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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 9/17/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 5.150
~~First~~ PR

Attachment

2 cys

PD-AAW-705

5300

FIRST PROGRESS REPORT

ALCOHOLIC FERMENTATION OF SUGAR CANE IN

A HIGHLY SOLID STATE SYSTEM (5.150)

Rec'd in SCI SEP 17 1987

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FIRST PROGRESS REPORT

ALCOHOLIC FERMENTATION OF SUGAR CANE IN A HIGHLY SOLID STATE SYSTEM (5.150)

AID PROJECT NUMBER: 936-4452

PRESENTED BY ICAITI TO

ROCAP

OFFICE OF THE SCIENCE ADVISOR, AID/WASHINGTON

GUATEMALA, SEPTEMBER, 1987

1. INTRODUCTION

This report describes the activities which have been undertaken in the project "Alcoholic fermentation of sugar cane in a highly solid state system (5.510)" sponsored by the Office of the Science Advisor of AID Washington, since the beginning of activities in August, 1985.

The purpose of this project is to study and improve the alcoholic fermentation by yeast of sugar cane in the solid state using a minimum amount of water. Specifically the research addresses the handling of the moist solids in a way to secure a homogeneous system; enhancing the rate limiting step of sucrose diffusion and/or the penetration of the yeast in the moist solid matrix; and avoiding bacterial contamination which may decrease ethanol yield. Additionally, the effect of cane preservation techniques on the preservation of fermentable sugars is being considered. This work builds upon the experience in ICAITI in developing the FX-FPRM process for the simultaneous extraction and fermentation of sugar cane.

The activities which are described in this report cover the work which has been carried out on the base studies of direct fermentation of sugar cane prepared in the mill; modification of the cane preparation to ease handling and to improve diffusion of the sugar; systems for cane preservation, including ensilage, steam explosion, use of sodium bisulfite, and a combination of these; improvement of fermentation conditions to enhance yeast fermentative capacity and inhibit metabolic activity of contaminants; study of ethanol toxicity of the yeast strains used; study of the kinetics of dextran production by contaminants isolated in the studies described above; design of a fermentation system which can be used on a pilot level. Also included are the reports of the consultant, Dr. Arthur Humphrey, financial statements, and program of coming activities.

The contents of this report are as follows:

1. Introduction

2. Report on research activities

    2.1 Initial fermentation studies using cane from the processing line
    2.2 Cane preservation
    2.3 Improved fermentation conditions
    2.4 Ethanol toxicity studies
    2.5 Dextran production kinetics

3. Equipment design

    3.1 Integrated system
    3.2 Mill
    3.3 Fermentation
    3.4 Pressing
    3.5 Ethanol recovery

4. Consultant's visit

5. Visits

6. Financial statement

7. Program of coming activities

2. REPORT ON RESEARCH ACTIVITIES

2. REPORT ON RESEARCH ACTIVITIES

The research activities undertaken during the development of this project include, first of all, base line studies on the fermentation of sugar cane as it is prepared in the mill prior to sugar extraction, with no extra water added. Here we found that the long ribbons of cane were difficult to handle so we looked into alternative forms of cane preparation, such as use of a mill to prepare a more finely ground material resembling saw dust. This material not only proved to be much easier to handle but also provided more surface area to improve sugar diffusion. Since cane variety and fiber content have been taken into consideration as important process variables in the sugar industry, we also looked into the fermentation of three different varieties of cane. Detail of these activities are described in trials 1, 2, and 3 in part 2.1 of this report. At the end of part 2.1 the reader will find a short set of conclusions for this work.

Cut sugar cane, particularly shredded or ground, is very susceptible to deterioration by microbial contaminants with a resulting loss of sugar. In order to conserve the sugars present in the cane for use during the off season, different preservation techniques were studied. First of all, we looked into ensilage of the cane ribbons with and without the addition of a lactic bacteria inoculum. Then we studied the steam explosion of the cane ribbons, and the ensilage of the exploded cane. Following this, we continued on to the use of sodium bisulfite, commonly used in the wine industry to control bacterial contaminants. Detail of these activities are described in trials 4, 5, and 6 in part 2.2 of this report. At the end of part 2.2, the reader will again find a short set of conclusions for this work.

As we saw the difficulties encountered in working with cane from the mill's patios, the research took more into account techniques for favoring yeast fermentation over metabolic activities of contaminants. And at this time processing techniques were modified to reduce time between grinding of the cane and addition of yeast inoculum and control of fermentation temperatures to levels above those reported to be favorable for dextran formation. We studied the effect of using higher levels of inoculum in the fermentations. We also studied the addition of water and use of agitation to

induce mixing of the cane and yeast cells and to improve diffusion of the sugar from the internal cells of the sugar cane particles. Detail of these activities are described in trials 7 through 10 of part 2.3 of this report. Then a section where the conversion parameters have been calculated for the data is presented, and compared for similar data calculated from the literature.

It is important to point out that the sugar industry in Central America is in a point of change, with mechanical harvesting taking place of much of the harvesting by hand as traditional in the area. For example, the mill which we worked with during the activities described in this report, Pantaleon in Guatemala's south coast, now processes about 80% mechanically harvested cane and 20% hand harvested cane. A few years ago, this relationship was the other way around. The mechanically harvested cane is burned in the fields to remove leaves which would interfere with the cutting process and later milling. The burning leaves wounds on the cane stalk, making it more susceptible to microbial penetration. Delays often occur in the field and in the patio, also increasing the opportunity for microbial deterioration. During the 1986-1987 harvest season, we encountered contamination levels that we had not seen in earlier work during the last ten years on EX-FERM.

Part 2.4 goes on to describe a study on the ethanol tolerance of the yeasts which form part of the ICAITI Culture Collection and which are being used in this study. It is particularly important for a comercial process that the yeast culture used be tolerant to ethanol at temperatures commonly found in the sugar cane areas of the coastal regions.

Part 2.5 describes very briefly the work being done on the dextran production kinetics of cultures isolated in the sugar cane during the 1986-1987 season. We are looking for ways to delay or inhibit the activity of the dextran-producing contaminants.

2.1 Initial fermentation studies using cane from the mill processing line.

Trial No. 1.
February 10-20, 1986

Low Moisture Fermentation of Ground Sugar Cane, 1.

Feasibility of ground sugar cane fermentation with no addition of water was studied. Thinking of future scale-up of the process, we chose to work with chopped cane as prepared in the mill.

Materials:

Sugar cane was obtained from Ingenio Pantaleon, Santa Lucia Cotzumalguapa. Cane was taken from the process line after going through four choppers, just before entering the mill. Appearance of chopped cane was irregular. Shredded ribbons were of different lengths, the longest being 20 cm. Cane was taken from the mill to ICAITI in plastic containers (large garbage cans) and left in refrigeration overnight.

Yeast: a Saccharomyces cerevisiae strain, L-181, was used. Inoculum was grown in a fermentor for 20 h. Yeast concentration in the fermentor reached 10^7 cells ml^{-1}. The medium used for inoculum growth was 4% sucrose, 1.17% $(NH_4)_2SO_4$, 0.043% $Na_2HPO_4 \cdot 2H_2O$, 0.03% $MgSO_4$ and 0.1% yeast extract.

Method:

Plastic bags were prepared containing 5 kg of the chopped sugar cane. Inoculum at the 5% (w/w) was added proportionally after every kg of cane was put in the bag. Bags were closed, pressing the cane to reduce air bubbles trapped inside. Each 5 kg bag was put inside another plastic bag in which CO_2 was injected to provide an anaerobic environment. Fermentation temperature was 28 C.

The bags were opened 3, 8 and 10 days after fermentation started. Sugar and ethyl alcohol analyses were made on the juice obtained by pressing a representative sample (around 250 g) of fermented cane. Residual sugar in pressed bagasse was also determined. Sugar analyses were made using the Luff-Schoorl method.[1] Ethanol was determined by gas chromatography.[2] Moisture of bagasse was determined by drying

1. Browne, C. A. and F. W. Zerban. 1955. Physical and chemical methods of sugar analysis. Third edition. New York: John Wiley & Sons. pp.830-833.
2. Lie, S., A. Haukeli and J. J. Gether. 1970. Gas chromatographic determination of ethanol. Brygmesteren 11: 281-289.

to constant weight at 60 C. Reported data are the average of two samples.

Microbiological counts were made in the fermented cane pieces. A weighed portion of cane was blended with sterile phosphate buffer, and decimal dilutions were then prepared. Aliquotes were plated in malt agar, and the plates were incubated at 28 C for 48-72 h. Flora was differentiated by microscopical observation.

Results:

1. There exists strong evidence that delay in adding yeast inoculum to sugar cane favored another fermentation process, presumably a lactic fermentation. It can be seen in Table 1 that initial sugar content was 69% lower than the average value of 20%. Data in Table 2 shows that no ethanol was formed before cane was inoculated, and pH values shown in Table 3 are low compared with a value of 5.0 considered as average in fresh sugar cane juice.

2. A fermentation time of three days was necessary to convert 81% of fermentable sugars to ethanol. The average value of $Y_{p/s}$ yield was 0.44 g ethanol/ g sugar consumed.

3. Trials with uninoculated sugar cane showed a loss of 65% of the fermentable sugars after a storage period of eight days. As observed in Table 2, ethanol was not produced.

4. Data shown in Table 4 correspond to residual sugar found in pressed bagasse. A mass balance showed that 17% of original sugar was still unfermented after three days. This residual sugar was distributed in the following way: pressed bagasse contained 3.3% and juice contained 13.7%. After a fermentation period of eight days, unfermented sugar was 7.4% of original, 1.3% contained in pressed bagasse and 6.1% was recovered in cane juice. After ten days of fermentation, unfermented sugar lowered to 6.0% of original sugar, 1.0% in pressed bagasse and 4.9% in the juice. Results show that sugar retained in bagasse is negligible compared to sugar content of juice.

5. Preliminary microbiological counts showed the presence of high levels of bacteria in both cane treatments. We assume that the bacteria are responsible for the low pH and the rapid initial loss of sugar. The source of bacterial contamination was not determined but could have been from the original flora in cane, flora which developed during delay in the mill's patios, contaminants in the process line, and/or contaminants that developed due to favorable conditions during transport to the Institute and the overnight delay.

Table 1: Total sugar (g/100 cm^3 juice)

Time (days)	Inoculated cane	Cane without inoculum
0	13.86	13.86
3	2.64	nd
8	0.99	4.87
10	0.80	nd

nd = not determined

Table 2: Ethanol produced (g/100 cm^3 juice)

Time (days)	Inoculated cane	Cane without inoculum
0	0.09	0.09
3	4.83	nd
8	5.78	0.00
10	2.43	nd

nd = not determined

Table 3: pH value in juice

Time (days)	Inoculated cane	Cane without inoculum
0	3.80	3.80
3	3.77	nd
8	3.60	3.03
10	3.37	nd

nd = not determined

Table 4: Residual sugar in the pressed bagasse (g/100 g wet bagasse)

Time (days)	Inoculated cane	Cane without inoculum
0	8.52	8.52
3	1.10	nd
8	0.38	3.53
10	0.31	nd

nd = not determined

Table 5: Microbial counts in the fermented sugar cane (10^6 cells/g)

Time (days)	Inoculated cane	Cane without inoculum	Flora present
2	22	nd	yeast
6	8	nd	yeast
	46	nd	bacteria
8	nd	0.5	yeast
	nd	81	bacteria

nd = not determined

Trial No. 2
February 18-24, 1986

Low Moisture Fermentation of Ground Sugar Cane. 2.

Two different sized particles were tested in the fermentation of sugar cane: ground and shredded.

Materials:

Sugar cane: Shredded sugar cane was obtained from the process line of the mill as before. The smaller particle size was obtained using a laboratory grinder that belonged to the mill. This machine had several parallel sawdisks which ground sugar cane to tiny fibers, 1-2 cm long. The material resembled sawdust.

Yeast inoculum was prepared as before.

Method:

Ground and chopped sugar cane was inoculated the same as described before. Samples were taken at 0, 2 and 6 days of fermentation. Analytical procedures are those described previously.

Results:

Table 6 shows the sugar concentration data. Initial sugar content of cane juice was lower than expected. Since sugar cane was left in refrigeration overnight, it is assumed that bacterial contaminants and wild yeasts consumed fermentable sugar before tests began. After two days of fermentation, 82% of initial sugars had been consumed. Theoretical conversion of sugar to ethanol was 65% for chopped cane and 48% for ground cane.

As seen in Table 7, chopped cane fermentation reached higher ethanol concentrations than ground cane fermentation. A reason for this behavior could be the extended surface area that ground cane offers to microbial attack. Contaminants grow better in this environment, converting sugar to their own metabolic products. Low $Y_{p/s}$ values shown in Table 8 and bacterial counts of Table 9 support this idea.

The pH values for both treatments are similar, as can be seen in Table 10.

With regards to the microbial flora present, data can be found in Table 9. Again bacterial contaminants predominate, particularly in the case of ground cane where the ratio of bacteria to yeast at 6 days is 9.6 to 1.

Table 6: Total sugars (g/100 cm^3 juice)

Time(days)	Shredded cane	Ground cane
0	16.37	16.20
2	2.97	5.02
6	1.02	1.94

Table 7: Ethanol produced (g/100 cm^3 juice)

Time(days)	Shredded cane	Ground cane
2	4.05	2.66
6	5.39	3.97

Table 8: $Y_{p/s}$ values (g ethanol produced/g sugar consumed)

Time(days)	Shredded cane	Ground cane
2	0.44	0.32
6	0.44	0.34

Table 9: Microbial counts in fermented cane

Cane	Time (days)	Count (10^4 cells/g)	Flora present
Shredded	2	22	yeast[1]
	6	8	yeast
		46	bacteria
Ground	2	30	yeast[1]
	6	14	yeast
		135	bacteria

Bacterial count was not determined.

Table 10: pH values

Time(days)	Shredded cane	Ground cane
2	3.60	3.75
6	3.55	3.75

Trial 3
April 9-12, 1986

low moisture fermentation of ground sugar cane. 3.

Trials were made using three sugar cane varieties with different fiber content.

Materials:

Sugar cane: Three different varieties of sugar cane were provided by the Agricultural Department of Ingenio Pantaleón. Cane varieties were identified as follows:

P-33-11, a low fiber content variety with about 10% fiber

PINDAR, with about 13% fiber

BJ-67-32, a variety whose fiber content ranges between 15 and 17%.

Yeast: Saccharomyces cerevisiae strain (ICAITI L-181) was used. Inoculum was grown in a fermentor for 20 h as described previously. Medium used for inoculum growth was the same as the two previous experiences: 4% sucrose, 1.17% $(NH_4)_2SO_4$, 0.043% $Na_2HPO_4 \cdot 2 H_2O$, 0.03% $MgSO_4$ and 0.1% yeast extract.

Method:

Sugar cane was ground in the laboratory grinder as described before. Particle size was about 2 cm long and general appearance resembled sawdust.

Bags were prepared with 5 kg each of ground cane. Inoculum was added at 5%(w/w), adding a portion after each kg of cane was put in the bag.

Fermentation was carried out at room temperature, 23-27 C.

Analysis:

Sugar profile was determined using high pressure liquid chromatography.

Results:

1. Tables 11 and 12 show data on sugar and ethanol concentrations during fermentation. An F test showed that there is no significative difference between the three sugar cane varieties tested. Fiber content did not affect sugar conversion to ethanol.

2. Ethanol production was not as high as that obtained with chopped cane in previous experiences, but handling of ground cane is much easier than handling of chopped cane.

3. Table 13 shows yeast and bacterial counts. It can be observed that the bacterial count is 100 times greater than the yeast count. Also, bacterial contamination is 10 times greater than that observed in the chopped cane fermentation (see previous trials). Fiber content does not seem to have an effect on bacterial growth as discussed before.

4. Table 14 shows the sugar profile for the three cane types, before and during fermentation. The sum of the sugars sucrose, fructose and glucose correspond well with the results shown in Table 11 for total sugars determined by the Luff procedure. In terms of distribution of sugars, the fresh cane shows sucrose to be the predominant sugar as would be expected in fresh cane. The fermented canes show almost equal portions of glucose and fructose, product of the action of the yeast invertase.

Table 11: Sugars (g/100 cm^3 juice)

Time	Fiber content, %		
h	10	13	15-17
0	20.5	20.2	18.6
24	13.9	15.1	15.0
48	11.2	14.4	10.6

Table 12: Ethanol (g/100 cm^3 juice)

Time	Fiber content, %		
h	10	13	15-17
0	0	0	0.1
24	1.2	1.0	1.3
48	2.8	1.7	2.1

Table 13: Microbial counts in the ground cane of different fiber levels

Fiber content %	Time h	Yeast count (10^4 cells/g)	Bacterial count (10^4 cells/g)
10	0	0.3	nd
	24	14	1305
	48	21	1150
13	0	60	610
	24	17	805
	48	20	1315
15-17	0	100	700
	24	55	2070
	48	20	965

nd = not determined

TABLE 14

SUGAR PROFILE FOR THREE DIFFERENT CANES BEFORE AND DURING FERMENTATION

	TIME (h)	FRUCTOSE (9/100 cm^3)	GLUCOSE (9/100 cm^3)	SUCROSE (9/100 cm^3)	TOTAL	FRUCTOSE (%)	GLUCOSE (%)	SUCROSE (%)
Low fiber content	0	0.25	0.17	20.22	20.64	1.2	0.8	98.0
Sugar cane (10%)	24	2.22	3.42	8.13	13.77	16.1	24.8	59.0
-P - 33 - 11-	48	2.84	2.12	6.49	11.45	24.8	18.5	56.7
Medium fiber content	0	1.85	0.74	16.46	19.05	9.7	3.9	86.4
Sugar cane (13%)	24	2.06	1.53	11.71	15.30	13.5	10.0	76.5
-PINDAR-	48	2.01	1.52	11.15	14.68	13.7	10.4	76.0
High fiber content	0	1.28	0.55	17.08	18.91	6.8	2.9	90.3
Sugar cane (15 - 17%)	24	1.18	0.94	12.57	14.69	8.0	6.4	85.6
-Bj - 67 - 32 -	48	3.23	2.47	5.16	10.86	29.7	22.7	47.5

Conclusions for trials 1, 2 and 3.

Low moisture fermentation of ground sugar cane

1. Bacterial contamination is a major problem in using sugar cane prepared in the mill for ethanol fermentation trials. Delay in processing of sugar cane favors growth of contaminants and reduces sugar conversion to ethanol.

2. The smaller size particle obtained with the grinder is easier to work with. Samples can be mixed more throughly with ground cane than with shredded cane.

3. Cane variety and fiber content appear to have no effect on fermentation of sugar to ethanol.

Recomendations:

Control of contaminants can be achieved by:

- use of good quality cane,
- reduced delay between grinding of sugar cane and inoculation with yeast,
- improved yeast inoculum level,
- use of chemical preservatives to control bacterial growth, and/or
- modification of fermentation conditions to favor yeast growth over that of contaminants by:
    - addition of water,
    - temperature control.

2.2 Cane preservation

Trial No. 4
March-September 1986

Sugar cane preservation:ensilage

Preservation of fermentable sugars was studied in sugar cane which had been ensiled for one and five months. The effect of adding lactic inoculum to the sugar cane previous to the ensilage period was also studied. In both treatments, the fermentability of remaining sugars to ethanol was tested.

Materials:

Sugar cane: Shredded sugar cane from the process line of Ingenio Pantaleon was used.

Lactic inoculum: A **Lactobacillus plantarum** strain (ICAITI 35) was used. Inoculum was grown in tomato juice medium for 24 hours prior to use.

Yeast: A **Saccharomyces cerevisiae** strain (ICAITI L-181) was used. The medium used for inoculum growth was the same described before.

Method:

Five-kg bags of shredded sugar cane were inoculated with **Lactobacillus plantarum** inoculum at the 5% level(w/w). A set of bags of uninoculated cane was also prepared. Bags were closed trying to avoid air bubbles trapped inside and left at room temperature for one to five months.

Fermentation of ensiled material was started with a 5% (w/w) yeast inoculum. One kg bags were prepared from each five kg ensiled bag in order to have samples at 0, 24, and 48 hours.

Ethanol and sugar analysis were made on pressed juice from the cane samples. Reported data are an average of at least three experimental units.

Sugar profiles show the results of high pressure liquid chromatography analysis. The total acids were determined by titration with NaOH. Lactic and acetic acids were determined by gas-liquid chromatography.[1]

1. L. V. Holdeman, E. P. Cato and W. E. Moore. 1972. Anaerobe Laboratory Manual. Fourth edition. Virginia Polytechnical Institute State University, Blacksburg, Va. p. 117-142.

Results:

1. Data of sugar concentration in cane juice is shown in Table 15. It can be seen that lactic silage trials did not appear to be very different from the uninoculated trials. The silage procedure, either with or without lactic inoculum, was not effective in preserving the sugar.

2. Fermentation after silage was not effective, since after one month ensilage, 94% of the original sugar had already been consumed.

3. Use of lactic acid bacteria was effective in preserving the ethanol which had been produced during ensiling. As can be seen in Table 16, where the level of ethanol reached at one month in both cane treatments was greater than 5 g/ 100 cm^3 juice. By five months, the alcohol level dropped to 1g/100 cm^3 in the uninoculated cane, yet remained at 4.7 g/100 cm^3 of juice in the cane which had received the lactic inoculum.

4. The pH values of the expressed cane juice are given in Table 17. The original value of the fresh cane is rather low, at pH 5.2. All ensiled and fermented samples are similar in pH value, with the average value at one month being pH 3.6 and after five months, pH 3.5. The alcoholic fermentation did not change the pH value.

5. Tables 18 through 21 show the results of the colony counts made on the different cane samples. At one month, bacteria predominated in both cane treatments, with the inoculated cane (Table 18) showing counts four times higher than uninoculated cane (Table 19). By five months, the counts had dropped considerably and fungi appeared. The samples showed a definite aroma of volatile acids, particularly the uninoculated cane (Table 21). These observations, as well as the loss of ethanol demonstrated in Table 16, indicate that a secondary fermentation had taken place, converting the ethanol to other metabolites.

6. The sugar profiles of fresh, fermented, ensiled and fermented ensiled cane are shown in Table 22. The results for fresh cane are as expected, 91.5% sucrose and the rest monosaccharides. After 48 hours fermentation, the monosaccharides predominate, with fructose being slightly higher again as expected. The sucrose had been hydrolyzed by yeast invertase, and the glucose is fermented slightly more rapidly than fructose.

The ensiled cane presents very interesting results, also shown in Table 22. No sucrose is present, indicating invertase activity present in the flora. Monosaccharides are present in low levels, with glucose predominating, being present at levels of about 1%. We suspect that the flora in the ensilage was everchanging, with the last being certain

lactic acid bacteria unable to use glucose and instead, ferment fructose. See Rainbow (1975) for further discussion. The lactic acid bacteria are able to produce acetic acid as well as lactic acid. The high levels of acetic acid found (Table 23) would be toxic to yeasts and most bacteria (see Morjanoff and Gray, 1987, and Pons et al.,1986, for a discussion), which would explain residual glucose present.

7. Acid profiles are shown in Table 23 for silage at one month and in Table 24 for silage at 5 months. Here data for total acids and lactic acid are given. A very interesting point is that the lactic acid is a minor component of total acidity, representing 5% at five months when no lactic bacteria had been added, and 16% when the cane had been inoculated with a lactic culture. For the one-month samples, acetic acid was determined, showing it to be the principal component of total acids. This would indicate certain activity on the part of the lactic acid bacteria added. Alli and Baker (1982) studied ensilage of chopped cane, including leaves and tops, and reported lactic acid as the principal acid, but by 10 days of ensiling, the molar ratio of lactic acid to acetic acid was only 1.1 to 1. From their data, we calculated the juice acidity (sum of lactic and acetic acids both calculated as acetic acid) to be 0.73 g/100 cm^3, which is much lower than the values we observed. How do we explain this? The final pH is too low to permit activity by most Clostridium. Many lactic acid bacteria are capable of producing acetic acid and ethanol as well as lactic acid. Yeasts also produce acids.

Rainbow, C. 1975. Beer spoilage lactic acid bacteria. pp 149-158 in J. G. Carr, C. V. Cutting, and G. C. Whiting, eds. Lactic Acid Bacteria in Beverages and Food. London:Academic Press.

Morjanoff, P. J., and P. P. Gray. 1987. Optimization of steam explosion as a method for increasing susceptibility of sugar-cane bagasse to enzymatic saccharification. Biotechnology and Bioengineering 29 733-741.

Pons, Marie-Noelle, Ali Rajab and Jean-Marc Engasser. 1986. Influence of acetate on growth kinetics and production control of Saccharomyces cerevisiae on glucose and ethanol. Applied Microbiology Biotechnology 24 193-198.

Alli, Inteaz, Bruce Baker, and Gary Garcia. 1982. Studies on the fermentation of chopped sugarcane. Animal Feed Science and Technology 7 41-4[illegible]

Table 15: Fermentation of ensiled cane : sugar concentration, g/100 cm^3 juice

Cane	Fresh	Ensiled one month		Ensiled five months	
Lactic inoculum	-	+	-	+	-
Time (h)					
0	18.4	1.1	1.4	0.8	1.0
24	nd	0.9	1.2	1.0	1.0
48	8.1	1.0	1.4	1.0	1.0

nd = not determined

Table 16: Fermentation of ensiled cane: ethanol concentration g/100 cm^3 juice

Cane	Fresh	Ensiled one month		Ensiled five months	
lactic inoculum	-	+	-	+	-
time (h)					
0	0.1	5.3	5.1	4.7	1.0
24	1.1	5.3	4.9	4.5	0.9
48	3.4	5.2	4.8	3.8	1.0

Table 17: pH values in juice during fermentation[1]

Cane	Ensiled one month		Ensiled five months	
Lactic inoculum	+	-	+	-
Time (h)				
0	3.6	3.7	3.6	3.5
24	3.6	3.7	3.5	3.5
48	3.6	3.7	3.5	3.5

1. pH value of fresh cane juice was 5.2 before ensilage

Table 18: Fermentation of cane ensiled for one month with lactic inoculum: microbial counts. (10^6 CFU/g)

Sample	Time (h)	Count	Predominant flora present
	0	450	
1	24	465	bacteria
	48	243	
	0	186	
2	24	149	bacteria
	48	118	
	0	236	
3	24	158	bacteria
	48	113	

Table 19: Fermentation of cane stored for one month without lactic inoculum:microbial counts (10^4 CFU/g)

Sample	Time (h)	Count	Predominant flora present
	0	37	
1	24	127	bacteria
	48	64	
	0	21	
2	24	66	bacteria
	48	37	
	0	59	
3	24	38	bacteria
	48	86	

Table 20: Fermentation of cane ensiled with lactic inoculum for five months: microbial counts (10^6 CFU/g)

Sample	Time (h)	Count	Predominant flora present
	0	0.008	
1	24	0.011	bacteria
	48	5.7	
	0	0.38	yeast, fungi and bacteria
2	24	nd	count refers to yeast
	48	0.18	
	0	0.17	yeast, fungi and bacteria
3	24	0.0003	count refers to yeast
	48	0.05	

nd = count was not determined because of fungal contamination

Table 21: Fermentation of uninoculated cane stored for five months: microbial counts (10^4 CFU/g)

Sample	Time (h)	Count	Predominant flora present
	0	0.01	
1	24	0.01	bacteria
	48	0.09	
	0	0.02	yeast and fungi
2	24	0.002	yeast and fungi
	48	0.0005 0.0018	yeast bacteria
	0	nd	
3	24	0.0014 0.007	yeast and fungi bacteria
	48	0.004	yeast and fungi

nd = count was not determined because of fungal contamination

(Samples had a characteristic odor of volatile acids, particularly butyric acid.)

TABLE 22

SUGAR PROFILE DURING THE FERMENTATION AND ENSILAGE OF SUGAR CANE

	TIME (h)	FRUCTOSE (9/100 cm^3)	GLUCOSE (9/100 cm^3)	SUCROSE (9/100 cm^3)	TOTAL	FRUCTOSE (%)	GLUCOSE (%)	SUCROSE (%)
Fresh cane	0	0.9	0.7	17.3	18.9	4.8	3.7	91.5
	48	4.54	3.84	0.12	8.50	53.4	45.2	1.4
ONE MONTH ENSILED	0	0.04	1.00	0	1.04	3.8	96.2	0
Cane with lactic	24	0.06	0.81	0	0.87	6.9	93.1	0
Inoculum	48	0.02	0.94	0	0.96	2.1	97.9	0
CANE KEPT ONE MONTH	0	0	1.42	0	1.42	0	100.0	0
Without	24	0.06	1.15	0	1.21	5.0	95.0	0
Lactic inoculum	48	0.22	1.18	0	1.40	15.7	84.3	0

TABLE 23

ACID PRODUCTION (g/100 cm^3 JUICE) -ONE MONTH ENSILAGE-

CANE	TIME (h)	ACETIC ACID (g/100 cm^3)			LACTIC ACID (g/100 cm^3)			TOTAL ACIDS (g lactic/acid 100 cm^3)			ACETIC ACID / TOTAL ACIDS AS ACETIC ACID (%)	LACTIC ACID / TOTAL ACIDS AS LACTIC ACID (%)	NOT DETERMINED ACIDS (%)
				$\bar{X}$			$\bar{X}$			$\bar{X}$			
Ensiled with	0	0.75	0.73	0.74	0.95	1.26	1.11	2.79	2.97	2.88	43.3	38.5	18.2
Lactic	24	1.00	0.94		0.84	0.84		2.75	2.72				
Inoculum		0.84	0.73	0.88	1.05	1.16	0.97	2.79	2.75	2.75	53.4	35.3	11.3
	48	0.90	0.91		1.00	0.89		2.84	2.61				
		0.60	0.60	0.75	1.26	0.89	1.01	2.79	2.79	2.76	47.2	36.6	16.2
Ensiled	0	1.37	1.38	1.38	0.63	0.50	0.56	2.66	2.48	2.57	71.9	21.8	6.3
Without lactic	24	1.31	1.27		0.45	0.74		2.57	2.52				
Inoculum		1.19	1.86	1.41	0.55	0.56	0.58	2.43	2.34	2.47	77.0	23.5	-
	48	1.44	1.18		0.42	0.76		2.75	2.48				
		1.37	1.34	1.33	0.47	0.58	0.74	2.31	2.03	2.39	72.3	31.0	-

TABLE 24

ACID PRODUCTION (9/100 cm^3 juice) - FIVE MONTHS ENSILAGE -

CANE	TIME (h)	LACTIC ACID (9/100 cm^3)		$\bar{X}$	TOTAL ACIDS (9 lactic acid/100 cm^3)		$\bar{X}$	LACTIC ACID / TOTAL ACIDS AS LACTIC ACID (%)
Ensiled with Lactic inoculum	0	0.40			8.29			
		0.99	1.13	0.83	3.96	3.78	5.29	15.6
	24	0.45	0.45		8.29	7.75		
		0.91	0.94	0.84	3.42	3.60	5.10	16.5
		1.02	1.33		3.96	3.86		
	48	0.41	0.43		8.65	8.29		
		0.70	0.93	0.93	3.42	3.99	5.23	17.8
		1.54	1.64		3.60	3.72		
Ensiled without Lactic inoculum	0	0.22			5.95			
		0.45	0.51	0.39	7.75	9.73	7.73	5.0
	24	0.18	0.23		5.76	5.58		
		0.74	0.83	0.53	8.83	8.11	6.87	7.7
		0.59	0.64		6.49	6.85		
	48	0.25	0.22		7.21	9.91		
		0.49	0.49	0.44	9.07	8.83	8.05	5.6
		0.63	0.60		6.70	7.06		

Trial No. 5
March - September, 1986

Sugar cane preservation by steam explosion

Steam explosion of shredded sugar cane was tested as a method for preserving sugar cane during non-harvest periods.

Materials:

Shredded sugar cane was obtained from the process line of the mill.

Yeast and medium used were the same as described before.

Method:

Shredded sugar cane was steam exploded in a digester, keeping addition of water to the minimum. Internal temperature was raised to 170 C before being released. Heating time was thirty minutes.

Five kg plastic bags were prepared with the cooled exploded sugar cane. Five conditions were tested:

- Direct fermentation of exploded cane.
- Fermentation of exploded cane after a one month period of ensiling with a lactic acid bacteria inoculum.
- Fermentation of exploded cane ensiled for one month without addition of the lactic inoculum.
- Fermentation of exploded cane after a five month period of ensiling with a lactic acid bacteria inoculum.
- Fermentation of exploded cane ensiled for five months without addition of the lactic inoculum.

Yeast and lactic inocula were added to exploded cane at the 5% (w/w) level. Fermentation of the cane which had been ensiled for one and five months was also initiated with a yeast inoculum at the 5% level. The bags were closed as in the preceeding trials, trying to eliminate air bubbles. Fermentation and ensilage were carried out at room temperature (25-27 C). Reported data are the average of two experimental results.

Analytical techniques are the same as those described earlier.

Results:

1. Steam explosion as carried out in the laboratory did not prove to be a satisfactory method for preservation of the sugar. The long heating time favored unwanted reactions such as the Maillard browning. It was found that 36% of the original sugars were lost during steam explosion and the remainder could not be fermented by yeast as seen in Table 25. Presumably, some toxic components were formed during the explosion process.

2. The sugar profile is given in Table 26. Results for the freshly exploded cane are not consistent with the rest which show most of the sugar to be inverted with the amounts of glucose and fructose being equal. Inversion of sucrose during explosion is expected, given the severe reaction conditions and the liberation of acetate during the explosion. The observation that the yeast activity was inhibited as cited above is reinforced here because of the obvious availability of fermentable monosaccharides.

3. Later fermentation of ensiled cane did not produce much ethanol. Table 27 shows that freshly exploded cane had slightly better ethanol production that ensiled trials. Also the lactic acid bacteria favored ethanol production. Nevertheless sugar degradation and ethanol production are negligible.

4. Ensilage with or without lactic acid bacteria reported a sugar loss of 25% from the initial sugar levels indicating some degradation activities even though the products were not detected.

5. pH values were all low, 3.4 to 3.7, including the freshly exploded cane (Table 28).

6. The microbial counts obtained are shown in Tables 29 through 33. Microscopic observation of the growth obtained on the plates revealed yeast growth. No bacterial growth was observed. Nevertheless, the counts are a fraction of those observed in the previous experience (Tables 18-21). This reinforces the hypothesis that toxic products were formed during explosion.

7. Acid profile for the silage at five months is given in Table 34. Acid levels are much lower than those shown in Table 25, indicating inhibition of microbial activity. Lactic acid is present at about 25% of total activity in the ensiled unfermented cane.

Table 25: Sugar concentration during the fermentation of steam exuloded sugar cane (g/100 cm^3 juice)

Cane	Freshly exploded	Ensiled one month		Ensiled five months	
Lactic inoculum	-	+	-	+	-
Time(h)					
0	11.7[1]	8.7[1]	7.4[2]	7.8[1]	7.2[2]
24	10.5	9.5	9.6	5.7	5.3
48	10.4	8.6	8.9	5.8	4.4

1. Sugar content of the cane juice was 15.6 g/100 cm^3 before steam explosion. Explosion reduced sugar content to an average of 9.4 g/100 cm^3, a reduction of 40%.

2. Sugar concentration of the cane juice was 18.4 g/100 cm^3 before steam explosion. Explosion reduced sugar content to an average of 7.3 g/100 cm^3, a reduction of 60%.

Table 26: Please see next page.

Table 27: Ethanol concentration during the fermentation of steam exploded sugar cane (g/100 cm^3 juice)

Cane	Freshly exploded cane	Cane ensiled one month		Cane ensiled five months	
lactic inoculum	-	+	-	+	-
Time(h)					
0	0.1	1.2	0.03	0.18	0.50
24	1.1	0.3	0.04	1.26	0.42
48	3.7	0.6	0.08	1.12	0.63

TABLE 26

SUGAR PROFILE DURING THE FERMENTATION AND ENSILAGE OF STEAM EXPLODED CANE

	TIME (h)	FRUCTOSE (9/100 cm^3)	GLUCOSE (9/100 cm^3)	SUCROSE (9/100 cm^3)	TOTAL	FRUCTOSE (%)	GLUCOSE (%)	SUCROSE (%)
Exploded cane	0	4.1	2.0	0.6	6.8	61.1	30.2	8.7
	24	4.5	4.8	1.2	10.5	43.1	45.3	11.6
	48	4.2	5.1	1.0	10.3	40.9	49.7	9.4
One month ensiled	0	4.7	4.6	0	9.2	50.5	49.5	0
Exploded cane with	24	4.8	4.8	0	9.6	59.7	50.3	0
Lactic inoculum	48	3.9	4.5	0	8.4	46.2	53.8	0
	0	4.0	3.7	0	7.6	51.9	48.1	0
Exploded cane kept	24	5.5	4.6	0	10.1	54.9	45.1	0
One month Without lactic inoculum	48	4.8	4.1	0	8.9	53.8	46.2	0

Table 28: pH values in juice of exploded and fermented cane

Cane	Freshly exploded cane	Exploded cane ensiled one month		Exploded cane ensiled five months	
Lactic inoculum	-	+	-	+	-
Time(h)					
0	3.4	3.6	nd	3.6	3.6
24	3.7	3.5	3.5	3.5	3.5
48	3.5	3.5	3.4	3.4	3.6

nd = not determined

Table 29: Microbial counts during the fermentation of freshly exploded cane (10^6 CFU/g)

Time (h)	Yeast	Bacteria
0	0.06	0.01
24	2.8	0.50
48	0.1	0.39

Table 30: Microbial counts during the fermentation of steam exploded cane which had been ensiled for one month with lactic inoculum (10^6 CFU/g)

Time (h)	Count	Predominant flora
0	0.01	yeast
24	1.35	yeast
48	2.56	yeast

Table 31: Microbial counts during the fermentation of steam exploded cane which had been ensiled for five months with lactic inoculum (10^6 CFU/g)

Time (h)	Count	Predominant flora
0	1	yeast
24	4	yeast
48	6	yeast

Table 32: Microbial counts during the fermentation of steam exploded cane which had been ensiled for one month without the addition of lactic inoculum (10^6 CFU/g)

Time (h)	Yeast	Bacteria
0	0.0012	0.0012
24	0.0012	0.0030
48	0.0016	0.0020

Table 33: Microbial counts during the fermentation of steam exploded cane which had been ensiled for five months without the addition of lactic inoculum (10^6 CFU/g)

Time (h)	Count	Predominant flora
0	nd	fungi
24	16	yeast
48	30	yeast

nd = not determined

TABLE 34

ACID PRODUCTION -FIVE MONTHS ENSILAGE-

	LACTIC ACID (g Acetic Acid/ 100 cm^3)			TOTAL ACIDS (g Acetic Acid/ 100 cm^3)			LACTIC ACID (g/100 cm^3)			TOTAL ACIDS (g Lactic Acid/ 100 cm^3)			LACTIC ACID / TOTAL ACIDS
Steam exploded cane			$\bar{X}$			$\bar{X}$			$\bar{X}$			$\bar{X}$	
. With lactic inoculum													
0h	0.31		0.31	1.20		1.20	0.47		0.47	1.80		1.80	25.8
24h	0.30	0.31	0.31	1.08	1.44	1.26	0.45	0.47	0.46	1.62	2.16	1.89	24.6
48h	0.23	0.23	0.23	1.56	1.20	1.38	0.35	0.35	0.35	2.34	1.80	2.07	16.7
. Without lactic inoculum													
0h	0.19		0.19	0.84		0.84	0.29		0.29	1.29		1.29	22.6
24h	0.13	0.10	0.12	0.84	0.96	0.90	0.19	0.15	0.17	1.26	1.44	1.35	13.3
48h	0.14	0.07	0.11	1.42	0.55	0.99	0.21	0.11	0.16	2.13	0.83	1.48	11.1

Trial No. 6
January 26-30, 1987, and March 2-7, 1987

Fermentation and ensilage of chopped sugar cane using sodium bisulfite as inhibitor of bacterial contaminants.

Usefulness of sodium bisulfite as inhibitor of contaminants of ethanol fermentation was evaluated. Two concentrations were studied, 0.15 and 0.30%(w/w). A series of samples without bisulfite was used for comparative purposes.

Methods:

The sugar cane, from Ingenio Pantaleon, Santa Lucia Cotz., was ground in the pilot plant of ICAITI using an experimental mill which has ten round blades parallel to each other with teeth 0.25 inch long and a motor of 1 hp. Please see Section 3 of this report for a description of the mill.

The ground sugar cane and the sodium bisulfite were mixed in a ribbon blender. After 15 minutes of mixing, the yeast inoculum (Saccharomyces cerevisiae L-181) was added. The inoculum contained 10^7 cells ml^{-1}, and was added in such a way as to obtain a final concentration of 10^6 cells g^{-1} ground cane. Mixing was continued for 10 minutes more. As little as possible water was added with the sodium bisulfite and the inoculum. We estimated that the amount of water added was less than 10% of the weight of the ground cane. Portions of the mixture, weighing 1 kg, were placed in plastic bags. The bags were closed after removing the free air, pressing moderately the mass of ground cane in the bag before tying the bags. The bags were then placed in thicker plastic bags. The fermentation was carried out at room temperature (25-28 C). Enough bags were prepared to have samples at 0, 24, 48 and 72 h of fermentation. Reported values are the average of two experimental data points.

Ensilage was prepared in the same way as described previously. Five-kg plastic bags were prepared with the cane. Ensilage was carried out at room temperature for one month. After this period, the cane was fermented with a yeast inoculum added at the 5%(w/w) level, samples being taken at 0, 24, 48 and 72 h of fermentation. Results are the average of two experimental units.

Results:

1. Tables 35 and 36 show data for sugar and ethanol concentrations during the alcoholic fermentation. It can be observed that addition of sodium bisulfite reduced ethanol production and sugar consumption. The two levels of sodium bisulfite used were inhibitors of yeast growth, the 0.15% (w/w) level being the less toxic.

Table 37 shows theoretical conversion of sugar to ethanol in the trials. The conversions reached in the cane which had no sodium bisulfite and that which had 0.15% sodium bisulfite added are very similar(70.5 and 63.9%), while addition of 0.30% shows a clearly inhibitory effect with a theoretical conversion of only 9.7%. $Y_{P/S}$ values also show the same behaviour, a reduction of yield at the 0.30% level of bisulfite.

The pH values given in Table 38 are higher than those observed previously in this project. The microbial counts are given in Table 39. The cane without sodium bisulfite had mixed flora, bacteria and yeast, while the sodium bisulfite treated cane had only yeast present. The pH levels are related to the microbial counts: The more rapid decrease in pH in the untreated cane correlates with the high counts at 24 h. In cane treated with 0.15% sodium bisulfite, the reduction is gradual, reaching a lower pH and higher count at 72 h. pH decreased slightly and the yeast count raised slightly in the cane treated with 0.30% sodium bisulfite. Similar comparisons can be made with sugar commsumption (Table 35) and ethanol production (Table 36), reinforcing the observation that sodium bisulfite exerts an inhibitory effect not only on the bacteria but also on the yeast.

2. Data in Tables 40-43 show the results for the microbial counts in the ensiled cane. The results labeled 0 h are those of the analysis carried out on the cane after one month's storage with or without sodium bisulfite. Sodium bisulfite at 0.15% did not inhibit celular growth completely since sugar consumption was 90% of the original value. Sugar consumption of the cane with no added sodium bisulfite was 96.9% of the original value before ensilage, 21.11 g/100 cm^3 juice. Addition of 0.30% (w/w) sodium bisulfite did work as a preservative, conserving 85% of the original sugars after one month.

Theoretical conversion data and $Y_{P/S}$ values shown in Table 37 revealed that addition of sodium bisulfite at the 0.15% provided good conditions for ethanol production by the wild yeast present in the sugar cane. The lower pH values (Table 43) also indicate microbial activity. The microbial counts after one month of silage (Table 43, 0 h) are higher than those for the fresh cane (Table 39, 0 h), 10^8 times in the case of untreated cane, 12 times in the case of cane treated at the 0.15% level, and 4 times in the cane treated at the 0.30% level.

3. Data from the fermentation of sodium bisulfite ensiled cane are shown in Tables 40-45. Cane at 0 and 0.15% (w/w) sodium bisulfite were already fermented by wild yeast. Cane at 0.30% (w/w) sodium bisulfite could not be fermented by neither wild or inoculated yeast cultures.

Table 35: Fermentation of fresh cane treated with sodium bisulfite. Sugars (g/100 cm^3 juice)

Time	Sodium bisulfite added, %(w/w)		
h	0	0.15	0.30
0	21.1	21.1	21.1
24	15.3	18.3	19.4
48	3.8	12.7	19.8
72	0.7	2.1	18.1

Table 36: Fermentation of fresh cane treated with sodium bisulfite. Ethanol (g/100cm^3 juice)

Time	Sodium bisulfite added, %(w/w)		
h	0	0.15	0.30
0	0	0	0
24	1.6	0.3	0
48	6.7	3.5	0.3
72	7.6	6.9	1.0

Table 37: Fermentation of fresh cane treated with sodium bisulfite. Theoretical conversion and yield

Time (h)	Sodium bisulfite added, % (w/w) 0	0.15	0.30
	Theoretical conversion		
24	14.9	3.2	0.1
48	62.5	32.5	2.6
72	70.5	63.9	9.7
$Y_{p/s}$	0.35	0.30	0.19

$$\text{Theoretical conversion} = \frac{\text{g ethanol produced}}{\text{g theoretical ethanol}} \times 100 \quad \text{where}$$

$$\text{Theoretical ethanol} = 0.51(\text{g original sugar})$$

$$Y_{p/s} = \frac{\text{g ethanol produced}}{\text{g sugar consumed}}$$

Table 38: pH values of juice during fermentation of sodium sulfite treated ground sugar cane

Time(h)	Sodium bisulfite, % (w/w) 0	0.15	0.30
0	3.8	4.3	4.3
24	3.6	4.0	4.0
48	3.6	3.9	4.0
72	3.5	4.0	4.0

Table 39: Microbial count during fermentation of sodium bisulfite treated sugar cane (10^6 cells/g)

	Sodium bisulfite ,% (w/w)		
Time (h)	0	0.15	0.30
0	0.1	0.1	0.1
24	310	13	0.9
72	215	142	17

Table 40: Fermentation of sugar cane stored for one month. Sugars, g/100 cm^3 juice

	Sodium bisulfite, % (w/w)		
Time(h)	0	0.15	0.30
0	1.0	0.9	15.9
24	0.9	0.1	19.1
48	0.9	0.1	18.9
72	1.0	0.2	nd

Initial sugar content of fresh sugar cane juice before ensilage was 21.11 g/100 cm^3

nd = not determined

Table 41: Fermentation of sugar cane stored for one month. Ethanol, g/100 cm^3 juice

	Sodium bisulfite, % (w/w)		
Time(h)	0	0.15	0.30
0	3.4	6.8	0
24	3.5	7.7	0.1
48	3.6	7.7	0.2

Table 42: pH values of juice during fermentation of ground sugar cane stored for one month

	Sodium bisulfite, % (w/w)		
Time (h)	0	0.15	0.30
0	3.8	4.3	4.3
24	3.6	4.0	4.0
48	3.6	3.9	4.0
72	3.5	4.0	4.0

Table 43: Microbial count during fermention of sugar cane ensiled with sodium bisulfite (10^4 cells/g)

	Sodium bisulfite, % (w/w)		
Time (h)	0	0.15	0.30
0	11	1.2	0.4
24	51	0.8	0.001
72	11	3.7	0.003

Table 44: Fermentation of sugar cane stored for one month with sodium bisulfite. Calculated data: theoretical conversion and yield

Time (h)	Sodium bisulfite added, % (w/w) 0	0.15	0.30
	Theoretical conversion		
0	31.5	62.7	0.3
24	32.9	71.7	1.3
48	38.2	82.3	1.6
$Y_{p/s}$	0.17	0.34	0.03

Theoretical conversion = $\frac{\text{g ethanol produced}}{\text{g theoretical ethanol}}$ X 100 where

Theoretical ethanol = 0.51 (g original sugar)

$Y_{p/s}$ = $\frac{\text{g ethanol produced}}{\text{g sugar consumed}}$

Conclusions for trials 4, 5 and 6: Sugar cane preservation techniques

1. Steam explosion

Steam explosion of sugar cane resulted in loss of sugar and formation of inhibitory compounds which made this technique unapplicable as a method for preserving sugar cane for further use as a fermentation feedstock.

2. Ensilage

2.1 During silage, sugar was consumed with some production of ethanol. Subsequent fermentation was not justified due to the low levels of residual sugar left in the stored cane.

2.2 Scale up of the procedure for ensiling cane and, at the same time, permitting recovery of the ethanol produced appears to be difficult.

2.3 The use of lactic acid bacteria to foster proper ensilage conditions did not improve the results. Most sugar was still converted to ethanol. Silages were the same with or without lactic inoculum.

3. Addition of sodium bisulfite

3.1 Addition of sodium bisulfite was effective in controlling bacterial growth during storage and during fermentation of ground sugar cane.

3.2 However, sodium bisulfite also inhibited activity of the yeast cultures which were added for the ethanol fermentation.

3.3 The inhibitory effect of the sodium bisulfite on the yeast was present even after storage.

Recommendations:

1. Another alternative for cane storage must be found. From previous work at ICAITI, we know that drying is an effective means for preserving the cane and at the same time, the drying affects the cells structure in such a way as to improve the diffusion of the sucrose from the cane cells during the fermentation. However, an appropiate drying process which would also preserve all the fermentable sugars is an expensive process. Future research should contemplate defining the maximum amount of water permisible in the cane while still assuring preservation.

2. Use of sodium bisulfite still needs further research. If this technique is to be used, yeast strains resistant to bisulfite should be tested. Likewise, process design considerations would have to take into account the toxicity of the sodium bisulfite as well as its possible corrosiveness.

2.3 Improved fermentation conditions

Trial No. 7
February 2-8, 1987

Effect of inoculum level and addition of water on the fermentation of ground sugar cane.

Three inoculum levels (10^5, 10^7, and 10^9 yeast cells/g cane) and three levels of added water (0, 50 and 100 % w/w) were tested.

Materials:

Sugar cane was brought from Ingenio Pantaleon in Santa Lucia Cotzumalguapa. It was ground in a pilot mill provided with 10 parallel sawdisks and a 1-hp engine. Final appearance of the ground sugar cane resembled sawdust.

Yeast: Saccharomyces cerevisiae strain ICAITI L-181 was used.

Medium: composition of the medium used for inoculum growth was 2% glucose, 0.5% yeast extract and 1.0% peptone (Jimenez and van Uden, 1985).

Method:

Sugar cane was ground in the laboratory mill. Inoculum was prepared by centrifuging the content of a 10-liter fermentor with 24 h growth and diluting the yeast cream with distilled water to a concentration of 10^{10} cells per ml. This was then adjusted with water to provide the appropriate inoculum levels. Water addition was calculated on fresh cane weight basis. Levels of 0, 50 and 100% (w/w) were used.

Fermentation was carried out in plastic bags containing 200g of ground sugar cane. The water and inoculum mixture was poured into each bag containing cane. In order to achieve complete mixing of cane and liquid, bags were kneaded several times until homogeneous composition was observed. Bags were closed trying to avoid entrapped air bubbles. Fermentation temperature was set at 30 C. Each bag represented one sample. Samples were taken at 0, 6, 12, 18 and 24 h of fermentation. Reported values are the average of triplicate units. Ethanol and total sugar determinations were made on the pressed juice.

Jimenez, J., and N. van Uden. 1985. Use of extracellular acidification for the rapid testing of ethanol tolerance. Biotechnology and Bioengineering 27(11)1596-1598.

Results:

1. Dextran formation was observed during this trial with the highest levels ocurring in the 12 h sample of 10^5 and 10^7 inoculum levels. When pressed, these samples exuded a slimy liquid instead of clear juice. Much less dextran formation was observed with the 10^9 inoculum.

2. Tables 45 to 50 summarize analytical data obtained during fermentation of ground sugar cane. For comparative purposes, data were converted to per cent theoretical conversion. Calculated values for inoculum levels of 10^5 and 10^7 cells/g cane are shown in Tables 51 and 52. Values are very similar and denote a complete inhibition of fermentation. Table 53 shows theoretical conversion reached with the inoculum level of 10^9 cells/g cane. Values are six times better than those obtained with the lower inoculum levels. An F test with a 95% confidence limit made on the theoretical ethanol conversion data showed that inoculum level has a significant effect on fermentation while water level did not.

3. Sugar consumption was highly dependent on inoculum level. Figure 1 shows that inoculum of 10^9 cells/g cane is able to ferment almost all sugar when 50 or 100% water is added.

4. Supporting the idea of a significant growth of contaminants are the data shown in Table 54, where calculated $Y_{p/m}$ values are given. Inoculum levels of 10^5 and 10^7 cells/g cane yielded extremely low values, which can be explained by sugar consumption by the contaminants not producing ethanol. This agrees with the visual inspection of contaminated samples. $Y_{p/m}$ values for trials with inoculum levels of 10^9 cells/g cane are higher. It seems that the contaminants' growth is overwhelmed by the large number of yeasts and their activity. It also seems that the addition of water favored yeast activity, since $Y_{p/m}$ values are better for trials with 50 and 100% (w/w) of water added than in ground cane alone.

Table 45: Fermentation of ground cane with added water and inoculum level set at 10^5 cells/g cane

Ethanol production (g/100 cm^3 juice)

	Water addition, % (w/w)		
Time (h)	0	50	100
0	0.04	0.02	0.02
6	0.04	0.01	0.02
12	0.10	0.08	0.12
18	0.31	nd	0.20
24	0.70	0.58	0.37

nd = not determined

Table 46: Fermentation of ground cane with added water and inoculum set at 10^5 cells/g

Total sugars (g/100 cm^3 juice)

	Water addition, % (w/w)		
Time (h)	0	50	100
0	21.23	14.16	10.62
24	12.28	5.13	6.41

Table 47: Fermentation of ground cane with added water and inoculum level set at 10^7 cells/g cane

Ethanol production (g/100 cm^3 juice)

	Water addition, % (w/w)		
Time (h)	0	50	100
0	0.06	0.04	0.02
6	0.02	0.06	0.06
12	0.14	0.17	0.18
18	0.48	nd	0.34
24	0.82	0.70	0.52

nd = not determined

Table 48: Fermentation of ground cane with added water and inoculum set at 10^5 cells/g

Total sugars (g/100 cm^3 juice)

	Water addition, %(w/w)		
Time (h)	0	50	100
0	21.23	14.16	10.62
24	10.57	5.87	5.25

Table 49: Fermentation of ground cane with added water and inoculum level set at 10^7 cells/g cane

Ethanol production (g/100 cm^3 juice)

	Water addition, % (w/w)		
Time (h)	0	50	100
0	0.06	0.04	0.02
6	0.93	1.02	0.96
12	2.06	1.95	1.79
18	4.46	4.66	3.43
24	4.88	4.46	3.28

Table 50: Fermentation of ground cane with added water and inoculum set at 10^7 cells/g cane

Total sugars (g/100 cm^3 juice)

	Water addition, % (w/w)		
Time (h)	0	50	100
0	21.23	14.16	10.62
24	1.24	0.15	0.07

Table 51: Fermentation of ground cane with added water and inoculum level set at 10^8 cells/g cane

Theoretical conversion, %

	Water addition,% (w/w)		
Time (h)	0	50	100
0	0.4	0.3	0.3
6	0.4	0.1	0.4
12	0.9	1.1	2.2
18	2.9	nd	3.7
24	6.5	8.0	6.8

nd = not determined

$$\text{Theoretical conversion} = \frac{\text{g ethanol produced}}{\text{g theoretical ethanol}} * 100 \text{ where}$$

Theoretical ethanol = 0.51(g original sugar)

Table 52: Fermentation of ground cane with added water and inoculum level set at 10^7 cells/g cane

Theoretical conversion, %

	Water addition, % (w/w)		
Time (h)	0	50	100
0	0.6	0.5	0.4
6	0.2	0.8	1.1
12	1.3	2.4	3.3
18	4.4	nd	6.3
24	7.6	9.7	9.6

nd = not determined

$$\text{Theoretical conversion} = \frac{\text{g ethanol produced}}{\text{g theoretical ethanol}} * 100 \text{ where}$$

Theoretical ethanol = 0.51 (g original sugar)

Table 53: Fermentation of ground cane with added water and inoculum level set at 10^9 cells/g cane

Theoretical conversion, %

	Water addition, % (w/w)		
Time (h)	0	50	100
0	0.6	0.5	0.4
6	8.6	14.1	17.7
12	19.0	27.4	33.0
18	41.2	64.5	63.3
24	45.1	61.8	60.6

nd = not determined

$$\text{Theoretical conversion} = \frac{\text{q ethanol produced}}{\text{q theoretical ethanol}} * 100 \text{ where}$$

Theoretical ethanol = 0.51 (g original sugar)

FIGURE I Effect of addition of water on residual sugars

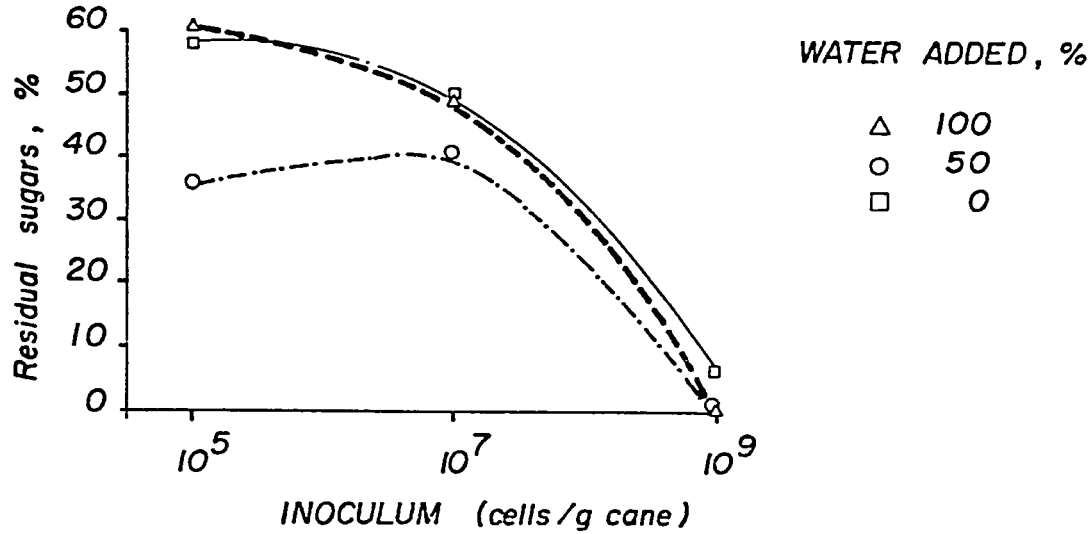

Table 54: Comparative Yp/s values

Inoculum (cells/g cane)	Water addition, % (w/w) 0	50	100
10^5	0.08	0.06	0.09
10^7	0.08	0.08	0.10
10^9	0.24	0.33	0.33

$$Y\ p/s = \frac{\text{g ethanol produced}}{\text{g sugar consumed}}$$

Trial No. 8.
February 23-27, 1987

Effect of addition of water on the fermentation of ground sugar cane

Since the previous experience showed that a 50 and 100% addition of water and a high inoculum gave the best results, these conditions were studied further. Three levels of water addition (75, 100 and 125% w/w) were tested. Inoculum remained constant at 10^7 yeast cells/g cane.

Method:

Inoculum for ground cane was prepared the same as before.

Procedure: To reduce the Leuconostoc mesenteroides contamination, a new procedure was tried. Inoculum was added to the cane immediately after grinding, mixing both in a ribbon blender for 15 minutes. After mixing, 400 g portions of inoculated ground cane were put in plastic bags and water was added. The water had been preheated to 35-40 C in order to raise the cane temperature above optimal growth temperature of L. mesenteroides (25-28 C). The bags were kneaded until a homogeneous composition was observed and then were closed avoiding trapped air bubles. Fermentation temperature was 30 C.

Samples were taken at 0, 8, and 20 h. Reported values are the average of triplicate samples.

Results:

1. Data for sugar concentration and ethanol production obtained in ground sugar cane fermentation are shown in Tables 55 and 56. Table 58 shows values of calculated theoretical conversions. Response is very similar for the three levels of water added. Statistical analysis using the F test with a 90% confidence level shows that there is no significant difference between them.

2. Comparison of calculated $Y_{p/s}$ yields also show no difference between the treatments. Values are presented in Table 59, which also includes the percent residual sugars present. Residual sugar values are higher than expected given the experience from past trials, but growth of contaminants was not evident.

3. Levels of 75, 100 and 125 % water had been chosen because higher levels showed weeping or separation of the liquid and solid phases. Also, calculations showed that the addition of

more water diluted the sugar in the cane to such an extent that the maximum ethanol values obtainable would be 5 % w/v or less.

Table 55: Ethanol produced in the fermentation of ground sugar cane with the addition of water (g/100 cm^3 juice)

Time	Water added, % (w/w)		
h	75	100	125
0	0	0	0
8	0.76	0.73	0.65
20	2.84	2.86	2.23

Table 56: Total sugars present during the fermentation of ground sugar cane with the addition of water. (g/100 cm^3 juice)

Time	Water added, % (w/w)		
h	75	100	125
0	12.03	10.52	9.36
20	3.59	1.93	2.54

Table 57: Theoretical conversion, %, during the fermentation of ground sugar cane with the addition of water

Time	Water added, % (w/w)		
h	75	100	125
0	0	0	0
8	12.4	13.6	13.6
20	46.3	53.3	46.7

$$\text{Theoretical ethanol conversion} = \frac{\text{g ethanol produced}}{\text{g theoretical ethanol}} * 100$$

where theoretical ethanol = 0.51 (g original sugar)

Table 58: Yield, $Y_{p/s}$, during the fermentation of ground sugar cane with the addition of water

	Water added, %		
	75	100	125
$Y_{p/s}$	0.34	0.33	0.33
Residual sugars, %	29.8	18.3	27.1

$$\text{where} \quad Y_{p/s} = \frac{\text{g ethanol produced}}{\text{g sugar consumed}} * 100$$

$$\text{and} \quad \text{residual sugars} = \frac{\text{final sugar}}{\text{original sugar}} * 100$$

Trial No. 9
March 9-13, 1987

Effect of agitation and addition of water on ground sugar cane fermentation

Influence of agitation and addition of water (50 and 100%) were evaluated.

Materials:

Agitation of the ground sugar cane was carried out using a leather curing laboratory chamber. This unit was provided with two parallel rollers in the bottom of the chamber. One was driven by a small motor, and the other was free to revolve. The reaction chambers were glass cylinders which had three fixed internal baffles. When the cylinders were placed on the rollers, the action of the motor driven one caused the cylinders to revolve at a constant rate. A timer was used to set movement at three minutes each fifteen minutes. The chamber was closed, and internal chamber temperature was set at 30 +/- 2 C.

Method:

Inocula and sugar cane preparation were the same as in the preceeding trial. Warm water (35-40 C) and 200 g of inoculated ground sugar cane were mixed in plastic bags until the mixture had a homogeneous appearance. The mixture was then placed in the glass containers which were closed with sponge stoppers and placed in the chamber.

Samples were taken at 3, 8, and 20 h of fermentation. Reported values are an average of at least 2 units.

Results:

1. Product and substrate concentration during fermentation is presented in Tables 59 and 60. It can be seen that sugar was consumed completely, but ethanol production was not as high as expected.

2. In order to compare responses to different water levels, theoretical conversion was calculated. These values are shown in Table 61. F test with a confidence level of 90% shows that a 100% addition of water gave significantly better conversions than a 50% addition.

3. Table 61 also shows $Y_{p/s}$ values and residual sugar percentages. Residual sugar values were very low, the same as the $Y_{p/s}$ value for the trial with 50% addition of water.

This implies that a contaminant consumed sugar as its carbohydrate source. It seems that the contaminant growth was adversely affected in some way by addition of more water, since $Y_{p/m}$ value for a 100% addition of water is not low even though theoretical conversion was 63%. This means that sugar consumed was converted to ethanol, although sugar consumption was lower than expected.

4. Agitation provided by the revolving containers was not complete. By the time the first samples were taken, ground cane had already packed against the container wall. Only at the beginning of the run was proper agitation provided to fermenting cane. Then the cane particles began to stick to the container wall.

Table 59: Fermentation of ground cane in rotating cylinders
Ethanol produced (g/100 cm^3 juice)

Time	Water added, %(w/w)	
h	50	100
3	0.69	0.52
8	2.22	2.17
20	2.02	2.94

Table 60: Fermentation of ground cane in rotating cylinders
Total sugars (g/100 cm^3 juice)

Time	Water added, % (w/w)	
h	50	100
3	12.05	9.04
8	4.31	2.08
20	0.11	0.08

Table 61: Fermentation of ground cane in rotating cylinders Theoretical conversion, $Y_{p/s}$ values and residual sugars

Time	Water added, % (w/v)	
h	50	100
	Theoretical conversion, %	
3	11.2	11.3
8	36.1	47.1
20	32.9	63.8
	$Y_{p/s}$	
20	0.224	0.413
	Residual sugars, %	
20	0.61	0.88

Where Theoretical conversion = $\frac{\text{g ethanol produced} * 100}{\text{g theoretical ethanol}}$

Theoretical ethanol = 0.51 (g original sugar)

$Y_{p/s}$ = $\frac{\text{g ethanol produced}}{\text{g sugar consumed}}$

Residual sugars = $\frac{\text{g final sugar} * 100}{\text{g original sugar}}$

Trial No. 10.
April 20-24, 1987

Effect of agitation and addition of water on fermentation of ground sugar cane

In order to reconfirm the data obtained in the previous experiences, another trial was carried out with the fermentation of ground sugar cane with two levels of addition of water, 50 and 100 %, and with or without agitation.

Method:

The same procedure as described for the previous trial was used. Samples were taken at 0, 8, and 21 h of fermentation. Reported data are the average of two experimental units.

Results:

1. Table 62 and 63 summarize product and substrate concentration during fermentation. Table 64 shows calculated theoretical conversions. It can be observed that final conversion is very similar for all conditions under study. An F test showed that there is no significant difference between the final theoretical conversions. The disagreement of these results with those obtained in previous experiences could be explained by growth of contaminants. Dextran formation was observed but not quantified.

2. Comparison of $Y_{p/s}$ values and per cent residual sugars (also shown in Table 64) pointed out that the agitated trial with a 100 % addition of water reached a higher $Y_{p/s}$ value than the other conditions tested, implying a better conversion of sugar to ethanol The use of water and agitation both should improve diffusion of the sugar from the interior of the cane to the particle surface where it is more readily available for the yeasts.

3. The pH values shown in Table 65 are low, indicating again bacterial contamination.

Table 62: Effect of agitation and water addition on the fermentation of ground sugar cane. Ethanol produced (g/100 cm^3 juice)

Agitation	Time	Water added, % (w/w)	
	h	50	100
agitated	0	0	0
	8	1.19	0.89
	21	2.84	2.58
static	0	0	0
	8	1.54	1.12
	21	3.52	2.50

Table 63: Effect of water addition and agitation on the fermentation of ground sugar cane. Total sugars (g/100 cm^3 juice)

Agitation	Time	Water added, % (w/w)	
	h	50	100
agitated	0	15.8	11.8
	8	12.0	7.5
	21	4.1	4.7
static	0	15.8	11.8
	8	11.0	6.7
	21	4.1	3.1

Table 64: Effect of water addition and agitation on the fermentation of ground sugar cane. Calculated values: Theoretical conversion, $Y_{p/s}$ values and residual sugars.

Agitation	Time	Water added, % (w/w)	
	h	50	100
	Theoretical conversion, %		
agitated	0	0	0
	8	14.8	14.7
	21	35.3	42.6
static	0	0	0
	8	19.2	18.5
	21	43.7	41.5
	$Y_{p/s}$		
agitated	21	0.24	0.36
static	21	0.30	0.20
	Residual sugars, %		
agitated	21	26.1	39.4
static	21	25.9	26.3

Where Theoretical conversion = $\frac{\text{g ethanol produced} * 100}{\text{g theoretical ethanol}}$

Theoretical ethanol = 0.51 (g original sugar)

$Y_{p/s}$ = $\frac{\text{g ethanol produced}}{\text{g sugar consumed}}$

Residual sugars = $\frac{\text{g final sugar} * 100}{\text{g original sugar}}$

Table 65: pH values in juice

Agitation	Time	Water added, % (w/w)	
	h	50	100
agitated	0	5.4	5.4
	8	4.1	4.2
	21	3.6	3.6
static	0	5.4	5.4
	8	4.1	4.2
	21	3.7	3.7

Conclusions for trials 7 through 10

Conversion parameters calculated for this work.

In the following Table 66, we present the conversion parameters calculated for this work. Here we have calculated the theoretical conversion for each of the four trials, taking into account agitation or static conditions, and level of water addition. Likewise, the per cent residual sugars, yield ($Y_{p/s}$), and specific rate of ethanol production are given. A statistical evaluation follows, as well as comparative values from the literature.

Table 66: Comparison of calculated values from this work.

Agitation	Time h	Trial	Water added, % (w/w) 0	50	75	100	125
			Theoretical conversion				
static	18	7	41.2	64.5		63.3	
	20	8			46.3	53.3	46.7
	21	10		43.7		41.5	
agitated	20	9		32.9		63.8	
	21	10		35.3		42.6	
			Residual sugars, %				
static	18	7	5.8	1.1		0.7	
	20	8			29.8	18.3	27.1
	21	10		25.9		26.3	
agitated	20	9		0.6		0.9	
	21	10		26.1		39.4	
			$Y_{p/s}$				
static	18	7	0.24	0.33		0.33	
	20	8			0.34	0.33	0.33
	21	10		0.30		0.20	
agitated	20	9		0.22		0.41	
	21	10		0.24		0.36	
			Specific rate of ethanol production at t = 0, g/l-h				
static	18	7	0.223	0.418		0.353	
	20	8			0.162	0.136	0.125
	21	10		0.310		0.219	
agitated	20	9		0.352		0.345	
	21	10		0.238		0.181	

Where Theoretical conversion = $\frac{\text{g ethanol produced} * 100}{\text{g theoretical ethanol}}$

Theoretical ethanol = 0.51 (g original sugar)

$Y_{p/s}$ = $\frac{\text{g ethanol produced}}{\text{g sugar consumed}}$

Residual sugars = $\frac{\text{g final sugar} * 100}{\text{g original sugar}}$

Statistical analysis was made using the least significant difference method (lsd) from Steel and Torrie (1960).

Trial No. 7: Effect of inoculum level and addition of water.

Theoretical conversions during the first 24 h of fermentation were analyzed. Trials with inoculum level of 10^6 and 10^7 cells/g cane showed no significant differences in the fermentation of the ground sugar cane with or without addition of water. An inoculum of 10^8 cells/g cane showed a different response: theoretical conversion was significantly higher in the fermentation of sugar cane with a 50 or 100% water addition than it was in the fermentation of ground sugar cane alone, without added water. No significant difference exists between results of the fermentation of cane with 50 and 100 % of water added at this inoculum level, which suggests that the addition of water is necessary but that the critical amount to be added is probably less than the levels tried.

Trial No. 8: Effect of addition of water.

Results showed that there is no significant difference in the initial theoretical conversion of sugar cane fermented with 75, 100 or 125% (w/w) of water.

Trial No. 9: Effect of agitation and addition of water.

Statistical analysis of results of agitated trials showed that there is a significant difference between adding 50 or 100% of water to the sugar cane before fermentation. Higher conversions were attained with a 100% addition of water.

Trial No. 10: Effect of agitation and addition of water.

In this trial, however, a 50 or 100 % addition of water did not affect the theoretical conversions. Both additions attained similar conversions. The effect of agitation was also confused since agitated and static trials reached similar conversions.

Since the above statistical analyses originated some contradictions, further analyses were made pooling the data from the four trials.

Steel, Robert G. D. and James H. Torrie. Principle and procedures of statistics. 1960. Mc Graw - Hill book Company, Inc., New York. p. 99-112.

1. In static or unagitated trials, theoretical conversions were similar in the fermentation of ground sugar cane with addition of 50, 75, 100 and 125 % (w/w) of water. The only significantly lower conversions were attained with a 50% addition of water in trials No. 9 and No. 10. The similar conversions support the idea of a limit to the addition of water, above which no appreciable improvement in fermentation occurs.

2. Theoretical conversions from trials 7 and 9 with a 100% addition of water are not different from each other but are significantly higher than those obtained in the other trials. This agrees with the fact that cane quality varies from time to time and can not be standardized. With this respect, sugar content and initial microbial load are different every time.

3. Analysis of the specific rates of ethanol production showed that trials 7 and 9 reached significantly higher values than other trials.

This information makes us believe that cane quality (defined as sugar content and microbial load) had a definite effect on the results of the fermentations, independent of any experimental condition.

Comparison with values from the literature[1]:

Rates of ethanol production seems to be highly dependent on inoculum level as discussed before. In the fermentation of sweet sorghum, Kargi et al. (1985) observed that higher inoculum gave higher rates of ethanol production and reduced lag periods. Data given in Table 67 show that an inoculum of 7×10^9 cells/g raw sorghum yielded a specific rate of ethanol production of 5.43 g/l-h while the inoculum of 10^6 cells/g raw sorghum only reached 0.58 g/l-h. They worked with sweet sorghum that had been peeled and fiber removed before being cut into segments and dried. This indicates that pretreatment of the fermentable material had an important effect on the availability of sugar, when we compare the data obtained with our experimental average value of 0.37 g/l-h. Earlier work on dried sugar cane in ICAITI had also indicated superior results when the cane had been dried previously (Cabrera, 1983).

Moisture content of rehydrated sorghum was also studied by Kargi et al. (1985), finding that 78.2% of moisture gave better results. Inoculum used was 10^7 cells/g sorghum.

Saucedo et al. (1985) also worked with sweet sorghum segments which were dried to less than 6 % moisture before fermentation. Volumetric rate of ethanol production was 0.256 g/l-h.

1. References cited will be given at the end of this section.

Contamination was the main problem in the fermentation of fodder beets as observed by Gibbons et al. (1987). They worked with a semi-continuous fermenter where thawed, washed and sliced beets were fermented. Inoculum used was 2.5 x 10^7 cells/g cane which was too low to overcome growth of contaminants. They used sodium meta-bisulfite and H_2SO_4 to control contamination, but even so the rates of ethanol production were low: 0.113 and 0.202 g/l-h respectively.

Fermentation of grape pomace was studied by Wang et al. (1986) at two temperatures. At 25 C, rate of ethanol production was 1.1 g/l-h, and at 18 C it was 0.17 g/l-h.

Gibbons and Westby (1983), working with prehydrolized corn and whey mixtures and an inoculum of 6 x 10^8 cells/l, obtained an initial rate of ethanol production of 1.18 g/l-h.

Er-el et al. (1981) worked with sugar cane segments, carrying out fermentation in three cycles. Each cycle started with fresh cane segments and the fermented beer was recycled. The first cycle with an inoculum of 6 x 10^8 cells/l yielded a high rate of ethanol production, 3.66 g/l-h.

Data given in Table 67 shows the importance of inoculum level in the fermentation of unsterilized solids. The studies where high inocula were used reached higher rates of ethanol production than those where lower levels of inocula, 10^7 and 10^4 cells/g, were used. Higher inocula also contributed toward reducing growth of contaminants, probably through competition for the substrate and/or production of unfavorable conditions for the contaminants growth, such as through anaerobic conditions or lower pH. As observed in Gibbon's experiences (1987), low inocula needed an external agent to control microbial growth during fermentation.

Higher rates of ethanol production are also dependent on pretreatment given to the fermentable material. Results obtained by Kargi et al. (1985) using sweet sorghum which was peeled, removing the outer fiber, are higher than those obtained with the untreated material. Drying also has an impact on the fermentability of the substrate.

Table 67: Comparative calculations from data reported in the literature

Substrate	Pretreatment	Temperature C	Initial rate of etanol production (g/l-h)	Reference
Fodder beets	Beets were thawed, washed and sliced. Inoculum was 2.5 x 10⁷ cells/g. Contamination was controlled with sodium meta bisulfite.		0.113	Gibbons & Westby, 1987
Fodder beets	As above, but contamination controlled with H_2SO_4.		0.202	Gibbons & Westby, 1987
Grape pomace	Inoculum at 4gDW/kg	25	1.014	Hang et al.,1986
Sugar cane	Cane cleaned and cut into segments. Fermentation in 3 cycles,with fresh segments and recycled beer. High inoculum at 12% v/v			Er-el et al.,1981
	first cycle		3.652	
	second cycle		0.828	
	third cycle		1.655	
Sugar cane	Cane cut into segments and dried. EX-FERMentation in 3 cycles:	30		Cabrera, 1984
	Yeast 178: first cycle		3.440	
	second cycle		1.924	
	third cycle		1.023	
	Yeast 181: first cycle		1.500	
	second cycle		0.723	
	third cycle		0.611	

Continuation of Table 67

Substrate	Pretreatment	Temperature C	Initial rate of etanol production (g/l-h)	Reference
Sweet sorghum	Sorghum cleaned, cut into segments and dried at 50-60 C to less than 6% moisture.	32	0.256	Saucedo et al., 1985
Sweet sorghum	Sorghum peeled, outer fiber removed, center chopped into small pieces, then fermented in the following ways:	28		Kargi et al., 1985
	presqueezed sorghum solids and juice		2.100	
	juice alone		1.500	
	unsqueezed sorghum		2.700	
	72.2% moisture content, yeast inoculum 6 x 107 cells/g sorghum		1.892	

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Ethanol toxicity studies

Rate of ethanol production is related to many factors: yeast strain, fermentation temperature, substrate composition and concentration, pH, free water level and ethanol concentration. Furthermore, these factors are interrelated. Since, in the present research project, the fermentation conditions are quite favorable to the yeast. Substrate is fixed as a mixture of predominately sucrose with some glucose and fructose, at a total maximum concentration of around 21%, and temperature at 30-35 C. Thus, the toxicity of ethanol to the yeast strain becomes a determinant factor for fermentation rate. The purpose of these studies, therefore, is to characterize the ethanol toxicity for the yeast strains in the ICAITI Culture Collection, in order to select for the most promising strains. Two temperatures are used in the characterization studies: 25 C to compare data obtained with literature values, and 35 C to simulate plant conditions in the warm Guatemalan Coast.

Testing of the strains continues. Thus results are given for those tested, with no final conclusions.

Methods:

1. Each yeast was grown on YM agar for 24 h at 32 C.

2. The yeast growth was washed from the agar surface with 2 ml of distilled water and transferred to a baffled shake flask containing 100 ml of sterile liquid medium. This was then incubated at 25 C for 24 h with agitation fixed at 200 rpm.

3. Ten ml of the yeast was used to inoculate 500 ml of sterile broth distributed in five baffled shake flasks. These were then incubated for 24 h at 25 C with agitation fixed at 200 rpm.

4. The yeast broth was then centrifuged at 10 000 rpm for 10 min, washed two times with sterile distilled water, and resuspended in 10 ml of sterile distilled water.

5. Aliquotes of 0.5 ml of this suspension were transfered to tubes containing 0.5 ml of a glucose solution at 20% and 3.5 ml of the required ethanol solution. Ethanol concentrations used were 0, 2, 4, 6, 8, and 10 % (V/V). The tubes had been tempered to the temperature to be used in the test.

6. The tubes were then closed with screw caps and incubated at 25 or 35 C for 75 min with gentle mechanical agitation.

7. At the end of the incubation period, the pH of the cell suspension was measured with a digital pH meter (Digital

pH/temperature meter, Kernco Instruments Co. LTD., Model 671). A pencil-thin electrode was used to facilitate pH measurement directly in the tube (SIGMA No. E-5259 pH electrode).

Materials used:

1. Storage medium for the yeast strains - YM Agar

Yeast extract	0.3%
Malt extract	0.3%
Peptone	0.5%
Glucose	1.0%
Agar	2.0%

2. Liquid growth medium (Jimenez and van Uden, 1985)

Glucose	2.0%
Yeast extract	0.5%
Peptone	1.0%

3. Glucose solution, 20% in distilled water

4. Ethanol solutions:

% ethanol,v/v	Molarity (# moles/ l)
0	0
2	0.35
4	0.69
6	1.04
8	1.39
10	1.74

Calculations used:

When the yeast cell is suspended in the glucose solution, internal protons are excluded and the extracellular pH falls to a final value. This acidification of the solution can be measured, and represents an equilibrium between the active extrusion of protons balanced by the pasive influx of protons.

The concentration of ethanol in the solution exponentially increases the diffusion constant of the influx of protons according to the following equation:

$$C_x = C_o e^{kx}$$

where: C_x = difusion constant at an alcohol concentration of x
C_o = difusion constant at an alcohol concentration of 0
k = exponential constant of increase

The ethanol concentration increases the final pH acording to:

$$pH_f = (\log_{10} C_o - \log_{10} K_p) + kX \log_{10} e$$

where: k_p = the rate of active proton extrusion
X = the alcohol concentration in molarity.

Under the condition that $(\log_{10} C_o - \log_{10} k_p)$ is a constant, or nearly so over the range of experimental pH_f values, the equation, if valid, is transformed in a linear equation and k can be estimated from the slope.

EXAMPLE

The constant, k, was determined at 25 C and 35 C for yeast I-187, and the experimental data obtained is given in the following table:

Molarity	pH_f 25 C	pH_f 35 C
0	3.84	4.13
0.35	3.99	4.22
0.69	4.14	4.41
1.04	4.27	4.67
1.39	4.36	4.88
1.74	4.45	5.08

The constants k for 25 C and 35 C were the following:

25 C	k =	0.85236
	b =	3.86904
	r =	0.99275
35 C	k =	0.57440
	b =	4.06619
	r =	0.99323

Where: $b = (\log_{10}C_o - \log_{10}k_p)$
r = correlation coefficient
$k = (\text{molarity})^{-1} = (\#\ \text{moles}/l)^{-1} = (1/\#\ \text{moles})$

Criteria for establishing which yeast strains are more tolerant to ethanol.

In order to establish which yeast strains listed in Table 68 were more tolerant to ethanol, the constants described above were calculated and can be found in Table 69. Average values for the exponential constant (k) of ethanol-enhanced proton influx are presented as well in the form of a bar graph in Figure 2.

Jimenez and van Uden (1985) established that low values of k correspond to a greater ethanol tolerance in the yeast. In other words, the ethanol tolerance of the yeast is inversely proportional to the value of k.

Additionally, another important factor which should be considered is the temperature. In Figure 2, the values for k are given for both 25 and 35 C. Here it can be seen that the constant k is greater at 35 C than at 25 C. This means that an increase in the temperature is accompanied by a decrease in the ability of the yeasts to tolerate ethanol, as reported by Gray in 1941. Other authors have reported that the ethanol toxicity to **Saccharomyces cerevisiae** and other similar yeasts can be increased by an increase in the temperature, and this increase in the toxicity can induce a growth repression and a loss of viability of the yeast population (Lee et al.,1980; Troyer, 1953; Oliver and Sugden, 1983). Other similar observations regarding the dependence of ethanol toxicity on the temperature have been reported for other yeasts, namely **Candida krusei** (Kilian et al., 1981), **Kluyveromyces fragilis** (Sa-Correia and van Uden, 1982 and 1983) and **K. marxianus** (Hughes et al., 1984; Watson et al., 1986).

The great sensitivity of the yeasts to ethanol when the temperature increases can be tied to the rapid acumulation of intracelular ethanol (Moulin et al.,1984) and the loss of potassium (Sa-Correia and van Uden, 1986), since various authors have reported that the intracelular concentration of ethanol during batch fermentations is much higher than the extracellular concentration or that in the medium (Novak et al., 1981; Lee et al., 1983; Navarro and Duran, 1978; Rose and Beavan, 1981; Thomas and Rose, 1979; Gonde et al., 1985; and Strehiano and Goma, 1983). Work on the kinetics of the diffusion of ethanol across the plasma membrane have confirmed this hypothesis.

In addition, the increase in the toxicity of ethanol affects not only the viability of the cell but also the minimum and maximum growth temperatures. As the ethanol concentration increases, the maximum growth temperature decreases and the minimum growth temperature increases when temperature profiles are studied (Sa-Correia and van Uden, 1983).

The yeasts which have shown an exponential constant at 25 C to be approximately equal to the constant at 35 C are considered to be thermoresistant, since there is little difference between the two. This indicates that the temperature does not affect viability within a certain range of temperature.

References:

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TABLE 68

IDENTIFICATION OF YEAST CULTURES

ICAITI CULTURE COLLECTION NUMBER	IDENTIFICATION OF CULTURE	RECEIVED FROM	ORIGINAL NUMBER	SOURCE
L-106	Saccharomyces ellipsoideus	A	1089	Vino Vernaccia de Aristano
L-108	Saccharomyces ellipsoideus	A	1104	Vino Nuragus de Sandati
L-110	Saccharomyces ellipsoideus	A	1111	Vino Nuragus de Sandati
L-114	Saccharomyces ellipsoideus	A	1136	Vino Vermentido de Monti
L-115	Saccharomyces ellipsoideus	A	1139	Vino Vermentino de Monti
L-116	Saccharomyces ellipsoideus	A	1142	
L-117	Saccharomyces ellipsoideus	A	1154	Vino Cannonau de Oliena
L-118	Saccharomyces ellipsoideus	A	1156	Vino Cannonau de Oliena
L-119	Saccharomyces ellipsoideus	A	1160	Vino Cannonau de Oliena
L-120	Saccharomyces ellipsoideus	A	1163	Vino Moscato de Sorso
L-127	Saccharomyces cerevisiae	Y	0762	
L-130	Saccharomyces sp	Z	Equilait - LG 78	
L-130	Saccharomyces sp	Z	Equilait - LG 78	
L-131	Saccharomyces cerevisiae	B	CCY - 22 - 7 - 1	
L-140	Saccharomyces sp	AA	B	
L-142	Saccharomyces carlsbergensis	C	No. 18	
L-144	Saccharomyces sp	D		
L-149	Saccharomyces cerevisiae	BB	14/44	
L-150	Saccharomyces ellipsoideus	E	Montrachet	
L-150	Saccharomyces ellipsoideus	E	Montrachet	
L-151	Levadura de destilería	F	NRRL Y - 567	
L-151	Levadura de destilería	F	NRRL Y - 567	
L-153	Saccharomyces uvarum	F	NRRL Y - 1347	
L-158	Saccharomyces diastaticus	G	PRL-77	
L-159	Saccharomyces cerevisiae	H	NCYC 1026	
L-162	Saccharomyces cerevisiae	H	NCYC 1119	
L-162	Saccharomyces cerevisiae	H	NCYC 1119	
L-164	Saccharomyces cerevisiae	H	NCYC 1256	
L-165	Saccharomyces cerevisiae	I	HYBRID 1	
L-166	Saccharomyces cerevisiae	I	HYBRID 2	
L-168	Saccharomyces cerevisiae	I	HYBRID 4	
L-169	Saccharomyces cerevisiae	I	HYBRID 5	
L-170	Saccharomyces cerevisiae	I	HYBRID 6	

TABLE 6A

IDENTIFICATION OF YEAST CULTURES
(continued)

ICAITI CULTURE COLLECTION NUMBER	IDENTIFICATION OF CULTURE	RECEIVED FROM	ORIGINAL NUMBER	SOURCE
L-171	Saccharomyces sp	J		
L-172	Saccharomyces sp	K	Geisenheim 49	
L-174	Saccharomyces cerevisiae	J		
L-175	Saccharomyces cerevisiae	L	NO. 729	
L-177	Saccharomyces cerevisiae	M	CBS 2962	
L-178	Saccharomyces cerevisiae	M	CBS 1394	
L-179	Saccharomyces cerevisiae	M	CBS 1196	
L-180	Saccharomyces cerevisiae	M	CBS 2959	
L-180	Saccharomyces cerevisiae	M	CBS 2959	
L-181	Saccharomyces cerevisiae	M	CBS 1242	
L-181	Saccharomyces cerevisiae	M	CBS 1242	
L-182	Saccharomyces cerevisiae	M	CBS 2961	
L-183	Saccharomyces cerevisiae	M	CBS 1235	
L-187	Saccharomyces cerevisiae	N	156	
L-191	Saccharomyces cerevisiae	O		
L-192	Saccharomyces uvarum	O		
L-194	Saccharomyces cerevisiae	O		
L-196	Saccharomyces	O	Levadura port	
L-198	Saccharomyces	O	Levadura tokay	
L-201	Saccharomyces	O	Levadura bordeaux	
L-202	Saccharomyces	O	Levadura audovin	
L-203	Saccharomyces	O	Levadura beer	
L-206	Saccharomyces	O	Levadura de panificación	
L-207	Saccharomyces	O	Levadura de destilería	
L-208	S. cerevisiae + S. carlsbergensis	P	I - S. C. / 194	
L-209	Saccharomyces cerevisiae	P	1 - S. C. / 39	
L-211	Saccharomyces sp	V	CFCC 3	
L-213	Saccharomyces sp	U	Ysa 6	
L-216	Saccharomyces sp	U	Ysa 22	
L-217	Saccharomyces uvarum	U	Ysa 23	
L-218	Saccharomyces	SQUIBB		
L-222	Rhodosporidium dibovatum	Q	NCYC 778	
L-226	Torulopsis magnoliae	Q	NCYC 742	
L-232	Candida brumptii	Q	NCYC 39	
L-240	Saccharomyces fructum	Q	28	
L-243	Saccharomyces cerevisiae	Q	AWY 350 K	
L-244	Saccharomyces cerevisiae	Q	Montrachet	
L-245	Saccharomyces cerevisiae	Q	NCYC 1119	

TABLE 6B

IDENTIFICATION OF YEAST CULTURES
(continued)

ICAITI CULTURE COLLECTION NUMBER	IDENTIFICATION OF CULTURE	RECEIVED FROM	ORIGINAL NUMBER	SOURCE
L-247	Saccharomyces bayanus	Q	Champ U. S. A.	
L-248	Saccharomyces cidri	Q	NCYC 775	
L-250	Saccharomyces bailii	Q	NCYC 464	
L-251	Saccharomyces bailii	Q	BYC 22	
L-252	Saccharomyces bayanus	Q	WS / 36	
L-255	Hansenula canadensis	Q	NCYC 497	
L-256	Hansenula anomala	Q	NCYC 18	
L-260	Brettanomyces sp	Q	BR/USA/ 2	
L-261	Brettanomyces anomalus	Q	NCYC 449	
L-274	Candida krusei	M	CBS 573	
L-278	Candida shehatae	S	FPL 050	
L-279	Candida sp	R		Melaza de caña, Guatemala
L-280	Candida tropicalis	M	CBS 6948	
L-282	Candida utilis	T	70167	
L-283	Candida utilis	T	2361	
L-284	Candida utilis	T	70163	
L-285	Debaryomyces sp	R		Melaza de caña, Guatemala
L-286	Endomycopsis fibuligera	M	CBS 6310	
L-287	Hansenula sp	R		Melaza de caña, Guatemala
L-288	Kloeckera sp	R		Melaza de caña, Guatemala
L-289	Kloeckera sp	R		Melaza de caña, Guatemala
L-290	Kloeckera sp	R		Melaza de caña, Guatemala
L-291	Kloeckera sp	R		Melaza de caña, Guatemala
L-293	Kluyveromyces fragilis	T	70343	
L-298	Pachysolen tannophilus	G	NRRL Y-2460 NRC 2507	
L-299	Pachysolen tannophilus	S	FPL N03-N03-4	
L-300	Pachysolen tannophilus	S	NRRL-Y-2460 FPL 240	
L-301	Saccharomyces bayanus	M	CBS 380	
L-302	Saccharomyces cerevisiae	Q	Montrachet	
L-303	Saccharomyces cerevisiae	Q	AWY 350R	
L-304	Saccharomyces sp	R		Melaza de caña, Guatemala
L-305	Saccharomyces sp	R		Melaza de caña, Guatemala
L-306	Saccharomyces sp	R		Melaza de caña, Guatemala
L-307	Saccharomyces sp	R		Melaza de caña, Guatemala
L-308	Saccharomyces sp	R		Melaza de caña, Guatemala
L-309	Saccharomyces sp	R		Melaza de caña, Guatemala
L-310	Saccharomyces uvarum	M	CBS 395	
L-311	Schizosaccharomyces pombe	T	DSM 70576	

TABLE 68

IDENTIFICATION OF YEAST CULTURES
(continued)

ICATT CULTURE COLLECTION NUMBER	IDENTIFICATION OF CULTURE	RECEIVED FROM	ORIGINAL NUMBER	SOURCE
L-314	Schwanniomyces castelli	B	CCY 47-3-1	
L-319	Saccharomyces cerevisiae	F	NRRL Y-11572	
L-321	Pichia stipitis	F	NRRL Y-11544	
L-322	Pichia stipitis	F	NRRL Y-11545	
L-323	Pachysolen tannophilus	F	NRRL Y-2460	
L-325	Candida shehatae	F	NRRL Y-12857	
L-329	Candida curvata	W	CBS 570	
L-332	Hansenula saturnus	T	70278	
L-335	Saccharomyces lipolytica	T	70561	
L-336	Hansenula ciferri	T	70180	
L-338	Saccharomyces lipolytica	T	DSM 70562	
L-339	Saccharomyces lipolytica	T	DSM 1345	
L-340	Hibrido de S. rouxii	X		
L-341	Hibrido de S. diastaticus	X		
L-342	Saccharomyces cerevisiae	T	ASM 3774	

Table 68, continuation

Source of yeast strains.

A. Dr. Fabrizio Fatichenti, Instituto di Microbiologia Agaria e Tecnica "A.Capriotti", Universita degli Studi de Sassari, Sassari, Sardegna, Italia.

B. Kockova-Kratochvilova, Institute of Chemistry of the Slovak Academy of Science, Dubraskacesta, Bratislava, Czechoslovakia

C. K. Mayer, Eidgenossische forschungsanstalt fur Obst, Wein und Gartenbau, Wandeswil, Switzerland.

D. Destileria de Alcolholes y Rones, DARSA, Santa Lucia Cotzumalguapa, Escuintla, Guatemala.

E. Emil Wick. WRRL/USDA/ARS. Berkley, California, USA.

F. C. P. Kurtzman, NRRL /USDA/ARS, Peoria, Illinois, USA.

G. R. H. Hanskins, National Research Council, Prarie Regional Laboratory, Saskatoon, Canada.

H. National Collection of Yeast Cultures. The Brewing Research Foundation , Lyhel Hall, Nutfield, Redhill, Surry, England.

I. S. Burrows, the Destillers Company Limited, Yeasts Food Division, Menstrie, Clackmannshire, Scotland.

J. R. B. Gilliland. Arthur Guiness Son & Co. Ltd. The Research Laboratory, Dublin, Ireland.

K. Institute fur Mikrobiologie und Biochemie der Forsklungsanhalt, fur Weinbau Gartenbau, Getranketechnologie und Landespflege, Geisenheim am Rhein, FDR

L. Andre Hool. The Autralian Wine Research Institute, Glen Osmond, South Australia.

M. Dr. D. Yarrow, Centraal Bureau voor Schimmel-Cultures. Yeast Division, Delft, Holand.

N. Dr. G. G. Stewart. Labatt Breweries of Canada, Ltd. London, Ontario, Canada.

O. Dr. Ricardo Molins, Private colletion.

P. Institut Przemyslu, Permentacyjnego, Warsaw, Poland.

Q. Dr. R. Davenport, Long Ashton Research Station, Long Ashton, Bristol, BS18 9AF, England.

Table 68, continuation

R. ICAITI Culture Collection, isolated locally.

S. T. Jeffries, USDA, Madison, Wisconsin, U. S. A.

T. Deutsche Sammlung von Microorganismen Grisebachstrasse 8, 3400 Gottingen, Germany

U. R. H. Tilburg. Tate and Lyle Ltd. Reading, U. K.

V. M. Thorpe, Department of Biological Sciences. University of Aston, Gosta Green, Birmingham, England

W. Dr. Ronnie Glatz, Dairy Industry Building, Iowa State University, Ames, Iowa 50011, U. S. A.

X. Dr. Frank Spencer, Biological Sciences Dept., Goldsmith College, University of London, Rachel McMillan Building, Creek Road, SE 83 BV London, U. K.

Y. Dr. S. Windisch. Institute fur Garungswerbe und Biotechologie, 1 Berlin, 65, Seestrasse 13, Germany.

Z. E. Peynaud, Station Agronomique et Vinologique de Bordeaux, 351 Cours de la Liberacion, 33405, Talence, France.

AA. Joseph E Seagram & Sons, Inc., 7th Street Road, Louisville, Kentucky 40201, USA

BB. Institute of Hygiene and Epidemiology, Czechoslovak National Collection of Type Cultures. Prague 10, Skobarova 48, Czechoslovakia.

CC. Dr. Alojz Veros, Director, Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia, Matustrova 21.

AVERAGE VALUES FOR THE EXPONENTIAL CONSTANT (K) OF ETHANOL-ENHANCED PROTON INFLUX

ICAITI CULTURE COLLECTION NUMBER	TEMPERATURE 25°C RUN NO. 1	TEMPERATURE 25°C RUN NO. 2	TEMPERATURE 35°C RUN NO. 1	TEMPERATURE 35°C RUN NO. 2	OBSERVATIONS
L-106	K 0.20145 r 0.97705	K 0.14215 r 0.99162	K 0.36246 r 0.99587	r 0.42646 r 0.99931	K25 0.1718 K35 0.39446
L-108	K 0.17591 r 0.97694	K 0.15784 r 0.99316	K 0.26708 r 0.99490	K 0.27203 r 0.98256	K25 0.16688 K35 0.26956
L-110	K 0.21792 r 0.95799	K 0.2056 r 0.95961	K 0.57867 r 0.98735	K 0.52616 r 0.97217	K25 0.21176 K35 0.55242
L-114	K 0.3469 r 0.92344	K 0.26816 r 0.91686	K 0.39910 r 0.83019	K 0.41968 r 0.91991	K25 0.30753 K35 0.40939
L-115	K 0.17099 r 0.98318	K 0.15935 r 0.88310	K 0.28638 r 0.95179	K 0.26314 r 0.98198	K25 0.16517 K35 0.27476
L-116	K 0.12117 r 0.96971	K 0.17357 r 0.93477	K 0.16811 r 0.97796	K 0.17600 r 0.86611	K25 0.14737 K35 0.18205
L-117	K 0.19571 r 0.97416	K 0.09526 r 0.98644	K 0.49648 r 0.98533	K 0.50313 r 0.98297	Repeat run 25°C
L-118	K 0.14382 r 0.95403	K 0.17266 r 0.98110	K 0.47278 r 0.99127	K 0.3866 r 0.92274	Repeat run 35°C
L-119	K 0.17236 r 0.85797	K 0.21295 r 0.97237	K 0.42884 r 0.97906	K 0.65521 r 0.98410	Repeat run 35°C
L-120	K 0.16525 r 0.95958	K 0.129206 r 0.99063	K 0.12167 r 0.98422	K 0.15455 r 0.98583	K25 0.14727 K35 0.13811
L-127	K 0.10606 r 0.95163	K 0.11341 r 0.93706	K 0.46701 r 0.95429	K 0.45387 r 0.97760	K25 0.10974 K35 0.46044
L-130	K 0.07645 r 0.97715	K 0.10848 r 0.99141	K 0.17340 r 0.99634	K 0.18491 r 0.99722	Flocculent yeast K25 0.09247 K35 0.17916
L-130	K 0.12891 r 0.96764	K 0.13869 r 0.92907	K 0.28033 r 0.98223	K 0.2868 r 0.9975	Flocculent yeast K25 0.13380 K35 0.28357
L-131	K 0.13234 r 0.9943	K 0.13229 r 0.99661	K 0.16608 r 0.97493	K 0.15867 r 0.96430	K25 0.13232 K35 0.16238
L-140	K 0.33872 r 0.97533	K 0.34866 r 0.97008	K 0.54999 r 0.98128	K 0.56157 r 0.9795	K25 0.34369 K35 0.55578
L-142	K 0.12499 r 0.97838	K 0.09637 r 0.93025	K 0.38754 r 0.91897	K 0.34709 r 0.94111	K25 0.11068 K35 0.36732
L-144	K 0.11755 r 0.98256	K 0.12164 r 0.99637	K 0.25903 r 0.96874	K 0.23513 r 0.98814	K25 0.11960 K35 0.24708
L-149	K 0.26314 r 0.97558	K 0.28106 r 0.99158	K 0.64519 r 0.9882	K 0.65922 r 0.99246	K25 0.2721 K35 0.65220
L-150	K 0.17103 r 0.96239	K 0.18327 r 0.93157	K 0.38276 r 0.89490	K 0.61132 r 0.73425	K25 0.19505 K35 0.32671
L-150	K 0.18085 r 0.993370	K 0.21125 r 0.99654	K 0.30852 r 0.89276	K 0.3449 r 0.84552	K25 0.16012 K35 0.31909
L-151	K 0.17607 r 0.9860	K 0.15197 r 0.93623	K 0.3035 r 0.95685	K 0.33465 r 0.98733	K25 0.16102 K35 0.31908
L-151	K 0.21294 r 0.96629	K 0.18001 r 0.98211	K 0.22601 r 0.9945	K 0.20643 r 0.94633	K25=K35 K25 0.19648 K35 0.21622
L-153	K 0.37051 r 0.98735	K 0.29678 r 0.9838	K 0.54503 r 0.99032	K 0.54252 r 0.99329	K25 0.33365 K35 0.54378
L-158	K 0.15447 r 0.97111	K 0.15851 r 0.98418	K 0.25407 r 0.97228	K 0.2377 r 0.9595	K25 0.15649 K35 0.24589
L-159	K 0.21383 r 0.95554	K 0.21868 r 0.94460	K 0.24293 r 0.85383	K 0.29442 r 0.92942	K25 0.21626 K35 0.26868

TABLE 69

AVERAGE VALUES FOR THE EXPONENTIAL CONSTANT (K) OF ETHANOL-ENHANCED PROTON INFLUX

(continued)

ICAITI CULTURE COLLECTION NUMBER	TEMPERATURE 25°C RUN NO. 1	TEMPERATURE 25°C RUN NO. 2	TEMPERATURE 35°C RUN NO. 1	TEMPERATURE 35°C RUN NO. 2	OBSERVATIONS
L-162	K 0.07637 r 0.63934	K 0.20442 r 0.07211	K 0.15959 r 0.86432	Non linear response	Flocculent yeast
L-162	K 0.09197 r 0.79950	K 0.28711 r 0.90154	K 0.30012 r 0.8228	K 0.2725 r 0.8176	Flocculent yeast
L-164	K 0.16033 r 0.97219	K 0.16687 r 0.98213	K 0.2245 r 0.95032	K 0.23765 r 0.94809	K25 0.1636 K35 0.25169
L-165	K 0.18001 r 0.98769	K 0.17346 r 0.97708	K 0.15213 r 0.94629	K 0.16291 r 0.8995	K25> K35 K25 0.1767 K35 0.15752
L-166	K 0.22275 r 0.98648	K 0.17912 r 0.99047	K 0.38436 r 0.93722	K 0.38947 r 0.99202	K25 0.20094 K35 0.35692
L-168	K 0.23157 r 0.98085	K 0.26740 r 0.93117	K 0.22350 r 0.99110	K 0.22163 r 0.99898	K25> K35 K25 0.24949 K35 0.22267
L-169	K 0.12659 r 0.99662	K 0.11503 r 0.99999	K 0.16113 r 0.98402	K 0.16929 r 0.99368	K25 0.12081 K35 0.16521
L-170	K 0.08044 r 0.96836	K 0.08873 r 0.99180	K 0.11085 r 0.92540	K 0.11514 r 0.94320	K25 0.08459 K35 0.11600
L-171	K 0.07551 r 0.92383	K 0.08050 r 0.97134	K 0.24997 r 0.97596	K 0.23516 r 0.97323	K25 0.08201 K35 0.24257
L-172	K 0.078002 r 0.97020	K 0.08621 r 0.97678	K 0.21712 r 0.95930	K 0.2088 r 0.96747	K25 0.08211 K35 0.21296
L-174	K 0.14308 r 0 97964	K 0.13889 r 0.99196	K 0.35627 r 0.9034	K 0.38175 r 0.85025	Flocculent yeast K25 0.14049 K35 0.35901
L-175	K 0.07072 r 0.97492	K 0.07236 r 0.96892	K 0.4140 r 0.86076	K 0.4271 r 0 89822	K25 0.07164 K35 0.42955
L-177	K 0.11432 r 0.96517	K 0.11604 r 0.92240	K 0.78346 r 0.93845	K 0.77261 r 0.97680	K25 0.11518 K35 0.77804
L-178	K 0.16439 r 0.98670	K 0.14798 r 0.98658	K 0.53572 r 0.89904	K 0.53160 r 0.88315	K25 0.15619 K35 0.52366
L-179	K 0.11923 r 0.98374	K 0.10274 r 0.97522	K 0.41390 r 0.88153	K 0.43290 r 0.86639	K25 0.11099 K35 0.4234
L-180	K 0.12906 r 0.98020	K 0.14887 r 0.97345	K 0.12599 r 0.88707	K 0.14164 r 0.89175	K25 0.13397 K35 0.13391
L-180	K 0.3041 r 0.9881	K 0.2649 r 0.9838	K 0.4777 r 0.8057	K 0.4227 r 0.9722	K25 0.28450 K35 0.4552
L-181	K 0.20384 r 0.99389	K 0.1503 r 0.9446	K 0.39445 r 0.99940	K 0.29452 r 0.98332	K25 0.17707 K35 0.34410
L-181	K 0.20570 r 0.97792	K 0.16105 r 0.96996	K 0.37784 r 0.99399	K 0.40591 r 0.9903	K25 0.16338 K35 0.39183
L-182	K 0.13816 r 0.96767	K 0.11992 r 0.97941	K 0.5568 r 0.9491	K 0.5479 r 0.94017	K25 0.12904 K35 0.55235
L-183	K 0.12819 r 0.99765	K 0.14387 r 0.94849	K 0.30203 r 0.90253	K 0.30947 r 0.86793	K25 0.13503 K35 0.30575
L-187	K 0.35236 r 0.99275	K 0.36467 r 0.99433	K 0.57344 r 0.99323	K 0.58856 r 0.99213	K25 0.35352 K35 0.58100
L-191	K 0.28120 r 0.97571	K 0.31076 r 0.96477	K 0.80631 r 0.99169	K 0.79710 r 0.99426	K25 0.29593 K35 0.80171
L-192	K 0.20546 r 0.99548	K 0.19902 r 0.97811	K 0.42094 r 0.98157	K 0.42689 r 0.96397	K25 0.20224 K35 0.42392
L-194	K 0.11678 r 0.96482	K 0.10692 r 0.95123	K 0.36439 r 0.93865	K 0.37023 r 0.93027	K25 0.11185 K35 0.36731

TABLE 69

AVERAGE VALUES FOR THE EXPONENTIAL CONSTANT (K) OF ETHANOL-ENHACED PROTON INFLUX

(continued)

ICAITI CULTURE COLLECTION NUMBER	TEMPERATURE 25°C RUN NO. 1	TEMPERATURE 25°C RUN NO. 2	TEMPERATURE 35°C RUN NO. 1	TEMPERATURE 35°C RUN NO. 2	OBSERVATIONS
L-196	K 0.15294 r 0.97000	K 0.13724 r 0.98731	K 0.47280 r 0.97526	K 0.5155 r 0.97462	K25 0.14509 K35 0.49415
L-198	K 0.18671 r 0.95930	K 0.22211 r 0.94402	K 0.53873 r 0.95574	K 0.55102 r 0.95528	K25 0.20441 K35 0.54488
L-201	K 0.19738 r 0.95316	K 0.18012 r 0.95746	K 0.45804 r 0.95773	K 0.48091 r 0.97634	K25 0.18875 K35 0.46948
L-202	K 0.19246 r 0.95281	K 0.16525 r 0.95352	K 0.67452 r 0.94728	K 0.58801 r 0.98533	K25 0.17886 K35 0.63127
L-203	K 0.40428 r 0.77778	K 0.40917 r 0.78934	K 0.75726 r 0.83957	K 0.79013 r 0.84755	K25 0.40673 K35 0.7737
L-206	K 0.51111 r 0.80275	K 0.47737 r 0.80036	K 0.74899 r 0.85504	K 0.75214 r 0.87485	K25 0.49424 K35 0.75057
L-207	K 0.35738 r 0.77430	K 0.3649 r 0.75604	K 0.8558 r 0.85458	K 0.8576 r 0.8653	K25 0.36114 K35 0.85670
L-208	K 0.43785 r 0.82964	K 0.41489 r 0.80869	K 0.80052 r 0.90998	K 0.85144 r 0.92717	K25 0.42637 K35 0.82598
L-209	K 0.6129 r 0.91327	K 0.59142 r 0.91765	K 0.85117 r 0.93984	K 0.84303 r 0.94684	K25 0.6022 K35 0.8471
L-211	K 0.39756 r 0.83864	K 0.42468 r 0.84714	K 0.60640 r 0.913360	K 0.61113 r 0.94899	Flocculent yeast K25 0.41112 K35 0.60877
L-213	K 0.12405 r 0.94473	K 0.1199 r 0.9919	K 0.26737 r 0.94136	K 0.28291 r 0.9754	K25 0.12199 K35 0.27514
L-216	K 0.16523 r 0.97892	K 0.15867 r 0.96599	K 0.40946 r 0.98351	K 0.38501 r 0.97085	K25 0.16195 K35 0.39724
L-217	K 0.16124 r 0.90701	L 0.1677 r 0.9786	K 0.43062 r 0.9738	K 0.39864 r 0.96340	K25 0.16447 K35 0.41463
L-218	K 0.26967 r 0.97581	L 0.2705 r 0.96387	K 0.73573 r 0.98877	K 0.74302 r 0.99321	K25 0.27009 K35 0.73938
L-222	K 0.22035 r 0.97024	L 0.21458 r 0.97785	L 0.60908 r 0.99123	K 0.62618 r 0.99068	K25 0.21747 K35 0.61763
L-226	K 0.2186 r 0.93148	K 0.08868 r 0.60853	K 0.65955 r 0.96455	K 0.72420 r 0.95463	Abundant growth
L-232	K 0.2472 r 0.72184	K 0.09211 r 0.73758	K 0.3782 r 0.98978	K 0.47785 r 0.97001	Abundant growth Flocculent yeast
L-240	K 0.12171 r 0.92125	K 0.13481 r 0.9788	K 0.45382 r 0.97636	K 0.5229 r 0.97768	K25 0.12826 K35 0.48836
L-243	K 0.2688 r 0.97704	K 0.27769 r 0.9928	K 0.60662 r 0.99056	K 0.60592 r 0.98505	K25 0.27325 K35 0.60627
L-244	K 0.1676 r 0.9955	K 0.16686 r 0.98311	K 0.63001 r 0.95505	K 0.62424 r 0.95805	K25 0.16723 K35 0.62713
L-245	K 0.2171 r 0.9596	K 0.24513 r 0.9566	K 0.36091 r 0.98061	K 0.3575 r 0.99321	Flocculent yeast K25 0.23112 K35 0.35921
L-247	K 0.2434 r 0.98132	K 0.2821 r 0.9631	K 0.6563 r 0.96773	K 0.63902 r 0.9685	K25 0.26275 K35 0.64766
L-248	K 0.2294 r 0.94037	K 0.2318 r 0.9819	K 0.37898 r 0.9859	K 0.42748 r 0.9896	K25 0.2306 K35 0.40323
L-250	K 0.10503 r 0.97162	K 0.1989 r 0.9602	K 0.13587 r 0.82432	K 0.16124 r 0.8959	K25 0.19237 K25>K35 K35 0.14856
L-251	K 0.3865 r 0.9432	K 0.4344 r 0.9297	K 0.3765 r 0.9866	K 0.3576 r 0.98316	K25 0.4105 K25>K35 K35 0.36705

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TABLE 69

AVERAGE VALUES FOR THE EXPONENTIAL CONSTANT (K) OF ETHANOL-ENHACED PROTON INFLUX
(continued)

ICAITI CULTURE COLLECTION NUMBER	TEMPERATURE 25°C RUN NO. 1	TEMPERATURE 25°C RUN NO. 2	TEMPERATURE 35°C RUN NO. 1	TEMPERATURE 35°C RUN NO. 2		OBSERVATIONS
L-252	K 0.35170 r 0.99131	K 0.3764 r 0.9899	K 0.43499 r 0.96910	K 0.4908 r 0.98705		K25 0.36405 K35 0.46290
L-255	K 0.30166 r 0.9894	K 0.3043 r 0.9633	K 0.33231 r 0.9445	K 0.3232 r 0.93624		K25 0.30298 K25=K K35 0.32776
L-256	K 0.3905 r 0.9738	K 0.44902 r 0.9726	K 0.40617 r 0.9874	K 0.40432 r 0.9754		Yeest goes to bot
L-260	K 0.1653 r 0.9755	K 0.1390 r 0.9755	K 0.4297 r 0.9831	K 0.4320 r 0.9766		K25 0.15215 K35 0.43099
L-261	K 0.2589 r 0.9746	K 0.2901 r 0.9894	Non linear response	Non linear response		K25 0.2745
L-274	Non linear response	Non linear response	K 0.16522 r 0.90268	K 0.2041 r 0.86742		K35 0.18466
L-278	Non linear response	Non linear response	K 0.5035 r 0.9564	K 0.5147 r 0.9798		K35 0.5091
L-279	K 0.1620 r 0.9776	K 0.1907 r 0.9774	K 0.4521 r 0.9907	K 0.4456 r 0.9859	Osmofilic yeast	K25 0.1764 K35 0.4489
L-280	K 0.2441 r 0.9940	K 0.2620 r 0.9931	K 0.3145 r 0.8881	K 0.3068 r 0.9224		K25 0.25305 K35 0.31065
L-282	K 0.2557 r 0.8030	K 0.2397 r 0.8052	K 0.6047 r 0.98827	K 0.5176 r 0.99694		K25 0.2477 K35 0.56115
L-283	K 0.2961 r 0.93157	K 0.3051 r 0.95462	K 0.49314 r 0.98740	K 0.5045 r 0.9945		K25 0.3006 K35 0.4988
L-284	K 0.12967 r 0.8088	K 0.0892 r 0.71801	K 0.2811 r 0.9838	K 0.2736 r 0.98420	Abundant growth	K25 0.1094 K35 0.27735
L-285	K 0.19235 r 0.9306	K 0.1573 r 0.8533	K 0.4464 r 0.9873	K 0.4470 r 0.99202	Osmofilic yeast	K25 0.1748 K35 0.4467
L-286	K 0.1052 r 0.6726	K 0.05589 r 0.45498	K 0.3382 r 0.79795	K 0.3720 r 0.84315	Abundant growth	K25 0.08053 K35 0.3551
L-287	K 0.2492 r 0.9931	K 0.2508 r 0.9894	K 0.5757 r 0.9682	K 0.5773 r 0.9637	Abundant growth	K25 0.2500 K35 0.5765
L-288	K 0.1092 r 0.9918	K 0.1288 r 0.9740	K 0.3525 r 0.9986	K 0.3092 r 0.9909	Osmofilic yeast Abundant growth	K25 0.11900 K35 0.33085
L-289	K 0.0840 r 0.9069	K 0.0814 r 0.8529	K 0.3050 r 0.9825	K 0.3149 r 0.9668	Osmofilic yeast	K25 0.0827 K35 0.30995
L-290	K 0.1027 r 0.9920	K 0.1028 r 0.8730	K 0.2869 r 0.9900	K 0.2917 r 0.9894	Osmofilic yeast	K25 0.10275 K35 0.2893
L-291	K 0.1605 r 0.9039	K 0.1760 r 0.8989	K 0.3680 r 0.9960	K 0.3599 r 0.9908		K25 0.16825 K35 0.36395
L-293	K 0.1142 r 0.9705	K 0.1316 r 0.9285	K 0.3320 r 0.9887	K 0.3484 r 0.9962		K25 0.1229 K35 0.3402
L-298	Non linear response	Non linear response	Non linear response	Non linear response		
L-299	Non linear response	Non linear response	Non linear response	Non linear response		
L-300	Non linear response	Non linear response	Non linear response	Non linear response		
L-301	K 0.1636 r 0.9757	K 0.1809 r 0.9366	K 0.5476 r 0.9810	K 0.5665 r 0.9797	Floculent yeast	K25 0.1723 K35 0.5571
L-302	K 0.2819 r 0.9663	K 0.2343 r 0.9865	K 0.5747 r 0.9813	K 0.5428 r 0.9616		K25 0.2581 K35 0.5586
L-303	K 0.1571 r 0.9098	K 0.13990 r 0.9279	K 0.3641 r 0.9829	K 0.3585 r 0.9855		K25 0.1481 K35 0.3613

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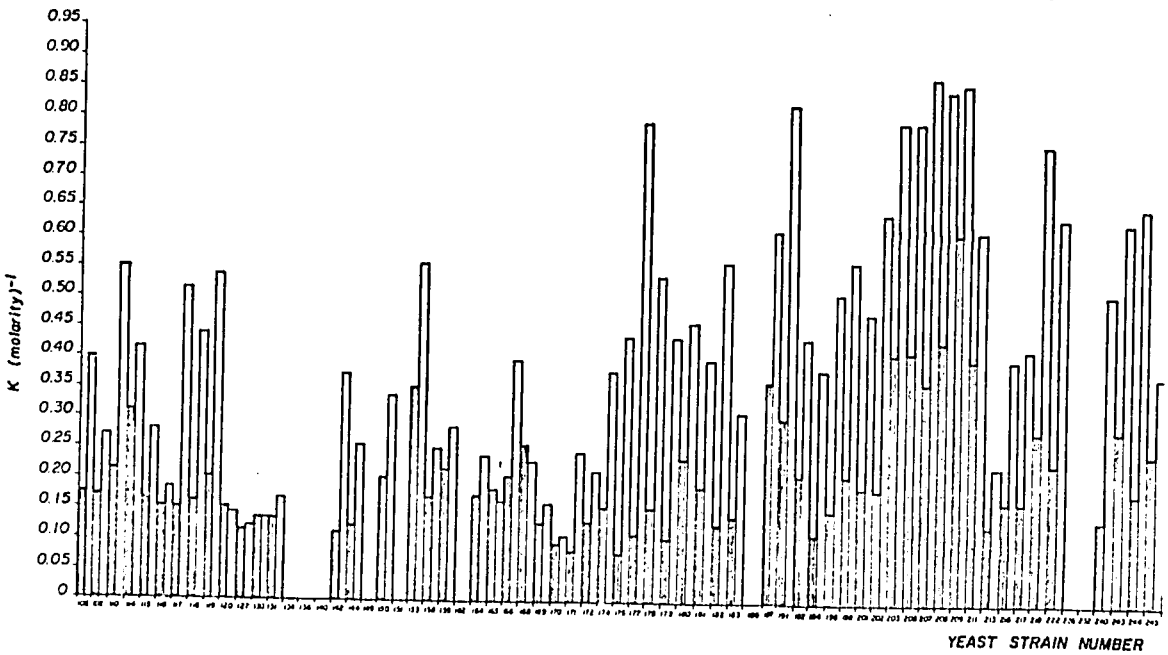
TABLE 69

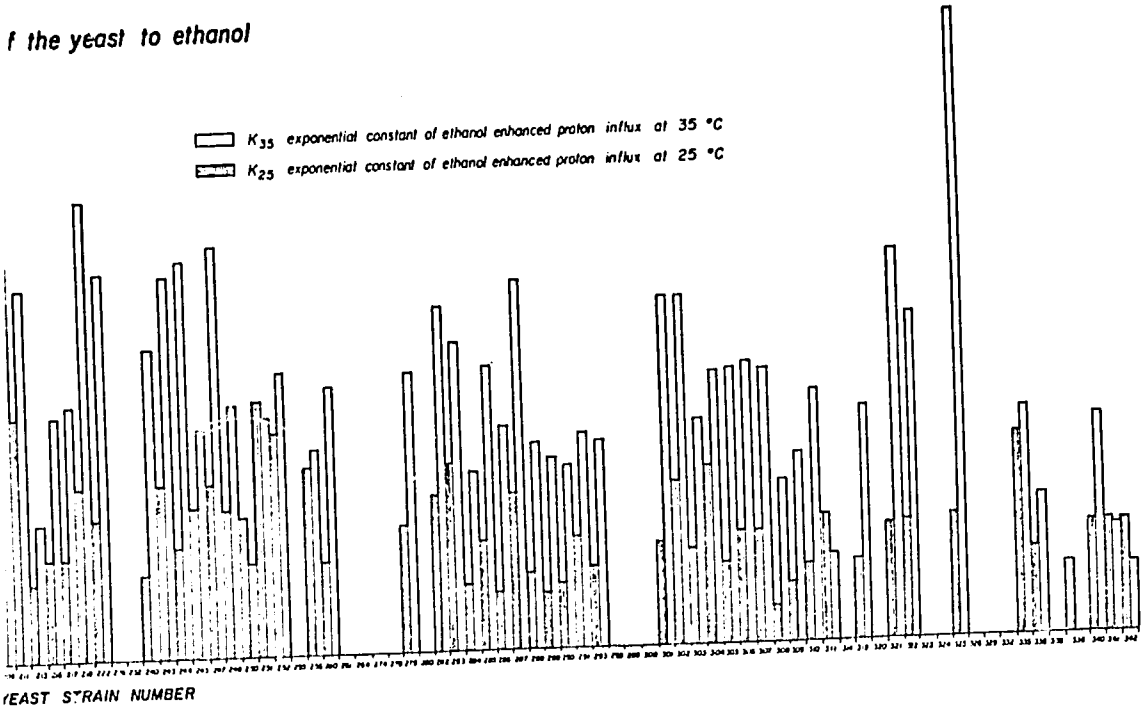
AVERAGE VALUES FOR THE EXPONENTIAL CONSTANT (K) OF ETHANOL-INDUCED PROTON INFLUX

(continued)

ICAITI CULTURE COLLECTION NUMBER	TEMPERATURE 25°C RUN NO. 1	TEMPERATURE 25°C RUN NO. 2	TEMPERATURE 35°C RUN NO. 1	TEMPERATURE 35°C RUN NO. 2		OBSERVATIONS
L-304	K 0.2704 r 0.9589	K 0.2969 r 0.9704	K 0.4313 r 0.9744	K 0.4489 r 0.9807	Osmofilic yeast	K_{25} 0.2836 K_{35} 0.4401
L-305	K 0.1415 r 0.9396	K 0.1275 r 0.9378	K 0.3715 r 0.9987	K 0.3715 r 0.9960	Osmofilic yeast	K_{25} 0.1345 K_{35} 0.3715
L-306	K 0.1661 r 0.9643	K 0.1767 r 0.9492	K 0.4548 r 0.9630	K 0.4474 r 0.9564	Osmofilic yeast	K_{25} 0.1714 K_{35} 0.4511
L-307	K 0.1815 r 0.9975	K 0.1955 r 0.9986	K 0.4469 r 0.9972	K 0.4380 r 0.9910	Osmofilic yeast	K_{25} 0.1885 K_{35} 0.4425
L-308	K 0.0494 r 0.8503	K 0.065 r 0.9147	K 0.2575 r 0.9435	K 0.2721 r 0.9496	Osmofilic yeast	K_{25} 0.0572 K_{35} 0.2648
L-309	K 0.0880 r 0.8897	K 0.0978 r 0.9470	K 0.3050 r 0.9883	K 0.3108 r 0.9683	Osmofilic yeast	K_{25} 0.0929 K_{35} 0.3079
L-310	K 0.1135 r 0.9610	K 0.1339 r 0.9399	K 0.3855 r 0.9613	K 0.4084 r 0.9822	Osmofilic yeast	K_{25} 0.1237 K_{35} 0.3970
L-311	K 0.1997 r 0.9953	K 0.2069 r 0.9842	K 0.1364 r 0.9754	K 0.1471 r 0.9780	Osmofilic yeast	K_{25} 0.2083 K_{35} 0.1418
L-314	Non linear response	Non linear response	Non linear response	Non linear response		
L-319	K 0.1250 r 0.9916	K 0.1398 r 0.9557	K 0.3857 r 0.9537	K 0.3659 r 0.9531		K_{25} 0.1324 K_{35} 0.3758
L-321	K 0.1867 r 0.9632	K 0.1874 r 0.9091	K 0.6464 r 0.9069	K 0.6104 r 0.9255		K_{25} 0.1871 K_{35} 0.6284
L-322	K 0.1956 r 0.9683	K 0.1801 r 0.8677	K 0.5242 r 0.9100	K 0.5206 r 0.9221		K_{25} 0.1879 K_{35} 0.5224
L-323	Non linear response	Non linear response	Non linear response	Non linear response		
L-325	K 0.2110 r 0.9683	K 0.1872 r 0.7988	K 1.0102 r 0.9828	K 1.0717 r 0.9577		K_{25} 0.1991 K_{35} 1.0410
L-329	K 0.4034 r 0.9425	K 0.3327 r 0.7988	Non linear response	Non linear response	Abundant growth	K_{25} 0.36805
L-332	Non linear response	Non linear response	K 0.2959 r 0.8493	K 0.1991 r 0.9385		K_{35} 0.2475
L-335	K 0.3633 r 0.9671	K 0.2943 r 0.9357	K 0.3301 r 0.9807	K 0.3926 r 0.9595		K_{25} 0.3288 K_{35} 0.3614
L-336	K 0.1523 r 0.9326	K 0.1358 r 0.8481	K 0.2163 r 0.9660	K 0.2295 r 0.8311		K_{25} 0.1441 K_{35} 0.2229
L-338	Non linear response	Non linear response	Non linear response	Non linear response		
L-339	K 0.1102 r 0.9191	K 0.1620 r 0.9191	Non linear response	Non linear response		K_{25} 0.1361
L-340	K 0.1914 r 0.9841	K 0.2319 r 0.9494	K 0.4053 r 0.9690	K 0.3043 r 0.8855	Bakers yeast	K_{25} 0.2117 K_{35} 0.3548
L-341	K 0.2039 r 0.9588	K 0.2155 r 0.9604	K 0.2228 r 0.9304	K 0.1641 r 0.9146	$K_{25} = K_{35}$	K_{25} 0.2097 K_{35} 0.1935
L-342	K 0.2155 r 0.9421	K 0.2172 r 0.9338	K 0.1290 r 0.9392	K 0.1243 r 0.8688	$K_{35} > K_{25}$°C	K_{25} 0.2164 K_{35} 0.1267

FIGURE 2. Tolerance of the yeast to e:




f the yeast to ethanol
K_{35} exponential constant of ethanol enhanced proton influx at 35 °C
K_{25} exponential constant of ethanol enhanced proton influx at 25 °C
YEAST STRAIN NUMBER

2.5 Dextran prodution kinetics

Since the production of dextran by contaminating microorganisms has been the principal problem with contamination in this project, the consultant, Dr. Arthur Humphrey, suggested that the kinetics of dextran production by the contaminants be studied taking into account the effect of temperature, pH, and ethanol in the fermentation medium.

To carry out this activity, dextran-producing microorganisms were isolated from the sugar cane from Ingenio Pantaleon. The cultures were purified and dextran production was studied first on agar plates containing Drew medium, a sucrose-rich medium designed for detecting **Leuconostoc mesenteroides** in the sugar mills. Those cultures which produced the most mucoid colonies were tested for dextran production at the shake flask level in the laboratory. At present, continuous culture experiments are being carried out to define the effect of pH, ethanol, and temperature on the kinetics of dextran production.

3. EQUIPMENT DESIGN

One of the main purposes of this project is to study the problems involved in materials handling during the fermentation of the ground sugar cane. This can not be done on the bench scale in the laboratory, and so we have proceded to take the process to the pilot plant level. Figure 3 shows a flow diagram for the process which we hope to try. And, in the following section, we will be describing the technical characteristics for each of the components which are being developed as part of this project.

The central unit operation of the flow diagram is the fermentation process which will be carried out in a semi-continuous fermentor designed in the Institute. A model is presented in this section. Additionally, the cane grinder or mill will be presented, as will the inoculum and make water preparation, and the process for steam stripping of the bagasse. The pressing will be done in a Vincent screw press with a maximum capacity of 1 metric ton per hour. Distillation itself will not be studied as part of this project.

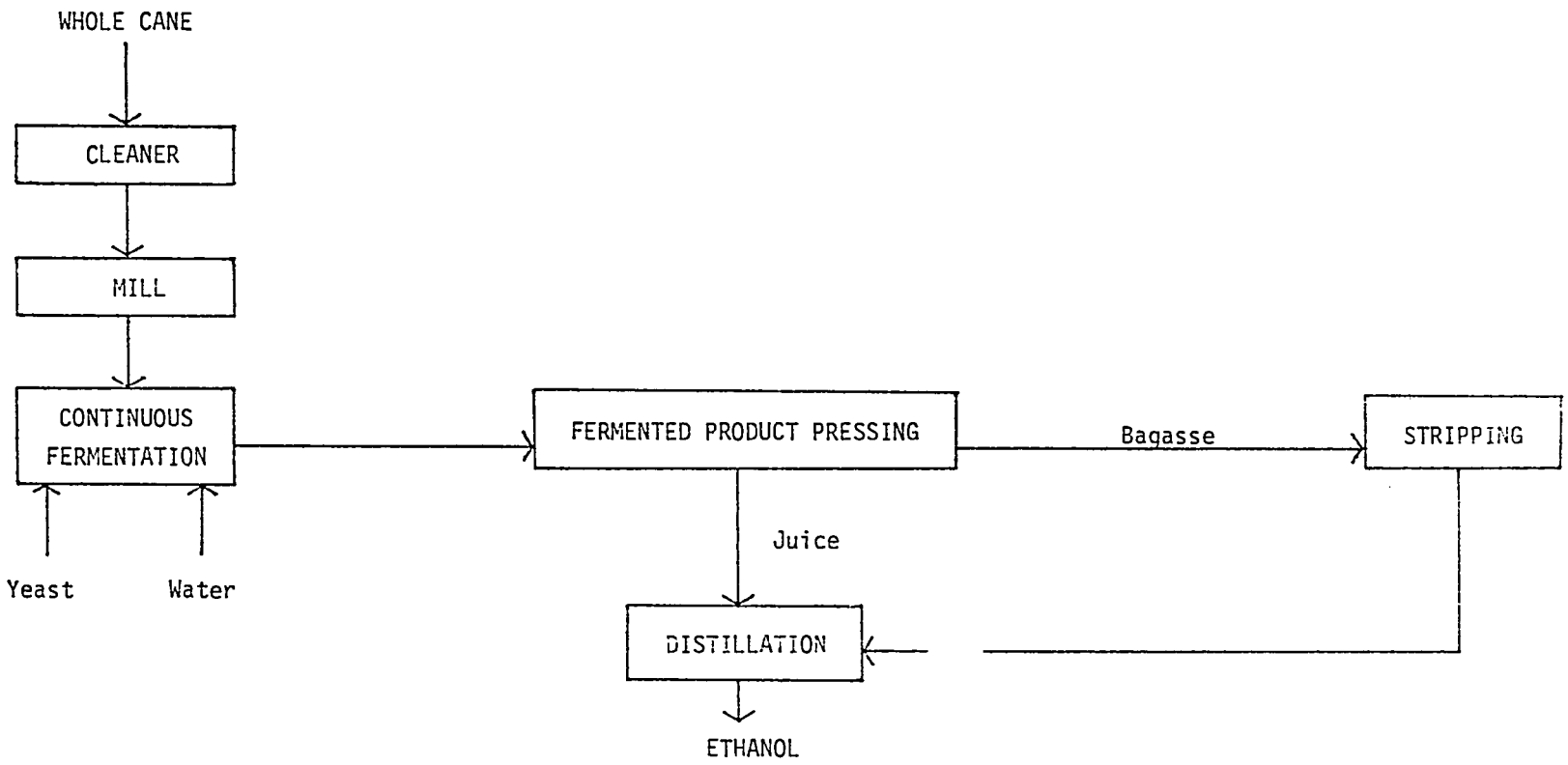
Description of the equipment to be used:

3.1 Grinding of fresh cane.

In order to work with ground cane at the laboratory level during the 1986-1987 harvest season, a cane mill was designed, modifying the experimental unit in the laboratoy at the Pantaleon mill. Figure 4 shows various views of the mill. Here one can see the feeding trough made from galvanized metal, and which can be moved from a horizontal to a 45 degree angle. The cane is introduced manually, one or several canes at a time, according to the thickness of the cane being studied. The trough leads to a set of 10 parallel sawdisks, each 10 inches in diameter which spin at 3450 rpm. The blades are placed on the axel in such a way so that as they spin, they move to cover the entire space. The separation between the blades is 1 cm, and this combined with the movement back and forth results in a completely shredded material which resembles sawdust.

The blades are powered by an industrial 2 hp Baldor motor which works at 1725 rpm, 6.5 amperes, and 208 volts. The portion of the blades exposed above the work table is covered by a movable shield to protect the operator and to permit a thorough cleaning of the mill. This shield will be fitted with a system for injecting liquids so that the inoculum and added water can be mixed with the cane at this point. The movement of the blades permits a complete dispersion of the liquid with the ground material, which provides a good mixture of liquids with solids.

FIGURE 3. PROPOSED PILOT PLANT SUGAR CANE FERMENTATION PROCESS



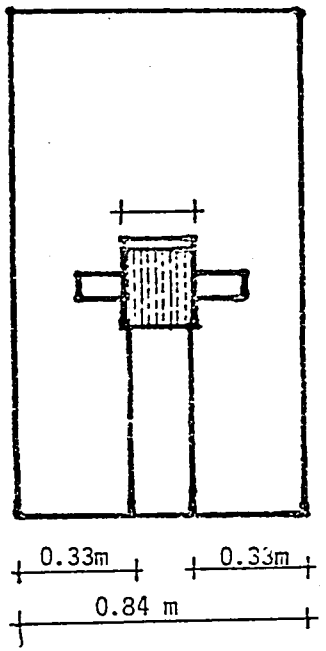


FIGURE 4a

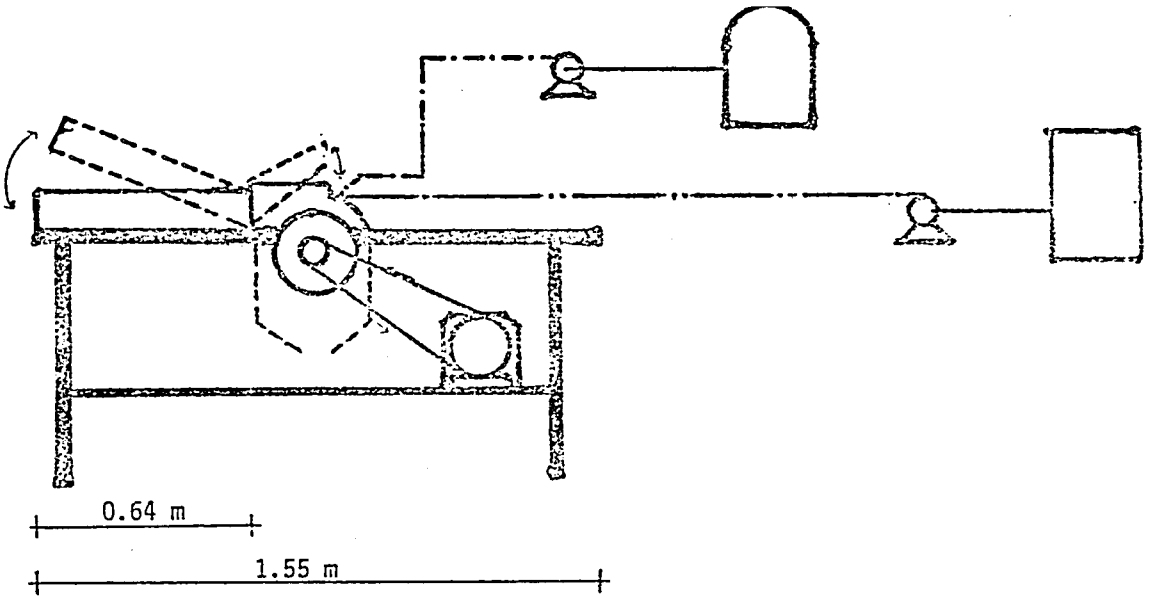


FIGURE 4b

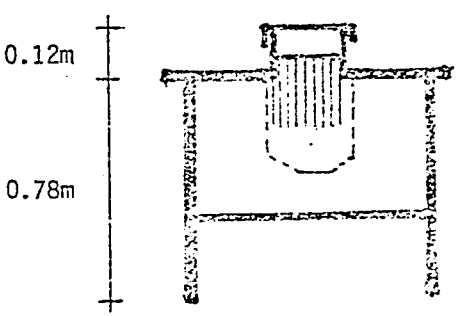


FIGURE 4c

FIGURE 4. FRESH SUGAR CANE MILL.

The ground material is expelled through a hopper found in the lower part of the mill at a rate of 2 kg/min. The density of the ground material is 456.3 kg/m³. Energy consumed is 49713 Joules/kg ground cane or 13.80 kw-h/t cane. One worker is needed to operate the mill.

3.2 Prepation of inoculum and process water.

A Saccharomyces cerevisiae culture, L-181, will be used as inoculum. It will be added to the ground cane together with the process water through the protective shield of the mill at the time the cane is ground. The liquids will be injected with variable flow pumps so that the proportion of each of the liquids can be changed in relation to the material which will be fermented. This is shown in Figure 4 b in more detail.

3.3 Fermentation unit

Due to the need to handle solids during the process and the different levels of water which must be tested, the fermentor should be provided with a means for mixing the contents. This mixing should be variable either in terms of speed or periodic use, so that the movement caused by the mixer governs the residence time of the material inside the fermentor without altering its dimensions. Also, we should be able to use the fermenter for batch fermentations.

Feeding and discharge should be simultaneous and proportional so that the volume being fermented is kept constant. The activity and rest periods of the system are synchronized with the mixing system of the fermenter, providing the fermentation system with the variable parameters which will determine the optimal residence time.

In order to follow the fermentation closely, the fermenter must be provided with sampling ports.

The temperature inside the fermenter should be kept in the range of 30-35 C, so a temperature control system will be needed.

3.3.1 Fermenter capacity

In order to obtain representative data at the pilot plant level, the working volume of the fermenter must be sufficiently large and controlable by simple methods. Additionally, it must be of such a configuration that scale up is possible, or at least, that reliable data can be obtained for scale up to a similar unit.

Given these requirements, we propose that the fermenter have a working volume of 1 m^3, which would correspond to a scale up of approximately 1:1000 when compared to the scale used in the laboratory. The working volume should be kept constant during the fermentation cycle. This means that the feed and harvest of the fermenter must be carried out proportionately, at a rate of 50 kg/h for example. The capacity of the cane mill is aproximately 2 kg/min, which means that to work at the rate described above, the mill would have to operate 25 min/h.

3.3.2 Construction materials

Due to the nature of the material which will be fermented and the conditions inside the fermenter during fermentation (30 - 35 C, pH 3.5), we propose that the fermenter be constructed from cast iron with a protective coating of epoxy resin in the interior to control corrosion. The rest of the fermentor which does not come into direct contact with the fermenting cane can be built without the coating or, if the strength required so permits, of galvanized metal.

3.3.3 Geometric shape and dimensions

The physical characteristics of the material to be fermented and the scale at which we will be working require equipment of simple design, easy to control, and accessible for thorough cleaning and disinfection. We have suggested that the fermenter have a cylindrical shape with the following dimensions:

Height	3.00 m
Diameter	1.50 m
Volume	5.30 m.

In order to provide several stages for fermentation and accessibility for control, the interior of the fermenter will be provided with trays so that the cane can fall periodically from one stage to another. Figure 5 shows a diagram of this fermenter, and Table 70 gives a series of the dimensions relative to the fermenter and its trays.

3.3.4 General characteristics

The fermenter will be placed vertically in order to take advantage of the force of gravity for the falling cane. In the inside of the fermenter, there are a series of trays. The fermenting material will fall from tray to tray as the fermentation cycle progresses. The solids will pass from one tray to the next lower tray through a free space in the tray, a discharge slot. The material is pushed towards the slot with a rotary arm. Another arm set at a certain distance above the tray will spread the cane evenly over the surface, and also provide certain mixing at each stage. The periods of movement, and/or the velocity of the arms will be synchronized to provide the necessary residence time in the fermenter.

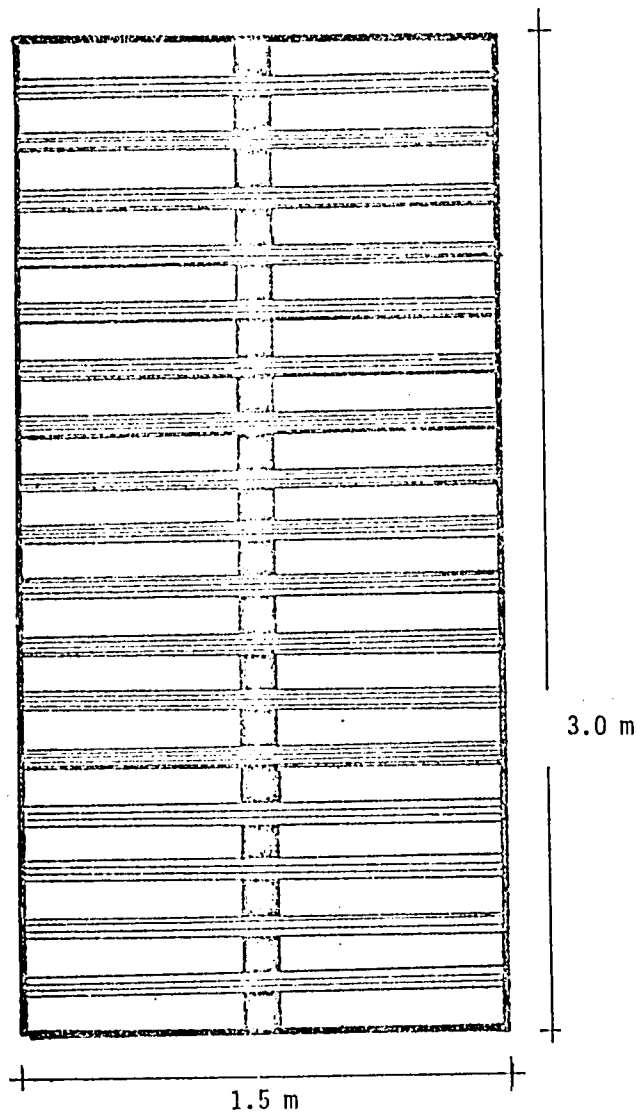


FIGURE 5. PROPOSED FERMENTOR DESIGN

Table 70: General dimensions of the fermenter

Height	3.00 m
Diameter	1.50 m
Volume	5.30 m^3
Total working volume	1.00 m^3
Working volume per tray	0.1414 m^3
Height of each tray	0.08 m
Weight per tray	143.38 kg
Number of trays	8
Space between trays	0.30 m

The trays are arranged in such a way that the slots do not coincide with those in the tray that is immediately above or below. Rather, they are located so that the cane makes a complete round on each tray before falling to the next tray. Figures 6 and 7 show details of the design.

Since mixing or movement of the fermenting cane is a parameter which must be studied further, consideration has been given to the design of the rotating arms. One set will spread the cane over the trays and another set will scrap the ground cane and push it to the opening in the tray so that it can fall to the next stage. An alternative is only one set of arms which make one round spreading out the cane and then lower to the surface of the tray to push the cane to the next level. Please see Figure 8 for details.

Equipment similar to the fermenter proposed here have been used previously by industry for gentle drying of labile biochemicals, extraction of antibiotics, adsorptions, ion interchange, and so forth. The diameters of the towers can vary from 0.30 to 2.25 m, and the internal plates or trays are at distances no greater than 30 cm from each other.

3.3.5 System for feeding and harvest

The feeding of the fermenter will occur directly from the cane mill to the feed hopper which will dose the material to enter through a hermetic port located in the upper part of the fermenter. The harvest will occur in the lower part of the fermenter, using a receiving hopper or a screw which will remove the material from the inside of the fermenter.

Sampling and temperature control is relatively easy given the distribution of the material inside the fermenter on the several trays. According to the height of the tray, the residence time of the cane can be calculated. The sampling can be carried out through control ports or windows which are placed in the wall of the fermenter.

3.4 Pressing of fermented material

The pilot plant at ICAITI has a Vincent press with a 2 hp motor and a working capacity compatible with the process described here. The press will be sealed as much as possible to avoid loss of ethanol through evaporation. The fermented material will be received from the harvest hopper of the fermenter in a closed container on wheels, and then transported to the press. The press liquor will be available for distillation, and the bagasse or press cake will be received in containers for the stripping process to remove residual ethanol. Samples of the liquor, the press cake and the spent bagasse will be analyzed for ethanol, residual sugar, yeast biomass, and other pertinent tests.

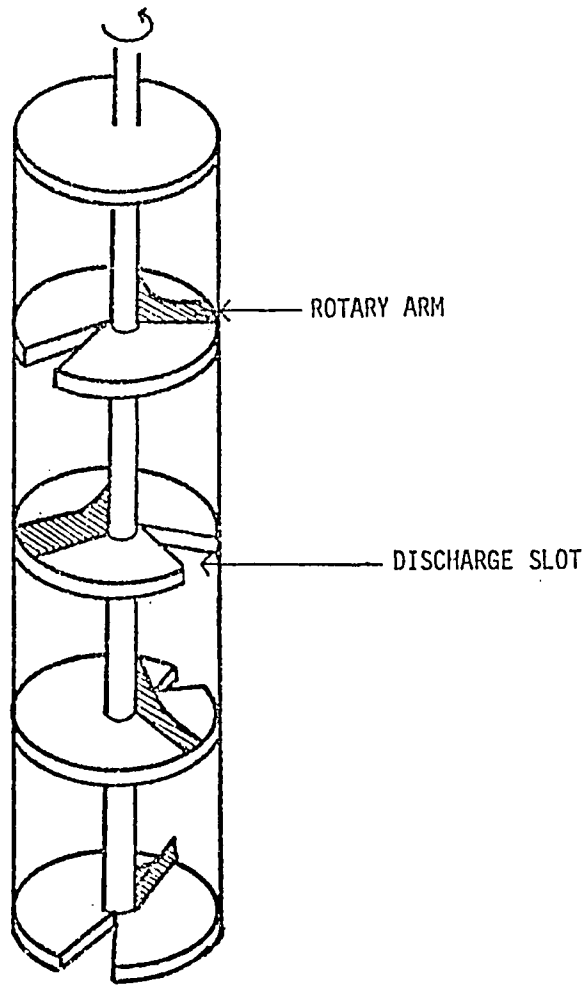


FIGURE 6. DISTRIBUTION OF TRAYS INSIDE FERMENTOR

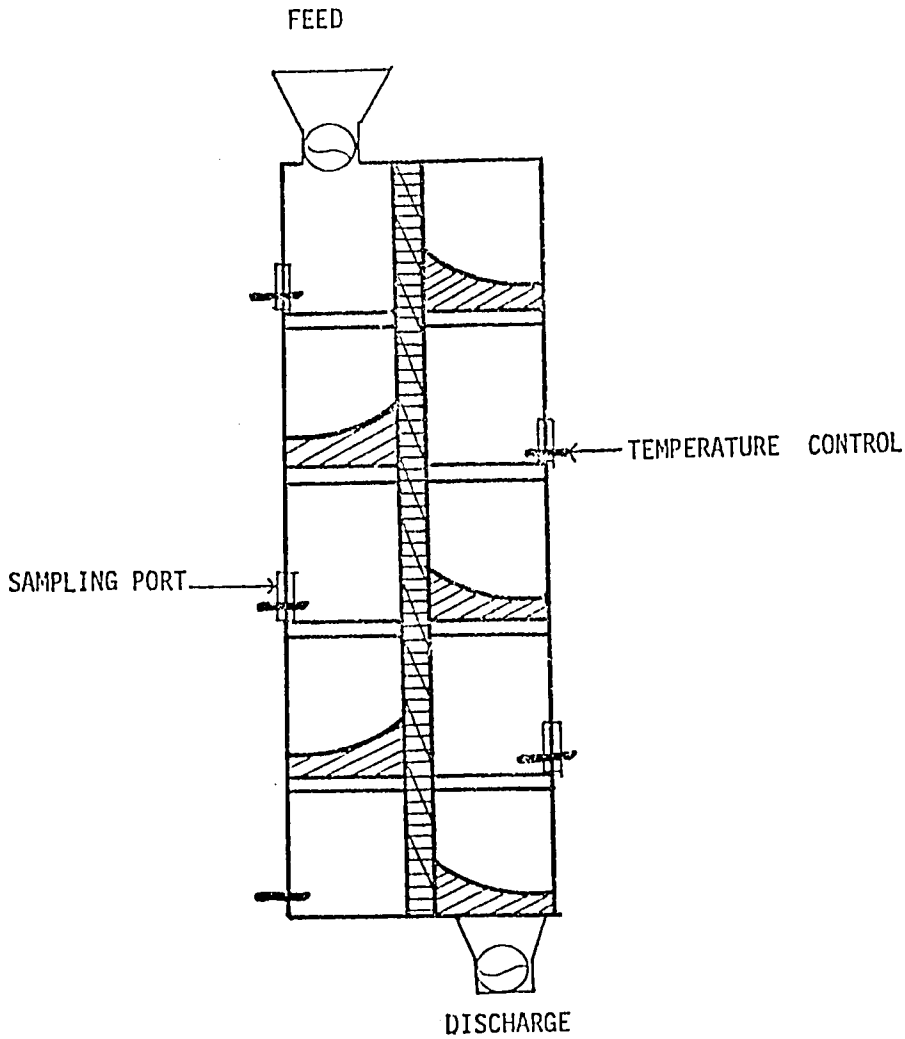


FIGURE 7. FRONTAL VIEW OF FERMENTOR

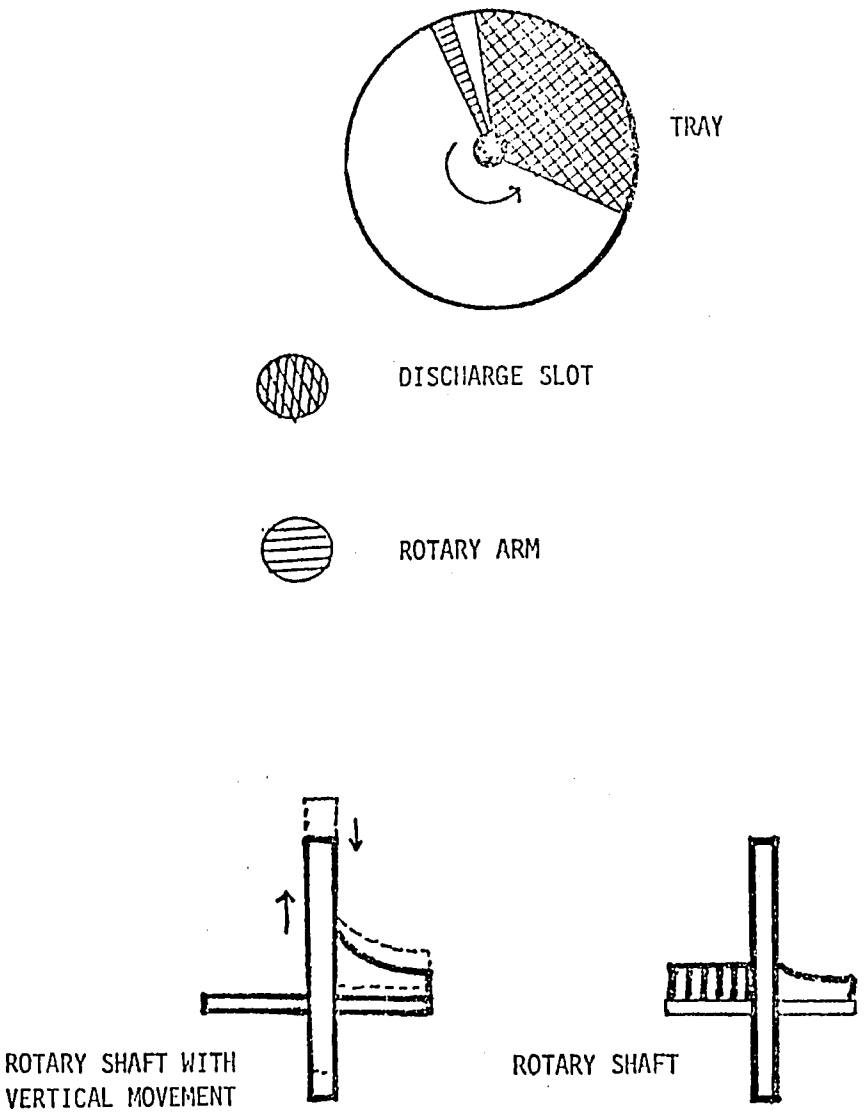


FIGURE 8. TRAY DETAIL AND MIXING MECHANISMS.

3.5 Stripping of bagasse

A container similar to those used for receiving the fermented cane will be used to collect the press cake. Coils for steam will be found in the bottom of the container. The lid will be provided with a connection to the distillation column. The spent bagasse, after stripping, will be discarded.

Figure 9 shows a diagram of the sugar cane fermentation equipment: mill, fermenter and press.

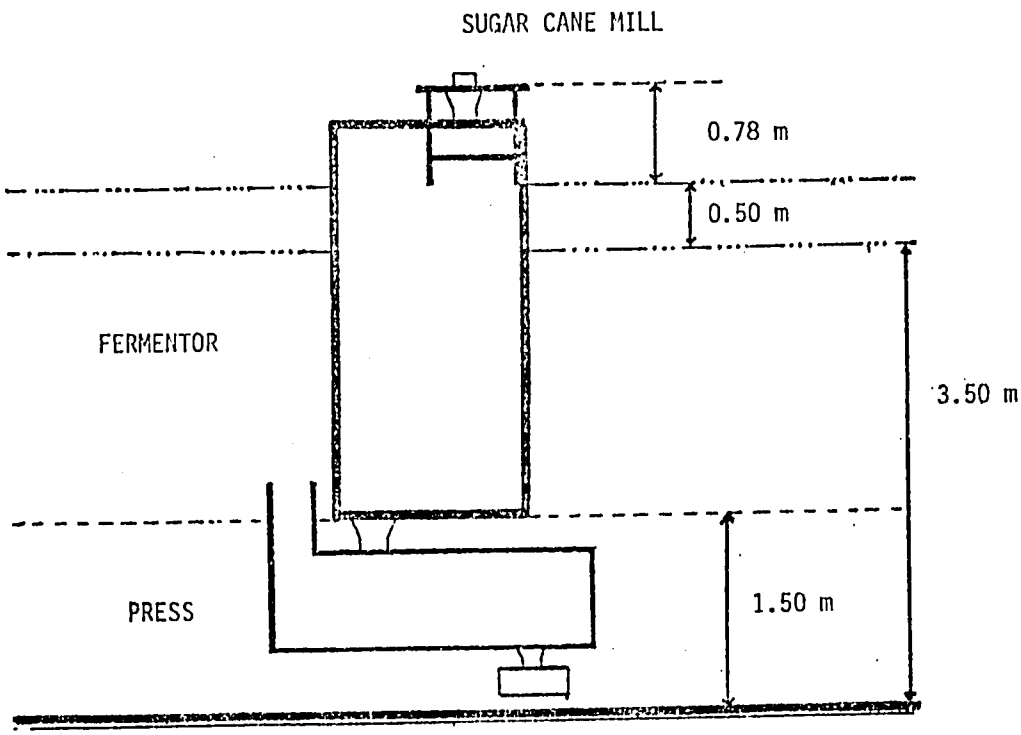


FIGURE 9. DIAGRAM OF SUGAR CANE FERMENTATION EQUIPMENT.

4. CONSULTANT'S REPORT

SUMMARY REPORT

OF

A TECHNICAL REVIEW

by

Arthur E. Humphrey
Director, Biotechnology Research Center
Lehigh University
Bethlehem, PA 18015 USA
February 23, 1987

OF

AN AID SUPPORTED PROJECT AT ICAITI
ENTITLED

"Innovative Scientific Research (Low Moisture Processing of Solid State Bagasse)"

On February 16 and 17, 1987 the author visited ICAITI and met with various researchers for the purpose of reviewing and offering scientific advice on the US Aid supported project on "Low Moisture Processing of Solid State Bagasse."

The aim of the Project as described is to study and improve the processing of chip or fragmented cane by yeast for the production of alcohol using a minimum amount of water. The research is to specifically address (1) the handling of moist solids in a way to achieve a homogeneous system; (2) identifying and enhancing the rate limiting step, believed to be either sucrose diffusion from the interstices of the cane to the surface fluid or the penetration of yeast into the cane matrix and (3) avoiding bacterial contamination that could decrease ethanol yields.

Process Objectives

Reasonable process objectives, within the constraints of the project aims, are to achieve a 5-6% alcohol concentration in the cane juice within a 12-18 hour batch processing cycle and to do this with a greater than 80% conversion of fermentable sugars. The attainment of these process objectives should result in the chance of attaining an economically viable process.

Results/Observation

The following results/observation were reported to me during my visit.

1. Sawdust, when compared to cane chips, gives a much higher alcohol production rate. Increases in the fermentation rates are linear with time, strongly suggesting a diffusional process rather than a biological process as the rate limiting step. This is somewhat surprising in view of the fine character of the sawdust. An understanding of this phenomenon will be important to bioreactor design considerations.

2. Addition of water to the sawdust (50% and 100% by weight of the ground sawdust) produces significantly faster fermentation rates than the ground sawdust alone.

3. Higher yeast innoculant (10^9 yeast cells/g of cane sawdust) produces higher overall fermentation.

4. Only 65% of the theoretical sugar conversion (based on 55% conversion of fermentable sugar to alcohol) is achieved within 24 hours with the best conditions to date.

5. Cane juice assays 20% fermentable sugars. This can vary among different cane sources by as much as $\pm 2\%$. A rough (approximate) analysis of the cane sawdust is 72% moisture, 13% fiber and 15% soluble solids (sugars).

Recommendations

On the basis of these observations and as a result of my discussions with the various researchers at ICAITI (in particular with Sheryl de Cabrera and Silvia Saravia) I would offer the following comments and recommendations.

1. I strongly urge that the project be extended through a 3rd cane season. Unfortunately the project funding and initiation was begun just after the sugar cane season ended in 1984-1985. Consequently, only two seasons of cane have been tested. By the end of this present season (the 2nd one during this project contract) sufficient basic knowledge will exist to suggest several potential pilot scale reactor designs. However, proof of concept (using cane from a 3rd season) is needed in order to have the necessary data for an economic evaluation of the project.

2. The reasons for the linear increase in fermentation rates with juice must be established. It appears likely that the rate limiting step is diffusional, i.e., extraction of sugar from within the interstices of the sawdust. For this reason water addition and bulk mixing could be important both to yields and rates. Suggestions were given as to how this might be done. (I would be delighted to review the results if sent to me for comment when they are obtained.)

3. 125% water addition appears to be an upper limit if the objective of 5-6% alcohol containing juice is to be obtained. This level of water addition should be studied as an "upper limit" experiment.

4. It appears that the sawdust cannot absorb more than 100% water in addition to the moisture that occurs naturally in the cane. This means that with the higher solution additions, e.g. 125%, there should be some free juice available for recirculation and mixing.

5. It is important to inoculate the cane sawdust with yeast as soom as possible after the grinding process. It is believed that this will increase the overall fermentation rate and minimize growth of competing micorflora and, in some instances, the dextran formation.

6. Use of bisulfite does not enhance the reaction rate. Consequently, I feel that studies on optimal size of inoculum should have priority over those involving use of biocides.

7. In order to formulate a reactor design, a comparison test should be run at 100% and 125% water addition. In one set of runs there should be no solids mixing other than that which occurs initially. In another set of runs these should occur every ½ hour mixing of the solids and free solution by kneading the plastic bag containing the fermenting mass.

8. Tests should be undertaken to see if the juice from pressed spent cane is a viable source of yeast inoculum. This could impinge on the reactor design concept.

9. Included in future tests should be the use of hot water additions (37-45°C) as a way to inhibit bacterial infection in the juice and cane as well as a way to reduce the lag time of the fermentation.

10. Three reactor designs appear feasible
    a.) rotating drum
    b.) screw
    c.) packed bed with free solution recycle.

11. A fluidized bed is not a reasonable reactor design concept because of compacting of the moist solids and drying which will lower fermentation rates.

12. Although the scope of the project is limited to handling moist solids (cane) including ensiled and steam exploded cane (both of which do not appear very promising), ultimately a question will be raised about the possibility of extended processing through utilization of storable cane. Some thought should be given to ways of handling cane other than ensiled, exploded, or freshly harvested cane. One should determine how dry (<10%?) cane must be in order to store without detrimental changes. Also, are there economical alternatives to drying?

13. investigations should be continuing on characterizing cane (sugar) deterioration on storage including measuring the microflora, dextran, etc. content of cane on handling and storage.

14. Studies should be initiated to determine the effect of alcohol concentration on inhibiting dextran formation and bacterial (L. mesintroides) growth on cane as a means to understanding and controlling this problem.

15. An obvious off-shoot to this project would be a new study on converting the spent cane solids to paper pulp. Such a project might include a study of

a.) mechanical/microbial/enzyme depithing
b.) organo-solvent delignification

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5. VISITS

5. Visits

5.1 Visit to the United States

In March of 1987, Sheryl de Cabrera was invited by the Microbiology Department of the University of South Dakota at Brookings to present a seminar on the studies on the alcoholic fermentation of sugar cane carried out at ICAITI, the present project being part of these activities. The visit to Brookings gave her an opportunity to visit with Dr. Carl Westby and Bill Gibbons, the doctorial candidate who did the work on the solid state fermentation of sugar beets. She also saw their pilot plant facilities which are no longer in use.

As part of the activities in this project, Sheryl de Cabrera attended the 87 th Annual Meeting of the American Society for Microbiology which was held in Atlanta, Georgia, on March 1-6, 1987. This provided an oportunity to learn more about recent research in the United States in the area of alcohol fermentation and microbial loads in sugar processing. Related to this project, three presentations should be pointed out with particular interest:

The first is the Eli Lilly Award Address given by Prof. Randy Schekman of the University of California, Berkeley, entitled "Genes and organelles that compose the yeast secretory pathway", a stimulating lecture on the cellular view of the activities in the yeasts and how these relate to normal function and to genetically manipulated cells.

Secondly, scientists from the Department of Microbiology and Cell Science, University of Florida, Gainsville, presented an interesting poster "Glycolytic enzyme levels, internal pH and membrane potential in **Saccharomyces cerevisiae** during growth and fermentation." In conversations with them, they pointed out the great effect that cell age has on the sensitivity to ethanol gradients, the younger cells appearing to be much more sensitive to ethanol than the older cells. Prof. L. O. Ingram heads this research group.

Thirdly, David Otts from the Department of Microbiology and the Audobon Sugar Institute, Louisiana State University, Baton Rouge, presented an interesting poster "Determination of the proton motive force in **Leuconostoc mesenteroides**". The work dealt in part with the singular effects that pH has on the metabolic activities of this bacteria which has an optimum pH of 5.5, no dextran production at pH 7.5, and no growth at pH 4.0.

5.2 Participation in the VII Central American Congress of Sugar Technologists

Silvia Saravia and Eva Mejía attended the VII Central American Congress of Sugar Technologists which was held from August 31 through September 5, 1987, in San Salvador, El Salvador. At this time they presented a paper on the work being carried out in this project. The paper was titled "Fermentación directa de caña de azúcar para la producción de ethanol". We believe that this gave us an excellent opportunity to share with the industry in Central America our work and to pave the way for future development of the process in the local industry.

6. FINANCIAL STATEMENTS

INSTITUTO CENTROAMERICANO DE INVESTIGACION
Y TECNOLOGIA INDUSTRIAL - ICAITI
PROJECT: LOW MOISTURE PROCESSING OF SOLID STATE BAGASSE
NUMBER: AID 936-4452 - ICAITI Nr. 85/4.6.1860
PERIOD: APRIL THROUGH JUNE, 1987

	Total Budget	EXPENDITURES This Period	Prior Period	Accumulated	Total Budget availability
Salaries	58 500.00	3 067.97	23 993.36	27 061.33	31 438.67
Equipment and Materials	38 000.00	2 559.60	19 400.14	21 959.74	16 040.26
Travel and Per Diem	5 500.00	10.13	2 802.86	2 812.99	2 687.01
TOTAL DIRECT COSTS	102 000.00	5 637.70	46 196.36	51 834.06	50 165.94
Indirect Costs	46 800.00	1 691.32	13 953.61	15 644.93	31 155.07
T O T A L US$	148 800.00	7 329.02	60 149.97	67 478.99	81 321.01

"The undersigned hereby certifies: 1. that payment of the sum claimed on the voucher is proper and due under the terms of the agreement; and 2. that the information on the fiscal report is correct and such detailed supporting information as ROCAP may require will be furnished promptly on request".

Mario A. Santos, Chief
Budget & Cost Control Division

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7. PROGRAM OF COMING ACTIVITIES

Coming activities will be focussed at finishing the experimental activities which are now underway, construction of the pilot plant fermentation unit, and operation of the unit during the coming harvest season.

Activity	Time schedule
Ethanol toxicity studies	Jan.-Oct., 1987
Dextran production kinetics	June-Dec., 1987
Design of fermenter	May-Sept., 1987
Construction of fermenter	Sept.- Dec., 1987
Installation of the system in the ICAITI pilot plant	Dec. 1987-Jan. 1988
Trials with drying of cane to determine maximum water content permitted for conservation	Dec. 1987-Feb. 1988
Fermentation trials in the pilot plant to determine effect of water addition and agitation	Jan. - Apr. 1988
Evaluation of data and preparation of final report	April- Sept. 1988

This research in this project is being carried out by the scientists, research assistants and technicians of the Applied Research Division of the Central American Institute for Research in Industry. This report was prepared by Sheryl de Cabrera and Silvia Saravia, with the help of Eva Mejia and Dina Melgar, and Graciela Saravia, secretary.