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Fuel Alcohol Production

Conversion of cellulose to glucose

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FARM-SCALE CONVERSION OF CELLULOSE TO GLUCOSE FOR FUEL ALCOHOL PRODUCTION

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Cellulose (a component of plant tissue) is the most abundant organic material on earth (15). Farmers produce, often without subsequent use, more cellulose than any other group of people. This exists as corn and wheat stover, beet tops, grain dust, and other forms of crop residues which are of limited economic value except when returned to the soil.

Cellulose, which is a complex sugar, can be broken down through saccharification by some types of microorganisms that typically inhabit the soil. Saccharification yields glucose. If the glucose is subjected to further action by different microorganisms, it may be converted to ethyl alcohol (grain alcohol) for use as a fuel (6,8). The discussion that follows deals with some work that we have done at South Dakota State University (SDSU) to ascertain the feasibility of this process in a farm or community-scale fuel alcohol production facility.

Saccharification is carried out by specialized proteins called enzymes that act as biological catalysts to hasten the breakdown of cellulose to glucose. Actually, this process is carried out by a complex of three enzymes, collectively called cellulase, that act together to achieve the degradation process. This complex of enzymes is most frequently obtained from strains of the mold *Trichoderma reesei* chosen for their ability to produce large amounts of cellulase (6,8,9). *T. reesei* also holds the greatest promise for use in commercial fuel alcohol production facilities.

Presently, the limiting factor in obtaining fuel alcohol from cellulose wastes is the cost of producing the enzyme complex. Studies have shown that 50 to 60% of the cost of producing glucose is directly related to the efficiency of cellulase production (2,12). The only viable options a plant manager has are either to buy the enzyme, which is expensive, or to produce his own, which is difficult, as the following discussion reveals.

To date, improvements in enzyme production have been made only in the research laboratory. They involve incorporating additives (5,7) to the production medium, pH cycling and temperature profiling, and using continuous culture processes (4,11). An owner-operator system that would convert cellulose to glucose has yet to be economically developed.

The fuel alcohol production facility at SDSU is similar in size to that which might be used by an individual owner-operator. This study sought to determine how effective it would be to scale-up from the laboratory production level to a rudimentary pilot plant.

T. reesei cellulase in our studies at SDSU was produced in a stainless-steel dairy culture vessel with a working capacity of 74 gallons (280 liters) and fitted with a stirring paddle, a pH monitor and control device, heating-cooling coils, and a sterile air supply. A polyethylene reservoir provided ammonium hydroxide (2N NH_4OH) for pH adjustment. This fermentor doubled as a vessel for both enzyme production and cellulose saccharification.

The mold was grown by a submerged culture technique where it was mixed throughout the culture vessel to achieve maximum production of the saccharifying enzyme. As *T. reesei* grows, the pH of the culture medium drops to about 3.0, usually within a 24- to 72-hour time span. It is at this pH that enzyme

production begins and must be maintained (up to a pH of 3.5) for optimum yields. After a period of a week to 10 days, the pH will rise rapidly, indicating the end of the production process (7,12,14).

The cellulose substrate employed for saccharification was Solka Floc SW-40 (Brown Co, Berlin, NH). Solka Floc SW-40 is a purified form of alpha-cellulose derived from spruce wood. (It is typically the most difficult to break down; therefore it is used as a standard for comparative studies.) It is approximately 95% pure and is ground to 40 mesh fibers (3,8). Saccharification experiments were performed on both the laboratory and pilot plant scales.

Enzyme production was monitored by observation of the pH profile illustrated in Figure 1. The pH dropped rapidly after 24 to 72 hours and was maintained at 3.0 with the addition of ammonium hydroxide (13,14). After several days the pH rose. This is attributed to the release of ammonium ions and is indicative of the end of enzyme production (12,14). Data observed in both the laboratory and pilot plant facility depict this type of profile (Fig. 1).

There was some variance in the amount of time it took for the pH to drop initially for some of the pilot plant experiments, but they still followed the same basic pattern. These variances of time were probably due to the lack of control over process parameters. For example, although temperature was constantly monitored, it was necessary to adjust it manually. Consequently, temperature fluctuated over the production period as much as 3 to 4°C. Control of temperature to achieve optimal production is considered critical (10,12).

Another variable was inoculum size.

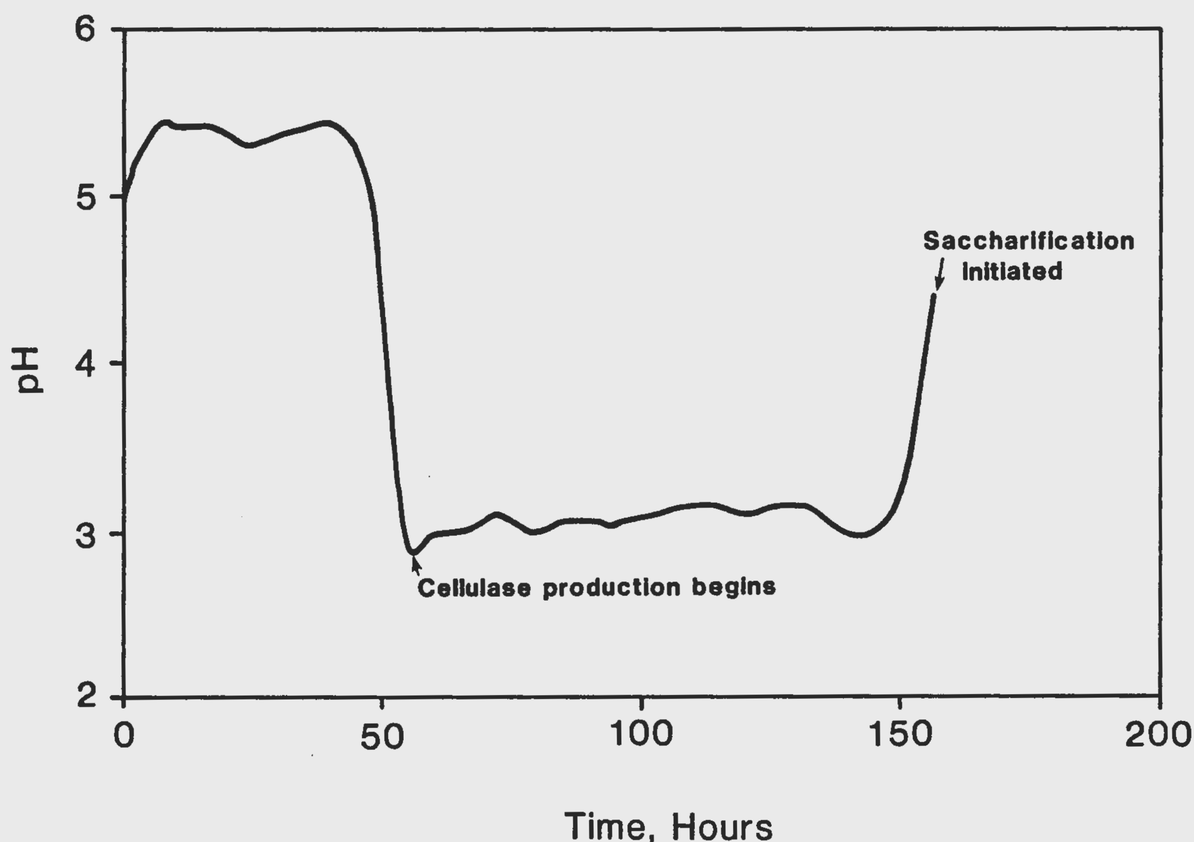

Figure 1. Typical pH profile of a plant experiment.

There is no rapid method for estimating inoculum size. Therefore, even though all experiments were treated alike, there were no adequate means to ensure that the inoculum size was maintained consistently between experiments. This variation affected the initial lag period (when the microorganisms are multiplying). Of all variables, it affected cellulase production most seriously; lag time should be kept to a minimum, if possible.

Saccharification was not initiated until the pH began to rise on its own (6 to 9 days in our studies). This increase in pH indicates the end of enzyme production as well as additional enzyme release from the cell wall on the organism's death (1,7,13,14).

Saccharification efficiency of the Solka Floc SW-40 substrate showed poor results for pilot plant experiments when compared to a typical laboratory experiment (Fig. 2). The pilot plant experiments showed an increase in saccharification for the first four trials. This suggested that problems associated with the scale-up and the process con-

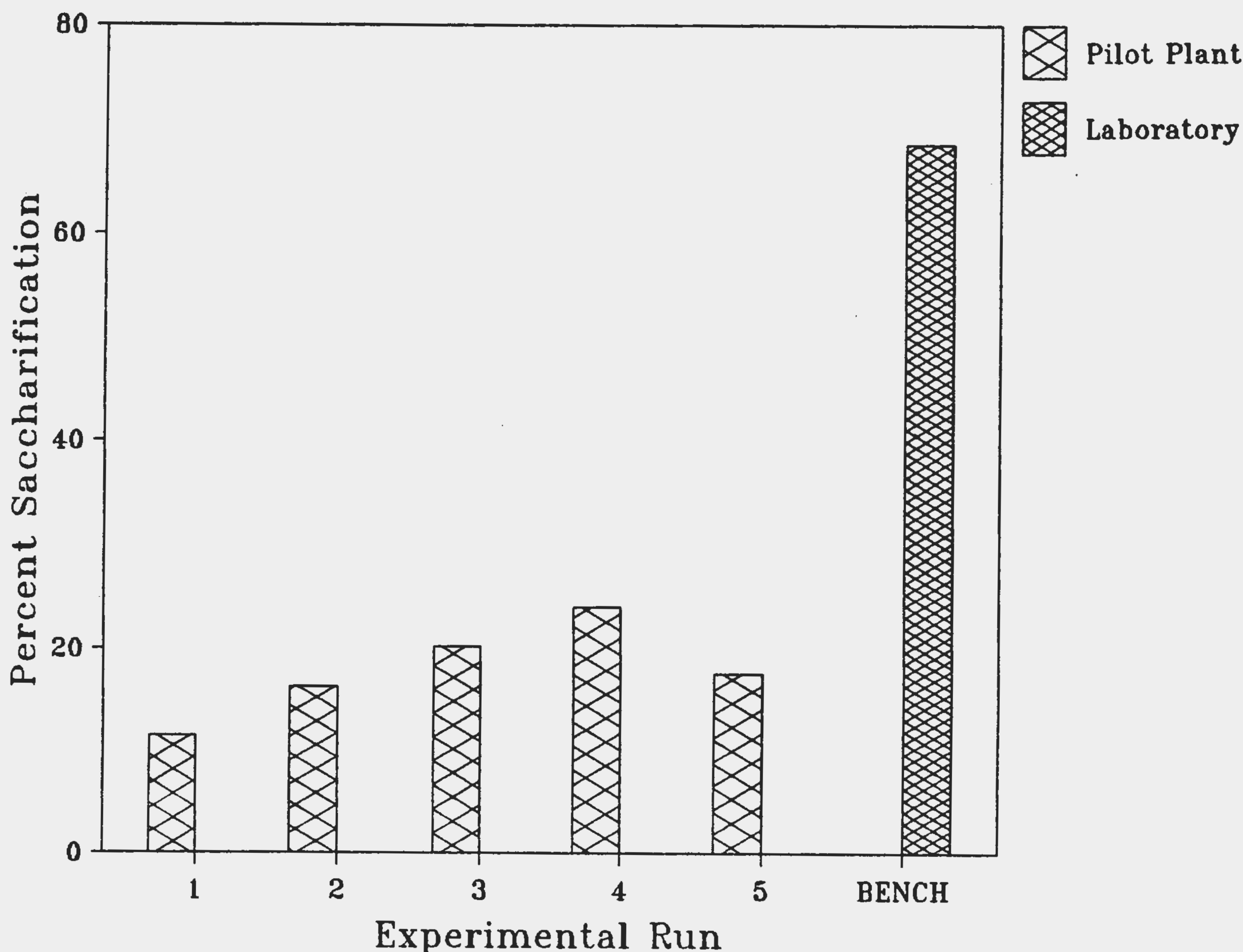

Figure 2. Saccharification* of Solka Floc SW-40 (5% w/v) by *Trichoderma reesei* QM 9414 cellulase.

*Saccharification period for pilot plant and laboratory experiments were Run 1, 2 days; Run 2, 3.5 days; Run 3, 2.6 days; Run 4, 4.3 days; Run 5, 2 days; Bench, 5.5 days.

trol in the pilot plant were diminishing and overall improvement was being achieved. The final pilot plant experiment (#5) showed a decrease in saccharification efficiency, which was most likely attributed to a small inoculum size.

Statistical analyses were performed to determine if characteristics of saccharification efficiency differed from trial to trial. An analysis of variance (ANOVA) test established that there was a highly significant difference (0.01 level) between plant and laboratory experiments. In other words, pilot plant saccharifications were nowhere as successful in achieving conversion of the cellulose to glucose. As a consequence, not as much glucose for fermentation to fuel alcohol was produced in the scaled-up process as was theoretically possible.

To ascertain how the individual pilot plant trials compared to one another, a least significant difference statistical test was computed. It showed that trials 2 and 5 were the only two plant scale trials which were not significantly different from one another. In other words, although the saccharification efficiency was poor when compared to laboratory data, efficiency improved as more experiments were performed, with the exception of the last one which had a low starting inoculum.

Our data confirm the variability of saccharification efficiencies between experiments performed in the enzyme production facility. The extent to which saccharification occurs is proportional to the amount of enzyme produced. The differences in efficiency between individual plant experiments and between plant and laboratory data can be attributed to a variety of problems.

The major problem was the need for improved process controls including temperature, pH, aeration, agitation, and process sterility. Ideally, this production procedure should be controlled by a microprocessor or other computerized system. This would eliminate error associated with manual operation of parameters and ensure that processes would be adjusted more frequently and probably more economically. Furthermore, it would release personnel to carry out other necessary activities.

Our results confirm that process control is critical during the saccharification process. The optimum temperature of 50°C was difficult to maintain manually. This fluctuation can lead to a decline in enzyme activity as well as saccharification efficiency (10,12).

Saccharification periods varied from plant experiment to plant experiment as depicted (Fig. 2). The time periods listed were the amount of time for which maximum glucose was produced. All plant experiments showed a severe reduction in saccharification efficiency after those times. This decrease was most likely due to contamination by bacteria and other microorganisms. The source for contamination could have originated from the substrate, Solka Floc, which was not sterilized prior to its addition. Sterilization of the substrate would be energy intensive, and therefore, it would not be an economically viable option.

Removal of samples from the production-saccharification vessel for analysis could not be achieved without introducing contamination. Consequently, airborne microorganisms could have gained entry and caused contamination. Saccharification conditions (50°C, pH = 4.8) are not extreme enough to retard bacterial growth by the majority of organisms.

A problem may also have been associated with glucose, the product of conversion. This undoubtedly provided an essential growth substrate for any of the contaminating organisms, including any viable _T. reesei_ cells.

The total production period from enzyme production through saccharification was generally 1 1/2 to 2 weeks. Over this amount of time it is not

surprising that contamination would occur, especially with such rudimentary facilities. There was no contamination associated with laboratory experiments, suggesting that rigorous control over process parameters in the plant is essential.

Summary

We have determined that, with the owner-operator facility at SDSU, enzyme production is not feasible. Updating the facility for optimal enzyme production requires a large amount of money. Along with more sophisticated equipment, a microprocessor or computer-driven system would be necessary if the process is to operate smoothly and without constant supervision. Furthermore, enzyme production and substrate saccharification require personnel with extensive training in microbiology, chemistry, and engineering.

Other conversion methods such as acid degradation are currently being compared with enzymatic breakdown at SDSU. At present, the owner-operator system for enzyme production and cellulose saccharification appears not to be feasible without a substantial investment in equipment and technical expertise, which is impractical for a farm or community-scale operation. An alternative to *in situ* production would be to purchase the enzyme, but presently this is not cost effective.

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