IN-SITU BIODEGRADATION STUDY USING ^{36}Cl LABELED BLEACHING FILTRATES

A thesis submitted by

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SUMMARY

A procedure has been developed for mapping the biodegradation of AOX in the water column of secondary treatment systems. The field work for this research was done at two southeastern U.S. mills, one operating an aerated stabilization basin (ASB) and the other operating an activated sludge system. It was found that only 30% of the total AOX reduction achieved in the water column of the ASB was attributable to biodegradation. While, 60% of the total AOX reduction achieved in the water column of the activated sludge system was attributable to biodegradation. In both systems, however, the initial AOX was reduced by 30-40% by mechanisms other than biodegradation. Available evidence points to sorption/settling and chemical degradation as the dominant modes for the unaccounted AOX removal. This opens up the possibility of enhancing sorption/settling through chemical means and chemical degradation by physical means. Finally, a new methodology for measuring BOD biodegradation in secondary treatment systems was developed. This information can then be used to optimize treatment ponds for BOD removal.

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LIST OF ABBREVIATIONS

ASB	Aerated Stabilization Basin
AOX	Adsorbable Organic Halides
BOD	Biochemical Oxygen Demand
Ci	Curie
COD	Chemical Oxygen Demand
BKME	Bleached Kraft Mill Effluent
D	Dalton
DO	Dissolved Oxygen
DPM (dpm)	Disintegrations Per Minute
HMW	High Molecular Weight (>1000 Daltons)
HRT	Hydraulic Retention Time
K_{ow}	Octanol:Water Partition Coefficient
LMW	Low Molecular Weight (<1000 Daltons)
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
MP	Metal Post Near ASB Center Used for Placement of Nonaerated Probes
MW	Molecular Weight
PCTE	Polycarbonate Track Etched
PPM (ppm)	Part Per Million
SOC	Soluble Organic Carbon
TAC	Total Active Carbon
TOC	Total Organic Carbon
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids

BACKGROUND

ENVIRONMENTAL CONSIDERATIONS

The pulp bleaching process, the removal of residual lignin by oxidation, results in the formation of large quantities of various lignin decomposition products. When using chlorine containing bleaching agents, chlorinated organic compounds are formed. Kraft bleaching effluents are of environmental concern due, not only to their high organic load, but also to their toxicity, mutagenicity, and potential for bioaccumulation.[1] For softwood pulps, organic loadings are on the order of 80 kg/ton chemical oxygen demand (COD) and chlorinated organic loadings are on the order of 1 kg/ton adsorbable organic halides (AOX) for 100% ClO_2 substitution, and 4 kg/ton AOX for 100% Cl_2.[2,3,4] Post treatment AOX loads are in the range of 0.2-0.5 kg/ton for ClO_2 bleached softwood pulp and 2.5 kg/ton for Cl_2 bleached softwood pulp.[1,2,5,6] This represents a large amount of chlorinated organic material (400-1000 kg/day for a 2000 ton/day mill) being discharged into receiving waters on a continual basis.

Recently, the US Environmental Protection Agency proposed new regulations (Cluster Rules) for the effluent from pulp and paper mills which limit levels of AOX discharge to 0.46 kg AOX/ton.[7] In order to meet these new guidelines, and reduce the overall environmental impact of mill effluents, considerable importance has been placed on processes which reduce AOX loads in mill effluents. Two of these processes, secondary treatment and chlorine dioxide substitution, will be addressed in this review. Although total AOX is the primary pollution control parameter utilized, other characteristics of AOX may be of more importance in assessing the environmental impact of bleach plant effluents. Some of the characteristics of particular environmental interest include: toxicity, mutagenicity, bioaccumulation, and recalcitrance.[1,8,9,10,22] Measurement of these characteristics for AOX is necessary to more accurately establish the relationship between AOX discharged and environmental impact.

As many chlorinated phenolic compounds are known to be toxic, and have an affinity for fatty tissue,[11] much concern exists over the discharge of chlorinated organic material from pulp and paper mills. A complete review of the literature available on the toxicity of bleached mill effluent is beyond the scope of this thesis, therefore, only a few basic concepts will be introduced. Bleach plant effluents have been shown to exhibit acute toxicity,[1,12,13] sublethal effects,[5,14,15,16] and mutagenicity.[17,18] The toxicity of the overall effluent is generally attributed to specific compounds such as chlorolignins, chlorophenolics, resin compounds, and fatty acids.[1,19,20,21] AOX, the parameter by which chlorinated organic discharges will be monitored, does not differentiate between toxic and nontoxic compounds and has not been shown to have a direct correlation with toxicity.[5,22,23,24] Chlorination stage effluent toxicity reaches a maximum when the chlorinated phenolic content is at a maximum, and is also higher at low final chlorination pH's where more chlorinated phenolics are produced.[25] This is attributable to a shift in the equilibrium between chlorine and hypochlorous acid to chlorine in the low pH range.

The degree of substitution of chlorine on the AOX compounds greatly impacts their potential for biodegradability and toxicity. Typically, with organic compounds, as the degree of chlorination increases, so do toxicity and bioaccumulation.[26,27,28] Increasing chlorine substitution on organic compounds reduces reactivity and water solubility, and increases acidity. The reduction in activity, especially toward oxidation, has been shown to reduce the biodegradability of some compounds.[8,28] Many chlorinated organic compounds are relatively resistant to decomposition in nature and are, therefore, of significant environmental concern.[29] Compounds with molecular weight above 1000 D also tend to be recalcitrant due to their size. Compounds having minimal internal cross sections of 1 nm or larger are believed to be unable to cross cellular membranes and, therefore, have negligent bioconcentration factors.[30] Due ti their large size, the high molecular weight component of AOX was previously believed to be recalcitrant and was, therefore, considered harmless to the aquatic environment.[4,23,24,26] In light of recent evidence that high molecular mass material is unstable under conditions similar to those of receiving waters, this material cannot simply be dismissed as biologically inactive.[14,29,31]

It has been well documented that persistent hydrophobic organic chemicals can biomagnify, causing concentrations to increase with every step in the ecological food chain.[32,33] The higher the K_{OW} of the contaminant, the longer the exposure time required to reach equilibrium between surrounding water and fish lipid (for a log K_{OW} of 3.3, it would take 5 days; for a log K_{OW} of 6, it would take 7 years).[33] This lag time in equilibrium has significant implications for environmental monitoring. Measurement of contaminant levels in organisms may not reflect the ultimate bioconcentration potential based on current pollutant levels. Additionally, ecological effects from current pollutant discharges may not be fully realized for years to come. Bioassay studies with fish have demonstrated that some of the low molecular weight chlorinated compounds, especially the phenols and guaiacols, tend to bioaccumulate.[34,35,36]

As chlorine dioxide tends to form less highly chlorinated AOX compounds during bleaching,[28] chlorine dioxide substitution would be expected to play a role in reducing effluent toxicity and bioconcentration potential, and increasing biodegradability, but the exact effects remain unclear. Tests conducted on the effect of chlorine dioxide substitution show mixed results for acute toxicity, as conditions of the studies vary, indicating the importance of careful documentation of procedures, effluent preparation, and toxicity test parameters.[37,38,39] Research at the Pulp and Paper Research Institute of Canada, along with other sources,[37,40] indicates that increasing chlorine dioxide substitution significantly reduces the acute toxicity of combined chlorination and extraction stage effluents.[28] Other research has indicated little effect of ClO_2 substitution on toxicity.[38,41] The decrease in the extent of substitution on the phenolic and other compounds, is a reasonable explanation for the reduction in toxicity found with chlorine dioxide substitution.

Most North American pulp and paper mills employ some type of secondary treatment system for the reduction of BOD and AOX emissions. However, biological treatment, anaerobic, aerobic or aerobic and anaerobic combined, remove only some of the methanogenic toxicity of bleached kraft mill effluent (BKME). The continued methanogenic toxicity of the BKME after aerobic treatment, even after achieving 50% AOX reduction, could be attributed to the methylation of chlorinated compounds or to the breakdown of high molecular weight compounds, resulting in more toxic compounds.[1] Studies have shown that 5-day aerated lagoon treatments do not completely eliminate toxicity up to 20% of the time.[42] Although not completely effective in eliminating toxicity, microbiological treatment is the best available technique for reducing the toxicity of bleached kraft mill effluent.[43]

PULP BLEACHING

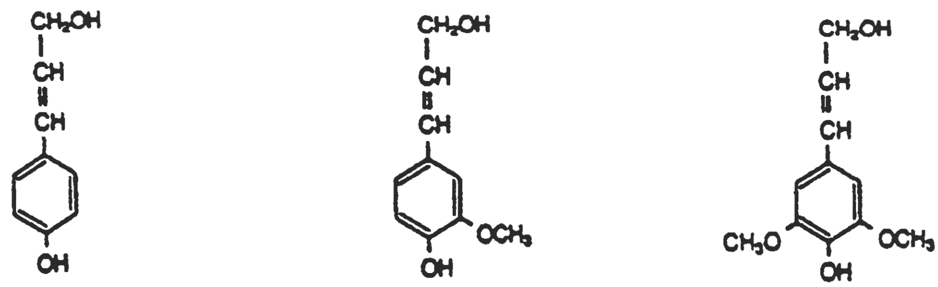
Introduction to Bleaching

The bleaching of chemical pulp is a brightening process, including removal of residual lignin and destruction of chromophores. One of the keys to effective bleaching is removal of the maximum amount of remaining lignin, while avoiding cellulose degradation. In the past, chlorine has been used as the primary bleaching agent for the paper industry because it is both an effective and selective chemical for removing lignin. However, chlorine forms maximum amounts of chlorinated organics,[44] and, because of this, alternatives are being sought.[45,46]

AOX discharge can be reduced by making any of a variety of changes in the bleaching process including oxygen predelignification, extended delignification, improved washing, chlorine dioxide substitution, low chlorine multiple, ozone bleaching, hydrogen peroxide bleaching, oxidative extraction, pH control, improved mixing, and split chlorine addition.[47,48,49] By employing totally chlorine free bleaching, mills can eliminate the concern over AOX discharge limits, but switching to this process is capital intensive and is not currently in wide practice in the US.[26,50] As an alternative, the majority of US mills are employing substitution of chlorine dioxide for chlorine, if not practicing elemental chlorine free bleaching, as a means to lower AOX emissions. In order to investigate the impact of chlorine dioxide substitution on the biodegradability of bleaching effluents, some review of the types of compounds formed by the reaction of chlorine and chlorine dioxide with residual lignin is necessary.

Lignin, essentially an aromatic polymer, is formed in wood by an enzyme-initiated dehydrogenative polymerization of a mixture of three different 4-hydroxy-arylpropenyl alcohols (Fig. 1).[4]

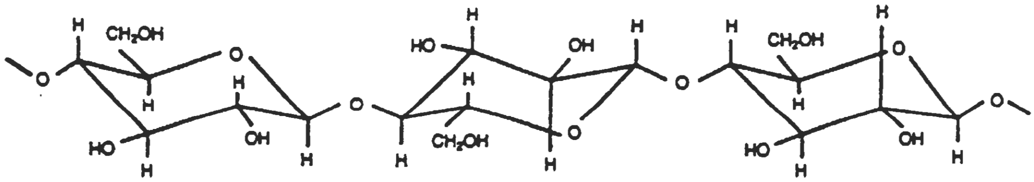
Figure 1. Lignin Precursors.[4]



p-coumaryl alcohol coniferyl alcohol sinapyl alcohol

It differs from cellulose (Fig. 2) in that cellulose is a linear series of glucose molecules containing only single carbon-carbon bonds. Lignin, on the other hand, is a branched structure consisting of phenolic type compounds. It is the aromatic nature of the lignin that provides for the ability to find reagents that readily react with lignin while remaining relatively unreactive toward cellulose.

Figure 2. The structure of cellulose.[4]



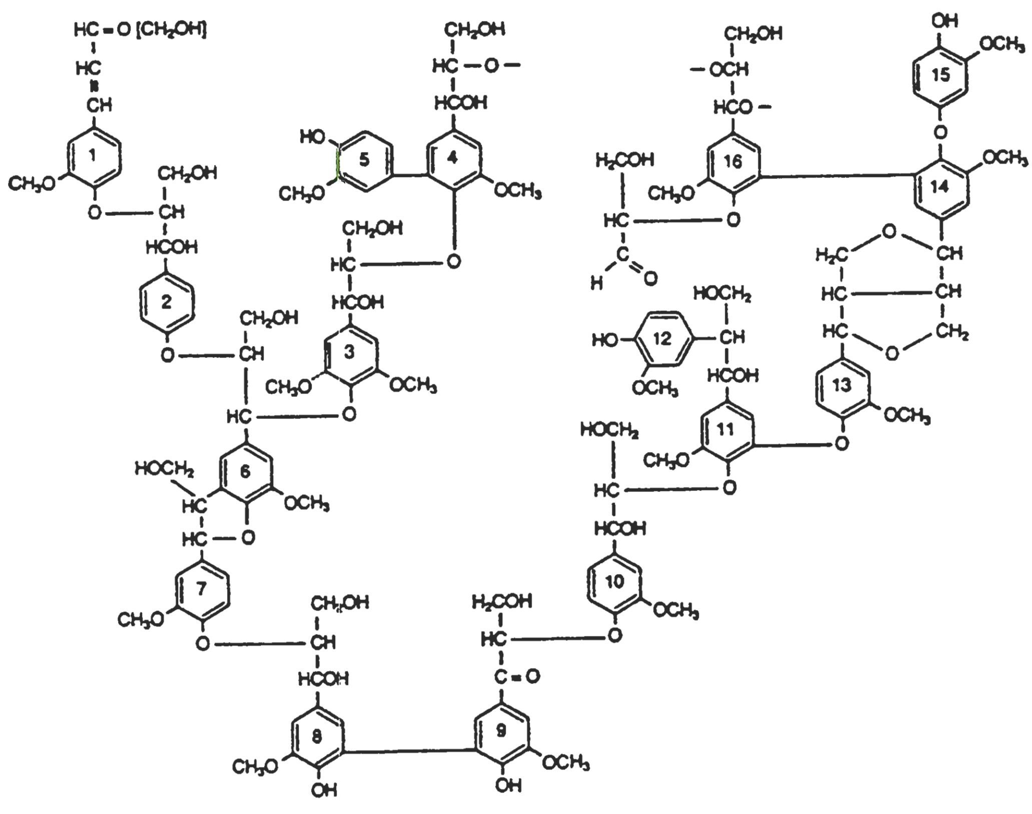
Chlorine dioxide and chlorine are both oxidizing agents; they accept electrons during delignification. On an oxidizing equivalent basis, 1 kg of chlorine dioxide is equivalent to 2.63 kg of chlorine.[28] The improved bleaching efficiency of chlorine dioxide reduces the total equivalent chlorine required to reach a given extracted kappa number.[28] As chlorine dioxide is substituted for chlorine in the chlorination stage, with equivalent chlorine demand remaining the same, the total elemental chlorine consumed, as well as the amount of chlorinated organic compounds formed, decreases.[5,26,28,37,44] In conjunction with other AOX reducing process, chlorine dioxide substitution can contribute up to a ten-fold reduction in AOX.[8] In a study by Aprahamian *et. al.*,[26] 6.5 times more AOX was observed to be generated in a mill using a CEHDED bleaching sequence than in a mill using a ODCED sequence.

Specific compounds may undergo varying degrees of formation and substitution under differing degrees of ClO_2 substitution. Chlorinated phenolic reductions have been reported in many cases as a result of ClO_2 substitution,[5,51] and, often, the highly chlorinated materials are decreased to a greater extent than the total chlorinated phenolics.[40] The higher chlorinated guaiacols are practically eliminated by using a ClO_2 substitution of about 75% or more.[2,3,45] Increasing ClO_2 substitution, at a constant active Cl_2-multiple, significantly decreases the formation of TCDD and TCDF.[28] An increase in the total chlorinated phenolics can be noticed up to about 50% ClO_2 substitution. A further increase in the proportion of ClO_2 drastically decreases the amount of chlorinated phenolics.[52] Increasing chlorine dioxide substitution minimizes the formation of chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans in bleached pulp.[28] The conversion of an oxygen delignified softwood bleach plant from 10% to 60% ClO_2 substitution reduced the total chlorinated phenolics by 23% in the chlorination stage, and 11% in the overall final treated effluent.[28]

Reactions Related to Bleaching

As stated earlier, lignin is an aromatic polymer of a mixture of three different 4-hydroxyarylproprenylalcohols (Fig. 1). The structure of lignin is very complicated and not completely known. A simplistic model of lignin based on the known bonding configurations is depicted in Figure 3.

Figure 3. Softwood Lignin.[4]

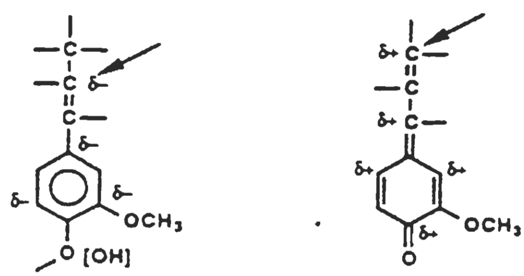


The very complex system of pulp bleaching can be simplified by dividing the bleaching reactions into three categories with respect to the initial reacting species:[53]

1. Cationic reactions under acidic conditions with Cl^+, HO^+, or their precursors H_2O^+Cl, H_2O^+OH as the reacting species.
2. Radical reactions under acidic, neutral, or alkaline conditions with free radicals as the reacting species.
3. Anionic reactions under alkaline conditions with ClO^- or HOO^- as the reacting species.

As chlorine dioxide falls into category 2 and chlorine in category 1, only these groups of reactions will be discussed in detail. The cationic and oxidative radical bleaching processes are electrophilic, attack occurs at the electron-rich aromatic and alkene structures, and they make up the initial steps of lignin-degrading bleaching. These bleaching compounds preferentially attack at the positions *ortho-* and *para-* to the phenolic hydroxyl group and at the b-carbon in ring-conjugated structures (Figure 4).

Figure 4. Sites of attack by electrophiles (δ-) and nucleophiles (δ+).[53]

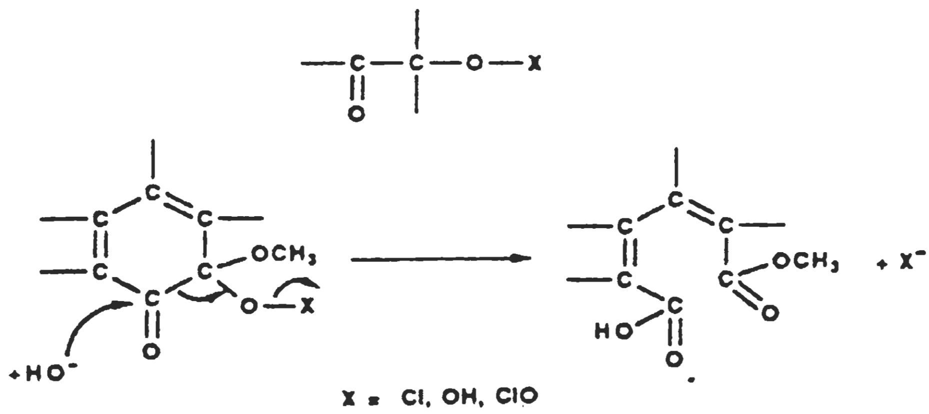


The oxidative radical bleaching process begins by forming a complex between the active bleaching species and the aromatic nuclei. The next step in the process is pH dependent. In acidic media, the radical complex is protonated followed by HX elimination and formation of a cationic radical. If the aromatic structure was phenolic, it will lose a proton and re-aromatize. Otherwise, it will exist as a mesomeric oxonium ion type.[53] In alkaline media, the electrophile (as a negative ion) is replaced by a hydroxide ion resulting in a hydroxycyclohexadienyl radical. If the aromatic structure was a phenol, it will lose H_2O and re-aromatize. Otherwise, it will remain as a hydroxycyclohexadienyl radical.[53] In neutral media, one step is eliminated. If the compound originated from a phenol, it will eliminate HX and re-aromatize while all others form a substituted cyclohexadienyl radical.[53]

In contrast to the radical bleaching agents, cationic bleaching agents (Cl_2) react with aromatic structures by electrophilic aromatic substitution. The initial reactive species rapidly forms a nonspecific complexation with the π-electron system of the aromatic ring. Rearrangement products will be determined by the position of the electrophilic substitution. Addition to an unsubstituted carbon, or to a carbon substituted with a good leaving group, will result in the loss of a proton or side chain and rearomatization to form a substituted aromatic compound. If the addition occurs at a carbon with an oxygen containing substituent, hydrolysis will take place resulting in the formation of quinonoid structures. In both cases, formation of radical intermediates occurs.

These radical intermediates will further react with radical bleaching compounds to form quinonoid type structures, muconic acid type structures, or substitution products. The unique feature of the radical process is its ability to break open the aromatic ring as detailed in Figure 5.[53]

Figure 5. Opening of aromatic ring.[53]



Other than reacting with another radical bleaching agent, the radical complexes can react with a radical scavenger, such as oxygen or superoxide, to form peroxy radical or peroxy anion compounds. These can then undergo further oxidative degradation.[53] The combination with other radical complexes to form larger lignin compounds is also possible.

During bleaching, chlorine dioxide is reduced to chlorite and hypochlorous acid. Chlorite ions decompose in acidic medium to form hypochlorous acid and chlorate. The hypochlorous acid can react with organic material to form chlorinated organic material, or react with HCl to form Cl_2. In the more typical ClO_2 bleaching range of pH 2-3, hypochlorous acid is responsible for the formation of the majority of chlorinated organics produced by ClO_2 bleaching. Hypochlorous acid and chlorine react differently with lignin components. Hypochlorous acid reacts with double bonds to form chlorohydrins, which undergo extensive chlorine elimination during the alkaline stages of bleaching. Chlorine reacts with double bonds to form dichlorinated products. The dichlorinated products are much more stable during alkaline hydrolysis than chlorohydrins.[8]

The numerous possible reactions between bleaching agents and lignin yield a wide variety of products formed by substantial depolymerization of the lignin, as well as, the introduction of chlorine and various acidic groups onto the lignin structure.[4] As a result of this, some of the lignin will dissolve in the chlorination liquor and leave as waste products. The dissolved lignin fragments consist of a wide variety of chlorinated and nonchlorinated compounds with a large molecular weight distribution. The lower molecular weight compounds have been studied more extensively and many compounds have been identified, however, there are still many compounds in this group that have yet to be defined. The structure of a substantial group of products, the high molecular weight compounds (chlorolignins), is not readily known and may vary widely from mill to mill. Although the exact structures of the compounds formed from chlorine and chlorine dioxide bleaching are not known, the likelihood of bleaching resulting in substitution on the aromatic ring to form stable chlorinated organic compounds is higher for Cl_2 than for ClO_2.[53]

AOX DEGRADATION

Introduction to Biodegradation

In order to reduce the level of AOX and BOD discharged to the environment, many mills employ secondary biological treatment of the bleach plant effluent. This type of system is based on the principle of biodegradation, the biological transformation of organic compounds by living organisms.[54] Biodegradation does not necessarily lead to complete breakdown (mineralization) nor does it always have a benign outcome.[54] Instead, it has the potential to convert an innocuous chemical into a toxic one, change a readily metabolizable compound into one that is difficult to destroy, or alter the toxicity of a chemical so that it acts against another organism.[54,55] Despite these drawbacks, biological treatment is the most common form of secondary treatment at US mills.

Once a chemical is introduced into a microenvironment, three conditions are possible:

(a) one or more of the micro-organisms present has the required enzymes, and is present in a high enough concentration, to effect immediate biodegradation;
(b) acclimation of the microorganisms may be necessary, this could be signaled by a lag period between addition of chemical and the onset of biodegradation;
(c) a microorganism capable of degrading the chemical is absent.[56]

Since effluents are discharged into established treatment ponds, case (a) will be the dominant system. However, changes in the bleaching sequence which effect the products formed, may have significant impacts on treatment efficiencies. For effective treatment, a sufficient population of healthy microorganisms must be maintained.

Microorganisms require energy to maintain themselves; that is, they must carry out oxidation reactions at a rate sufficient to create the energy and carbon they need. A supply of readily degradable organic material capable of meeting their nutritional needs must be available to the microorganisms.[57] The organic compounds that best serve as a food source for microorganisms contain oxygen in the form of hydroxyl and carboxyl groups.[54,55] As microorganisms convert the organic substrate to inorganic products (CO_2, H_2O, CH_4, and Cl^-), the responsible populations make use of the carbon in the substrate and convert it to cell constituents. As a consequence, mineralization is typically a growth-linked process.[55] This indicates the existence of a specific chemical concentration (organic substrate) below which the system will no longer support the microorganisms. This is the minimal concentration to which biological treatment is effective in degrading that chemical or mixture of chemicals. This may be the reason that compounds having very low water solubility are also recalcitrant when considered individually.[55] It should be noted that only the concentration of the compounds which support growth of the microorganisms is important. For mill effluents, the level of BOD is several orders of magnitude higher than AOX. This would suggest that BOD load is the critical parameter for maintaining a viable microbial population. Except for the unlikely event that the organism(s) responsible for AOX biodegradation cannot exist primarily on BOD elements, there will be no minimum concentration of AOX necessary for biodegradation to occur.

The factors governing the ability of a microorganism to degrade a natural or synthetic compound are:

(a) the compound must be able to reach the organism or the enzyme site;
(b) the compound must not be lethal;
(c) the enzymes necessary to alter the chemical must be present or able to be induced;
(d) the environmental conditions must permit the operation of the enzymes.[54,55,56,57,58,59]

If the secondary treatment system is well mixed and maintains a sufficient microbial population, condition (a) will not be a limiting factor. One factor which seems to be missing from this list is that the compound must be capable of undergoing the required reactions necessary for degradation. This will be discussed in more detail later. Processes or conditions affecting the transport and distribution of substrate in the treatment system, i.e. fines movement and concentration, will impact the degradation rates. Microbial acclimation, the process by which the microorganisms develop the ability to produce necessary enzymes, may show a significant lag time before biodegradation occurs. Other parameters such as pH and temperature will affect the enzymatic reactions of the microbes.

After reaching a microorganism, a chemical may be:

(1) degraded to serve as a source of carbon and/or energy;
(2) degraded by a cometabolic processes;
(3) slightly modified and then stored or excreted;
(4) stored or excreted without chemical alteration.[56,57]

Cases (1), (2), and (3) will cause a reduction in substrate concentration, while case (4) has the potential to lead to an apparent concentration reduction, without any real reduction in the amount of chemical present. The cometabolic process has been used to explain the degradation of chemicals which are known not to support the growth of microbes, and may be a significant contributor to the reduction of AOX.[54,55] The cometabolic pathway provides a means for degradation of compounds which are not known to be degraded by any single organism through the cooperative efforts of multiple bacteria strains. In addition to actual compound degradation or uptake, adsorption of substrate to other material, such as fines or soil, will cause a reduction in the apparent chemical concentrations.

A major factor determining the susceptibility of an organic compound to microbial attack is the length of time the compound has been on Earth. Most naturally occurring compounds have been present for millions of years, and thus there are organisms present in the biosphere which can initiate their mineralization. On the other hand, there exist chemicals in use by industry today that have been present only a mere instant on the evolutionary time scale; these compounds are referred to as xenobiotic. Many xenobiotic compounds are closely related to biogenic compounds and thus are biodegradable. However, most xenobiotic compounds do not have biogenic counterparts and tend to be recalcitrant. These chemicals pose a major problem because their novelty can cause deleterious effects on living systems and they tend to accumulate in the environment.[54]

One mechanism by which it is thought that bacteria attack xenobiotic compounds is known as gratuitous metabolism. This involves the bonding of cellular enzymes to xenobiotic groups which are similar to natural substrates.[54] The success of gratuitous metabolism depends on several factors. It depends, to a large degree, on the similarity in structure between the xenobiotic compound and the natural substrate.[54,55] If the xenobiotic compound is incapable of causing induction of the requisite enzyme, then its biodegradation will only occur in the presence of the natural inducer. Another important factor is the nature of the product resulting from the enzymatically catalyzed reaction. The reaction product has the potential to be more toxic than the original, and it can be less susceptible to microbial attack, thereby making it more likely to persist and bioaccumulate.[54,55,56]

The production of metabolites by cometabolic attack on a xenobiotic compound may not result in their bioaccumulation within the medium, if the organism performing the cometabolism is growing in a mixed microbial community. In this case, there exists a good chance that the cometabolic metabolite can be degraded by another species of organism in the community. In this way, concerted attack of many microbes in a community can lead to complete degradation of a xenobiotic compound, even when no organism in the community individually is able to totally use the substrate. In the event that no organism is present that can degrade the cometabolic metabolites, a buildup of compounds not necessarily found in the original effluent may occur.[54]

An important consideration when interpreting biodegradation data is whether persistence is due to recalcitrance or to unsatisfactory conditions in the environment in question. If it is due to recalcitrance, then appropriate steps may be required to limit release of the compound. If it is due to environmental conditions, an evaluation must be made to determine the factors preventing biodegradation from occurring. Labeling a compound as recalcitrant is a difficult process, as recalcitrance can not be proven; rather, it is deduced by the absence of biodegradation under applied test conditions.[54] The recalcitrance of compounds thought to be biodegradable may result from lack of an essential nutrient, substrate concentration, substrate inaccessibility, or the presence of toxicants.[54,55] As a result, it demands that every effort be made to ensure that some factor in the testing protocol is not causing the compound to persist when it is in fact biodegradable. It also requires a tiered approach to testing, going to more complex and expensive methods only when the chemical has proven to be persistent in the simpler and more frugal procedures. This view must also be extended to interpreting data from biological treatment plants. Lack of degradation of a compound does not confirm it as recalcitrant; rather, it implies the absence of conditions necessary for biodegradation of the compound in question.[54]

Microorganisms in secondary treatment systems are classified into three categories based on their use of oxygen. Aerobic bacteria require oxygen as an electron acceptor and can not function in the absence of oxygen. Anaerobic bacteria use nitrate or sulfate as an electron acceptor and are generally found only under conditions where oxygen is not present. Facultative organisms can function either with or without oxygen. In the presence of oxygen, the more energetically favorable aerobic metabolism will be used, while in the absence of oxygen, they will revert to anaerobic metabolism. Reaction mechanisms for these organisms will be discussed in more detail in the following sections.

AOX Degradation, Biological

The theoretical pathway for removal of chlorinated organic compounds in secondary treatment includes volatilization; biosorption with subsequent settling in the benthal (transitional phase between aerobic and anaerobic zones) zone; anaerobic degradation and dehalogenation in the benthal zone; and chemical degradation.[27,60,61] As proposed by Amy and Bryant,[60,62,63] the majority of AOX reduction occurs in the benthal zone of the treatment system. Based on their proposal, biosorption is a necessary transport step required for anaerobic degradation and dehalogenation in the benthal layer.[62] Theoretically feasible anaerobic dehalogenation mechanisms (reductive dehalogenation) are available to support their claims.[64,65,66] In a recent study by Bryant *et. al.*,[6] however, it was determined that sorption to biomass did not correlate with AOX removal and, therefore, their earlier hypothesis on the removal mechanism for AOX was probably in error. The exact mechanisms for AOX removal in secondary treatment systems is unclear at this time.

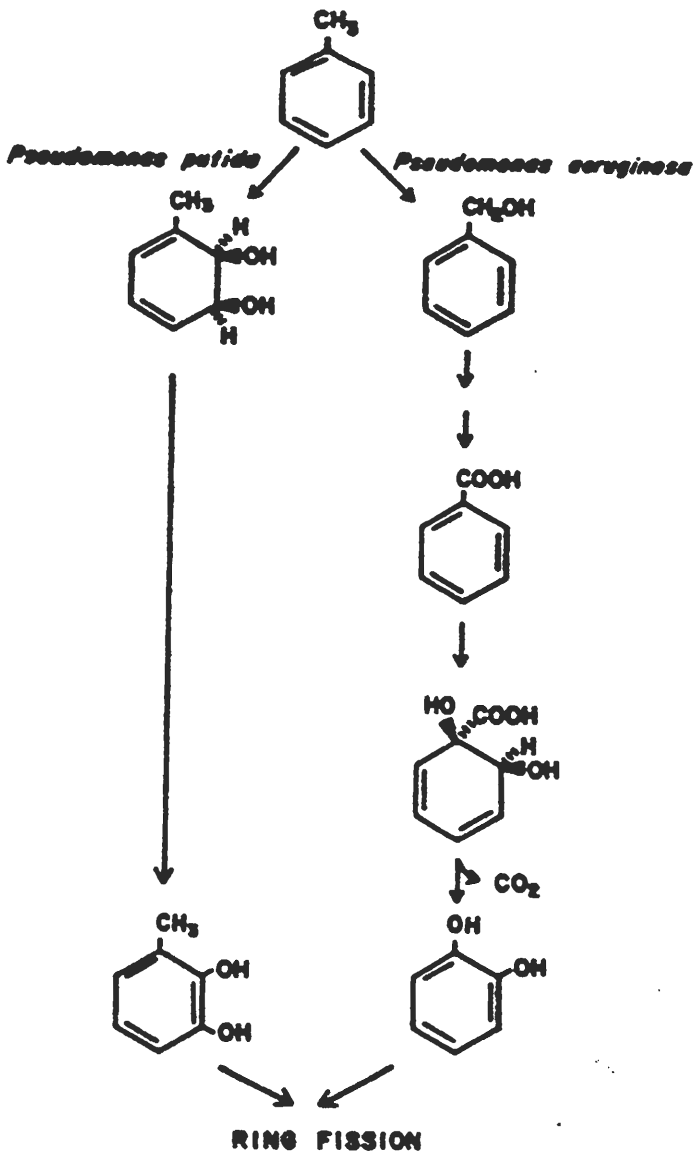
Microorganisms are typically capable of degrading compounds of MW of <500. Larger compounds, those with minimal internal cross sections exceeding 0.96 nm, are unable to diffuse through cell membranes and require involvement of active transport systems or extracellular enzymes to facilitate biodegradation.[30] This would imply that only the fraction of AOX below MW of ~500 would be readily available for biodegradation. The process of relating the fraction of HMW material to biodegradation is, however, a very complicated issue. Measurement of the HMW fraction differs greatly according to the method used, and can vary from 90% reported below MW 500 to reports of 90% of the AOX being above MW 1000.[14,26,61,67,68] In addition, the HMW fraction has been found to be unstable, and is believed by some to be comprised of aggregates of LMW compounds.[14,31,69,70,71,72,73] The importance of the fate of HMW material can not be discounted in determination of the environmental impact of mill effluent.

Aerobic Degradation

Aerobic secondary treatment systems are the most common secondary treatment systems used in North America. The initial reaction in the aerobic degradation of an organic compound is almost exclusively the incorporation of molecular oxygen into the substrate molecule. Reactions of this type are catalyzed by enzymes of the class oxygenase. The next step is also an oxidation reaction involving the same class of enzymes. The oxidation of toluene is depicted in Figure 6.

The enzymatic fission of the aromatic ring requires the presence of at least two hydroxyl groups, generally located adjacent to each other. A requirement for biodegradation is, therefore, the ability of the substrate to be oxidized to a dihydroxy structure. Often, multiple substituted aromatic compounds lack the available sites necessary for the oxidation reactions to take place and are thereby recalcitrant. As chlorine bleaching leads to a higher degree of ring substitution than does chlorine dioxide bleaching, it is reasonable that chlorinated organic compounds in effluents from chlorine bleaching of pulp would be more resistant to biodegradation.

Figure 6. Mechanisms for the oxidation of toluene.[74]



Due to the wide variety of dihydroxy aromatic compounds available as substrates, a diverse metabolic reaction sequence exists for the further degradation of these compounds. The reactions in these sequences are often times quite similar and are dictated by the site and type of substituents. Several pathways for the cleavage of the aromatic rings are depicted in Figures 7-9. Aerobic ring cleavage can be expected to simply transform organically bound chlorine from one type of chlorinated organic compound to another.[60] Once the aromatic rings are opened up, the compounds will then follow the reaction mechanisms for the degradation of alkane type compounds (Fig. 10). The linear alkane must undergo two oxidation reactions at the carbon on the free end in order to biodegrade. As with opening the phenolic rings, the more highly chlorinated the structure, the more resistant it should be toward biodegradation.

Anaerobic Biodegradation

Microbial methanogenesis, complete anaerobic degradation, is the conversion of organic matter to methane and carbon dioxide in the absence of oxygen. Methanogenesis is a multiple step process involving three distinct groups of bacteria; namely the acidogens, acetogens, and methanogens (Fig. 11). The acidogens convert complex organic molecules, such as cellulose and protein, to high-molecular weight fatty acids. From these intermediate products, the acetogens produce acetic acid which is then converted to methane and carbon dioxide by the methanogenic bacteria.[75] The first step involves the hydrolysis and fermentation of organic compounds by acid forming bacteria to yield fermentation products such as organic acids, alcohols, neutral compounds, hydrogen, and carbon dioxide. This is followed by the conversion of the organic acids, neutral compounds, and alcohols to acetate, hydrogen, and carbon dioxide by the hydrogen-producing acetogenic bacteria. A third class of bacteria convert the hydrogen, carbon dioxide, and formate to acetate. The final step involves the conversion of the acetate to methane by the methanogenic bacteria.[76]

Figure 7. Microbial degradation of protocatechuic acid and catechol.[74]

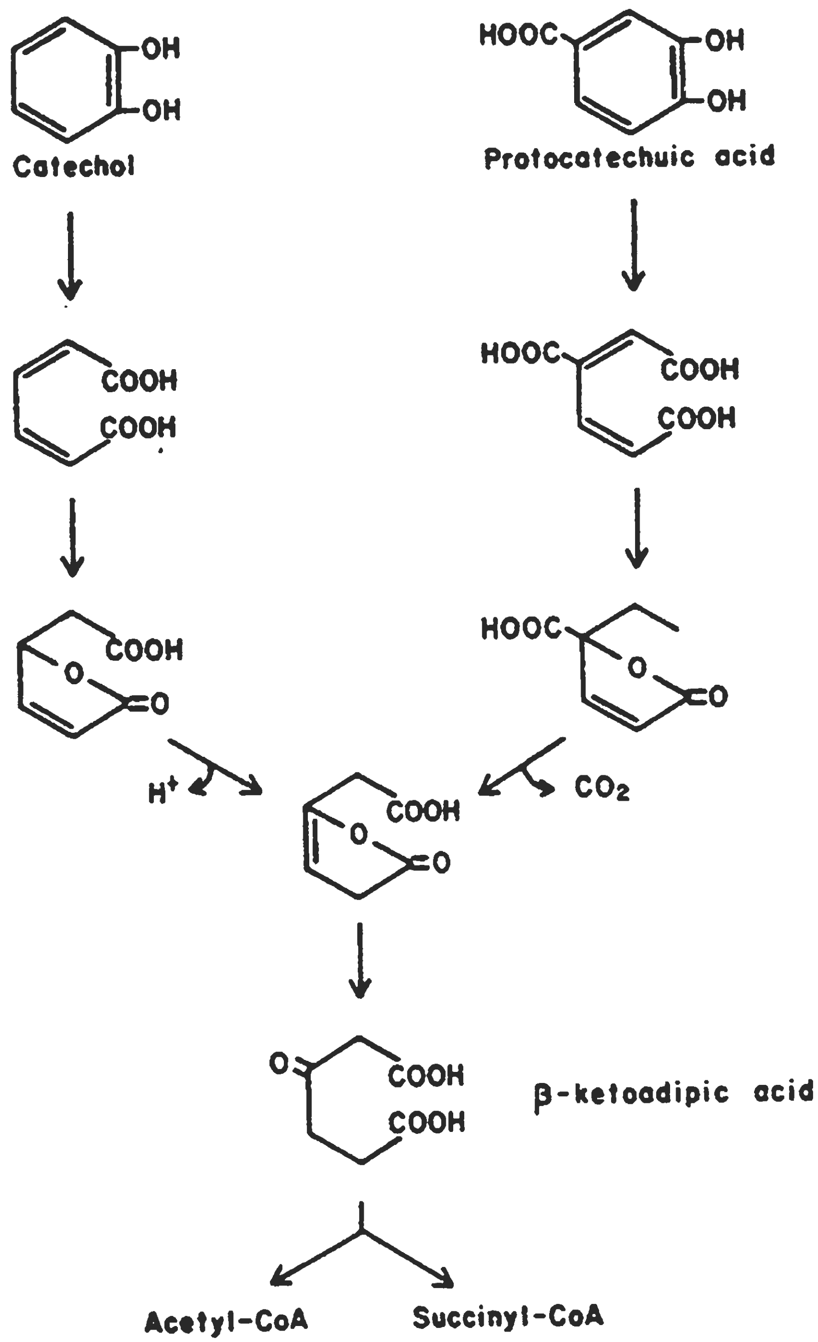


Figure 8. Microbial degradation of catechols.[74]

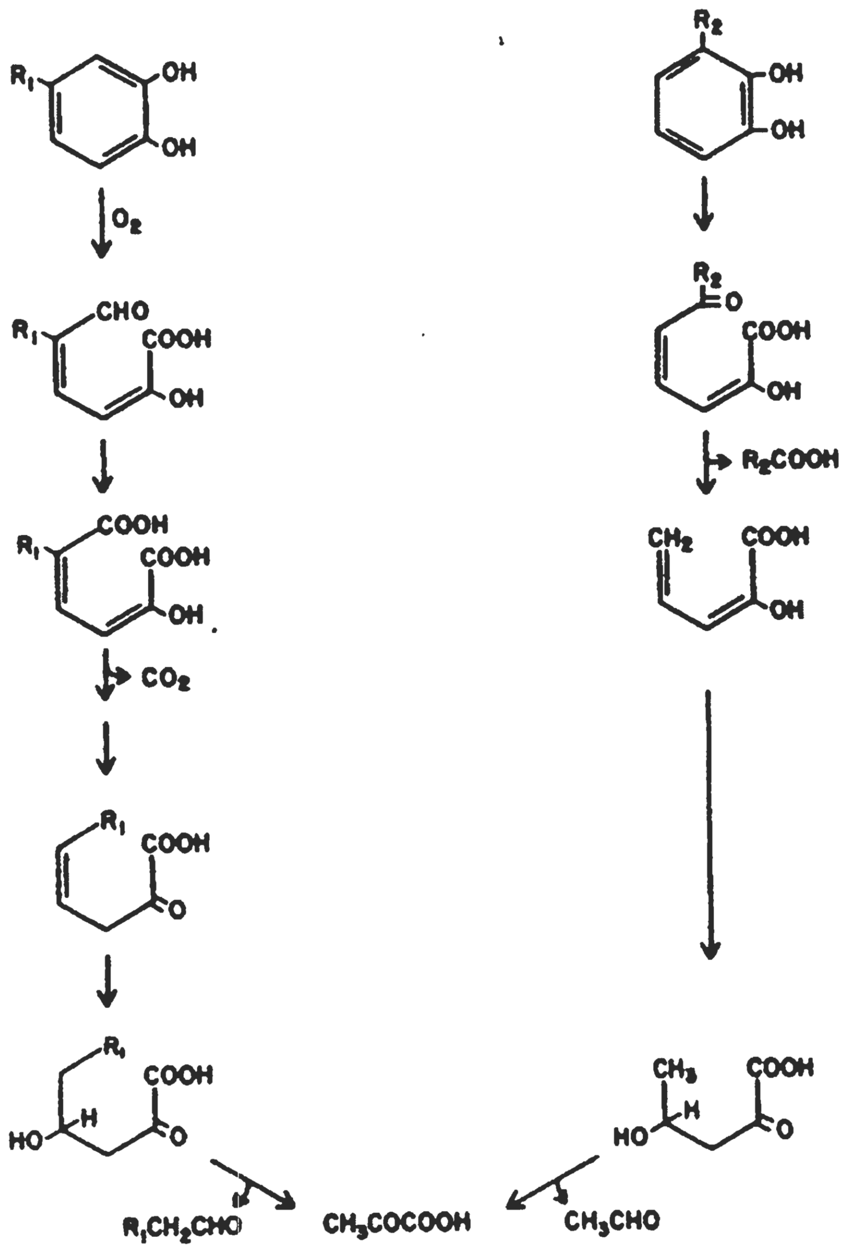


Figure 9. Degradation of multiple ring structures.[74]

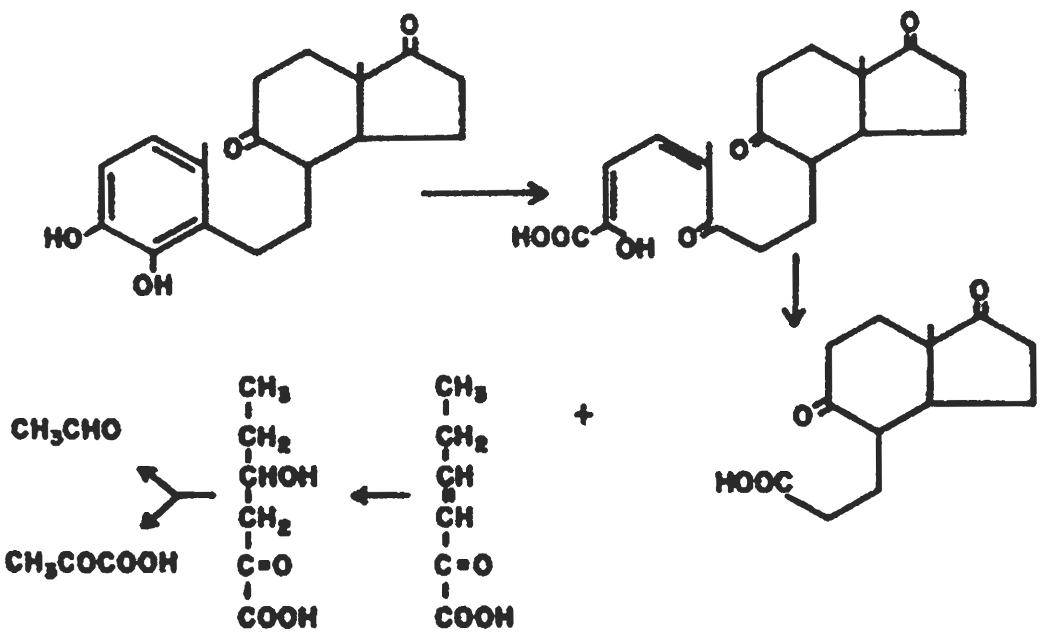


Figure 10. Degradation of linear alkanes.[74]

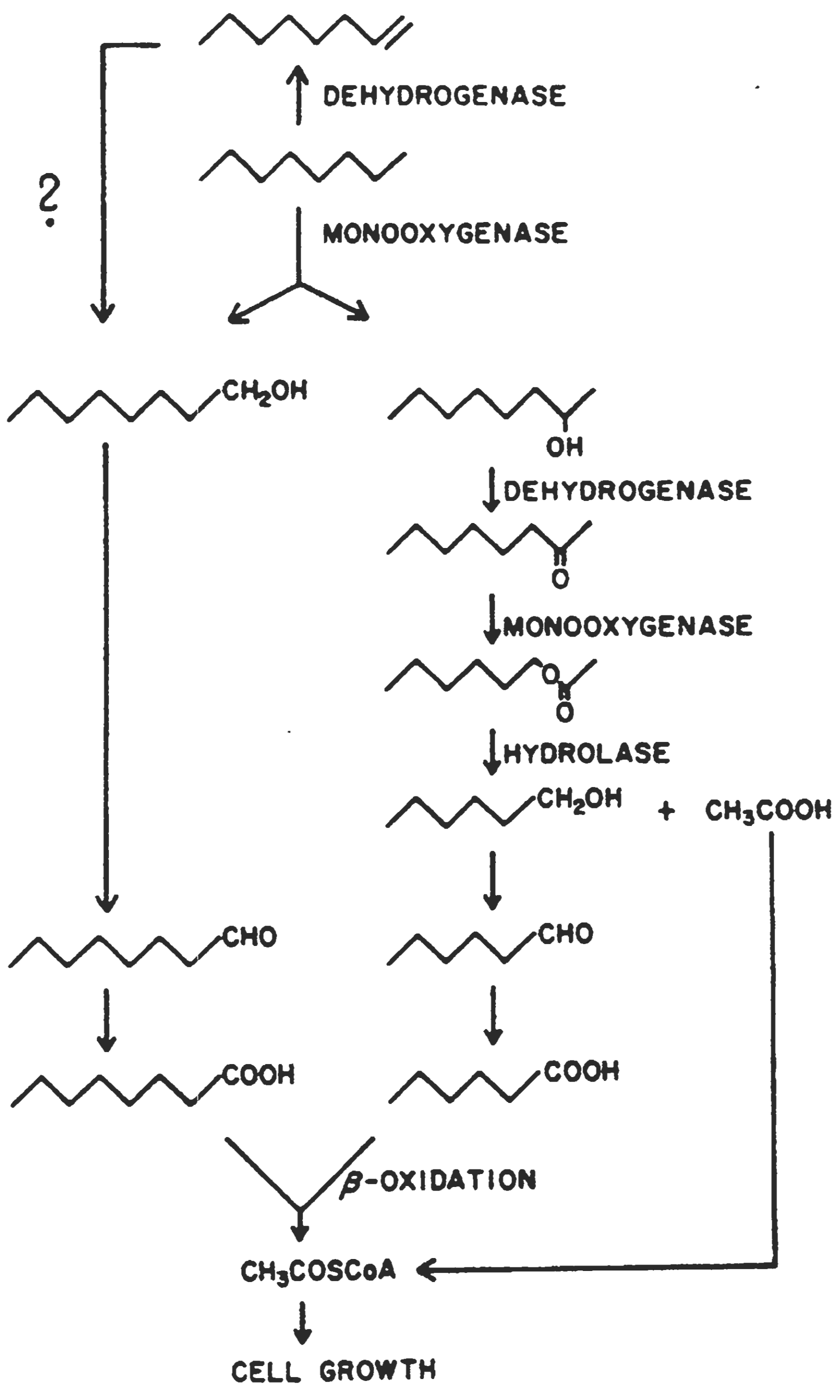
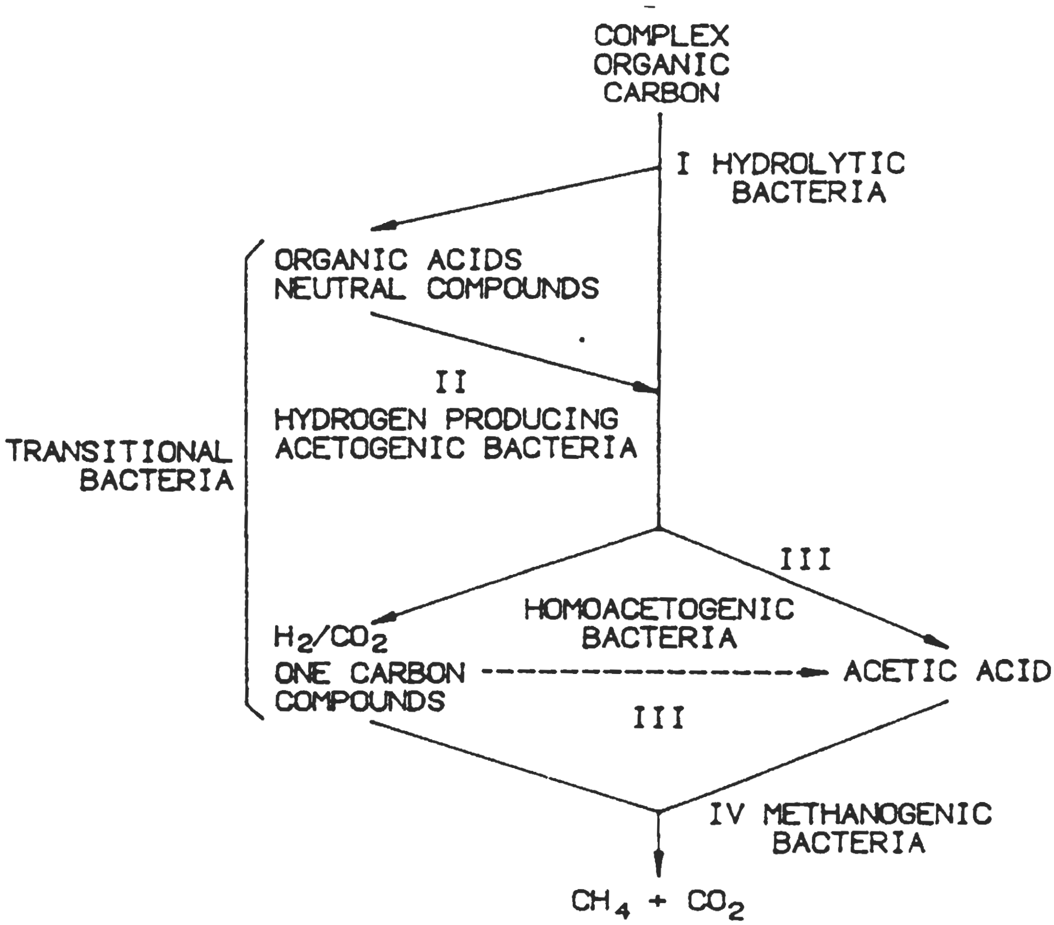
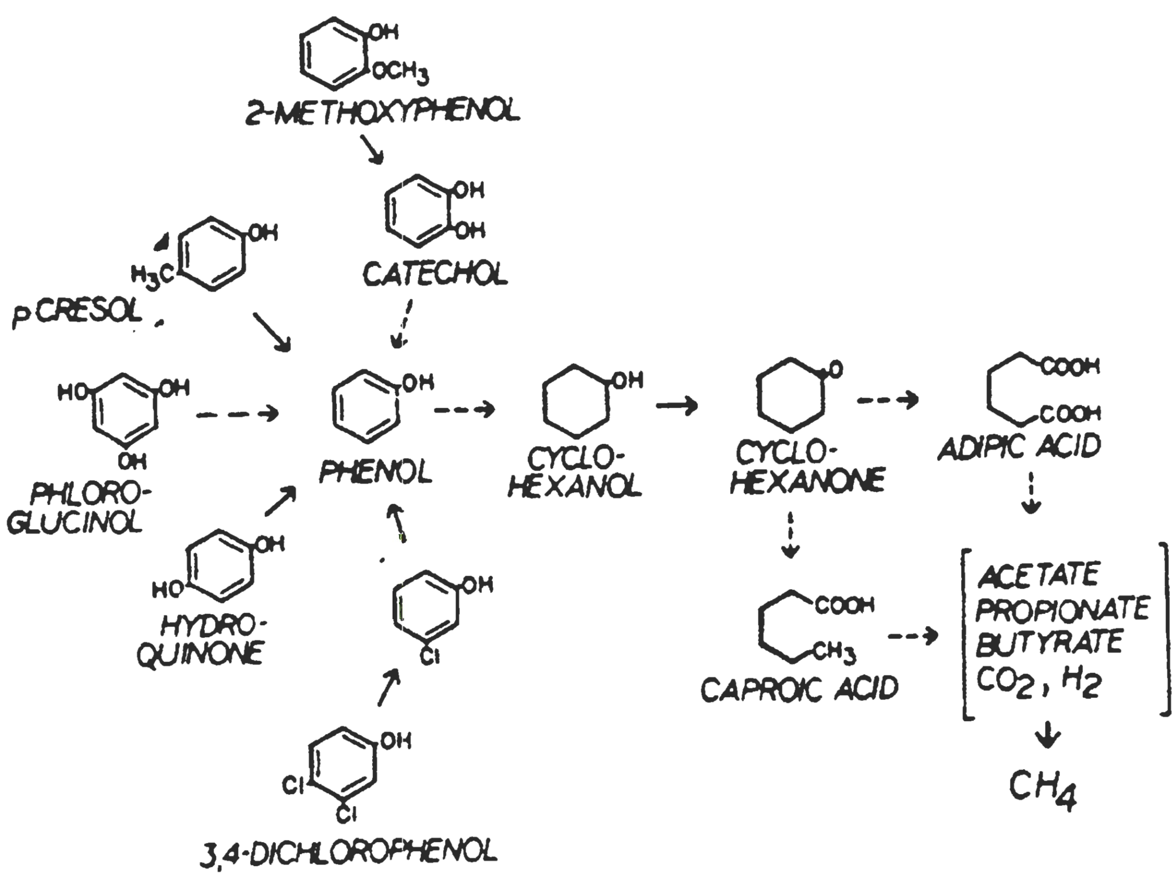


Figure 11. Microbial Methanogenesis.[76]



For substituted aromatic compounds the process is more complicated. Prior to the oxidation of the aromatic ring, the substitution groups are removed to convert the compound to phenol (Fig. 12). This can be accomplished through reductive dehalogenation, the transfer of one electron to the chlorinated compound and the subsequent release of chlorine atoms as Cl^-. The compound is then oxidized according to the above procedure. The degradation rate will be influenced by the degree of substitution on the parent compound. Again, as with aerobic degradation, the less substituted compounds expected for chlorine dioxide substitution would be expected to show more extensive biodegradation.

Figure 12. Metabolic fate of substituted phenols.[77]



Degradation Rate

Biodegradation rates have been typically interpreted through the Monod Equation (Equation 1), which is analogous to the Michaelis-Menten equation used in enzyme kinetics.[56]

$$\mu = \mu_m \frac{[S]}{Ks + [S]} \qquad \text{Equation 1}$$

The parameters μ and μ_m refer to the specific growth rate in the presence of substrate concentration S and the maximum specific growth rate, respectively, and K_s is the half-velocity coefficient.

The degradation rate of a substrate can be expressed by Equation 2,

$$-\frac{d[S]}{dt} = \frac{\mu_m [S][B]}{Y(Ks + [S])} \qquad \text{Equation 2}$$

where B represents biomass and Y is the growth yield factor. The Monod equation assumes that the compound of interest sustains growth and is the only source of carbon. The equation also ignores toxicity and microbial decay and makes no provision for acclimation.

Experimentally, rates are measured either at low substrate concentrations where $K_s > [S]$ and Eq. 2 simplifies to Eq. 3, or at high substrate concentrations where $[S] > K_s$ and Eq. 4 follows from Eq. 2.

$$-\frac{d[S]}{dt} = \frac{\mu_m}{YKs}[S][B] \qquad \text{Equation 3}$$

$$-\frac{d[S]}{dt} = \frac{\mu_m}{Y}[B] \qquad \text{Equation 4}$$

For the former case, which is environmentally more relevant and applies in the system of study, the rate obeys first order kinetics with respect to substrate and biomass. In the case of a bleach plant effluent studied as a unit as will be done here, it is not meaningful to try to calculate exact rate constants for each case. The complexity of the system, the possibility of cometabolism, and the fact that individual chemicals are not being monitored invalidate the use of these equations for this case based on the assumptions made.[56]

AOX Degradation, Abiotic

Recent research has indicated that much of the AOX reduction observed across wastewater treatment systems may be due to chemical degradation, however, the percentage of AOX removal attributed to abiotic mechanisms varies significantly from one study to another.[14,29,75,78,79,80] A fraction of AOX in bleach plant effluent is unstable and can be degraded by effluent neutralization and alkaline hydrolysis as the acid and caustic sewers combine.[27,78] The amount of degradation that occurs appears to be a function of pH, temp, and time.[14,75,78,79,80,81] While significant AOX reductions can be attributed to chemical degradation, virtually no organic matter is removed from the wastewater during neutralization or alkaline hydrolysis.[79] The lack of COD removal is explained by the fact that no significant oxidation of organic matter by oxygen takes place in the absence of microorganisms at the conditions prevailing in these processes.[79]

Extensive research has recently been conducted in the area of abiotic degradation of AOX material. AOX removal by non-biological means was measured by Dorica and Elliot[75] under conditions similar to actual treatment conditions. Effluent streams from a Canadian mill using softwood pulp and a CDEH bleaching sequence were individually collected and stored at 4°C. The effluents were combined to simulate actual mill effluent and tested for BOD and AOX removals by a non-biological reactor consisting of a 4L upflow glass column without packing or biological activity. All tests were conducted at a temperature of 50°C, an initial pH of 10, and using an initial effluent AOX value calculated from the components prior to combination. For this system, 24% and 32% AOX reductions were measured for one and two day treatment periods respectively.[75]

Recently it was reported by Dorica[82] that AOX in BKME undergoes an immediate (complete within 15 minutes) and significant (up to 25%) mineralization to Cl^- in response to mixing the C- and E-stage effluents. In another study, Dorica and Elliot[75] found that about 23% of AOX was removed from a hardwood BKME by simply mixing the CD and E effluent streams, with up to 40% AOX reduction achieved by mixing and storage at 4°C for 24 hours. Both temperature and alkaline component were shown to have large impacts on the amount of AOX reduction. Dorica[82] treated softwood BKME with NaOH, $Ca(OH)_2$, and weak black liquor to a pH of 11 at 62°C for 45 min. For NaOH, AOX reduction occurred over the pH range 5.5 to 11, with a maximum reduction of 37%. For $Ca(OH)_2$, AOX reduction occurred over the pH range 4 to 11, with a maximum reduction of 67%. All of the AOX reduction induced by NaOH treatment could be accounted for by Cl^- generation. However, for the $Ca(OH)_2$ treatment, both alkaline hydrolysis and precipitation played major roles. Weak black liquor produced a 30% to 40% reduction in AOX across the pH range of 4 to 11. Temperatures over the range of 110°C to 150°C produced AOX reductions of 50% to 80% for a softwood BKME treated for 15 min. at pH = 7.

Bottger *et. al.*[83] found that treatment of an industrial C-stage effluent at pH 10.5 over a temperature range of 25-70°C for 2 hours resulted in AOX reductions of 37-54%. When alkali treatment (pH 11, 60°C, 1 h) yielding a 50% AOX reduction was followed by biological treatment in an aerobic biological fixed-film reactor, an additional 20% reduction in AOX could be achieved. This indicates that chemical degradation and biological degradation, to some extent, affect different compounds. Additionally, they found that removal of AOX achieved by adjusting a kraft softwood bleach plant effluent (100%D) to a pH of 7, and holding at 60°C for 4 hours, was 35%. Holding the effluent at this temperature for 20 hours yielded a reduction of ~50%.[83]

Barton and Drake[81] measured the effects of CD- and E-stage effluent mixing and pH adjustment on AOX reduction of a softwood BKME at both low ClO_2 substitution CDEOPHD and high ClO_2 substitution CDEOD. CD- and E- stage effluents (collected from the mill site and stored separately at 4°C with out preservation) were mixed to simulate actual mill flow ratios. Weak black liquor was then added to bring the simulated effluent to a BOD level of that of the actual mill effluent. They reported only a 10% reduction in AOX at low ClO_2 substitution (pH 9.4) and a 20% reduction in AOX at high ClO_2 substitution (pH 9.8) due to mixing of CD and E effluents followed by 24 hour storage at 22°C. The low values reported are most likely due to their definition of initial AOX as the AOX level of the mixed filtrates immediately after mixing, rather than computing the initial AOX level from the components of the feed mixture. Other studies[75] have indicated that alkaline hydrolysis is a rapid process complete within 5-15 minutes. Setting the initial AOX value by this method establishes an inaccurate initial AOX value, as some degree of hydrolysis certainly occurs during the mixing and sampling process. Combination of the alkaline hydrolysis values with the biotreatment values measured for the same effluent, brings the laboratory reduction values to almost exactly those reported by the mill. This seems to indicate that AOX removal by secondary treatment is due to a combination of chemical degradation (alkaline hydrolysis) and biodegradation.

Shariff and Houde[80] studied the effects of pH and temperature on a simulated BKME. They combined CD-, E-, and H-stage effluents collected from a softwood bleached kraft mill using a CDEOHDED bleaching sequence in a 3:1:1 ratio. The initial simulated effluent, pH 2.0, was used as the basis for comparison of the effects of pH adjustment with sodium hydroxide. At room temperature, increase in pH to 8.0 and 10.0 produced AOX reductions of 21% and 36%, respectively, with final pH values of 7.7 and 8.4 after 24 hours. At a temperature of 60°C (relatively that of a bleach plant effluent), increase in pH to 9.3 and 11.1 produced AOX reductions of 36% and 48%, respectively, with final pH values of 6.9 and 8.2 after 24 hours.

Studies by Archibald and Roy-Arcand[14] indicate that much, if not all, of the HMW AOX (prepared by dialysis using a 1 kD MW cutoff membrane) can be mineralized to chloride ions, principally by the actions of time and light. Color, AOX and UV absorbence of an effluent from a Canadian softwood mill using a CDEHDED bleaching sequence all showed substantial decreases upon exposure to sunlight (samples contained in 500 mL flasks with gas-permeable foam plugs were placed in a west-facing window at 20-23°C) over a 16 week period. The reduction in HMW AOX was attributed to mineralization, as all the lost organochlorine was accountable for by the appearance of chloride ions. Removals as high as 59% for HMW color, and 66% for HMW AOX, were found. Incubation in the dark over the same period, at 20-23°C and 4°C, produced AOX reductions of 38% and 13.5% respectively. This indicates that the majority of AOX mineralization is by light induced mechanisms.[14] This result does not appear to explain abiotic AOX degradation in secondary treatment systems, given that the extremely dark color of BKME will prevent light penetration into the treatment system. Samples inoculated with microbes from the mill ASB did not show significant differences in color, or an increase in AOX reduction from equivalent samples in sterile environments,[14] although sufficient microbial growth to produce visual turbidity did occur. During the 16 week incubation period, a new low molecular weight fraction containing 20 to 38% of the residual AOX appeared. This new LMW AOX seemed to be produced via mechanisms independent of light, oxygen, and microbial activity, as light exposed samples at 20-23°C, samples kept in the dark at 4°C, and samples inoculated with microbes from the mill ASB produced equivalent levels of LMW AOX.[14] The new LMW AOX also had the same AOX/TOC ratio as the initial HMW AOX. Appearance of the LMW AOX is most likely indicative of aggregates of LMW AOX, that had been separated as HMW AOX, disassociating during the study.

The effect of an increase in pH on AOX in bleach plant effluents can be explained by the fact that many chlorinated organic compounds undergo substitution reactions in which hydroxyl ions replace chlorine, resulting in the formation of alcohols and in the release of organically bound chlorine.[79] Separate investigations by Dorica *et. al.*,[75] Archibald *et. al.*,[14] and Yu *et. al.*[79] revealed that AOX removed in the abiotic treatments was by conversion of organic chlorine to inorganic chloride by alkaline hydrolysis. Analysis of suspended and settlable solids showed that AOX was not being removed by precipitation in the abiotic treatment systems.[75,79] Sorption to the settlable material coming in with the influent and precipitation accounted for only 1% of removed AOX in the systems studied by Dorica *et. al.*.[75]

Two important conclusions can be drawn from the abiotic treatment data. First, chemical degradation is responsible for significant AOX mineralization. Second, the AOX degraded by chemical and biological mechanisms consists, to some degree, of different compounds. This second point indicates that a system designed to enhance both chemical degradation and biological treatment is most likely to produce maximum AOX reductions.

TREATMENT OF BKME

Bleach Plant Effluents

Effluents from bleached kraft pulp and paper mills are very complex, containing a variety of organic and chlorinated organic material, additives from the paper production process, pulp fines, defoamers, biocides, etc. Effluents from the bleaching, extraction, and subsequent washing stages supply the organic compounds necessary for the growth of the microbial colonies in the treatment systems. The chlorination stages are the main source of low molecular weight chlorinated organics, while the alkali extraction effluents are reported as the main source of high molecular weight chlorinated organics.[60] The existing treatment facilities at paper mills were designed to control conventional pollutants, such as suspended solids and biochemical oxygen demand. Only 30-70% of the AOX in chlorine bleaching wastewater is removed by aerated stabilization basins or activated sludge systems.[5,84,85] Approximately 10% of the initial chlorine applied as bleaching chemicals can be found as AOX in the bleach plant effluent.[5]

The bulk, as much as 80% or more, of the chlorinated organic matter which is dissolved in the spent wash liquors from the bleaching of softwood kraft pulp is thought by some to be comprised of relatively high molecular weight chlorinated material, commonly referred to as chlorolignin.[86] Literature on AOX degradation gives mixed results on the biodegradability of the high molecular weight material. Results ranging from a significant preference for biodegradation of low molecular weight material to no preference for low molecular weight material can be found in the literature.[19,20,26,60,67] The fate of high molecular weight constituents in the treatment process is not well understood, but is important since these compounds may degrade/dissociate into lower-molecular-weight, potentially toxic chlorophenolic derivatives. In addition to persisting in the environment, some of these compounds exhibit toxicity or mutagenicity.[60,72,87]

In a study by Eriksson,[72] the high relative molecular mass material was found to be chemically unstable under conditions that prevail in receiving water systems. The material slowly decomposed in the environment to products that included various chlorinated catechols and guaiacols.[72,86] These results were interpreted to mean that the high molecular weight chlorolignin material was "chemically degrading" to form various low molecular weight chlorinated phenolic compounds. Two observations have been made which raise doubts about the likelihood of such a process occurring.[86] First, due to the near absence of aromatic structure in the chlorolignin,[86] it is inconceivable that this material could extensively break down to low molecular weight polychlorinated aromatic compounds. Second, it is puzzling that the degradation takes place under very mild conditions, since the previous conditions were much more severe. More work in the area of understanding the structure and fate of the chlorolignin compounds is needed.[72]

It was concluded by O'Conner and Voss,[86] that the appearance of low molecular weight compounds in the studies on HMW AOX stability was due to the desorption of monomeric chlorinated phenolic compounds associated with the dissolved chlorolignin material. They suggest that the high MW chlorolignin was actually an aggregate of smaller compounds. The fact that the AOX/TOC ratio for the HMW and LMW compounds were the same in the cases where measured, supports the theory that the LMW material is a result of dissociation rather than degradation. This fits in well with studies by Bryant *et. al.*[20,62] in which they observed a decrease in HMW AOX with a concomitant increase in LMW AOX across the ASB, but no change in AOX/TOC ratio. Recent studies by Jokela and Salonen[31] and Schwantes,[50] employing new analytical techniques, have shown the majority of AOX in BKME to have a molecular weight less than 1000, indicating that the molecular weight determined for AOX may be dependent on the measurement technique used.

Resent research has shown that the HMW fraction of AOX is not stable and biologically inactive as previously believed. Doubt has been raised as to the mere existence of this material. Regardless of the fraction of AOX contained in the HWM range, or the mechanisms for HMW AOX decomposition, this material must be given equal consideration to the LMW AOX components when evaluating BKME for environmental impacts.

Aerated Stabilization Basinsh

Aerated stabilization basins represent a common treatment system for the removal of BOD, AOX, and suspended solids from paper mill waste waters.[63] The processes involved in the removal of chlorinated organic compounds in a lagoon include:

- volatilization;
- chemical degradation;
- aerobic degradation;
- sorption onto biomass within the aerobic layer of the lagoon;
- deposition of biomass onto the benthal layer;
- anaerobic degradation and dehalogenation of chlorinated organic compounds within the benthal layer.[20,63]

It has been reported in the literature that removal of chlorinated organics occurs under anaerobic conditions within the benthal zone, while the upper region of the lagoon functions as an aerobic system for the decrease in biochemical oxygen demand.[26,27,31,60,62,63,87,88]

In studying an active ASB system, Banerjee *et. al.*[61] found an average inlet total COD of 1196 ppm, with 43% <500D. Assuming that the less than 500D portion represents biodegradable compounds, the outlet COD should be about 626 ppm. This correlated well with the measured average outfall COD of 600 ppm. The ratio of total COD to <500D COD decreased significantly across the treatment system, as would be expected for removal by biodegradation.[61] Only 11% of the inlet AOX was found to be above 500D. This indicates that 89% of the total AOX would be available for biodegradation. However, the mill reports less than 50% AOX reduction for their treatment system. The ratio of total AOX to ultrafiltered AOX decreased to a much smaller extent than did the COD values. The small decrease in the inlet:outfall ratio translates to about 15% biodegradation of AOX in the water column.[61] To confirm this value, the treatment pond was sampled at the inlet, the entry to the aeration zone, and the pond outlet (Fig. 21). Only 13% of the 50% total AOX reduction occurred from the aeration zone inlet to the pond outlet, whereas 37% AOX reduction occurred by the end of the pre-settling basin.

The ~15% value for AOX biodegradability in ASB systems is further supported by findings from a variety of other studies. Bryant *et. al.*[60] sampled AOX from a pulp and paper mill ASB over one week periods on four occasions. Samples were taken at the inlet, three places in the aeration zone, and at the outlet. AOX decrease between inlet and first aeration point averaged 35%, while AOX reduction from this point to the outlet was only 9%. On the other hand, TOC removals across the system were more consistent with TOC removals between the inlet and the first aeration point of 13% and removal between this point and the outlet of 11%. Oleszkiewicz *et. al.*[89] tested the biodegradability of raw wastewater collected after equalization tanks from a bleached kraft mill. The waste was stored at 4°C. The cold storage deterioration was found to be less than 3% for AOX and soluble organic carbon. For a lab scale aerated stabilization basin with a MLVSS level of

170 mg/L and a HRT of 1.5 days, the BOD removal was 65%, the SOC removal was 35%, and the AOX removal was 19%.

Dorica and Elliot[75] tested combined anaerobic/ASB systems for AOX removal under conditions similar to actual treatment conditions. Effluent streams from a Canadian mill using softwood pulp and a CDEH bleaching sequence were individually collected and stored at 4°C. Effluents were combined to simulate actual mill effluent and tested for BOD and AOX removals by single and double stage anaerobic reactors followed by two aerobic reactors, simulating ASBs with a total HRT of 3 days. All tests were conducted at a temperature of 50°C, an initial pH of 10, and using an initial effluent AOX value calculated from the components prior to combination. Softwood AOX removals of 34% and 40% were measured for the single and double stage anaerobic reactors, respectively. When combined with the aerobic reactors, AOX removals for the single and double stage anaerobic systems showed only a small increase to 35% and 43%, respectively. In contrast to the AOX results, BOD removal doubled upon addition of the aerobic stage increasing from 30-50% for the anaerobic only systems to > 92% for the treatment systems including the aerobic stages. This study indicates that AOX degradation is roughly equivalent in both anaerobic and aerobic treatment systems. Monitoring of the operation of the biological treatment system over a two year period led to the conclusion that AOX reduction was through biodegradation and not through absorption and settling.[75]

ASBs represent the most common secondary treatment systems currently in use in North America. These systems typically achieve AOX reductions of 30-50%. Although the exact mechanisms for AOX removal in these systems is still not understood, abiotic mechanisms appear to be responsible for the majority of AOX removal. Only about 15% of the AOX removal can be attributed to biodegradation.

Activated Sludge Systems

Lagoon treatment systems are typically less efficient than the activated sludge plants, where AOX reductions of as much as 70% have been recorded.[3,5,6] Activated sludge systems consist primarily of an aeration basin, secondary clarification basin, and pumping and aeration equipment. Mill waste is introduced into the aeration basin where it is mixed with microorganisms and DO. Effluent from the aeration basin is further treated in a secondary clarifier to remove the microorganisms. A portion of the microorganisms are returned to the aeration basin to maintain the desired biomass levels, with the remainder processed as waste sludge. Advantages of activated sludge systems as compared to ASB systems include:

- more easily controlled biomass levels;
- higher DO levels;
- lower space requirements;
- higher efficiency of BOD and AOX removal;
- shorter start-up periods;
- typically less odor.

The increased efficiency is mainly due to the more enriched bacterial culture found in the activated sludge system and the ability to maintain sufficient levels of DO for aerobic biodegradation to occur. The reduced odor is primarily attributable to the lack of anaerobic zones in activated sludge systems as compared to ASBs.[90]

The variation in levels of AOX removal reported by activated sludge systems is high, with values ranging from 28 to 70%, while corresponding reductions of chlorophenolics are consistently high at 70 to 95%.[5,68,91,92,93,94] The effect of operating parameters on removal efficiencies also appears to be inconsistent. Amy *et. al.*[62] found a linear relationship between AOX sorption and biomass levels, and calculated the corresponding Freundlich constants. Based on the sorption isotherms, and the theory that AOX removal is primarily due to sorption to settling biomass,[63] a linear relationship should exist between mixed liquor suspended solids (MLS) and AOX removal. A study by Oleszkiewick *et. al.*,[89] in which they found a linear relationship between AOX removal in lab scale activated sludge reactors and MLSS, offers solid evidence to support this theory. However, in several other studies[5,6,95] on lab scale and industrial activated sludge and ASB systems, no relationship was found between biomass/MLSS levels and AOX removal efficiency.

Several studies on the effects of chlorine dioxide substitution on the biotreatability of BKME in activated sludge systems have recently been conducted. Gergov *et. al.*[5] found AOX removal to be in the range of 48-57% for a softwood BKME, using a DCEDED bleaching sequence, treated in an activated sludge system with a HRT of 12 hours, temperature of 33°C, and sludge age of 8 days. Chlorophenolics removal for the system ranged from 86-95%. Less than 10% of the AOX could be accounted for in the excess sludge; leading to the conclusion that AOX biodegradation was the major contributor to AOX removal. Oleszkiewicz *et. al.*[96] tested the biodegradability of raw wastewater collected after equalization tanks from a bleached kraft mill. For a lab scale aerated stabilization basin, MLVSS level of 170 mg/L and HRT of 1.5 days, BOD removal was 65%, SOC removal was 35%, and AOX removal was 19%. When the effluent from the stabilization basin was further treated by a lab scale activated sludge system and facultative lagoon in series, only an additional 1-11% reduction in AOX was obtained. The removal of AOX in the secondary reactors was directly proportional to biomass concentrations.

Barton and Drake[81] measured the AOX reduction of a softwood BKME at both low ClO_2 substitution and high ClO_2 substitution. CD- and E-stage effluents (collected from the mill site and stored separately at 4°C with out preservation) were mixed to simulate actual mill flow ratios. Weak black liquor was then added to bring the simulated effluent to a BOD level of that of the actual mill effluent. They treated the simulated mill effluent (after storage for 24 hours to allow completion of alkaline hydrolysis) in parallel lab scale activated sludge reactors. Biotreatment was conducted at 20°C with a HRT of 12 hours and a mean cell residence time of 8 days. For the low ClO_2 substitution effluent, they measured a 20% AOX reduction due to biotreatment as compared to mill reduction values of 33%. For the high ClO_2 substitution effluent, they measured an 11% AOX reduction due to biotreatment as opposed to mill reduction levels of 28%. Combination of the alkaline hydrolysis values with the biotreatment values, 29% AOX reduction for low ClO_2 substitution and 31% AOX reduction for high ClO_2 substitution, brings the laboratory reduction values to almost exactly those reported by the mill.

The rate and level of chemical degradation of AOX is dependent on the conditions under which the acid and caustic sewers are mixed. Therefore, collection and storage procedures for obtaining AOX samples from mill sites have a large impact on biodegradation test results. This is evident from a study by Saunamaki *et. al.*[85] in which wastewater collected after the equalization basin of a kraft mill was tested in two lab scale activated sludge systems. Initial tests resulted in 85-95% BOD reduction, 35-45% COD reduction, 35-85% chlorinated phenol reduction, and 25-35% AOX reduction. Later studies combining an anaerobic stage either before or after the activated sludge system, using the same wastewater sample, but after prolonged storage, resulted in 95-97% BOD reduction, 38-42% COD reduction, 59-91% chlorophenol reduction, and only 14-18% AOX reduction.

Activated sludge systems typically achieve higher AOX removals than ASBs, although removals vary significantly from one treatment system to another. As with the ASBs, the mechanisms of AOX removal are not wholly understood. The contribution of biodegradation to AOX removal is significantly higher in these systems, with values in the range of 20-40%. Attempts to measure the contribution of biodegradation to AOX removal yield highly variable results caused, in part at least, by differences in effluent collection and handling. Regardless of where over the 20-40% range the contribution of biodegradation lies, abiotic mechanisms are still responsible for a significant portion of AOX removal.

Mechanisms for AOX Removal

A key question in developing mechanisms for organic pollutant removal from water by microbial biomass is whether the biomass metabolically degrades the compounds or whether the process involves purely physical absorption. Studies done with nonviable biomass indicate that adsorption by dead biomass is equal to or greater than adsorption by live biomass, suggesting that the adsorption process is caused by physical adsorption, and not active uptake or biodegradation.[62] Aerobic biomass was shown to effectively absorb both high and low molecular weight organic halide fractions. Biomass loadings of up to 85,000 μg/g were measured in equilibrium with a AOX of 30,000 μg/L at 23°C. Biomass levels used in the study were 3000, 1500, & 300 mg/L.[62] The levels of AOX and biomass used in this study were relatively high, as compared to levels typically found in treatment systems (500-6000μg/L for AOX and 50-500mg/L for biomass).[20,62] However, AOX removal can not be accounted for simply by physical sorption. In the activated sludge systems reviewed in the literature, less than 10% of the AOX removed from the water column was shown to remain associated with the sludge.[5,20,95]

Bryant and Amy[62,63] indicated that adsorption of the organochlorines to settling biomass and subsequent biodegradation in the benthic layer of the lagoon, is the major factor in organochlorine removal. The transport mechanism necessary for benthal dehalogenation under anaerobic conditions is biosorption onto settling biomass within the aerobic zone of the ASB.[20] Mass balances for AOX across biological treatment systems have shown that loss of AOX cannot be accounted for in the sludge and water phase of the sludge.[5,20] These finding support the assumption that a large part of the organically bound chlorine is cleaved during activated sludge treatment, and if significant sorption of AOX to biomass occurred, AOX was dehalogenated in the benthic zone.

Barkley and Bryant[97] previously hypothesized that AOX was removed from paper mill wastewater by absorption onto the biomass generated from soluble BOD in treatment systems. If absorption onto biomass was primarily responsible for the removal of AOX in these treatment systems, the extent of absorption would be affected by the influent AOX from the mill, temperature, and the amount of biomass present. In a study on AOX removal by different types of treatment systems, Bryant *et. al.*[6] found that biomass generated across some treatment systems was much higher than others. However, the higher biomass levels did not correlate with any appreciable increase in AOX removal. They concluded that although their previous laboratory and field research had demonstrated biosorption of AOX to be the key step in AOX biodegradation, the data from this study indicated that the extent of AOX removal is not controlled by biosorption.[6]

Biodegradation has been reported to be the major mechanism for AOX removal in secondary treatment systems. A laboratory study by Randle *et. al.*[95] tested a simulated combined mill effluent for AOX and BOD removal by an activated sludge system, a facultative stabilization basin, and an aerated stabilization basin. At a retention time of 10.4 hours and 40°C, AOX reductions for the three systems, activated sludge, facultative stabilization basin, and aerated stabilization basin were 15%, 56%, and 42%, respectively. No significant levels of AOX were found in the off gases, and less than 10% of the removed AOX could be accounted for in either the waste sludge or with effluent suspended solids. They concluded that biodegradation was the most significant AOX removal mechanism, accounting for 67 to 96% of the AOX removed during biological treatment. It seems odd, however, that AOX reductions in the facultative and aerated stabilization basins were approximately three times that of the activated sludge system, even though the biomass level in the activated sludge system was ten-fold that of the other two systems.

In a study by Banerjee[61] on an ASB treating BKME, it was determined that AOX decreases by ~40% between the inlet and first bank of aerators, while reduction across the aerated portion of the basin was only 15%. Kemeny *et. al.*[98] measured a 53% increase in chloroform as it entered the waste treatment system from the acid sewer, indicating chemical conversion of some of the chlorinated organic material. Gergov *et. al.*[5] also found that chloroform concentrations in the combined inlet stream were above that accountable for in the two individual bleach plant effluent streams. Upon mixing with the alkaline stream, trichloromethyl-α-carbonyl groups present in compounds from the acid sewer are probably converted to chloroform through the haloform reaction.[98]

Tests carried out under controlled laboratory conditions showed that changes in the aeration section (temp., pH, or chemical addition) had little effect on the removal of AOX, but a considerable effect on the removal of chlorinated phenols and BOD.[85] The changes in chlorophenol reduction closely followed those in BOD reduction, indicating that the chlorophenol compounds were biodegrading while the majority of the AOX reduction was by another mechanism.[85]

Some combination of chemical degradation and settling (either precipitation or sorption) occurring in the presettling basin appears to be responsible for significant AOX removal, around 30-40%. There appears to be some portion of AOX that is biologically degradable (15% in ASBs and 20-40% in activated sludge), but the majority of AOX reduction is due to abiotic processes. The overall mechanism for AOX removal by secondary effluent treatment must, therefore, be a complex association of chemical and/or physical degradation, settling (by sorption or precipitation), and biological degradation.

THESIS OBJECTIVES

- The primary goal of this research was to develop a system to measure the biodegradation of AOX in operating secondary treatment systems.
- This information would be used to determine the conditions most favoring biodegradation of AOX, compare different secondary treatment systems, and contribute to the understanding of the mechanisms of AOX removal.
- A secondary goal of the project was to determine the difference, if any, in the biotreatability of AOX produced from chlorine and chlorine dioxide bleaching.
- Additionally, the in-situ measurement system designed was to be tested for its applicability in measuring the biodegradation of BOD model compounds.

EXPERIMENTAL APPROACH

The objective of this research was to develop a mechanism by which the biodegradation of AOX could be measured in the field under normal treatment conditions. To accomplish this, AOX which could be distinguished from the AOX existing in the treatment systems had to be generated. This was accomplished by the use of ^{36}Cl labeled bleaching agents. The radioactive AOX material could not be analyzed with the available AOX measurement instrumentation, thereby, requiring the development of novel AOX analysis techniques. Finally, a system for deploying the labeled AOX in the field and methods to evaluate the field results had to be developed. All of these aspects of the field study, along with several side experiments, will be discussed in detail in the following sections.

PREPARATION OF ^{36}Cl LABELED AOX

Introduction

Background AOX levels in the treatment ponds posed a significant challenge to in-situ measurement of AOX biodegradation. To overcome this, AOX containing a ^{36}Cl tag was generated in the laboratory and used for the field studies. The radioactive tag allowed for measurement of only the AOX of interest without interference from AOX present in the secondary treatment systems. Methods for the preparation of $^{36}Cl_2$ had previously been published and are quite simple. Generation of $^{36}ClO_2$ was a much more complicated task. The ^{36}Cl isotope, purchased as $^{36}Cl^-$, had to be transferred to chlorate prior to use in chlorine dioxide generation. A high transfer efficiency of $^{36}Cl^-$ to $^{36}ClO_3^-$ could not be achieved and posed problems throughout the study.

Methods

$^{36}Cl_2$ Generation

$^{36}Cl_2$ was prepared according to a procedure published by Diercks.[99] Briefly, 100 μCi of $H^{36}Cl$ was added to 200 mL of chlorine water and stirred for a few hours. The isotope exchanges into Cl_2 through equilibria such as

$$Cl_2 + Cl^- \Leftrightarrow Cl_3^- \qquad \text{Equation 5}$$

$$Cl_2 + H_2O \Leftrightarrow HOCl + HCl \qquad \text{Equation 6}$$

Exchange between $^{36}Cl^-$ and Cl_2 was confirmed by reacting the labeled chlorine solution with an aqueous phenol solution as described by Diercks.[99] A phenol to chlorine molar ratio of 50:1 was used to insure consumption of active chlorine. The solution was mixed at room temperature for one hour, adjusted to pH 2 with nitric acid, then extracted with toluene. The toluene extract was analyzed for ^{36}Cl. If all the added ^{36}Cl was converted to chlorine, then a distribution of 1:1 for activity in the aqueous and organic phases would be expected. A distribution of approximately 2:3 was determined experimentally. This indicates that the majority of the ^{36}Cl was incorporated into chlorine through the equilibrium reactions.

$^{36}ClO_2$ Generation

The first step in the production of labeled chlorine dioxide was to prepare $K^{36}ClO_3$. Tagged potassium chlorate was prepared through the equilibrium

$$3\ ClO_2^- \Leftrightarrow 2\ ClO_3^- + Cl^- \qquad \text{Equation 7}$$

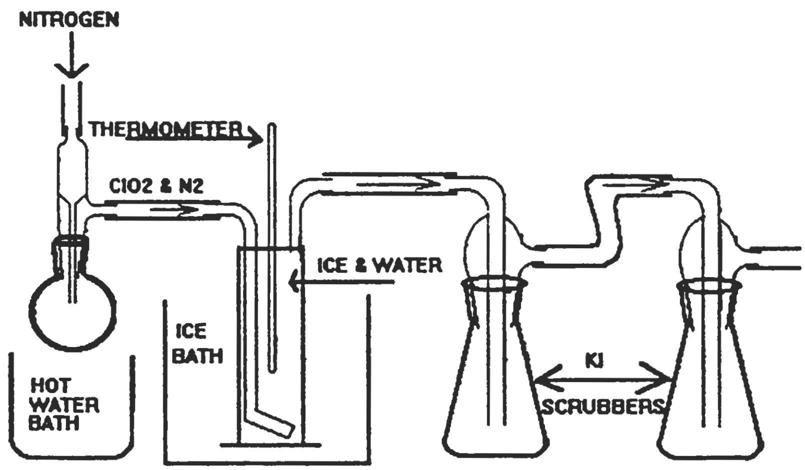
Addition of $^{36}Cl^-$ to a solution of sodium chlorate under neutral conditions leads to isotope incorporation into the chlorate. $H^{36}Cl$ (300 μCi, specific activity 13 mCi/g) was added to a solution of 10 g $NaClO_3$ in 20 mL water. The solution was slowly cycled between 60°C and room temperature over a 4h period on four occasions. As the disproportionation reaction only occurs to any reasonable extent at the higher temperature, heating was necessary. However, chlorate solutions are not stable at high temperature, and therefore, the chlorate solutions was never kept at high temperature for extended periods of time. Solid KOH (5.5g) was then added to the solution at 60°C, and the solution was chilled, upon which $K^{36}ClO_3$ crystallized out. The crystals were washed three times by adding 5 mL of water and stirring for 30 min. with low heat to remove traces of $^{36}Cl^-$. A total of 9.4 g. $KClO_3$ (24 μCi) was obtained indicating an 82% recovery for ClO_3^- and a transfer of 8% of the initial ^{36}Cl activity. Ion chromatographic analysis of recovered chlorate showed that less than 2% by mass of chloride was contained in the precipitate.

Labeled ClO_2 was prepared by the Bray method[100] from tagged potassium chlorate and oxalic acid according to the equation

$$2\,K^{36}ClO_3 + 2\,C_2O_4H_2 \Rightarrow 2\,^{36}ClO_2 + 2\,CO_2 + C_2O_4K_2 + 2\,H_2O \qquad \text{Equation 8}$$

In a typical experiment, $K^{36}ClO_3$ (9.4 g, 24 μCi) and oxalic acid (33 g) were mixed in a round bottom flask. The flask was connected to a trap containing 200 mL of ice water to collect the $^{36}ClO_2$ (Fig. 13). In order to prevent release of radioactive gas, the outflow from the trap was fed to two gas scrubbers containing 200 mL of 150 g/L and 100 g/L of KI respectively. The flask was placed in a 60°C constant temperature bath, swept continuously with nitrogen, and the reaction was initiated by adding 5 mL of water. The reaction was run for 3 hours; longer reaction did not materially increase yield, probably because of ClO_2 loss from the trap. The ClO_2 yield was 1.4 g (3.1 μCi) in 200 mL. A side reaction generates $^{36}Cl_2$ at about 4% of the ClO_2 mass.

Figure 13. ClO_2 Generation Apparatus.



Various scrubber solutions, placed in the line between the reaction vessel and the ClO_2 collector, were tested for their ability to remove residual chlorine (Table 1). As no positive results were achieved through the use of scrubbers, $NaClO_2$ (2.6 g. per g. of Cl_2) crystals were added to the ClO_2 solution to quench the trace level of Cl_2 in the final product. The radioactive Cl_2 is converted to sodium chloride according to the equation

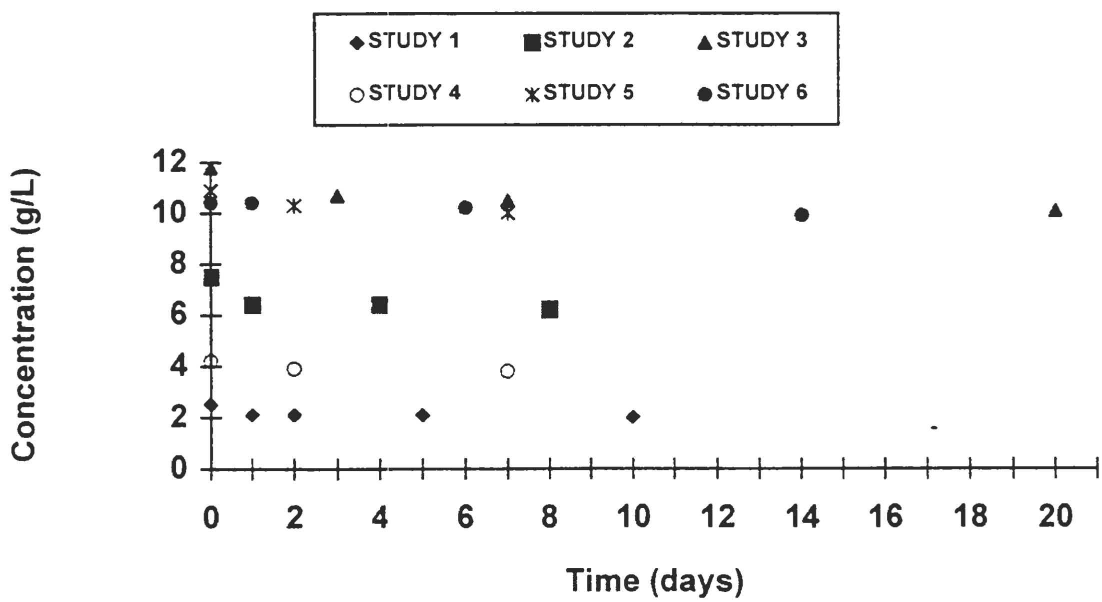
$$2NaClO_2 + {}^{36}Cl_2 \Rightarrow ClO_2 + 2\,Na^{36}Cl \qquad \text{Equation 9}$$

leaving ClO_2 as the only radioactive bleaching reagent. Six of the ClO_2 samples prepared in the trials using the different scrubber solutions (Table 1) were stored in the dark at 4°C and monitored for changes in ClO_2 concentration. Results for the decomposition of chlorine dioxide with time and their corresponding study number are given in Figure 14. For the samples stored the longest periods, sample 3 for two weeks, and sample 6 for three weeks, the concentration decreased by 5% and 14%, respectively (Fig. 14).

Table 1. Scrubber Solutions.

STUDY #	SCRUBBER	pH of ClO_2 COLLECTOR	ClO_2 (g/l)	Cl_2 (g/l)	% Cl_2 (wt./wt.)
1	10% NaOH, 25 mL	2	2.5	0.3	12
2	25 mL H_2O	3	7.5	0.5	7
3	NONE	2.5	11.8	0.9	7
4	10% NaOH, 25 mL	7	4.2	0.4	8
5	25 mL H_2O	7	10.9	0.7	6
6	NONE	7	10.4	0.6	5
7	0.1 M $NaClO_2$, 20 mL	7	10.4	0.9	8

Figure 14. ClO_2 Stability.

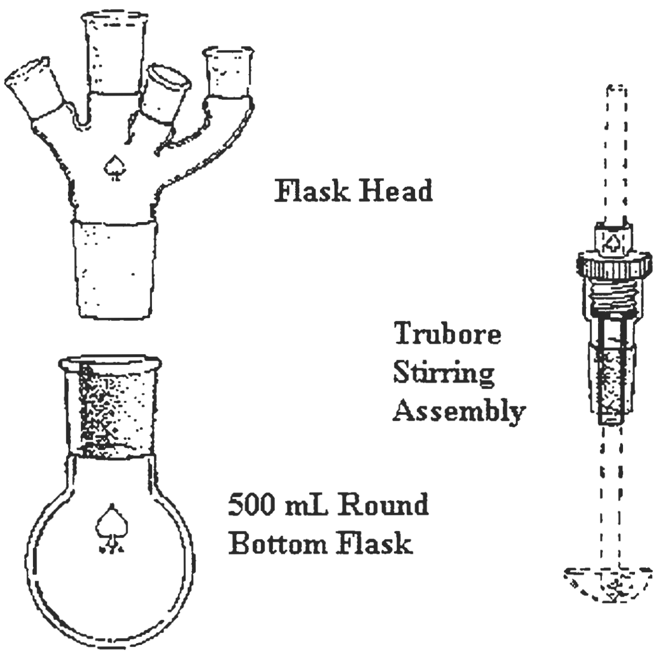


PULP BLEACHING

Bleaching Reactor

A special bleaching reactor vessel was constructed for bleaching with radioactive compounds (Fig. 15). A 500 mL round bottom flask with a 45/50 single neck was fitted with a four necked flask head, with the center position containing a Trubore stirring assembly with a glass shaft and Teflon paddle, and the remaining three ports sealed with Teflon coated septa. The septa allowed for introduction of bleaching chemicals into the sealed reactor. Good pulp mixing was observed for this system at consistencies up to 3%.

Figure 15. Bleaching Reactor.



Pulp

Kappa number 24 pulp from the G-P Brunswick mill was used for the ASB studies and kappa number 27 pulp from G-P Leaf River mill was used for the activated sludge studies. Pulp samples were collected from the brown stock washers at the respective mills. Pulp samples were washed thoroughly at the mill site before packaging into 5 gallon buckets for transportation to IPST. Pulp was stored at 4°C until used. Due to the use of the pulp bleaching filtrates in biodegradation studies, no biocide treatment was applied to the pulp. Prior to the start of the bleaching experiments, the pulp was extensively washed, vacuum filtered, and analyzed for consistency[101] and Kappa number.[102]

Bleaching

Bleaching was done with 100% ClO_2 at a chlorine multiple of 0.25, and 100% Cl_2 at a chlorine multiple of 0.21. Both the bleaching and extraction stages were done at a consistency of 2%. A single D-stage bleach was conducted at 50°C (30 minutes) followed by a single E-stage extraction of 2.4% NaOH at 60°C for 1 hour. Residual chlorine in the D-stage filtrate was <0.07 g/L TAC. Post bleaching Kappa numbers were 5.4 and 6.2 for ClO_2 and Cl_2, respectively, for the Brunswick pulp and 4.4 and 5.5 for ClO_2 and Cl_2 bleaches, respectively, for the Leaf River pulp. In a typical run, the distribution of radioactivity corresponding to both organic and inorganic chlorine in the effluent and pulp was:

	ClO_2-bleaching	**Cl_2-bleaching**
D-stage filtrate:	91%	71%
E-stage filtrate :	8%	12%
pulp:	2%	3%
unaccounted:		14%

Concentration of Bleaching Filtrates

The D- and E-stage filtrates were preserved (addition of 1 mL 0.1 M sodium thiosulfate to D-stage and pH adjustment to 2 for E-stage) and stored separately at 4°C until concentrated. The D- and E-stage samples were mixed 1:1 in a 250 mL round bottom flask to a total volume of 100 mL and frozen in a dry ice-ethanol bath. Once frozen, the samples were placed under vacuum for freeze drying. Samples were concentrated 5-10 fold over a period of 2-3 days each. After concentration, samples were allowed to thaw and were transferred to a 250 mL amber bottle and stored at 4°C until used in the field. All of the concentrate for each bleaching sequence was added to the same storage bottle.

ANALYSIS OF RADIOACTIVE AOX

Since analysis of our radioactive samples with a conventional AOX analyzer would require dedication of an instrument to radioactive use, an alternate procedure was developed. In the conventional Method 1650A of AOX determination, the sample is sorbed on granular charcoal, the inorganic chloride is displaced from the charcoal with a nitrate rinse, the charcoal is combusted converting the AOX to HCl, which is then measured through argentometric titration. In our adaptation of this method, we eliminated the combustion step. Instead, we counted the original solution (prior to charcoal addition) and the nitrate rinsate (which contained the chloride fraction), and determined the AOX by difference.

Methods

The sample (5 mL) was mixed with water (10 mL) and 2 mL of a nitrate solution containing 20 g. KNO_3 and 1.4 mL HNO_3 per liter. Two aliquots (2 mL each) were withdrawn and counted. This provides the total ^{36}Cl in the system from both AOX and chloride ions. Granular activated charcoal was then added (500 mg), the sample was shaken for two hours and then filtered through a 0.45 μm filter. The charcoal sorbs both organic and inorganic chlorine. Chloride ion was removed from the charcoal by rinsing it with 2 mL of the nitrate solution. The filtrate and rinsate were counted, and the AOX sorbed on the charcoal was obtained by difference. Recovery, determined by from processing 9 $^{36}Cl^-$ samples, was 103%; σ = 1%.

For samples of very low activity, as with many of the ClO_2 bleach filtrate samples, the sample volume was adjusted to 10 mL and the water volume adjusted to 5 mL. To ensure that all of the AOX in the samples was removed by the carbon added, cold bleach filtrates were subjected to the test method, with the filtrate being analyzed by conventional AOX analysis. AOX removal by this test method exceeded 97% over the range of 20 to 70 mg/L Cl^-.

Scintillation Counting

Determination of sample activity was done by liquid scintillation counting in a Beckman LS 3801 liquid scintillation counter. The samples to be counted were mixed with 15ml Scintiverse E ® (xylene based scintillation cocktail), shaken, stored in the counter for at least 1 hour, and then counted. Storage in the counter was to allow the fluor molecules excited by fluorescent lighting to return to their ground states. Samples were counted in a window of 400 to 1720 keV for 20 minutes. In order to correct for quenching, a quench curve for ^{36}Cl was prepared by mixing 20 $^{36}Cl^-$ samples with increasing amounts of nitromethane. Samples ranging in H-number (difference in channels between the inflection points of the unquenched standard and the sample) from 93 to 342 were used in preparation of the quench curve. Sample activity values were reported in DPM, the ratio of counts per minute (CPM) to counting efficiency.

In order to determine the effect of high water volumes (water leads to chemical quenching) used in sample analysis, aliquots of 0.5, 1.0, 2.0, & 4.0 mL from a ^{36}Cl water solution were counted. The results indicated no significant difference in DPM/mL calculated for the solution from the four sample sizes. Background radioactivity was measured by counting a series (5 or more samples) of vials containing only 15 mL of scintillation cocktail. The values for the series were averaged, with the mean used for background subtraction and 2σ used for background variability. Background radioactivity levels were measured each time samples were counted.

Relationship of Radioactive AOX Analysis to Conventional Analysis

In order to establish a link between radioactive AOX analysis and conventional AOX analysis, pulp was bleached under identical conditions using both radioactive and nonradioactive bleaching agents. Filtrates from radioactive bleaching were analyzed for total DPM and percent DPM attributable to AOX. Filtrates from cold (bleached with nonradioactive bleaching solutions) bleaching were analyzed for AOX and TOC.

The counts in the D- and E-stage filtrates are associated with AOX as well as with labeled chloride. Results averaged from four radioactive bleaches showed that 6% of the counts in the D-stage were AOX and 35% of the counts in the E-stage were AOX. Total counts found in the D- and E-stage filtrates are relative to the specific activity of the $^{36}ClO_2$ used, and will vary with each batch of $^{36}ClO_2$ prepared. The ratio of total AOX counts in the D-stage to total AOX counts in the E-stage was 4.1, and is independent of total activity of the filtrates.

Results averaged from three cold bleaches indicated a level of 19.4 ppm AOX in the D-stage and 7.5 ppm in the E-stage. Combined bleaching filtrates had an AOX value of 13.4 ppm, representing 1.34 kg AOX per ton OD pulp. This compares favorably with values of 1.5 kg AOX/ton expected for 100% ClO_2 bleaching.[103] The ratio of total AOX in the D-stage to total AOX in the E-stage, as calculated above for radioactive AOX, was 4.1. This indicates an excellent agreement between the methods used for radioactive AOX analysis and conventional AOX analysis. AOX:TOC ratios for combined cold bleach filtrates averaged to 0.08 mg/mg. This indicates a chlorination level of 1 chlorine atom per 37 carbon atoms. The AOX:TOC ratio determined here was not far outside the range of values (0.10-0.177 g AOX/g TOC) reported for treatment pond inlet AOX:TOC.[19,20,26]

In order to relate the radioactive bleaching filtrates to conventional bleaching parameters, one batch each of cold and radioactive bleaching was done for each of the bleaching sequences. Filtrates were collected, stored, and concentrated in exactly the same manner, for both the radioactive and cold bleaches. Results of the analysis for both chlorine dioxide and chlorine bleaching sequences are given below:

Concentrated ClO_2 Bleaching Filtrate

Radioactive

- Concentrated 10 fold
- 38,350 DPM/mL Total Activity
- 13% (4985 DPM/mL) of the Total Activity was as AOX

Cold

- Concentrated 10 fold
- AOX of 187 mg/L
- Cl^- of 1288 mg/L

Relationship of Results

- Ratio of counts in nonadsorbable fraction to mg Cl^- = 26,000 DPM $^{36}Cl^-$/mg cold Cl^-
- Ratio of Counts as AOX to mg AOX = 26,650 DPM labeled AOX/mg cold AOX

Concentrated Cl_2 Bleaching Filtrate

Radioactive

- Concentrated 8.3 fold
- 281.435 DPM/mL Total Activity
- 8% (22,514 DPM/mL) of the Total Activity was AOX

Cold

- Concentrated 8.3 fold
- AOX of 240 mg/L
- Cl^- of 1875 mg/L

Relationship of Results

- Ratio of counts in nonadsorbable fraction to mg Cl^- = 133,000 DPM $^{36}Cl^-$/mg cold Cl^-
- Ratio of Counts as AOX to mg AOX = 93,800 DPM labeled AOX/mg cold AOX

MEASUREMENT OF K_{ow} FOR AOX

An important factor in evaluating the environmental impact of a chemical is its tendency to bioconcentrate. Bioconcentration is predicted for most compounds based on their octanol:water partitioning coefficient (K_{ow}) where:

$$K_{ow} = \text{concentration in octanol} / \text{concentration in water} \qquad \text{Equation 10}$$

The radioactively labeled bleaching filtrates provided a means by which the K_{ow}s for D- and E-stage AOX could be measured. The K_{ow} for combined filtrates were calculated based on the results for the separate stages.

Methods

Bleaching filtrates were prepared by bleaching kappa #33 softwood pulp with $^{36}ClO_2$ at a chlorine multiple of 0.21 and a consistency of 3%. D- and E-stage filtrates were analyzed independently for total counts and percent counts attributable to AOX. Six percent of the total ^{36}Cl in the D-stage was determined to be AOX, while a value of 35% was determined for the E-stage. The concentration of AOX was calculated by multiplying the DPM/mL of the filtrate by the percent counts as AOX to get DPM AOX/mL.

K_{ow} measurements were conducted in 2 mL microcentrifuge tubes. Distilled octanol and filtrate in the proportions 1:1, 2.6:1, & 1:2.6 were added to the tubes . Six samples were prepared for each filtrate at each ratio. Tubes were vortex mixed and then centrifuged to separate the phases. A quantitative sample was then withdrawn from the octanol phase for analysis. The remainder of the octanol phase was then removed and a quantitative sample of the water phase taken.

To determine the partitioning of inorganic chloride into the octanol phase, six samples were prepared using octanol and a $^{36}Cl^-$ solution in a 1:1 ratio. The levels of radioactivity in the octanol phase of all samples was found to be within 1 standard deviation of background, indicating that less than 1% of the chloride partitions into the octanol. Based on this analysis, all the activity in the octanol phase of the filtrate samples was attributed to AOX material.

Total DPM AOX in each sample was determined by multiplying the DPM/mL of the filtrate used by its percent AOX and the volume added. The total DPM for the octanol phase was subtracted from total DPM AOX, and this value was assigned as DPM AOX in the water phase. The DPM in the octanol phase was divided by DPM AOX in the water phase to get K_{ow}. The K_{ow} values for combined filtrates were calculated from the values for the separate filtrates rather then direct measurement. The average values for the samples tested are given in Table 2.

Table 2. K_{ow} Results.

Filtrate	Octanol (mL)	Water (mL)	K_{ow}	Standard Deviation
D-stage	0.9	0.9	0.4	0.02
D-stage	1.3	0.5	0.3	0.02
D-stage	0.5	1.3	0.6	0.04
E-stage	0.9	0.9	0.3	0.01
E-stage	1.3	0.5	0.2	0.01
E-stage	0.5	1.3	0.7	0.05
Combined	0.9	0.9	0.4	0.02
Combined	1.3	0.5	0.3	0.02
Combined	0.5	1.3	0.6	0.03

Results

As can be seen by the low K_{ow} values for the pulp bleaching filtrates, AOX as a whole, has a very low potential for bioaccumulation. The low K_{ow} values also indicate that the majority of the components of AOX are hydrophilic compounds. A high content of carboxylic groups in the AOX, as suggested by Osterberg and Lindstrom,[68] is also consistent with these results. Approximately one-third of the AOX material does partition into to the organic phase. This fraction is likely comprised of the higher molecular weight and/or more highly chlorinated material. The low K_{ow} value for AOX as a whole does not indicate the absence of specific compounds of high K_{ow} and of significant environmental concern.

In a previous study by Diercks and Banerjee,[99] K_{ow} values were measured for Cl_2 bleaching filtrates. A K_{ow} of 0.02 was reported for C-stage filtrates and a K_{ow} of 0.002 was reported for E-stage filtrates. Variations in Cl_2-multiple, percent caustic applied, and chlorine dioxide substitution did not make a significant difference in the values of K_{ow}. While the K_{ow} values reported in their study were lower than those reported here, both studies determined that, as a whole, AOX has a low potential for bioconcentration.

DEVELOPMENT OF A SYSTEM FOR IN-SITU MEASUREMENTS

Once it had been determined that ^{36}Cl labeled AOX could be produced, a system for deploying this material in the field had to be developed. In designing the field testing system, the following items proved challenging:

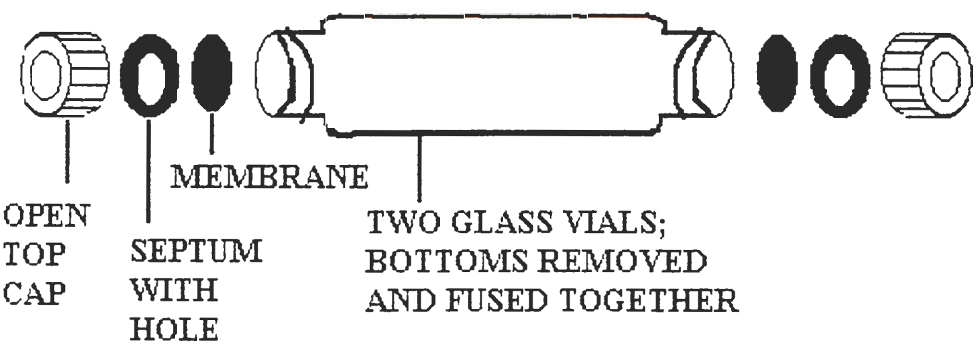
- design of a cell to hold the radioactive AOX and pond sample;
- design a vehicle to suspend samples across the depth of the pond that was portable yet rugged enough to withstand pond conditions over prolonged exposure;
- design a pond water sampler to collect precise depth samples;
- selection of a membrane to retain AOX while maintaining the sample at pond conditions.

Each aspect of the design will be discussed in detail in the sections to follow.

Sample Cell Construction

To make assembly in the field possible, the sample cells had to be easily capped, on both ends, with semipermeable membranes under various conditions. The caps also needed to be easily removable for sample collection. At the same time, the membranes had to be securely attached so as to not come loose in the turbulent water around aerators. To accomplish this, two 20 mL borosilicate glass vials were cut in half. The top halves of the two vials were then fused together (Fig. 16) to form a sample cell with a threaded mouth at each end. Membranes could then be attached to either end using open top phenolic closures. To ensure a tight seal between the membrane and the lip of the cell, a Teflon coated silicone septa with a hole equivalent to the diameter of the opening in the cap was used between the cap and the membrane.

Figure 16. Sample Cell Construction.



Construction of Sample Cell Holder

The sample cells needed to be suspended at specified intervals across the depth of the treatment system. Given that variation in depth can be significant from one position to another in a secondary treatment system, the suspension system had to be adjustable to accommodate various depths. The system also had to be portable, as transportation from IPST to field sites on a routine basis was necessary. As treatment pond environments tend to be corrosive, the materials used had to be noncorrosive. Due to extreme turbulence in positions near operating aerators, the system had to be very strong and durable.

A rigid probe system in which the sample cells could be inserted through the probe and locked into place was constructed. 11/2" Schedule 80 PVC was chosen as the probe material. The probes were constructed in 5 foot sections and connected together by PVC unions. Each probe consisted of one section with an 8" by 10" metal base plate, two middle sections, and one section with a PVC cap and radioactive material marker (Fig. 17). The number of middle sections could be altered to achieve any length needed (in five foot intervals).

One inch holes were drilled through the sections of pipe at one foot intervals. In the middle sections of selected probes, a series of five holes were drilled close together (2 inch spacing) to be used for duplicate samples. Small holes was drilled through the pipe perpendicular to the one inch holes, at positions corresponding to the center line of the one inch holes. The small holes were threaded and nylon screws inserted. The sample cells could be inserted through the pipe in the large holes and the nylon screws tightened against them to lock them in place.

Pond Water Sampler Construction

As each sample cell was intended to measure biodegradation at a discrete location, a mechanism to collect samples from any given position in the pond without mixing the sample with water from other parts of the pond had to be developed. This was accomplished by attaching a glass bottle to a sampling pole via an aluminum bracket and strapping clamp (Fig. 18). A spring loaded stopper was affixed above the bottle so that the stopper rested firmly against the mouth of the bottle. The sampling pole was marked in one foot intervals to indicate the depth from which the sample was taken. Upon submersion of the bottle to the appropriate depth, the bottle was opened by pulling a string attached to the stopper. Once the bottle was full, the string was released to reseal the bottle.

Figure 17. Probe for Holding Sample Cells.

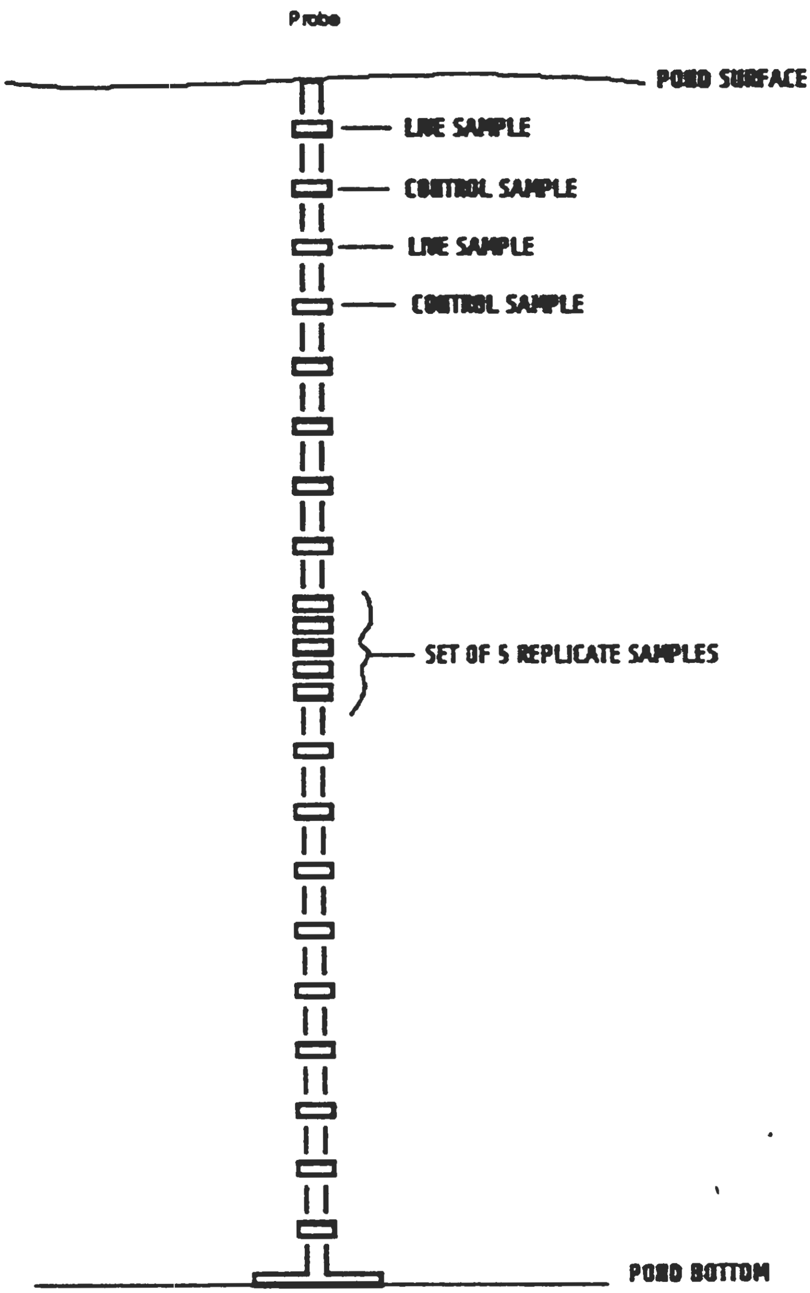
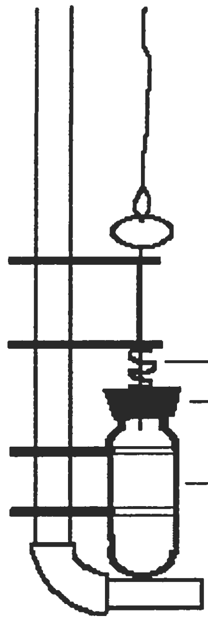


Figure 18. Pond Water Sampler.



Membrane Selection

One of the most critical aspects in the design of the field testing system was the selection of a semipermeable membrane for capping the sample cells. Ideally, the membrane would retard the diffusion of AOX material while, at the same time, allow sufficient flow of Cl^-, ClO_3^-, and pond nutrients (such as O_2). A series of experiments were run to test a variety of membranes for their applicability to field testing (Table 3). Sample cells were prepared which contained 2 mL concentrated filtrate and deionized water for the various membranes. Sample cells were then individually placed into 1 L buckets. The water in each bucket was changed daily. Sample cells were recovered after seven days and analyzed. Membranes showing the most potential were then subjected to simulated field conditions in a flow through chamber (10 L container with a constant flow of fresh water passing through). Sample cells were analyzed for retention of AOX, Cl^-, and ClO_3^- after seven days.

Results

Both the 0.01 μm PCTE and 500D dialysis membranes had very similar diffusion characteristics for all species tested (Table 3). The decision to use the PCTE membranes was based on the physical properties of the membrane as well as availability. The PCTE membranes are much stronger and more tear resistant than the cellulose ester membranes. The cellulose ester membranes require constant submersion in water and refrigeration, a serious complication for field testing. The PCTE membranes required no special handling procedures and can be exposed to air for extended periods with no detrimental effects. The 0.01μm PCTE membrane was tested under simulated field conditions to estimate AOX losses during field studies. For the simulated field tests, diffusion was measured in a constant flow chamber. The flow chamber consisted of a sealed 10 L polyethylene chamber with a constant flow of water passing through it. After seven days, retention of AOX was 35%, retention of Cl^- was 6%, and retention of ClO_3^- was 17%. Although these values were not ideal (AOX retention was too low and ClO_3^- retention was too high), this is probably the best that can be achieved given the molecular weight range for AOX material.

Table 3. Membrane Analysis.

MEMBRANE	% AOX RETAINED	% Cl^- LOST	% ClO_3^- LOST
0.45 μm PCTE*	19	Equilibrium (98)	
0.22 μm PCTE	19	Equilibrium (98)	
0.05 μm PCTE	40		
0.01 μm PCTE	45	86	63
1000 D**	37		
500 D	45	87	59
100 D	87	15	

* Polycarbonate Track Etched Membranes from Poretics.

** Cellulose Ester Dialysis Membranes from Spectra/Por.

FIELD STUDIES

Approach

The objective was to study AOX biodegradation in the field under normal treatment conditions. This was achieved by means of the radioactive AOX concentrate used in conjunction with the field sampling system previously described. Samples were drawn at one foot intervals through the depth of the pond at each location to be tested. The pond water samples were added to the sample cells and then spiked with concentrated radioactive AOX. The sample cells were capped at each end with a semipermeable membrane. The membranes restrict the microorganisms and the tagged AOX inside the vials, but allow flow of DO and nutrients. The addition of the radioactive AOX increases the AOX level inside the vial to 3-4 times pond level for the ASB study, and 4-5 times pond level for the activated sludge study. The vials were then inserted in a sample probe at 1-foot intervals corresponding to the position in the pond from which they were taken. The sample probe was placed in the treatment system for a period equal to the retention time in the pond. Thus, the radioactive AOX experienced the conditions prevalent at a given x,y,z coordinate in the pond. A unique feature of our approach is that the radioactive AOX added can be distinguished from the AOX already present in the pond. The high precision and sensitivity associated with radioactivity measurements allows a controlled experiment to be conducted in the field.

Theory

The membrane holds the microorganisms captive in the vial, but permits partial leakage of the AOX. Ideally, one would prefer all the AOX to be contained in the vial, but a membrane of pore size small enough to achieve this also restricts flow of inorganic ions. Thus, we compromised on a membrane that allowed transport of the nutrients and dissolved oxygen (DO), but which also allowed potential outflow of radioactive AOX from the vial, and inflow of AOX present in the lagoon into the vials. The non-radioactive AOX in the lagoon does not interfere since we only measure radioactivity.

In addition to biodegradation, AOX loss from the vials can occur through diffusion, sorption, and chemical degradation. In order to account for nonbiological losses, every second vial was sterilized by adding formalin to its contents (15 mL formalin was mixed with 200 mL pond water prior to addition to the sample cells). Now, the difference in AOX concentrations between adjacent vials more nearly reflects biodegradation. A final complication is that AOX leakage is not necessarily uniform across the vials in a sampler, since, for example, the outer surface of the membranes may be partially clogged by particulate in the pond. This was compensated for by using the $^{36}Cl^-$ present in the solution as a product of bleaching as a conservative tracer. Since $^{36}Cl^-$ will principally be lost through diffusion, the rate of $^{36}Cl^-$ removal can be used to normalize the rate of AOX diffusion across the length of the sampler. By assuming that diffusion of both AOX and $^{36}Cl^-$ follow first-order kinetics, the AOX lost through biodegradation could be obtained as follows.

The ratio of first-order rate constants for diffusional loss of AOX and $^{36}Cl^-$ (chloride) from a sterilized vial is

$$\frac{k_{AOX}}{k_{chloride}} = \frac{\ln[AOX]_o - \ln[AOX]}{\ln[chloride]_o - \ln[chloride]} \qquad \text{Equation 11}$$

This ratio is averaged for a pair of sterile vials straddling a sample vial. The rate constant for diffusional AOX loss from a "live" vial is

$$(k_{AOX})_{diff} = (k_{AOX}/k_{chloride})_{sterile}(k_{chloride})_{live} \qquad \text{Equation 12}$$

where $(k_{chloride})_{live}$ represents loss of chloride from the live vial. If $k_{AOX}/k_{chloride}$ for diffusional loss remains constant for both live and sterilized vials, then the above equation normalizes small differences in AOX diffusivity caused by variability in membrane permeability. AOX loss from the live vial due to diffusion can be expressed as

$$[AOX]_{diff} = [AOX]_o exp(-k_{AOX})_{diff} t \qquad \text{Equation 13}$$

$$= [AOX]_o exp-(k_{AOX}/k_{chloride})_{sterile} (k_{chloride})_{live}\, t \qquad \text{Equation 14}$$

$$= [AOX]_o\, exp- (k_{AOX}/k_{chloride})_{sterile} ln[chloride]_o/[chloride]_{live}$$

Equation 15

Hence, the amount of AOX biodegraded is

$$[AOX]_{bio} = [AOX]_{diff} - [AOX] \qquad \text{Equation 16}$$

where [AOX] is the concentration remaining in the unsterilized vial. Final biodegradation results are given as percent degradation and were calculated according to the following

$$\%AOX\ biodegraded = [AOX]_{bio}/[AOX] * 100 \qquad \text{Equation 17}$$

In summary, the approach consists of the following steps.

1. Samples are drawn from various depths at a given location in the pond. Each sample is amended with [^{36}Cl] AOX prepared from either $^{36}Cl_2$ or $^{36}ClO_2$ bleached pulp.

2. The amended samples are placed in vials capped on both ends with semi-permeable membranes. The membranes permit free flow of oxygen and nutrients, holds the organisms in place, and partially restricts outflow of AOX.

3. The vials are placed in samplers at 1-foot intervals extending to the bottom of the pond. Every second vial is sterilized. The vials are retrieved after a period equal to the hydraulic retention time of the pond.

4. The [^{36}Cl]AOX and $^{36}Cl^-$ are determined in each vial. In order to minimize variability in diffusivity for membranes attached to the various vials in a sampler, the ^{36}Cl[AOX] in each vial is normalized with respect to the $^{36}Cl^-$ remaining in that vial. Comparison of the AOX in a live vial to that in an adjacent sterilized vial provides the amount of AOX biodegraded.

Confirmation of First Order Diffusion

A key assumption in the theory behind the calculations was that diffusion for both $^{36}Cl^-$ and AOX followed first order kinetics (*d[conc]/dt = -kt[conc]*). To confirm this assumption, diffusion from sample cells was monitored over a period of 12 days for both chlorine and chlorine dioxide bleaching filtrates in the continuous flow chamber (described earlier). Data from these experiments is plotted in Figures 19-20. As can be seen by the two plots. AOX from both bleaching sequences, as well as the Cl^-, follow first order kinetics for diffusion over the range of interest. This is an important aspect in confirming the applicability of the equations developed to real systems.

Figure 19. Diffusion for $^{36}Cl_2$ bleaching filtrates.

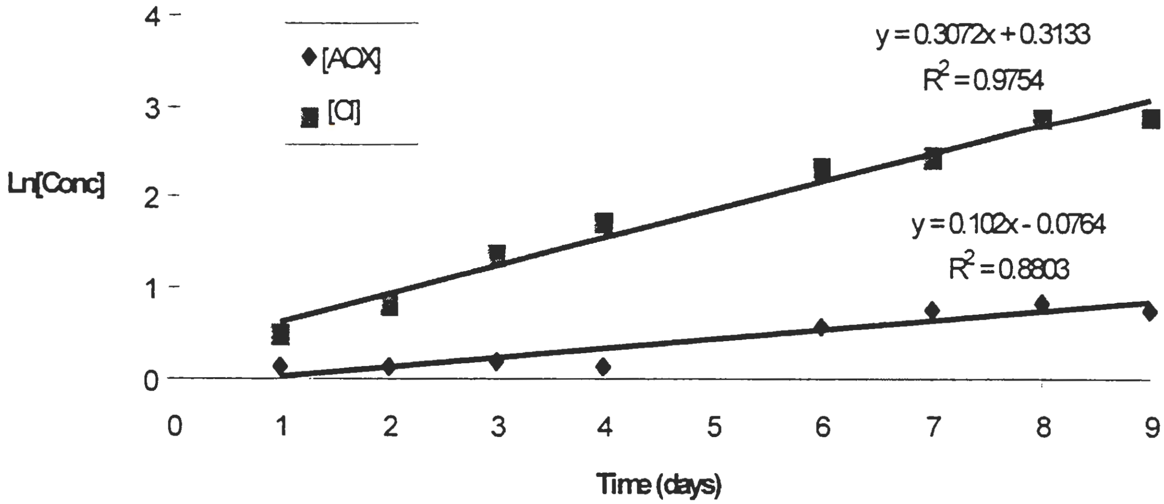
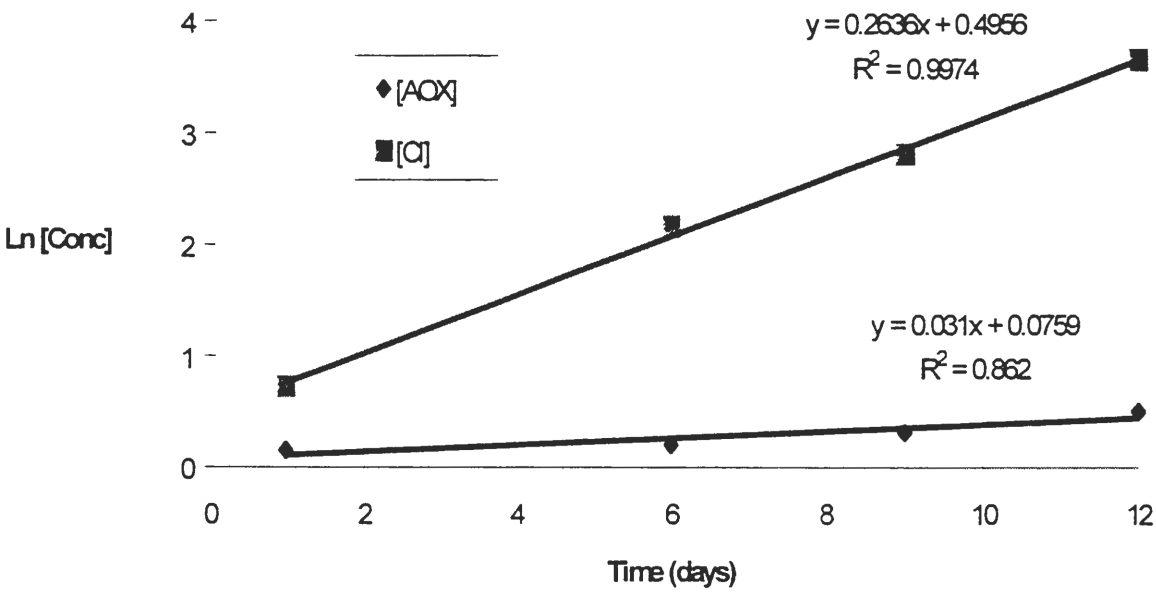


Figure 20. Diffusion for $^{36}ClO_2$ bleaching filtrates.



Field Experimental Procedures

Samples were prepared according to the following procedures then loaded into probes and submerged in the treatment pond. Water samples were withdrawn from the pond at one foot intervals across the depth of the pond. If the depth at the site was not in the range 12-30 feet, an alternative site was chosen if one was available in close proximity to the initial location. Pond samples were immediately sealed in 500 mL buckets after collection. Sample cells were prepared one at a time while at the sampling site according to the following procedure:

1. Add 200 mL of pond sample from alternating position to 250 mL amber jars containing 15 mL formalin then shake (control samples);
2. Cap one end of a sample cell with a 0.01 μm PCTE membrane;
3. Add 10 mL pond sample (from the amber jars for the control samples) to the sample cell;
4. Add 2 mL concentrated radioactive AOX;
5. Fill cell with pond sample (from the amber jars for the control samples);
6. Cap open end with membrane;
7. Place sample cell into pond sample bucket until placed into probe.

Once all the sample cells for a site were prepared, they were placed into a probe and lowered into the pond. The amount of time that either pond samples or completed sample cells were exposed to ambient conditions was kept to a minimum. Sample cell preparation was conducted at the sampling site to minimize the time between pond sampling and sample cell preparation.

After submersion in the pond for a period equivalent to the pond retention time, probes were extracted and sample cells placed into the 500 mL buckets. Samples were immediately returned to shore for processing. Sample cell contents were transferred into 30 mL vials containing 1.5 mL formalin each. Contents from four sample cells (2 live and 2 control) were transferred to vials without formalin to be tested for microbial viability. The vials were labeled then stored on ice for transport back to IPST. Once at IPST, samples were stored at 4°C until analyzed.

As the ASB study progressed, it became evident that returning the pond samples to the dock house for sample cell preparation was more efficient than on site preparation, due to poor working conditions experienced on the pond. After the first field study, steps 1-6 were conducted on the dock rather than on site. Samples for the activated sludge system study were all taken to a truck parked next to the pond for processing. The time cycle for sample collection, sample cell preparation, and sample deployment was much shorter at the activated sludge system than at the ASB, due mainly to reductions in transport times for pond samples between the collection point and the preparation area.

Solids levels were noticeably higher in the activated sludge system than in the ASB (300 mg/L as opposed to 100 mg/L) and the solids tended to settle out rather quickly in the ASB samples. Samples had to be thoroughly mixed before each aliquot was taken. Achieving a solids level in the sample cell equal to that of the corresponding pond position added an additional area for variability. Unless the majority of AOX reduction is attributable to a biomass dependent process, small variations is biomass will not significantly effect the results obtained.

Microbial Viability

One live and one control sample from the ClO_2 filtrate study at aerator 21 and the Cl_2 filtrate study at the Metal Post position were collected in vials without formalin. These four samples were plated on potato dextrose agar and incubated at 30°C. After one day, both of the live samples indicated microbial growth (appearance of colonies of white cells) while neither of the control samples displayed any signs of growth. After two days, both live samples showed increases of the white colonies and the appearance of orange colonies. After a three week period, neither of the control samples displayed any signs of growth.

This confirms that the microbes survived the treatment with labeled AOX and their residence time in the sample cells. It also confirms the success of the control measures used.

Calculation of ^{36}Cl Recoveries

Aliquots (2 mL) were taken from each vial, mixed with 15 mL scintillation cocktail, and then counted to determine DPM/mL. The samples were then analyzed for percent AOX by the method previously described. Based on the results for DPM/mL and percent AOX measurements, recoveries for tagged AOX and $^{36}Cl^-$ were calculated as follows:

Calculation of labeled AOX concentration:

$$\text{DPM/mL recovered} * \text{\% AOX} = \text{DPM AOX/mL} \qquad \text{Equation 18}$$

Calculation of total recovered labeled AOX:

$$\text{DPM AOX/mL} * (\text{Cell Volume} + 1.5\text{ mL}) = \text{DPM AOX}_{(REC)} \qquad \text{Equation 19}$$

Calculation of percent labeled AOX recovered:

$$(\text{DPM AOX}_{(REC)}/\text{DPM AOX}_{(INIT)}) * 100 = \text{\% AOX}_{(REC)} \qquad \text{Equation 20}$$

Calculation of $^{36}Cl^-$ concentration:

$$\text{DPM/mL recovered} * (1\text{-\% AOX}) = \text{DPM } {}^{36}Cl^-/\text{mL} \qquad \text{Equation 21}$$

Calculation of total $^{36}Cl^-$ recovered:

$$\text{DPM } {}^{36}Cl^-/\text{mL} * (\text{Cell Volume} + 1.5\text{ mL}) = \text{DPM } {}^{36}Cl^-_{(REC)} \qquad \text{Equation 22}$$

Calculation of percent $^{36}Cl^-$ recovered:

$$(\text{DPM } {}^{36}Cl^-_{(REC)}/\text{DPM } {}^{36}Cl^-_{(REC)}) * 100 = \text{\% } {}^{36}Cl^-_{(REC)} \qquad \text{Equation 23}$$

The values calculated for percent tagged AOX recovered and percent $^{36}Cl^-$ recovered were then inserted into the equations developed in the theoretical section to calculate values for AOX biodegradation.

Statistical Analysis of Samples

In some probes, several samples differed significantly from the others in the amount of activity recovered. This is most likely indicative of either leakage around the membrane or blockage of the membrane depending on the sign of the deviation. In order to determine which samples experienced significantly different diffusion rates, recovery values for $^{36}Cl^-$ for each cell were tested against the average for the probe.[104] Samples for which $^{36}Cl^-$ recovered tested positive as an outlier were considered as invalid, and excluded from consideration in percent degradation calculations. Each of the probes contained some invalidated sample cells (either by outlying $^{36}Cl^-$ recovery values or physical damage to the cell). In the cases where the lost cells were control samples, the next closest control sample was used in place of the lost sample for biodegradation calculations.

Sets of five replicate samples were deployed in selected probes. These sample replicates were used to calculate 95% confidence intervals for determination of percent biodegradation. Calculations were based on the pivotal method[105], developed for use with small sample sets. In cases where more than one of the five samples was invalidated, the entire set was considered invalid. The largest confidence interval observed in each study was used to set the confidence limits for the study.

Field Studies at an ASB in Brunswick, Georgia

The Brunswick facility pulps both hardwood and softwood with an operating capacity of 2150 tons/day. This facility produces bleached kraft market pulp, along with linerboard and plate stock. The bleaching sequence used is DEOPDEPD. The D-stage is done at 4% consistency, 90-95°F, for 15-25 min. The E-stage has a retention time of 30 min. and is done at 165°F. Washing is counter current with machine white water or fresh water to the fifth stage. Only half the wash at the first stage (top shower) is recycle, with the bottom shower being fresh water. Of the 40 mgd of fresh water used by the mill, the bleach plant sewers 30 mgd.

The treatment system was designed to remove effluent BOD in order to meet guidelines set by the state of Georgia for release into the adjacent Turtle River. The first stage in the Brunswick pond is primary treatment of the alkaline and process sewers in a Dorr-Oliver clarifier completed in July, 1970. Its dimensions are 320 ft. diameter, 14 ft. sidewall depth, and 27 ft. center depth. It has a holding capacity of 12.3 million gallons, a flowing capacity of 54 million gallons, and is operated at a rate of 20-24 million gallons/day. The effluent from the primary settling tank is mixed with the acid sewer in a small channel leading to the secondary treatment system (Fig. 21). Secondary treatment occurs in a 6 acre presettling basin (8.6 hr retention), an 88 acre aeration lagoon (5.14 day retention), and a 6 acre settling lagoon (8.6 hr retention). At the time of this study, the aeration lagoon had 39 Ashbrook (75 or 100 HP) and two Aire-O2 100 HP surface aerators. Total flow to the river is 40 mgd. BOD removal was 90,000 lbs/day which represents a 90% removal rate. Average AOX was 6.5 ppm at the exit and 13.8 ppm at the inlet, representing a reduction of 53%.

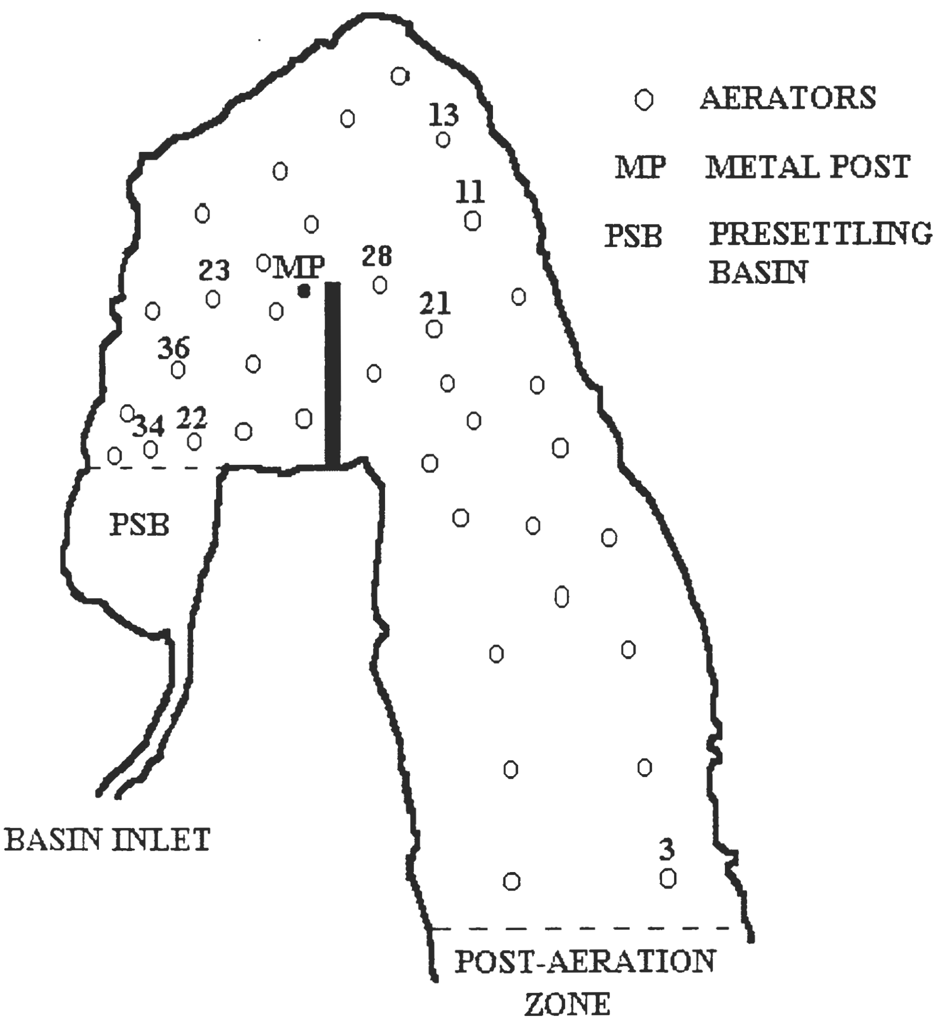
Three field studies were conducted at the ASB. Major characteristics of the ASB system pertaining to biodegradation in the water column include:

- retention time of 5 days;
- low levels of dissolved oxygen in the pond;
- low TSS values except in the bottom foot of the pond (sludge blanket);
- no method employed to control microbial population;
- very non-uniform pond conditions;

- single introduction point for inlet flow.

Field studies were conducted in September of 1994, January of 1995, and September of 1995. Over the series of field studies, sample probe deployment was designed to give a representative sampling of both aerated and non-aerated sites across the entire pond. The intent of the studies carried out at this facility was to measure the biological degradation of AOX with respect to position within the treatment system.

Figure 21. Diagram of the Brunswick mill ASB pond.



Deployment Scheme

Due to the vast expanse of the ASB (approximately 100 acres), measurements were made at different locations in a series of field exercises. Locations for measurements were chosen based on their position in the pond relative to the inlet and outlet points as well as their distance from active aerators. The intent was to measure biodegradation at evenly spaced intervals from the inlet to the outlet in both aerated and nonaerated regions. In the first field study, sample probes were deployed at aerators 21 & 36 and a metal post near the middle of the pond. In the second field study, sample probes were deployed at aerators 34 & 3, presettling zone (non-aerated), and the post aeration (PA) zone (Fig. 21). For the first two field studies, probes containing samples for ClO_2 and Cl_2 bleaching filtrates were deployed side by side at all positions. The third field study at the Brunswick facility consisted of one probe for ClO_2 bleaching filtrate deployed at aerator 22. The location of the sampling positions are depicted in Figure 21.

Background Variability Study

In scintillation counting, the DPM value measured for any sample includes counts due to background radioactivity. The counts attributable to background must be subtracted from the measured value. Therefore, the accuracy with which the activity of a sample can be measured is dependent on the accuracy with which the background radioactivity can be determined and the activity level of the sample (at sufficiently high activity levels background radioactivity is insignificant). Background radioactivity levels in unknown samples can be affected by many factors including exposure to UV light, water content, chemical content, and solids content. To most accurately determine background radioactivity, sample blanks should resemble unknown samples as closely as possible. Only in the case of very low level samples, where precision is imperative, does the composition of the sample blank become significant.

Due to the low level of activity in the $^{36}ClO_2$ bleaching filtrates, it was necessary to precisely determine the background variability induced by the pond water. To accomplish this, ten samples of pond water were counted both before and after carbon filtering (2 mL sample in 15 mL scintillant). In addition, 4 samples each of $^{36}Cl^-$ solutions in DPM/mL ranges of 100, 1000, and 20,000 were analyzed to determine effects of background variation at different DPM levels. Background radioactivity for the ASB samples was determined to be 53.4 DPM ±1.4 DPM at a 95% confidence interval. In the 100 DPM/mL range, representative of the low end of the range for recovered $^{36}ClO_2$ bleaching filtrate samples, analysis of variance by the Pivotal Method[105] indicated confidence limits of ±2% for a 95% confidence interval. For samples with DPM/mL values of 1000 and above, confidence limits were less than ±1% for a 95%confidence interval.

Pond Water Analysis

Pond water samples were collected and analyzed for a variety of properties. Samples were analyzed at Georgia Southern University for DO, temperature, TSS, BOD, COD, and Total Kjeldahl Nitrogen. Results from these analysis are given in Appendix A. Samples were analyzed at IPST for AOX, Cl^-, and ClO_3^-. AOX analysis was conducted with a Dhormann DX-20A/B TOCl analyzer according to Scandinavian Pulp, Paper, and Board Testing Committee Method SCAN-W 9:89. Chloride and chlorate analysis were conducted with a Dionex Ion Chromatography system according to TAPPI Method T 699 om-87. Results for these analysis are given in Table 4.

Table 4. Pond Water Analysis.

Position	Depth (ft.)	AOX (ppm)	Cl^- (ppm)	ClO_3^-(ppm)
Aerator 21	2		508	936
Aerator 21	10	5.7	527	1411
Aerator 21	15		527	1680
Aerator 3	6	5.8	635	1640

Sorption to Pond Solids

One of the theories proposed for the removal of AOX by secondary treatment systems, is the sorption of AOX to pond solids followed by settling to the benthic zone.[60] ASB samples were analyzed for sorption of AOX to solids material. Random pond samples collected from the ASB were mixed with concentrated radioactive filtrates from both chlorine and chlorine dioxide bleaching. Samples were then filtered through a 0.45 μm PCTE membrane. The membranes were then analyzed for DPM values. Control samples of $^{36}Cl^{-}$ mixed with pond samples were analyzed to determine to sorption of inorganic chloride to pond solids. Finally, samples of the concentrated filtrates were diluted 1:10 then filtered through the 0.45μm PCTE membrane.

No activity above background was found on the filters from the control samples, indicating no sorption of inorganic chloride to pond solids. No significant (DPM values on filters were less than 2 standard deviations above background) sorption of AOX from chlorine dioxide bleaching occurred in any of the samples. A higher level of activity was present on the filters from the chlorine bleaching filtrate concentrate samples, ~6% of the initial DPM added. However, filtration of the diluted chlorine bleaching concentrate alone indicated a value of ~6% for residual counts on the filter. Therefore, no additional removal of AOX from the chlorine bleaching filtrates occurred upon reaction with pond solids. The activity removed by filtration of the diluted chlorine bleaching filtrate concentrate is due to a small amount of particulate matter that forms upon concentration.

Field Studies at an Activated Sludge System in New Augusta, Mississippi

The Leaf River mill began operations in 1984 and is in the market bleached kraft subcategory. Total pulp production is approximately 1,500 ton/day. The mill uses a single continuous Kamyr dual-vessel hydraulic unit. The mill has one five stage bleach line that swings between softwood and hardwood. The bleaching sequence employed is DEOPDEPD with counter current washing. Pulp dryer whitewater was used as wash water for the final chlorine dioxide stage and hot water was used for half the wash on the first stage. Approximately 20 million gallons of water are taken daily from the Leaf River, treated by coagulation in a clarifier and passed through gravity filters before use.

Wastewater from the chemical recovery area, the pulping area, the pulp dryer, the woodyard, and the power boiler, as well as storm water, enters the wastewater treatment system through the bar screen. This water is clarified, mixed with the acid and caustic sewers, and introduced into an equalization basin. The equalization basin is aerated with ten 40 HP floating aerators, has an area of 237,864 square feet, and a retention time of 16.5 hours. Following equalization, nutrients (ammonia and phosphoric acid) are added, and the wastewater flows to a cooling tower which discharges to a 48 million gallon aeration basin (Fig. 22). The aeration basin has a retention time of 45 hours and is aerated by five 700 HP blowers. After aeration, the wastewater is clarified in secondary clarifiers, 9.4 hour retention time and a 40% recycle of sludge, then discharged to a holding pond prior to release into the Leaf River. The mill discharges approximately 18 million gallons per day of treated wastewater to the Leaf River. The average 1994 AOX at the inlet was 16.3 ppm and at the outlet was 5 ppm, indicating a reduction of 69%.[90]

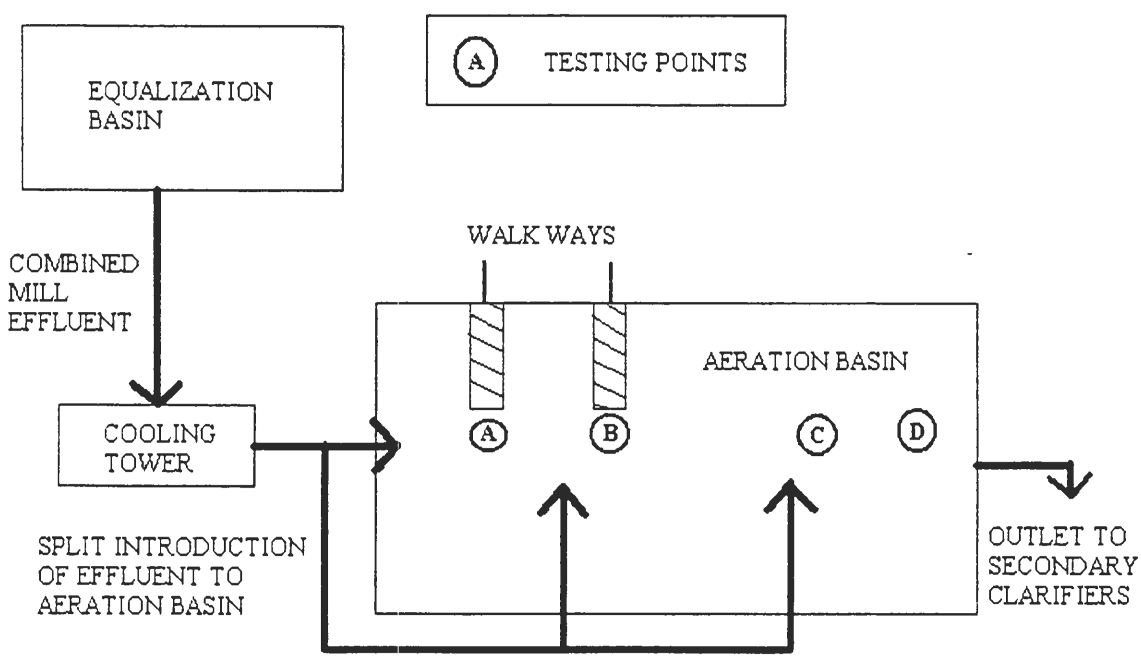
The activated sludge system is comprised of an aeration basin, secondary clarification basins, and aerating equipment. Microorganisms eliminate and partly respire the organic matter contained in the waste water, or convert the organic matter into sedimentary biomass. The activated sludge is mixed with dissolved oxygen and waste water through air injection ports on the basin floor. This system ensures that microorganisms in the activated sludge are kept in constant suspension, are maintained in constant contact with organic pollutants, and have sufficient dissolved oxygen. Pumps are provided to recirculate the required sludge from secondary sedimentation tanks to maintain a sufficient quantity of biomass. This system allows for much greater control over the conditions in the treatment system as well as requiring much less space. On the other hand, the capital investment for an activated sludge system is significantly higher than that for an aeration basin. As regulation on effluents becomes more stringent and land becomes less available, many more mills will progress toward activated sludge systems.

Major differences between this system and the ASB at the Brunswick facility include:

- pond dissolved oxygen level maintained at approximately 6 mg/L;
- a TSS level of 300 mg/L (about three times that of the ASB);
- considerably higher microbial population (40% of secondary sludge is recycled to the aeration basin;
- very uniform conditions throughout the basin;
- split introduction of inlet waste water.

Unlike the ASB system, little variation in DO, solids, or mixing conditions was expected with pond position and, therefore, fewer positions were tested. Tests were primarily conducted to determine the percentage of the observed AOX removal that could be attributed to biodegradation.

Figure 22. Diagram of the Leaf River Activated Sludge System.



Deployment Scheme

The activated sludge system is much more compact than the ASB and, therefore, required fewer sampling points. In addition, being an activated sludge system with a split inlet system, variation with respect to position or depth was much less likely than in the ASB system. Four sampling points were chosen, one after each inlet point and one near the pond outlet. Sampling locations are depicted in Figure 22 as positions A, B, C, & D. One probe for Cl_2 bleaching filtrates and one probe for ClO_2 bleaching filtrates was deployed at each of the sampling locations.

Background variability study

It was necessary to determine the precise level of background variation caused by the solids present in the pond water for the activated sludge system, as the solids level in this system was significantly higher than that of the ASB system. The procedures used were exactly as in the background variability study for the ASB system. Confidence limits for the determination of background radioactivity at a 95% confidence interval were ±7% for the activated sludge samples as opposed to ±1.3% for the ASB samples. The high variation in background causes a high degree of variability in the low DPM samples. For the 100 DPM/mL range, representative of the $^{36}ClO_2$ bleaching filtrates, confidence limits for counting unknown samples were ±6.3% at a 95% confidence interval. This raised concern as to the accuracy of percent AOX determination in the low DPM samples. Determination of percent AOX for 100 DPM/mL samples, at the 50% AOX level (most accurately measured value), will have confidence limits of 50(±8)% at a 95% confidence interval. Random variation of ±8% for percent AOX determination will make any samples in the 100 DPM/mL range unreliable. As DPM/mL values reach 1000 and above, the confidence limits drop to 0.4% at a 95% confidence interval. These results confirm the validity of the results for the chlorine bleaching filtrates while discounting any results for the chlorine dioxide bleaching filtrates. Due to this, no further discussion of chlorine dioxide bleaching filtrates will be included for the activated sludge study.

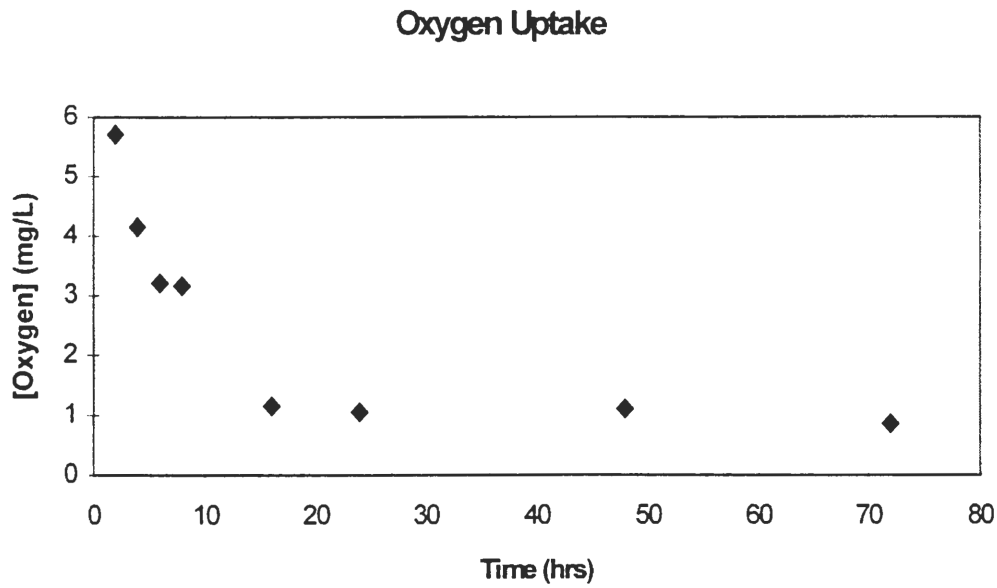
Sorption to Pond Solids

Samples from the activated sludge system were analyzed for sorption of AOX material to suspended solids. The procedures used were identical to those described for the ASB study. As was also found in the ASB study, no detectable sorption of AOX or chloride to the filterable solids in the activated sludge samples was found. With the activated sludge samples at a TSS level of about 300 mg/mL (three times that of the ASB) not showing any detectable sorption of AOX, it seems fairly conclusive that sorption of AOX to settling biomass/solids in the pond does not appreciably contribute to the removal of AOX in secondary treatment systems. It must be kept in mind, however, that no tests were conducted on the sorption to solids material from the primary clarifiers or presettling zones in either study.

O_2 Diffusion Into Cells

The aeration basin at Leaf River maintained a consistent DO level of 6 mg/L during this study. In order to relate conditions inside the sample cells to the basin, it was necessary to determine the rate at which O_2 diffused into the sample cells. To accomplish this, sample cells (prepared as in the field study) were submerged in a container of effluent held at 7(±1) mg/L DO. Sample cells were tested for change in DO level inside the cells with time, over a range of 2 hours to 72 hours. The DO level inside the cells dropped over the first 16 hours, and then held steady at a level of 1 mg/L. Results for this study are given in Figure 23.

Figure 23. O_2 diffusion into sample cells.



These results indicate that the DO level inside the sample cell does not remain the same as the aeration basin over the course of the study. The DO content in the sample cells results from the difference between the oxygen supply and the oxygen consumption. Since the DO level inside the cells remains consistent at 1 mg/L after 16 hours, a sufficient supply of DO was diffusing into the cells to support aerobic biodegradation. Dissolved oxygen levels in excess of the required 1-3 mg/L do not promote increased oxidation of organic substances.[90]

Oleic Acid Biodegradation

The success of the in-situ measurement system in determining AOX biodegradation prompted interest in use of this technique for the mapping of ponds for biodegradation of BOD components. To determine the applicability of the existing methods for measuring biodegradation of BOD model compounds, ^{14}C labeled oleic acid samples were tested in the activated sludge treatment system. Oleic acid was chosen as the model compound due to its relatively large size and its similarity to resin acids released in effluents from pulping operations. In order to obtain a measure of diffusional variation between cells, $^{36}Cl^{-}$ was added to the sample cells in addition to the ^{14}C labeled oleic acid.

Dual Isotope Scintillation Counting

The use of different isotopes for diffusion monitoring and biodegradation measurement is a tremendous advantage where sample analysis is concerned. The liquid scintillation counter has the ability to measure DPM values of two different isotopes simultaneously. This eliminates the need to physically separate the two components, a major contributor to experimental variation in the AOX study as well as being excessively time consuming.

Quench curves were established for ^{36}Cl and ^{14}C based on prepared quench standards. Two counting windows were established for dual isotope counting; one covering the entire range of the lower energy isotope and the other covering from the upper limit of the low energy isotope to the upper limit of the high energy isotope. The scintillation counter stores information on the percentage of counts for each isotope in the two windows as H-number varies. During unknown sample counting, the scintillation counter automatically corrects for both quench and channel spill over giving results in DPM for both isotopes.

Sample Preparation and Deployment

Stock ^{14}C labeled oleic acid was prepared by dissolving concentrated radioactive oleic acid (55μCi/mmol) in methanol to a concentration of 0.72 ppm and then adding $^{36}Cl^{-}$ (50 mCi/mmol) to a concentration of 8.0×10^{-5} ppm. Aliquots of the stock solution (250 μl) were added to field samples yielding concentrations of 3.6×10^{-2} and 4×10^{-6} for ^{14}C oleic acid and $^{36}Cl^{-}$ respectively. Due to their extremely low concentrations, the added radioactive materials should have no significant impact on the biological or physical environments to which they were added.

Field BOD samples were prepared identically to the AOX samples except that a 250 µl stock oleic acid solution was used for BOD biodegradation measurement in place of the 2 mL concentrated bleaching filtrate used in the AOX studies. The BOD study was conducted at the Leaf River secondary treatment facility. Samples were placed in positions A & B as depicted in Figure 22. Due to the higher rate of diffusion of oleic acid over the labeled AOX, samples were left in the treatment system for a shorter period of 24 hours. The liquid phase of the recovered samples was transferred to 30 mL amber vials containing 1.5 mL formalin. The membranes and sample cells were placed into 250 mL amber bottles containing 20 mL of ethanol. The ethanol solutions were counted to determine the level of oleic acid sorbed to the surface of the cells and membranes.

RESULTS AND DISCUSSION

Biodegradation of AOX

ASB Treatment System

Of the 15 probes (8 for ClO_2 bleaching filtrate and 7 for Cl_2 bleaching filtrate) deployed over the course of this study, 3 probes (2 for ClO_2 and 1 for Cl_2) were invalidated. Two of the invalidated probes consisted of ClO_2 and Cl_2 probes deployed near the presettling basin. In January of 1995 the presettling basin at the ASB was almost completely filled with solids. The probes designated for the presettling basin were, therefore, deployed as close as possible to the presettling zone without being close to an aerator (Fig. 21). At this location, the pond depth was only 8 feet and the solids content was high. This location may have also been outside of the main flow of the pond. Recoveries for both labeled AOX and $^{36}Cl^-$ were inconsistent for the control samples making data interpretation meaningless. In order for the equations developed in the theoretical section to be valid, the ratio K_{AOX}/K_{INORG} must remain relatively constant for the control samples over the depth of the pond. This was not true with the probes in the presettling zone and therefore these probes were considered as invalid.

The other invalidated probe was a ClO_2 probe deployed at the post aeration zone near the end of the pond. The $^{36}Cl^-$ recovery values for the live samples in this probe were extremely high, at 5 to 10 times that of the control samples. This indicates that some type of membrane blockage occurred in the live samples. The large amount of BOD contained in the concentrated bleach filtrate sample may have lead to significant microbial growth in these samples. Microbial growth concentrated on the membrane surface would explain the extremely high levels of $^{36}Cl^-$ recovered. However, the labeled AOX recoveries for the live samples were approximately the same as for the control samples. A significant removal of AOX by means other than diffusion had to have occurred in these samples. The equations developed for calculating biodegradation are based on first order diffusion for both AOX and $^{36}Cl^-$. In cases of extensive membrane blockage, as must exist in these samples, first order diffusion cannot be confirmed. Therefore, the amount of biodegradation occurring in the sample cells for this probe cannot be calculated.

Results for AOX biodegradation measurements in the September 1994 ASB study are given in Figures 24-26. Biodegradation was measured at aerators 36 & 21 and at a metal post (MP) near the midpoint of the pond. During this study, all sample preparation was done on a boat at the location of sampling. This minimized the time between collection of the pond samples and placement of the prepared samples into the pond (2-3 hours). Average biodegradation values for the aerated locations were 9% for both ClO_2 and Cl_2 bleaching filtrates. Average biodegradation for the non-aerated zone was 12% for ClO_2 and 13% for Cl_2.

Figure 24. Biodegradation at Aerator #36, Near the Front of the ASB.

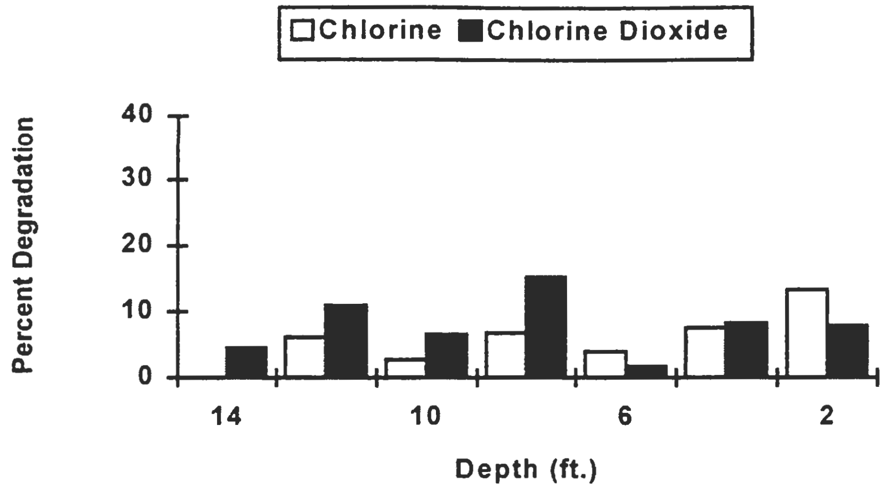


Figure 25. Biodegradation in the Non-Aerated Zone in the Middle of the ASB.

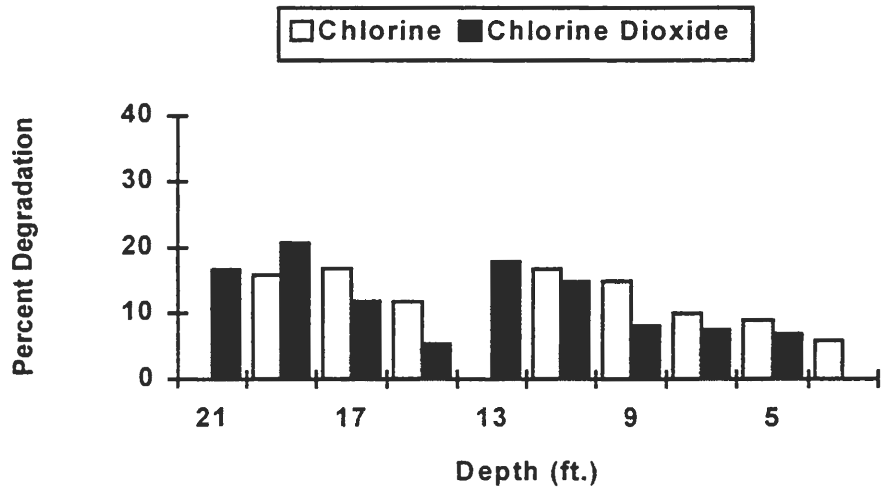
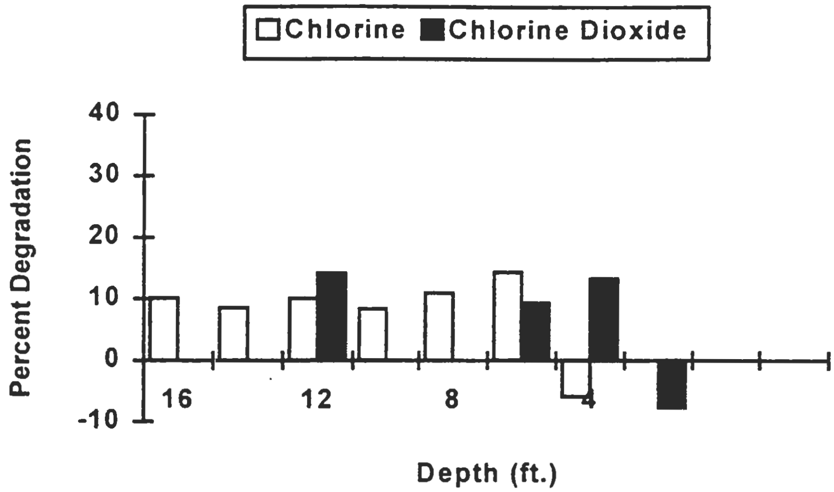


Figure 26. Biodegradation at Aerator #21, Aerator in the Middle of the ASB.



Results for AOX biodegradation measurements in the January 1995 ASB study are given in Figures 27-29, and the results were consistent with those of trip one. Biodegradation was measured at aerators 34 & 3 and in the presettling and post aeration (PA) zones. Due to the inclement weather during this study, all sample preparation was done in the deckhouse. This slightly increased the time between collection of the pond samples and sample preparation, but greatly reduced the time necessary for sample preparation. The actual effect on the time between pond sample collection and placement of the prepared samples into the pond (3-4 hours in this case) was not extensive. Average biodegradation values for the aerated locations were 19% for ClO_2 and 15% for Cl_2 bleaching filtrates. The average biodegradation for the non-aerated zone was 18% for Cl_2.

Figure 27. Biodegradation at Aerator #34, First Aerator in the ASB.

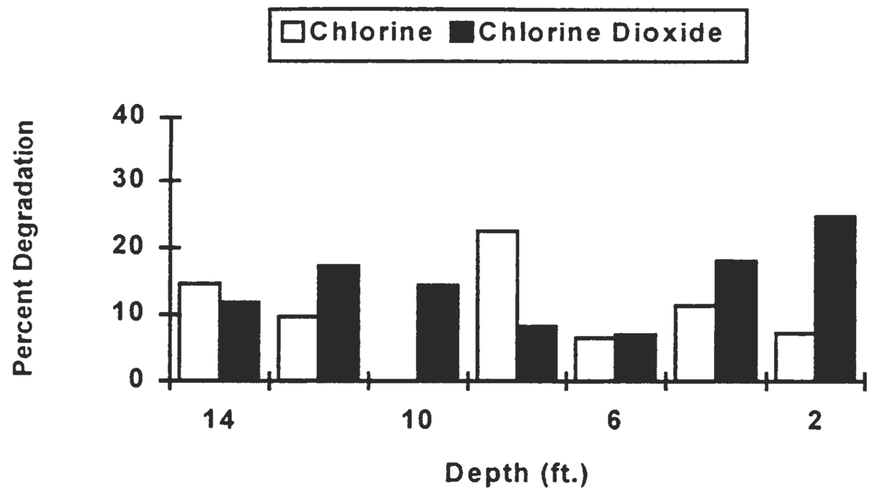


Figure 28. Biodegradation at Aerator #3, Last Aerator in the ASB.

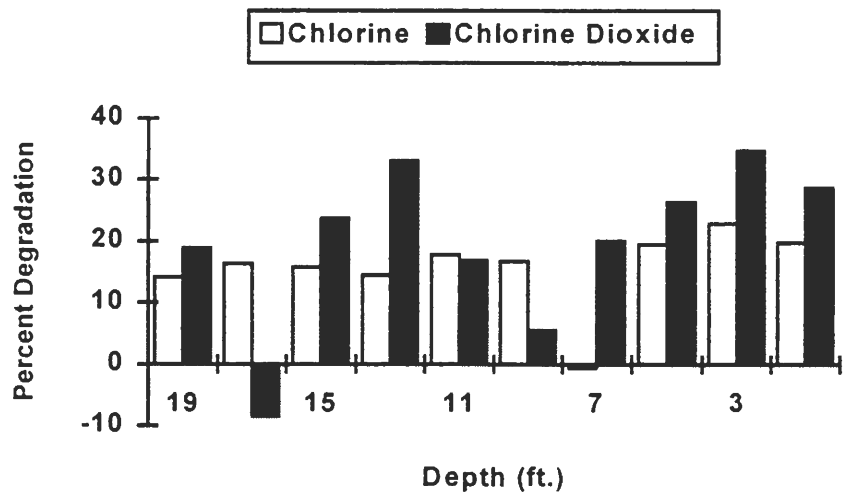
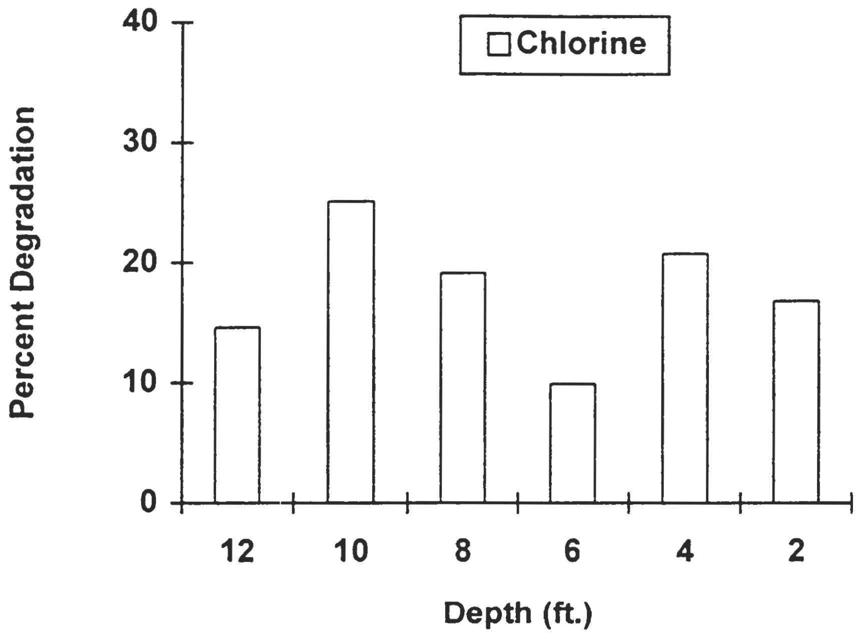
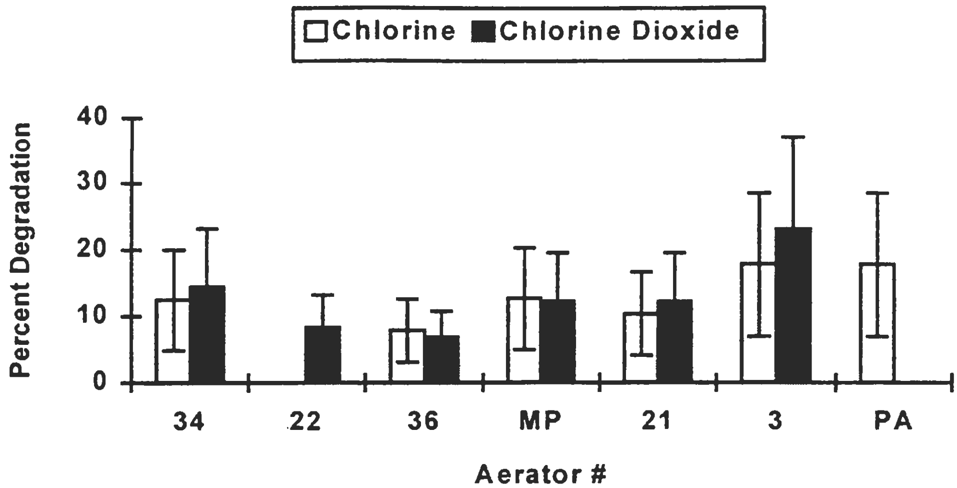


Figure 29. Biodegradation in the Post Aeration Zone.



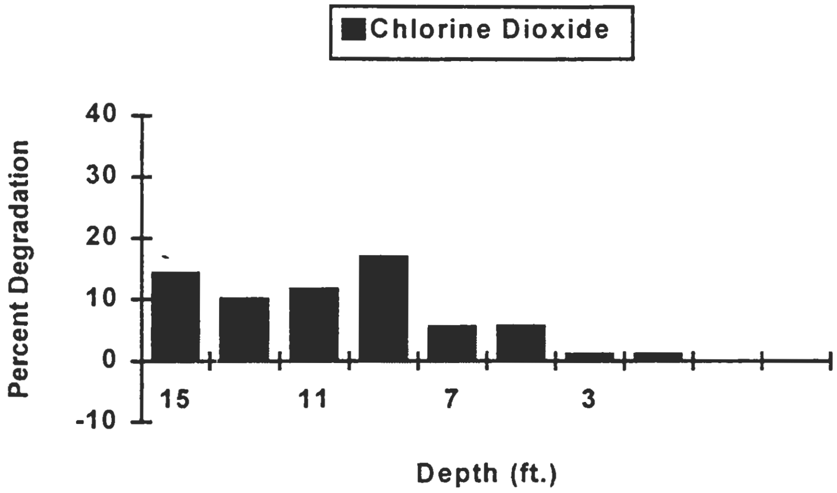
A total of five sets of duplicate samples (series of five samples placed close together) were deployed in this field exercise. Three of these yielded acceptable data (no more than one lost sample out of five) and were used to determine confidence limits for biodegradation values at a 95% confidence interval.[105] Values for the confidence limits calculated from the three sets were 13% (Cl_2 bleach filtrate), 23% (ClO_2 bleach filtrate) and 61% (Cl_2 bleach filtrate), with 61% being used for the determination of significance between values. The average biodegradation values with their 95% confidence interval for the different pond sites are given in Figure 30.

Figure 30. Average Biodegradation Values with 95% Confidence Intervals.



The third ASB field study was conducted to check the biodegradability of the ClO_2 bleaching filtrate used in the activated sludge study. Sample preparation was the same as in the second study at this site. This filtrate had not appeared to biodegrade when tested in the activated sludge system, a result that did not seem possible. One sample probe was deployed at aerator 22. Average biodegradation for the filtrate was 8%, well within the 95% confidence limits for ClO_2 biodegradation in the ASB (Fig. 31).

Figure 31. Biodegradation at Aerator #22, Aerator in the Front of the ASB.



The initial intent of this study was to develop a three dimensional model for AOX biodegradation occurring in the ASB. Analysis of the data at each of the sampling sites for trends between depth and biodegradation indicated that only the ClO_2 filtrate samples at aerator 22 showed any depth dependency. In this case, biodegradation decreased near the surface, opposite of the results expected for an O_2 dependent process. Although the probe located at the metal post appears to show some depth dependence, there was no significant difference between the samples at a 95% confidence level. In the case of the positions closest to the aerators, dissolved oxygen (DO) levels would be expected to differ over the depth of the pond based on the design of the aerators (floating surface aerators). DO measurements indicated that immediately after shutting down an aerator, DO levels were near zero around the aerator. Values for DO in the turbulent water around operating aerators could not be obtained. The lack of increase in biodegradation near the surface of the samples in the aerated zones, indicates that AOX biodegradation measured in this system is not an O_2 dependent process.

The fact that biodegradation is not depth dependent allows average values for each probe to used in site comparisons. As would be expected, based on the depth dependency results, no significant differences existed between samples in aerated and nonaerated zones (Fig. 38). ClO_2 filtrate samples averaged 13% biodegradation in aerated zones and 12% biodegradation in non-aerated zones. The Cl_2 filtrate samples averaged 12% biodegradation in aerated zones and 13% biodegradation in non-aerated zones. This again supports the theory that biodegradation of AOX in the ASB is not an O_2 mediated process. Dissolved oxygen levels at aerator sites immediately falling to near zero when the aerator is shut off indicates a very rapid consumption of DO by aerobic microorganisms. Therefore, the lack of a relationship between aeration and biodegradation for AOX may be representative of ineffective aeration (DO residence time in the pond too short for DO to diffuse into the sample cells) rather than recalcitrance of AOX in aerobic systems. However, results from a recent study by Talat Mahmood,[106] using the same sampling system, show a definite fall-off of oleic acid biodegradation as the pond is traversed, suggesting that O_2 diffusion into the sample cells is representative of pond conditions.

Comparison of biodegradation values measured at aerators spanning the length of the pond indicated no significant difference in AOX biodegradation with location (Fig. 38). As a higher concentration of biodegradable material exists at the head of the pond, microbial populations would be expected to be higher at the head of the pond.[107] Measurement of BOD and TSS[108] indicate that both parameters are higher at the head of the pond than at the pond outlet. The consistency of biodegradation levels across the span of the basin indicates that AOX biodegradation in this ASB is not limited by microbial population. These results apply to the ASB from the first aerator to the pond outlet and do not include the presettling zone. As no relationship was found between position and AOX biodegradation, generation of a three dimensional map for AOX biodegradation was of no benefit.

The average value of all pond measurements for the biodegradation of AOX from both chlorine and chlorine dioxide bleaching was 12%. No significant difference was found between biodegradation values for ClO_2 and Cl_2 bleaching filtrates when averaged across the pond. The average biodegradation for AOX from ClO_2 bleaching was 13% and from Cl_2 bleaching was 12%. Biodegradation of AOX from both bleaching sequences increased or decreased similarly as AOX biodegradation varied from site to site across the pond (Fig 30). No significant difference was found between the biodegradation of AOX from chlorine or chlorine dioxide bleaching at any of the sites.

Samples in the bottom one or two positions had an extremely high sludge content at several of the sites studied. This sludge had a very dark black color and, even at 20 fold dilution, imparted significant color in solution. Due to the nature of liquid scintillation counting (very sensitive to color quenching), samples containing sludge were not able to be accurately counted. Attempts were made to develop a color quench curve to correct for color quenching caused by the sludge, however a consistently reproducible curve could not be generated. This is due to the combined effects of color quench, chemical quench, and quench due to the high solids content of the sludge. Samples containing sludge were considered as lost samples due to the inability to evaluate them.

Brunswick reports a 50% removal efficiency for AOX, yet our values are much lower. The obvious explanation is that most of the AOX is removed non-biologically. This was confirmed in a parallel project by Banerjee *et. al.*,[108] where ratios of high molecular weight and low molecular weight AOX and COD were measured at the inlet to and outlet from the pond. The molecular weight cut-off used was 500 D, and samples were taken every weekday for five weeks. The ratio for COD increased from pond inlet to outlet since the low molecular weight COD was preferentially removed due to biodegradation. The ratio for AOX increased by only 8% across the pond. While the total AOX decreased as the material traversed the pond, the high molecular weight and low molecular weight material decreased at the same rate. It is highly unlikely that the decrease reflects biodegradation, since the low molecular weight AOX would be degraded much more rapidly. Totally different approaches were used in the two studies; yet, the biodegradation efficiency results were comparable at 8% and 12%.

In addition to the above study, other studies have determined biodegradability of mixed BKME in ASB treatment systems to be in the 10% to 20% range. Bryant *et. al.*[60] determined the drop in AOX, from the end of the primary settling basin to the outlet, for an ASB treating BKME to be 9%. Oleszkiewicz *et. al.*[89] tested a BKME, collected after the equalization tanks, for AOX removal in a lab scale aerated stabilization basin. They determined AOX removal to be 19%, with no accumulation of AOX in the sludge or settled solids. These studies support the value of 12% for the biodegradation of AOX in ASB systems.

There are, however, a number of studies supporting a much higher level of AOX removal in some ASB systems. Cook[109] measured AOX levels across an ASB treating BKME over a six month period. He found that AOX levels dropped 37% from the bleach plant to the end of the primary settling basin. The AOX level dropped an additional 31% from the outlet of the primary settling basin to the treatment system outlet, yielding a total AOX removal for the system of 68%. Aprahamian and Stevens[26] measured AOX removal across the ASBs of two bleached kraft mills. Although the mills employed very different bleaching sequences, CEHDED and ODCED, AOX removal at both mills was 40%, as measured from pond inlet to outlet. Tomar and Allen[19] monitored the AOX removal by an ASB treating a BKME over five months. AOX removal varied from 15-47% during the study, as measured from the primary clarifier outlet to the final effluent. While AOX removal does not appear to depend heavily on the bleaching sequence used, AOX removal by ASB systems (as measured after combination of the bleach plant effluent streams) does vary significantly from one mill to another. This suggests that the mechanism for AOX removal may be a complex of physical, chemical, and biological pathways.

It is clear that mechanisms other than biodegradation are responsible for much of the AOX removal. A small part of the AOX will strip into the atmosphere, but volatile AOX only constitutes a small fraction of the total AOX, less than 5%.[20,95] Chemical degradation, one possibility for the removal of AOX, is supported by several recent studies.[14,29,75,78,79,80]

AOX reductions of over 50% have been measured upon mixing D(C)- and E-stage effluents under conditions similar to those in a mill. This reduction has been attributed to alkaline hydrolysis of the AOX compounds in the D(C)-stage upon treatment with the alkaline extraction stage. Chemical degradation could certainly explain the initial drop in AOX between the bleach plant and ASB inlet found in all treatment systems.

An alternative mechanism is sorption to pond particulates and subsequent settling. Results from the Banerjee study[108] appear to support this view. First, it was found in that the AOX concentration at an aerator situated at the front end of the lagoon was virtually identical to the AOX level in the final effluent; i.e. the pond is ineffective for AOX removal shortly after the pond inlet. Since the total suspended solids (TSS) also drops from this point on, the potential for sorption decreases. All of the AOX reduction occurs in the presettling zone, where the solids level is the highest. Second, the solids level in activated sludge system is much higher than that in an ASB, which could explain why an activated sludge system performs better than an ASB in removing AOX from the water column. This sorption of AOX to biomass followed by settling and subsequent anaerobic degradation was proposed by Bryant *et. al.*[60] to be the principal mechanism for AOX removal in secondary treatment systems.

While sorption has been implicated as the principal means of AOX removal in several studies, it should be recognized that the propensity for AOX to sorb to biomass is actually quite low. Sorption to biomass is favored for hydrophobic compounds, and the octanol:water partition coefficient is typically used as an index of hydrophobicity. Generally, a compound is considered to have appreciable sorption potential if the partition coefficient exceeds 1000. For AOX, this value is less than one, as measured in this study and in prior work.[99] Tomar and Allen[19] have confirmed this, as we also have in the present study. Additionally, Bryant *et. al.*[6] found no relationship between biomass levels and AOX removal in an extensive study of secondary treatment systems. Hence, for sorption to be important for AOX, there needs to be high levels of solids present in the system. However, this may be the case in the pre-settling zones and primary clarifiers, which accounts for the drop in AOX across these regions.

Although 12% of the AOX can biodegrade in the water column, there is no reason to believe that any of it actually does; the biodegradable AOX fraction could sorb and settle out. Although this work does not address the fate of AOX in sludge, there is evidence that these compounds degrade in soil,[110,111] which suggests that biodegradation in sludge rather than in the water column is a likely route for the removal of these compounds. Since AOX biodegrades only minimally (if at all) in the treatment system, efforts to enhance biological treatability are probably fruitless. Since the majority of AOX removal appears to be by abiotic means, it seems more fruitful to address AOX removal in the bleach plant effluent streams, before they are diluted into the pond, by altering conditions to promote chemical degradation and/or sorption and settling.

Activated Sludge System

As discussed earlier, all of the data for the ClO_2 samples was discounted based on the level of random variability in counting low level radioactive samples. Just as in the ASB study, the data for the Cl_2 samples was analyzed for outlying $^{36}Cl^-$ recovery values. Only one sample cell was found to have an outlying $^{36}Cl^-$ recovery. This is very different from the ASB study in which most probes had several cells with outlying $^{36}Cl^-$ recovery values. This difference can primarily be attributed to the high degree of consistency in the activated sludge system, especially with respect to the mode of aeration. The remainder of the lost cells in the activated sludge study can be attributed to physical problems such as cell breaks or membrane leakage. As with the ASB study, where lost control samples occurred, values for the next closest control sample were substituted in biodegradation calculations. No Cl_2 probes were lost in the activated sludge study.

Results for the activated sludge study conducted in June of 1995 are given in Figures 32-35. Biodegradation was measured after each of the effluent inlet points (positions A-C) and at the pond outlet (position D). Due to the design characteristics of the activated sludge system, no variation with either depth or position was expected. Air is introduced into the system by a forced air injection system on the basin floor. This creates a very uniform DO profile for the basin (~6 mg/L) as well as providing excellent vertical mixing. Introducing the influent as three streams delivers an even feed rate across the length of the basin. The average value from all positions tested for the biodegradation of AOX from chlorine bleaching was 40%. There was no significant difference in biodegradation between any of the sites. No relationship between depth and biodegradation was found. This is exactly as expected based on the design characteristics of the activated sludge system.

Figure 32. AOX Biodegradation at Leaf River, Site A.

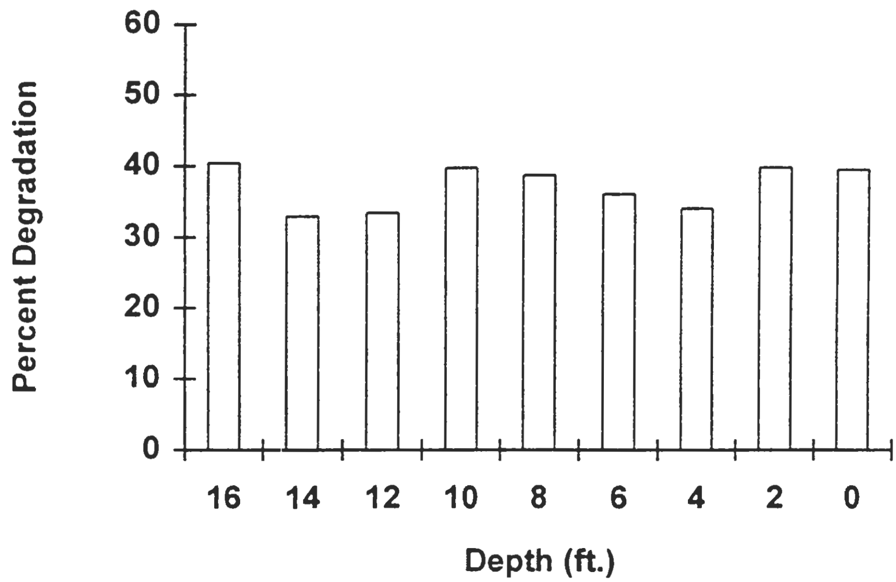


Figure 33. AOX Biodegradation at Leaf River, Site B.

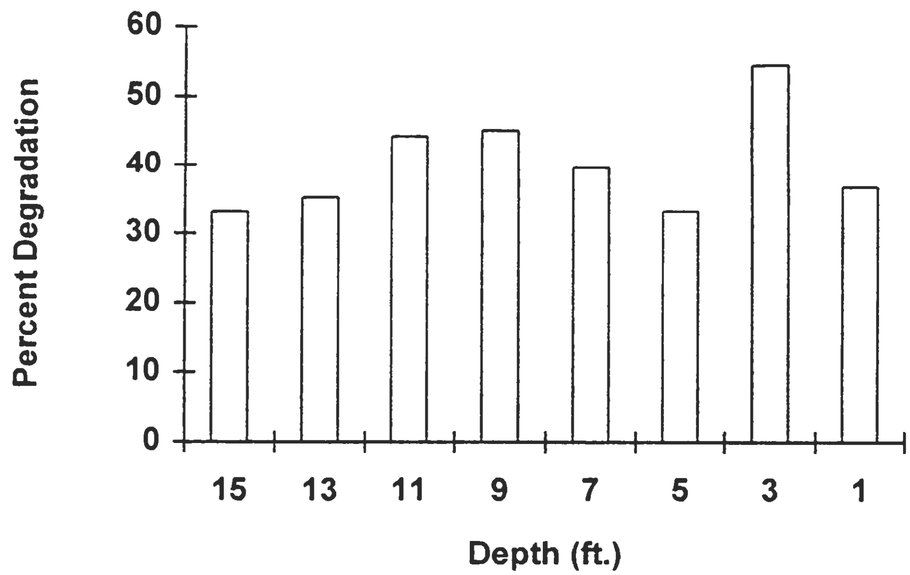


Figure 34. AOX Biodegradation at Leaf River, Site C.

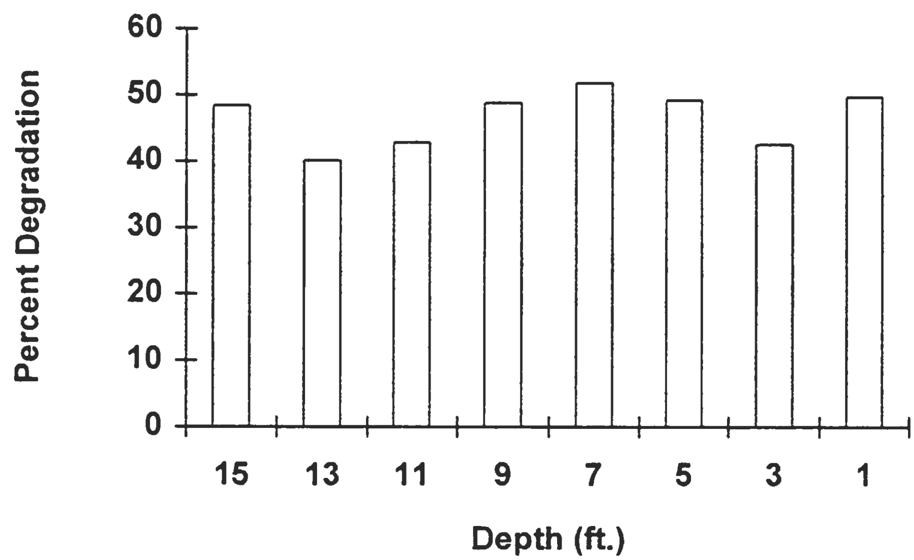
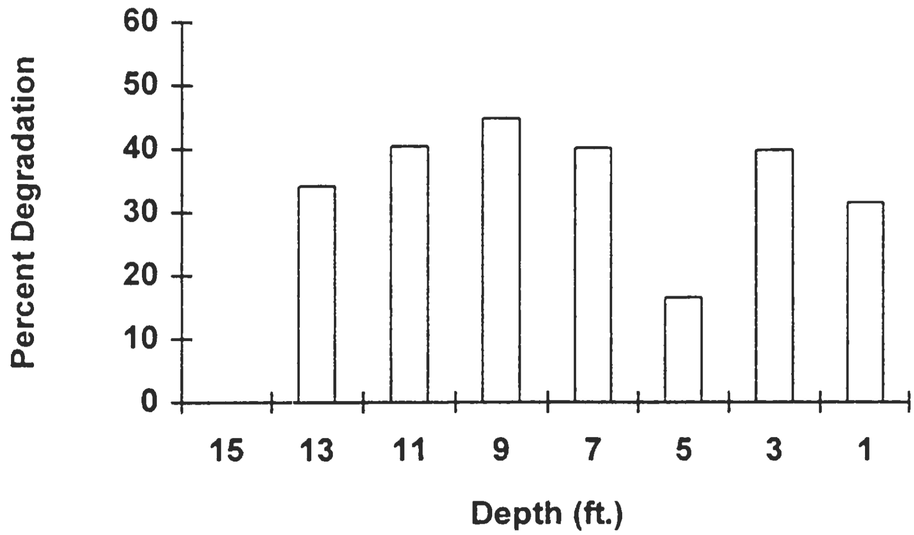
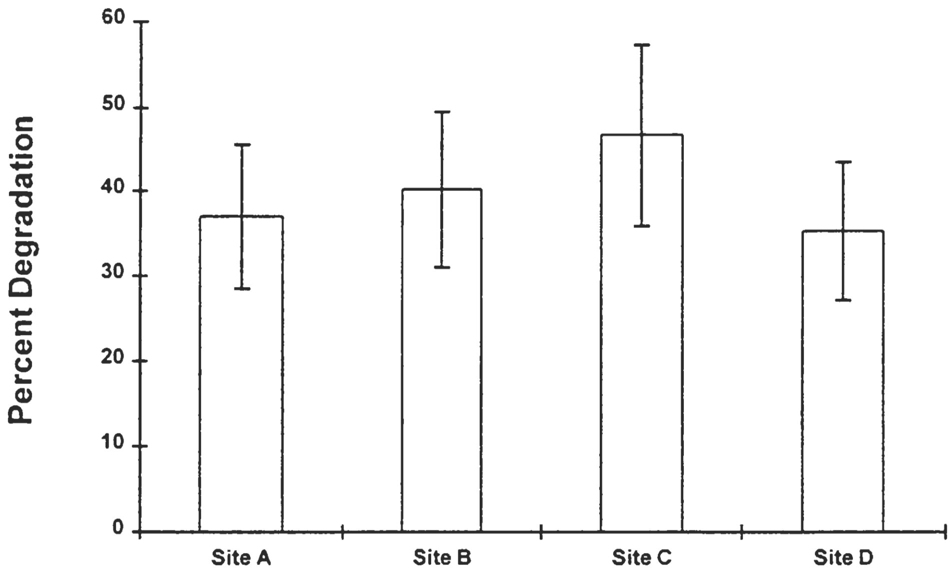


Figure 35. AOX Biodegradation at Leaf River, Site D.



Three sets of duplicate samples using chlorine bleaching filtrates were included in the activated slude study. Confidence limits expressed as a percent of the average value for percent biodegradation established at a 95% confidence interval were 10%, 20%, and 23%. As with the ASB study, the highest value was used for establishing confidence limits. The lower limits of uncertainty for the activated sludge system are most likely attributable to the more consistent conditions found with the system. Average biodegradation values with their 95% confidence interval for the different pond sites are given in Figure 36.

Figure 36. Average biodegradation values with 95% confidence intervals.



In contrast to the ASB study, the intent of this study was to measure the level of AOX biodegradation occurring in the system, rather than mapping the ASB for AOX biodegradation. Given the design of the ASB, no differences in biodegradation were expected with respect to depth or basin location. The results from the four probes deployed in the system confirmed this, as they showed no relationship between AOX biodegradation and position within the basin.

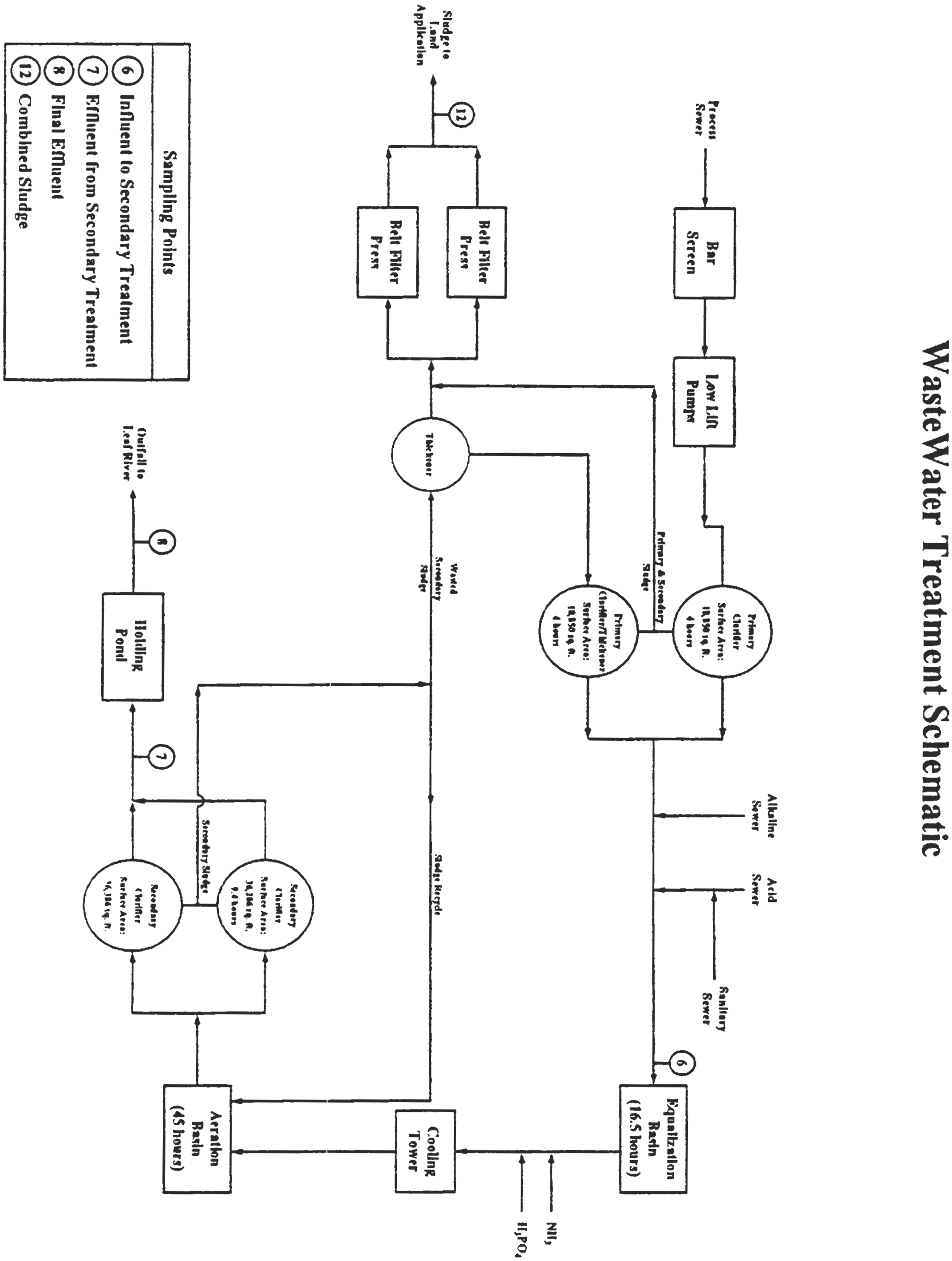
As with the Brunswick mill, the Leaf River mill reported an higher AOX reduction (70%) than measured in this study (40%). The majority of the AOX reduction reported appears to be by biological degradation, leaving a balance of 30% attributable to abiotic means. Data on AOX levels at specific locations across the secondary system (i.e. equalization basin outlet or cooling tower outlet, Figure 37) were not available. This prevented determination of the exact location of abiotic degradation in the secondary treatment system. Additionally, with the split inlet system employed by the activated sludge system, it was impossible to track the reduction of AOX as it traversed the aeration basin as was done with the ASB. The equalization basin in the activated sludge system should act similar to the presettling basin in the Brunswick system, at least as far as chemical degradation and settling are concerned. From the bleach plant to the end of the presettling basin, approximately 40% reduction in AOX occurs in the ASB. It seems reasonable, therefore, that a 30% (or more) reduction in AOX could occur between the bleach plant and the inlet to the aeration basin in the activated sludge system.

Numerous laboratory studies have been conducted in attempt to measure the biodegradation of AOX in ASB type systems. Studies by Saunamaki *et. al.*,[85] Nevalainen *et. al.*,[112] and Yu *et. al.*[79] on AOX biodegradation under controlled laboratory conditions in simulated ASBs have indicated biodegradation levels of 25-35%, 22%-40% and 43-48% respectively. In all three studies, initial AOX values were determined by analysis of the prepared effluent just prior to its introduction into the treatment system. This is an important factor, in that studies basing initial AOX on values from individual waste streams prior to combination tend to report higher values for AOX biodegradation. These findings are in excellent agreement with the value of 40% AOX biodegradation measured by this study.

The biodegradation rate in wastewater treatment depends on many factors, specifically on organic load, sludge volume, number of organisms, oxygen supply, and temperature. Two major differences between the systems studied, relating to AOX biodegradation, are DO concentration and microbial population. The dissolved oxygen concentration in the ASB is very low, except for in the immediate vicinity of operating aerators and near the lagoon outlet. In contrast, the activated sludge system maintains a DO level of ~6 mg/L. This is a significant difference in that aerobic degradation rates are related to DO levels only in cases where DO levels are below 1 mg/L.[90] The samples studied in the activated sludge system are initially at a DO level of ~6, and (based on the O_2 diffusion study) remain above a DO of 1 mg/L for the duration of the field study. As much of the aeration lagoon is operating at DO levels below 1 mg/L (even at the aerators when they are off), the sample cells for the positions studied will have initial DO levels below 1 mg/L. Under these conditions, aerobic biodegradation will not be occurring at optimal efficiency. The activated sludge system would, therefore, be expected to show significantly higher levels of AOX biodegradation.

In addition to a higher DO level, the activated sludge system maintains a significantly (~ 3 fold) higher microbial population. Even at the high TSS levels of this system, sorption of AOX to pond filterable solids is negligible. This indicates that sorption of AOX to biomass in the aeration basin is not the major mechanism of abiotic AOX removal. As microbial population is directly proportional to biodegradation efficiency, the higher population maintainable in activated sludge systems makes them capable of a higher rate of biodegradation than ASBs.[90] The combination of increased microbial population and higher DO level results in a 3 fold increase in the biodegradation of AOX for the activated sludge system as measured by this study. While biodegradation potential appears to be greater in activated sludge systems than ASBs, overall AOX removal efficiencies for the two systems vary widely from mill to mill, depending on operating parameters of the mill and design of the treatment system.

Figure 37. Schematic of the Leaf River waste treatment system.

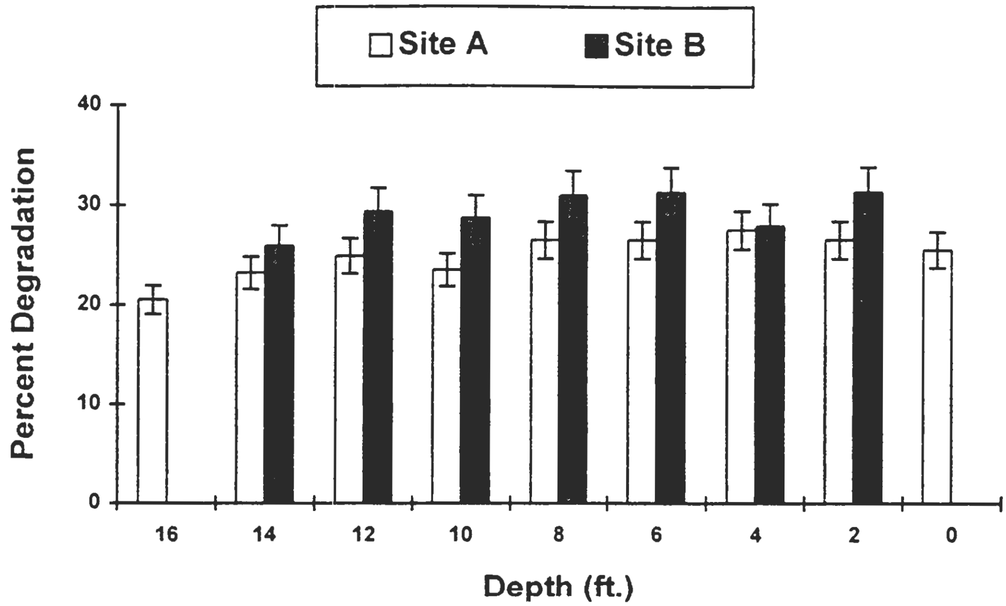


Biodegradation of Oleic Acid

Recovery of $^{36}Cl^-$ was used to measure variations in diffusion with depth as in the AOX studies. Values for $^{36}Cl^-$ recovery for all cells were analyzed for outlying values as described previously. None of the sample cells were found to have outlying $^{36}Cl^-$ recovery values. This was consistent with the activated sludge study in which only one sample had an outlying $^{36}Cl^-$ recovery value. The lost cells in the oleic acid study were attributed to physical problems or prolonged residence above the water level. As with the other studies, where lost control samples occurred, values for the next closest control sample were substituted in biodegradation calculations. No probes were lost in the oleic acid study.

As was expected based on the simplicity of analysis, results for oleic acid biodegradation were much more consistent than for AOX biodegradation. Analysis of variance at the 95% confidence limit resulted in a confidence interval of ±26% for the five samples placed together. Only four of the five repeat samples were useable, leading to an artificially high confidence interval. If all of the live samples on each probe are taken into consideration (valid due to the consistent conditions in activated sludge systems) confidence limits drop to ±7% and ±8% for sites A and B respectively. Average biodegradation values for positions A and B were 25% and 30% respectively, and are depicted in Figure 38 with their 95% confidence intervals.

Figure 38. Oleic Acid Biodegradation at Sites A and B with 95% Confidence Limits.



Kinetic studies to develop an equation for the relationship between pond exposure time and biodegradation are necessary to accurately extrapolate the 24 hour biodegradation values to the actual pond retention time. By assuming that biodegradation follows first order kinetics,

$$\frac{Ln[AOX]}{Ln[AOX]_0} = -Kt \qquad \text{Equation 24}$$

the 24 hour results can be extrapolated to 2.5 days (retention time of the activated sludge basin) to yield an average degradation value of 55%. This value is considerably lower than the 96% BOD loss reported by the mill, which includes chemical degradation, settling and volatilization of the VOC compounds. In addition, mill effluent contains significant amounts of easily degraded material such as methanol. Therefore, BOD removal rates for the mill effluent would be expected to exceed the biodegradation rate of oleic acid.

CONCLUSIONS

Biodegradation of AOX

ASB Treatment System

- Successfully developed method for in-situ measurement of AOX biodegradation.
- Found no difference in AOX biodegradation between Cl_2 and ClO_2 bleaching filtrates.
- Of the 50-55% AOX reduction reported for the secondary treatment system at the Brunswick mill, only about 13% reduction can be accounted for by biodegradation.
- No relationship found between pond location and biodegradation.
- The sorption of AOX to the solids material in the aeration basin was below detection levels.
- Chemical degradation has the potential to be a significant factor in AOX reduction upon combination of the acid and caustic sewers.
- If sorption and/or settling is a significant factor in AOX removal, it is occuring in the presettling zone prior to any aeration.
- Mills looking to improve the removal of AOX in their ASB treatment systems may be well advised to pursue improvements in conditions promoting sorption and settling (addition of chemicals) or chemical degradation (high temperature and increased alkali charge) rather than investing in improvements designed to promote biological activity.

Activated Sludge System

- No relationship was found between AOX biodegradation and position in the basin.
- The activated sludge system is capable of 40% AOX biodegradation at a retention time of three days.
- The activated sludge system has a 3 fold greater capacity for AOX biodegradation than the ASB, at the retention time for each system as operated.
- Abiotic degradation for both systems appears to be on the order of 30-40%.
- Sorption to biomass, followed by settling and anaerobic degradation, does not appear to be the mechanism responsible for biodegradation.

- Increased biomass and DO are the most likely factors for the increased AOX biodegradation in the activated sludge system.

Biodegradation of Oleic Acid

- The system, as developed for AOX biodegradation measurement, is effective in measuring biodegradation of BOD model compounds.
- Biodegradation of oleic acid over a 24 hour period was 25-30%.
- Due to the simplicity in sample preparation and analysis, mapping of treatment systems for BOD model compound biodegradation has greater industrial applicability than mapping for AOX biodegradation.

SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK

The major goal of this research was to develop a system to measure in-situ biodegradation of AOX and compare the biodegradation of AOX from Cl_2 and ClO_2. This was accomplished for an ASB treatment system in Brunswick, Georgia and an activated sludge system in Leaf River, Mississippi. No statistically significant differences in AOX biodegradation were found between any of the locations tested. Generation of a three dimensional plot for biodegradation was determined to be of no benefit.

No statically significant differences were noted between AOX biodegradation from Cl_2 and ClO_2 bleaching filtrates in the ASB study. Of the 50% AOX removal observed across the ASB treatment system, only 10-15% AOX reduction was found to be attributable to biodegradation in the water column. This clearly demonstrates, that for this system, abiotic degradation mechanisms, chemical degradation and settling (via sorption or precipitation), account for the majority of AOX reduction. Investigations to measure the contribution of each of the different abiotic mechanisms would be of great benefit in determining the optimum conditions for AOX removal in ASB systems.

The in-situ sampling system was also successful in measuring AOX biodegradation in an activated sludge system. AOX biodegradation was much higher in the activated sludge system, 40%, representing 57% of the total AOX removed. In both the ASB and activated sludge systems, abiotic processes accounted for removal of ~30% of the initial AOX. As abiotic processes are responsible for a large portion of the total AOX removal for activated sludge systems as well, investigation into conditions promoting abiotic AOX removal would be of benefit in optimize operating conditions.

The in-situ biodegradation measuring equipment was demonstrated to be effective for the evaluation of biodegradation of BOD model compounds. The three dimensional mapping concept has more applicability for these compounds, as biodegradation has been correlated with location in the treatment system.[106] Information gained from these types of studies can be used to optimize aerator placement and evaluate the aerators in operation for their BOD removal efficiency. During periods requiring reduction in power consumption (shutdown of a portion of the aerators), the aerators having the least effect on BOD removal can be selected for shutdown, thus minimizing the effect on BOD discharges.

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APPENDIX A

Pond Water Analysis for the ASB

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(12-1-93)

LOCATION (aerator #-depth)	DISSOLVED OXYGEN (mg/l)	TEMPERATURE °C
36-2'	0.4	28
36-7'	0.2	27
36-12'*	0.2	26.8
13-2'	0.6	24
13-7'	1.0	23.5
13-15'*	0.6	24

* The DO probe did not have sufficient weight to insure that it was dropping vertically into the pond under the windy conditions on the day of the sampling. The 12' and 15' depth readings maybe at shallower depths.

OXYGEN CONSUMPTION RATES
(12-1-93)

SAMPLE ID (aerator #-depth)	RATE (mg/l/min)
13-2'	0.024 @ 25°C
13-7'	0.018 @ 22.5°C
36-2'	0.135 @ 24°C
36-2'	0.122 @ 23.5°C
36-7'	0.138 @ 27.2°C
36-12'	0.246 @ 22.6°C

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(4-25-94 @ 4:30 pm)

LOCATION (aerator #-depth)	DISSOLVED OXYGEN (mg/l)	TEMPERATURE °C
11-1'	0.1	32.2
11-3'	0.1	32.2
11-5	0.1	32.1
11-7'	0.1	32
11-9'	0.1	31.9
11-11	0.1	31.8
11-13	0.1	31.3
11-15	0.1	31.2
11-17	0.1	31
11-19	0.1	30.8
11-21	0.1	30.4
11-22.5	0.1	30.1

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(4-25-94 @ 10:00 am)

21-2'	0.1	31
21-4'	0.1	30.5
21-6	0.1	30
21-8'	0.1	30
21-10'	0.2	30
21-12	0.2	30
21-14	0.1	30
21-16	0.1	30

At the surface with the probe just barely submerged, the DO reading was 1.7 mg/l. At 1 inch under DO= 0.2 mg/l.

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(9-8-94 @ 10:00 am)

LOCATION (aerator #-depth)	DO (mg/l)	TEMPERATURE °C
21-0.5'	0.15	30.2
21-2'	0.15	30.5
21-4'	0.15	30.8
21-6'	0.15	31
21-8'	0.2	31
21-10'	0.2	31
21-12'	0.1	31.1
21-14'	0.1	31.5
21-16'	0.3	32

At the surface with the probe just barely submerged, 1 inch under, the DO = 0.3 mg/l.

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(9-9-94 @ 1:10 pm)

MP-0.5'	0.3	34.8
MP-1.5'	0.2	34.8
MP-3.5'	0.1	34.4
MP-5.5'	0.1	34.2
MP-7.5'	0.08	34
MP-9.5'	0.05	34
MP-11.5'	0.05	33.8
MP-13.5'	0.03	33.8
MP-15.5'	0.02	33.8
MP-17.5'	0.03	33.8
MP-19.5'	0.02	33.7
MP-21.5'(sludge)	0	33.2

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(12-28-94 @ 4:00 pm)

LOCATION (aerator #-depth)	DISSOLVED OXYGEN (mg/l)	TEMPERATURE °C
EFF-0'(surface)	1.6	20.5
EFF-1'	1.4	20.5
EFF-2'	1.1	20.5
EFF-3'	1.1	20.5
EFF-4'	0.8	20.25
EFF-5'	0.8	20
EFF-6'	0.4	20
EFF-7'	0.3	20
EFF-8'	0.2	19.8
EFF-9'	0.2	19.5
EFF-10'	0.2	19.2
EFF-11'	0.2	19
EFF-12'	0.2	19
EFF-13'(sludge)	0.1	19

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(9-9-94 @ 10:40 am)

LOCATION (aerator #-depth)	DO (mg/l)	TEMPERATURE ^0C
36-0.5'	0.4	36
36-2.5'	0.2	35.5
36-4.5'	0.1	35
36-6.5'	0.1	34.9
36-8.5'	0.1	34.9
36-10.5'	0.1	34.9
36-11.5'	0.05	35.1
36-12.5'(sludge)	0	35

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(12-28-94 @ 10:50 am)

PS-0'(surface)	0.5	28
PS-1'	0.6	29.5
PS-2'	0.2	29
PS-3'	0.2	28
PS-4'	0.2	28.5
PS-5'	0.2	28.1
PS-6'	0.2	28.1
PS-7'	0.2	26.1
PS-8'	0.15	25.2
PS-8.5'(sludge)	0.1	25.2

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(12-29-94 @ 10:45 am)

LOCATION (aerator #-depth)	DISSOLVED OXYGEN (mg/l)	TEMPERATURE ^0C
A34-0'(surface)	0.25	36
A34-0.5'	0.25	36
A34-1.5'	0.2	34
A34-2.5'	0.2	31
A34-3.5'	0.19	30.5
A34-4.5'	0.19	30
A34-5.5'	0.19	30
A34-6.5'	0.18	29.5
A34-7.5'	0.17	29
A34-8.5'	0.16	29
A34-9.5'	0.15	29
A34-10.5'	0.15	29
A34-11.5'	0.14	29
A34-12.5'	0.12	29
A34-13.5'	0.11	29
A34-14.5'	0.11	29
A34-15.5'(sludge)	0.1	29

DISSOLVED OXYGEN AND TEMPERATURE PROFILE
(12-30-94 @ 10:00 am)

LOCATION (aerator #-depth)	DISSOLVED OXYGEN (mg/l)	TEMPERATURE ^0C
A3-0'(surface)	0.6	22
A3-1'	0.6	22
A3-2'	0.5	22
A3-3'	0.4	22.2
A3-4'	0.3	22.4
A3-5'	0.25	22.4
A3-6'	0.2	22.4
A3-7'	0.2	22.2
A3-8'	0.2	22.2
A3-9'	0.2	22
A3-10'	0.2	22
A3-11'	0.18	22
A3-12'	0.15	22
A3-13'	0.15	22
A3-14'	0.15	22
A3-15'	0.12	22
A3-16'	0.12	22
A3-17'	0.12	22
A3-18'	0.1	22
A3-19'	0.1	22
A3-20'(sludge)	0.1	22

SOLIDS CONCENTRATION
(12-1-93)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	SOLIDS CONCENTRATION (mg/l)
36-2'	35	77
36-2'	40	142
36-7'	30	130
36-7'	35	117
36-12'	(bottom sludge approx. 4 ft. deep no solids test on this sample)	
13-2'	40	83
13-2'	50	100
13-7'	35	83
13-7'	40	83
13-15'	20	38570
	(bottom sludge approx. 3 ft.deep)	

SOLIDS CONCENTRATION
(4-25-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	SOLIDS CONCENTRATION (mg/l)	PERCENT VOLATILE
11-5'	30	170	65
11-7'	30	93	57
11-9'	30	143	51
11-13'	30	123	43
11-15'	30	120	9
11-17'	30	133	40
11-19'	30	137	56
11-21'	45	71 *	31
11-22.5'	50	4766	23

* This value seems low. Visually the sample appeared to have more solids that the sample bottles at the upper depths.

SOLIDS CONCENTRATION
(4-25-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	SOLIDS CONCENTRATION (mg/l)	PERCENT VOLATILE
21-2'	30	163	53
21-4'	35	109	71
21-6'	30	177	51
21-8'	25	144	53
21-10'	30	103	61
21-12'	25	128	59
21-14'	25	150	62
21-16'	50	302	32

SOLIDS CONCENTRATION
(Sampling date: 9-8-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	SOLIDS CONCENTRATION (mg/l)
21-0.4'	35	86
21-4.4'	36	156[‡]
21-8.4'	30	73
21-11.4'	30	93
21-14.4'	26	88
21-15.4'	26	69

[‡] This value appears to be out of line with others at aerator 21.

SOLIDS CONCENTRATION
(Sampling date: 9-9-94)

MP-0.4'	33	79
MP-1.4'	30	83
MP-5.4'	30	73
MP-10.4'	30	87
MP-14.4'	25	68
MP-20.4'	25	88
MP-21.4'(sludge)	50	43524
MP-23.4'(sludge)	50	86930
36-0.4'	33	558[‡]
36-5.4'	25	100
36-8.4'	30	143
36-11.4'	22	109
36-12.4'(sludge)	50	44570
36-13.4'(sludge)	50	66262

[‡] This value seems high. Visually the sample appeared to have no more

SOLIDS CONCENTRATION
(Sampling date 12-28-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	SOLIDS CONCENTRATION (mg/l)
PS-1'	23	135
PS-3'	27	163
PS-6'	30	213
PS-8(sludge)	50	45336
EFF-1'	29	76
EFF-4'	25	60
EFF-7'	30	87
EFF-10'	31	77
EFF-13(sludge)	50	29264

SOLIDS CONCENTRATION
(Sampling date: 12-30-94)

A34-1.5'	23	104
A34-4.5'	21	119
A34-10.5'	27	133
A34-15.5'	18	944
A34-15.5'	50	4506*

SOLIDS CONCENTRATION
(Sampling date: 12-29-94)

A3-1'	26	88
A3-7'	28	82
A3-14'	25	68
A3-17'	25	72
A3-20'	24	92

* Sample A34-15.5' is total solids done on a steam table in an evaporating dish.

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(12-1-93)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)
36-2'	5	102
36-2'	10	111
36-2'	15	126
36-7'	5	150
36-7'	10	128
36-7'	15	139
36-12'	5	192
36-12'	10	240+
13-2'	5	72
13-2'	10	92
13-2'	15	80
13-7'	5	54
13-7'	10	54
13-7'	15	55
13-15'	5	500+
13-15'	5	500+

+ indicates that DO in BOD test at 5 days was less than 1.0 mg/l and the BOD_5 reported is questionable.

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(4-25-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)	AVERAGE BOD_5 (mg/l)
21-2'	5	27	
21-2'	10	31	34
21-2'	15	44	
21-6'	5	27	
21-6'	10	31	39
21-6'	15	60	
21-12'	5	36	
21-12'	10	31	37
21-12'	15	43	
21-14'	5	21	
21-14'	10	28	27
21-14'	15	32	
21-16'	5	24	
21-16'	10	28	28
21-16'	15	31	
11-5'	5	39	
11-5'	10	51	53
11-5'	15	69	
11-9'	5	48	
11-9'	10	49	54
11-9'	15	66	
11-13'	5	66	
11-13'	10	36	42
11-13'	15	39	
11-17'	5	36	
11-17'	10	32	40
11-17'	15	51	
11-21'	2	67	
11-21'	5	36	46
11-21'	10	36	
11-22.5'	1	105	
11-22.5'	3	75	91
11-22.5'	5	93	

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(Sampling date: 9-8-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)	AVERAGE BOD_5 (mg/l)	COD (mg/l)
21-0.4'	5	69		
21-0.4'	15	70	70	667
21-4.4'	5	57		
21-4.4'	15	68	63	
21-8.4'	5	33		
21-8.4'	10	41	37	692
21-11.4'	5	33		
21-11.4'	10	33	33	692
21-14.4'	1	75		
21-14.4'	3	35	55	
21-15.4'	1	105		
21-15.4'	3	45	75	

NOTE: COD test was performed on selected samples.

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(Sampling date: 9-9-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)	AVERAGE BOD_5 (mg/l)	COD (mg/l)
36-0.4'	5	84		
36-0.4'	15	139	111	769
36-2.4'	5	60		
36-2.4'	15	126	93	
36-5.4'	5	66		
36-5.4'	10	84	75	769
36-8.4'	5	66		
36-8.4'	10	82	74	769
36-11.4'	3	80		
36-11.4'	6	62	71	795
36-12.4'	0.5	870		
36-12.4'	1	930	900	
MP-0.4'	5	78		
MP-0.4'	15	93	86	718
MP-1.4'	5	87		
MP-1.4'	15	79	83	
MP-5.4'	5	69		
MP-5.4'	10	87	78	744
MP-10.4'	5	69		
MP-10.4'	10	62	65	744
MP-14.4'	5	69		
MP-14.4'	10	59	64	
MP-20.4'	3	85		
MP-20.4'	6	60	73	718
MP-21.4'	0.5	1770		
MP-21.4'	1	1440	1605	

NOTE: COD test was performed on selected samples.

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(Sampling date: 12-28-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)	AVERAGE BOD_5 (mg/l)	COD (mg/l)
PS-1'	5	96		
PS-1'	10	123	109	1008
PS-3'	5	93		
PS-3'	10	118	106	1044
PS-6'	2	97		
PS-6'	5	84	91	900
PS-8'	1	840		
PS-8'	3	620	730	
EFF-1'	5	30		
EFF-1'	10	25	28	792
EFF-4'	5	30		
EFF-4'	10	24	27	792
EFF-7'	5	27		
EFF-7'	10	23	25	774
EFF-10'	5	30		
EFF-10'	10	21	25	792
EFF-13'	1	540		
EFF-13'	3	760	650	

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(Sampling date: 12-29-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)	AVERAGE BOD_5 (mg/l)	COD (mg/l)
A34-1.5'	5	189		
A34-1.5'	10	186	188	1062
A34-4.5'	5	120		
A34-4.5'	10	153	136	1008
A34-10.5'	3	125		
A34-10.5'	6	97	111	1008
A34-15.5'	2	180		
A34-15.5'	5	129	154	1728

BIOCHEMICAL OXYGEN DEMAND (BOD_5)
(Sampling date: 12-30-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	BOD_5 (mg/l)	AVERAGE BOD_5 (mg/l)	COD (mg/l)
A3-1'	5	27		
A3-1'	10	45	36	810
A3-7'	5	33		
A3-7'	10	36	34	774
A3-14'	5	42		
A3-14'	10	48	45	810
A3-17'	5	30		
A3-17'	10	58	44	792
A3-20'	5	45		
A3-20'	10	51	48	792

TOTAL KJELDAHL NITROGEN
(4-25-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	TKN (mg/l)
21-2'	150	2.1
21-8'	150	2.6
21-16'	150	10.9
11-5'	150	10.6
11-22.5'	150	61.3*

* This value may be in error. There was boil over through the condenser in the final distillation.

TOTAL KJELDAHL NITROGEN
(Sampling dates: 9-8/9-94)

SAMPLE ID (aerator #-depth)	SAMPLE VOLUME (ml)	TKN (mg/l)
21-11.4'	150	3.7
36-8.4'	150	2.2
36-12.4'(sludge)	80	63.0
MP-1.4'	150	3.2
MP-21.4'(sludge)	75	144.0