On the Fermenting Ability of Sand Cultures of Acetone-Butanol Organisms Stored for Long Years

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One of the authors¹⁾ has previously reported on the fermenting ability of the sand cultures of acetone-butanol organisms stored for long years. The fermenting ability was tested in sand cultures of three strains (B. Y., B. G. P. and Rokusho No. 1) of *Clostridium acetobutylicum*, and it was found that even the sand culture stored at room temperature for 38 years had still maintained normal fermenting ability. All of these sand cultures tested had been prepared according to Weizmann's method.

In the present paper the results of fermentation test of the sand cultures stored at room temperature for 34~46 years long are described.

A small amount of the sand culture was

inoculated to 10 ml of 3 per cent sterilized mash of unpolished rice in a test tube and incubated anaerobically at 37~38°C. A small portion of the germinated mash was then inoculated to 10 ml of 4 per cent sterilized mash of unpolished rice in a test tube (the rate of inoculant was about 5 per cent) and after three transfers at 24 hr intervals, the 10 ml of fermented mash was transferred to 140 ml of about 9 per cent mash of unpolished rice in a 300 ml Erlenmyer flask. The mash in an Erlenmyer flask was prepared as follows: To 12 g of unpolished rice meal 140 ml of tap water was added and the mixture was sterilized at 130°C for 90 min under pressure. The starch value of unpolished rice used was

TABLE I. FERMENTATION DATA

Sand culture tested			Results of analyses of fermented mashes				
Strains	Year of sand culture preparation	Years of storage	Solvents produced			Total solvents	
			Acetone mg/100 ml	n-Butanol mg/100 ml	Ethanol mg/100 ml	mg/100 ml	% yield based on starch value
B. Y.	(a) 1934	34	700	1139	266	2105	34.7
	(b) 1924	44	600	1246	229	2075	34.8
	(c) 1960	8	720	1184	304	2208	35.7
B. G. P.	(a) 1930	38	484	903	147	1534	24.8
	(a') 1930		634	1127	316	2077	35.3
R1*	1922	46	573	1199	207	1979	33.2

*R1 is the same to Rokusho No. 1 in the previous report.

1) B. Rokusho, *Nippon Nogeikagaku Kaishi*, **34**, 942 (1960).

71.5 per cent.

All fermentations were ended in 48 hr and the mashies were then analyzed.

Acetone was determined by Messinger's method, and *n*-butanol and ethanol were assayed by Christensen's method.

The results of the analyses of the fermented mashies are presented in the following table.

The sand cultures of B. Y. (b) and B. Y. (a) have been stored for 44 years and 34 years, respectively. The both strains vigorously fermented as the strain B. Y. (c) stored for 8 years. The strain B. G. P. (a) stored for 38

years produced smaller quantities of solvents than others, but after one treatment of "heat shock" (100°C for 1 min), it recovered normal fermenting ability and produced normal quantities of solvents as shown in B. G. P. (a') of the table. The strain R1 stored for 46 years still maintains the normal fermenting ability.

Based on the above data, it is plausible that the sand culture of acetone-butanol bacteria maintain their fermenting ability for considerably long years, if the sand culture is properly prepared.