

BIOLOGICAL TREATMENT SCHEMES FOR PREVENTING OXIME INHIBITION
OF NITRIFICATION

by

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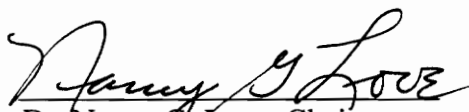
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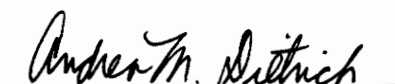
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Department of Environmental Engineering

(ABSTRACT)

The purpose of this research was to develop a single sludge multi-environment anoxic/aerobic biological treatment scheme that could achieve oxime degradation and nitrification in the same treatment process. Aerobic and anoxic batch experiments were initially performed to determine degrees of nitrification inhibition caused by three oximes, acetaldehyde oxime (AAO), aldicarb oxime (ADO), and methyl ethyl ketoxime (MEKO), and to investigate the fate of these oximes under anoxic, denitrifying conditions. Results from aerobic batch studies showed that MEKO was the only oxime which caused significant nitrification inhibition at concentrations expected in the industrial client's waste streams. Nitrification rates were reduced by 31% at MEKO concentrations as low as 2 mg/L and were almost completely inhibited above 9 mg/L. Results from anoxic batch studies demonstrated that MEKO was biologically degraded under nitrate limiting conditions, although the microorganism(s) responsible were not explicitly identified. Similar degradation trends were seen for AAO, but at significantly lower rates. ADO, however, appeared to be stable under all anoxic conditions examined. Results from batch studies were utilized to determine operational conditions for a single

sludge multi-environment anoxic/anaerobic/aerobic sequencing batch reactor supplied with a synthetic organic wastewater containing up to 40 mg/L MEKO and 56 mg/L AAO. The system was able to achieve complete oxime degradation and nitrification when operated on a one day cycle with a twelve hour anoxic/anaerobic reaction phase and a nitrate:carbon ratio below 0.15 mg NO₃-N/mg TOC.

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I. INTRODUCTION

As federal regulations become increasingly more stringent, many municipal and industrial dischargers are faced with removing inorganic nutrients from their waste streams. Biological nitrification of municipal wastewater treatment systems has proven to be an efficient method of removing ammonium ($\text{NH}_4^+\text{-N}$) from such waste streams and operational parameters have been studied and optimized (Argaman, 1982). However, biological nitrification of industrial wastewaters has proven to be more difficult as many industrial compounds have been shown to inhibit nitrification. Investigating the effects of specific organic compounds on nitrification, Hockenbury and Grady (1977) showed that many industrial organic compounds, particularly nitrogen containing organic compounds, caused significant inhibition of nitrification. The “catch 22” for these industries is that manufacturers of such nitrogen containing organic compounds are the ones who will most likely be forced to consider using nitrogen removal processes such as nitrification.

A caprolactam manufacturer in the Chesapeake Bay area (hereafter referred to as “the Industry”) is faced with the situation described by Hockenbury and Grady (1977). As the largest producer of caprolactam in the world, the Industry produces several million pounds of ammonium per day. As a result, it discharges large quantities of ammonium into its waste streams. This wastewater is discharged to the Regional Wastewater Treatment Facility (RWTF), which receives 85% of its influent from industrial facilities. Waste flows from the Industry constitute 13% of the total waste flow

at the RWTF. Although the wastewater treatment facility does not presently treat nitrogen in the wastewater, implementation of the Virginia Water Quality Standards Regulation of 1992 will require nitrogen removal in the future (Smith, 1994). As a result, both the Industry and the RWTF are investigating biological nitrogen removal as a feasible treatment technology for removing ammonium from their waste streams.

The RWTF receives the majority of its total flow from six major industrial facilities. After dilution, the influent ammonium level at the RWTF typically is in the range of about 14 - 52 mg/L-N, although spills from the Industry can lead to ammonium spikes exceeding 100 mg/L-N.

Operational conditions at the RWTF are atypical compared to conditions at most municipal plants. The RWTF utilizes an UNOX pure oxygen activated sludge process to achieve secondary treatment. It is anticipated that biological nitrogen removal at the RWTF will prove to be difficult to achieve. Specific operational conditions that may interfere with nitrification include the following:

- Covered activated sludge basins that retain carbon dioxide (CO_2) acidity produced by microorganisms can cause the pH to drop below the acceptable range of 7 - 9 for nitrifier growth (Grady and Daigger, unpublished);
- Summer temperatures of the influent wastewater often exceed 40° C, which may be detrimental to nitrifier growth (Painter, 1970);
- The elevated temperatures and slightly alkaline pH levels encountered in the influent wastewater may cause free ammonia (NH_3) concentrations to exceed inhibitory levels for *Nitrobacter* (Eckenfelder, 1991);
- The six different industries discharging to the RWTF may all be potential sources of nitrification inhibition.

As the main source of ammonium to the RWTF, the Industry has considered treating wastes from its ammonium sulfate production processes prior to discharge to the RWTF. Previous studies considered physical treatment technologies for the most highly concentrated ammonium waste streams (Smith, 1994). In 1988, preliminary biological nitrification studies were performed on the caprolactam wastewater, which contains approximately 30% of the total ammonium discharged from the Industry. Results from this study led to the conclusion that the caprolactam waste stream contained a strong nitrification inhibitor (Smith, 1993). In 1994, physical and biological treatments of the caprolactam wastewater were further investigated. Air stripping was partially effective at stripping the ammonium, but only at high air:liquid ratios. Therefore, biological nitrogen removal, if possible, would be the more economical solution. However, little to no nitrification was achieved during the treatability study. It was found that one of the specialty oximes produced by the Industry, methyl ethyl ketoxime (MEKO), was acting as a nitrification inhibitor at concentrations as low as 14 milligrams per liter (mg/L) (Smith, 1994). Although Smith (1994) concluded that another oxime present in the caprolactam wastewater, cyclohexanone oxime, was not inhibitory to nitrification, the Industry does produce several other specialty oximes throughout the plant that could possibly also pose problems to nitrification.

This research was undertaken to investigate alternative single sludge biological treatment schemes for their effectiveness in degrading oximes and preventing nitrification inhibition. Three oximes were included in this research: acetaldehyde

oxime (AAO), aldicarb oxime (ADO), and methyl ethyl ketoxime (MEKO). The specific objectives of this work were to:

1. identify the degree to which AAO, ADO, and MEKO impact the rate of nitrification in a laboratory scale activated sludge system,
2. determine the fate of each oxime under denitrifying, anoxic conditions and the rate of oxime degradation under these conditions,
3. demonstrate the potential for a multi-environment anoxic/aerobic single-sludge activated sludge treatment process to transform or biodegrade the oximes prior to the aerobic zone, thus enabling oxime degradation and nitrification in the same treatment process.

Objectives (2) and (3) were performed by the primary graduate researcher.

Objective (1) was performed by Kevin Gilmore in fulfillment of an undergraduate research project; a summary of the results from this work are presented as well.

II. LITERATURE REVIEW

Introduction

In 1988, the Industry performed initial nitrification pilot studies on their caprolactam wastewater. As cited in Smith's 1993 memo, "Report of Findings - Nitrifier Inhibition of VT-31 Wastewater" the operator noted that "nitrifying conditions are difficult to establish and maintain using conventional activated sludge process when the feed is VT-31 [caprolactam] wastewater...The mother liquor evidently contains strong inhibitory compounds." Further research by Smith (1994) led to the identification of methyl ethyl ketoxime (MEKO) as the potent inhibitor in the caprolactam wastewater.

Smith (1994) found that MEKO was inhibitory to nitrification at concentrations as low as 14 milligrams per liter (mg/L). In addition, inhibition was controlled by pH, with stronger inhibition occurring at more neutral pH conditions as compared to slightly basic conditions. This phenomena was explained by the fact that MEKO can chemically break down into degradable components, methyl ethyl ketone and hydroxylamine, under basic conditions (March, 1985). Therefore, MEKO inhibition could be controlled by stringent, basic pH control of an activated sludge system. However, such stringent pH control would be costly and potentially cause free ammonia inhibition problems.

A possible alternative solution to the MEKO inhibition problem would be the utilization of a single sludge anoxic/aerobic activated sludge system. Sensitive nitrifiers can be protected from exposure to toxic chemicals in the aerobic basin if such chemicals can be degraded in alternative, nonaerobic environments. Many organic compounds

which are aerobically recalcitrant, such as heavy halogenated and oxygen-rich compounds, can be degraded under anaerobic conditions (Haas and Vamos, 1995). In addition, as explained by Zitomer and Speece (1993), “the sequential exposure of microorganisms to anaerobic, anoxic, and aerobic environments that facilitate biological nitrogen and phosphorus removal shows promise as an effective process for removing a broad range of chemicals.”

While some research has been performed on aerobic degradation of oximes, only a few researchers have examined the fate of oximes under anoxic conditions (Castignetti and Hollocher, 1982; and Omura, in Nason and Takahashi, 1958, cited in Painter, 1970). Therefore, the following literature review was designed to encompass general aspects of single sludge multi-environment treatment schemes as they relate to removal of toxic organic chemicals and improved nitrification. A brief overview of the nitrogen cycle is presented and topics of nitrification inhibition relevant to the Industry and the RWTF are discussed. In addition, single sludge multi-environment systems designed to treat industrial wastewaters are examined. The chemical characteristics of oximes are covered, and the environmental significance of oximes are discussed. Finally, biological studies on oxime degradation are described. Because oximes can potentially hydrolyze into hydroxylamine, pertinent studies on hydroxylamine are included.

Nitrogen Transformations in Biological Treatment Systems

Painter (1970) provides an excellent overview of biological nitrogen transformations. Figure 1 shows the main biological transformations of nitrogen in

wastewater treatment processes. Although the complete nitrogen cycle is much more complex, the focus of this review is placed on ammonium oxidation and nitrate reduction.

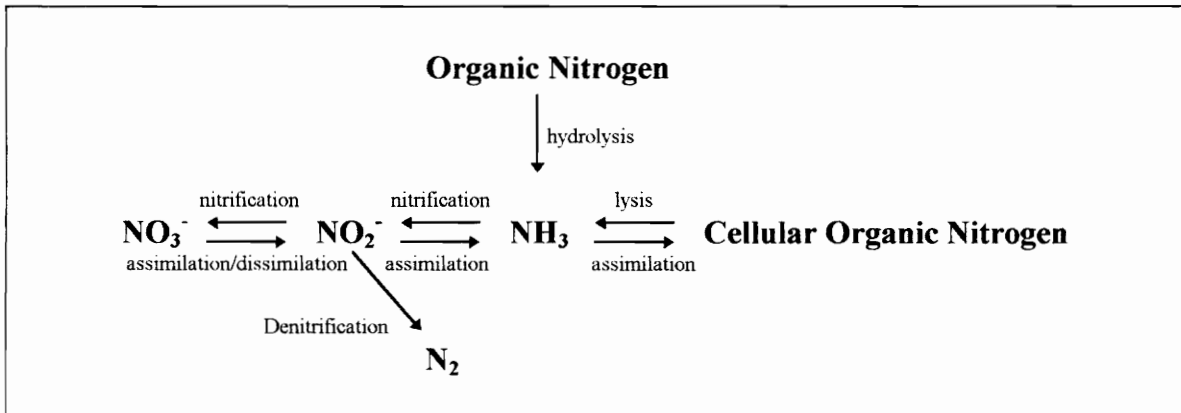


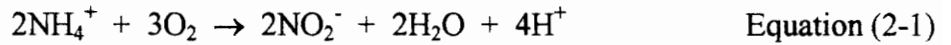
Figure 1. Biological transformations of inorganic nitrogen in wastewater treatment systems (Adapted from Painter, 1970; Metcalf & Eddy, 1991).

Nitrification

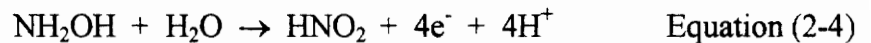
Nitrification is described as the oxidation of ammonium ($\text{NH}_4^+\text{-N}$) to nitrite ($\text{NO}_2^-\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$). Typically, nitrification is carried out by autotrophic organisms, which utilize inorganic nitrogen as an energy source and carbon dioxide (CO_2) or bicarbonate (HCO_3^-) as a carbon source. Some heterotrophic organisms are capable of nitrifying; however, typically their ammonium oxidation rates are much lower than autotrophic rates (Painter, 1970).

A number of autotrophic organisms are capable of nitrification; however, *Nitrosomonas* and *Nitrobacter* are considered the dominant autotrophs responsible for nitrification in wastewater treatment systems (Painter 1970). Nitrification by *Nitrosomonas* and *Nitrobacter* is considered a two step process. In the first step,

Nitrosomonas oxidizes ammonium to nitrite as shown in Equation (2-1). Nitrite is then oxidized to nitrate by *Nitrobacter* as described by Equation (2-2). Because *Nitrosomonas* exhibits lower oxidation rates, the first step of nitrification is generally considered the rate limiting step (Eckenfelder, 1991).



The first step of nitrification, that is, the conversion of ammonium to nitrite by *Nitrosomonas*, is postulated to be a three step process involving hydroxylamine (NH_2OH) as an intermediate compound. Equations (2-3) - (2-5) describe the three intermediate steps of ammonium oxidation. The sum of these three equations yields Equation (2-1) (Yang and Alleman, 1992).



Nitrate Reduction

Nitrate can be reduced in an assimilatory or dissimilatory manner as shown in Figure 2. Painter (1970) described differences between assimilatory and dissimilatory nitrate reduction. During assimilatory nitrate reduction, nitrate is reduced to ammonium, which can then be used for biosynthesis. This form of nitrate reduction can occur under both aerobic and anaerobic conditions; however, assimilation occurs at a much slower rate than dissimilation. In dissimilatory nitrate reduction, nitrate is utilized as a terminal

electron acceptor for carbon oxidation and is subsequently reduced to nitrite, ammonium, nitrous oxide (N₂O), or nitrogen gas (N₂). If nitrate is reduced to nitrous oxide or nitrogen gas, then the process is known as denitrification. Dissimilatory nitrate reduction can only occur in the absence of molecular oxygen (O₂), as oxygen is inhibitory to the process (Painter, 1970).

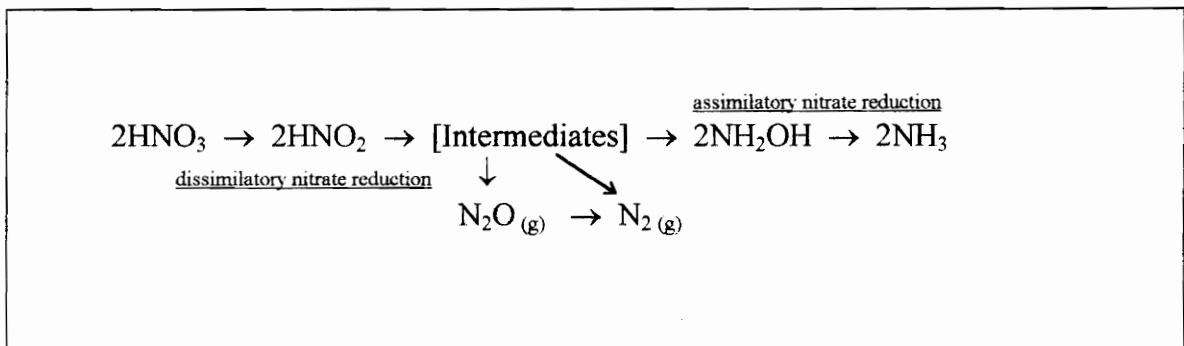


Figure 2. Description of assimilatory and dissimilatory nitrate reduction (Adapted from Painter, 1970).

Assimilatory and dissimilatory nitrate reduction are closely related as depicted in Figure 2. It is important to note that hydroxylamine is an intermediate of assimilatory nitrate reduction. In fact, the conversion of hydroxylamine to ammonium was noted in several studies cited by Painter (1970). In addition, in one study cited in Painter's literature review, Verhoeven (1957) detected hydroxylamine during dissimilatory nitrate reduction in a pure culture of *Denitrobacillus licheniformis*. Fewson and Nicholas (1961, cited in Painter, 1970) initiated the theory that denitrification evolved from assimilatory nitrate reduction.

Role of Nitrification and Denitrification in Biological Treatment Systems

Nitrification and denitrification, as described above, can be manipulated in wastewater treatment systems to achieve biological nitrogen removal. In fact, these processes often provide the most economical solution for total nitrogen removal in domestic and industrial wastewater treatment systems (Argaman, 1982). Operational conditions for biological nitrogen removal for domestic wastewaters have been studied and general design parameters have been established; however, these general parameters usually cannot be applied for industrial wastewaters. Therefore, pilot studies are often necessary to determine design needs for a particular industrial wastewater (Argaman, 1982).

Biological nitrogen removal systems are usually designed as multiple sludge or single sludge systems. Argaman (1982), Metcalf & Eddy (1991), and Grady and Daigger (unpublished) describe these systems. Figure 3 illustrates the general design for a multiple sludge system. In this treatment scheme, biodegradable organics are degraded aerobically in the first reactor, while ammonium is converted to nitrate via nitrification. Effluent from the first clarifier, which contains nitrate from nitrification, is sent to an anoxic basin, where, with the addition of an external organic carbon source, the nitrate is converted to nitrogen gas via denitrification. Note that in this scheme, two separate activated sludge populations are maintained.

Figure 4 illustrates a single sludge treatment scheme. Note that for this treatment, only one activated sludge population is maintained. Organic carbon is degraded

anoxically in the first basin, utilizing residual nitrate recirculated from the aerobic basin. In the aerobic basin, any remaining organic carbon is degraded aerobically, and influent ammonium is converted to nitrate via nitrification.

Argaman (1982) and Metcalf and Eddy (1991) highlight some of the advantages of a single sludge system over a multiple sludge system. First, external carbon requirements are eliminated by placing the anoxic zone at the front of the treatment scheme. Second, since nitrate is being utilized as a electron acceptor in the first basin, oxygen requirements in the aerobic basin are decreased, resulting in a savings in aeration costs. Finally, because nitrification consumes alkalinity, pH control is often required in a multiple stage system; however, in a single stage system, denitrification helps to produce alkalinity, preventing pH changes in the nitrifying zone.

In this research, a single sludge multi-environment anoxic/aerobic reactor was operated as a sequencing batch reactor (SBR). Haas and Vamos (1995) described the SBR as the “simplest suspended-growth process.” Orhon *et al.* (1986) explained that the SBR can often be an excellent alternative to a conventional treatment system because it provides a high degree of flexibility in its operation. In these systems, biological reactions and biomass settling are performed in a single reactor. Biological nitrogen removal can be achieved by modifying aeration schemes (Haas and Vamos, 1995). Therefore, the same sequence of processes illustrated in Figure 4 are employed in an SBR incorporating biological nitrogen removal. In this case, however, denitrification and

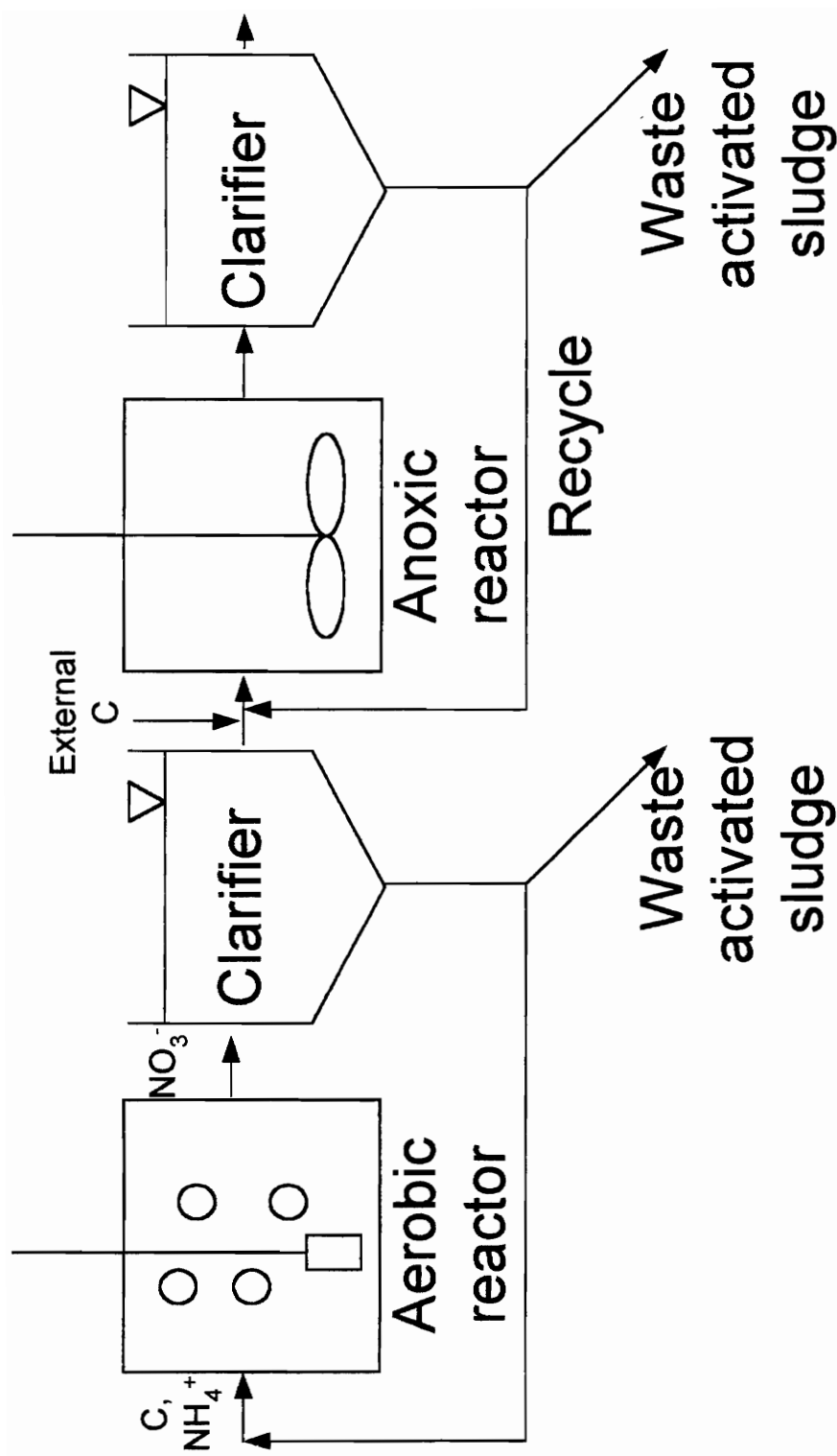


Figure 3. Illustration of multiple sludge denitrification/nitrification treatment scheme (Adapted from Givens *et al.*, 1991 and Argaman, 1982).

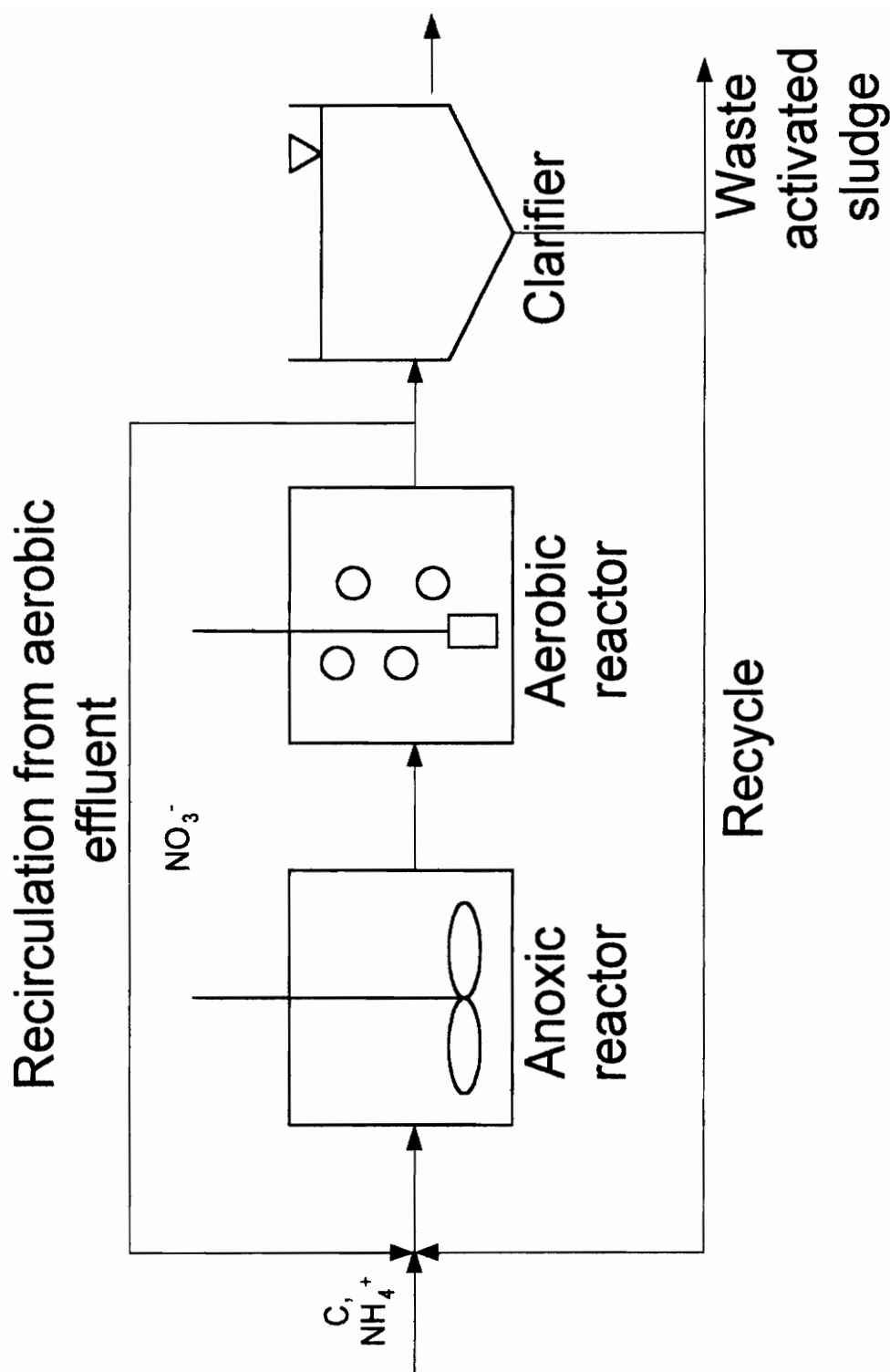


Figure 4. Illustration of single sludge denitrification / nitrification treatment scheme (Adapted from Argaman, 1982, and Givens *et al.*, 1991).

nitrification can occur in the same reactor. Figure 5 illustrates operational cycles for an SBR utilizing single sludge biological nitrogen removal.

Orhon *et al.* (1986) investigated microbial kinetics in several SBR's. They concluded that organic degradation occurs more rapidly in these systems than in conventional activated sludge systems. Their results on substrate removal over the reaction time illustrated that concentration profiles more closely resemble those in a plug flow reactor than in a complete mixed system. However, in this case, a concentration gradient occurs over time in the completely mixed system rather than over the length of the reactor as in a plug flow treatment scheme.

Nitrification Inhibition

As described by Groeneweg *et al.* (1994), nitrification is considered “the principal bottle neck” in biological nitrogen removal systems. In addition, Shammas (1986) explained that nitrification is “least reliable phase in the process sequence” of an anoxic/aerobic treatment system. Design of biological treatment schemes which include biological nitrogen removal, therefore, is often limited by nitrifiers which are characterized by low maximum specific growth rates and tend to be more prone to upsets caused by either operating conditions, such as pH and temperature, or presence of toxic organic compounds (Hockenbury and Grady, 1977; Shammas, 1986).

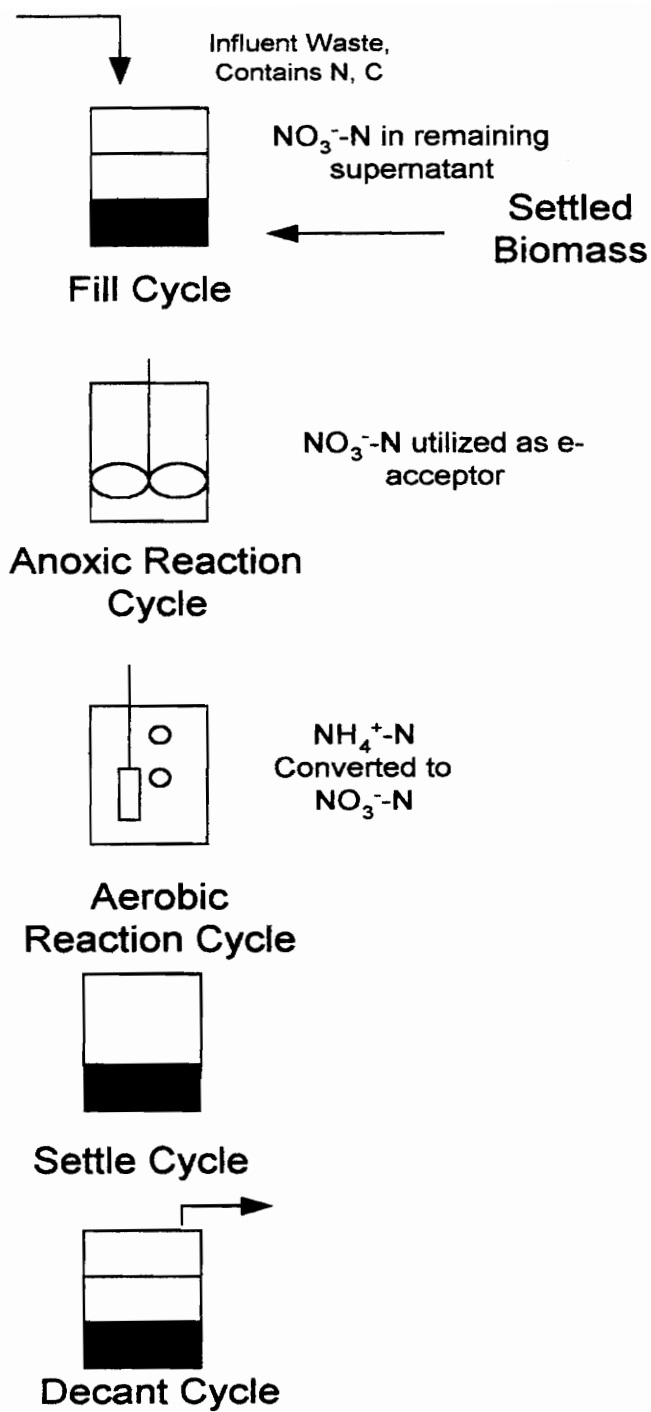


Figure 5. Operation of a single sludge SBR incorporating biological nitrogen removal.

Yang and Alleman (1992) and Painter and Loveless (1983) identified several factors that play a role in nitrification inhibition. These factors included the following:

- dissolved oxygen (DO) levels
- temperature
- pH
- free ammonia
- presence of inhibitory compounds
- solids retention time (SRT)
- loading to system.

Of these factors, temperature, pH, and presence of inhibitory compounds are discussed in detail as they relate specifically to problems that may be encountered at both the Industry and the RWTF. Although not examined separately, potential free ammonia problems are mentioned as they relate to elevated temperature and pH.

Temperature Effects

Most temperature studies on nitrification have focused on the effects of low temperatures. Because nitrifiers exhibit a higher temperature coefficient than heterotrophs, decreased rates of nitrification during winter months are more severe for these autotrophs (Eckenfelder, 1991). In general, most researchers found that nitrification rates increased with temperature with the Arrhenius relationship, $K = K_{20^{\circ}\text{C}} \Theta^{(T-20)}$, usually holding for the mesophilic temperature range (Shammas, 1986). Temperature inhibition of nitrification has been reported to occur at temperatures below

10 ° C for overall nitrification and below 12 to 14 ° C for *Nitrobacter* (Painter, 1970; Shammas, 1986).

Because aqueous waste streams at the Industry and RWTF are at elevated temperatures, nitrification inhibition at high temperatures should be considered. Unfortunately, few studies focus on the effects of high temperatures on nitrification. Optimum temperatures for nitrification have been cited from 28 to 42° C (Painter, 1970; Shammas, 1986). Several researchers have found that nitrification rates plateau at a maximum rate. For example, Shammas (1986) found that nitrification rates did not increase from 25 to 33° C and cited another study (Borchardt, 1966) in which maximum rates were observed from 15 to 35° C. An upper temperature limit for nitrification has been cited by Painter (1970) to occur at 45° C.

pH Effects

The rate of nitrification is optimum at a pH range of 7.2 to 9.0 (Metcalf & Eddy, 1991). In comparing results from other nitrification studies, Painter and Loveless (1983) found the optimum range to be around pH 7.5 to 8.5; further, nitrification was completely inhibited below pH 6.5 and above pH 10. In their own study, Loveless and Painter discovered the optimum pH range to be 7.5 to 8.0 in the majority of their experiments; they were unable to achieve nitrification at pH 6.0. In comparing nitrification rates at pH 7.0, 7.7, and 8.3, Shammas (1986) concluded that nitrification was greatest at pH 8.3 and lowest at pH 7.0. Examining ammonium oxidation rates of *Nitrosomonas europaea* for a

pH range of 5 to 11, Groeneweg *et al.* (1994) found that maximum rates occurred between pH 7.5 and 8.0 for 5 mg/L $\text{NH}_x\text{-N}$.

Smith (1994) concluded that MEKO inhibition of nitrification was pH dependent with decreasing inhibition at elevated pH levels. She postulated that MEKO inhibition could be prevented by maintaining pH levels in the activated sludge system above pH 8.2 to 8.5 to foster the breakdown of MEKO into hydroxylamine and methyl ethyl ketone. Although this pH range occurs within the optimum for nitrifiers (Groeneweg *et al.*, 1994, Metcalf & Eddy, 1991; Painter and Loveless, 1983), at the elevated temperatures encountered at the Industry, free ammonia (FA) levels could potentially cause inhibition problems. Eckenfelder (1991) noted that *Nitrobacter* can be inhibited by ammonia (NH_3) at concentrations as low as 0.1 - 1 mg/L. To put this concentration of FA into perspective, at 30° C and pH 8.5, the ratio of $\text{NH}_3/\text{NH}_x\text{-N}$ would be 0.20 (Groeneweg *et al.*, 1994). If the FA inhibition threshold limit were 1 mg/L, then *Nitrobacter* would be inhibited if the total ammonium concentration exceeded 5 mg/L! Therefore, it is evident that using elevated pH to control MEKO inhibition may lead to another form of nitrification inhibition.

Inhibitory Compounds

In addition to environmental conditions, nitrifiers can also be affected by the presence of specific organic and inorganic compounds. Although nitrifiers can be inhibited by many inorganic compounds, such as heavy metals and nitrite (Eckenfelder, 1991), it is unlikely that such inhibition would be encountered at the Industry. Therefore,

the following sections focus on inhibition caused by organic chemicals and hydroxylamine.

Several methods exist for defining nitrification inhibition due to the presence of organic and inorganic chemicals. Hockenbury and Grady (1977) described inhibition as a greater than 10% reduction in the nitrate production rate, β . This definition of inhibition is utilized throughout this report

Specific Organic Chemicals. In their investigation of nitrification inhibition, Hockenbury and Grady (1977) explained that although many studies have determined the effects of operational conditions, including pH and temperature, on nitrification, significantly less is known about the inhibitory potentials of specific organic compounds. Several researchers have reported the effects of specific organic compounds on nitrification (Hockenbury and Grady, 1977; Wood *et al.*, 1981; Joel and Grady, 1977; Tomlinson *et al.*, 1966); however, the problem lies in the fact that the number of compounds tested is minimal compared to the number of compounds that exist.

Some general trends have been identified in studies on nitrification inhibition by specific organic compounds. First, it has generally been found that *Nitrosomonas* is more sensitive to inhibition by organic compounds than *Nitrobacter* (Wood *et al.*, 1981; Hockenbury and Grady, 1977). Second, two types of inhibition have been identified, competitive inhibition and non competitive inhibition. In competitive inhibition, the degree of inhibition is a direct function on the ammonium concentration, with increased inhibition at lower ammonium/inhibitor ratios. In the case of non competitive inhibition,

the degree of inhibition is not a function of the concentration of ammonium, but rather a function of the concentration of the organic compound, itself (Hockenbury and Grady, 1977).

Some trends in nitrification inhibition have been identified with certain classes of organic compounds. For example, as noted by Wood *et al.* (1981), compounds containing the N-C-S group, such as thiourea and its analogues, were particularly toxic to nitrification, even at very low concentrations. Hockenbury and Grady (1977) found that many industrial nitrogen containing organic compounds were inhibitory to nitrification. Some of the most potent inhibitors included dodecylamine, aniline, and n-methylaniline. Interestingly, they found that the majority of these compounds did not exhibit competitive inhibition. Conversely, Smith (1994) found that MEKO, another industrial nitrogen containing organic compound, was acting as a competitive inhibitor against nitrification.

As a final note, in a study on aniline, Joel and Grady (1977) reported that because aniline was degraded aerobically, nitrification could be achieved in both single stage and double stage systems. These results are important to note because, like MEKO, aniline is inhibitory at very low concentrations, causing greater than 50% inhibition at 1 mg/L. Thus, by degrading the toxic organic compound, inhibitory effects were eliminated. The researchers concluded that both completely mixed systems and plug flow systems could be used successfully to degrade aniline and achieve nitrification. However, they noted that the completely mixed system would require an equalization basin to prevent shock

loads which would upset nitrification. Furthermore, they noted that an effective solids retention time (SRT) for the nitrifiers in the plug flow reactor must be based on the relative reaction time remaining after aniline had been completely degraded.

Hydroxylamine. Hydroxylamine has been found to be bacteriostatic to many microorganisms at concentrations exceeding 33 mg/L (Resenkranz and Bendich, 1963; Amarger and Alexander, 1968). In addition, two studies have reported that hydroxylamine severely inhibited *Nitrobacter*. In one study, Stuvén *et al.* (1992) discovered that hydroxylamine inhibited *Nitrobacter* at 1 mg/L. In another study, Yang and Alleman (1992) postulated that *Nitrobacter* inhibition experienced during low DO conditions was actually the result of elevated concentrations of hydroxylamine. Concentrations detected were on the order of 1 to 2 mg/L.

Multi-Environment Systems as Solution for Preventing Nitrification Inhibition

As explained in Joel and Grady's report (1977), nitrification inhibition caused by the presence of toxic organic compounds can be eliminated if the compound can be successfully degraded. However, Joel and Grady considered only the aerobic degradation of aniline in their study; therefore, since nitrification could occur only after aniline had been nearly completely degraded, retention times had to be significantly increased to achieve good ammonium removal. Such increased retention times result in increased basin volumes and, subsequently, increased treatment costs. Conversely, if an inhibitory compound can be degraded in an alternative environment, such as an anoxic or anaerobic

environment, then nitrification can be achieved without increasing retention times in the aerobic basin.

The following section examines the potential for degrading toxic organic chemicals in alternative environments and considers the advantages of utilizing a single sludge multi-environment system for the prevention of nitrification inhibition.

Treatment of Toxic Organic Compounds

Unfortunately, few studies have been performed on the anoxic degradation of toxic organic compounds. More studies have been performed on the anaerobic degradation of toxic organic compounds. For example, Kupferle *et al.* (1995) reported 76% removal of methyl ethyl ketone, a degradation product for MEKO, in an anaerobic pretreatment scheme. Zitomer and Speece (1993) provided an excellent overview on the role of alternative environments on the fate of toxic organic compounds. As explained in their review, many compounds which cannot be degraded aerobically can be successfully degraded under anoxic or anaerobic conditions. For example, highly chlorinated compounds, which are often aerobically recalcitrant, can be dehalogenated under anaerobic conditions. In addition, many compounds have been found to degrade most effectively when subjected to alternating oxidative and reductive environments.

Zitomer and Speece (1993) described the anoxic fate of several specific organic compounds. They explained that generalizations on anoxic degradation of specific groups of organic compounds cannot be made. For example, while some aromatic organics, such as phenol and toluene, are biodegradable under anoxic conditions, others,

such as benzene and naphthalene, are either anoxically recalcitrant or degrade very slowly under anoxic conditions. The fact that generalizations cannot usually be made for the fate of groups of organic compounds under anoxic conditions highlights the importance of investigating the fate of specific organic compounds before considering multi-environments as a possible treatment scheme. For example, prior to this research, the fate of oximes under alternative environments had not been well elucidated. Therefore, the investigation of oxime degradation under anoxic conditions played a vital role in determining the possibility of utilizing a multi-environment treatment scheme for preventing oxime inhibition of nitrification.

Advantages and Disadvantages of Single Sludge Multi-Environment Systems

Utilizing a single sludge multi-environment treatment scheme can provide several advantages over conventional activated sludge treatment. First, as explained earlier, Joel and Grady (1977) reported that increased retention times were necessary to achieve good nitrogen removal when using only an aerobic treatment process for degrading aniline. Therefore, utilization of a multi-environment treatment scheme for degradation of a nitrification inhibitor can eliminate the need for an increased retention time in the aerobic basin. In addition, exposure of microorganisms to alternative environments has been shown to improve sludge settling (Zitomer and Speece, 1993). Also, because nitrate is being utilized as a terminal electron acceptor for the majority of the carbon utilization, oxygen requirements are often decreased for the aerobic basin (Argaman, 1982). Further, with an anoxic basin as the first step in the treatment scheme, nitrifiers are often

protected against toxic shocks (Joel and Grady, 1977). Finally, anoxic degradation of volatile organic compounds can prevent stripping, which is often encountered in aerobic systems (Haas, 1995).

Argaman (1982) and Metcalf & Eddy (1991) list a few disadvantages of single sludge multi-environment systems. First, as explained by Argaman (1982), although single sludge systems often reduce aeration costs, they maintain higher mixed liquor pumping requirements. In addition, while single sludge systems can eliminate the need for increased retention times in the aerobic basin, overall structure requirements can be augmented by the incorporation of an anoxic zone (Metcalf & Eddy, 1991).

Oximes

Chemical Characteristics

Oximes are characterized by a hydroxyl group double bonded to a carbonyl compound as shown in Figure 6 (Carex, 1987). For aldehyde oximes and ketoximes with unsymmetrical carbon chains, there exist *syn* and *anti* isomers depending on the location of the hydroxyl group. *Syn* and *anti* isomers for AAO are illustrated in Figure 7. Note that for the *syn* isomer, the hydroxyl group is located on the same side as the hydrogen on the carbon chain, while for the *anti* isomer, the hydroxyl group is situated on the opposite side of the hydrogen on the carbon chain (Gilman, 1943).

Oximes are formed by reacting hydroxylamine with their respective carbonyl compounds. This reaction occurs under basic conditions and at high temperatures. The following chemical reaction represents the oximation of acetaldehyde to AAO

(Dazeley, 1965):



Just as carbonyl compounds react with hydroxylamine to form oximes, oximes can also hydrolyze into hydroxylamine and their respective ketone or aldehyde. This hydrolysis occurs naturally under acidic and basic conditions or with the addition of specific reagents, such as thallium(III) nitrate (March, 1985). Oxime hydrolysis has been noted by several researchers. Smith (1994) postulated that decreased MEKO inhibition at elevated pH's was caused by the hydrolysis of MEKO into methyl ethyl ketone and hydroxylamine between pH 8.2 and 8.5. In one heterotrophic nitrification study, Castignetti *et al.* (1985) noted that chemical degradation of several oximes occurred in uninoculated flasks. For example, after a 21 day study, half of the original acetone oxime concentration had decomposed as measured by increased hydroxylamine, nitrite, and nitrate concentrations in uninoculated flasks. Finally, Amarger and Alexander (1968) concluded that oxime degradation occurred as a result of heterotrophic microorganisms utilizing its hydrolysis product, hydroxylamine, as a nitrogen source.

Oximes Studied

Three oximes, acetaldehyde oxime (AAO), aldicarb oxime (ADO), and methyl ethyl ketoxime (MEKO) were included in the scope of this research. Figure 8 gives the chemical structures for these three oximes. General chemical characteristics for each oxime are shown in Table 1.

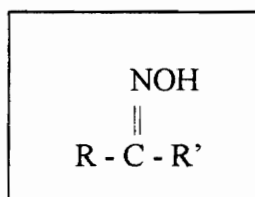


Figure 6. General chemical structure for an oxime compound.

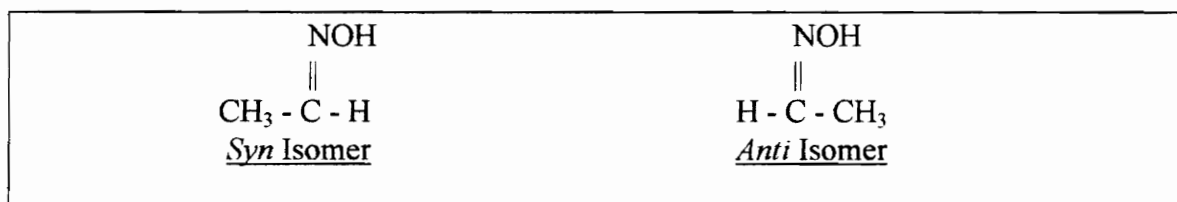


Figure 7. Illustration of *syn* and *anti* isomers for AAO (Adapted from Gilman, 1943).

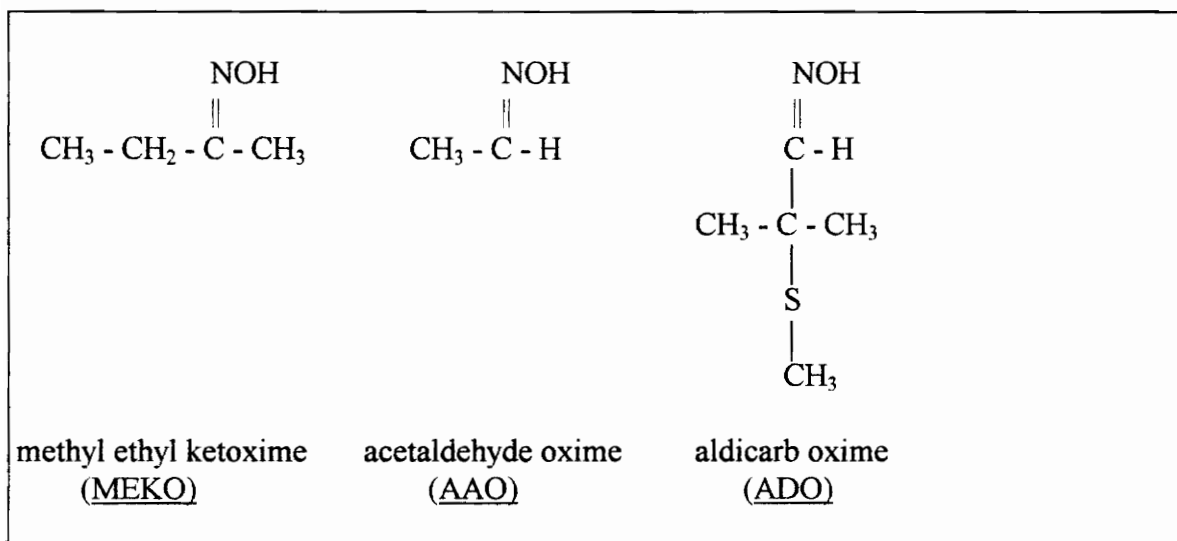


Figure 8. Chemical structures for the three oximes studied.

Table 1. Chemical characteristics of the oximes studied.

Oxime Name	Chemical Formula	Molecular Weight	Specific Gravity (g/mL)	Solubility (%) [*]	Boiling Point (°C)	Melting Point (°C)
Acetaldehyde oxime, acetaldoxime, AAO	C ₂ H ₅ NO	59	0.9656	Complete	107 [*]	-
Aldicarb oxime, [*] 2-methyl-2-(methylthio) propionaldehyde oxime, ADO	C ₅ H ₁₁ SNO [*]	133 [*]	1.15 [*]	2.5 (22 °C) [*]	210 [*]	21 [*]
methyl ethyl ketoxime, 2-butanone oxime, MEKO	C ₄ H ₉ NO	87	0.9232	Moderate	152	-29.5

Table information adapted from Astle and Weast, 1985, except when marked with (^{*}).

^{*}Adapted from MSDS for oximes.

Uses and Environmental Significance

AAO. AAO is a specialty oxime produced by the Industry. Waste streams from AAO production at the industry are stripped and incinerated causing AAO to partially volatilize. Residual aqueous waste streams are sent to an equalization holding basin, where hydroxylamine and oxime concentrations are monitored. Because AAO is partially volatilized during hazardous waste treatment, final concentrations of this oxime in the equalization basin do not typically exceed 10 mg/L (Smith, 1995).

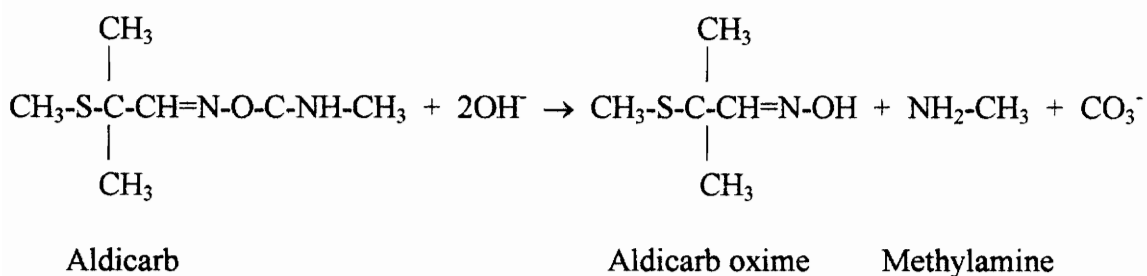
AAO also has been found to occur naturally in the environment. In one study by Mulvaney and Hageman (1984), AAO was identified as the main nitrogen product formed from nitrate reduction in soybean leaves during an *in vivo* nitrate reductase assay. The researchers concluded that further studies are required to determine the role of this oxime in nitrate dissimilation.

Although AAO is considered only moderately toxic, laboratory testing of the compound led to the conclusion that occupational exposure can cause potential health problems. As noted in the Environmental Protection Agency's (EPA's) Final Test Rule for MEKO (1989), AAO caused positive results in a lymphoma mutagenicity study on mice. However, tumors were discovered only in male rats.

ADO. Aldicarb oxime is produced and sold by the Industry to manufacturers of the insecticide, aldicarb. Like AAO, ADO waste streams are stored in an oxime holding pond for oxime and hydroxylamine monitoring. Under normal operating conditions, average aqueous ADO concentrations are approximately 50 mg/L (Smith, 1995).

Although little research has been performed on aldicarb oxime, many researchers have consider the fate of aldicarb, one of the most potent insecticides known. Pirbazari *et al.* (1989) reported an LD₅₀ for aldicarb of 0.9 mg/kg. Upon application, aldicarb rapidly undergoes either oxidation in the environment to form aldicarb sulfoxide and aldicarb sulfone, or hydrolysis to form aldicarb oxime and aldicarb nitrile (Mateen, 1994). The sulfoxide and sulfone metabolites can then further degrade, hydrolyzing into their corresponding sulfoximes and nitriles (Pirbazari *et al.*, 1989). Although the hydrolysis products of aldicarb show significantly lower toxicities, aldicarb sulfoxide and aldicarb sulfone posses similar toxicities to aldicarb (Mateen *et al.*, 1994). Because aldicarb has leached into groundwater and contaminated drinking water systems in over 16 states, maximum contaminant levels (MCLs) in drinking water supplies have been set for aldicarb (0.01 mg/L), aldicarb sulfoxide (0.01 mg/L), and aldicarb sulfone (0.04 mg/L) (Pirbazari *et al.*, 1989).

Equation (2-7) illustrates the hydrolysis of aldicarb into aldicarb oxime (US EPA, 1988):



Equation (2-7);

Oxidation of aldicarb into sulfone and sulfoxide metabolites is considered the dominant reaction over hydrolysis in moist, neutral soil environments (US EPA, 1988). Hydrolysis into aldicarb oxime is slow under neutral conditions, but rapidly increases with increasing pH and temperature (US EPA, 1988). Aldicarb hydrolysis has also been mediated in anaerobic environments (Mateen *et al.*, 1994; Trehy *et al.*, 1984). Trehy *et al.* (1984) found that one half of the aldicarb in a sterile anaerobic sample degraded into aldicarb oxime after 43 days at pH 8.2. The aldicarb oxime did not further degrade, remaining stable for 2 weeks, even at pH 11. In the presence of microorganisms, however, the insecticide was converted rapidly to aldicarb nitrile with 85% degradation after two weeks.

MEKO. MEKO is utilized as an alternative corrosion inhibitor exhibiting a lower toxicity than its predecessor corrosion inhibitor, hydrazine (Rumpf and Gonzalez, 1993). This oxime is often added to oil based paints or used as an oxygen scavenger in boilers with mechanical deaerators. In a corrosion control study for boiler systems, Rumpf and Gonzalez (1993) found that MEKO performed similarly, if not better than hydrazine, hydroquinone, and carbohydrazide as an oxygen scavenger.

The Industry produces MEKO as a corrosion inhibitor for the previously mentioned applications. During Smith's investigation of biological treatment of the Industry's caprolactam wastewater (1994), average MEKO concentrations were approximately 90 mg/L. Since then, process modifications have resulted in lower

aqueous MEKO concentrations. Under normal operating conditions, average MEKO concentrations are approximately 50 mg/L (Smith, 1995).

Because MEKO is structurally similar to AAO, the U.S. EPA expressed concerns for occupational exposure to MEKO. In 1989, the U.S. EPA issued a final test rule for MEKO under the Toxic Substances Control Act (TSCA). This ruling, which requires manufacturers of MEKO to perform routine health effects tests, was the result of several previous tests on MEKO indicating that it may be mutagenic and carcinogenic (U.S. EPA, 1989).

Biological Degradation of Oximes

As mentioned previously, little research has been performed on the biological fate of oximes. Most research on oxime degradation has focused solely on its role in heterotrophic nitrification. Fewer studies have been performed on the anoxic fate of oximes. Alternatively, hydroxylamine, an oxime hydrolysis product, has been investigated in many research projects.

Heterotrophic Nitrification Studies. Typically ammonium oxidation, or nitrification, is associated with chemoautotrophic organisms. However, it has been found that some heterotrophic organisms are capable of oxidizing ammonium to nitrite. Unlike autotrophs which utilize carbon dioxide as a carbon source, heterotrophic nitrifiers utilize organic carbon for growth. In addition, they can utilize both inorganic and organic nitrogen (Castignetti *et al.*, 1985).

Several studies have led to the conclusion that certain heterotrophic organisms have the ability to oxidize oximes and/or hydroxylamine to nitrite under aerobic conditions (Amarger and Alexander 1968; Castignetti and Gunner, 1980; Castignetti and Hollocher, 1982; Castignetti and Hollocher, 1984; Castignetti *et al.*, 1985; Castignetti *et al.*, 1986; Verstraete and Alexander, 1972). In 1973, Verstraete and Alexander became the first researchers to observe heterotrophic nitrifier behavior in environmental samples, such as water, soil, and sludge samples. They concluded that although heterotrophic microorganisms existed in the environment that were capable of oxidizing nitrogen, oxidation rates for these microorganisms were much lower than for their autotrophic counterparts.

In 1980, Castignetti and Gunner discovered an *Alcaligenes* strain capable of nitrifying pyruvic oxime (PO), a product from animal and plant tissue decay. This heterotrophic microorganism produced significantly larger quantities of nitrite than had been previously reported for other heterotrophs. Only small quantities of nitrate were produced. The researchers noted that growth occurred concurrently with oxime degradation, concluding that the oxime carbon was being utilized for growth. This conclusion conflicted with an earlier study (Obaton *et al.*, 1968, cited in Castignetti and Gunner, 1980), where an external organic carbon source was needed in order for *Pseudomonas aeruginosa* to successfully oxidize AAO.

Several other strains of heterotrophic microorganisms have been found to utilize pyruvic oxime. In 1984, Castignetti and Hollocher examined twelve heterotrophic

denitrifiers for their ability to utilize PO and hydroxylamine under aerobic conditions. Results from this study are summarized in Table 2. Six of these microorganisms generated nitrite in the presence of PO, and eight were capable of utilizing hydroxylamine. No distinct pattern was noted in oxidation, as some microorganisms were able to preferentially oxidize PO over hydroxylamine, while some could oxidize only hydroxylamine.

Table 2. Comparison of the abilities of different heterotrophic microorganisms to oxidize pyruvic oxime and hydroxylamine under aerobic conditions.

Microorganism	Oxidized PO (Y/N)	Oxidized NH ₂ OH (Y/N)	Comments ¹
<i>Alcaligenes faecalis</i>	Y	N	
<i>Chromobacterium violaceum</i>	N	Y	
<i>Flavobacterium</i> sp.	N	Y	
<i>Neisseria sicca</i>	N	N	Failed to grow
<i>Paracoccus denitrificans</i>	N	N	
<i>Pseudomonas aeruginosa</i> PAO1	Y	Y	NH ₂ OH > PO
<i>Pseudomonas aureofaciens</i>	Y	Y	NH ₂ OH > PO
<i>Pseudomonas denitrificans</i>	Y	Y	NH ₂ OH > PO
<i>Pseudomonas fluorescens</i> 400	Y	Y	NH ₂ OH > PO
<i>Pseudomonas fluorescens</i> 401	N	N	
<i>Pseudomonas fluorescens</i> 402	Y	Y	NH ₂ OH > PO
<i>Pseudomonas stutzeri</i>	N	Y	

¹NH₂OH > PO, indicates the ability of a microorganism to oxidize hydroxylamine at a faster rate than PO. (Data adapted from Castignetti and Hollocher, 1984).

Several researchers have tried to identify other oximes utilized by heterotrophic nitrifiers. Amarger and Alexander (1968) discovered that *P. aeruginosa* was able to utilize acetaldehyde oxime (AAO), acetone oxime, butyraldoxime, and butanone oxime (MEKO) under aerobic conditions. Interestingly, microorganisms grown on AAO were able to oxidize hydroxylamine to nitrite, yet those grown in the absence of oximes could

not oxidize hydroxylamine. Later, Castignetti *et al.* (1985) investigated the ability of an *Alcaligenes* sp. to oxidize seven oximes, including acetohydroxamic, AAO, butyraldehyde oxime, acetone oxime, α -ketobutyric oxime, and α -ketoglutaric oxime. Of these oximes, the *Alcaligenes* sp. was only able to oxidize α -ketobutyric oxime and α -ketoglutaric oxime. It is important to note that in this study no external carbon was added; therefore, the heterotrophs had to rely on the oxime as the sole carbon and nitrogen source. Unlike *P. aeruginosa*, which was able to utilize hydroxylamine in a growth medium (Amarger and Alexander, 1968), the *Alcaligenes* sp. grown in an α -ketoglutaric oxime medium could not oxidize hydroxylamine.

Pathways for oxime oxidation were postulated in several studies (Amarger and Alexander, 1968; Castignetti and Hollocher, 1982; Castignetti *et al.*, 1983; Castignetti *et al.*, 1985; Castignetti *et al.*, 1986). Figure 9 depicts the two major pathways proposed. Amarger and Alexander (1968) believed that aerobic oxime degradation occurs via its hydrolysis product, hydroxylamine. They supported this conclusion by observations that heterotrophic cell free extracts more readily oxidized hydroxylamine than oxime nitrogen and that the quantity of nitrite generated from oximes by these cell free extracts was similar to the quantity of hydroxylamine present as a hydrolysis product in the oxime medium. According to Amarger and Alexander's hypothesis, the oxime provides a constant source of hydroxylamine, which is continually oxidized by heterotrophic nitrifiers. Interestingly, Amarger and Alexander found that at concentrations greater than 1 mM hydroxylamine inhibited growth of *P. aeruginosa*.

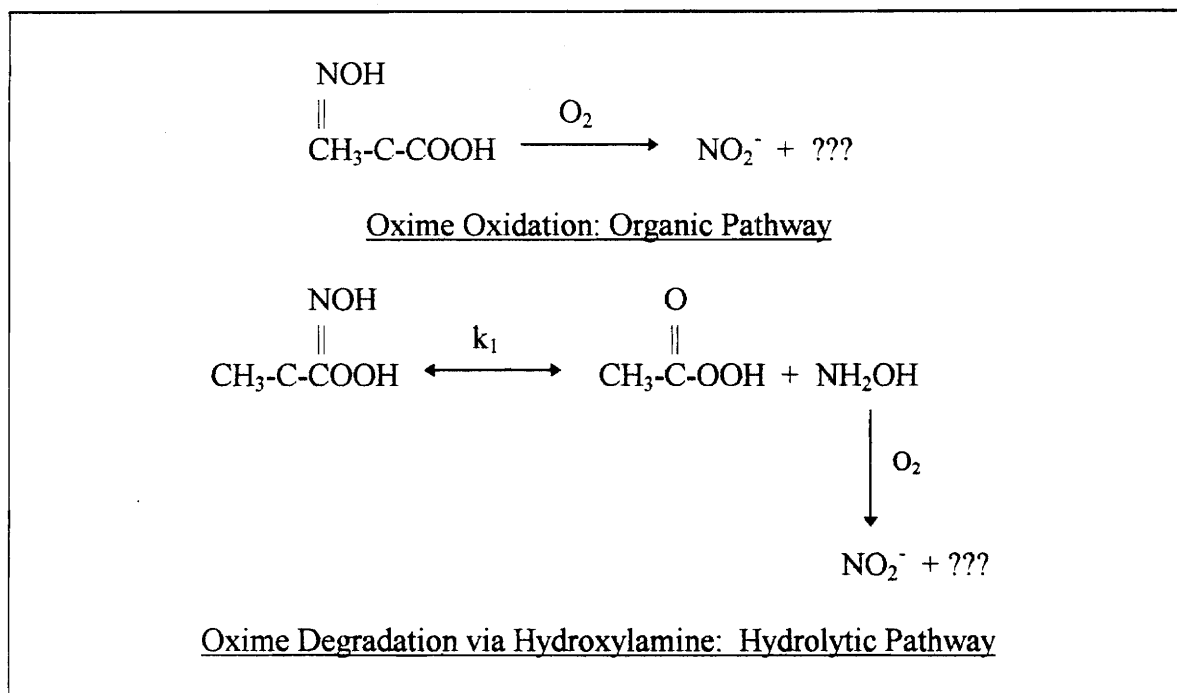


Figure 9. Proposed pathways for aerobic oxime degradation. (Adapted From Castignetti and Hollocher, 1982).

Castignetti concluded that aerobic oxime degradation did not occur via hydroxylamine, but rather through an organic pathway as shown in Figure 9. In this pathway the nitrogen is oxidized prior to its cleavage from the carbon. This conclusion was supported by the fact that an *Alcaligenes* sp. utilized PO at a rate 20 times faster than it oxidized hydroxylamine (Castignetti and Hollocher, 1982). This conclusion was supported in all of Castignetti's heterotrophic nitrification studies (Castignetti and Hollocher, 1982; Castignetti *et al.*, 1983; Castignetti *et al.*, 1985; Castignetti *et al.*, 1986) except one (Castignetti and Hollocher, 1984). When investigating if other heterotrophic microorganisms were capable of utilizing PO, Castignetti and Hollocher found that some heterotrophs were able to oxidize hydroxylamine preferentially over PO (See Table 2).

They concluded that, for the five heterotrophs that preferentially utilized hydroxylamine, both the organic and hydrolytic pathways are possible, but that further research is required to clarify which pathway is utilized.

Anoxic Studies. Very little research has been performed on the anoxic fate of oximes. In a literature review on inorganic nitrogen metabolism, Painter (1970) cited a study by Omura in which AAO, pyruvic acid oxime, and hydroxylamine inhibited nitratase preparations. In 1982, Castignetti and Hollocher investigated the ability of a denitrifier *Alcaligenes* sp. to utilize PO; however, although *Alcaligenes* sp. was grown as a denitrifier in the absence of oxygen, the experiment was performed under aerobic conditions. Therefore, caution should be taken in interpreting these results as the fate of oximes under truly anoxic conditions. Castignetti and Hollocher found that the denitrifier was unable to utilize PO under aerobic conditions; however, it was able to convert hydroxylamine to equimolar concentrations of nitrous oxide and ammonium.

Additional Hydroxylamine Studies. From the previous discussion on oxime degradation it is evident that, as a hydrolysis product for oximes, hydroxylamine may play an important role in oxime degradation. Hydroxylamine is highly toxic to microorganisms, showing bacteriostatic actions at concentrations as low as 1 mM (Resenkranz and Bendich, 1963; Amarger and Alexander, 1968). However, several researchers demonstrated that, at lower concentrations, hydroxylamine can be aerobically oxidized to nitrite by both autotrophic and heterotrophic nitrifiers (Amarger and Alexander, 1968; Bruijn *et al.*, 1995; Castignetti and Hollocher, 1984).

Two studies investigated the fate of hydroxylamine under anoxic conditions. In a novel study, Bruijn *et al.* (1995) discovered that *Nitrosomonas europaea* converted hydroxylamine to nitrous oxide, utilizing nitrite as the terminal electron acceptor. In another study, Kurcera and Skladal (1990) concluded that hydroxylamine was utilized as the terminal electron acceptor for the cytochrome c region of a denitrifier, *Paracoccus denitrificans*.

In addition to serving as an electron donor, and, possibly, as an electron acceptor, hydroxylamine can also be utilized in an assimilatory manner. In his literature review, Painter (1970) commented that the conversion of hydroxylamine to ammonium is commonly noted, as hydroxylamine is an intermediate of assimilatory nitrate reduction. Interestingly, one researcher (Verhueven, 1956, cited in Painter, 1970) detected hydroxylamine during dissimilatory nitrate reduction. This finding led to the postulation that assimilatory and dissimilatory nitrate reduction are closely linked. However, in their 1982 oxime study, Castignetti and Hollocher noted that no nitrogen gas was produced during hydroxylamine degradation by a denitrifier subjected to aerobic conditions. This finding insinuated that hydroxylamine was being utilized in an assimilatory manner and not in a dissimilatory manner.

Summary

As noted in the review of the literature, the biological fate of oximes under anoxic conditions has not been well elucidated. No studies were found in which the degradation of oximes was investigated under strictly anoxic conditions. However, several studies

did consider the degradation of hydroxylamine under such conditions (Bruijn *et al.*, 1995; Kurcera and Skladal, 1990). The fact that no previous research has considered anoxic degradation of oximes highlights the importance of this research in determining the anoxic fate of oximes. Because oximes do have the ability to hydrolyze into hydroxylamine, care must be taken to examine this route of degradation. Ammonium and nitrous oxide production should be examined as hydroxylamine has been noted to reduce to these compounds during assimilatory nitrate reduction (Painter, 1970). Also, hydroxylamine should be considered as a potential alternative electron acceptor under anoxic conditions as noted in Kurcera and Skladal's study (1990).

III. MATERIALS AND METHODS

The purpose of this research was to investigate the effectiveness of alternative single sludge environments in preventing oxime inhibition of nitrification. This section describes the methods employed to achieve this goal. The first section explains the experimental approach adopted for this research. In the second section, specific details of sampling and analyses are provided. Operation of three SBR's is described. In addition, experimental procedures for aerobic and anoxic batch studies are provided. Finally, specific analysis techniques are explained.

Experimental Approach

Specific research objectives, as described in the introductory chapter, were achieved in two distinct phases. Phase one consisted of a series of anoxic and aerobic batch experiments to investigate the fate of oximes in the presence of a denitrifying culture and to determine the degree of nitrification inhibition caused by each oxime. Activated sludge for batch experiments was obtained from two master SBR's; one was aerobic, and one was anoxic. Separate reactors were maintained since Smith (1994) had previously identified MEKO as a potent inhibitor of nitrification. The aerobic SBR was fed a broad range of biogenic organics, ammonium, phosphorus, and micronutrients, so that it could develop a robust activated sludge culture with a strong nitrifier community for conducting oxime batch studies. The intent of the second reactor was two fold. First, this reactor acted as a master activated sludge source for anoxic and anaerobic batch experiments. Second, this reactor was fed oximes in addition to a range of biogenic

organics. Oxime concentrations fed to the anoxic master reactor were slowly increased over time in an attempt to attain an acclimated oxime-degrading culture.

Results from phase one of this research were vital for the commencement of phase two. The purpose of this second phase was to combine the anoxic and aerobic activated sludges from the SBR's operated in the first phase to investigate the ability of a single sludge anoxic/anaerobic/aerobic SBR to achieve nitrification using a wastewater containing oximes. Operational parameters, such as anoxic/anaerobic detention times, were determined based on results from phase one of this research. Daily monitoring of this system helped to improve operation, so that complete ammonium removal could be achieved. This activated sludge was used for a few additional anoxic and anaerobic batch studies, which were performed to further define mechanisms involved in MEKO degradation.

Materials

Test Compounds

Oximes included in the scope of this research were AAO, ADO, and MEKO. Chemical characteristics of these oximes were shown previously in Table 1. Oxime solutions supplied to the master SBR's and utilized in batch experiments were prepared from stocks obtained from the Industry. While ADO and MEKO stocks were considered pure, the AAO stock was approximately a 50 - 54% solution. For quality assurance purposes, oxime standards were prepared from pure (99%) stocks obtained from Aldrich (Milwaukee, WI). Note that because aldicarb oxime was not sold by other

manufacturers, ADO standards were made from the pure solution provided by the Industry.

Initial Biomass Inocula

Original biomass for the master SBR's was obtained from a secondary treatment system for one of Eastman Chemical Company's production plants. This industrial activated sludge contained a diverse culture of microorganisms acclimated to xenobiotic compounds. In addition, the aerobic reactor was supplied with half of its initial biomass from a local municipal wastewater treatment plant which nitrifies regularly.

Biogenic Organic Solutions and Mineral Salt Medium (MSM)

Reactors were supplied with a synthetic biogenic organic feed adapted from Sock (1993) as shown in Table 3. In addition, the anoxic and multi-environment SBR's were fed AAO, ADO, and MEKO. Two liters of each biogenic stock solution were prepared periodically, stored at 4 °C, and diluted to make reactor feed. A 1:200 dilution of the three biogenic stock solutions provided a Chemical Oxygen Demand (COD) of approximately 1,100 milligrams per liter (mg/L). Sterile techniques, as cited by Rosalie and Gherna (1994), were adopted in preparing biogenic organic stocks. Protein stocks were autoclaved for 20 minutes (at 121 °C and 15 PSI), while organic acid and sugar stocks were filter sterilized using 0.2 µm bottle-top vacuum filter units (VacuCap™ 60). All stock solution bottles were autoclaved prior to use.

Table 3. Composition of synthetic biogenic organic wastewater

Compound	Stock Concentration (g/L)	Feed COD (Diluted 1:200) (mg/L)
Proteins		462
Beef Extract	25.6	
Bacto-Casitone	13.6	
Phytone™	13.6	
Yeast Extract	41.6	
Sugars		156
Fructose	15.6	
Galactose	15.6	
Glucose	15.6	
Organic Acids	(mL/L)	459
Acetic Acid	98	
Glycerol	14	
Total COD (mg/L)		1100

In addition to organics, the reactors were also provided with macro and micro nutrients. Composition of the mineral salt medium (MSM) was adapted from Porter (1993). Individual nutrient concentrations are shown in Table 4. Mineral salt chemicals, which were obtained from Fisher Scientific, were reagent grade or better. Separate chloride, ammonium, phosphate, and sulfate stocks were prepared. In addition, calcium chloride (CaCl_2) was prepared as a single stock to avoid precipitation in the chloride stock. Five milliliters (mL) of each stock solution was diluted into one liter of tap water to prepare the MSM supplied to master SBR's. MSM for batch experiments was one-fifth the concentration for SBR operation. Note that a phosphate stock solution was utilized for the anoxic and multi-environment reactors, while a pH 8 phosphate buffer

Table 4. Composition of mineral salt medium (MSM) for Sequencing Batch Reactor and batch experiments¹.

Compound	Stock Concentration (g/L)	Stock Molarity (mM)	Feed Concentration (mg/L)	Feed Molarity (mM)
Chlorides				
CaCl ₂ ·2H ₂ O ²	5.3	36	27	0.18
FeCl ₃	1.8	11	9.0	0.055
CoCl ₂ ·6H ₂ O	0.30	1.3	1.5	0.0063
ZnCl ₂	0.30	2.2	1.5	0.011
CuCl ₂ ·2H ₂ O	0.10	0.59	0.50	0.0029
H ₃ BO ₃	0.03	0.49	0.15	0.0024
MgCl ₂ ·6H ₂ O	18.9	93.0	94.5	0.465
Sulfates				
MgSO ₄	3.4	28	17	0.142
MnSO ₄ ·H ₂ O	0.90	5.3	4.5	0.027
	0.10	0.41	0.50	0.0021
NaMoO ₄ ·2H ₂ O				
Ammonium				
(NH ₄) ₂ SO ₄ ³	7.5	56.8	30 as N, aerobic 20 as N, multi- environment	2.1 aerobic, 1.4 multi- environment
Phosphates⁴				
Stock Solution				
KH ₂ PO ₄	136	1000	155 as P	5.00
Buffer - pH 8⁵				
K ₂ HPO ₄	15.2	100 total	380	2.5 total
KH ₂ PO ₄	1.6		40	

¹ Stock solutions were diluted 1:200 to make feed solutions and 1:1000 to make salt solutions for batch experiments.

² CaCl₂·2H₂O was made as a separate stock to avoid precipitation.

³ Amount of ammonium stock added was varied for each reactor.

⁴ A Phosphate stock solution was used as a nutrient solution for the anoxic and multi-environment reactors; pH 8 Phosphate buffer was used as nutrient solution and for pH control for aerobic reactor.

⁵ Buffers were diluted 1:40.

was used for the aerobic reactor. Both the phosphate stock solution and the pH 8 buffer were used to provide the reactors with phosphorus. In addition, the pH 8 buffer was utilized to maintain the pH in the aerobic reactor between 7.5 and 8.0. Also note that no ammonium salt was added to the anoxic reactor only because the biogenic organic feed provided ample organic nitrogen for growth.

Methods

Operation of Master SBR's

Three master SBR's, an aerobic, an anoxic, and a multi-environment anoxic/aerobic, were operated throughout this research. All reactors were run with the following cycles (See Figure 5): feed, react, settle, and decant. Anoxic and multi-environment SBR operation was automated by ChonTrol® XT- 4 timers (San Diego, CA), whereas aerobic maintenance was manual. Cycles varied for each reactor and are explained in detail in the following subsections for individual reactors.

Aerobic and anoxic SBR's were operated in 5 L Plexiglas cylinders (53 cm x 15 cm diameter). Because biomass tended to accumulate on the walls of these reactors, a 4.5 L New Brunswick Scientific glass reactor (New Brunswick, NJ) was utilized for operation of the multi-environment SBR. Figure 10 illustrates the anoxic and multi-environment SBR arrangement.

Aerobic SBR. Table 5 summarizes operational parameters for the aerobic SBR. Cycles were maintained manually as described by Gilmore (1995). Biogenic organic stock solutions were diluted daily at 1:100 with MSM to prepare one liter of feed with

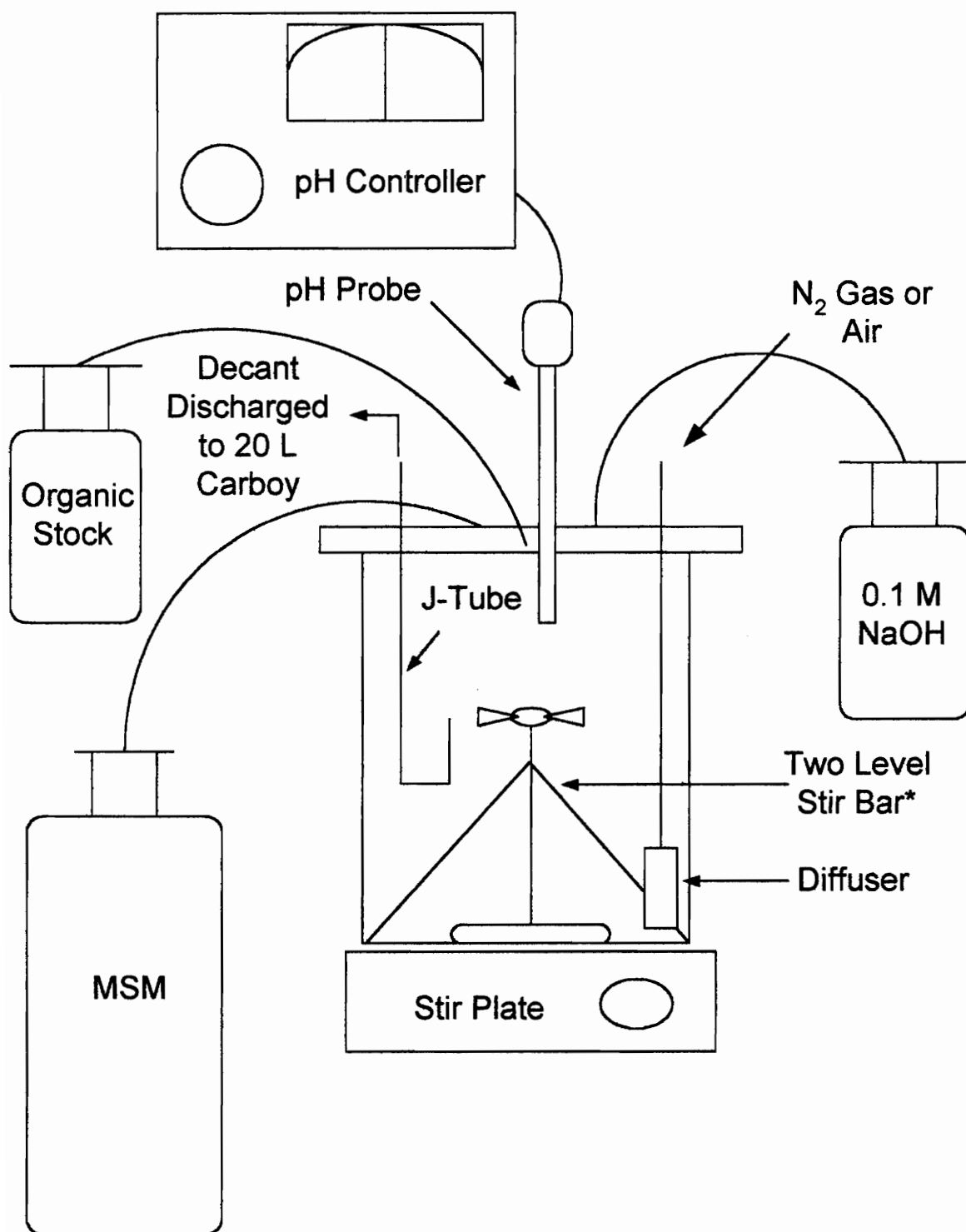


Figure 10. SBR reactor configuration for anoxic and multi-environment anoxic/aerobic systems.

* Mechanical mixer utilized for multi-environment SBR.

a COD of approximately 2,200 mg/L. One liter of feed was added manually to three liters of supernatant and settled mixed liquor remaining after the decant cycle. A whisper® 500 aquarium pump (Willinger Bros., Inc., Oakland, NJ) was operated throughout the 21.5 hour reaction cycle to maintain a dissolved oxygen concentration near saturation. Additional mixing was provided by a magnetic stir plate. Upon completion of the reaction cycle, both the air pump and stir plate were turned off, and the reactor was allowed to settle for approximately two hours. One liter of supernatant was decanted by pumping through a 1/4 inch J-shaped glass tube which prevented loss of settled biomass. Steady state was maintained by wasting approximately 250 mL (adjusted to effluent suspended solids) daily through a reactor side port prior to settling.

Table 5. Operational parameters for the aerobic SBR.

1 cycle / day: 21.5 hour reaction phase
2.0 hour settle phase
0.5 hour decant phase
Influent COD: 2,200 mg/L (Diluted 1:4 in the reactor)
Initial NH ₄ -N (in the reactor): 30 mg/L
Solids retention time (SRT): 16 days
Effective Hydraulic retention time (HRT): 4 days
pH maintained at 7.5 - 8.0 using pH 8 phosphate buffer
Maintained at room temperature

Anoxic Reactor. The anoxic SBR configuration, shown in Figure 10, was adapted from another SBR study by Liu *et al.* (1994). Operation of the anoxic reactor was automated by two ChonTrol® XT-4 timers (San Diego, CA) using a series of interval programs. Table 6 summarizes the six hour cycle utilized for this reactor. Operational parameters are shown in Table 7. Note that, in this case, one liter of synthetic

wastewater was supplied over a fifteen minute period. Sterilized biogenic organics supplemented with the desired amount of each oxime were supplied to the reactor through a Masterflex® peristaltic pump (Cole Parmer Instrument Co., Chicago, IL) at a rate of 6.7 mL/min. Acidified MSM was simultaneously added at a rate of 60 mL/min., providing approximately a 1:10 dilution of the organic stock. Nitrogen gas (N₂), controlled electrically by a two way solenoid valve, was purged for the first thirty minutes of the SBR cycle to ensure anoxic conditions existed. Mixing during the four hour reaction time was accomplished by a magnetic stir plate. A rodged propeller attached to the stir bar and supported by a stainless steel tripod helped provide complete mixing. Upon completion of the reaction phase, the mixed liquor was allowed to settle for 1.5 hours. One liter of supernatant was then decanted at 33.3 mL/min. through a J- shaped tube (described previously) using a Masterflex® peristaltic pump (Cole Parmer Instrument Co., Chicago, IL). Steady state was achieved by manually wasting biomass through a reactor side port.

Two liter biogenic organic feed stocks were prepared every five days. Because it was diluted 1:10 during the feed cycle, the organics stock had to be prepared at ten times the 1,100 mg/L COD feed concentration. Stock jars and feed tubing were steam sterilized for 20 minutes (at 121 ° C, 15 PSI) prior to preparing the stock. A sterilized bacterial air vent (Gelman Sciences, Ann Arbor, MI) was attached to an air vent for the feed rubber stopper to avoid airborne contamination. Bacterial growth in the feed tubing

was prevented by using a drip tube which provided an air gap between the feed line and the reactor influent.

Table 6. Description of the anoxic SBR cycle.

Stage	Occurrences during stage	Time
Feed	Decant pump off	0:00
	Mixer on	0:00
	N ₂ purges	0:00
	pH controller on	0:01
	Feed pumps start	0:02
React	Feed pumps stop	0:17
	N ₂ purging stops	0:30
Settle	Mixer off	4:00
	pH controller off	4:00
Decant	Decant pump starts	5:30

The complete cycle time was 6 hours. The reactor was operated at 4 cycles per day.

Table 7. Operational parameters for the anoxic SBR.

4 cycles/day:	4.0 hour reaction phase
	1.5 hour settle phase
	0.5 hour decant phase
Reactor volume: 4 L	
Influent COD: 1,100 mg/L (Diluted 1:4 in reactor)	
Solids retention time (SRT): 16 days	
Hydraulic retention time (HRT): 1 day	
pH maintained at 7.0 ± 0.5 using pH controller	
Maintained at 32° C using Digital Circulating Batch	
Oxime concentrations slowly increased over time (1 - 18 mg/L each oxime)	

MSM was prepared as described previously in 9 liter Pyrex® glass jars. In addition, sodium nitrate (NaNO_3) was added to the MSM to act as a terminal electron acceptor for denitrification. The amount of nitrate added initially varied until a carbon to nitrogen ratio was established. To prevent precipitation, 25 mL of 2 M phosphoric acid (H_3PO_4) was added to each MSM jar. As mentioned earlier, ammonium salts were not added to MSM for the anoxic reactor as reactor analysis indicated that organic nitrogen present in the biogenic organic feed provided ample ammonium for heterotrophic bacterial growth.

A pH controller (Cole Parmer Instrument Co., Model 5997-20, Chicago, IL) provided one sided pH control to maintain the reactor pH between 6.5 and 7.5. Because the MSM was acidified, only base addition was necessary to maintain a neutral pH. When the reactor pH dropped below 6.5, a Masterflex® peristaltic pump (Cole Parmer Instrument Co., Chicago, IL) supplied 0.1 M sodium hydroxide (NaOH) from a two liter glass jar until a desirable pH was restored.

The reactor was maintained at an elevated temperature of 32° C since similar temperatures were expected for wastewater at the Industry. Temperature control was accomplished by a Fisher Scientific Digital Circulating Bath (Model 9001, Fisher Scientific, Niles, IL). The water bath operated in a closed loop circulation mode pumping heated water at 15 liters per minute (LPM) through Tygon® tubing wrapped around the anoxic reactor.

In addition to biogenic organics, the anoxic reactor was also supplied with oximes. Oxime concentrations were gradually increased over time as shown in Table B-39 in Appendix B. During its operation from July, 1995 to February, 1996, oxime concentrations were increased from 1 mg/L to 18 mg/L.

Multi-Environment Anoxic/Anaerobic/Aerobic SBR. The multi-environment SBR was operated similarly to the anoxic SBR. Again, ChonTrol® XT-4 timers (San Diego, CA) were utilized to automate SBR operation. In this case, a ten hour aerobic stage was incorporated into the reactor cycle following the anoxic/anaerobic stage. Dissolved oxygen was supplied through a Whisper® 600 aquarium air pump (Willington Bros., Inc., Oakland, NJ). In order to ensure complete degradation of MEKO, anoxic detention times were increased initially to 10 hours and eventually to 12 hours. Therefore, the reactor was operated at one cycle per day. Due to the long nonaerobic detention times, anaerobic conditions existed for the latter part of anoxic cycle. For this reason, the anoxic cycle is referred to as the “anoxic/anaerobic cycle” throughout this report. Table 8 illustrates the reaction program.

As mentioned previously, a 4.5 L glass New Brunswick Scientific reactor replaced the 5 L Plexiglas reactor for operation of the multi-environment SBR. This reactor provided several advantages. First, the glass walls decreased biofilm growth significantly. Second, it provided mechanical mixing at 100 rpm. Operational parameters are described in Table 9. Most of these operational conditions were explained in the previous section. Because the hydraulic retention time was increased

Table 8. Description of the anoxic/anaerobic/aerobic SBR cycle.

Stage	Occurrences during stage	Time
Feed	Decant pump off	0:00
	Mixer on	0:00
	N ₂ purges	0:00
	pH controller on	0:01
	Feed pumps start	0:02
React (Anoxic/Anaerobic Phase)	Feed pumps stop	0:17
	N ₂ purging stops	0:30
React (Aerobic Phase)	Air pump on	12:00*
Settle	Mixer off	22:00
	pH controller off	22:00
	Air off	22:00
Decant	Decant pump starts	23:50

The complete SBR cycle was 24 hours.

*The anoxic/anaerobic phase was originally 10 hours long, but had to be increased to 12 hours to ensure complete degradation of MEKO.

Table 9. Operational parameters for the anoxic/anaerobic/aerobic SBR.

1 cycle/day: 22 hour reaction stage: 12 hour anoxic/anaerobic, 10 hours aerobic
1.5 hour settle stage
0.5 hour decant stage

Reactor volume: 4 L

Influent COD: 2,200 mg/L (Diluted 1:4 in the reactor)

Solids retention time (SRT): 16 days

Hydraulic retention time (HRT): 4 days

pH maintained at 7.0 ± 0.5 using pH controller

Maintained at 32° C using Digital Circulating Batch

Initial ammonium concentration: 20 mg/L-N in the reactor

Supplemental nitrate added: initial NO₃-N = 20 - 30 mg/L

Oxime concentrations slowly increased over time (18 mg/L - 56 mg/L)

four fold, the influent organic concentration was increased to 2,200 mg/L to avoid low Food to Microorganism ratios (F/M). For this reactor, ammonium was added to the MSM as ammonium sulfate. Initial ammonium concentrations were gradually increased to 20 mg/L-N (in the reactor). In addition, although nitrifiers were generating nitrate during the aerobic stage of treatment, additional nitrate was added for the anoxic cycle. Care was taken to ensure that the majority of the biogenic organics would be utilized during the anoxic stage, but that nitrate limiting conditions would occur early in the anoxic reaction stage. To obtain an initial nitrate concentration of approximately 20 to 30 mg/L-N (in the reactor), 5.4 g of sodium nitrate were added to the nine liter MSM jar.

Oxime concentrations were again increased over the three month operation of the multi-environment SBR. ADO was not supplied to this reactor because it appeared to cause color and odor problems in the anoxic SBR. MEKO was increased gradually from 18 to 40 mg/L, while AAO was increased from 18 to 56 mg/L.

SBR Sampling and Monitoring. Both effluent and completely mixed SBR samples taken over the reaction cycle were monitored throughout this research for the following constituents: nitrate, nitrite (NO_2^- -N), ammonium, total organic carbon (TOC), and oximes. Effluent samples were taken during the 30 minute decant phase. Completely mixed samples were obtained manually through a side port on the reactor. These samples were centrifuged at $2,200 \times g$ for 5 minutes, and the resulting supernatant was utilized for analysis. Both types of samples were filtered through a $0.45 \mu\text{m}$ membrane filter (Supor® - 450, Gelman Sciences, Ann Arbor, MI) prior to analysis.

Batch Experiments

Batch tests were performed to determine the degree of nitrification inhibition caused by each oxime and to investigate the fate of oximes in alternative environments. Activated sludge for these experiments was derived from the master SBR's. Sludges were centrifuged at 12,100 x g for five minutes, rinsed and resuspended in carbonless MSM two times prior to commencement of the experiment. For each experiment, electron acceptors (oxygen, nitrate) and donors (ammonium, oximes) were added at time zero; completely mixed samples were obtained over the reaction times, centrifuged for five minutes at 2,200 x g, and filtered through 0.45 μ m membrane filters (Supor - 450, Gelman Sciences, Ann Arbor, MI). Batch test analyses included nitrate, nitrite, ammonium, oximes, total organic carbon, and hydroxylamine.

Aerobic experiments. Aerobic batch studies were performed by an undergraduate researcher, Kevin Gilmore, in fulfillment of an independent research requirement. More specific details for aerobic batch experiments are provided his final report (1995).

Aerobic batch studies were performed in 125 mL Erlenmeyer flasks. Samples were obtained over the reaction time through 10 mL syringes attached to 4 1/2 inch 20 gage syringe needles. These studies were performed at room temperature. A pH 8 phosphate buffer was used for pH control during the studies. Dissolved oxygen was provided via humidified air to reduce evaporation effects encountered in the mechanical hood.

Aerobic batch studies were designed to compare nitrate production in activated sludge containing no oximes and in activated sludge subjected to various concentrations of oximes. In each study, 30 mg/L-N ammonium was provided to each batch flask. Initial oxime concentrations ranged from 7 mg/L to 33 mg/L for AAO, 7 mg/L to 45 mg/L for ADO, and 2 mg/L to 10 mg/L for MEKO. Samples were analyzed over a 24 hour period.

Anoxic Studies. Anoxic batch studies were performed in 125 mL sealed Erlenmeyer flasks with side arm ports. Samples were obtained by attaching 10 mL syringes to 4 1/2 inch 20 gage syringe needles which penetrated through septa in the side arm ports. Batch flasks were mixed on a multiple unit stir plate placed underneath a 17 1/2 inch x 12 1/8 inch x 7 3/4 inch plastic water bath. An extension of the water bath circulation tubing was attached to a 1/4 inch coiled copper U-shaped piece and placed in the plastic storage unit to maintain a temperature of 32° C. In later batch experiments, batch flasks were mixed and maintained at 32° C in a Gyrotory® Water Bath Shaker (Model G76, New Brunswick Scientific, New Brunswick, NJ). All batch flasks were purged with nitrogen gas for 20 - 30 minutes prior to the start of the experiment. Experiments were performed at a neutral pH (pH 6.5 to 7.5).

Anoxic batch studies were performed to determine the fate of oximes under different conditions. Often results from a previous batch experiment would assist with the design of subsequent studies. In all cases, oximes and nitrate, if added, were supplied

at the start of the experiment; oxime degradation was then tracked over time. Details for specific batch experiments are provided in the following subsections.

Initial Anoxic Batch Studies. In initial batch experiments, ample nitrate (10 to 15 mg/L-N), in addition to oximes, was added at the start of each study to act as a terminal electron acceptor. A control batch flask was provided with an equivalent amount of nitrate, but no oximes, to determine endogenous nitrate oxidation.

Statistical Batch Experiment. Early in the research project, an anoxic batch experiment was performed to determine variability encountered in procedural techniques. Equal concentrations of ADO (25 mg/L) and nitrate (15 mg/L-N) were added to three experimental batch flasks. Again, a control flask was provided with ample nitrate in the absence of ADO. Oxime and nitrate mean concentrations and corresponding standard deviations were calculated to determine relative experimental error encountered in batch studies.

MEKO Batch Tests Under Nitrate Limiting Conditions. Several batch tests were performed in the complete absence of nitrate or in the presence of a limiting amount of nitrate. In one study, biogenic organics were added in addition to MEKO to determine if co-metabolic effects influenced MEKO degradation. Controls for these experiments included batch flasks supplied with MEKO in the presence of ample nitrate. In addition, the chemical stability of MEKO was investigated by monitoring MEKO degradation in a batch flask with no microorganisms.

Sulfate Limited Conditions. In one MEKO batch experiment performed under nitrate limiting conditions, sulfate was completely eliminated from the MSM. The purpose of this experiment was to investigate the role of sulfate reducers on MEKO degradation.

Batch Studies in the Presence of a Denitrification Inhibitor. In one batch experiment, initial attempts were made to determine if MEKO was possibly being utilized as an alternative electron acceptor. Tungsten (W), a known inhibitor of the nitrate reductase enzyme (Chauret and Knowles, 1991; Wu and Knowles, 1994), was added to one batch flask as sodium tungstate ($\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$) at a concentration of 25 mM. Nitrate degradation was compared to removal in a control flask not subjected to tungsten. Note that biogenic organics were added to the batch flasks rather than oximes in order to first determine if the mixed heterotrophic culture from the anoxic SBR could be inhibited by Tungsten. As explained in the following chapter, nitrate degradation rates were not affected by the presence of Tungsten. Therefore, this technique was not utilized in MEKO batch experiments.

Hydroxylamine studies. Hydroxylamine was successfully monitored in two MEKO batch studies. Because hydroxylamine samples degraded rapidly, special sampling techniques were utilized during batch experiments. One mL of filtered batch sample was immediately added to a glass vial spiked with 10 μL of 3-pentanone (99% grade, Mallinckrodt, Paris, KY) and mixed vigorously for fifteen seconds. In this way,

unstable hydroxylamine would react with 3-pentanone to form pentanone oxime.

Pentanone oxime was stable for a minimum of 24 hours.

Aerobic Side Study

During operation of the anoxic/anaerobic/aerobic SBR, an aerobic side study was performed in response to a nitrification upset in the master SBR. The objectives of this side study were as follows: to investigate the inhibitory potential of the SBR effluent; and to determine if nitrification could be restored in the master reactor.

Three small scale (400 mL) manual aerobic SBR's were operated in 500 mL Erlenmeyer flasks. Reactors were operated aerobically on a 24 hour cycle as described previously in the aerobic SBR subsection (See Table 5). Note that since the reactors were operated at 400 mL, corresponding feed and decant volumes were one-tenth the volume utilized for the master aerobic SBR. Dissolved oxygen was supplied via humidified aeration and additional mechanical mixing was provided by a multiple unit magnetic stir plate. The reactors were maintained at room temperature and pH was maintained between 7.5 and 8.0 by utilization of a pH 8 phosphate buffer. Each reactor was supplied with 15 mg/L-N ammonium.

Activated sludge for the first reactor was obtained from the multi-environment SBR. The purpose of this experiment was to determine if nitrification could be recovered in the absence of oximes. Therefore, the reactor was supplied with only biogenic organics diluted in aerobic MSM. Note that the biomass was not washed and rinsed prior to commencement of the study so that the effect of accumulated nitrite on

nitrification recovery could be investigated. Reactor effluent was monitored daily for a period of two weeks. Samples were monitored for nitrate, nitrite, and ammonium.

The two other reactors were operated to compare nitrate production in a nitrifying activated sludge culture exposed to the multi-environment SBR effluent to a control nitrifying activated sludge culture not exposed to the multi-environment SBR effluent. Biogenic organic feeds for these reactors were identical to wastewater supplied to the aerobic SBR, except that, for one experimental flask, half of the MSM was replaced with effluent from the master multi-environment SBR. Reactors were operated for one week prior to analysis to allow the systems to stabilize. Effluent samples were then monitored for one week for nitrate, nitrite, and ammonium.

Analytical Methods

This section describes the analyses performed for this research. *Standard Methods for the Analysis of Water and Wastewater* (1992) were utilized if available or appropriate. Detailed descriptions are provided for analyses not following a standard method.

Chemical Oxygen Demand. Chemical Oxygen Demand (COD) was determined using Standard Method 5220C, the closed reflux, titrametric method. This method was utilized for determining COD concentrations of biogenic organic stock solutions; however, chloride concentrations in SBR samples interfered with COD measurements, requiring another method for analyzing organic carbon in the reactor.

Total Organic Carbon. Total Organic Carbon (TOC) was utilized as a surrogate for COD measurements for reactor samples. Samples were analyzed according Standard Method 5310C, the persulfate-ultraviolet oxidation method, using a Dohrmann® DC-80 TOC analyzer (Santa Clara, CA). Inorganic carbon was removed prior to analyses by external sparging. For samples with TOC concentrations of less than 20 mg/L, a 10 mg/L output range was utilized, while for samples with TOC levels above 20 mg/L, a 400 mg/L output range was used.

Chloride concentrations in the reactor were reduced in the anoxic SBR to establish a COD/TOC ratio. Hourly samples were analyzed over the reaction time for COD and TOC. Based on four hourly samples, the COD/TOC ratio was calculated to be 4.1 ± 0.3 .

Total and Volatile Suspended Solids. Analysis for total and volatile suspended solids were performed as per Standard Methods 2540D and 2540E, respectively. Total suspended solids for mixed liquor and effluent samples were analyzed every other day to determine daily wastage volumes required to maintain the reactor at steady state. Volatile suspended solids were performed for the master reactor during batch experiments so that results from different batch experiments could be compared.

Anion Analysis. Nitrate (NO_3^- -N), nitrite (NO_2^- -N), and sulfate (SO_4^{--}) were analyzed by Ion Chromatography (I.C.) according to Standard Method 4110B using a Dionex System 2010I Ion Chromatograph with a Dionex 4270 Integrator (Sunnyvale,

CA). A 1.8 mM sodium carbonate (NaCO_3) / 1.7 mM sodium bicarbonate (NaHCO_3) eluent was utilized at a flow rate of 2.0 mL/min.

Ammonium Analysis. Although many Standard Methods exist for ammonium analysis, options were limited by the small volumes (generally less than 2 mL) from batch experiments available for analysis. Therefore, ammonium was analyzed by ion chromatography (I.C.). A Dionex System 2010I Ion Chromatograph with a Dionex 4270 Integrator (Sunnyvale, CA) was utilized. A 20 mM methane sulfonic acid solution was used as eluent at a flow rate of 1.0 mL/min. As this method was not considered standard, six point standard curves were developed for a concentration range of 0 - 25 mg/L-N. A linear trend was found to exist above 5 mg/L with a non-zero intercept and below 2.5 mg/L with a zero intercept. An example of a standard ammonium curve for I.C. analysis is shown in Figure A-1 in Appendix A.

Oxime Analysis. Oxime analysis was accomplished by gas chromatography (GC) utilizing a method adapted from a GC protocol provided by the Industry. A Tremetrics 9001 GC (Finnegon Corporation, Austin, TX) with a Hewlett Packard 3396 Series II Integrator and $\mu\text{Gold}^{\text{TM}}$ Flame Ionization Detector (FID) was used to perform the analyses. A J & W Scientific (Rancho Cordoba, CA) 0.5 μm thick DB-wax fused silica capillary column (15m x 0.25 mm) was found to give the best overall results for all three oximes. Table 10 lists approximate gas flowrates and the temperature program for this method.

Standard curves for AAO, ADO, and MEKO are shown in Figure A-2 in Appendix A. Note that peak heights were measured rather than peak areas. This method proved to give more reproducible results and a lower detection limit.

Method Detection Limits (MDL's) for oxime analysis were determined according to Standard Method 1030. For each oxime, an analysis of a 10 mg/L standard was repeated seven times. The MDL was then calculated by multiplying the standard deviation of the seven samples by 3.14, the 99% confidence coefficient. Analysis showed that lower limits of detection were approximately 1 mg/L.

Table 10. GC/FID method for Oximes.

Parameter	Program Setting
Column flowrate	3.2 mL/min.
Makeup gas (Helium) flowrate	22 mL/min.
Hydrogen (H ₂) flowrate	20 mL/min.
Air	175 mL/min.
Injector port temperature	130° C
Initial oven temperature	50° C
Detector temperature	220° C
Initial hold time	1 minute
Temperature rise rate	20° C/min.
Final oven temperature	130° C
Final hold time	1 minute
Injection size	1 µL
Injection type	splitless
Column	DB-wax capillary column (15 m x 0.25 mm x 0.5 µm)
Detector	FID

Hydroxylamine. Several attempts were made to analyze hydroxylamine (NH₂OH) during batch studies and reactor monitoring. Two methods were investigated. The first method was a colormetric one developed by Magee and Burris (1954). Unfortunately,

oximes proved to interfere with this method. The second method investigated was adapted from a GC/FID protocol provided by the Industry. Operational parameters were identical to those listed in Table 10. Again, the J & W Scientific DB-wax capillary column was utilized in a Tremetrics 9001 GC with a Hewlett Packard 3396 Series II Integrator and μ Gold™ Flame Ionization Detector (FID). In this method, excess 3-pentanone was added to hydroxylamine samples to form pentanone oxime. Development of this method using the capillary DB-wax column led to the discovery that a biogenic organic compound detected by the FID interfered with the pentanone oxime peak. Further modifications of the method, however, finally resulted in successful analysis. The final temperature program and 3-pentanone to hydroxylamine sample ratio are provided in Table 11.

Table 11. Operational parameters for the GC/FID hydroxylamine method¹.

Parameter	Program Setting
Injector port temperature	130° C
FID temperature	220° C
Initial hold time	1 minute
Initial oven temperature	35° C
Temperature rate rise	20°/min.
Final oven temperature	135° C
Final hold time	1 minute
Injection type	splitless
3-pentanone/sample ratio (vol./vol.)	1:100

¹Operational conditions not listed are identical to those utilized for oxime analysis.

Hydroxylamine peaks were detectable to approximately 1 mg/L; however, a formal method detection limit was not determined for this analysis. Note that in the presence of higher concentrations of MEKO, pentanone oxime was still detectable but only qualitatively and not quantitatively.

Statistical Analysis. Statistical analysis of data were performed using Data Analysis Tools in Microsoft Excel 5.0. Analysis tools utilized included descriptive statistics, linear regressions, and one-tailed student t-tests.

IV. RESULTS AND DISCUSSION

In this chapter, results from nitrification studies, anoxic studies and multi-environment SBR studies are presented and discussed.

Nitrification studies, which included aerobic batch tests, were performed by an undergraduate researcher, Kevin Gilmore (Gilmore, 1995). Data from these studies which indicate the effect of oximes on biological nitrification are shown. In addition, the aerobic fates of these oximes in the presence of an unacclimated culture are described.

Results from anoxic studies are presented and include data describing the fate of oximes under nonaerobic conditions, as investigated during anoxic and anaerobic batch tests. In addition, data from the master anoxic SBR are provided. Finally, possible pathways for MEKO degradation under nitrate limiting conditions are discussed and data from batch tests where hydroxylamine was monitored to assess its role in oxime degradation are presented.

The results from the single sludge multi-environment anoxic/anaerobic/aerobic SBR experiment are included in this chapter. Data were obtained from daily monitoring and include information on nitrification efficiency and oxime degradation. In addition, results from an aerobic side study investigating the inhibitory potential of the SBR effluent on a nitrifying culture are explained. Finally, kinetic information of the rates of oxime degradation under nitrate limiting conditions are discussed.

Nitrification Studies

Investigation of Oxime Inhibition of Nitrification

Inhibitory potentials of AAO, ADO, and MEKO on nitrification were investigated during aerobic batch tests using sludge from the aerobic master SBR. Inhibition was determined by comparing nitrate (NO_3^- -N) generated in a control flask containing activated sludge not subjected to oximes to nitrate produced in batch flasks containing activated sludge exposed to various concentrations of oximes. Note that the activated sludge used in these experiments had not been previously exposed to oximes.

Ammonium (NH_4^+ -N) degradation was also monitored throughout the batch test.

Ammonium data are not reported in the section, but are provided in Tables B-10 and B-11 in Appendix B.

Initial aerobic batch tests were performed to determine the effect of each oxime at a concentration below 10 milligrams per liter (mg/L) on the nitrifying activated sludge culture. Figure 11 shows the rate at which nitrate was generated in each batch flask over a 24 hour period. Note that nitrate concentrations in the AAO and ADO fed flasks did not differ significantly from the control flask. However, nitrate concentrations were significantly lower in the MEKO flask, indicating that MEKO was strongly inhibiting nitrification at 9 mg/L.

Since MEKO was the only oxime causing inhibition in the first aerobic batch study, a subsequent experiment was performed in which aerobic nitrifying activated

sludge was exposed to the following concentrations of MEKO: 0 mg/L, 3 mg/L, 6 mg/L, and 10 mg/L. Results from this batch test are presented in Figure 12. Note that the y-axis scale is normalized to mixed liquor volatile suspended solids (MLVSS) so that results from this batch test and the previous batch test can be compared. Note that MEKO showed inhibition at all concentrations investigated when compared to the controls. In addition, as shown by the low levels of nitrate generated in the presence of 9 and 10 mg/L MEKO, severe inhibition was encountered above 9 mg/L.

A final batch test was performed to determine the effect of higher concentrations of AAO and ADO on a nitrifying activated sludge culture. Nitrate generation during the 24 hour batch test are shown in Figure 13. In this case, both AAO and ADO caused decreased rates of nitrification at 33 mg/L and 45 mg/L, respectively. Note, however, that the degree of nitrification inhibition is significantly less than for MEKO.

Hockenbury and Grady's definition of nitrification inhibition (1977) as the percent decrease in the rate of nitrate production was utilized for determining the degree of nitrification inhibition. Table 12 summarizes the percent inhibition caused by each concentration of oxime based on rates of nitrate generation. Nitrate generation rates were calculated based on the linear portion of the nitrate curves from aerobic oxime batch tests. As shown in Figure 11, nitrate production in the batch flasks showed a linear trend throughout the 24 hour study. However, as shown in Figures 12 and 13, linear

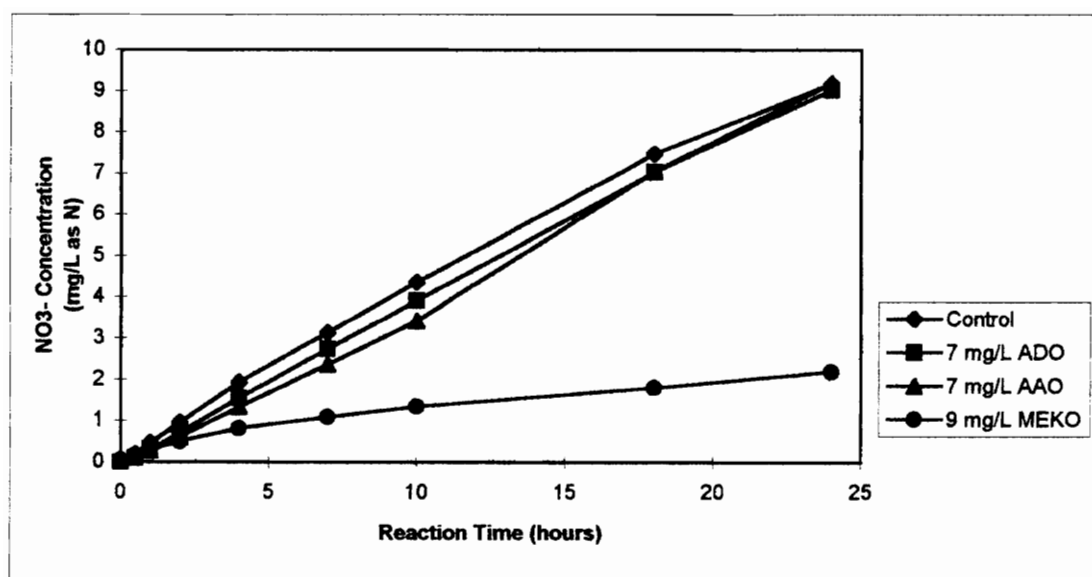


Figure 11. The effect of each oxime on nitrification at a concentration less than 10 mg/L. Oximes were not fed to the control. Each batch flask was amended with 30 mg/L $\text{NH}_4^+\text{-N}$ (Gilmore, 1995).

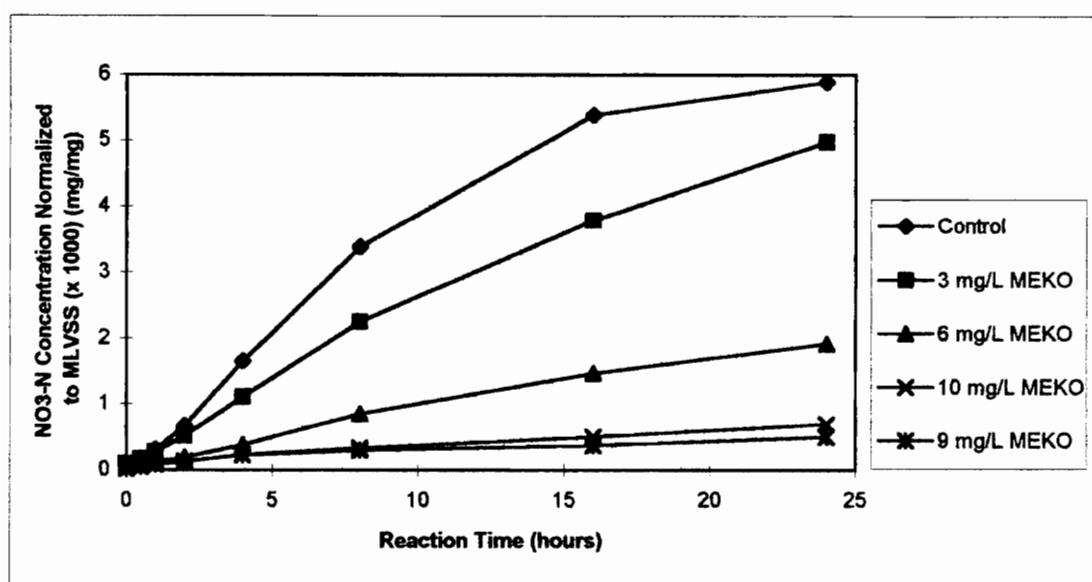


Figure 12. The effect of MEKO concentration on the degree of nitrification inhibition. Note that the y-axis values are normalized to MLVSS. Data for the 9 mg/L MEKO case was taken from a previous batch test. Oximes were not fed to the control. Each flask was amended with 30 mg/L $\text{NH}_4^+\text{-N}$ (Gilmore, 1995).

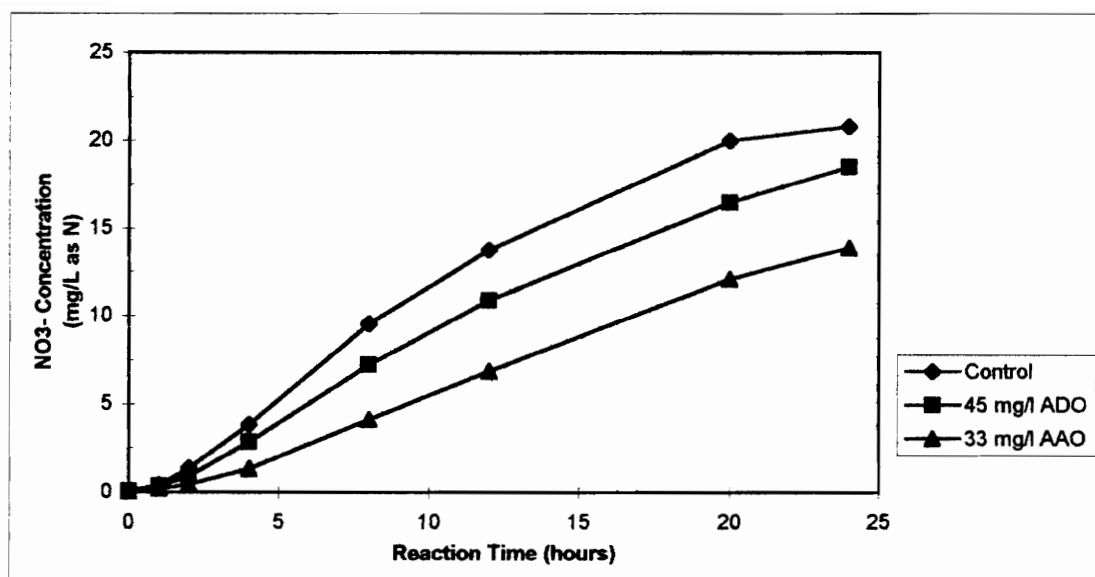


Figure 13. The effect of 33 mg/L AAO and 45 mg/L ADO on nitrate generation by a nitrifying activated sludge culture. Oximes were not fed to the control. Each batch flask was amended with 30 mg/L $\text{NH}_4^+\text{-N}$ (Gilmore, 1995).

Table 12. The effect of oxime concentrations on the degree of nitrification inhibition.

Oxime	Theoretical Concentration (mg/L)	Measured Initial Concentration (mg/L)	% Inhibition*
MEKO	2	2.8	31
	5	5.8	79
	10	9	85
	15	10	90
AAO	10	7	<10 (not inhibitory)
	50	33	58
ADO	10	7	<10 (not inhibitory)
	50	45	24

*Inhibition was calculated based on nitrate production in the control run during the same experiment. Oximes were considered inhibitory if the rate of nitrate generation decreased by $\geq 10\%$.

trends in nitrate generation for the latter two aerobic batch studies were only valid for the first part of the experiment. Therefore, rate constants were calculated based on initial nitrate generation rates. This procedure was also adopted by Hockenbury and Grady (1977). Resulting oxidation rate constants are provided in Table B-4.

Results of MEKO inhibition agreed with Smith's findings (1994) that MEKO acted as a competitive inhibitor and caused severe inhibition at concentrations as low as 14 mg/L. In addition, this research illustrated that MEKO was inhibitory down to 2 mg/L. Therefore, MEKO can be considered a potent nitrification inhibitor like aniline, which caused greater than 50% inhibition at 1 mg/L (Joel and Grady, 1976). The implications of this oxime's inhibitory characteristics are that complete degradation of MEKO must be accomplished in order to achieve nitrification.

As shown in Table 12 and Figures 11 through 13, AAO and ADO were not as severely inhibitory as MEKO. Because it is structurally similar to MEKO, one might speculate that AAO would also act as an inhibitor of nitrification. This speculation is justified by the fact that many structurally similar nitrogen compounds have been found to possess similar characteristics in their ability to inhibit nitrification (Hockenbury and Grady, 1977). In addition, because MEKO was identified as a competitive inhibitor (Smith 1994), it is possible that the structurally similar AAO could also bind to active enzyme sites and prevent ammonium utilization (Rawn, 1989). However, AAO caused a much lower degree of inhibition than MEKO. As explained in the following section, AAO degraded much more readily than MEKO under aerobic conditions. Therefore, its

inhibitory effects may be reduced or eliminated by its degradation. This theory agrees with Joel and Grady's conclusion (1976) that nitrification could occur in a wastewater containing aniline, another nitrogen containing organic compound, because inhibitory effects were eliminated by its aerobic degradation.

ADO caused the smallest degree of inhibition of the three oximes studied in this research. One might note that it has a significantly different structure than MEKO and AAO (See Figure 8). Smith's finding (1994) that cyclohexanone oxime did not cause nitrification inhibition suggests that not all oximes cause nitrification inhibition. In addition, both cyclohexanone oxime and ADO have more complicated structures than MEKO. Therefore, the larger structure of ADO may make it difficult for ADO molecules to interfere with nitrifying enzymes. It should be noted, however, that many other sulfur containing organic compounds have been identified as potent inhibitors of nitrification (Hockenbury and Grady, 1977).

Oxime Degradation During Aerobic Batch Tests

Oxime degradation was monitored throughout the 24 hour aerobic batch tests. It is important to note that this aerobic culture had not been previously exposed to oximes.

Figure 14 describes the degradation of AAO, ADO, and MEKO during the first batch test. AAO completely degraded during the experiment; however, ADO remained stable, exhibiting little to no degradation during the experiment. MEKO degraded partially, with approximately 54 % degradation over the 24 hour period.

Figure 15 illustrates the degradation of MEKO at the three different concentrations investigated in the second aerobic batch test. MEKO degraded partially at all three concentrations. Note that MEKO degradation in this batch test showed similar trends to MEKO removal in the first batch test.

Figure 16 depicts AAO and ADO degradation during the final batch test. Both oximes degraded partially during the batch study. Note that in this batch experiment AAO partially degraded, while in the previous batch test it completely degraded. This result suggests that AAO may impose some degree of substrate inhibition on its own biodegradability at higher AAO concentrations. Furthermore, AAO did not cause inhibition at 7 mg/L, but did cause inhibition at 33 mg/L.

As explained earlier, a relationship between the relative ability of AAO to degrade and its degree of inhibition may exist. When AAO degraded completely, it did not cause inhibition; however, when higher concentrations of AAO were present throughout the batch experiment and it degraded only 55%, AAO did cause inhibition of nitrification. It is important to note that, typically, aqueous concentrations of AAO at the Industry do not exceed 10 mg/L. At such low concentrations, AAO would most likely degrade aerobically and not cause inhibition.

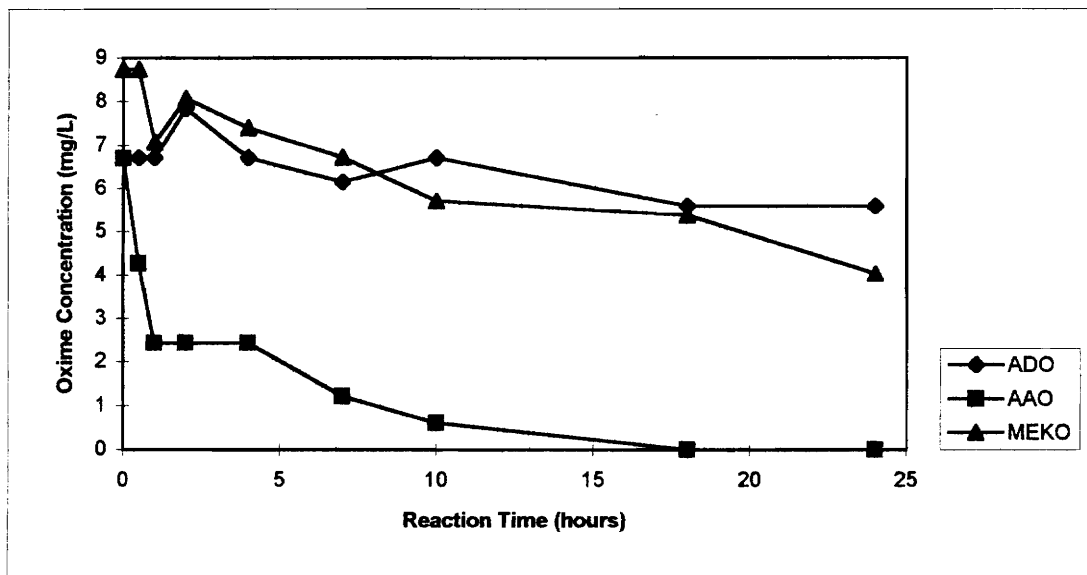


Figure 14. Oxime degradation during an aerobic batch test. Initial oxime concentrations were as follows: AAO = 7 mg/L; ADO = 7 mg/L; MEKO = 9 mg/L (Gilmore, 1995).

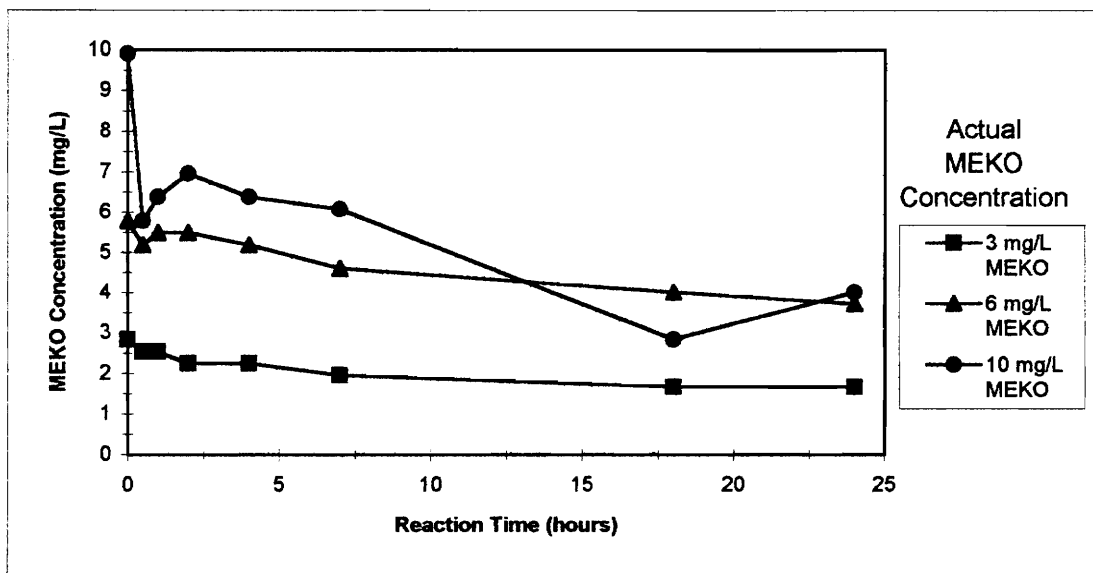


Figure 15. MEKO degradation during an aerobic MEKO batch test. Actual MEKO concentrations are shown in the legend. The theoretical MEKO concentrations were as follows: 2 mg/L, 5 mg/L, and 15 mg/L. (Gilmore, 1995).

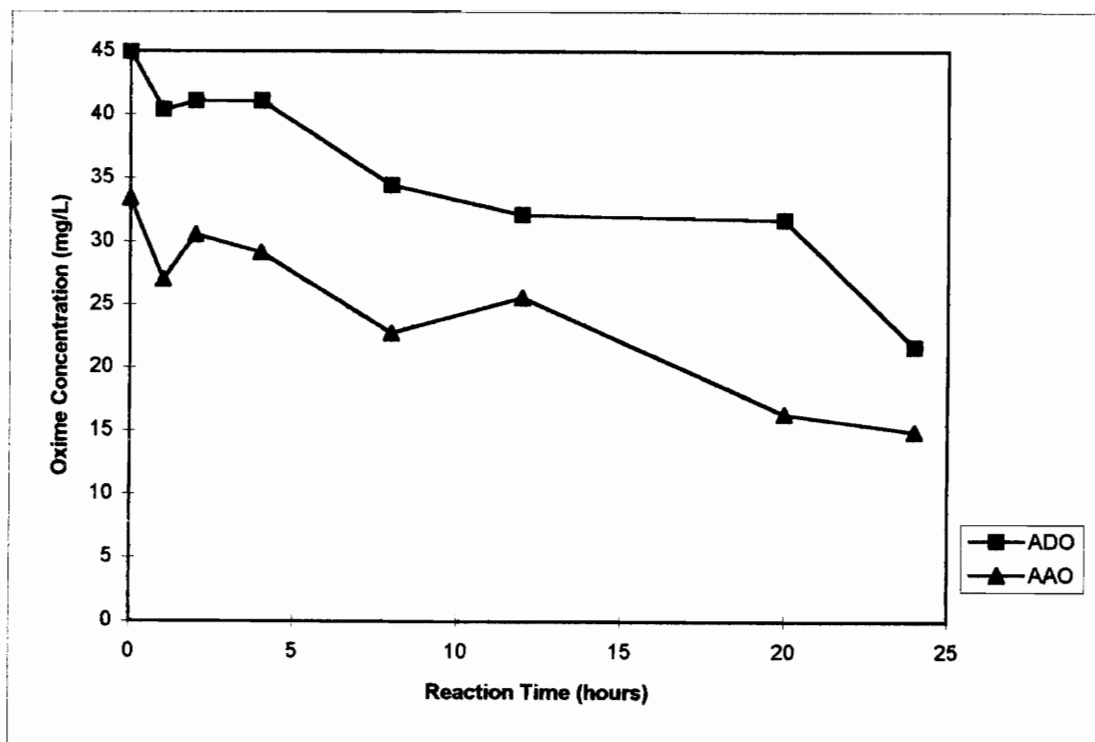


Figure 16. AAO and ADO degradation during an aerobic batch test.
Initial oxime concentrations were as follows: AAO = 33 mg/L and
ADO = 45 mg/L (Gilmore, 1995).

Aerobic oxime degradation results in this research were similar to those reported in other studies. Smith (1994) also reported that MEKO degraded partially under aerobic conditions, but over long detention times. In addition, Obaton *et al.* (1968, cited in Castignetti and Gunner, 1980) and Amarger and Alexander (1968) found that AAO could be degraded aerobically by heterotrophic nitrifiers. Amarger and Alexander (1968) discovered that MEKO could also be degraded aerobically by a pure culture of heterotrophic nitrifiers; however, because this was a pure culture study, the degree of MEKO degradation cannot be compared to results from this research.

Anoxic Studies

Initial Anoxic Batch Tests

As noted earlier, initial anoxic batch tests were performed to determine the fate of oximes under anoxic conditions. As shown in Figure 17, these initial anoxic batch tests showed little to no degradation of oximes under anoxic conditions. However, larger amounts of nitrate were utilized in some batch flasks fed oximes than in the control flask fed no oximes. This difference in nitrate uptake is illustrated in Figure 18.

A batch test was performed to determine if differences in nitrate uptake between the control and the experimental batch flasks could be explained by the procedural variability in batch experiments. Statistical analyses for this batch test are shown in Tables B-19 and B-20 in Appendix B. Figure 19 shows the resulting nitrate uptake in each flask. Again, differences could be noted in nitrate uptake between some oxime fed flasks and the control; however, as depicted in Figure 20, by comparing the mean and standard deviation for the nitrate concentration in the three batch flasks fed ADO to the nitrate concentration in the control, one finds that the amount of nitrate uptake observed in the control falls within the error (one standard deviation) observed in the replicate oxime fed flasks.

As shown in Figure B-1 in Appendix B, this statistical experiment showed that little variability was encountered in monitoring the ADO concentration throughout the test. Unfortunately, some of the concentrated ADO stock seeped out of the lure lock during injection into one of the batch flasks. Therefore, data from only two flasks could

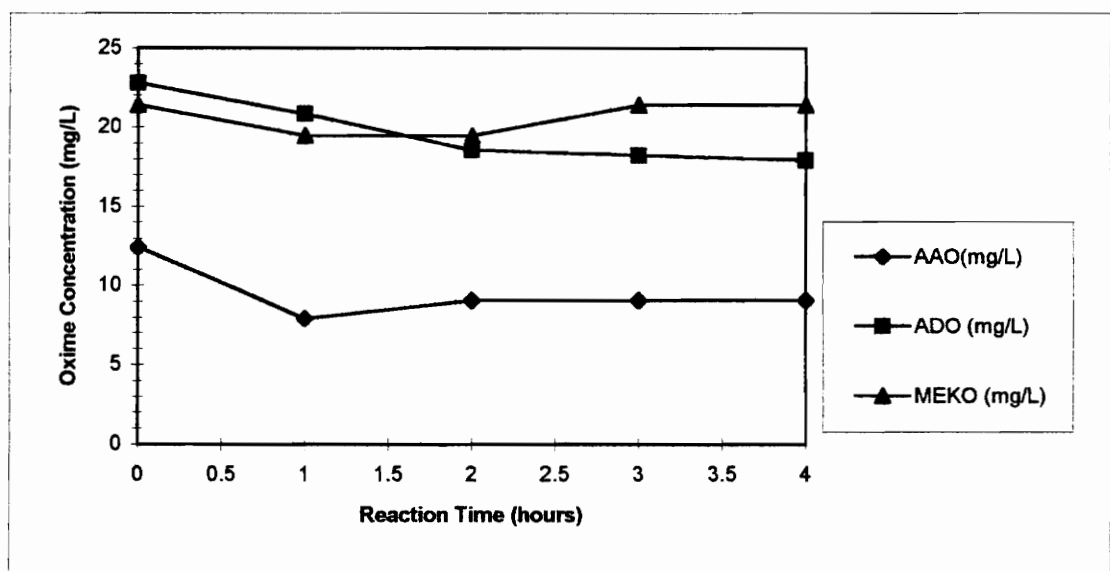


Figure 17. An investigation of oxime degradation during an anoxic batch test. Each batch flask contained 12 mg/L NO_3^- -N initially. No oximes were added to a control batch flask (not shown). The approximate oxime concentration was 25 mg/L for ADO and MEKO, and 12.5 mg/L for AAO.

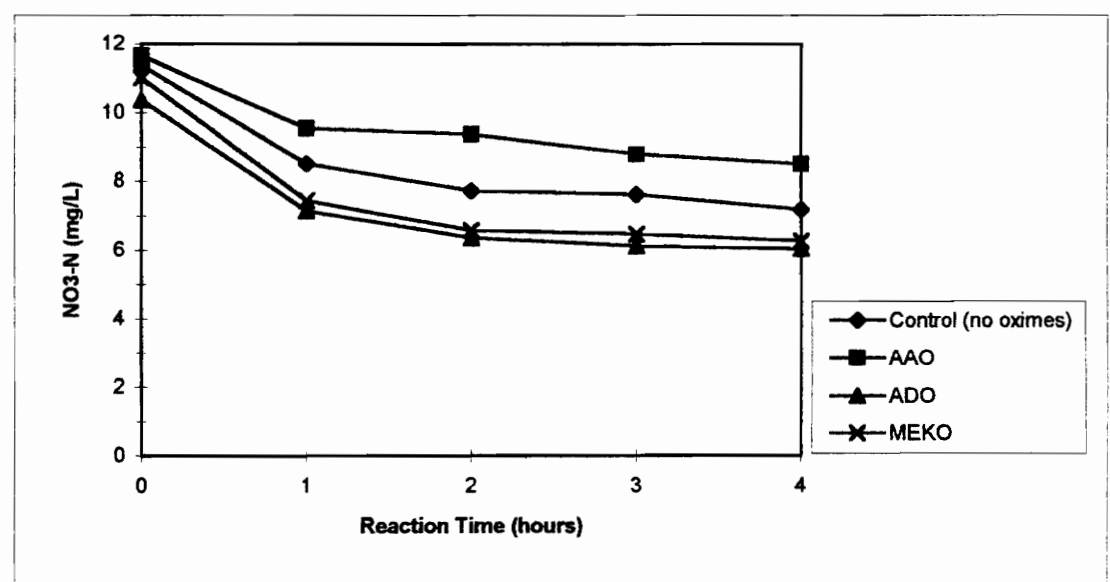


Figure 18. Nitrate uptake during an anoxic oxime batch study. Each batch flask contained 12 mg/L NO_3^- -N initially. No oximes were added to a control batch flask. The theoretical initial oxime concentration was 25 mg/L for ADO and MEKO, and 12.5 mg/L for AAO.

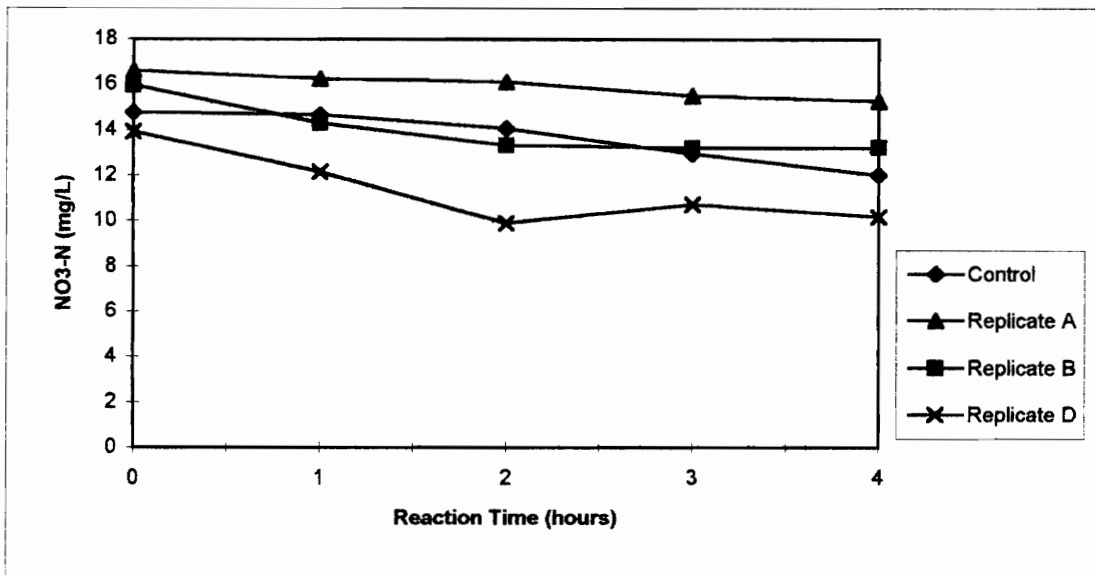


Figure 19. A comparison of nitrate uptake in three replicate anoxic batch flasks fed 25 mg/L ADO to a control flask provided with no oximes. Each batch flask contained approximately 15 mg/L NO_3^- -N initially.

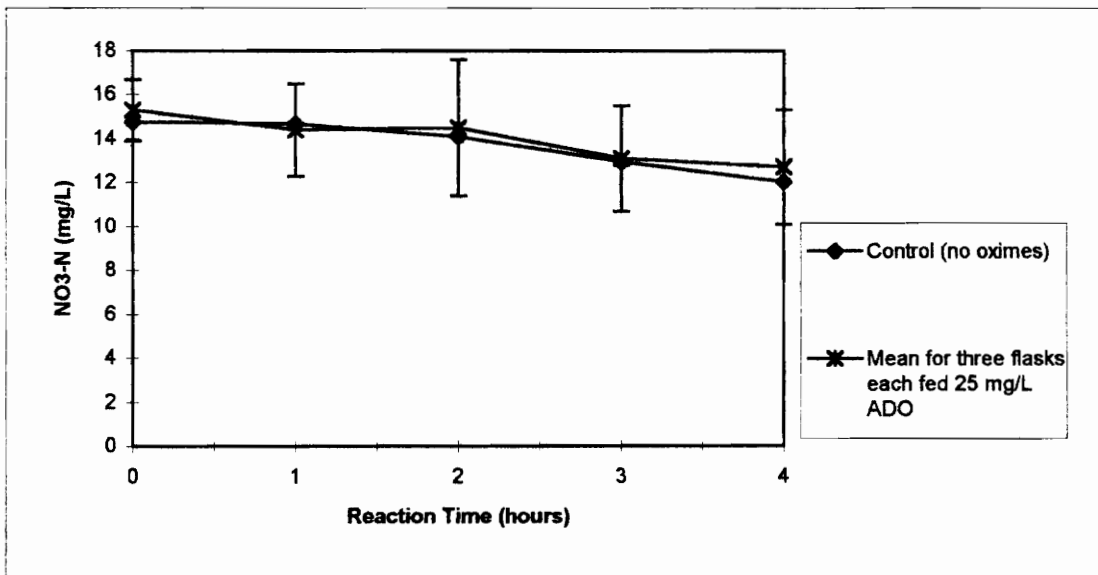


Figure 20. The statistical variability in nitrate uptake encountered in anoxic oxime batch tests. The mean nitrate curve was derived from three batch flasks each fed 25 mg/L ADO (See Figure 19). Error bars indicate plus or minus one standard deviation. Each batch flask contained approximately 15 mg/L NO_3^- -N initially. A summary of the statistical analysis is provided in Table B-20.

be utilized for calculating variability in determining ADO concentrations in experimental procedure. Therefore, caution should be employed in interpreting these results.

Variability in procedural techniques would appear to be the cause of differences in nitrate uptake in the control and experimental batch flasks. As explained in detail later, it was unlikely that any nitrate was being utilized for oxime degradation. In fact, as became apparent in later studies, the presence of ample nitrate may have actually prevented the oximes from degrading in these early batch tests.

Anoxic SBR Monitoring

Because initial batch tests showed little to no oxime degradation, focus was then directed toward acclimating the culture in the anoxic SBR to the oximes.

Routine Monitoring. Routine monitoring of the anoxic SBR was performed from August, 1995 to February, 1996. Data from this monitoring are provided in Tables B-31 through B-40 in Appendix B.

Figure 21 shows the mixed liquor suspended solids (MLSS) concentrations for this time period. From this figure, it can be noted that steady state for MLSS was encountered after mid August, 1995. In addition, a gradual increase in MLSS concentrations was encountered during the operation of the anoxic reactor. This trend was caused by routine scraping of biofilm that tended to grow on the reactor walls, but was eliminated when the Plexiglas reactor was replaced with a New Brunswick glass reactor.

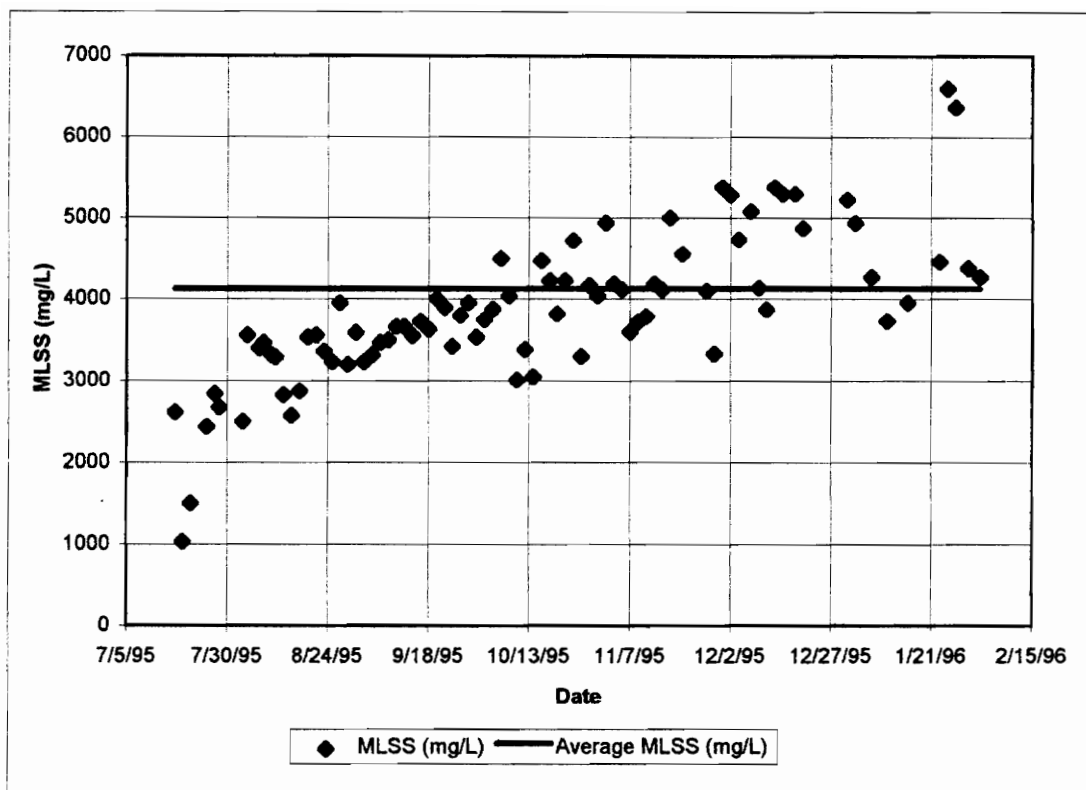


Figure 21. Mixed liquor suspended solids concentrations over the operation time period for the anoxic SBR. A steady state for MLSS was achieved by mid-august, 1995.

As part of the routine monitoring for the anoxic SBR, samples were taken over the SBR reaction time and analyzed for nitrate ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), ammonium ($\text{NH}_4\text{-N}$), total organic carbon (TOC), and oximes. Raw data for these analyses are provided in Table B-31 through B-37 in Appendix B.

Overall averages for TOC and nitrate uptake over the four hour reaction cycle are shown in Figures 22 and 23, respectively. Error bars represent standard error of the mean concentration. Note that the majority of the biogenic organics and nitrate were utilized within the first hour of the reaction cycle. Since SBR's provide a concentration gradient

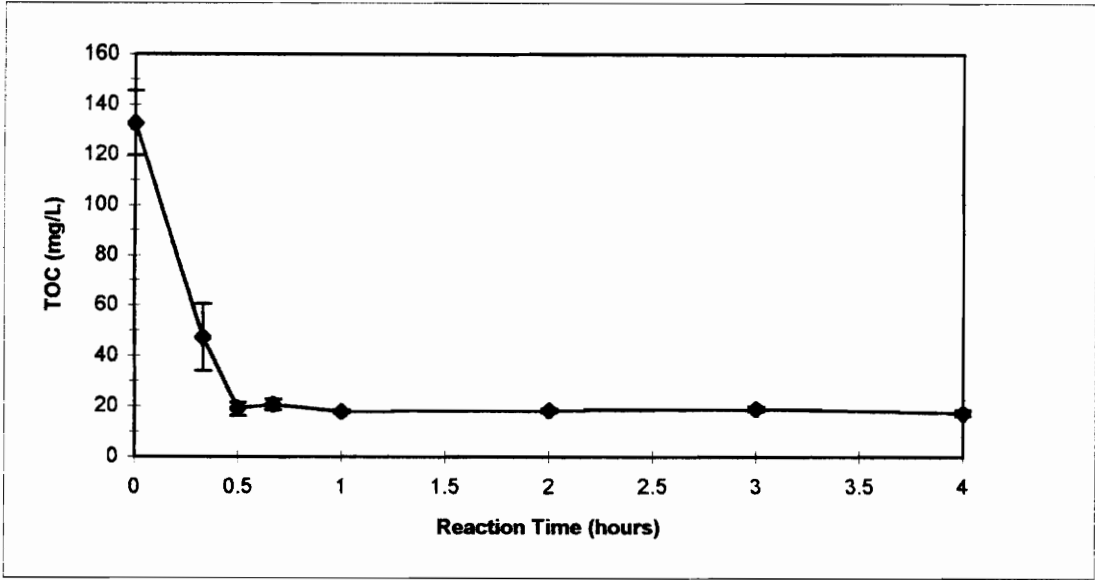


Figure 22. Average total organic carbon concentrations over the anoxic SBR cycle. Error bars represent standard error of the mean.

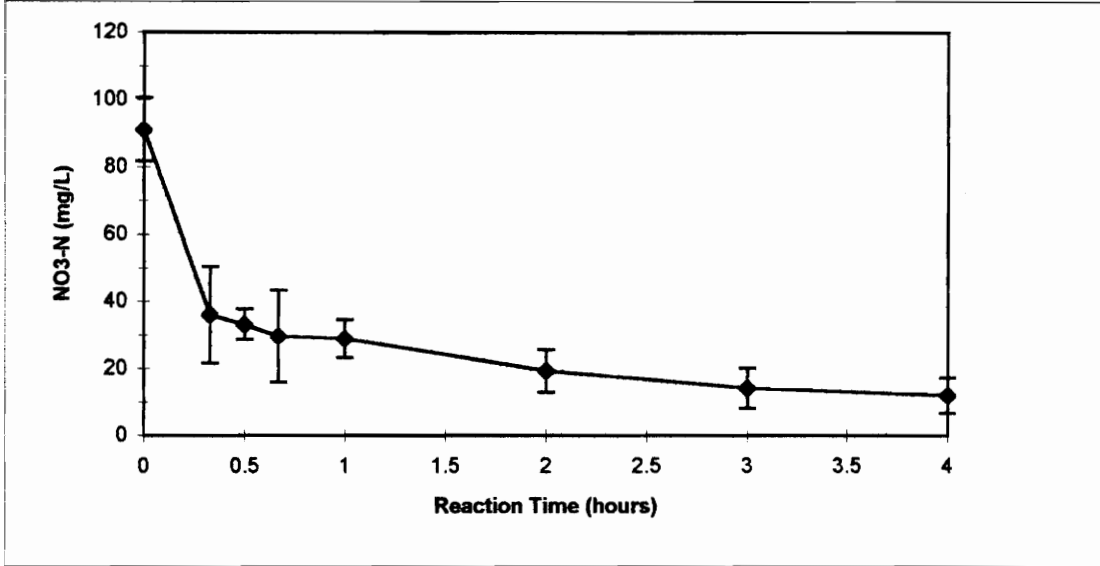


Figure 23. Average nitrate concentrations over the anoxic SBR cycle. Error bars represent standard error of the mean.

over the reaction time, highest growth rates can be expected during the beginning of the reaction cycle. Orhon *et al.* (1986) reported similar trends in carbon consumption during their operations of several SBR's.

Ammonium monitoring over the reaction cycle showed that the ammonium concentration was fairly stable over time. It should be noted that the biogenic organic feed contained several proteins, such as beef extract, with high levels of organic nitrogen. Because this organic nitrogen could potentially be converted to ammonium during the reaction cycle, it is difficult to interpret ammonium results.

Nitrite was generally low or not detectable during the anoxic reaction cycle. In a few cases, when the reactor was fed manually to obtain accurate initial oxime concentrations, nitrite did accumulate during the first few samples, but was oxidized later in the reaction cycle after nitrate had been utilized. It is postulated that in cases where the reactor was fed manually, the activated sludge was temporarily inhibited by sudden pH drops and high organic loadings.

Oxime Degradation. During the first few months of operation of the anoxic SBR, oxime concentrations remained stable over the reaction cycle as they had during the initial anoxic batch studies. However, in mid-September, MEKO degradation was seen for the first time. Unfortunately, during the next few analyses over the reaction cycle, MEKO degradation appeared sporadic, sometimes degrading and sometimes remaining stable. The only factor that seemed to play a role in MEKO degradation was the nitrate concentration remaining at the end of the reaction cycle. MEKO appeared to degrade

only if nitrate was completely utilized during the anoxic oxidation of biogenic organics. If residual nitrate was present throughout the entire reaction cycle, then MEKO did not degrade during that particular SBR cycle.

Figure 24 shows a comparison of nitrate consumption and MEKO degradation over the anoxic reactor cycle. Because this graph contains a bar chart, please observe that it is not to scale. Note that nearly all the nitrate had been utilized for oxidizing the biogenic organics within the first two hours of the reaction cycle. After the nitrate had been utilized and nitrate limiting conditions existed, MEKO degraded throughout the rest of the reaction cycle. Although MEKO did not degrade completely in this particular cycle, complete degradation of MEKO was recorded on several occasions. Other cases of MEKO degradation under nitrate limiting conditions are provided in Table B-33 in Appendix B.

Similar degradation patterns to MEKO were noted for AAO; however, AAO degradation proved to be slower than MEKO degradation. Conversely, ADO appeared to remain stable under all conditions. Overall averages for degradation of each oxime over the reaction cycle for a constant influent concentration of oxime (approximately 18 mg/L ADO and MEKO, and 9 mg/L AAO, diluted 1:4 in the reactor) are illustrated in Figures 25 through 27. Again, note that these bar charts are not to scale. Error bars represent standard error of the mean.

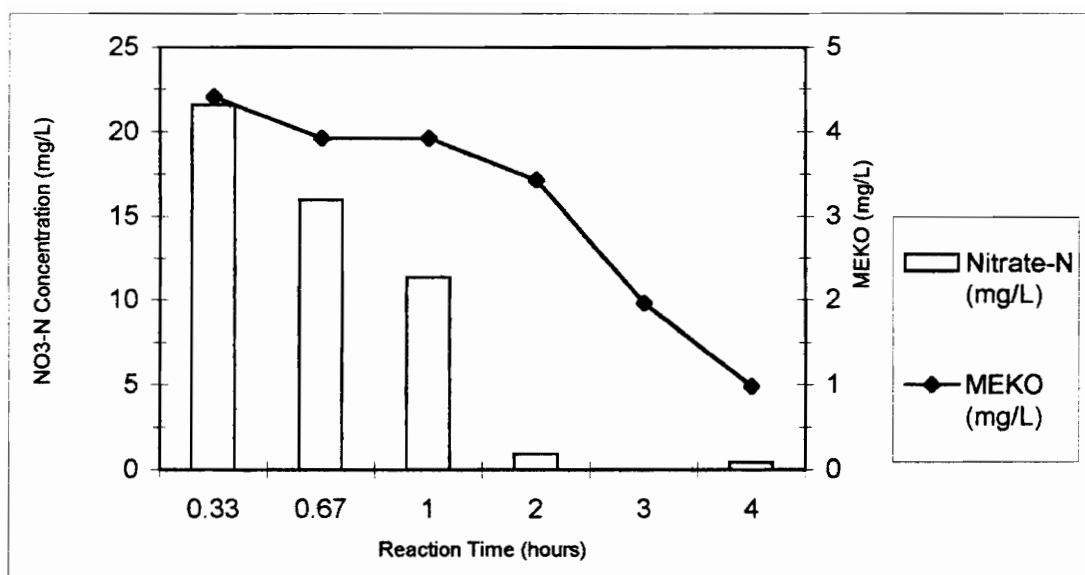


Figure 24. A comparison of MEKO degradation to residual nitrate concentration during an anoxic reactor cycle. Note that the chart is not to scale. Data are from the 10/17/95 reactor cycle.

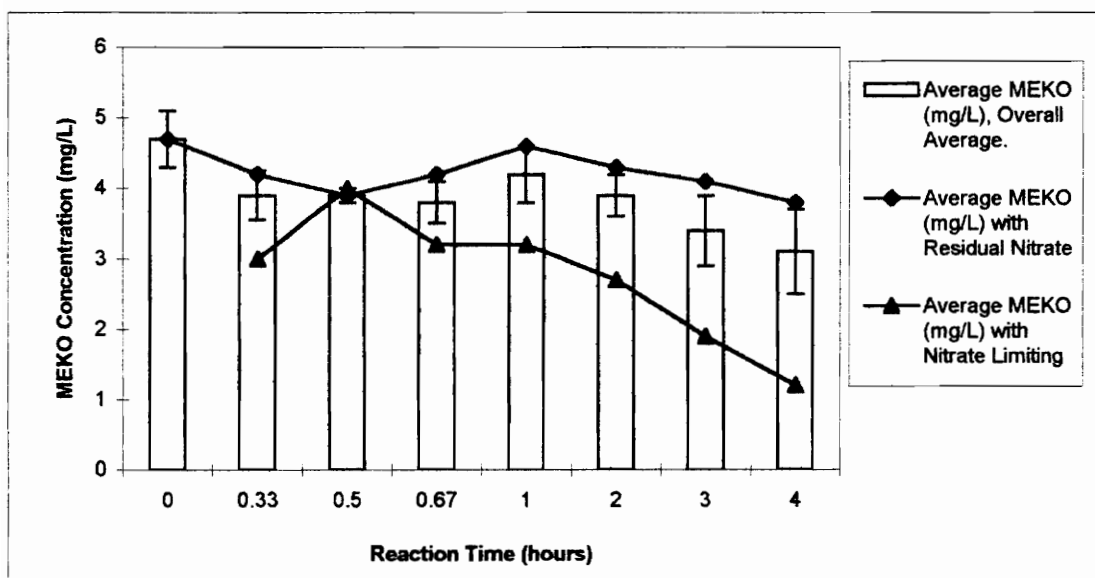


Figure 25. Average MEKO concentration during anoxic SBR cycles. Error bars represent standard error of the mean. Data included are from 9/22/95 to 1/16/96 ($n = 7$ for 1, 2, 3, and 4 hour samples; $n = 4$ for 0.33 and 0.67 hour samples; $n = 2$ for 0.5 hour sample; and $n = 3$ for 0 hour sample). The theoretical influent MEKO was approximately 17.5 mg/L (diluted 1:4 in the reactor)

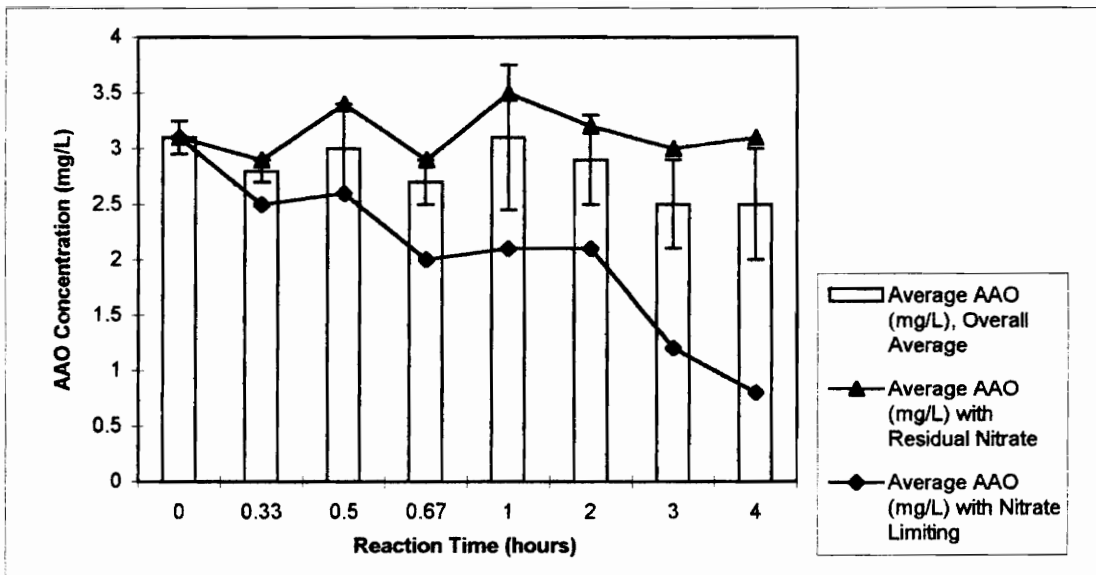


Figure 26. Average AAO concentrations during anoxic SBR cycles. Error bars represent standard error of the mean. Data are from 9/22/95 to 1/16/96 ($n = 7$ for 1, 2, 3, and 4 hours samples; $n = 4$ for 0.33 and 0.67 hour samples; $n = 2$ for 0.5 hour sample; and $n = 3$ for 0 hour sample). Theoretical influent AAO = 9 mg/L (diluted 1:4 in the reactor).

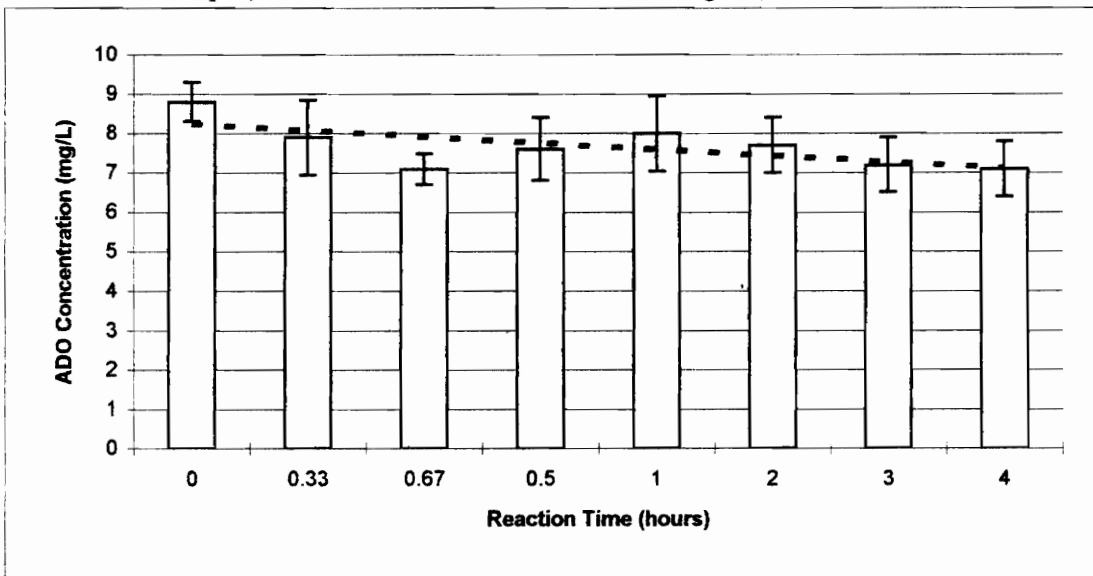


Figure 27. Average ADO concentrations during anoxic SBR cycles. Error bars represent standard error of the mean. Data are from 9/22/95 to 1/16/96 ($n = 7$ for 1, 2, 3, and 4 hours samples; $n = 4$ for 0.33 and 0.67 hour samples; $n = 2$ for 0.5 hour sample; and $n = 3$ for 0 hour sample). The dashed line represents a best line fit for the average data. The theoretical influent ADO was 17.5 mg/L (diluted 1:4 in the reactor).

As shown in the Figures 25 and 26, overall average MEKO and AAO concentrations over the reaction cycles showed little degradation. However, if data are split into two categories, degradation when ample nitrate was available throughout the reaction cycle and degradation when nitrate was limiting after the first hour or two of the reaction cycle, one can note that AAO and MEKO degradation was encountered only in the latter case. Again, although these figures show that AAO and MEKO degraded to only about 1 mg/L, complete degradation of both oximes, to well below the method detection limit, were recorded (See Tables B-32 and B-33 in Appendix B).

As shown in Figure 27, ADO remained very stable throughout the reaction cycle. Although not shown, no differences were noted in ADO degradation under nitrate limiting conditions and ample nitrate conditions. The dashed line shown on the graph represents a linear regression for the ADO data. Note that this line is fairly horizontal, illustrating the stability of ADO under anoxic conditions.

In addition to appearing resistant to biodegradation under anoxic conditions, ADO appeared to cause the reactor to turn a distinct army green and produced an unpleasant stale sulfur odor on several occasions. For this reason, ADO was eliminated from the anoxic SBR feed in December, 1995. Unfortunately, no operational conditions were identified that appeared to initiate this color change and odor generation. On two occasions, the reactor pH was slightly elevated (pH 7.5 to 7.8), but on another occasion the pH was more neutral (pH 6.95). Managers at the Industry commented that the odor could possibly originate from a degradation product, methyl mercaptan.

Since MEKO and AAO appeared to be degrading only after all available nitrate had been utilized, an attempt was made to establish a nitrogen/carbon ratio needed to ensure nitrate limiting conditions early in the reactor cycle. Figure 28 depicts different $\text{NO}_3\text{-N/TOC}$ ratios encountered during operation of the anoxic SBR. As noted in the figure, nitrate limiting conditions appeared to occur in cases when this ratio was below 0.66. Alternatively, in all cases where the ratio was above 0.66, residual nitrate was present throughout the entire reaction cycle. It should be noted that this ratio only applies to the synthetic biogenic organic feed used in this research (see Table 4). A similar investigation would be required to determine the minimum nitrogen/carbon ratio for the Industry's wastewater. This analysis does, however, seem to prove that such a ratio could be established for the Industry's wastewater.

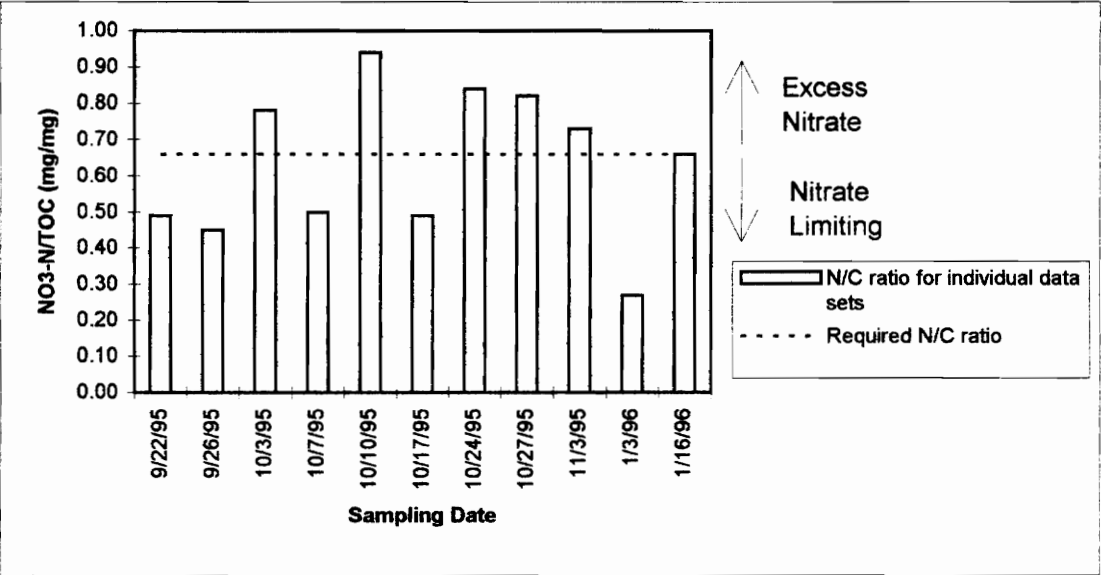


Figure 28. History of the applied $\text{NO}_3\text{-N/TOC}$ ratio for the synthetic wastewater used in the master anoxic SBR. The maximum ratio allowed before nitrate-sufficient conditions were experienced in the reactor was 0.66 $\text{NO}_3\text{-N/TOC}$ (mg/mg). Data are provided in Table B-38.

Oxime Batch Tests Under Nitrate Limiting Conditions

Preliminary results illustrating degradation of MEKO and AAO under nitrate limiting conditions led to several postulations about oxime degradation. A series of batch tests, as described in detail in the following section, were performed in an effort to identify the mechanism(s) responsible for MEKO and AAO degradation. Several hypotheses were established in an attempt to explain the mechanism(s) responsible for MEKO and AAO degradation under nitrate limiting, anaerobic conditions.

Hypothesis 1. AAO and MEKO are degraded cometabolically, only after responsible enzymes are induced in the presence of biogenic organic compounds.

Hypothesis 2. AAO and MEKO chemically break down under anaerobic conditions.

Hypothesis 3. AAO and MEKO are utilized as alternative electron acceptors and, possibly, electron donors.

Hypothesis 4. AAO and MEKO biochemically degrade under low redox conditions that are generated by anaerobic cultures.

Hypothesis 5. AAO and MEKO are biologically degraded by anaerobic bacteria, such as fermentative or sulfate reducing bacteria.

Hypothesis 6. AAO and MEKO hydrolyze into hydroxylamine, which is then converted to ammonium during assimilatory nitrate reduction, or is utilized as a terminal electron acceptor.

Other researchers performing oxime and hydroxylamine studies have noted chemical oxime degradation (Castignetti *et al.*, 1985), assimilatory reduction of hydroxylamine (Painter, 1970; Castignetti and Hollocher, 1982), and utilization of hydroxylamine as an alternative electron acceptor (Kurcera and Skladal, 1990).

Several batch tests were performed to (i) confirm the degradation of MEKO under nitrate limiting conditions and (ii) confirm or eliminate the mechanistic hypotheses listed above as being responsible for the loss of MEKO observed in the anoxic SBR. Batch tests were not performed on AAO, since it was found that AAO would not cause nitrification inhibition at concentrations encountered at the Industry. Results for MEKO degradation, TOC utilization, ammonium generation, and hydroxylamine detection are discussed.

Figure 29 illustrates the results from the first MEKO batch test performed under nitrate limiting conditions. In one batch flask, anoxic microorganisms were subjected to 8 mg/L MEKO in the absence of nitrate. In the other flask, both MEKO, at approximately 7 mg/L, and biogenic organics, at approximately 100 mg/L COD, were added in the presence of a limiting amount of nitrate. In fact, all nitrate had been consumed by denitrifiers within the first fifteen minutes of the reaction time. In both cases, as shown in Figure 29, MEKO degraded substantially under nitrate limiting conditions. As a control, approximately 10 mg/L MEKO was added to an uninoculated flask containing MSM and subjected to the same operating conditions as the inoculated flasks. As shown in Figure 29, MEKO did not appear to chemically degrade under the test conditions. In addition, a final sample (not shown) from the uninoculated flask proved that MEKO was still chemically stable after 24 hours.

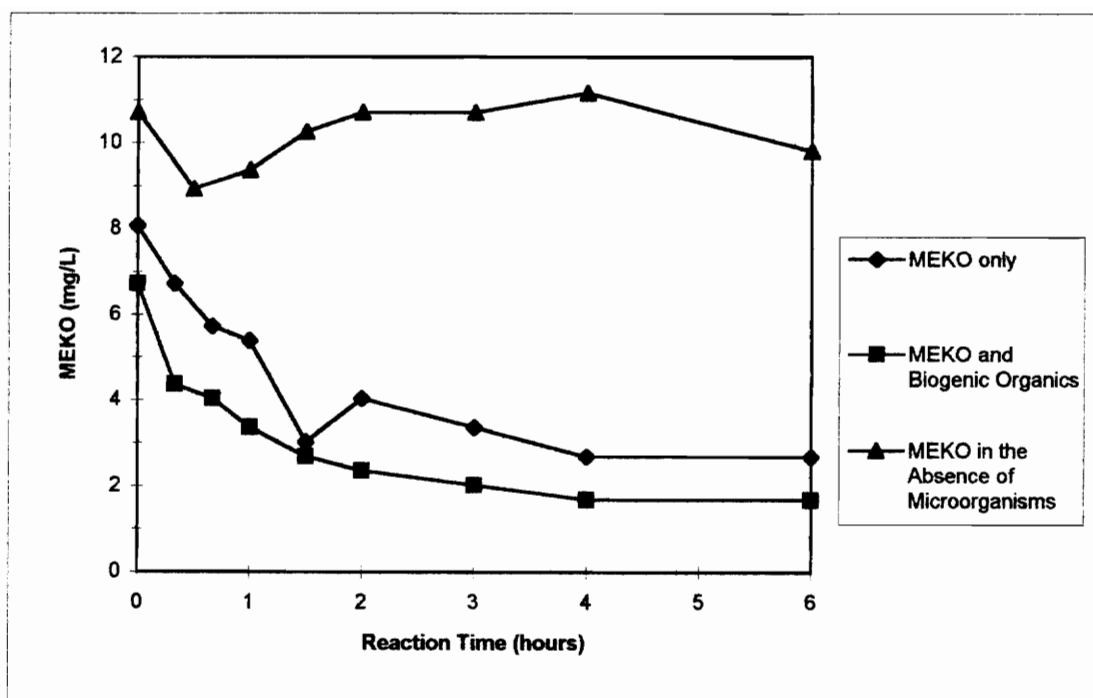


Figure 29. Confirmation of MEKO degradation under nitrate limiting conditions. A control flask was run under the same test conditions, but in the absence of microorganisms.

This initial batch test under nitrate limiting conditions helped provide some direction in determining mechanisms playing a role in MEKO degradation. First, the fact that MEKO degraded both alone and in the presence of biogenic organics provided evidence that MEKO degradation was not co-metabolic, thereby eliminating hypothesis 1. Second, the stability of MEKO in the uninoculated flask suggested that MEKO degradation under anaerobic conditions was not purely chemical, thereby eliminating hypothesis 2.

Both of these results can be compared to findings from heterotrophic nitrification studies. Oxime degradation in the absence of an external carbon source had also been

accomplished aerobically by others (Castignetti and Gunner, 1980; Castignetti *et al.*, 1985). Conversely, Amarger and Alexander (1968) and Obaton *et al.* (1968, cited in Castignetti and Gunner, 1980) found heterotrophic nitrifiers could not utilize oximes as a carbon source under aerobic conditions. Also, results illustrating the chemical stability of MEKO conflict with Castignetti *et al.*'s study (1985) where it was found that several oximes, including AAO, substantially degraded in control flasks containing no microorganisms. This discrepancy may be explained by the relative lengths of the batch experiments; batch experiments in this research varied from four to 24 hours, while Castignetti's tests were performed over a 21 day period. Oxime hydrolysis could also have occurred as the result of acidic or basic conditions, (March, 1985) but pH conditions in Castignetti's study were not published.

One might note that MEKO did not degrade completely during this batch experiment and was remaining fairly stable toward the latter part of the study. However, MEKO was found to degrade more rapidly and efficiently under nitrate limiting conditions in the anoxic SBR. It was postulated that perhaps oxygen was slowly entraining into the anoxic batch flasks during sampling. In order to minimize such entertainment, batch flasks were periodically purged in the following batch experiments.

Figure 30 shows the effect of purging on anoxic cultures subjected to three different concentrations of MEKO under nitrate limiting conditions. Notice that oxime degradation occurred most rapidly immediately after purging and was impeded immediately following samplings that occurred between purgings. This trend suggests

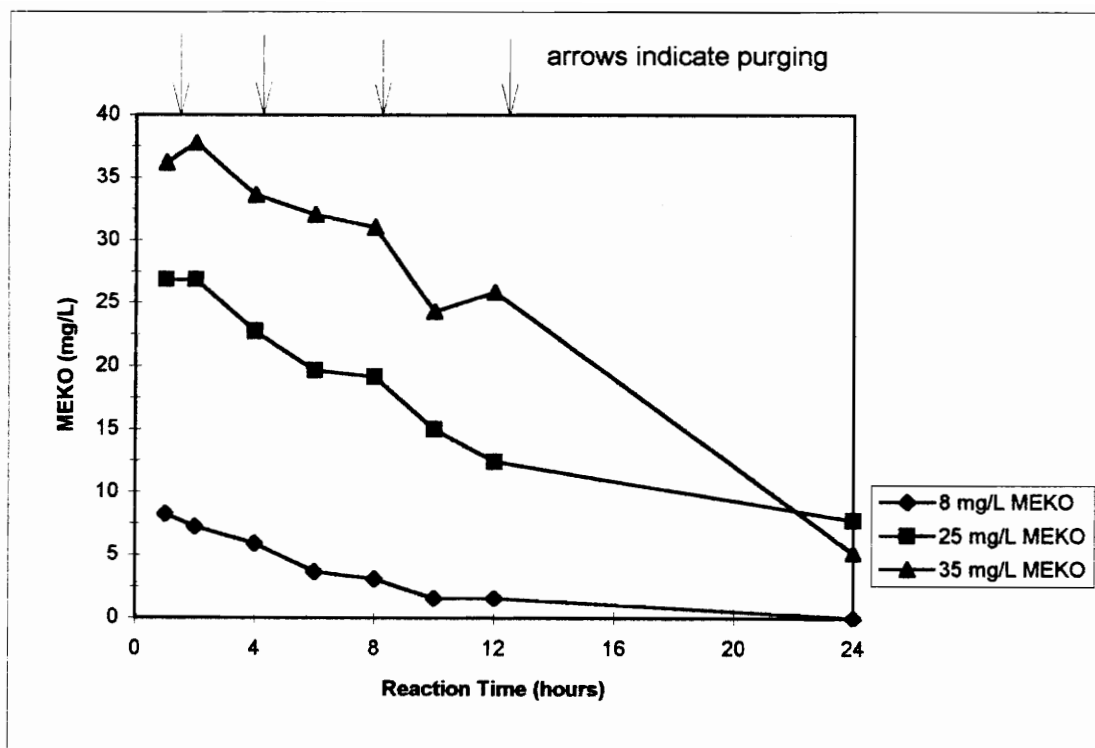


Figure 30. The effect of N₂ purging on MEKO degradation under nitrate limiting conditions.

that MEKO degrades most rapidly in the complete absence of oxygen. Three possible explanations for this phenomenon are that (i) MEKO is utilized as an alternative electron acceptor and its use is strictly regulated by molecular oxygen, (ii) MEKO is biochemically degraded under low redox conditions, or (iii) MEKO is utilized as an electron donor and biologically degraded under nonaerobic conditions. These explanations support hypotheses 3, 4, and 5, respectively. Note that hypothesis 5 considers degradation of MEKO only by anaerobic bacteria. If MEKO were utilized as an alternative electron acceptor and MEKO nitrogen cleaved from its carbon backbone, then the remaining organic carbon could potentially serve as an electron donor for this

MEKO degrader. Biological MEKO carbon removal in this manner is considered in hypothesis 3.

Results from initial anoxic oxime batch tests also directly support hypotheses 3 and 4 listed above. For example, if MEKO were utilized as an alternative electron acceptor, as described by hypothesis 3, then a preferential electron acceptor, such as nitrate, would inhibit its utilization. Alternatively, if MEKO were biochemically degraded under low redox conditions, as explained by hypothesis 4, then the presence of nitrate would poise the redox potential at a higher level and prevent such degradation.

If MEKO were biologically utilized under anaerobic conditions, as suggested by hypothesis 5, then fermentative or sulfate reducing bacteria would be the most likely microorganisms responsible for MEKO degradation. Two sets of batch tests were designed to determine if these microorganisms were degrading MEKO. In the first set of these batch experiments, organic carbon was monitored to determine if MEKO carbon was being utilized under nitrate limiting, anaerobic conditions. In the second set of batch experiments, the role of sulfate reducers on MEKO degradation was investigated by performing an anaerobic MEKO batch test in the absence of sulfate (SO_4^{2-}).

Figure 31 compares TOC degradation monitored in a batch flask throughout a previously described MEKO batch experiment performed under nitrate limiting conditions to theoretical carbon uptake corresponding to measured MEKO degradation. Although not shown, similar trends between theoretical and measured organic carbon concentrations were observed in other instances (See Tables B-23, B-26, and B-27 in

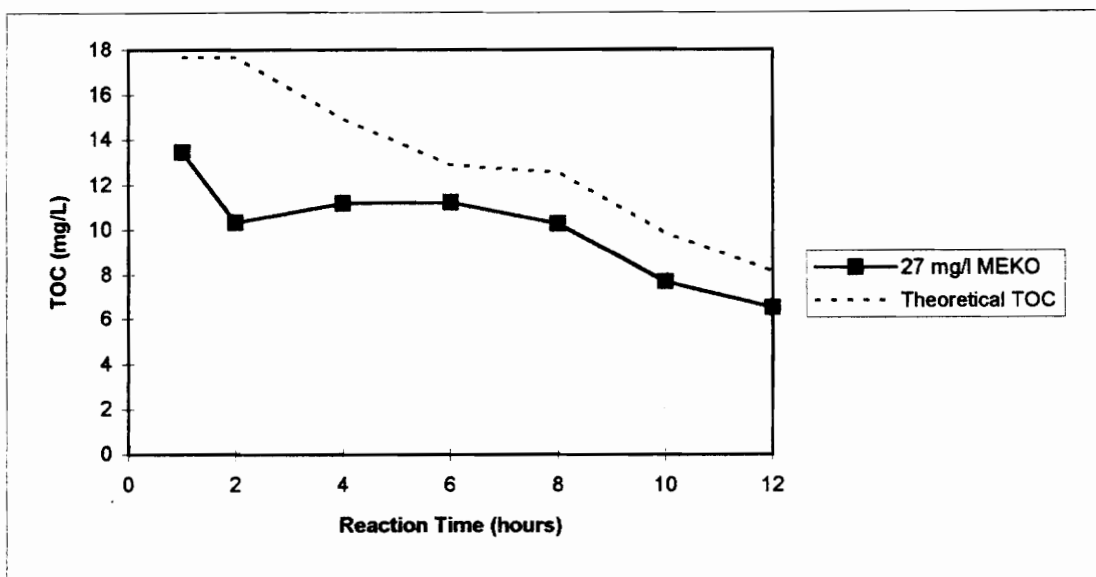


Figure 31. A comparison of measured and theoretical TOC concentrations during an anoxic MEKO batch test under nitrate limiting conditions. The theoretical TOC was calculated based on measured MEKO concentrations from the 3/27/96 batch test shown in Figure 30.

Appendix B). The dashed line represents the theoretical TOC level for the remaining MEKO concentration measured in the batch flask. Note the similarities between actual TOC utilization and theoretical TOC uptake corresponding to MEKO degradation.

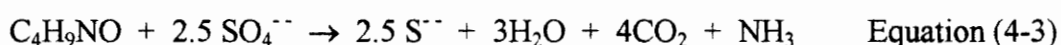
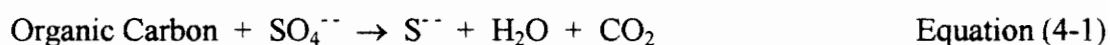
Similar trends between theoretical and actual TOC degradation during MEKO batch tests under nitrate limiting conditions suggest that MEKO carbon was being utilized by the activated sludge during nonaerobic batch experiments. These results could possibly eliminate the feasibility of fermentative degradation of MEKO as fermentative bacteria utilize carbon both as electron donors and acceptors and, thus, do not cause a significant net decrease in the concentration of organic matter. However, because TOC concentrations were generally less than 20 mg/L and batch experiments

were run over long periods of time, endogenous metabolism may have influenced dissolved organic carbon concentrations; therefore, further studies are required to confirm these results.

Results from the sulfate limiting batch test are provided in Table B- 29 in Appendix B. In this study, MEKO was added to the batch flask at a concentration of approximately 7 mg/L. The batch flask was continually purged with nitrogen to ensure completely anaerobic conditions. Analysis of an initial and six hour sample showed that MEKO had completely degraded. Despite washing and rinsing the activated sludge two times, a small amount of sulfate was detected in the first sample (1.3 mg/L). By the sixth hour of the experiment, all of the available sulfate had been utilized.

Decreases in sulfate over the SBR reaction cycle had been monitored on several occasions, demonstrating that sulfate reducers were present in the master SBR. However, evaluation of the amount of sulfate needed to degrade 7 mg/L of MEKO in the batch experiment previously described showed that more sulfate would have been required to degrade MEKO than was present in the batch flask. Equations (4-1) and (4-2) represent the general chemical equations used to describe microbial sulfate reduction (Metcalf and Eddy, 1991). Equation (4-3) illustrates a mass balance for sulfate reduction using MEKO as the carbon source. From this chemical equation, one would calculate that 2.75 mg of sulfate are required to degrade 1 mg of MEKO. Therefore, approximately 19 mg/L of sulfate would have been required to completely degrade the MEKO present in the batch flask. This calculation provides evidence against the

degradation of MEKO by sulfate reducing bacteria, and therefore, with the above discussion on TOC removal during MEKO batch experiments, eliminates hypothesis 5. The disappearance of the small amount of sulfate present during the batch test was probably due to endogenous decay of sulfate reducing bacteria present in the mixed activated sludge culture.



Preliminary experiments were performed to investigate if MEKO was being utilized as an alternative electron acceptor as described by hypothesis 3 listed above. As explained in the Materials and Methods chapter, tungsten (W), a known dissimilatory nitrate reductase inhibitor (Chauret and Knowles, 1991; Wu and Knowles, 1994), was added to batch flasks as sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) in an attempt to inhibit denitrification. It was postulated that if MEKO nitrogen were being utilized in a dissimilatory manner, then MEKO degradation would be inhibited in the presence of high concentrations of sodium tungstate due to the inactivation of nitrate reductase, the enzyme responsible for the first step in nitrate reduction. Unfortunately, initial control experiments showed that even the presence of over 6000 mg/L sodium tungstate (25 mM as W) did not cause a decrease in nitrate utilization rates in anoxic activated sludge from the master SBR (see table B-30 in Appendix B). Chauret and Knowles (1991) noted that because it acts as a competitive inhibitor against molybdenum (Mo), some pure cultures

may not be inhibited by tungsten unless grown in a molybdenum deficient solution. Therefore, the failure to inhibit denitrification in this experiment can probably be explained by the fact that the mixed activated sludge culture had been provided ample molybdenum throughout the operation of the master SBR.

Ammonium.

Because it was postulated that MEKO could potentially be degrading via a hydrolytic pathway, as explained in hypothesis 6, ammonium concentrations were monitored throughout batch tests in an effort to identify assimilatory production of ammonium from hydroxylamine. No general trends in ammonium production could be identified in any of the batch experiments (See Tables B-22 and B-25 in Appendix B). For the most part, ammonium appeared relatively stable, although in a few cases it increased slightly during the first part of the experiment. However, such small increases could likewise result from endogenous decay.

Hydroxylamine. As noted in the Materials and Methods chapter, difficulties were encountered throughout this research in analyzing hydroxylamine. Unfortunately, hydroxylamine was analyzed successfully in only two batch tests. In the first of these batch tests, previously described in Figure 30, hydroxylamine was not detected in any of the samples taken throughout the batch experiment. However, the highest concentration of MEKO for this batch test was approximately 35 mg/L. If hydroxylamine were acting as a hydrolytic intermediate, then the expected concentrations for this compound would be low at a starting MEKO concentration of 35 mg/L. It was concluded that any dynamic

increases in hydroxylamine due to a sudden increase in MEKO to 35 mg/L may not have been detected.

A final batch test was performed at a spiked MEKO concentration of approximately 350 mg/L under nitrate limiting conditions. Hydroxylamine was not detected at any point during this test. To avoid oxygen entrainment, the batch flask was continuously purged throughout the study. Unfortunately, this continuous purging increased evaporation rates; therefore, experimental data are only presented for the first four hours when evaporation was assumed to be minimal. However, during those four hours, hydroxylamine was not detected. Furthermore, MEKO still degraded partially to below 200 mg/L despite its high initial concentration.

If hydroxylamine was being formed from MEKO degradation, it is reasonable to assume that it might have been detected in the latter batch test where the initial MEKO concentration was at 350 mg/L. Therefore, results from hydroxylamine monitoring during MEKO batch tests seem to suggest that MEKO is not degrading via a hydrolytic pathway, but rather through an organic one, thereby discounting hypothesis 6. This postulation is further supported by similarities between trends in measured and theoretical organic carbon uptake rates as described previously. In addition, findings from several heterotrophic nitrification studies (Castignetti and Hollocher, 1982; Castignetti *et al.*, 1983; Castignetti *et al.*, 1985; Castignetti *et al.*, 1986) have also provided evidence for oxime degradation occurring via an organic pathway.

One should not completely exclude hydroxylamine as possibly playing a role in MEKO degradation. Results from this research can suggest only that MEKO is not hydrolyzing into quantifiable levels of hydroxylamine; it is still possible that hydroxylamine could be an intercellular intermediate in microbial MEKO degradation. Thus, additional studies are required to further define the role of hydroxylamine in MEKO degradation.

Investigation of Single Sludge Anoxic/Anaerobic/Aerobic Treatment Scheme

After the degradation of MEKO under nitrate limiting conditions was confirmed through extensive batch studies, the reactor cycle was modified, as described in the Materials and Methods chapter, to include both anoxic and aerobic phases. Note that due to the 12 hour anoxic reaction time, nitrate limiting, anaerobic conditions existed after the first few hours of the anoxic reaction cycle. After modification of the reactor cycle, the reactor was initially seeded with one liter of activated sludge from the aerobic, nitrifying SBR. Daily monitoring of the reactor for both oxime degradation and nitrification are discussed in this section. Because nitrification was upset earlier in the operation of the multi-environment reactor, a side study was performed to determine if the SBR effluent was inhibitory to an aerobic, nitrifying activated sludge culture. Results from this study are also presented. Finally, oxime degradation over the anoxic reaction cycle is shown.

Operation of Anoxic/Anaerobic/Aerobic Reactor: MLSS and NO_x-N Species

The single sludge multi-environment reactor was monitored routinely during its operation from February to April, 1996, for the following constituents: MLSS, ESS, TOC, nitrate, nitrite, ammonium, and oximes. Figure 32 shows the results from MLSS data for this operational period. MLSS concentrations were relatively stable after February 14, 1996 (Day 9). This point established a pseudo steady state. However, nitrate and nitrite concentrations profiles (shown in Figure 33) suggest that the system was not truly stable until February 25, 1996 (Day 20).

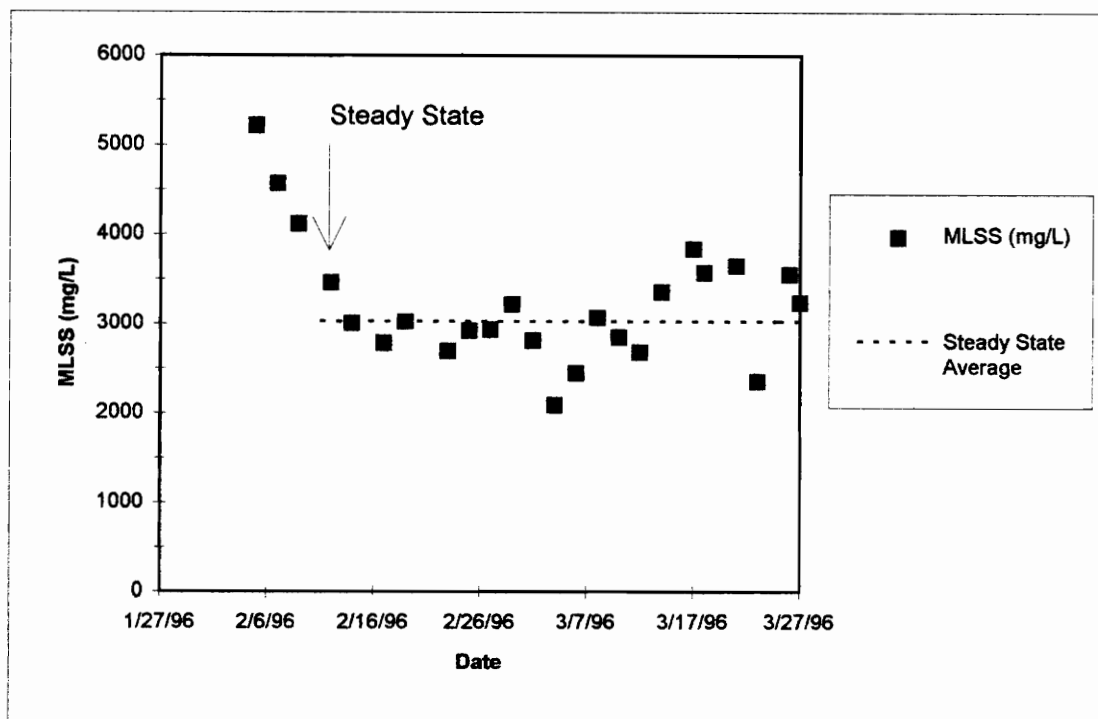


Figure 32. MLSS concentrations for the multi-environment anoxic/aerobic SBR during its operation from February through March, 1996. Note that a steady state for MLSS was achieved by February 14, 1996.

Nitrification was monitored daily by analyzing effluent samples for nitrate, nitrite, and ammonium concentrations. Figure 33 shows the daily effluent nitrite and nitrate concentrations recorded. As illustrated in Figure 33, nitrification was upset on several occasions as noted by decreases in effluent nitrate and accumulations of nitrite. However, during these upset periods, residual oxime concentrations were either not detectable or low enough to not cause inhibition (see Table B-47 in Appendix B). Also, results from batch tests showing that hydroxylamine was not detectable during MEKO degradation suggested that hydroxylamine, a potent inhibitor of *Nitrobacter* (Stuven *et al.*, 1992; Alleman, 1992), was not causing nitrification inhibition.

As shown by the operational remarks provided in Figure 33, upsets appeared to coincide with operational problems. For example, in two cases, nitrite accumulation coincided with aeration failures. Yang and Alleman (1992) reported that low dissolved oxygen (DO) concentrations have been found to cause nitrite accumulation in nitrifying activated sludge. Consequently, it is reasonable to assume that loss of complete nitrification was most likely the direct result of aeration failures.

Although nitrite accumulation could be explained in two of three cases by low DO conditions, the nitrite build up experienced shortly after seeding the multi-environment reactor with 1 liter (L) of aerobic nitrifying activated sludge cannot be explained in the same way because normal dissolved oxygen concentrations were maintained.

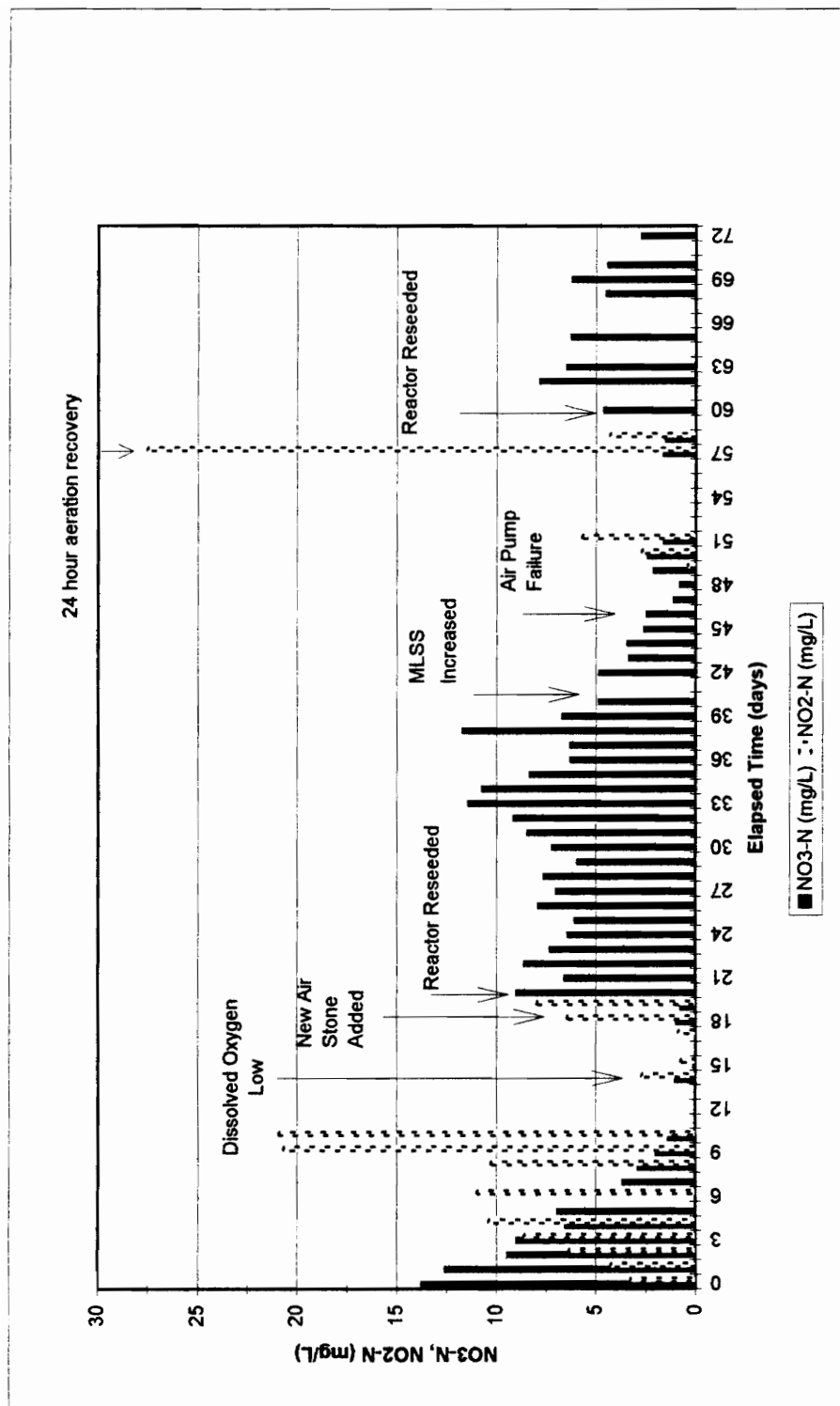


Figure 33. Nitrate and nitrite generated during the aerobic treatment cycle in the anoxic/anaerobic/aerobic reactor. Feed MEKO concentrations were increased gradually from 17.5 mg/L to 40 mg/L during the reactor operation. Feed AAO concentrations were increased gradually from 17.5 mg/L to 56 mg/L during the reactor operation. The reactor was monitored during its operation from February through April, 1996. Note that breaks in the data are due to days when effluent samples were not taken.

A side study was performed to determine the cause of nitrification inhibition encountered after the first seeding. In this experiment, three aerobic batch activated sludge cultures were maintained. Two of these activated sludge cultures were derived from the aerobic master SBR; the third activated sludge culture was taken from the multi-environment master SBR. The purpose of the two aerobic, nitrifying activated sludge cultures was to investigate the effect of the multi-environment SBR effluent on a nitrifying culture. The objective of the aerobic flask containing activated sludge from the multi-environment reactor was to determine if nitrification could be recovered. A description of these experiments was described previously in the Methods and Materials chapter.

Figure 34 shows the comparison of nitrate generation in the control flask fed only biogenic organics diluted in MSM and the experimental flask fed biogenic organics diluted in SBR effluent. Batch flasks were operated for several days prior to sampling to allow the systems to stabilize. A one-tailed student t-test was performed to determine if nitrate generation in the experimental flask was significantly lower than nitrate generation in the control flask (See Table B-49, Appendix B). As is evident in Figure 34, the hypothesis that nitrate generation in the experimental flask was greater than or equal to nitrate generation in the control held true; therefore, no inhibition was noted.

Results from this side study suggested that nitrification inhibition was not caused by the presence of oximes or oxime degradation products. Thus, inhibition in the master SBR was more likely to be the result of a seeding failure, as occurred in the first case of

nitrite accumulation, or operational conditions, as occurred in the latter two cases.

Possible operational conditions, as described by Yang and Alleman (1992) and Painter and Loveless (1983) included pH, temperature, dissolved oxygen concentrations, and ammonia levels. As illustrated by daily operational conditions (Table B-46 in Appendix B), both pH and temperature were within suitable ranges for nitrifier growth (Painter, 1970; Painter and Loveless, 1983; Shammas, 1986). In addition, at ammonium levels in the reactor (generally 20 - 30 mg/L N), free ammonia should not have been at a high enough concentration to inhibit *Nitrobacter* (Groeneweg *et al.*, 1994; Eckenfelder, 1991).

Figure 35 shows the nitrate and nitrite monitored daily in the third aerobic batch flask containing activated sludge from the multi-environment SBR. Note that the activated sludge was not washed and rinsed prior to the start of the study so that residual nitrite was present in the batch flask. As is evident from the figure, although the reactor was fed only biogenic organics diluted in MSM solution, nitrate generation never fully recovered. In addition, nitrite levels decreased over time, but at a slow rate. These results suggest that the *Nitrobacter* population had been adversely affected during the initial seeding of the anoxic/aerobic SBR. Further, it appeared that this population of nitrite oxidizers was unable to fully recover.

It is postulated that the nitrifiers needed a longer acclimation period to adjust to the new reaction cycle with a long anoxic/anaerobic retention time. Therefore, the master reactor was reseeded with nitrifying activated sludge on February 24, 1996, (Day 19) and

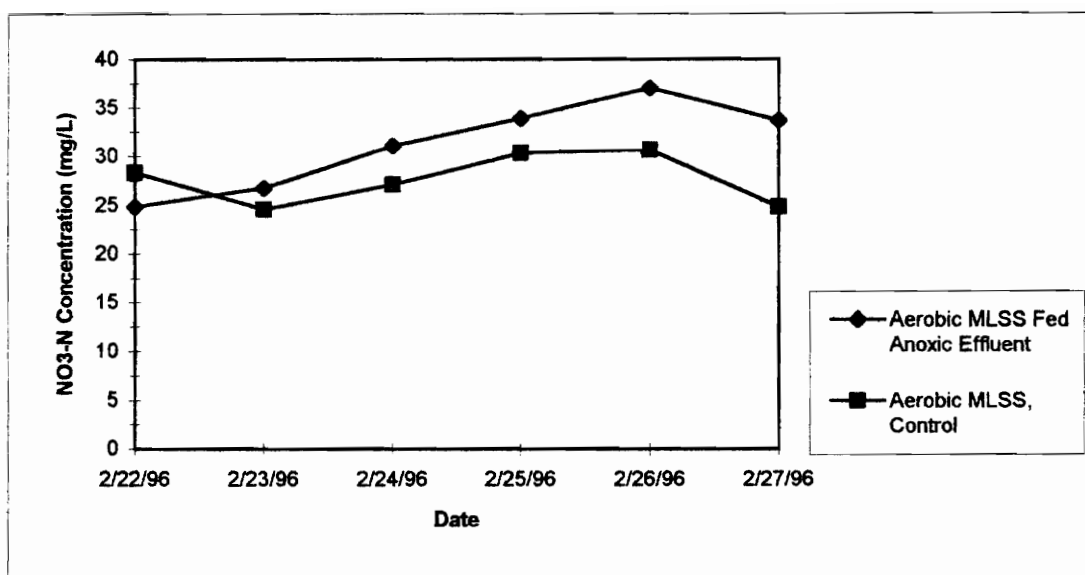


Figure 34. An investigation of the inhibitory potential of multi-environment SBR effluent on an nitrifying activated sludge culture. Sampling began one week after the experiment was started to allow time for the systems to stabilize. Statistical analyses of data provided in Table B-49.

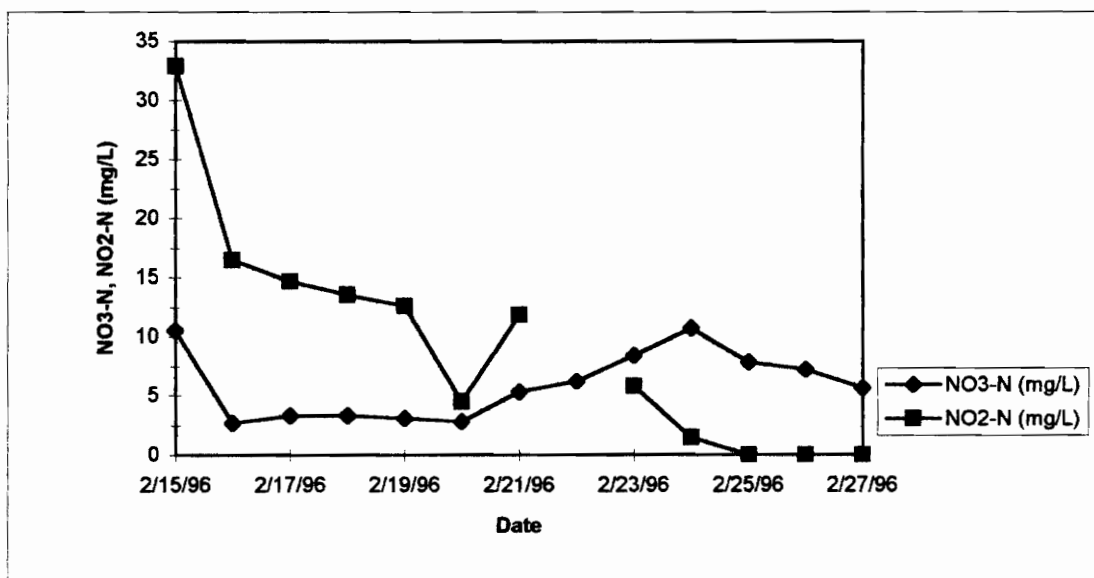


Figure 35. An investigation of the ability of multi-environment activated sludge to nitrify in the absence of oximes. Note that activated sludge was not rinsed prior to the start of the experiment.

a more gradual seeding process was adopted to allow ample time for nitrifiers to adjust to the new system. Again, one liter of aerobic activated sludge was added to the multi-environment reactor as an initial seeding; however, in this case, a small volume of the multi-environment reactor's activated sludge was replaced with aerobic activated sludge daily for a period of one week. The volume of aerobic activated sludge added each day was gradually decreased so that the final seeding schedule was as follows: 1 L, 60 milliliters (mL), 60 mL, 40 mL, 30 mL, 20 mL, and 10 mL.

As shown in Figure 33, after the gradual reseeded of the reactor, the reactor achieved complete nitrification until an air pump failure occurred. During this time period, no residual ammonium was detected (see Table B-47 in Appendix B). In addition, no effluent nitrite was detected. After the air pump failure, the reactor was reseeded with aerobic activated sludge on April 4, 1996 (Day 59), and complete nitrification was again achieved.

Operation of the single sludge multi-environment anoxic/anaerobic/aerobic SBR illustrated the feasibility that such a system can be used to prevent oxime inhibition of nitrification. The successful operation of this system supports Zitomer and Speece's statement (1993) that utilization of multi-environment systems "shows promise as an effective process for removing a broad range of chemicals." In addition, the successful nitrification of a wastewater containing MEKO agrees with Joel and Grady's study (1976) in which they found that nitrification inhibition by aniline could be prevented by

its biological degradation; however, Joel and Grady utilized completely aerobic treatment schemes.

Operation of anoxic/aerobic SBR: Oxime Degradation

As noted previously, samples were periodically taken over the SBR anoxic/anaerobic reaction cycle to track oxime degradation and nitrate utilization for denitrification. Raw data for oxime and nitrogen balances are provided in Tables B-50 through B-54 in Appendix B.

Some general trends can be noted in MEKO and AAO degradation during the anoxic/anaerobic reaction cycle. Oxime degradation usually occurred during the latter part of the anoxic cycles after nitrate had been completely utilized to oxidize biogenic organics and nitrate limiting conditions existed. MEKO degradation generally occurred more rapidly than AAO degradation; however, with a few minor exceptions, AAO and MEKO were never detected in the reactor effluent. In addition, long detention times were required for oxime degradation. For example, when the influent MEKO concentration was increased to approximately 32 mg/L (on March 12, 1996, Day 36), the anoxic reaction time had to be increased from 10 to 12 hours to ensure complete degradation of MEKO during the anoxic/anaerobic cycle.

Results from oxime monitoring over the anoxic/anaerobic reaction cycle of the master SBR showed similar trends to results from MEKO batch tests, with these oximes degrading only in the absence of residual nitrate. As noted earlier, oximes appeared to be removed more rapidly in the master SBR than during batch experiments. As explained in

detail in the previous section on anoxic studies, this phenomenon was probably due to oxygen entraining into flasks during batch experiments. With the presence of a high concentration of biogenic organics, the master SBR easily maintained a zero DO concentration throughout the entire anoxic/anaerobic reaction cycle.

It is unknown why MEKO appeared to degrade more rapidly than AAO under anoxic/anaerobic conditions. In fact, because AAO has a simpler structure than MEKO (see Figure 8), one would believe that the opposite would hold true. Under aerobic conditions, AAO did indeed degrade more quickly than MEKO. However, often residual AAO would be present at the end of the anoxic/anaerobic cycle, while MEKO almost always completely degraded. Concentrations of AAO bleeding through the anoxic reactor were low enough, though, to ensure complete aerobic degradation of the residual AAO.

Oxime degradation during the anoxic/anaerobic SBR cycle showed two basic trends. In some cases, AAO and MEKO degradation over time appeared linear, illustrating zero order kinetics. In other cases, however, oxime degradation rates appeared to be first order. Figures 36 and 37 show examples of data sets illustrating zero and first order degradation trends. The lines shown on these graphs illustrate predicted oxime concentrations based on the calculated rate constants.

Table 13 shows ranges of first order and zero order degradation rate constants for MEKO and AAO. Note that these constants have been normalized to MLSS. This procedure was adopted in an attempt to normalize the rates because it was suspected that

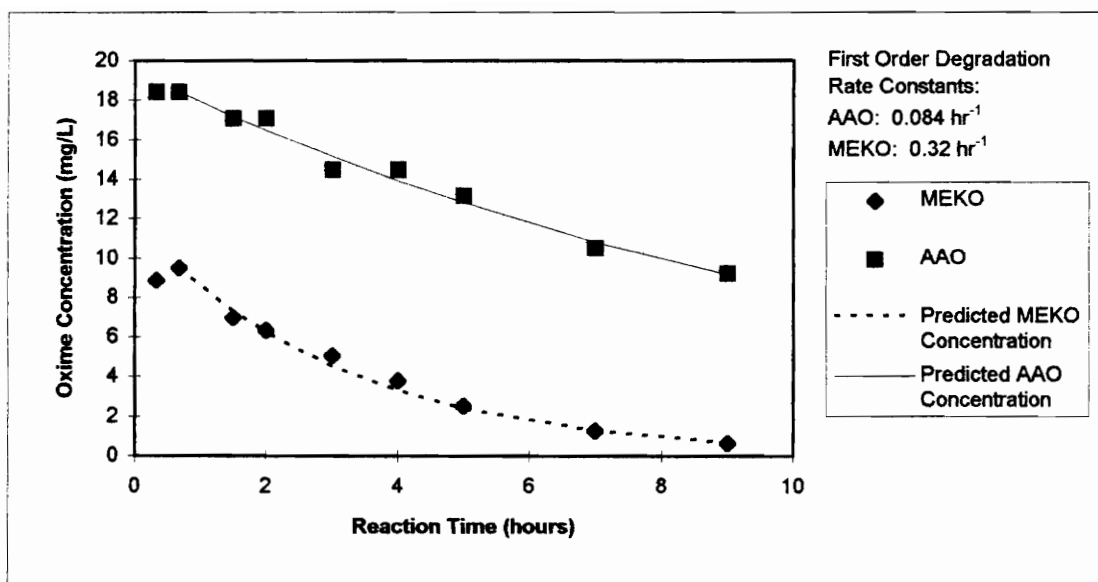


Figure 36. An example of a case where oxime degradation over the anoxic/anaerobic reaction cycle appears to be first order. Data are from the 4/2/96 reactor cycle. Note that the first order model predicts oxime concentrations only after nitrate limiting conditions exist.

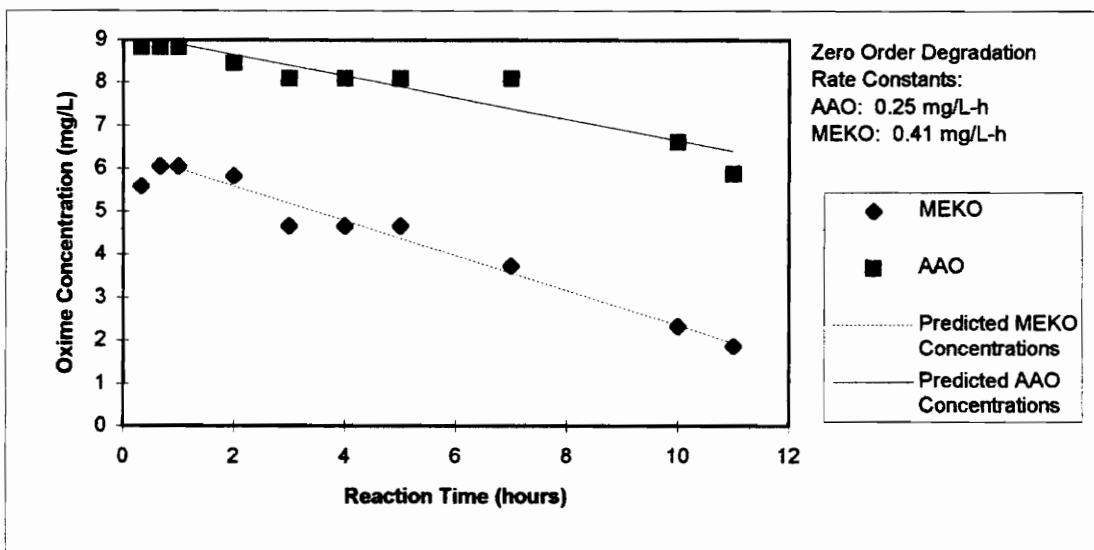


Figure 37. An example of a case where oxime degradation over the anoxic/anaerobic reaction cycle appears to be zero order. Data are from the 3/7/96 reactor cycle. Note that the model only predicts oxime concentrations after nitrate limiting conditions exist.

increases in degradation rates corresponded to increases in MLSS concentrations in the multi-environment SBR.

Both first and zero order kinetics are commonly used to describe microbial degradation rates (Eckenfelder, 1991; Metcalf and Eddy, 1991). Typically, only one model can be used to describe a particular set of activated sludge data, unless the substrate concentration is close to the concentration of its half saturation constant. As shown in Figures 36 and 37, oxime degradation data over the anoxic/anaerobic SBR reaction cycle can be accurately described by both models. If MEKO and AAO biochemically degrade under low redox conditions (hypothesis 4 listed previously), one would expect to encounter similar degradation patterns for different sets of data. More complex degradation kinetics can be expected if MEKO and AAO can be utilized as alternative electron acceptors and donors (hypothesis 3 listed previously) and a dual substrate limitation occurs. Also, fluctuations between first and zero order degradation trends could have resulted if oxime concentrations in the master SBR were close to their respective half saturation concentrations.

Table 13. Ranges of degradation rate constants estimated for AAO and MEKO degradation over the anoxic/anaerobic reaction cycle in the multi-environment SBR.

Oxime	Estimated zero order rate constant (mg/L-h per mg/L MLSS)	Estimated first order rate constant (hr ⁻¹ per mg/L MLSS)
AAO	0.08 - 0.24	0.01 - 0.061
MEKO	0.10 - 0.24	0.034 - 0.087

Degradation rate constants are normalized to MLSS. Range of rate constants were estimated from 5 sets of data taken over the anoxic/anaerobic reaction cycle; three sets of data showed a first order trend, while two sets showed a linear trend.

V. SUMMARY AND CONCLUSIONS

Summary

The purpose of this research was to investigate the effectiveness of alternative environments in degrading oximes and to develop a single sludge multi-environment anoxic/aerobic biological treatment scheme that could achieve oxime degradation and nitrification in the same treatment process. Aerobic and anoxic batch experiments were initially performed to determine the degree of nitrification inhibition caused by acetaldehyde oxime (AAO), aldicarb oxime (ADO), and methyl ethyl ketoxime (MEKO) and the fate of these oximes under anoxic, denitrifying conditions. Results from these batch studies were utilized to determine operational conditions for a single sludge multi-environment anoxic/anaerobic/aerobic sequencing batch reactor supplied with a synthetic organic wastewater containing up to 40 mg/L MEKO and 56 mg/L AAO. By utilizing 10 to 12 hour anoxic detention times and maintaining low nitrate:organic carbon ratios, this system was able to achieve oxime degradation and nitrification in the same treatment process.

Conclusions

From the results of nitrification studies, anoxic studies, and multi-environment reactor studies, the following conclusions can be drawn:

Nitrification Studies

1. AAO and ADO are partially inhibitory to nitrification at higher concentrations; AAO should not pose problems to nitrification at concentrations encountered at the

Industry because it is readily biodegradable under aerobic conditions at the low concentrations expected. Results from ADO batch studies suggest that this oxime will cause approximately a 24% decrease in nitrate production rates at typical concentrations encountered in the Industry's aqueous waste streams.

2. In accordance with Smith's findings (1994), MEKO proved to be a potent inhibitor of nitrification. Results from batch studies suggest that MEKO is inhibitory at concentrations as low as 2 mg/L and causes almost complete inhibition above 9 mg/L. Because aerobic degradation of this oxime is slow, pre-treatment of MEKO containing wastewaters would be required in order to achieve nitrification in an aerobic treatment stage.

Anoxic Studies

1. ADO degradation is minimal under anoxic conditions. MEKO and AAO can be efficiently degraded, but their removal appears to be a direct function of residual nitrate ($\text{NO}_3\text{-N}$) concentrations. Degradation of these oximes occurs only after all residual nitrate is utilized during denitrification and nitrate limiting conditions exist. Therefore, low nitrate:organic carbon ratios are required to ensure the removal of AAO and MEKO in anoxic treatment schemes.
2. Results from MEKO batch studies provide some insight into the mechanisms responsible for MEKO degradation under nitrate limiting conditions. Chemical degradation, including hydrolysis into hydroxylamine, can be eliminated as degradation pathways. Rather, it is postulated that MEKO degradation occurs via an

organic pathway, as it appears that MEKO carbon is utilized by heterotrophic microorganisms during MEKO degradation. Further studies are required to determine the role of MEKO nitrogen as a potential alternative electron acceptor.

Multi-Environment Anoxic/Anaerobic/Aerobic Studies

1. Degradation of MEKO and AAO under nitrate limiting conditions can be described by both linear and zero order kinetics. Because degradation rates are low, detention times in excess of 12 hours are required for complete removal of MEKO; however, degradation under these conditions proves to be more efficient than in aerobic treatment processes.
2. A single sludge anoxic/anaerobic/aerobic treatment scheme does prove to be a feasible method for preventing oxime inhibition of nitrification. An equalization basin, such as the oxime holding pond, should be utilized to avoid toxic shock loads caused by MEKO spills.

Engineering Significance

In recent years, more and more wastewater treatment facilities have upgraded or are considering upgrading their treatment schemes to incorporate anoxic/aerobic treatment. Operation of these systems have demonstrated some advantages over conventional aerobic systems, particularly in the area of municipal wastewater treatment. Less knowledge is available on the their effectiveness in industrial wastewater treatment. Results from this research provide further insights toward some of the roles that anoxic zones may play in the treatment of industrial wastewaters. First, this research

demonstrated that incorporation of an anoxic zone prior to an aerobic treatment stage may help improve nitrification. Second, as demonstrated by MEKO and AAO degradation under nitrate limiting conditions, the utilization of anoxic environments can play a vital role in the degradation of some xenobiotic compounds. As regulations grow increasingly stringent, the fate of many synthetic organic compounds (SOC's) in wastewater treatment systems must be considered. Anoxic degradation of xenobiotics provides an advantage over aerobic degradation in that air stripping is less likely in the first treatment scheme.

Because a high degree of variability exists among different industrial wastewaters, individual studies must be performed to determine the applicability of a single sludge anoxic/aerobic system for treatment of a wastewater produced by a particular industry. Results from this research provide initial evidence that a single sludge multi-environment anoxic/anaerobic/aerobic treatment system is a feasible treatment technology for the Industry and, possibly, for the RWTF. Successful operation of this treatment scheme plays an important role because the RWTF has recently submitted a request for proposals to develop a comprehensive plan that addresses the community's long term wastewater management strategy, which includes identifying technologies that will enable nitrogen removal from its wastewaters.

Finally, results showing the nonaerobic degradation of MEKO and AAO are considered novel as no research was identified that considered the fate of these compounds under alternative environments. These findings demonstrate that microbial

cultures can be acclimated to degrade some xenobiotic compounds under alternative environments.

Recommendations

Results from this research have raised many new questions regarding oxime degradation under nitrate limiting conditions and the feasibility of successfully applying the single sludge multi-environment anoxic/anaerobic/aerobic system utilized in this research toward treating the actual wastewater produced by the Industry.

Although this research provided preliminary evidence that supports certain mechanisms that may be involved in MEKO degradation under nitrate limiting conditions, additional experiments are required to determine the degradation pathway for this oxime. Isolation and characterization of the MEKO degrader may help with the identification of mechanisms involved in MEKO degradation. Initial steps for this work are being undertaken by an undergraduate researcher, Mary Rust.

This research played a fundamental role in determining the degradability of AAO, ADO, and MEKO under anoxic conditions. Now that conditions required for the successful degradation of oxime nitrification inhibitors have been determined, steps can be taken toward optimizing a single sludge system for treating actual wastewater produced by the Industry.

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APPENDIX A
Standard Curves

Standard Curves for Ammonia and Oximes

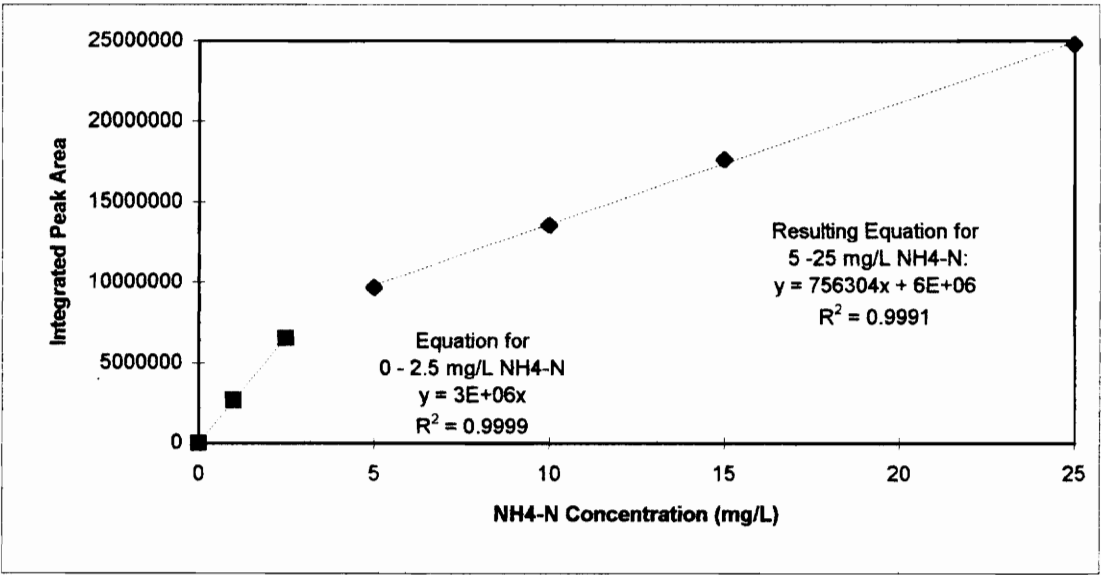


Figure A-1. An Ammonia standard curve from I.C. Analysis.

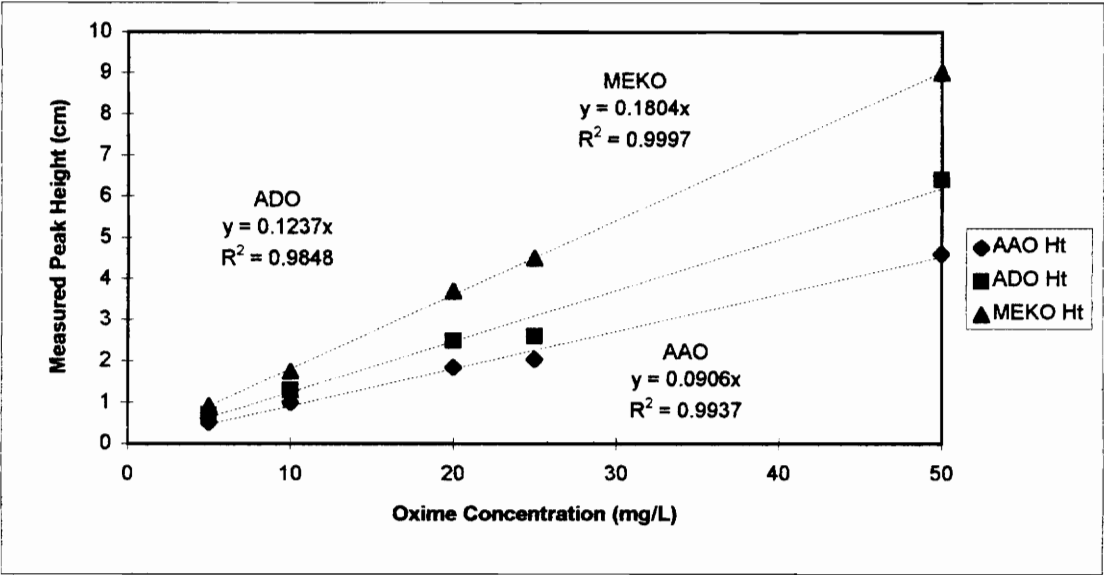


Figure A-2. Oxime standard curves using GC/FID analysis. Note that the AAO peak height was taken as the sum of syn and anti isomer peaks.

APPENDIX B

Raw Data and Statistical Analyses

Data for Aerobic Batch Experiments

Table B-1. Effect of Each Oxime on Nitrification at a Concentration Less Than 10 mg/L.

Time (hours)	Nitrate Concentration, mg/L -N with:			
	Control	ADO	AAO	MEKO
0	0.0	0.0	0.0	0.1
0.5	0.2	0.1	0.1	0.2
1	0.5	0.3	0.3	0.3
2	1.0	0.7	0.6	0.5
4	1.9	1.5	1.3	0.8
7	3.1	2.7	2.4	1.1
10	4.4	3.9	3.4	1.3
18	7.5	7.1	7.1	1.8
24	9.2	9.0	9.2	2.2

Table B-2. Effect of MEKO Concentration on Degree of Nitrification Inhibition.

Sample Time, hours	Nitrate Concentration, mg/L-N with MEKO at:			
	Control	2 mg/L	5 mg/L	15 mg/L
0	0.0	0.4	0.3	0.3
0.5	0.6	0.6	0.4	0.3
1	1.1	1.0	0.5	0.4
2	2.5	1.9	0.7	0.5
4	6.0	4.1	1.4	0.9
8	12.4	8.2	3.1	1.2
16	19.7	13.8	5.4	1.8
24*	21.5	18.2	7.0	2.5

* 24 hour sample for Control disregarded when determining zero order rate

Table B-3. Effect of 33 mg/L AAO and 45 mg/L ADO on Nitrification.

Time hours	NO ₃ - Concentration, mg/L as N		
	Control	ADO	AAO
0	0.1	0.1	0.1
1	0.4	0.4	0.2
2	1.4	0.9	0.4
4	3.9	2.9	1.3
8	9.5	7.2	4.1
12	13.7	10.9	6.9
20	20.0	16.5	12.1
24	20.8	18.5	13.9

* 24 hour sample for Control disregarded when determining zero order rate

Tables B-4, B-5, and B-6: Linear regressions for Nitrification Batch Experiments.

Table B-4. MEKO Nitrification Batch Test.

Oxime	Concentration (mg/L)	Nitrate Production Zero Order Rate Constant (mg NO ₃ -N/mg MLVSS)/hours	R ²	% Inhibition
Control	0	0.39	0.98	
MEKO	2	0.27	0.98	31
MEKO	5	0.08	0.97	79
MEKO	10	0.06	0.94	85
MEKO	15	0.04	0.97	90

Note that nitrate production rates were based on samples taken during the first four hours of the experiment because nitrate production was linear throughout this time period.

Table B-5. Effect of Each Oxime on Nitrification at a Concentration Less Than 10 mg/L.

Oxime	Concentration (mg/L)	Nitrate Production Zero Order Rate Constant (mg/L NO ₃ -N)/hours	R ²	% Inhibition
Control	0	0.4	0.99	
AAO	7	0.38	0.99	5
ADO	7	0.38	0.999	5
MEKO	9	0.086	0.95	79

Note that nitrate production rates were based on samples taken over the entire 24 hour period because nitrate production was linear throughout this time period.

Table B-6. Effect of Higher Concentrations of AAO and ADO on nitrification.

Oxime	Concentration (mg/L)	Nitrate Production Zero Order Rate Constant (mg/L NO ₃ -N)/hours	R ²	% Inhibition
Control	0	1.1	0.97	
AAO	33	0.84	0.96	24
ADO	45	0.46	0.94	58

Note that nitrate production rates were based on samples taken during the first eight hours of the experiment because nitrate production was linear throughout this time period.

Tables B-7, B-8, and B-9. Oxime degradation during aerobic batch tests.

Table B-7.

Time (hours)	ADO (mg/L)	AAO (mg/L)	MEKO (mg/L)
0	6.7	6.7	8.7
0.5	6.7	4.3	8.7
1	6.7	2.4	7.1
2	7.8	2.4	8.1
4	6.7	2.4	7.4
7	6.2	1.2	6.7
10	6.7	0.6	5.7
18	5.6	0.0	5.4
24	5.6	0.0	4.0

Table B-8.

Time, hours	ADO (mg/L)	AAO (mg/L)
0	44.9	33.4
1	40.4	27.0
2	41.1	30.5
4	41.1	29.1
8	34.4	22.7
12	32.1	25.6
20	31.7	16.4
24*	21.6	15.0

Table B-9.

Time (hours)	Actual MEKO Concentrations for		
	2 mg/L	5 mg/L	15 mg/L
0	2.8	5.8	9.9
0.5	2.5	5.2	5.8
1	2.5	5.5	6.4
2	2.3	5.5	7.0
4	2.3	5.2	6.4
7	2.0	4.6	6.1
18	1.7	4.0	2.8
24	1.7	3.7	4.0

Table B-10. Ammonia Utilization During Aerobic Batch Test with Oximes at less than 10 mg/L.

Sample Time, (hours)	Ammonium Concentration, mg/L-N with:			
	Control	7 mg/L ADO	7 mg/L AAO	9 mg/L MEKO
0	35.8	30.5	33.8	33.2
0.5	32.8	27.0	32.3	28.7
1	31.0	26.5	31.5	27.4
2	29.4	26.9	29.6	43.0*
4	27.4	24.8	27.6	24.6
7	24.3	33.5*	24.9	22.9
10	21.8	21.3	23.4	22.9
18	18.6	15.6	21.7	24.0
24	13.8	19.6	21.3	22.6

*Ammonia Peaks Integrated incorrectly on I.C.

Table B-11. Effect of MEKO Concentration on Ammonia Utilization During Aerobic Batch Test.

Sample Time, hours	Control	NH ₄ -N Concentration, mg/L with MEKO at:		
		2 mg/L	5 mg/L	15 mg/L
0	30.0	30.7	31.9	32.5
0.5	27.9	27.6	28.4	29.8
1	27.4	25.2	28.1	28.6
2	24.1	25.1	27.0	27.7
4	16.1	20.6	25.2	28.4
8	12.3	12.8	23.7	26.2
16	1.8	6.4	20.4	22.7
24	0.1	4.9	19.2	25.7

Data for Anoxic Batch Tests

Table B-12. MLVSS for Batch Tests.

Date	MLVSS (mg/L)
8/2/96	N/A
8/30/96	N/A
12/18/96	4390
1/30/96	5140
3/27/96	2925
4/20/96	3885

8/2/96 Anoxic Batch Test Results

Table B-13. Oxime Degradation Under Anoxic Conditions, 8/2/96.

Time (hours)	AAO (mg/L)	ADO (mg/L)	MEKO (mg/L)
0	12.5	22.8	21.4
1	7.9	20.8	19.5
2	9.1	18.6	19.5
3	9.1	18.2	21.4
4	9.1	17.9	21.4

Table B-14. Nitrate Utilization During Anoxic Oxime Batch Test, 8/2/96.

Time (hours)	NO ₃ -N (mg/L)			
	Control	12.5 mg/L AAO	25 mg/L ADO	25 mg/L MEKO
0	11.4	11.7	10.4	11.0
1	8.5	9.5	7.2	7.4
2	7.7	9.4	6.4	6.6
3	7.6	8.8	6.1	6.5
4	7.2	8.5	6.0	6.3

Table B-15. Ammonia Utilization During Anoxic Oxime Batch Test, 8/2/96.

Time (hours)	NH ₄ -N (mg/L)			
	Control	12.5 mg/L AAO	25 mg/L ADO	25 mg/L MEKO
0	30.4	31.6	29.8	29.7
1	30.8	32.1		29.7
2	31.2	31.8	30.1	29.8
3	31.4	32.2	29.9	30.5
4	46.4	33.1	44.8	32.4

Table B-16. Statistical Anoxic Batch Test: 25 mg/L ADO provided to Flask A, B, and D.

Time (hours)	ADO (mg/L)		
	A (mg/L)	B (mg/L)*	D (mg/L)
0	22.4	16.7	22.4
1	19.7	15.6	20.9
2	23.0	13.5	20.9
3	21.5	15.0	21.5
4	22.7	13.8	19.1

*Note: Some of ADO stock seeped out side of lure lock for syringe during injection

Table B-17. Statistical Anoxic Batch Test: Nitrate Uptake Over Reaction Time.

Time (hours)	NO ₃ -N (mg/L)			
	Control (No oximes)	25 mg/L ADO Each		
		A	B	D
0	14.8	16.6	16.0	13.9
1	14.7	16.3	14.3	12.2
2	14.1	16.1	13.3	9.9
3	13.0	15.5	13.2	10.7
4	12.0	15.3	13.3	10.2

Table B-18. Statistical Anoxic Batch Test: Ammonia Uptake Over Reaction Time.

Time (hours)	NH ₄ -N (mg/L)			
	Control (No oximes)	25 mg/L ADO Each		
		A	B	D
0	26.3	19.3	16.3	21.0
1	28.8	20.4	23.7	33.3
2	19.3	32.5	15.4	20.4
3	29.0	21.9	24.6	34.3
4	13.0	34.5	25.1	34.5

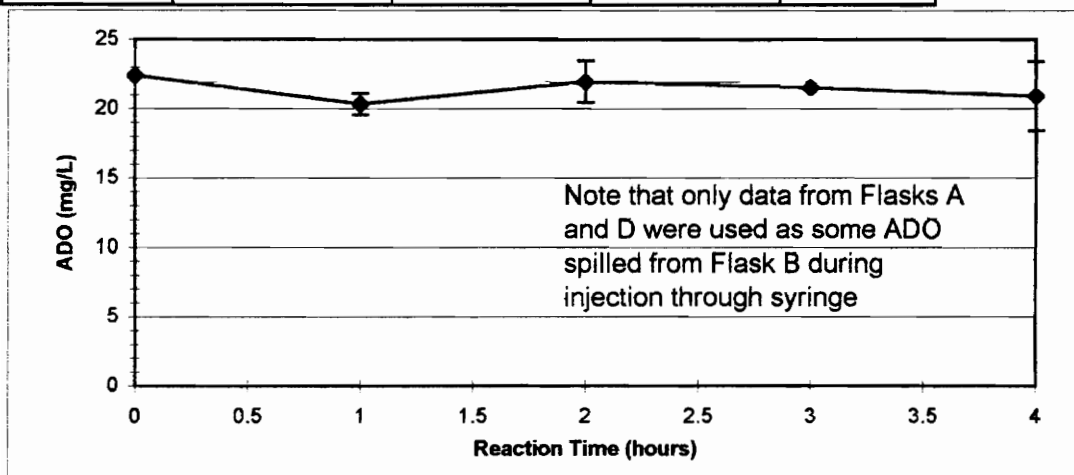


Figure B-1. Average Aldicarb Oxime Concentrations during Anoxic Batch Test: 8/15/95 Batch Test, Statistical Analysis. Error bars represent plus or minus one standard deviation.

Table B-19. Statistical Summary for ADO in Flasks A and D from Statistical Anoxic Batch Test, 8/15/96.

0 hour sample			1 hour sample			2 hour sample			3 hour sample		
Mean	22.4	Mean	20.3	Mean	22.0	Mean	21.5	Mean	21.5		
Standard Error	0.0	Standard Error	0.6	Standard Error	1.0	Standard Error	0.0	Standard Error	0.0		
Standard Deviation	0.0	Standard Deviation	0.8	Standard Deviation	1.5	Standard Deviation	0.0	Standard Deviation	0.0		
Sample Variance	0.0	Sample Variance	0.7	Sample Variance	2.2	Sample Variance	0.0	Sample Variance	0.0		
Minimum	22.4	Minimum	19.7	Minimum	20.9	Minimum	21.5	Minimum	21.5		
Maximum	22.4	Maximum	20.9	Maximum	23.0	Maximum	21.5	Maximum	21.5		
Count	2.0	Count	2.0	Count	2.0	Count	2.0	Count	2.0		
Confidence Level(95.000%)	#NUM!	Confidence Level(95.000%)	1.2	Confidence Level(95.000%)	2.1	Confidence Level(95.000%)	#NUM!	Confidence Level(95.000%)	#NUM!		

4 hour sample		
Mean	20.9	
Standard Error	1.8	
Standard Deviation	2.5	
Sample Variance	6.4	
Minimum	19.1	
Maximum	22.7	
Count	2.0	
Confidence Level(95.000%)	3.5	

Table B-20. Statistical Summary for Nitrate in Batch Flasks A,B, and D During Statistical Anoxic Batch Test, 8/15/96.

	0 hour sample	1 hour sample	2 hour sample	3 hour sample
Mean	15.5	14.3	13.1	13.2
Standard Error	0.8	1.2	1.8	1.4
Standard Deviation	1.4	2.1	3.1	2.4
Sample Variance	2.0	4.2	9.8	5.8
Minimum	13.9	12.2	9.9	10.7
Maximum	16.6	16.3	16.1	15.5
Count	3.0	3.0	3.0	3.0
Confidence Level(95.000%)	1.6	2.3	3.5	2.7

4 hour sample	
Mean	12.9
Standard Error	1.5
Standard Deviation	2.6
Sample Variance	6.6
Minimum	10.2
Maximum	15.3
Count	3.0
Confidence Level(95.000%)	2.9

12/18/96 MEKO Batch Test Results

Table B-21. MEKO Degradation Under Nitrate Limiting Conditions, 12/18/95 Batch Test.

Time (hours)	MEKO Only MEKO (mg/L)	MEKO + Organics MEKO (mg/L)	Control* MEKO (mg/L)
0	8.1	6.7	10.7
0.33	6.7	4.4	
0.5			8.9
0.67	5.7	4.0	
1	5.4	3.4	9.4
1.5	3.0	2.7	10.3
2	4.0	2.4	10.7
3	3.4	2.0	10.7
4	2.7	1.7	11.2
6	2.7	1.7	9.8

*Control contained no microorganisms to investigate chemical stability under test conditions

Table B-22. NH₄-N Utilization During MEKO Batch Test Under Nitrate Limiting Condition, 12/18/95.

Time (hours)	MEKO Only NH ₄ -N (mg/L)	MEKO + Organics NH ₄ -N (mg/L)
0	6.1	6.7
0.33	8.5	6.0
0.67	8.8	6.4
1	9.3	7.0
1.5	9.4	7.6
2	9.5	8.3
3	8.8	7.8
4	8.4	6.4
6	7.7	6.5

Table B-23. TOC Utilization During MEKO Batch Test Under Nitrate Limiting Condition, 12/18/95.

Time (hours)	MEKO Only TOC (mg/L)	MEKO + Organics TOC (mg/L)	Theoretical TOC* TOC (mg/L)
0	9.5	15	6.2
0.33	8.5	10.2	5.2
0.67	6.8	8.6	4.4
1	7.3	7	4.1
1.5	6.2	6.6	2.3
2	5.8	6.2	3.1
3	6.1	7.8	2.6
4	6.5	6.8	2.1
6	6.6	6.6	2.1

* Theoretical TOC calculated based on MEKO degradation in batch flask containing only MEKO

3/27/96 MEKO Batch Test Results

Table B-24. Effect of Periodic Purging on MEKO Degradation During Anoxic Batch Tests Under Nitrate Limiting Conditions, 3/27/96 Batch Test.

Time (hours)	8 mg/l MEKO MEKO (mg/L)	25 mg/l MEKO MEKO (mg/L)	35 mg/l MEKO MEKO (mg/L)	Time (hours)	Control* MEKO (mg/L)
1	8.3	26.9	36.2	0	13.2
2	7.2	26.9	37.7	0.5	12.8
4	5.9	22.8	33.6	1	12.4
6	3.6	19.6	32.1	1.5	13.2
8	3.1	19.1	31.0	2	13.2
10	1.6	15.0	24.3	3	13.2
12	1.6	12.4	25.9	4	13.7
24	0.0	7.8	5.2	6	14.1

*Control provided ample nitrate; from 1/30/96 batch test.

Hydroxylamine not detected in any of the above samples.

Table B-25. NH₄-N Utilization During MEKO Batch Test Under Nitrate Limiting Conditions, 3/27/96.

Time (hours)	8 mg/l MEKO NH ₄ -N (mg/L)	25 mg/l MEKO NH ₄ -N (mg/L)	35 mg/l MEKO NH ₄ -N (mg/L)
0	7.2	7.5	7.4
1	7.0	7.1	7.0
2	7.8	7.9	8.2
4		7.1	8.7
6	7.5	8.8	8.1
8	7.2		8.3
10	6.8	8.4	8.4
12	7.4	8.2	8.2
24	N/A	N/A	N/A

Table B-26. TOC Utilization During MEKO Batch Test Under Nitrate Limiting Condition, 3/27/96.

Time (hours)	8 mg/l MEKO TOC (mg/L)	25 mg/l MEKO TOC (mg/L)	35 mg/l MEKO TOC (mg/L)
1	6.2	13.5	17.0
2	6.6	10.3	18.2
4	7.4	11.2	14.7
6	4.8	11.2	13.4
8	6.1	10.3	14.5
10	4.8	7.7	15.5
12	4.2	6.5	13.9
24	N/A	N/A	N/A

Table B-27. Theoretical TOC for MEKO Batch Test Under Nitrate Limiting Conditions, 3/27/96 Batch Test.

Theoretical TOC (mg/L)*		
8 mg/l MEKO TOC (mg/L)	25 mg/l MEKO TOC (mg/L)	35 mg/l MEKO TOC (mg/L)
5.4	17.7	23.8
4.8	17.7	24.8
3.9	15.0	22.1
2.4	12.9	21.1
2.0	12.6	20.4
1.0	9.9	16.0
1.0	8.2	17.0
N/A	N/A	N/A

* Theoretical TOC calculated based on MEKO degradation in each batch flask

4/22/96 MEKO Batch Test

Table B-28. Investigation of Hydroxylamine Formation in Presence of High Concentrations of MEKO.

Time (hours)	Spiked MEKO MEKO (mg/L)	Hydroxylamine (mg/L)
0	350	N/D
1	281	N/D
2	263	N/D
3	247	N/D
4	129	N/D

Table B-29. Investigation of MEKO Degradation Under Nitrate Limiting Conditions in Absence of SO₄²⁻.

Time (hours)	MEKO (mg/L)	SO ₄ ²⁻ (mg/L)
0	6.7	1.3
6	0	0.3

Table B-30. Effect of 25 mM Tungsten and Molybdenum Deficient Salt Solution on Denitrification.

Time (hours)	NO ₃ -N (mg/L)		
	Control	25 mM Tungsten	Mo-Limited MSM
0	15.7	17.2	12.3
0.5	9.6		8.9
1	6.1	5.4	5.8
1.5	2.9		3.0
2	0.7		1.2
3	0.1		0.0
4	0.1		0.0
6	0.1	0.1	0.1

Data for Anoxic SBR Monitoring

Table B-31. Anoxic SBR Data for ADO Concentration (mg/L) over Reaction Time.

Theoretical Influent		Reaction Time (hours)							
Date	Oxime Concentration (mg/L)	0*	0.33	0.5	0.67	1	2	3	4
9/15/96	10.0					6.2	6.2	6.6	6.6
9/22/96	10.0			5.4		5.4	5	4.2	3.7
9/26/96	12.5					4.3	3.9	3.1	3.1
10/3/95	17.5					12.1	8.9	7	6.4
10/7/95	17.5			7.5		6.7	6.7	6.7	6.2
10/10/95	17.5			6.7		5.4	6	6.4	6.7
10/17/95	17.5					5.4	5.1	4.1	4.1
10/24/95	17.5	8.1	8.1		5.1	7.4	7.8	7.8	8.1
10/27/95	17.5	8.6	9.3		8.2	8.6	8.6	8.6	8.6
11/3/95	17.5	9.7	8.9		8.9	10.5	10.5	10.1	9.7
Averages for 17 mg/L ADO		8.8	7.9	7.1	7.6	8.0	7.7	7.2	7.1

* Obtained only when reactor was fed manually.

Table B-32. Anoxic SBR Data for AAO (mg/L) Concentration over Reaction Time

Date	Theoretical Influent Concentration (mg/L)	Time (hours)							
		0*	0.33	0.5	0.67	1	2	3	4
9/15/95	5					3.7	3.7	3.7	3.7
9/22/95	5			2.1		1.4	0.95	0	0.95
9/26/95	6.3					0.98	0.5	0.98	0.5
10/3/95	8.8					6.8	4.9	3.9	3.9
10/7/95	8.8			2.6		2.6	2.1	1.9	1
10/10/95	8.8			3.4		2.3	3.4	3.4	3.4
10/17/95	8.8		2.5		2	1.5	2	0.5	0.5
10/24/95	8.8	3.4	2.9		2.9	2.5	2	2	2
10/27/95	8.8	3.0	3		3	3	3	3	3.5
11/3/95	8.8	2.9	2.9		2.9	2.9	2.9	2.9	2.9
Averages for 8.8 mg/L		3.1	2.8	3.0	2.7	3.1	2.9	2.5	2.5

* Obtained only when reactor was fed manually.

Table B-33. Anoxic SBR Data for MEKO Concentration (mg/L) over Reaction Cycle.

Date	Theoretical Influent Concentration (mg/L)	Reaction Time (hours)							
		0*	0.33	0.5	0.67	1	2	3	4
9/15/95	10					3.1	3.3	3.3	3.1
9/22/95	10			1.4		0.8	0.7	0.4	0
9/26/95	12					1.3	1.1	0.52	0
10/3/95	17.5					5.4	5.4	4.7	4.1
10/7/95	17.5			4	3.8	3.8	3.1	2.5	1.8
10/10/95	17.5			3.8		3.8	3.8	3.8	3.2
10/17/95	17.5		3		2.6	2.6	2.3	1.3	0.67
10/24/95	17.5	4.0	4		4	4.3	4	4	4
10/27/95	17.5	5.4	4.7	4	4.7	5.4	4.7	4.7	4.7
11/3/95	17.5	4.6	3.8		3.8	4.2	3.8	3.1	3.1
Averages for 17.5 mg/L		4.7	3.9	3.9	3.8	4.2	3.9	3.4	3.1

* Obtained only when reactor was fed manually

Table B-34. Anoxic Reactor Data for TOC Concentration (mg/L) over Reaction Cycle.

Date	0	Reaction Time (hours)							
		0.33	0.5	0.67	1	2	3	4	
9/22/95	92.3		15.2		14.9	18	19.1	13	
9/26/95	137.6		15.3		16.5	17.9	19.7	15.1	
10/3/95	122.4				16.5	16.3	16.2	15.9	
10/7/95	134.5		28.3		20.2	19.6	20	19.1	
10/10/95	111.8		17.1		17.8	20.6	18.6	17.6	
10/17/95	127.4	70.6		20.5	19.8	19.2	20.4	18.4	
10/24/95	114.1	46.7		24.7	20.2	20.8	23.94	22.91	
10/27/95	165.7	150		129.5	120.6	79.8	24	20.1	
11/3/95	110.6	24.6		20.3	17	17.9	14.6	14.4	
1/3/96	140.3	83.5	20	84.7	78.6	78.8	85.1	54.7	
1/16/96					8.1	5.9	14.2	10.1	

Data sets where activated sludge were inhibited by manual feeding were excluded in calculating averages.

Table B-35. Anoxic SBR Data for NO₃-N Concentration (mg/L) over Reaction Cycle.

Date	Reaction Time (hours)							
	0	0.33	0.5	0.67	1	2	3	4
9/22/95	45.4		4.8		0.36	0.13	0.14	0.15
9/26/95	62.3		5.3		0.2	0	0	0
10/3/95	95.1				40.1	34.5	30.1	27.9
10/7/95	67.7		28.7		19.8	8.32	0.45	0.13
10/10/95	104.6		37.6		33.6	25.2	21.6	18.2
10/17/95	63	21.6		16	11.4	0.9	0	0.4
10/24/95	95.4							
10/27/95	136.3							
11/3/95	80.9	50.3		43.1	39.4	27.4	18.6	13.3
1/3/96	37.3	1.8		0	0	0	0	0
1/16/96	87		45.8		31.7	14.6	5	0.84

Data sets where activated sludge were inhibited by manual feeding were exclude in calculating averages.

Table B-36. Anoxic SBR Data for NH₄-N Concentration (mg/L-N) over Reaction Cycle.

Date	Reaction Time (hours)							
	0	0.33	0.5	0.67	1	2	3	4
9/26/95			5.2		4.8	5.1	5.1	5.1
10/3/95					4.3	4.2	4.3	4.3
10/7/95			4.3		4.6	4.2	N/A	N/A
10/10/95			3.5		3.9	3.2	3.5	N/A
10/17/95		N/A		N/A	7.5	7.4	7.4	7.4
10/24/95	3.7	4.4		4.6	4.6	5	N/A	N/A
10/27/95	4.5	5.1		5.2	5.7	6.4	N/A	6.8
11/3/95	12.9	14.8		14.7	16	13.4	6.6	6.3
1/3/96	9	N/A		N/A	10.1	10.7	10.8	N/A
1/16/1996*								

N/A indicates that ammonia concentration not available because peak was integrated incorrectly by I.C.

* Ammonia analysis not performed on 1/16/96.

Table B-37. Anoxic SBR Data for NO₂-N (mg/L-N) over Reaction Cycle.

Date	Reaction Time (hours)							
	0	0.33	0.5	0.67	1	2	3	4
9/22/96			0		0	0	0	0
9/26/95			0		0	0	0	0
10/3/95					4.1	0	0	0
10/7/95			12.2		4.6	0	0	0
10/10/95			12.2		8.5	1.9	0	0
10/17/95		4.25		0	0	0	0	0
10/24/1995*	1.3	26.5		53	55.9	58.6	55	51.9
10/27/1995*	6.9	4.1		2.8	1.7	0	0	0
11/3/1995*	0	0		0	0	0	0	0
1/3/1996*	0.7	16.4		0	0	0	0	0
1/16/96			8.2		13.9	8.4	0	0

* Reactor fed manually to obtain accurate initial concentration; pH adjustment of feed performed on 11/3/96 to avoid nitrite accumulation

Table B-38. Establishment of Nitrate/TOC Ratio.

Date	NO ₃ -N/TOC (mg/mg)	Theoretical N/COD (mg/mg)	Nitrate Limiting?
9/22/95	0.49	0.12	Y
9/26/95	0.45	0.11	Y
10/3/95	0.78	0.19	N
10/7/95	0.50	0.12	Y
10/10/95	0.94	0.23	N
10/17/95	0.49	0.12	Y
10/24/95	0.84	0.21	N
10/27/95	0.82	0.20	N
11/3/95	0.73	0.18	N
1/3/96	0.27	0.07	Y
1/16/96	0.66	0.16	N

Table B-39. Daily Operation Parameters for Anoxic SBR.

Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)*
7/17/95	0	2620	44			183	15.99	
7/19/95	2	1030	83					
7/21/95	4	1500	150					
7/25/95	8	2440	222					1
7/27/95	10	2840	30			208	15.98	1
7/28/95	11	2680	97.5					1
8/3/95	17	2510	90					1
8/4/95	18	3560	40		7.7	211	15.63	1
8/5/95	19			32	7.6	250		1
8/6/95	20				7.6	250		1
8/7/95	21	3400	25	31	7.4	220	16.04	1
8/8/95	22	3470	55	29	7.2	187	15.97	1
8/9/95	23			31	7.8	250		1
8/10/95	24	3320	70			166	15.98	1
8/11/95	25	3290	20	33		225	16.04	1
8/12/95	26							1
8/13/95	27	2830	50			225	13.53	1
8/14/95	28			33	7.7	180		1
8/15/95	29	2580	25	35	7.4	250	13.85	1
8/16/95	30				7.3	211		1
8/17/95	31	2870	120	34	7.2	83	15.98	1
8/18/95	32			32	7.1	211		1
8/19/95	33	3530	95	32	6.9	83	20.98	1
8/20/95	34			32	6.9			1
8/21/95	35	3560	3			246	16.04	1
8/22/95	36			33		246		1
8/23/95	37	3360	27	32		217	16.06	1
8/24/95	38							1
8/25/95	39	3230	37	33		204	16.01	1
8/26/95	40			30		204		1
8/27/95	41	3960	28	31		204	17.22	1
8/28/95	42			33		222		5

Table Continued

Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)
8/29/95	43	3200	28	33		250	14.04	5
8/30/95	44			33		215		5
8/31/95	45	3590	28	35		219	15.99	5
9/1/95	46			32	7	219		5
9/2/95	47	3230	30	30	6.9	213	15.99	5
9/3/95	48			32	7	213		5
9/4/95	49	3320		32	6.9	213		5
9/5/95	50			33	7	213		5
9/6/95	51	3470	40	32	6.75	204	15.99	5
9/7/95	52			32	6.7	204		5
9/8/95	53	3500	37	34	6.9	208	15.98	7
9/9/95	54			32	7.1	208		7
9/10/95	55	3660	20	33	6.7	228	16.01	7
9/11/95	56			32	6.7	228		7
9/12/95	57	3660	44	33	6.7	202	15.99	7
9/13/95	58							7
9/14/95	59	3550	68	34	6.9	173	16.02	10
9/15/95	60			33		173		10
9/16/95	61	3730	43	31	7	204	15.99	10
9/17/95	62			32	6.7	204		10
9/18/95	63	3630	47	31		198	16.01	10
9/19/95	64			32	7.8	198		10
9/20/95	65	4010	13.3	32.5	7.3	237	15.98	10
9/21/95	66			32	7.2	237		10
9/22/95	67	3900	47	30	6.9	208	15.61	10
9/23/95	68							10
9/24/95	69	3420	27	30.5	6.9	218	16.03	12.5
9/25/95	70			35	6.9	218		12.5
9/26/95	71	3800	40	33.5	6.8	208	15.99	12.5
9/27/95	72				6.8	208		12.5
9/28/95	73	3960	44	31.5	7.1	206	15.97	12.5

Table Continued								
Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)
9/29/95	74			33	7.3			17.5
9/30/95	75	3530	47	33.5	7.3			17.5
10/1/95	76							17.5
10/2/95	77	3750	30	35	7	218	16.00	17.5
10/3/95	78					218		17.5
10/4/95	79	3880	33	33		216	16.00	17.5
10/5/95	80			34	7.2	216		17.5
10/6/95	81	4500	60	34	7.1			17.5
10/7/95	82			32	6.95	197		17.5
10/8/95	83	4040	17	30	6.8	233	16.01	17.5
10/9/95	84			31	6.85	233		17.5
10/10/95	85	3010	20	31	6.9	398	9.42	17.5
10/11/95	86			33	6.9	68		17.5
10/12/95	87	3380	28	32	6.9	217	15.99	17.5
10/13/95	88			32	6.5	217		17.5
10/14/95	89	3050	12	30	7.05	234	16.02	17.5
10/15/95	90			25	7.1	234		17.5
10/16/95	91	4470	30	34	6.9	223	16.01	17.5
10/17/95	92			37	6.95	223		17.5
10/18/95	93	4230	17	32	6.8	234	16.00	17.5
10/19/95	94			33	6.7	234		17.5
10/20/95	95	3820	33	34	6.7	215	16.03	17.5
10/21/95	96			30	7	215		17.5
10/22/95	97	4230	16	34	6.85	235	15.99	17.5
10/23/95	98			35	7	235		17.5
10/24/95	99	4730	40	34	6.7	245	14.35	17.5
10/25/95	100			32	6.6	187		17.5
10/26/95	101	3300	20	33	6.6	226	15.98	17.5
10/27/95	102			32	6.8	226		17.5
10/28/95	103	4170	52	31	6.7	200	16.01	17.5
10/29/95	104			31	7.1	200		17.5

Table Continued								
Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)
10/30/95	105	4040	32	32	7.5	218	16.02	17.5
10/31/95	106			33	7.5	218		17.5
11/1/95	107	4940	8	34	6.75	242	16.10	17.5
11/2/95	108			34	6.9	242		17.5
11/3/95	109	4190	24	30	7	227	16.01	17.5
11/4/95	110			28	6.7	227		17.5
11/5/95	111	4120	36	32	6.75	215	16.00	17.5
11/6/95	112			32	6.65	215		17.5
11/7/95	113	3600	27		6.65	218	16.13	17.5
11/8/95	114			32	6.7	218		17.5
11/9/95	115	3720	18			232	15.91	17.5
11/10/95	116							17.5
11/11/95	117	3790	38	34	7	210	15.99	17.5
11/12/95	118			31	6.9	210		17.5
11/13/95	119	4190	24	32	6.9	210	17.17	17.5
11/14/95	120			33	7	210		17.5
11/15/95	121	4120	36	32	6.9	223	15.51	17.5
11/16/95	122			30	6.7	223		17.5
11/17/95	123	5010	18	32	6.9	236	15.98	17.5
11/18/95	124			33	7	236		17.5
11/19/95	125			33	6.8	236		17.5
11/20/95	126	4560	46	31	7	210	15.98	17.5
11/21/95	127			33.5	6.9	210		17.5
11/22/95	128			32.5	6.8	210		17.5
11/23/95	129			32.5	6.8	210		17.5
11/24/95	130					210		17.5
11/25/95	131			32	6.8	210		17.5
11/26/95	132	4100	18	31	6.9	232	16.03	17.5
11/27/95	133			32	6.9	232		17.5
11/28/95	134	3330	28	34	6.9	232	15.06	17.5
11/29/95	135			33	6.8	216		17.5

Table Continued

Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)
11/30/95	136	5390	7	32	6.8	243	16.12	17.5
12/1/95	137							17.5
12/2/95	138	5290	24	32	6.9	232	15.99	17.5
12/3/95	139			33	6.9			17.5
12/4/95	140	4740	8	31	6.9	243	16.02	17.5
12/5/95	141			32	6.9	243		17.5
12/6/95	142			31	7	243		17.5
12/7/95	143	5090	3	32.5		248	15.98	17.5
12/8/95	144			32	6.9	248		17.5
12/9/95	145	4140	11	30	6.8	239	16.02	17.5
12/10/95	146			31	6.9	239		17.5
12/11/95	147	3870	70	32	7	177	16.04	17.5
12/12/95	148			32	7	177		17.5
12/13/95	149	5390	70	33	7.2	198	16.00	17.5
12/14/95	150			33		198		17.5
12/15/95	151	5300	75	32	7.8	198	15.71	17.5
12/16/95	152			31.5	7.7	193		17.5
12/17/95	153			32.5	7.3	193		17.5
12/18/95	154	5300	84		8	210	14.63	17.5
12/19/95	155				7.5	210		17.5
12/20/95	156	4880	58	30.5	7.5	203	15.97	17.5
12/21/95	157			32	7.5	406		17.5
12/22/95	158			32	7.4	0		17.5
12/23/95	159			32	7.6	203		17.5
12/24/95	160			32	7.4	203		17.5
12/25/95	161			32	7.6	203		17.5
12/26/95	162			32	7.5	203		17.5
12/27/95	163			32	7.6	203		17.5
12/28/95	164			31	7.6	203		17.5
12/29/95	165					0		17.5
12/30/95	166			31.5	7.6	406		17.5

Table Continued

Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)
12/31/95	167	5230	110	31.5	7.7	203	13.93	17.5
1/1/96	168			31.5	7.4	177		17.5
1/2/96	169	4940	110	33	6.95	161	16.00	17.5
1/3/96	170			33	7.1	161		17.5
1/4/96	171				6.9	153		17.5
1/5/96	172			33	6.7	153		17.5
1/6/96	173	4270	83	32.5	6.75	172	16.02	17.5
1/7/96	174			32	6.8	172		17.5
1/8/96	175					203		17.5
1/9/96	176			31	6.7	203		17.5
1/10/96	177	3730	13	32	7	236	16.00	17.5
1/11/96	178			33	6.85	236		17.5
1/12/96	179			32	6.8	236		17.5
1/13/96	180			31	6.8	215		17.5
1/14/96	181			32	6.8	215		17.5
1/15/96	182	3955	33	34	6.9	217	15.98	17.5
1/16/96	183			33	6.9	217		17.5
1/17/96	184			33		300		17.5
1/18/96	185			33	6.8	132		17.5
1/19/96	186				6.8	230		17.5
1/20/96	187			31	6.75	230		17.5
1/21/96	188			31	6.7	220		17.5
1/22/96	189			31	6.7	220		17.5
1/23/96	190	4460	0			230	17.39	17.5
1/24/96	191					250		17.5
1/25/96	192	6600	30	32.5	6.8	232	15.99	17.5
1/26/96	193			33	6.8	232		17.5
1/27/96	194	6370	15	31.5	6.8	241	15.97	17.5
1/28/96	195					241		17.5
1/29/96	196			35	7	217		17.5
1/30/96	197	4585	8	33	6.9	400		17.5

Table Continued

Date	Time Elapsed (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT	Oxime (mg/L)
1/31/96	198			33	6.75	117		17.5
2/1/96	199				6.9	242		17.5
2/2/96	200	4270	48	33	6.2	205		17.5

* Oximes included AAO, ADO, and MEKO. ADO was eliminated from the reactor in January, 1996. AAO concentration was approximately one half the concentration of ADO and MEKO until January, 1996. AAO concentrations were then equivalent to MEKO.

Steady State Averages (After 8/19/95)	4129	36	32	7	215	16
Minimum	3010	0	25	6.2		
Maximum	6600	110	37	8		

Table B-40. MLVSS for Anoxic Reactor.

Date	Elapsed Time (days)	MLVSS (mg/L)	MLSS (mg/L)	% VSS
10/26/95	101	2820	3300	85
11/9/95	115	3240	3720	87
11/20/95	126	3890	4560	85
12/15/95	151	4390	5300	83
1/15/96	182	3470	3955	88
1/30/96	197	4005	4585	87
Averages		3636	4237	86

Statistical Analysis for Anoxic SBR

Table B-41. ADO		0.0			
		0.33 hours		0.5 hours	0.67 hours
Mean Standard Error Standard Deviation Minimum Maximum Count		1 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		8.8 Mean	7.9 Mean	7.1 Mean	7.6
		0.5 Standard Error	1.0 Standard Error	0.4 Standard Error	0.8
		0.8 Standard Deviation	1.9 Standard Deviation	0.6 Standard Deviation	1.7
		8.1 Minimum	5.1 Minimum	6.7 Minimum	5.1
		9.7 Maximum	9.3 Maximum	7.5 Maximum	8.9
		3.0 Count	4.0 Count	2.0 Count	4.0

Mean Standard Error Standard Deviation Minimum Maximum Count		1 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		8.0 Mean	7.7 Mean	7.2 Mean	7.1
		1.0 Standard Error	0.7 Standard Error	0.7 Standard Error	0.7
		2.5 Standard Deviation	1.9 Standard Deviation	1.9 Standard Deviation	1.8
		5.4 Minimum	5.1 Minimum	4.1 Minimum	4.1
		12.1 Maximum	10.5 Maximum	10.1 Maximum	9.7
		7.0 Count	7.0 Count	7.0 Count	7.0

Table B-42. AAO		0.0			
		0.33 hours		0.5 hours	0.67 hours
Mean Standard Error Standard Deviation Sample Variance Maximum Count		1 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		3.1 Mean	2.8 Mean	3.0 Mean	2.7
		0.2 Standard Error	0.1 Standard Error	0.4 Standard Error	0.2
		0.3 Standard Deviation	0.2 Standard Deviation	0.6 Standard Deviation	0.5
		0.1 Sample Variance	0.0 Sample Variance	0.3 Sample Variance	0.2
		3.4 Maximum	3.0 Maximum	3.4 Maximum	3.0
		3.0 Count	4.0 Count	2.0 Count	4.0

Mean Standard Error Standard Deviation Minimum Maximum Count		1 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		2 hours		3 hours	4 hours
		0.33 hours		0.5 hours	0.67 hours
		3.1 Mean	2.9 Mean	2.5 Mean	2.5
		0.6 Standard Error	0.4 Standard Error	0.4 Standard Error	0.5
		1.7 Standard Deviation	1.0 Standard Deviation	1.1 Standard Deviation	1.3
		1.5 Minimum	2.0 Minimum	0.5 Minimum	0.5
		6.8 Maximum	4.9 Maximum	3.9 Maximum	3.9
		7.0 Count	7.0 Count	7.0 Count	7.0

Table B-43. MEKO

	0.0		0.33 hours		0.5 hours		0.67 hours	
	1 hours	2 hours	3 hours	4 hours				
Mean	4.7 Mean	3.9 Mean	3.9 Mean	3.2 Mean			3.8	
Standard Error	0.4 Standard Error	0.3 Standard Error	0.3 Standard Error	0.5 Standard Error			0.3	
Standard Deviation	0.7 Standard Deviation	0.7 Standard Deviation	0.7 Standard Deviation	1.2 Standard Deviation			0.8	
Minimum	4.0 Minimum	3.0 Minimum	3.0 Minimum	1.3 Minimum			2.6	
Maximum	5.4 Maximum	4.7 Maximum	4.7 Maximum	4.7 Maximum			4.7	
Count	3.0 Count	4.0 Count	4.0 Count	6.0 Count			5.0	
Mean	4.0 Mean	3.6 Mean	3.6 Mean	3.2 Mean			2.9	
Standard Error	0.4 Standard Error	0.3 Standard Error	0.3 Standard Error	0.5 Standard Error			0.6	
Standard Deviation	0.9 Standard Deviation	0.8 Standard Deviation	0.8 Standard Deviation	1.2 Standard Deviation			1.5	
Minimum	2.6 Minimum	2.3 Minimum	2.3 Minimum	1.3 Minimum			0.7	
Maximum	5.4 Maximum	4.7 Maximum	4.7 Maximum	4.7 Maximum			4.7	
Count	6.0 Count	6.0 Count	6.0 Count	6.0 Count			6.0	

Table B-44. TOC

	0.0		0.33 hours		0.5 hours		0.67 hours	
	1 hours	2 hours	3 hours	4 hours				
Mean	132.7 Mean	47.3 Mean	47.3 Mean	19.2 Mean			20.7	
Standard Error	12.8 Standard Error	13.3 Standard Error	13.3 Standard Error	2.4 Standard Error			2.2	
Standard Deviation	25.7 Standard Deviation	23.0 Standard Deviation	23.0 Standard Deviation	5.5 Standard Deviation			3.9	
Minimum	110.6 Minimum	24.6 Minimum	24.6 Minimum	15.2 Minimum			17.0	
Maximum	165.7 Maximum	70.6 Maximum	70.6 Maximum	28.3 Maximum			24.7	
Count	4.0 Count	3.0 Count	3.0 Count	5.0 Count			3.0	
Mean	18.0 Mean	18.4 Mean	18.4 Mean	19.0 Mean			17.4	
Standard Error	0.7 Standard Error	0.8 Standard Error	0.8 Standard Error	1.0 Standard Error			1.2	
Standard Deviation	2.0 Standard Deviation	2.1 Standard Deviation	2.1 Standard Deviation	2.9 Standard Deviation			3.2	
Sample Variance	3.9 Sample Variance	4.6 Sample Variance	4.6 Sample Variance	8.1 Sample Variance			10.2	
Maximum	20.2 Maximum	20.8 Maximum	20.8 Maximum	23.9 Maximum			22.9	
Count	8.0 Count	8.0 Count	8.0 Count	8.0 Count			7.0	

Table B-45. NO3-N

0.0		0.33 hours			0.5 hours			0.67 hours					
		1 hours			2 hours			3 hours			4 hours		
Mean		91.9	Mean		36.0	Mean		33.2	Mean		29.6		
Standard Error		9.4	Standard Error		14.4	Standard Error		4.5	Standard Error		13.6		
Standard Deviation		24.8	Standard Deviation		20.3	Standard Deviation		6.3	Standard Deviation		19.2		
Minimum		63.0	Minimum		21.6	Minimum		28.7	Minimum		16.0		
Maximum		136.3	Maximum		50.3	Maximum		37.6	Maximum		43.1		
Count		7.0	Count		2.0	Count		2.0	Count		2.0		
Mean		28.9	Mean		19.3	Mean		14.2	Mean		12.0		
Standard Error		5.7	Standard Error		6.3	Standard Error		6.0	Standard Error		5.3		
Standard Deviation		12.7	Standard Deviation		14.1	Standard Deviation		13.4	Standard Deviation		11.9		
Minimum		11.4	Minimum		0.9	Minimum		0.0	Minimum		0.1		
Maximum		40.1	Maximum		34.5	Maximum		30.1	Maximum		27.9		
Count		5.0	Count		5.0	Count		5.0	Count		5.0		

Table B-46. Operational Parameters for Anoxic/Aerobic SBR.

Date	Elapsed Time (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT (days)
2/5/96	0	5210	67.5	33	7	237	16
2/6/96	1					237	
2/7/96	2	4570	75		7	234	16
2/8/96	3			31	7.05	234	
2/9/96	4	4111	65	32	7.05	234	16
2/10/96	5			31	6.9	234	
2/11/96	6			32	7	234	
2/12/96	7	3455	30	32	7	234	
2/13/96	8			32.5	7.05		
2/14/96	9	3000	18	35	7.1	280	
2/15/96	10			33	7.1	244	
2/16/96	11			32	7.1	244	
2/17/96	12	2780	10	33	7.1	246	16
2/18/96	13			31	7	246	
2/19/96	14	1780	0	33	7.7	250	16
2/20/96	15				7.4	250	
2/21/96	16	3020	11	33	7.7	250	16
2/22/96	17			34	7.7	246	
2/23/96	18	2690	30	30	7.3	239	16
2/24/96	19			26	7.6		
2/25/96	20	2920	72	27	7.3	225	16
2/26/96	21			32	7.5	225	
2/27/96	22	2930	44	32	7.4	235	16
2/28/96	23			30	7.6	235	
2/29/96	24	3220	25	31	7.2	242	16
3/1/96	25			32	7.3	242	
3/2/96	26	2810	16	30	7.4	244	16
3/3/96	27			32	7.1	244	
3/4/96	28	2090	17	32	7.5	242	16
3/5/96	29			32	7.1	242	
3/6/96	30	2450	33	33	7.3	236	16
3/7/96	31			32	7.6	236	

Table Continued							
Date	Elapsed Time (days)	MLSS (mg/L)	ESS (mg/L)	Temp. (C)	pH	Wastage	SRT (days)
3/8/96	32	3070	17.5		7.4	244	16
3/9/96	33			31	7.3	244	
3/10/96	34	3040	15	32	7.4	244	16
3/11/96	35			32.5	7.3	244	
3/12/96	36	2680	15		7.3	240	16
3/13/96	37			32	7.3	244	
3/14/96	38	3355	25	33	7.7	243	16
3/15/96	39			32	7.3	243	
3/16/96	40					243	
3/17/96	41	3840	24	33	7.5	244	16
3/18/96	42			33	7.1	0	
3/19/96	43	3265	11		7.1	400	
3/20/96	44			32	7.6	247	
3/21/96	45	3645	13	32	7.5	247	16
3/22/96	46			31	7.4	244	
3/23/96	47			31	7.2	247	
3/24/96	48			32	7	247	
3/25/96	49			33	6.8	247	
3/26/96	50				7.2	0	
3/27/96	51			33	7.1	500	
3/28/96	52				7.1	249	
3/29/96	53					249	
3/30/96	54			29	6.95	249	
3/31/96	55			32	6.6	249	
Averages		2917	22	32	7		16

Table B-47. Daily Effluent Analysis for Anoxic/Aerobic SBR.

Date	Elapsed Time (hours)	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	AAO (mg/l)	MEKO (mg/L)
2/5/96	0	13.7	3.3		0.0	0.0
2/6/96	1	12.6	4.3		0.0	0.0
2/7/96	2	9.4	6.3		0.0	0.0
2/8/96	3	9.0	8.6		0.0	0.0
2/9/96	4	6.5	10.4		0.0	0.0
2/10/96	5	7.0			0.0	0.0
2/11/96	6		11.0		0.0	0.0
2/12/96	7	3.7			0.0	0.0
2/13/96	8	2.9	10.3		1.9	0.6
2/14/96	9	1.9	20.7		2.5	0.4
2/15/96	10	1.4	20.9		1.9	0.4
	11				0.0	0.0
	12				0.0	0.0
	13				0.0	0.0
2/19/96	14	1.0	2.7		0.0	0.0
2/20/96	15	0.1	0.7		4.4	0.8
2/21/96	16	0.0	0.0		3.1	0.4
2/22/96	17		0.9		4.4	1.3
2/23/96	18	1.0	6.5		4.4	1.7
2/24/96	19	0.7	7.9		3.1	0.8
2/25/96	20	9.0	0.0		2.5	1.3
2/26/96	21	6.6	0.0		0.0	0.8
2/27/96	22	8.6	0.0		0.0	0.4
2/28/96	23	7.3	0.0		0.0	0.0
2/29/96	24	6.4	0.0	N/D	0.0	0.0
3/1/96	25	6.0	0.0	N/D	0.0	0.0
3/2/96	26	7.9	0.0		0.0	0.0
3/3/96	27	7.0	0.0	N/D	0.0	0.0
3/4/96	28	7.6	0.0		0.0	0.0
3/5/96	29	5.9	0.0	N/D	0.0	0.0
3/6/96	30	7.2	0.0		0.0	0.0
3/7/96	31	8.5	0.0	N/D	0.0	0.0

Table Continued						
Date	Elapsed Time (hours)	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	AAO (mg/l)	MEKO (mg/L)
3/8/96	32	9.1	0.0	N/D	0.0	0.0
3/9/96	33	11.4	0.0	N/D	0.0	0.0
3/10/96	34	10.7	0.0		0.0	0.0
3/11/96	35	8.3	0.0	N/D	0.0	0.0
3/12/96*	36	6.3	0.0	N/D	0.0	0.8
3/13/96	37	6.3	0.0	N/D	0.0	0.0
3/14/96	38	11.7	0.0		0.0	0.0
3/15/96	39	6.7	0.0	N/D	0.0	0.0
3/16/96	40	4.8	0.0	N/D	0.0	0.0
	41				0.0	0.0
3/18/96	42	4.8	0.0	N/D	0.0	0.0
3/19/96	43	3.4	0.0	N/D	0.0	0.0
3/20/96	44	3.4	0.0	N/D	0.0	0.0
3/21/96	45	2.6	0.0	N/D	0.0	0.0
3/22/96	46	2.4	0.0	N/D	0.0	0.0
3/23/96	47	1.1	0.0	N/D	0.0	0.0
3/24/96	48	0.8	0.0	N/D	0.0	0.0
3/25/96	49	2.1	0.4	N/D	0.0	0.0
3/26/96	50	2.4	2.7	N/D	0.0	0.0
3/27/96	51	1.6	5.7		0.0	0.0
3/28/96	52	0.0	0.0	29.9	0.0	0.0
	53				0.0	0.0
	54				0.0	0.0
	55					
	56					
4/2/96	57	1.6	27.5		0.0	0.0
4/3/96	58	1.5	4.3		3.1	0.0
	59					
4/5/96	60	4.6	0.0			
	61					
4/7/96	62	7.8	0.0			

Table Continued						
Date	Elapsed Time (hours)	NO3-N (mg/L)	NO2-N (mg/L)	NH4-N (mg/L)	AAO (mg/l)	MEKO (mg/L)
4/8/96	63	6.5	0.0			
4/10/96	64					
	65	6.2	0.0			
	66					
	67					
4/13/96	68	4.5	0.0	N/D		
4/14/96	69	6.2	0.0			
4/15/96	70	4.4	0.0	N/D		
	71					
4/17/96	72	2.7	0.0	N/D		

* Anoxic/anaerobic reaction cycle increased from 10 to 12 hours.

Table B-48. Effluent Analysis for the Batch Flasks Operated During the Aerobic Side Study.

Date	Flask A: Anoxic MLSS		Flask B: Aerobic MLSS		Flask C: Aerobic MLSS, Control	
	NO3-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)
2/15/96	10.5	32.9				
2/16/96	2.7	16.5				
2/17/96	3.3	14.7				
2/18/96	3.3	13.6				
2/19/96	3.1	12.6				
2/20/96	2.9	4.5				
2/21/96	5.3	11.8				
2/22/96	6.2		24.9	0.0	28.4	0.0
2/23/96	8.4	5.8	26.8	0.0	24.6	0.0
2/24/96	10.7	1.5	31.1	0.0	27.2	0.0
2/25/96	7.8	0.0	33.9	0.0	30.4	0.0
2/26/96	7.2	0.0	37.1	0.0	30.7	0.0
2/27/96	5.6	0.0	33.7	0.0	24.9	0.0

Table B-49. Student's t-Test Used to Determine if Anoxic/Aerobic SBR Effluent was Inhibitory to Nitrification.

	<i>Flask Fed Anoxic/Aerobic SBR Effluent</i>	<i>Control</i>
Mean	31.3	27.7
Variance	21.5	6.8
Observations	6.0	6.0
Hypothesized Mean Difference	0.0	
df	8.0	
t Stat	1.6	
P(T<=t) one-tail	0.1	
t Critical one-tail	1.9	

T-test assumes unequal variances. Null hypothesis is as follows: Nitrate generated in experimental flask is greater than or equal to nitrate generated in the control flask. Because t statistic is less than t critical, the null hypothesis is accepted.

Tables B-50 through B-54. Oxime Degradation Over Anoxic/Anaerobic Cycle in Sequential SBR

Table B-50. 2/6.

Time (hours)	MEKO (mg/L)	AAO (mg/L)
0.33	2.8	5.9
1	2.3	4.4
2	1.4	2.9
3	0.9	2.2
4	0.5	2.2

Table B-51. 2/2.

Time (hours)	MEKO (mg/L)	AAO (mg/L)
0.33	3.7	8.8
0.67	3.3	7.4
1	3.3	7.4
2	3.0	7.4
3	3.0	7.4
4	2.8	6.6
5	2.3	6.6
7	1.4	5.1
10	0.7	4.4

Table B-52. 3/7.

Time (hours)	MEKO (mg/L)	AAO (mg/L)
0.33	5.6	8.8
0.67	6.1	8.8
1	6.1	8.8
2	5.8	8.5
3	4.7	8.1
4	4.7	8.1
5	4.7	8.1
7	3.7	8.1
10	2.3	6.6
11	1.9	5.9

Table B-53. 3/12/96.

Time (hours)	MEKO (mg/L)	AAO (mg/L)
0.33	5.7	10.8
0.67	5.7	10.8
1	5.7	10.8
2	4.6	10.8
3	4.1	10.0
4	3.6	9.2
5	3.1	9.2
7	1.5	7.7
10	1.0	8.4
12	0.0	6.9

Table B-54. 4/2/96

Time (hours)	MEKO (mg/L)	AAO (mg/L)
0.33	8.9	18.4
0.67	9.5	18.4
1.5	7.0	17.1
2	6.3	17.1
3	5.1	14.5
4	3.8	14.5
5	2.5	13.2
7	1.3	10.5
9	0.6	9.2

VITA

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