PD-AAX-36
54773

PROGRESS REPORT

(June - November 1985)

THAILAND-USAID PROJECT

on

Utilization of Cassava and Cassava Waste Through Fermentation Technology

Introduction

This is the third second progress report of the research activities within the frame work of the project on Utilization of cassava and cassava waste through fermentation technology. The report covers the work period of June-November 1985. The techical work includes three main parts namely enzyme studies , selection for thermotolerant fungi which could grow under anaerobic condition and final selection of yeasts based on various desirable characteristics. In the first catagory , work initiated during the last reporting period concerning activity of the enzyme obtained from mixed culture koji as well as that using combination of enzyme prepared by individual culture against various cassava products (starch, chips and solid waste was completed. The results thus provide an information for a more appropriate use of the enzyme and substrate combination. Physicochemical properties of the crude enzyme was made. For the second head line a few strains of Rhizopus spp. was selected. Under the third catagory final decission on selection of the most appropriate yeast culture could be made.

Also included in this report is a summary on activities besides technical works conducted since the starting of the project

Comparison of enzymes produced by individual culture to that produced by mixed cultures

Koji preparation using the two strains either individually or in a mixed cultures system was carried out for comparative study. Hydrolysis of various substrates including raw starch, raw cassava waste and raw cassava chips by the enzymes was performed. Data tabulated in Table 1 compiles the results obtained during this reporting period with those shown in the last report. It indicates that individual enzyme of Aspergillus J_8 gave higher amount of fermentable sugars than those obtained either by activity of the mixed enzymes or the mixed-culture enzyme when raw starch or raw cassava chips was used as substrate. On the contrary, hydrolysis of cassava waste (containing ca.50% starch and 20% crude fibre) by enzyme of Aspergillus J_{10} which was strong in cellulase activity either individually or in combination with Aspergillus J_8 enzyme liberated higher sugars than that produced by the latter alone. Similar result was obtained when mixed-culture koji extract was used. It seemed possible then that between the two macerating enzymes, cellulase gave more contribution that the xylanase did. Moreover parts of glucose found in hydrolysate of cassava waste using enzymes of Aspergillus J_{10} might have come from its B-D-glucosidase activity on cellulase containing in

Table 1. Hydrolysis of raw starch cassava solid waste and cassava chips by mixed enzyme extract of individual culture and enzyme extract of mixed cultures of *Aspergillus* J_8 and J_{10}

substrate	koji extract from strain	Hydrolyzed products (mg%)			
		Dextrin	Maltose	Glucose	Total fermentable[1/] sugars
starch	J_8	70.66	21.47	2,223.94	2,245.41
	mixed enzymes	102.58	19.54	1,985.51	2,005.05
	mixed cultures	105.78	26.43	1,825.44	1,851.87
cassava waste	J_8	114.81	34.7	1,019.19	1,053.89
	J_{10}	114.61	71.98	1,047.70	1,119.68
	mixed enzymes	33.09	90.52	1,054.06	1,144.58
	mixed cultures	105.98	88.41	998.74	1,087.15
cassava chips	J_8	98.02	46.69	1,107.56	1,153.69
	J_{10}	229.19	41.25	511.12	552.35
	mixed enzymes	78.1[illegible]	41.63	1,051.25	1,092.88
	mixed cultures	115.15	40.39	886.91	937.30

1/ : mastose + glucose

Properties of crude enzyme of Aspergillus J_8

The data obtained from studies of optimal cultural conditions were used for preparing the koji. A mixture of wheat bran (11% moisture content) , rice husk and water (9:1:10) pH 5.0 was used as substrate , the culture was incubated at 35°C for 48 hours. Crude enzyme was extracted as previously described.

Optimum pH : Optimum pH for raw starch digestion was studied using 0.05 M acetate buffer of pH range from 3.0-6.0. The results as shown in Table 2 and Fig 1. indicated that the enzyme had broas range of working pH 3.0-4.5 with its maximum activity at 4.0. There was a singificant drop of activity at pH 5.0.

Optimum temperature : Raw starch digestion by the crude enzyme was carried out at various temperatures ranging from 35-55°C. Although hydrolysis performed at 45°C liberated highest amount of fermentable sugar (Table 3 , Fig 2) , the activity carried out at 35 , 45 and 50°C gave only 2-5% less yield.

The mentioned properties would make it more possible for a practical application of the enzyme in a prolong hydrolysis of noncooked cassava under condition unfavorable for contaminances.

Table 2 Effect of pH on raw starch digestibility of crude enzyme of *Aspergillus* J_8

pH	Hydrolysis products (mg%)[1/]			
	Dextrin	Maltose	Glucose	Total Fermentable sugar
3.0	84.16	0	2281.39	2281.39
3.5	75.97	0	2308.63	2308.63
4.0	108.96	34.82	2580.37	2615.19
4.5	226.54	27.54	2308.63	2336.17
5.0	285.62	97.85	1547.73	1645.58
5.5	146.14	81.29	827.65	908.94
6.0	401.60	115.04	583.09	698.13

1/ : mg% of hydrolysis products of the reaction mixture containing 1 ml enzyme , 3 ml water , 0.1 M citrate buffer and 0.1 g raw cassava starch.

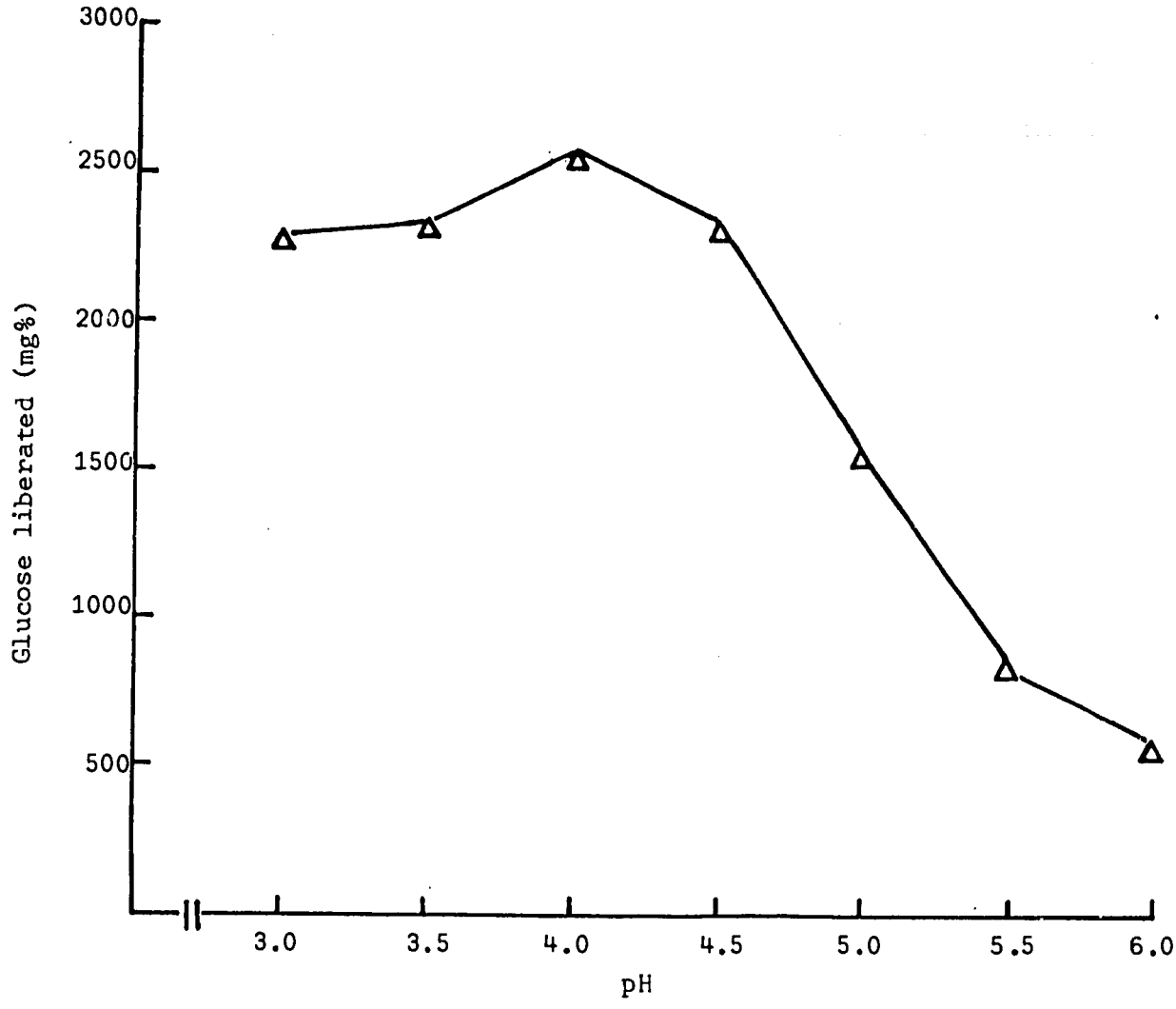

Fig 1. Effect of pH on raw starch digestibility of crude enzyme of <u>Aspergillus</u> J_8

Table 3 Effect of temperature on raw starch digestibility of crude enzyme of *Aspergillus* J_8

Temperature (°C)	Hydrolysis products (mg%)[1/]			
	Dextrin	Maltose	Glucose	Total Fermentable sugar
35	108.96	34.82	2580.37	2615.19
40	45.54	0	2613.24	2613.24
45	58.18	0	2725.49	2725.49
50	76.00	23.23	2661.55	2684.78
55	186.19	106.23	2263.31	2369.54

1/ : mg% of hydrolysis products of the reaction mixture containing 1 ml enzyme , 3 ml water , 1 ml 0.1 M citrate buffer and 0.1 g raw cassava starch.

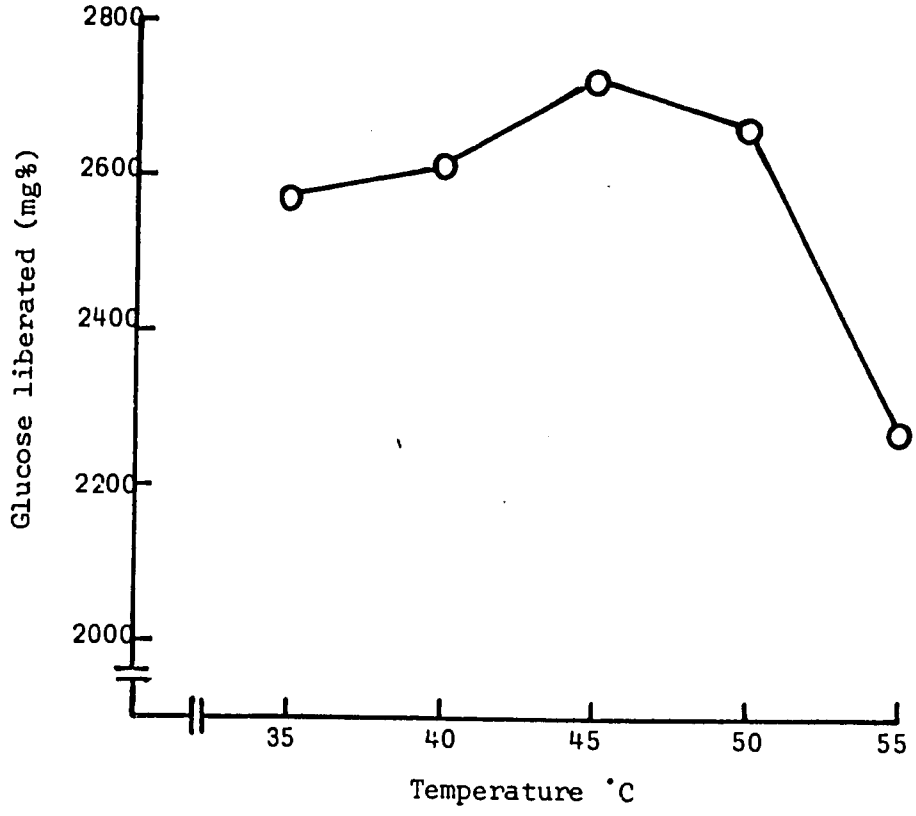

Fig 2. Effect of temperature on raw starch digestion

strate. Complete hydrolyzation was reported to be 3-5 days. We therefore considered that in order to be practically used , the enzyme should be stable under its working temperatures for a period of time. Studies were made for both crude enzyme extract and the koji.

While crude extract was kept at various temperature ranging from 4-45°C for 48 hours , the koji was held at 4°C and 27°C for 48 hours and sun dry (50-55°C) for 5 and 10 hours. The so treated enzymes were determined for their activities against raw starch. Table 4 and Fig 3 show that the enzyme extract storaged at 4 and 27°C retained its activity as the fresh enzyme while those kept at 40 and 45°C lost 10% and 12% activity. Data tabulated in Table 5 and Fig 4 indicate that enzyme which was kept in form of koji lost 13% and 17% activity at 4°C and 27°C respectively , whereas 20% was lost in the 5 hour sun dried koji. Drying of koji for 10 hours cause 26% drop of activity. Moisture content of the koji dried for 5 hour decreased to 5.86% which was the level that limitted to growth of any contaminants. The so conducted experiment was made with a plan in mind to search for the most appropriate and least costly method in preserving the enzyme as well as to determination of its stability. However before a more definit conclusion on validity of the method could be made , data

Table 4 Temperature stability of crude enzyme of *Aspergillus* J_8

Temperature[1/] (°C)	Hydrolysis products (mg%)[2/]			
	Dextrin	Maltose	Glucose	Total Fermentable sugar
4	101.04	84.96	2700.41	2785.37
27	92.81	79.46	2704.92	2784.38
40	80.79	78.00	2436.75	2514.75
45	80.90	68.79	2344.17	2412.96

1/ : The enzyme was kept at various temperature for 48 hours.

2/ : mg% of hydrolysis products of the reaction mixture containing 1 ml enzyme, 3 ml water, 1 ml 0.1 M citrate buffer and 0.1 g raw cassava starch.

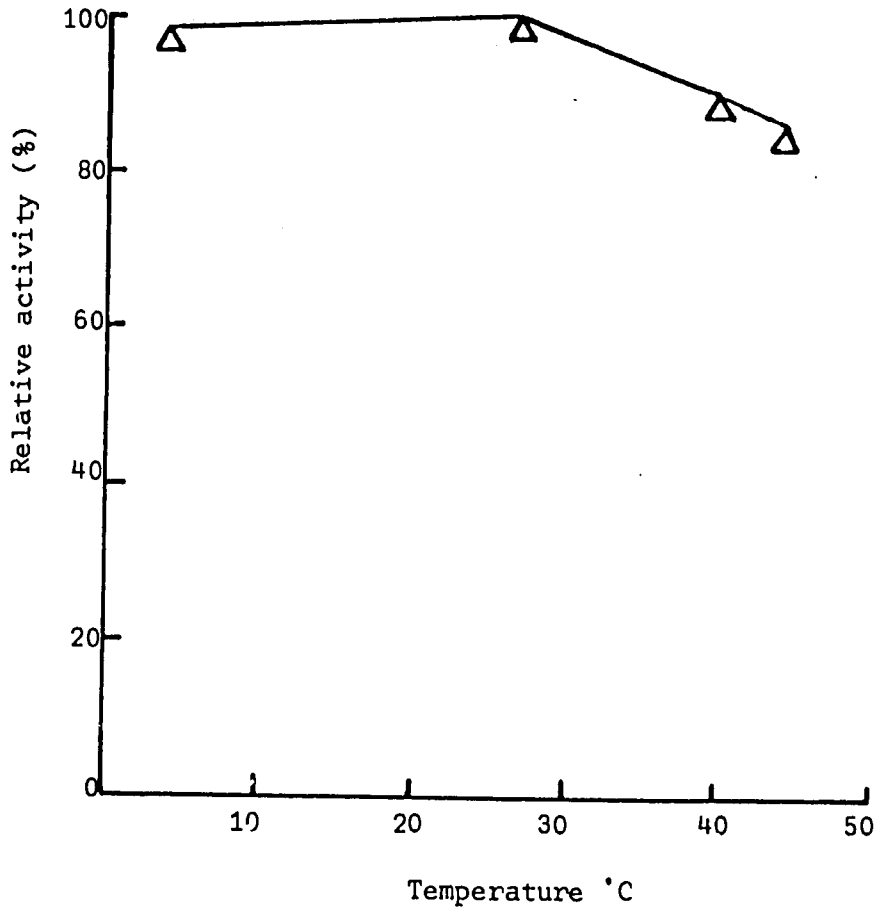

Fig 3. Temperature stability of crude enzyme of <u>Aspergillus</u> J_8 after storage for 48 hours

Table 5 Temperature stability of koji of *Aspergillus* J_8

Storage condition	Moisture of koji (%)	Glucose liberated[1/]	
		mg%	mg/g dry koji
Fresh koji	62.24	2702.05	268.34
4°C , 48 h	62.24	2608.65	235.95
27°C , 48 h	59.13	2432.65	223.21
Sun dried 5 h	5.86	2710.78	218.99
Sun dried 10 h	4.81	2747.47	201.16

1/ = Glucose liberated was determined by using glucostat

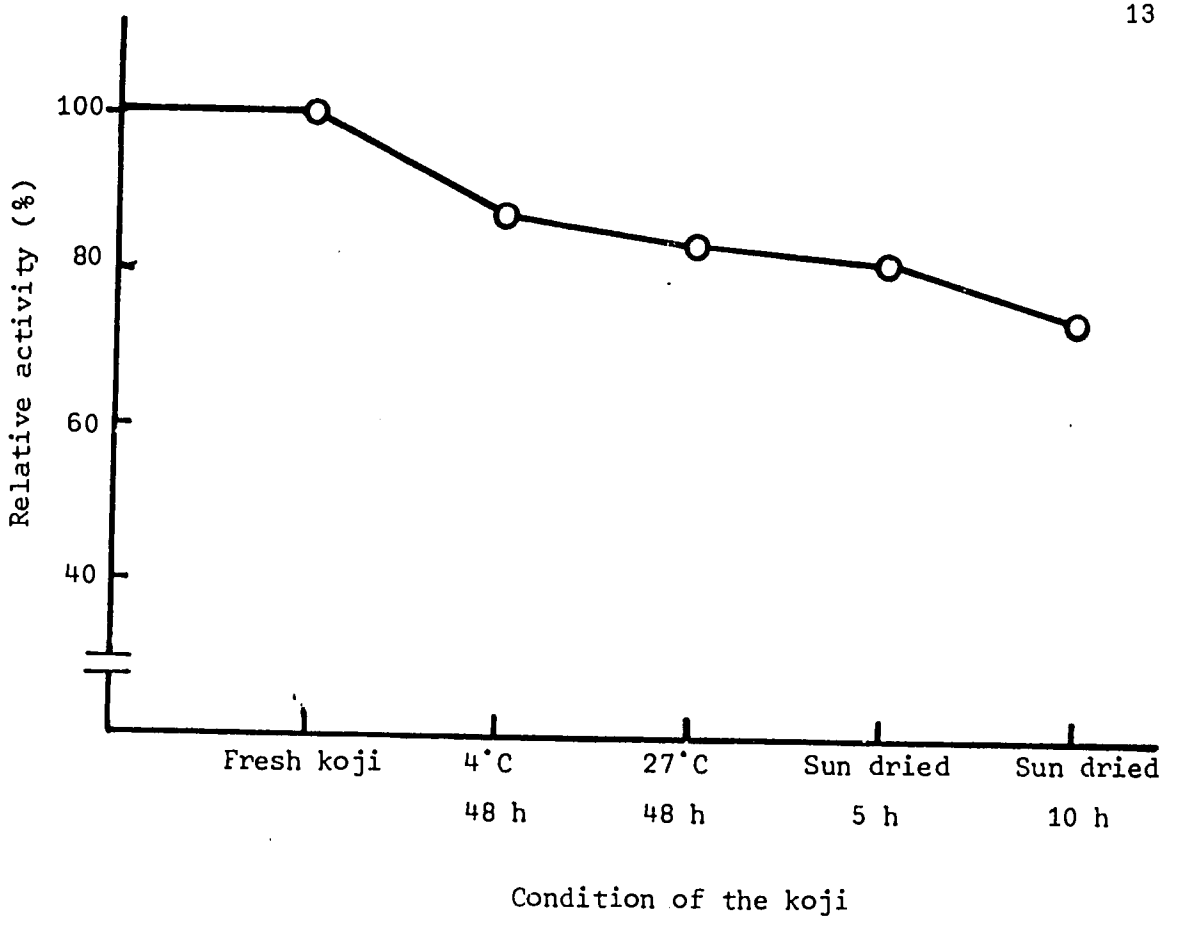

Fig 4. Relative activity of enzyme of Aspergillus J_8 in from of koji storaged under various condition

3.0-6.0 and kept at 4°C for 48 hours. Assaying of enzyme activity was done after the pH was readjusted to its optimum pH (4.0) with citric acid or sodium citrate. It was found that the enzyme retained full activity at pH 5.5 while there was approximately 10% drop at pH 3.0 , 3.5 , 4.0 , 4.5 , 5.0 and 6.0 (Table 6 , Fig 5).

Raw starch digestion of Rhizopus spp. at different pH

As shown in the second progress report that among 20 potent strains of fungi , Aspergillus J_8 was selected basing on its ability to liberate highest yield of fermentable sugars from raw starch at pH 3.5. However in reviewing more literatures we found that there were several Rhizopus enzyme that had sharp maximum activity at higher pH while worked poorly at pH 3.5. A aring of discarding of some effective Rhizopus strains , we therefore recheck for raw starch digestibility of 7 strains of Rhizopus spp which had passed the primary screening. Enzyme activity of crude extract of these similarly grown as that Aspergillus J_8 was assayed at various pH. Data tabulated in Table 7 and Fig. 6 revealed that like other Rhizopus enzyme , the enzyme under study had very low activity at pH 3.5. Of the 7 strains tested two (Rhizopus N_{27} and Rhizopus 102 R)

Table 6 pH stability of crude enzyme of *Aspergillus* J_8

pH	Hydrolysis product (mg%)			
	Dextrin	Maltose	Glucose	Total fermentable sugar
3.0	0	74.47	2729.27	2803.94
3.5	0	88.98	2715.82	2804.80
4.0	0	80.57	2727.48	2808.05
4.5	119.30	108.12	2816.82	2924.94
5.0	91.71	93.81	2731.41	2825.22
5.5	112.27	118.61	3124.82	3243.43
6.0	103.93	112.55	2813.15	2925.70

1/ : mg% of hydroylsis products of the reaction mixture containing 1 ml enzyme , 3 ml water , 1 ml 0.1 M citrate buffer and 0.1 g raw cassava starch

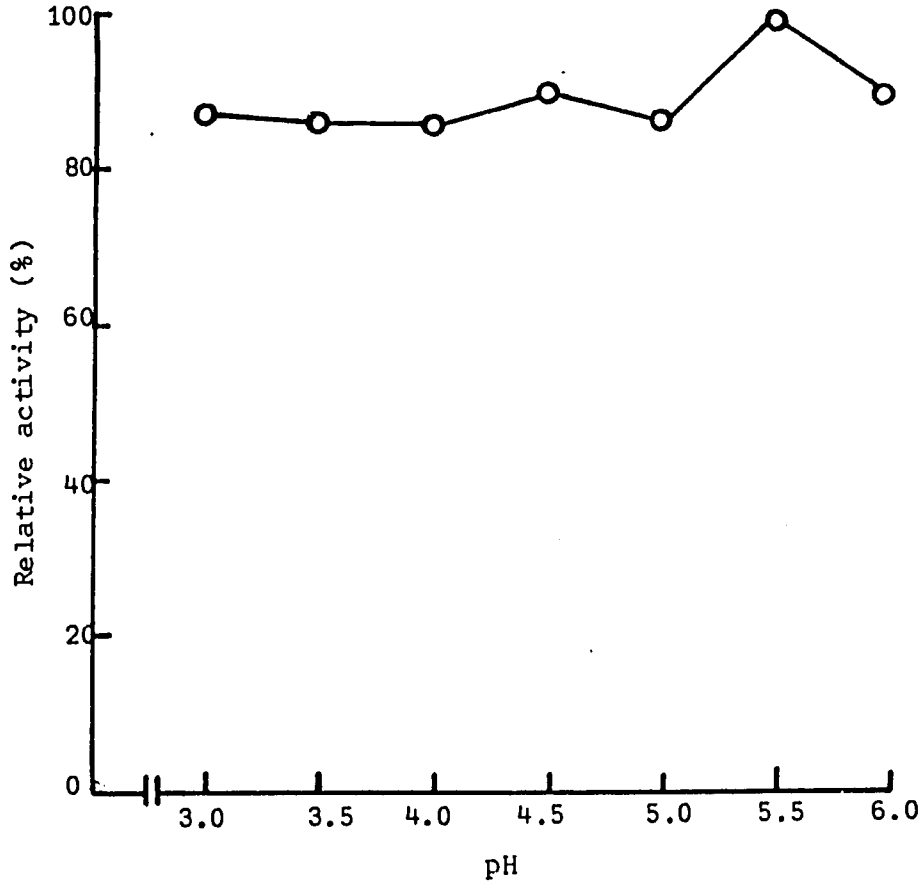

Fig 5. pH stability of crude enzyme of Aspergillus J_8 after 48 hours storage at 4°C

Table 7 Raw starch digestibility of enzymes of *Rhizopus* spp at different pH

STRAIN NO	pH	Hydrolysis product[1/] glucose (mg%)
A 25975	3.5	1485.76
	4.0	1859.18
	4.5	1918.81
	5.0	1985.77
Kuch 1	3.5	1259.16
	4.0	1487.76
	4.5	1455.61
	5.0	1472.93
N_{37}	3.5	1429.75
	4.0	2181.505
	4.5	2301.98
	5.0	2081.95
26 R	3.5	1182.40
	4.0	1271.85
	4.5	1830.41

Table 7 (cont.)

STRAIN NO	pH	Hydrolysis product[1/] glucose (mg%)
RH	3.5	827.07
	4.0	1650.84
	4.5	2027.46
	5.0	2175.18

1/ : mg% of hydrolysis products of the reaction mixture containing 1 ml enzyme , 3 ml water, 1 ml 0.1 M citrate buffer and 0.1 g raw cassava starch.

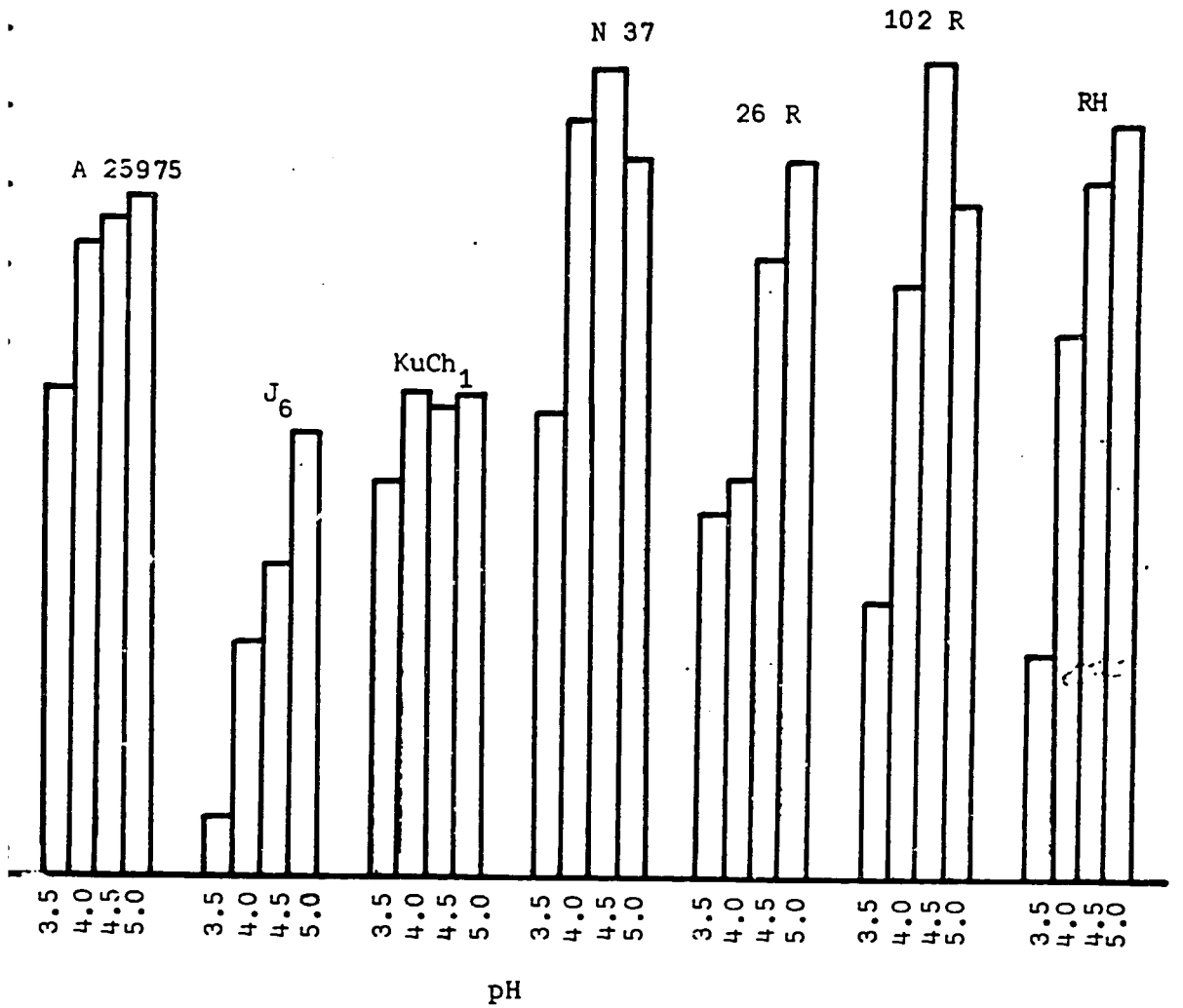

Fig 6. Raw starch digestibility of enzyme of Rhizopus spp at various pH

strains to be applied for starch hydrolysis.

Selection for strains of Rhizopus spp. capable in growing under anaerobic condition and tolerate to high temperature

One of the major objective of this project is to develope a method to produce protein rich (8-10%) silage for animal feed. Two attempts were proposed in order to obtain the appropriate procedure in protein enrichment. One is that using koji as enzyme source for starch digestion followed by culturing of yeast. The other approach is directly growing the fungi on raw cassasava under unaerated fermentation condition so that the system would be easier to control in earge scale production. A selection scheme for competitive strains possessing the following characteristics namely capability in growing under condition limitting in oxygen and toleration to high temperature were performed. A number of Rhizopus spp taking from various sources were grown using single point inoculation in plates containing medium compossing of 0.03% yeast extract , 0.1% $(NH_4)_2SO_4$, 0.05% $MgSO_4$ 7 H_2O , 0.05% KH_2PO_4 and 1.5% agar. An amount of 1.0% raw cassava starch was added and the pH was adjusted to 3.5 using H_2SO_4. Cultures were incubated under aerobic and anaerobic conditions at 35 , 37 and 40°C

Table 8 Growth of Rhizopus spp on starch agar under aerobic and anaerobic condition at 35 and 37°C

Temperature °C	Strain No	Condition	
		Aerobic	Anaerobic
35	3007	+2	+2
	3032	+4	+3
	3033	+3	+1
	3052	+4	+3
	3054	+2	+2
	3057	+2	+2
	3058	+3	+1
	3059	+2	+2
	3079	+2	+2
	3099	+2	+2
	3136	+1	-
	3138	+3	+2
	3139	+3	+2
	3181	+3	+3
	3188	+2	+2
	3190	+3	+2

Table 8 (cont.)

Temperature °C	Strain No	Condition	
		Aerobic	Anaerobic
	KuS_3	+3	+2
	KuS_3	+3	+2
	$KuCh_1$	+4	+3
	$KuCh_2$	+3	+1
	N_{37}	+4	+3
	26 R	+4	+2
	102 R	+4	+2
	RH	+3	+3
37	3032	+3	+2
	3052	+3	+2
	3181	+2	+2
	3188	+2	+2
	A 25975	+3	+3
	$KuCh_1$	+3	+3
	N_{37}	+4	+3
	26 R	+3	+1
	102 R	+4	+1

Table 8 (cont.)

Temperature °C	Strain No	Condition	
		Aerobic	Anaerobic
	26 R	+3	-
	102 R	+4	-
	RH	+3	-
	N_{37}	+3	+1

Relative growth was reported using +4 for maximum growth to - for no growth

could grow anaerobically at 35 and 37°C and that at these temperatures , strain No. A 25975 , KuCh and N_{37} gave as good growth under aerobic as that under anaerobic condition. Aerobic growth of strain No. A 3188 , A 25975 , KuCh , 26 R , 102 R , RH and N_{37} at 40°C was found comparable to that at 37°C. While most of the cultures could not grow anaerobically at 40°C , strain No. A 3188 and N_{37} showed scantly growth. Based on these results , strain No. A 25975 , KuCh , N_{37} , R 26 , 102 R , and RH were primarily selected for further tests.

Determination of ability of the selected cultures to hydrolyse cassava starch and to increase the protein was made. The fungi were cultured on 60 g substrate containing cassava waste , wheat bran and water in a ratio of 10.6:0.4:19 in plastic bags. For each culture, substrate in a quardrupicate bags , after inoculation , was pressed tightly to simulate condition limitting of aer , while that in another quardruplicate was loosely incubated. After cubation at 37 and 40°C for 72 hours , material containing in each duplicate bags was placed into a flask and 60 ml of water was added. The mixture was shaked for 1 hour. The extract was then filtered through a cheese-cloth and centrifuged at 5,000 rpm for 10 minutes the supernatant was deter-

mixed with 9.5 ml NaOH and 9.5 ml H_2O. The mixture was digested by boiling for 30 minutes. The extract was diluted and determined for protein using lowry method. Fig 7 , show that among the 6 strains tested , Rhizopus No. R 6 and 102 R were inferior to the others regarding their ability to increase substrate protein at 40°C. Strain No. 102 R and A 25975 gave highest protein yield at 37°C under aerobic condition whereas No. N_{37} , KuCh and RH were considered efficient at 40°C. Results shown in Fig. 8 indicated that the strain KuCh besides growing well and produced high protein could liberated highest amount of sugar at 40°C. Basing on these properties , strain N_{37} would be placed in the second rank. Ability to produce considerable amount of sugar is considered important since it provides substrate for lactic acid fermentation.

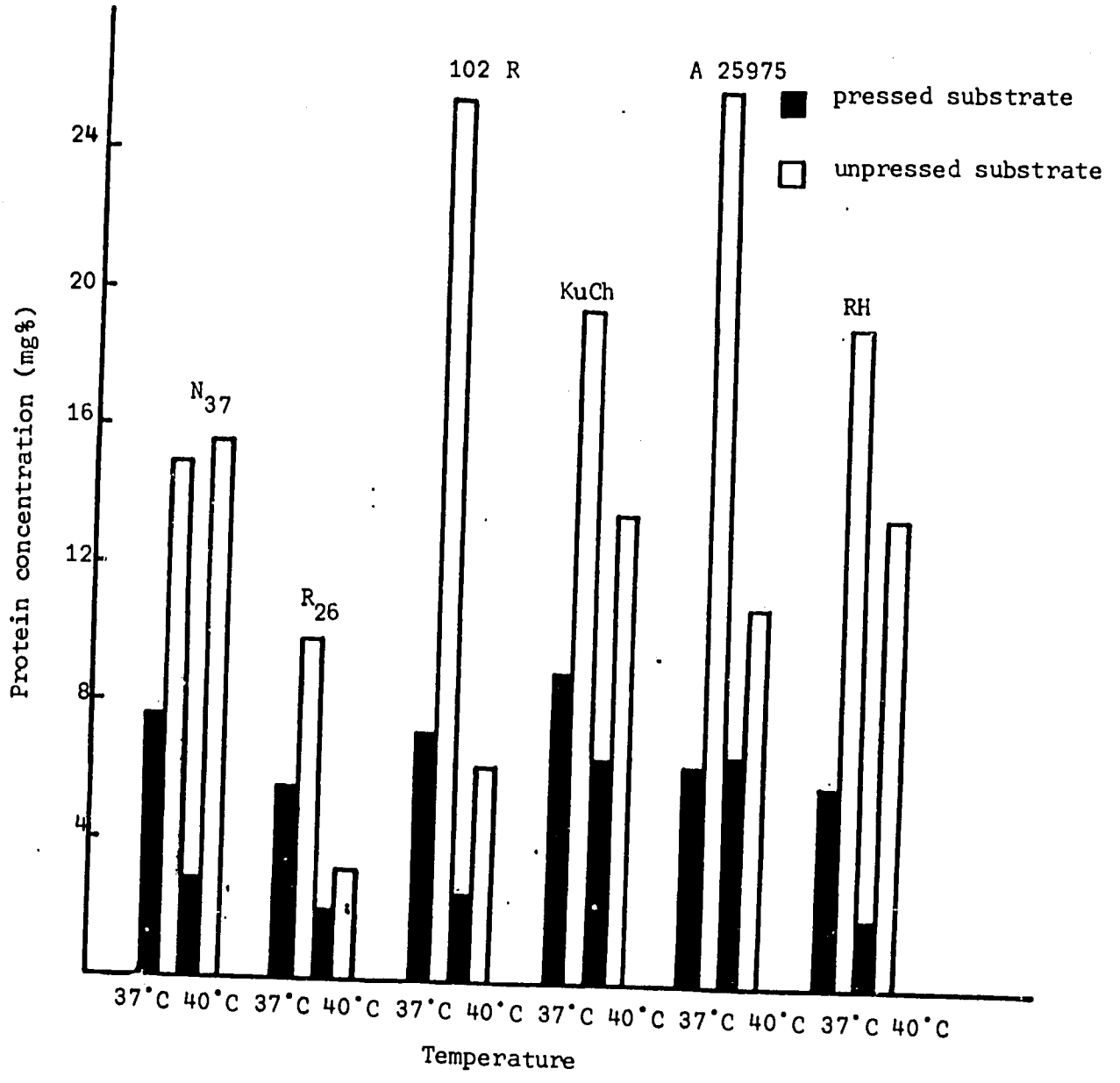

Fig 7. Increase of protein in by growing Rhi...

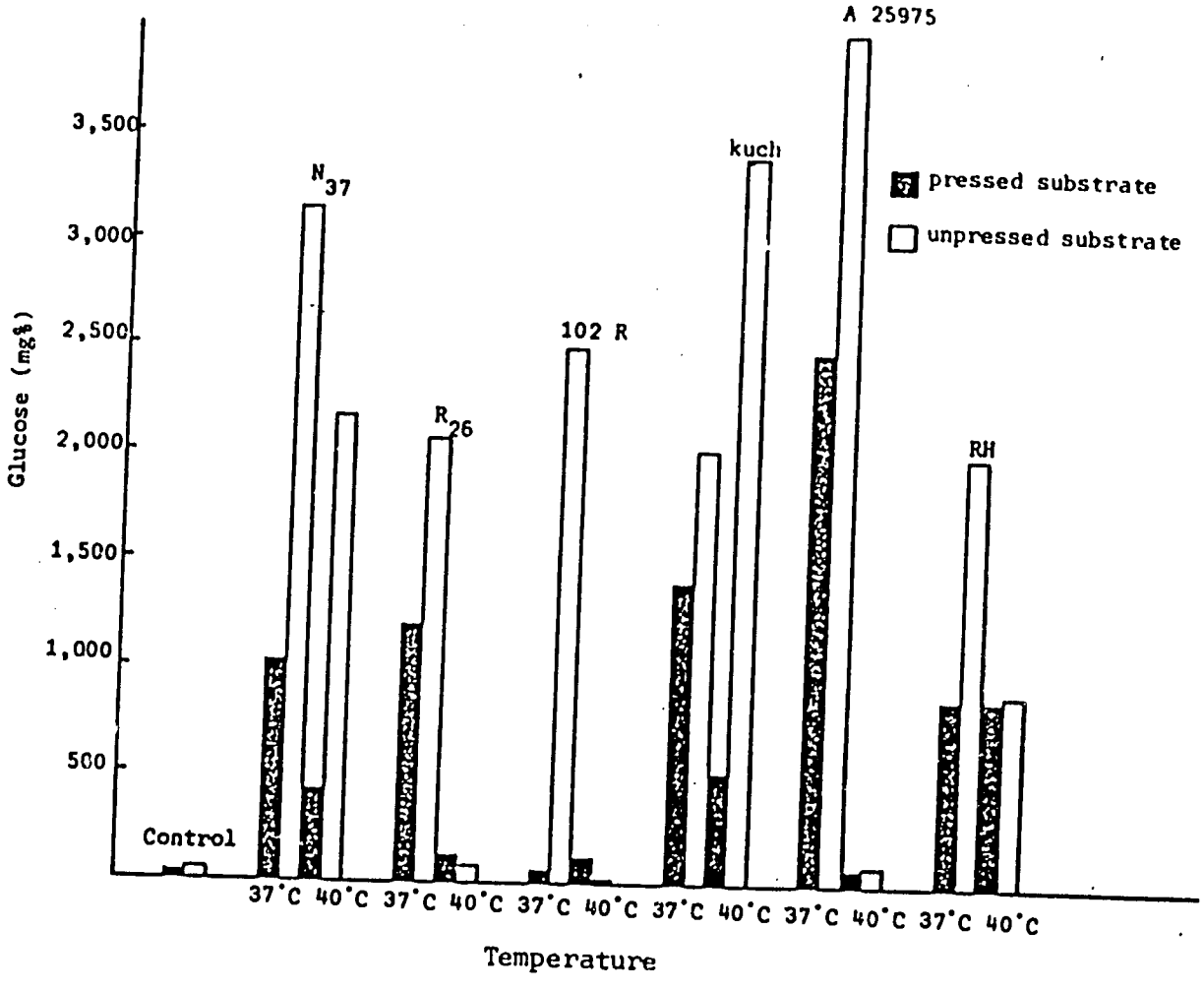

Fig 8. Glucose liberation by Rhizopus spp. growing at 37 and 40°C for 72 hours in lightly pressed and unpressed cassava waste substrate

OPTIMIZATION OF YEAST FOR ALCOHOL AND SILACE FERMENTATION

Preliminary screening of nine collected yeast strains indicated that four strains , i.e , M-30 , AM-12 , 191 and Sc 90 showed promising fermentation efficiency in cassava hydrolysate. All of them yielded ethanol on the average of 7.5% W/V within 24 hours and residual glucose of about 1% from the initial concentration of about 16%. The number of viable yeast cells during course of fermentation was considerably high (1.8×10^8 cells/ml). Therefore , they were selected for further study on their performances under two critical parameters , pH and temperatures.

Fermentation for both alcohol and silage should be done with initial pH as low as possible. Since alcohol fermentation will be carried out from cassava hydrolysate of non-cooking system and , hence , heat sterilization of medium would not be possible. Acidic medium would reduce chances of bacterial contamination. In silage fermentation system , several microorganisms were involved and some of them prefered low pH especially amylase producing fungi and lactic acid bacteria.

In this study , Three-way Layout was used as a statistical

40°C , and 42°C by an incubator shaker. Initial pH of the medium was adjusted to 3.5 , 4.0 , 4.5 and 5.0 by lactic acid.

Yeasts which were selected for the study could be devided into two groups. They were flocculating and non-floculating yeasts. The first group consisted of M-30 and AM-12 while 191 and Sc. 90 were in the second. However , all of them were Saccharomyces cerevisiae.

Comparision of performances was made on per cent glucose utilization because initial substrate concentrations were varied , but they are in the range of 17-18%. Ethonol yield was expressed as per cent conversion efficiency which was calculated from the factor of available glucose time 0.511. The constant 0.511 was derived from Guy-Lussac Equation where 1 mole of glucose yielded 2 moles of ethanol.

Results from Table 9 , 11 , and 13 revealed that yeast strains AM-12 and Sc. 90 from each group performed better in terms of glucose utilization , ethanol conversion efficiency and viable cell numbers. At lower temperature (35°C) both yeasts were able to utilized about 80% of the available glucose and yielded approxi-

Table 9. Percent glucose utilization of selected yeast strains (A) at different pH levels (B) and temperatures (C) after 24 hours fermentation in cassava hydrolysate.

Strain (A)	pH (B)	Temp (°C) 35	37	40	42
M-30	3.5	75.65	72.51	44.69	7.78
	4.0	75.22	69.09	47.20	16.36
	4.5	76.40	71.62	49.53	25.11
	5.0	76.29	68.20	49.42	27.54
AM-12	3.5	80.86	76.47	49.18	19.17
	4.0	80.37	77.81	52.68	23.97
	4.5	81.04	76.82	55.31	35.31
	5.0	81.21	77.86	55.66	34.83
191	3.5	62.80	68.85	12.78	9.88
	4.0	63.98	70.18	15.46	13.77
	4.5	64.45	69.79	21.12	16.79
	5.0	63.93	74.74	21.07	0.86
Sc. 90	3.5	86.91	68.20	27.07	19.22
	4.0	83.96	77.86	41.72	16.85

Mean B	pH 3.5	=	48.88	pH 4.0	=	51.66
	pH 4.5	=	53.63	pH 5.0	=	53.47
Mean C	35°C	=	75.97	37°C	=	72.70
	40°C	=	39.36	42°C	=	18.99

Table 10. Analysis of variance of per cent glucose utilization of selected yeast strains (A) at different pH levels (B) and temperatures (C) after 24 hours fermentation in cassava hydrolysate.

SOV	d.f.	SS	MS	F-cal	F table	
					0.05	0.01
Treatment	63	41024.43	651.18	38.55	1.78	2.29
strain (A)	3	3121.42	1040.47	61.60	2.96	4.60
pH (B)	3	234.77	78.26	0.22	2.96	4.60
Temperature (C)	3	35384.98	11794.00	698.35	2.96	4.60
AB	9	61.22	6.80	0.40	2.25	3.14
AC	9	1467.81	163.09	9.66	2.25	3.14
BC	9	298.21	33.13	1.96	2.25	3.14
ABC	27	456.02	16.89			

Table 11. Ethanol conversion efficiency of selected yeast strains (A) at different pH levels (B) and temperatures (C) after 24 hours fermentation in cassava hydrolysate.

Strain (A)	pH (B)	Temperature (°C)			
		35	37	40	42
M-30	3.5	74.17	69.54	57.25	9.09
	4.0	80.63	70.70	67.20	14.79
	4.5	79.44	73.32	76.34	14.90
	5.0	77.52	79.34	79.97	12.79
AM-12	3.5	79.56	76.13	57.02	6.34
	4.0	82.22	70.02	65.60	21.03
	4.5	78.42	78.56	69.25	19.34
	5.0	80.86	78.38	74.28	21.24
191	3.5	68.79	58.87	9.14	0.00
	4.0	67.83	70.70	19.31	5.28
	4.5	65.67	68.38	19.88	3.38
	5.0	62.61	76.33	21.83	6.02
Sc. 90	3.5	56.55	79.34	20.57	3.59
	4.0	78.99	78.38	48.91	4.33

Mean A	M-20	= 58.55		AM-12	= 59.89
	191	= 39.00		Sc. 90	= 49.57
Mean B	pH 3.5	= 45.37		pH 4.0	= 52.87
	pH 4.5	= 52.90		pH 5.0	= 55.93
Mean C	35°C	= 74.75		37°C	= 73.55
	40°C	= 49.20		42°C	= 9.51

Table 12. Analysis of variance of ethanol conversion efficiency of selected yeast strains (A) at different pH levels (B) and temperatures (C) after 24 hours fermentation in cassava hydrolyate.

SOV	d.f.	SS	MS	F-cal	F table	
					0.05	0.01
Treatment	63	55331.15	878.27	32.23	1.78	2.29
Strain (A)	3	4443.19	1481.06	53.34	2.96	4.60
pH (B)	3	980.70	326.90	0.08	2.96	4.60
Temperature (C)	3	44777.08	14925.69	547.67	2.96	4.60
AB	9	129.12	14.35	0.53	2.25	3.14
AC	9	3791.89	421.32	15.46	2.25	3.14
BC	9	473.34	52.59	1.93	2.25	3.14
ABC	27	735.54	27.25			

Table 13. Viable cells of selected yeast strains at different pH levels and temperature after 24 hours fermentation in cassava hydrolysate.

Strain	pH	Temperature (°C)			
		35	37	40	42
M-30	3.5	8.30	6.24	2.70	0.21
	4.0	9.95	4.73	4.13	0.26
	4.5	7.37	4.06	3.40	0.29
	5.0	7.35	4.51	3.37	0.14
AM-12	3.5	19.10	10.70	5.80	0.22
	4.0	20.70	11.80	7.05	0.47
	4.5	24.30	12.50	7.10	0.46
	5.0	18.80	13.00	6.88	0.43
191	3.5	16.20	5.74	0.47	0.10
	4.0	12.60	8.80	0.56	0.08
	4.5	15.30	6.79	0.46	0.05
	5.0	13.30	6.60	0.84	0.06
Sc. 90	3.5	11.60	9.98	1.49	0.44
	4.0	13.60	1.18	3.05	0.28

glucose medium. Therefore , it was not surprised that this strain showed outstanding characteristics among the group. For yeast strain Sc. 90 , it has been used among alcohol distilleries in Thailand where cane molasses was the principal carbon source. Various published papers revealed that this strain had high adaptability to several carbohydrate sources.

At 37°C , AM-12 exhibited little changes in growth , substrate utilization and ethanol yield. Cell concentration was still at the level of 10^8 cells/ml which was common for alcohol fermentation. At 40°C yeast growth decreased considerably to the cell concentration of about $7x10^7$ cells/ml which certainly effect glucose consumption and ethanol production. At 42°C , growth almost ceased with very low fermentation activity. Cell numbers were in the range of $4x10^6$ cells/ml.

Sc. 90 at various tamperature showed similar performances as compare to AM-12. However , the rates and cell concentrations were lower , especially at higher temperature.

Surprising by , strain M-30 and 191 did not result in satisfactory growths and ethanol yields. This was probably due to short fermentation time (24 hrs). It has been reported that M-30 and 191 could yield 10.3 and 9.8% v/v ethanol from cassava hydrolysate after 40 hrs. However , being low specific rates had its

own advantage in terms of simultaneous fermentation. Since hydrolysation of raw starch was expected to be quite low in both alcohol and silage fermentation.

Concerning initial pH of the medium , its effect was not evidence. Statistical analysis showed non- significant difference among each treatment. Effect of strains and temperature were highly significant for both glucose utilization and ethanol yield. According to the result , it seemed that lower the temperature higher the fermentation rates.

However , it would be too early to conclude and select final strain for alcohol and silage fermentation. Since the best strain would be the one that could grow parallelly to starch hydrolysis. Therefore , next studies will be carried on all four strains for their kinetic behavior in the fermentar.

Other activities

This is a summary of activities besides technical works which was made since starting of the project to the end of this reporting period. The activities was itemized as followed.

1. Remodeling of the laboratory : Using part of the grant , a teaching laboratory was remodeled to an aerconditioning research laboratory with the builtin working bench , cabinets and shelves.

2. Procurement for main items of equipments was completed. The following is the list of equiptments supported by the grants.

2.1 One complete set of High Performance Liquid Chromatography (Waters Associates , Inc). consisting of : Injector - U6k S/n , U6 K025936 , 2 sets of pumps M-510 s/n 510126253 , 510126266, Gradient controller M-680 , s/n 680003179 , Variable UV-Visible Detector M-481 s/n 481003397 , Refractive Index M-401 s/n 401007696 , Data module M-730 s/n 730007767 and accessories.

2.2 Fraction collector with single channel recorder , chart paper ,waterjacket chromatography column , recorder pump.

2.3 Lab Thermo Water bath

2.4 Local made freezer

2.5 Refrigerator (2)

2.6 Hotpack Refrigrated Bath/Circulator to be equiped

with the existing incubator.

2.7 Temperature gradient incubator with standard accessories

2.8 Cutting mill

2.9 Pipett washer

3. Seminar attendance : The principal investigator attended the "AID/SCI Biomass/Biogas Network meeting" held in Boston , Massachusettes during October 31 to November 2, 1984. Present and planned work of the project was presented. A post seminar scientific visit was made at the Cereal Science and Food Laboratory and the Culture Collection of the Northern Region Research Laboratory of the Agricultural Research Center , USDA, Peoria , Illinois. All financing for this trip was included in the grant.