VINEGAR BASE PRODUCTION FROM WASTE PINEAPPLE JUICE

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SUMMARY

The effects of sugar concentration, aeration, addition of the yeast nutrients ammonium phosphate and ammonium sulphate, and variation in the number of yeast cells in the inoculum on fermentation rate and efficiency and yeast growth in the alcoholic fermentation process were studied.

Aeration gave a substantial initial increase in the rate of cell growth and the rate of alcohol production, but after 24 hr the amount of alcohol produced was the same as with no aeration.

Addition of ammonium phosphate or ammonium sulphate had no effect on alcohol production or cell population.

When inoculum size was increased sixfold to 54 x 10^6 cells per ml, the time for completion of fermentation of juice with and without added sugar was reduced, in the former case from 40 hr to 16 hr.

The rapid fermentation method involving large inoculum was not assessed on flavour of the vinegar stock produced.

I. INTRODUCTION

In Queensland, the skins and cores of pineapples are comminuted and pressed to extract most of the juice, which is blended with flesh juice and the mixture then processed by bottling or canning. However, the pressed skins and cores, at present used for stock food, still contain juice which could be extracted by a second, more severe pressing.

The use of pineapple juice or pineapple waste for vinegar manufacture has been mentioned by Cruess (1958), Hromatka and Ebner (1959) and Collins (1960).

Since the sugar content of pineapples used for canning in Queensland ranges from 9 per cent. in winter to 13 per cent. in summer (Leverington 1962), and as Joslyn (1955) and Cruess (1958) reported that any fruit with a sugar content of more than 8 or 9 per cent. can be used for the manufacture of vinegar, it appeared that the juice from a second extraction could be utilized for the manufacture of a fermented product such as vinegar. A project was accordingly undertaken to establish conditions for the fermentation of pineapple juice for vinegar production.

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The two processes involved are alcoholic fermentation of the juice and acetification of the resultant stock. The former process usually consists of a primary fermentation taking 3-7 days and a secondary fermentation of several weeks' duration. Usually a wine yeast (*Saccharomyces ellipsoideus*) is used to ferment the sugar and a temperature of 24-27°C is maintained to encourage yeast activity. A large population of yeast cells in the mash is necessary for rapid production of ethyl alcohol, and there must be a sufficient supply of oxygen for the growth and multiplication of yeast cells (White and Munns 1951). The prepared stock should preferably have a reducing sugar content below 0·3 per cent. (Joslyn 1955). This is a prerequisite for rapid and extensive oxidation of ethyl alcohol to acetic acid by the acetic acid bacteria, which have been found to be dependent on carbohydrates for growth initiation. Once this has occurred, ethyl alcohol is utilized as a source of carbon and energy (Rao and Stokes 1953). The concentration of alcohol in the stock is important. Low concentrations of 1-2 per cent. ethyl alcohol are unsatisfactory, as aroma and flavour suffer and insufficient alcohol is present to produce the legal minimum content of 4 g acetic acid per 100 ml on acetification. On the other hand, concentrations higher than 14 per cent. alcohol result in incomplete oxidation of ethyl alcohol and inhibit the growth of acetic acid bacteria (Prescott and Dunn 1959).

This paper reports a study of the effects of sugar concentration, aeration, addition of the yeast nutrients ammonium phosphate and ammonium sulphate, and variation in the number of yeast cells in the inoculum on fermentation rate and efficiency and yeast growth in the alcoholic fermentation process.

II. MATERIALS AND METHODS

(i) *Pineapple Juice.*—As at present no second extraction of pineapple residues is carried out in Queensland, juice of the exact composition expected to be used eventually was not available. However, some residue was pressed a second time between battens and cloths in a hydraulic press and about 5 gal of this juice was obtained. This was combined with 7 gal of first-extraction juice, pasteurized, and used for the majority of the fermentation experiments. Other fermentations were carried out on pasteurized first-extraction juice. In each case the juice was centrifuged to give less than 1 per cent. insoluble solids. Pasteurization was carried out by passing the juice through a heat exchanger at 82-85°C for 0·5 sec and allowing the hot juice to cool slowly. The juice was stored frozen in ½-gal polythene bottles at −18°C until required. "Pineapple juice" in this paper means pasteurized pineapple juice.

(ii) *Yeasts.*—Three strains of wine yeast (*Saccharomyces ellipsoideus*)—Nos. 318, 138 and 150—obtained from the Australian Wine Research Institute were used. They were maintained as slope cultures on Wickerham's malt-extract yeast-extract agar (Wickerham 1951). Fermentations and cultivation of the yeast were carried out at 28°C, except where otherwise stated. As Strain 318 gave the highest average yield of alcohol under the conditions of the earlier experiments in the series, it was subsequently adopted as the standard strain.

(iii) *Preparation of Inocula.*—Inocula for fermentations were prepared from 2½-day-old agar slope cultures. The growth from the slope cultures was washed off with pineapple juice into pineapple juice in sterile flasks. These pineapple juice cultures were incubated for 20 hr and then used as inocula. When large quantities of inoculum were required, the pineapple juice cultures were built up with tenfold increments in volume. To produce inocula with extra-high cell counts, quantities of pineapple juice yeast cultures were built up as described, and then the yeast cells were concentrated by allowing them to settle in the culture flask and decanting the supernatant, counting the cells in the sediment and resuspending in a volume of supernatant calculated to give the required cell count.

(iv) *Aeration.*—The equipment used for aerating the pineapple juice was a rotary shaker housed in an incubator maintained at 28°C. This shaker held 24 x 500-ml erlenmeyer flasks and was operated at a speed of 240 r.p.m. The flasks were plugged with cotton wool, which was held on by rubber bands so that it could not work out of the flasks during shaking.

(v) *Fermentation Procedure.*—For those experiments using the rotary shaker, 130-ml quantities of pineapple juice in sterile 500-ml erlenmeyer flasks were inoculated with 10 ml yeast cultured in pineapple juice as described. The flasks were fixed on the shaker, which was then set in motion, and samples were taken at predetermined times by removing duplicate flasks. To prevent the continuation of fermentation after the time of sampling, 1 ml of 1:80 aqueous mercuric chloride solution was added to the samples to kill the yeast and the samples were frozen until the time of analysis. Samples for cell counts were made by removing 1 ml of the fermenting liquid and adding to this sample an equal volume of 10 per cent. (v/v) formalin. This preserved the yeast cells until counts could be made.

(vi) *Determinations.*—Alcohol concentrations in distillates from fermentation samples were determined by the methods of the Association of Official Agricultural Chemists (1960, pp. 105, 140).

Hydrometer readings were found to be inaccurate, but alcohol determinations from refractive index and from specific gravity measured by pycnometer weighings were found to correspond well.

The chief sugars in pineapple juice are sucrose, glucose and fructose (Winton and Winton 1949). Estimations of fermentable sugars were made by determining the total reducing sugars in the juice (Association of Official Agricultural Chemists 1960, p. 424; Cruess 1949, pp. 413-6). Reducing sugar determinations by the Lane and Eynon method were then carried out after inversion. This method was found to be accurate for low concentrations of reducing sugars, but not for higher concentrations.

Cells were counted in a counting chamber with Improved Neubauer ruling. The chamber was 0·1 mm deep and the area of the smallest square was 0·0025 sq. mm.

III. EXPERIMENTS AND RESULTS

(a) Control Test

Three 2-1 erlenmeyer flasks each containing 1 1 of pineapple juice (11·9 per cent. reducing sugars) were inoculated with yeast Strains 318, 138, and 350 respectively, and were allowed to ferment at room temperature (20-28°C). After one week the yeast had settled to the bottom of the flask, and active fermentation as evidenced by the evolution of carbon dioxide had almost stopped. After 14 days, reducing sugar determinations were carried out; in all cases the sugar content was less than 0·2 g per 100 ml. However, the fermented juice had a stale taste and odour and had darkened to a greyish brown colour. Although the yeast had settled, the juice was not clear. On microscopic examination some small spherical yeast cells were found, distinct from the inoculated yeast, but attempts to isolate this contaminant were unsuccessful.

(b) Addition of Cane Sugar

Using the three strains of yeast, a group of fermentations was carried out with the sugar content of the pineapple juice increased to 20° Brix by the addition of cane sugar. The fermentations were done in a room at 21°C, as incubation at 28°C was not then practicable. Within 16 hr, fermentation had commenced, and it continued actively for four days. After six days most of the yeast had settled, and the supernatant juice was decanted into sterile 1-l flasks containing 5 ml of a 0·1 per cent. tannic acid solution intended to precipitate suspended matter in the juice. The suspended matter was not completely removed, but discolouration was not so evident as in the control test. Alcohol determinations were not carried out on this fermentation, but reducing sugar determinations made after four weeks were as follows:

Yeast Strain	*Reducing Sugars (g/100 ml)*
138	1·35
318	0·6
350	0·4

In a further experiment, 9 l of pineapple juice to which cane sugar had been added to give a concentration of 19 g sugar per 100 ml was fermented by Strain 350 at room temperature. Tannic acid at 2 g per l added before inoculation precipitated much of the suspended matter in the juice. After five days the fermentation was becoming less vigorous, so the juice was siphoned off the yeast sediment into another vessel, thus aerating the liquid and reactivating the suspended yeast. Alcohol determinations were made at the intervals shown in Table 1.

The yield of 10·6 per cent. (v/v) alcohol after 24 days represents 86 per cent. of the theoretical yield of alcohol from 19 g sugar per 100 ml. The fermented product was of poor quality, however, because the tannic acid was in excess and that not precipitated out of the juice gave it a very astringent taste. The product became cloudy and very dark on storage.

TABLE 1

INCREASE OF ALCOHOL CONCENTRATION WITH TIME

(Sugar content 19 g/100 ml)

Time After Inoculation (days)	Alcohol (% v/v)
7	8·5
12	9·6
20	10·1
24	10·6

(c) Aeration

Fermentations following 10-hr preliminary aeration and using the three strains of yeast were observed. Alcohol and reducing sugar determinations were made 50 hr after the time of inoculation. Results are shown in Table 2.

TABLE 2

FERMENTATION AFTER AERATION

(Preliminary aeration 10 hr ; estimations 50 hr after inoculation)

Yeast Strain	Flask No.	Ethyl Alcohol (% v/v)	Reducing Sugars (g/100 ml)
318	1	5·5	
	2	5·8	
	3	5·8	0·13
	4	5·5	
138	1	4·8	
	2	5·8	
	3	4·8	0·10
	4	4·8	
350	1	4·1	
	2	4·8	0·11
	3	4·8	

The reducing sugar determinations in this case were made on the pooled juice from the three or four flasks of each yeast after removal of the samples for alcohol determination. The best yield of alcohol was 5·8 per cent. (v/v) from Strain 318. This is equivalent to 90 per cent. yield from the sugar in the juice (10·0 g reducing sugar per 100 ml).

Using an inoculum of Strain 318, 24 flasks were aerated for 10 hr. The progress of fermentation was followed by taking samples at 5-hr intervals and determining alcohol and reducing sugars. In this case the sugars in the juice were not inverted before the sugar determinations were carried out, so the true course of sugar utilization was not followed. The results are shown in Table 3 and Figure 1.

TABLE 3

FERMENTATION AFTER AERATION

(Preliminary aeration 10 hr ; estimations 5–72 hr after inoculation)

Time After Inoculation (hr)	Alcohol (% v/v)	Reducing Sugars (g100ml)
10	2·0	2·75
10	1·3	2·25
15	1·6	1·15
15	2·8	1·55
15	2·3	1·40
20	3·2	0·45
20	3·2	1·25
20	3·25	1·61
25	3·8	0·61
25	4·5	0·60
25	4·0	0·51
30	4·0	0·26
30	4·0	0·16
30	4·4	0·37
35	4·8	0·57
35	5·2	0·56
40	4·2	0·45
40	5·5	0·36
45	5·2	0·26
45	5·2	0·18
50	5·2	0·17
72	5·7	0·13

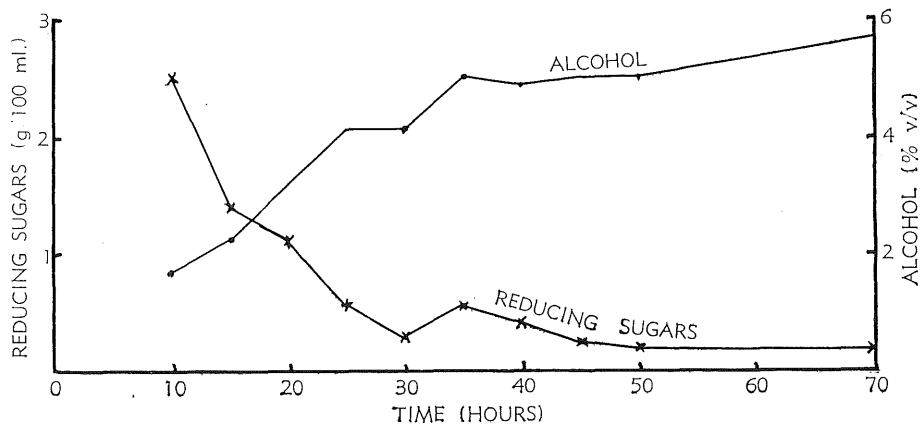

Fig. 1.—Changes in reducing sugars and alcohol with time after aeration for 10 hr.

The effects of various other periods of aeration on the rate of alcohol production were also observed. Reducing sugar determinations were not carried out. The results are shown in Table 4 and Figure 2.

TABLE 4

FERMENTATION AFTER AERATION

(Preliminary aeration 0–15 hr ; estimations 5–50 hr after inoculation)

Time After Inoculation (hr)	Alcohol (% v/v)			
	Aeration Period (hr)			
	0	5	10	15
5		0·7		
		0·7		
10			—	
			2·1	
12	1·9	2·7	2·7	
	1·6	2·8	2·7	
15				3·7
				3·2
24	4·6	5·6	4·7	
	4·5	5·2	4·7	
36	—	5·6	5·5	5·2
	5·7	5·8	4·9	4·8
51	5·9	5·6	5·8	
	5·8	5·9	5·0	

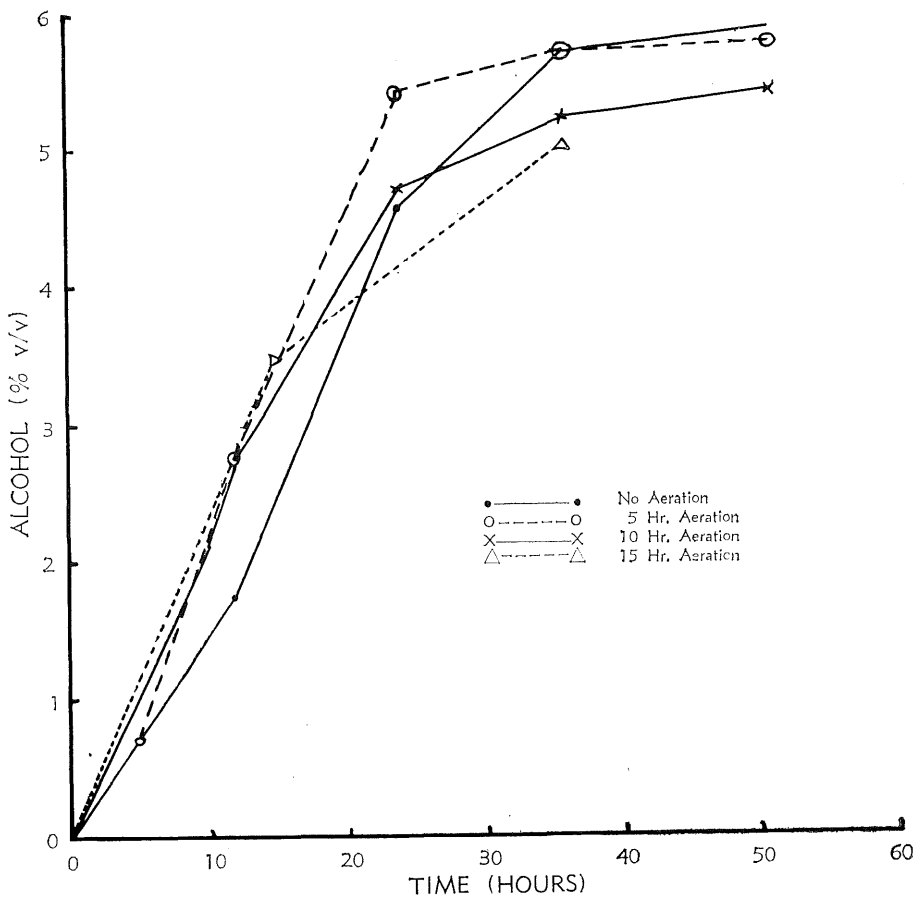

Fig. 2.—Effect of aeration for various periods on alcohol production.

In further comparisons, also with Strain 318, cell counts were made and alcohol was determined for duplicate samples at each sampling time. Reducing sugar determinations were made at the cessation of aeration and at 24 hr. Determinations after 48 hr were made on 5-hr and 10-hr aeration samples. Results are shown in Tables 5 and 6 and depicted graphically in Figures 3 and 4.

TABLE 5

FERMENTATION AND YEAST CELL DEVELOPMENT AFTER AERATION

(Preliminary aeration 0–10 hr ; estimations 0–48 hr after inoculation)

Time After Inoculation (hr)	No Aeration			Aeration 5 hr			Aeration 7½ hr			Aeration 10 hr		
	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol
0	8	10·4	0·0									
	8	9·6	0·3									
2½	13		0·1	14	9·9	0·0						
	14		0·1	13	9·0	0·2						
5	21		0·2	53	8·1	0·7						
	22		0·1	45	8·0	0·8						
7½	53		0·8	97		1·6	133	7·7	1·4			
	38		0·8	85		0·8	120	6·6	1·4			
10	48		1·1	141		2·0	170		2·0	171	5·8	2·4
	48		1·0	126		1·9	186		2·2	234	6·8	2·4
24	143	2·5	4·5	152	1·8	4·7	202	2·0	4·5	317	1·8	4·5
	148	2·7	4·3	135	2·3	4·7	189	2·0	4·4	238	1·8	4·5
48				212	0·6	5·4				267	0·11	5·4
				238	0·4	5·4				258	0·23	5·0

Cells = millions of cells/ml.

R.S. = g reducing sugar/100 ml.

Alcohol = % alcohol (v/v).

TABLE 6

FERMENTATION AFTER AERATION

(Preliminary aeration 0–10 hr ; estimations 24 and 48 hr after inoculation)

Period of Aeration (hr)	Period of Fermentation (hr)	Initial Sugar Content (g/100 ml)	Alcohol Content (g/100 ml)	Yield of Alcohol (%)
0	24	7·4	3·6	95
5	48	9·5	4·3	89
7½	24	8·0	3·5	85
10	48	9·8	4·3	86

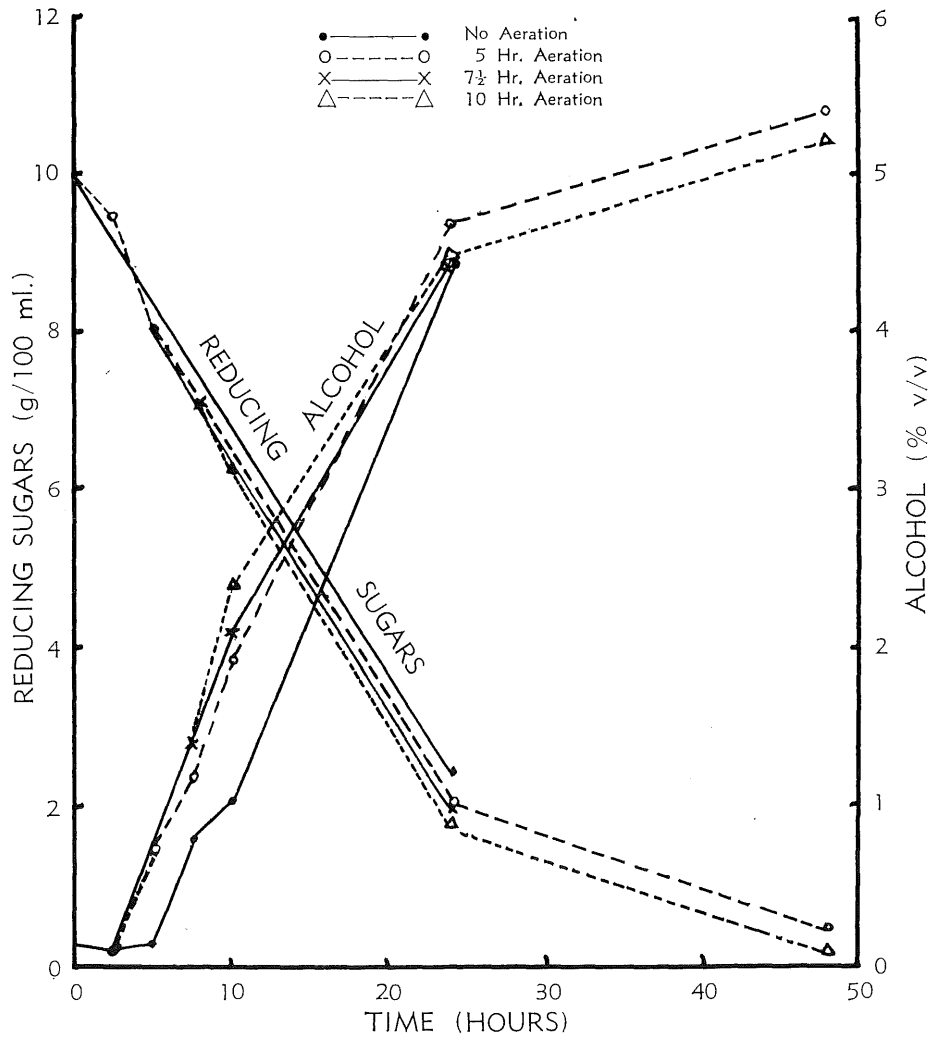

Fig. 3.—Effect of aeration for various periods on reducing sugars and alcohol concentrations.

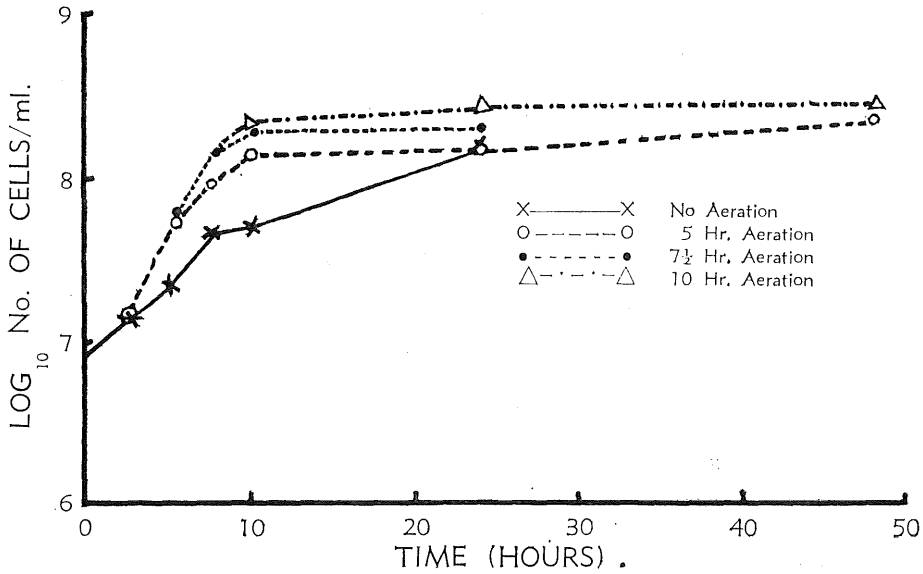

Fig. 4.—Effect of aeration for various periods on cell population.

(d) Addition of Phosphorus and Nitrogen

Cane sugar and diabasic ammonium phosphate were added to pineapple juice to see the effect on fermentation with 7½ hr aeration. Cane sugar was added to give an increase of 5 g per 100 ml in the total reducing sugar content of the juice.

Pineapple juice contains on an average 90 p.p.m. phosphorus and 640 p.p.m. nitrogen by weight (Tressler and Joslyn 1954). The amount of 400 p.p.m. diabasic ammonium phosphate added to the juice in this experiment gave an increase of 108 p.p.m. phosphorus and 49 p.p.m. nitrogen, representing a substantial increase in phosphorus concentration but not in nitrogen content.

Cell counts and alcohol and reducing sugar determinations, carried out in duplicate, are set out in Table 7 and shown graphically in Figures 5 and 6.

TABLE 7

EFFECT OF ADDITION OF NUTRIENTS TO FERMENTING PINEAPPLE JUICE

Time After Inoculation (hr)	Pineapple Juice			Pineapple Juice + $(NH_4)_2HPO_4$			Pineapple Juice + Sugar			Pineapple Juice + $(NH_4)_2HPO_4$ + Sugar		
	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol
0	3·7	9·9	0				3·5	15·0	0			
	3·5	9·6	0				3·1	14·4	0			
7½	63	9·0	0·75	68	8·0	0·75	48	14·0	0·55	72	13·6	0·55
	80	8·6	0·75	55	8·2	0·55	68	13·8	0·55	57	13·0	0·55
24	215	1·9	4·6	113	1·8	4·6	239	5·1	4·5	207	3·9	4·6
	226	2·0	4·6	181	1·7	4·6	208	5·4	4·5	200	4·2	4·6
48	74	0·3	5·6	243	0·3	5·4	175	0·9	7·4	213	1·0	7·6
	184	0·3	5·6	184	0·3	5·4	175	1·5	6·4	209	1·0	7·4

Cells = millions of cells/ml.
R.S. = g reducing sugars/100 ml.
Alcohol = % alcohol (v/v).

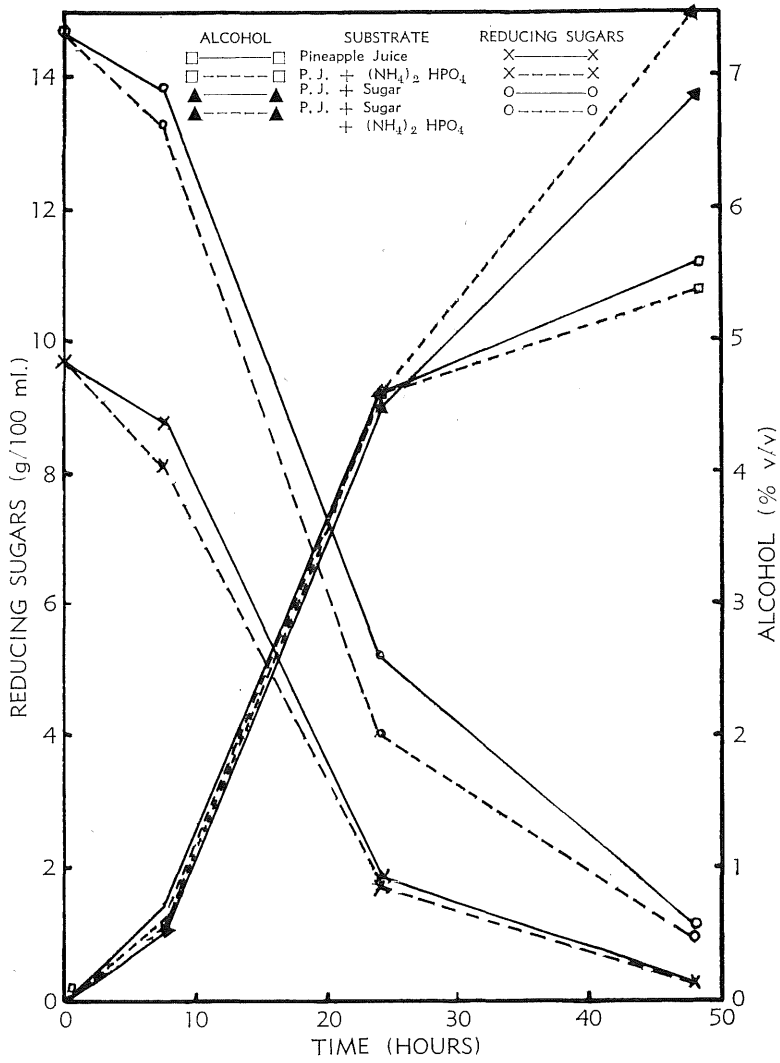

Fig. 5.—Effect of addition of nutrients on reducing sugars and alcohol concentrations.

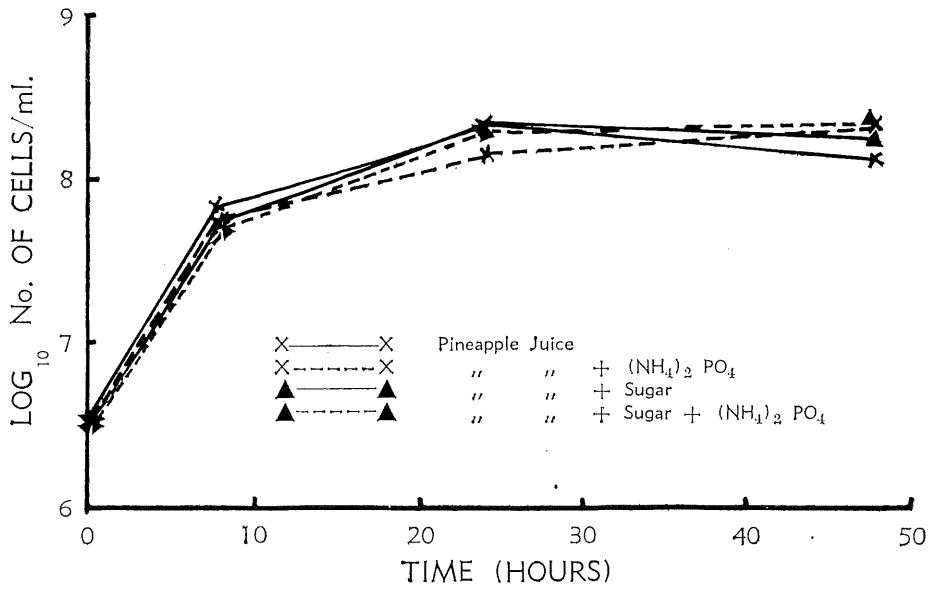

Fig. 6.—Effect of addition of nutrients on cell population.

With added sugar the fermentation is not complete in 48 hr, but on the amount of sugar used in this time a yield of 79 per cent. of the theoretical yield of alcohol is obtained. The yield from the juice without added sugar is 86 per cent.

The effect on fermentation of added ammonium sulphate with and without added sugar was observed. At inoculation, the cell count was 4 x 10^7 cells per ml. The approximate average nitrogen content of the pineapple juice was 640 p.p.m., and this was increased by 300 p.p.m. by the addition of ammonium sulphate. Cane sugar was added to one series at the rate of 6·4 g per 130 ml. Duplicate flasks from each series were taken as samples at various periods up to 40 hr after inoculation. The results are shown in Table 8 and Figure 7.

The addition of ammonium sulphate had little effect on the yield of alcohol where there was no added sugar in the juice, but gave a much higher yield of alcohol with added sugar. However, the yield without added sugar was lower than that previously obtained from the same juice using the same conditions. It appeared that the yeast had become less vigorous, so the experiment was repeated, going back to the original culture for preparing the inoculum. The results are shown in Table 9 and Figure 8.

TABLE 8

EFFECT OF ADDITION OF NUTRIENTS TO FERMENTING PINEAPPLE JUICE (1ST SERIES)

Time After Inoculation (hr)	Pineapple Juice				Pineapple Juice + Sugar				Pineapple Juice + $(NH_4)_2SO_4$				Pineapple Juice + Sugar + $(NH_4)_2SO_4$			
	Alcohol	R.S.	Cells	pH	Alcohol	R.S.	Cells	pH	Alcohol	R.S.	Cells	pH	Alcohol	R.S.	Cells	pH
0	0·4	8·5	45	—	0·4	12·4	37	3·3	0·4	8·2	53	3·3	0·5	12·6	50	3·3
	0·5	8·4	46	3·35	0·4	12·8	39	3·3	0·4	8·2	39	3·3	0·5	12·9	40	3·35
6	1·5	6·8	59	3·25	1·1	12·1	51	3·2	1·5	6·4	81	3·2	1·3	10·6	72	3·15
	1·5	6·4	78	3·2	1·1	12·0	73	3·2	1·5	7·0	80	3·2	1·1	11·4	82	3·2
16	3·2	5·1	82	3·2	2·8	7·8	80	3·2	3·1	2·9	112	3·15	3·0	8·5	97	3·1
	3·3	5·3	77	3·1	*	*	73	*	3·2	2·6	106	3·1	3·3	7·3	90	3·1
20	4·0	1·5	52	3·2	3·9	7·1	84	3·15	4·0	2·1	82	3·1	4·4	5·8	110	3·15
	4·3	1·5	144	3·15	4·2	6·6	92	3·1	4·2	1·8	92	3·1	3·7	6·4	100	3·1
24	4·3	1·4	144	3·2	4·6	5·8	95	3·15	3·8	2·2	110	3·4	6·1	4·8	86	3·35
	4·1	1·5	85	3·2	4·7	5·2	109	3·15	4·0	1·3	125	3·4	6·5	5·4	111	3·4
40	—	—	—	—	4·7	0·84	180	3·4	—	—	—	—	6·1	2·0	109	3·4
	—	—	—	—	4·5	2·2	126	3·35	—	—	—	—	6·5	2·6	145	3·4

* Accident to sample

Alcohol = % alcohol (v/v)

R.S. = g reducing sugar/100 ml

Cells = millions of cells/ml.

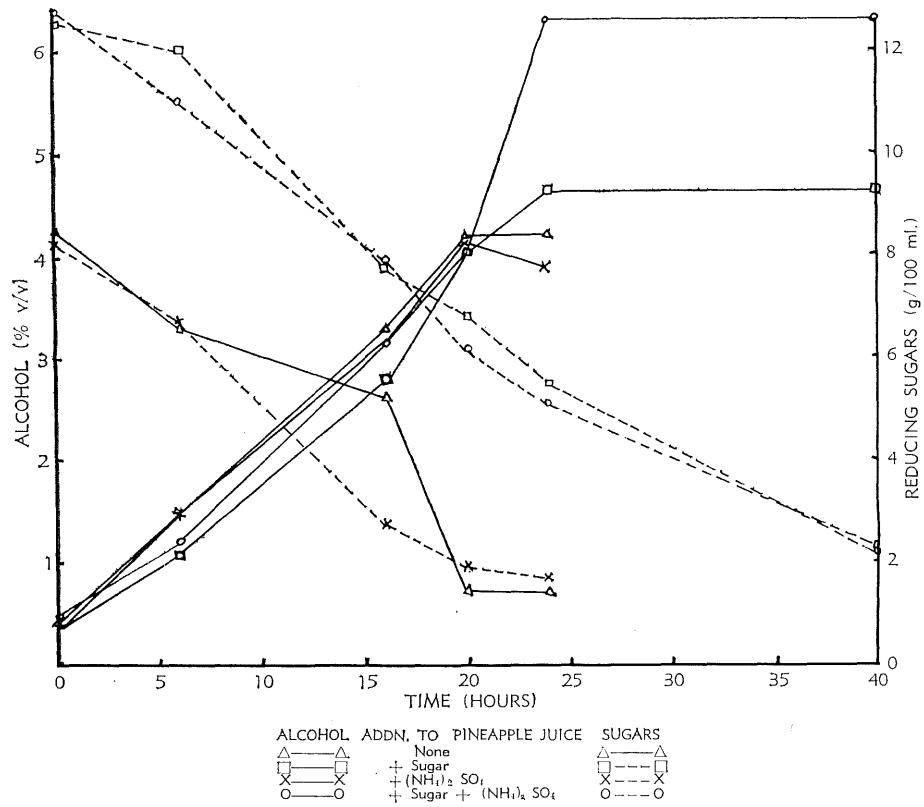

Fig. 7.—Effect of addition of nutrients on reducing sugars and alcohol concentrations.

TABLE 9

EFFECT OF ADDITION OF NUTRIENTS TO FERMENTING PINEAPPLE JUICE (2ND SERIES)

Time After Inoculation (hr)	Pineapple Juice			Pineapple Juice + Sugar			Pineapple Juice + $(NH_4)_2SO_4$			Pineapple Juice + Sugar + $(NH_4)_2SO_4$		
	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells
0	6·7	0·5	57	11·6	—	55	7·1	0·5	54	12·3	0·4	38
	6·7	0·5	58	10·9	0·4	46	7·2	0·5	57	11·2	0·5	61
16	2·1	4·0	107	6·3	4·2	101	1·4	3·8	112	5·8	4·5	89
	1·5	4·1	95	4·8	4·6	126	1·6	3·9	99	6·2	4·5	95
20	1·1	4·5	129	3·5	5·6	156	1·2	4·3	119	5·2	5·0	106
	1·0	4·3	147	3·4	5·6	161	1·5	4·4	124	3·2	4·8	90
24	1·0	4·6	106	3·3	5·3	128	—	4·3	134	3·6	5·3	108
	0·7	4·6	129	—	5·7	100	—	4·7	127	1·6	5·4	108
40	0·2	4·8	127	1·1	6·8	132	0	4·8	130	0·9	6·8	126
	0·4	4·8	121	1·2	6·8	104	0	4·8	126	1·4	6·8	122

R.S. = g/100 ml reducing sugars.

Alcohol = % alcohol (v/v).

Cells = millions of cells/ml.

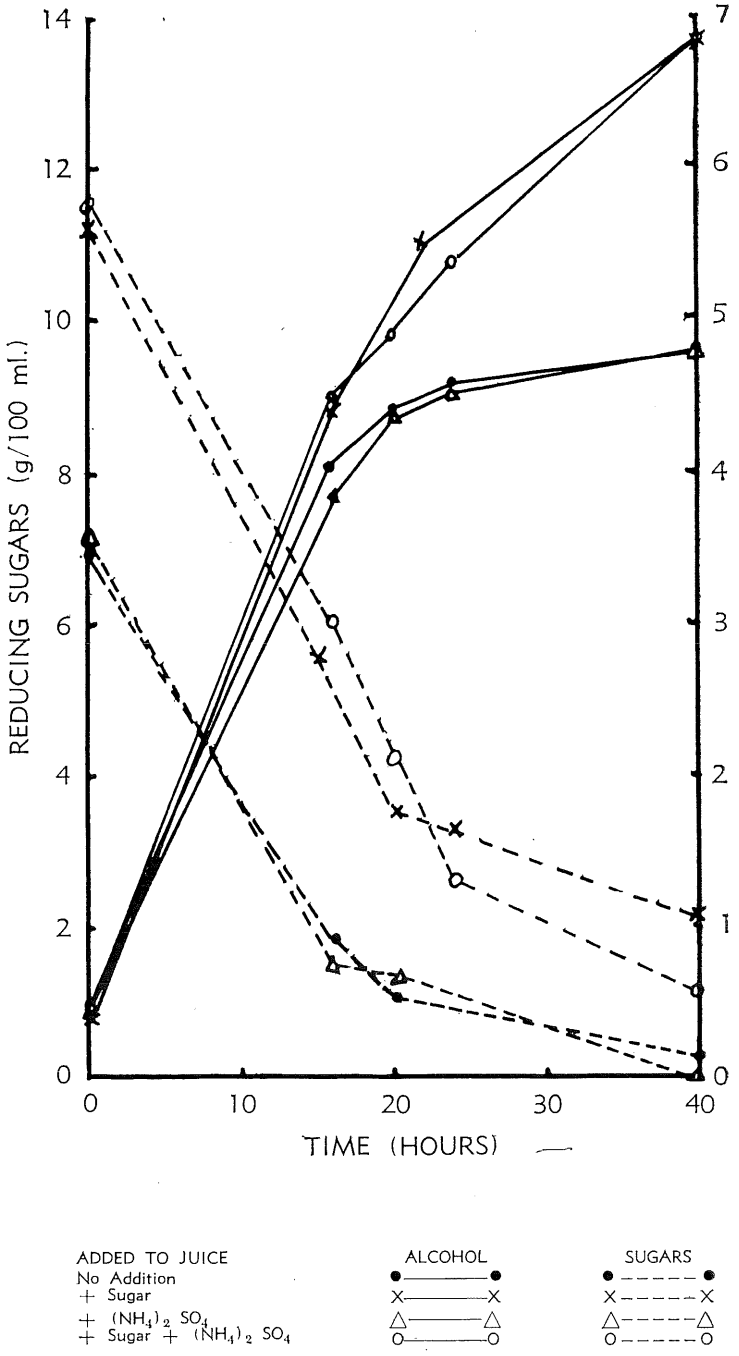

Fig. 8.—Effect of addition of nutrients on reducing sugars and alcohol concentrations.

(e) Variation in Number of Yeast Cells

An approximately fivefold increase in the number of cells in the inoculum was compared with a normal inoculum in juice with and without added sugar. Cell counts were made and alcohol and reducing sugars were determined in duplicate. Cane sugar was added to give an increase of 5 g per 100 ml in the total reducing sugars (6·4 g cane sugar to 130 ml pineapple juice). Reducing sugar determinations gave a lower reading than the calculated figure. The cause of this discrepancy is not known. Results are shown in Tables 10 and 11 and depicted graphically in Figures 9 and 10.

TABLE 10

EFFECT OF INOCULUM SIZE AND ADDED SUGAR ON ALCOHOL PRODUCTION

Time After Inoculation (hr)	Normal Inoculum			Large Inoculum			Large Inoculum with Added Sugar		
	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells	R.S.	Alcohol	Cells
0	9·3	0·3	10	9·3	0·5	65	12·4	0·5	53
	9·3	0·3	9	9·1	0·4	53	12·9	0·5	54
16		3·1	130	0·9	5·3	208		6·1	232
		3·0	115	0·6	5·5	185		6·0	202
24		4·5	171		5·5	224		7·5	261
		4·6	142		5·4	238		7·6	280
40	0·4	5·4	163	0·3	5·6	225	0·5	8·1	226
	0·4	5·55	162	0·3	5·55	222	0·4	8·2	275
48		5·6			5·55			8·2	
		5·6			5·55			8·1	

R.S. = g reducing sugar/100 ml.
Alcohol = % alcohol (v/v).
Cells = millions of cells/ml.

TABLE 11

EFFECT OF INOCULUM SIZE AND ADDED SUGAR ON YIELD OF ALCOHOL

Time After Inoculation (hr)	Initial Sugar Content (g)	Alcohol Content (g)	Yield of Alcohol (%)	Inoculum Used
40	8·9	5·3	93	Normal
16	8·45	4·95	91	Large
40–48	14·25*	8·15	90	Large + sugar

* Calculated value allowing for dilution by the inoculum.

As all fermentations appeared complete at 40 hr as shown by no increase in alcohol content at 48 hr, it was considered unnecessary to carry out sugar determinations and cell counts on the 48-hr samples.

The application of the method just described to fermentation on a larger scale was tested, 1·6 l of inoculum with a cell count of 4·6 x 10^8 cells per ml being added to 13·6 l of pineapple juice with total reducing sugar content of 9·0 g per

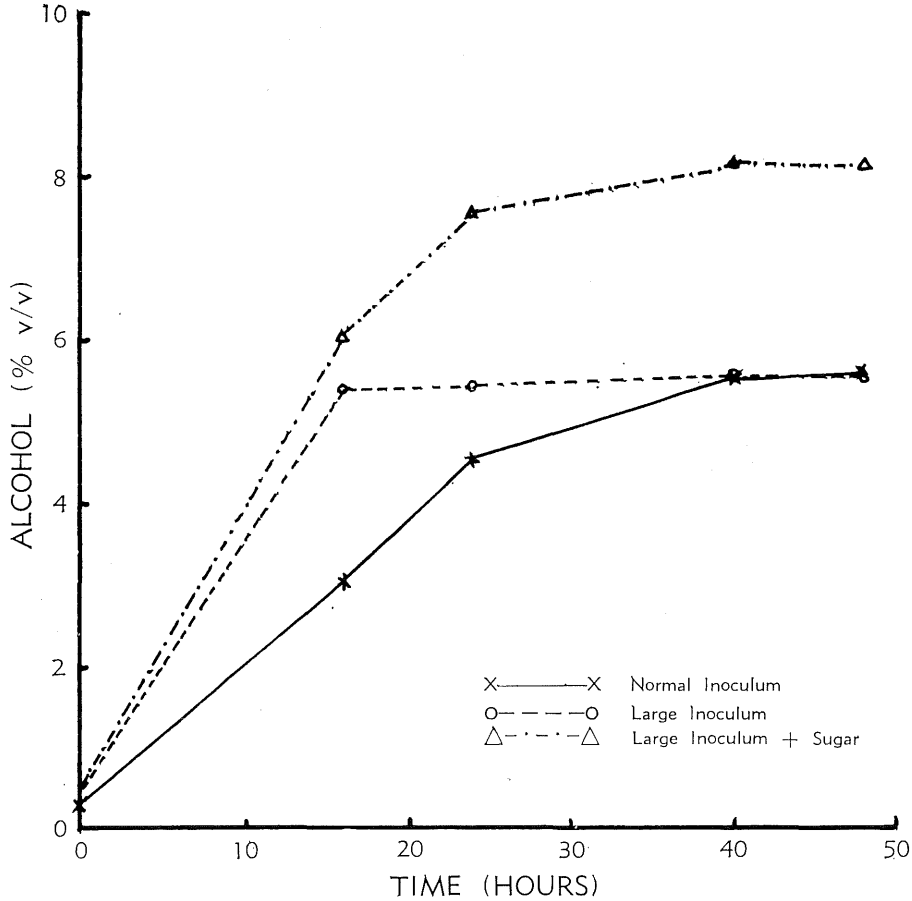

Fig. 9.—Effect of inoculum size and added sugar on alcohol production.

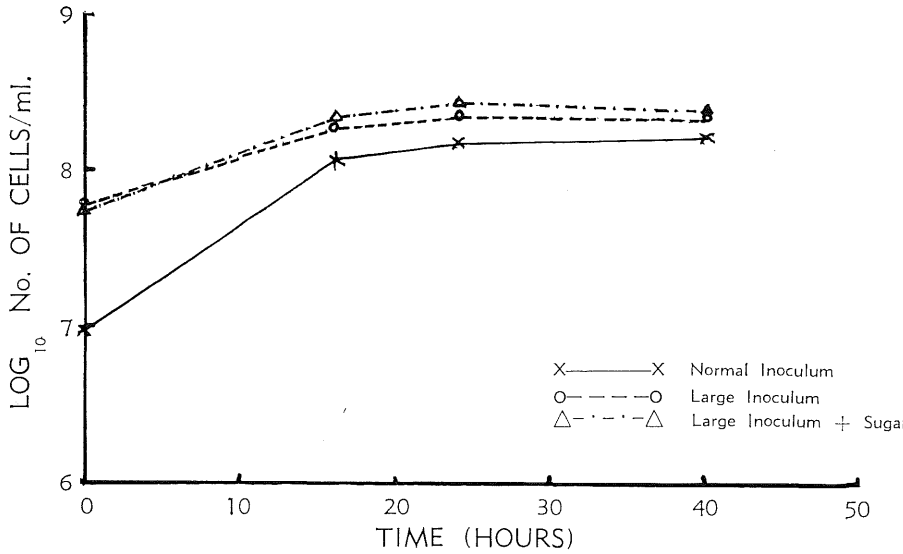

Fig. 10.—Effect of inoculum size and added sugar on cell population.

100 ml. The reducing sugar content of inoculated juice was then 8·2 g per 100 ml and the alcohol 0·6 per cent. (v/v). After 16 hr fermentation the reducing sugar content was 0·3 g per 100 ml and the alcohol content 5·0 per cent. (v/v). That is, in 16 hr, 4·4 ml alcohol was produced from 7·9 g reducing sugars, which is equivalent to a yield of 87 per cent. After 24 hr fermentation the reducing sugar content was 0·12 g per 100 ml and the alcohol content 5·15 per cent. (v/v), equivalent to a yield of 88 per cent.

IV. DISCUSSION

A typical two-stage alcoholic fermentation—rapid alcohol production during 3-7 days after inoculation, followed by a slow increase in alcohol content for a further two weeks—occurred in trials in which only the original sugar content of the juice was varied.

Though Cruess (1958) reported that the first stage is usually conducted commercially at 24-27°C, which is regarded as the optimum range for yeast activity, in these experiments slow fermentation at temperatures well below 28°C did not give a product of good flavour or appearance.

The aeration procedure employed gave a substantial initial increase in the rate of cell growth and the rate of alcohol production. Continued aeration stimulated growth and multiplication of the yeast cells, but also resulted in increased alcohol production in comparison with unaerated controls and samples which had a shorter aeration period (Figures 3 and 4). The degree of aeration achieved apparently was insufficient to allow the cells to assimilate sugar aerobically, and so alcoholic fermentation took place. Evolution of carbon dioxide and the cotton-wool plug acting as a barrier to gas diffusion probably had some effect on the sufficiency of aeration.

After 24 hr the amounts of ethyl alcohol produced following various periods of aeration were approximately the same as that produced with no aeration.

The addition of 400 p.p.m. ammonium phosphate had no effect on alcohol production in pineapple juice with or without added sugar (Figure 5 and Table 7), though a slight increase in the rate of sugar utilization was observed. There was no effect on cell population as compared with the controls (Figure 6), and there was little increase after 24 hr. The drop in cell numbers in juice to which no ammonium phosphate had been added was probably due to autolysis. Therefore a phosphate deficiency was not a limiting factor to cell growth or to fermentation. The addition of ammonium sulphate had no effect on alcohol production, sugar utilization or growth of yeast cells. An increase in the sugar concentration had the effect of lengthening the time for completion of fermentation. In this case the fermentation of 14·7 g sugar per 100 ml was not complete in 48 hr, though without added sugar fermentation was practically complete in 40 hr.

When the initial inoculum size was increased from an average of 9 x 10^6 to 54 x 10^6 cells per ml, that is sixfold, it was found that the time for completion of fermentation of juice without added sugar was reduced from 40 hr to about 16 hr and that juice with added sugar was fermented in 40 hr compared with more than 48 hr with a small initial inoculum.

It took about 10 hr for a sixfold increase in cell numbers when a small initial inoculum was used and there was no aeration. With aeration, the time for sixfold increase was halved, but 40 hr was still required for completion of fermentation.

With the large inoculum there was still considerable growth of yeast, which stopped soon after 16 hr even in the case with added sugar, where alcohol production continued for a further 24 hr. Oxygen was presumably the factor limiting growth in this case, as there was still adequate sugar present. The large inoculum produced a larger final population than the small inoculum. Use of a large inoculum on an increased volume of pineapple juice resulted in a slightly reduced yield, though the fermentation was complete in 16 hr as in the small-scale fermentation. A different batch of juice was used for the large-scale experiment and this may have been the reason for the fall in yield.

The results of experiments with the use of different numbers of cells in inocula were in agreement with White and Munn's (1951) conclusion that large inocula gave more rapid sugar utilization and also gave considerable growth of yeast. Therefore more rapid fermentation was achieved by using a large initial inoculum than by aerating the juice, after inoculation with a smaller number of cells, to build up the yeast population.

Under industrial conditions the cells from the lees of one batch could be resuspended in the following one and used as a starter, provided that appropriate controls on the purity of the yeast were maintained.

The effect of accumulation of carbon dioxide on fermentation rates was not investigated. No attempt was made to remove carbon dioxide at any stage in the experiments.

Most fermentations for the preparation of vinegar stock are carried out slowly in order to obtain a satisfactory flavour. The quality of the vinegar stock produced from pineapple juice by a rapid fermentation method has not been assessed on flavour.

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