Straw has many uses, as feed, roughage, animal bedding, but these requirements are not sufficient to utilize the available production. In areas where grass seed is produced, post-harvest straw is burned in the field to help prevent crop disease the following year. This treatment is very wasteful in terms of straw utilization and also produces considerable air pollution. Straw can be incorporated in the soil, but this process requires added nitrogen to prevent reduced crop productions the following year. Obviously, new uses are needed to assist in the profitable elimination of excess straws.

One possibility would be to alter the straw photolytically to produce a material more readily decomposable by soil or rumen microorganisms. Increased microbial utilization would rapidly return to the soil many basic chemical components taken out during crop production. If the level of energy derived from straw in the rumen
could be increased significantly by photolytic treatment, a nutritive feed could be produced. A study of the effects of ultraviolet light on the soil and rumen microbial utilizability of straw consequently might be of considerable value.

In this study, finely ground Newport Kentucky Bluegrass (*Poa pratensis*) straws or Annual Ryegrass (*Lolium multiflorum*) straws were mixed with distilled water in 0.5% w/v concentrations and exposed to ultraviolet light emitted from a mercury-vapor lamp. Gaseous oxygen or 30% hydrogen peroxide were used as electron acceptors in the photooxidation process.

Growth studies, with an *Aspergillus* sp. isolated from local soil, on the liquid portion of irradiated straw mixtures were used to evaluate the effect of ultraviolet irradiation in the presence of oxygen. Straws irradiated with ultraviolet light in the presence of varying concentrations of 30% hydrogen peroxide were used to evaluate soil and rumen microbial responses.

Fungal responses to the liquid portion of photolyzed straw showed a positive correlation with a carbonyl compound utilization. Fungal growth was depressed in liquid portions removed from hydrogen peroxide irradiated straws where negligible carbonyl containing compounds were found.

Soil microbial activity increased significantly when straws irradiated in the presence of 30% hydrogen peroxide were mixed
with soil in vitro. The increased activity corresponded with the utilization of hemicellulose-like compounds formed on the exposed surfaces of straw particles by the photooxidation process. When these newly formed compounds were decomposed the microbial activity decreased to the same level as non-treated straws.

Hydrogen peroxide photooxidized straws exhibited decreased rumen digestibility when tested in vitro. When these photooxidized straws were washed with distilled water and oven dried before digestion studies, the reduction in digestibility was significantly greater.

The primary action of ultraviolet light on straw appears to be with the molecules found on the straw particle surfaces. The role of oxygen appears to be very important in the photooxidation process since recombination of ruptured bonds is interfered with by activated oxygen, resulting in new compound formation. Many of these compounds are water soluble and are retained in solution during irradiation with oxygen. When hydrogen peroxide is used the availability of oxygen active species is much greater and the soluble compounds are rapidly decomposed photolytically to carbon dioxide and water, thus reducing the availability of nutrients for microbial growth in the liquid portions.

Apparently, many complex aromatic and polymeric straw structures are ruptured photolytically, followed by combination with oxygen to form open chain and cyclic products. Soil incorporation studies
indicate that many of these compounds are similar to hemicelluloses.

Rumen studies indicate that either optimum in vitro parameters were not maintained or some photolytic compound or compounds are formed which are inhibitory to the rumen microflora. Further investigation is required in order to obtain conclusive results for future rumen applications.

All data, except those from rumen studies, indicate that treatment of straw with ultraviolet light in the presence of oxygen does alter the molecular structure in such a way as to increase its microbial utilizability. This process, therefore, has promising possibilities for new uses of straw and warrants further investigation.
Soil and Rumen Microbial Responses
to Photooxidized Grass Straw

by

Jerry Eugene Park

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CURRICULUM VITAE

Jerry Eugene Park was born in Carlsborg, Washington, October 9, 1935. He attended high schools in Idaho and Oregon, graduating from Hill Military Academy High School, Portland, Oregon in June 1953. Undergraduate work was undertaken at the College of Idaho, Caldwell, Idaho and completed at Utah State University, Logan, Utah. He received a B. S. degree in electronic engineering, March 1965. Masters degree studies in microbiology were begun in September 1968 at Oregon State University and the requirements completed September 1970.
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INTRODUCTION

Straw has many uses, but none are extensive enough to utilize all the straw produced annually in the United States. Currently, in areas where grass seed is produced, the post-harvest straw residues are burned in the field to help prevent crop disease the following year. This treatment, while very effective, has created considerable air pollution and should be discontinued if at all possible. If crop disease prevention can be obtained without burning excess straw then new uses must be found to help dispose of the straw.

Straws have been pelleted for animal feed and treated chemically to produce papers and fiber materials, but these approaches from economic process standpoints, are not able to utilize all available straw. One additional approach could be to alter the straw composition photolytically to produce a material readily decomposable by soil or rumen microorganisms. This treatment would allow the producer to add straw to the soil and rapidly return to his fields the basic chemical components removed during crop growth. Also, a high energy animal feed could possibly be produced for use in feed lot operations.

It was the purpose of this work to survey the effects of ultra-violet light on fungal, soil and artificial rumen microbial straw
utilization. The photolytic decomposition of straw in the presence of gaseous oxygen vs. 30% hydrogen peroxide was also considered.
LITERATURE REVIEW

Characteristics of Straw

The degradation of straw materials in nature by microorganisms is of considerable importance to agricultural scientists and grain and grass producers.

Straw is extremely resistant to microbial decomposition, with approximately 30% of the plant, by weight, being lignin (1), a substance that serves as an incrusting material of the plant consisting mainly of phenylpropane building blocks (11). The lignin content can be as low as 5% of the total weight when the plant is young but when the plant ages the lignin percentage increases rapidly (1), resulting in a high lignin material very resistant to microbial decomposition. The remainder of the plant contains such organic constituents as cellulose, hemicellulose, fats, oils, waxes, resins, and proteins along with a water-soluble fraction containing simple sugars, amino acids, and aliphatic acids which contribute 5 to 30 percent of the plant tissue weight (1).

Straw Decomposition in Soil

Many investigators (12, 36, 37, 38, 41, 52, 56, 58, 59, 62) have shown that the lignin portion of a plant is highly resistant to
microbial decomposition while the other organic constituents are readily utilized. In 1938, Waksman (57) investigated changes in the chemical composition of Rye straw added to soil for a two month period. He found that the cellulose content of the straw decreased 56 percent and the pentosan content 60 percent, but only 11 percent of the plant lignin was decomposed in this time.

Broadbent (12) noted a major weight loss in a straw incorporated into soil for six months. An investigation of the chemical properties of the straw after exposure disclosed a marked reduction in the polysaccharide content and retention of lignin.

Plant residue decomposition time in soil is dependent on soil conditions as well as chemical composition. Some of the major factors governing decomposition in soil are the organic matter level, cultivation, temperature, moisture, pH, depth of residue and soil aeration (1).

An important consideration in microbial decomposition of straw residues is the availability of utilizable nitrogen in the straw or surrounding soil. A number of workers (8, 19, 30, 32, 35, 41, 43) have studied the effect of nitrogen on microbial decomposition of organic materials. They found that if the plant material is rich in organic nitrogen, nitrogen mineralization proceeds rapidly. When organic nitrogen is not added to the soil artificially, the microflora will not maintain a high level of activity and organic residue
decomposition can be retarded (31).

Harmsen and Van Schreven (21) reported that the nitrogen content of an organic material, below which no nitrogen mineralization may be expected, is 1.5 to 2.0 percent of the dry matter, which corresponds to a C/N ratio of 20 to 25. Similar values were also reported by Broadbent (13) and Allison (2).

Parker (34) compared the decomposition of surface-applied and buried cornstalks. The nitrogen content of the surface-applied cornstalks did not change but the C/N ratio narrowed from 57 to 30. Buried cornstalks decomposed 65 percent in 140 days, with the C/N ratio narrowing from 57 to 22. The narrowing of the C/N ratio was due to carbon loss rather than from nitrogen increase in the residue. At the lower C/N ratios, nitrogen was lost from the residue. Smith and Douglas (50) in their studies on wheat straw decomposition, noted similar losses of carbon, resulting in reduced C/N ratios.

Straw Rumen Decomposition

The ruminant and the rumen microbial population exist in a reciprocally beneficial relationship in which many of the plant materials consumed by the animal are digested and fermented by the rumen microbes to form carbon dioxide, methane and volatile acids (18, 51). The level of these substances is dependent on the type of forage used and the quantity ingested.
The study of how a material is utilized in the rumen is difficult at best when the analysis is carried out in vivo. To simplify this type of analysis the artificial rumen technique was developed and has been successfully used by many workers (7, 9, 10, 22, 24, 40, 44, 46, 53, 55). The results obtained generally encourage the use of artificial rumen techniques to assess the nutritive value of ruminant feeds, including hay and straw.

The major parameters investigated in artificial rumen studies are the percent digestibility of dry matter, the percent cellulose digestibility and determination of volatile fatty acids. The dry matter determination simply requires weighing a forage sample before and after the fermentation period to determine percentage dry matter utilized by the rumen microorganisms. For cellulose determinations (15) the dry matter remaining after fermentation is treated with a boiling mixture of acetic and nitric acid; then washed with ethanol, dried and ignited in a muffle furnace. The sample is weighed again and the percentage of cellulose remaining after fermentation determined by weight loss.

In a study by Bowden and Church (9) the mean digestibility of dry matter for a dried alfalfa was 57.8 percent. The mean cellulose digestibility of the dried alfalfa was 54.8 percent, indicating that the major portion of the alfalfa utilized in the rumen was cellulosic in nature.
Volatile fatty acids such as valeric, butyric, propionic and acetic acids are generally determined by column or gas chromatography (62). Samples of the rumen inoculum are analysed and compared with the analysis of the post-fermentation material. Any acids found in excess of the base levels are attributed to microbial decomposition of the substrate.

Many factors must be considered when analysing a forage material being utilized by a ruminant. El-Din and el-Shazly (16) found that variations of the C/N ratio, rumen liquor dilution, and substrate concentration in the rumen can affect the rate with which a feed is utilized. A wheat straw mixed with a feed concentrate in a 3:1 ratio was used, and the maximum fermentation rate, in vivo, occurred immediately after feeding. The presence of fermentable carbohydrates in the feed concentrate was evidently responsible for this response. Eight hours later a second fermentation burst occurred corresponding to utilization of the fibrous cellulosic material in the straw. The highest net growth rates of the straw-concentrate ration were found when the C/N ratio was between 16.5 and 17.5 and close to the ratio of the feed consumed by the animal just prior to the test (16), indicating microbial adaptation.

The presence of soluble carbohydrates is also important when testing a forage material in vitro. The rate of cellulose digestion can be stimulated by the addition of small amounts of soluble
carbohydrates, however, if an excess is added a reduction of fermentation occurs (5, 22).

In addition to cellulose and carbohydrates, the effect of rumen liquor dilution with mineral solutions in the artificial rumen must be considered. With wheat straw and a feed concentrate a dilution of three parts mineral solution to one part rumen liquor has been found to give satisfactory microbial activity. Greater dilution caused a slight drop in the activity because of the reduced concentration of fermentation end products (16).

Substrate concentration in the artificial rumen also influences microbial activity, according to el Din and el Shazly (16). When 15 grams of feed were used for every 50 grams of rumen sample, the highest microbial activity was obtained. Variations in concentration either way from the ideal level reduced the activity.

It is evident from these findings that studies with the artificial rumen can only be of value when one considers the substrate concentration, the C/N ratio and the rumen liquor dilution in relation to the forage being tested. If these parameters are correctly selected the activity in the artificial rumen can be increased five fold (16) and give results closely approximating conditions within the rumen.
Photolysis

Physico-chemical Fundamentals

Molecules absorbing radiation become excited and highly reactive (33), 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 (33) 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 impractical.

Plant Polysaccharides

Treating a refractory molecule with light of wavelength below 350 nm will rupture existing bonds and form new compounds (4, 6, 14).
Cellulose films irradiated by ultraviolet light with wavelengths of 253.7 nm and 185 nm exhibit a variety of chemical changes. If irradiated with 253.7 nm, carboxyl groups are mainly formed. If the wavelength is lowered to 185 nm, the primary end products contain aldehyde groups (25).

The presence of oxygen during the irradiation of cellulose also effects the breakdown pattern at these two wavelengths. At 185 nm a primary dissociation occurs in cellulose because of its strong absorption characteristics at this wavelength. This reaction is independent of the presence of oxygen (26). Early in the irradiation period a direct scission of glucosidic linkages predominates, but on prolonged exposure 2,3-dialdehyde is formed by splitting off hydrogen atoms from secondary carbon atoms of the molecule. At 253.7 nm the presence of oxygen becomes important in the degradation because cellulose is transparent to visible and ultraviolet light having a wavelength longer than 200 nm and the oxygen molecule, therefore, serves as the absorption molecule (26). When irradiation of cellulose is done at 253.7 nm in the presence of oxygen, the oxygen molecule is raised to an excited state and is capable of abstracting a hydrogen atom from the 1-carbon atom of the pyranose ring to form a cellulose radical. The radicals formed react with oxygen and other cellulose molecules to form cellulose peroxide. The peroxide is decomposed photochemically to form a δ-lactone end group which is hydrolysed photochemically to form a hydroxy-carboxylic acid end group (26).
Cellulose films irradiated by ultraviolet light with wavelengths greater than 300 nm did not cause photodecomposition.

Other investigators (28, 29) have noted chemical changes occurring in the polysaccharidic portions of woody material upon exposure to light. They noted a significant increase in the copper number of groundwood after exposure to ultraviolet light in the presence of oxygen. This was postulated as being due to depolymerization and/or oxidation of the polysaccharides. They also noted a reduction in pentosan and cellulose content and an increase in ethanol-benzene solubility and 1% sodium hydroxide solubility.

Lignin and Related Compounds

Lignified materials such as wood, high-yield pulps and paper products are also modified when exposed to ultraviolet light. The most noticeable effect is a yellowing of the material when exposed to 355-500 nm light. It was suggested by Leary (27) that demethoxylolation, resulting from the photolytic degradation of lignin, was involved in the yellowing process.

As noted with cellulose, the presence of oxygen during light exposure of lignified materials also plays an important role in the degradation rate and the end products formed. Free radicals are generated first, as was the case with cellulose, which combine rapidly with oxygen to oxidize the lignin (23). These findings support the
suggestion that photoinduced oxidation is an important mechanism in the degradation of lignified materials.

Some of the products isolated from photooxidized wood materials are vanillin, syringaldehyde, some higher-molecular weight phenolic degradation products (48), carbon dioxide, carbon monoxide, water and methanol (23). Forman (20) irradiated spruce wood meal for 710 hours with ultraviolet light and isolated small amounts of vanillin and a high-molecular weight degradation product extractable with ethanol. The extractables amounted to 4.5 percent of the weight of the irradiated sample.

A recent study by Rockhill (47) has shown that a sodium ligninsulfonate can be photochemically altered with ultraviolet light in the presence of oxygen. Thin layer chromatography revealed three new components formed from irradiation and three other components existing before irradiation increasing in concentration with increased irradiation time. Rockhill noted that photochemical modifications of the ligninsulfonate occur most efficiently when (a) the irradiation solution is acidic, (b) sufficient oxygen is present and (c) the light wavelength was less than 210 nm. Ligninsulfonate photolysis was found to be temperature independent and oxygen dependent, showing a true primary photodecomposition mode.
Microbial Utilization of Phototreated Ligninsulfonate

Irradiation of a sulfonated lignin with ultraviolet light and subsequent growth studies on the irradiated end products has shown that lignin containing materials, normally highly resistant to microbial decomposition, can be photochemically modified to allow increased microbial growth (47).

Studies with an *Aspergillus* isolate (47) and with a *Pseudomonas* sp. (17) on a ligninsulfonate have shown that approximately 55 percent of the residual carbon remaining in the ligninsulfonate after irradiation with ultraviolet light in the presence of oxygen is rapidly utilized by these microorganisms under described laboratory test conditions. This treatment very effectively modified the lignin material and increased its biological availability by approximately ten-fold when compared to a nonirradiated material (47).

Lack of specific information to date on the microbial utilizability of straw exposed to intense ultraviolet light in the presence of oxygen led to the development of the present study.
MATERIALS AND METHODS

Substrates

Newport Kentucky Bluegrass (*Poa pratensis*) straw and Annual Ryegrass (*Lolium multiflorum*) straw were used for all irradiation studies. Straws from 1969 crops were provided by the Department of Farm Crops, Oregon State University, Corvallis, Oregon. The following analysis of the two straws was determined by the Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon.

<table>
<thead>
<tr>
<th>Newport Kentucky Bluegrass Straw</th>
<th>%</th>
<th>μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter</td>
<td>85.5</td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>8.35</td>
<td>4.0</td>
</tr>
<tr>
<td>Crude fat</td>
<td>1.16</td>
<td>8.0</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>34.12</td>
<td>48.0</td>
</tr>
<tr>
<td>Ash</td>
<td>3.36</td>
<td>91.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.16</td>
<td>≤1.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.16</td>
<td>≤5.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>µg/ml</td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>Dry matter</td>
<td>84.7</td>
<td>Copper</td>
</tr>
<tr>
<td>Crude protein</td>
<td>3.05</td>
<td>Zinc</td>
</tr>
<tr>
<td>Crude fat</td>
<td>0.91</td>
<td>Manganese</td>
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<tr>
<td>Crude fiber</td>
<td>37.50</td>
<td>Iron</td>
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<tr>
<td>Ash</td>
<td>5.88</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Sodium</td>
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<tr>
<td>Calcium</td>
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<td>Nickel</td>
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<tr>
<td>Potassium</td>
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<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.10</td>
<td></td>
</tr>
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</table>

Samples of both straws were pulverized in a shatterbox (serial #6306, Spex Industries, Inc., Metuchen, New Jersey). The pulverized straw was placed in a 150 mesh screen container on a Porter Sand Shaker (serial #39744, Braun Corp., Los Angeles, California) to remove all 150 mesh and smaller size straw. These 150 mesh straws were prepared in 0.5 percent w/v mixtures at pH 6.9 for all irradiation studies.
Photolysis

Ultraviolet Lamp

A type "A" medium pressure 500 watt mercury-vapor lamp (model #673A-36, Engelhard-Hanovia Co., Newark, New Jersey), powered by a step-up transformer (model #20651-1, Engelhard-Hanovia Co., Newark, New Jersey), 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 (42).

Immersion Well and Photolysis Hardware

A double wall quartz immersion well with a 60/50 male ground glass joint (model #19434, Engelhard-Hanovia Co., Newark, New Jersey) was used to contain the lamp. The mixture to be irradiated was circulated around the outside of the well while coolant water was circulated through the double wall to maintain the desired substrate temperature, usually set at 37°C.

The sample irradiation vessel, with inlet tube near the base and outlet tube at the top, was designed to fit externally over the immersion well and seal with the well by a 60/50 ground glass female joint. The volume of the vessel was approximately 350 ml. The
vessel was connected to a two liter external reservoir via a pump to allow continuous circulation of any volume sample desired. For this purpose, a centrifugal polyethylene-housing pump (Chemical Rubber Co., Cleveland, Ohio) was used to circulate the straw-water mixture through the reaction vessel at eight liters per minute.

**Oxygenation System**

Compressed oxygen gas (NCG) was used as the electron acceptor during irradiation of Newport Kentucky Bluegrass Straw. The oxygen was passed through a needle valve and flowmeter (#10, Gilmont Instruments, Inc., Great Neck, New York) allowing flow rates of 0.90 ml/min and then passed through a fritted disc gas dispersion tube submersed in the straw-water mixture in the external reservoir.

**Hydrogen Peroxide System**

Hydrogen peroxide (30%-Reagent A. C. S., Allied Chemical) was used to provide oxygen as an electron acceptor during irradiation of Annual Ryegrass straw. The amount of hydrogen peroxide required for each irradiation study was added to the prepared straw sample in the external reservoir at the rate of 0.33 ml/min from a 35 ml syringe mounted on a syringe pump (model #234-2, Sage Instruments, Inc., White Plains, New York). The dispensing rate was maintained
by controlling the "on" time of the syringe pump with a variable
timer (custom made) which could interrupt the pump power supply
periodically.

**Irradiation of Newport Kentucky Bluegrass**

A 1000 ml volume of 0.5% straw-water mixture was added to
the two liter external reservoir of the irradiation system and stirred
with a magnetic stirrer for a minimum of 15 minutes before beginning
irradiation to assure homogeneity of the mixture. The stirring was
maintained throughout the irradiation period to retard foaming which
occurs when oxygen is added to the reservoir and circulated through
the irradiation vessel. A fritted disc gas dispersion tube was placed
in the reservoir near the outlet port to allow a 5 ml/min oxygen flow
to be added to the system during irradiation. The oxygen flow was
started five minutes before lamp activation so the flow rate could
equilibrare.

Coolant water was circulated through the double wall immersion
well at a sufficient rate to maintain a mixture temperature of 37 ± 1 °C.
The end of the tubing, used to return the irradiation sample to the
reservoir, was placed below the liquid surface so the rapid swirling
action of the magnetic stirring bar could help break up any foam pro-
duced by the oxygen and irradiation. All foaming stopped after two
hours of irradiation.
Each irradiation run was discontinued at a predetermined time to give a series of irradiation exposures, the sample removed from the reservoir and centrifuged at 9750 x g for 20 minutes to separate the straw residue and liquid supernatant. Each portion was stored at -10°C for future growth studies. After each irradiation run the entire system was flushed out with four liters of distilled water. At the completion of four consecutive runs the entire system was dismantled and cleaned with chromic acid and detergent.

**Irradiation of Annual Ryegrass**

The samples of Annual Ryegrass straw used for irradiation were prepared and circulated through the irradiation system by the same method used with the Newport Kentucky Bluegrass straw. During irradiation, 30% hydrogen peroxide was added to the straw sample in the reservoir at a rate of 0.33 ml/min from a 35 ml syringe until the desired amount had been added for that particular run. The percent hydrogen peroxide remaining in the irradiation sample was determined periodically by titrating with KMnO₄ (61). When the peroxide concentration decreased to 0.03% v/v the irradiation was terminated.

In some of the irradiation runs the percent soluble oxygen in the sample was determined with a Beckman Oxygen Analyser (Model #1777, Beckman Instruments, Inc., Fullerton, Calif.) and recorded
on a strip chart recorder (Serva-riter, model PWD, Texas Instruments, Inc., Houston, Texas). The oxygen analyser was adjusted to read 20% soluble oxygen in a nonirradiated sample. The sample was treated with $\text{H}_2\text{O}_2$ and exposed to ultraviolet light until the percent soluble oxygen decreased to zero, then irradiation was terminated.

After irradiation the samples were removed from the system and evaporated to dryness on a flash-evaporator (Model FE-2, Buchler Instruments, Fort Lee, New Jersey) at 40°C. The straw residue was oven-dried for 48 hours at 60°C and sealed in a desiccator until needed for soil incorporation and artificial rumen studies.

**Thin Layer Chromatography (TCL)**

Thin layer chromatography was used to analyse ether extracts and 2, 4 dinitrophenylhydrazine derivatives prepared from the liquid phases removed from irradiated samples of both types of straw. The ether fractions were prepared by extracting 50 ml of the liquid phase with three 50 ml volumes of diethylether, after reducing the acidity of the liquid phase to pH 2 with 1 N HCl. The liquid phase and the ether were mixed together vigorously on a magnetic stirrer for three hours and then placed in a glass stoppered collection flask for removal of the ether layer. After removal, the ether layer was placed in a 30°C water bath and evaporated to dryness in vacuo. The
residue remaining after evaporation was dissolved in 2 ml of 95% ethanol and then used for TLC.

The 2, 4 dinitrophenylhydrazine derivatives were prepared by mixing 5 ml of 0.4% 2, 4 dinitrophenylhydrazine (DNPH), in 2 N HCL (54), with 5 ml of the liquid phase and storing the solution at 25°C for 24 hours. The solution was then centrifuged and the supernatant decanted off leaving a 2, 4 DNPH precipitate. The precipitate was washed with three 10 ml volumes of 2N HCl and three 10 ml volumes of distilled water, then was dissolved in 1 ml of methanol for TLC application.

Chromatographic plates were prepared by spreading a 0.250 mm layer of 50 percent w/v silica gel G (American Optical Corp., #08075) in water on 8” x 8” glass plates. The plates were allowed to air dry for two hours and then activated at 105°C for 30 minutes (45). About 5 mm of the silica gel was trimmed off the bottom edge of the plate to help produce a uniform solvent front and reproducible Rf values.

Solvents (45) were prepared in 100 ml volumes from benzene/dioxane/acetic acid (90:25:4), and benzene/methanol/acetic acid (45:8:4) and used in rectangular glass tanks with closed 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 then placing against the sides of the tank with the lower edge of the paper submerged in the solvent. The solvent and saturation papers were
replaced after running five plates.

The sample to be tested by TLC was applied to the plate in 20 µl volume spots approximately 5 mm above the edge of the adsorbent and then dried with forced air. The solvent front was allowed to travel approximately ten centimeters from the spot before the plate was removed from the tank and air dried in a fume hood prior to spraying with a reagent.

The following spray reagents were used:

1. **Concentrated sulfuric acid**--the plate was heated at 105 °C to visualize the compounds by charring.

2. **Tetrazotized benzidine (45)**--After spraying, the plates were dried at 105 °C for a few minutes until the spots were clearly visible. Used for the detection of phenolics.

3. **2, 4-DNPH**--A 0.4% w/v solution in 2N HCl was sprayed on the plates to detect carbonyl group-containing compounds, indicated by yellow to red spots.

4. **Methyl red**--Used for detection of carboxyl group-containing compounds, red spots indicating acidic character.

**Fungal Growth**

**Mineral Salts Solution**

A basic Bushnell-Haas mineral salts solution was used in all liquid phase growth studies. The solution contained the following
mineral concentrations per liter of tap water: magnesium sulfate, 0.2 grams, calcium chloride, 0.02 grams, potassium phosphate (monobasic), 1.0 gram, potassium phosphate (dibasic), 1.0 gram, ammonium nitrate, 1.0 gram, and ferric chloride, 0.05 grams. The growth medium contained 5 ml of the mineral salts solution and 25 mls of liquid sample.

Growth System Using Liquid Phase of Irradiated Bluegrass

A fungus belonging to the form-genus *Aspergillus*, ATCC #20243, was used for all growth studies. The growth responses analysed were on the liquid portions of a non-irradiated control and on 0.5 to 12.0 hour irradiated 0.5% w/v mixtures of Newport Kentucky Bluegrass straw. The samples, after mineral salts addition and pH adjustment to 6.9, were filter sterilized, aseptically divided into four 30 ml volumes and transferred to sterile 125 ml Erlenmeyer flasks. A 0.01 ml platinum loop was used to transfer conidia from a sterile distilled water suspension to each flask. Each flask was closed with sterile plastic foam plugs after inoculation. The same method was used to prepare non-irradiated straw and water controls.

The flasks were aerated on a shaker at 27° ± 1°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 (Van Waters & Rogers #28153020). The supernatant removed from
each flask was preserved at -10°C for chemical analysis. Adhering solids were removed from the flasks with distilled water and then the filter discs containing the collected solids were washed with 100 ml of distilled water before removal from the filter holder. The filter discs were dried at 105°C to constant weight. Growth responses were expressed on the basis of fungal dry weight per ml of growth medium.

**Soil Incorporation**

**Metabolic Carbon Dioxide Trap**

The trap was designed to provide carbon dioxide free air to a soil environment containing a sample substrate and, after an incubation period, collect the carbon dioxide produced by microbial utilization of the treated straws.

The air used in the growth system was passed through the following sequence of flasks and materials to remove any foreign matter, moisture, and atmospheric carbon dioxide: an air trap to remove any rust or dirt particles in the air line; a concentrated sulfuric acid bath to remove moisture and organic materials; a second air trap to prevent the passage of sulfuric acid to the remainder of the system; one disorber cartridge (#24-923-01, Burrell Corp., Pittsburgh, Pa.) containing anhydrous magnesium perchlorate to remove all remaining moisture; one disorber cartridge containing a color indicating
carbon dioxide absorbant (Ascarite-20 to 30 mesh, Arthur H. Thomas Co., Philadelphia, Pa.) to remove all carbon dioxide from the air; and a distilled water bath to add moisture back to the air and help prevent air drying of the soil sample. The purified, carbon dioxide-free, moist air was distributed to a series of 500 ml, wide mouth Erlenmeyer flasks containing soil and straw, then passed into 20 x 150 mm culture tubes containing 20 ml volumes of 1 N sodium hydroxide for absorption of metabolic carbon dioxide produced in the growth flasks.

An air flow of 50 to 60 bubbles per minute through the culture tubes containing sodium hydroxide was maintained by restricting the growth flask air input tubes with clamps. Excess air pressure was vented into a 1 x 24 inch pyrex glass tube bubble chamber.

Growth System - Annual Ryegrass

Washed and non-washed Ryegrass straw residues taken from 10, 20, 30, 40 and 50 ml 30% hydrogen peroxide-treated irradiations were added in 200 mg quantities to 100 g of Cloquoto Fine Sandy Loam soil in 500 ml wide-mouth Erlenmeyer flasks. The moisture of the soil was maintained at 65-70% of water holding capacity and the temperature held at 27 ± 1 °C. Four flasks were prepared for each irradiated washed residue along with four flasks for non-irradiated washed straw and four soil controls without straw. A series of 25 day growth
studies were conducted on these residues with metabolic carbon dioxide produced measured every 24 hours.

**Carbon Dioxide Determination**

The culture tubes containing the 1N sodium hydroxide were removed from the growth system every 24 hours and immediately sealed with rubber stoppers. New tubes of 1N sodium hydroxide were prepared just prior to the change and inserted into the system for the next 24 hour determination.

The quantity of metabolic carbon dioxide absorbed was calculated in mg/unit time from values obtained by titrating with standardized 0.093 N hydrochloric acid. Phenolphthalein and methyl orange indicators were used to determine the end points required for the calculations (39).

The standardized acid and 1N sodium hydroxide were prepared in eight liter carboys containing syphons and sealed with rubber stoppers to prevent evaporation. A disorber cartridge containing Ascarite (Arthur H. Thomas Co., Philadelphia, Pa.), a carbon dioxide absorbent, was inserted in the air inlet side of the 1N sodium hydroxide syphon to remove atmospheric carbon dioxide from air exchanged when samples of the solution were removed.
Artificial Rumen

Straw Preparation

A series of Annual Ryegrass straw residues, taken from the 10 through 50 ml 30% hydrogen peroxide treated irradiated samples, were washed three times in 100 ml volumes of distilled water before being dried at 60 °C for 48 hours. A second series of irradiated residues were dried without washing to remove water soluble residues. All dried residues were sealed in snap-top polycarbonate tubes and stored in a desiccator until required for the rumen study.

Artificial Rumen Digestion

The fermentation studies were carried out by the Animal Science Department, Oregon State University, Corvallis, Oregon according to the method outlined in a master's thesis written by Daniel C. Anderson (3). The methods described for this study are a modification of Anderson's work.

A Hereford steer fitted with a permanent rumen fistula was used as the rumen liquor donor in this study. The steer received a poor quality Ryegrass hay diet for six weeks prior to the first liquor collection.

A portion of the rumen ingesta from the posterior region of the dorsal sac of the rumen was removed by hand to obtain the rumen
liquor. The ingesta was compressed between two layers of cheesecloth and the liquid removed was collected in prewarmed thermos bottles. The bottles were immediately taken to the laboratory where the liquor was strained twice through a double layer of cheesecloth into a tall beaker. The strained liquor was placed in a 39 °C incubator for 45 minutes after which the bottom liquid was removed. The standing period permitted a large percentage of the solid materials to rise to the surface, thereby lowering the dry matter content of the liquor removed from the bottom of the beaker.

Fermentation studies were done in 100 ml beakers to which one gram of the dried irradiated straws were added. An artificial saliva was prepared and warmed to 39 °C in a water bath while carbon dioxide was bubbled through it. The saliva was prepared the night before the fermentation study and was composed of the following materials:

<table>
<thead>
<tr>
<th></th>
<th>Concentration (grams/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃</td>
<td>9.8</td>
</tr>
<tr>
<td>NaHPO₄·7H₂O</td>
<td>7.0</td>
</tr>
<tr>
<td>KCl</td>
<td>0.57</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.47</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.12</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Concentration (grams/liter)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Buffer Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5.5 grams/100 ml</td>
<td>Add 5 ml/300 ml</td>
</tr>
<tr>
<td>Urea</td>
<td>5.5 grams/100 ml</td>
<td>Add 5 ml/300 ml</td>
</tr>
</tbody>
</table>

The inoculum was prepared by mixing 10 ml of rumen liquor with 35 ml of the artificial saliva. To each 100 ml beaker containing straw was added 45 ml volumes of inoculum. Carbon dioxide was bubbled into the beakers for approximately 30 seconds before being fitted with stoppers containing valves for the escape of fermentation gases.

Samples were incubated at 39°C for 24 hours before treating with 1 ml of 2N sulfuric acid to arrest microbial activity. The contents of each beaker were filtered and washed several times with distilled water through sintered glass crucibles in vacuo. The crucibles were dried at 70°C for 24 hours, cooled in a desiccator, and weighed. Residues remaining in the crucibles were assumed to be undigested dry matter of the straw.

After each run the crucibles were cleaned by rinsing in tap water, and soaking in a sulfuric acid-dichromate cleaning solution for 24 hours. Finally they were rinsed with tap and distilled water and dried in an oven for approximately four hours.
RESULTS

Irradiation Effect on Physical Condition of Straw

Samples of Newport Kentucky Bluegrass straw in 0.5% w/v mixtures were irradiated initially at pH 6.9 with an oxygen flow rate of 5 ml/min for 64 hours and the physical changes of the straw observed periodically. The color of the straw residue gradually lightened as irradiation proceeded until after approximately eight hours, the residue remained colorless. Beyond eight hours irradiation a noticeable decrease of straw volume occurred, until at 64 hours all solids had disappeared and a clear liquid remained.

Another series of samples were irradiated in the presence of 30% hydrogen peroxide and similar physical changes took place in a shorter period. The straw residues were colorless within four hours and had been solubilized in 24 hours. These observations indicate that oxygen availability and form, either diatomic in the gaseous state or nascent from hydrogen peroxide, greatly influences the photolytic decomposition rate of the straw particles.

Variations of pH Due to Irradiation

The pH variation for a 0.5% w/v sample straw in distilled water irradiated with ultraviolet light in the presence of oxygen for 72 hours is shown in Figure 1. The pH of the straw mixture decreases linearly
Figure 1. Effect of ultraviolet irradiation with oxygen on the pH of a 0.5% w/v straw-water mixture.
from 6.9 to 3.3 in four hours and then rises again at almost the same rate to a constant value of approximately pH 7.6.

Similar responses from the irradiation of straw in the presence of 30% hydrogen peroxide are shown in Figure 2. The sample irradiated in the presence of 10 ml 30% hydrogen peroxide exhibited a different type of response curve with a lower leveling-off pH than the 20 through 50 ml samples. As the concentration of hydrogen peroxide is increased the curve tends to level off earlier and move closer together until at the 40 and 50 ml hydrogen peroxide additions the curves are coincident.

**Fungal Growth Responses**

*Aspergillus* A-17-30 (47) growth responses to 25 ml volumes of the liquid phases from 0.5% w/v mixtures of phototreated Newport Kentucky Bluegrass straw are expressed in relation to photolysis time in Figure 3. Fungal responses as determined by dry weight in relation to 2, 4 dinitrophenylhydrazone formation before and after growth are considered.

Figure 3 curves show that maximum fungal growth responses as well as maximum 2, 4 DNPH derivative formation from carbonyl compounds occur within the first 12 hours of irradiation. The production of carbonyl compounds decreases rapidly from 12 to 18 hours irradiation and ceases after 18 hours. Fungal growth continues to
Figure 2. Effect of ultraviolet irradiation with $H_2O_2$ concentration on the pH of a 0.5% w/v straw-water mixture.
Figure 3. Fungal utilization of liquid portions from photooxidized straw.
decrease after 12 hours and stabilized near 18 hours irradiation at approximately 0.02 mg/ml. Extended irradiation did not alter this lower level.

Similar studies were conducted on the liquid phases of 0.5% w/v mixtures of Annual Ryegrass straw irradiated in the presence of varying concentrations of 30% hydrogen peroxide. Five samples containing 10, 20, 30, 40 and 50 ml of 30% hydrogen peroxide respectively, were irradiated until all traces of hydrogen peroxide were gone, as determined by potassium permanganate titration. Irradiated straw liquid phases, tested to determine the presence of carbonyl compounds, revealed only traces in the 10 and 50 ml hydrogen peroxide treated samples. All other samples tested did not contain detectable carbonyl compounds.

Fungal growth responses on the samples lacking carbonyl compounds were also essentially negative. The 10 and 50 ml hydrogen peroxide treated samples supported growth to the extent of 0.04 mg/ml, indicating a dependence on carbonyl compounds for the growth of this Aspergillus under these laboratory conditions. Since the concentration of nascent oxygen in the hydrogen peroxide system is much higher than in the gaseous oxygen system, it appears that any compounds formed by photon interaction with the straw particles are not retained in solution but rapidly degraded to carbon dioxide.
Thin Layer Chromatography

Thin layer chromatography using a benzene/dioxane/acetic acid solvent and sulfuric acid char for spot detection on diethyl ether extracts taken from the liquid phases of irradiated and non-irradiated 0.5% w/v Newport Kentucky Bluegrass straw, revealed several new zones. These zones were found at Rf values of 0.34-0.43 in samples irradiated two hours in the presence of gaseous oxygen, 0.37-0.42 in samples irradiated four hours, and 0.37-0.39 in samples irradiated six hours. A sample irradiated eight hours revealed a single spot at 0.37 and a new zone at 0.68-0.80. Samples irradiated 16 and 64 hours revealed two very light spots at 0.37 and 0.80.

When a developed TLC plate was sprayed with methyl red indicator, acidic zones were detected at Rf values of 0.40-0.43 on the two, four and six hour irradiated samples, indicating compounds containing carboxyl groups. These zones did not appear on the eight, 18 or 64 hour irradiated samples or on the non-irradiated samples, however, a very intense red spot did appear at Rf 0.79 on the eight hour irradiated sample.

Light red zones appeared at the same Rf values on all irradiated samples, when sprayed with tetrazotized benzidine, as appeared with the sulfuric acid char plates, indicating the presence of phenolic compounds. Parallel chromatography of ten phenolic compounds
considered related to the basic monomeric building blocks of the lignin molecule did not agree with any of the compounds formed by photolysis.

Yellow zones were detected on samples taken from the six hour irradiated straw, using the benzene/methanol/acetic acid solvent, at Rf values of 0.14, 0.28, 0.61 and 0.98 when a developed TLC plate was sprayed with 2,4 DNPH, indicating the presence of carbonyl compounds.

Thin layer chromatography of 2,4 DNPH derivatives from samples irradiated six hours using benzene/methanol/acetic acid solvent taken before growth with the *Aspergillus* isolate revealed five zones at Rf values of 0.63, 0.58, 0.44, 0.35 and 0.28 all in a heavy streak. After growth the zone intensities decreased significantly and all streaking had disappeared.

**Soil Incorporation Responses**

Growth responses in Cloquoto Fine Sandy Loam soil to straw residues taken from 0.5% w/v mixtures of Annual Ryegrass straw and distilled water treated with ultraviolet light and 30% hydrogen peroxide are shown in Figure 4. Cumulative values of metabolic carbon dioxide collected in relation to days of growth for non-irradiated and irradiated washed straws are considered.

Increased microbial activity is shown for all irradiated straws
Figure 4. Soil microbial responses to H$_2$O$_2$ photooxidized straw.
by the fifth day of incubation. A pronounced increase in the activity of the 20-50 ml hydrogen peroxide treated samples over the 10 ml hydrogen peroxide treated sample and the non-irradiated control is noted by the tenth day of incubation, indicating changes in the straw resulting from photolytic interaction which made portions of the straw more accessible to the soil organisms. The 30 ml hydrogen peroxide treated sample produced the most active response of all samples tested, and was exceeded only by the 50 ml hydrogen peroxide treated sample eight days into the growth period. After ten days incubation the microbial activity stabilizes and proceeds at the same rate for all samples.

Artificial Rumen Responses

The results of artificial rumen digestibility studies done on washed and non-washed residues from irradiated straws treated with 30% hydrogen peroxide are shown in Figure 5. Percent digestibility values in relation to straws irradiated in the presence of 30% hydrogen peroxide and a comparison of non-washed straws to straws washed in distilled water after irradiation are considered.

These studies show a decreased digestibility of both the washed and non-washed straws treated with ultraviolet light in the presence of 30% hydrogen peroxide. The non-washed straw gave higher digestibility values and demonstrated more erratic responses than the washed
straws. The reduction in digestibility for the non-washed straws varied between 4.2% and 23.4% when compared to the non-irradiated straw digestibility. Washing the straws not only affected the overall digestibility of the control straw, as noted by a 13% reduction, but seriously reduce the digestibility of the irradiated straws. The reduction in digestibility for the washed straws, compared with the control straw, varied between 55.7% and 67.3%.
Figure 5. Effect of ultraviolet irradiation and water washings on the digestibility of Ryegrass straw.
DISCUSSION

The purpose of this study was to determine if ultraviolet light would alter grass straws sufficiently to allow increased decomposition by soil and rumen organisms in their respective environments.

An evaluation of the results obtained should be preceded by information on the spectral properties of light from the mercury arc lamp in order to explain some of the changes noted. One of the major spectral lines emitted from the lamp is at 280.3 nm (42) and, based upon quantum energy calculations (49), this wavelength would contain sufficient energy to rupture the common bonds found in the lignin and cellulose molecules, such as C-C, C-O, and C-H bonds, which make up a major portion of straw.

The energy distribution of the lamp used in these studies is reported to have approximately 6.9% of its spectral output lying below 280 nm (42). Many of the longer wavelengths, even though they contain lower energy potential, still have sufficient energy to slowly degrade some of the structures found in the lignin molecule and, therefore, cannot be overlooked completely when considering the effects of photolysis. Oxygen is activated by approximately 34.9 Kcal/mole and is readily influenced by wavelengths as long as 819 nm. The output of this lamp, therefore, provides many wavelengths with sufficient energy to activate the oxygen used with photolysis (47).
The total power output below 219 nm, when blocked with Vycor, is 0.53% (42). Even though most of the energy produced by the lamp exists at wavelengths longer than 280 nm and does cause some lignin and cellulose bond cleavage, most of the rapid bond cleavage occurs below 280 nm and is probably a result of secondary reactions not apparent at longer wavelengths (48).

Photooxidation is evidently limited by the availability of molecular and other activated oxygen species capable of combining with the substrate after quantum absorption (33). The use of gaseous oxygen in these studies did cause pronounced physical changes in the straw, but at a slower rate than when hydrogen peroxide was used, indicating that gaseous oxygen, even though easier to work with, is not the most efficient oxidant available for this process. However, from the standpoint of saving water-soluble photolytic intermediates for chemical uses or for use as an efficient microbial growth substrate, the gaseous oxygen system appears to be superior to the hydrogen peroxide. During photolysis most of the intermediates were destroyed rapidly using hydrogen peroxide, presumably because of the greater availability of nascent oxygen. When in solution with the irradiation mixture, the probability of photon interactions with the hydrogen peroxide, producing an active species of oxygen, is much greater than with gaseous oxygen. With gaseous oxygen the molecules can be assumed to be contained in small bubbles and can only be
activated by photon interaction with the surface of the bubble.

If the straw itself is to be used after irradiation, a treatment with hydrogen peroxide or some other efficient oxidant, together with sensitizing agents such as ferric ions and uranyl salts (6), to accelerate the degradation process during photolysis, could produce larger amounts of straw residues in shorter periods of time. Photolysis itself is not the limiting factor when considering an efficient straw irradiation system because sufficient energy is available at the proper wavelength to cleave any organic bond. The more important problem to be solved is the integration of certain parameters such as electron acceptors, irradiation time, and concentration and pH values of the irradiation mixture in such a way to optimize the efficiency during photolysis.

Detailed mechanistic information regarding lignin photodegradation is not available as yet, however, some of the findings of Rockhill (47) in recent work with a ligninsulfonate phototreated with ultraviolet light and gaseous oxygen are helpful in analysing some of the results of this study. He observed that carboxyl and carbonyl groups were formed during photolysis, as evidenced by the isolation of one new acidic and two new carbonyl components. The formation of three new acidic compounds and three new carbonyl components from the irradiation of straw in the presence of gaseous oxygen indicates that some parts of the lignin molecules in the straw may have been
degraded. This is also supported by the bleaching of the straw, noted by irradiation and is consistent with the findings of Leary (27) who observed a yellowing of wood when exposed to ultraviolet light which he attributed to demethoxylation of the lignin molecule. These straws contain somewhat less lignin than the woody material used by Leary (27) and were irradiated with ultraviolet light approaching 180 nm wavelength instead of the 355-400 nm light which produced the yellowing. The results of the more intense treatment is a rapid bleaching of the straw to give white residues.

The formation of carboxyl compounds would also be expected from the photooxidation of the cellulose molecule. Earlier studies (26) have shown that 253.7 nm light in the presence of gaseous oxygen will cleave a hydrogen atom from the 1-carbon atom of the pyranose ring in cellulose to form a cellulose radical which combines with other oxygen molecules to form cellulose peroxide. The peroxide is further degraded to produce hydroxycarboxylic acid end groups. The decreasing pH during photolysis of the straw also suggested that acidic components were being formed.

The carbonyl compounds formed during the photolytic degradation of straw apparently plays an important role in the growth of the Aspergillus isolate used on the liquid phases of irradiated straw. The maximum growth response, as determined by fungal dry weight, corresponded very closely to the disappearance of the carbonyl
compounds. As noted in Figure 3, the formation of 2,4 dinitrophenyl-hydrazone from carbonyl compounds increased rapidly on samples irradiated for eight to twelve hours but the growth response continued to decrease even though the utilization of carbonyl compounds increased. This response indicates that the fungus is not only using carbonyl compounds as a growth substrate in the samples irradiated up to eight hours but it also seems to be growing on at least one other non-carbonyl substrate. After eight hours irradiation the non-carbonyl compound is apparently destroyed and growth is sustained at a less efficient rate by the carbonyl compounds formed. This would account for the apparent increase in carbonyl compound utilization in the eight and 12 hour samples without an increase in the fungal growth.

The physiological significance of these compounds formed in the liquid phase of an irradiated straw in relation to photolysis time must be more completely understood, and will be important in possible development of single cell protein production processes where maximum cell yields must be obtained.

The soil incorporation studies reveal a considerable improvement in microbial utilization during the early stages of growth on a straw irradiated in the presence of 30% hydrogen peroxide. The responses noted for the non-irradiated straw during the first ten days of growth has been reported by several workers to be due primarily to hemicellulose utilization. The irradiated straws show a significant
increase in this response and can probably be accounted for on the basis of photon interaction with the molecules making up the surface cells of the straw particles. Photon interaction with the phenolic structures of lignin and the glucosidic chains of cellulose, as well as many other structures present in straw, would break carbon-carbon bonds and form new open chain structures, with the activated oxygen present, which would be more accessible to attack by microorganisms. This hypothesis is further supported by the isolation of several phenolic compounds, by thin layer chromatography, resulting from straw photolysis.

Additional evidence for the formation of more utilizable substrates from complex molecules is seen in the growth response for the 10 ml hydrogen peroxide treated sample. In this sample the initial concentration of hydrogen peroxide is 0.19%, therefore, not many of the cleaved structures will be in the vicinity of activated oxygen to form new compounds. As the concentration of hydrogen peroxide increases so does the probability of finding activated oxygen and bond cleavage occurring in the same vicinity to form a new structure.

When the amount of hydrogen peroxide added exceeds 20 ml much of the nascent oxygen recombines before it has an opportunity to interact with the activated species of the straw molecules. This reaction is demonstrated by the large increase of utilization of the
20 ml hydrogen peroxide treated straw over the 10 ml sample. Addi-
tions of peroxide in excess of 20 ml gives decreased microbial activi-
ty.

After ten days incubation all the responses have stabilized,
indicating that the newly formed compounds on the surface of the
straw particles have been utilized and now the organisms must be-
gin utilizing the more complex lignin and cellulose molecules which
were not affected by irradiation because of their physical location
beneath the surface of the straw particle.

The increased overall activity shown with the 50 ml hydrogen
peroxide treated sample may be the result of physical reduction in
particle size. These straws visibly decreased in size and coarse-
ness when irradiated with higher concentrations of hydrogen perox-
ide and seemed to be semi-colloidal since longer times were re-
quired for the residue to settle out than was noted with the 10, 20
or 30 ml treated samples.

A third study done to survey the artificial rumen responses to
an irradiated straw resulted in a reversal of the expected response.
Based upon the responses obtained with the Aspergillus growth stud-
ies on the liquid phases of irradiated straws and the responses ob-
tained when irradiated straws were incorporated into soil, it was
assumed that a positive response would also be obtained in the rumen.
However, on the basis of these preliminary studies, the digestibility
is reduced considerably by treating with ultraviolet light in the presence of hydrogen peroxide.

The general decrease in digestibility could be due to a number of variable conditions in the artificial rumen system such as temperature, pH, concentration of the rumen liquor, or it could be due to the photolytic formation of compounds in the straw which are inhibitory to some rumen microflora living in a symbiotic relationship with other organisms needed to efficiently degrade straw-like material.

Further investigation is required in order to obtain any conclusive results from the rumen studies which might be useful in future applications of this photolysis system for more efficient straw utilization by ruminants.

An achievement of a more complete understanding of the role of oxygen and oxygen activated species in photolysis, and of specific wavelength effects on lignin and cellulose structures will allow development of more efficient photolysis conditions to maximize the production of readily utilizable substrates from materials like straw which are difficult to degrade biologically. To achieve such an understanding, factors influencing the photochemical modification of these substrates and the optimum growth conditions must be elucidated.

Based upon the present study, the use of photolysis for improving the biological availability of grass straws has promising possibilities and warrants further investigation. This approach should
be considered for use on many waste products in our environment
which could be rendered to materials readily utilizable by micro-
organisms.
SUMMARY

Oregon field straws treated with ultraviolet light in the presence of oxygen were used to evaluate changes in microbial availability. Liquid portions from treated straws were used for fungal growth studies to evaluate microbial responses to soluble photolytic by-products. The remaining straw residues were used to determine the effects of ultraviolet light on microbial availability in the soil and rumen environment.

Fungal activity showed a positive correlation with the presence of carbonyl compounds in the liquid portions. Carbonyl function concentration increased with irradiation time and appeared to represent a major portion of substrates supporting fungal growth.

Soil microbial activity increased significantly after addition of photolysed straw residues, in comparison with unmodified control materials. The activity corresponded to the utilization of hemicellulosic type compounds formed on the surfaces of straw particles after exposure to ultraviolet light. After these photolytic by-products were utilized the microbial activity decreased to rates given by controls. Apparently, many complex aromatic and polymeric straw structures are ruptured photolytically, followed by combination with oxygen to form open chain and cyclic products similar to hemicelluloses.

Straw digestibility in the rumen, when evaluated by artificial
rumen techniques, decreased considerably after exposure to photooxidized straw residues. These results indicate that photolytic by-products may have been formed to which the rumen organisms were not able to adapt efficiently. Also, optimum fermentation conditions may not have been obtained during the in vitro studies, thus reducing the overall digestibility. Additional study is required to further evaluate factors influencing rumen utilization of these materials.

All fungal response and soil incorporation data indicate that ultraviolet light treatment of straw in the presence of oxygen does alter the molecular structure and increase microbial availability. Although considerable research is required to optimize the efficiency of the system, this process has been shown to effectively convert recalcitrant molecules to more readily biodegradeable substrates and warrants consideration as a treatment which could be utilized in developing additional uses for straw.
BIBLIOGRAPHY


