MASTER

**ENERGY AND PROTEIN PRODUCTION
FROM PULP MILL WASTES**

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ABSTRACT

During the past quarter significant progress was achieved in increasing yeast protein production from ozonated desugared spent sulfite liquor (OSSL). In addition, the prime factors influencing the rate of methanogenesis of OSSL were studied in depth with two factors appearing to be dominate. Continuous fermentation experiments made to corroborate earlier batch studies achieved yields of 5.0 g/l of yeast cells at a residence time of 1.8 days. Improved strain selection and increased aeration rate were responsible for the approximate doubling of prior productivities. All experiments were conducted on DSSL which had been ozonated with 0.8 g/l. This value appears to be close to optimum.

The most important parameters influencing the rate of methanogenesis in continuous fermentation appear to be the microflora, sufficient trace growth factors, and adequate precursor concentrations. Although efforts to date at independently varying these three parameters have been unsuccessful outstanding results have been obtained when the three were varied concurrently. Methane rates which averaged 8 cc/hr were increased to over 100 cc/hr in several exploratory experiments. A concerned effort will be expended during the next quarter to identify the exact parameter responsible for the order of magnitude increase in productivity.

BACKGROUND

Nearly three million tons of lignin derivatives and other soluble organic compounds are generated a waste by-product annually by the sulfite pulping process¹.

Conventional mechanical treatment of the waste liquor to prevent pollution consists of evaporation of the water and burning of the dried solids. Although some cooking chemicals are recovered and energy is generated by the combustion, the evaporation consumes 20 to 50 percent of the heat generated, the burning creates air pollution, and the evaporate has an objectionably high BOD due to the presence of volatile organic compounds.

Conventional biological treatments such as the activated sludge process, storage oxidation and aerated stabilization² seek to avoid water removal. Although these methods effectively remove the low molecular weight organics, thus lowering the BOD, the levels of lignosulfonates and sulfur are not significantly reduced³.

A newer biological treatment is fermentation by Torula yeast for protein production⁴. Although this consumes the pentose and hexose sugars the levels of lignosulfonates and sulfur are not affected.

Research conducted at Michigan Technological University has attempted to degrade the lignosulfonates by continuous digestion with a mixed microbial culture containing the sulfur-reducing anaerobe Desulfovibrio Desulfuricans⁵. This research indicated that the lignosulfonate molecule or its sulfur component is not readily metabolized.

The general lack of success with biodegradation of lignosulfonates suggests that a chemical pretreatment may be desirable. Stern and Gasner⁶ showed that ozonation of kraft mill waste liquor degraded its lignin derivatives and increased their susceptibility to fermentation. The Barber-Colman Company⁷ has shown that wet oxidation of sulfite paper-pulp

waste also produces lower molecular weight compounds.

Both yeast growth and fermentation with Desulfovibrio should be aided by chemical pretreatment. In addition, methane-producing anaerobes seem ideally suited for subsequent fermentation. Benson and Partansky⁸ have estimated that is 25% of the carbon in desugared spent sulfite liquor (DSSL) could be converted by methanogenesis, the caloric value of the annual production would be equivalent to that of forty billion cubic feet of natural gas. Many anaerobic bacteria (i.e. Methanobacterium ruminantium, methanococcus, Methanosarcina, Methanosperillum hungatii, Methanobacterium omelianskii) are methanogenes. Since 1975 work in this laboratory on the development of a process for converting the pollutants present in sulfite pulp mill waste to useful fuels and protein has been supported by a grant from the Energy Research and Development Administration (now the Department of Energy⁹). The process as currently visualized is shown in Diagram 1. The primary yeast fermenter converts wood sugars and other assimilable organics into protein and CO₂. The ozonator modifies sulfur bonds and breaks down high molecular weight organics. The ozonated fragments are fed to the anaerobic digester where a mixed bacterial culture is employed because only a few specific organics can be utilized by methane bacteria and therefore other organisms (i.e. Desulfovibrio) are needed to develop the substrate for methane synthesis. A secondary yeast fermentation is included to permit digestion of any unassimilated ozonated fragments that can be utilized by Torula yeast. Any BOD remaining is removed by standard treating processes such as the activated sludge process or soil infiltration. During the anaerobic digestion some organic

sludge, biological solids and lignin will be produced. The solids are recovered in a thickener and can, after dewatering, be burned as fuel if no higher value alternative is available. By virtue of the work thus far completed, we believe the following conclusions are justified:

1. Desugared spent sulfite liquor reacts readily with ozone to produce lower molecular weight organic fragments which can be metabolized by a variety of microorganisms. Ozonation removes color bodies and increases BOD.

2. Production of protein through growth of Torula yeast on DSSL is enhanced by ozonation. Longer ozonation times reduce this effect.

3. Production of methane by anaerobic fermentation is enhanced by ozonation. Longer ozonation times increase this effect.

STAFFING

The return of Dr. Patton to the University in May allowed him to resume full-time participation on the project. He was able to devote three full months to the project before returning to New Mexico State University. It is planned that he will continue to participate when needed as consultant. Due to conflicting commitments, Dr. Jurgensen was able to devote only 30% of his time to the project during the summer months. In order to maintain the necessary progress, a new chemical engineer, Miss Robin Swartz, was employed part-time to provide additional staffing. Mr. Craig Bremmon, research associate, and Sushil Dugar, Ph. D. candidate, continued their commendable work on the project. Mr. Brian Delaney, M. S. candidate in chemical engineering, was added to the project in June to assume responsibility for the protein optimization study.

RESULTS AND DISCUSSION

Methane Production

Prior work has established that oxidative fragmentation of DSSL using ozone is effective in producing a substrate useful in methanogenesis. Exploratory experiments generated gas production in the range of 5 to 40 cc/hr from a continuous fermentation vessel. Since gas production from unozonated DSSL was essentially nil, there is no question as to the effectiveness of ozonation. However, the random variation in yields indicated that much more understanding is necessary for the process to be optimized.

A group of statistically designed experiments were undertaken utilizing different concentrations of OSSL coupled with a variety of residence times. These experiments indicate that ozonation increases methanogenesis approximately fifty-fold. Further, the maximum specific growth rate for the mixed culture appeared to be 0.3 days⁻¹. At dilution rates less than maximum rate, productivity is about constant and at rates above 0.3 days⁻¹ washout occurs and gas production gradually diminishes.

In the second stage of experiment the fermenter was reinoculated with an anaerobic culture obtained from the digester in Lanse, Michigan. This reinoculation produced negligible results. Similarly, when methyl alcohol together with a liberal amount of required vitamins and minerals were added to the fermentation vessel no increase in gas production was noted. However, when the methyl alcohol-nutrient mixture was added simultaneously with reinoculation methanogenesis increased dramatically, in some cases reaching a production rate as high as 180 cc/hr. An analysis of this gas

shows it to be the same high quality methane, 80%, that has been consistently obtained in earlier experiments.

Since other workers studying the production of methane by fermentation of cellulose consistently produce gas continually 50% methane an explanation for the differences was sought. The three likely methanogenesis reactions are shown below. Only in the case of acetic acid is the heat of reaction inadequate to sustain cell metabolism and hence a nonthermodynamic explanation seems appropriate. The obvious answer appears to lie in the stoichiometry of the reactions. It can be observed that the production of methane from alcohol should produce a gas that is at least 75% methane while methane produced from acetic acid of cellulose should be expected to be only 50% methane. This is consistent indeed with existing experimental data and one should suspect that the precursors of methanogenesis are alcohols or similar products generated during ozonolysis of DSSL. These types of products would not be expected in cellulosic or acetate mixtures.

Yeast Production

Additional continuous fermentation experiments to produce yeast from OSSL (containing 0.8 g/l ozone) were continued during the quarter. Analysis of cultures taken from the continuous fermenter indicated that the yeast strain had possibly undergone some type of mutation and was much more difficult to grow than cultures used initially in batch fermentation studies. A concerted effort directed at isolating a reliable strain were successful and the continuous fermentation experiments that were conducted using this new isolate. Yeast production increased immediately, however, the growth still appeared to be limited by a substrate deficiency. The air rate was increased to approximately 0.3 vol/vol/min and an immediate

response increased yeast production was noted. Cell concentrations of 5.0 g/l were obtained at residence time of 1.8 days. This is in good agreement with the earlier batch experiment and represents the maximum yields obtained to date on OSSL.

Additional work was also undertaken to better define the optimum degree of ozonation for the production of protein. Batch growth studies were conducted on OSSL with ozone dosages ranging from 0.5 to 1.0 g/l. In addition DSSL was reacted with pure oxygen for a period of 20 minutes in the ozonation reactor. The results of these studies show that yields of 5.0 g/l can be obtained on 20 minute oxygenated DSSL in the absence of ozone. Yields obtained with ozonated product were lower at the 0.5 g/l and at the 1.6 g/l dosages. Additional work is planned to better define the optimum ozone dosages. It would appear on these preliminary experiments, however, that oxygen treatment would be more economic than ozone. This is not the same conclusion which can be reached with respect to methanogenesis.

SUMMARY AND CONCLUSION

Results obtained during the past three months are quite encouraging with respect to the viability of the concept of producing methane and protein from pulp mill wastes. The project is exactly on schedule with respect to the milestone chart, however, dedicated effort must be expended in the coming months in order to achieve the degree of optimization required in the time framework of this project. The order of magnitude increase in methane production is a real breakthrough and can be expected to make a significant contribution to the overall success of this project. The demonstrated rates are definitely commercial and the job remaining will

be to obtain them without the addition of any artificial precursors.

The yields of yeast grown OSSL are only barely encouraging and additional increases in productivity must be achieved in order to have a commercially attractive process. The fact that the yeast grow better on very lightly oxidized DSSL indicates that the protein and methane fermentations are not competitive for the same substrate. This could be important in the development of an integrated overall process.

A large number of requests for preprints of the paper on protein production presented at the 85th National AIChE Meeting in Philadelphia indicates the strong and continuing interest on the part of both industry and university researchers in the project. An exchange of ideas and information with those requesting preprints could provide additional insights into understanding and optimizing the process.

PLANS FOR THE FUTURE

During the coming year work will continue on improving the rate of methane production from ozonated desugared spent sulfite liquor, OSSL. It is hoped that the additional experimentation will identify a single parameter most responsible for controlling methanogenesis. Work will also continue on improving the growth of yeast on OSSL. In as much as the fermentate from the methane reactor is rich in acetate, studies will also be undertaken to determine the suitability of acetate for the production of protein in this system.