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USE OF SOLAR ENERGY FOR WASTEWATER
DISINFECTION FOR CROP IRRIGATION
BARD-RESEARCH PROJECT #US-613-83

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USE OF SOLAR ENERGY FOR WASTEWATER DISINFECTION

FOR CROP IRRIGATION

BARD-RESEARCH PROJECT #US-613-83

FINAL REPORT

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PART I

USA CONTRIBUTION

by

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ABSTRACT
USE OF SOLAR ENERGY FOR WASTEWATER DISINFECTION FOR CROP IRRIGATION

The objectives of the project were to determine the feasibility of sensitized photooxidation for wastewater disinfection and detoxification in a full scale field situation; screen a variety of dyes and stains for potential sensitizers; and develop mathematical models to predict and optimize disinfection and detoxification efficiencies.

Sensitized photooxidation was effective for wastewater disinfection and detoxification in full scale systems. Influent coliform values ranging from 500,000 to 5,000,000 per 100 mL were consistently reduced to less than 500 per 100 mL under the following conditions: pH value above 9, a methylene blue concentration of 1 mg/L, and a detention time of 2 hours. When turbidity and suspended solids were low, coliform were reduced to less than 10 per 100 mL. Disinfection of coliform was dependent on pH value, and a pH value above 9 was necessary to ensure maximum reaction rates. A methylene blue concentration of 1 mg/L was sufficient for disinfection; however, higher reaction rates occurred at higher concentrations. Degradation of bromacil was directly related to percent ionization, and both were dependent on pH value. A pH value above 9 was necessary to ensure maximum reaction rates. A methylene blue concentration in the range from 1 to 10 mg/L was sufficient to photooxidize a 30 mg/L bromacil solution.

Reaction rates for coliform and bromacil removal were adequately described by first order kinetics; however, the sensitized photooxidation of coliform and bromacil is more complex and is dependent on pH, methylene blue concentration, dissolved oxygen, turbidity, and sunlight intensity. Non-linear models were developed to incorporate these parameters.

Screening of 69 potential sensitizing agents showed that thiazine, alloxazine, and xanthene dyes were the most effective sensitizers.

INTRODUCTION

In areas where abundant water supplies are available, treated wastewater is normally discharged into a nearby creek, river, ocean, or suitable waterway. In arid, semi-arid, and other regions, the demands on water for domestic, industrial, and agricultural use at times exceed supplies, and strict rationing and conservation measures are necessary. Water is recognized as too valuable a resource to be used only once before being surrendered to the hydrologic cycle for natural treatment (Bulla and Edgerley, 1968). In these areas where water shortage problems exist, treated wastewater represents a much needed source of water for municipal, industrial and agricultural use.

The requirements for wastewater reuse are that the treated effluent pose no ecological or health problems. These requirements can be met by disinfection and detoxification of the wastewater effluent. Chemical oxidation is presently used for wastewater disinfection. Chlorination is the most widely used method, and ozonation is being used more frequently. The limiting factor in chemical treatment is usually its high cost (ozonation) or the unacceptable buildup of toxic chlorinated organics (Brungs, 1973 and Christensen, 1974). For contact times of two hours, chlorine concentrations of 40 mg/L were required to disinfect virus, and chlorine concentrations of 15 mg/L were needed to reduce coliform bacteria to a few tens per 100 mL for oxidation pond effluents (Kott, 1973). Some organic materials which do not react thermally with chlorine under normal conditions can react photochemically with chlorine under the

influence of the UV light available in sunlight and produce chlorinated organics (Oliver and Carey, 1977). Furthermore, the inability of chlorine to act as an effective disinfectant against viruses, specifically enteroviruses, in wastewater and the possible production of toxic and carcinogenic chlorinated compounds has led to a call for the development of alternate disinfectants (Gerba et al, 1977). If reclamation of wastewater for potable use is to become a reality, a disinfection method must be developed which is capable of achieving large reductions of pathogenic microorganisms.

Many wastewater treatment processes are unable to produce suitable effluents because of increased loading and the demands for a greater degree of treatment. Refractory organic compounds are among the materials adding to the problems of wastewater treatment (Bulla and Edgerley, 1968). Some of these compounds are toxic, incompatible with biological wastewater treatment, persist for long periods in the environment, and accumulate in body fat of fish and higher organisms. Carcinogenic or mutagenic organic compounds in industrial waste streams, if present in even trace amounts, may pose a public health hazard.

Many chemicals are resistant to microbial degradation (Nemerow, 1978). Ketones are more resistant than alcohols, aldehydes, or acids, but less resistant than ethers (Ludzack and Ettinger, 1960). Alkyl benzenes, diols, 2,4,5-trichlorophenol, and morpholines with a nitrogen-containing heterocyclic ring are resistant or inhibitory to microbial oxidation. Halogenated ethers and aromatics, larger polycyclic aromatic hydrocarbons,

polychlorinated biphenyls, organochlorine pesticides, phenolic wastes, and refractory pesticides pose potential problems to biological industrial waste treatment (Nemerow, 1978).

Physicochemical means of industrial waste treatment are increasingly used to deal with these problem wastes. Activated carbon is sometimes a cost effective treatment for removing toxic and refractory organic compounds from industrial wastes (Becker and Wilson, 1978). Other physicochemical treatments such as chlorination, ozonation, ultraviolet light treatment (Bauer, 1972), and acid or alkaline hydrolysis (Hackman, 1978; Ferguson, 1975) are sometimes used. Incineration is also gaining widespread attention, particularly for extremely toxic and recalcitrant wastes such as polychlorodibenzo-p-dioxins and polychlorinated biphenyls (Hackman, 1978). These methods are all effective in the proper setting; however, they often have high operation and maintenance costs and are therefore used on a limited basis (Siegrist, 1983).

A new approach to wastewater disinfection and detoxification is the use of sunlight and photosensitizers. Sunlight or solar ultraviolet (UV) radiation is a good disinfectant by itself (Metcalf and Eddy, 1978), and makes a substantial contribution to the natural purification of surface water (Grigsby and Calkins, 1980; Bitton et al, 1979). In direct photodegradation, the molecules excited by sunlight lose their absorbed light energy and are thus decomposed or altered by dissociation, dehalogenation, isomerization, or other deactivation routes (Plimmer, 1971). The problem with direct photodegradation or photolysis is that many organic pollutants and fundamental

biological compounds absorb light primarily in the UV region.

The energy necessary to break chemical bonds between C and C or C and O amounts to 80 and 88 kcal/mole, respectively, and only UV radiation with energy higher than these values and with wavelengths less than 325 nm can disrupt these bonds in a photolytic reaction. Solar UV radiation below 270 nm is absorbed by ozone and air, and does not reach the earth's surface (Meier, 1971). Because less energy reaches the earth's surface in the UV region than in the visible region, there is often insufficient sunlight for direct photodegradation or photolysis by UV radiation (Thorington, 1980). For photochemical reactions, it is usually visible light between 400 and 700 nm and UV radiation between 390 and 350 nm which are active. Furthermore, UV light, unless produced artificially (i.e. lamps), would be ineffective on wastewater effluents because of the absorption by organic matter at the surface of the water (Gerba et al, 1977). Longer wavelengths (e.g. methylene blue at 670 nm) are not absorbed by organics and can penetrate to much greater depths.

Photochemical activity with visible light is associated with sensitizers or dyes. Sensitizers absorb light in the visible region of solar radiation and transfer the absorbed energy either directly or indirectly to a substrate, which results in the photodecomposition of the substrate. Sensitized photooxidation, photodynamic action, photodynamic inactivation, photodynamic oxidation, indirect photolysis, and photosensitization are terms used to denote the oxidation of a substrate in the presence of molecular oxygen and an appropriate sensitizer. The term

photodynamic action was coined to distinguish these reactions from the sensitization of photographic plates by dyes and is applied by biologists to the killing or damaging of biological systems by visible light, sensitizers and molecular oxygen (Spikes and Straight, 1967). The term photosensitization, rather than sensitized photooxidation, is sometimes preferred for reactions occurring in the absence of oxygen or under anaerobic conditions.

In areas where water shortage problems exist, treated wastewater represents a much needed source of water for municipal, agricultural, and industrial use. The advantages of sensitized photooxidation for wastewater disinfection and detoxification are several. Because sensitizers absorb energy in the visible spectral region, sunlight serves as a no cost energy source for the process, and energy costs for such systems are minimal. The sensitizer is regenerated in the process, and only trace amounts are required. Unlike chlorination, toxic chlorinated organics are not formed. Structural facilities (ponds) and instrumentation associated with these systems are relatively inexpensive and are simple to design, construct, operate, and maintain. In addition, the required contact time for disinfection and detoxification is relatively short.

Sensitized photooxidation for wastewater disinfection and detoxification has proved to be effective in small scale laboratory systems; however, the process has not been evaluated in a full scale system. The objective of this study was to develop information that could be used to design a full scale sensitized photooxidation system. The specific objectives were

to: determine the feasibility of sensitized photooxidation for wastewater disinfection and detoxification in a full scale field situation; determine the effects of mixing, sensitizer concentration, sunlight intensity, pH value, reactor depth, dissolved oxygen concentration, temperature, suspended solids, and detention time on disinfection and detoxification efficiency; document operational and process control difficulties and develop remedial measures for these problems; screen a variety of dyes, stains, and indicators for potential sensitizing agents and determine the effect of pH value on the reaction rates for these dyes; and develop mathematical models to predict and optimize disinfection and detoxification efficiency.

LITERATURE REVIEW

INTRODUCTION

With the absorption of light, a molecule rises from its ground state of lowest energy to an excited state of higher energy in which one of the electrons is at a higher energy level (Meier, 1971). The activated molecule, with its raised electron, expends the energy of excitation in one of several ways. The molecule may either dissipate the absorbed energy as heat, release the energy as light (i.e., fluoresce or phosphoresce), or convert the energy into chemical energy by dissociation, by intramolecular rearrangement, by redox processes with neighbor molecules, or by other photochemical reactions.

The absorbed energy may also be transferred to another molecule which becomes excited and ready for photochemical reactions. In the energy transfer process, the absorbing molecules act as photosensitizers or photocatalysts, which displace or transfer photochemical reactions to the longer wavelengths of the visible region. Without the sensitization effect of dyes, many important photoreactions such as assimilation in plants (photosynthesis), vision in the eye, and vaccine purification would not be possible (Meier, 1971).

In certain reactions, dyes are predestined sensitizers because the most reactive triplet state (3D) is produced with high efficiency by intersystem crossing from the first excited singlet state (1D). 3D is reactive not only in redox reactions, but 3D can also transfer the energy of the triplet state to other molecules and initiate specific reactions (Meier, 1971). With

few exceptions, photosensitized reactions proceed by way of the triplet sensitizer, probably because the triplet state has a much greater lifetime than the excited singlet state (Foote, 1968). The most effective sensitizers are, therefore, those which give a long-lived triplet state in high quantum yield.

Most proposed mechanisms suggest the triplet sensitizer (3D) can react via several possible pathways (Rizzuto and Spikes, 1977). The sensitizer triplet can react initially with oxygen (a sensitizer-oxygen, or oxygen transfer or Type II mechanism) or with a substrate (a sensitizer-substrate or hydrogen abstraction or Type I mechanism), or with other triplet or ground state dye molecules.

MECHANISMS

SENSITIZER-SUBSTRATE, HYDROGEN ABSTRACTION, TYPE I MECHANISM

A sensitizer-substrate interaction occurs when dyes with strong electron or hydrogen abstracting groups react with compounds with removable hydrogen (Meier, 1971). These dyes are highly reactive against reducing agents or hydrogen donors, and are mostly vat dyes or derivatives of anthraquinone, indanthrone, flavanthrone, etc. They have a triplet (n, π^*) as the lowest excited state. The photoexcited dye is reduced by the oxidizable reactant via a transfer of electrons or hydrogen, and the resulting semireduced dye radical is reoxidized to the ground state by oxygen (Meier, 1971).

After the initial reaction between the dye triplet and the substrate, molecular oxygen can enter the process via several different mechanisms (Nilsson et al, 1972). Molecular oxygen may reoxidize semireduced dye intermediates or may act as an electron acceptor and cause irreversible formation of oxidized products by electron transfer from the half-oxidized substrate molecule A[·].

At low O₂ or high dye concentrations, the triplet dye can transfer electrons to the ground state dye (Foote, 1968).

The complexity of the sensitizer-substrate mechanism is due to the variety of secondary reactions available to the initially formed redox pair. These reactions are often followed by a rapid reverse reaction regenerating the ground state sensitizer, so that the only observable effect is quenching of the excited sensitizer. A second important reaction is the combination of radical species produced in Type I reactions. Instead of combining, intermediate radicals may disproportionate giving equal amounts of leuco dye and starting dye.

Intermediate radicals can react rapidly with oxygen to form peroxy radicals, which in turn, abstract hydrogen to yield hydroperoxides (not to be confused with the formation of allylic hydroperoxides by singlet oxygen). Depending on the source of

hydrogen, new radicals may or may not be produced, and may result in conventional chain radical autooxidations.

Bleaching, Fading, and Reduction

The loss of the long wavelength absorption maximum of a sensitizer (photofading) is due to two types of reactions (Knowles, 1971). The first, photoreduction, results from hydrogen abstraction and gives the colorless leuco-dye, which can be reoxidized by admission of air to the solution. The second, photobleaching, cannot be reversed by oxidation by air. The nature of the reactions which cause photobleaching is uncertain; however, the reaction may involve a degree of irreversible reduction.

In the absense of oxygen, the semireduced dye is transformed to the leuco dye, (i.e., photobleached) and is not regenerated. This sensitized oxidation can also lead to a sensitized photodestruction of a dye without any special substrate. In these cases, the dye serves as an oxidizable substrate, as in the bleaching of aerated chlorophyll by high intensities of light. The photoreduction of excited dye molecules by solvent or solute may also occur (Meier, 1971). The solute or solvent is oxidized with a simultaneous reduction and consequent photobleaching of the dye.

The above reaction involves a hydrogen atom or an electron transfer from the reducing (electron-donating) agents to the dye.

Both the hydrogen atom transfer (which can also be discussed as

an electron transfer followed by a proton transfer) and the electron transfer require an electrophilic center in the reduced species.

For the photobleaching of a dye, usually two hydrogen atoms (or two electrons and two protons) are added to a double bond of the conjugated system of the dye.

The photoreduction of dyes (e.g., riboflavin) proceeds in two one-electron steps, giving a semiquinone $DH^\cdot$ which by autooxidation is transformed into the leuco form of the dye. With the reducing agent Fe^{2+} , the following occurs.

The reduction of the dye may be reversed by hydrogen peroxide or atmospheric oxygen.

In a series of combinations of dyes and reductants, the recovery of the photoreduced dye by oxidation may be so rapid that photoreduction and photobleaching, respectively, are not at all observed (Meier, 1971).

For some amino acids, a correlation existed between the bleaching effect and the ease of sensitized oxidation (Knowles and Gurnani, 1972). Tryptophan and cysteine readily underwent both reactions while alanine, phenylalanine, and cystine did not bleach the excited dye and were thought to be resistant to sensitized oxidation. The reactive amino acids provided a source of electrons or hydrogen atoms and would thereby by partially

oxidized in the anaerobic system.

If the irradiation is carried out in the absence of oxygen, the behaviour of the sensitizer should indicate which mechanism is involved. The hydrogen abstraction mechanism will proceed until all of the dye is reduced, while in the Type II mechanism neither sensitizer nor substrate will react since there is no oxygen present to form the intermediate (Knowles, 1971). Methylene blue was reduced when irradiated in an oxygen free solution with secondary and tertiary amines, and the reaction was strongly dependent on the pH value. The photofading reaction was not only dependent upon the pH value but also on the buffer. Addition of phosphate or borate buffer caused an increase in the photofading rate of the sensitizer, even though the buffers were not acting as reducing agents.

Phosphate buffer had a salt effect which accelerated the protonation of the dye triplet (Knowles, 1971). Xanthosine monophosphate (XMP), which was most sensitive to the photodynamic action of methylene blue, was also effective in reducing the triplet of the dye. Other nucleotides which were resistant to photooxidation did not act as reducing agents. The quantum yield of the sensitized oxidation of XMP was directly related to the anaerobic photoreduction of methylene blue, and it was concluded that the primary step of the photooxidation was by electron or hydrogen abstraction from the nucleotide by the triplet excited methylene blue.

In bacterial suspensions of *Proteus mirabilis* VI, a decrease in the concentration of oxidized (colored) thiopyronine was

observed during aeration (Jacob, 1974). The reduction of the dye to leucothiopyronine was counteracted to only a small extent by aeration with oxygen since thiopyronine was irreversibly reduced by the bacteria. The decrease of thiopyronine concentration resulted in a decrease in the rate of photodynamic inactivation. In suspensions of bacteria containing methylene blue, addition of oxygen inhibited reduction of the oxidized dye. In a similar study, corrections for the photooxidative bleaching of eosin Y during irradiation indicated that bleaching also caused a significant reduction in the apparent rate of inactivation (Cohn and Tseng, 1977).

SENSITIZER-OXYGEN INTERACTION, OXYGEN TRANSFER PROCESS, TYPE II MECHANISM

A sensitizer-oxygen interaction occurs for dyes with a higher reactivity for oxygen than for reducing agents (Meier, 1971). These dyes include the acridines, xanthenes, thiazines, thioxanthenes, and alloxazines, and appear to have the common feature of a (π , π^*) as the lowest excited state. Different mechanisms are presented for this process in which oxygen is transferred to an oxidizable substrate; however, all the mechanisms are initiated by the excitation of the dye to the singlet state, followed by intersystem crossing to the triplet state.

Dye-oxygen complex

The first mechanism, proposed by Schoenberg and advocated by Schenk and others, was once widely accepted (Foote, 1968; Meier, 1971). The reactive species is a metastable dye-oxygen complex

produced between the triplet excited dye and oxygen (Meier, 1971).

This complex then reacts with acceptor A to give the product peroxide AO_2 and to regenerate the sensitizer. Experimentally, it is difficult to distinguish between the sensitizer-oxygen complex and singlet oxygen, and at one time most workers preferred the dye-oxygen complex (Foote, 1968).

Superoxide anion

Kautsky, Egerton, and others showed that sensitized photooxidation can occur in systems with separated dyes and substrates (Meier, 1971). This indicated the production of an oxidizing volatile species and seemingly contradicted the previous concept. This volatile species may be either hydrogen peroxide (in the presence of water) or a semireduced oxygen molecule $O_2^{\cdot-}$, also known as superoxide anion, which acts as an intermediate in the production of hydrogen peroxide.

The reaction between molecular oxygen and dye leads to a semioxidized dye radical which is rereduced to the ground state by the oxidizable reactant.

This semioxidized dye is also produced by an electron dismutation reaction between dye molecules in the excited triplet state and dye molecules in the ground state. As in the

dismutation process, a semireduced dye is formed which rapidly reacts with oxygen; the reduced $O_2^{\cdot-}$ also results from this indirect reaction (Meier, 1971).

In aqueous solution, $O_2^{\cdot-}$ exists in equilibrium with the hydroperoxy radical $HO_2^{\cdot}$ (Khan, 1978). A pK of 4.88 for the ionization was determined. At a neutral pH value, the ratio $[HO_2^{\cdot}]/[O_2^{\cdot-}]$ was roughly 1:100. A second protonation occurs at lower pH, yielding OOH_2^+ (pK 1.2). In acid and neutral pH ranges, the decay of the $O_2^{\cdot-}$ and $HO_2^{\cdot}$ species is due to the disproportionation proceeding through the $HO_2^{\cdot}$ radical.

The interest in the biochemical and chemical properties of the superoxide radical $O_2^{\cdot-}$ largely due to the discovery of the enzymatic role of superoxide dismutase (Cadet and Teoule, 1978). The enzyme, which dismutates $O_2^{\cdot-}$ to hydrogen peroxide and ground state oxygen, is ubiquitous in aerobic organisms. A large number of enzymatic systems and autooxidation agents produce $O_2^{\cdot-}$. In addition, superoxide radicals may be generated in sensitized photooxidation and gamma irradiation of aerated solutions. The participation of $O_2^{\cdot-}$, the reactivity of which is controlled by its anionic character and reduction potential, has been demonstrated in a large number of reactions.

Singlet Oxygen

Kautsky, Egerton, and others discuss another possibility, the production of an activated oxygen species by the excitation of the dye (Meier, 1971). In this mechanism, originally proposed by Kautsky in 1931, energy is transferred from the triplet sensitizer to oxygen to give excited singlet molecular oxygen (Foote, 1968). The singlet oxygen then reacts with an acceptor to give the peroxide.

The oxidation products should be the same as in the first mechanism; however, instead of the dye-peroxide complex, oxygen eventually, after diffusing over relatively large distances, reacts as the active species. This activated oxygen may be vibrationally excited oxygen O_2^* .

The addition of the excitation energy to molecular oxygen changes its ground triplet state 3O_2 to the first excited singlet state ${}^1O_2({}^1D_g)$ which has a higher energy by 22.5 kcal/mol (Acher and Rosenthal, 1977). When more energy is imparted to the oxygen molecule, another state is formed, ${}^1O_2({}^1S_g)$, which corresponds to a level of 37.5 kcal/mol above ${}^1O_2({}^1D_g)$. The ${}^1O_2({}^1D_g)$ state is long-lived and survives at least 10^8 collisions with methanol in the vapor phase, whereas the ${}^1O_2({}^1S_g)$ state survives no more than 10 collisions under the same conditions (Foote, 1968). It appears likely that only ${}^1O_2({}^1D_g)$ is important in solution

oxidations.

The pH value of the solution plays an important role in singlet oxygen production. The production of singlet oxygen by energy transfer from triplet methylene blue in air saturated solutions varied by a factor of approximately 5 for the pH region between 5 and 9, with a maximum change around a pH value of 7 (Bonneau et al, 1975). In strongly buffered solutions, the yield of singlet oxygen varied by a factor of two when the pH was changed from 6.5 to 7.5. The concentration of the buffer is also important. In unbuffered or weakly buffered solutions, the quenching of the non-protonated methylene blue species by oxygen was much faster than the protonation rate for any pH values greater than 5, and the yield of singlet oxygen was similar to that obtained at a pH value of 8. For solutions in the pH range of 5 to 6, the yield of singlet oxygen will be 4 to 5 times smaller in strongly buffered solutions than in non-buffered solutions. The above magnitudes of singlet oxygen production variations with pH value and buffer effect were determined for air-saturated solutions.

The various reactions which have to be considered in any attempt to evaluate the role of singlet oxygen in sensitized photooxidation are indicated below (Nilsson et al, 1972)

where ^3S and $^1\text{S}_0$ denote excited triplet and the ground singlet state sensitizer, respectively. $^1\text{O}_2$ is $^1\Delta$ oxygen, A is the oxidizable substrate, and AO_2 is some oxidation product.

The addition of singlet oxygen to dienes and aromatic hydrocarbons gives endoperoxides and the addition to olefins gives hydroperoxides (Figure 1) (Foote, 1968; Kearns and Khan, 1969). The peroxides are often thermally unstable with respect to release of molecular oxygen and regeneration of the parent hydrocarbon. Many studies have shown that singlet oxygen can oxidize a variety of organic substances including biologically important compounds such as amino acids and pollutants such as polycyclic aromatic hydrocarbons and pesticides (Zepp et al, 1977). Singlet oxygen is likely an important intermediate in the transformation of certain pollutants and biologically important substances in lakes and rivers. It is also possible that the peroxides formed in singlet oxygen reactions may initiate the free radical oxidation of aquatic pollutants. Among the various forms of molecular oxygen, singlet oxygen is of particular physiological importance because of its relatively long lifetime in aqueous solutions (Houba-Herlin et al, 1982).

TYPE I AND/OR TYPE II (SUPEROXIDE AND/OR SINGLET OXYGEN)

The question of which species of oxygen, superoxide anion or singlet oxygen, is generated in the sensitizer-oxygen interaction is complicated because singlet oxygen can also be produced (probably in low yield) from $\text{O}_2^{\cdot-}$ or $\text{HO}_2^{\cdot}$ and could also be

Figure 1. Sensitizer interactions. (Foote, 1968)

produced via the electron transfer path or a Type I mechanism (Foote, 1968). Evidence is accumulating that $^1\text{O}_2$ and $\text{O}_2^{\cdot-}$ are interconvertible (Khan, 1978). Experimental results indicated that $^1\text{O}_2$ was generated in the electron transfer reactions of $\text{O}_2^{\cdot-}$; however, the reverse process, the reduction of $^1\text{O}_2$ to $\text{O}_2^{\cdot-}$ was suggested. The complex relationship between these two forms of activated oxygen is illustrated by the capacity for $^1\text{O}_2$ to be simultaneously generated and quenched in the disproportionation of the superoxide anion. Many photooxidation reactions previously ascribed to the presence of singlet oxygen in water may be due to hydroxy radicals (Draper and Crosby, 1981); however, the mechanism of formation of both $\text{OH}^\cdot$ and $^1\text{O}_2$ from $\text{O}_2^{\cdot-}$ remains open to question (Cadet and Teoule, 1978).

The question of which mechanism, Type I or Type II, governs in a given system may not always be obvious. Photooxidation reactions sensitized by thionine follow a Type I mechanism; however, the reaction can be switched to a Type II (singlet oxygen) mechanism (Kramer and Maute, 1973). For the system thionine and 9,10-dimethylanthracene (DMA), photooxidation at low DMA concentration occurs according to a Type II mechanism, but in oxygen free solutions, thionine is partially photoreduced by DMA, which is typical of Type I mechanisms. For the systems thionine and allylthiourea (ATU) in pyridine solution, the reaction proceeds via radicals at high ATU concentrations and via singlet oxygen at low ATU concentrations.

To complicate matters further, it is possible to conceive of situations where both pathways, sensitizer-substrate and

sensitizer-oxygen, are operative, especially in systems involving the oxidation of complex molecules such as proteins and nucleic acids where many alternative reaction sites of differing chemical character exist (Nilsson et al, 1972). The sensitized photooxidation of guanosine proceeds by both singlet oxygen and Type I mechanisms, and the efficiency of the singlet oxygen mechanism is strongly dependent on the photosensitizer type (Saito et al, 1975). In complex biological systems, binding of the sensitizer to the oxidizable substrate (protein, polynucleotide, etc.) is also expected to influence the course of the reaction, favoring sensitizer-substrate interactions even at high O_2 concentrations (Foote, 1968).

The fundamental mechanism of biological systems (DNA, RNA, protein, lipid, carbohydrate) can involve either a free radical (Type I) or a singlet oxygen (Type II) intermediate, or both concurrently (Roberts, 1981). Sensitized photooxidation of amino acids may proceed via both Type I and Type II reactions at the same time, the relative proportions of the reactions varying with substrate concentration. It is difficult to establish the mechanisms of photosensitized oxidations in these complex systems (Bagno et al, 1979; Kraljic et al, 1978). The relative importance of the different mechanisms in a particular system depends on the relative values of the oxygen and substrate concentrations, the rates of reaction of sensitizer triplet with substrate (k_s) and with oxygen (k_{O_2}), and the rate of triplet decay (k_d) (Foote, 1968).

In addition, photooxidation reactions can also proceed via radical forms of the sensitizer, which are produced by triplet-

triplet, triplet-oxidant, triplet-reductant as well as triplet-ground state dye interactions (Rizzuto and Spikes, 1977). Reactions between substrate and these radicals may play an even more important role in oxidation of the substrate than substrate reactions involving the dye triplet state.

SENSITIZERS

Studies have shown that the nature of the sensitizer will influence the mechanism of sensitized photooxidation on chemical and biological systems from amino acids (Ghiron and Spikes, 1965) to bacteria (Bagchi and Basu, 1979) to virus (Yamamoto, 1958) to pesticides (Watts, 1985). The mechanism of disinfection and detoxification is not unique and depends on pH value, dissolved oxygen, buffer, sensitizer, and substrate. Not all sensitizers require oxygen. For example, 8-methoxypsoralen is ineffective and destroyed in the presence of oxygen (Matthews, 1963).

For inactivating phage, the most effective sensitizers were the thiazine, oxazine, and acridine dyes (Figures 2-3)(Yamamoto, 1958). These active dyes are basic (cationic) heterotricyclic compounds with two chargeable nitrogen atoms situated at comparable positions on both sides of the skeleton (Figure 4). The distances of the two nitrogens are almost equal in all active dyes, and conjugated double bond systems lie between the two atoms. The dyes possess an affinity for nucleic acids and the association is of an ionic nature.

These active dyes can all be derived from the fundamental skeleton by suitable variations in X and Y where X is nitrogen or carbon and Y is sulfur, oxygen, or nitrogen. The cations of

Proflavine

Acridine Yellow

Acriflavine

Quinacrine

Acridine Orange

Aminoacridine

Figure 2. Structure of acridine sensitizing dyes.

THIAZINES

Toluidine Blue

THIAZINES

Thionin

THIOXANTHINES

Thiopyryronine

ALLOXAZINES

Lumichrome

ALLOXAZINES

Riboflavin

THIAZINES

Methylene Blue

Figure 3. Structure of sensitizing dyes.

these dyes are a resonance hybrid of the structures, with the cationic charge located either on one of the two nitrogen atoms or on the heteroatom in the heterocyclic ring. The cationic charge associates with an anionic charge of the phage. The uncharged nitrogen atom in the side chain forms a coordination bond with a cationic charge or an empty orbital via the lone pair of electrons. These active dyes may associate with the substance-X of the phage through a bridge-like structure as shown in Figure 4 (Yamamoto, 1958).

Sensitizers interact with the cell in specific ways depending on the chemical properties (Ito, 1980). Acridine orange binds to DNA resulting in DNA damage upon illumination as a major cause of cell killing. Toluidine blue, on the other hand, appears to act from outside of the cell without penetrating deep inside the cell. Toluidine blue does not sensitize genetic material and a major intermediate involved in these photodynamic actions is probably singlet oxygen (Ito and Kobayashi, 1977). Three xanthene, three thiazine, three acridine dyes and ethidium bromide were surveyed with respect to cell inactivation and induction of genetic changes in yeast cells. Rhodamine B in the first group and methylene blue in the 2nd group were inactive and were found to be non-penetrable into the cell. However, since toluidine blue was active, non-penetrability was not a determining factor in photodynamic action. Ethidium bromide was inactive, even though it was penetrable into the cell. The survey showed that the dye must be bound to DNA in order to be active in the induction of genetic change. All dyes which were

Figure 4. The common structures of active dyes. Ia (top left), Ib (top right), Ic (center): resonance. II(bottom): suggested mechanism of association with phage substance-X. (Yamamoto, 1957)

active in either inactivation or induction or both were modified by the addition of N_3^- (suppression) and in deuterated medium (enhancement), indicating that the sensitization mechanism involved singlet oxygen.

Lumichrome sensitized the photooxidation of deoxyguanylic acid and xanthine, while thiopyronin sensitized the photooxidation of guanine, 2-aminopurine, 2,6-diaminopurine, xanthine and isoguanine, but not the photooxidation of adenine, hypoxanthine, and 8-azaguanine (Spikes and Straight, 1967). Toluidine blue, thionine, rose bengal, riboflavin, and eosin Y were also efficient sensitizers for guanine derivatives; fluorescein and neutral red showed some activity; and acridine orange, alizarin red, crystal violet, Janus green, methyl orange, proflavin, pyronin, and safranin O showed little activity. With methylene blue, the rate of photooxidation of guanine derivatives increased with pH value, suggesting that the anionic forms were more sensitive than the neutral species.

For the detoxification of bromacil, the most effective sensitizers were found to be methylene blue, riboflavin, eosin Y, rose bengal, dichlorofluorescein, fluorescein, phloxine B, and Wright's Stain (a compound of eosin with oxidized and partly demethylated methylene blue) (Eisenberg et al, 1986).

In addition to introduced sensitizers, natural photosensitizers may play an important part in the environmental transformations of xenobiotics (Ross and Crosby, 1973). Sensitizers such as riboflavin and chlorophyll occur in natural waters, and agricultural waters can sensitize the photolysis of xenobiotics. The influence of natural photosensitizers will have

to be considered in future investigations into the environmental photochemistry of pesticides and related products.

pH VALUE

The pH value of the solution may play an important role in the efficiency of the sensitizer. The pH dependence of the methylene blue and eosin Y sensitized inactivation of trypsin was distinctly different from that of the flavin sensitized reaction (Ghiron and Spikes, 1965). The flavin sensitized reaction occurred at measurable rates over the pH range from 2 to 9.5, with a maximum at a pH value of approximately 8. With methylene blue and eosin Y, however, there was little or no measurable photoinactivation below a pH value of 6; the rate of inactivation increased rapidly over the pH range from 6 to 8 and leveled off at a pH value of 9. Disc electrophoresis studies on photodynamically inactivated trypsin showed a progressive loss of protein in the main trypsin band with flavins but not with methylene blue and eosin Y as sensitizers. In addition, the stoichiometric relationship between oxygen consumption and trypsin inactivation appeared to be different with different sensitizers. In studies of the photodynamic inactivation of ribonuclease T_1 , only the histidine residues were destroyed with riboflavin as sensitizer, while with methylene blue both histidine and tryptophan residues were changed. The damaged class of trypsin molecules may be produced by destruction of different proportions of susceptible amino acids, depending on the type of sensitizer and the experimental condition.

The rate of protein inactivation typically increased with pH

value regardless of the charge characteristics of the dye; however, flavins showed activity at low pH values and often had an optimum in the middle pH range (Spikes and Straight, 1967). Inactivation rates increased sharply with dye concentration, went through a maximum, and then decreased. Certain classes of dyes sensitized photodynamic action, while other dyes did not. Inactive types included the azo, indophenol, nitro, oxazine, thiazole, and triarylmethane dyes. Active dyes included acridines, flavins, azines, thiazines, anthraquinones, and xanthenes, and certain porphyrin and chlorophyll derivatives. A number of materials such as aromatic amino acids, reducing agents, other proteins, ions of certain metals, iodide ion, etc. "protected" proteins against photodynamic destruction. In some cases the effect was highly specific for a given dye.

A study of the kinetics of sensitized photooxidation as a function of pH in anionic micellar systems using cationic dyes as sensitizers showed that adsorption of the dye slowed the protonation step of the triplet state (Bagnò et al, 1979). Using cationic dyes as sensitizers, the rate of photooxidation for the same pH value was different depending whether or not the medium allowed adsorption of the dye. In aqueous solutions the rate of photooxidation by singlet oxygen from thiazine dyes was pH dependent in a range critical to many biological systems. This was caused by the acid-base equilibrium occurring in the triplet state of the dyes. Monocationic triplet methylene blue ($^3\text{MB}^+$) can be protonated in nearly neutral media yielding $^3\text{MBH}^{2+}$.

Both triplet species are photosensitizers, and the pH dependence is due to differences in their lifetimes and their energies with respect to $^1\text{O}_2$.

SINGLET OXYGEN

Singlet oxygen production at a pH value of 9.0 was analyzed for different sensitizers, and the rates (in arbitrary units per min) were: 71 with rose bengal, 70 with methylene blue, 61 with eosin Y, 18 with thiopyronine, 10 with proflavine and 9 with acridine yellow (Houba-Herlin et al, 1982). Production of $^1\text{O}_2$ was not observed with 9-aminoacridine, acridine orange, quinacridine and ethidium bromide.

Some sensitizers acted simultaneously as producers as well as quenchers of singlet oxygen during the photoinactivation of lysozyme (Schmidt et al, 1981). The quantum yield of the photodynamic inactivation of lysozyme increased in the sequence acridine orange, methylene blue, proflavine and acriflavine (1:5:6:12). For protein concentrations upto 0.1 mM, singlet oxygen was responsible for the inactivation of the enzyme. For methylene blue, acriflavine, and proflavine, the quantum yield decreased with increasing dye concentrations. In the case of methylene blue, the effect was mainly caused by the quenching of singlet oxygen. For the acridine sensitizers, both singlet oxygen and dye triplet quenching processes had to be considered.

Thiazine dyes are often employed as sensitizers in the production of singlet oxygen (Bonneau et al, 1975). The lifetimes of the acidic and basic methylene blue triplet species differ by a factor of approximately 20. The redox properties

undergo similar variations, and the rate of singlet oxygen production may also vary as a function of pH, owing to the various ionic species present. The production of singlet oxygen by thiazine dyes, as measured by the rate of photooxidation of tryptophan, was found to be very sensitive to changes in pH value in the ranges from 5 to 9. For methylene blue in aerated solutions, the production of $^1\text{O}_2$ is approximately five times more efficient in basic than in acidic medium and is related to the pK's of the triplet dyes.

METHYLENE BLUE

Methylene blue is a widely use photosensitizer due to its high effectiveness, low cost, ready availability, ease of removal via adsorption, and penetrability into high turbidity wastewater (Gerba et al, 1977; Hobbs et al, 1977; Watts, 1983; Eisenberg et al, 1985). The dye has a low toxicity and is administered orally or intravenously in the treatment of certain human diseases and as an antidote. The effectiveness of methylene blue for virus inactivation increased with increasing pH, probably because of an increase in the non-ionized form of the dye, which allows for a greater penetration rate into a virus particle as compared to the ionized form (pK = 11) (Gerba et al, 1977). Methylene blue is a weak base, and the percent of the undissociated or unionized form of methylene blue increases with increasing pH value (Hobbs et al, 1977). The reaction rate for the photooxidation of deoxyguanosine, the active moiety for the inactivation of DNA type nucleic acids, increased 30 times with a changes in pH value from 7 to 10.5.

The wavelength of maximum absorption for methylene blue (670 nm) was found to penetrate clarified wastewater with 15 FTU turbidity to depths of 50 mm with greater than 80% transmission (Gerba et al, 1977). The light penetration of methylene blue into wastewater and other colored waters is greater than systems using other dye/light combinations or to an UV light source (Hobbs, et al, 1977). A large part of energy near the 670 nm wavelength contained in light from the sun passes through the atmosphere and reaches the surface of the earth. Photometer readings indicated that during midday the 670 nm energy reaching the Houston, Texas area exceeded 100 W/m^2 ; even on completely overcast days, a range of 10 to 20 W/m^2 was observed. Exposure of sensitized virus to sunlight indicated that the source of energy was very effective in the photoinactivation of virus in both wastewater and tapwater.

XANTHENE DYES AND HALOGENATION (Figure 5)

Although only certain classes of dyes sensitize photodynamic action, the sensitizing efficiency of a given dye can be altered by changing substituents on the molecule (Wade and Spikes, 1971). Increasing the halogenation of fluorescein (FL) dyes increased the triplet state ^3D production and decreased the singlet excited state ^1D formation, apparently from enhanced spin orbital coupling due to the heavy atom effect of the halogens. Since most mechanisms for photodynamic action involve the long-lived triplet excited state of the sensitizing dye, increasing the halogenation presumably increased the sensitizing efficiency of fluorescein dyes. The sensitizing efficiency and the binding of halogenated fluorescein dyes to trypsin both increased with

Rose Bengal

Eosin Y

Fluorescein

Phloxine B

Dibromofluorescein

Dichlorofluorescein

Figure 5. Structure of xanthene sensitizing dyes.

increasing number and atomic weight of the substituted halogen atoms. A direct correlation was found between the efficiency of a dye in sensitizing the photoinactivation of trypsin and the quantum yield for the formation of the triplet state of the dye; however, there was no direct correlation between binding and photodynamic efficiency. FL, FLCl₂, FLCl₄, and FLBr₂ did not appear to bind to trypsin, although there a 10 fold increase in sensitizing efficiency in going from FL to FLBr₂. FLBr₄ and FLI₂ bound only slightly, yet showed a high photodynamic efficiency. From the dissociation constants calculated, FLCl₄I₄ bound to trypsin greater than ten times more efficiently than FLI₄, yet its photodynamic quantum efficiency was only slightly greater. In some systems, binding decreased the photodynamic efficiency of sensitizing dyes. In the study cited, the ability of halogenated fluorescein dyes to sensitize the photodynamic inactivation of trypsin was primarily a function of the efficiency of formation of triplet state dye and not of the ability of the dye to bind to trypsin.

ADDITIONAL APPLICATIONS

In addition to use as photosensitizing agents, sensitizers have a wide range of applications. The efficiency of salt water evaporation for the manufacture of common salt and other salts is improved with the addition of a dye to increase the amount of absorbed radiation (Venkaataraman, 1952). Rose bengal, and eosine are used as adsorption indicators in volumetric analyses in the titration of halides with silver nitrate (Venkaataraman, 1952). The intense green fluorescence of fluorescein has been

used for the detection of leaks in drains and passage of water through subterranean channels. During World War II fluorescein, which could be seen at great distances by rescuers, was released into the water by airmen forced down into the sea.

Methylene blue (the principle sensitizer employed in this study) has a host of non-photosensitizing applications: oxidation reduction indicator, selective staining of nerve cells, examination of pathogenic organisms such as tubercular and cholera bacilli, reagent for testing tubercular infection in milk, analgesic and urinary antiseptic, antimalarial agent, weak and slow acting antiseptic, external applicant in skin diseases, and an antidote for cyanide and nitrate poisoning (Venkaataraman, 1952; Merk Index, 1983; Gerba et al, 1977)

SUMMARY

Sensitizers which are effective in sensitized photooxidation for wastewater disinfection and detoxification must meet the following criteria: induce a reaction with visible light; be chemically stable during the radiation (refractory to auto-oxidation and resistant to degradation); be aesthetically acceptable to end-consumers; not be toxic to or taken up by plants; not cause toxicity problems in the environment or on return to the wastewater treatment plant (be free of reactive functional groups which can react with chemicals present in sewage and inactivate it; have a good light absorption capacity (be effective at low concentration); be soluble in water but easy to remove; (Acher and Rosenthal, 1977; Cheung et al, 1986). The most efficient sensitizers are xanthene (e.g. eosin, rose

bengal), thiazine (e.g. methylene blue, thiopyronine), acridine (e.g. acriflavine, proflavine), azine (e.g. safranin, neutral red), isoalloxazine (e.g. luminflavin, riboflavin) dyes, certain porphyrins and polycyclic aromatic hydrocarbons (e.g. benzpyrene) (Yamaototo, 1958; Meier, 1971; Acher and Rosenthal, 1977; Eisenberg et al, 1986; etc).

BIOLOGICAL SYSTEMS

INTRODUCTION

Photodynamic action has been known since about 1890 but the phenomenon was first clearly described by Raab in 1900 (Hobbs et al, 1977). Raab showed that paramecia suspended in an acridine solution were killed by ordinary daylight in the presence of oxygen but were unaffected in the dark under the same conditions (Spikes and Glad, 1964; Wallis and Melnick, 1965; Spikes and Straight, 1967). Detailed studies on inactivation of proteins, nucleic acids, bacteria, and viruses were not performed until the 1920's and the early 1930's; however, assay methods were still crude and the results reported were not quantitative (Clifton, 1931; Perdau and Todd, 1933; Burnet, 1933). Quantitative studies of the kinetics of photodynamic inactivation of bacteriophages in the 1950's confirmed that the relative sensitivities of phage strains to photodynamic action could be correlated with serological groupings (Welsh and Adams, 1954; Yamamoto, 1958). Studies on photodynamic inactivation increased rapidly and results showed that almost any type of biological system (plant, animal, cells, viruses, biologically important molecules) could be sensitized to photochemical damage and destruction by

appropriate dyes.

The mode of inactivation for viruses is an attack on the nucleic acid which prevents replication of the viral nucleic acid in the host cells (Gerba et al, 1977). The mode of inactivation for bacteria and fungi is destruction of metabolic enzymes in addition to attack on the nucleic acid. All major types of animal viruses are sensitive to photodynamic action. Bacteria found in wastewater, such as coliforms, and pathogenic fungi, such as *Candida albicans*, are easily photoinactivated. Because cell division of bacteria and replication of viral nucleic acid in host cells is prevented, the photodynamic inactivation process may allow for a more positive control of infectious agents in wastewater than either halogens or ozonation (Hobbs et al, 1977).

AMINO ACIDS AND RELATED COMPOUNDS

Dye sensitized photooxidation of the amino acids in proteins or the bases in nucleic acids, is believed to be the basis for most of the phenomena classified under photodynamic action, such as the killing of cells by illumination in the presence of certain dyes (Matheson et al, 1975).

Aromatic amino acids tend to be susceptible to photodynamic action, whereas the aliphatic amino acids are resistant (Spikes and Straight, 1967). Histidine, methionine, tryptophan, and tyrosine were photooxidized rapidly with a wide variety of sensitizers. Dihydroxyphenylalanine was very sensitive to photooxidation, while phenylalanine was oxidized only very slowly. Cysteine was photooxidized with thionine, while cystine

was rather insensitive.

Studies of pH dependence with histidine indicated that sensitized photooxidation occurred only when the imidazole group was unionized, while tyrosine was photooxidized only when the phenolic group was ionized (Spikes and Straight, 1967). The rates of photooxidation of methionine, tryptophan, and tyrosine were not dependent on the extent of ionization of the amino and carboxyl groups. Both the rate and the extent of amino acid photooxidation increased with temperature.

With histidine, tryptophan, and tyrosine, oxygen uptake was associated with destruction of the aromatic ring (Spikes and Straight, 1967). Methionine sulfoxide was reported as the sole oxidation product of methionine, but prolonged illumination produced methionine sulfone and perhaps homocysteic acid. FMN-sensitized photooxidation of sarcosine produced CO_2 , methylamine and formaldehyde. Methylene blue sensitized photooxidation of histidine yielded 4 unidentified products while photooxidation of tryptophan gave 7 organic products, one of them dioxindolylalanine. Deiodination occurred when iodothyronines and iodotyrosines were illuminated in the presence of sensitizers and oxygen. L-histidine and glycyl-L-histidine were photooxidized at essentially the same rate with the same pH dependence with methylene blue; similar results were observed with L-tryptophan and glycyl-L-tryptophan, L-methionine and glycyl-DL-methionine, and L-tyrosine and glycyl-L-tyrosine. The tripeptide glutathione was also photooxidized. In no case was the rupture of the peptide bond observed during the photodynamic treatment of peptides.

Phenylalanine was not photooxidizable, but substitution of electron-donating groups such as $-OH$ (as in tyrosine) or $-NH_2$ (as in p-aminophenylalanine) resulted in rapidly photooxidized derivatives (Rizzuto and Spikes, 1977). However, substituting deactivating groups such as $-Cl$ (as in p-chlorophenylalanine) or weakly activating groups such as $-OCH_3$ (as in 4-methoxyphenylalanine) resulted in non-photooxidizable derivatives. Substitution of additional activating groups to the ring of hydroxy-substituted phenylalanines resulted in increased rates of photooxidation, whereas additional deactivating groups resulted in decreased photooxidation rates.

The rate-determining step in the phenylalanine photooxidization mechanism was dependent on the presence of an electron donating substituent on the benzenoid ring (Rizzuto and Spikes, 1977). Both singlet oxygen and hydrogen abstraction mechanisms were involved in the eosin sensitized photooxidation of hydroxy-substituted phenylalanines (e.g. tyrosine); however, the hydrogen abstraction mechanism probably predominated at pH values of 8 and 11.

Flash and laser photolysis experiments were conducted to distinguish between singlet and non-singlet oxidation mechanisms in the methylene blue photooxidation of amino acids. With histidine, the rate constants ruled out an oxidation mechanism involving a direct reaction with the excited dye. With the other amino acids, the oxidation rates might be accounted for by either a Type I or a Type II mechanism. Tryptophan, methionine, and histidine photooxidation were shown to involve singlet oxygen.

The rate of photooxidation of some of the amino acids measured in terms of oxygen uptake showed a marked dependence on pH value (Matheson and Lee, 1979). This was particularly evident in the case of histidine where the rate had a pK around 6.5, the same pK as for the dissociation of imidazole.

The photooxidation of cysteine to cysteic acid was sensitized by crystal violet and hydrogen peroxide was formed as an end product (Gennari et al, 1974). The participation of singlet oxygen in the photoreaction was ruled out, since exposure of cysteine to singlet oxygen gave only cystine as a product. The photoreaction was inhibited by radical scavengers such as hydroquinone and allylic alcohol. A mechanism was proposed involving hydrogen abstraction by the triplet dye from the thiol group of cysteine.

In the presence of flavin mononucleotide (FMN), visible light was shown to cause the photosensitized deamination of a number of amino acids including methionine, tyrosine, tryptophan, phenylalanine, serine, histidine, glycine, and alanine (Bowen and Yang, 1975). The reaction products from methionine, glycine and alanine were identified as 3-methylthiopropionaldehyde, formaldehyde and acetaldehyde, respectively. In the case of methionine, 3-methylthiopropionaldehyde was further degraded into ethylene. Relative rates of deamination of eight amino acids were reported. For the sulfur containing amino acids such as methionine and S-methylcysteine, the sulfur atom was the predominant initial site of electrophilic attack followed by an intramolecular electron transfer and subsequent decarboxylation and deamination of the amino acid substrates.

The photooxidation of histidine, tryptophan, and tyrosine with methylene blue and eosin as sensitizers resulted in a loss of primary amine reactivity (Straight and Spikes, 1978). Glycine was not sensitized to photooxidation and no change in primary amine reactivity was observed. Primary amine reactivity of the N-acetyl derivatives of these amino acids was negligible and the reactivity increased slowly with N-acetylhistidine and N-acetyltryptophan while the reactivity of N-acetylmethionine and N-acetyltyrosine did not change. Imidazole and indole also showed a slow increase in primary amine reactivity. Radical scavengers did not affect rates which indicated that free radical intermediates were not involved. The oxygen uptake rate and the loss of primary amine reactivity during photooxidation of histidine and methionine was increased in deuterium oxide which indicated that singlet oxygen was involved.

PROTEINS

Photodynamic inactivation of proteins results primarily in the destruction of certain amino acid side chains, especially tryptophan and histidine (Spikes and Glad, 1964). The breakage of peptide bonds does not appear to occur. Secondary alterations observed in photodynamically treated proteins include changes in absorption spectra, antigenicity, specificity, hormonal activity, viscosity, solubility, enzyme activity, etc. Soon after the discovery of photodynamic action, it was shown that sensitizing dyes increased the inactivation of the enzymes papain, invertase and diastase by sunlight. The photodynamic inactivation of a number of enzymes has been investigated, including α -amylase,

carbonic anhydrase, catalase, chymotrypsin, cytochrome-c, dehydrogenases, enolases, hyaluronidase, lysozyme, myosin, pepsin, peroxidase, phosphoglucomutase, urease, ribonuclease, ribonuclease T₁. Methylene blue sensitized photooxidation of carboxypeptidase A increased its esterase activity and decreased its peptidase activity. In most of these studies on enzymes, photodynamic action was used primarily to destroy certain amino acid side chains in the enzyme in order to determine their role in the catalytic mechanism.

Studies have been made on all of the standard categories of enzymes as well as on protein hormones, and a variety of other proteins (Spikes and Straight, 1967). Of all the proteins examined, only radish peroxidase and bacterial alkaline phosphatase were resistant to photodynamic treatment. Damage results in the destruction of amino acid chains which rupture peptide bonds. Histidine, tryptophan, tyrosine, methionine and cysteine residues are the principal loci of photodynamic damage in proteins, and the tryptophan and histidine residues are typically destroyed most rapidly. The disappearance of tyrosine residues is delayed and often is not significant. In some proteins methionine is destroyed as well as cystine. Not all of the residues of a given amino acid in a protein are photooxidized at the same rate. Proteins are folded such that some residues will be at the surface and therefore more available for photooxidation than those which are buried.

A variety of physical chemical changes occur in proteins as a result of photodynamic treatment: characteristic alterations of

the UV absorption spectrum, conformational changes, and changes in electrophoretic mobility, solubility, viscosity, surface tension, mechanical properties, ultracentrifuge sedimentation behavior, optical rotation, coenzyme binding behavior, metal-binding behavior, sensitivity to digestion by proteolytic enzymes and temperature sensitivity (Spikes and Straight, 1967). Photodynamic treatment also produces a number of changes in the biological properties of proteins including loss or increase in catalytic activity, changes in immunological properties, loss of hormonal activity, loss of toxicity of bacterial toxins, and loss of toxicity of snake venom.

The time-course of inactivation or denaturation is typically first order (Spikes and Straight, 1967). The rate of inactivation is directly proportional to light intensity, and the quantum yield for inactivation is independent of light intensity. The temperature dependence is usually small. The rate of inactivation typically increases with pH value regardless of the charge characteristics of the dye, except for flavins, which show activity at low pH and often have an optimum in the middle pH ranges. The rate typically increases sharply with oxygen concentration and then levels out. Quantum yields for protein destruction increase with increasing protein concentration. Inactivation rates increase sharply with dye concentration, go through a maximum and then decrease. A number of materials such as aromatic amino acids, reducing agents, other proteins, ions of certain metals, iodide ion, etc. protect proteins against photodynamic destruction.

Quantum requirement studies on trypsin with methylene blue

and riboflavin-5'-phosphate (FMN) were conducted (Spikes and Glad, 1964). The quantum requirement (i.e. inverse of quantum yield) of inactivation with both sensitizers decreased rapidly with increasing dye concentration, reached a minimum, and then increased. Both sensitizers showed increased quantum requirements of inactivation with increasing enzyme concentration, and both were independent of light intensity. FMN-sensitized inactivation showed large quantum requirements at acid pH values and low requirements at basic pH values. Methylene blue showed no activity at acid pH values and high activity at basic pH values. Quantum requirements decreased slightly with temperature from 4 C to 33 C. Both dyes showed different efficiencies for light energy under similar conditions, suggesting that different mechanisms were acting in each case.

The kinetics of trypsin photoinactivation with methylene blue and eosin Y were investigated (Ghiron and Spikes, 1965). A urea-sensitized 'damaged class' of trypsin molecule was not produced with either dye but was produced in another study with flavins as sensitizers. The results indicated that the mechanism of inactivation of enzymes might be different with different photosensitizing dyes.

The kinetics of the flavin sensitized photoinactivation of trypsin were investigated (Ghiron and Spikes, 1965). Similar results were obtained with riboflavin and riboflavin-5'-phosphate (FMN) as sensitizers, while flavin adenine dinucleotide had a very small sensitizing effect. The rate of inactivation reaction was directly proportional to light intensity over the range

examined, and the time-course of the trypsin inactivation was fitted to first order kinetics. The t_{37} value for trypsin inactivation increased linearly with dye concentration. The trypsin concentration dependence of the reaction was found to be different in acid and basic solution. Trypsin also inhibited the rate of photobleaching of the dye. The results did not conform with any one of the several mechanisms that have been proposed to explain photodynamic action.

NUCLEIC ACIDS AND NUCLEIC ACID BASES

During the late 1950's and early 1960's, mechanistic studies were made on the inactivation of free nucleic acids and on intact viruses (Hobbs, et al, 1977). The results of these studies indicated that the site of the attack for viruses was the nucleic acid and involved the purine base guanine, a heterodicyclic carbon-nitrogen compound. Studies also showed that the rates of inactivation of the guanine moiety of the nucleic acid was much faster in the presence of oxygen and that one mole of oxygen was consumed per mole of guanine photooxidized when sensitized with methylene blue.

Purines such as uric acid, guanine, hypoxanthine, and xanthine were photooxidized on illumination in the presence of appropriate sensitizers (Spikes and Straight, 1967). Guanine residues were destroyed during the methylene blue sensitized photooxidation of DNA. With free nucleotides and methylene blue as a sensitizer at a pH value of 8.5, deoxyguanylic acid was photooxidized rapidly, thymidylic acid was photooxidized very slowly, and deoxyadenylic, deoxycytidylic, and uridylic acids

were not significantly affected. In parallel studies with the corresponding ribonucleotides, nucleosides, and base, only the guanine derivatives were degraded rapidly. With methylene blue, the rate of photooxidation of guanine derivatives increased with pH value, suggesting that the anionic forms were more sensitive than the neutral species. Studies of a large variety of purines pyrimidines, and related compounds indicated that bases must have an imidazole ring and a lactim structure involving the N-1 and N-3 positions in order to be photooxidized.

Studies indicated that both ring systems of guanosine were broken on photodynamic treatment, giving as the main products ribosylurea, ribose, urea, and guanidine (Spikes and Straight, 1967). When guanosine-5'-phosphate was treated photodynamically in Tris buffer, three products resulted, two of which had Tris incorporated into their structure.

Two distinct lesions were identified in proflavine photosensitized DNA (Houba-Herlin et al, 1982). Single strand breaks were dependent upon OH radical induction while altered guanine residues probably resulted from $^1\text{O}_2$ action. Each lesion could be preferentially induced depending upon the conditions of the proflavine and light treatment. Questions arose whether or not sensitizers other than proflavine operated along the same two photochemical pathways and caused the same two types of DNA damage.

From the sedimentation behaviour and T-jump relaxation of in vitro photodynamically treated DNA, it was shown that not only photooxidation of guanine but also single- and double- strand breaks could occur (Berg et al, 1972).

When frozen solutions of complexes of DNA and a dye of the acridine family were exposed to visible light, free radicals were induced in the DNA (Calberg-Bacq and Van de Vorst, 1974). Two types of radicals were shown to exist in the DNA constituents tested: an $R^{\cdot}$ type induced through a two-photon mechanism and similar to those produced by the direct effect of ionizing radiations and an $ROO^{\cdot}$ type due to a photodynamic process.

The binding of acridine dyes by DNA involved two modes of fixation: an intercalation of the dye molecule between two pairs of bases and a binding of the dye cations to the phosphate groups external to the DNA double helical structure (Calberg-Bacq and Van de Vorst, 1974). The second mode of association predominated after saturation of the intercalation process. The relative importance of the two types of fixation was greatly dependent upon the ionic strength of the medium.

MICROORGANISMS

The mode of photodynamic inactivation is not unique and varies from dye to dye (Bagchi and Basu, 1979). For the inactivation of yeast cells, three major categories were identified. The variation in the mode of inactivation was due to the differences in the penetrability, the binding ability and the nature of the binding of the dye molecules with DNA, and the role of chromosomal proteins in modifying the binding. For non-penetrating dyes, damage to regions of the cell envelope or to the cytoplasm resulted in the rupture of the cell envelope and loss of cytoplasm associated with photochemical functions. For non-penetrating dyes, it was presumed that the singlet oxygen

formed outside the cell diffused over a short distance inside the cell and brought about the photodynamic inactivation.

In contrast, dyes that penetrated inside the cell, in particular the acridines, bound to the polynucleotides and sensitized photodynamic damage to DNA (Bagchi and Basu, 1979). Singlet oxygen was involved in causing the damage but was formed inside the cell near the DNA. For penetrating dyes, the possibility of either damage to the cell envelope or to the DNA via a singlet oxygen mechanism existed. The effect of the penetrating dye acriflavine remaining outside *E. coli* B/r cells during irradiation on photodynamic inactivation was found to be nil or negligible.

Temperature, pH value, dye concentration, and sensitization time all had an effect on the amount of coliforms and poliovirus that could be inactivated for a given light exposure (Gerba et al, 1977). Coliforms were far more sensitive to dye and photoinactivation than poliovirus. Significant reductions in bacterial numbers often occurred in the dark by the dye alone.

After a 1 hr exposure in the dark at 37 C at a pH value of 9 in 10^{-4} M proflavine, microbial floras (other than virus) from stool and throat specimens were irreversibly photosensitized (Wallis et al, 1965). The dye was removed by cationic resins, and the photosensitized bacterial and fungal contaminants were inactivated in the presence of white light. Under the same conditions, enteroviruses and rhinoviruses were found to be photoresistant, although some loss of rhinovirus occurred as a result of adsorption to the resin.

Complete destruction of algae was obtained following 2 weeks

of incubation in the presence of 0.25, 0.15, and 0.75 mg/L methylene blue or 0.5, 0.8 and 0.8 mg/L riboflavin after exposure to solar radiation for 30 - 60 min in *Peridinium*, *Pediastrum* and *Cosmarium* cultures respectively (Acher and Elgavish, 1980).

Light, eosin Y and oxygen were all required for substantial inactivation of wild-type diploid yeast (Cohn and Tseng, 1977). Long periods of irradiation were required for greater than 90 percent inactivation, and binding and uptake of the dye by yeast were not detected. The photooxidative bleaching of eosin Y caused a significant reduction in the apparent rate of inactivation. The results suggested that eosin Y acted as an extracellular sensitizer where the likelihood of damage to the cell envelope was enhanced. The lifetimes of photodynamically generated damaging agents, such as singlet molecular oxygen, might be shorter than the times required for diffusion into the cell and damage might be localized to the biological membranes and membrane associated biochemical processes.

The presence of carotenoid pigments protected *S. lutea* cells from lethal photosensitization by toluidine blue but had no protective effect in photosensitization with 8-MOP (Mathews, 1963). Photosensitization by toluidine blue resulted in damage to the protein of the cell membrane, whereas photosensitization by 8-MOP resulted in damage to cellular DNA. The carotenoid pigments of the wild type of the organism were capable of quenching singlet oxygen as well as free radicals in in vitro photosensitization by toluidine blue (Mathews-Roth, 1977). It was concluded that several mechanisms might be involved in the

photodynamic inactivation.

COLIFORMS

For coliform bacteria sensitized in the dark from 10 min to 4 hr at a pH value of 10, the sensitization of bacteria by dye was highly temperature dependent and could be increased by short exposures to elevated temperatures (Gerba et al, 1977). The bacteria could be sensitized to light with methylene blue at 0.01 mg/L at elevated temperatures. At 10 mg/L methylene blue and at elevated temperatures, bacteria was inactivated in the dark. By increasing the preincubation time of the dye bacteria mixture and lengthening the exposure time of mixture to light, larger number of bacteria could be inactivated with low concentration of dye at room temperature. Greater than 8 logs of coliform were inactivated with 60 sec exposure to 20 W/m^2 of 670 nm light with 1 mg/L methylene blue after 4 hr sensitization, while after 8 hr sensitization, less than 10 sec was required to inactivate the same number of bacteria. The sensitization of microorganisms was dependent on pH value and a pH value lower than 10 was not as effective. Only after prolonged exposure to light (10 min with 4 hr sensitization time) were large reductions in bacterial numbers achieved.

The effects of methylene blue (0 to 10 mg/L), radiation times (0 to 2 hr), and sunlight intensities [0, 68, and 2,030 $\text{uE}/(\text{m}^2 \text{ sec})$] were investigated (Acher and Juven, 1977). In laboratory-scale experiments, $1.3 (10^9)$ coliforms in 100 mL of wastewater at pH values from 7 to 8 containing 0.5 mg methylene blue were destroyed in about 30 min. Similar results were

obtained with nonchlorinated potable water. It was concluded that the intensity of the solar radiation might not be a limiting factor and that after disinfection the sensitizer could be removed from water by adsorption onto bentonite.

The photodynamic inactivation of *E. coli* by visible light and O_2 was found to occur in the presence of the sensitizer rose bengal immobilized by covalent bonding to polystyrene beads (Bezman et al, 1978). The absence of dissolved rose bengal in solution indicated that an inactivation mechanism based on penetration of sensitizer molecules into the cell's interior could not be operating. Survival curves typically exhibited induction periods followed by rapid exponential death, with 99.99% kill requiring 1 to 2 hr depending on conditions.

The photodynamic inactivation of *E. coli* by methylene blue, rose bengal, and eosin covalently immobilized on polystyrene beads and by methylene blue coated on granular activated carbon, silica gel and XAD-2 (polystyrene resin) was studied (Savino and Angeli, 1985). After 30 min treatment time, 97.5, 92.2, and 81.6 percent of *E. coli* were inactivated by the immobilized methylene blue, rose bengal and eosin dyes respectively. Methylene blue was the most active dye. Methylene blue coated on granular activated carbon, on silica gel and on XAD-2 was still active. Activated carbon proved to be the best support both for the amount of dye adsorbed (2 mg/g) and the amount of *E. coli* inactivated and did not require the complex, expensive immobilizing techniques.

E. coli cells were inactivated with visible light in the presence of toluidine blue as a photosensitizer, and the

photodynamic effect was partially protected with α -tocopherol (Wakayama et al, 1981). The pH value, the concentration of the buffer during irradiation, and cultivation and irradiation temperature also affected survival. The addition of osmotic stabilizers such as KCl, glycerol and polyethyleneglycol to the buffer increased the survival; however, the difference in singlet oxygen production could not be related to the stabilizers. The results with E.coli supported the notion that one of the primary targets of toluidine blue sensitized photodynamic inactivation was the cytoplasmic membrane, although factors other than the cytoplasmic membrane also influenced the survival of the cells.

Some studies on different E. coli strains have failed to reveal any significant difference in the sensitivity to photodynamic damage between radiation resistant E. coli B/r and radiation-sensitive E. coli B_{s-1} strains suggesting the absence of a repair mechanism (Das et al, 1974). Other studies, however, have shown that B/r can repair photodynamic damage whereas B_{s-1} does not have this ability.

VIRUSES

In 1958 the first quantitative studies on the photodynamic inactivation of bacterial virus were reported (Yamamoto, 1958). The photodynamic inactivation of coliphage was determined to be an exponential function of time with an initial somewhat variable lag. The inactivation rate increased as the pH value increased and the salinity decreased. The velocity constant under anaerobic conditions was negligibly small compared to aerobic conditions, and the relative sensitivity of phage strains was

correlated with their serological classification. Both active dyes and inhibiting dyes had a strong affinity for nucleic acid. The experiments indicated that the photodynamic susceptibility of phage strains was correlated with their permeability to dye molecules.

The effects of light upon several different animal viruses suspended in different dyes were reported (Hiatt, 1960; Hiatt et al, 1960). Adenovirus, vaccinia virus, SV40 papovirus, and reovirus were photosensitive at a pH value of 7 in the presence of toluidine blue. It was assumed that the dye combined with the nucleic acid, which made the virus photosensitive. Under these conditions vaccinia virus proved to be reversibly photosensitized (i.e. when the dye was removed from the virus by binding on cation resins, the virus effluent was photoresistant). The studies indicated that the RNA containing viruses were less permeable to the dye than the DNA containing viruses and were therefore less sensitive to the photodynamic effects of the dye. The dye combined with one or more critical sites on the virus particle under the proper conditions to yield a dye-virus complex. On irradiation, the complex absorbed light energy and attained an excited state. The excited complex then combined with oxygen as the energy was released in a reaction that resulted in loss of infectivity. The dye was assumed to penetrate the virus coat and to be bound to the nucleic acid.

Acridine orange was shown to localize in the nucleic acid structures of living cells, which were photosensitive to visible light (Hackett, 1962). The principal damage was probably

mediated through the DNA, suggesting that the mutagenic action of the acridine dyes might be related to the photodynamic effects.

Enteroviruses and non-enteroviruses were the two major groupings of animal viruses based on photodynamic inactivation (Wallis and Melnick, 1965). Enteroviruses, including polio, echo, and coxsackie viruses, were resistant to photosensitizers in the presence of organic medium; however, under certain conditions these viruses could be sensitized, but the effects were reversible. Enteroviruses not only differed from other virus groups in their photosensitivity, but also differed among themselves. Enteroviruses could be photosensitized only after purification through anion resins to remove organic medium, or by growing virus in a simple salt-glucose medium. Under these conditions, the effects were reversible, and after removal of dye, the virus suspension became photoresistant. Non-enteroviruses were highly sensitive to dyes and in most cases could be irreversibly photosensitized even in the presence of organic medium. These included viruses of the pox-, myxo-, reo-, herpes-, and papovavirus groups.

The reaction between viruses and photoreactive dyes was reported to result in a tightly bound, photosensitive, virus-dye complex (Wallis and Melnick, 1964). Following incubation of herpes virus in the dark at a pH value of 9 at 37 C in 10^{-4} M neutral red, proflavine, or toluidine blue, free dye was removed from the virus-dye mixture by cationic resins to yield a water clear virus preparation of the original titer. Subsequent exposure of the herpes virus to visible light for a few minutes rendered it noninfectious.

Herpesvirus and measles virus were rendered totally photosensitive by each of the above three dyes under the above conditions (Wallis and Melnick, 1964). Reovirus and influenza virus were completely photosensitized by toluidine blue or proflavine, but parainfluenza, vaccinia, and papova SV40 viruses only by toluidine blue. Picorna viruses could not be photosensitized under these conditions. Adenoviruses could not be completely photosensitized with neutral red or proflavine, but were inactivated by toluidine blue in the dark. Optimal pH and temperatures were found necessary for the effective binding of dye to viruses. Subsequent changes in pH value and temperature could not reverse the attachment and render the sensitized viruses resistant to light. If the excess free dyes were not removed, all the viruses studied except the picornaviruses were photosensitized by neutral red, proflavine, or toluidine blue.

Enteroviruses, usually resistant to photodynamic inactivation, were rendered completely photosensitive to proflavine at pH values from 9 to 10 following purification by filtration through an anionic resin (Wallis and Melnick, 1965). Enteroviruses grown in cells maintained in a salt-glucose medium were also susceptible to photosensitization. Of the 38 enteroviruses tested, 10 were rendered completely photosensitive to proflavine or toluidine blue or both, and the remaining 28 viruses were sensitized but to a lesser degree. The binding of the dye to the viruses could be reversed by lowering the pH value.

Earlier studies were confirmed and ways were found to bind

dyes irreversibly to non-enteroviruses and to render enteroviruses sensitive to heterotricyclic dyes (Wallis and Melnick, 1965). Enteroviruses are normally resistant to photodynamic inactivation; however, enteroviruses can be made photosensitive in the presence of added dyes. It was found that the organic components present in tissue culture harvests prevented attachment of heterotricyclic dyes to enteroviruses. Purified poliovirus was photosensitive in 10^{-4} M heterotricyclic dyes, especially proflavine. Although toluidine blue and neutral red were also capable of photosensitizing poliovirus, they were less effective.

The poliovirus-dye complex was readily reversible (Wallis and Melnick, 1965). Poliovirus treated at pH values of 8 to 10 became photosensitive but could be rendered photoresistant by lowering the pH value between 6 and 7 before exposure to light. Purified poliovirus could be rendered photoresistant by addition of the organic components of tissue culture medium to the virus prior to addition of dye. However, if the dye were added first, followed by the organic components, the virus remained photosensitive. The enteroviruses were are not irreversibly bound by the basic heterotricyclic dyes (Wallis and Melnick, 1965). When the virus was treated with proflavine, neutral red or toluidine blue and the dye was removed on cation resins, the virus suspension became photoresistant.

The inactivation reaction for viruses was described by a three step reaction scheme (Hobbs et al, 1977). The virus (V) combines reversibly with the photoreactive dye (D) to form a dye-virus complex (D·V).

Irradiation of the dye-virus complex allows for the absorption of a photon of the proper quantum energy ($h\nu$) to produce an excited complex.

The excited complex then dissociates in the presence of oxygen to form an inactive virus particle V_1 and an unaltered dye molecule.

Poliovirus added to tapwater, wastewater or seawater was readily photoinactivated by methylene blue and visible light (Gerba et al, 1977). Typically, almost 2.5 logs of virus could be inactivated upon a 5 min exposure to 670 nm light (20 W/m^2) in solutions containing 13 μM methylene blue at a pH value of 10. A biphasic inactivation curve was produced for poliovirus, regardless of dye concentration, pH value, temperature, sensitization time, nature of suspending solution or sequence of light exposure. These results indicated that a multi-hit inactivation event was occurring. Preincubation of the dye-virus mixture at 24 C increased the rate of virus photoinactivation. Dye concentrations above 26 μM had little advantage in increasing the amount of virus photoinactivated. Significant inactivation of the virus in the dark occurred at high dye concentrations (52-130 μM).

Poliovirus was inactivated in wastewater at a pH value of 10, a methylene blue concentration of 5 mg/L, and a 24 hr sensitization time (Gerba et al, 1977). The amount of virus inactivated continued to increase upto a methylene blue

concentration of 10 mg/L. Higher concentrations resulted in a gradual reduction in the amount of virus that could be inactivated. This effect was observed before with vaccinia virus and was attributed to the absorption of the light by the dye in the upper layers of the fluid. Almost 70 percent of the light energy was absorbed after passage through a buffer containing 10 mg/L dye. A doubling of light intensity for a given exposure time resulted in an approximate doubling of the $\log_{10}$ amount of virus that could be inactivated, when the energy of the light source exceeded 2.5 W/m^2 . A marked increase in the amount of virus inactivated occurred between pH values of 9.5 and 10.

For poliovirus at a pH value of 10, a methylene blue concentration of 5 mg/L and a 24 hr sensitization before light exposure, inactivation was initially slow but gradually became logarithmic after longer light exposures (Gerba et al, 1977). For a 5 min exposure, the amount of virus inactivated increased upto 10 mg/L methylene blue and then gradually decreased. Varying light intensity and exposure time indicated that doubling of light intensity for a given exposure time resulted in approximate doubling of the $\log_{10}$ amount of virus that could be inactivated. The effect of pH value after 3 min exposure showed that a marked increase in the amount of virus inactivated occurred between a pH value of 9.5 and 10.0.

Solar radiation may inactivate viruses in the absence of any photosensitizers (Bitton et al, 1979). Naturally occurring substances (i.e. humic substances) may well play an important role in the photoinactivation of viruses. Singlet oxygen may

bring about inactivation of bacteria and viruses in natural waters. The T_{90} for poliovirus was greater than 3 hours and was higher than the T_{90} (20 min) observed for coliform bacteria. Nontronite, a three-layer clay, displayed protective effect against thermal and light inactivation. The photoinactivation of virus in lake water was retarded by presence of blue green algae.

An overview of studies on sensitized photooxidation of biological systems is presented in Table 1. The substrate, sensitizer, results and author(s) are given for each study. The results presented in the Table 1 may not be complete, and the reader is directed to review the original paper for more complete information.

CHEMICAL SYSTEMS

Organic compounds in biological systems which undergo sensitized photooxidation include: alcohols, aldehydes, amines, amino acids, carbohydrates, esters, indoles, ketones, nitrogen heterocyclics, phenols, plant growth regulators, proteins, purines, pyrimidines, pyrroles, and steroid hormones (Spikes and Straight, 1967). Compounds able to undergo autooxidation under a given set of conditions will probably also be oxidized in dye-sensitized photoreactions under the same conditions. Saturated and unsubstituted aliphatic compounds are resistant to sensitized photooxidation, whereas benzenoid and aromatic heterocyclic compounds tend to be sensitive. The introduction of amino or hydroxyl groups on the benzene ring greatly increases the sensitivity to photooxidation, while the introduction of cyano, halogen, or nitro groups has little effect. The susceptibility

ble 1. Studies on sensitized photooxidation of biological systems.

ALGAE	Sensitizer*	Results	Reference
peridinium pediastrum cosmarium	MB, RB	complete destruction 30-60 min exposure sunlight, pH 7.4, 2 mg/L MB, and 2 week incubation	Acher and Elgavish, 1980
BACTERIA	Sensitizer*	Results	Reference
Candida albicans	PF	irreversibly photosensitized at pH 9, 1 hr incubation	Wallis and Melnick, 1965
coliform	PF	irreversibly photosensitized at pH 9, 1 hr incubation	Wallis and Melnick, 1965
coliform	MB	cost effective, attractive for arid zones with irrigation	Acher, 1985
coliform	MB, RB	RB not as effective as MB, solar radiation not limiting; 1.3 E09 coliform destroyed in 30 min, pH 7 - 8, MB 5 mg/L	Acher and Juven, 1977
coliform	MB, RB	100% reduction, 30 min, pH 7.7, 3-5 mg/L MB, low sunlight	Acher and Rosenthal, 1977
coliform	AF	damage to regions near cell envelope or to DNA	Bagchi and Basu, 1979
coliform	RB	99.99% kill in 1-2 hr, RB on polystyrene beads, singlet oxygen	Bezman et al, 1978
coliform		solar UV important factor in natural purification	Calkins et al, 1976
coliform	MB	pilot scale plant, 2ndary effluent, pH>9, MB=1ppm, t=2 hr	Eisenberg et al, 1986
coliform	AO, AF	B/r can repair photodynamic damage, Bs-1 cannot	Das et al, 1974
coliform	MB	pH > 9 required, greatest effect at 9.5 - 10; temp. depend.; 8 log drop w/ 60 sec exposure, 1 mg/L MB, 4 hr sensitization	Gerba et al, 1977
coliform		< .01% survival, 1 d sun; uv contributes substantially	Grigsby and Calkins, 1980
coliform		near UV inhibits growth similar to artificial sources	Jagger, 1975
coliform		review of near UV effects on microorganisms	Jagger, 1981
coliform	MB	sensitiz time affects rate; effective at pH 10, 5 mg/L MB	Hobbs et al, 1977
coliform	UA	pH 7 w/ total kill in 5 hr, penetration in cell interior reqd	Neely et al, 1971
coliform	MB, RB, E	MB > RB > E, activated carbon best insoluble support, pH ?	Savino and Angeli, 1985
coliform	MISC	review	Spikes and Straight, 1967
coliform		uvrA and recA gene products repair portion of solar DNA damage	Tyrrell and Souza-Neto, 1981
coliform	TB	vitamins, stabilizers protect; pH, temp, buffer important	Wakayama et al, 1980
proteus	MB, TP, P	TP irreversibly reduced by bacteria; adsorption by and penetration into cell (< 1 min)	Jacob, 1974

Table 1 (cont.). Studies on sensitized photooxidation of biological systems.

<i>S. domonas</i>	PF	irreversibly photosensitized at pH 9, 1 hr incubation	Wallis and Melnick, 1965
<i>Sarcina lutea</i>	MP, TB	damage to DNA w/ MP, to protein with TB	Mathews, 1963
<i>Sarcina lutea</i>	TB	both whole cell and membrane inactivated; histidine protects	Prebble and Huda, 1973
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VIRUS	Sensitizer*	Results	Reference
enterovirus (38)	PF, TB	10 complete inactiv, 28 partial; reversed by lowering pH	Wallis and Melnick, 1965
entero, non-entero	PF, NR, TB	review; entero resist. in organic medium, non-entero sensitive	Wallis and Melnick, 1965
herpes, measles, reo, influenza, vaccinia, papova SV40, adeno, parainfluenza, picorna	PF, NR, TB	TB most effective, all except picorna inactivated by all dyes	Wallis and Melnick, 1964
OX174	PF	inactivation increased w/ PF bound to DNA as PF increased or ionic strength decreased	Calberg-Bacq et al, 1977
OX174	MISC	singlet oxygen: RB 71 > MB 70 > E 61 > TP 18 > PF 10 > AY 9	Houba-Herlin et al, 1982
OX174	PF	guanine residue target for PF in DNA, high rate of alteration	Piette and Moore, 1982
OX174	PF	thiols and disulfides protect phage and DNA,	Piette et al, 1977
OX174	PF	RS radicals decrease peroxide radicals in photosensitized DNA	Piette et al, 1978
polio		solar w/o sensitizer had effect, clay and algae retard inactiv.	Bitton et al, 1978
polio	MB	2.5 log drop, 5 min exposure, 13 uM MB, pH 10, 20 W/sq.m 670 nm	Gerba et al, 1977
polio	MB	pH > 9 required, greatest effect at 9.5 - 10, MB 1 - 10 mg/L inactivation in dark at [MB] = 52 - 130 uM	Gerba et al, 1977
polio	MB	attack on nucleic acid chain, effective at pH 10 and 5 mg/L MB; rate increase w/ sensitization time	Hobbs et al, 1977
T-series	MISC	review	Spikes and Straight, 1967
T-series	MISC	thiazine, oxazine, acridine dyes active; reversibly dissociable	Yamamoto, 1958
vesicular exanthema	A0	lethal effect after light exposure	Hackett, 1962
<hr/>			
YEAST	Sensitizer*	Results	Reference
<i>S. cerevisiae</i>	TB, A0	sensitizing action A0 increase w/ 20 sec til 10 min; TB small	Ito, 1980
<i>S. cerevisiae</i>	A0	singlet oxygen major intermediate, pH 6.8	Kobayashi and Ito, 1976
<i>S. cerevisiae</i>	HP	singlet oxygen increases & inactivation efficiency decreases w/ increase in pH	Stenstrom et al, 1980

Table 1 (cont.). Studies on sensitized photooxidation of biological systems.

type diploid	E	long irradiation period, no binding or uptake of dye	Cohn and Tseng, 1977
yeast cells	XNT,MB,TB,TH	ascorbate enhances (not w/ XNT), independence	and Ito, 1982
yeast cells	MISC	dye must be bound to DNA, RH and MB inactive, singlet oxygen	Ito and Kobayashi, 1977
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ENZYMES	Sensitizer*	Results	Reference
lysozyme	MB	no peptide cleaved, more susceptible to trypsinolysis	Gurnani, 1968
lysozyme	MISC	AD < MB < PF < AF; (1:5:6:12) quantum yield	Schmidt et al, 1981
trypsin	MB, E	1st order rates; inactivation different with different dyes	Ghiron and Spikes, 1965
trypsin	RF, FMN	1st order kinetics; rate proport. to light intensity and [dye]	Ghiron and Spikes, 1965
trypsin	MB, FMN	quantum requirements: decrease w/ increase [dye] and then increase; increase w/ increase [enzyme]; light independent	Spikes and Glad, 1964
trypsin	FL	efficiency increases w/ increasing # and atomic weight; no correlation w/ binding and inactivation	Wade and Spikes, 1971
<hr/>			
AMINO ACIDS	Sensitizer*	Results	Reference
general	PF	only Met and Trp photooxid in formic and acetic acids	Benassi et al, 1967
general	FLV	anaerobic yield keto acids and NH ₃ ; rate dependent on structure	Byrom and Turnbull, 1967
general	MISC	review	Jori, 1975
general	MB	anaerobic: sensitive amino acids reduce MB, insensitive don't	Knowles and Gurnani, 1972
general	MB	1st order, effect of imidazole ring determined	Lukton et al, 1965
general		quenching rate in D ₂ O, pD 8: Ala .2, Met 3, Try 9, His 17, carbonic anhydrase 85, lysozyme 150, SOD 260, apoSOD 250	Matheson et al, 1975
general		rate constant (1E7/(M sec)): His 10, Try 3, Met 1.7, Tyr 0.8 Ala 0.2; no quenching in addition to chemical rx	Matheson and Lee, 1979
general	FLV	review	McCormick, 1977
general	MB	singlet oxygen for Met, Trp, His; others either type I or II	Nilsson et al, 1972
general	MISC	review	Spikes and Straight, 1967
general	MB, E	loss of primary amine activity for His, Met, Trp, Tyr	Straight and Spikes, 1978
Ala, Cys, Met, Ser	FMN	Met-sulfoxide & non S amino acids deaminated at slower rate	Bowen and Yang, 1975
	CV	yields cysteic acid & H ₂ O ₂ ; inhibited by radical scavenger	Gennari et al, 1974

Table 1 (cont.). Studies on sensitized photooxidation of biological systems.

, Met, Trp	MB, CT	types of photosensitized oxidations in biological systems	Foote, 1968
His	PS	distinguish free radical and singlet oxygen w/o quantum yield	Kraljic et al, 1978
His	MB	products at pH 7 & 9, none pH 4; yield increase w/ irradi. time	Gurnani and Arifuddin, 1966
His	FLV	anaerobic yields keto acids and ammonia; FMN more effective	Johnson et al, 1975
Met	PF	reaction to Met sulfoxide inhibited by Cu(II) and Mn(II)	Jori and Cauzzo, 1970
Phe, Tyr	E	Phe not sensitive but subst of OH or NH ₂ rapid photooxidation	Rizzuto and Spikes, 1977
Trp	THZ	5 times more efficient in basic than in acid, pK related	Bonneau et al, 1975
Trp	MB	Indole oxygenated to dioxindole derivatives	Gurnani et al, 1966
Tyr	FL, E	Type I and II	Kasche, 1967
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NUCLEIC ACIDS NUCLEOSIDES NUCLEOTIDES	Sensitizer*	Results	Reference
DNA	PF	free radicals; low sensitiz. w/o oxygen, increase w/ increase NaCl, free radicals are peroxides	Calberg-Bacq and Van de Vorst, 1974
DNA & RNA	PF	synthesis inhibited, free radical or singlet oxygen	Roberts, 1981
deoxyguanosine	MISC	singlet oxygen: RB 71> MB 70> E 61> TP 18> PF 10> AY 9	Houba-Herlin, 1982.
guanine derivatives	MB, CT	mechanisms, singlet oxygen and radical chemistry	Foote, 1968
guanosine-5-monophosphate	MB	hydrogen abstraction mechanism	Nilsson et al, 1972.
guanosine	TP	oxidation potential of donor between triplet and radical	Gollmick and Berg, 1972
nucleic acids	MISC	review	Spikes and Straight, 1967
nucleosides	PF	new compound formed from cytosine	Roberts, 1981
nucleotides	LF	initial reaction with triplet	Knowles, 1971
nucleotides	LF	quantum yield increase with O ₂ at low O ₂	Knowles and Mautner, 1972
nucleotides	PF	influenced by relative [dye] & [substrate]	Van de Vorst and Lion, 1975
xanthosine monophosphate (XMP)	MB	hydrogen abstraction, buffer accelerates protonation and reduction of dye, sensitive nucleotides act as reducing agents	Knowles, 1971.

SENSITIZER KEY

AF=acriflavin, AO=acridine orange, AO=acridine orange, CT=carotenoids, CV=crystal violet, E=eosin, FL=fluoresceins, FMN=flavomononucleotide, FLV=flavins, HP=hematoporphyrin, LF=lumiflavin, MB=methylene blue, MP=methoxypsoralen, NR=neutral red, P=pyronine, PF=proflavine, PS=phenosafranin, RB=rose bengal, RF=riboflavin, RH=rhodamine, TB=toluidine blue, TH=thionine, THZ=thiazines, TP=thiopyronine, UA=uranyl acetate, XNT=xanthenes, MISC=miscellaneous

of simple amines to sensitized photoautoxidation decreases in the following order: tertiary > secondary > primary > quaternary.

Free-radical oxidations of chemicals exposed to sunlight were reported to occur in natural waters and not in pure water (Mill et al, 1980). The photolysis of natural organics in many water bodies generates free radicals (e.g. alkylperoxy $RO_2^{\cdot}$, hydroxyl $HO^{\cdot}$) and oxidants such as singlet oxygen. These photolysis products contribute to the transformation of some synthetic chemicals in water. In general, $RO_2^{\cdot}$ transformed only the most reactive chemicals (e.g. phenols, aromatic amines, hydroxylamines, hydroquinones), resulting in half-lives of a few days or less. Oxidation by $HO^{\cdot}$, despite its high reactivity, will not be an important degradation pathway for most organic compounds because of the low $HO^{\cdot}$ concentration in aquatic systems. The half-life for oxidation by $HO^{\cdot}$ of even the most reactive structure will be at least 80 days.

Substrates such as dienes, dienoid heterocycles, and polycyclic aromatic compounds add oxygen to give endoperoxides, whereas olefins react to give hydroperoxides (Figure 1) (Foote, 1968). The reactions are analogous to the Diels-Alder reaction, and the Alder 'ene' reaction respectively. Alcohols act as hydrogen donors for excited ketones and quinones and (much less efficiently relative to reaction with oxygen) for dyes. Phenols and amines are much more efficient reductants than alcohols, and are capable of reacting with excited dyes at rates competitive with that of oxygen.

A second important reaction is the combination of radical species produced in the electron hydrogen-atom transfer

reactions (Foote, 1968). Instead of combining, intermediate radicals may disproportionate. For example, semiquinone radicals (or radical anions) yield quinone and hydroquinone (Figure 6). This process is very common with dyes, producing equal amounts of leuco dye and starting dye.

Intermediate radicals can react rapidly with oxygen to form peroxy radicals, which in turn, abstract hydrogen to yield hydroperoxides (a process not to be confused with the formation of allylic hydroperoxides by the singlet oxygen mechanism) (Foote, 1968). Depending on the source of the hydrogen, new radicals may or may not be produced, or may result in a conventional radical chain autooxidation. An example of a reaction which does not go by way of a chain is the reaction with oxygen of hydroxyisopropyl radicals, formed by hydrogen abstraction from isopropyl alcohol by triplet benzophenone to give peroxyradicals. These are reduced by the semireduced benzophenone radicals to give the final product hydroxyhydroperoxide and regenerate benzophenone. Electron or hydrogen atom transfer reactions of the primarily produced radical species are often very important.

Singlet oxygen degrades compounds with Diels-Alder reaction centers, or high electron rich double bonds, or easily oxidizable functional groups, such as alkyl sulfides and thiones or some types of phenolate ions (Haag and Holgne, 1986). In wastewater singlet oxygen oxidizes unsaturated compounds to peroxides, which decompose thermally or photochemically to initiate further free radical oxidation (Acher and Rosenthal, 1977). However, radical

hydroxyisopropyl radicals (VIII), formed by hydrogen abstraction from
 isopropyl alcohol by triplet benzophenone, react with oxygen to give
 peroxyradicals (IX), which are reduced by the semireduced benzophenone
 radicals to give the final product hydroxyhydroperoxide (X).

Figure 6. Sensitizer interactions. (Foote, 1968)

reactions or direct interactions between the substrate and the excited triplet state sensitizer may also occur and produce indirect phototransformations. An example is thiobencarb, which is oxidized by peroxide radicals and not by singlet molecular oxygen (Draper and Crosby, 1981).

The photooxidation of phenols is important both biologically and commercially (Thomas and Foote, 1978). $^1\text{O}_2$ was the active agent in the sensitized photooxidation of phenols. Several schemes were proposed, the two most plausible schemes being the cycloaddition of $^1\text{O}_2$ with the phenol and subsequent rearrangement of an unstable endoperoxide, and a charge-transfer reaction between $^1\text{O}_2$ and phenol leading to products by means of superoxide and phenol radical cation.

Both $^1\text{O}_2$ and triplet dye mechanisms were involved in the eosin sensitized photooxidation of hydroxy-substituted phenylalanines (Rizzuto and Spikes, 1977). In the singlet oxygen mechanism, $^1\text{O}_2$ formed by reaction with the triplet dye reacted with the substrate to give an intermediate oxidation product that rearranged into an endoperoxide, hydroperoxide, etc. or decomposed into final products (Kraljic and Lindqvist, 1974).

Results from studies of the $^1\text{O}_2$ oxidation of olefins, dienoid compounds and polydienes showed that $^1\text{O}_2$ attacked double bonds, forming allylic hydroperoxide groups and shifting double bonds according to the 'ene' type process (Rabek and Ranby, 1979). Studies of $^1\text{O}_2$ reactions with low molecular weight model compounds (e.g. 2-methyl-2-hexene or 4-methyl-octene) showed the formation of a mixture of allylic hydroperoxides. The reaction of singlet oxygen with polydiene polymers produced hydroperoxides

by the typical 'ene' type reaction. The observed chain scission process could not be explained by the photodecomposition of the hydroperoxide formed by visible light, because these hydroperoxides do not absorb light in the visible region. Spectroscopic and EPR studies of the dye-solvent systems showed the formation of reactive free radicals, which were probably responsible for the abstraction of hydrogen from the polymer molecules. The next step was the well known free radical oxidation mechanism which was responsible for the chain scission reactions.

The photosensitized destruction of carcinogens by several triplet sensitizers, with enhanced damage in the presence of oxygen was reported (Greenstock and Wiebe, 1978). The reactions were catalyzed by micelles which concentrated polycyclic hydrocarbons in a non-polar environment. The exclusion of water within the micelles possibly increased the lifetimes of the reactive species. The model photochemical system mimicked the aerobic metabolic activation of chemical carcinogens, which may be mediated by the various forms of activated oxygen.

For many precarcinogens (e.g. polycyclic aromatic hydrocarbons, aromatic amines, nitro and nitroso compounds), the activated oxygen species were strong electrophiles. The electrophiles reacted with the nucleophilic centers within important cellular target macromolecules to produce irreversible chemical damage and ultimately neoplastic transformations (Greenstock and Wiebe, 1978). An attempt was made to distinguish between the possible mechanisms of carcinogen photooxidation.

Although the results were consistent with the hypothesis that $^1\text{O}_2$ was the activating species, peroxy radical formation could not be ruled out.

The sensitized photooxidation of several lignin compounds with alkylated paraphenolic groups was examined using riboflavin, rose bengal and methylene blue (Kutsuki et al, 1983). Riboflavin effected transformations via a Type I hydrogen abstraction mechanism rather than a Type II singlet oxygen mechanism. Results indicated that riboflavin was an inefficient generator of $^1\text{O}_2$ and was not effecting oxidation of lignin model compounds via a type II mechanism.

In the photooxidation of 2,4-D by riboflavin, riboflavin was not regenerated, but rather was completely destroyed with no appreciable degradation of 2,4-D (Silber et al, 1976). In the photooxidation of 2,4,5-T, however, riboflavin caused a drastic increase in the rate of photodecomposition. The riboflavin concentration was greater than the concentration of the herbicide and no measurements on riboflavin decomposition were made (Crosby and Wong, 1973).

Surfactants caused an increase in the photodegradation rate of herbicides with low water solubilities, chloro substituent(s) on the aromatic ring, and triplet energies lower than that of the added surfactant. When the above conditions were not met, however, the addition of the surfactant had a variable effect on the photodegradation rate of the herbicides in aqueous solution (Tanaka et al, 1981). Nonionic surfactants can either enhance or suppress herbicidal photodegradation. By careful selection of surfactants in formulation, herbicidal lifetimes with respect to

photodecomposition in aqueous media can apparently be either encouraged or discouraged.

The sensitized photooxidation of bromacil has been extensively studied (Acher and Dunkelblum, 1979; Acher, 1982; Acher and Saltzman, 1981; Watts et al, 1983; Eisenberg et al, 1985; Eisenberg et al, 1986). Bromacil is practically stable in sunlight. The prolonged irradiation (4 mo) of bromacil to sunlight, resulted in only a 2.2% yield of the dealkylated product, 5-bromo-6-methyluracil (Moilanen and Crosby, 1974). With the addition of methylene blue (a known singlet oxygen generator), however, a fast chemical transformation occurred (Acher and Dunkelblum, 1979). The rate of the reaction was pH dependent, being faster at higher pH values (9.4) and almost negligible at lower pH values (5.0). The rate of the reaction also closely followed the percent ionization of bromacil which was pH dependent (Eisenberg et al, 1986). No photoreaction products were detected when 3-sec-butyl-methyluracil was irradiated under identical conditions, indicating the necessary presence of bromine for photooxidation to take place. A mechanism was proposed based on a reaction of singlet oxygen with the double bond followed by a fast OH^- catalyzed rearrangement with loss of bromine. Photooxidation of terbacil and bromacil in frozen solutions resulted in the loss of the halogen atoms (Acher, 1982). The released halogen atoms formed hydroacids, attacked the bicarbonate and carbonate present, and formed carbon dioxide bubbles.

A model was developed to predict sensitized photooxidation

rate as a function of lagoon depth (Watts et al, 1983). The model was based on light intensity, sensitizer extinction coefficient, and an applied quantum yield, all of which were integrated over the wavelengths of visible light. The model served as the basis for sizing photooxidizing lagoons.

An overview of studies on the sensitized photooxidation of chemical systems is presented in Table 2. The substrate, sensitizer, results and author(s) are given for each study. The results presented in the Table 2 may not be complete, and the reader is directed to review the original paper for more complete information.

Table 2. Studies on sensitized photooxidation of chemical systems.

PESTICIDE	Sensitizer*	Results	Reference
general	MISC and 175 pest.	aromatic amines sensitized DDT and chlorinated cyclodienes, anthraquinone showed broadest spectrum	Ivie and Casida, 1971
general	rotenone & chromanones	sensitizers accelerate photoalteration	Ivie and Casida, 1971
amide, phenylurea, triazine, carbamate	surfactant	increase in degradation w/ solubility of substrate in water	Tanaka et al, 1981
aniline, triazine uracil	MB	cost effective, attractive for arid and semiarid zones	Acher, 1985
carbamates, phenoxy, organophosphates, etc.	H2O2	H2O2 promoted rxs at conc. occurring in irrigation and surface waters	Draper and Crosby, 1984
hexaethoxylated 2,6,8-trimethyl-4-nonanol	MISC & herb	6 herbicides sensitized degradation, products identified	Tanaka et al, 1986
amides			
chlorthiamid	RF	singlet oxygen, chlorthiamid yields dichlobenil and benzonitrile	Pillai, 1977
anilines			
aniline	HA	azobenzene, $t(1/2) = 4 - 8$ hr (May)	Zepp et al, 1981
chloroanilines	FMN	photoproducts azobenzenes and anilinazobenzenes	Rosen et al, 1970
chloroanilines	HA	H abstract., yield less in lab soln than in natural water	Miller et al, 1980
propachlor	RF,MB	RF efficient, MB not; pH 3-4, 12 hr complete degrad	Rejto et al, 1984
benzimidazoles			
MBC	RF,AC	oxidation of benzene, less than 10% was lost, greater w/ sensitizer added	Fleeker and Lacy, 1977
carbamates			
butocarboxim, methiocarb	soil	rapid rates to sulfoxides, disulfoton most rapid, methiocarb slowest	Gohre and Miller, 1986
CIPC	AC	rate increased 30-fold w AC	Guzik, 1978
2-dimethylamino-6'-dimethylpyrimidin-4-ol	RB	increased rate at high pH due to increase in singlet oxygen singlet oxygen production	Dixon and Wells, 1983
thiobencarb	H2O2, MB, Trp	H2O2 and peroxide radicals active oxidants, not singlet oxygen	Draper and Crosby, 1981.

Table 2 (cont.). Studies on sensitized photooxidation of chemical systems.

thiobencarb	Trp,Tyr	amino acids oxidant generators, oxidiz. power natural waters general	Ross and Crosby, 1985
chlorinated hydrocarbons			
aldrin, DDE	Trp,Tyr	amino acids oxidant generators, oxidiz. power natural waters general	Ross and Crosby, 1985
dienachlor	Cl	t(1/2) < 1 h, photoproducts perchloro ketones	Quistad and Mulholland, 1983
kepone	amines	78% degraded in soil test after 10 days exposure	Christensen and Weimer, 1980
insect growth regulator			
methoprene	RB,MeOH	rapid degradation and many photoproducts	Quistad et al, 1975
nitrile			
dichlobenil	RF	singlet oxygen, chlorthiamid yields dichlobenil and benzonitrile	Pillai, 1977
organophosphate			
disulfoton fenthion	soil	rapid rates to sulfoxides, disulfoton most rapid, methiocarb slowest	Gohre and Miller, 1986
disulfoton	HA	disulfoton sulfoxide, t(1/2) = 4 (summer) to 10 d (winter)	Zep et al, 1981
mephosfolan	AC	accelerates photolysis of mephosfolan and products	Ku et al, 1979
Na phenyl phosphate	MB,RB,TH	release of orthoPO ₄ enhanced w/ dyes, bleaching, singlet O ₂	Halmann and Levy, 1979
parathion	MB	no reaction	Grunwell and Erickson, 1973
organotin			
triphenyltin OH	AC, RB	product diphenyltin oxide, sensitizers increased rate	Soderquist and Crosby, 1980
phenoxy			
2,4-D	RF	RF decomposes and not regenerated; undegraded after 22 hr	Silber et al, 1976
2,4,5-T	RF, AC	11 fold increase w/ sensitizers, products chlorinated phenols chlorinated resorcinols	Crosby and Wong, 1973
2,4,5-T	HA,Fe,H ₂ O ₂	HA effective; Fe & OOH contribute to 2,4,5-T in acidic waters	Skurlatov et al, 1983
picolinic acid			
loram	HA,Fe,H ₂ O ₂	HA ineffective	Skurlatov et al, 1983

Table 2 (cont.). Studies on sensitized photooxidation of chemical systems.

triazine			
ametryne, atraton, atrazine, simazine, propazine	RF	deethylated and acetamido-s-triazines, reaction rate higher at lower pH	Rejto et al, 1983

urea			
ethylenethiourea	RF, AC	stable sunlight, sensitizers to ethyleneurea and glycineSO4	Ross and Crosby, 1973
ethylenethiourea	Trp, Tyr	amino acids oxidant generators, oxidiz. power natural waters general	Ross and Crosby, 1985
fluometron	MB, RF	basic pH reqd MB, RF was pH & substrate dependent, model developed	Watts, 1983
monuron	surfactants	increase rate, eliminate ring hydroxylation rxns, enhance dechlorination, rxns occur in organic phase of micelles	Tanaka et al, 1979

uracil			
bromacil, terbacil	RF, MB	best results at alkaline pH, at pH 7 practical for terbacil	Acher, 1982
bromacil, terbacil,	MB, RF	basic pH reqd for MB, RF was pH & substrate dependent, model developed	Watts, 1983
bromacil	MB	pH dependent, diastereoisomers of butylacetylhydroxyhydantoin	Acher and Dunkelblum, 1979
bromacil	MISC	RB, E, MB, RF effective, rate dependent on % ionization and pH (max at alkaline), anaerobic rate 1-2 orders less	Eisenberg et al, 1986
bromacil	MB	settling enhanced removal, 1st order kinetics, pH>9, [MB] 4-10ppm, sun 300-900 suffic., lab vs. indust. MB equiv.	Eisenberg et al, 1985
bromacil	MISC	MB & RF most effective, RB, HA, CHL less; pH dependent (faster at alkaline), rx complete after 1 hr	Acher and Saltzman, 1980
terbacil	MB, RF	MB ineffective under acid condit., RF effective down to pH 3,	Acher et al, 1981

ORGANIC	Sensitizer*	Results	Reference
acetylaminofluorene, benzopyrene, dimethylbenzanthracene, roquinolineoxide	AO, E, MB, PF	enhanced damage in presence of O2, catalyzed by micelles	Greenstock and Wiebe, 1978
allylthiourea dimethylanthracene	TH	For DMA Type II at low conc and Type I w/o O2. For ATU, Type I at high conc and Type II at low conc in pyridine soln	Kramer and Maute, 1973

Table 2 (cont.). Studies on sensitized photooxidation of chemical systems.

allylthiourea, allylurea, thiourea	PS	for allylthiourea thiourea group site of singlet oxygen, while for allyl moiety neither quenches nor reacts	Kraljic and Kramer, 1978
aniline, resorcinol naphthol, bromophenol sulfanilic acid	E	aromatic radicals formed, quench E triplet	Chrysochoos and Grossweiner, 1968
cholesterol	RF,MB,F,E,L	singlet oxygen, yield epoxy ketone and enone	Kearns et al, 1967
chlorinated organics	Ti, Zn, Fe	photocatalysis, rx const over most of day; chloroolefins > chloroparaffins > chloroacetic acids	Ollis, 1985
chloroform, NH ₃ , methanol, phenol, dimethylamine	Ti,Zn,Fe oxide	Fe ineffective, NH ₃ not destroyed, organics showed 30 to 50 % removal in 6 hr	Peyton and Deberry, 1981
COD, MBAS	RB,MB	MB better, decreases in COD and MBAS of 67 and 90%, pH 8, MB=12ppm, MB removed w/ 6 bentonite: 1 MB	Acher and Rosenthal, 1977
COD, MBAS	MB	cost effective, attractive for arid and semiarid zones	Acher, 1985
dienes, olefins	H ₂ O ₂ ,MOCL	singlet oxygen generation identical to dye photosensitization	Foote, 1968
dienes	?	singlet oxygen,free radicals, hydrogen abstraction, yields hydroperoxides by typical 'ene' reaction	Rabek and Ranby, 1979
dimethylantracene, surfactants	THZ	adsorption of dye slows protonation, rate for same pH dependent on adsorption of dye	Bagno et al, 1979
dimethylfuran	HA	reaction 1st order, proportional to intensity and [HA], independent of pH (5 to 10)	Zepp et al, 1981
dimethylfuran, tetramethylethylene	soil	photoproduct indicate singlet oxygen is formed on soil surfaces and may contribute to indirect photooxidative processes	Gohre and Miller, 1983
general	TiO ₂	polar and N or S compounds susceptible, limiting factors low quantum efficiency and wide band gap for TiO ₂	DeBerry et al, 1983
general	MB, RB	singlet oxygen: Diels-Alder, electron rich double bonds low quantum yield suggests sensitizer lower cost as tertiary treatment	Haag and Holgne, 1986 Schorr et al, 1971
general		rapid generation singlet oxygen in inland and coastal waters	Zepp et al, 1977
lignin	RF,RB,MB	RF via H abstraction, cleavage at alph & beta carbons	Kutsuki et al, 1983
PBB	amines	49 % degradation after 4 days sunlight exposure	Christensen and Weimer, 1980
secondary effluent	Cl ₂	sensitized rate 20 times greater than nonsensitized	Hancil and Smith, 1971
TNT	p-acetobenzene sulfonic acid	photolysis rapid with t(1/2) < 30 min in natural waters	Mabey et al, 1983

Table 2 (cont.). Studies on sensitized photooxidation of chemical systems.

dyes

cyanines	MB,RB	singlet oxygen, most esp. w/ low oxidation potentials attacked	Byers et al, 1976.
luminol	MB	quantum yield dependent on Temp, pH and [luminol]	Matheson and Lee, 1970
xanthenes		degraded by visible light, products non-toxic	Heitz and Wilson, 1978

phenols

Cl-phenols	RB	RB bound to beads, effective oxidizing phenols, rates practical	Cherry and Jessen, 1982
cresol	MB	5-10 mg/L MB for 10-300 cresol, 1st order, 3 times faster in aerobic than anaerobic	Sargent and Sanks, 1974
cresol	MISC	MB & RB effective but NR, MG, DCF, AO ineffective; 1st order; 5 ppm dye; pH 10; $t(1/2) < 3$ min	Sargent and Sanks, 1976
dichlorophenol toluidine	TH,PS	gelled alginate, acceptability depends on cost, time, stability of sensitizer, & elimination of toxic products	Seely, 1978
phenol	metal-NO3	first order reaction, ferric nitrate most effective catalyst	Schrader and Hwang, 1982
phenols		different ratios of reaction w/ and quench.of singlet oxygen	Thomas and Foote, 1978

SENSITIZER KEY

AC=acetone, E=eosin, F=fluorescein, FMN=flavomononucleotide, HA=humic acid, L=lumichrome, MB=methylene bue, PF=proflavine, PS=phenolsafranine, RB=rose bengal, TH=thionine, THZ=thiazine dyes

METHODOLOGY

COLIFORM BENCH SCALE STUDIES

Bench scale studies in 208 L (55 gal) drums were conducted to determine the disinfection potential of sensitized photooxidation on total and fecal coliform. Coliform densities in wastewater were monitored over time under different operating conditions. The effects of mixing, sensitizer concentration, pH value and chemical used for pH adjustment, depth, suspended solids, temperature, influent coliform density, sunlight intensity, solids settling and detention time on coliform disinfection were determined.

Experimental Procedure

Secondary unchlorinated conventional activated sludge effluent from the City of Livingston, Tennessee Wastewater Treatment Plant was the source of water for the bench scale studies. The water was pumped from the overflow weirs of the secondary clarifier into a series of 208 L (55 gal) containers. Experiments were conducted during the period of December 23, 1983 to September 27, 1984, during which time 26 batch experiments were performed. A total of 140 combinations of parameters were investigated using predetermined pH values, (adjusted to desired value with either lime, caustic soda, or soda ash), sample depths, methylene blue concentrations, and mixing regimes, and random initial coliform densities, temperatures, sunlight intensities, dissolved oxygen, solids settling, and suspended solids (Table 3).

Table 3. Initial parameter values for bench scale coliform experiments.

MB	pH	INFLUENT	SUN	PAR	MIX	CHEM	HT	NOTE
0.00	8.1	4600000	119	FC	S	C	60	
0.50	8.2	4600000	119	FC	S	C	60	
1.00	8.1	4600000	119	FC	C	C	60	
1.00	8.1	4600000	119	FC	P	C	60	
1.00	8.1	4600000	119	FC	S	C	60	
0.00	8.7	11000000	272	FC	S	C	60	
0.50	8.7	11000000	272	FC	S	C	60	
1.00	8.7	11000000	272	FC	C	C	60	
1.00	8.7	11000000	272	FC	P	C	60	
1.00	8.8	11000000	272	FC	S	C	60	
1.00	8.4	46000	370	FC	S	C	60	
1.00	8.5	46000	370	FC	S	C	60	
1.00	8.7	46000	370	FC	P	C	60	
1.00	8.8	46000	370	FC	C	C	60	
1.00	8.8	46000	370	FC	S	C	60	
0.00	9.1	240000	485	FC	S	C	60	GT
0.00	9.2	240000	485	FC	S	C	60	GT
2.00	7.3	240000	485	FC	S	C	60	GT
2.00	7.4	240000	485	FC	S	C	60	GT
2.00	9.2	240000	485	FC	S	C	60	
2.00	9.3	240000	485	FC	S	C	60	
1.00	9.0	240000	560	FC	S	C	40	20>
1.00	9.0	240000	560	FC	S	C	40	DK&20>
5.00	7.4	240000	560	FC	S	C	40	GY&20>
5.00	8.1	240000	560	FC	S	C	40	GY>
5.00	9.0	240000	560	FC	S	C	40	GY>
0.10	9.2	240000	599	FC	C	C	60	X>
0.50	9.1	240000	599	FC	C	C	60	X>
1.00	8.4	240000	599	FC	S	C	60	X>
1.00	8.4	240000	599	FC	P	C	60	X>
1.00	8.4	240000	599	FC	C	C	60	X>
1.00	9.2	240000	599	FC	C	C	60	DK&X>
1.00	8.0	4600000	642	FC	S	C	60	
1.00	8.1	4600000	642	FC	S	C	60	
1.00	8.8	4600000	642	FC	C	C	60	
1.00	8.8	4600000	642	FC	P	C	60	
1.00	8.9	4600000	642	FC	S	C	60	
0.10	8.8	240000	688	FC	S	C	60	GT
0.50	8.8	240000	688	FC	S	C	60	GT
1.00	8.8	240000	688	FC	S	C	60	GT
1.00	8.8	240000	688	FC	P	C	60	GT
1.00	8.8	240000	688	FC	C	C	60	GT
5.00	8.8	240000	688	FC	S	C	60	GT

Table 3 (cont.). Initial parameter values for bench scale coliform experiments.

MB	pH	INFLUENT	SUN	PAR	MIX	CHEM	HT	NOTE
0.00	8.8	43000	751	FC	S	C	60	
2.00	8.8	43000	751	FC	S	C	60	
2.00	8.8	43000	751	FC	C30	C	60	
2.00	8.8	43000	751	FC	C60	C	60	
2.00	8.8	43000	751	FC	C90	C	60	
0.00	7.2	240000	803	FC	S	C	60	GT
0.00	7.6	240000	803	FC	C	C	60	GT
0.00	8.8	240000	803	FC	S	C	60	GT
1.00	8.8	240000	803	FC	C	C	60	GT
1.00	8.9	240000	803	FC	S	C	60	GT
1.00	8.9	240000	803	FC	C	C	60	GT
0.10	7.4	240000	882	FC	S	C	60	GT
0.10	9.1	240000	882	FC	S	C	60	GT
0.50	7.4	240000	882	FC	S	C	60	GT
0.50	9.1	240000	882	FC	S	C	60	GT
1.00	7.4	240000	882	FC	S	C	60	GT
1.00	9.1	240000	882	FC	S	C	60	GT
0.00	8.9	23000	930	FC	S	C	60	
1.00	8.9	23000	930	FC	S	C	60	
1.00	8.9	23000	930	FC	C30	C	60	
1.00	8.9	23000	930	FC	C60	C	60	
1.00	8.9	23000	930	FC	C90	C	60	
0.00	8.9	890000	55	TC	S	L	60	
2.00	8.2	890000	55	TC	S	L	60	
2.00	8.5	890000	55	TC	S	L	60	
2.00	9.3	890000	55	TC	S	L	60	
2.00	9.3	890000	55	TC	P	L	60	
2.00	9.5	890000	55	TC	C	L	60	
1.00	7.5	300000	104	TC	C90	C	0	
1.00	8.6	300000	104	TC	C90	C	0	
1.00	9.2	300000	104	TC	C90	C	0	
0.00	9.5	2800000	120	TC	S	L	60	
2.00	8.2	2800000	120	TC	S	L	60	
2.00	8.6	2800000	120	TC	S	L	60	
2.00	9.5	2800000	120	TC	S	L	60	
2.00	9.7	2800000	120	TC	P	L	60	
2.00	10.0	2800000	120	TC	C	L	60	
1.00	7.1	340000	240	TC	C90	C	0	
1.00	7.7	330000	240	TC	C90	C	0	
1.00	9.0	220000	240	TC	C90	C	0	
1.00	8.4	600000	290	TC	C90	C	0	
1.00	8.4	760000	290	TC	C135	C	0	

Table 3 (cont.). Initial parameter values for bench scale coliform experiments.

MB	pH	INFLUENT	SUN	PAR	MIX	CHEM	HT	NOTE
1.00	8.4	3300000	290	TC	C45	C	0	
0.00	8.1	500000	320	TC	S	C	0	
2.00	8.1	500000	320	TC	C135	C	0	
2.00	8.1	500000	320	TC	C90	C	0	
2.00	8.1	500000	320	TC	C45	C	0	
2.00	8.1	500000	320	TC	S	C	0	
2.00	8.1	500000	320	TC	S	C	0	DK
1.00	8.8	300000	359	TC	S	S	60	
1.00	8.8	300000	359	TC	S	S	60	
1.00	9.0	300000	359	TC	S	C	60	
1.00	9.0	300000	359	TC	S	C	60	
1.00	9.1	300000	359	TC	S	L	60	
1.00	9.2	300000	359	TC	S	L	60	
0.00	8.9	6700000	551	TC	S	C	0	
0.00	8.9	6700000	551	TC	S	C	60	
0.25	8.8	6700000	551	TC	S	C	0	
0.25	8.8	6700000	551	TC	S	C	60	
0.50	8.8	6700000	551	TC	S	C	0	
0.50	8.8	6700000	551	TC	S	C	60	
1.00	8.8	6700000	551	TC	S	C	0	
1.00	8.8	6700000	551	TC	S	C	60	
2.50	8.8	6700000	551	TC	S	C	0	
2.50	8.8	6700000	551	TC	S	C	60	
5.00	8.9	6700000	551	TC	S	C	0	
5.00	8.9	6700000	551	TC	S	C	60	
2.00	7.4	360000	560	TC	C90	C	0	
2.00	8.0	260000	560	TC	C90	C	0	
2.00	9.3	140000	560	TC	C90	C	0	
0.00	8.9	6300000	615	TC	S	C	60	
0.25	8.9	6300000	615	TC	S	C	60	
0.50	8.9	6300000	615	TC	S	C	60	
1.00	8.9	6300000	615	TC	S	C	60	
2.50	8.8	6300000	615	TC	S	C	60	
5.00	8.9	6300000	615	TC	S	C	60	
2.00	9.4	1900000	630	TC	C45	C	0	
2.00	9.4	2200000	630	TC	C90	C	0	
2.00	9.4	2500000	630	TC	C135	C	0	
0.00	8.9	4400000	738	TC	S	C	0	
0.00	8.9	4400000	738	TC	S	C	60	
0.25	8.8	4400000	738	TC	S	C	0	
0.25	8.8	4400000	738	TC	S	C	60	
0.50	8.8	4400000	738	TC	S	C	0	
0.50	8.8	4400000	738	TC	S	C	60	

Table 3 (cont.). Initial parameter values for bench scale coliform experiments.

MB	pH	INFLUENT	SUN	PAR	MIX	CHEM	HT	NOTE
1.00	8.8	4400000	738	TC	S	C	0	
1.00	8.8	4400000	738	TC	S	C	60	
2.50	8.8	4400000	738	TC	S	C	0	
2.50	8.8	4400000	738	TC	S	C	60	
5.00	8.9	4400000	738	TC	S	C	0	
5.00	8.9	4400000	738	TC	S	C	60	
0.00	8.9	7600000	749	TC	S	C	60	X
0.25	8.9	7600000	749	TC	S	C	60	X
0.50	8.9	7600000	749	TC	S	C	60	X
1.00	8.9	7600000	749	TC	S	C	60	X
2.50	8.8	7600000	749	TC	S	C	60	X
5.00	8.9	7600000	749	TC	S	C	60	-X
2.00	7.3	510000	793	TC	S	C	0	
2.00	8.1	350000	793	TC	S	C	0	
2.00	9.4	470000	793	TC	S	C	0	

pH: pH value

INFLUENT: coliform density/100 mL

SUN: sunlight intensity (W/sq.m)

PAR: FC = fecal coliform and TC = total coliform

MIX: S = mixed once (initially) and allowed to settle

P = mixed just prior to taking sample

C = mixed continuously at 45, 90, or 135 rpm

CH: chemical used for pH adjustment

L = lime

C = caustic soda

S = soda ash

HT: sample depth (cm)

NOTE: X = atypical feedwater

GT = coliform density was greater than listed

DK = experiment conducted in absense of sunlight

Materials

Methylene blue was obtained from TRICON COLORS INCORPORATED as basic blue 9 or methylene blue 2B powder. The TRICON methylene blue was colorimetrically measured against Fisher certified biological stain methylene blue at 660 nm and found to be 82 percent of the methylene blue concentration of the biological stain (Figure 7). Methylene blue concentrations reported in the study were based on the TRICON methylene blue. The caustic soda, lime, and soda ash, used for adjustment of pH value, were reagent grade chemicals.

Analytical

Total coliform densities were determined according to the Standard Methods membrane filter procedure. Three replicates per dilution were analyzed, and the average of the those replicates with acceptable coliform counts was reported as the total coliform density. Fecal coliform densities were determined according to the Standard Methods MPN procedure (A-1 Medium)(TENTATIVE). Three fermentation tubes per dilution were used, and the MPN/100 mL was calculated from the "MPN Index And 95% Confidence Limits For Various Combinations of Positive Results When Three Tubes Are Used Per Dilution."

Sunlight intensity was measured at ten minute intervals using an Eppley Radiometer Model 8-46 and an Instrulab 2000 Datalogger. Temperature and dissolved oxygen were measured concurrently using a Yellow Springs Instrument Model 57 dissolved oxygen meter. A Fisher Accumet pH Meter Model 800 was used to measure the pH value. Suspended solids were measured

FIGURE 7. COMPARISON BETWEEN SENSITIZERS.
FISHER VS TRICON METHYLENE BLUE
METHYLENE BLUE CONCENTRATION = 10 mg/L

according to Standard Méthods.

BROMACIL BENCH SCALE STUDIES

Bench scale studies in 208 L (55 gal) drums were conducted to determine the degradation potential of sensitized photooxidation on the refractory herbicide bromacil. Herbicide concentrations in wastewater were monitored over time under different operating conditions. The effects of mixing, dye concentration, pH value, dissolved oxygen, initial bromacil concentration, suspended solids, light intensity, and detention time on bromacil degradation were determined.

Experimental Procedure

Secondary unchlorinated activated sludge effluent from the City of Livingston, Tennessee Wastewater Treatment Plant was the source of water for the bench scale studies. The water was pumped from the overflow weirs of the secondary clarifier into a series of six 208 L (55 gal) containers. Experiments were conducted during the period of March 20 to April 18, 1985, during which time 6 batch experiments were performed. A total of 36 combinations of parameters were investigated using various pH values (adjusted to desired value with 6N NaOH), methylene blue concentrations, initial bromacil concentrations and mixing regimes, and random effects of sunlight intensity, dissolved oxygen and suspended solids (Table 4).

Materials

Bromacil [5-bromo-3-sec-butyl-6-methyluracil] was obtained

Table 4. Initial parameter values for bench scale bromacil experiments.

MB	pH	Co	SUN	MIX	DO	SS
0.0	9.5	29	294	S	6.2	28
2.0	7.3	28	294	S	6.2	25
2.0	9.5	29	294	S	6.2	32
2.0	9.5	32	294	C90	6.2	30
2.0	9.6	31	294	C30	6.2	21
2.0	9.6	34	294	MS	6.2	35
4.0	10.0	3	393	S	6.0	60
4.0	10.0	5	393	S	6.0	64
4.0	10.0	10	393	S	6.0	44
4.0	10.0	17	393	S	6.0	52
4.0	10.0	38	393	S	6.0	34
4.0	10.0	84	393	S	6.0	54
0.0	9.7	39	446	S	.	98
0.5	9.8	42	446	S	.	90
1.0	9.7	39	446	S	.	90
2.0	9.6	39	446	S	.	96
4.0	9.7	37	446	S	.	90
8.0	9.5	39	446	S	.	84
2.0	7.3	28	819	S	6.1	.
2.0	7.6	32	819	S	6.1	.
2.0	8.0	34	819	S	6.1	.
2.0	8.9	31	819	S	6.1	.
2.0	9.7	28	819	S	6.1	.
2.0	10.2	32	819	S	6.1	.
0.0	9.7	22	854	S	6.0	.
2.0	9.7	23	854	S	6.1	.
4.0	9.6	24	854	S	6.1	.
8.0	9.7	23	854	S	6.0	.
13.0	9.7	26	854	S	6.2	.
32.0	9.6	25	854	S	6.1	.
4.0	10.0	31	876	S	6.6	.
6.0	10.0	34	876	S	6.6	.
8.0	10.0	32	876	C90	6.6	.
8.0	10.0	33	876	MS	6.6	.
8.0	10.0	34	876	S	6.6	.
10.0	10.0	36	876	S	6.5	.

pH: pH value

Co: Bromacil at t = 0 (mg/L)

SUN: sunlight intensity (W/sq.m)

DO: dissolved oxygen (mg/L)

SS: suspended solids (mg/L)

MIX: S = mixed initially & allowed to settle

C = mixed continuously at listed rpm

MS = mixed prior to taking sample

as DuPont HYVAR X-L, a miscible liquid containing 240 g/L (2 lb/gal) bromacil. The active ingredient is a 21.9 percent lithium salt of bromacil, and the inert ingredients are 78.1 percent. The bromacil concentration of HYVAR X-L was verified against bromacil reference standards obtained from EPA and was found to agree with the manufacturer's claim. Methylene blue was obtained from TRICON COLORS INCORPORATED and caustic soda was obtained as reagent grade as described previously.

Analytical

Quantitative analysis of the bromacil solutions involved liquid-liquid extraction of the compounds from aqueous solution followed by gas chromatographic measurement using a flame ionization detector. The pH value of all samples and standards were lowered to a neutral pH value prior to extraction. Methylene chloride (CH_2Cl_2) was used to extract the bromacil from the water samples. A 10 mL portion of CH_2Cl_2 was added to 100 mL of the herbicide solution in a 125 mL separatory funnel. The funnel was vigorously agitated for two minutes, inverted and opened to release pressure. The procedure was repeated again, and the funnel was set aside to allow the CH_2Cl_2 and water phases to separate. The denser CH_2Cl_2 bromacil phase was then drawn-off into a 14 mL glass bottle. Standard bromacil solutions were prepared in the same manner using EPA reference bromacil.

The gas chromatograph was a Varian Model 3700 C with a Varian CDS 111 Recorder. A 61 cm x 2 mm (ID) glass column packed with 1 percent SP-2250 on 80/100 Supelcort was used. Operating conditions for the chromatograph were an injector port

temperature of 250 C, an initial oven temperature of 160 C for 2 minutes, a program rate of 20 C/min, a detector temperature of 260 C, a nitrogen carrier flow rate of 30 mL/min, a hydrogen flow rate of 30 mL/min, and an air flow rate of 300 mL/min. The volume of sample injected was 1 uL. Rubber septa with teflon coatings were required to avoid bleeding of the septum into the injector port of the gas chromatograph.

Measurements of sunlight intensity, temperature, dissolved oxygen, pH value, and suspended solids were as previously described. Chemical oxygen demand was measured using Oceanographic International Standard Range COD Twistubes and reading optical density at 600nm. COD standard solutions were verified against an EPA Demand Series #4 reference.

COLIFORM PILOT SCALE STUDIES

Pilot scale studies were conducted in two plug flow reactors (each 2.44 m wide by 9.75 m long by 1.22 m deep) to determine the disinfection potential of sensitized photooxidation on total coliform. Coliform densities in wastewater were monitored over time under different operating conditions. The effects of sensitizer concentration, pH value, depth, turbidity, temperature, influent coliform density, sunlight intensity, solids settling, and detention time on coliform disinfection were determined.

Design and Construction

The site of the pilot plant was the City of Livingston, Tennessee, Wastewater Treatment Plant. Construction on the pilot plant was started in March, 1985. The construction site was first

graded with 100 tons of low-quality gravel. The reactors were constructed from: AC Exterior 4' x 8' x 3/4" plywood sheets; 2" x 4", 2" x 8", and 4" x 4" pressure treated pine; and 6" angle iron. Factory cut and seamed 10 x 10 denier Hypalon membrane liners were used to line and seal the interiors of the reactors. The mixing and constant head tanks were fabricated from 4' x 8' x 11/16" galvanized aluminum. Influent distribution lines were PVC Schedule 40 3" and 4" pipe, and effluent distribution lines were PVC DWV 3" and 4" pipe. A Zoeller Model D295 submersible pump was used to pump unchlorinated secondary effluent into the system. The influent flow was split prior to entering the system, and 3" gate valves were used to throttle down excess flow into the constant head tanks. Construction was completed in August, 1986, and the BARD Solar Wastewater Disinfection Plant was officially dedicated on August 22, 1986. Schematics and pictures of the pilot plant are shown in Appendix A. †

Hydraulic Characteristics

Tracer studies were conducted to evaluate the flow characteristics of both reactors. Rhodamine WT was used as the tracer dye, and a Turner Designs Fluorometer Model 10-005 was used to measure dye concentrations. Two mL of a 0.2 mg/mL concentration was injected into the mixing tank of each reactor. Samples were collected at one-fourth and one-half the distance of each reactor and at the outlet of each reactor. Samples were taken from the mid-width of the channel and at one-half the water depth.

Experimental Procedure

Secondary unchlorinated activated sludge effluent from the City of Livingston, TN Wastewater Treatment Plant was the source of water for the pilot scale studies. The water was pumped from the overflow weirs of the secondary clarifier into two parallel plug flow reactors. Experiments were conducted during the period of November 2, 1986 to April 1, 1986, during which time 15 experiments were performed. A total of 81 combinations of parameters were investigated using predetermined pH values (adjusted to desired values with a 40 percent industrial grade NaOH), sample depths, and methylene blue concentrations, and random initial coliform densities, temperatures, sunlight intensities, dissolved oxygen and turbidities (Table 5).

Materials

Methylene blue was obtained from TRICON COLORS INCORPORATED as previously described. Caustic soda was obtained as 50% bulk solution from the City of Livingston, Tennessee, Water Treatment Plant and diluted to a 20% solution.

Analytical

Total coliform densities, sunlight intensity, temperature, and dissolved oxygen were measured as previously described. Turbidity was measured on a Hach Ratio Turbidimeter Model 189000. The turbidity in the wastewater samples due to methylene blue was adjusted by subtracting out the turbidity of distilled water with methylene blue present. The methylene blue concentration present in the sample was determined by measuring absorbance at 660 nm

Table 5. Initial parameter values for pilot plant coliform experiments.

MB	pH	INFLUENT	SUN	NTU	DATE
0.5	10.2	403333	127	43	11-02-85
2.0	10.0	390000	127	60	11-02-85
0.0	8.7	7400000	160	200	11-13-85
1.1	8.6	5800000	160	100	11-13-85
0.0	9.0	333333	315	200	11-13-85
1.1	9.1	640000	315	170	11-13-85
0.5	8.8	7066666	124	200	11-19-85
1.2	9.0	9466666	124	200	11-19-85
0.0	9.2	3400000	394	133	11-19-85
0.7	9.3	2800000	394	130	11-19-85
0.2	9.2	343333	110	32	11-26-85
0.5	8.8	443333	110	23	11-26-85
0.0	9.1	656667	360	39	11-26-85
0.4	9.3	560000	360	40	11-26-85
0.2	9.3	343333	130	32	11-30-85
0.3	8.9	443333	130	10	11-30-85
0.0	9.1	656667	405	39	11-30-85
0.3	9.2	560000	405	40	11-30-85
0.0	9.0	566667	.	15	12-15-85
0.0	9.1	363333	.	5	12-15-85
0.0	9.1	363333	.	19	12-15-85
0.0	9.2	566667	.	17	12-15-85
0.5	9.1	346667	.	9	12-15-85
0.9	9.1	323333	.	21	12-15-85
0.0	9.1	420000	137	19	12-16-85
0.0	9.4	346667	137	30	12-16-85
0.7	9.1	420000	137	20	12-16-85
0.9	9.2	346667	137	15	12-16-85
0.0	8.6	420000	342	23	12-16-85
0.6	8.5	33000	342	9	12-16-85
0.8	8.8	146667	342	23	12-16-85
0.0	9.1	446667	89	8	12-27-85
0.0	9.2	446667	89	8	12-27-85
0.6	9.1	2650000	89	13	12-27-85
0.0	9.0	6600000	155	7	12-27-85
0.0	9.1	6600000	155	10	12-27-85
0.6	9.1	6600000	155	12	12-27-85
0.0	7.3	1300000	.	5	01-08-86
0.0	8.2	1833333	.	8	01-08-86
0.0	8.8	473333	.	31	01-08-86
0.0	9.4	1466667	.	46	01-08-86
0.9	7.7	1833333	.	10	01-08-86
0.9	8.7	1466667	.	15	01-08-86

Table 5 (cont.). Initial parameter values for pilot plant coliform experiments.

MB	pH	INFLUENT	SUN	NTU	DATE
1.0	7.9	473333	.	11	01-08-86
1.1	7.5	1300000	.	11	01-08-86
0.0	8.7	513333	221	13	01-11-86
0.0	8.8	503333	221	15	01-11-86
0.9	8.4	513333	221	15	01-11-86
1.3	8.9	503333	221	20	01-11-86
0.0	7.9	280000	463	6	01-11-86
0.0	8.8	423333	463	15	01-11-86
0.9	7.8	280000	463	10	01-11-86
1.4	8.8	423333	463	27	01-11-86
0.7	9.0	573333	269	33	01-15-86
1.1	8.7	336667	269	14	01-15-86
0.0	8.8	298333	406	15	01-15-86
0.5	9.0	283333	406	29	01-15-86
0.8	8.8	313333	406	14	01-15-86
1.0	8.8	320000	134	27	01-16-86
2.0	8.8	366667	134	17	01-16-86
0.0	9.3	261667	374	18	01-16-86
0.5	8.9	160000	374	20	01-16-86
1.3	8.6	363333	374	17	01-16-86
1.4	9.5	600000	463	14	03-22-86
3.0	9.6	653333	463	18	03-22-86
0.0	9.8	435000	739	19	03-22-86
0.6	9.4	526667	739	16	03-22-86
1.2	9.5	343333	739	13	03-22-86
1.2	9.6	223333	572	21	03-26-86
2.0	9.5	1333333	572	15	03-26-86
0.0	9.6	916667	639	25	03-26-86
1.1	9.7	833333	639	21	03-26-86
1.4	9.6	1000000	639	26	03-26-86
0.0	10.1	2383334	674	22	04-01-86
0.0	11.1	2383334	674	54	04-01-86
0.9	9.5	2400000	674	22	04-01-86
1.0	9.8	2366667	674	25	04-01-86
0.0	7.0	1456667	736	7	04-01-86
0.0	9.6	1456667	736	12	04-01-86
0.7	8.6	813333	736	17	04-01-86
0.8	7.1	2100000	736	15	04-01-86

MB: methylene blue (mg/L)

pH: pH value

INFLUENT: coliform density/100 mL

SUN: sunlight intensity (W/sq.m)

NTU: turbidity (NTU)

with a Bausch and Lomb Spectronic 1001 Spectrophotometer. Absorbance due to turbidity was adjusted by subtracting the absorbance of samples with same turbidity measurements but without methylene blue.

LABORATORY SCALE STUDIES: SENSITIZERS

Laboratory scale studies were conducted to evaluate 69 different dyes, stains, and indicators as potential photosensitizing agents for the degradation of bromacil. The effects of sensitizer, pH value, and dissolved oxygen were determined.

Experimental Procedures

Water from a type 1 reagent-grade water system was used. A stock 0.00383 M (1 g/L) bromacil solution in ethanol was used to prepare bromacil standards and samples. A stock 1 g/L dye solution in water or ethanol (if solubility posed a problem) was used to prepare 10 mg/L dye concentrations. The effects of pH and buffer system on ionization of bromacil were determined by liquid-liquid extraction of 15 mg/L solutions of bromacil adjusted to pH values from 3 to 11 with acetate, diphosphate, and carbonate buffers. Quantitation was by comparison of peak area of the sample chromatogram with chromatograms of standards prepared in CH_2Cl_2 .

The effects of pH value and different dyes on reaction rates were determined by testing 69 potential photosensitizing agents at pH values of 4, 7, and 10. Samples and standards at pH 4 were buffered with 0.05 M $\text{CH}_3\text{CO}_2\text{H}$ and 0.01 M $\text{CH}_3\text{CO}_2\text{Na}$, at pH 7 with

0.0194 M NaH_2PO_4 and 0.0194 M Na_2HPO_4 , and at pH 10 with 0.0249 M NaHCO_3 and 0.0249 M Na_2CO_3 . Solutions were aerated prior to sunlight exposure to assure an initial saturation of dissolved oxygen. All experiments were conducted outdoors in sunlight. Solutions of dyes and bromacil were placed outdoors in Pyrex or Klimax vessels. No covering was placed on the sides or the bottoms of the vessels.

Samples were collected at selected time intervals and stored in the dark at 4 C until CH_2Cl_2 extractions were performed. Quantitation was by comparison of peak area of the sample chromatogram with chromatograms of standards. Dyes, which were found to be effective sensitizers, were also tested under reduced conditions by adding 126 mg/L Na_2SO_3 to the samples, omitting aeration, and following the above described procedure.

Materials

1. Bromacil [5-bromo-3-sec-butyl-6-methyl-uracil] was obtained as DuPont technical bromacil, a wettable powder containing 95% bromacil. The bromacil concentration was verified against bromacil reference standards obtained from EPA and was found to agree with the manufacturer's claim.

2. Textile dyes were obtained from TRICON COLORS INCORPORATED. Non-textile dyes and stains were obtained from chemical and biological supply companies. A list of the dyes, stains, and indicators tested is presented in Table 6.

Analytical

Quantitative analysis of the bromacil solutions involved

Table 6. Dyes, stains, and indicators tested in laboratory scale bromacil studies.

Common Name	Color Index Name	Source*
Acetone		CBS
Acid Red WA		TDM
Acridine Orange		CBS
Acridine Yellow		CBS
Alizarin Cyanine Green GN	Acid Green 25	TDM
Alizarine Red		CBS
Alizarol Cyanine RC		CBS
Alphazurine FGND	Acid Blue 9	TDM
Aniline Blue	Acid Blue 93	CBS
Auromine O Conc. 130%	Basic Yellow 2	TDM
Azorubin Extra Conc. 133%	Acid Red 14	TDM
Bismark Brown 53	Basic Brown 4	TDM
Brilliant Yellow	Direct Yellow 4	CBS
Bromocresol Green		CBS
Bromocresol Purple		CBS
Bromophenol Blue		CBS
Bromothymol Blue		CBS
Carmin (Alum Lake)		CBS
Chrysoidin 3R	Basic Orange 1	TDM
Congo Red		CBS
Cresol Red		CBS
Crystal Violet		CBS
Curcumin		CBS
Diazophenyl Blue		CBS
Dichlorofluorescein		CBS
Eosin Y	Acid Red 87	CBS
Erie Congo 4B (Congo Red)	Direct Red 28	TDM
Erie Fast B Conc.	Direct Brown 31	TDM
Erie Fast Orange A	Direct Orange 26	TDM
Erie Green WT	Direct Green 1	TDM
Eriochrome Black T		CBS
Ethyl Orange		CBS
Ethyl Red		CBS
Evans Blue		CBS
Fast Green FCF		CBS
Fluorescein	Acid Yellow 73	CBS
Fuchsin Acid	Acid Violet 19	CBS
Gentian Violet		CBS
Grand Yellow		CBS
Malachite Green	Basic Green 4	CBS
Marshall's Reagent		CBS
Methyl Red		CBS
Methyl Violet	Basic Violet 1	CBS
Methyl Violet 2B	Basic Violet 1	TDM
Methylene Blue (Fisher)	Basic Blue 9	CBS
Methylene Blue (Tricon)	Basic Blue 9	TDM
Methylene Blue 2B Powder	Basic Blue 9	TDM
Neutral Red	Basic Red 5	CBS
Nigrosin	Acid Black 2	CBS

Table 6 (cont.). Dyes, stains, and indicators tested in laboratory scale bromacil studies.

Common Name	Color Index Name	Source*
Phenol Red		CBS
Phloxine B	Acid Red 92	CBS
Propyl Red		CBS
Resorcine Brown R 167%	Acid Orange 24	TDM
Rhodamine B		CBS
Rhodamine WT		CBS
Rhodamine B Conc. 500%	Basic Violet 10	TDM
Rose Bengal	Acid Red 94	CBS
Safranin O		CBS
Solantine Turquoise G	Direct Blue 86	TDM
Solantine Yellow 4GL	Direct Yellow 44	TDM
Tartrazine 118%	Acid Yellow 23	TDM
Thionin 1%		CBS
Thymol Blue		CBS
Thyodene		CBS
Victoria Green WB	Basic Green 4	TDM
Victoria Pure Blue BO	Basic Blue 7	TDM
Wool Orange A	Mordant Orange 6	TDM
Wool Violet 4BN	Acid Violet 49	TDM
Wrights Stain		CBS

*

CBS = chemical and biological supply company
TDM = textile dye manufacturer

liquid-liquid extraction of the compounds from aqueous solution followed by gas chromatographic measurement using a flame ionization detector. The pH value of all samples was adjusted to below 4.0 with HNO_3 to ensure that the bromacil in solution was not ionized, and therefore, extractable in methylene chloride (Eisenberg et al, 1986). Methylene chloride (CH_2Cl_2) was used to extract the bromacil from the water samples. A 10 mL portion of CH_2Cl_2 was added to 100 mL of sample in a 125 mL separatory funnel. The funnel was inverted, opened to release pressure and closed, agitated, and set aside to allow the CH_2Cl_2 and water phases to separate. The denser CH_2Cl_2 -bromacil phase was drawn off into a 40 mL beaker, Na_2SO_4 was added to absorb water, and the CH_2Cl_2 -bromacil phase was poured into 1 mL reaction vials and stored at 4 C. Standard bromacil solutions were prepared in the same manner using EPA reference bromacil.

The gas chromatograph was a Hewlett Packard HP 5880A with a Hewlett Packard HP 7673A automatic sampler and tray. A 61 cm x 2 mm (ID) glass column packed with a 1 percent SP-2250 on 80/100 Supelcoport was used. Operating conditions for the chromatograph were an injector port temperature of 250 C, an initial oven temperature of 160 C for 2 minutes, a program rate of 20 C/min, a detector temperature of 260 C, a nitrogen carrier gas flow of 30 mL/min, a hydrogen flow rate of 30 mL/min, and an air flow rate of 400 mL/min. A 2 uL sample was injected. Sunlight intensity and pH value were measured as described previously.

LABORATORY SCALE STUDY: LIGHT INTENSITY

A laboratory scale study was carried out to determine the

effect of light intensity, methylene blue and pH value on the degradation of bromacil.

Experimental Procedure

Water from a type 1 reagent-grade water system was used. A stock 0.00383 M (1g/L) bromacil solution in ethanol was used to prepare bromacil standards and samples. A stock 1.0 g/L methylene blue solution was used to prepare the 1.0 mg/L methylene blue solutions used in the experiment. The effects of light intensity and pH value on reaction rate were determined by testing 30 mg/L solutions of bromacil at pH values of 7, 8, 9, and 10, at light intensities of 61, 39, 19, and 0 W/sq.m, at methylene blue concentrations of 0 and 1 mg/L methylene blue, and at a 60 minute detention time (Table 7).

Samples at a pH value of 7 were buffered with 0.05 M KH_2PO_4 + 0.0291 M NaOH, at a pH value of 8 with 0.05 M KH_2PO_4 + 0.0461 M NaOH, at a pH value of 9 with 0.0125 M $\text{Na}_2\text{B}_4\text{O}_7$ + 0.0046 M HCl, and at a pH value of 10 with 0.0125 M $\text{Na}_2\text{B}_4\text{O}_7$ + 0.0107 M NaOH. Solutions were aerated prior to exposure to light to assure an initial saturation of dissolved oxygen. The experiment was conducted indoors using Duro Test VitaliteTM lamps as the light source. Duro Test VitaliteTM lamps are 91 percent corrected to the quality of natural sunlight.

The bromacil solutions were placed at distances from the lamps yielding light intensities of 61, 39, 19, and 0 W/sq.m. Sample volumes of 200 mL were placed in 250 mL Pyrex vessels. No covering was placed on the side or the bottoms of the vessels. Samples were collected at 0 and 60 min and stored in the dark at

Table 7. Initial setup to determine effect of light intensity on reaction rate for degradation of 30 mg/L solutions of bromacil.

SAMPLE	pH	MB	SUN	TIME
1	7.0	1.0	61	60
2	7.0	1.0	39	60
3	7.0	1.0	19	60
4	7.0	1.0	0	60
5	7.0	0.0	61	60
6	7.0	0.0	0	0
7	7.8	1.0	61	60
8	7.8	1.0	39	60
9	7.8	1.0	19	60
10	7.8	1.0	0	60
11	7.8	0.0	61	60
12	7.8	0.0	0	0
13	9.0	1.0	61	60
14	9.0	1.0	39	60
15	9.0	1.0	19	60
16	9.0	1.0	0	60
17	9.0	0.0	61	60
18	9.0	0.0	0	0
19	9.9	1.0	61	60
20	9.9	1.0	39	60
21	9.9	1.0	19	60
22	9.9	1.0	0	60
23	9.9	0.0	61	60
24	9.9	0.0	0	0

pH: pH value

MB: methylene blue (mg/L)

SUN: sunlight intensity (W/sq.m)

TIME: detention time (min)

4 C until the CH_2Cl_2 extractions were performed. Quantitation was by comparison of peak area of the sample chromatogram with chromatograms of standards.

Materials

1. Bromacil [5-bromo-3-sec-butyl-6-methyl-uracil] was obtained as DuPont technical bromacil, a wettable powder containing 95 percent bromacil.

2. Methylene blue was obtained from Fisher Chemical as a biological stain.

Analytical

Quantitative analysis of the bromacil solutions involved liquid-liquid extraction with methylene chloride followed by gas chromatographic measurement using a flame ionization detector as previously described. The gas chromatograph was a Hewlett Packard HP 5890 with a Hewlett Packard HP 7637A automatic sampler and tray. A 5 m x 0.53 mm (ID) fused silica capillary column with HP-1 (similar to OV-101) was used. Operating conditions for the chromatograph were an injector port temperature of 250 C, an initial oven temperature of 160 C for 2 minutes, a program rate of 20 C/min, a detector temperature of 260 C, a nitrogen carrier gas flow of 20 mL/min, a nitrogen auxiliary gas flow of 10 mL/min, a hydrogen flow of 30 mL/min, and an air flow of 400 mL/min. A 1 uL sample was injected. Light intensity and pH value were measured as described previously.

RESULTS

COLIFORM STUDIES

Hydraulic Characteristics

Samples which were collected near the surface of the reactors proved to be unrepresentative of the hydraulic characteristics of the reactors and were not used for data analysis. Samples collected at mid-depth of the reactors were used to determine the parameters described below. The results from the tracer studies were analyzed using:

T = theoretical detention time

t_i = time to initial indication of tracer

t_p = time to peak concentration of tracer

t_g = time to centroid of tracer curve

t_{10} , t_{50} , t_{90} = time to 10, 50, and 90 percent of tracer curve, respectively

t_i/T = index of short circuiting

t_p/T = index of modal detention time

t_g/T = index of average detention time

t_{50}/T = index of mean detention time

t_{90}/t_{10} = Morrill dispersion index

d = chemical engineering dispersion index from the variance of the tracer curve.

Excluding the chemical engineering dispersion index, the other indices may not be the most accurate indication of hydraulic performance since they reflect either single points or portions of the tracer curve. These indices are commonly used,

however, and are useful in comparing data with literature values. In contrast, the chemical engineering dispersion index includes all points on the tracer curve and is reported to have the strongest statistical probability of correctly describing the hydraulic performance of a reactor (Marske and Boyle, 1973).

The results of the tracer studies including the performance indices are shown in Figures 8-10. The mean, average, and modal detention time indices in the pilot plant reactors did not coincide, indicating that dispersion is not absent; however, the indices did not differ greatly from one another, suggesting that dispersion was not high. The mean, average, and modal detention time indices for both north and south pilot plant reactors were also within the range reported by Marske and Boyle (1973) for a reactor approximating plug flow conditions with a length to width ratio of 40:1. The Morril index and the dispersion index both decreased, and more closely approached plug flow with distance or time through the reactors. Although the indices did not indicate perfect plug flow conditions, they did indicate that the reactors approached plug flow more closely than most contact tanks currently in use (Deaner, undated).

Problems With Coliform Data

Initial coliform densities which were measured as "greater than" some upper value were excluded from all statistical analyses. Coliform densities at time t, which were either measured as "greater than" some upper value or which were associated with an initial coliform density with a "greater than" value, were likewise excluded from all statistical analyses. In

FLOW CURVE, SOUTH REACTOR

SAMPLE POINT AT $L/4 = 7.32$ m

FLOW CURVE, NORTH REACTOR

SAMPLE POINT AT $L/4 = 7.32$ m

Figure 8. Hydraulic performances at one-fourth distance.

FLOW CURVE, SOUTH REACTOR

SAMPLE POINT AT L/2 = 14.63 m

FLOW CURVE, NORTH REACTOR

SAMPLE POINT AT L/2 = 14.63 m

Figure 9. Hydraulic performances at one-half distance.

FLOW CURVE, SOUTH REACTOR

SAMPLE POINT AT L = 29.26 m

FLOW CURVE, NORTH REACTOR

SAMPLE POINT AT L = 29.26 m

Figure 10. Hydraulic performances at outlet.

the bench scale studies, a "greater than" value resulted in roughly 20 percent of the data being excluded. In the pilot plant studies, "greater than" values were not encountered.

Initial Conditions

The variability of the random effects of sunlight intensity influent coliform density, dissolved oxygen, temperature, suspended solids, and turbidity is presented in Tables 8 and 9.

Table 8. Variability of random initial pilot scale coliform parameters.

VAR	MEAN	STD DEV	MINIMUM	MAXIMUM
CO	1307318.0	1920164.0	33000.0	9466666.0
TURB	15.1	22.4	5.0	200.0
SUN	360.0	212.8	89.0	739.0
DO	5.2	3.1	1.0	8.4

Table 9. Variability of random initial bench scale coliform parameters.

VAR	MEAN	STD DEV	MINIMUM	MAXIMUM
CO	3095594.8	3131537.1	23000.0	11000000.0
SUN	572.9	226.7	55.0	930.0
DO	2.0	1.3	1.0	7.0
TEMP	22.4	2.1	15.0	25.0
SS	8.1	5.1	3.0	22.0

In the bench scale studies, sunlight intensity averaged 572 ± 226 W/sq.m with a range from 55 to 930. In the pilot scale studies, sunlight intensity averaged 360 ± 213 W/sq.m with a range from 89 to 739. The minimum sunlight intensities occurred predictably on overcast cloudy days. It is interesting to note that the minimum sunlight intensities recorded for the bench scale and pilot scale studies (55 and 89 W/sq.m, respectively) did not differ greatly

from the 61 W/sq.m maximum light intensity recorded with the Duro Test VitaliteTM lamps. In fact, the mean sunlight intensities recorded in the bench and pilot scale experiments were many times greater than the maximum light intensity of the laboratory lamps.

In the bench scale studies, the influent wastewater temperature averaged 22.4 ± 2.1 C with a range from 15 to 25. Low temperatures were predictably associated with cold winter days and high temperatures were associated with hot summer days. In the bench scale studies, suspended solids averaged 8.1 ± 5.1 mg/L with a range from 3 to 22. The suspended solids data were taken from the operating logs of the wastewater treatment plant and at best, these data are suspect. In the bench scale studies, dissolved oxygen averaged 2.0 ± 1.3 mg/L with a range from 1.0 to 7.0. In the pilot scale studies, dissolved oxygen averaged 5.2 ± 3.1 mg/L with a range from 1.0 to 8.4. It is suspected that the higher dissolved oxygen in the pilot scale studies was due to increased aeration from the higher pumping rate (i.e. 400 L/min for the pilot scale pump vs 75 L/min for the bench scale pump) and additional turbulence and aeration from the constant head and mixing tanks preceding the reactors.

The remaining parameters, i.e. influent coliform density and turbidity, reflect the performance or lack of performance of the wastewater treatment plant and secondary clarifier. In the bench scale studies, influent coliform densities averaged $3,095,594 \pm 3,131,537$ per 100 mL with a range from 23,000 to 11,000,000. No significant difference was observed between influent fecal and total coliform densities. In the pilot scale studies, influent

coliform densities averaged $1,307,318 \pm 1,920,164$ per 100 mL with a range from 33,000 to 9,466,666. In the pilot plant studies, influent turbidity averaged 15.1 ± 22.4 NTU with a range from 5.0 to greater than 200.

High influent turbidity and coliform were associated with both poor settling in the secondary clarifier and high suspended solids in both the effluent of the wastewater plant and alas, unfortunately, the influent to the pilot plant. On numerous occasions, the influent into the pilot plant reactors was so high in suspended solids that the reactors merely served as settling basins. The methylene blue was totally adsorbed onto the solids and removed from the system. Under these conditions, the operation of the pilot plant was suspended until a more favorable influent became available.

Linear Relationships Between Variables

Pearson correlation coefficients were calculated to measure what degree of linear relationship existed between initial coliform density, coliform density at time t , time, pH value, methylene blue concentration, sample depth, sunlight intensity, dissolved oxygen, temperature, suspended solids, and turbidity. In addition to the above linear relationships, Pearson correlation coefficients were also calculated for time versus C - C_0 , $\ln(C/C_0)$, and $(1/C_0) - (1/C)$ to determine what order reaction (i.e. zero, first, or second) best described the sensitized photooxidation of coliform. The rate equation $dC/dt = kC^n$, when solved for $n = 0, 1$ and 2 , have the following solutions for zero, first, and second order reactions:

$$C - C_0 = kt$$

$$\ln(C/C_0) = kt$$

$$(1/C_0) - (1/C) = kt.$$

Pearson correlation coefficients and alpha levels of significance are presented in Tables 10 and 11.

Dissolved oxygen was inversely related to influent coliform density, sample depth, temperature, and pH value. High values of dissolved oxygen were associated with low values of the above parameters, and conversely, low dissolved oxygen concentrations were associated with high values of the parameters. Correlation coefficients for dissolved oxygen were: with initial coliform density, -0.85203 and -0.45244 for the pilot and bench scale experiments, respectively; with pH value, -0.49250 and -0.43604 for the pilot and bench scale experiments, respectively; with sample depth, -0.38387 for the bench scale study; and with temperature, -0.90290 for the bench scale study. The above correlation coefficients were all significant at an alpha level of 0.0001.

The inverse relationships of the above parameters with dissolved oxygen were for the most part not surprising. Higher coliform densities exert higher oxygen demands. The saturation concentration of dissolved oxygen in water varies inversely with temperature. Dissolved oxygen concentrations are generally higher at the water surface than at subsurface depths. The inverse relationship with pH value is suspected to be due to higher photooxidation rates at higher pH values exerting higher oxygen demands.

In the pilot scale experiments, turbidity was directly

Table 10. Pearson correlation coefficients / probability > |R| under H:RHO=0 for pilot scale coliform experiments.

	CO	C	t	pH	MB	TURB	HT	SUN	DO	DC0	DC1	DC2
CO	1.00000 0.0000	0.39533 0.0001	-0.01110 0.7919	0.06297 0.1339	-0.09209 0.0282	0.15441 0.0002	0.04239 0.3133	-0.19486 0.0001	-0.85203 0.0001	-0.88132 0.0001	-0.18078 0.0001	0.03197 0.4470
C	0.39533 0.0001	1.00000 0.0000	-0.34370 0.0001	-0.27961 0.0001	-0.17032 0.0001	0.53978 0.0001	-0.09855 0.0188	-0.04773 0.2966	-0.23220 0.0567	0.08562 0.0414	0.31884 0.0001	0.03408 0.4175
t	-0.01110 0.7919	-0.34370 0.0001	1.00000 0.0000	0.37317 0.0001	0.23776 0.0001	-0.19947 0.0001	0.15224 0.0003	0.01770 0.6989	0.02232 0.8566	-0.16477 0.0001	-0.50980 0.0001	-0.09929 0.0179
pH	0.06297 0.1339	-0.27961 0.0001	0.37317 0.0001	1.00000 0.0000	0.23401 0.0001	0.00387 0.9268	0.21099 0.0001	-0.12729 0.0052	-0.49250 0.0001	-0.21214 0.0001	-0.56105 0.0001	-0.16737 0.0001
MB	-0.09209 0.0282	-0.17032 0.0001	0.23776 0.0001	0.23401 0.0001	1.00000 0.0000	-0.00671 0.8732	0.28704 0.0001	0.02386 0.6020	0.39951 0.0007	0.01228 0.7703	-0.39611 0.0001	-0.01111 0.7916
TURB	0.15441 0.0002	0.53978 0.0001	-0.19947 0.0001	0.00387 0.9268	-0.00671 0.8732	1.00000 0.0000	-0.10211 0.0149	-0.06286 0.1691	-0.18236 0.1366	0.11019 0.0086	0.06986 0.0963	-0.06805 0.1052
HT	0.04239 0.3133	-0.09855 0.0188	0.15224 0.0003	0.21099 0.0001	0.28704 0.0001	-0.10211 0.0149	1.00000 0.0000	-0.05403 0.2374	-0.04288 0.7284	-0.09667 0.0212	-0.16040 0.0001	-0.03225 0.4430
SUN	-0.19486 0.0001	-0.04773 0.2966	0.01770 0.6989	-0.12729 0.0052	0.02386 0.6020	-0.06286 0.1691	-0.05403 0.2374	1.00000 0.0000	0.02570 0.8352	0.18682 0.0001	0.08765 0.0550	0.10333 0.0236
DO	-0.85203 0.0001	-0.23220 0.0567	0.02232 0.8566	-0.49250 0.0001	0.39951 0.0007	-0.18236 0.1366	-0.04288 0.7284	0.02570 0.8352	1.00000 0.0000	0.76291 0.0001	0.45908 0.0001	0.09698 0.4314
DC0	-0.88132 0.0001	0.08562 0.0414	-0.16477 0.0001	-0.21214 0.0001	0.01228 0.7703	0.11019 0.0086	-0.09667 0.0212	0.18682 0.0001	0.76291 0.0001	1.00000 0.0000	0.36010 0.0001	-0.01715 0.6834
DC1	-0.18078 0.0001	0.31884 0.0001	-0.50980 0.0001	-0.56105 0.0001	-0.39611 0.0001	0.06986 0.0963	-0.16040 0.0001	0.08765 0.0550	0.45908 0.0001	0.36010 0.0001	1.00000 0.0000	0.31773 0.0001
DC2	0.03197 0.4470	0.03408 0.4175	-0.09929 0.0179	-0.16737 0.0001	-0.01111 0.7916	-0.06805 0.1052	-0.03225 0.4430	0.10333 0.0236	0.09698 0.4314	-0.01715 0.6834	0.31773 0.0001	1.00000 0.0000

Co = influent coliform density at t = 0
C = coliform density at time t
t = time
pH = pH value
MB = methylene blue
TURB = turbidity

HT = sample depth
SUN = sunlight intensity
DO = dissolved oxygen
DC0 = C-Co
DC1 = ln(C/Co)
DC2 = (1/Co)-(1/C)

Table 11. Pearson correlation coefficients / probability > |R| under H:RHO=0 for bench scale coliform experiments.

	CO	C	t	pH	MB	HT	SUN	DO	TEMP	SS	DCO	DC1	DC2
CO	1.00000 0.0000	0.41167 0.0001	-0.15657 0.0150	0.09375 0.1468	0.11040 0.0872	-0.17878 0.0054	0.01295 0.8414	-0.45244 0.0001	0.14333 0.0261	0.13736 0.0785	-0.72315 0.0001	-0.33271 0.0001	-0.01070 0.8687
C	0.41167 0.0001	1.00000 0.0000	-0.32465 0.0001	0.01168 0.8569	-0.01130 0.8614	-0.07449 0.2493	-0.02321 0.7199	-0.17603 0.0061	0.05412 0.4029	0.07102 0.3647	0.33176 0.0001	0.46780 0.0001	0.09652 0.1352
t	-0.15657 0.0150	-0.32465 0.0001	1.00000 0.0000	-0.22910 0.0003	-0.04009 0.5357	-0.19938 0.0019	0.13104 0.0421	0.25977 0.0001	-0.26059 0.0001	-0.04663 0.5520	-0.08397 0.1939	-0.29638 0.0001	-0.10943 0.0901
pH	0.09375 0.1468	0.01168 0.8569	-0.22910 0.0003	1.00000 0.0000	0.03069 0.6355	0.24388 0.0001	0.00630 0.9225	-0.43604 0.0001	0.39444 0.0001	-0.12735 0.1031	-0.08819 0.1724	-0.22643 0.0004	-0.05876 0.3637
MB	0.11040 0.0872	-0.01130 0.8614	-0.04009 0.5357	0.03069 0.6355	1.00000 0.0000	-0.10129 0.1168	0.00937 0.8850	-0.00067 0.9918	-0.08171 0.2062	-0.11522 0.1406	-0.12285 0.0569	-0.31002 0.0001	-0.10959 0.0896
HT	-0.17878 0.0054	-0.07449 0.2493	-0.19938 0.0019	0.24388 0.0001	-0.10129 0.1168	1.00000 0.0000	-0.13127 0.0417	-0.38387 0.0001	0.57415 0.0001	-0.09012 0.2497	0.12861 0.0461	0.02107 0.7449	-0.07061 0.2749
SUN	0.01295 0.8414	-0.02321 0.7199	0.13104 0.0421	0.00630 0.9225	0.00937 0.8850	-0.13127 0.0417	1.00000 0.0000	-0.27531 0.0001	0.18657 0.0037	-0.55759 0.0001	-0.03100 0.6320	-0.18247 0.0045	-0.10466 0.1051
DO	-0.45244 0.0001	-0.17603 0.0061	0.25977 0.0001	-0.43604 0.0001	-0.00067 0.9918	-0.38387 0.0001	-0.27531 0.0001	1.00000 0.0000	-0.90290 0.0001	0.08798 0.2611	0.33494 0.0001	0.25749 0.0001	0.06416 0.3212
TEMP	0.14333 0.0261	0.05412 0.4029	-0.26059 0.0001	0.39444 0.0001	-0.08171 0.2062	0.57415 0.0001	0.18657 0.0037	-0.90290 0.0001	1.00000 0.0000	-0.07934 0.3111	-0.10735 0.0964	-0.12253 0.0575	-0.07473 0.2478
SS	0.13736 0.0785	0.07102 0.3647	-0.04663 0.5520	-0.12735 0.1031	-0.11522 0.1406	-0.09012 0.2497	-0.55759 0.0001	0.08798 0.2611	-0.07934 0.3111	1.00000 0.0000	-0.07640 0.3294	0.07453 0.3414	0.03088 0.6938
DCO	-0.72315 0.0001	0.33176 0.0001	-0.08397 0.1939	-0.08819 0.1724	-0.12285 0.0569	0.12861 0.0461	-0.03100 0.6320	0.33494 0.0001	-0.10735 0.0964	-0.07640 0.3294	1.00000 0.0000	0.69895 0.0001	0.08423 0.1925
DC1	-0.33271 0.0001	0.46780 0.0001	-0.29638 0.0001	-0.22643 0.0004	-0.31002 0.0001	0.02107 0.7449	-0.18247 0.0045	0.25749 0.0001	-0.12253 0.0575	0.07453 0.3414	0.69895 0.0001	1.00000 0.0000	0.37196 0.0001
DC2	-0.01070 0.8687	0.09652 0.1352	-0.10943 0.0901	-0.05876 0.3637	-0.10959 0.0896	-0.07061 0.2749	-0.10466 0.1051	0.06416 0.3212	-0.07473 0.2478	0.03088 0.6938	0.08423 0.1925	0.37196 0.0001	1.00000 0.0000

Co = coliform density at time t = 0
C = coliform density at time t
t = time
pH = pH value
MB = methylene blue concentration
HT = sample depth
SUN = sunlight intensity

DO = dissolved oxygen
TEMP = temperature
SS = suspended solids
DCO = C-Co
DC1 = $\ln(C/Co)$
DC2 = $(1/Co)-(1/C)$

related to coliform density, which was not unusual since turbidity has been used to measure biomass concentration (Gaudy and Gaudy, 1980). The correlation coefficient for turbidity with coliform density was 0.53978, and the alpha level was significant at 0.0001.

Methylene blue appeared directly related to dissolved oxygen and sample depth in the pilot scale experiments but not in the bench scale experiments. The absence of a correlation between methylene blue with dissolved oxygen and sample depth in the bench scale studies is perhaps due to the fact that methylene blue concentrations were measured prior to running the experiments. In the pilot scale studies, methylene blue concentrations were measured at the time the sample was taken, and the concentrations recorded reflect the possible effects of bleaching, fading, adsorption to suspended solids, etc. The correlation coefficients for methylene blue with sample depth and dissolved oxygen for the pilot scale studies were 0.28704 and 0.39951, and the correlation coefficients were significant at the 0.0001 alpha level.

At low dissolved oxygen concentrations, methylene blue is reduced to a colorless or leuco-methylene blue, and with reaeration is returned back to its normal state. The higher methylene blue concentrations at lower depths may be due to bleaching of methylene blue at the water surfaces, adsorption onto colloidal material and subsequent settling out, and possible thermal gradients.

Coliform density was directly related to initial coliform

density and inversely related to time in both pilot and bench scale studies. Low coliform density was associated with low influent coliform density, and the density decreased with time. Correlation coefficients for coliform density at time t with initial coliform density were 0.39533 and 0.41167 for the pilot and bench scale studies, respectively. Correlation coefficients for coliform density with time for the pilot and bench scale studies were -0.34370 and -0.32465. The correlation coefficients were significant at an alpha level of 0.0001.

Correlation coefficients were highest in both pilot and bench scale studies for $\ln(C/C_0)$ versus time, and the reaction rate, dC/dt , followed first order kinetics better than zero or second order kinetics. Further evidence of a first order reaction will be presented shortly. The correlation coefficients for the pilot and bench scale studies were -0.50980 and -0.29638, and the correlation coefficients were significant at an alpha level of 0.0001.

Some correlations could not be explained. For example, sample depth appeared directly related to the influent wastewater temperature, i.e. the greater the temperature of the wastewater, the deeper the sample was taken. The correlation coefficient was 0.57415, and the correlation coefficient was significant at the 0.0001 alpha level. Suspended solids also appeared inversely related to sunlight intensity, i.e. the greater the sunlight intensity, the less the suspended solids. The correlation coefficient was -0.55759, and the correlation coefficient was significant at the 0.0001 alpha level. The variables in the above relationships are independent of one another, and the

correlations are coincidental, at best.

Individual Bench Scale and Pilot Scale Experiments

Results of the bench scale experiments are shown in Figures 11-24. The effect of pH value on sensitized photooxidation is shown in Figures 11, 13-16, and 23. Inactivation of coliform was most effective at pH values of close to 9.0 and above. Inactivation was not effective in the pH range from 7.0 to 8.0; however, a pH range from 7.0 to 8.0 has been reported effective in other studies (Acher and Rosenthal, 1977; Acher and Juven, 1977; Acher and Elgavish, 1980; Acher, 1985; Kraus, 1987).

The discrepancy in what is the effective pH range for methylene blue sensitized photooxidation (i.e. a pH range of 7.0 to 8.0 versus a pH range of 9.0 and above) merits discussion. If a pH range of 7.0 to 8.0 were effective for the methylene blue sensitized photooxidation of wastewater, then no pH adjustment would be required to treat municipal wastewater; however, if a pH value of close to 9.0 were required, then chemical addition (i.e. caustic soda, lime, etc.) would be needed.

Wallis and Melnick (1964) reported that vaccinia and other nonenteroviruses could be rendered irreversibly photosensitive only at pH values of 9.0 and above. Wallis and Melnick (1964) stated that enteroviruses, usually resistant to photodynamic inactivation, could be rendered completely photosensitive with proflavine at pH values of 9.0 to 10.0. Wallis et al (1965) reported that microbial floras (other than virus) from a large number of stool and throat specimens were found to be irreversibly photosensitized by proflavine at a pH value of 9.0.

TOTAL COLIFORM VS TIME, 12/3/83

SUN = 793 W/sq.m, DEPTH = 0 cm
METHYLENE BLUE = 2 mg/L

Figure 11. Effect of pH.

FECAL COLIFORM VS TIME, 7/25/84

SUN = 930 W/sq.m, DEPTH = 60 cm
pH = 8.9

Figure 12. Effect of mixing regime.

FECAL COLIFORM VS TIME, 8/5/84

SUN = 803 W/sq.m

Figure 13. Effect of mixing, pH, and methylene blue.

FECAL COLIFORM VS TIME, 8/10/84

SUN = 882 W/sq.m, DEPTH = 60 cm

REACTORS MIXED INITIALLY 5 min

Figure 14. Effect of methylene blue and pH.

FECAL COLIFORM VS TIME, 8/26/84 SUN = 642 W/sq.m, DEPTH = 60 cm METHYLENE BLUE = 1 mg/L

Figure 16. Effect of mixing regime and pH.

FECAL COLIFORM VS TIME, 8/19/84 SUN = 485 W/sq.m, DEPTH = 60 cm

Figure 15. Effect of methylene blue and pH.

TOTAL COLIFORM VS TIME, 9/7/84
 SUN = 738 W/sq.m, DEPTH = 0 cm
 pH = 8.9, SETTLE

Figure 17. Effect of methylene blue and depth.

TOTAL COLIFORM VS TIME, 9/7/84
 SUN = 738 W/sq.m, DEPTH = 60 cm
 pH = 8.9, SETTLE

Figure 18. Effect of methylene blue and depth.

TOTAL COLIFORM VS TIME, 9/7/84
 SUN = 551 W/sq.m, DEPTH = 0 cm
 SETTLE, pH = 8.9

Figure 19. Effect of methylene blue and depth.

TOTAL COLIFORM VS TIME, 9/7/84
 SUN = 551 W/sq.m, DEPTH = 60 cm
 SETTLE, pH = 8.9

Figure 20. Effect of methylene blue and depth.

TOTAL COLIFORM VS TIME, 9/13/84
 SUN = 749 W/sq.m, DEPTH = 60 cm
 pH = 8.9, POOR SETTLING

Figure 21. Effect of methylene blue and poor settling.

TOTAL COLIFORM VS TIME, 9/13/84
 SUN = 615 W/sq.m, DEPTH = 60 cm
 pH = 8.9

Figure 22. Effect of methylene blue.

TOTAL COLIFORM VS TIME, 9/27/84

SUN = 120 W/sq.m, DEPTH = 60 cm

Figure 23. Effect of settling, methylene blue and pH.

TOTAL COLIFORM VS TIME, 9/23/84

SUN = 359 W/sq.m, DEPTH = 60 cm

METHYLENE BLUE = 1 mg/L, pH = 9.0

Figure 24. Effect of pH adjustment chemical.

Gerba et al (1977) reported that the photosensitization of coliforms and poliovirus by methylene blue appeared to require a pH value of at least 9.0, but that the greatest effect on sensitization occurred between pH values of 9.5 and 10.0.

The sensitized photooxidation studies which were performed in a pH range of 7.0 to 8.0 for sensitized photooxidation were laboratory studies and were conducted under controlled conditions in small reactors less than 1 L in size. Results in full scale operating systems often differ from laboratory results. The bench scale studies and the pilot scale studies reported here were carried out in 208 L batch reactors and 24,000 L continuous flow reactors, and were subject to the daily and hourly variations of a small rural wastewater treatment plant.

The effect of methylene blue on the sensitized photooxidation of coliform is shown in Figures 12-15 and 17-23. A reduction in coliform density occurred at pH values ≥ 8.8 , even in the absence of methylene blue. The reduction was due to the disinfective properties of ultraviolet radiation from sunlight and to the improved settling and removal of suspended matter at higher pH values.

The addition of methylene blue to the wastewater (pH value ≥ 8.8) resulted in significant increases in the removal of coliform, sometimes greater than 5 logs. The rate of inactivation appeared to occur in two phases, a sharp initial drop of up to 4.5 logs in the first 5 min followed by a gradual drop over the remaining detention time. Inactivation rates in the first phase increased with an increase in methylene blue

concentration; whereas, rates in the second phase appeared somewhat independent of the sensitizer concentration. A minimum concentration of methylene blue was required for effective inactivation rates; however, concentrations greater than this minimum value did not significantly improve the rate. After a two hour exposure time at a pH value > 8.8 and a methylene blue concentration of 1.0 mg/L, influent coliform densities > 10^6 /100 mL were consistently reduced to < 100 coliform/100 mL and in some instances to < 10 coliform/100 mL.

The effect of mixing on the inactivation of coliform is shown in Figures 12, 13, 16, and 23. Three mixing patterns were investigated: continuous mixing at 30, 60, and 90 rpm; mixing for 5 min prior to sampling to resuspend settleable solids; and an initial mixing for 5 min followed by settling for the duration of the experiment. When the reactors were stirred just prior to taking a sample, inactivation rates were lowest due to resuspension of coliforms which had settled to the bottom of the reactors. Inactivation rates were highest for initial mixing followed by settling. Inactivation rates for continuous mixing were between the above described mixing schemes. While mixing did not appear to affect the dissolved oxygen concentration, it did ensure that all the initial coliform density was exposed for photooxidation.

The effect of depth on sensitized photooxidation is shown in Figures 17-20. Coliform densities at a 60 cm depth were generally within one log of the coliform densities at the water surface, and at times no significant difference was observed. The wavelength of maximum absorption for methylene blue has been

reported to penetrate clarified wastewater with 15 FTU turbidity to depths of 50 mm with greater than 80 percent transmission (Gerba et al, 1977).

The effect of poor settling and turbidity on the inactivation of coliform is shown in Figures 21 and 22. Final coliform density for a poorly clarified influent was about 3 logs greater than the final coliform density for a properly clarified influent, even though the sunlight intensity for the properly clarified influent was 134 W/sq.m less than the light intensity for the poorly clarified effluent. The importance of a properly clarified feedwater into a sensitized photooxidation treatment process cannot be overstated. Effective inactivation rates, high removals, and low effluent coliform density do not occur with a feedwater high in suspended solids and turbidity. The effect of the pH adjustment chemical on the inactivation of coliform is shown in Figure 24. Best results were obtained with lime due to the improvement in sedimentation and removal of suspended solids.

Some results of the pilot scale experiments are shown in Figures 25-38. The methylene blue concentrations and pH values listed in the figures are the averages of the samples taken at time = 0, 30, 60, 90 and 120 min. Higher methylene blue concentrations and pH values were sometimes observed at both mid-depth and the inlet and outlet of the reactors. These occurrences were associated with warmer air and wastewater temperatures and higher sunlight intensities. The results were unexpected since the hydraulic studies indicated no signs of density or temperature gradients in the reactors. The hydraulic

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 137 W/sq.m
DECEMBER 16, 1985

Figure 25. Pilot plant study, 12/16/85.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 342 W/sq.m, DO > 8.0 mg/L
DECEMBER 16, 1985

Figure 26. Pilot plant study, 12/16/85.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 221 W/sq.m
JANUARY 11, 1986

Figure 27. Pilot plant study, 1/11/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 463 W/sq.m
JANUARY 11, 1986

Figure 28. Pilot plant study, 1/11/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 269 W/sq.m
JANUARY 15, 1986

Figure 29. Pilot plant study, 1/15/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 406 W/sq.m
JANUARY 15, 1986

Figure 30. Pilot plant study, 1/15/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 134 W/sq.m
JANUARY 16, 1986

Figure 31. Pilot plant study, 1/16/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 374 W/sq.m
JANUARY 16, 1986

Figure 32. Pilot plant study, 1/16/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 463 W/sq.m
MARCH 22, 1986

Figure 33. Pilot plant study, 3/22/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 739 W/sq.m
MARCH 22, 1986

Figure 34. Pilot plant study, 3/22/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 572 W/sq.m
MARCH 26, 1986

Figure 35. Pilot plant study, 3/26/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 639 W/sq.m
MARCH 26, 1986

Figure 36. Pilot plant study, 3/26/86.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 124 W/sq.m
NOVEMBER 19, 1985

Figure 37. Pilot plant study, 11/19/85.

COLIFORM VS TIME: PILOT PLANT STUDIES

SUN = 130 W/sq.m
NOVEMBER 30, 1985

Figure 38. Pilot plant study, 11/30/85.

studies, however, were performed with a slug dose of Rhodamine WT; whereas, the pilot plant studies were carried out with a continuous input of sensitizer.

It is suspected that methylene blue increased the heat absorption capacity of water, and that with higher sunlight intensity and higher ambient and water temperatures, the effect became even more pronounced. A study is in progress to determine the extent of temperature and density gradients in the pilot scale reactors; if necessary, the baffling system in the reactors will be changed. It is suspected that heat losses and gains in a lagoon system would be less than in an above ground reactor system due to the insulating properties of the ground.

Coliform removals were more sensitive to methylene blue concentration, pH value, sunlight intensity and depth in the pilot plant studies than in the bench scale studies. The effect of pH value on sensitized photooxidation is shown in Figures 27, 28, 35 and 36. Inactivation of coliform was again most effective at pH values close to 9.0 and above. At lower pH values, inactivation was not as effective. At methylene blue concentrations of 1 mg/L, coliform removal was increased by 3 logs when the pH value was raised 1 unit.

The effect of methylene blue concentration on coliform removal is shown in Figures 27, 28, 30-34, 35, and 37. The addition of methylene blue again resulted in significant increases in the removal of coliform; however, the removals were not as dramatic as in the bench scale studies. After a two hour exposure time at pH values ≥ 8.8 and methylene blue concentrations of 1.0 mg/L, influent coliform densities $> 10^6/100$

mL were reduced to < 500 coliform/100 mL.

The effect of depth on sensitized photooxidation in the pilot plant studies was similar to those in the bench scale studies. Removals were generally higher at the surface than at mid-depth; however, the differences in removals between the two depths were generally within 1 log unit.

The effects of sunlight intensity on coliform removal are shown in Figures 25 and 26. An increase in sunlight intensity from 137 W/sq.m to 342 W/sq.m resulted in a > 2 log increase in coliform removal; however, at higher light intensities, the effect of an increase in sunlight did not appear to significantly improve the inactivation of coliform. A minimum of sunlight is required for effective removal rates. Coliform removal in control samples (i.e. dark bottles at the same pH value and methylene blue concentration as the reactors) was significantly less than coliform removal in the reactors. It should be noted, however, that significant reductions were observed in the dark controls at methylene blue concentrations > 5 mg/L.

Gerba et al (1977) reported that the amount of 670 nm light from sunlight was many times that available from an artificial light source and that large reductions of poliovirus in both turbid wastewater and tapwater were easily accomplished, even under heavily overcast skies. Eisenberg et al (1985) also stated that while no doubt a minimum value of light intensity was necessary to initiate the sensitized photooxidation reaction, the light intensities available from sunlight appear to be above this minimum value.

Grouped Bench Scale and Pilot Scale Experiments

Summaries of coliform removals for the bench and pilot scale studies are presented in Tables 12 and 13. In the bench scale studies, coliform removals ranged from 99.042 to 99.999 percent for pH values ≥ 8.8 , methylene blue concentrations ≥ 0.25 mg/L, and detention times of 2 hr. In the pilot scale studies, coliform removals ranged from 83.383 to 99.999 percent for pH values ≥ 8.8 , methylene blue concentrations ≥ 0.3 mg/L, and detention times of 2 hr. The poorer removals in the pilot scale studies were suspected to be due to short circuiting in the reactors and occasional deterioration in feedwater quality.

Model Development

First order reaction rate constants were determined for the pilot plant data by modeling $\ln(C/C_0)$ versus time by the method of least squares. The data were grouped into 3 sets and the reaction rate constants were determined for fixed ranges of methylene blue concentrations, fixed ranges of pH values, and fixed ranges of methylene blue concentration paired with pH value.

For the data grouped by ranges of methylene blue concentrations (Table 14), the first order reaction rate constant was predictably lowest at methylene blue concentrations of 0.0 mg/L ($k = 0.0279/\text{min}$) and highest at methylene blue concentrations > 1.0 mg/L ($k = 0.0642/\text{min}$). The coefficient of determination (r^2) ranged from 0.6443 to 0.8540 and was significant at an alpha level of 0.0001.

For the data grouped by ranges of pH values (Table 15), the

ble 12. Results of coliform disinfection (pilot scale studies).

HDT = 0.0 min (influent)										
MB	<----pH----->		<---NTU--->		<-----DO----->		<----SUN----->		<-----COLIFORM----->	
	range	med	range	med	range	med	range	med	range	med
0.0	7.0-8.6	7.6	1-200	35	2.2-8.2	4.9	89-739	341	33000-9466667	1436430
HDT = 30.0 min										
HT	<----MB----->		<-----pH----->		<---NTU--->		<-----COLIFORM----->		<-----PERCENT REMOVAL----->	
	range	med	range	med	range	med	range	med	range	med
0	0.0-0.0	0.0	7.1- 7.9	7.5	5-10	6.3	183333-2466667	627291	0.0000-86.2500	38.2260
45		0.0		7.6	4- 8	6.6	55667- 623333	323133	0.9708-65.2081	49.1657
0		0.0	8.0- 8.5	8.4	5- 7	6.0	203333- 710000	456666	61.2727-75.6000	68.4364
0		0.0	8.6- 8.9	8.7	6-20	13.6	3867- 323333	131650	35.7616-97.3634	71.8288
45		0.0		8.9		2.0		7400000		0.0000
0		0.0	9.0- 9.3	9.1	4-33	15.2	240000-1900000	6580000	0.0000-71.2121	29.6842
45		0.0		9.1	2- 7	5.3	26000-5700000	1130380	0.0000-92.0000	65.1146
0		0.0	9.4- 9.9	9.6	12-42	23.1	33333-1433333	446944	1.6019-87.2613	61.4172
0		0.0	10.0-11.1	10.6	21-51	36.0	5400- 193333	99366	91.8881-99.7734	95.8308
0	0.1-0.5	0.3	7.1- 7.9	7.5	1-14	7.6	41667- 840000	325666	45.8331-86.9791	69.8691
45		0.3	8.6- 8.9	8.9		10.0		266667		39.8495
0		0.3	9.0- 9.3	9.1	2-18	8.4	553- 653333	151160	0.0000-99.9136	81.7107
45		0.3		9.1	5-13	7.6	2233- 273333	72176	78.7378-99.6511	90.7645
0		0.5	9.4- 9.9	9.4		8.0		47333		99.5000
45		0.5		9.5		5.0		710		99.9746
0	0.5-0.9	0.8	7.1- 7.9	7.8	10-14	11.3	300000- 550000	415555	0.0000-70.0000	27.8205
45		0.8		7.5	9-14	11.7	236667-2400000	748333	0.0000-82.7273	34.5281
0		0.9	8.0- 8.5	8.4		15.0		276667		46.1037
45		0.8		8.4	11-13	12.0	7100- 363333	185216	29.2208-78.4848	53.8528
0		0.7	8.6- 8.9	8.8	9-25	17.4	7506- 430000	202929	14.2857-97.1831	63.4887
45		0.8		8.8	7-26	15.0	800-1466667	344852	0.0000-99.4545	63.7627
0		0.8	9.0- 9.3	9.0	8-21	14.4	100- 403333	149753	57.7318-99.9964	78.0464
45		0.7		9.0	7-15	11.0	9933- 463333	273311	86.9182-96.9279	92.2753
45		0.7	9.4- 9.9	9.5	11-20	15.0	13333- 153333	64999	70.8862-99.4366	88.9743
0	1.0-1.9	1.1	7.1- 7.9	7.5	9-10	9.5	330000- 636667	483333	30.2816-51.0256	40.6536
0		1.1	8.0- 8.5	8.2	6- 9	7.5	2367- 17333	9850	95.2295-99.3545	97.2919
0		1.1	8.6- 8.9	8.8	14-23	18.5	2533- 120000	61266	57.6470-99.4968	78.5718
45		1.4		8.7	14-99	32.5	4133-5800000	1012111	0.0000-98.8728	71.0394
45		1.2	9.0- 9.3	9.1	14-40	24.0	3467- 386667	138267	95.0993-99.0458	96.6869
45		1.3	9.4- 9.9	9.6	14-15	14.5	17333- 133333	75333	77.7778-92.2389	85.0084
45	2.0-5.0	2.3		9.4		16.0		24667		98.1499

Table 12 (cont.). Results of coliform disinfection (pilot scale studies).

HDT = 60.0 min

HT	<----MB----->		<-----pH----->		<---NTU--->		<-----COLIFORM----->		<-----PERCENT REMOVAL----->	
	range	med	range	med	range	med	range	med	range	med
0	0.0-0.0	0.0	7.1- 7.9	7.5	4- 7	5.7	54667-233333	481619	0.0000-87.5000	62.0165
0		0.0	8.0- 8.5	8.3	5- 8	6.5	14667- 496667	255667	72.9091-98.2400	85.5745
0		0.0	8.6- 8.9	8.7	13-31	17.7	7333- 320000	139722	32.3943-98.2678	68.4181
45		0.0		8.8	5- 6	5.5	115006- 433333	274169	93.4343-98.4459	95.9401
0	0.0	0.0	9.0- 9.3	9.1	5-19	9.1	110000-1100000	443333	0.0000-83.3333	51.1943
45		0.0		9.0	3-12	6.2	59000- 575000	180555	0.0000-98.1569	69.2895
0		0.0	9.4- 9.9	9.6	12-46	24.0	27000- 563333	174222	61.3276-97.5909	81.4449
0		0.0	10.0-11.1	10.6	20-51	35.5	4667- 56333	30500	97.6364-99.8042	98.7203
0	0.1-0.5	0.3	7.1- 7.9	7.6	3-10	6.8	76667- 490000	295000	41.8033-79.5833	63.0103
0		0.2	8.6- 8.9	8.8	8-21	14.3	2100- 286667	104366	35.3382-99.2588	75.8593
45		0.3		8.8	8-20	12.0	4567- 243333	163744	45.1128-97.1456	62.4571
0		0.3	9.0- 9.3	9.1	2-18	7.3	250- 165000	57909	62.7819-99.9974	89.1863
45	0.3	0.3		9.1	3- 9	9.1	230- 46667	21275	92.3076-99.9820	97.4345
0		0.2	9.4- 9.9	9.4		7.0		4133		98.1494
45		0.2		9.4		9.0	61333- 69333	65333	79.8059-82.1360	80.9709
0		0.9	7.1- 7.9	7.6	8-15	10.5	60000- 770000	360833	21.4288-88.3117	62.9048
45	0.8	0.8		7.5	8-14	10.7	233333- 843333	478888	16.6668-80.3636	52.2908
0		0.7	8.0- 8.5	8.4	9-16	12.3	5067- 270000	104244	22.1155-88.2291	64.9967
45		0.7		8.4	8-17	12.5	3167- 313333	154041	21.1540-91.7709	60.5722
0		0.7	8.6- 8.9	8.8	12-15	13.3	2000- 57333	21711	86.3493-99.7549	94.9135
45	0.8	0.8		8.7	11-25	16.8	517- 650000	187921	20.0819-99.6475	71.4325
0		0.7	9.0- 9.3	9.1	10-33	17.0	2300- 460000	148966	87.2956-99.5988	94.0166
45		0.7		9.2	6-16	10.7	47- 293333	133441	88.0555-99.9983	93.6684
0		0.7	9.4- 9.9	9.6	7- 8	7.5	67- 42000	21033	93.0000-99.9976	96.9498
45	0.7	0.7		9.5	12-19	14.8	4267- 50333	16991	90.4431-99.8155	97.1880
0		1.0	7.1- 7.9	7.6	8-15	11.3	296667- 510000	376666	37.3283-77.9546	58.6825
45		1.1		7.7	9-11	10.0	213333- 466667	340000	54.9296-64.1025	59.5161
0		1.0	8.0- 8.5	8.0		11.0		4567		98.7430
45	1.0	1.0		8.3		18.0		35667		97.5682
0		1.3	8.6- 8.9	8.7	12-16	14.0	967- 11000	4666	96.7327-99.8088	98.6620
45		1.3		8.8	15-26	18.8	667- 54000	18233	83.9604-99.8675	95.3997
45		1.2	9.4- 9.9	9.5	11-21	15.0	4733- 44667	18422	92.5555-98.2912	96.2425
45	2.0-5.0	2.4		9.6		15.0	397- 750	573	99.9392-99.9437	99.9415

ble 12 (cont). Results of coliform disinfection (pilot scale studies).

HDT = 90.0 min

HT	<----MB----->		<-----pH----->		<---NTU--->		<-----COLIFORM----->		<-----PERCENT REMOVAL----->	
	range	med	range	med	range	med	range	med	range	med
0	0.0-0.0	0.0	7.1- 7.9	7.5	3- 6	4.9	39333-2200000	433571	0.0000-96.3077	70.6845
0		0.0	8.0- 8.5	8.3		7.0		310000		83.0909
0		0.0	8.6- 8.9	8.8	6-30	15.6	5333- 203333	67999	59.6027-98.7402	85.0728
45		0.0		8.9		1.0		37000		89.8165
0		0.0	9.0- 9.3	9.2	3-42	16.0	27000- 693333	272933	0.0000-97.6591	70.1904
45		0.0		9.1	2- 7	4.9	4233- 185000	75699	72.0813-99.1802	93.9578
0		0.0	9.4- 9.9	9.6	4-24	14.5	38667- 233333	126833	80.0004-89.3577	85.3464
0		0.0	10.0-11.1	10.5	20-54	37.0	3800- 53667	28733	97.7482-99.8406	98.7944
0	0.1-0.5	0.2	7.1- 7.9	7.5	2-10	6.0	32333- 600000	267083	71.4286-88.7500	80.8259
0		0.4	8.0- 8.5	8.2		12.0		350000		56.9672
0		0.3	8.6- 8.9	8.9	6-21	16.0	533- 55000	28066	91.5816-99.9070	95.0075
45		0.2		8.9		2.0		24000		99.6604
0		0.3	9.0- 9.3	9.2	2-29	9.2	27- 72000	23997	79.0291-99.9990	95.0455
45		0.3		9.1	4-20	9.1	150- 57000	17661	83.3980-99.9820	95.6034
0		0.3	9.4- 9.9	9.6		9.0		453		99.9456
45		0.1		9.4		7.0		52333		84.7574
0	0.6-0.9	0.8	7.1- 7.9	7.7		9.0		356667		80.5454
45		0.9		7.8	8- 9	8.5	226667- 253333	240000	19.0475-86.1818	52.6147
0		0.8	8.0- 8.5	8.2	7-15	11.0	2133- 41667	21900	85.1189-93.5364	89.3276
45		0.8		8.4	12-15	13.5	2733- 186667	94700	63.6363-91.7182	77.6772
0		0.8	8.6- 8.9	8.8	6-23	12.4	397- 160000	40092	53.8462-99.7455	88.8358
45		0.8		8.7	8-25	14.9	247- 470000	83235	42.2131-99.8316	86.6633
0		0.7	9.0- 9.3	9.1	10-14	12.6	400- 346667	119786	90.9434-99.8763	96.5701
45		0.7		9.1	7-16	10.4	3933- 273333	109253	88.6111-99.8384	94.2279
0		0.7	9.4- 9.9	9.6	6-10	8.0	93- 1967	1030	99.1193-99.9990	99.5591
45		0.7		9.6	6-21	12.5	27- 48333	13715	90.8229-99.9990	97.5668
0	1.0-1.9	1.0	7.1- 7.9	7.9		10.0		253333		46.4789
45		1.1		7.3	8-15	11.5	390000- 816667	603333	61.1111-70.0000	65.5555
0		1.0	8.0- 8.5	8.0		8.0		40667		92.0778
45		1.0		8.3	12-15	13.5	17667- 193333	105500	59.1549-98.7954	78.9752
0		1.3	8.6- 8.9	8.8	11-14	12.8	240- 1333	696	99.9365-99.9523	99.8161
45		1.3		8.7	13-24	16.8	330- 42000	11724	85.1765-99.9221	95.9624
45		1.3	9.0- 9.3	9.1		20.0		360		99.9285
45		1.3	9.4- 9.9	9.6	12-14	13.3	523- 39000	11247	93.5000-99.8477	97.9046
0	2.0-5.0	2.0	9.0- 9.3	9.0		7.0		34000		99.4138
45		2.5	9.4- 9.9	9.6	15-18	16.5	370- 623	497	99.9434-99.9533	99.9483

Table 12 (cont). Results of coliform disinfection (pilot scale studies).

HDT = 120.0 min										
HT	<----MB----->		<-----pH----->		<---NTU--->		<-----COLIFORM----->		<-----PERCENT REMOVAL----->	
	range	med	range	med	range	med	range	med	range	med
0	0.0-0.0	0.0	7.1- 7.9	7.4	4- 7	5.7	5667- 146667	499111	0.0000-99.5641	63.5452
0		0.0	8.0- 8.5	8.2		7.0		1267		99.9309
0		0.0		8.5		15.0		5500		99.9257
0		0.0	8.6- 8.9	8.8	12-30	17.3	3667- 153333	61857	63.4921-99.1338	86.3062
0		0.0	9.0- 9.3	9.2	8-50	19.5	2000- 643333	203833	0.0000-99.6591	79.9744
45		0.0		9.1	2- 9	5.7	3000- 323333	85142	27.6121-99.1000	83.5757
0		0.0	9.4- 9.9	9.7	8-23	15.5	7000- 90000	52250	88.7356-97.9808	93.1433
0		0.0	10.0-11.1	10.6	24-32	28.0	3400- 33333	18366	98.6014-99.8573	99.2294
0	0.1-0.5	0.1	7.1- 7.9	7.2		10.0		456667		78.2540
0		0.3	8.0- 8.5	8.3	3-11	6.7	23- 256667	87974	68.4426-99.9303	88.7042
45		0.2		8.4		6.0		29000		91.5534
0		0.5	8.6-8.9	8.9		25.0		163		99.9716
45		0.4		8.7	3-23	13.0	10- 54333	27171	90.5233-99.9697	95.2465
0		0.3	9.0-9.3	9.1	2-18	6.9	103- 73667	19519	83.3834-99.9955	95.3330
45		0.3		9.1	2- 8	5.1	82- 52333	10846	84.7473-99.9872	97.3044
0	0.6-0.9	0.8	7.1- 7.9	7.7	7- 8	7.5	10667- 26667	18667	94.8051-99.4182	97.1117
45		0.7		7.5	7-12	9.7	2667- 566667	259778	25.0000-99.8545	65.9568
0		0.9	8.0- 8.5	8.0		10.0		203333		57.0422
45		0.8		8.3	13-17	15.3	59333- 310000	172000	61.8852-88.4416	73.1136
0		0.8	8.6- 8.9	8.8	10-17	13.2	187- 3533	2650	98.9901-99.8725	99.5235
45		0.7		8.8	7-20	13.2	40- 160000	35476	92.7779-99.9852	96.3613
0		0.7	9.0- 9.3	9.1	5-33	11.1	17- 253333	68274	81.7708-99.9998	95.7517
45		0.7		9.1	5-27	12.9	60- 216225	41088	87.1766-99.9993	95.8646
0		0.7	9.4- 9.9	9.5	11-17	14.0	667- 17000	8833	99.2917-99.9200	99.6058
45		0.7		9.5	6-20	11.6	23- 33000	7246	93.7342-99.9992	98.7105
0	1.0-1.9	1.0	7.1- 7.9	7.2		9.0		33000		97.4615
45		1.0		7.3		8.0		14333		98.8975
0		1.1	8.0- 8.5	8.1		7.0		3667		98.6904
0		1.3	8.6- 8.9	8.9	11-17	13.0	50- 523	285	99.8574-99.9901	99.9235
45		1.3		8.8	13-15	14.0	303- 25000	8580	92.5743-99.9284	97.4608
45		1.2	9.0- 9.3	9.0		18.0		210		99.9583
0		1.1	9.4- 9.9	9.5	8-12	10.0	1133- 1200	1166	99.4927-99.8000	99.6463
45		1.2		9.6	10-13	11.5	2567- 26333	14450	95.6117-98.8501	97.2309
45	2.0-5.0	2.0	8.6- 8.9	8.8		17.0		2633		99.2819
0		2.1	9.0- 9.3	9.1		21.0		22667		99.6092
0		3.3	9.4- 9.9	9.6	13-15	14.3	210- 21667	6341	98.3750-99.9679	99.4878
45		3.7		9.7	12-14	13.0	253- 2900	1152	99.7100-99.9536	99.8633

Table 13. Results of coliform disinfection (bench scale studies).

HDT	MB	pH	SUN	Influent Coliform	Effluent Coliform	% Removal
5	0.00	8.9 - 9.5	55 - 738	890,000 - 6,700,000	220,000 - 3,330,000	8.989 - 92.142
	0.25	8.8 - 8.9	551 - 615	6,300,000 - 6,700,000	70,000 - 430,000	93.582 - 98.889
	0.50	8.8 - 9.1	551 - 738	240,000 - 6,700,000	430 - 93,000	96.875 - 99.991
	1.00	8.8 - 9.2	104 - 803	240,000 - 6,700,700	23 - 64,000	85.667 - 99.990
	2.00	8.8 - 9.3	55 - 738	240,000 - 6,700,000	640 - 24,000	90.000 - 99.990
	5.00	8.9 - 9.2	551 - 738	4,000,000 - 6,700,000	180 - 7,500	96.875 - 99.996
30	0.00	8.9 - 9.2	55 - 615	890,000 - 6,300,000	530,000 - 6,300,000	34.921 - 40.449
	0.50	8.9 - 9.1	615 - 882	240,000 - 6,300,000	4,300 - 4,600	98.083 - 99.317
	1.00	8.8 - 9.1	272 - 882	46,000 - 11,000,000	590 - 46,000	97.978 - 99.950
	2.00	9.2 - 9.5	55 - 630	240,000 - 2,200,000	730 - 43,000	96.629 - 99.741
60	0.00	8.8 - 9.5	120 - 803	240,000 - 6,700,000	21,000 - 520,000	91.250 - 95.714
	0.25	8.8 - 8.9	551 - 738	4,400,000 - 6,700,000	3,400 - 63,000	99.060 - 99.923
	0.50	8.8 - 9.1	551 - 882	240,000 - 6,700,000	220 - 11,000	96.125 - 99.995
	1.00	8.8 - 9.1	370 - 930	23,000 - 11,000,000	3 - 9,300	98.130 - 99.999
	2.00	8.8 - 9.5	120 - 793	43,000 - 6,700,000	40 - 3,500	99.860 - 99.999
	5.00	8.8 - 8.9	551 - 738	240,000 - 6,700,000	40 - 2,300	99.041 - 99.999
120	0.00	8.8 - 8.9	551 - 738	4,400,000 - 6,700,000	150,000 - 430,000	90.227 - 95.671
	0.25	8.8 - 8.9	551 - 738	4,400,000 - 6,700,000	280 - 5,400	99.957 - 99.996
	0.50	8.8 - 9.1	551 - 882	240,000 - 6,700,000	10 - 430	99.821 - 99.997
	1.00	8.8 - 9.2	104 - 930	23,000 - 6,700,000	9 - 990	99.348 - 99.999
	2.00	8.8 - 9.4	485 - 738	43,000 - 6,700,000	10 - 2,300	99.042 - 99.999
	5.00	8.8 - 8.9	551 - 738	240,000 - 6,700,000	10 - 930	99.613 - 99.999

HDT = hydraulic detention time (min)

MB = methylene blue concentration (mg/L)

SUN = sunlight intensity (W/sq.m)

Coliform = coliform density/100 mL

Table 14. Summary of pilot scale order reaction rate constants ($dC/dt=-kC$) grouped by methylene blue concentrations.

ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
0	yes	0.0-0.0	0.0	7.0-11.1	8.4	167	0.6789	0.02793847
0	no	0.1-0.5	0.3	7.1-10.2	8.8	71	0.6457	0.04999339
0	no	0.6-1.0	0.8	7.1- 9.6	8.7	77	0.6908	0.04634450
0	no	1.1-4.6	1.7	7.3-10.4	9.0	26	0.8540	0.06420241
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
45	no	0.0-0.0	0.0	7.4- 9.3	8.8	37	0.7769	0.03252134
45	no	0.1-0.5	0.3	8.4-10.2	9.2	49	0.6851	0.05546479
45	no	0.6-1.0	0.8	7.1- 9.7	8.7	93	0.6443	0.04110890
45	no	1.1-4.6	1.6	7.1-10.4	9.1	48	0.8006	0.06036864

Table 15. Summary of pilot scale first order reaction rate constants ($dC/dt=-kC$) grouped by pH values.

ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
0	yes	0.0-1.1	0.2	7.0- 8.1	7.6	117	0.7144	0.02278653
0	yes	0.0-1.5	0.4	8.2- 8.7	8.5	44	0.7387	0.04319772
0	yes	0.0-2.0	0.4	8.8- 9.3	9.0	129	0.6835	0.04481559
0	yes	0.0-4.6	0.6	9.4-11.1	9.8	51	0.6910	0.05673280
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
45	yes	0.0-1.1	0.7	7.1- 8.0	7.6	25	0.5688	0.01920043
45	yes	0.0-1.7	0.9	8.2- 8.7	8.5	34	0.6724	0.03603415
45	yes	0.0-2.0	0.5	8.8- 9.3	9.0	121	0.7264	0.04530888
45	yes	0.1-4.6	1.3	9.4-10.4	9.6	47	0.7771	0.06958745

first order reaction rate constant was lowest in the pH range from 7.0 to 8.1 ($k = 0.0192/\text{min}$) and highest in the pH range ≥ 9.4 (0.06959). The coefficient of determination ranged from 0.5688 to 0.7771 and was significant at an alpha level of 0.0001.

For the data grouped by fixed ranges of pH values and fixed concentrations of methylene blue (Table 16), the reaction rate constant was lowest in the pH range from 7.1 to 7.4 and at methylene blue concentrations of 1.1 mg/L ($k = 0.0200/\text{min}$). The reaction rate constant was highest in a pH range from 9.4 to 10.4 and a methylene blue concentration from 0.2 to 0.5 mg/L. The coefficient of determination ranged from 0.5547 to 0.9671 and was significant at an alpha level of 0.0001. The lowest value of "k" was expected at 0.0 mg/L and not 1.1 mg/L methylene blue which suggested that the effect of methylene blue concentration at low pH values was not significant.

The data for methylene blue concentrations ≥ 8.8 mg/L and pH values ≥ 8.8 were fitted to the 25 equations shown in Table 17. The equations were linearized and the coefficients of the linear forms were determined by the method of least squares. The independent variable X was detention time, and the dependent variable Y was either C_0/C or $\ln(C_0/C)$. The results of the linearized equations with the highest coefficients of determination are presented in Table 18. The equations which yielded the best fits were $\ln(C/C_0) = -kt$ and $\ln(C/C_0) = -k_1t$.

The data for methylene blue concentrations > 0.0 mg/L were fitted to the 25 equations shown in Table 17. The equations

Table 16. Summary of pilot scale reaction rate constants ($dC/dt=-kC$) grouped by methylene blue concentrations & pH value.

ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
0	yes	0.0-0.0	0.0	7.0- 8.0	7.5	79	0.6539	0.02076459
0	no	0.1-0.5	0.2	7.1- 8.1	7.5	15	0.8244	0.02141581
0	no	0.7-1.0	0.9	7.1- 8.0	7.7	20	0.6991	0.02454222
0	no	1.1-1.1	1.1	7.3- 8.1	7.7	3	0.9370	0.03163008
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
45	no	0.0-0.0	0.0	7.4- 7.8	7.6	5	0.7897	0.02555321
45	no	0.6-1.0	0.8	7.1- 8.0	7.6	17	0.5547	0.02009628
45	no	1.1-1.1	1.1	7.1- 7.4	7.3	3	0.9671	0.01287017
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
0	yes	0.0-0.0	0.0	8.2- 8.7	8.5	20	0.7626	0.03858597
0	no	0.1-0.5	0.3	8.2- 8.7	8.4	4	0.5964	0.03409445
0	no	0.6-0.9	0.8	8.2- 8.7	8.6	14	0.8270	0.04290895
0	no	1.1-1.5	1.2	8.4- 8.7	8.6	6	0.8701	0.07405316
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
45	no	0.2-0.5	0.3	8.4- 8.7	8.6	3	0.7209	0.03591505
45	no	0.6-1.0	0.8	8.2- 8.7	8.5	23	0.6074	0.02827051
45	no	1.1-1.7	1.4	8.6- 8.7	8.6	7	0.8641	0.05876698
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
0	yes	0.0-0.0	0.0	8.8- 9.3	9.0	40	0.7239	0.02353985
0	no	0.1-0.5	0.3	8.8- 9.3	9.1	45	0.7118	0.0512387
0	no	0.6-1.0	0.7	8.8- 9.3	9.0	36	0.7343	0.05232051
0	no	1.1-4.6	1.6	8.8- 9.3	8.9	8	0.9622	0.06935062
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
45	no	0.0-0.0	0.0	8.8- 9.3	9.0	31	0.7776	0.03071144
45	no	0.1-0.5	0.3	8.8- 9.3	9.0	38	0.7717	0.05208002
45	no	0.6-1.0	0.7	8.8- 9.3	9.0	36	0.6966	0.04450535
45	no	1.1-2.0	1.3	8.8- 9.2	9.0	16	0.8377	0.06024973
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
0	yes	0.0-0.0	0.0	9.4-11.1	9.9	28	0.7146	0.03577794
0	no	0.2-0.5	0.4	9.4-10.2	9.7	7	0.9079	0.1132467
0	no	0.6-1.0	0.7	9.4- 9.6	9.5	7	0.7656	0.06692252
0	no	1.1-4.6	2.4	9.4-10.4	9.8	9	0.8024	0.06346712
ht	controls	MB range	mb mean	pH range	pH mean	n	RSQ	k
45	no	0.1-0.5	0.4	9.4-10.2	9.8	8	0.6969	0.09497056
45	no	0.6-1.0	0.7	9.4- 9.7	9.5	17	0.8223	0.06455087
45	no	1.1-4.6	2.0	9.4-10.4	9.7	22	0.8328	0.06690988

Table 17. Equations fitted to coliform data.*

equation	SAS statement	description
$Y=A+B \cdot X$	$Y=X$	straight line
$Y=B \cdot X$	$Y=X/\text{NOINT}$	line through origin
$Y=1/(A+B \cdot X)$	$(1/Y)=X$	reciprocal straight line
$Y=A+B \cdot X+C/X$	$Y=X (1/X)$	line and reciprocal
$Y=A+B/X$	$Y=(1/X)$	hyperbola
$Y=X/(A \cdot X+B)$	$(1/Y)=(1/X)$	reciprocal hyperbola
$Y=A+B/X+C/X \cdot X$	$Y=(1/X) 1/(X^{**2})$	2nd order hyperbola
$Y=A+B \cdot X+C \cdot X \cdot X$	$Y=X (X^{**2})$	parabola
$Y=A \cdot X+B \cdot X \cdot X$	$Y=X (X^{**2})/\text{NOINT}$	parabola at origin
$Y=A \cdot X^B$	$\log(Y)=\log(X)$	power
$Y=A \cdot B^X$	$\log(Y)=X$	modified power
$Y=B^{(1/X)}$	$\log(Y)=(1/X)/\text{NOINT}$	root
$Y=A \cdot X^{(B \cdot X)}$	$\log(Y)=X \cdot \log(X)$	super geometric
$Y=A \cdot X^{(B/X)}$	$\log(Y)=(1/X) \cdot \log(X)$	modified geometric
$Y=A \cdot e^{(B \cdot X)}$	$\log(Y)=X$	exponential
$Y=A \cdot e^{(B/X)}$	$\log(Y)=1/X$	modified exponential
$Y=A+B \cdot \ln(X)$	$Y=\log(X)$	logarithmic
$Y=1/(A+B \cdot \ln(X))$	$(1/Y)=\log(X)$	reciprocal logarithmic
$Y=A \cdot B^X \cdot X^C$	$\log(Y)=X \log(X)$	Hoerl function
$Y=A \cdot B^{(1/X)} \cdot X^C$	$\log(Y)=(1/X) \log(X)$	modified Hoerl function
$Y=A \cdot e^{(((X-B)^2)/C)}$	$\log(Y)=(X^{**2}) X$	normal
$Y=A \cdot e^{((\ln(X)-B)^2/C)}$	$\log(Y)=\log(X)^{**2} \log(X)$	log normal
$Y=A \cdot X^B \cdot (1-X)^C$	$\log(Y)=\log(X) \log(1-X)$	beta
$Y=A \cdot (X/B)^C \cdot e^{(X/B)}$	$\log(Y)=X \log(X)$	gamma
$Y=1/(A \cdot (X+B)^2+C)$	$(1/Y)=(X^{**2}) X$	Cauchy

* where $Y = C/Co$ and $X = \text{time}$
 $Y = \log(C/Co)$ and $X = \text{time}$
or
 $Y = (C/Co)/t$ and $X = \text{pH}$
 $Y = [\log(C/Co)]/t$ and $X = \text{pH}$

Table 18. Results of equations fitted to pilot scale coliform data (MB>0.0, pH >=8.8).

depth = 0 & 45, observations = 248				
MODEL	RSQUARE	INTERCEPT	COEFFICIENT 1	COEFFICIENT 2
$\ln(Co/C) = t$	0.7444		0.05770093	
$\ln(Co/C) = t^{**2} t$	0.7688		-0.000441593	0.10289850
depth = 0, observations = 111				
MODEL	RSQUARE	INTERCEPT	COEFFICIENT 1	COEFFICIENT 2
$\ln(C/Co) = t$	0.7469		0.05865996	
$\ln(C/Co) = t^{**2} t$	0.7698		-0.000455112	0.10616261
depth = 45, observations = 137				
MODEL	RSQUARE	INTERCEPT	COEFFICIENT 1	COEFFICIENT 2
$\ln(C/Co) = t$	0.7423		0.05681395	
$\ln(C/Co) = t^{**2} t$	0.7692		-0.000442101	0.10123578

Table 19. Results of equations fitted to pilot scale coliform data (MB>0.0).

depth = 0 & 45, observations = 248				
MODEL	RSQUARE	INTERCEPT	COEFFICIENT 1	COEFFICIENT 2
$[\ln(C/Co)]/t = 1/pH$	0.8852		-84.4063	
$[\ln(C/Co)]/t = pH$	0.9052		-1.00597	
$[\ln(C/Co)]/t = pH^{**2} pH$	0.9166		-0.306259	1.82539767
depth = 0, observations = 111				
MODEL	RSQUARE	INTERCEPT	COEFFICIENT 1	COEFFICIENT 2
$[\ln(C/Co)]/t = 1/pH$	0.8836		-87.0125	
$[\ln(C/Co)]/t = pH$	0.9027		-1.04162	
$[\ln(C/Co)]/t = pH^{**2} pH$	0.9140		-0.320247	1.91137392
depth = 45, observations = 137				
MODEL	RSQUARE	INTERCEPT	COEFFICIENT 1	COEFFICIENT 2
$[\ln(C/Co)]/t = 1/pH$	0.8880		-82.2767	
$[\ln(C/Co)]/t = pH$	0.9092		-0.977344	
$[\ln(C/Co)]/t = pH^{**2} pH$	0.9217		-0.306351	1.86077291

were linearized and the coefficients of the linear forms were determined by the method of least squares. The independent variable X was pH value, and the dependent variable Y was either $(Co/C)/t$ or $[\ln(Co/C)]/t$. The results of the linearized equations with the highest coefficients of determination are presented in Table 19. The equations which yielded the best fits were $[\ln(C/Co)] = k/pH$, $[\ln(C/Co)] = k \cdot pH$ and $[\ln(C/Co)] = k_1 \cdot (pH^2) + k_2 \cdot pH$.

BROMACIL STUDIES

Results from the bromacil studies are given in papers which were presented at the 1985, 1986, and 1987 Purdue Industrial Waste Conferences and the 1986 IAWPRC Biennial Conference, and in papers which will be presented at the 1987 National WPCF Conference, the 1987 ACS Environmental Chemistry Conference, and the 1987 International Conference on "Water Management in 2000 for Developing Countries." Copies of these papers are enclosed in the Appendices B-H.

SUMMARY AND CONCLUSIONS

COLIFORM STUDIES

Sensitized photooxidation is effective for wastewater disinfection in full scale systems. Influent coliform values ranging from 23,000 to 11,000,000 per 100 mL were consistently reduced to less than 500 per 100 mL under the following conditions: pH value above 9, a methylene blue concentration of 1 mg/L, and a detention time of 2 hr.

If turbidity and suspended solids are low, influent coliform values are reduced to less than 10 per 100 mL. The importance of a properly clarified feedwater into a sensitized photooxidation treatment process cannot be overstated. Effective inactivation rates, high removals, and low effluent coliform density do not occur with a feedwater high in suspended solids and turbidity.

The sunlight intensities encountered during the study, 55 - 930 W/sq.m, were sufficient for disinfection. Increases in reaction rate were most sensitive at lower light intensities; however, - at higher light intensities, the effect of an increase in sunlight did not appear to significantly improve the inactivation of coliform. A minimum value of light intensity is necessary to initiate the sensitized photooxidation reaction; however, the light intensities available from sunlight appear to be above this minimum value.

Air temperatures (0 - 35 C) and wastewater temperatures (10 - 25 C) did not appear to affect inactivation rates. Disinfection of coliform was dependent on pH value, and a pH value above 9 was necessary to ensure maximum reaction rates.

Inactivation was not effective in the pH range from 7.0 to 8.0. A reduction in coliform density occurred at pH values ≥ 8.8 , even in the absence of methylene blue. The reduction was due to the disinfective properties of ultraviolet radiation from sunlight and to the improved settling and removal of suspended matter at higher pH values.

A methylene blue concentration of 1 mg/L is sufficient for disinfection; however, maximum reaction rates occur at higher concentrations. Textile grade methylene blue was as effective as reagent grade methylene blue and considerably less expensive. A dissolved oxygen concentration greater than 2 mg/L was required for effective disinfection. Some removal of coliform and methylene blue was due to adsorption onto and subsequent settling out of suspended solids.

Higher methylene blue concentrations were observed at lower depths and may be due to bleaching of methylene blue at the water surfaces, adsorption onto colloidal material and subsequent settling out, and possible thermal gradients. The addition of methylene blue to the wastewater (pH value ≥ 8.8) resulted in significant increases in the removal of coliform, sometimes greater than 5 logs.

The rate of coliform inactivation in the bench scale studies appeared to occur in two phases, a sharp initial drop of up to 4.5 logs in the first 5 min followed by a gradual drop over the remaining detention time. Inactivation rates in the first phase increased with an increase in methylene blue concentration; whereas, rates in the second phase appeared somewhat independent of the sensitizer concentration. A minimum concentration of

methylene blue was required for effective inactivation rates; however, concentrations greater than this minimum value did not significantly improve the rate. After a two hour exposure time at a pH value > 8.8 and a methylene blue concentration of 1.0 mg/L, influent coliform densities > 10^6 /100 mL were consistently reduced to < 100 coliform/100 mL and in some instances to < 10 coliform/100 mL.

Inactivation rates were adequately described by first order kinetics in both the pilot and bench scale studies; however, the sensitized photooxidation of coliform is more complex and is dependent on pH, methylene blue concentration, dissolved oxygen, turbidity, and sunlight intensity. Linear and non-linear models incorporating these parameters were developed.

Coliform removals were more sensitive to methylene blue concentration, pH value, sunlight intensity and depth in the pilot plant studies than in the bench scale studies. Removals were generally higher at the water surface than at mid-depth in the pilot scale reactors; however, the differences in removals between the two depths were generally within 1 log unit. Poorer removals in the pilot scale studies are suspected to be due to short circuiting in the reactors and occasional deterioration in feedwater quality.

BROMACIL STUDIES

Sensitized photooxidation appears to be effective for detoxification of bromacil in full scale systems. The degradation is directly related to percent ionization, and both

are dependent on pH value. A pH value above 9 is necessary to ensure maximum reaction rates using methylene blue as a sensitizer. A methylene blue concentration in the range from 1 to 10 mg/L is sufficient to photooxidize a 30 mg/L bromacil solution. Reaction rates are highest at 10 mg/L, and at higher concentrations reaction rates begin to decline.

As a first approximation, reaction rates for bromacil removal are best described by first order kinetics; however, the sensitized photooxidation of bromacil is more complex and is dependent on pH, methylene blue concentration, dissolved oxygen, turbidity, and sunlight intensity.

The sunlight intensities encountered during the study, 294 - 876 W/sq.m, are sufficient for degradation of bromacil. A minimum value of sunlight intensity is necessary to initiate the photooxidation reaction (i.e. dark controls showed no degradation); however, the sunlight intensities encountered during the study appear to be above the minimum value.

Air temperatures (0 - 35 C), and wastewater temperatures (10 - 25 C) do not affect bromacil removal rates. Textile grade methylene blue is as effective as reagent grade methylene blue and considerably less expensive. A dissolved oxygen concentration greater than 2 mg/L is required for effective reaction rates.

Little or no bromacil degradation is observed at a pH value of 4, an increase in reaction rate occurs at pH value of 7, and reaction rates are highest at pH value $\geq$ 10. Reaction rates under anoxic conditions are generally 1 to 2 orders of magnitude less than reaction rates under aerobic conditions. Thiazine,

alloxazine, and xanthene dyes are the most effective sensitizers.

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APPENDIX A

Pilot Plant Studies: Coliform, Virus, And Bromacil

Figure A1. Flow diagram and photograph of the USA Pilot Plant located at Livingston, Tennessee, USA.

APPENDIX E

Poster Presented at the 13th Biennial International Conference of the International Association on Water Pollution Research and Control, Rio de Janeiro, 17-22 August 1986

SENSITIZED PHOTOOXIDATION FOR WASTEWATER

DISINFECTION AND DETOXIFICATION

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INTRODUCTION

A new approach to disinfection and detoxification of wastewater is the use of sunlight and photosensitizers. The sensitizer absorbs energy in the visible spectral region and transfers this energy either to the substrate or to molecular oxygen. Unsaturated compounds are oxidized to peroxides, which in turn generate free radical oxidation reactions. The effect on microorganisms is that cell constituents are irreversibly altered and cell reproduction is inhibited. The effect on certain toxic organics is that the chemical structure is changed, rendering the compound non-toxic.

OBJECTIVES

The objective of this study was to provide information to design a full scale sensitized photooxidation system by performing the following tasks: determine the feasibility of sensitized photooxidation for wastewater disinfection and detoxification in a full scale field situation; determine the effects of mixing, sunlight intensity, pH value, reactor depth, sensitizer and sensitizer concentration, initial substrate concentration, dissolved oxygen concentration, water and air temperature, suspended solids and turbidity, and hydraulic detention time on disinfection and detoxification efficiency; screen a variety of dyes and stains for potential sensitizers and determine the

effect of pH value on the reaction rate for the sensitizers; develop mathematical models to predict and optimize disinfection and detoxification efficiencies; document operational and process control difficulties and develop remedial measures for these problems.

RESEARCH PLAN

Unchlorinated secondary treated effluent was used. Bench scale studies (208 L batch reactors) were first conducted to determine the feasibility of sensitized photooxidation for wastewater disinfection of coliform and detoxification of bromacil. The results from the bench scale studies were used to design and construct a pilot scale plant. The pilot plant consists of two parallel plug flow reactors, each treating 200 L/min. Samples were taken at locations in the reactors corresponding to hydraulic detention times of 0, 30, 60, 90, and 120 min at depths of 0.0 and 0.46 m. Laboratory scale studies (batch 250 mL clear beakers) were carried out to determine the effects of sensitizer (69 textile, biological, and chemical dyes and stains), pH value (4, 7, and 10) and dissolved oxygen on bromacil degradation.

RESULTS

Results from the bench and pilot scale studies showed that sensitized photooxidation was an effective treatment process for wastewater disinfection and detoxification. Influent coliform ranging from 500,000 to 5,000,000 per 100 mL were consistently reduced to less than 500 per 100 mL under the following conditions: pH value above 9, a methylene blue concentration of 1 mg/L, and a detention time of 2 hr. With low influent turbidity and suspended solids, coliform were reduced under the same conditions to less than 10 per 100 mL. Disinfection of coliform was dependent on pH value, and a pH value above 9 was necessary to ensure maximum reaction rates. A methylene blue concentration of 1 mg/L was sufficient for disinfection; however, higher

reaction rates occurred at higher concentrations.

Degradation of bromacil was directly related to percent ionization, and both were dependent on pH value. A pH value above 9 was necessary to ensure maximum reaction rates using methylene blue as a sensitizer. A methylene blue concentration in the range from 1 to 10 mg/L was sufficient to photooxidize a 30 mg/L bromacil solution. Reaction rates were highest at 10 mg/L, and at higher concentrations began to decline.

The sunlight intensities encountered during the study, 200 - 900 W/sq.m, were sufficient for disinfection and detoxification. Air temperatures (0 - 35 C), and wastewater temperatures (10 - 25 C) did not affect reaction rates. Textile grade methylene blue was as effective as reagent grade methylene blue and considerably less expensive. A dissolved oxygen concentration greater than 2 mg/L was required for effective reaction rates. Some removal of coliform, bromacil, and methylene blue was due to adsorption onto and subsequent settling out of suspended solids. No significant difference between coliform or bromacil removals at 0.0 and 0.46 m depth was observed. As a first approximation, reaction rates for coliform and bromacil removal were adequately described by first order kinetics; however, the sensitized photooxidation of coliform and bromacil is more complex and is dependent on pH, methylene blue concentration, dissolved oxygen, turbidity, and sunlight intensity. Non-linear models were developed to incorporate these parameters.

In the laboratory studies to screen potential sensitizing agents, little or no bromacil degradation was observed at a pH value of 4. An increase in reaction rate was seen at pH 7, and reaction rates were highest at pH 10 with no bromacil detected at 4 min for phloxine B, at 8 min for rose bengal, methylene blue, and eosin Y, and at 16 min for Wright's Stain. Reaction rates under anoxic conditions were generally 1 to 2 orders of magnitude less than

reaction rates under aerobic conditions. Thiazine, azine, and xanthene dyes were predictably the most effective sensitizers.

CONCLUSIONS

In areas where water shortage problems exist, treated wastewater represents a much needed source of water for both agricultural and municipal use. The advantages of sensitized photooxidation for wastewater disinfection and detoxification are several. Because sensitizers absorb energy in the visible spectral region, sunlight serves as a no cost energy source for the process, and energy costs for such systems are minimal. The sensitizer is regenerated in the process, and only trace amounts are required. Unlike chlorination, toxic chlorinated organics are not formed. Structural facilities (ponds) and instrumentation associated with these systems are relatively inexpensive and are simple to design, construct, operate, and maintain. In addition, the required contact time for disinfection is relatively short. Sensitized photooxidation is a low cost, low technology treatment process that can help alleviate water shortage problems.

SENSITIZED PHOTOOXIDATION

coliform
virus

MICROORGANISMS

herbicide
pesticide
refractory
organic

ORGANIC MATTER

DYE

col if orm
vi r us

DEAD MICROORGANISMS

her bic ide

pes tic ide

ref ract ory

or gan ic

OXIDIZED ORGANICS

PROPOSED MECHANISMS

HYDROGEN ABSTRACTION

OXYGEN TRANSFER

Schoenberg, Schenk, et.al.

Kautsky, Egerton, et.al.

Kautsky, Egerton, et.al.

D = dye or sensitizer

A = substrate

$h\nu$ = sunlight in visible spectrum

PHOTOSENSITIVE SUBSTRATES

Nucleic Acids Nucleosides Nucleotides N-Heterotricyclics

Indoles Phenols Proteins Purines Pyrimidines Pyrroles

Benzenoid and Aromatic Heterocyclics

Amino or Hydroxyl Group on Benzene Increases Sensitivity

Amines [Tertiary > Secondary > Primary > Quaternary]

Aromatic Amino Acids [Histidine, Methionine, Tryptophan]

PHOTORESISTANT SUBSTRATES

Saturated and Unsubstituted Aliphatics

Benzene with Cyano, Halogen, or Nitro

Aliphatic Amino Acids

BENEFITS OF SENSITIZED PHOTOOXIDATION FOR POLLUTION CONTROL

1. Free Energy Source With Minimal Energy Costs

2. Structural Facilities And Instrumentation Easy To Design, Build, Operate, and Maintain

3. Relatively Short Contact Times For Disinfection And Detoxification

4. Sensitizers Or Dyes Inexpensive, Trace Amounts Required, Regenerated

5. No Chlorinated Organics

RESEARCH PLAN

1. Pilot Plant Studies: Coliform, Virus, And Bromacil

Secondary Unchlorinated Effluent

RESEARCH PLAN (cont.)

2. Bench Scale Studies: Coliform (Fecal and Total) and Bromacil
3. Laboratory Scale Studies: Survey of Potential Photosensitizing Agents

Secondary Unchlorinated Effluent

Variables:

pH

Methylene Blue

Substrate

Mixing

Sunlight

DO

208 L (55 gal)

pH = 4, 7, 10

Sensitizer = 10 mg/L

Bromacil = 30 mg/L

Textile Dyes

<p>Methyl Violet</p> <p>Erie Congo</p> <p>Wool Violet</p> <p>Wool Orange</p> <p>Alphazurine</p> <p>Chrysoidine</p> <p>Erie Fast Orange</p> <p>Victoria Green</p> <p>Alizarine Cyanine Green</p> <p>Acid Red WA</p>	<p>Azorubine</p> <p>Solentine Yellow</p> <p>Blamark Brown</p> <p>Erie Green</p> <p>Solentine Turquoise</p> <p>Resorcine Brown</p> <p>Rhodamine B</p> <p>Auromine O</p> <p>Victoria Pure Blue</p> <p>Methylene Blue</p>
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Chemical and Biological Dyes

<p>Acridine Orange</p> <p>Acridine Yellow</p> <p>Alizarine Red</p> <p>Alizarol Cyanine</p> <p>Aniline Blue</p> <p>Brilliant Yellow</p> <p>Bromocresol Green</p> <p>Bromocresol Purple</p> <p>Bromophenol Blue</p> <p>Carmine</p> <p>Congo Red</p> <p>Cresol Red</p> <p>Crystal Violet</p> <p>Curcumin</p> <p>Diaphenyl Blue</p>	<p>Dichlorofluorescein</p> <p>Eosin Y</p> <p>Eriochrome Black T</p> <p>Ethyl Orange</p> <p>Evans Blue</p> <p>Fast Green FCF</p> <p>Fluorescein</p> <p>Fuchsin Acid</p> <p>Gentian Violet</p> <p>Grand Yellow</p> <p>Malachite Green</p> <p>Marshall's Reagent</p> <p>Methyl Orange</p> <p>Methyl Red</p> <p>Methyl Violet</p>	<p>Methylene Blue</p> <p>Neutral Red</p> <p>Nigrosin</p> <p>Phenol Red</p> <p>Phloxine B</p> <p>Propyl Red</p> <p>Rhodamine B</p> <p>Rhodamine WT</p> <p>Riboflavin</p> <p>Rose Bengal</p> <p>Safranin O</p> <p>Thionin</p> <p>Thymol Blue</p> <p>Thyodene</p> <p>Wright's Stain</p>
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FECAL COLIFORM VS. TIME

SUN = 882 W/sq.m, SAMPLE DEPTH = 60 cm

AUGUST 8, 1984

Effect of Methylene Blue and pH on coliform removal.

TOTAL COLIFORM VS. TIME

SUN = 615 W/sq.m, pH = 8.9

SEPTEMBER 13, 1984

Effect of Methylene Blue on coliform removal

BROMACIL VS. TIME

SUN = 819 W/sq.m, Methylene Blue = 2 mg/L

April 11, 1985

Effect of pH value on Bromacil removal

BROMACIL VS. TIME

SUN = 854 W/sq.m, pH = 9.7

April 17, 1985

Effect of Methylene Blue on Bromacil removal

MODELS USED TO DESCRIBE BROMACIL DATA

Reaction Order	Constant Reaction Rate	Michaelis-Menten Reaction Rate
	$dS/dt = -kS^n$	$dS/dt = -[uS/Ks+S]S^n$
0th	$So - S = kt$	$t = [Ks/\hat{u}] \ln[So/S] + [So-S]/\hat{u}$
1st	$\ln[So/S] = kt$	$t = [Ks/\hat{u}][1/S - 1/So] + [1/\hat{u}] \ln[So/S]$
2nd	$[1/S - 1/So] = kt$	$t = [Ks/2\hat{u}][1/S^2 - 1/So^2] + [1/\hat{u}][1/S - 1/So]$

n = order of reaction [0, 1, 2]; t = time; S = Bromacil at time t; So = Bromacil at time 0; k = constant reaction rate; u = maximum reaction rate; Ks = Bromacil at 0.5u

ANOVA FOR CONSTANT REACTION RATES

Order	MB	R-Square	Prob>F	k	Df
0th	0.5-32	0.6400	0.0001	0.1584	1,111
1st	0.5-32	0.8489	0.0001	0.0142	1,111
2nd	0.5-32	0.3391	0.0001	0.0003	1,111

MB = range of methylene blue; R-Square = coefficient of determination; Prob>F = alpha value of F-Test; DF = degrees of freedom for regression and error.

ANOVA FOR MICHAELIS-MENTEN REACTION RATES

Order	MB	R-Square	Prob>F	Ks	u	DF
0th	0.5-32	0.8491	0.0001	473.043	7.140	2,110
1st	0.5-32	0.8491	0.0001	0.100	0.015	2,110
2nd	0.5-32	0.8491	0.0001	0.504	-0.002	2,110

PREDICTED VS. OBSERVED BROMACIL

8.8 < pH < 10.2

0.25 < Methylene Blue < 13 mg/L

Reaction Rate Constant and Half-Life for Sensitized Photooxidation of
50 mg/L Bromacil: pH = 10, Reactor = 1 L, Sensitizer = 10 mg/L,
Sun = 853 W/sq.m; Dissolved Oxygen = saturated.

Sensitizer	-k [1/min]	A = Co exp[INTCPT]	t[1/2] [min]	Relative Sensitizing
Dichlorofluorescein	0.0297	48.56	22	0.30
Fluorescein	0.0087	47.15	73	0.09
Riboflavin	0.0659	46.14	9	0.67
Safranin O	0.0050	48.03	131	0.05
Solantine Turquoise	0.0049	49.07	138	0.05
Wrights Stain	0.0988	53.33	8	1.00

Reaction Rate Constant and Half-Life for Sensitized Photooxidation of
30 mg/L Bromacil: pH = 10, Reactor = 125 mL, Sensitizer = 10 mg/L,
Sun = 849 W/sq.m, Dissolved Oxygen = saturated.

Sensitizer	-k [1/min]	A = Co exp[INTCPT]	t[1/2] [min]	Relative Sensitizing
Safranin O	0.0081	28.34	79	0.01
Tricon MB	0.5154	27.43	1	0.47
Fisher MB	0.5079	27.57	1	0.46
Riboflavin	0.1152	31.15	6	0.10
Rose Bengal	0.7756	31.04	1	0.70
B. Blue 9	0.6866	30.62	1	0.62
D. Blue 86	0.0142	31.26	52	0.01
Dichlorofluorescein	0.0491	29.47	14	0.04
Eosin Y	0.7264	32.26	1	0.66
Fluorescein	0.0304	30.41	23	0.03
Phloxine B	1.1080	29.23	1	1.00
Wrights Stain	0.3306	28.55	2	0.30

RESULTS AND CONCLUSIONS

Pilot Plant Studies (Reactor Volume = 22000 L, Flow = 180 L/min)

1. Influent Total Coliform was consistently reduced from 500,000 - 5,000,000 to less than 500 Coliform/100 mL (HDT = 2 hr, pH = 9, Methylene Blue = 1 mg/L) and less than 10 Coliform/100 mL if influent solids and turbidity were low.
2. The sunlight intensities encountered, 200 - 900 W/sq.m, were sufficient for sensitized photooxidation.
3. Air temperature, 0 - 35 C, and wastewater temperature, 10 - 25 C, did not affect reaction rates.

Bench Scale Studies (Reactor Volume = 208 L)

1. Ionization of Bromacil, disinfection of coliform, and detoxification of bromacil were all pH dependent. A pH value above 9 was necessary to ensure maximum reaction rates.
2. Methylene Blue dye concentration of 1 mg/L was sufficient for disinfection and detoxification; however, maximum reaction rates were at 10 mg/L.
3. Textile grade Methylene Blue was as effective as reagent grade Methylene Blue and less expensive.
4. Dissolved Oxygen concentration > 2 mg/L was required for effective reaction rates.
5. Some removal of Coliform and Bromacil was due to solids settling.

RESULTS AND CONCLUSIONS (cont.)

Bench Scale Studies (cont.)

6. Mixing in the reactor ensured all substrate was exposed.
7. No significant difference occurred between removals of substrate at surface and removals at 0.5 m depth.
8. As first approximation, first order kinetics adequately described reaction rate; however, reaction mechanism is more complex and is dependent on pH, Methylene Blue, solids settling, DO residual, etc.
9. Some removal of methylene blue was due to solids settling.

Laboratory Scale Studies (Potential Photosensitizing Agents)

1. The most effective sensitizers were Methylene Blue, a basic cationic thiazine dye; Safranine O, Neutral Red, and Riboflavin (Vitamin B-2), basic cationic azine dyes; Eosin Y, Rose Bengal, Dichlorofluorescein, Fluorescein, and Phloxine B, acid anionic xanthene fluorane derivatives; and Wright's Stain, a compound of Eosin with oxidized and partly demethylated Methylene Blue.
2. Reaction rates under anoxic conditions were 1 to 2 orders of magnitude less than rates under aerobic conditions.