ENCLOSURE (B) 5

STUDIES ON BUTANOL FERMENTATION

(In Four Parts)

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Research Period: 1941-1944

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PART I

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PART III

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PART I

by

CHEM. ENG. LT. COMDR. T. UMEMURA

Research Period: 1941

SUMMARY

The production of butanol in quanitity from the butanol fermentation of molasses was studied, and 9.6% acetone and 21.6% butanol were produced from the total sugar by the regulation of PH to 7, and using the proper nitrogen source such as soya bean cakes, etc.

I. INTRODUCTION

A. History of Project

In the past, many butanol bacteria were selected by the agricultual department of Tokyo Imperial University. The bacteria best fitted for the butanol fermentation of molasses of the above mentioned bacteria was found to be the No. 314 bacteria.

Therefore, using No. 314 bacteria, the conditions of butanol fermentation of molasses were investigated.

B. Key Reasearch Personnel Working on Project

Chem. Eng. Lt. Comdr. T. UMEMURA

II. DETAILED DESCRIPTION

A. Test Procedures

1. Analytical Methods.

Acetone: Acetone is determined by Messinger's method.

Butanol and Ethanol: Butanol and Ethanol is determined by Johnson's method.

Sugar: Directly reduced sugar is determined as glucose by Bertran's method. After saccharification by acid, total sugar is determined as glucose by Bertran's method.

pH: Measured with pH test paper.

2. Cultivation Method. This is in accordance with Weizman's method. The molasses mash is adjusted to a sugar concentration of 7-8%, and is sterilized for 30 minutes a day for 3 successive days. It is innoculated from a pure culture of butanol bacteria which may be maintained in corn mash. After innoculation, it is cultivated at 37°C in the incubator.

B. Experimental Results

1. Analysis of Molasses.

~~Directly reduced sugar~~	
Total sugar	43.12 gm/100cc
Specific gravity (20°/4°)	64.52 gm/100cc
Ash	0.8122
Nitrogen	4.71%
	0.225%

2. Selection of Bacteria: In accordance with Weizman's method suitable bacteria for molasses are selected from the butanol bacterias which are kept in the Agricultual department of Tokyo Imperias University. Bacteria No. 314 was found to be suitable for molasses as indicated in Table I(B)5.

3. Effect of pH: Experiments were carried out and the following results were obtained.

a. pH change for sterilization. Molasses mash containing ca. 8% sugar as total sugar and 5% soya bean cakes in total sugar was sterilized for 30 minutes a day, continuing for 3 days. The changes of pH were obtained and are recorded in Table II(B)2.

b. Relation between pH-change and fermentations: Molasses mash containing 8% sugar as total sugar and 5% soya bean cakes in total sugar was sterilized for 30 minutes a day, continuing for 3 days. The molasses mash was adjusted to the desired pH by the addition of a measured quantity of alkali, and then innoculated with bacteria No. 314. The results obtained are listed in Table III(B)5 and IV(B)5.

4. Relation between Sugar Concentration and Fermentation: The molasses mash is adjusted to the desired sugar concentration and to a pH of 7 by the addition of a measured quantity of alkali.

Other treatment is the same as previously described. The results are tabulated in Tables V(B)5 and VI(B)5.

5. Relation between Nitrogen and Fermentation. The data showing the relation between the amount of nitrogen and fermentation are shown in Tables VII(B)5 and VIII(B)5. The molasses mash is adjusted to the desired ratio of soya bean cakes to total sugar.

Other treatment is the same as mentioned above.

III. CONCLUSIONS

Butanol bacteria for molasses fermentation were selected, and the best conditions of fermentation were studied.

It was found that No. 314 bacteria is suitable for molasses fermentation.

It has been found that it is best to adjust the pH to 7 and to use the proper nitrogen source such as 5% concentration of soya bean cakes to total sugar.

A sugar concentration of 5% in the molasses fermentation is suitable for the formation of butanol.

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Table I(B)5
EFFECT OF DIFFERENT BACTERIA ON MOLASSES FERMENTATION

Bacteria No.	pH		Directly Reduced Sugar	
	Before Fermentation	After Fermentation	Before Fermentation	After Fermentation
31	4.8	4.2	4.968	1.412
74	5.2	4.8	4.950	1.487
138	5.0	4.4	4.542	1.166
173	5.0	4.8	4.542	0.933
314	4.8	4.2	4.968	1.254

Table II(B)5
CHANGES IN pH DURING STERILIZATION

The Initial pH	The 1st Sterilization	The 2nd Sterilization	The 3rd Sterilization
4.8	4.7	4.6	4.6
6	5.4	5.2	5.0
7	5.6	5.3	5.3
8	5.8	5.6	5.6
9	5.4	5.6	5.6

Table III(B)5
EFFECT OF INITIAL pH ON FERMENTATION

(Analysis of mash before fermentation.)

No. of Experiment	Initial pH	Nitrogen Source	Acidity cc.	Total Sugar (%)
1	4.6	Soya bean cakes	2.57	8.569
2	6.2	"	0.78	7.626
3	6.9	"	0.02	7.550
4	8.3	"	"	7.626
5	6.2	none	0.88	7.335
6	7.2	"	"	7.339
7	8.1	"	"	7.345

Table IV (B)5
EFFECT OF INITIAL pH ON FERMENTATION
(Analysis of mash after fermentation.)

No. of Experiment	Final pH	Acidity	Fermented Sugar (gm)	Fermented Sugar Based on Total Sugar	Produced Acetone (Mg/100cc)	Produced Butanol (Mg/100cc)	Produced Ethanol (Mg/100cc)
1	4.3	5.10	3.124	36.4	77		
2	4.4	4.16	4.638	60.8	226		
3	4.7	4.90	5.694	75.4	332	762	135
4	4.6	4.99	6.481	84.9	310	719	127
5	4.5	3.92	3.042	41.4	134		
6	4.6	4.38	2.236	30.4	82		
7	4.6	4.41	2.896	30.4	94		

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Table V(B)5
EFFECT OF SUGAR CONCENTRATION ON FERMENTATION

No. of Exp.	pH	Total Sugar (gm/100 cc)
1	7	4.445
2	"	5.850
3	"	6.321
4	"	7.018
5	"	9.588
6	"	10.392

Table VI(B)5
EFFECT OF SUGAR CONCENTRATION ON FERMENTATION

(Analysis of mash after fermentation)

No. of Exp.	Final pH	Acidity (cc)	Fermented Sugar (mg)	Fermented Sugar % Based on Total Sugar	Produced Acetone mg/100 cc	Produced Butanol mg/100 cc	Produced Ethanol mg/100 cc
1	4.8	2.56	4.032	90.7	274		
2	4.7	3.45	5.428	92.7	352	824	143
3	4.6	4.46	5.518	84.6	367	850	152
4	4.6	4.72	5.402	76.9	392	896	158
5	4.8	4.60	6.392	66.6	362	855	159
6	4.8	4.80	6.594	63.4	358	824	150

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Table VII (B)5
EFFECT OF AMOUNT OF NITROGEN ON FERMENTATION

(Analysis before Fermentation)

No. of exp.	Amount of Soya bean Cakes (gm/100cc)	Soya bean Cakes % based on Total Sugar	pH	Total sugar (mm/100cc)
1	0.5	8	7	6.172
2	0.25	4.5	"	5.591
3	0.05	0.7	"	6.651

Table VIII(B)5
EFFECT OF AMOUNT OF NITROGEN ON FERMENTATION

(Analysis after Fermentation)

No. of Exp.	Final pH	Acidity cc	Fermented Sugar (gm)	Fermented Sugar, % Based on Total Sugar	Produced Acetone (mg/100cc)	Produced Butanol (mg/100cc)	Produced Ethanol (mg/100cc)
1	4.6	4.75	4.316	69.9	232		
2	"	"	4.248	75.9	406	917	132
3	"	"	4.368	65.6	350	820	136

ENCLOSURE (B)5

PART II

by

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Research Period: 1942

SUMMARY

Various bacteria for the acetone butanol fermentation were selected from different soils. Several effective bacteria were found and the following results were obtained:

1. When dried strips of sweet potatoes were fermented, using 21.4% of rice bran as a nitrogen source, the results were as tabulated in Table IX (B)5

2. When dried strips of sweet potatoes were fermented, using 1.86% of $(NH_4)2SO_4$ and 1.4% of $CaCO_3$ as a nitrogen source, the results were as shown in Table X (B)5

I. INTRODUCTION

A. History of Project

Strong bacteria for acetone butanol fermentation were selected from various soils in order to ascertain which bacteria were the most suitable.

B. Key Research Personnel Working on Project

Engineer S. SHIMADA

II. DETAILED DESCRIPTION

A. Test Procedure

1. Examination of Bacteria

a. Collecting the Soil. Most of the soils were obtained from cane sugar or sweet potato farms.

b. Selection Method. Samples containing 7% dried strips of sweet potato mash (water 50cc dried strips of sweet potatoes 3.5gm, rice bran 0.5) were placed in a 3 x 2.5cm test tube, and digested for 40 min. under 2kg/cm^2 pressure. The test soil was added to this mash, heating for 3 min. on a water

bath. After cooling, it was cultivated at 37°C under 15mm-20mm Hg - pressure for 120 hr, and then a part of the cultivating mash was added to the new mash. This treatment was repeated 5~10 times, and the product was tested by the Iodoform reaction. Promising samples were then subjected to plate culture.

2. Plate Culture. The components of the artificial culture solutions were as follows:

Distilled water	100cc
K_2HPO_4	0.5gm
KH_2PO_4	0.5gm
$MgSO_4$	0.2gm
NaCl	0.0.gm
$FeSO_4$	0.01gm
$MnSO_4$	0.01gm
Peptone	5.0gm

An extract of yeast (50cc), glucose (15gm), and agar (6gm) was added to this artificial culture solution. This mash was sterilized 30 minutes a day for three successive days. After operating plate culture as in ordinary methods, the mash was kept at 37°0 under 15mm-20mm Hg - pressure for 5 - 7 days.

3. Sand Culture. Sand is boiled in acid and alkali, washed with water, and dried. A small quantity of this sand is put in a sterilized test tube, sealed with cotton in a $CaCl_2$ - dessicator and then sealed with glass.

When using this sand for an experiment, the glass seal is broken, the sand is added to the unhulled rice mash, and kept at 37°0.

B. Test of Fermentation

1. Preparation of Mash and Analytical Methods. A mixture of sweet potato (21gm), rice bran (3gm), and water (300cc) were placed in a 500cc bottle, mixed well and digested for 40 minutes under 2kg/cm² pressure in an autoclave. The mash volume was about 290cc, and when mash was analyzed, the total volume was put in a mortar and pulverized sufficiently.

a. Quantitative analysis of starch. A 20cc sample of starch was placed in a 300cc flask, 180cc of distilled water and 20cc HCl (specific gravity 1.125) were added, and the solution heated for 3 hrs. on a water bath. After cooling, it was neutralized with alkali and diluted to a total volume of 500cc with water. The total sugar in the mash, measured by Bertran's method, was about 20cc of the above-mentioned sample. The starch value in 100cc of mash was 0.9 times as much as the quantity of sugar.

b. Acidity. A volume of 20cc of mash were diluted with 100cc of distilled water in a 300cc flask, and titrated with N/10 NaOH, using phenolphthalein as an indicator. The acidity is expressed as cc of N/10 NaOH required to neutralize 100cc of mash.

c. pH. The pH was determined with pH test paper.

2. Analysis Method After Fermentation. The mixture was analyzed as follows 96 hrs. after innoculation.

a. Rested starch. After stirring the fermentation bottle, 20cc of mash in a 150cc flask were diluted with 80cc distilled water and 10cc HCl (specific gravity 1.125), saccharified for 3 hrs. on a water bath, cooled, neutralized, and then diluted to 200cc with water. The total sugar in the mash was measured by Bertran's method on a 20cc portion of above-mentioned sample. The starch value in 100cc of mash is 0.9 times as much as the quantity of sugar.

b. Acidity. After filtering the fermented mash, 10cc of filtrate is titrated with N/10 NaOh, using phenolphthalein as indicator.

Acidity is expressed as cc of N/10 NaOH to neutralize 100cc of fermented mash.

c. pH. Same as previously.

d. Acetone. Acetone was determined by Goodwin-Messinger's method, and is expressed as grams in 100cc of mash.

e. Butanol and Ethanol. Determined by Christansen's method.

3. Results. The results are as tabulated in Table XI(B)5.

C. Test of Fermentation Using $(NH_4)_2SO_4$ and $CaCO_3$ as Nutriments

The following results were obtained.

1. Dried strips of sweet potatoes (21gm), 0.39gm of $(NH_4)_2SO_4$, 0.3gm of $CaCO_3$, and 300cc water in a 500cc bottle were digested for 40 hrs. under 20kg/cm^2 pressure. The molar ratio of $(NH_4)_2SO_4$ to $CaCO_3$ was determined by experiment to be 1 : 1.

2. Result. The initial mash analysis was as follows:

Acidity.....10.0
pH.....5.4
Starch.....5.0

The results of the experiment are tabulated in Table XII(B)5.

III. CONCLUSIONS

Bacteria for acetone-butanol fermentation were selected from various soils and the effectiveness of each was examined.

Ten excellent bacteria for the acetone-butanol fermentation were found.

Using rice bran or $(NH_4)_2SO_4$+$CaCO_3$ as the nitrogen source, the following results were obtained.

Butanol for starch.....about 20%
Acetone for starch.....about 10%

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~~Table IX(B)5~~

RESULTS OF NEW SPECIES OF BACTERIA
IN BUTANOL FERMENTATION
(NITROGEN SOURCE: RICE BRAN)

Name of Bacteria	Yield of Butanol	Yield of Acetone	Time of Fermentation hr
KN 1	19.96	10.21	120
KN 12	19.46	10.24	120
KN 16	20.01	10.98	120
KN 20	20.89	11.26	120
KN 21	20.46	11.75	120
KN 26	18.71	9.74	96
Weizmann	19.26	9.72	120

Table X(B)5
RESULTS OF NEW SPECIES OF BUTANOL BACTERIA
IN BUTANOL FERMENTATION*

Name of Bacteria	Yield of Butanol	Yield of Acetone	Time of Fermentation hr
KN 1	20.29	10.86	72
KN 19	21.00	10.78	48
KN 46	20.40	10.55	72
Weizmann	20.29	10.52	96

*Nitrogen source: $(NH_4)_2SO_4+CaCO_3$

Table XI(B)5
ANALYSIS OF FERMENTED MASH USING RICE-BRAN
AS NITROGEN SOURCE

Bacteria	pH	Acidity (cc)	Rested Starch (gm)	Analysis of Fermented Mash: Acetone mg/100cc	Ethanol mg/100cc	Butanol mg/100cc
KN 1	4.4	46	0.5	0.5513	0.0972	1.0779
KN 12	4.4	56	0.5	0.5532	0.1050	1.0509
KN 14	4.4	46	0.8	0.5538	0.0754	0.9916
KN 16	4.6	38	0.6	0.5930	0.0384	1.0808
KN 19	4.2	75	0.7	0.5476	0.0480	0.9698
KN 20	4.6	40	0.6	0.6081	0.0469	1.1274
KN 21	4.6	40	0.6	0.6346	0.0352	1.1600
KN 23	4.4	51.5		0.3300	0.0409	0.5863
KN 27	4.4	58.0	0.8	0.5476	0.0384	0.9933
KN 46	4.3	59.0	0.8	0.5259	0.0570	1.0101

Original mash analysis
Starch 5.4gm
Acidity 10cc
pH 5.2

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Table XII(B)5

ANALYSIS OF FERMENTED MASH USING $(NH_4)_2SO_4$ AND $CaCO_3$ AS SOURCE OF NITROGEN

Bacteria	Acidity (cc)	pH	Rested Starch (gm)	Ethanol mg/100cc	Acetone mg/100cc	Butanol mg/100cc
KN 1	39.5	4.4	0.38	0.0649	0.5428	1.0147
KN 12	38.0	4.4	0.33	0.0574	0.5239	0.9436
KN 14	43.5	4.3	0.81	0.0639	0.4464	0.8517
KN 16	39.0	4.4	0.38	0.0654	0.3366	0.5732
KN 19	32.5	4.6	0.40	0.0876	0.5390	1.0502
KN 20	50.0	4.2	0.43	0.0978	0.4728	0.9572
KN 21	37.0	4.4	042	0.0538	0.1646	0.2387
KN 23	43.0	4.3	0.33			
KN 27	35.0	4.4	0.44	0.0796	0.3102	0.5073
KN 46	41.0	4.3	0.45	0.0632	0.5277	1.0199
Weizmann	34.5	4.4	0.41	0.1223	0.5258	1.0145

ENCLOSURE (B)5

P A R T III

by

CHEM. ENG. LT. COMDR. T. UMEMURA

CHEM. ENG. LIEUT. M. TAKAHASHI

Research Period: 1944.

A means of increasing or strengthening the fermenting power of clostridium acetobutyricum was conducted by the use of physical stimulations such as an electric lamp, x-rays and super sonics.

The significant results were as follows:

1. Super sonics slightly improved the fermenting power of organisms with short time exposure of the stimulant.

2. Incandescent lamps decreased the fermenting power of the organisms.

3. X-rays had varying influences in accordance with the wave length of the ray: viz, long waves increased the fermenting power of the organisms.

I. INTRODUCTION

A. History of Project

A device for increasing or strengthening the fermenting power of butanol bacteria was Weizmann's method, but the effect of physical stimulation was not studied. In this report various physical effects on fermenting power were investigated.

B. Key Research Personnel Working on Project

Chem. Eng. Lieut. M. TAKAHASHI.

II. DETAILED DESCRIPTION

A. Description of Test Apparatus

1. Sand Culture. A test tube, half filled with dry pure sand, was sterilized by dry heating, and was then innoculated with clostridum Acetobutyricum.

2. The test tube containing the nourishing medium contained sucrose (3%) and soya bean cakes (0.3%).

3. A vacuum flask was evacuated to about 20 or 30mm pressure.

4. A flask containing 500cc of the culture medium consisting of 20gm sucrose and 2gm soya bean cakes.

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B. Details of Test Procedures and Conditions

As mentioned above, three physical stimulating agencies were used and the times of exposure of the sand culture of bacteria were 10, 30, and 60 seconds.

These cultures were subjected to the physical stimulation along with the control culture, and were cultured in an evacuated apparatus, at 20mm to 30mm pressure and at a temperature of 37°C for 24 hours. After 24 hours, these cultures were poured in a new medium containing sucrose (4%) and soya bean cakes (0.4%) at 37°C. After 48 hours, the butanol and acetone contents were determined. Other cultures in the medium containing sucrose (6%) and soya bean cakes (0.6%) were held at 37°C for 48 hours, and the effect was determined as above.

C. Experimental Results

1. Yields. In the case of the super sonics or W-anticathode X-ray, products were formed in the ratio of 6 parts of n-butanol, 3 parts of acetone, and one part of ethanol, just as in the normal butanol-acetone fermentation. However, the incandescent lamp and Cu-anticathode X-ray influence yielded an abnormal ratio of acetone butanol. In the former case, 120 grams of mixed solvent was obtained from 342 grams of sucrose. Detailed data are presented below.

2. Details of Tests.

a. Effect of super sonics.

Super sonics; 480 K.C., 300 Watts

Exposure of the sand cultures to super sonics was conducted as above mentioned, for 10, 30, and 60 seconds. In the case of 4% sugar mash, 10 seconds' exposure showed yields of butanol comparable to those obtained with the control, but 30 or 60 second exposures resulted in inferior yields. It was almost the same in the case of 6% sugar mash, except that the effect of 10 seconds' exposure was to give a considerable increase in in the yield of butanol.

The influence of super sonics in increasing the fermenting activity of organisms was not too promising. The results are tabulated in Table XIII(B)5.

b. The effect of incandescent lamp. A 320 Watts mercury lamp was used. The time of exposure of the sand culture to the incandescent lamp was 10, 30, and 60 seconds. The activity of fermentation was decreased in accordance with the duration of exposure. The results are tabulated in Table XIV(B)5.

c. The effect of X-rays.

(1) Cu-anticathode (3000 Volts 10 ma). All exposures to Cu-anticathode x-rays decreased the fermenting ability of the organism. This was especially true of the exposure to the rays for 60 seconds which gave extremely poor fermentation yields. The results are tabulated in Table XV(B)5.

(2) W-anticathode (65000 V, 3 ma). In exposing the sand cultures to W-anticathode for 30 seconds or 60 seconds, the activity of the organism increased and gave a better yield than the control in the culture of 4% sugar mash. The same was true of the effect of exposure on the 6% sugar mash. The yield of butanol after an exposure of 60 seconds was much superior to that obtained after 30 seconds' exposure or to that of the control. (See Table XVI(B)5.)

d. Summary of data. The percentage yields of butanol under the various stimulations studied are tabulated in Table XVII(B)5.

III. CONCLUSIONS

The effects of three types of physical stimulation, super sonics, incandescent lamps and x-rays, upon the sand culture of clostridum acetobutyricum were studied. Super sonics increased the fermenting activity of the organism slightly after 10 seconds' exposure, but decreased the activity after longer exposures.

Incandescent lamps showed an extremely bad influence on the fermentation activity. This influence increased with increasing time of exposure.

In the case of x-rays the results are different for Cu-anticathode and W-anticathode.

The Cu-anticathode rays decreased the fermenting ability, while W-anticathode was effective in increasing the activity of bacteria, viz; exposures of 30 seconds and 60 seconds gave an increased fermenting ability over the control. The 60 seconds' exposure was extremely effective in increasing bacterial action.

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Table XIII(B)5
INFLUENCE OF SUPER SONICS

(480 kc, 300 watts)

Time of Exposure (sec)	pH	Acidity (cc)	Sugar Content (%)	Ratio of Consumed Sugar to Total Sugar (%)	Acetone (mg/100cc)	Butanol (mg/100cc)	Time of Fermentation (hr)
0	4.2	2.7	4	95.4	420	820	48
0	4.4	2.8	4	95.4	432	825	"
30	4.4	3.2	4	94.2	398	725	"
60	4.3	3.1	4	94.1	446	756	"
0	4.1	5.7	6	74.7	440	926	48
10	4.2	2.7	6	85	520	1209	"
30	4.0	5.9	6	18.2	109	-	"
60	4.0	5.0	6	51.6	344	882	"

Table XIV(B)5
INFLUENCE OF INCANDESCENT LAMP

Time of Exposure (sec)	pH	Acidity (cc)	Sugar Content (%)	Ratio of Consumed Sugar to Total Sugar (%)	Acetone (mg/100cc)	Butanol (mg/100cc)	Time of Fermentation (hr)
0	4.4	2.5	4	92.5	427	779	48
10	4.6	2.4	4	90	404	715	"
30	4.4	3.9	4	92.4	227	405	"
60	4.6	2.2	4	92.4	248	455	"
0	4.6	2.4	6	64.5	412	959	48
10	4.2	3.1	6	58	387	893	"
30	4.2	3.2	6	32.9	208	593	"
60	4.6	3.2	6	32.7	228	673	"

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Table XV(B)5
INFLUENCE OF CU-ANTICATHODE

(3000 V, 10 ma)

Time of Exposure (sec)	pH	Acidity (cc)	Sugar Content (%)	Ratio of Consumed Sugar to Total Sugar (%)	Acetone (mg/100cc)	Butanol (mg/100cc)	Time of Fermentation (hr)
0	4.4	4.7	4	75	386		48
10	4.4	2.2	4	60	364	427	"
30	4.4	4.8	4	30	287	427	"
60	3.6	7.6	4	26			"
0	4.4	2.3	6	65.5	447	872	48
10	4.4	2.9	6	70	462		"
30	4.4	3.2	6	25	202	1015*	"
60	4.0	7.4	6	53			"

*Probably in error by infection.

Table XVI(B)5
INFLUENCE OF W ANTICATHODE

(65000 V, 3 ma)

Time of Exposure (sec)	pH	Acidity (cc)	Sugar Content (%)	Ratio of Consumed Sugar to Total Sugar (%)	Acetone (mg/100cc)	Butanol (mg/100cc)	Time of Fermentation (hr)
0	4.4	2.4	4	74	340	667	48
30	4.4	2.9	4	87.5	385	703	"
60	4.4	3.0	4	95	400	856	"
0	4.4	3.4	6	76.6	477	946	48
30	4.2	3.8	6	76.6	476	961	"
60	4.6	2.9	6	96.5	586	1355	"

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Table XVII(B)5
YIELD OF BUTANOL

(Fermentation Time 48 Hours)

	Conc. of Mash Exposure Time	4% Sugar Mash				6% Sugar Mash			
		0 sec	10 sec	30 sec	60 sec	0 sec	10 sec	30 sec	60 sec
Percentage Yield of Butanol	Super Sonics	20.5	20.6	18.1	18.9	15.0	20.0	-	13.3
	Incandescent Lamp	19.5	17.0	10.0	11.0	16.0	14.0	9.8	11.3
	Cu-Anticathode	-	10.0	10.0	-	14.0	-	16.0*	-
	W-Anticathode	16.0	-	17.0	21.0	15.1	-	16.0	22.0

* Probably in error by infection.

ENCLOSURE (B)5

P A R T IV

by

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Research Period: 1941-1942

SUMMARY

A promoter for the acetone-butanol fermentation of dried strips of sweet potatoes was desired.

It was found that rice bran, peanut bean cakes, soya bean cakes, banko beans, and the powdered Formosan brewer's grains on which Ryzopus Delemer has been bred, and $(NH_4)_2SO_4$+$CaCO_3$ gave excellent results as promoters of butanol fermentation from dried Formosen sweet potatoes.

I. INTRODUCTION

A. History of Project

In the acetone-butanol fermentation from dried strips of sweet potatoes, a good result can not be obtained because of the lack of nitrogen and other nutriments for the bacteria.

In regard to this, the question arose as to what kind of materials should be added to the principal raw materials. At present, rice bran is considered to be the best fitted for the purpose, but it has disadvantages in the following respects:

1. It must be used in large quantities (20% based on raw material).

2. The rice bran is a good fodder, and is difficult to obtain in large quantity.

3. According to the literature, fermentation is retarded when rice bran is added.

4. Rice bran is comparatively expensive and is difficult to preserve since it contains oil. Hence, it is considered to be desirable to find the material having as few of the above disadvantages as possible in considering the fermentation efficiency on an industrial scale.

B. Key Research Personnel Working on Project

Eng. of Formosan Central Research S. SHIMADA

II. DETAILED DESCRIPTION OF FERMENTATIONS TESTS

A. The Testing of Rice Bran

The fermentation was carried out in the usual manner by adding rice bran to a mash containing 7% dried strips of sweet potatoes.

The percentage of rice bran is the percentage based on the total amount of mash. The results are as tabulated in Table XVIII(B)5. In the following experiments, rice bran is used as a control.

B. The Testing of Peanut Bean Cakes

The fermentation was carried out by adding different percentage of peanut bean cakes. The addition of 0.5% cake was sufficient for normal fermentation. The results are as tabulated in Table XIX(B)5.

C. The Testing of the Soya Bean Cakes

The fermentation was, in general, unsatisfactory. The optimum results were obtained with 0.5% soya bean cakes. This quantity is 7% of the sweet potato content. The results are tabulated in Table XX(B)5.

D. The Testing of Black Sesame Seed Cakes

The fermentation was carried out by adding 0.1, 0.3, 0.5, 0.8, and 1.0% black sesame seed cakes to the mash.

In this case the fermentation was poor, and the cake was not effective as a promoting material.

E. The Testing of Banko Beans

Banko beans were tested in the same manner and the adequate quantity of banko beans was found to be 0.5 or 0.8%. (This is 7 or 10% based on the sweet potatoes.)

The results are tabulated in Table XXI(B)5.

F. The Testing of the Rice Bran with Fungi Powder

1. The fermentation was carried out using rice bran and Asp. Oryzae or Rhyzopus. Delemer.

The fermentation was normal, when 0.5% rice bran and 0.5% Asp. Oryzae, or 0.5% of rice bran and 0.5% Rhyz. Delemer was added. The results are tabulated in Table XXII(B)5.

2. The Testing of Rice Bran With the Extract of Fungi. When the extract of the fungi was used, the fermentation was poor. The results are tabulated in Table XXIII(B)5.

The extracts were made as follows: 45 gr. of the fungi were boiled in 250cc of water for 2 hrs., filtered, and concentrated to 100cc.

G. The Testing of Formosan Brewer's Grains

1. The filtrate of Formosan brewer's grains was used instead of water, both with and without rice bran.

In every case, acidity was very high, and the fermentation failed.

2. Formosan brewer's grains were ineffective as the promoting material. The results are tabulated in Table XXIV(B)5.

3. Formosan Brewer's Grains on which Rhyz. Delemer had been bred were used. The results are tabulated in Table XXV(B)5. Using 1% of the grain, the fermentation was normally carried out and the yield of the solvent was satisfactory.

H. The Testing of the Green Tea Waste

The results using green tea waste were unsatisfactory, since the fermentation was very poor.

I. The Testing of $(NH_4)_2SO_4$+$CaCO_3$ or $(NH_4)_2SO_4$ + Rice Bran

1. Using only $(NH_4)_2SO_4$ as a nitrogen source, the fermentation was not normal, while using both $(NH_4)_2SO_4$ and $CaCO_3$, the result was comparatively good and was superior to the control in respect to the rested starch.

The results are tabulated in Table XXVI(B)5.

2. Varying amounts of $(NH_4)_2SO_4$- $CaCO_3$ were used, and it was found that the yield of solvent decreased with increasing amounts of $(NH_4)_2SO_4$- $CaCO_3$. In all cases, the acidity was low and the rested starch was small. Since Calcium was used, it appears that Ca-salts are formed by the acid produced during the fermentation process.

If this is the case, an excess of $CaCO_3$ should be advantageous in lowering the acidity. The results are tabulated in Table XXVII(B)5.

3. Varying ratios of $CaCO_3$ to $(NH_4)_2SO_4$ were used. It was found that the optimum ratio was 1 : 1.

The results are tabulated in Table XXVIII(B)5.

4. Consideration of Experiments on use of $CaCO_3$- $(NH_4)_2SO_4$.

a. In the case of the 7% mash of dried strips of sweet potatoes, the addition of 0.08 or 0.1% of $(NH_4)_2SO_4$ (1.14 or 1.43% based on the sweet potato content) and 0.08 or 0.12% $CaCO_3$ (1.14 or 1.71% based on the sweet potato content) is considered to be adequate.

b. Adding $(NH_4)_2SO_4$ and $CaCO_3$, if the $(NH_4)_2SO_4$ was in excess, the yield of acetone was increased and the butanol yield was decreased.

If $CaCO_3$ was in excess, the yield of ethanol was increased.

5. The Testing of Fertilizer in Place of Pure $(NH_4)_2SO_4$.

$(NH_4)_2SO_4$ used as fertilizer can be used as effectively as chemically pure $(NH_4)_2SO_4$. The results are tabulated in Table XXIX(B)5.

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6. The Testing of a new species of Bacteria with $(NH_4)_2SO_4$+$CaCO_3$. Using the new species of soil bacteria developed in this laboratory and reported herewith, the superiority of the new species was recognized and the characteristic effect of $(NH_4)_2SO_4$ and $CaCO_3$ was obtained.

The results are tabulated in Table XXX(B)5.

III. CONCLUSIONS

The best material as a nitrogen source in the fermentation of dried strips of sweet potatoes was found to be rice bran.

Satisfactory materials for use as nitrogen source, in order of effectiveness, are listed below.

The following materials were found to be unsatisfactory:

Black sesame bean
Extract of Asp. Oryzae
Extract of Rhyzopus Delemer
Formosan brewer's grains
Green tea waste

When $(NH_4)_2SO_4$ and $CaCO_3$ were used, the yield of acetone was increased and the yield of butanol was decreased when the $(NH_4)_2SO_4$ was in excess.

When $CaCO_3$ was in excess, the yield of ethanol was increased.

Using the new species bacteria, adding 0.13% $(NH_4)_2SO_4$ and 0.1% $CaCO_3$, the superiority of the new species was recognized and the characteristic effect of $(NH_4)_2SO_4$ and $CaCO_3$ was obtained.

Table XVIII(B)5
EFFECT OF RICE BRAN IN SWEET POTATO FERMENTATION

Percent of Rice bran	After fermentation*					
	acidity** (cc)	pH	Rested starch gm/100cc	Ethanol gm/100cc	Acetone gm/100cc	Butanol gm/100cc
0.5	33.0	4.2	2.75	0.265	0.375	0.744
1.0	40.0	4.2	2.05	0.342	0.401	0.825
1.5	32.0	4.6	0.67	0.391	0.570	1.082
2.0	30.5	4.4	0.71	0.434	0.582	1.099

* Before fermentation; acidity 8.0cc, pH 5.0, Starch 4.94 gm/100cc.
** cc of 0.1 N NaOH required to neutralize 10cc of product.

ENCLOSURE (B)5

Table XIX(B)5
EFFECT OF PEANUT BEAN CAKES IN SWEET POTATO FERMENTATION

Exp.No.	Percent of Peanuts' Bean cakes	After fermentation*					
		Acidity (cc)	pH	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
	0.5	26.5	4.6	0.47	0.276	0.506	1.012
1	1.0	27.5	4.6	0.46	0.419	0.509	1.047
	1.5	25.5	4.7	0.45	0.361	0.588	1.077
	Rice bran 1.5	27.0	4.6	0.65	0.285	0.596	1.131
2	0.1	39.0	4.3	4.31	0.052	0.036	0.074
	0.3	19.5	4.7	0.52	0.392	0.486	1.033
	0.5	26.5	4.3	1.17	0.294	0.429	0.853
	Rice bran 1.5	25.0	4.8	0.23	0.371	0.552	1.047

* Before Fermentation; acidity 9.0cc, pH 5.0, Staroh 4.94 for Exp. No. 1
Before Fermentation; acidity 8.5cc, pH 5.0, Starch value 5.06 for Exp. No. 2

Table XX(B)5
EFFECT OF SOYA BEAN CAKES IN SWEET POTATO FERMENTATION

Exp.No.	Percent of Soya Bean Cakes	After fermentation*				
		Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
1	0.1	24.5	3.38	0.078	0.208	0.396
	0.3	22.0	1.50	0.189	0.429	0.820
	0.5	27.0	1.01	0.200	0.502	0.918
	Rice bran 1.5	33.0	1.86	0.185	0.492	0.875
2	0.5	19.0	0.42	0.376	0.515	1.017
	0.8	24.0	0.44	0.429	0.495	1.008
	1.0	25.0	0.50	0.457	0.479	1.002
	Rice bran 1.5	27.0	0.59	0.432	0.547	1.125

* Before Fermentation; Acidity 9.5cc, Starch 5.06 for Exp. No. 1
Before Fermentation; Acidity 8.0cc, Starch 4.79 for Exp. No. 2

ENCLOSURE (B)5

Table XXI(B)5
EFFECT OF BANKO BEAN CAKES IN SWEET POTATO FERMENTATION

Percent of Banko beans	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
0.1	24.0	2.86	0.206	0.212	0.409
0.3	23.0	1.08	0.282	0.416	0.885
0.5	20.0	0.65	0.360	0.458	1.023
0.8	20.5	0.58	0.322	0.483	1.056
1.0	21.0	0.54	0.355	0.496	1.055
Rice bran 1.5	27.0	0.55	0.344	0.480	1.049

* Before Fermentation: acidity 9.5cc, starch 5.09

Table XXII(B)5
EFFECT OF RICE BRAN AND FUNGI IN SWEET POTATO FERMENTATION

Percent of rice bran & Fungi	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice 1.5	32.0	0.48	0.364	0.581	0.999
Rice 0.5 A 0.5	26.5	0.52	0.278	0.546	1.104
Rice 1.0 A 0.5	18.5	0.51	0.343	0.550	1.029
Rice 0.5 R 0.5	26.0	0.43	0.315	0.575	1.062
Rice 1.0 R 0.5	21.0	0.44	0.287	0.538	1.094

A: Asp. Oryzae R: Rhyzopus Delemer
* Before Fermentation:- acidity 10.5cc, starch 4.79

ENCLOSURE (B)5

~~Table XXIII(B)5~~

EFFECT OF RICE BRAN AND THE EXTRACT OF FUNGI IN SWEET POTATO FERMENTATION

Percent of Rice Bran & Fungi extract	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice bran 1.5	35.0	0.57	0.506	0.533	1.002
Rice bran 1.5 AE 10cc	27.0	0.52	0.461	0.615	1.037
AE 10cc	25.0	2.21	0.300	0.311	0.615
Rice bran 1.5 RE 10cc	38.0	0.86	0.466	0.492	0.929
RE 10cc	26.0	2.72	0.315	0.258	0.467

AE: Extract of Asp. Orz. RE: Extract of Rhyz. Delemer
* Before Fermentation: acidity 9.5cc, Starch 5.03

Table XXIV(B)5

EFFECT OF FORMOSAN BREWER'S GRAINS IN SWEET POTATO FERMENTATION

Percent of Formosan brewer's grain	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
0.3	18.5	2.95	0.087	0.258	0.523
0.5	19.5	2.35	0.102	0.331	0.669
1.0	20.5	2.67	0.111	0.317	0.637
Rice bran 1.5	29.5	0.48	0.242	0.587	1.175

* Before Fermentation: acidity 10.25cc, starch 5.15

Table XXV(B)5

EFFECT OF FORMOSAN BREWER'S GRAINS ON WHICH RHYZOPUS DELEMER HAS BEEN BRED IN SWEET POTATO FERMENTATION

Percent of Formosan brewer's grains with Rhyz. Delemer	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice bran 1.5	34.5	0.59	0.095	0.514	1.049
R.E.b.g. 1.0	24.0	0.49	0.134	0.555	1.040
" 0.5	17.0	0.42	0.144	0.465	0.885
" 0.3	17.0	1.41	0.106	0.346	0.703

R.E.b.g. Formosan brewer's grains on which Rhyzopus Delemer has been bred.
* Before Fermentation: acidity 10.5cc, starch 4.89.

ENCLOSURE (B)5

Table XXVI(B)5
EFFECT OF $(NH_4)_2SO_4$ $CaCO_3$ OR $(NH_4)_2SO_4$ * RICE BRAN IN SWEET POTATO FERMENTATION

Percent of Material	After fermentation*				
	Acidity (cc)	Rested Starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice bran 1.5	44.5	0.70	0.138	0.580	1.185
$(NH_4)_2SO_4$ 0.1	70.0	4.17		0.027	
$(NH_4)_2SO_4$ 0.3	77.0	4.44		0.011	
$(NH_4)_2SO_4$ 0.1	30.5	0.31	0.167	0.456	1.095
$CaCO_3$ 0.2					
$(NH_4)_2SO_4$ 0.3	38.5	0.26	0.132	0.432	0.954
$CaCO_3$ 0.5					
Rice bran 0.5 $(NH_4)_2SO_4$ 0.07	55.5	3.69		0.161	

* Before Fermentation: acidity 11.3cc, Starch 5.02 gm.

Table XXVII(B)5
EFFECT OF $(NH_4)_2SO_4$ AND $CaCO_3$ IN SWEET POTATO FERMENTATION

Percent of Material	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice bran 1.5	33.0	0.504	0.161	0.597	1.264
$(NH_4)_2SO_4$ 0.05 $CaCO_3$ 0.1	26.5	0.509	0.203	0.479	1.036
$(NH_4)_2SO_4$ 0.10 $CaCO_3$ 0.2	23.5	0.333	0.259	0.463	1.003
$(NH_4)_2SO_4$ 0.2 $CaCO_3$ 0.4	22.0	0.223	0.201	0.444	0.965
$(NH_4)_2SO_4$ 0.3 $CaCO_3$ 0.6	23.5	0.311	0.105	0.412	0.927
$(NH_4)_2SO_4$ 0.5 $CaCO_3$ 1.0	38.0	0.245	0.064	0.312	0.722

* Before Fermentation: acidity 4.25cc, starch 4.89

ENCLOSURE (B)5

Table XXVIII(B)5
EFFECT OF $(NH_4)_2SO_4$ AND $CaCO_3$ IN SWEET POTATO FERMENTATION

Percent of material	After fermentation*				
	Acidity (cc)	Rested starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice bran 1.5	35.0	0.502	0.267	0.608	1.153
$(NH_4)_2SO_4$ 0.1 $CaCO_3$ 0.05	56.0	4.187		0.029	
$(NH_4)_2SO_4$ 0.1 $CaCO_3$ 0.01	56.0	4.139		0.020	
$(NH_4)_2SO_4$ 0.1 $CaCO_3$ 0.05	25.0	0.578	0.203	0.525	1.028
$(NH_4)_2SO_4$ 0.1 $CaCO_3$ 0.1	20.0	0.372	0.297	0.571	1.088
$(NH_4)_2SO_4$ 0.1 $CaCO_3$ 0.15	24.0	0.430	0.324	0.530	1.102

* Before Fermentation: acidity 9.00cc, Starch 4.98 gm.

Table XXIX(B)5
EFFECT OF $(NH_4)_2SO_4$ FOR FERTILIZER IN SWEET POTATO FERMENTATION

Percent of material	After fermentation*					
	Acidity (cc)	pH	Rested Starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
Rice bran 1.5	33.0	4.5	0.81	0.214	0.5487	1.006
$(NH_4)_2SO_4$ 0.08 $CaCO_3$ 0.1	22.5	4.6	0.39	0.194	0.5325	1.061
$(NH_4)_2SO_4$ 0.1 $CaCO_3$ 0.1	25.5	4.5	0.48	0.159	0.5619	1.012
$(NH_4)_2SO_4$ 0.13 $CaCO_3$ 0.1	29.5	4.4	0.40	0.080	0.6054	1.038

* Before Fermentation: acidity 9.5cc, Starch 5.18 gm.

ENCLOSURE (B)5

Table XXX(B)5

THE EFFECT OF $(NH_4)_2SO_4$ AND $CaCO_3$ USING NEW SPECIES OF BACTERIA*

Test No.	Species No.	After fermentation					
		Acidity (cc)	pH	Starch (gm)	Ethanol (gm)	Acetone (gm)	Butanol (gm)
1	KN1	39.5	4.4	0.38	0.065	0.543	1.015
2	KN12	38.0	4.4	0.33	0.057	0.524	0.944
3	KN14	43.5	4.5	0.81	0.064	0.446	0.852
4	KN16	39.0	4.4	0.38	0.065	0.337	0.573
5	KN19	32.0	4.6	0.40	0.088	0.539	1.050
6	KN20	50.0	4.2	0.43	0.098	0.473	0.957
7	KW21	37.0	4.4	0.42	0.054	0.165	0.239
8	KW23	43.0	4.3	0.33			
9	KW27	35.0	4.4	0.44	0.080	0.310	0.507
10	KW46	41.0	4.3	0.45	0.063	0.528	1.020
11	Weizmann	34.5	4.4	0.41	0.122	0.526	1.015

* $(NH_4)_2SO_4$ 0.13%
$CaCO_3$ 0.1%
Acidity 10.0cc

Table XXXI(B)5

NITROGEN SOURCE MATERIALS

Order	Material	Optimum Concentration (based on Mash)
1	Rice bran	0.5
2	$(NH_4)_2SO_4$ $CaCO_3$	0.1 0.1
3	Peanut bean cakes	0.1 - 1.5
4	Rice bran*	0.5
5	Banko bean	0.5 - 0.8
6	Soya bean cakes	0.5

* Plus Aspergillus Oryzae or Rhyzopus Delemer