THE UTILIZATION OF AGRICULTURAL BYPRODUCTS IN THE PRODUCTION OF PROPIONIC ACID BY FERMENTATION [1]

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INTRODUCTION

The production of propionic acid from agricultural byproducts by the propionic acid bacteria offers commercial possibilities which have not been adequately investigated.

Sherman and his coworkers (*3, 5, 6*)[2] have considered the commercial merits of the propionic fermentation, especially with reference to the utilization of the lactose of whey and milk. They obtained the most satisfactory results when the propionic acid bacteria were grown in association with other organisms, principally members of the lactic acid and proteus groups. However, under their most satisfactory conditions the fermentation was rather slow. They indicated that industrial application was doubtful.

Van Niel (*1*) has shown that the propionic acid bacteria are limited in their utilization of nitrogen from various sources. Vigorous fermentations were obtained only when relatively high concentrations of yeast were used. The ratio of propionic acid to acetic acid as well as the yield of volatile acids was found to vary with the concentration of yeast extract.

Of interest from an industrial standpoint is van Niel's isolation of a starch-fermenting species, *Propionibacterium technicum*. He experimented briefly with this strain, but the fermentation obtained was slow. Van Niel suggested a method of speeding up the propionic acid fermentation. He observed that with many strains the propionic acid bacteria settle to the bottom of the flask after fermentation is complete, leaving a relatively clear supernatant liquid. By removing the liquid above the bacteria and adding sterile medium a 2-percent sugar solution was completely fermented within 48 hours.

Wilson, Fred, and Peterson (*7*) worked on the fermentation of malted corn, blackstrap molasses, and hydrolyzed starch by several strains of propionic acid bacteria grown in association with *Lactobacillus casei*. The fermentation of molasses and hydrolyzed starch was practically complete in 8 to 10 days. The yield of volatile acids was low, however, ranging from 45 to 59 percent of the fermented sugar. Of some industrial significance is the fact that residues of the acetone-butyl alcohol fermentation were found to serve as a source of nitrogen in these fermentations.

Recently a number of patents have been granted in the United States pertaining to the production of propionic acid by fermentation, indicating that commercial production is under investigation.

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[2] Reference is made by number (italic) to Literature Cited, p. 1024.

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This brief survey of the literature indicates that there are three problems that require solution before extensive industrial production will be feasible.

(1) *The nitrogen requirements.*—Yeast extract is the only satisfactory source of nitrogen which has been reported for this group. Utilization of residues from the butyl-acetone fermentation in which pure cultures were used has not been reported. A cheap and satisfactory source of nitrogen is essential for commercial production.

(2) *The carbohydrate.*—The most satisfactory source of carbohydrates for this fermentation has not been definitely established. A knowledge of the number of materials which can be used satisfactorily and the merits of each is essential.

(3) *The period of fermentation.*—The prolonged period of fermentation is one of the present difficulties in the way of commercial utilization of the propionic acid bacteria.

The object of this investigation was: (1) To determine the utilization made of natural carbohydrate materials as well as carbohydrate byproducts of industrial processes by *Propionibacterium arabinosum*; (2) to determine the rate of fermentation of these materials; and (3) to determine the effect of different sources of nitrogen.

PRELIMINARY EXPERIMENTS

Preliminary investigations indicated that *Propionibacterium arabinosum* possesses strong fermentative powers. Table 1 shows typical results. Fermentation of glucose was vigorous; a 3-percent solution was utilized in 9 days under favorable conditions. Of special note is the high yield of propionic acid. The fermentation of starch, although active, was much slower than that of glucose.

TABLE 1.—*Production of volatile acids (percentage by weight) by fermentation of glucose or starch*

[Medium: Yeast extract, carbohydrate (3 percent) and $CaCO_3$ (3 percent); glucose, 9-day fermentation; starch, 14-day fermentation at 30° C.]

Carbohydrate	Yeast extract[1]	Carbohydrate fermented	Volatile acid per liter of medium	Fermented sugar as volatile acids	Volatile acid as— Propionic	Acetic	Formic
	Percent	*Percent*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Glucose	1	53.0	8.7	54.6	84.7	12.8	2.5
	5	98.9	16.9	56.9	82.7	17.3	0
	10	97.4	19.5	66.7	80.0	20.0	0
Starch	10	57.6	11.1	64.6	73.4	23.0	3.6
	10	50.3	9.9	65.6	73.0	26.0	1.0

[1] Water extract of a quantity of dried yeast equivalent to the indicated percentage by weight of the fermentation medium.

Further study indicated that corn gluten serves as a source of nitrogen for the propionic acid bacteria. In preliminary fermentations of glucose in which gluten was used as a source of nitrogen, yields were comparable to those obtained with yeast.

These preliminary experiments indicated that a nitrogen source had been found suitable for the growth of the propionic acid bacteria. Further investigation concerning the industrial application of this organism seemed of value.

PROCEDURE AND METHODS

Approximately, 1,600 cc of medium was used in each fermentation. Three fermentations of each carbohydrate were conducted. The first contained no source of nitrogen other than that present in the raw material; to the second 100 g of dried yeast was added, and to the third 100 g of corn gluten. Fifty grams of $CaCO_3$ was used as buffer in each flask. In those fermentations in which hydrol, whey, milk, or blackstrap molasses served as the raw material, a sufficient quantity was added to supply 80 g (5 percent) of carbohydrate. The carbohydrate content was 48 g (3 percent) in the corn-meal, potato, and artichoke media. Fermentation of yeast (100 g) and of corn meal (100 g) with no addition of carbohydrate material was also run.

The media were autoclaved for 1 hour at 20 pounds pressure and were still sterile after 3 days' trial incubation.

Inoculations were made by using 60 cc of 7-day cultures of *Propionibacterium arabinosum* (strain described by Werkman and Brown (*4*)) grown in a medium consisting of 0.5 percent of Bacto yeast extract, 2 percent of glucose, and 1 percent of $CaCO_3$. The fermentations were incubated at 30° C. No attempt was made to control the degree of anaerobiosis. Cotton plugs were used as stoppers. The media were thoroughly shaken twice daily to aid the buffer action of the $CaCO_3$.

Eight hundred cubic centimeters of the fermented medium was removed from each flask after 7 days' incubation. The remaining 800 cc was fermented during an additional 7-day period. The cultures were checked microscopically for purity by the Gram stain. The volatile acids were determined by the partition method (*2*) as described by Osburn, Wood, and Werkman.

EXPERIMENTAL RESULTS

The yields of volatile acids obtained from the raw materials are summarized in table 2. The results of the fermentation of yeast and of gluten meal are presented in table 3.

The yield is calculated on the basis of 1 l of medium. The volatile acids in grams per 100 g of carbohydrate were determined on the basis of carbohydrates present in the raw material. The total carbohydrate utilized was not determined. It is apparent that these calculations are only comparable for fermentations within the groups containing 3 percent (or 5 percent) of added carbohydrate. No correction has been made for carbohydrates which may have been added with the nitrogen source, since the primary object was to determine the amount of volatile acids obtainable from a given quantity of material in a definite period. Fermentation of the nitrogen source, particularly of gluten meal, indicates the presence of considerable quantities of fermentable material. In the case of corn gluten this is largely cornstarch.

Hydrol and whey were the most satisfactory of the carbohydrate materials examined. The theoretical yield of volatile acids in the propionic acid fermentation is approximately 75 g per 100 g of glucose utilized. The recovery of 69 g in the hydrol-yeast medium indicates that utilization of the 5 percent of carbohydrate was practically complete in 14 days. It is interesting to note that in fermentations

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in which pure cultures of propionic acid bacteria were used there have been obtained in this laboratory substantial yields of the nonvolatile acids, succinic and lactic. It is thus possible that the sugar utilization was more complete than is indicated by the yields of volatile acids.

TABLE 2.—*Production of volatile acids from various byproducts by Propionibacterium arabinosum (calculated on a basis of 1,000 cc of medium) without the addition of a nitrogen source, and with yeast or gluten added as nitrogen sources*

NO SOURCE OF NITROGEN—14-DAY FERMENTATION

Byproducts and the acids formed	Volatile acid produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate
	Cubic centimeters	*Grams*	*Percent*	*Grams*
Corn meal: [1]				
Propionic				
Acetic				
Formic				
Total	21.4			
Artichoke: [1]				
Propionic	85.8	6.34	75.6	21.2
Acetic	33.1	1.99	23.7	6.6
Formic	1.3	.06	.7	.2
Total	120.2	8.39		28.0
Potato: [1]				
Propionic	39.1	2.89	71.5	9.6
Acetic	13.4	.80	19.8	2.7
Formic	7.6	.36	8.7	1.2
Total	60.1	4.05		13.5
Hydrol: [2]				
Propionic	24.4	1.81	74.1	3.6
Acetic	5.0	.30	12.3	.6
Formic	7.4	.33	13.5	.7
Total	36.8	2.44		4.9
Whey: [2]				
Propionic	31.2	2.39	63.1	4.8
Acetic	18.6	1.12	29.6	2.2
Formic	6.0	.27	7.3	.5
Total	55.8	3.78		7.5
Milk: [2]				
Propionic	47.2	3.49	74.6	7.0
Acetic	19.6	1.14	24.4	2.3
Formic	1.0	.04	1.0	.1
Total	67.8	4.67		9.4
Blackstrap: [2]				
Propionic	84.0	6.22	80.3	12.5
Acetic	15.8	.94	12.2	1.9
Formic	12.6	.58	7.5	1.1
Total	112.4	7.74		15.5

[1] Medium contained 3 percent carbohydrate.
[2] Medium contained 5 percent carbohydtate.

TABLE 2.—*Production of volatile acids from various byproducts by Propionibacterium arabinosum (calculated on a basis of 1,000 cc of medium) without the addition of a nitrogen source, and with yeast or gluten added as nitrogen sources*—Continued

GLUTEN ADDED AS SOURCE OF NITROGEN

Byproducts and the acids formed	7-day fermentation				14-day fermentation			
	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate
	Cubic centimeters	*Grams*	*Percent*	*Grams*	*Cubic centimeters*	*Grams*	*Percent*	*Grams*
Corn meal: [1]								
Propionic	95.3	7.05	70.2	23.5	154.8	11.46	70.8	38.2
Acetic	48.8	2.93	29.1	9.7	78.2	4.68	29.0	15.6
Formic	1.5	.07	.7	.2	.9	.04	.2	.1
Total	145.6	10.05		33.5	233.9	16.18		53.9
Artichoke: [1]								
Propionic	110.0	8.15	70.5	27.1	147.4	10.91	66.8	36.4
Acetic	55.5	3.33	28.9	11.1	87.1	5.21	31.9	17.4
Formic	1.7	.07	.6	.5	4.7	.22	1.3	.7
Total	167.2	11.55		38.7	239.1	16.34		54.5
Potato: [1]								
Propionic	50.2	3.71	65.3	12.4	83.0	6.14	67.6	20.5
Acetic	32.2	1.93	34.0	6.4	47.6	2.86	31.4	9.5
Formic	.9	.04	.7	.1	2.0	.09	1.0	.3
Total	83.3	5.68		18.9	132 6	9.09		30.3
Hydrol: [2]								
Propionic	121.5	8.99	70.7	18.0	222.2	16.46	81.6	32.9
Acetic	61.9	3.72	29.3	7.4	59.4	3.56	17.6	7.2
Formic	(3)				3.7	.17	.8	.3
Total	183.4	12.71		25.4	285.4	20.19		40.4
Whey: [2]								
Propionic	61.6	4.50	74.7	9 0	143.3	10.61	71.7	21 2
Acetic	24.5	1.47	24.3	3.0	66.6	4.00	27.2	8.0
Formic	1.4	.06	1.0	.1	3.2	.16	1.1	.3
Total	87.5	6.03		12.1	213.1	14.77		29.5
Milk: [2]								
Propionic	69.2	5.12	70.3	10.2	175.0	12.93	75.5	25.9
Acetic	34.9	2.09	28.8	4.2	68.5	4.12	24.0	8.2
Formic	1.5	.07	.9	.1	2.2	.10	.5	.2
Total	105.6	7.28		14.5	245.7	17.15		34.3
Blackstrap: [2]								
Propionic	209.5	15.51	85.0	31.0	311.7	23.03	82.1	46.1
Acetic	43.2	2.59	14.2	5.2	80.1	4.82	17.1	9.6
Formic	3.3	.15	.8	.3	5.1	.24	.8	.5
Total	256.0	18.25		36.5	396.7	28.10		56.2

[1] Medium contained 3 percent carbohydrate.
[2] Medium contained 5 percent carbohydrate.
[3] Trace.

TABLE 2.—*Production of volatile acids from various byproducts by Propionibacterium arabinosum (calculated on a basis of 1,000 cc of medium) without the addition of a nitrogen source, and with yeast or gluten added as nitrogen sources*—Continued

YEAST ADDED AS SOURCE OF NITROGEN

Byproducts and the acids formed	7-day fermentation				14-day fermentation			
	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate
	Cubic centimeters	*Grams*	*Percent*	*Grams*	*Cubic centimeters*	*Grams*	*Percent*	*Grams*
Corn meal: [1]								
Propionic	42.4	3.13	71.8	10.4	73.7	5.45	73.0	18.2
Acetic	19.7	1.18	27.1	4.0	32.8	1.97	26.3	6.6
Formic	1.1	.05	1.1	.2	1.1	.05	.7	.2
Total	63.2	4.36		14.5	107.6	7.47		24.9
Artichoke: [1]								
Propionic	113.3	8.38	70.7	28.0	129.2	9.56	68.3	31.9
Acetic	55.1	3.31	28.0	11.0	70.6	4.24	30.2	14.1
Formic	3.3	.15	1.3	.5	4.5	.21	1.5	.7
Total	171.7	11.84		39.5	204.3	14.01		46.7
Potato: [1]								
Propionic	53.3	3.95	73.7	13.2	78.7	5.82	71.2	19.4
Acetic	23.4	1.40	26.3	4.7	38.9	2.33	28.5	7.8
Formic	(3)				.6	.03	.3	.1
Total	76.7	5.35		17.8	118.2	8.18		27.3
Hydrol: [2]								
Propionic	242.3	17.93	72.8	35.9	338.9	25.10	72.7	50.2
Acetic	108.0	6.48	26.2	13.0	154.0	9.24	26.8	18.5
Formic	5.0	.23	1.0	.4	4.0	.18	.5	.3
Total	355.3	24.64		49.3	496.9	34.52		69.0
Whey: [2]								
Propionic	140.1	10.37	72.9	20.7	350.9	25.95	76.9	51.9
Acetic	62.8	3.78	26.5	7.6	128.6	7.71	22.8	15.4
Formic	1.8	.08	.6	.1	1.9	.09	.3	.2
Total	204.7	14.23		28.4	481.4	33.75		67.5
Milk: [2]								
Propionic	204.2	15.11	75.1	30.2	303.5	22.47	76.4	44.9
Acetic	80.7	4.84	24.1	9.7	113.8	6.83	23.2	13.7
Formic	3.4	.16	.8	.3	2.5	.11	.4	.2
Total	288.3	20.11		40.2	419.8	29.41		58.8
Blackstrap: [2]								
Propionic	207.4	15.36	72.6	30.7	256.7	18.99	75.3	38.0
Acetic	85.6	5.13	24.3	10.2	100.2	6.01	23.8	12.0
Formic	14.4	.66	3.1	1.3	5.1	.23	.9	.5
Total	307.4	21.15		42.2	362.0	25.23		50.5

[1] Medium contained 3 percent carbohydrate.
[2] Medium contained 5 percent carbohydrate.
[3] Trace.

TABLE 3.—*Production of volatile acids (calculated on the basis of 1,000 c c of medium) by the fermentation of gluten and yeast for 7- and 14-day fermentations*

Acids	Production for 7-day gluten fermentation			Production for 14-day gluten fermentation			Production for 7-day yeast fermentation			Production for 14-day yeast fermentation		
	As normal acid	Portion by weight of total acid		As normal acid	Portion by weight of total acid		As normal acid	Portion by weight of total acid		As normal acid	Portion by weight of total acid	
	Cubic centimeters	*Grams*	*Percent*	*Cubic centimeters*	*Grams*	*Percent*	*Cubic centimeters*	*Grams*	*Percent*	*Cubic centimeters*	*Grams*	*Percent*
Propionic	22.0	1.63	57.2	39.5	2.92	54.5	19.9	1.48	74.7	21.2	1.58	77.5
Acetic	17.6	1.05	36.9	33.4	2.01	37.4	8.0	.48	24.3	6.0	.36	17.6
Formic	3.6	.17	5.9	9.4	.43	8.1	.7	.02	1.0	2.1	.10	4.9
Total	43.2	2.85	------	82.3	5.36	------	28.6	1.98	------	29.3	2.04	------

It is evident that there was no source of nitrogen in any of the raw materials examined suitable to support a vigorous propionic fermentation. Comparison of yeast and gluten as nitrogen sources for the propionic acid fermentation is made difficult by the large quantity of acids obtained in the fermentation of the gluten meal (table 3). The large yield of acids obtained in the fermentation of blackstrap molasses indicates, however, the value of corn gluten as a source of nitrogen. Further study will be required to explain the low yields obtained from carbohydrates with gluten meal as a nitrogen source as compared with yeast. Further study will be required to explain the low yields obtained from carbohydrates with gluten meal as a nitrogen same as compared with yeast.

The percentages of propionic acid obtained in the fermentation of raw materials were somewhat lower than those found in glucose fermentations.

Results which are closely related to those described above have been obtained in this laboratory in the case of propionic acid fermentations in which steep water was utilized as a nitrogen source. Data obtained with glucose-steep water fermentations with *Propionibacterium arabinosum* are summarized in table 4. Similar data have been obtained with other strains of propionic acid bacteria. These results are in most respects similar to those obtained with glucose-yeast extract fermentations (table 1). Concentrations of 5 to 10 percent of steep water appear adequate to fulfill the nitrogen requirements of the propionic acid bacteria. Since steep water is a byproduct resulting from the commercial production of corn sugar, it seems possible that it can be utilized as a nitrogen source in commercial fermentations by the propionic acid bacteria. Further studies with larger fermentations in which carbohydrate materials with commercial possibilities are used will be necessary to determine the value of steep water as a nitrogen source. The results reported indicate, however, that steep water is a potential and cheap source of nitrogen for the propionic acid fermentation.

TABLE 4.—*Production of volatile acids (percentage by weight) by 14-day fermentation at 30° C. of glucose (3 percent), $CaCO_3$ (2 percent), and various percentages by weight of steep water*

Steep water used	Sugar fermented	Volatile acid produced per 1,000 cc of medium	Fermented sugar as volatile acids	Volatile acid as—	
				Propionic	Acetic
Percent	*Percent*	*Grams*	*Percent*	*Percent*	*Percent*
0.5	58.3	7.8	44.7	84.5	15.5
1.0	66.4	12.9	64.7	84.1	15.9
5.0	89.4	18.4	68.4	80.5	19.5
10.0	96.2	21.6	74.8	79.6	20.4

SUMMARY AND CONCLUSIONS

The utilization of hydrol, whey, milk, blackstrap molasses, artichoke tubers, and potatoes in the production of propionic acid by fermentation has been determined together with an evaluation of corn gluten meal and yeast as nitrogen sources. A summary is presented of investigations on the fermentation of glucose in which steep water was used as a source of nitrogen.

The results of the experiments have led to the following conclusions:

Hydrol, whey, milk, blackstrap molasses, artichoke tubers, potatoes, and corn meal contain carbohydrates which are utilized by *Propionibacterium arabinosum*. Hydrol and whey are the most satisfactory of the materials tested.

Corn gluten meal and steep water serve as sources of nitrogen for the propionic acid bacteria.

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