PRODUCTS FROM THE FERMENTATION OF GLUCOSE AND ARABINOSE BY BUTYRIC ACID ANAEROBES[1]

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As part of a comprehensive survey of a large number of anaerobic, butyric-acid forming bacteria and related types, the fermentation products from the decomposition of glucose and arabinose were investigated. A bacteriological study of part of the group has previously been reported by McCoy, et al. (1930).

Butyric acid and butyl alcohol (Pasteur (1861), Fitz (1882), Gruber (1887), Botkin (1892), Beijerinck (1893), Grimbert (1893), Winogradsky (1902)) have long been distinguished and accepted as fermentation products characteristic of at least some of the group. Beijerinck listed four distinct types of fermentation, while Bredemann (1909) recognized only one with varying amounts of lower-boiling alcohols and acids. Acetone (Schardinger (1905), Breaudat (1906)) and isopropyl alcohol (Pringsheim (1906), Morikawa and Prescott (1927)) were later recognized as commonly-occurring fermentation products. In addition, certain other compounds such as isobutyl alcohol (Grimbert (1893), Folpmers (1920), Canonici (1932)) and amyl alcohol (Perdrix (1891)) have been reported, but their occurrence as direct products of carbohydrate decomposition is still uncertain.

EXPERIMENTAL

Cultures

The cultures investigated are designated as strains 1 to 5 inclusive, 7 to 26, 28 to 33, 36 to 44 and 46 to 57. Of these the cul-

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tures numbered 1 to 33 are those reported by McCoy, et al. (1930) and the numbers correspond to their designation. In addition to these, new isolations include strains 36, 40, 53, 54, 56 and 57. Cultures from other sources not previously reported are:

STRAIN	DESCRIPTION	SOURCE
37	F. B. Fernbach	Collection of Sir Frederick Andrewes
38	F. B. BB	
39	*B. butylicus* Fitz	
42	Pink BY	
43	German Maize	
44	Feeble pink white	
46	*B. butylicus*	
A	*B. butylicus* x 160	
B	*B. butylicus* Bakonyi	
41	*Cl. felsineum*	Collection of Dr. A. J. Kluyver
H	*B. butylicus* (Hall BY Cat. No. 3625)	American Type Culture Collection
T	*B. butylicus* (Thaysen BY Cat. No. 4259)	
51	*Cl. multifermentans* (Cat. No. 3538)	
52	*Cl. pectinovorum* (Cat. No. 859)	

According to cultural characteristics the organisms studied may be grouped as follows: Strains 1 to 7 inclusive are of the *Cl. Pasteurianum* type, which does not attack starch. The *B. saccharobutyricus* type, which partially hydrolyzes starch includes strains 8 to 28, 36, 44, 46 and 57. Strains 29 to 33 are starch-fermenting plectridia, while strains 37 to 39 are of the *B. butylicus* type. The starch-fermenting butyl alcohol producers include strains A, B, H and T, which are of the *Cl. acetobutylicum* type, strain 40, which is an unknown pectin-fermenting type, strain 41, *Cl. felsineum*, and strains 42 and 43, *Cl. roseum*.

The inoculum used in the fermentations was prepared as follows: 10 cc. of 6 per cent corn mash in a tube was inoculated with a spore culture in soil. This tube was pasteurized in boiling water for one minute, cooled and incubated at 37°C. When this culture showed vigorous activity, as it did after a period varying from eighteen to twenty-four hours, it was transferred to duplicate tubes of 3 per cent glucose in double-strength yeast water. One of these tubes was used as the inoculum at an age of eighteen to twenty-four hours.

Analytical methods

The determinations of glucose and arabinose were carried out according to the method of Stiles, Peterson and Fred (1926). For the determination of titrable acidity, 10 cc. of the culture was brought to a boil to drive off carbon dioxide, cooled and titrated with 0.1 N sodium hydroxide to the phenolphthalein endpoint.

Butyl, ethyl and isopropyl alcohols and acetone were determined on distillates of the fermented culture. For this purpose 15 cc. of the culture was placed in a 38 x 200 mm. Pyrex test tube, made alkaline with 2 cc. of N sodium hydroxide and 5 to 8 cc. was distilled off and collected under carbon-dioxide-free water in a 50 cc. volumetric flask. The distillate was then made up to 50 cc. with carbon-dioxide-free water. To prevent bumping during the distillation a few glass beads were added, and to reduce foaming small amounts of sodium sulphate and magnesium sulphate were used. Butyl and ethyl alcohols were determined on an aliquot by the method of Johnson (1932). Acetone was determined by a modification of Goodwin's method (1920).

Isopropyl alcohol was determined according to a procedure for the dichromate method developed in these laboratories by R. J. Allgeier and E. L. Tatum as follows: A sample is placed in a 38 x 200 mm. Pyrex test tube together with 10 cc. of 1.5 N potassium dichromate in 5 N sulphuric acid. The volume is made up to exactly 25 cc. with distilled water and three glass beads are added. A rubber stopper is then wired in and the tube is placed in a boiling water bath for exactly three minutes, after which it is removed and cooled in running water. The sample is then distilled from the tube, the distillate being collected under the surface of 15 cc. of N sodium hydroxide in a 125 cc. Erlenmeyer flask. When about half of the liquid has been distilled off, 5 cc. of 0.2 N iodine is added to the distillate by rapid dropping from a pipette. After standing for five to ten minutes the sample so treated is made acid with 20 cc. of N sulphuric acid and the liberated iodine is titrated with 0.1 N sodium thiosulphate from a microburette. A blank for the purpose of standardizing the iodine solution is also titrated, and each cc. of 0.1 N iodine used by the sample is equivalent to 0.967 mgm. of acetone. Of the

isopropyl alcohol originally present in the sample, 85 per cent is recovered in the distillate as acetone, while 96 per cent of the original acetone is recovered as such.

For the determination of butylene glycol the extraction method as used by Fulmer, et al. (1933) was employed, except that, instead of drying the extract for two days in a desiccator, it was dried to constant weight, which required a period of about two weeks. Acetylmethylcarbinol was determined by a modification of the method employed by Wilson, et al. (1927).

Selection of medium

A group of representative cultures was cultivated on four different media for the purpose of choosing the most suitable. The media were:

1. Single strength yeast water—the clear water extract of fresh, starch-free yeast, 100 grams per liter of tap water.
2. Double-strength yeast water—the clear water extract of fresh, starch-free yeast, 200 grams per liter of tap water.
3. A mineral-salts peptone medium (McCoy, et al. (1930)).
4. Mineral-salts peptone medium to which had been added 25 cc. of potato juice per liter.

To each of the above, 3 per cent of glucose was added.

The cultures (11, 14, 16, 22, 32, 36 and 40) chosen for this preliminary study were started in corn mash and after eighteen hours were transferred to fresh tubes of the same medium. This procedure was repeated for a third transfer.

It was found that in the plain mineral-salts peptone medium all cultures died out after the third transfer, and in that containing potato juice only two cultures were still active. In single-strength yeast water five cultures were still active, as was the case in double-strength yeast water. However, the double-strength yeast water medium appeared to be the most generally favorable since in it those cultures which did not appear active after twenty-four hours became so after another day of incubation.

In order to study further the effect of variations in the medium, it was attempted to ferment 5 per cent of glucose in media of various compositions as follows:

Number 1. Single-strength yeast water.
Number 2. Double-strength yeast water.
Number 3. Quadruple-strength yeast water.
Number 4. Double-strength yeast water + 0.5 per cent NaH_2PO_4.
Number 5. Double-strength yeast water + 0.5 per cent $(NH_4)_2SO_4$.
Number 6. Double-strength yeast water + 0.5 per cent peptone.

All media were adjusted to pH 7.0.

These media, each with 5 per cent of glucose, were inoculated with a selected group of cultures. One series of tubes inoculated with cultures 8, 11, 14, 16, 22, 32, 36, 40, 41 and 42 was incubated at 37°C. for a period of five days, while another series inoculated with cultures 8, 22, 32, 36, 40, 41 and 42 was incubated at 30°C. for six days. The cultures were then analyzed for residual glucose.

While in some cases a modified medium appeared to be preferable, in no case did all cultures respond to a single medium. Destruction of more than four-fifths of the sugar was obtained in 7 per cent of the cases with medium number 1, 59 per cent with medium 2, 65 per cent with medium 3, 39 per cent with medium 4, 35 per cent with medium 5 and 52 per cent with medium 6. From these results it is apparent that the most suitable basic medium is either medium 2 or 3 (double or quadruple-strength yeast water), and also that complete fermentation of a 5 per cent glucose concentration could not be expected. Therefore, on the basis of these results and in order to eliminate any effects due to sugar concentration, it was decided to reduce the glucose content. The basic medium finally chosen was a double-strength yeast water containing 2.5 per cent glucose and with the pH adjusted to 7.0.

Products of fermentation

Of the medium chosen, 40 cc. portions were placed in calibrated 1 x 8 inch tubes which were plugged with cotton and sterilized for forty-five minutes at 15 pounds pressure. Immediately after cooling, a 2.5 per cent inoculum was added to duplicate tubes and

these were incubated at 37°C. After four days of incubation the tubes were analyzed for residual glucose, acidity, butyl, ethyl and isopropyl alcohols and acetone.

On the basis of the analytical data the cultures have been grouped as will be brought out in the following discussion.

Group 1. Cultures producing isopropyl alcohol. Data for the cultures producing isopropyl alcohol are recorded in table 1.

TABLE 1

Fermentations characterized by production of isopropyl alcohol

CULTURE NUMBER	GLUCOSE FERMENTED	ACIDITY 0.1 N NaOH IN 10 CC.	NEUTRAL VOLATILE PRODUCTS (BASED ON GLUCOSE FERMENTED)				
			Butyl alcohol	Ethyl alcohol	Isopropyl alcohol	Acetone	Total
			Group I				
	per cent	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
21	94.9	3.95	19.2	2.5	4.7	0.5	26.9
22	94.9	3.70	22.2	2.2	4.4	0.7	29.5
36	94.1	3.75	19.3	2.3	5.2	0.9	27.7
18	78.0	5.30	17.8	2.1	4.5	0.6	25.0
24	75.1	4.85	16.6	2.8	6.1	0.8	26.3
			Group II				
20	94.8	4.30	15.7	2.4	3.2	1.6	22.9
30	93.1	3.65	21.0	1.7	3.8	1.3	27.8
46	94.5	3.30	17.1	2.4	3.8	3.7	27.0
			Group III				
*	40.5–27.2	5.35–5.20	11.2–0.5	1.8–1.3	1.3–0.3	0.5–0.2	14.8–2.4

* Includes cultures 3, 4 and 57.

Strain 57 is included on the basis of an earlier experiment in which isopropyl alcohol to the extent of 21.5 per cent of the neutral products was found. In addition, strains 3 and 4, which also gave low results, may only be tentatively grouped as isopropyl-alcohol-producing types. It will be noted that of the eight definite isopropyl types reported, strains 18, 21, 22, 24 and 36 produce very little acetone while strains 20, 30 and 46 produce acetone and isopropyl alcohol in varying proportions.

Group II. Cultures producing acetone. This group contains the majority of the cultures studied and includes those in which the products, butyl and ethyl alcohols and acetone, are found. Table 2 presents data for those cultures which transform 20 per cent or more of the carbohydrate to neutral volatile products, while data for the remainder are presented in table 3. From these cultures butyl alcohol is the chief product, acetone is second in importance and ethyl alcohol is least important. It appears from examination of the data that certain bacteria of this group produce neutral volatile products amounting to about 30 per cent of the fermented glucose (strains 10, 11, 13, 14, 16 and perhaps 8). On the other hand strains 7, 12, 15, 17, 19, 25, 38, 39 and 41 produce about 20 per cent of neutral volatile products on the basis of the glucose fermented. Of the latter group certain strains (15, 17, 19, 25, 41) produce abnormally high final acidity and this may explain the reduced production of neutral volatile products. In the remaining four cultures, 7, 12, 38 and 39, some other compound which was not detected by analysis may have been produced, or there may have been an unusually high production of gas.

The remaining cultures, which are tabulated in table 3, include those which convert a low percentage of the carbohydrate to neutral volatile products, and a group which may be called "poor fermenters," which utilize less than 30 per cent of the glucose.

It should be kept in mind that modification of the medium may serve to improve the fermentations produced by some of the cultures, but for purposes of comparison it is essential that the same medium be used in all fermentations. For instance, strains A, B, C, H and T which, on the basis of cultural behavior, are strains of *Cl. acetobutylicum*, the industrial butyl alcohol organism, give poor fermentations on the medium used, as may be seen from table 4. It is well known that this organism ferments corn mash vigorously and completely, and will ferment glucose well in a synthetic, mineral-salts peptone medium. However, the observation that this organism shows poor fermentation on a yeast water medium serves as a useful means of comparison with related organisms.

Group III. Cultures producing ethyl alcohol and butylene glycol.

TABLE 2

Acetone-producing types which convert over 20 per cent of the glucose to neutral volatile products

CULTURE NUMBER	GLUCOSE FERMENTED	ACIDITY 0.1 N NaOH IN 10 CC.	NEUTRAL VOLATILE PRODUCTS (BASED ON GLUCOSE FERMENTED)			
			Butyl alcohol	Ethyl alcohol	Acetone	Total
	per cent	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
12	95.4	3.75	15.8	1.3	2.5	19.6
38	95.0	3.20	17.0	1.6	2.8	21.4
8	94.7	4.00	19.2	2.5	2.9	24.6
7	93.7	3.50	15.9	2.1	3.2	21.3
10	93.5	3.75	22.4	2.5	4.1	29.0
11	93.5	3.50	23.6	2.8	3.8	30.2
16	93.3	3.30	20.8	2.5	6.2	29.5
39	93.2	3.30	16.6	1.2	3.5	21.3
13	92.2	3.70	24.5	2.8	4.6	31.9
14	92.2	3.50	25.6	2.0	3.6	31.2
41	79.6	6.70	14.4	1.8	4.4	20.6
19	69.8	5.25	14.5	3.0	3.2	20.8
17	61.5	6.00	17.8	3.2	0.8	21.8
15	59.9	5.20	14.4	2.8	2.9	20.1
25	53.9	4.35	17.8	1.7	1.2	20.8

TABLE 3

Acetone-producing types which give low yields of neutral products from glucose

CULTURE NUMBER	GLUCOSE FERMENTED	ACIDITY 0.1 N NaOH IN 10 CC.	NEUTRAL VOLATILE PRODUCTS (BASED ON GLUCOSE FERMENTED			
			Butyl alcohol	Ethyl alcohol	Acetone	Total
	per cent	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
5	62.2	9.25	5.1	1.2	0.7	7.0
1	58.6	9.30	7.3	1.3	0.7	9.3
2	57.6	9.80	7.7	1.9	0.6	10.2
42	56.5	9.85	4.6	3.5	1.5	9.6
43	46.5	9.05	2.8	3.1	1.0	6.9
23	39.6	7.00	7.0	4.0	0.5	11.5
28	37.2	9.40	4.6	3.6	2.0	10.2
37	33.0	6.60	1.7	2.4	0.8	4.9
55	32.2	6.05	9.1	1.9	1.0	12.1
*	26.2–2.7	6.45–3.65	14.8–0.3	11.5–2.4	4.9–0.5	31.2–3.5

* Includes cultures 9, 26, 29, 31, 32, 33, 40, 44, 51 and 52.

A third distinct group which differs markedly from those already described is presented in table 5. These organisms are not strictly anaerobic but are facultative and cause pellicle formation in the yeast water medium. According to the analytical methods employed the presence of ethyl alcohol and acetone was indicated. However, since oxidation with acid potassium dichromate caused

TABLE 4

Behavior of various strains of Cl. acetobutylicum on yeast water medium

CULTURE NUMBER	GLUCOSE FERMENTED	ACIDITY 0.1 N NaOH IN 10 CC.	NEUTRAL VOLATILE PRODUCTS (BASED ON GLUCOSE FERMENTED)			
			Butyl alcohol	Ethyl alcohol	Acetone	Total
	per cent	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
A	43.2	8.25	3.2	3.8	1.2	8.2
B	46.8	9.45	3.4	2.1	1.2	6.7
H	41.5	9.40	1.7	2.2	1.0	4.9
T	42.3	9.25	1.8	2.2	1.3	5.3
C*	55.5	9.70	4.6	4.1	2.0	10.7
B*	50.8	8.80	6.5	4.5	2.7	13.6

* These determinations were made on 4-liter fermentations of the same medium. Culture C is an authentic strain of *Cl. acetobutylicum*.

TABLE 5

Butylene glycol types

CULTURE NUMBER	GLUCOSE FERMENTED	ACIDITY 0.1 N NaOH IN 10 CC.	PRODUCTS (BASED ON GLUCOSE)			
			Butylene glycol	Acetylmethylcarbinol	Ethyl alcohol	Total
	per cent	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
53	89.3	2.30	6.0	3.8	14.7	24.5
56	89.7	2.35	7.1	6.9	18.0	32.0
54	20.2	2.80			24.7	24.7

the acetone figure to disappear, it was concluded that this product could not be present.

A 20-liter fermentation was then set up, using strain 53, to determine the character of the products formed. The fermented material was repeatedly distilled in order to concentrate the volatile constituents. The concentrated material (about 600 cc.) was then fractionated between 77° and 99°C. through a col-

umn. The fractions were then dried overnight with anhydrous potassium carbonate and the top layers obtained were combined and refractionated.

Fractions were collected as follows: I. 76° to 76.5°C.—5 cc. II. 76.5° to 77°C.—9 cc. III. 77° to 77.3°C.—14 cc. IV. 77.3° to 79°C.—10 cc.

Fraction I gave the iodoform reaction, but no precipitate was obtained with phenylhydrazine. The derivative made by treatment by 3, 5-dinitrobenzoyl chloride had a melting point of 89° to 91°C. The derivative made with ethyl alcohol melted at 91°C. and a mixture of the two melted at 91° to 91.5°C. The refractive index was determined on the two fractions, I and III, at room temperature, and was found to be 1.3610 and 1.3600 respectively. The value reported for ethyl alcohol at 25°C. is 1.360.

To test for the presence of acetylmethylcarbinol the method of Lemoigne as modified by Kluyver, et al. (1925) was used, in which the compound is converted to the insoluble red nickel salt of dimethylglyoxime. This test was found to be negative when applied to a composite sample of the various fractions, but was positive with the residue from the first fractionation. The presence of 2, 3-butylene glycol was demonstrated in the residue from the original distillation after first removing the acetylmethylcarbinol by oxidation with ferric chloride and distillation, after which the glycol remaining in the residue was oxidized by bromine to diacetyl and the latter was converted to the characteristic red salt.

The production of ethyl alcohol, 2, 3-butylene glycol and acetylmethylcarbinol is characteristic of a species which Donker (1926) has called *Aerobacillus polymyxa*. The organisms reported in table 5 are similar in cultural and metabolic behavior to the species described by Donker, and are evidently related to it. Strain 54 has repeatedly exhibited poor fermentation of glucose, but on the basis of cultural characteristics is included with the more vigorous types, strains 53 and 56. However, because of the weak fermentation, no conclusive analytical results on butylene glycol and acetylmethylcarbinol could be obtained for strain 54.

For these fermentations with strains 53, 54 and 56, 500 cc.

flasks containing 300 cc. of medium were used; except for size these experiments were carried out in exactly the same way as the other fermentations. The increased volume of medium was necessary to supply samples for the butylene glycol and acetylmethylcarbinol determinations.

Arabinose fermentation

On the basis of their behavior on glucose, a series of 20 of the cultures were selected for study with respect to their products

TABLE 6

Products from the fermentation of arabinose

CULTURE NUMBER	ARABINOSE FERMENTED	ACIDITY 0.1 N NaOH IN 10 CC.	NEUTRAL VOLATILE PRODUCTS (BASED ON ARABINOSE FERMENTED)				
			Butyl alcohol	Ethyl alcohol	Isopropyl alcohol	Acetone	Total
	per cent	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
40	95.8	2.9	13.7	1.5	2.2	5.2	22.6
20	44.3	4.9	17.8	1.4	1.7	0.6	21.5
57	30.9	5.1	1.4	1.6	0.6	0.6	4.2
42	95.5	6.8	10.7	3.5		7.9	22.1
8	44.1	4.7	9.9	1.1		0.6	11.6
12	40.8	4.9	13.4	1.2		1.0	15.6
28	37.6	5.2	5.5	2.9		0.5	8.9
7	36.1	4.8	13.0	0.4		0.8	14.2
13	35.3	5.0	14.0	0.5		0.7	15.2
25	33.3	5.3	5.5	2.9		0.5	8.9
19	30.0	4.9	7.1	2.4		0.9	10.4
C	35.3	11.4	1.3	1.7		0.8	3.8
*	29.1–8.5	5.9–2.8	11.9–1.4	5.0–1.3	1.0–0.0	1.1–0.2	14.4–4.0

* Includes cultures 15, 16, 17, 21, 24, 26, 32, 44 and 46.

from the fermentation of *l*-arabinose. The sugar was sterilized separately in aqueous solution and added aseptically to the yeast water to produce a medium of 2.5 per cent arabinose. The *l*-arabinose used had a specific rotation of 106°C. Except for the separate sterilization of the sugar solution exactly the same procedure was employed for the arabinose fermentations as for the glucose fermentations.

Table 6 shows that, in general, the fermentation of arabinose

was poorer than that of glucose, although strains 40 and 42 gave better results on this substrate. Van der Lek (1930) found that with some members of the group poor fermentation of arabinose was associated with a high proportion of butyl alcohol and butyric acid, and he ascribed the poor fermentation to the high proportion of butyric acid present. On the other hand, Johnson, et al. (1931) found that in the arabinose fermentation with *Cl. acetobutylicum* the proportion of oxidized products, acetone and acetic acid, increased markedly at the expense of the reduced products, butyl alcohol and butyric acid. In the present experiments a strain of *Cl. acetobutylicum*, designated as culture C, was found to give very poor fermentation, with a very high final acidity and a very low production of neutral volatile products. In the distribution of neutral volatile products from this organism, the butyl alcohol and acetone were depressed with an increase in the proportion of ethyl alcohol, as was found also to be the case in the glucose fermentation.

It will be noted that in general the acid production was higher in the arabinose fermentation than in the glucose fermentation, and that the increased acidity accompanies a lower yield of neutral volatile products. Even when good fermentations were obtained, as with strains 40 and 42, the production of neutral volatile products was lower than was obtained in good fermentations of glucose. In the fermentation with strain 42 this condition is undoubtedly due to the high acid formation, while with strain 40 it may be due to high production of gas or of some other product which was not determined in these experiments.

DISCUSSION

It is especially notable that decreased yields of neutral products are in general associated with high final acidity. This is to be expected if the neutral products are assumed to arise from the reduction of corresponding acids (Peterson and Fred (1932)). When the mechanism is interrupted or the conditions become unsuitable for such a reduction, it may be expected that acids will accumulate with consequent inhibition of further activity. If the acids were neutralized fermentation of glucose would probably

continue, but the end products would then consist largely of acids with few or no neutral products.

The occurrence of isopropyl alcohol among the fermentation products of this group of bacteria seems to be a variable characteristic. For instance, strain 40, when cultivated on glucose which it fermented poorly, was not found to produce isopropyl alcohol, but, when cultivated on arabinose which was vigorously fermented, isopropyl alcohol was obtained as a product. A somewhat similar result is reported by Van der Lek (1930) who found that in one fermentation with *Cl. felsineum* large amounts of isopropyl alcohol were obtained, while in a second experiment a small amount of this product was obtained, and in a series of subsequent experiments no evidence of the presence of isopropyl alcohol could be found. It therefore seems probable that some of the organisms which are tabulated among the poor fermenters of the acetone-producing group may, under more favorable conditions of fermentation, prove to be capable of producing isopropyl alcohol.

The behavior of *Cl. acetobutylicum* (table 4), the industrial butyl alcohol organism, is unusual, but can be explained. It will be noted that the distribution of neutral volatile products is different from that normally obtained, the fermentation is incomplete and there is a high final acidity. The results here reported show a much higher proportion of ethyl alcohol than is normal in the distribution of neutral products or "solvents" obtained with a suitable substrate. However, on the basis of carbohydrate decomposed, the weight of ethyl alcohol obtained in these fermentations is approximately the same as that obtained in the normal fermentation, while the production of butyl alcohol as well as of acetone is much reduced. These facts lead to the conclusion that ethyl alcohol is more readily formed by the organism from some central intermediate product. If that intermediate is acetaldehyde as has been suggested by several investigators (Peterson and Fred (1932)), such a conclusion obviously has a sound foundation, since the formation of butyl alcohol and acetone requires condensation reactions while ethyl alcohol may be formed through the operation of a simple Cannizzaro reaction (Peterson and Fred (1932)).

Comparison of the isopropyl alcohol-producing cultures with corresponding acetone producers brings out an interesting relationship. In the case of isopropyl-alcohol producers the ratio of butyl alcohol to total neutral volatile products is lower than in the case of acetone producers, indicating the manner in which oxidized products are balanced against reduced products. Since isopropyl alcohol corresponds to acetone in its reduced form, the production of that compound results in a decreased production of butyl alcohol, which is the most reduced of all the products.

SUMMARY

1. Fifty-two butyric acid-forming bacteria and related cultures have been studied with respect to fermentation of glucose and arabinose and production of neutral products.

2. After comparison of several media a yeast water medium containing 2.5 per cent of glucose was selected as the most generally favorable.

3. Low yields of neutral products are associated with high concentrations of acid.

4. Lower yields of butyl alcohol are obtained when isopropyl alcohol replaces acetone as a product.

5. The formation of ethyl alcohol in fermentations proceeds more readily than does the formation of acetone and butyl alcohol.

6. Arabinose is only poorly fermented by most of the cultures with production of high acidity and, generally, smaller amounts of neutral products.

7. On the basis of glucose fermented and products formed, the cultures may be grouped as follows:

I. Isopropyl alcohol producers: 11 strains.
   - A. Cultures producing isopropyl alcohol and little or no acetone: 5 strains.
   - B. Cultures producing both isopropyl alcohol and acetone: 3 strains.
   - C. Poor fermenters: 3 strains.

II. Acetone producers: 38 strains.
   - A. Cultures converting over 20 per cent of the glucose to neutral volatile products: 15 strains.

B. Cultures fermenting more than 30 per cent of the glucose but producing less than 20 per cent of neutral volatile products: 13 strains.

C. Poor fermenters; cultures fermenting less than 30 per cent of the glucose: 10 strains.

III. Polymyxa type: 3 strains.

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