

**RESTRICTED**

**ENCLOSURE (B)**

ENCLOSURE (B) 1

S T U D I E S   O N   E T H A N O L   F E R M E N T A T I O N

by  
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Research Period: 1944

Prepared for and Reviewed with Authors  
by U. S. Naval Technical Mission to Japan

December 1945

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SUMMARY

Various oil cakes were decomposed with protein decomposed fungi, and the decomposed matter was used as the nutriment for the alcohol fermentation of sugar.

The following significant results were obtained:

Nutriments	Percent (based on sugar) %	Ethyl- alcohol %	Ferment- ing time (days)
Soya bean cakes	3	86.4	3
Peanut bean cakes	5	92.7	4
Cotton seed cakes	3.5	87.0	4
Castor bean cakes	14	88.2	3
Cupura seed cakes	20	88.2	3
Pupa oil cakes	14	88.2	4

Note: In all cases the sugar concentration is 11.6%.

I. INTRODUCTIONA. History of Project

It was hoped that soya bean cakes or other oil cakes might be used as nutriments in the alcohol fermentation of sugar, but the nitrogen is mostly in the protein form, which is unsatisfactory for use as nutriments.

Decomposition of the cakes by acid or alkali is difficult because of the apparatus and materials required. Therefore, it was thought that various oil-cakes might be decomposed by protein decomposed fungi, and the decomposed matter used as nutriments for the alcohol fermentation of sugar.

B. Key Research Personnel Working on Project

Kazuo SHIBAZAKI

II. DETAILED DESCRIPTION

A. A protein of soya bean cakes is decomposed by bacteria, in the following manner:

A 2gm sample of powdered soya bean cakes and 10cc water in a 40cc erlenmeyer flask was digested for an hour under 13.6 kg/cm<sup>2</sup> pressure. After digestion it was inoculated with Bac. mesentericus Trevisan var. Proteolyticus II nov. var., and kept at 37°C. The sample was analyzed after 24 hours, 36 hours, and 48 hours, and the results obtained are shown in Table I(B)1.

B. The ethanol fermentation of sugar was studied, using the decomposed products of soya bean cakes as the nutriments.

The details of the test were as follows:

Powdered soya bean cakes (3 or 5% for total sugar) were diluted with five times as much water as the weight of soya bean cakes in a 40cc erlenmeyer flask. This was digested for an hour under 13.6 kg/cm<sup>2</sup> pressure. After digestion it was inoculated with Bac. mesentericus Trevisan var. Proteolyticus II nov. var., and cultivated at 37°C for 48 hours. After this

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treatment, the decomposed products and 18gm of sugar in a 200cc erlenmeyer flask were diluted to a total volume of 150cc, and digested for 15 minutes under 6.8 kg/cm<sup>2</sup> pressure.

After cultivation at 30°C., the results tabulated in Table II(B)1 were obtained.

C. Studying the ethanol fermentation of sugar by the same method, but using the decomposed products of various oil cakes as the nutrients, the results tabulated in Table III(B)1 were obtained.

### III. CONCLUSIONS

A. Using the decomposed products of soya bean cakes as the nitrogen source, (1.5% or 3.0%, vs. total sugar), and then fermenting mash of 11.58% sugar concentration for five days, the yield of alcohol obtained was 86% of the theoretical value. On the contrary, using 3% non-treated soya bean cakes (vs. total sugar), the yield of alcohol obtained was only 32.1% of the theoretical value for five days.

B. Using the decomposed products of other oil cakes as the nitrogen source, the results were as shown on Table III(B)1.

It is possible to increase the yield of alcohol and to shorten the fermenting time compared with contrast (using non-decomposed product of soya bean cakes as nitrogen source.).

C. The results of these findings have not been extended to commercial practice.

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Table I(B)1  
NITROGEN ANALYSIS OF DECOMPOSED MATTER

Time of Culture	pH	Ammonia Type Nitrogen (%)		Amino Type Nitrogen (%)	
		In Soya Bean Cakes	Total	In Soya Bean Cakes	Total
0	5.7	0.165	2.30	0.185	2.59
24	6.4	0.250	3.51	0.685	9.56
36	7.4	0.635	8.80	0.850	11.83
48	7.8	1.110	15.45	1.475	20.51

Table II(B)1  
SOYA BEAN CAKES IN ALCOHOL FERMENTATION

Soya Bean Cakes for Sugar (%)	pH	acidity (cc)	Sugar (%)		Alcohol Produced (%)	Fermentation		
			Rested	Fermented		Percent	Time *	
Digested	3	3.9	2.24	7.94	31.4	2.5	32.1	—
	5	3.8	2.61	2.32	80.0	4.4	56.3	—
Bacteria Treatment	3	3.8	2.79	0.05	100	6.7	86.4	5
	5	3.8	2.65	0.05	100	7.0	89.8	3.5

Note. In all cases total sugar is 11.58% \*Days

Table III(B)1  
YIELDS FOR VARIOUS CAKES

	Amount Total Sugar Used (%)	Yield of Alcohol*	Ferment Time (days)
Peanut oil cakes	5	92.7	4
Cotton seed oil cakes	3.5	87.0	4
Castor bean cakes	14	88.2	3
Cupura seed cakes	20	88.2	3
Pupa oil cakes	14	88.2	4

\* Percent of theoretical value

Table IV(B)1  
 VARIOUS OIL CAKES AS NITROGEN SOURCE IN SUGAR FERMENTATION

Oil Cakes	Total N %	Treatment	Cakes based on Sugar %	Total Sugar (gm)	pH	Acidity cc	Residual Sugar (gm)	Fermented Sugar (%)	Produced Alcohol %	Fermentation %	Fermented Time (days)
Peanut Oil Cakes	6.61	Digest only	3	12.44	3.6	2.24	7.78	37.46	2.1	25.41	
			5	12.44	3.6	2.25	5.80	53.34	3.5	41.85	
		Bacteria	3	12.44	3.3	2.52	1.38	88.90	5.3	62.78	5
			5	12.44	3.4	2.54	0.22	98.23	7.8	92.69	4
			3.5	12.44	4.0	2.12	7.82	37.14	1.6	19.43	
Cotton Seed Oil Cakes	3.72	Digest only	7	12.44	3.9	2.24	5.25	57.79	3.5	41.85	
			3.5	12.44	3.4	2.12	0.06	99.47	7.3	86.69	4
		Bacteria	7	12.44	3.4	2.70	0.06	99.47	7.4	88.19	2.5
			7	12.44	4.1	2.20	8.16	34.45	2.1	25.41	
			14	12.44	4.0	2.98	5.42	56.43	3.0	35.87	
Castor Bean Cakes	5.43	Digest only	7	12.44	3.2	2.60	3.08	75.24	4.8	56.80	5
			14	12.44	3.6	3.08	0.01	100.00	7.4	88.19	3
		Bacteria	10	12.44	4.2	1.90	7.88	36.65	2.5	29.89	
			20	12.44	4.1	2.24	5.21	58.12	3.7	43.34	
			10	12.44	3.6	2.52	0.25	97.99	4.8	56.80	5
Cupura Oil Cakes	29.1	Bacteria	20	12.44	3.5	2.12	0.01	100.00	7.4	88.19	3
			7	12.44	4.0	2.24	8.47	31.91	1.8	20.93	
		Digest only	14	12.44	4.0	2.80	6.05	51.36	2.9	34.38	
			7	12.44	3.2	3.18	1.74	86.01	5.2	61.29	5
			14	12.44	3.4	2.12	0.01	100.00	7.4	88.19	4
Pupa Oil Cakes	4.85	Bacteria	14	12.44	3.4	2.12	0.01	100.00	7.4	88.19	4