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Sodium ligninsulfonate, a water soluble wide-range molecular weight polymeric by-product of the paper pulping industry, is added as waste effluent to water ways or burned, causing extreme pollution. The polymer is highly resistant to biodegradation. Therefore, modification of the structure to a more readily biodegradable form would be desirable, to be able to use this material for economic recovery, such as single cell protein (SCP), or other useful products. The ideal method of alteration would be one which did not in itself lead to other unwanted waste products such as would be inherent in a chemical process.

In this study a sodium ligninsulfonate, Marasperse CB, was phototreated using a mercury-vapor ultraviolet lamp. Parameters studied during photolysis were oxygen effects, initial starting pH,

temperature and wavelength ranges. Analytical methods included total carbon, thin layer chromatography, spectrophotometry, and fungal dry weight. The photolyzed ligninsulfonate was then used as a growth substrate for an Aspergillus isolated from soil enrichment.

Definitive improvement of ligninsulfonate biological availability was demonstrated after photo-treatment of initial pH 3, 7 and 12 solutions. The response of the Aspergillus isolate was substantiated by assays of substrate carbon before and after growth, dry weights in relation to photolysis treatment time, and semiquantitative thin layer chromatography. The highest fungal yield came from the initial pH3 solution followed closely by the initial pH 12 solution. The pH 7 solution was approximately 50 percent of the former two. The greatest loss of carbon after growth occurred in the pH 3 and 12 solutions. Thin layer chromatography showed that during irradiation three new components were formed and three existing components increased in concentration as irradiation times increased. All of these components were reduced below detection limits after use of the solution as a growth medium.

Carbon retention after irradiation was greatest in the initial pH 3 and 12 solutions as compared to the pH 7 solution. Photolysis occurred most efficiently in the pH 3 solution with regard to time, fungal yield, carbon loss after growth, and substrate utilization

efficiency (fungal dry weight/carbon utilized). Wavelengths less than 210 nm allowed the most rapid modification with wavelengths greater than 280 nm causing essentially no short-term change. Photolysis of the ligninsulfonate was found to be temperature independent and oxygen dependent, showing a true primary photodecomposition mode.

Photo-treatment of ligninsulfonate and definitive fungal growth response to the irradiated solution suggests that this treatment could be applied to improve biodegradation of other recalcitrant structures as well.

Fungal Growth Responses to a Photooxidized Sodium Ligninsulfonate

by

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INTRODUCTION

Lignin, the strengthening component in plants, is sulfonated in the bisulfite paper pulping process to form water-soluble lignin-sulfonates. Lignin is a large polymer for which no definite structure has been established. However, it is now assumed that the basic building units are of the phenylpropanoid type, mainly syringyl, quaiacyl, and p-hydroxybenzyl moeities depending on the species of plant from which the lignin is derived.

Lignin and its sulfonated derivatives are difficult to biodegrade with one class of white-rot Basidiomycetes, for example, utilizing approximately twenty-eight percent of these components in three months. This condition of high resistance to biodegradation has been termed recalcitrance.

Commercial chemical processes to modify the lignin structure are used to produce a variety of compounds such as vanillin. However, the low demand for these degradation products as opposed to the large production of lignin derivatives leads to disposal of the excess as effluent into water ways or by burning with concomitant pollution of natural surroundings. Some efficient method of treatment to eliminate the lignin derivatives coupled with the economic recovery

of useful by-products would be desirable.

Light, usually wavelengths in the ultraviolet, has been shown to degrade or polymerize molecular structures depending on the conditions imposed. This process is termed photolysis and is being used in numerous commercial applications.

Photolysis produces a "clean" reaction because the photon-molecule interaction is selective, since one photon is absorbed by a bond only if the photon is of sufficient energy to rupture the bond. If the photon is of lower energy it is dissipated and has no effect on the substrate. Higher energy photons can cause secondary reactions but these can be eliminated by utilizing monochromatic wavelengths of the desired energy. Photolysis involves only vibrational modes for activation and allows traverse of energy levels not possible by thermal means. It also eliminates disposal of the undesirable reaction products inherent in chemical processes.

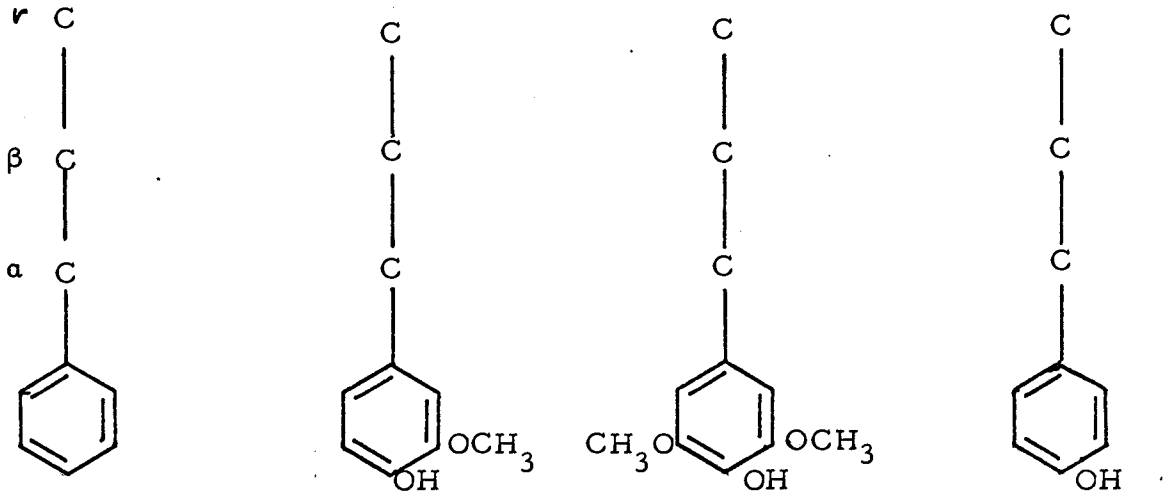
This study was designed to establish and describe parameters necessary to photolytically modify a sodium ligninsulfonate to reduce recalcitrance to biodegradation and to evaluate the growth responses of a fungal isolate.

LITERATURE REVIEW

Chemical Nature of Lignin

Lignin has been defined by Brauns (9) "as that incrusting material of the plant which is built up mainly, if not entirely, of phenylpropane building stones; it carries the major part of the methoxyl content of the wood; it is unhydrolyzable by acids, readily oxidizable, soluble in hot alkali and bisulfite, and readily condenses with phenols and thio compounds."

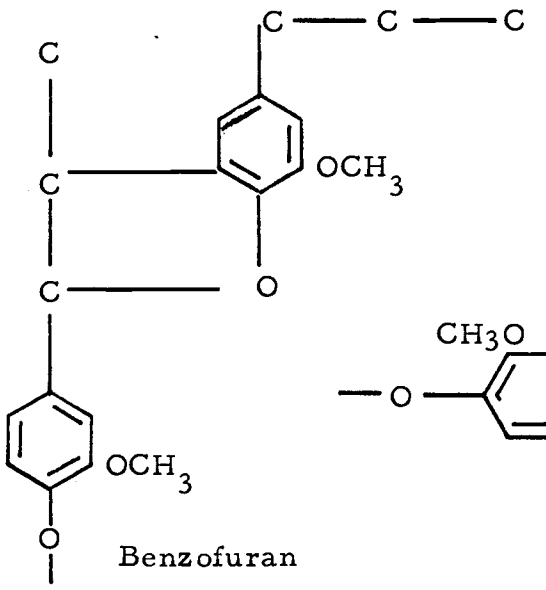
At the present time there is no single "lignin" (10) and no method has been developed for the isolation of the protolignin macromolecule which is considered to be a random rather than an ordered structure. Although no definite chemical structure has been determined, lignin now appears to be a polymer built up by condensing a few similar structural units of the phenylpropane (C-6, C-3) type (12, 14, 49). Gymnosperm wood appears to possess the guaiacyl unit whereas angiosperms contain both guaiacyl and syringyl units as the phenol part of the phenylpropane structure. In the Gramineae (grasses) and some angiosperms, the p-hydroxyl-phenyl unit is present (14).



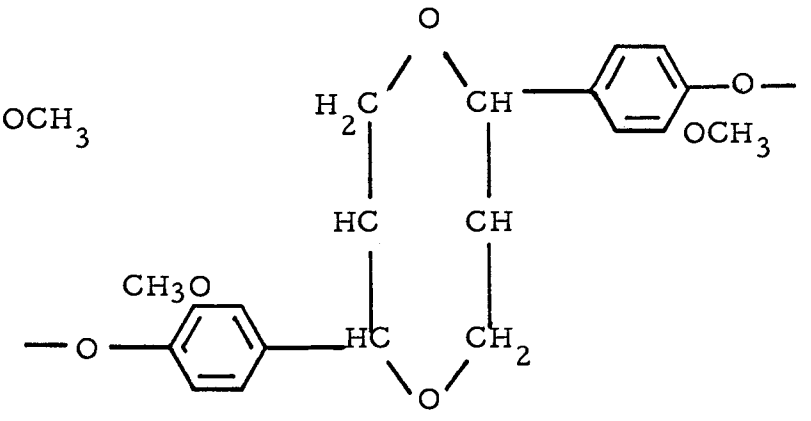
Phenylpropane Guaiacyl Syringyl p-Hydroxy-phenyl

The monomeric phenylpropane units are presumably linked together forming the lignin macromolecule, by various linkages. Namely, two ether linkages, β-4' and alpha-alkyl and four carbon to carbon linkages β-5', alpha-alpha', β-β', and 5-5' (14).

Multiple monomeric bonding may also be demonstrated by the benzofuran and pinoresinol structures. Benzofuran represents an α-4' linkage and pinoresinol an α-gamma' type (14)



Benzofuran



Pinoresinol

Phenolic substances comprise approximately 20-30 percent of wood material, being mainly tannins, phlobaphenes, coloring matter, lignans, and lignin. These wood components vary among species and are attributed to genetic ecological factors (13).

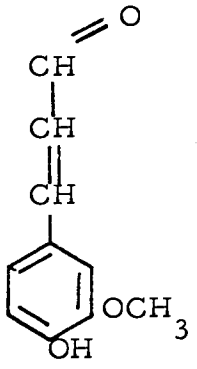
Early investigations by Klason (36, 37), indicated that lignin represented the condensation product of coniferyl and hydroxyconiferyl alcohols and resulted from the polymerization of the basic building block, R-C-C-C, where R was the 4-hydroxy-3-methoxy-phenyl group. He knew that the cambial sap of sprucewood contained

coniferin, a glucoside of coniferyl alcohol. Coniferin was regarded as a dimer of coniferyl alcohol and the glucoside as a dimer of hydroxyconiferyl alcohol. His studies showed that the coniferyl alcohol polymerized easily, was unsaturated, and readily formed bisulfites resulting in sulfonic acids. Thus it possessed many properties of lignin and ligninsulfonic acid.

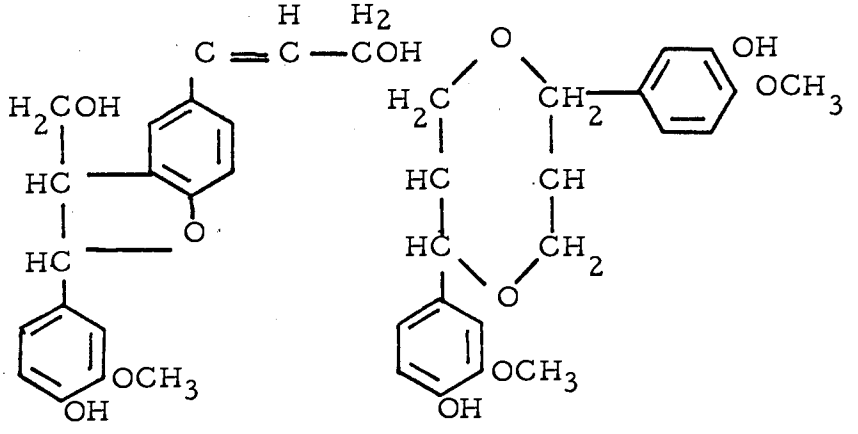
With the advent of Klason's hypothesis that lignin was a condensation polymer of coniferyl alcohol, Erdtman (20) suggested that lignin was formed by oxidative polymerization of phenolic precursors. This was deduced from studies of phenolpropane derivaties during dehydrogenative oxidation suggesting that the unsaturated side chain of p-hydroxyphenylpropane compounds initiated coupling reactions on oxidation in the ortho position to the phenolic hydroxyl group and the β-carbon atom of the side chain. It appeared that quaiacylpropane units were first oxidized in the side chain with subsequent dehydrogenation. Lignin therefore appeared to be formed from precursors similar to coniferyl alcohol.

Proof (30) of the aromaticity of lignin came when high-pressure hydrogenation produced 4-n-propylcyclohexanol, 4-n-propylcyclohexan-1-2-diol and 3-(4-hydroxycyclohexyl)-1-propanol. The cyclohexylpropane structure showed the aromatic nature of lignin and also that it was made from polymerization of guaiacylpropane monomers.

In 1943 Freudenberg (26) reacted crude mushroom extract, containing laccase and peroxidase enzymes, and coniferyl alcohol, obtaining dehydrogenation polymers consisting of coniferyl aldehyde, dehydrodiconiferyl alcohol, D, L, - pinoresinol and guaiacylglycerol-β-coniferyl ether.

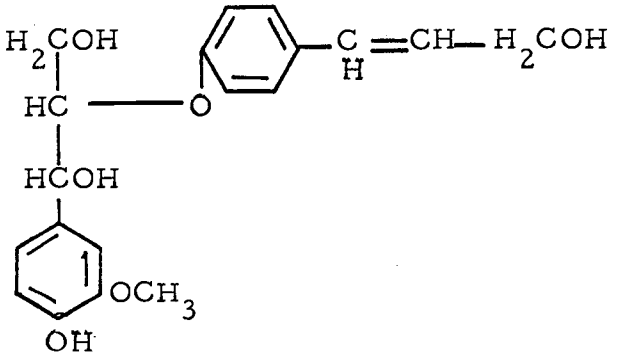


Coniferyl aldehyde



Dehydrodiconiferyl alcohol

D, L-Pinoresinol

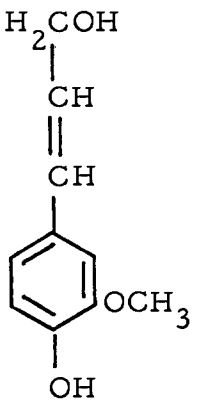


Guaiacylglycerol-β-Coniferyl Ether

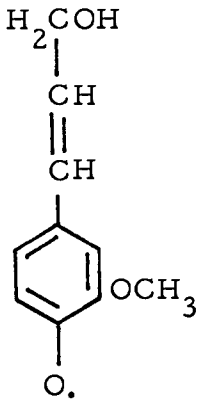
Additional amorphous polymers were obtained (26) by further enzymatic oxidation of D, L, -pinoresinol, guaiacylglycerol-β-coniferyl ether and dehydrodiconiferyl alcohol, supporting the hypothesis that coniferyl alcohol was the basic building unit responsible for the formation of the polymers and that the dimers were intermediates in the

reaction.

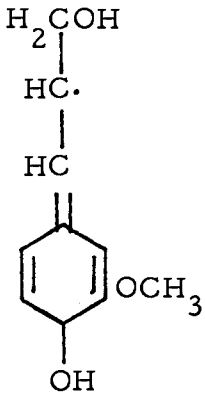
In further studies, Freudenberg (27) attempted to explain the reaction mechanism for the dehydrogenation polymerization of coniferyl alcohol by postulating that dehydrogenation of coniferyl alcohol occurred at the phenolic hydroxyl group, resulting in mesomeric forms leading to aroxyl, p-quinone methide and o-quinoid radicals:



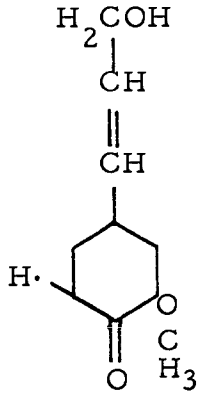
Coniferyl alcohol



Aroxyl



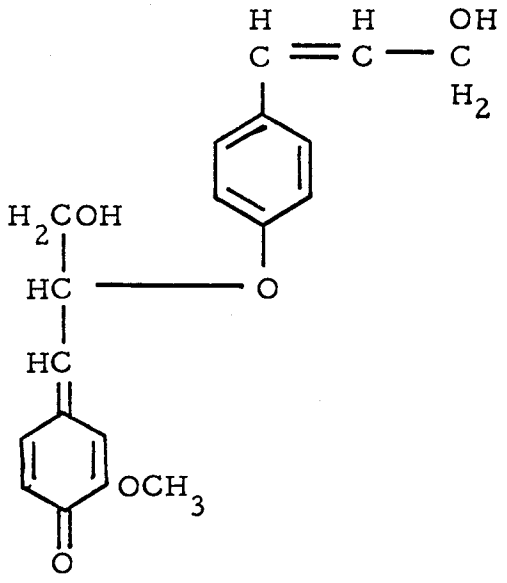
P-Quinone methide



O-Quinoid

Dimerization occurred by the combination of p-quinone methide and o-quinoid radicals to give a quinone methide and subsequently form dehydrodiconiferyl alcohol ty intramolecular prototrophic stabilization. Two p-quinone methide radicals thus formed a double quinone methide which by a double prototrophic shift resulted in pinoresinol, and finally p-quinone methide and aroxyl radicals reacted forming a

quinone methide that could not react intramoleculary with hydroxyl groups. Reaction of this molecule with water gave guaiacylglycerol-β-coniferyl ether.



Quinone methide

These dimeric compounds are now called dilignols, monomeric units lignols, trimeric units trilignols, and polymeric units oligolignols, and represent states of interrupted polymerization of coniferyl alcohol. Oligolignols were prepared and isolated by further dehydrogenation of coniferyl alcohol (50), showing that molecular growth occurred from enzymatically dehydrogenated phenolic compounds. Sugar, as well as water and phenols, adds to the quinone methide, being incorporated in the molecular growth of the dehydrogenated coniferyl alcohol (50).

Biosynthesis of Lignin

Based on the investigations of chemical synthesis of lignin-like compounds, it has been possible to elucidate lignin biosynthesis in plants with a large part of the information derived from radioisotope studies.

Wheat plants grown in $C^{14}O_2$ (64) and harvested regularly every two days until mature were subjected to nitrobenzene oxidation. Vanillin, syringaldehyde, and o-hydroxybenzaldehyde were obtained and all showed $C^{14}O_2$ incorporation. The incorporation was greatest after twenty four hours of growth and remained constant to maturity. It was concluded that the end product of carbon dioxide fixation was lignin.

In additional studies (2), D-glucose-1-C^{14} and D-glucose-6-C^{14} were fed to spruce trees. Lignin was isolated and subjected to nitrobenzene oxidation. Vanillin was obtained and the distribution of radioactivity found mainly in this compound.

Ishakawa and Takaichi (33) postulated that lignin biosynthesis was correlated with the shikimic acid pathway through which aromatic amino acids are synthesized. They found that CO_2 and light resulted in more lignin, shikimic acid, phenylalanine, and tyrosine in cultivated plants. The shikimic acid was formed from glucose by the condensation of a resulting triose with a tetrose from the pentose

phosphate pathway (63); namely, phosphoenolpyruvate from glycolysis and D-erythrose-4-phosphate from the pentose phosphate pathway. To confirm this concept, C^{14}-labelled shikimic acid was found to be a precursor to phenylalanine and tyrosine in Salvia (44). Shikimic acid then proceded to phenylalanine or tyrosine, these going to p-coumaric acid. The p-coumaric acid formed p-hydroxy-cinnamyl alcohol, coniferyl alcohol or sinapyl alcohol, depending on the growing species, and were incorporated into lignin (57).

Ligninsulfonate Formation

Investigations on the formation of lignin have led to at least a basic understanding on the common chemical linkages involved in this random structure. This knowledge has led to further application of lignin conversion to a water-soluble product, ligninsulfonate.

Sulfonation of wood chips by bisulfite and sulfurous acid at elevated temperature and pressure results chiefly in cellulose, hemicellulose, soluble sugars, resins, extractives and lignin-sulfonates (20). The digestion reaction is thought to procede by three distinct reactions; (1) formation of alkyl sulfonate groups, (2) hydrolysis, and (3) condensation. Formation of the sulfonate appears to occur by replacement of alkyl hydroxyl groups, cleavage of aryl-alkyl or alkyl-alkyl ether linkages and addition at an unsaturated alkyl position (29).

Sulfonation of phenylpropane derivatives (38, 68), to determine the position of sulfonation during bisulfite cooks, showed that in most instances the sulfonic acid group was formed at the alpha-alkyl carbon atom in the side chain. There is not complete agreement with this, as ligninsulfonate fully sulfonated at the same carbon atom is still not conclusively observed (11).

Wood delignification (2) during sulfite cooking results in the hemicelluloses being hydrolyzed faster into monomeric sugar units than celluloses. Protolignin is hydrolyzable to a limited extent but sulfonation is primary causing solubilization as ligninsulfonate. As sulfonation nears completion (60), the soluble ligninsulfonates are formed and cellulose hydrolysis increases due to reduced protection by lignin. One sulfonic acid group is considered necessary per four building blocks to render a ligninsulfonate soluble in water (10). Delignification is dependent on the type of cation used (60) because sulfonated lignin acts as a cation exchanger; the rate occurring faster with monovalent cations in comparison with divalent cations and trivalent cations. The neutralization of the sulfonic acid groups by the cations reduces the hydrolytic activity by reducing the acidity of the solution.

Ligninsulfonate Separation and Commercial Use

The commercial separation of ligninsulfonate, from an estimated three million tons of spent sulfite liquor produced yearly in the United States (21), is achieved by the Howard Process (58). Initially, sulfite and sulfate are removed by lime addition to pH 10.5. More lime is added giving a basic calcium ligninsulfonate which precipitates. The precipitate is collected by filtration, and sulfuric acid and magnesium or sodium sulfate added. Calcium sulfate precipitates and the liquid is evaporated and dried. The molecular weight distribution of the ligninsulfonates varies, depending on the assay procedure used, dialysis (23), light scattering (46), or ethanol precipitation (22, 28). In general, the molecular weight has been shown by these assays to lay in the range between 400-150,000.

Ligninsulfonate is used as a roadbinder, for animal-feed pelletizing, cement products, industrial cleaners, trace element chelation, leather tanning, metallic complexing, oil well drilling (75 million pounds annually), and vanillin (1.1 million pounds annually) (51). However, the very small amount of ligninsulfonate used as compared to that produced leaves a very large excess for disposal (52).

Microbial Utilization of Lignin and Ligninsulfonates

Based upon the large production of ligninsulfonate and the small demand for its used, studies have been made to find a procedure to modify the structure to gain economically useful products as well as help delineate the structure of lignin. Microbial assimilation and modification have been extensively studied.

Ishakawa, et al., (32), found that three basidiomyetous white-rot fungi, possessing polyphenoloxidase activity were able to degrade soft-wood lignin to 15 identifiable compounds. The principle degradation products obtained were coniferyl alcohol, guaiacylglycerol and guaiacylglycerol-β-coniferyl ether. These were further metabolized by side chain reduction and converted to protocatechuic acid or catechol, focal points in the metabolism of aromatic compounds by well established pathways (31). Brown-rot fungi, containing minimal polyphenoloxidase activity were unable to decompose lignin as the sole carbon source (61). Fungal lignin degradation results in removal of some side chains, methoxyl decrease, and phenolic hydroxyl and carboxyl group increases (4). Degradation is also influenced by temperature, nitrogen availability, anerobiosis, and nutritive components. Under optimum laboratory conditions, one third of the lignin was metabolized in a six month period, and only one half in a year, the rate depending on the species of plant producing

the lignin (4).

Studies related to microbial degradation of lignin have also been extended to ligninsulfonates. In a recent study (23) enrichments from soil sludge samples resulted in the isolation of 32 yeast fungi, 23 mold fungi, and 5 bacteria which grew using a commercial sodium ligninsulfonate containing between 0.6 and 0.8 w/w of carbohydrate. Adsorption of the ligninsulfonate on the cells was obviated by repeated washing of the growth sediment with sodium hydroxide. Molecular weight fractionation indicated that growth occurred only on fractions below 5000 and that some of the growth occurred on non-aromatic functions. The highest overall growth response yields were obtained with Fusarium and Cephalosporium. No change in substrate 280 nm absorption was observed.

Marasperse CB, a commercial sodium ligninsulfonate, was used to determine growth response of acclimated soil bacteria (1). Several quantitative procedures were used to monitor ligninsulfonate utilization, and the study established that rates of removal were dependent on the quantitative procedure used. However, a finite ligninsulfonate decrease was attributed to metabolism. Again, chains appeared to be attacked rather than aromatic moeities. Inadequacies of analytical techniques to specifically monitor lignin breakdown and a lack of knowledge regarding the fate of sulfur in the sulfonate were

emphasized (1).

Further studies have emphasized the slow utilization rates observed. Analysis by ultraviolet spectrophotometry (3) indicated that sodium ligninsulfonate was 4 percent decomposed after 47 days when inoculated with several wood-rot basidiomycetous fungi, and a study using Biological Oxygen Demand (BOD)(55) showed less than 50 percent of the Kraft lignin effluent added to a river water was oxidized in one hundred days.

Continuous culture of sodium ligninsulfonate and an acclimated bacterial population for 10-15 days resulted in slight and variable ligninsulfonate decomposition using total carbon and sephadex gel filtration as the analytical techniques (45).

Thus the accumulated data relating microbial utilization of lignin and ligninsulfonates have shown consistently slow and variable rates of degradation. The very large amounts of ligninsulfonates produced compared to the small amounts used for industrial application would suggest that a method to modify the recalcitrant structure to one more readily attacked by microbial populations would be desirable. In this way, microbial growth could possibly occur in a shorter time with greater substrate incorporation into cellular constituents. This could then be used as a possible source of single cell protein as a food supplement. Other products also could possibly be

claimed such as chemicals or antibiotics and, of equal or greater importance, the pollutant would be eliminated from the environment.

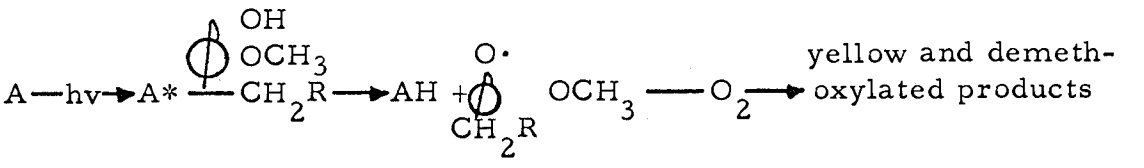
Ideally, molecule modification should not result in additional unwanted by-products, as given in many chemical processes. One such means could possibly be by photolysis whereby the energy input would be "pure" and once used not lead to unwanted chemicals since the energy of a photon is dissipated after collision with a bond.

Photolysis

Lignin and Other Structures

Lignified products such as newspaper and wood were yellowed during exposure to 355-400 nm light (41), and the rate of yellowing is related to demethoxylation, resulting from the photolytic degradation of lignin. The groups considered responsible for chromophoric formation are carbonyl, hydroxyl, and methoxyl moeities. Oxygen is an essential element for yellowing and if in high enough concentration during photolysis, will allow bleaching. Irradiation of the lignin-containing components in the presence of purified nitrogen, carbon dioxide or vacuum produces no visible yellowing and only a loss of 0.08 percent methoxyl content after 500 hours of exposure to ultraviolet radiation, whereas a control irradiated in air lost 0.43 percent methoxyl content after the same period of time. Acetylation

and methylation of phenolic groups reduced the yellowing of the lignin-containing components, thus indicating active phenolic participation in yellowing of lignin during photolysis. Acetylation and methylation apparently acted as blocking groups and prevented the phenolic groups from being photooxidized. It would appear that phenolic groups are the reactive sites for yellowing since yellowing and demethoxylation were apparent only when free phenolic groups are present. The postulated mechanism (35, 41) is one of phenolic hydrogen abstraction to form a free phenoxy radical. This radical could then form quinones, quinone methides, or cyclohexadienones by further degradation of the peroxy radical resulting in colored compounds:



"A" is an unidentified component which acted as a primary absorber of light and was not affected by acetylation or methylation showing lack of phenolic participation. "A" was photolytically destroyed only when free phenolic groups were present. The degree of sulfonation (41) of the lignin influences yellowing by ultraviolet irradiation, apparently by increasing the degree of chromophore formation.

Small amounts of vanillin and a high-molecular weight lignin degradation product extractable by ethanol have been detected (25)

after irradiating sprucewood meal for 710 hours with an ultraviolet lamp. The extractables amounted to 4.5 percent of the weight of the irradiated sample.

Further studies on the photolysis of other substrates not directly related to lignin or ligninsulfonates have added additional support for the use of this technique to modify recalcitrant structures. Mosier (47) irradiated DDT in hexane and as a solid with a 15-watt low-pressure ultraviolet lamp emitting most wavelengths at 253.7 nm. It was found that DDT was degraded to two components when solid and four components when dissolved in hexane.

Workers at the Midwest Research Institute (6) photolyzed highly nitrified sewage at a pH of 5 from a treatment plant with an ultraviolet lamp using chlorine and hypochlorous acid as the oxidants. The chemical oxygen demand (COD) was decreased 88 percent in 15 minutes. Different pH values altered the acid concentration and reduced the degradation rate.

Armstrong, Williams, and Strickland (7) irradiated sea water under oxygenation using a 1200 watt ultraviolet mercury-vapor lamp and were able to destroy all organic carbon present, forming carbon dioxide. Other organics were oxidized to completion, among those being humic acid. It was also shown that inorganic phosphate was liberated from RNA, and other organo-phosphate molecules. It was

concluded that organic compounds could be decomposed to carbon dioxide by ultraviolet irradiation.

Kujirai (40) irradiated cellulose films with 185.0 nm and 253.7 nm wavelengths. Oxygen was necessary for degradation at the 253.7 nm wavelength but not at 185.0 nm. The primary process, showing temperature independence, occurred at 185.0 nm with the formation of 2,3 dialdehyde groups from glucosidic linkage rupture. Photolysis at 253.7 nm was temperature dependent and was considered to be due to the effect of the light on the oxygen molecule which subsequently reacted with the cellulose, forming autooxidized cellulose peroxide which decomposed concomitant with direct ring scission at the glucosidic linkages. Wavelengths greater than 300.0 nm did not cause photodecomposition.

Chou and Jellinek (18) found that the viscosity of polymethacrylic acid decreased after irradiation with 253.7 nm radiation. The rate was pH dependent and was carried out under anerobic conditions by nitrogen gas purging. It was concluded that the carboxyl groups absorbed the light quantum producing carboxyl radicals and chain scission; the scission being dependent on radical production.

It was also found (34) that polyacrylonitrile was degraded randomly during irradiation at 253.7 nm, with degradation occurring in the presence and absence of oxygen. The intrinsic viscosity was

reduced and was dependent on irradiation time.

Irradiation of aldrin, dieldrin, and endrin (16), so-called refractory organic compounds, with 253.7 nm light reduced toxicity to fish fingerlings. These workers developed equations for photodegradation as a function of depth of solution, time of irradiation, and power. The reaction was temperature independent and was therefore considered a primary type where the rate was dependent on the amount of refractory compound present.

Gamma irradiation can also be used with a similar effect. Fleischman and Price (24), using 54,000 rads of gamma irradiation, destroyed 98 percent of an alkyl benzene sulfonate solution in approximately 40 hours. Ferrous ion increased the yield of primary radicals by reaction with hydrogen peroxide and hydroperoxides formed by photolysis of the water solvent. The free radicals formed then were able to cause autooxidation of the alkyl benzene sulfonate. Decreasing the pH, with a pH less than 3 optimum, increased the ferrous ion concentration and consequently the formation of hydroxyl and hydroperoxy radicals. Above pH 3, these radicals decomposed to a proton and oxygen radical, reducing the rate of alkyl benzene sulfonate degradation.

All of these studies demonstrated that a large array of different structures were altered physically after irradiation; the end

products being dependent on the imposed conditions such as wavelength, aerobiosis, pH and temperature.

Basic Photochemical Mechanisms

Relating irradiation to chemical alteration necessarily leads to the need for a basic understanding of wavelength-molecular interactions inherent in photochemistry. The two basic laws of photochemistry can be stated as: 1) only radiations which are absorbed by a reacting system are effective in producing chemical change, and 2) each molecule taking part in a chemical reaction induced by exposure to light absorbs one quantum of radiation, causing the reaction. The photo-excited species has to be more reactive chemically than the ground state to effect chemical changes (59).

Photoactivation occurs by the absorption of a photon or quantum of radiation (54) with the energy of the photon expressed as $E = h\nu$ where E = ergs, h is Planck's constant and ν = frequency in sec^{-1}. The proper wavelength of radiation for a photoprocess raises only the electronic energy of the molecule to a particular energy state resulting in free radicals, ions, or excited species (19). This does not disrupt the rotational or translational levels whereas thermal energy does.

Molecules absorbing radiation become excited and highly

reactive (48), and can take either primary or secondary reaction paths. There are three possible primary modes for an excited molecule to take: 1) form ground state products through a chemical reaction, 2) lose energy through radiation emission, and 3) lose excess energy by transfer to another molecule. Secondary photochemical reactions can be divided into approximately six categories, 1) elimination and decomposition, 2) additions and dimerization, 3) atomic abstraction, 4) rearrangements, 5) substitution, and 6) chain reactions. The quantity of energy necessary for bond rupture of most organic molecules ranges from about 1 to 5 ev (48), with differences in a given molecule of only a few tenths of an electron volt. However, the molecular decomposition mode is greatly influenced by these small differences in energy. This selectivity makes reactions possible that are thermodynamically impracticable.

The usual wavelength range for photochemical processes is between 200-700 nm (48). Electronic transitions occur within this range and are referred to as π-π^* and n-π^* excitations. The π-π^* excitation occurs when one electron is elevated to the π^* antibonding orbital. When the elevated electron retains its spin in the π^* orbitol it is termed a singlet state and when spin inversion occurs, it is termed triplet. Excitation from a singlet to a triplet state is forbidden from a quantum mechanics standpoint and must occur by a

secondary process (i. e. decay from a singlet state to a triplet state). The n-π^* excitation occurs with heteroatoms such as sulfur, oxygen, and nitrogen. The heteroatoms possess unshared electron pairs. Photolytic excitation can raise one of these electrons from the n level to the π^* level. Most organic molecules exist as the singlet in the ground level state. The n-π^* transition requires less energy than the π-π^* transition because of the non-bonded electrons. Fluorescence results from the reversion of the singlet π^* electron to the singlet ground level whereas phosphorescence occurs when a triplet π^* electron decays to the ground level.

Several commercial photolytic processes based on those principles are the production of aniline from benzene and ammonia, alkyl sulfonates from hydrocarbons and sulfur dioxide, and the formation of ascorbic acid (48).

MATERIALS AND METHODS

Substrate

All irradiation studies were carried out with Marasperse CB, a partially desugared and desulfonated sodium exchanged sodium ligninsulfonate (American Can Company, Nennah, Wisconsin). The following analysis was given by the company:

pH - 3% solution	8.5-9.0
Total sulfur as S (%)	1.70
Sulfate Sulfur as S (%)	trace
Sulfite Sulfur as S (%)	-
CaO (%)	0.20
MgO (%)	0.02
Na_2O (%)	10.00
R_2O_3 (%)	trace
Reducing Sugars (%)	none
OCH_3 (%)	12.64

Solutions of the sodium ligninsulfonate for irradiation had initial concentrations of 0.05 percent and 0.5 percent and were adjusted to pH 3, 7, and 12 during preparation of the solution using 1 N HCl or NaOH. Ten liter volumes were prepared in precalibrated carboys by dissolution of the sodium ligninsulfonate in approximately 9.9

liters of distilled water, pH adjustment, and final water addition to the required 10 liter mark.

Photolysis

Ultraviolet Lamp

A type "A" medium pressure 500 watt mercury-vapor lamp (model #673A-36, Engelhard-Hanovia Company, Newark, N. J.), powered by a transformer (model #20651-1, Hanovia Company), was used as the irradiation source. The lamp emitted approximately 29 percent of its radiation between 184.9 and 404.5 nm, 16 percent between 435.8 and 623.4 nm and 55 percent in the infrared region (54).

Immersion Well

A double wall quartz immersion well with a 60/50 male ground glass joint (model #19434 Engelhard-Hanovia Company, Newark, N. J.) was used to contain the lamp. The irradiated solution was circulated around the outside of the well. Coolant water to control the irradiated solution temperature was circulated through the double wall.

Solution Irradiation Vessel

The vessel with inlet tube at the bottom and outlet tube at the

top, was designed to fit externally to the immersion well and integrally with it by a 60/50 ground glass female joint. Oxygen capability was maintained through a fine-pore (4.0-5.0 μ) glass sparger fused in the bottom. The volume of the vessel was approximately 350 ml and integral hookup to an external reservoir connected to the outlet tube and pump connected to the inlet tube allowed any desired volume to be irradiated.

Pump

A centrifugal polyethylene-housing pump (Chemical Rubber Co., Cleveland, Ohio) circulated the sodium ligninsulfonate through the reaction vessel at 8 liters per minute.

Oxygenation System

Compressed oxygen gas (NCG) was passed through a needle valve and flowmeter (#10, Gilmont Instruments, Inc., Great Neck, New York) allowing flow rates of 0-90 ml/min. The oxygen was then passed through the fine pore glass sparger in the reaction vessel into the sodium ligninsulfonate solution.

Wavelength Filtering System

Filtering sleeves, fitting integrally around the lamp, were

made out of pyrex, absorbing all wavelengths of 280.0 nm and less, and vycor, absorbing all wavelengths of 210.0 nm and less. These values were confirmed using a Cary model 11 Spectrophotometer within the range of 190.0-900 n m. The sleeves were made by slicing 10.9 cm long, 2.7 cm I.D. x 3.1 cm O.D., pyrex and vycor tubes longitudinally, making a small cutout in one of the two halves to fit around the evacuation nib present on all lamps, and fitting around the lamp. Two additional sleeves were sliced in the same manner, but without the nib cutout, and placed around the first two, to cover the joints, giving total lamp coverage.

Procedures for Sodium Ligninsulfonate Irradiation

Sodium ligninsulfonate solution, 500 ml, was added to a 2 liter external reservoir and circulation commenced through the system. The desired oxygen flow rate, 5 ml/min was used in all cases except oxygen dependent studies, was equilibrated with the Marasperse solution for at least five minutes prior to firing the lamp. Coolant water was adjusted to give the desired sodium ligninsulfonate temperature during photolysis. Except for temperature studies, all irradiation was done with the solution at 37^{o}C. Foam accumulation in the reservoir was prevented by directing the incoming ligninsulfonate solution parallel to and below the surface of the solution where

rapid swirling action broke up the foam. When the optical density reached less than 0.5 there was no further foam formation.

Each irradiation was taken to a desired endpoint, the lamp turned off, the solution collected in a chemically clean dry flask, and stored at 4°C until further use. The entire system was then purged with three 1-liter washings of distilled water, air dried, and the next run commenced. After each complete irradiation of a series at a given concentration and pH the entire system was dismantled, cleaned in chromic acid, washed with detergent, rinsed in distilled water and dried.

Thin Layer Chromatography (TLC)

TLC was performed from ether extracts and 2,4 dinitrophenylhydazine derivaties of sodium ligninsulfonate. The ether preparations were made by extracting 100 ml of the ligninsulfonate with three 100 ml volumes of diethyl ether after acidification to pH 2 with 1 N HCl. Each extraction was done by vigorously mixing the two phases on a magnetic stirrer for two hours, allowing to separate, and decanting the ether layer into a glass stoppered collection flask. A total of 280 ml of ether was recovered. The ether was evaporated at 30°C in vacuo and the dry residue remaining was dissolved in 2 ml of 95 percent ethanol for TLC application. The 2,4 dinitrophenylhydrazine derivatives were prepared by mixing 5 ml of 0.4 percent

2,4 DNPH in 1 N HCl (67) with 5 ml of the ligninsulfonate sample, holding at 27°C for 24 hours, centrifuging and decanting the supernatant. The precipitate was washed with three 10 ml volumes of 1 N HCl and one 10 ml volume of distilled water. The precipitate was dissolved in 2 ml of methyl alcohol for TLC application.

Thin layer plates were prepared by spreading to a thickness of 0.250 mm a 50 percent W/V suspension of silica gel G (American Optical Corp., #08075) in water on 8" x 8" glass plates, allowing to air dry for two hours and activating the layer at 105°C for 30 minutes (55). About 5 mm of the silica gel was then trimmed off each edge of the plate, this resulting in a more uniform solvent front and better Rf reproducibility.

Solvents (55) were prepared in 100 ml volumes from benzene/dioxane/acetic acid (90:25:4) and benzene/methanol/acetic acid (45:8:4) and added to rectangular glass tanks closed with glass lids. Tank saturation was maintained by immersing two 7" x 7" and two 4" x 7" pieces of Whatman #1 filter paper in the solvent and placing against the sides of the tank with lower edge of the papers touching the bottom of the tank through the solvent. Five plates were run per solvent addition before renewing solvent and saturation paper.

Sample volumes of 20μl were applied 5 mm above the edge of the adsorbent and dried with forced air. Spots were not allowed

to exceed 5 mm in diameter. The ascending method of development was used. The solvent front was allowed to travel approximately ten centimeters above the edgeand the TLC plate taken from the tank and air dried for at least five hours in a fume hood before spraying.

The following spray reagents were used:

1. H_2SO_4 (concentrated). The plate was heated to 105°C after spraying.
2. 2,4 DNPH-A 0.4% solution in 1 N HCl was used to detect carbonyl group-containing compounds, resulting in yellow to red spots when present.
3. Methyl Red - This reagent was used for detection of carboxyl group containing compounds, red spots indicating acidic character.
4. Iodine vapor - Used to detect unsaturation resulting in brown spots on a yellow background.

Spectrophotometry

Chromophoric change of the irradiated sodium ligninsulfonate solution was determined using a spectrophotometer (spectronic 20, Bausch and Lomb) set at 430 nm. The initial 0.05 percent solutions were read directly but the initial 0.5 percent solutions had to be

diluted 10 fold until a direct reading of less than 1.0 optical density unit was attained after irradiation. However, to facilitate plotting of points all samples from the 0.5 percent solution were diluted 10 fold and recorded on the basis of the diluted sample value.

Total Carbon Analysis

Total organic carbon analysis (TC) was considered essential to determine the fate of carbon after photolysis and biological growth response. Carbon analysis capability was achieved by construction of a unit capable of handling one 20 μl sample every thirty seconds.

The parts list in order of their integration into the TC unit consisted of the following:

1. Oxygen flowmeter and needle valve (0-2.5 l min, FEP Co. No. 91-150-70)
2. Injection port and combustion tube containing asbestos and platinum mesh as catalysts (custom made) according to Minear (45).
3. Pyrometer Controller (Wheelco Instruments Div., Barber-Coleman Co., Model 293)
4. U-tube moisture condenser (custom made)
5. High efficiency small volume filter, particles filtered down to 0.3 microns, (Mine Safety Appliance Co.,

Pittsburgh, Pa.)

6. Infrared analyzer (LIRA, Mine Safety Appliance Co., Pittsburgh, Pa. Model 300, standardized for 0-600 μg/ml carbon as CO_2.)
7. Servo-Riter recorder (Texas Instrument Co., Inc., Huston, Texas, 0-5 MV input, Model PWD)
8. Electric furnace (Hevi-Duty Electric Co., Milwaukee, Wis., Type 77)
9. Cabinet (custom made)

Combustion in the furnace to carbon dioxide was done with a flow of 500 ml 0_2/min and a temperature of 950°C. The asbestos and platinum surfaces tended to catalyze the reaction and the platinum provided oxidation of any difficulty oxidizable compounds (45).

Standards were prepared from acetic acid to represent 10-1000 μg/ml carbon by weighing 2.500 grams of glacial acetic acid to the nearest 0.1 mg and diluting to 1000 ml with CO_2 free distilled water. This gave the equivalent of 1000 μg/ml of carbon. Serial dilutions were prepared from this stock to give 100 μg/ml decrements to 100 μg/ml then 10 μg/ml decrements to zero. A standard plot of points was made of μg/ml carbon versus LIRA meter reading. A linear response was obtained between 50 and 900 μg/ml. When possible samples for combustion were diluted to a total carbon

concentration of between 100 and 500 μg/ml.

A warm-up period of at least two hours was needed for LIRA unit and recorder stabilization. The furnace was maintained at 950°C with total combusion being independent of temperature between 900°C-1000°C. Also, flow rates between 500 and 1000 ml were optimal and peak height was independent of flow within this range. Twenty microliter samples were injected using a 3-inch long 22 gauge needle on a Hamilton 50 μl syringe. The needle was inserted through the injection port needle and a rapid syringe plunger depression made, followed by immediate capping of the injection port after needle withdrawal. Flow through time of the sample from injection to peak formation and final return to zero vaied with flow rate, being about 1 minute at 500 ml/min O_2 and 30 sec at 1000 ml/min. Five injections were made per sample and the peak heights averaged.

Evaluation of Fungal Growth Response

Mineral Salts Solution

A basic mineral salts solution was prepared to result in concentrations of phosphate, nitrogen, iron, magnesium and sulfate to give adequate growth levels, but contain no carbon. The concentration of the solution was made so that 1 liter of the final growth medium would contain the following; sodium nitrate, 3.0 grams, magnesium

sulfate, 0.5 grams, potassium phosphate (dibasic) 1.0 gram, and ferrous sulfate, 0.01 gram. This was accomplished by doubling the weight of each salt and dissolving in enough distilled water to make 100 ml. The growth system consisted of 5 ml of the concentrated mineral salts solution and 95 ml of ligninsulfonate solution. This gave adequate mineral nutrients without excessive dilution of the lignin sulfonate substrate.

Enrichment for Fungal Response

Agar pour plates were prepared from 5 ml of concentrated mineral salts and 95 ml of 0.5 percent non-irradiated and 10 hour irradiated sodium ligninsulfonate solution that had a pre-irradiated pH of 7, adjusted to pH 5 with 0.1 N HCL or NaOH and dissolution of 3 grams of agar at 70°C. An inoculum of 0.01 ml of an aqueous suspension of soil was spread over the surface of each plate and allowed to incubate at 27°C. Heavy fungal growth was present after four days on the irradiated ligninsulfonate plates and minimal growth on the nonirradiated samples, with one morphologically-distinct type predominating over several others. The major varieties of fungal-like growth were transferred to Czapek's Solution Agar (Difco) for monoculture preparation. The predominate fungal growth was isolated in monoculture after one transfer and was kept on Czapek's Solution

Agar slants as inoculum source. The fungus has been identified as belonging to the form-genus Aspergillus (65) and further keying-out is in progress. An ATCC number of 20243 has been assigned to the fungus. However, the number originally given to the fungus after monoculturing was A-17-30 and will be carried in this thesis.

Aspergillus Conidial Inoculum Preparation

Czapek's Dox Broth (Difco, B338) was inoculated with a seed of the Aspergillus growth from Czapek Solution Agar. The culture was statically incubated at 27°C for one week for development of a well-formed mycelial mat. Abundant conidia were produced during this time. Conidia were collected by using a sterilized fine-pore fritted glass sparger under negative pressure acting as a "vacuum cleaner", which was passed very close to and over the surface of the mycelial mat while gently agitating the mat with a sterile glass rod. The conidia were collected on the surface of the sparger in large numbers and finally suspended in 50 ml of sterile distilled water and stored at 4°C. This technique resulted in an inoculum source free from residual nutrients.

Each ligninsulfonate growth system was inoculated by immersion of a flame sterilized 0.01 ml platinum wire loop in the aqueous spore suspension, followed by transfer to the growth medium.

The spore count was maintained at approximately 10^8 per ml. Thus approximately 10^6 spores were added per inoculum loop.

Sodium Ligninsulfonate Growth System

Fungal growth response to non-irradiated and irradiated sodium ligninsulfonate was performed on initial 0.5 percent marasperse CB. The solutions, after mineral salts addition and pH adjustment to 5.0 were filter sterilized and aseptically split into five 50 ml volumes, transferred to sterile 125 ml Erlenmeyer flasks, and closed with sterile plastic foam plugs. Four of the five volumes were inoculated, saving the 5th as a noninoculated control. Distilled water blanks were prepared in a similar manner.

After inoculation the flasks were rotated at 250 RPM to ensure adequate aeration at $27^{\circ} \pm 1^{\circ}C$ for seven days. The solid contents of each flask were collected on tared, glass filter discs (S & S No. 24) held in a borosilicate filter holder (VW & R #28153020). The supernatant was collected in chemically clean, dry vacuum flasks and transferred to screw cap tubes for further analysis. The growth flask was then washed free of any adhering solids with 50 ml distilled water and the total filter collected solids washed with 500 ml of distilled water. The filter discs and collected residue were dried to constant tare at $105^{\circ}C$ and weighted. Growth responses based on 50 ml

culture volumes were expressed on the basis of fungal dry weight per ml of growth medium. The utilization coefficient (15) was calculated as the mg/ml of fungal dry weight produced per mg/ml of carbon removed from the growth system.

RESULTS

Irradiation effects on a 0.05 percent sodium ligninsulfonate solution, initially at pH 7, with wavelength transmission through quartz, is shown in Figure 1. Oxygen availability greatly influenced the decoloration rate. Essentially no decrease in absorbance was noted without oxygen flowing into the solution. However, the slight decoloration noted indicated that some type of photolytic action was taking place, possibly as a result of atmospheric oxygen dissolved in the solution through circulation.

Increasing the oxygen flow to one ml/min resulted in a sharp initial increase in absorbance, which reached a maximum thirty minutes after irradiation, then decreased, indicating destruction of chromophoric structures absorbing at 430 nm.

The character of the decoloration curve changed when oxygen was increased to 5 ml/min and greater. The increased absorbance noted at 1 ml/min disappeared. The slopes of the curves increased also with increased oxygenation, reducing the time necessary for decoloration. The effect of gaseous oxygen on the decoloration rate appeared to approach a maximum value at 40 ml/min under these test conditions. The use of 30 percent hydrogen peroxide showed that the decoloration rate could be greatly increased above that of the gaseous oxygen system. This observation indicated that oxygen availability

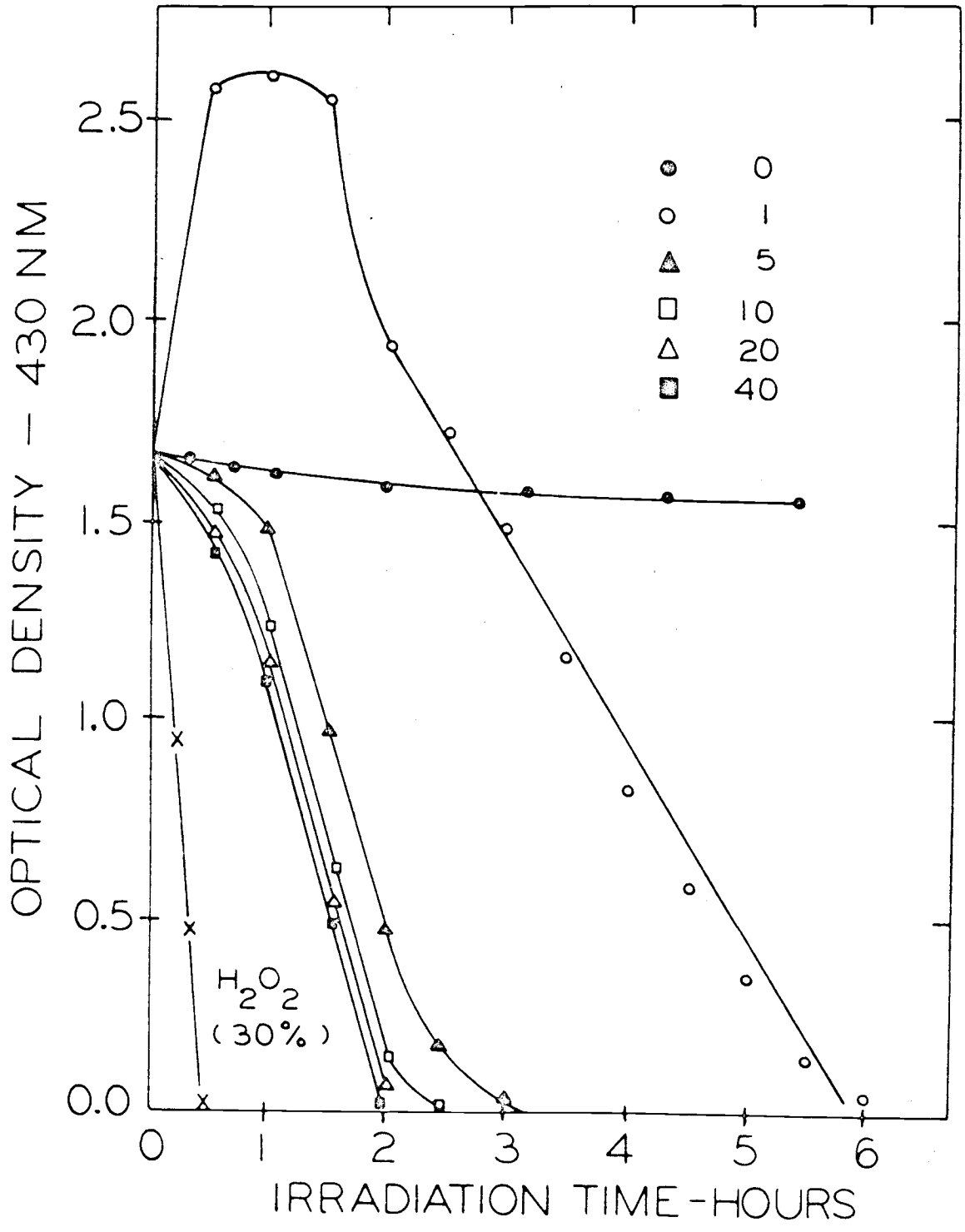


Figure 1. Effect of oxygen flow rate (ml/min) on decoloration of 0.05 w/v sodium ligninsulfonate solution

and form, whether diatomic, in the gaseous state, or nascent from the hydrogen peroxide, influenced the chromophoric loss.

pH Effects

The initial starting pH of the sodium ligninsulfonate had a marked effect on the rate of decoloration during irradiation. Figure 2 shows the optical density relationships for pH values 3, 7 and 12. Solutions decolorized markedly faster at pH 3 and 12 than at pH 7. At pH 12 the solution had a higher initial absorbance because of ionization of the phenolic hydroxyl groups to phenoxy ions (52), but still required the same length of time to be decolorized as the pH 3 solution.

At pH 3 and 12 decoloration was linear from time of irradiation initiation but at pH 7 exhibited an initial lag phase. The pH values of all solutions varied as irradiation time increased. The pH 3 and 12 solutions decreased linearally to final values of 2.5 and 10, respectively. However, the pH 7 solution exhibited a sigmoidal shape with the inflection point at one hour of irradiation. The pH value then reached a minimum value of 3.2 at two hours of irradiation increasing to a value of 4.5 at the end of the three hour irradiation period.

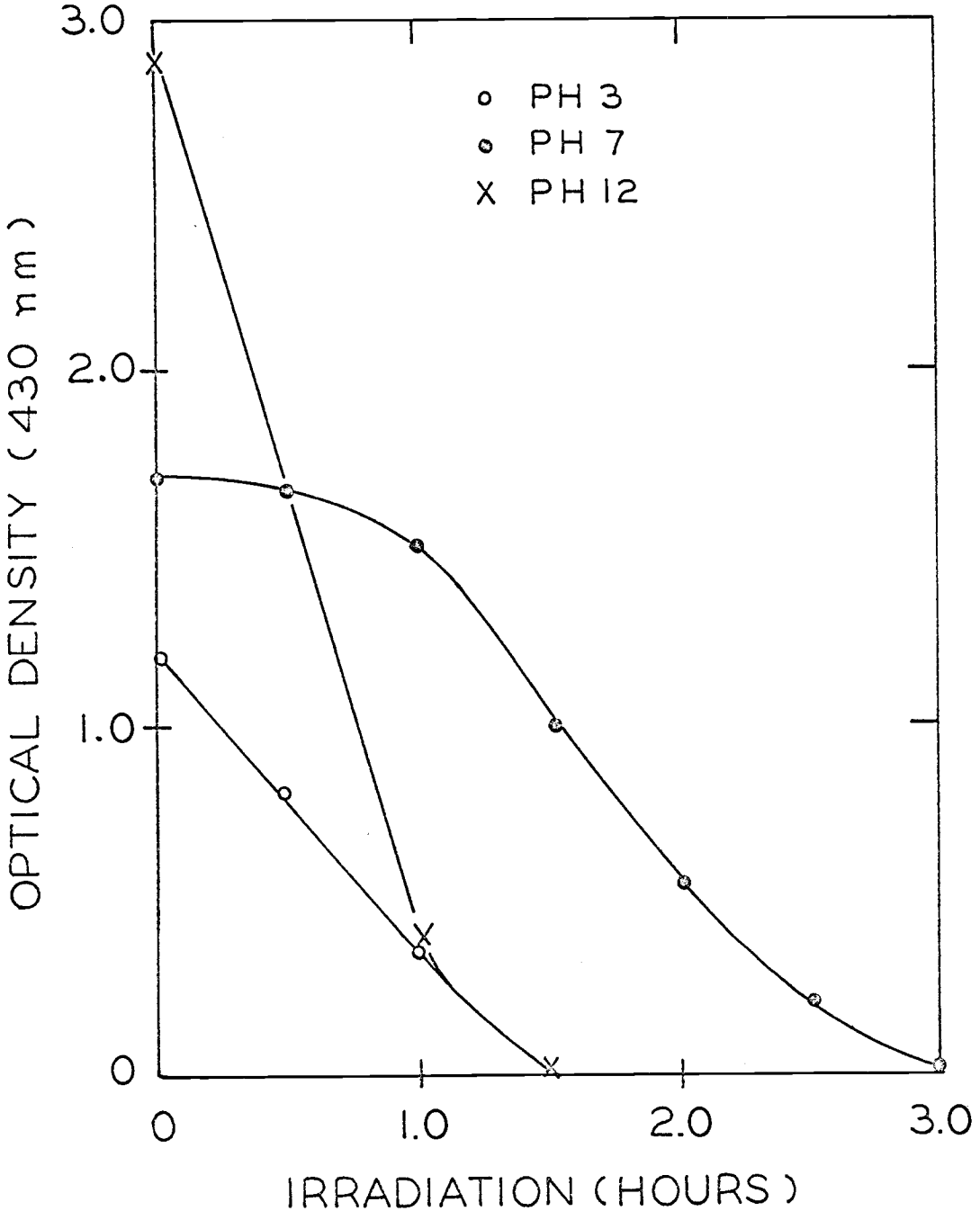


Figure 2. Effect of initial pH on decoloration of 0.05% w/v sodium ligninsulfonate

Wavelength and Temperature Effects on Decoloration and Total Carbon

Figure 3 shows that filtering out wavelengths of 280.0 nm and less essentially eliminated decoloration through the six hour irradiation interval tested for the 0.05 percent w/v ligninsulfonate sodium. An optical density decrease of about 0.2 units was observed and the gradual slope of the line would suggest that total decoloration would eventually occur. However, the total carbon content did not change from the initial value, indicating that while decoloration took place, total carbon was not lost from the system.

When wavelengths of 210.0 nm and less were filtered, using vycor, Figure 4, a greater decoloration rate compared to the pyrex filtered solution was noted. Total carbon also decreased after a lag of two hours, indicating loss from the solution, possibly as carbon dioxide. Under these conditions, the total carbon loss began at approximately the same optical density reached by the pyrex filtered solution after the six hour period.

When no filter was used, Figure 5, extending the effective wavelength range to 184.9 nm, a marked increase in decoloration rate and total carbon loss was noted. Approximately sixty-five percent of the original carbon was lost from the system after three hours of irradiation. Again the beginning of total carbon decrease

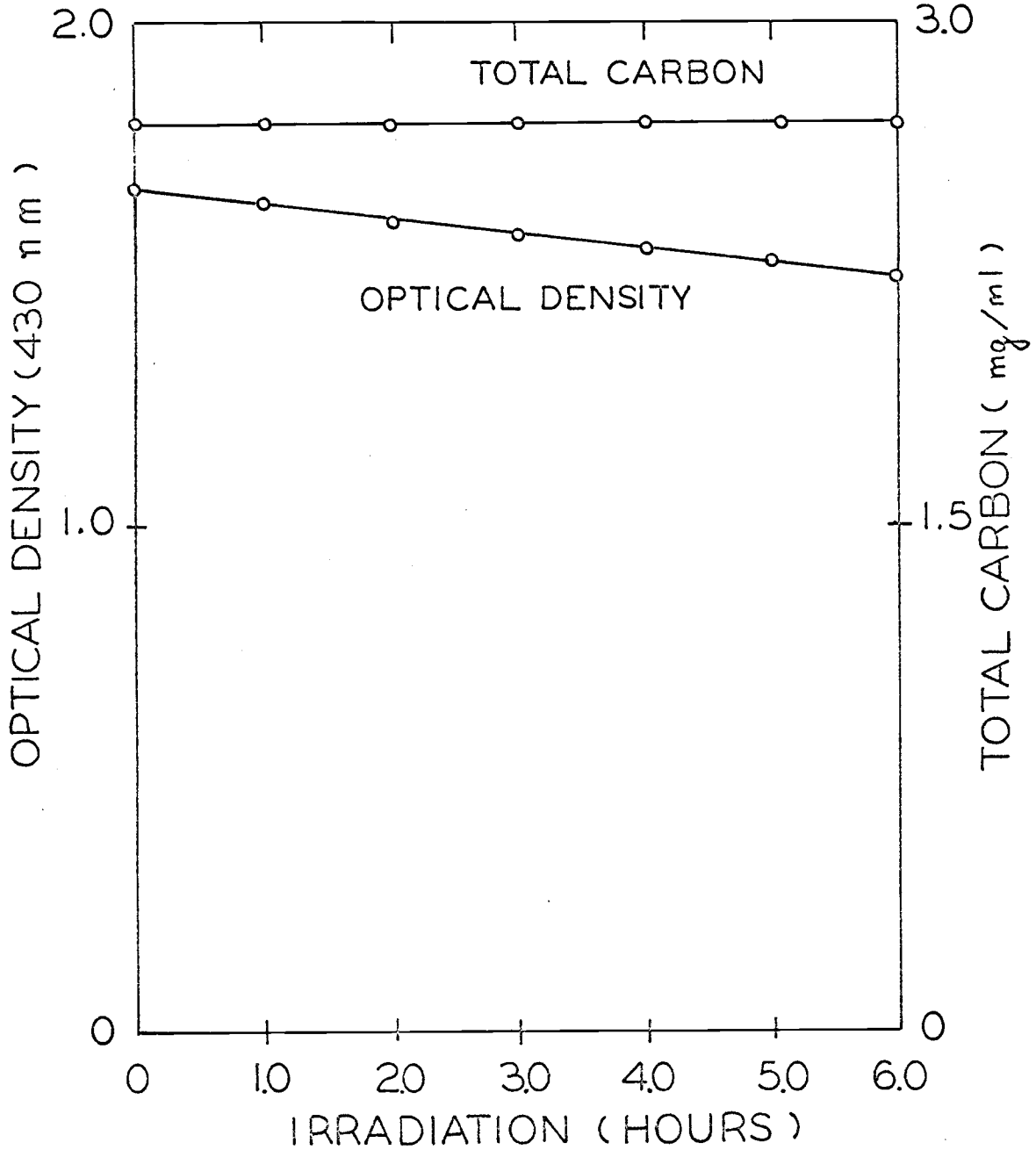


Figure 3. Effects of wavelengths longer than 280.0 nm on decoloration and total carbon of 0.05% w/v sodium ligninsulfonate solution

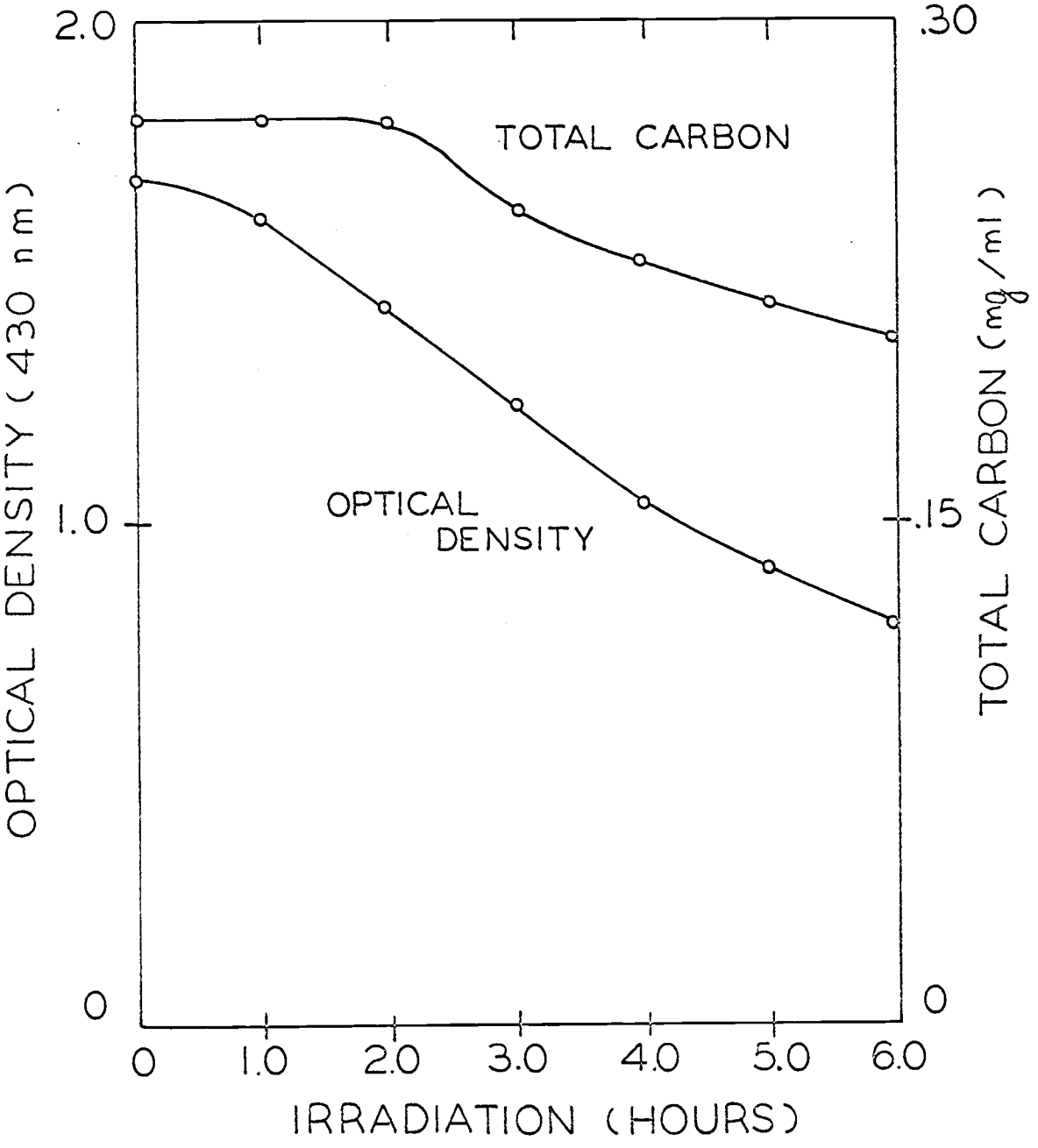


Figure 4. Effects of wavelengths longer than 210.0 nm on decoloration and total carbon of 0.05% w/v sodium ligninsulfonate solution

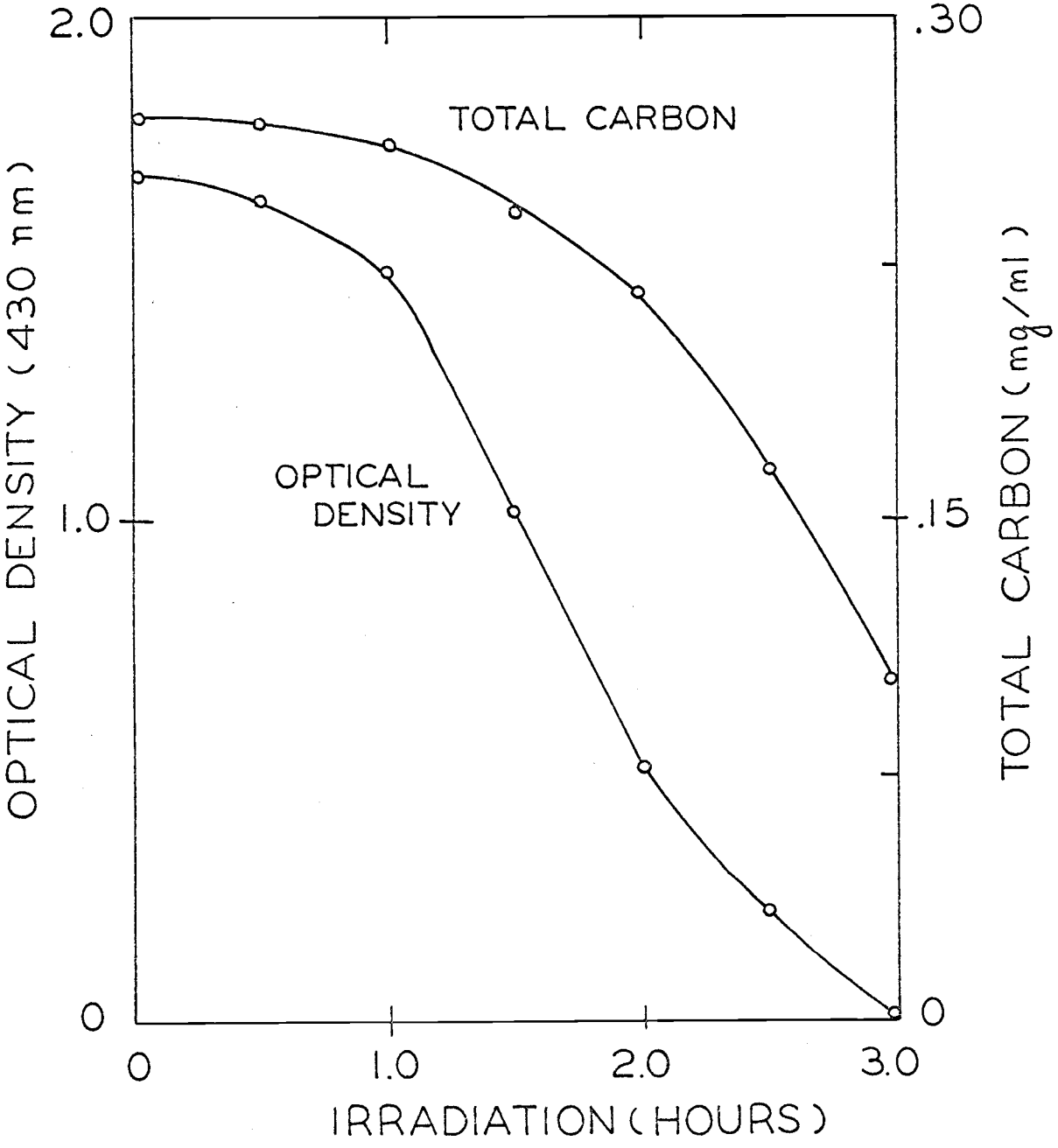


Figure 5. Effects of wavelengths longer than 184.9 nm on decoloration and total carbon of 0.05 w/v sodium ligninsulfonate solution

occurred at approximately the same optical density as with the vycor filtered solution.

Studies of temperature effects on photolysis rates, using quartz transmission, at 37, 42, 55, and 90°C showed no change in decoloration or total carbon concentration.

Fungal Growth Responses

Aspergillus A-17-30 growth responses to 0.05 percent w/v solutions phototreated at the initial pH values of 3, 7, and 12, respectively are given in Figures 6, 7, and 8, and are expressed in relation to photolysis time. Fungal dry weight response in relation to total carbon levels before and after growth, the optical density of the solution, and the utilization efficiency, or yield of fungal dry mass in relation to the loss of carbon from the medium are considered, in addition to pH and optical density.

Figure 6 shows the growth response of the Aspergillus isolate to ligninsulfonate photolysis, using a solution with an initial pH of 3.0. Under these conditions, maximal growth response occurred after achievement of decoloration, at approximately 15 hours of photolysis. At this time the total carbon decreased from an initial level of 2.5 mg/ml to approximately 0.97 mg/ml at the 15 hour time and carbon loss was linear after the initial period, which correlated with

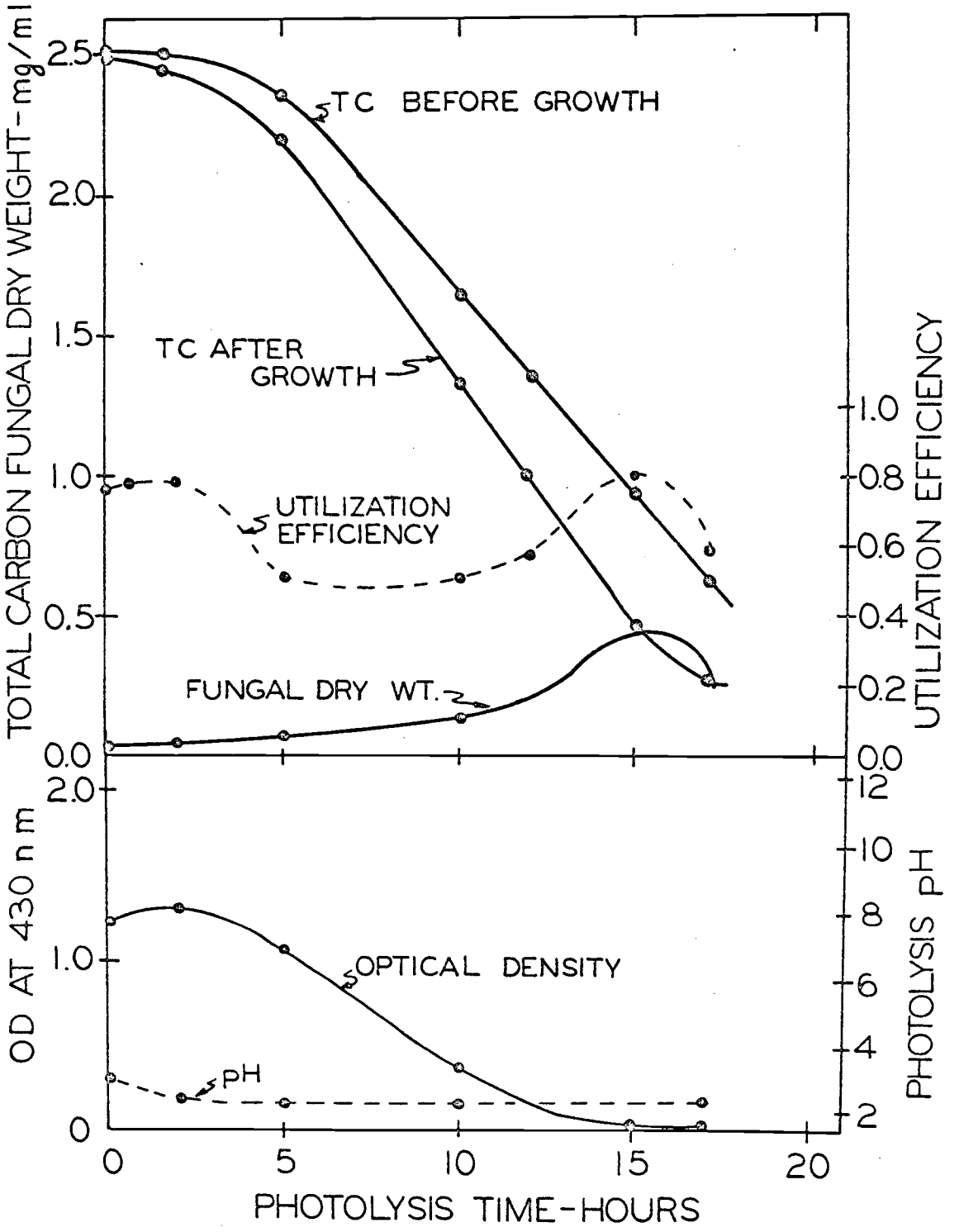


Figure 6. Fungal growth response to initial pH 3.0, 0.5 percent w/v, sodium ligninsulfonate solution

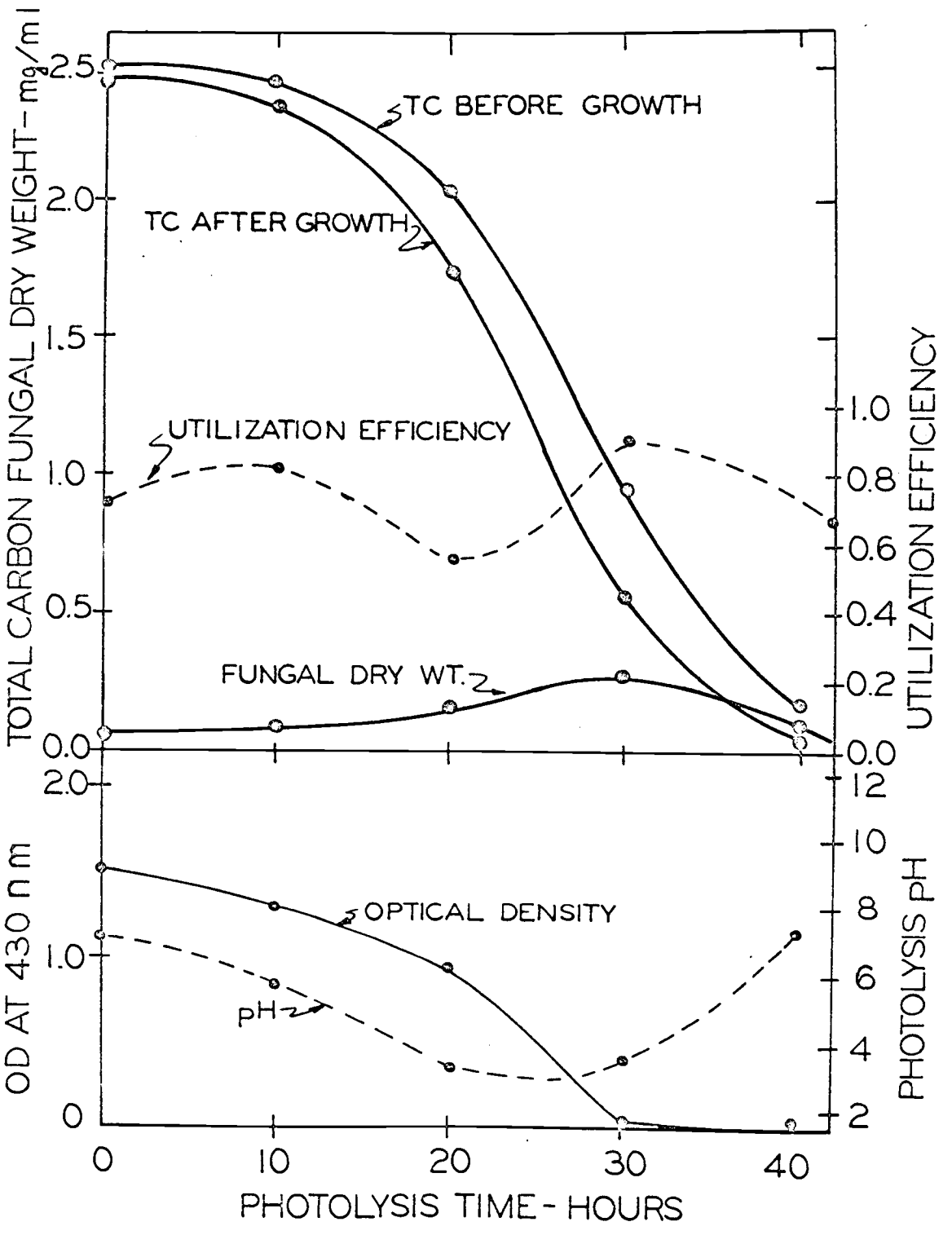


Figure 7. Fungal growth response to initial pH 7.0, 0.5 percent w/v, sodium ligninsulfonate solution

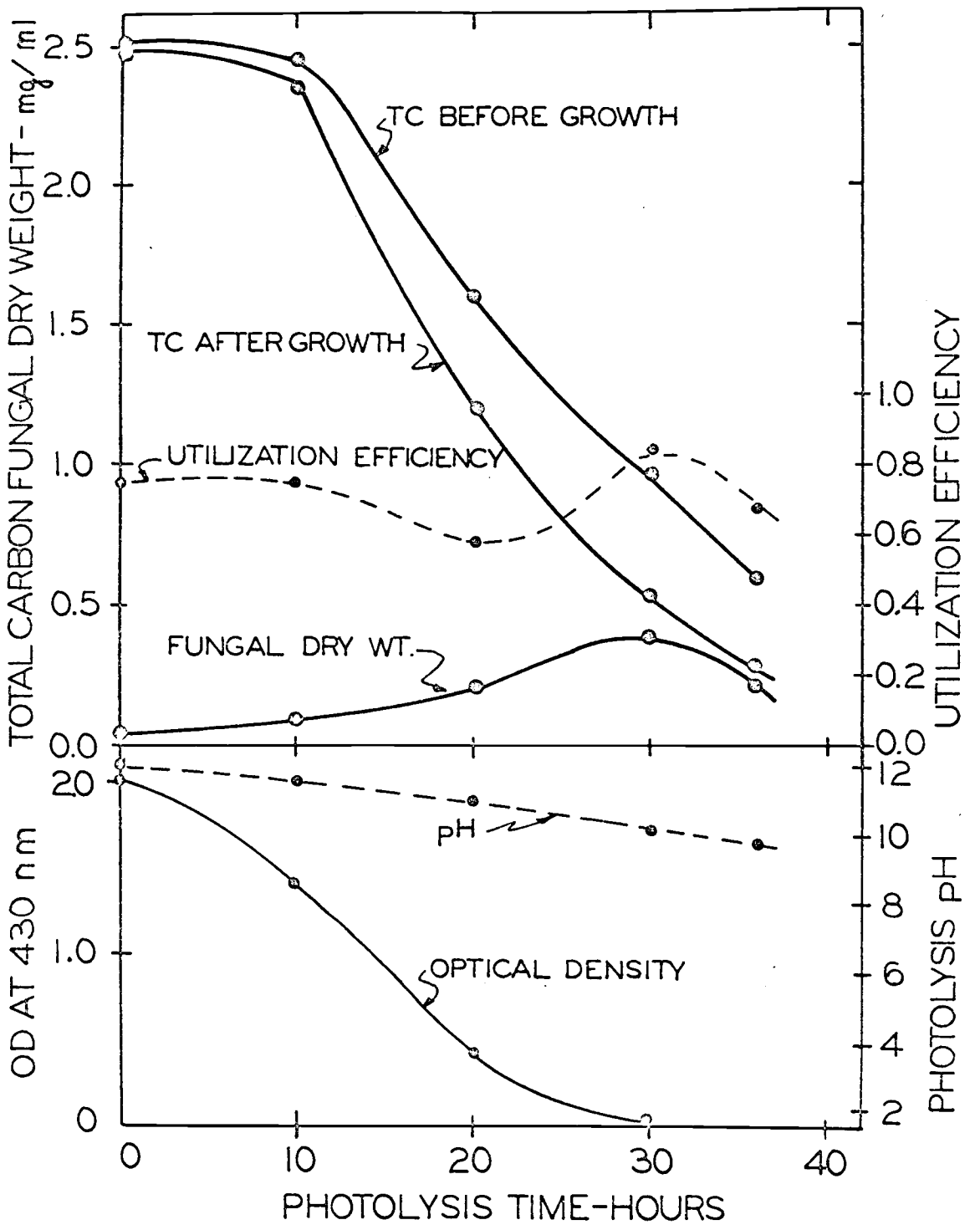


Figure 8. Fungal growth response to initial pH 12.0, 0.5 percent w/v sodium ligninsulfonate solution

achievement of a stable pH value of 2.3. However, under these conditions approximately 55 percent (0.5 mg/ml of the residual carbon was capable of being utilized by the fungal isolate, yielding a final fungal dry weight of 0.39 mg/ml. On the basis of the zero time results, only 0.05 mg/ml of the original carbon was available for transformation to fungal cell mass, implying that the ligninsulfonate biological availability had been increased approximately ten times, even with the loss of a significant portion of the original substrate carbon in photolysis, presumably as carbon dioxide. The utilization coefficient indicates a higher level of substrate conversion to cell mass during the initial period, and the latter period after decoloration of the substrate.

The photolysis of an initial pH 7 solution is shown in Figure 7. Under these conditions, the time required before achievement of decoloration was approximately 30 hours, and during this period the pH was observed to undergo a shift to the acidic range followed by an increase to pH 7.5 after decoloration. During this entire photolysis process, the total carbon level was observed to decrease, but without the rapid achievement of straight-line conditions observed with irradiation at pH 3. As observed earlier, the point of maximal fungal response was at the point where the solution had become decolorized. The maximal final yield observed, approximately

0.27 mg/ml, was not as high as the levels achieved with the pH 3 solutions. With the pH 3 system, at the point of decoloration, the system still had approximately 50 percent of the original carbon remaining, while in the pH 7 system, only approximately 40 percent of the carbon was retained, perhaps resulting in the somewhat lower total yield of fungal dry mass observed with the pH 7.0 irradiation.

The results for irradiation at an initial value of pH 12 are given in Figure 8. Irradiation under these conditions allowed a retention of approximately 50 percent of the original carbon at the time of decoloration. The fungal growth response, being approximately 0.37 mg/ml at the maximum level, also indicated the increased ability of this substrate to support fungal growth, being comparable to pH 3 photolysis, althoug requiring approximately twice the photolysis time to achieve a substrate having the same level of growth response. As in the earlier photolysis experiments at pH values of 3 and 7, the utilization coefficient also shows a maximal reponse at the initiation and termination of the photolysis period.

Thin Layer Chromatography

Thin layer chromatography using the benzene/dioxane/acetic acid solvent and sulfuric acid char for spot detection of the pH 3.0, 0.5 percent w/v, ligninsulfonate solution irradiated through

a 17 hour period showed zones at Rf values of 0.00 - 0.43, 0.49, 0.64, and 0.77 in the nonirradiated control and an additional new zone at 0.86. This latter zone had formed by five hours of irradiation. The 0.00 - 0.43 streak decreased in intensity by 10 hours of irradiation, becoming a 0.00 - 0.31 streak at 12 hours and was not detectable at the 17 hour assay. After 1.5 hours of irradiation, the 0.49 zone was eliminated and the 0.64 and 0.77 zones increased in intensity. These latter two zones reached a maximum intensity at 15 hours of irradiation decreasing at 17 hours. The 0.86 zone reached a maximum intensity at 15 hours of irradiation then decreased at 17 hours.

Yellow zones were detected at Rf values of 0.00 - 0.43, 0.00 - 0.31, 0.64, and 0.77 when a developed TLC plate was sprayed with 2,4 dinitrophenylhydrazine, indicating carbonyl containing components. These spot intensities followed the same pattern as the sulfuric acid charred TLC plate. The zone at 0.86 gave a red color when sprayed with methyl red, indicating an acidic component and all zones responded for unsaturation in iodine vapor. Parellel chromatohraphy of fifty phenolic compounds (56) considered related to the basic monomeric building blocks of the lignin molecule did not agree to any of the above Rf values.

TLC using the benzene/methanol/acetic acid solvent of the

hydrazones from the nonirradiated control resulted in four detectable zones at Rf values of 0.00, 0.09, 0.38, and 0.66 and the formation of two new components, one at Rf value of 0.19 at five hours of irradiation and the other at 0.04 at 15 hours of irradiation. The material at 0.00 remained dense to 15 hours of irradiation, decreasing in intensity after this time. The 0.09 and 0.19 zones increased to the 17 hour sample, while the 0.38 component increased throughout irradiation. The 0.66 zone agreed with the 2,4 dinitrophenylhydrazine used to form the hydrazone derivatives. The 0.04 zone was of decreased intensity at 17 hours of irradiation.

After growth of the Aspergillus isolate, definitie changes in TLC - detectable components were observed, both by 2,4 dinitrophenylhydrazine derivatization and ether extraction.

Derivatization with 2,4 DNPH followed by TLC showed that spots with Rf values of 0.00, 0.04, 0.09, 0.19, and 0.38 were not detectable after growth. The 0.66 spot remained at the same intensity before and after growth response. Ether extracts of the growth systems, TLC, and sulfuric acid char or 2, 4 DNPH spray showed that spots with Rf values of 0.49, 0.64, 0.77, and 0.86 were not detectable after growth response. The 0.00 - 0.43 and 0.00 - 0.31 streaks also were very light after growth.

DISCUSSION

In comparing the relationship of wavelength effects to decoloration rates, information on the spectral properties of light from the mercury arc lamp should be considered. With use of the pyrex blocking filter, the first major spectral line available from the lamp would be at 280.3 nm (54), and based on quantum energy calculations as related to wavelength (59) would contain sufficient energy to rupture the common bonds found in the lignin molecule, such as C-C, C-O, and C-H. Based on physical data, the energies necessary to rupture these bonds could be given by wavelengths as long as 328 nm. Considering the energy distribution of this type of lamp (54) approximately 6.9 percent of the spectral output lies below 280 nm, and the remaining longer wavelengths, although of lower energy potential, have an ability to slowly degrade the ligninsulfonate solution. Oxygen, requiring approximately 34.9 Kcal/mole for activation, is readily influenced by wavelengths as long as 819 nm. Thus, plentiful wavelengths are available for activation of this species.

The total power output below 210 nm, as blocked by Vycor, is 0.53 percent (54). However, the energy found in the 184.9 nm mercury vapor lamp spectral line is great enough to rupture any bond to be found in the ligninsulfonate molecule. Although wavelengths above

280 nm result in chromophore group cleavages, the additional added energy of wavelengths below 280 nm and less results in a much faster rate, probably by causing many secondary reactions not apparent at longer wavelengths.

Further modifications and improvements in photodegradation of ligninsulfonate will be derived from the use of monochromatic light sources active in the 185-210 nm region which would allow maximal decoloration and depolymerization while retaining a greater portion of substrate carbon than is now possible.

As discussed by Marcus, Kent and Schenk (43), the use of monochromators for the production of an efficient narrow spectrum photolytic input from mercury arc light sources is not considered to be practical in development of large scale or commercial processes. However, there are several potential sources of monochromatic light which should be considered in the future for the production of spectrally uniform light in the ultraviolet lasers (60) and of perhaps greater interest, the use of microwave-driven inert gas ultraviolet-emitting devices (5). These latter devices appear to be tunable, and can have energies in the UV region which can be higher than those available with conventional sources.

Photooxidation appears to be limited by the availability of molecular and other activated oxygen species capable of combining

with the substrate after quantum absorption (48). The observation that photolysis decoloration rates are accelerated in the presence of hydrogen peroxide indicates that under the test conditions used, gaseous oxygen may not be the most efficient oxidant. There is, however, the possibility that sensitization as with ferric ion and uranyl salts (7) may be capable of accelerating this process. In regard to treatment of ligninsulfonate, use of the atmosphere as a source of oxygen would be desirable from a process development viewpoint.

Photolysis itself is not a limiting factor because energy is available, when the correct wavelength is used, to cleave any organic bond. The problem arises in integrating a group of parameters such as election acceptors (oxygen, organic, inorganic), irradiation time, and concentration and pH values of the irradiated solution in such a way that optimum efficiency during photolysis is maintained.

Prior investigations have shown that anerobiosis during ultraviolet irradiation at wavelengths near 460.0 nm prevented formation of chromophoric groups in newspaper (66) and ground pulp wood (41, 42). However, under aerobic conditions, chromophores formed from quinones, quinone methides or cyclohexadienone lead to yellowing (39, 41, 42, 66). The mechanism was one of hydrogen abstraction from the hydroxyl groups and oxidation of the resulting phenoxy radicals

to peroxy radicals followed by decomposition of the peroxy radical to these colored compounds. Evidence to support this mechanism came when acetylation (17, 42), benzoylation (53, 62), and methylation (53) of free phenolic hydroxyl groups acted to block the formation of phenoxy radicals during photolysis, eliminating yellowing.

Thus it appeared that the chromopore groups being formed at 1 ml/min oxygen flow might have been generated via the phenoxy radical.

Although detailed mechanistic information regarding lignin photodegradation is lacking, information on lignin chemical oxidation may aid in elucidating ligninsulfonate decoloration reactions. The energy input is different in each case however, carboxyl and carbonyl groups are formed during both photolysis and chemical oxidation (8) of ligninsulfonate. Acidic and carbonyl function formation in this study was fully demonstrated during photolysis since one new acidic and two new carbonyl components were detected. The decrease in pH of the photo-oxidized ligninsulfonate solutions also suggested that acidic components were formed.

The effects of pH on ligninsulfonate photolysis and growth responses observed in this study, substantiate, for this substrate, prior observations of irradiation and pH effects observed with other materials (24, 28). As it would appear that a low pH facilitates process

speed, substrate activation and carbon retention, it may be possible to combine these conditions with either fungal or yeast growth systems to avoid the need for excessive pH adjustment after phototreatment.

Although only preliminary observations have been made, there are indications that the pH decreases observed during photolysis may in part be due to modification or cleavage of sulfonate functions. As the sulfonate function has been implicated in the formation of a recalcitrant molecule (4), such modification could play a major part in preparing this substrate for microbial use.

Since growth response was at a maximum value at the point where low optical density values were first achieved, and more organic carbon was saved in the pre-irradiated pH 3 and 12 solutions than at pH 7.0, it would appear that maximizing organic carbon retention after essentially complete decoloration facilitates growth response of the Aspergillus.

The utilization coefficient provies a means of determining the ratio of substrate removed from the medium expressed as carbon, to that transformed to total dry weight cellular material. The relatively high utilization coefficients of the initial solutions probably result from assimilation of low molecular weight components present in the original wide-ranged molecular weight sodium ligninsulfonate.

By the first assay after initiation of irradiation, and until establishment of stable photolysis conditions these components are still present giving growth response at about the same rate as the nonirradiated control. This first period, in all cases, is where carbon loss has not reached maximal rates. During the period of maximal carbon loss rate, and until completion of decoloration, the data tend to indicate an interval during which the utilization coefficient is not as high, usually being in the range of 60 to 65 percent. Only as irradiation continues beyond decoloration do the utilization efficiencies again approach 80 percent. The physiological significance of varied substrate conversion efficiences in relation to photolysis time must be better understood, and will be of importance in possible development of single cell protein production processes, where maximum cell mass yields must be maintained.

An achievement of a more complete understanding of the role of oxygen and oxygen activated species in the photolysis, and of specific wavelength effects on ligninsulfonate structure will allow development of more efficient photolysis and photomodification conditions, to maximize carbon retention during photolysis. To achieve such an understanding, factors influencing the photochemical yield and the absorption coefficient of ligninsulfonates must be elucidated.

Based on the present study, the use of photolytic modification

for improving the biological availability of ligninsulfonates warrants further investigation, and this treatment approach should be considered for use with other recalcitrant materials.

SUMMARY

A commercial sodium ligninsulfonate, Marasperse CB, was phototreated using a mercury-vapor ultraviolet lamp. Parameters studied during photolysis were oxygenation effects, initial starting pH, temperature, and wavelength ranges. The photolyzed ligninsulfonate was then used as a growth substrate for a soil isolate of the form-genus Aspergillus.

Definitive improvement of ligninsulfonate biological availability was demonstrated after photo-treatment of initial pH 3, 7, and 12 solutions. The response of the Aspergillus isolate was substantiated by assays of substrate carbon before and after growth, dry weights in relation to photolysis treatment time, and semi-quantitative thin layer chromatography. Substrate utilization efficiencies, the ratio of fungal dry weight to carbon used during growth, were highest at the beginning and end of photo-treatment with fungal yields being greatest after essential decoloration of the substrate. The highest fungal yield developed from the initial pH 3 solution followed closely by the initial pH 12 solution. The pH 7 solution gave lower cell yields. The greatest loss of carbon after growth occurred in the pH 3 and 12 solutions. Thin layer chromatography showed that during irradiation three new components were formed and three existing components increased in concentration as irradiation times

increased. All of these compounds were reduced or disappeared entirely after exposure to the fungal culture.

Carbon retention after irradiation was greatest in the initial pH 3 and 12 solutions. With photolysis occurring most efficiently in the pH 3 solution with regard to time, fungal yield, carbon loss after growth, and substrate utilization efficiency. Wavelengths less than 210 nm allowed the most rapid modification with wavelengths greater than 280 nm causing essentially no short-term change. Ligninsulfonate photolysis was found to be temperature independent, and oxygen dependent, showing a true primary photodecomposition mode.

Based on the present study, the use of photolytic modification for improving the biological availability of ligninsulfonates warrants further investigation, and this treatment approach should be considered for use with other recalcitrant materials.

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