STUDIES ON THE ALCOHOL TOLERANCE OF YEASTS[1]

WILLIAM D. GRAY

Department of Botany, Miami University, Oxford, Ohio

Received for publication February 28, 1941

That microorganisms grown in culture will eventually cease growth and sometimes die is known to all who have carried on pure culture work. Cessation of growth may be due to one of three factors: (1) depletion of nutrients, (2) unfavorable environmental conditions, or (3) the so-called "staling effect" which is due to the accumulation of products of the organism's own metabolism. When cessation of certain physiological activities occurs even when environmental conditions are favorable and there is an ample supply of nutrient materials in the medium, it is evident that this cessation is due to the third of the above-mentioned factors. It is a well-known fact that in the commercial production of alcohol by fermentation there is a certain alcohol concentration which cannot be exceeded, regardless of the amount of sugar supplied to the yeast cell. It is obvious that after a certain period the yeast is no longer able to produce alcohol, and, since fermentation always stops about when the same alcohol concentration is attained (if the same yeast strain is always used), it is logical to assume that for every strain of yeast there is a maximum alcohol concentration above which the yeast ceases to function. A number of workers have observed that different yeasts will vary in the amount of alcohol they produce (e.g., distillery yeasts and wine yeasts); these differences might possibly be due to differences between the yeasts in their abilities to tolerate alcohol. Of course, during the alcoholic fermentation many substances other than alcohol are formed, but ethyl alcohol and carbon dioxide are

[1] Investigation conducted in the Microbiology Laboratory of Jos. E. Seagram and Sons, Inc.

produced in greatest quantity. Carbon dioxide can stop yeast growth when the concentration is too high (largely through the exclusion of oxygen), but this is much less likely to be a cause of stopping a fermentation than the building up of a high alcohol content, since we know that maximum alcohol content is obtained when oxygen concentration is low (Pasteur, 1859, 1860).

Guilliermond and Tanner (1920) as well as others have mentioned the differences in fermenting ability of different yeasts, so it is possible that these observed differences might be due to differences between the yeasts in their abilities to tolerate alcohol. For that reason, in this investigation a number of species and strains of yeast have been studied from the standpoint of their ability to ferment glucose under various conditions of alcohol concentration of the medium. Uniform conditions were maintained throughout, and the test fermentations which were run (particularly those in which there was a comparatively low initial alcohol content) roughly simulated the latter part of a normal fermentation in that sugar content was low and some alcohol was present. The yeasts used are listed below with the same number under which they are maintained in the Seagram Yeast Stock Culture Collection.

1. *Saccharomyces cerevisiae* Hansen. "D.C.L. Strain."
2. *Saccharomyces cerevisiae* Hansen. "S.C. Strain." Isolated from a ripe yeast tub originally inoculated with D.C.L..
3. *Saccharomyces cerevisiae* Hansen. "R Strain." A European strain reputed to be particularly good for rye fermentations.
4. *Saccharomyces cerevisiae* Hansen. "L.3 Strain." Probably another variety or strain of the D.C.L. culture.

15. "Black Yeast." Obtained from the Department of Agricultural Bacteriology, University of Wisconsin. Occurring as a contaminant of commercial yeast.
17. *Torula lactosa* Kluyver.
18. *Torula cremoris*
19. *Zygosaccharomyces soja B* Takahashi and Yukawa. Used in the preparation of Japanese rice beer.
20. *Willia anomala* Steuber (probably Variety IV, since it produces small amounts of ethyl alcohol). Used as a ripening yeast for Japanese rice beer.

21. "Yeast 21." Tokay wine yeast. Started from lees of an old fermentation.
22. "A Strain." A fission type which imparts a distinctive raspberry-like odor to mash after twenty-four hours.
23. "Yeast 23." A Burgundy wine yeast.
24. *Saccharomyces ellipsoideus* Hansen.
25. *Schizosaccharomyces pombé* Lindner. Used in the preparation of south African negro millet beer.
26. *Schizosaccharomyces mellacei* Jörgensen. Used in preparation of Jamaican rum from molasses.
27. *Schizosaccharomyces vordermani* (Went and Prinsen-Geerligs) ?.
28. *Saccharomyces cerevisiae* Hansen. "Rasse M Strain."
29. *Saccharomyces cerevisiae* Hansen. "Rasse XII Strain."
30. "Yeast 30." Occurred as a contaminant in Vitamin B_1 free wort.
31. *Saccharomyces cerevisiae* Hansen. Brown-Forman Strain.
32. "Yeast 32." Occurred as a contaminant in an open fermenter originally set with the D.C.L. strain.

MATERIALS AND METHODS

Test fermentations were run in 50 ml. Erlenmeyer flasks, each containing a final volume (medium, alcohol, and yeast suspension) of 25 ml. The medium used was 10 per cent yeast water with approximately 1.25 per cent glucose, adjusted to pH 4.30–4.38. Acidity measurements were made with a Leeds-Northrup pH Electrometer. The 10 per cent yeast water was prepared by autoclaving 100 gm. of fresh, starch-free distiller's yeast in 1000 ml. of distilled water and then centrifuging off the yeast cells. Alcohol (95.2–96.5 per cent neutral spirits) was added in increasing amounts to successive flasks, so that for a single series of flasks, the only variable was alcohol concentration. A control flask, to which no alcohol had been added, was run with each series.

Inoculum was prepared from large, seventy-two-hour slant cultures; the yeast was washed off quickly in distilled water, centrifuged, re-suspended so that the yeast concentration of the inoculum was 0.25 gm./ml., and immediately pipetted into the culture flasks. Each flask received 0.5 ml. of inoculum, so that the final yeast concentration in each flask was approximately 0.005 gm./ml.; sterile cultures were maintained throughout. Immediately after yeasting the initial sugar content was determined; with only three exceptions, the initial sugar content was between 0.911 and 1.267 per cent. Flasks were then incubated for 24 hours at 30°C. After twenty-four hours had elapsed, final sugar content of each flask was determined. The set-up for a typical test fermentation series is illustrated in table 1.

TABLE 1

Preparation of flasks for test fermentations

Each flask received 20 ml. of medium (1.25 per cent glucose in 10 per cent yeast water) and 0.5 ml. of yeast suspension (0.25 gm. yeast/ml.).

FLASK NUMBERS	NEUTRAL SPIRITS	PERCENTAGE ALCOHOL* (BY WEIGHT)	WATER
	ml.		*ml.*
1, 1A	1.25	4.76	3.25
2, 2A	1.50	5.71	3.00
3, 3A	1.75	6.66	2.75
4, 4A	2.00	7.61	2.50
5, 5A	2.25	8.57	2.25
6, 6A	2.50	9.52	2.00
7, 7A	2.75	10.47	1.75
8, 8A	3.00	11.42	1.50
9, 9A	3.25	12.38	1.25
10, 10A	3.50	13.32	1.00
11, 11A	3.75	14.28	0.75
12, 12A	4.00	15.23	0.50
13, 13A	4.25	16.18	0.25
14, 14A	4.50	17.14	0.00
Controls	0.00	0.00	4.50

* Determinations made with Zeiss Immersion Refractometer.

RESULTS

Fifteen flasks of medium were prepared as outlined in table 1. The results obtained with no. 1 (*Saccharomyces cerevisiae*) are presented in table 2.

As may be seen from table 2, Yeast 1 works efficiently with respect to glucose utilization in media containing ethyl alcohol in concentrations not exceeding 7.61 per cent by weight. At concentrations higher than this, the amount of glucose utilized is much smaller; roughly speaking, we may say that the per cent of glucose utilized varies inversely with alcohol concentration although it is certainly not a straight line function. Throughout the remainder of this paper a numerical value will be assigned to each yeast to designate its alcohol tolerance; alcohol tolerance is arbitrarily defined as "the maximum percentage of alcohol (by weight) at which percentage of glucose utilization is no more than one per cent below the percentage glucose utilization in the

TABLE 2

Effect of alcohol concentration upon percentage glucose utilization by yeast 1

Initial sugar content of each flask was 10 mg./ml.

FLASK NUMBER	PERCENTAGE ALCOHOL (BY WEIGHT)	FINAL (24-HOUR) SUGAR	PERCENTAGE GLUCOSE UTILIZED
		mg./ml.	
1	4.76	0.242	97.58
2	5.71	0.241	97.59
3	6.66	0.256	97.44
4	7.61	0.325	96.75
5	8.57	1.150	88.50
6	9.52	7.024	29.76
7	10.47	8.487	15.13
8	11.42	8.527	14.73
9	12.38	9.900	1.00
10	13.32	10.080*	0.00
11	14.28	10.210*	0.00
12	15.23	10.230*	0.00
13	16.18	10.340*	0.00
14	17.14	10.400*	0.00
Control	0.00	0.238	97.62

* Probably due to increase in sugar concentration brought about by slight evaporation of alcohol from flasks containing high alcohol concentrations.

control flask of the same series." Using this definition, an alcohol tolerance of 7.61 may be assigned to Yeast 1.

On the basis of their alcohol tolerances all of the yeasts investigated may be divided into six groups:

Group I. Yeasts of extremely low alcohol tolerance

Three yeasts may be classified in this group: nos. 32, 20, and 15. Data obtained from test fermentations are given in table 3.

It is evident that none of the yeasts of Group I could be used efficiently in a commercial alcoholic fermentation, because even at alcohol concentrations of only 4.76–4.82 per cent no. 32 (which is the most efficient of the three) can utilize only 77.2 per cent of the available glucose. The results in table 3 indicate a very erratic behavior for no. 15; this, however, may be ascribed to the difficulty of preparing an evenly distributed suspension of this particular yeast, since it forms an extremely gelatinous type of growth both on agar slants and in liquid media.

TABLE 3

Percentage utilization of glucose by yeasts of extremely low alcohol tolerance

PERCENTAGE ALCOHOL (BY WEIGHT)	NO. 32	NO. 20	NO. 15
4.76- 4.82	77.2	47.3	3.18
5.71- 5.79	71.3	27.5	7.27
6.66- 6.75	65.3	11.9	3.51
7.61- 7.72	45.6	10.6	24.36
8.56- 8.68	35.9	8.1	0.49
9.52- 9.65	28.0		2.11
10.47-10.61	27.5		1.06
11.42-11.58	17.3		0.00
12.37-12.54	15.5		0.00
13.32-13.51	15.4		0.00
14.28-14.47	12.9		0.00
15.23-15.44	10.9		0.00
16.18-16.40	7.6		0.00
17.13-17.37	10.9		0.00
Controls	97.1	96.6	3.51

A low alcohol tolerance could almost have been predicted for no. 20, since it produces little ethyl alcohol in its own metabolism. Test fermentations gave evidence of only mere traces of alcohol having been produced. Guilliermond and Tanner (1920) state that most of the genus *Willia* (of which no. 20 is a member) produce ethers.

Group II. Yeasts of low alcohol tolerance

Four yeasts may be placed in this group: nos. 2, 22, 19, and 17. All of the members of this group are much more efficient than any of the Group I types, but are scarcely suitable for use in an industrial fermentation with the possible exception of no. 22, which imparts a distinctive raspberry-like odor to mash. This yeast conceivably could be used in a fermentation in which it was desirable to sacrifice yield for flavor. Percentage glucose utilization by yeasts of Group II are presented in table 4.

TABLE 4

Percentage utilization of glucose by yeasts of low alcohol tolerance (Group II)

PERCENTAGE ALCOHOL (BY WEIGHT)	NO. 2	NO. 22	NO. 19	NO. 17
4.76– 4.82	97.4	96.4	94.7	92.6
5.71– 5.79	97.3	96.1	92.1	76.1
6.66– 6.75	95.1	95.8	91.8	64.5
7.61– 7.72	91.4	92.3	80.9	52.2
8.56– 8.68	76.7	57.4	56.7	50.8
9.52– 9.65	55.8	35.5	49.7	52.2
10.47–10.61	40.3	25.6		53.7
11.42–11.58	25.6	24.5		52.2
12.37–12.54	17.6	21.3		47.1
13.32–13.51	12.9	18.6		39.2
14.28–14.47	12.1	11.4		34.3
15.23–15.44	8.2	7.9		24.4
16.18–16.40	6.0	7.3		8.3
17.13–17.37		8.1		6.6
Controls	98.0	98.3	96.3	96.7

Group III. Yeasts of alcohol tolerance 6.66–7.72

In this group are placed three yeasts whose alcohol tolerances are high enough that they could scarcely be placed in the low alcohol tolerance and yet are still somewhat below the alcohol tolerances of those yeasts in Group IV (yeasts of average alcohol tolerance). The group is a rather diverse one in so far as species are concerned: no. 1 is the D.C.L. strain of *Saccharomyces cerevisiae*, no. 18 is *Torula cremoris*, and no. 26 is *Schizosaccharomyces mellacei*. The behavior of these three yeasts well-illustrates that alcohol tolerance classifications cannot be made on the basis of genera. Results of test fermentations conducted with yeasts of Group III are presented in table 5.

TABLE 5

Percentage utilization of glucose of yeasts of alcohol tolerance 6.66–7.72 (Group III)

PERCENTAGE ALCOHOL (BY WEIGHT)	NO. 1	NO. 26	NO. 18
4.76- 4.82	97.6	97.3	97.5
5.71- 5.79	97.6	97.3	97.5
6.66- 6.75	97.4	97.2	97.3
7.61- 7.72	96.8	96.7	94.3
8.56- 8.68	88.5	96.5	70.3
9.52- 9.65	29.8	94.9	57.4
10.47-10.61	15.1	85.2	37.5
11.42-11.58	14.7	68.1	37.3
12.37-12.54	1.0	59.5	36.7
13.32-13.51	0.0	53.9	32.9
14.28-14.47	0.0	55.7	23.2
15.23-15.44	0.0	47.9	12.6
16.18-16.40	0.0	39.9	9.4
17.13-17.37	0.0		7.3
Controls	97.6	97.6	97.7

TABLE 6

Yeasts of average alcohol tolerance (8.56–9.52)

PERCENTAGE ALCOHOL (BY WEIGHT)	NO. 24	NO. 30	NO. 25	NO. 21	NO. 29	NO. 4	NO. 23	NO. 27
4.76- 4.82	97.7	97.5	97.5	97.6	97.6	98.0	97.9	96.7
5.71- 5.79	97.7	97.4	97.4	96.4	97.7	97.9	97.9	96.8
6.66- 6.75	97.7	97.1	97.3	96.7	97.6	97.9	97.8	96.8
7.61- 7.72	97.7	96.4	97.3	96.9	97.5	97.8	97.7	96.6
8.56- 8.68	97.7	96.9	96.7	97.9	97.5	97.3	97.2	95.9
9.52- 9.65	97.0	95.4	87.2	62.4	96.2	93.8	95.0	95.4
10.47-10.61	93.7	92.5	77.5	36.4	78.4	75.7	94.5	95.6
11.42-11.58	79.6	81.4	84.1	14.4	51.4	52.7	69.1	92.0
12.37-12.54	70.8	67.8	53.1	6.4	35.3	37.2	42.4	64.4
13.32-13.51	52.1	66.2	41.5	0.0	25.1	49.3	43.0	61.0
14.24-14.47	40.6	58.3	18.6	2.2	16.7	64.7	31.6	60.3
15.23-15.44	38.8	52.3	14.1	0.6	10.7	26.3	15.6	46.8
16.18-16.40	34.0	50.4	3.4	0.0	13.5	32.3	14.8	33.1
17.13-17.37	15.4	39.8	4.9	0.0	9.5		17.2	24.5
Controls	97.9	97.8	97.5	97.1	97.7	98.0	97.8	96.8

Group IV. Yeast of average alcohol tolerance

Eight yeasts were found to have alcohol tolerances between 8.56 and 9.52 and are classified as yeasts of average alcohol tolerance because more forms fall into this particular group than into any other group. As in Group III several species of yeasts are represented. All of the yeasts of this group may be considered as being quite efficient and suitable for industrial use. Results of studies conducted with members of Group IV are summarized in table 6.

TABLE 7

Yeasts of high alcohol tolerance

PER CENTAGE ALCOHOL (BY WEIGHT)	NO. 28	NO. 3
4.76– 4.82	98.7	98.1
5.71– 5.79	98.5	98.0
6.66– 6.75	98.4	98.1
7.61– 7.72	98.5	97.8
8.56– 8.68	98.4	97.7
9.52– 9.65	98.4	97.8
10.47–10.61	97.7	97.5
11.42–11.58	46.6	94.5
12.37–12.54	43.1	78.7
13.32–13.51	28.3	55.2
14.24–14.47	17.2	38.5
15.23–15.44	8.9	31.0
16.18–16.40	0.0	8.3
17.13–17.37	0.0	14.7
Controls	98.6	98.1

Group V. Yeasts of high alcohol tolerance (10.47–10.61)

Two yeasts (strains of *S. cerevisiae*) are placed in this group. Yeast 3 is the "R Strain" reputedly good for rye fermentation, and no. 28 is the "Rasse M Strain"; both are of European origin. Their percentage glucose utilizations are given in table 7.

In addition to the yeasts classified above, one yeast (no. 31) exhibited an extremely high alcohol tolerance. This yeast was by far the best of all those studied, being able to utilize 96.8 per cent of the available glucose at an alcohol concentration of 11.58 per cent. A graphical representation of percentage glucose utilization of no. 31 is given in figure 1.

THE EFFECT OF TEMPERATURE ON ALCOHOL TOLERANCE

One series of fermentations was conducted at 35°C. in order to compare the behavior of a yeast at this temperature with that of

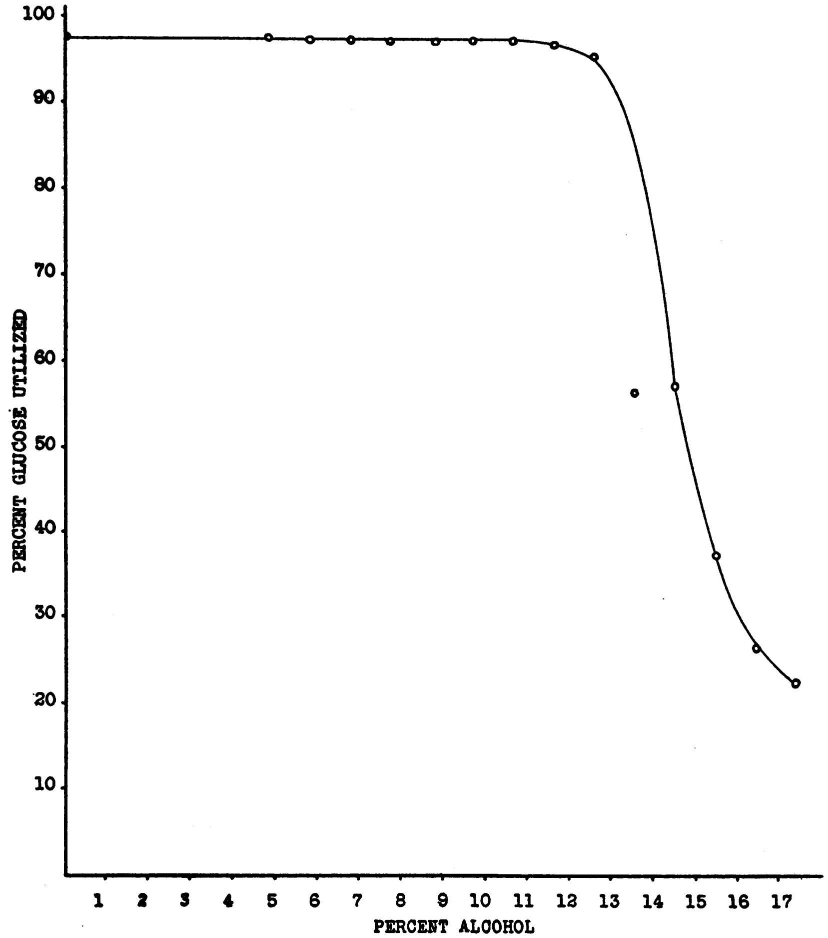

FIG. 1. THE EFFECT OF ALCOHOL CONCENTRATION ON PERCENTAGE GLUCOSE UTILIZATION BY YEAST 31

the same yeast operating at 30°C. Yeast 1 was used for this experiment, and it was found that at the higher temperature this yeast had a lower alcohol tolerance than it had at 30°C. Results are presented in table 8 and in graphical form in figure 2.

TABLE 8

The effect of temperature on alcohol tolerance of yeast 1

TEMPERATURE	FLASK NUMBER														CONTROL
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
°C.															
30	97.6	97.6	97.4	96.8	88.5	29.8	15.1	14.7	1.0	0.0	0.0	0.0	0.0	0.0	97.6
35	97.2	96.6	93.5	58.7	6.5	6.0	1.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0	97.5

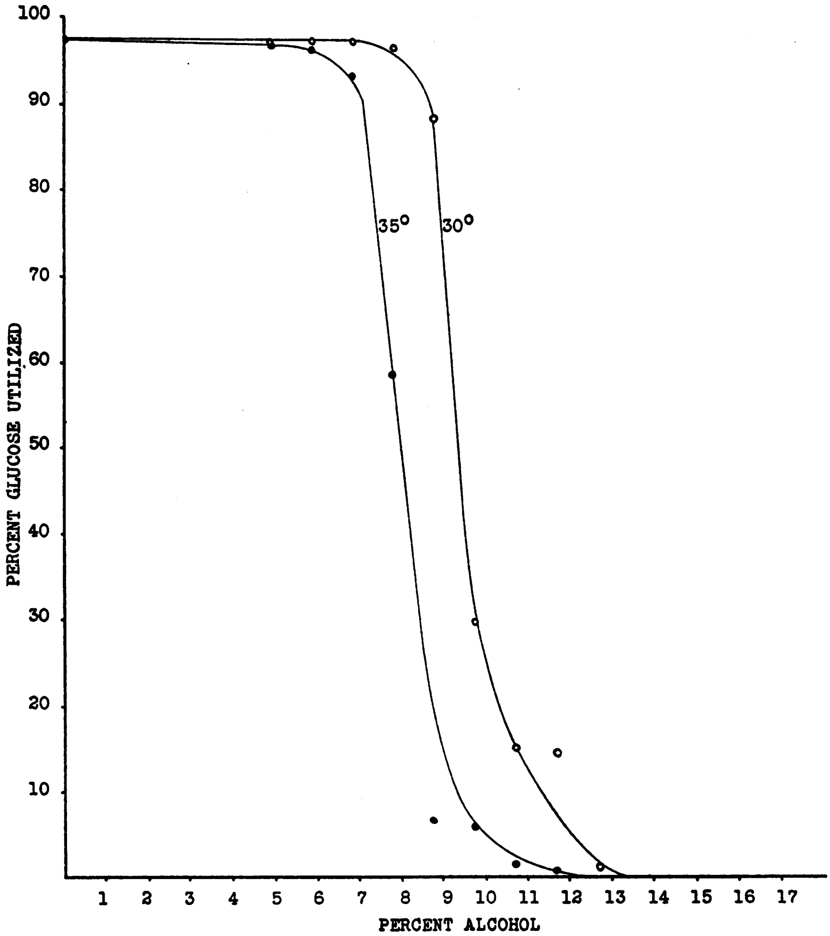

FIG. 2. THE EFFECT OF TEMPERATURE ON ALCOHOL TOLERANCE

Discrepancies in the lower parts of the curves are probably due to increase in sugar concentration due to slight evaporation from the flasks in which the initial alcohol was relatively high.

The above results more or less fit in with the findings of Müller-Thurgau as cited by Lutman (1929). This worker found that fermentation of one strain of yeast was stopped at 36°C. when the alcohol content (by weight) was 3.8 per cent, at 27° when the alcohol content was 7.5 per cent, at 18° when the alcohol content was 8.8 per cent, and at 9° when the alcohol content was 9.5 per cent.

THE EFFECT OF ALCOHOL ON FERMENTATION RATE

Since previous test fermentations had shown that at higher alcohol concentrations the ability to utilize glucose was diminished and also that considerable differences in alcohol tolerance existed between yeasts, it was thought advisable to run several experi-

TABLE 9

Effect of alcohol concentration on CO_2 output (expressed as average cmm./minute for the 30–60 minute period after inoculation)

YEAST NUMBER	ALCOHOL TOLERANCE	PER CENT NEUTRAL SPIRITS (95.2 PER CENT)								
		0	4	5	6	7	8	9	10	11
1	7.61	20.9	16.7	12.2	11.4	8.0	6.7	4.4	3.7	3.4
29	8.57	21.3	15.8	13.7	12.2	8.5	7.7	4.3	4.0	4.3
28	8.68	27.2	16.8	18.2	16.1	13.8	11.9	10.1	9.8	7.3

ments with the Warburg-Barcroft manometer in order to determine the effect of alcohol concentration on rate of fermentation as measured by CO_2 production. Nine flasks were run simultaneously in each experiment; the total volume of liquid in each flask was 2 ml. and was prepared as follows:

	ml.
10 per cent yeast water, 4 per cent glucose (pH 4.30–4.35)	1.0
Alcohol (4× desired concentration)	0.5
Yeast suspension (0.02 gm. yeast/ml.)	0.5

Yeasts employed in these experiments were nos. 1, 28, and 29, whose alcohol tolerances had been found to be 7.61, 8.68, and 8.57 respectively. Results of CO_2 output measurements made with these yeasts are summarized in table 9.

In the above table the yeasts are arranged in the order of increasing alcohol tolerance, and we see that with only two exceptions CO_2 production increased with alcohol tolerance. For any particular yeast, the higher the alcohol concentration the lower the rate of CO_2 production. It is evident that, since the results of these experiments parallel the results obtained by running test fermentations, CO_2 production could be used as an index to alcohol tolerance.

SUMMARY AND CONCLUSIONS

It is quite obvious that, under the conditions of these experiments, the yeasts differed widely in their toleration of alcohol. Thus, Yeast 31 utilized 97.5 per cent of the available glucose in an alcohol concentration of 4.67–4.82 per cent by weight, whereas no. 20 utilized only 47.3 per cent of the glucose at this same alcohol concentration. Definite numerical alcohol tolerance values were assigned to the various yeasts studied—primarily with the idea of industrial usability in mind. Of course, the alcohol tolerance of each yeast would be slightly higher than the assigned value, because in the twenty-four-hour fermentation some alcohol was produced; however, this would not exceed 0.5 per cent since the amount of glucose supplied was small.

It is evident that in selecting a yeast strain for a particular fermentation, alcohol tolerance should be considered or sugar losses may ensue. From the standpoint of yield alone the simplest solution is to select a yeast of known high alcohol tolerance, but this is not always feasible, particularly when a strain of yeast imparts an especially desired flavor. Losses can be prevented by determining the alcohol tolerance of the strain and then adjusting the glucose content of the material to be fermented so that it is not great enough to yield more alcohol than the yeast can tolerate. Observations on spent corn mash after distillation have shown losses of over five-hundred pounds of reducing sugar per fermenter (50,000-gallon capacity); when thirty such fermenters are set per week, the subsequent total loss of sugar is appalling. Quite possibly some of this sugar is not fermentable, but observations made by the writer have shown that at least 50 per cent of this residual sugar may be

attacked by the yeast cell (Yeast 1) when the fermented mash is stripped of its alcohol—this without adding anything to the spent mash or even readjusting the pH.

One experiment showed that rise in temperature is accompanied by decrease in ability to tolerate alcohol. Therefore, temperature is an important factor to be considered in fermentation work, if maximum yield is to be obtained.

Results obtained with fermentation rate measurements made by means of the Warburg-Barcroft manometer indicate that this apparatus may be used to determine alcohol tolerance, since CO_2 production varies inversely with alcohol concentration as does percentage glucose utilization, although in neither case did a straight line relationship exist.

High or low alcohol tolerance apparently is not peculiar to any particular genus or species, since both high and low alcohol tolerance yeasts may belong to the same genus. Likewise, one strain of a species may exhibit a high alcohol tolerance while another strain of the same species may have a low alcohol tolerance.

The writer is indebted to Dr. Paul Kolachov, Director of Research, and to Dr. William H. Stark, Director of the Microbiology Laboratory, Jos. E. Seagram and Sons, Inc., Louisville, Kentucky, for their helpful suggestions and courtesy throughout the course of this investigation.

REFERENCES

GUILLIERMOND, A., AND TANNER, F. W. 1920 The Yeasts. New York, John Wiley and Sons, Inc.

LUTMAN, B. F. 1929 Microbiology. New York, McGraw-Hill Company.

PASTEUR, L. 1859 Mémoire sur la fermentation alcoolique. Compt. rend. 48, 1149–1152. Also: Ann. chim. phys. 3-Ser., 58, 323–426. 1860.