.30	6.90	4.10	0.242	8.15	7.27	7.83
4	07-May-90	182	0.75	1.00	0.30	6.95	4.30	0.255	7.85	7.06	7.66
4	14-May-90	189	0.40	1.00	0.20	6.70	4.20	0.247	7.95	7.13	7.79
4	21-May-90	196	0.60	2.10	0.20	6.70	3.60	0.293	7.80	7.13	7.68
4	28-May-90	203	2.70	0.30	0.30	7.05	3.90	0.281	8.35	7.18	7.81
4	04-Jun-90	210	0.45	0.20	0.30	6.90	4.08	0.293	8.00	7.19	7.81
4	11-Jun-90	217	2.95	0.10	0.30	6.90	3.30	0.255	7.98	6.99	7.63
4	18-Jun-90	224	2.00	1.40	0.30	6.80	4.00	0.293	8.10	7.01	7.63
4	25-Jun-90	231	2.00	0.20	0.30	6.90	3.70	0.280	8.00	7.00	7.60
4	09-Jul-90	245	0.35	0.50	0.40	6.80	3.50	0.289	7.72	6.98	7.55
4	16-Jul-90	252	0.38	1.00	0.20	6.70	3.50	0.321	8.00	7.20	7.77
4	23-Jul-90	259	0.60	0.90	0.28	7.05	3.70	0.350	7.40	7.00	7.52
4	30-Jul-90	266	0.50	0.60	0.20	7.03	2.90	0.289	7.40	7.00	7.60
4	06-Aug-90	273	0.27	1.50	0.20	6.90	4.00	0.291	7.95	7.20	7.81
4	13-Aug-90	280	0.20	0.20	0.20	6.70	2.50	0.313	7.40	7.04	7.65
5	20-Aug-90	287	0.22	1.30	0.15	6.95	2.90	0.308	7.70	7.00	7.55
5	27-Aug-90	294	0.20	2.00	0.17	6.98	2.70	0.295	7.77	7.35	7.98
5	03-Sep-90	301	0.20	1.60	0.10	6.70	2.00	0.301	8.30	7.28	8.00
5	10-Sep-90	308	0.75	0.75	0.70	6.73	2.00	0.265	7.60	7.00	7.68
5	17-Sep-90	315	0.50	0.40	0.40	6.80	3.05	0.300	8.10	7.05	7.88
5	24-Sep-90	322	1.10	0.40	0.40	6.85	2.90	0.293	8.30	7.22	8.00
5	01-Oct-90	329	0.38	0.40	0.38	6.83	2.20	0.304	7.85	7.15	7.93
5	08-Oct-90	336	1.50	0.70	0.60	6.80	2.60	0.287	8.70	7.32	8.18

Suspended Solids

Phase	Date	Day	RAS TSS, mg/L	Ana TSS, mg/L	Aer TSS, mg/L	Eff TSS, mg/L	RAS VSS, mg/L	Ana VSS, mg/L	Aer VSS, mg/L	Eff VSS, mg/L
1	07-Nov-89	1	4850	3160	3410	8.8	4220	2730	2980	7.2
2	18-Dec-89	42	5760	2300	4160	53.3	5220	2100	3793	50.0
2	25-Dec-89	49	5120	3280	3050	76.0	4650	3020	2810	68.0
2	01-Jan-90	56	6100	3680	3520	19.0	5480	3340	3120	16.0
3	22-Jan-90	77	6220	3640	3680	32.0	5800	3440	3480	30.3
3	29-Jan-90	84	5700	3720	3560	52.0	5140	3400	3220	47.0
3	05-Feb-90	91	4540	2920	2940	24.5	4040	2660	2600	22.7
3	12-Feb-90	98	3920	2420	2600	26.0	3500	2220	2320	23.0
3	19-Feb-90	105	4060	2360	2440	26.0	3600	2180	2180	25.0
3	26-Feb-90	112	3340	2200	2420	18.0	2860	1880	2100	17.0
3	05-Mar-90	119	5280	2000	2280	42.9	4540	1800	2040	37.1
3	12-Mar-90	126	3880	3020	2820	34.5	3320	2720	2400	30.9
3	19-Mar-90	133	8720	2600	3320	30.0	7100	2260	2760	28.2
3	26-Mar-90	140	9620	2760	3520	18.2	7240	2260	2720	16.4
3	02-Apr-90	147	6480	3480	3920	28.2	4940	2840	3020	24.5
4	30-Apr-90	175	12180	2600	3140	33.6	10040	2200	2480	30.9
4	07-May-90	182	6480	3080	3820	30.0	4960	2480	2940	23.6
4	14-May-90	189	11520	3580	4320	45.5	8580	2760	3160	35.5
4	21-May-90	196	7060	4720	4420	46.4	5220	3720	3340	40.0
4	28-May-90	203	13780	4000	4520	60.9	9760	3020	3280	51.8
4	04-Jun-90	210	13140	4560	4920	70.9	8820	3120	3180	44.5
4	11-Jun-90	217	9760	3740	4440	69.1	7060	2940	3240	60.0
4	18-Jun-90	224	8300	4840	5140	43.6	6060	3660	3820	36.4
4	25-Jun-90	231	13520	4300	5040	21.8	9080	3180	3480	20.0
4	09-Jul-90	245	10660	4520	5060	11.8	7440	3280	3640	7.3
4	16-Jul-90	252	8960	4520	4920	17.3	6220	3340	3440	13.6
4	23-Jul-90	259	9200	4800	5040	17.3	6440	3480	3600	13.6
4	30-Jul-90	266	8560	4560	4960	16.4	5840	3340	3480	16.4
4	06-Aug-90	273	8600	4820	5280	20.9	5820	3480	3700	15.5
4	13-Aug-90	280	12380	4980	5080	21.8	8340	3640	3580	18.2
5	20-Aug-90	287	7200	5020	5120	18.2	5020	3700	3640	17.3
5	27-Aug-90	294	7000	4940	5060	24.5	5980	3560	3640	20.0
5	03-Sep-90	301	5600	4820	4920	9.1	4120	3640	3660	7.3
5	10-Sep-90	308	11140	4920	5160	23.6	7840	3580	3720	11.8
5	17-Sep-90	315	9760	4520	4720	8.2	7040	3400	3440	6.4
5	24-Sep-90	322	8580	4400	4480	10.0	5820	3040	3040	5.5
5	01-Oct-90	329	7960	4380	4540	3.6	5600	3340	3220	2.6
5	08-Oct-90	336	8420	4320	4620	9.0	5880	3180	3380	7.5

COD

Phase	Date	Day	Inf TCOD, mg/L	RAS TCOD, mg/L	Ana TCOD, mg/L	Aer TCOD, mg/L	Eff TCOD, mg/L	RAS FCOD, mg/L	Ana FCOD, mg/L	Aer FCOD, mg/L	Eff FCOD, mg/L
1	07-Nov-89	1	386	5288	3661	3966	56.9	56.9	211.5	40.7	32.5
2	18-Dec-89	42	544	6462	3744	3949	102.6	61.5	147.7	65.6	49.2
2	25-Dec-89	49	484	6353	4437	4235	121.0	56.5	173.4	36.3	40.3
2	01-Jan-90	56	434	7765	4689	4689	68.6	40.3	145.2	36.3	36.3
3	22-Jan-90	77	449	7916	4739	4538	74.6	38.3	199.7	34.3	30.3
3	29-Jan-90	84	420	7550	4725	4375	74.0	34.0	176.0	26.0	34.0
3	05-Feb-90	91	441	5842	3709	3660	64.2	32.7	184.0	36.7	31.9
3	12-Feb-90	98	443	5245	3309	3279	63.9	31.0	179.2	31.0	30.2
3	19-Feb-90	105	430				75.4	43.4	180.6	40.0	40.0
3	26-Feb-90	112	467	4007	2710	2720	51.1	44.8	195.6	35.4	31.4
3	05-Mar-90	119	454	5900	2348	2448	74.6	53.0	154.9	36.9	32.1
3	12-Mar-90	126	444	4447	2802	2995	76.8	35.9	133.1	25.1	28.2
3	19-Mar-90	133	433	9210	2965	3575	68.4	36.4	137.2	35.6	35.6
3	26-Mar-90	140	451	9360	2990	3530	52.8	33.6	94.4	24.0	25.6
3	02-Apr-90	147	437	6922	3621	3972	62.1	31.7	117.4	34.1	34.9
4	30-Apr-90	175	482	15329	2865	3244	70.6	35.5	125.6	29.1	29.1
4	07-May-90	182	467	6913	3378	3859	62.1	33.8	111.6	22.0	28.3
4	14-May-90	189	444	12468	3683	4454	72.2	35.5	101.9	25.4	25.4
4	21-May-90	196	436	7105	4552	4114	70.1	35.8	108.2	34.3	32.0
4	28-May-90	203	460	13412	3745	4137	91.8	32.2	105.1	27.5	25.1
4	04-Jun-90	210	440		3829	4216	77.5	30.2	93.8	23.3	27.9
4	11-Jun-90	217	446	9659	3811	4205	98.0	39.0	97.2	30.3	26.4
4	18-Jun-90	224	427	7675	4392	4325	68.9	33.7	97.2	32.2	30.6
4	25-Jun-90	231	449	12804	4181	4554	51.4	28.6	90.6	29.4	29.4
4	09-Jul-90	245	452	9911	4153	4458	35.1	28.8	80.8	20.9	19.3
4	16-Jul-90	252	468	8149	4250	4308	36.6	21.0	85.6	20.2	24.1
4	23-Jul-90	259	477	8990	4660	4722	49.5	68.5	92.4	86.6	31.3
4	30-Jul-90	266	429	8256	4331	4432	46.1	35.6	85.0	27.5	28.3
4	06-Aug-90	273	442	8265	4401	4649	47.7	28.6	76.3	25.4	23.0
4	13-Aug-90	280	472	11445	4607	4607	49.6	29.0	86.9	23.4	25.0
5	20-Aug-90	287	414	6745	4549	4382	42.4	24.3	73.7	23.5	22.7
5	27-Aug-90	294	437	6354	4510	4313	56.3	43.7	85.4	32.7	31.9
5	03-Sep-90	301	456	5481	4688	4512	36.0	32.1	80.7	25.8	26.6
5	10-Sep-90	308	479	10357	4309	4300	37.1	30.9	78.8	23.2	19.3
5	17-Sep-90	315	469	9079	4263	4303	38.7	30.0	78.9	30.8	26.8
5	24-Sep-90	322	457	8233	4414	4272	34.2	33.4	80.0	24.9	23.3
5	01-Oct-90	329	465	8118	4177	4256	33.9	40.2	88.3	35.5	26.0
5	08-Oct-90	336	474	8540	4140	4180	40.4	37.2	83.6	30.8	32.4

TKN and Anaerobic Reduced Gas Production

Phase	Date	Day	Inf TKN, mg/L	Ana TKN, mg/L	Aer TKN, mg/L	Eff TKN, mg/L	H2 partl. pr., atm	Henry diss. H2, mg/L	CH4 partl. pr., atm	Henry diss. CH4, mg/L
1	07-Nov-89	1								
2	18-Dec-89	42	67.9	321	351	6.8				
2	25-Dec-89	49	65.0	212	107	2.5	0.0001	0.0002		
2	01-Jan-90	56	58.7	198	164	2.0	0.0005	0.0008		
3	22-Jan-90	77	72.1	402	467	50.6				
3	29-Jan-90	84	71.8	399	419	59.1	0.0004	0.0007	0.0000	0.0000
3	05-Feb-90	91	65.3	329	380	53.4	0.0001	0.0001	0.0000	0.0000
3	12-Feb-90	98	64.8	338	298	50.1	0.0000	0.0000	0.0000	0.0000
3	19-Feb-90	105	64.1	211	149	67.9	0.0000	0.0000	0.0000	0.0000
3	26-Feb-90	112	62.7	123	106	46.2	0.0000	0.0000	0.0000	0.0000
3	05-Mar-90	119	66.3	98	111	50.1	0.0000	0.0000	0.0000	0.0000
3	12-Mar-90	126	63.7	146	140	49.0	0.0000	0.0000	0.0000	0.0000
3	19-Mar-90	133	64.2	277	313	50.6	0.0000	0.0000	0.0000	0.0000
3	26-Mar-90	140	64.2	327	391	48.5	0.0000	0.0000	0.0000	0.0000
3	02-Apr-90	147	64.3	373	404	48.4	0.0000	0.0000	0.0000	0.0000
4	30-Apr-90	175	68.2	305	346	47.7	0.0000	0.0000	0.0000	0.0000
4	07-May-90	182	56.4	325	393	45.1				
4	14-May-90	189	64.0	361	226	88.2	0.0000	0.0000	0.0000	0.0000
4	21-May-90	196	49.8	462	414	47.6				
4	28-May-90	203	63.5	364	417	48.1				
4	04-Jun-90	210					0.0000	0.0000	0.0000	0.0000
4	11-Jun-90	217	64.3	379	415	49.7	0.0000	0.0000	0.0000	0.0000
4	18-Jun-90	224	64.7	408	456	47.2	0.0000	0.0000		
4	25-Jun-90	231	64.0	413	469	45.9	0.0000	0.0000		
4	09-Jul-90	245	64.8	393	433	43.9	0.0000	0.0000		
4	16-Jul-90	252	67.4		440	45.9	0.0000	0.0000		
4	23-Jul-90	259	67.9	417	443	49.0	0.0000	0.0000	0.0000	0.0000
4	30-Jul-90	266	61.8	406	207	32.9	0.0000	0.0000	0.0004	0.0097
4	06-Aug-90	273	63.1	427	454	43.0	0.0000	0.0000		
4	13-Aug-90	280	67.8	451	429	48.1	0.0000	0.0000		
5	20-Aug-90	287	63.4	446	437	45.5	0.0000	0.0000	0.0000	0.0000
5	27-Aug-90	294	63.3	431	432	44.6	0.0000	0.0000		
5	03-Sep-90	301	63.5	448	450	42.9	0.0000	0.0000	0.0000	0.0000
5	10-Sep-90	308	64.7	421	427	44.6	0.0000	0.0000		
5	17-Sep-90	315	63.9	437	446	43.1	0.0000	0.0000		
5	24-Sep-90	322	63.3	416	418	42.6	0.0000	0.0001	0.0006	0.0146
5	01-Oct-90	329	46.8	415	420	45.9	0.0000	0.0000	0.0010	0.0226
5	08-Oct-90	336	63.7	397	404	43.8	0.0000	0.0000	0.0000	0.0000

Oxidized Nitrogen: Nitrite and Nitrate

Phase	Date	Day	Inf NO2-N, mg/L	RAS NO2-N, mg/L	Ana NO2-N, mg/L	Aer NO2-N, mg/L	Eff NO2-N, mg/L	Inf NO3-N, mg/L	RAS NO3-N, mg/L	Ana NO3-N, mg/L	Aer NO3-N, mg/L	Eff NO3-N, mg/L
1	07-Nov-89	1										
2	18-Dec-89	42	0.391	15.721	1.506	19.312	20.470	0.113	0.107	0.107	0.955	2.031
2	25-Dec-89	49	0.313	10.406	0.182	11.419	13.060	0.650	4.647	0.112	6.201	8.321
2	01-Jan-90	56	0.171	6.746	0.213	8.220	8.412	0.228	12.085	0.371	14.462	16.792
3	22-Jan-90	77	0.075	0.078	0.030	0.083	0.080	0.465	0.032	0.034	0.016	0.041
3	29-Jan-90	84	0.073	0.143	0.035	0.161	0.157	0.063	0.030	0.007	0.005	0.008
3	05-Feb-90	91	0.040	0.070	0.005	0.113	0.108	0.019	0.012	0.004	0.005	0.008
3	12-Feb-90	98	0.262	0.033	0.000	0.060	0.061	0.038	0.019	0.006	0.006	0.005
3	19-Feb-90	105	0.127	0.007	0.000	0.021	0.018	0.116	0.029	0.000	0.000	0.011
3	26-Feb-90	112	0.362	0.019	0.000	0.042	0.041	0.646	0.025	0.022	0.022	0.009
3	05-Mar-90	119	0.083	0.088	0.009	0.145	0.141	0.241	0.033	0.008	0.015	0.009
3	12-Mar-90	126	0.175	0.056	0.000	0.091	0.093	0.371	0.027	0.012	0.013	0.008
3	19-Mar-90	133	0.050	0.048	0.000	0.079	0.089	0.551	0.020	0.017	0.008	0.010
3	26-Mar-90	140	0.078	0.026	0.000	0.074	0.083	0.491	0.022	0.009	0.008	0.000
3	02-Apr-90	147	0.085	0.030	0.000	0.042	0.045	0.412	0.020	0.010	0.000	0.007
4	30-Apr-90	175	0.270	0.031	0.000	0.056	0.059	0.103	0.023	0.009	0.006	0.009
4	07-May-90	182	0.182	0.026	0.000	0.024	0.026	0.234	0.012	0.008	0.000	0.008
4	14-May-90	189	0.278	0.009	0.000	0.030	0.018	0.158	0.016	0.000	0.000	0.007
4	21-May-90	196	0.066	0.053	0.000	0.014	0.032	0.195	0.031	0.008	0.008	0.008
4	28-May-90	203	0.047	0.000	0.000	0.010	0.012	0.407	0.020	0.007	0.009	0.009
4	04-Jun-90	210	0.122	0.000	0.000	0.041	0.009	0.549	0.014	0.013	0.007	0.010
4	11-Jun-90	217	0.109	0.009	0.000	0.008	0.008	0.592	0.026	0.015	0.007	0.011
4	18-Jun-90	224	0.046	0.000	0.000	0.007	0.007	0.556	0.028	0.021	0.015	0.021
4	25-Jun-90	231	0.137	0.000	0.000	0.009	0.010	0.516	0.019	0.019	0.008	0.007
4	09-Jul-90	245	0.336	0.000	0.000	0.008	0.010	0.222	0.019	0.016	0.008	0.008
4	16-Jul-90	252	0.323	0.016	0.000	0.010	0.012	0.083	0.033	0.012	0.000	0.009
4	23-Jul-90	259	0.217	0.000	0.000	0.011	0.013	0.155	2.510	0.060	3.553	0.026
4	30-Jul-90	266	0.104	0.000	0.000	0.011	0.012	0.000	0.013	0.000	0.000	0.007
4	06-Aug-90	273	0.170	0.000	0.000	0.010	0.012	0.039	0.015	0.000	0.010	0.015
4	13-Aug-90	280	0.000	0.000	0.000	0.011	0.031	0.013	0.011	0.000	0.008	0.014
5	20-Aug-90	287	0.155	0.000	0.000	0.008	0.012	0.013	0.000	0.000	0.000	0.019
5	27-Aug-90	294	0.000	0.000	0.000	0.012	0.012	0.000	0.026	0.010	0.000	0.029
5	03-Sep-90	301	0.214	0.000	0.000	0.031	0.011	0.023	0.000	0.000	0.008	0.016
5	10-Sep-90	308	0.164	0.000	0.000	0.011	0.011	0.033	0.000	0.000	0.000	0.013
5	17-Sep-90	315	0.079	0.000	0.000	0.031	0.011	0.166	0.012	0.012	0.014	0.020
5	24-Sep-90	322	0.078	0.000	0.000	0.013	0.013	0.250	1.705	0.775	0.149	0.111
5	01-Oct-90	329	0.105	0.000	0.000	0.059	0.081	0.058	1.679	4.532	1.967	0.167
5	08-Oct-90	336	0.083	0.000	0.000	0.046	0.013	0.178	0.010	0.010	0.000	0.554

Phosphate and Sulfate

Phase	Date	Day	Inf FOP, mg/L	RAS FOP, mg/L	Ana FOP, mg/L	Aer FOP, mg/L	Eff FOP, mg/L	Inf SO4, mg/L	RAS SO4, mg/L	Ana SO4, mg/L	Aer SO4, mg/L	Eff SO4, mg/L
1	07-Nov-89	1										
2	18-Dec-89	42	15.0	14.9	16.7	15.0	14.8	31.9	38.1	35.1	37.0	36.9
2	25-Dec-89	49	13.7	12.9	15.2	12.8	13.0	25.9	29.6	30.4	31.4	43.5
2	01-Jan-90	56	13.3	13.3	15.8	13.1	13.5	27.0	32.0	27.9	33.4	41.2
3	22-Jan-90	77	13.8	12.4	18.4	11.8	13.0	25.9	33.8	27.8	31.2	31.4
3	29-Jan-90	84	13.5	10.8	22.7	10.8	11.3	26.2	31.6	23.7	29.7	63.5
3	05-Feb-90	91	14.0	12.8	26.3	12.6	12.7	24.9	31.3	24.1	28.9	28.3
3	12-Feb-90	98	13.7	10.0	23.8	10.6	11.4	25.1	29.6	23.2	29.5	42.6
3	19-Feb-90	105	13.6	10.5	25.9	10.3	10.4	25.6	29.4	25.0	28.5	30.6
3	26-Feb-90	112	13.0	10.3	26.5	9.9	10.5	98.9	62.1	54.9	61.2	78.6
3	05-Mar-90	119	13.0	7.7	27.8	7.5	7.9	26.0	28.3	19.9	29.7	38.5
3	12-Mar-90	126	11.9	5.3	29.8	6.5	7.0	27.1	28.1	20.3	28.2	52.0
3	19-Mar-90	133	12.1	6.1	32.7	6.1	5.7	25.2	31.0	25.3	31.7	40.8
3	26-Mar-90	140	12.7	3.6	38.9	3.8	3.7	24.7	29.4	22.4	28.8	29.8
3	02-Apr-90	147	12.6	0.8	35.1	0.6	0.9	24.4	30.0	23.4	29.9	36.5
4	30-Apr-90	175	23.0	17.0	43.9	16.7	18.2	25.1	27.7	23.0	27.4	33.0
4	07-May-90	182	22.7	7.3	41.5	8.2	8.5	25.1	27.0	20.7	26.0	38.9
4	14-May-90	189	23.0	7.9	41.4	8.3	8.4	24.2	25.4	20.3	24.7	28.2
4	21-May-90	196	23.9	10.2	42.7	10.1	10.2	24.5	25.5	19.2	24.8	26.1
4	28-May-90	203	22.3	8.5	41.4	7.6	7.9	24.7	27.0	22.1	27.6	61.7
4	04-Jun-90	210	22.8	2.8	37.2	1.5	2.0	28.1	31.0	24.2	29.2	38.6
4	11-Jun-90	217	22.8	12.8	44.4	9.3	11.5	29.3	30.0	25.8	30.6	41.0
4	18-Jun-90	224	23.0	5.6	42.5	5.2	6.1	26.2	27.9	26.7	27.6	35.2
4	25-Jun-90	231	21.4	7.2	39.3	5.0	6.2	28.5	24.1	22.4	24.2	33.5
4	09-Jul-90	245	21.6	7.0	51.3	4.9	6.1	29.2	32.2	22.4	27.1	26.6
4	16-Jul-90	252	22.1	6.5	43.5	5.5	6.0	26.0	28.4	20.7	25.8	24.9
4	23-Jul-90	259	22.7	5.8	40.9	5.2	5.7	25.0	28.4	19.2	27.8	30.3
4	30-Jul-90	266	22.8	6.5	39.9	4.2	5.1	24.3	31.2	20.0	26.5	34.7
4	06-Aug-90	273	23.4	7.1	42.9	6.1	5.7	26.1	27.3	22.6	26.9	39.9
4	13-Aug-90	280	23.9	18.1	45.4	7.0	7.2	24.1	29.8	20.5	28.2	29.8
5	20-Aug-90	287	17.9	5.0	41.4	4.5	5.1	27.1	27.2	22.6	27.2	34.2
5	27-Aug-90	294	17.9	3.0	35.1	2.5	3.1	23.5	26.4	20.5	26.8	38.9
5	03-Sep-90	301	17.9	4.0	43.2	4.1	4.1	28.2	28.0	26.3	28.6	39.4
5	10-Sep-90	308	18.1	7.8	41.8	2.5	2.6	25.7	27.1	22.8	27.6	36.6
5	17-Sep-90	315	17.6	5.6	42.7	3.0	3.1	27.0	26.3	22.0	26.7	34.0
5	24-Sep-90	322	17.0	2.8	40.7	1.3	1.7	27.7	29.6	23.3	26.7	38.1
5	01-Oct-90	329	18.3	4.5	42.8	2.0	2.2	25.5	27.1	20.9	27.6	34.7
5	08-Oct-90	336	17.8	5.7	46.6	2.4	2.8	27.4	27.4	25.0	27.5	41.6

Vita

Milind Vishnu Wable was born on August 1, 1963 in Pune, India. He attended the Hindustan Antibiotics School until the seventh grade, and the St. Vincent's High School and Junior College thereafter. In 1980, he received the Higher Secondary Certificate (equivalent to a high-school diploma) from the Maharashtra State Board of Secondary and Higher Secondary Education. He graduated with a Bachelor of Technology in Chemical Engineering from the Indian Institute of Technology in Bombay, India in 1984, and a Master of Engineering in Environmental Engineering from the Asian Institute of Technology in Bangkok, Thailand in 1986. After graduation from VPI&SU, he plans to work as a Wastewater Engineer with CH2M Hill in Corvallis, Oregon.