[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Effect of the Concentration of Sorbitol upon the Production of Sorbose by the Action of Acetobacter Suboxydans[1]

BY ELLIS I. FULMER, JOHN W. DUNNING, JAMES F. GUYMON AND L. A. UNDERKOFLER

A recent development of vitamin chemistry is the preparation of ascorbic acid (vitamin C) with sorbose as the starting material. The sorbose is generally prepared by the oxidation of sorbitol by means of *Acetobacter xylinum*. This organism was first employed by Bertrand.[2,3] Among other species of *Acetobacter* which have been found to oxidize sorbitol to sorbose are *A. aceti*,[4] *A. melanogenum*,[5,6] *A. suboxydans*,[5,7,8] *A. xylinoides*[9] and *A. gluconicum*.[9]

Kluyver and de Leeuw[8] studied the oxidizing tendencies of the following four species of Acetobacter, arranged in the order of increasing oxidizing power: *A. suboxydans, A. xylinum, A. rancens* and *A. aceti*. Their results indicate *A. suboxydans* to give higher rates of production and final yields of sorbose than do the other organisms although quantitative data are not given. This organism possesses a further advantage over *A. xylinum* in that it does not form a zoögleal mat, thus permitting a more convenient handling in the isolation of the sorbose. Böeseken and Leefers[7] have recently employed the above organism to produce sorbose for their conductivity studies.

In all studies so far reported the concentration of sorbitol used varied from 3 to 6%. It is evident that the use of higher concentrations of sorbitol would be advantageous in the large scale production of sorbose. In the present communication are presented data on the influence of the concentration of sorbitol upon the yield of sorbose by the action of *Acetobacter suboxydans*.

Experimental

The sorbitol sirup, furnished by E. I. du Pont de Nemours & Company, contained 62% sorbitol. It had been prepared by the reduction of dextrose. The organisms were obtained from the American Type Culture Collection. The sorbose was determined by the Schaffer–Hartmann[10] method using a factor of 1.25 for anhydrous sorbose. This factor was verified with pure sorbose isolated in these laboratories from fermentation of sorbitol. The crystalline sorbose contains one molecule of water of crystallization.

Preliminary experiments showed a temperature of 25 to 30° to be optimum and the experiments to be described were made at 25°. It was also found that disturbing the flasks to take samples materially reduced the yield of sorbose. Hence each datum presented represents the analysis of a separate flask. This procedure would disclose any variation in results due to possible differences in inoculation or other technique.

In Table I are given data comparing the action of *Acetobacter xylinum* and *A. suboxydans*. The medium contained, per 100 cc., 15.4 g. of sorbitol and 0.5 g. of yeast extract. One hundred-cc. portions of medium were used in 300-cc. Erlenmeyer flasks. The marked superiority of *A. suboxydans* is at once evident. The concentration of sorbitol is several fold that previously reported.

TABLE I

THE PRODUCTION OF SORBOSE BY THE ACTION OF *Acetobacter xylinum* (I) AND OF *A. suboxydans* (II) ON SORBITOL

Days	Conversion of sorbitol, % I	II
7	13	59
9	23	81
11	28	79
12	33	81

TABLE II

THE EFFECT OF SURFACE–VOLUME RATIO (SQ. CM. AREA PER 1 CC. VOLUME) UPON THE YIELD OF SORBOSE FROM 10.0% SORBITOL

Days	Surface–volume ratio 2.360	1.195	0.589	0.345	0.200	0.119
1.5	78	67	41	28	15	10
2.5	84	82	58	42	26	14
3.5	84	84	77	56	35	21
4.5	85	84	80	63	39	24
5.5	87	84	81	72	45	30
6.5	87	84	82	80	48	..
7.5	89	84	82	80	53	31

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(2) G. Bertrand, *Compt. rend.*, **122**, 900 (1896).
(3) G. Bertrand, *Ann. chim. phys.*, **8**, 3 (1904).
(4) R. Sazerac, *Compt. rend.*, **137**, 90 (1903).
(5) Hooft, visser't, "Bio chemurche onderzoekingen over het Geslacht Acetobacter," Thesis, Delft, 1925.
(6) H. J. Waterman, *Centr. Bakt. Parasitenk.*, [2] **38**, 451 (1913).
(7) J. Böeseken and J. L. Leefers, *Rec. trav. chim.*, **54**, 861 (1935).
(8) A. J. Kluyver and F. J. de Leeuw, *Tijdschr. verg. Geneesk.*, **10**, 170 (1924).
(9) K. Bernhauer and B. Görlich, *Biochem. Z.*, **280**, 375 (1935).
(10) P. A. Schaffer and A. F. Hartmann, *J. Biol. Chem.*, **45**, 365 (1920).

In Table II are given data for several surface–volume ratios (sq. cm. area per 1 cc. volume). The data show that both the rate of yield of sorbose and the final yield increase with increasing surface–volume ratio. These studies, together with aeration, are being extended.

In Table III are data for the action of the organism on varying concentrations of sorbitol at two surface–volume ratios (0.589 (a) and 1.195 (b)). It is evident that the rate of yield of sorbose is decreased with increasing concentrations of sorbitol while the final yields are not appreciably altered. The final yield of sorbose is practically independent of the concentration of sorbitol up to and including 35.0%. There is a marked drop in activity at 40%. At the former concentration of sorbitol the concentration of sorbose reaches the high value of 28 g. per 100 cc. of medium.

TABLE III

THE EFFECT OF VARIOUS CONCENTRATIONS OF SORBITOL UPON THE PRODUCTION OF SORBOSE AT TWO SURFACE–VOLUME RATIOS: (a) 0.589, (b) 1.159

Concn. sorbitol	Yield of sorbose after x days, %									
	2	3	4	5	6	7	8	9	11	14
5.0 (a)	72	74	76	78	78	78	80	..	..	80
7.6 (a)	59	70	76	83	83	83	83	..	..	83
10.0 (a)	67	79	79	79	79	79	79	..	..	79
10.0 (b)	80	83	..	..	..	84	..	..	..	..
12.6 (a)	34	56	62	79	79	79	80	79	82	83
15.0 (a)	24	47	54	77	77	81	81	81	82	81
15.0 (b)	72	..	..	..	70	86	..	..	85	84
17.5 (a)	19	36	45	70	65	74	76	76	79	81
20.0 (a)	16	31	43	65	..	71	74	76	79	81
20.0 (b)	..	64	..	..	..	80	..	..	83	83
25.0 (b)	..	49	..	..	..	73	..	..	82	85
30.0 (b)	..	34	..	..	..	62	..	..	76	78
35.0 (b)	16	..	..	..	..	67	..	..	81	80
40.0 (b)	3	..	..	..	..	9	..	..	14	14
45.0 (b)	2	..	..	..	..	3	..	..	4	4
50.0 (b)	2	..	..	..	..	..	..	..	2	2
55.0 (b)	2	..	..	..	..	..	..	..	2	2

Analysis of the data at the surface–volume ratio of 0.589 showed that, up to an 80% yield

$$\% \text{ yield} = m \log t + b \qquad (1)$$

The value of m proved to be independent of the concentration of sorbitol while the value of b varied according to the relation

$$b = c \times (\% \text{ sorbitol}) + d \qquad (2)$$

For the above surface–volume ratio, yields of sorbose up to 80% may be calculated, with reasonable accuracy, by the relation.

$$\% \text{ yield} = 80 \log t - 2.72 \times (\% \text{ sorbitol}) + 55.2 \qquad (3)$$

The time required to give an 80% yield at various concentrations of sorbitol, at the above surface–volume ratio, may be calculated by the equation

$$\log t = 0.034 \times (\% \text{ sorbitol}) + 0.31 \qquad (4)$$

Similar relations may be developed for other surface–volume ratios.

The sorbose is easily prepared from the above fermentation. The medium is filtered, evaporated in vacuum to a small volume and allowed to crystallize. The first fraction is about 95% pure sorbose. This material is easily purified by recrystallization.

Summary

Data have been presented concerning the production of sorbose by the action of *Acetobacter suboxydans* on various concentrations of sorbitol. The yield of sorbose is a linear function of log t up to a value of 80%. In concentration of sorbitol up to and including 40%, the rate of yield of sorbose is decreased by increased concentration of sorbitol while the final yield is little affected. In concentrations of sorbitol of 40% and above there is little action by the organisms. The employment of these high concentrations of sorbose affords an easy method for the large-scale production of sorbose with the handling of minimum volumes of liquids. The sorbose is readily prepared by filtering the medium and evaporating the filtrate to the required volume for crystallization.

AMES, IOWA

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