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FROM PULP MILL WASTES

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ABSTRACT

The goal of this research was to convert the organics and sulfur in sulfite spent liquor (SSL) now classified as pollutants from sulfite pulp mills, into synthetic methane and protein by means of a combination chemical-biological process. Ozonization was used to break the high molecular weight lignosulfonate molecules present in SSL into lower weight fractions which could be metabolized by methane-producing bacteria and protein-producing yeast. Ozonization experiments showed that this treatment is effective in partially oxidizing and fragmenting lignosulfonates into fermentable substrates. This process is initiated at low ozone concentrations and proceeds rapidly until nearly 30% of the Chemical Oxygen Demand (COD) has been consumed. The conditions under which ozonization is conducted greatly affects the degree of oxidation and the molecular weight of the cleaved fragments.

In spite of the appreciable oxidative cleavage of the lignosulfonate molecules, continuous-flow fermentation studies showed rather low yields of methane and yeast from ozonated SSL. Under optimum conditions, methane production averaged only 1.7 l/l of SSL or approximately 3% of the total organics present. Protein production was somewhat more favorable with 6% of the organics being converted to yeast biomass (~6g/l). Neither fermentation fully used all of the oxygenated fragments produced by ozonization, and thus, a two-stage process might yield better results.

Although it appears that ozonization is not a viable treatment of SSL under present economic conditions, with increased demand for energy and protein, it could become more competitive in the future. However, of possibly greater importance is the potential use of partial oxidation treatments to improve the biodegradability of organic wastes.

INTRODUCTION

Lignin derivatives and other soluble organic compounds of wood are the major waste products from the pulp and paper industry. The amount of pulp mill waste effluent produced each year is enormous. For example, the sulfite pulping industry alone accounts for nearly three million tons of organic waste material annually (9). The disposal of this spent sulfite liquor (SSL) is a significant source of water pollution. Mueller and Walden (16) estimate that 2,100 gallons of SSL are produced for every ton of sulfite pulp manufactured.

Various techniques have been developed to dispose of this large quantity of waste product. Originally the bulk of this material was discharged into the streams and lakes convenient to the pulping operations (3). The rivers and bodies of water which currently receive the SSL are overtaxed and cannot continue to accept even the current level of discharge without detrimental environmental effects. The expansion of this segment of the pulping industry has been curtailed because of the pollution problems, and the operation of many existing plants is now threatened.

Methods of treating pulping waste liquor to eliminate the pollution hazard can be generally classified as mechanical or biological. Mechanical methods generally separate the water, usually by vaporization, and dispose of the solids by burning, burying, or sales. The conventional treatment is the evaporation of the water followed by the burning of the organic solids. Besides eliminating water pollution, this process recovers some of the cooking chemicals and considerable energy is generated during the combustion of the solids. Nevertheless, the treatment does have its disadvantages. The burning of the organic solids creates air pollution. The evaporated water contains all

the volatile organics originally present in the effluent and thus, the condensate has an objectionably high biological oxygen demand (BOD) content. In addition, 20-50% of the energy recovered by burning the organic solids is consumed by the evaporation process, which detracts from the overall thermal efficiency of the operation. Growing demand for SSL solids for such uses as roadbinders and clay modifiers has allowed some mills to sell the residues rather than burn them.

Biological treatment of SSL seeks to avoid the expensive water removal step by enzymatic conversion of the waste effluent to a form which can be more efficiently removed. The activated sludge process, storage-oxidation and aerated stabilization methods have been extensively studied (27). In general, these treatments remove only the low molecular-weight, soluble organic fractions which effectively lowers the BOD of the SSL. The high levels of lignosulfonates and sulfur are not significantly reduced, and the full energy potential of this organic resource is not realized.

BACKGROUND

Many investigators have studied the possibility of using microbes to convert the fermentable substrates in SSL into usable products. Propionic, acetic, lactic and formic acids as well as thiamin, vitamin B₁₂, acetone and butanol have all been successfully produced biologically from SSL. By far the most significant product synthesized from SSL is protein, produced from the wood sugars by Torula yeast (10). Marketed for human consumption and as a cattle feed supplement, these yeasts are produced commercially in at least two plants in the United States. Unfortunately, the bulk of the organics present in SSL are not metabolized by any of these fermentation processes.

The general lack of success with biodegradation of lignosulfonates suggests that some pretreatment must be required to degrade SSL, or transform it into a state which could be metabolized. One such possibility is to break the lignosulfonate into smaller molecular weight fractions through ozone treatments. Stern and Gasner (26) have shown that such processing of kraft mill waste liquor did cause a shift in the molecular weight distribution of lignins to lower weight fractions. Ozonization also increased the susceptibility of the waste liquor to biological decomposition. It is likely the lignosulfonate present in SSL would be affected in a similar manner. This technique could be used prior to a yeast fermentation to increase protein yields or after to facilitate the removal of residual BOD and chemical oxygen demand (COD). The sulfur fraction of the lignosulfonate released by ozonization could be removed by stripping and/or bio-reduction.

The BOD remaining after ozonization is amenable to subsequent biological treatment. The methane-producing anaerobes seem ideally suited for such a role. These organisms use fatty acids and alcohols as substrates, which are the

readily available organic materials remaining in the SSL after yeast fermentation. Methane produced from SSL could then be used as a supplemental energy source in the processing plant.

Production of methane from organic residue by anaerobic digestion is well known. A variety of substrates ranging from activated sludge to cultured algae have been converted to methane by this process (14, 23). Considerable interest has recently developed on obtaining methane from animal wastes (7).

Methane fuel production from SSL was earlier considered as a promising fermentation possibility due to the ease with which the gaseous product could be recovered. Calculations by Benson and Partansky (2) based on incubation studies gave a heating value of 1,430,000 BTU/ton of pulp, assuming a 25% carbon removal, and incubation at 36°C. Cultures acclimatized to SSL by successive batch transfers or continuous fermentation were not used. Bannink and Muller (1) also reported significant production of methane from SSL, and Wiley (30) patented a process for use in sulfite pulp plants. The calorie value of the organic waste present in SSL amounts to approximately 40 trillion BTU's annually, equivalent to 40 billion cubic feet of natural gas (18). However, the relatively low cost of other fuels at that time discouraged subsequent research on methanogenesis using pulp mill substrates. This situation would now appear to have changed.

OBJECTIVES

The goal of this research was to convert the sulfur and organics in SSL now classified as pollutants into synthetic methane and protein by means of a combination chemical-biological process. A conceptual process flow diagram to accomplish this objective is shown in Figure 1. The process would use three fermentation stages coupled with ozone cracking, steam stripping, and centrifugation to synthesize and harvest useful products derived from the SSL. A flow diagram of this process is shown in Figure 1.

Waste liquor from the pulp mill enters the primary yeast fermentor where a large fraction of soluble wood sugars is converted to protein and carbon dioxide (CO_2). Effluent from the primary fermentor passes through a centrifuge to harvest the protein. The desugared spent sulfite liquor (DSSL) then passes to the ozonator where it is treated to modify the sulfur bonds and break down the high molecular weight organics. Ozone-cracked effluent is cooled and fed to the anaerobic digester. Here a mixed bacterial culture is used to convert additional organic compounds into synthetic methane. Since only a few specific organics can be utilized by methane bacteria, a mixture of other anaerobic bacteria are needed to transform the ozonated fragments into substrate for methane synthesis.

The effluent from the anaerobic digester may still contain unassimilated ozonated fragments which can be utilized by protein-producing Torula yeast. The effluent from the methane fermentor can, therefore, be fed to secondary yeast fermentors, and then to a centrifuge for yeast removal. Any BOD still remaining is removed by standard treating processes, such as activated sludge or soil infiltration.

During the anaerobic digestion, some organic sludge, biological solids and lignin will be produced. The effluent from the anaerobic digester is fed to a conventional thickener for the recovery of the precipitated organics. The sludge, after dewatering, can be utilized as fuel by burning directly if no higher value alternate is available.

A key factor in the success of this treatment process is the beneficial effects of ozonization on SSL biodegradability. Consequently, the main objectives of this study were to investigate: 1. the effect of ozone pretreatment on the fermentative properties of sulfite pulp mill waste; 2. the relationship between degree of ozonization and the growth of methanogenic bacteria and protein-producing yeasts in SSL; 3. the changes in chemical composition of ozonated SSL as related to biodegradability.

MATERIALS AND METHODS

Substrate Preparation

Desugared Spent Sulfite Liquor (DSSL)

The waste liquor used in the fermentation experiments was DSSL obtained from the Lake State Division of the St. Regis Paper Co., Rhineland, WI., a calcium-based sulfite pulp mill. The effluent from the pulping unit of this plant is fed to a protein production unit, where more than 90% of the wood sugars in the liquor are removed by Torula yeast (Candida utilis). Some of the important characteristics of the liquor are tabulated in Table 1. The data are averages of four batches obtained from the Rhineland mill over a one-year period. These properties vary depending on the type of wood used and cooking conditions employed for pulp manufacture.

Ozonization

Ozonization was carried out in a counterflow tubular reactor illustrated in Figure 2. Ozone was produced by a Welshbach Ozone Generator, Model T-816, which fed a 500 ml capacity 2.5 cm ID, 1.8 m tall glass column reactor packed with 1.25 berl saddles. All fittings for the ozonization apparatus were gas tight, and made of stainless steel to avoid corrosion problems. The gas stream produced by the ozone generator contained 2% ozone by weight and was introduced at the bottom of the reactor. DSSL was supplied to the top of the reactor by a variable speed feed pump which could be adjusted to obtain any residence time desired.

Preliminary batch ozonization experiments indicated that pH control was essential in order to obtain reproducible results. A pH meter and automatic titrator were used to monitor and control acidity changes with a 10% NaOH solution in an external recycling chamber. Ozone concentration of both influent

TABLE 1. Some Chemical Properties of Desugared SSL

	Value g/l	Standard deviation
Chemical oxygen demand (COD)	95.2	4.85
Biological oxygen demand (BOD)	9.2	0.71
Total sulfur - %	6.1	0.26
Total dissolved solids - g	97.9	---
Total organic carbon (TOC)	41.0	2.10
Volatile fatty acids	Traces	---
Sugars ^A - %	2.5	---

^ABased on 90% conversion of wood sugars in spent liquor (10)

FIGURE 2

EXPERIMENTAL OZONATION APPARATUS

and effluent gases were monitored by bubbling samples through gas absorbers containing a 2% aqueous solution of KI and titrating the free I_2 produced with a standard sodium thiosulfate solution.

Lignosulfonates present in DSSL have a natural surfactant quality that causes undesirable foaming in the reactor. It was necessary to employ a small quantity of anti-foam, and several commercial products proved effective. Dow Corning Antifoam A was selected for use in the bulk of the experiments.

During ozonization a small quantity of insoluble materials is produced. Prior to fermentation these solids were removed by filtering through a 0.45 micron Milipore filter.

Methane Production

All investigations on methane production from DSSL were carried out in continuous fermentation vessels. Three one-liter, magnetically stirred, stainless steel and glass fermentors equipped with Master-flex pumps were used. Ozonated and untreated DSSL supplemented with 0.5 g/l K_2HPO_4 and 4.0 ml/l NH_4OH (conc.) were continually fed into the fermentor from a 4°C refrigerated reservoir. Residence time of the DSSL substrates in the fermentor was controlled by the variable-flow input/output pumps. Fermentor pH was maintained near 7.2 by adjusting the feed pH to 8.0 with NaOH. This method of pH control was considered sufficient since fluctuation of pH was not greater than 0.2 pH units. The experimental flow diagram for this process is shown in Figure 3. Fermentation temperature was maintained at $35^\circ C \pm 1^\circ C$ in an environment chamber. Determinations of fermentor pH and redox potentials were performed daily using a glass combination pH electrode and a platinum redox electrode.

Inoculum Development

Mixed cultures of methane-producing bacteria were initially obtained by

DIAGRAM 3
EXPERIMENTAL FERMENTATION APPARATUS

adding 100 ml of fresh, fermenting sewage sludge to each of the fermentors as inoculum. A prereduced basal medium was added to give a final fermentor volume of 600 ml. This basal medium, described by Ferry *et al.* (8), was modified to include 2.5 g/l sodium formate, 2.5 g/l sodium acetate, 2.0 ml (60%) sodium lactate, 2.0 ml methanol, and 2.0 ml ethanol. The pH of the medium was adjusted to 7.2 with NaOH.

As soon as methane production began (in about 3 days), a solution of 75% basal medium and 25% ozonated DSSL was added continuously at a rate to provide a 3.5 day retention time. The ratio of basal medium to ozonated DSSL was decreased in stepwise fashion to 50:50 and then to 25:75 over a one-month period to acclimate the organisms to the ozonated DSSL. Eventually, the basal medium was eliminated and replaced by a DSSL medium supplemented with nitrogen, phosphorus and potassium.

Gas Production and Analysis

Gas production rates from each fermentor were recorded by water displacement in gas-measuring burets. Since these burets were not changed during the experiments, a correction for CO₂ dissolved in the collecting water was not necessary. Corrections were made for hydrostatic pressure, water vapor, and temperature to report gas at standard temperature and pressure (STP). Gas produced during fermentation was analyzed by a Varian Aerography Model 1800 gas chromatograph. A 1.8 m, 3 mm stainless steel column, packed with Porapak R run at 40°C with a N₂ carrier gas flow rate of 20 ml/min, was used for all gas sample analyses.

Bacteria Enumeration

Estimates of methanogenic bacteria populations in the fermentors were made using a modification of the Hungate roll tube technique (11). Samples for bacterial counts were collected by draining a few milliliters of fermentor

contents into a test tube continuously sparged by a stream of oxygen-free CO_2 . The test tube was then transferred to a three-place, swing-type cannula system (11) and 9.9 ml serial dilutions were made with the previously described broth. Each sample was prepared in triplicate, added to roll tubes containing the basal medium plus 2% agar, and incubated at 35°C for 28 days. Colony counts were made under a 40X binocular dissection microscope. Separate counts of light-colored and black-dark brown colonies were made to estimate the numbers of methanogenic and sulfur-reducing organisms.

The presence of anaerobic bacteria other than methanogens or sulfur-reducing bacteria were determined by plate counts using Brewer's anaerobic agar (Difco). Triplicate dilution plates were incubated at 35°C for 5 days in Torbal jars with an atmosphere of 80% N_2 , 15% CO_2 , and 5% H_2 . This lowered the OR potential below -75 mv as indicated by a clearing of resazurin present in the agar.

Yeast Production

The growth of two Torula yeast variants in DSSL were studied in batch fermentation studies. Both variants were isolated from yeast plant effluent obtained at the Lake States Yeast Plant, Rhinelander, Wisconsin, by streaking effluent samples on agar plates and incubating at 35°C . After 2-3 days of incubation, one rough and one smooth yeast colony type were observed, and each was isolated in pure culture.

Yeast inoculum for fermentation experiments were prepared by transferring several loops from a single colony into a flask containing peptone (0.08%), malt extract (.4%), glucose (.4%), K_2HPO_4 (.1%), NH_4Cl (.1%), and DSSL (10%). After two days, one ml of yeast culture was added to 125 ml Erlenmeyer flasks containing 100 ml of variously-ozonated DSSL, NH_4OH (.5%) and K_2HPO_4 (.5%).

Ten flasks were used in each yeast growth experiment. The flasks were

incubated on a NBS Gyrotory shaker, Model A-25 at 33°C. Two uninoculated blanks were also included in each experiment as controls in case some chemical precipitation occurred during the incubation period. Two culture flasks were removed every twelve hours and analyzed for yeast production. The yeast were harvested in a Sorval RC2-B refrigerated centrifuge at 15000 rpm for 20 minutes. The liquid was decanted and the cells resuspended in distilled water and recentrifuged. This technique was repeated a second time to ensure the removal of all soluble compounds from the harvested cells. A streak plate was made from each flask to check for yeast culture purity.

A similar procedure was performed on the uninoculated samples to determine the quantity of solids, if any, produced by non-biological effects. The cells or solids were then dried for one hour at 101°C. Yeast production was taken as the difference between the dry weight of solids obtained from yeast growth flasks minus dry weight of solids produced in the uninoculated flasks.

Effluent Analysis

Chemical oxygen demand and biochemical oxygen demand tests on DSSL were conducted as described in Standard Methods for the Examination of Water and Wastewater (25). An acclimated seed for the BOD₅ test was maintained by continual aeration and daily additions of 1:50 dilutions of an ozonated DSSL-water mixture. The ozonated DSSL in the DSSL-water mixture was supplemented with 0.1 g/l K₂HPO₄ and 1 ml/l NH₄OH. One-sixth of the entire acclimated seed was discarded once a week and replaced with raw sewage. Total sulfur was determined according to the procedure outlined by Salverson and Hogan (21). Organic and inorganic carbon was measured with a Beckman 915 Carbon Analyzer.

Volatile and non-volatile fatty acid contents were analyzed by gas chromatography according to the procedure given by Holderman and Moore (11). Fatty

acids were identified and quantified by comparison of retention times and peak heights with those of known standards. A 1.8 m, 6.3 mm stainless steel column packed with 15% SP-1220/1% H_3PO_4 on chromasorb WAW, a packing specifically developed by Supelco Inc. (Belefonte, PA) for fermentation product evaluation, was employed for these tests. Column conditions were 120°C with a helium gas flow of 60 cc/min.

EXPERIMENTAL RESULTS

Ozonization

Preliminary experiments were run to determine the effects of ozonization on the chemical properties of DSSL. Infra-red spectra taken for raw DSSL and 6-hour ozonated DSSL, shown in Figure 4, suggest that significant transformation of aromatics to carboxylic acids had been caused by ozonization. The BOD_5 test results of 6-hour ozonated DSSL indicated an appreciable increase of metabolizable organics when ozonization was conducted at pH 3.0, while there was very little increase in BOD_5 when ozonization occurred at alkaline pH. The COD of the ozonated sample was reduced the same amount, 23% whether acid or alkaline pH's were used. Tests indicated that there was a slight decrease in the sulfur content of the sample due to ozonization.

Additional ozonization experiments were conducted in both continuous and batch modes of operation, and results generally paralleled those obtained in the initial tests. The time required for ozone to appear in the effluent gas stream varied from 3 hours at pH 3 to about 6 hours at pH 8. Ozone consumption was approximately one-half at the lower pH, and the product was more easily assimilated by the microorganisms; consequently, all further testing was done with material ozonated at pH 3.

Reactivity of ozone with DSSL as a function of time is shown in Figure 5. During the early stages of ozonization the reaction rate was essentially constant; the ozone uptake efficiency was 100%. After approximately 3 hours of ozonization, the organics in solution showed resistance to further oxidation, and the rate of reaction slowed. The ozone uptake efficiency decreased as ozone consumed approached a value of approximately 15 g/l of DSSL treated. This was equivalent to a 3-hour ozonization time.

FIGURE 4 INFRA-RED SPECTRAL COMPARISON BETWEEN SPENT SULFITE LIQUOR (DSSL) AND 6 HR. OZONATED DSSL

Fig 5 Ozone/Desugared Spent Sulfite Liquor Reactivity

It has been reported that ozone has little effect on lignin in the absence of water (5), and oxidation is much faster in acidic media where an electrophilic attack by O_3H^+ is probable (22). The aromatic monomers of lignin are highly susceptible to electrophilic attack, which is catalyzed by the presence of Lewis acids (such as O_3H^+), to produce CO_2 , as well as formic, acetic, and oxalic acids. The last three products are biodegradable and thus contribute to an increase in BOD_5 . It was, therefore, not surprising to find the increase in BOD_5 on ozonization favored at low pH, as seen in Figure 6. However, the change in COD was independent of pH but in agreement with the consumption of ozone, which was a constant 6.6 g of ozone consumed/liter at all pH levels. The expected decrease in COD was calculated to be 6.6 g/l in all cases. At high pH the major products could either be CO_2 or other fragments which are not biodegradable and hence do not contribute to BOD_5 .

The decrease in COD on ozonization occurred linearly with ozone consumption. The decrease, because of degradation of lignin to oxygenated organic fragments, agreed with the theoretically expected value based on the quantity of ozone consumed. Thus, at an ozone consumption of 15.0 g/l, the theoretical expected decrease in COD was 15.0 g/l, while the experimentally found decrease was 16.2 g/l, with a standard deviation of 1.4 g/l. This difference was less than 2% based on the actual COD of 95.2 g/l of DSSL and was well within the accuracy of the test. However, the excess of 1.2 g/l could also result from free oxygen taking part in the reaction. Katuscak *et al.* (13) suggested that secondary reactions by oxygen with ozone acting as initiator are possible. This could not be experimentally verified. Extended ozonization up to 40 g/l of ozone consumption reduced COD by 39.8 g/l or 42%.

The increase in BOD_5 on ozonization (Figure 7) was likely the result of

Fig. 6 Hydrogen Ion Concentration Affects Chemical and Biochemical Oxygen Demands During Ozonation.

lignosulfonate breakdown into low-molecular-weight compounds such as formic, acetic, and oxalic acids. Stern and Gasner (26) have reported formation of such compounds on ozonization of lignin. The presence of these acids in ozonated liquor was detected by gas-liquid chromatography. Some of the organics in DSSL were completely oxidized to CO_2 , which was detected in the ozonization reactor gas stream by gas chromatography during the entire period of ozonization.

The most pronounced effect of the ozonization treatment was a change in DSSL color. The initial black color of the liquor changed to orange at 15 g/l and yellow at 40 g/l. The change in color, plotted in Figure 8, was monitored on a Spectronic 20 at 360 nm using dilute samples (1 part per 20 parts of water). The reduction in color resulted from destruction of the aromatic ring system together with the chromophores of lignin responsible for its color.

Changes in other characteristics, such as sulfur content, total dissolved solids (TDS), and total organic carbon, were recorded only for an ozone dosage of 15 g/l. A 7% decrease in sulfur content of the liquor was observed for this level of ozone consumption. This decrease was the result of calcium sulfate formation which precipitated out from the liquor. The overall effect of ozonization on TDS was complex. An increase caused by the production of non-volatile oxygenated compounds and a decrease caused by the loss of carbon as CO_2 and formation of a precipitate, identified as a mixture of calcium oxalate and sulfate, was expected. No net change in TDS was observed, indicating that the opposing mechanisms balanced out. Approximately a 5% decrease in total organic carbon was recorded.

Methane Production

Numerous studies were made with anaerobic fermentors to determine optimum conditions for producing methane from DSSL. Except for preliminary testing,

Fig. 7 Effect of Ozonation on Biological Oxygen Demand

Fig. 8 Effect of Ozonation on Color of Desugared Spent Sulfite Liquor

studies to determine methane yield from ozonated DSSL were done in continuous operation. Two fermentors were operated under identical conditions on a substrate consisting of 3-hour ozonated DSSL (15 g O_3 /l) diluted 25% (3:1) with tap water. The results obtained from the two reactors were identical. The working volume for the fermentors was varied from 500 to 700 mls to establish residence times between 2 and 6 days.

Steady state testing done on the fermentors over a 12-month period maintained an average gas production of 10 ml/hr at STP (Table 2).

TABLE 2. Gas Production from Continuous Fermentation of DSSL¹

	Retention Time (days)	Effluent Gas		
		Production (mls/hr)	Composition %CO ₂	%CH ₄
3-hr ozonated DSSL	3.3	10	22	78
Unozonated DSSL	3.3	2	86	14

¹All values are based on 75% concentration.

This is a total yield of 1700 mls per liter of feed for a 3.5-day retention time. The maximum gas production observed at any time during fermentation was 30-40 mls/hr. The average gas production of 10 mls/hr was equivalent to 2 liters of methane per liter of undiluted ozonated DSSL, or a carbon conversion of about 5%. Gas chromatography showed the average methane concentration for the steady state period over 75%, the balance being composed of CO₂. This methane concentration is higher than that reported in the literature (50-65%) from the fermentation of agricultural and municipal wastes.

The effect of DSSL ozonization on methane yield was evaluated by feeding one reactor unozonated 75% concentration DSSL. The results of this experiment

showed a general lack of metabolizable substrate in the unozonated effluent. Gas production was only a quarter of that obtained from ozonated DSSL reactors (approximately 2 mls per hour), and was composed mostly of CO_2 (Table 2). This result established that most of the methane produced from ozonated DSSL was due to compounds produced during ozonization, and not from organics remaining after the yeast fermentation. It was evident that prior yeast treatment has removed most of the sugars and lower weight compounds in DSSL to the point where it cannot support extensive anaerobic fermentation.

The average generation time for methane-producing organisms appeared to be about 2.3 days. When the retention time was brought below 3.0 days, gas production rates initially increased by about 10%, then rapidly dropped off. This indicated a washing out of the bacterial population. When the retention time was raised to greater than 3.5 days, a decrease in gas production was observed proportional to the increase in retention time, typifying a substrate limited condition.

A lower ratio of CH_4 to CO_2 was observed if fermentors were run at a pH below the optimum of 6.8 to 7.5, or if unozonated DSSL was used as a substrate. This range of pH along with oxidation-reduction (OR) potentials of -300 mv has been confirmed by a number of investigators (14, 29) to be optimal for methane production. Cappenberg reported best methane bacteria growth at OR potentials between -380 and -400 mv (4). In our fermentors OR potentials fell to as low as -420 mv during continuous operation and optimum gas production. It is probable that different fermentor feed had an effect on the net OR potentials during fermentation.

Fermenter Effluent Analysis

As indicated by the data in Table 3, a cumulative drop of 32% in COD values was found for a combined treatment of both ozonated and fermented DSSL.

TABLE 3. Average Values for COD, BOD, Total Sulfur, and Fatty Acids in Untreated and Treated DSSL

	Unozonated DSSL	Ozonated DSSL	Fermented & Ozonated DSSL
COD (mg/l) ¹	93,300	78,700	64,000
BOD (mg/l) ¹	9,200	10,900	8,500
Sulfur (g/l)	4.6	4.3	3.8
<u>Organic Acids</u> ²			
Formic (mM)	-	3	2
Acetic (mM)	2	8	66
Propionic (mM)	-	-	4
Butyric (mM)	-	-	4
Oxalic (mM)	-	4	-

¹Average of 10 replications. All others are averages of 5 replicates.

²Concentrations less than 1 mM not detectable

Fermentation of DSSL reduced the average COD value of the effluent by about 19%, while the average BOD₅ value dropped 23%. There is an initial increase in BOD of about 20% after ozonization, but this substrate is used up during fermentation. When unozonated DSSL was fermented under similar conditions, COD values dropped only 7% with no noticeable change in the BOD₅ value. A 11% loss in total sulfur content was observed after fermentation of ozonated DSSL. Fatty acid analysis indicated that approximately 66 mmol/liter of acetic acid were present in the effluent along with traces of propionic and butyric acids. The oxalic acid produced during ozonization was consumed during fermentation.

Analysis of reactor performance during this period indicated that the fermentation process appeared to be substrate limited. Various nutrient supplements

were added to 34 steady runs, lasting approximately 5 days each, to determine what the limiting substrate(s) may be. During these experiments varying quantities of methyl or ethyl alcohol, vitamins, minerals, salts, and salts of low molecular weight organic acids were added (in different amounts and combinations) in an attempt to stimulate methane production. The addition of alcohol, formate, or acetate alone had no measurable effect on gas production. The addition of a mixture of methyl alcohol with vitamins and minerals or vitamins alone increased gas production to only 15 cc/hr. The addition of sodium lactate with or without additional vitamins and minerals also tended to stimulate production only to 15 cc/hr.

Yeast Growth

Figures 9 and 10 show the effect degree of ozonization had on the growth of the rough and smooth variants of Torula yeast. As ozonization time was decreased from 120 minutes to 10 minutes, the maximum yield of the rough variant of Torula yeast increased approximately four fold. Although the smooth variant apparently has the same growth rate characteristics as the rough, its ability to utilize ozone generated substrate is much less. At an ozonization time of 10 minutes the smooth variant also showed a maximum production of cell mass; however, this production was only slightly greater than that obtained from the rough variant grown on an untreated DSSL. The ability of the smooth variant to utilize substrate generated from DSSL at ozonization times greater than 10 minutes was essentially nil. Only the rough variant of Torula yeast was used in subsequent experiments because of its higher yields.

All ozonization times produced some useful substrate as the yeast production on untreated DSSL was minimal. The data also indicated that at fermentation times exceeding 48 hours, some of the yeast cells lysed and total yield decreased.

FIGURE 9. GROWTH OF ROUGH TORULA VARIANT AFTER VARIOUS OZONATION TIMES

FIGURE 10. GROWTH OF SMOOTH TORULA VARIANT AFTER VARIOUS OZONATION TIMES.

Additional experiments were designed to show the dependence of yeast production on the pH of DSSL during ozonization. Results are shown in Figure 11 and Table 4. Greater amounts of substrate were available for yeast growth when DSSL was ozonated under acid conditions. These increases in carbon supply at low pH agrees with the results from BOD₅ tests discussed earlier.

TABLE 4. *Torula* Yeast Growth as Affected by Ozonization pH of DSSL

Fermentation Time (days)	DSSL pH		
	3.5	5.0	9.0
	----- g/l -----		
1	1.1	1.0	0.6
1½	1.8	1.1	2.2
2	3.7	3.5	3.0
2½	3.9	4.1	3.6
3	4.1	4.8	3.2

Initial experiments used liquid DSSL obtained from the St. Regis Paper Company. Midway in the project, the starting material was changed to DSSL in powder form obtained from St. Regis. This change was made to reduce variations in DSSL composition. The powder was mixed to match the composition of the initial liquid DSSL. Reconstituted DSSL appeared identical to the original liquid used with respect to both ozonization and fermentation properties.

Data comparing oxygen reactivity with ozone reactivity as affecting yeast yield are presented in Figure 12. The initial pH of 5.0 was not controlled during either ozonization or oxidation. The pH of the ozonated DSSL dropped to 3.5, while that of the oxygenated sample dropped to 4.5 over the 20-minute period. Similar results were obtained for pH controlled oxidations. The results for pH 5, which appears to be near optimum, are given in Figure 13.

FIGURE 11 YEAST PRODUCTION VARIES WITH PH OF OZONIZATION

FIGURE 12. OZONE VS. OXYGEN REACTIVITY

FIGURE 13. OXYGENATION TIME VS. YEAST GROWTH

Other DSSL Treatment

Since the efforts to increase methane production from ozonated DSSL by the addition of growth supplements or varying reactor operating conditions failed to isolate any sufficiently effective additive, the effects of other pre-treatments of DSSL prior to fermentation were examined. Research on sewage sludge has indicated that sonication greatly increased its biodegradability.^{1/} Therefore, a study was established in which ultrasonic treatment of DSSL was compared with or used in conjunction with ozonization.

All sonication treatments were performed in the same reactor having a starting volume of 300 ml of 100% yeast plant effluent. The reactor was placed in an icebath for temperature control, but substrate temperature did reach 60°C during the longer ultrasound treatments. A sound level of 41,000 hz was used in all experiments. Samples were removed from the reactor every half hour for COD and BOD₅ determinations.

The results of these tests showed that ultrasound treatment does have an appreciable effect on the BOD₅ and COD levels of DSSL, especially when used in conjunction with ozonization (Figure 14). Some of these changes may have resulted from the higher substrate temperature due to sonication or to the lowering of pH to 1.5 during the combined sound and ozone treatment. It is unclear why the combined sound-ozone treatment had such a marked effect.

Bacterial Populations

Methane bacteria counts in the fermentors were found to average 1.7×10^9 colony-forming units (CFU)/ml. This value was derived by subtracting the

^{1/}Personal communication. Mr. Craig Breannon. Dept. of Forestry, Michigan Technological University. 1976

Figure 14. Changes in COD and BOD vs. time for varied treatments of DSSL with ozone and sound.

counts of black-dark brown colonies (also numbering 1.7×10^9) from the total roll tube counts. The validity of this technique was verified when it was found that of 80 light-colored colonies randomly isolated from roll tubes, 78 were shown through gas chromatography to produce methane. Although all of these isolates may not have been pure cultures, there were definitely methanogens present in each isolate. None of 35 black-dark brown colonies were found to produce methane. Since the composition of the isolation medium selectively favored methanogens and sulfur-reducing bacteria, the presence of other anaerobes had a negligible effect on the total population estimates. However, counts from Torbal jars indicated an anaerobic population of at least 5.3×10^7 .

Methanogenic colonies larger than 0.5 mm in diameter were found to fluoresce in roll tubes when exposed to low frequency ultraviolet light. This is in accordance with the findings of Edwards and McBride (6). This fluorescence was not exhibited by any of the black-dark brown isolates. Although fluorescence by methane bacteria has been suggested as a method of enumeration, the subtraction method proved much easier and faster after it was shown through gas chromatography that nearly all the lighter-colored colonies were methanogens. Enumeration of methanogens by fluorescence proved to be unreliable because colonies smaller than 0.5 mm did not emit enough light for detection.

A microscopic examination was made of 135 randomly-picked, light-colored methanogenic colonies. Ninety-three percent of these were found to be Gram positive coccoids to short lancet-shaped rods, sometimes in chains, and various types of Gram negative rods, similar to descriptions for representatives of the genus Methanobacterium (24). This genus is present in many forms of anaerobic digestion (14, 28). The remaining 7% of the 135 light-colored colonies closely resembled the methogenic genus Methanococcus. These isolates were small Gram variable to Gram negative Cocci which grew in clumps, pairs, or as single cells.

Microscopic examination of the bacteria isolated from black-dark brown colonies revealed all were curved to sigmoid Gram negative, nonsporing rods. The production of H_2S by each of these isolates was readily detectable. These attributes indicated the presence of sulfur-reducing bacteria, probably Desulfovibrio spp. (20) since sulfate reducers such as Desulfotomaculum are spore formers. A culture of Desulfovibrio desulfuricans obtained from the American Type Culture Collection was comparable in microscopic and cultural characteristics to the 35 fermentor isolates.

The average number of methanogens present in ozone-treated DSSL fermentors is in the upper range commonly encountered in the digestion of other organic wastes. Reports by various authors show a range of 10^6 - 10^9 methanogens/ml in sewage, domestic refuse, and animal waste digestion (6, 14, 28). The high population of methanogens observed in ozonated DSSL fermentation as compared to sewage sludge may be due to less competition from other anaerobes in the low carbohydrate DSSL or to differences in counting media. Sulfur-reducing bacterial populations in fermenting DSSL were also much greater than the $3 - 5 \times 10^4$ counts/ml commonly found in sewage digesters (14, 28). The high sulfur content of the DSSL likely accounts for these higher numbers of sulfur-reducing bacteria.

ANALYSIS OF RESULTS

Methane Production

A fractional factorial screening analysis to evaluate the important variables in the production of methane from ozonated DSSL was run. The factors examined were: 1. concentration of the DSSL; 2. ozonization time; and 3. retention time of DSSL in the fermentor. The SPSS statistical package was used to analyze the fractional design data. The analysis was done with 21 treatment levels of DSSL concentration and retention time, and three ozonization times. The significance of each variable on methane production is shown below:

<u>Variable</u>	<u>Coefficient</u>	<u>F - Value</u>	<u>Significance</u>
Concentration	.35399	94.035	.00001
Retention time	.06125	.902	N.S.
Retention time x concentration	-.09964	4.920	.05

As would be expected, the concentration of DSSL is a very important factor for methane yield. DSSL concentrations used in these experiments ranged from 25% to 200%. All concentration values were expressed in terms of liquid DSSL obtained from the mill. DSSL levels over 100% were made with the powdered DSSL. Retention time beyond the washout period of 2.5 days was not significant. But there is the possibility that a retention time-concentration interaction exists.

Data was also gathered on the significance of ozonization time to gas production. The fractional factorial analysis on the fifteen treatment levels yielded the following results:

<u>Variable</u>	<u>Coefficient</u>	<u>F - Value</u>	<u>Significance</u>
Ozonization time	.32009	11.912	.01
Retention time	-.01254	.117	N.S.
Ozonization time x Retention time	.01910	.096	N.S.

Ozonization times were varied from 0.1 hours to 3.0 hours. Similar to DSSL concentration effects, ozonization time was also significant in increasing gas yield. Again, retention time and the ozonization time x retention time interreaction were not significant.

Ozonization

Ozone readily attacks DSSL in an acidic medium, converting it to low molecular weight compounds. Some of these have been identified as CO_2 , formic, acetic, and oxalic acids. The formation of these aliphatic acids as a function of ozone consumption is reported in Table 5. Based on the quantity of lignosulfonates degraded, these aliphatic acids do not account for all the oxygenated fragments produced. A number of other possible oxidation products of lignin have been reported (22), but these were not identified in this study. The oxygenated fragments, especially the aliphatic acids, are useful substrates for microorganisms, and thus an increase in BOD was expected. This was verified by actual BOD measurements as reported in Figure 6. Also found was a decrease in COD which agreed with the theoretically expected change based on the amount of ozone consumed.

Ozonization Time

Unozonated DSSL was found to be a very poor substrate for methane production. Only 350 mls of gas, mostly CO_2 , was produced from one liter of DSSL (Table 6). Formation of biodegradable oxygenated organic fragments was evident even with

TABLE 5. Low Molecular Weight Organic Acids Formed by Ozonization of DSSL

Ozone Consumption (g/l)	Formic	Acetic	Propionic (m moles/l)	Oxalic	Malonic
0.0	0.0	3.5	0.3	0.0	0.0
0.8	0.0	8.6	Trace (<0.1)	0.6	0.0
10.0	3.9	-	"	4.3	0.0
15.0	23.6	10.6	"	5.8	0.5
25.0	34.8	0.9	"	8.9	0.8
35.0	30.0	0.4	"	15.1	0.9
40.0	20.0	0.4	"	18.3	2.1

TABLE 6. Effect of DSSL Ozonization Time on Methane Production

Time (h)	Ozone Used (g/l)	Residence Time (days)	COD (% loss)	BOD (% loss)	Organic Acids		Gas Yield (ml/ml)	Gas Analysis	
					Acetic ---(m moles)---	Propionic		CO ₂ --(%)--	CH ₄
0	0	3.7	2.1	20.7	42	3.7	0.4	91	9
.5	4	3.3	12.2	39.6	36	1.8	0.6	34	66
		5.3	12.6	36.4	38	6.8	0.5	28	72
2	11	3.3	11.5	-	39	4.5	0.7	25	75
		6.0	20.1	36.6	38	8.2	0.4	-	-
3	15	3.3	26.0	47.0	43	2.5	1.8	21	79
		5.1	18.0	42.7	30	2.0	1.6	11	89
5	25	3.1	17.9	30.5	28	5.1	1.7	7	93
		5.0	25.7	31.7	42	6.4	1.6	11	89

mild ozonization (0.5 h) as shown by the increased quality of gas produced. Further ozonization improved the quality as well as quantity of gas produced with the optimum being a 3 h treatment which consumed 15 g/l of ozone. No further improvement in gas production was observed with higher ozonization levels. This is also evident in the BOD change which showed that the rate of biodegradable fragments produced on ozonization beyond 15 g O_3 /l was less than the rate of complete oxidation to CO_2 . On extended ozonization (5 h treatment), the quantity of substrate produced in earlier stages is further degraded and lost as CO_2 , thus, decreasing gas production. The destruction of substrates such as formic and acetic acid on extended ozonization was evident in Table 5.

DSSL Concentration

In order to study the effect of lignosulfonate concentration on methane production, DSSL dilution was done after ozonization; whereas, concentration was done prior to the treatment. The concentration of DSSL was regulated by diluting with water or increased by dissolving dry calcium lignosulfonate obtained from Rhineland, Wisconsin. The ozonization level was held constant at 0.15 g/g of dissolved solids present. Results of this study are shown in Table 7.

TABLE 7. Effect of Lignosulfonate Concentration in DSSL on Total Gas Production

Concentration (g/l)	Gas Production (ml/ml of feed)	Gas Analysis	
		CO_2 (%)	CH_4 (%)
20	0.3	-	-
40	0.5	-	-
61	1.2	13	87
81	1.8	21	79
121	2.6	10	90
162	2.2	35	65

Gas production values are based on a residence time of 3.3 days in the fermentors. No detrimental effect on gas production was observed up to a concentration of 121 g/l. A further increase in lignosulfonate concentration to 162 g/l decreased both the quantity and quality of the gas. This indicated that the anaerobic microorganisms acclimated to DSSL could tolerate higher molar concentrations of salts and sulfur than present during yeast fermentation at the St. Regis paper mill and reported in literature (10). The concentration of methane in the gas produced from the ozonated liquor averaged approximately 80%. This is greater than the 50 - 65% values reported in the literature from anaerobic digestion of agricultural and domestic refuse (15, 29). The explanation is found by examining the probable difference in stoichiometry for carbohydrates and alcohols as shown in equations (1) and (2):

Methane concentrations in excess of 75% indicated by equation (2) might be attributed to a substrate used more efficiently than methanol.

Chemical Oxygen Demand

Of the total COD loss of 11,200 mg O_2 /l DSSL, only 2900 mg can be accounted for by the production of methane during fermentation. This value is based on a gas production rate of 1100 ml/l of 61 g/l DSSL at a residence time of 3.3 days having a methane concentration of 80%. The CO_2 produced does not contribute to COD loss as it is already in the most oxidized state. The major portion of the unaccounted COD loss (8,200 mg O_2 /l of DSSL) was probably due to reactions in which the oxygen of the water was used by bacteria to degrade large organic molecules to smaller fractions, such as acetic and propionic acids. Such bacterial oxidation reactions which involve water as one of the reactants are known to occur during methanogenesis (22).

Some of the COD loss was also due to loss of sulfur as H_2S , hydrogen and a lowering of the dissolved oxygen content of the fermentor effluent as compared to the feed. Both H_2S and hydrogen in the product gas stream were below detection levels indicating all sulfides produced by sulfate reduction were precipitated by iron or other trace metals in the DSSL. Assuming that the total sulfur loss (0.42 g/l) during fermentation is converted to H_2S , this factor could contribute only 840 mg O_2 /l of DSSL to the decrease in COD. This further substantiates the belief that O_2 removed from water is largely responsible for the decrease in COD.

Residence Time

Residence time of ozonated DSSL in the fermentor appeared to have no net effect on gas production (Table 8). Since these were continuous cultures, the microbial growth rate and gas-producing rate was equal to the dilution rate. Thus, the size of the population was constantly limited by the concentration of the substrates in the inflowing medium. When the residence time was increased by decreasing the dilution rate, the rate of gas produced per unit time was decreased, but the net production of gas per unit of feed remained the same. This indicated that this system was very substrate limited. If there were ample or excess substrate, an increase in gas production/ml of feed would have been expected. The increase in residence time also showed no trend in changing gas composition. This may mean that essentially the same or similar gas yielding reactions are occurring at various residence times.

An increasing trend in BOD loss with increasing residence time (Table 9) may indicate a greater use of available substrate/of feed. This extra substrate likely was used for microbial cell maintenance rather than gas production, since no increase in gas produced/of feed was observed. A BOD loss of 55% at 8.4 days residence time would also be important for obvious ecological reasons.

TABLE 8. Effect of Fermentor Residence Time on Gas Production
from DSSL

Concentration (g/l)	Residence Time (Days)	Gas Production (ml/ml)	Gas Analysis	
			CO ₂ (%)	CH ₄ (%)
61	3.3	1.2	13	87
	3.7	1.1	9	91
	4.2	1.2	28	72
	5.2	1.2	7	93
	7.3	1.1	-	-
	8.4	1.4	37	63
81	3.2	1.8	21	80
	5.1	1.6	11	89
	6.1	1.4	14	86
	8.5	1.2	20	80
	11.8	1.3	6	94
121	3.5	2.6	11	89
	6.1	2.4	-	-
	8.2	2.3	12	88
162	3.5	2.2	35	65

TABLE 9. Effect of Fermentor Residence Time on DSSL Effluent Parameters

Concentration (g/l)	Residence Time (Days)	COD (% loss)	BOD (% loss)	Organic Acids	
				Acetic (m Moles)	Propionic (m Moles)
61	3.3	14.8	43.2	34	4.2
	3.7	16.2	48.8	43	5.7
	4.2	16.7	42.7	-	-
	5.2	19.5	58.3	31	1.9
	7.3	13.6	53.5	33	1.7
	8.4	18.1	55.2	36	0.3
<hr/>					
81	3.2	26.0	47.0	43	2.5
	5.1	18.0	42.7	30	2.0
	6.1	22.3	52.1	41	3.0
	8.5	21.1	53.4	34	1.4
	11.8	22.4	53.0	-	-
<hr/>					
121	3.5	23.0	23.0	32	4.0
	6.1	-	-	43	5.0
	8.2	-	19.6	38	4.4
<hr/>					
162	3.5	21.2	31.7	49	6.1

Organic Acids

As evident from Table 9, considerable amounts of acetic and propionic acids were produced during fermentation. Although acetic acid has been shown to be a substrate for methanogens (31), this study has shown at least a 37 mM concentration in the fermentor effluent stream. It is possible that during extended residence times of 20 - 30 days used in other systems, this acetic acid would be used in methane production. However, there was no trend of decreasing acetate concentration with the residence times studied of 3.3 - 11.8 days.

Since none of the formic, oxalic and malonic acids (shown in Table 5) remained after fermentation of the ozonated DSSL, these acids could be included as important substrates for gas production and cell maintenance. However, they would account for the major portion of BOD or COD loss, indicating that the majority of the gas-yielding substrate produced during ozonization of DSSL were other types of oxidation products.

Protein Production

Figures 9, 10, 11 and Table 4 related ozonization times to yeast production. The data showed that lowest ozonization times (5 or 10 minutes) gave the highest yeast production; approximately 5 gms/l for the rough variant. Increased yields at shorter ozonization times indicated that prolonged ozonization produces a medium less favorable for yeast production. Complete oxidation of substrate carbon to CO_2 would appear to be the mechanism responsible for the reduced yeast growth. Yeast yields for pure oxygen (Figures 12 and 13) support this concept and suggest that ozone may not be the most suitable oxidizing agent. Oxidation with low cost O_2 gave yeast yields (5.8 gms/l) comparable to the best results obtained with ozone.

As shown in Table 4 and Figure 11, optimum yeast productivity was obtained for DSSL ozonated at pH 5. This observation is not in agreement with COD and BOD data given in Table 10. Lower ozonization pH encourages more total oxidation

TABLE 10. COD and BOD₅ Vary with Ozonization pH

pH	BOD (g/l)	COD (g/l)	Yeast (g/l)
Raw DSSL	6088	100364	2.1
9.0	6713	103033	3.6
5.0	8284	93500	4.8
3.5	8675	91912	4.1
5 min. ozonization; 3 day fermentation			

and an increased production of substrate suitable for yeast growth. The question of what products are being formed at different oxidation conditions should be the major concern of further work.

An increase in BOD₅ over the first day or two of fermentation was found, particularly with the ozonated DSSL (Figure 15). This indicates that the Torula yeast breaks up the substrate it uses into smaller fragments during its growth phase, thus increasing the BOD₅. The maximum yield was obtained at three days which coincides with a lowering of the BOD₅. After this point the yeast begin to lyse and the yields drop.

Figure 15 EFFECT OF FERMENTATION TIME ON BOD
FOR OZONATED AND OXYGENATED DSSL

CONCLUSIONS AND RECOMMENDATIONS

The broad objective of this research project was to evaluate the feasibility of partial oxidation as a means to produce organic fractions which would be fermented to produce fuel gas or protein. Both desired end products were produced in significant yields; however, additional work will be required to elevate both yields and production rate to a commercial level.

The most significant result of the study was a demonstration of the ease with which the lignosulfonate molecule can be partially oxidized and fragmented to obtain fermentable substrate. This process, which might be described as oxidecracking, is initiated at very low oxygen concentrations and proceeds rapidly until approximately 30% of the COD has been consumed. During the process, very little carbon dioxide is produced, indicating the predominant reaction as one of oxidative cleavage of the macromolecule. Both oxygen and ozone are effective agents in this reaction.

As would be expected, the fragments produced vary in molecular weight and degree of oxidation. Not all of these fragments are useful substrates for any single fermentation. It was observed that methane bacteria required lower molecular weight carbon fragments and hence more oxidation than the Torula yeast used in the study. Ten minute reaction time with ozone or 20 minutes with oxygen produced a substrate which would support yeast growth up to almost six grams/liter of DSSL. On the other hand, methane fermentation conducted on ten-minute ozonate yielded only minor amounts of fuel gas. Longer ozonization times of up to three hours were found to be required to produce a substrate suitable for the production of methane.

The strong oxidizing power of ozone is particularly evident in the degree of COD reduction achieved for comparable reaction times. Although a treatment

with oxygen at room temperature and pressure was not particularly effective in reducing COD, treatment with ozone was very effective up to an ozonization time of three hours. COD reductions of 40% were easily obtained during this brief period. Prolonged treatment of DSSL with ozone resulted in a conversion of the oxygenated fragments to CO_2 , and the rate of COD removal decreased appreciably after these initial oxygenation reactions had been completed. The organics remaining in solution after this phase appeared to be quite refractory with respect to ozonization and more severe conditions would be required to oxidize the DSSL further.

In spite of the significant progress in oxidative cleavage of the ligno-sulfonate molecule, the results do not support the commercial viability of the conceptual process for producing protein or fuel gas from pulp mill waste. Under optimum conditions only 3% of the organics were converted to methane, which falls short of the commercial target by approximately a factor of 10. Protein production compared somewhat better with 6% of the organics being converted to yeast biomass. Neither fermentation fully utilized all of the oxygenated fragments produced by ozonization, and hence, the concept of utilizing a two-stage process still appears to be an attractive configuration. However, experiments demonstrated that the yeast were unable to ferment the organic acids present after the methane fermentation was completed. It would appear that the more viable process configuration would be to precede methane fermentation by a yeast production stage. Although no tests were made of the ability of the methane bacteria to use yeast fermentate, it is likely that the mixed anaerobic bacteria cultures would be able to assimilate many of the low molecular organics not used, or produced by the yeast. This hypothesis should be tested in any future work.

The fact that partial oxidation can produce useful substrate for protein or methane fermentation is a significant breakthrough which bears additional study. These results did indicate that oxidations conducted at slightly higher temperature and much higher pressure could be excitingly productive. The equipment used in this study was not suitable for studying ozonization at elevated pressures and only limited ability was available to study the effective temperature. A high pressure oxidizer commonly used in the Wet-Ox system is a type of reactor which should be employed in additional studies. Under these more severe conditions, it is possible that oxygen alone will be a sufficient agent to promote oxidative cleavage. This would mean that the much more expensive process of ozonization could be avoided without detriment to the results.

Future studies which are warranted would include the application of other forms of energy concurrent with oxidation to promote the desired fragmentation. Although it was shown that sonication was ineffective, other techniques cannot be disregarded a priori. In particular, the use of microwaves to break down the lignosulfonate molecule would appear to hold some promise in view of the recently published success in using this technique to desulphurize coal. In addition, the use of ultraviolet light to catalyze the oxidation reaction also appears to hold some promise.

In view of the serious energy input-output problems facing this process, much additional work is required to bring the concept to commercial fruition. However, the abundance of pulp mill wastes as a potential raw material is ample justification to persevere.

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