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ENERGY AND PROTEIN PRODUCTION FROM PULP MILL WASTES

Progress Report
for the Period June 15, 1977 to September 15, 1977

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ABSTRACT

Experiments conducted during this past quarter demonstrated the decided difference both in amount and composition of the gas produced from the fermentation of ozonated versus unozonated yeast-plant SSL. Gas from ozonated SSL averaged over 80% methane content while unozonated effluent was mostly carbon dioxide. Gas production rates and retention time studies indicated that the fermentation was substrate-limited. Preliminary tests using supplemental carbon sources have verified this. The success of the ozonation process in producing fermentable substrates was clearly shown by the appreciable yeast growth in the ozonated SSL. Of particular significance was the maximum yield obtained at the short ozonation time of 10 minutes as compared to the 2-hour treatment. It is possible that shortening the ozonation time could also increase the amount of substrate available for methane production. This would be very important in transferring this process to a commercial basis and reducing the operating costs.

BACKGROUND

The second year of this project began after accomplishing the majority of the milestones projected in our initial program plan. Methane-producing bacteria have been obtained from various sources and screened for their ability to grow on SSL. The effects of various ozonation treatments on the composition of SSL have been established. Anaerobic continuous flow fermentors were developed and used to demonstrate the feasibility of producing methane from SSL. During this past quarter emphasis was given to: (1) further work on optimizing both the ozonation and fermentation process and (2) evaluating SSL as a substrate for protein production by yeast.

STAFFING

During this quarter the project was fully staffed. Two full-time laboratory technicians were employed during the summer to aid in the numerous chemical and biological tests which were routinely conducted. In addition, Dr. Y. Lai, a lignin chemist in the Forestry Department, spent one month working on the changes in the organic fraction of SSL after ozonation. At the beginning of September Dr. Patton left on a nine-month leave of absence to New Mexico State University. He will continue working on the project through periodic trips to Houghton and by correspondence. This arrangement has been coordinated and approved by ERDA officials.

RESULTS AND DISCUSSION

SSL Fermentation

The anaerobic fermentors continued to be in operation during this entire period. Observations in the early part of this quarter showed unexplained fluctuations in gas production during each 24-hour period. It was finally realized that these daily variations were due to uncontrollable changes in the ambient temperature of the laboratory. Consequently, a constant temperature chamber was constructed which reduced temperature fluctuations to less than $\pm$ 1°C during the measuring period.

All the fermentors were fed 75% yeast-plant SSL which had been ozonated for 3 hours. The gradual decline in gas production noted in the last reporting period halted, and gas production stabilized at approximately 10 mls/hr. No explanation is evident to account for this lowering of gas output. The composition of the gas from the reactors still showed the high proportion of methane to carbon dioxide noted in earlier studies (Table 1). Continued decreases in OCD and BOD levels were also observed.

TABLE I. Properties of yeast plant SSL during continuous flow fermentation over a one-month period.

Average Time at Steady State (days)	Retention Time (days)	Effluent changes COD (%)	BOD (%)	SULFUR (%)	Effluent gas Production (mls/hr)	Composition CO_2 (%)	CH_4 (%)	Bacterial Counts Methane Producers ($x10^7$)	Desulfovibrio ($x10^7$)
Ozonated SSL *									
8	5.1	- 7.2	-33.7	-	12	19.4	80.6	50	60
6	3.4	-17.0	-37.5	-9.9	10	21.8	78.2	203	186
Unozonated SSL **									
2	3.0	- 9.9	-30.6	-9.2	4	85.7	14.3	183	60

* Yeast plant effluent ozonated for 3 hours at pH 3. Values are an average from two fermentors.

** Untreated yeast plant effluent. Values from one fermentor.

In order to establish the level of gas production which can be supported in untreated yeast-plant effluent, one reactor was fed a medium of 75% unozonated SSL. The results from this test showed the general lack of metabolizable substrate in unozonated effluent. Gas production was only a quarter of that obtained from ozonated SSL reactors and was composed almost entirely of carbon dioxide (Table 1).

Analysis of reactor performance during this period indicated that the fermentation process appeared to be substrate limited. Consequently, various nutrient supplements have been added to the SSL to determine what the limiting substrate or substrates may be. To date, vitamins, minerals and several organic acids and alcohols have been tested, several of which gave decided stimualtions of methane production. These studies will be continued in the following quarter.

Microbial Analysis

The populations of the anaerobic methane-producing and the sulfur-reducing bacteria were monitored during each reactor op ration. The gradual decrease in methane bacteria noted earlier appeared to halt and populations of both methane-producers and Desulfovibrio increased dramatically. In all of the reactors the populations of both methane-producers and sulfur-reducers were approximately equal, averaging nearly 5×10^9 organisms/ml at steady state conditions. Determinations were also run on non-methane producing anaerobes and facultative anaerobes using the standard plate count technique. Preliminary results of these tests indicated equal populations of each approximating 10^6 organisms/ml.

Our standard Hungate procedure used to monitor populations of methane-producing bacteria and Desulfovibrio was modified during this period. Based on the research results from the University of Illinois, the purging gas used in the preparation of the anaerobic Hungate tubes was changed from pure carbon

dioxide to one containing a mixture of 50% carbon dioxide and 50% hydrogen. Preliminary tests have indicated higher population counts by a factor of 10 using this technique. This method is now being routinely used in our laboratory.

Protein Production

The project initiated on protein production from yeast-plant treated SSL at the end of the last quarter was continued and expanded during the summer. Cultures of Torula yeast were inoculated into flasks of either unozonated or ozonated SSL. Various SSL ozonation times were used, which ranged from 10 minutes to 2 hours. In addition, nitrogen, phosphorous and potassium were added as supplements. The cultures were grown on a rotary shaker for up to eight days. Periodically duplicate flasks were centrifuged, dried and the residue weight determined. Yeast production was expressed as the amount of dry weight produced in each flask. The results of the tests are shown in Figure 1.

Some yeast development occurred in all the treatments. Yeast growth on the unozonated SSL was fairly low reaching a stable population after 3 days. Unexpected was the high yield found in the SSL ozonated for 10 minutes and the poor yeast development in the 2-hour ozonated SSL. The 10-minute treatment showed a dramatic production peak at 2 days and a very rapid decrease on the third day. This decrease is likely attributable to autolysis of the yeast cells. After 7 days there was very little difference in yeast yield among the various SSL treated media. It appears that the lower yeast yields after longer SSL ozonation treatments are related to a reduction in carbon available for yeast growth.

The growth of Torula yeast on ozonated yeast-plant SSL is appreciable, with yields approaching 10% of those obtained from yeast plants using raw SSL. Work is continuing on this process to more precisely determine the optimum ozonation time and growth conditions for maximum yeast production.

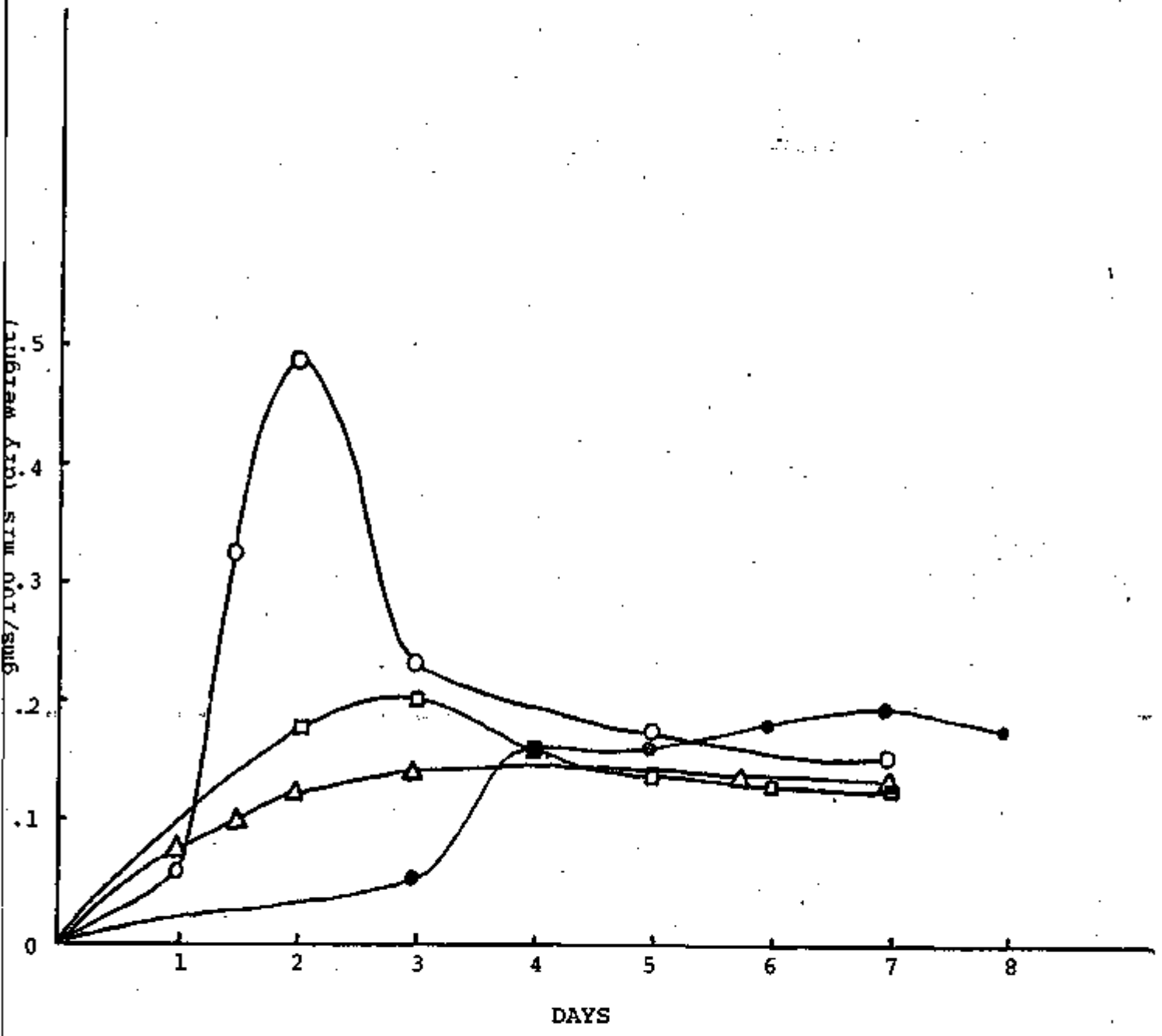

Figure 1. Yeast production vs. time for varied treatments with ozone on SSL. Unozonated SSL (Δ), SSL ozonated for 10 minutes (O), SSL ozonated for 1 hr. (□), and SSL for 2 hrs. (●).

SUMMARY AND CONCLUSION

Experiments in this quarter demonstrated the decided difference both in amount and composition of the gas produced from the fermentation of ozonated vs unozonated yeast-plant SSL. Gas production rates and retention time studies indicated that the fermentation was carbon-limited. Preliminary tests using supplemental carbon sources have verified this. The success of the ozonation process in producing fermentable substrates was clearly shown by the appreciable yeast growth in the ozonated SSL. Of particular significance was the maximum yield obtained at the short ozonation time of 10 minutes as compared to the 2-hour traatment. It is possible that reducing the ozonation time could also increase the amount of substrate available for methane production. This would be very important in transferring this process to a commercial basis and reducing the operating costs.

PLANS FOR THE FUTURE

During the coming quarter the characterization of optimum SSL ozonation time for protein production will be continued. In addition, the effect of SSL ozonation time on methane production will also be examined. More detailed experiments will be conducted to quantify the types and amounts of metabolizable substrates which are present in yeast-plant SSL after the various ozonation treatments.