

Study of the growth of yeast in ^{appr. 10} per cent ~~18~~ sucrose solution using ^{various concentration of} Urea as a source of nitrogen for it ~~under~~ ^{under} non-aerated condition -

Following substances were taken in each ^{flask} flask of 700cc Capacity.

- KH₂PO₄ - 1.8895 gms.
- K₂SO₄ - 0.1260 gms.
- NaCl - 0.0345 gm.
- CaCO₃ - 0.1205 gm.
- MgCO₃ - 0.220 gm.
- Water - 500 c.c.

The cultures contained 46.25 gms. of sugars obtained by 50 gms. of ~~sucrose~~ ^{sucrose} gur. The flasks were numbered and following amount of ammonium nitrate or urea was added in them.

- 1. — ammonium nitrate 5.7500 gms.
- 2 - Urea 2.1180 gms.
- 3 - Urea 1.0590 gms.
- 4 - Urea 0.5245 gm.
- 5 - Urea 0.2628 gm.

The cultures were made up to 500 c.c. with water and flasks were cotton plugged. Sterilisation was done at ¹⁰ lb. pressure for 20 min. and after cooling each culture ~~was~~ was seeded with a pure seed yeast. It required ~~two~~ nearly two months for complete fermentation and the temperature variation during this period was between 29.6 °C to 34.8 °C.

On analysis ~~the~~ culture gave the following results -

Growth of yeast using urea as nitrogen source.

Temp - 29.6°C to 34.8°C.

SN	gm. of urea present in the culture.	gm. mol of total acid produced.	gm. mol of Volatile acid produced.	gm. mol of non-volatile acid produced.	gm. of alcohol formed.	gm. of yeast produced.	yield of yeast in per cent of sugar fermented.
1	5.75 gms of ammonium nitrate.	4.0218	0.7240	3.2978	10.26	10.26	20.52
2	2.1180	16.4836	5.6840	10.7996	0.01	4.1120 2.5430	10.1 24.98
3	1.0590	16.2400	4.8720	11.3680	0.05	4.1720	9.01
4	0.5245	14.6160	3.2480	11.3680	0.06	4.2646	9.20
5	0.2648	4.8720	0.8120	4.0600	0.08	4.9936	10.89

Conclusion

If urea is used as source of nitrogen in the place of ammonium salts, ^{both} the growth of yeast and alcohol production ^{particularly alcohol formation is remarkably} ~~both~~ are lowered ^{lowered} ~~decrease~~ in the alcohol production ^{is} ~~remarkable~~ ^{decreased} so much ~~so~~ that at ~~20~~ 2.1180 gms in 500 c.c. ^{there is} ~~there is~~ almost no alcoholic formation. Acid production increases as the percentage of urea increases in the culture.

Study of growth of yeasts on the glucose solutions
obtained by the hydrolysis of barley, wheat and
potatoes.

Barley - Some barley flour was mixed with water and hydrolysed ~~with~~ by boiling for half an hour with hydrochloric acid. After hydrolysing for fairly a long period the solution was filtered and neutralised to bring ~~the~~ hydrogen ion concentration to pH 4.5. The amount of glucose now present in the solution was estimated.

Following substances were then added in the

solution	NH_4NO_3	-	1.53 gms.
	KH_2PO_4	-	0.5668 gm.
	$CaCl_2$	-	0.0308 gm.
	$MgSO_4$	-	0.0660 gm.
Total volume made to 400 c.c.			

F Flask containing the culture was ~~carefully~~ cotton-plugged and sterilised at 10 lb. pressure for 20 min. After cooling it was seeded with a little pure yeast.

~~Wheat~~ Wheat - Nearly 20 gms. of wheat flour was mixed with 300 c.c. of water and hydrolysed by hydrochloric acid ~~for~~ by boiling for half an hour. The solution was filtered and neutralised with sodium hydroxide to bring the pH near 4.5. Following substances were added in the culture.

K	KH_2PO_4	-	0.7558 gm.
K	$CaCl_2$	-	0.0482 gm.
	$MgCO_3$	-	0.0880 gm.
	NH_4NO_3	-	2.30 gm.

Total volume of the culture was made to ~~400~~ 400 c.c. and the flask after being cotton plugged was sterilised at 10 lb. pressure for 20 min. After cooling ~~the~~ culture was seeded with little pure yeast.

Potatoes — Nearly ~~100~~³⁰ gms. of potatoes were boiled, peeled, and pastefied. It was mixed with 300 c.c. of water and hydrolysed with hydrochloric acid by boiling for half an hour. After hydrolysis the solution was filtered and neutralised with alkali to bring the pH to 4.5. Following substances were added to it

- KH₂PO₄ — ~~0.38~~ 0.3779 gm.
- CaCO₃ — 0.0241 gm.
- MgCO₃ — 0.0440 gm.
- NH₄NO₃ — 1.15 gm.

Total volume of the whole bulk is made ~~up~~ to 400 c.c. and of flask after being cotton plugged was sterilised with at 10 lb. pressure of steam for 20 min.. After cooling the ~~the~~ culture ^{was} seeded with little amount of pure seed yeast.

After complete fermentation of the above cultures which took between 2 to 3 months, between 15.5°C to 20.4°C variation of temperature they were analysed and gave the following results.

Growth of yeast in glucose solutions obtained by the hydrolyses of barley, wheat and potatoes —

pH — 4.5

Temp 15.5°C to 20.4°C.

S.N.	experiment.	number of days taken in complete fermentation.	gm. of glucose originally present in the culture	gm. of alcohol formed after fermentation	gm. mol. of total acid produced.	gm. mol. of volatile acid formed.	gm. mol. of non-volatile acid produced.	gm. of yeast obtained	yield of yeast in % on the sugar fermented.
1	Barley	90	11.50	0.80	1.8250	0.0910	1.7340	2.2500	19.50
2	Wheat	90	12.00	0.10	1.2740	0.0091	1.2649	3.4900	29.08
3	Potatoes	61	8.00	0.50	1.5350	0.0728	1.4622	5.9680	73.75

CHAPTER I.

Comparative Study of Dhar yeast

I isolated this variety of yeast in 1946, when I was working for my D.Phil. degree, in the Chemistry Department of the University of Allahabad. I studied the properties and fermentation of this yeast and I was awarded D.Phil. degree on this work in 1949. I therefore need not give in detail the methods of its isolation, its morphological description and various fermentations which I have already given in my D.Phil. thesis. However for making this work complete I am giving here a short description.

Isolation of the yeast:

I, in collaboration with Prof. N.R. Dhar isolated Dhar Yeast from the toddy of Allahabad. The methods followed for this isolation were on the lines mentioned in the fifth last chapter of 'Isolation of Bacteria' of the book 'Practical Bacteriology', by Fred W. Tanner, Professor of Bacteriology and Head of the Department, University of Illinois.

~~Following procedure was adopted for the isolation of Dhar Yeast:~~

Procedure: A culture containing 0.2 gm. of calcium carbonate, 0.25 gm. of magnesium carbonate, 0.2 gm. of sodium chloride, 0.2 gm. of potassium sulphate, 0.2 gm. of disodium hydrogen phosphate was prepared. All these minerals were weighed in a flask, mixed with about 200 c.c. of water and the mixture was digested with dilute hydrochloric acid. To the clear solution was added 20 gms. of sucrose and the total volume was made

Conclusions

Potatoes are very good source for yeast production. In all the above cultures there is least production of alcohol but large amount of acids are formed. Wheat is a better source than barley for the yeast formation.

—X—

To study the ~~effect~~ growth of yeast formation in glucose solution obtained by the hydrolysis of potatoes.

50 gms. of potatoes were boiled, peeled and pastified. It was mixed with 300 c.c. of water and hydrolysed by dil. hydrochloric acid ~~for~~ by ~~for~~ boiling for half an hour. The solution was filtered and neutralised with sodium hydroxide till the pH becomes 4.5. Following substances were added in the ~~2~~ culture as mineral foods:

KH_2PO_4	-	0.3779 gm.
CaCO_3	-	0.0241 gm.
MgCO_3	-	0.0448 gm.
NH_4NO_3	-	1.15 gms.

Total volume of ^{each} ~~the~~ culture was made ~~up~~ to ~~400~~ 400 c.c. and after the flasks being cotton plugged they ~~it~~ ^{were} sterilised at 10 lb. pressure of steam for 20 min. After cooling the cultures ~~were~~ ^{were} seeded with pure yeast.

Two more ~~same~~ exactly the same similar cultures were prepared and seeded the same day. All the ~~three~~ cultures were kept ~~between~~ for fermentation between temperature variation of 30.4°C to 36°C .

The fermentation stopped with in a month and which was indicated by ~~the~~ the stopping of the growth of yeast ~~on~~ the surface of the culture. All the cultures were analysed and yielded the following results.

Growth of yeast on glucose obtained by potatoes analysis.

H.A.S.

temp. 30.4°C to 36°C.

S.N.	amount of the glucose in the culture before fermentation	days required for complete fermentation	gm. mol. of total acids produced.	gm. mol. of volatile acid generated	gm. mol. of non-volatile acids formed	gm. of alcohol produced.	gm. of yeast produced.	percentage of the yeast yield on the sugar fermented.
1.	10	27	2.4752	0	2.4752	0	8.4018	84.02
2.	10	27	2.1840	0	2.1840	0	8.5438	85.44
3.	10	27	2.1860	0	2.1860	0	8.3078	83.09

Above results confirm the fact that potatoes can be an ~~best~~ very good source of yeast.

Study of the growth of yeast in 5 per cent sucrose solution in presence of varying amount of cow's butter.

Three following cultures were prepared each containing the following substances.

Potassium dihydrogen phosphate	-	0.7358 gm.
Potassium sulphate	-	0.2016 gm.
Sodium chloride	-	0.0122 gm.
Calcium carbonate	-	0.0482 gm.
Magnesium carbonate	-	0.0880 gm.
Zinc sulphate	-	0.0500 gm.
Sucrose	-	20.00 gms.
Water	-	400 c.c.

All the substances were taken in ^{each} 700 c.c. flask and digested with little ~~am~~ dil. hydrochloric acid till the precipitates just dissolved. Total volume of the culture was 2.50 gms and 5.0 gms of cow's butter was added in two different flasks. Then made up to 400 c.c. and pH adjusted near 4.5. The flasks ~~was~~ ^{were} cotton plugged and sterilized at 10 lb steam pressure for 20 min.. After cooling they ~~was~~ ^{were} seeded with little amount of ~~seed~~ pure yeast.

After complete fermentation which took nearly one month the cultures were analyzed and ~~for~~ gave the following results -

Study of the growth of yeast in 3 per cent sucrose culture in presence of varying amount of cow's hilt & under non-aerated condition.

Temp. - 30.2°C to 35.2°C.

Serial number.	gms. of hilt added in the whole culture.	gms. ml. of total acid produced in the whole culture.	gms. ml. of volatile acid produced in the whole culture.	gms. ml. of non-volatile acid produced in the whole culture.	gms. of alcohol produced in the culture.	gms. of yeast formed in the fermentation.	number of days required for complete fermentation.
1	0.0 (Control)	0.4095	0.0364	0.3731	6.20	2.6536	32
2	2.50	2.9280	0.1220	2.8160	nil	6.2309	24
3	5.00	2.9262	0.0864	2.8398	nil	6.3806	24

Study of the growth of yeast ^{in oxygenation of the cultures} in one per cent sucrose solution ^{and} containing all other mineral salts, ~~or~~ oxygenation by bubbling air through it or adding hydrogen peroxide.

Three cultures were prepared containing the following substances.

Potassium dihydrogen phosphate	-	0.1889 gm.
Potassium sulphate	-	0.0504 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0120 gm.
Magnesium sulphate	-	0.0240 gm.
Zinc sulphate	-	0.0125 gm.
Ammonium nitrate	-	0.5730 gm.
Glucose Sucrose	-	5.00 gms.
Water	-	500 c.c.

Above substances were taken in ^{each} 700 c.c. flasks and digested with hydrochloric acid in 200 c.c. of water till the precipitates just dissolved. The total volume was made up to 500 c.c. and pH was adjusted near 4.5. The flasks were cotton plugged and sterilised under at 10 lb. steam pressure for 20 min.. After cooling they were seeded with pure yeast, and kept.

In one culture 2.0 c.c. of hydrogen peroxide was added. Another culture was kept as control and in the third sterilised air obtained by bubbling it through con. sulphuric acid and distilled water was ~~passed in steady stream~~ very slowly passed.

100 c.c. of cultures were taken out ~~by~~ each time for analysis and following results were obtained.

Effect of hydrogen peroxide and aeration of the growth of yeast -

Blank Culture. (non-aerated)

Temp. 22.5°C to 25.4°C .

S.N.	number of hours passed after seeding	gms. of sucrose fermented in each 100 c.c.	number of yeast cells in each view of microscope slide. #	gms. of yeast in 100 c.c. of culture.
1.	20	0.07	10	0.0057
2.	67	0.37	60	0.0348
3	92	0.88	175	0.1015

Hydrogen peroxide.

Temp. 22.0°C to 25.2°C .

S.N.	number of hours passed after seeding	gms. of sucrose fermented in 100 c.c.	number of yeast cells in in each view of microscope slide. #	gms. of yeast in 100 c.c. of the culture.
1	25	0.07	10	0.0058
2.	72	0.20	60	0.0346
3	95	0.88	250	0.1450.

Aerated Culture

Temp. 22.5°C to 25.0°C .

S.N.	number of hours passed after seeding	gms. of sucrose fermented in each 100 c.c. culture.	number of yeast cells in each view of microscope slide. #	gms of yeast in 100 c.c. of the culture.
1	19	0.01	10	0.0059
2.	73	0.13	80	0.0464
3	93	0.81	500	0.2901

Microscope - D. R. P. Leitz Wetzlar; Objective $\frac{1}{12}$ oc, Immersion Ernst Leitz Wetzlar, apert 1.30, 100x; Eye piece - Ernst Leitz Wetzlar Periplan O.K. 8x ..

Study of the effect of aeration of the growth of yeast in various concentration of sucrose solution.

Following cultures were prepared.

1. Two cultures containing following substances in 750 c.c. flasks.

K KH_2PO_4	-	2.2674 gm.
K_2SO_4	-	0.6048 gm.
NaCl	-	0.0266 gm.
$CaCO_3$	-	0.1446 gm.
$MgCO_3$	-	0.2640 gm.
$ZnSO_4$	-	0.1500 gm.
NH_4NO_3	-	2.30 gms.
Sucrose	-	60 gms.
water	-	600 c.c.

2. ~~Two~~ KH_2PO_4 cultures containing the following substances ~~in~~ 700 c.c. flasks ~~are~~ prepared.

Potassium dihydrogen phosphate	-	0.7558 gm.
Potassium sulphate	-	0.2016 gm.
Sodium chloride	-	0.0122 gm.
Calcium carbonate	-	0.0482 gm.
Magnesium carbonate	-	0.0880 gm.
Ammonium nitrate	-	2.30 gms.
Zinc sulphate	-	0.05 gm.
Sucrose	-	20 gms.
water	-	400 c.c.

3. Two cultures containing the following substances in 700 c.c. flasks were prepared.

Potassium dihydrogen phosphate	-	0.3779 gm.
Potassium sulphate	-	0.1008 gm.

Sodium chloride	-	0.0122 gm.
Calcium carbonate	-	0.0241 0.0440 gm.
Magnesium carbonate	-	0.0440 gm.
Zinc sulphate	-	0.0250 gm.
Ammonium nitrate	-	1.15 2.50 gms
Sucrose	-	10 20 gms.
Water	-	500 c.c.

4. Two cultures are prepared containing the following substances

Potassium dihydrogen phosphate	-	0.1889 gm.
Potassium sulphate	-	0.100 0.0504 gm.
Sodium chloride	-	0.0122 gm.
Calcium carbonate	-	0.0125 gm.
Magnesium carbonate	-	0.0220 gm.
Zinc sulphate	-	0.0175 gm.
Ammonium nitrate	-	0.5750 gm.
Sucrose	-	5 gms.
Water	-	500 c.c.

~~The~~ Each flask was first

The substances of each flask were digested with dil. hydrochloric acid till the whole ~~ppt~~ precipitate just dissolved and then pH ^{was} adjusted to 4.5. The 2 flasks were cotton plugged and sterilized at 5 lb. steam pressure for 20 min. and after cooling they were seeded with very little amount of pure yeast.

In one culture of each set ~~air~~ sterilised air ~~was~~ was passed through ^{very} slowly ~~current~~. Sterilisation of the air was done by ^{very} ~~passing~~ bubbling air through con. sulphuric acid and then to ~~ster~~ saturate it with water ~~vapour~~ vapours it was ~~passed~~ bubbled through distilled water.

The cultures were analysed after the fermentation completed and gave the following results.

Comparison of growth of yeast in aerated and non-aerated cultures of yeast at various concentrations using sucrose as the ^{Carbon} food of yeasts:—

S.N.	Percentage of the concentration of sucrose in the culture.	Qus. of total amount of sucrose present in the culture in (quantity)	Number of days required for complete fermentation.	Temperature	Condition	gm. vol. of total acid produced.	Percentage of acid formed.	Percentage of alcohol produced.	Percentage of acid production	gm. of yeast produced	Yield of yeast in 24 hours
1	10	60	45	28.5°C to 30°C	non-aerated	4.0218 6.66	6.66 20.00	20.00 33.33	33.33	12.26	20
2	10	60	31	28.5°C - 30°C	aerated	7.2063	12.00	16.20	27.00	15.25	25.40
1	5	20	35	26°C - 31°C	non-aerated	1.2640	6.020	4.42	22.10	4.1814	20.61
2	5	20	25	26°C - 31°C	aerated	2.0802	20.80	3.80	19.00	5.6840	28.42
1	2	10	20	25°C - 31°C	non-aerated	0.0882	0.88	2.01	20.10	2.7528	27.53
2	2	10	14	25°C - 31°C	aerated	2.0258	2.03	1.28	12.80	3.0821	30.82
1	1	5	12	28°C - 30°C	non-aerated	0.0402	0.82	0.86	16.20	1.5100	30.20
2	1	5	8	28°C - 30°C	aerated	0.075	1.50	0.29	5.8	1.7451	34.10 34.80

Conclusion

This aeration of yeast culture has beneficial effect on yeast growth. It decreases alcohol formation and minimises the fermentation period but increases acid production.

Analysis of the organic constituents of yeasts -

A sample of freshly prepared dried yeast was analysed and yield the following results.

Nitrogenous matter	- - - - -	41.84 %
Fatty matter	- - - - -	2.54 %
Cellulose	- - - - -	4.48 %
Starchy matter	- - - - -	41.84 %
Organic matter	- - - - -	2.08 %
mineral matter	- - - - -	5.68 %
miscellaneous	- - - - -	1.54 %

Another sample of the same yeast was analysed fresh without drying. It gave the following results.

Water	- - - - -	68.00 %
Nitrogenous matter	- - - - -	13.37 %
Fatty matter	- - - - -	0.81 %
Cellulose	- - - - -	1.43 %
Starchy matter	- - - - -	13.37 %
Organic matter	- - - - -	0.66 %
mineral matter	- - - - -	1.81 %
miscellaneous	- - - - -	0.55 %

Study of the temperature effect over yeast growth, alcohol formation and acid production in fermentation
pH - 4.5.

Temperature in °C.	Days needed for complete fermentation	gr. yeast in 100 cc of culture	gr. of yeast produced in 20 gr. of sugar	gr. of yeast cells or the yeast sugar fermented	gr. of alcohol produced in 100 cc of culture	Total gr. of alcohol produced by 20 gr. of sugar	% of alcohol produced on the sugar fermented	gr. of non-volatile acid in 100 cc of the culture	gr. of non-volatile acid in 20 gr. of sugar	% of non-volatile acid produced on the sugar	gr. of volatile acid in 100 cc of the culture
45	80	0.9325	3.7310	18.68	1.01	4.00	20.0	0.422	1.91	9.50	0.505
35	60	1.3925	5.5701	27.84	0.925	3.71	18.5	0.321	1.284	6.80	0.202
25	75	1.2025	4.8100	24.04	0.901	3.60	17.2	0.30	1.20	6.00	0.180
15	120	1.1175	4.4701	22.35	0.425	2.10	10.4	0.281	1.524	5.80	0.140
5	210	1.2420	8.01	25.05	0.400	1.60	8.0	0.240	1.060	5.21	0.110

Conclusion

Thus optimum temperature for the growth of the yeast is 35°C and the optimum temperature for the maximum production of alcohol is 45°C. It is interesting that as the temperature decreases the growth of yeast decreases up to 15°C but ^{below that} after that there is some increase in yeast production ^{at 5°C} but the time required to ferment a certain amount of sugar is double the time needed to ferment the same amount of sugar at 15°C. As the temperature decreases the production of ethyl alcohol in the culture also decreases.

Study of the effect of temperature on the growth of yeast, alcohol formation and acid production.

Several cultures containing the following substances were prepared.

- Potassium dihydrogen phosphate KH_2PO_4 -- 0.7558 gm.
- Magnesium sulphate $MgSO_4$ -- 0.0880 gm.
- Calcium carbonate $CaCO_3$ -- 0.0482 gm.
- Potassium sulphate K_2SO_4 -- 0.2016 gm.
- Ammonium sulphate $(NH_4)_2SO_4$ -- 2.30 gm.
- Sodium chloride $NaCl$ -- 0.0122 gm.
- Ferrous sulphate $FeSO_4$ -- 0.0048 gm.
- Zinc sulphate $ZnSO_4$ -- 0.0500 gm.
- Sucrose sugar -- 20 gm.
- Distilled water -- 400 c.c.

The cultures on above substances were ^{taken in each flask} ~~first~~ mixed ^{with} 200 c.c. of water and then digested ^{with dil. hydrochloric acid} till ~~the~~ all the precipitates just ~~of them~~ dissolved. ~~and~~ The pH of the culture was adjusted to 4.5 and the solution made up to 400 c.c. The flasks ^{were} properly cotton plugged and sterilised at 10 lb. pressure for 20 min. and after ~~seed~~ cooling they were carefully seeded with a pure variety of our yeast.

The cultures were kept at 5°c, ~~10°c~~, 15°c, 25°c, 35°c and 45°c. After the ~~the~~ As soon as the formation of fresh yeast stopped, which was seen by the growth of the yeast on the surface of the culture, the cultures were analysed. They gave the the following results on analysis.

Study of the effect of pH on the yeast growth

temp. 29.4°C

pH of the culture.	Range of temperature to which the culture was subjected	Days needed for the complete fermentation of the sugar.	gms. of yeast in 100 cc. of the culture.	gms. of yeast for 20 gm. of sugar.	% forcentage of yeast calculated on the sugar fermented.	gms. of alcohol in 100 cc. of the culture.	gms. of alcohol produced off the fermentation of 20 gm. of sugar.	% forcentage of alcohol produced calculated on the sugar fermented.	gms. of Total Acid in 100 cc. of the culture.	gms. of Total acid formed of sugar.	% forcentage of Acid formation calculated on the sugar fermented.	gms. wt of volatile acid produced in 100 cc. of culture.
3.5	29°C - 32.4°C	70	0.9625	3.8500	19.25	1.50	6.80	30	0.5600	2.8000 2.8000	9.01	.0602
4.5	29°C - 31.5°C	60	1.3781	5.5021	27.50	0.9 0.9	4.60	18	0.2812	1.1248	6.20	.0210
5.5	29°C - 33°C	90	0.850	3.4011	17.02	0.75	3.00	15	0.3601	1.4404	8.00	.0402
6.5	29°C - 34.2°C	100	0.5250	2.1000	10.50	0.50	2.00	10	0.4330	1.7320	10.00	.0711

Conclusion

Thus Optimum pH for the growth of yeast is 4.5. At this pH the culture requires minimum days in fermentation. As the pH decreases the growth of yeast decreases but the alcohol production increases. As the pH increases the growth of yeast decreases. It is least at pH 6.5. But it is surprising that as the pH ~~decreases~~ increases the alcohol production ~~decreases~~ increases.

Growth of yeast at different pH and study of alcohol and acid formation at these pH. at those hydrogen concentrations.

Each culture contained the following substances.

KH_2PO_4 -	0.7558 gm.
MgSO_4 -	0.088 gm.
CaCO_3 -	0.0482 gm.
$(\text{NH}_4)_2\text{SO}_4$ -	2.30 gm.
NaCl -	0.0122 gm.
K_2SO_4 -	0.2016 gm.
FeSO_4 -	0.0048 gm.
ZnSO_4 -	0.0500 gm.
Cane Sugar -	20 gm.
Distilled water -	400 c.c.

Each culture containing the above substances and nearly 200 c.c. of water was first digested with ^{dil.} hydrochloric acid till the whole ~~pp~~ ^{precipitates} ~~just~~ dissolved. The calculated amount of acetic acid and sodium acetate was added in each flask so as to give it the required pH. Then the whole volumes of the cultures were made 400 c.c. in each flask by adding requisite amount of distilled water. The flasks were carefully cotton-plugged and sterilised at 10 lb. pressure for 20 min. After cooling each ^{flask was} carefully seeded with a ^{little} required amount of pure yeast from an old culture and kept at a temperature of 29.4°C to 31.8°C . After the complete growth of the yeasts the cultures were analysed. The following pH were tried, 3.5, 4.5, 5.5 and ^{and} 6.5 and 7.5.

The cultures gave the following results on analysis:

Study of the efficiency of the process of conversion of carbon of glucose into the carbon of yeast at 2 per cent ~~carbon~~ carbon concentration in the culture ~~water~~ ^{under} aerated condition.

A culture containing the following substances was prepared.

Potassium dihydrogen phosphate	-	0.7558 gm.
Potassium sulphate	-	0.2016 gm.
Sodium chloride	-	0.0122 gm.
Calcium carbonate	-	0.0482 gm.
Magnesium carbonate	-	0.0880 gm.
Ammonium nitrate	-	2.30 gms.
Zinc sulphate	-	0.05 gm.
Glucose	-	20 gms.
Water	-	400 c.c.

All the above substances were taken in a 700 c.c. capacity flask and first mixed with 200 c.c. of water and digested with ^{dil.} hydrochloric acid till the whole precipitate just ~~disapp~~ dissolved. Then the volume was made to 400 c.c. and pH adjusted near 4.5. The flask was cotton plugged and sterilized at 10 lb. steam pressure for 20 min. After cooling ^{culture} it was seeded with little ~~yeast~~ yeast.

Arrangements were made to bubble sterilised air through this culture. Sterilisation of the air was done ~~or~~ by ~~passing~~ bubbling it through con. sulphuric acid and then through distilled water to ^{mixe} ~~stir~~ it with water ~~or~~ vapours.

100 c.c. of this culture was taken out at regular intervals of four days and analysed for its yeast, alcohol and acid contents. The variety of yeast we are using in our experiment contains 22.85 ^{of carbon} percent of its dry weight and by this help other calculations are done.

Study of the efficiency of the process of conversion of gly glucose carbon into the yeast carbon in 2 per cent ^{carbon concentration of} glucose culture under aerated condition. —
 Seeded on the 23rd Nov 1947. Temp. 23.8°C to 28.0°C.

S.N.	per centage of glucose carbon in the culture	per centage of glucose originally present in the culture	date of analysis	number of days past since the culture was seeded	gm. of glucose used up in fermentation	gm. mol. of lactic acid produced	gm. mol. of volatile acids produced	gm. mol. of non-volatile acids produced	gm. of alcohol present in the culture	per centage of alcohol produced on sugar fermented	gm. of yeast in the culture	per centage of yield of yeast	gm. of carbon present in the fermented glucose	gm. of carbon in the yeast produced	efficiency of yeast production phenomenon
1	2.0	5.00	27 th Nov. '48	4	0.8334	0.0402	0.0001	0.0401	0.20	23.55	0.1094	13.13	0.3334	0.0250	7.50
2	2.0	5.00	1 st Dec. '48	8	1.1339	0.0466	0.0002	0.0464	0.20 0.24	21.42	0.2100	18.52	0.4536	0.0479	10.53
3	2.0	5.00	3 rd Dec '48	12	1.8750	0.1817	0.0012	0.1805	0.35	18.61	0.3676	19.60	0.7500	0.0840	11.09
4	2.0	5.00	9 th Dec '48	16	3.4620	0.2016	0.0014	0.2002	0.40	11.42	0.6980	20.20	1.3848	0.1578	11.40

Study of efficiency of process of conversion of carbon of glucose
into the carbon of yeast ^{when} at 1.0 per cent of concentration of glucose
Carbon ^{is present} in the culture ^{under} aerated condition.

A culture containing following substances was prepared

Potassium dihydrogen phosphate	-	0.3779 gm.
Potassium sulphate	-	0.1008 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0241 gm.
Magnesium sulphate	-	0.0480 gm.
Ammonium nitrate	-	1.1500 gm.
Zinc sulphate	-	0.0250 gm.
glucose	-	10.00 gms.
Water	-	400 c.c.

All the above substances were taken in a 700 c.c. flask and mixed with 200 c.c. of water. The mixture was digested with hydrochloric acid till the precipitates just dissolved. Hydrogen ion concentration of ~~the~~ ^{the} culture was so adjusted as to give a pH value of 4.5. The flask was cotton plugged and sterilised at 10 lb. steam pressure for 20 min. and after cooling it was seeded with a little quantity of pure yeast.

The culture was kept continuously aerated by sterilised air. Sterilisation of air was achieved by passing it through con. Sulphuric acid and distilled water. 100 c.c. of this aerated culture was taken out after ~~each~~ ^{every} fourth ~~days~~ ^{day} and analysed. Following results were thus obtained.

Study of the growth of yeast efficiency of the process of conversion glucose carbon into the yeast carbon in 1 per cent glucose carbon concentration under aerated condition :-
 seeded on 23rd Nov. ~~1947~~ 1947
 Temp. 23.8°C to 28.0°C.

S.N.	Per cent of C originally present in the culture	Per cent of glucose originally present in the culture	gm. of glucose fermented.	Date of analysis	number of days passed since the culture was seeded	gm. mol of total acid in 100 of the culture.	gm. mol of volatile acid in 100 c.c. culture	gm. mol of non volatile acid in 100 c.c. culture	gm. of alcohol in 100 c.c. of the culture	Per cent of alcohol production in sugar used up	gm. of yeast formed in 100 c.c. of the culture	Per cent of yeast yield in the culture	gm. of carbon present in fermenting sugar	gm. of C present in the yeast produced	efficiency %
1.	1.0	2.50	0.7759	27 th Nov. '47	4	0.0324	0.0001	0.0323	0.17	21.71	0.1504	18.71	0.3104	0.0394	11.06
2	1.0	2.50	0.8338	31 st Nov. '47	8	0.0730	0.0002	0.0728	0.18	21.68	0.1654	20.00	0.3368	0.0378	11.51
3	1.0	2.50	1.0714	4 th Dec '47	12	0.1624	0.0008	0.1616	0.19	17.75	0.2300	21.49	0.4285	0.0528	11.52
4	1.0	2.50	2.2108	8 th Dec '47	16	0.1908	0.0011	0.1807	0.27	12.21	0.5940	27.12	0.8840	0.1130	12.78

acid

Study of efficiency of the process of conversion of Carbon of glucose into the carbon of yeast in a culture of glucose containing 0.5 per cent of glucose carbon ^{under} ~~to~~ ^{laserated} carbon dioxide

A culture was prepared containing the following substances

Potassium dihydrogen phosphate	-	0.1889 gm.
Potassium sulphate	-	0.0504 gm.
Sodium chloride	-	0.0061 gm.
Calcium Carbonate	-	0.0120 gm.
Magnesium sulphate	-	0.0240 gm.
Ammonium nitrate	-	0.5750 gm.
zinc sulphate	-	0.0125 gm.
Glucose	-	5.00 gms.
Water	-	400 c.c.

All the above substances were taken ⁱⁿ a 700 c.c. flask and mixed with 200 c.c. of water and digested with hydrochloric acid till the ~~precipitates~~ ^{precipitates} just dissolved. The hydrogen ion concentration ^{was ~~not~~ such} ~~was~~ adjusted as to give a pH value near 4.5.

The volume of this culture was made up to 400 c.c. The flask was cotton plugged and sterilised at 10 lb. steam pressure for 20 min. After cooling the culture was seed with little amount of ~~seed~~ pure yeast.

~~Air~~ Sterilised air was continuously bubbled through this culture. Sterilisation of air was achieved by bubbling it through con. sulphuric acid and distilled water to first sterilised it and ^{the second} ~~then~~ mixed it with some water vapours.

A ^{of 100 c.c.} sample of this culture was taken out after every ~~each~~ fourth days and analysed. ^{They} ~~It~~ gave the following results.

Study of the ~~pro~~ efficiency of the process of the conversion of glucose carbon into yeast carbon in a culture containing 0.05 per cent of glucose carbon under aerated condition ;

seeded on 24th Nov 1947

Temp. 23.8°C to 28.0°C.

Sl. No.	Percentage of Carbon in the culture.	Percentage of glucose originally present in the culture	Date of analysis	Number of days passed after seeding	gms. of sugar used up in fermentation in 100 c.c. culture	gm. ml. of total acid formed in 100 c.c. of culture	gm. ml. of Volatile acid generated in 100 c.c. of culture	gm. ml. of non-volatile acid produced in 100 c.c.	gms. of alcohol formed by fermentation in 100 c.c.	Percentage of alcohol production.	gms. of yeast produced in 100 c.c. of culture	Per cent of yeast yield.	gms. of Carbon present in the glucose fermented	gms. of Carbon in the yeast cells produced.	Efficiency
1.	0.5	1.25	28 th Nov. 47	4	0.3410	0.1047	0.0003	0.1044	0.09	26.49	0.0788	23.16	0.1364	0.0179	13.10
2	0.5	1.25	1 st Dec. 47	8	0.5147	0.1148	0.0004	0.1144	0.11	21.17	0.1203	23.53	0.2038	0.0279	13.60
3	0.5	1.25	5 th Dec '47	12	0.7738	0.1182	0.0004	0.1178	0.16	20.78	0.1882	24.67	0.3092	0.0430	14.26
4	0.5	1.25	9 th Dec '47	16	1.2500	0.0824	0.0003	0.0821	0.24	19.20	0.3680	29.59	0.6000	0.0830	13.84

66

Study of the efficiency of process of conversion of Carbon of yeast
glucose into the Carbon of yeast in a culture of glucose containing
^{0.20} ~~0.25~~ per cent of glucose carbon ^{under} aerated condition.

A culture containing the following substances was prepared.

Potassium dihydrogen phosphate	—	0.0944 gm.
Potassium sulphate	—	0.0252 gm.
Sodium chloride	—	0.0061 gm.
Calcium carbonate	—	0.0120 gm.
Magnesium sulphate	—	0.0240 gr.
Zinc sulphate	—	0.0125 gr.
Ammonium nitrate	—	0.5750 gm.
Glucose	—	2.00 grs.
Water	—	400 c.c.

All the above substances were taken in a flask of 700 c.c. capacity and all the precipitates were digested with ^{dil.} hydrochloric acid in 200 c.c. of water. The total volume was made up to 400 c.c. and pH was adjusted to near 4.5. The flask was then cotton plugged and sterilized at ^{pressure} 15 lb. of steam ^{pressure} and for ~~20~~ 20 min. and after cooling culture was seeded ~~a~~ with small quantity of yeast.

Sterilised air obtained by ~~to~~ bubbling it through conc. sulphuric acid and distilled water was passed through this culture continuously till the experiment was over.

100 c.c. of the culture was taken after ^{every} ~~each~~ fourth days and analyzed. Following results were thus obtained.

Study of the efficiency of the process of conversion of glucose carbon into yeast carbon in a 0.20 per cent glucose concentration under aerated condition.

Temp. 21.8°C to 24.5°C.

Seeded on the 20th Dec 1947

Serial number	percentage of Carbon of glucose originally in the culture	percentage of glucose glucose originally in the culture	date of analysis	number of days passed after seeding	gms. of glucose used up in fermentation in 100 c.c.	gms. mol. of total acid present in 100 c.c.	gms. mol. of Volatile acid in 100 c.c. of culture	gms. mol. of non-volatile acids in 100 c.c. of culture	gms. of alcohol produced in 100 c.c.	per centage of alcohol product from the sugar fermented	gms. of yeast produced by 100 c.c. of culture	per centage of yeast yield	gms. of Carbon present in the fermented glucose.	gms. of Carbon taken by yeast for its growth	efficiency
1	0.20	0.50	24 th Dec '47	4	0.1540	0.0732	0.0004	0.0728	0.01	6.66	0.0415	26.63	0.0616	0.0093	15.40
2	0.20	0.50	28 th Dec '47	8	0.8042	0.1024	0.0055	0.1019	0.04	5.00	0.4360	53.00	0.4000	0.0996	24.92
3	0.20	0.50	2 nd Jan. '48	12	1.0000	0.0624	0.0003	0.0621	0.05	5.00	0.5024	50.24	0.4020	0.1117	27.92
4	0.20	0.50	6 th Jan. '48	16	1.0000	0.0246	0.0002	0.0244	0.05	5.00	0.4008	40.00	0.4020	0.0914	25.63

Study of the growth of yeast in one percent aerated and one percent non-aerated cultures of 10% ethyl alcohol by volume.

Two cultures were prepared containing the following substances.

Potassium dihydrogen phosphate	-	0.1889 gm.
Potassium sulphate	-	0.0504 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.00 0.0120 gm.
Magnesium sulphate	-	0.0240 gm.
Ammonium nitrate	-	0.5750 gm.
Zinc sulphate	-	0.0125 gm.
Water	-	500 c.c.

All the above substances are weighed in each flask and then digested with ^{dil.} hydrochloric acid in 200 c.c. of water. The volume was finally made up to 500 c.c. and pH adjusted near 4.5. The flasks were cotton-plugged and sterilised at 10 lb. steam pressure for 20 min.. After cooling 5 c.c. of absolute ethyl alcohol was added in each flask and after carefully making the cultures homogenous by ~~stirring~~ they were seeded with little amount of pure yeast.

One culture was kept as such and in the other sterilised air, obtained by bubbling it through con. sulphuric acid and distilled water, was passed very slowly.

Aerated culture took 8 days for complete utilisation of alcohol and the non-aerated culture continued fermentation for 78 days.

The cultures were analysed after their complete fermentation and ~~the~~ following results were obtained.

Comparison of growth of yeast in aerated and non-aerated cultures of one per cent ethyl alcohol :-

Temp. 24°C to 28.4°C.

S.N.	Experimental Condition	Concentration of ethyl alcohol in number of c.c. for 100 cc. of culture.	number of days required for complete fermentation.	gm. mol. of total acid produced in 100 cc. of the culture.	gm. mol. of Volatile acid produced in 100 c.c. of the culture.	gm. mol. of non-volatile acid produced in 100 c.c. of the culture.	Relative density of the distillate.	gm. of alcohol in 100 c.c. of the culture.	gm. of yeast produced in 100 c.c. of the culture.	per centage of yield of yeast alcohol calculated on sugar used up in fermentation.
1.	non-aerated	1.00	78	0.0812	0.0772	0.0040	0.9998	0.10	0.1646	16.46
2.	aerated	1.00	8	1.0876	1.0842	0.0034	0.9999 nil	nil 0.3881	0.3881	38.81

Study of growth of yeast in ~~1.0~~ 1.0 per cent and 0.5 per cent ^{by volume} of ethyl alcohol solution ^{by volume} ~~under~~ aerated condition.

Two cultures were prepared containing following substances

Potassium dihydrogen phosphate	-	0.3779 gm.
Potassium sulphate	-	0.1008 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0241 gm.
Magnesium carbonate	-	0.0440 gm.
Ammonium nitrate	-	1.1500 gm.
Zinc sulphate	-	0.0250 gm.

One culture was prepared in 1 litre flask. It contained all the above substances. The substances were digested with dil. hydrochloric acid ~~in~~ in 400 c.c. of water till the whole precipitate ^{just} dissolved. Finally the volume was made to 750 c.c. and ~~of the flask~~ hydrogen ions concentration was so balanced as to give pH 4.5. The flask was ~~of~~ cotton plugged and sterilised at 10 lb. steam pressure for 20 min. After cooling 7.5 c.c. of absolute alcohol was added in the culture.

In the other culture which was prepared in 2 litre flask all the above substances were added together with and digested with dil. hydrochloric acid in 400 c.c. of water till the whole precipitate just dissolved. The total volume of the milk was made up to 1500 c.c. and pH adjusted near 4.5. The flask was cotton plugged and sterilised at 10 lb. steam pressure for 20 min. After cooling 7.5 c.c. of absolutely ethyl alcohol was added.

Both the cultures were seeded with little pure yeast and sterilised air, obtained by passing it ~~through~~ ^{continuously} through con. sulphuric acid and distilled water, was passed in the cultures ~~before~~ ^{until} ~~the~~ ^{the} cultures ~~were~~ ^{were} ~~analyzed~~ ^{analyzed} completely. On analyses of the cultures following results were obtained.

Growth of yeast in 1% and 0.5% cultures of ethyl alcohol by volume under aerated condition:-

pH - 4.5.

Temp. 25.2°C to 30.0°C.

S.N.	c.c. of absolute ethyl alcohol added per 100 c.c. of culture	c.c. of total ethyl alcohol present in the whole of the culture	number of days fermentation was allowed.	amount of absolute ethyl alcohol used up in fermentation in c.c. in the culture.	gm. mol. of total acid formed in the whole culture	gm. mol. of lactic acid produced in the culture	gm. mol. of mm. lactic acid produced in the whole culture	percentage of total acid produced.	gms. of alcohol still remaining in the culture.	gms. of yeast produced.	percentage of yeast yield.
1.	1.0	7.5	10	7.40	1.0876	0.0034	1.0842	15.51	0.10	2.8815	38.40
2.	0.5	7.5	5	5.52	0.8832	0.0026	0.8806	16.00	2.08	2.9024	52.50

Earlier Winogradsky claimed that microorganisms can also utilise the energy derived from the oxidation of inorganic materials for their growth. This first generalisation of its type was proved correct by Temple and Colmer who could grow *Thiobacillus ferrooxidans* autotrophically by means of energy derived from the oxidation of ferrous ions (13). Although such examples where an organism is found to utilise the energy obtained by ordinary inorganic chemical reactions are rare it has been seen that many organic compounds can easily be used as source of carbon by microorganism. Thus Starier (14, 15) has shown that ethyl alcohol is oxidised by *Pseudomonas fluorescens* to yield as much as 50 to 70 per cent of the calculated amount of acetic acid. Alcohol can also be oxidised to acetic acid liberating energy needed for the growth of *Streptococcus mastiditis* without the accumulation of hydrogen peroxide (16).

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→ The utilisation of ethyl alcohol as source of carbon is also seen in the case of several yeasts. We see that yeasts are able to live for many years in the liquids which have fermented. It is probable that they use the glycerol and succinic acid which are regarded by Laurent as being able to supply the need of yeast carbon. But never the less for certain species alcohol seems to be the source of carbon (11).

~~Researches of Kayser and Demolon (12)~~

and Trillat and Sauton (11) have long proved that yeast oxidises ethyl alcohol to acetaldehyde and this energy is probably utilised for the growth of yeast. Lindner and Cyiser (19)²⁰ found that ethyl alcohol can be used as the source of carbon for yeasts. Stockhausen (18)²¹ reported the *Saccharomyces membranefaciens* can take its carbon from ethyl alcohol but not from methyl alcohol.

It ~~has~~^{has been} seen that under certain conditions ~~that~~^{that} Dhar Yeast (19)²² and *Saccharomyces carlsbergensis* can grow in cultures containing only ethyl alcohol as source of carbon. In this ~~paper~~ experiment a comparative study of the above yeasts in cultures containing ethyl alcohol as the source of carbon under non-aerated conditions has been done.

Experimental: Several cultures as shown in the table were prepared each containing 0.2 gm. of calcium carbonate, 0.25 gm. of magnesium carbonate, 0.2 gm. of sodium chloride, 0.2 gm. of potassium sulphate, 0.2 gm. of disodium hydrogen phosphate and 2.5 gm. of ammonium sulphate were prepared. For this, first, all these minerals were weighed out into 750 c.c. flat-bottom Pyrex flask and 200 c.c. of distilled water were added to them. These were then digested with dilute hydrochloric acid. The clear solution thus obtained was then cooled and the total volume of each culture was made up to 400 c.c. with digested distilled water and pH adjusted to be 4.5.

These flasks were plugged with surgical cotton and sterilised at a pressure of 10 lbs. for 30 minutes in an autoclave. After cooling, ^{the} volume of absolute alcohol as mentioned in the table against them was added in each flask and then after giving the media a whirling motion they were seeded with a trace of activated samples of yeasts as described in the table.

One flask of each concentration of ethyl alcohol containing all the ingredients was kept as such without seeding to account for the loss of alcohol by evaporation during the period of fermentation. Thus for each concentration of alcohol three cultures were prepared ~~one for Dhar yeast~~, one for ~~Dhar~~ yeast, one for *S. carlsbergensis* and one for the finding out the loss of ethyl alcohol by evaporation during fermentation.

All these cultures were kept together at room temperature during the period of fermentation which lasted for 15 days. The temperature variation during this period ~~of~~ was between 25.5° ^{and} 29.2° C.

Observation:

The results obtained by the analysis of the above cultures are tabulated below. These results are per 100 c.c. of the culture:-

Alcohol consumption:

g. of ethyl alcohol in beginning.	Name of the yeast.	gm. of ethyl alcohol in the beginning.	gm. of alcohol lost by evaporation.	gm. of alcohol left unmeasured.	gm. of alcohol consumed in yeast growth.
0.50	Dhar Yeast	0.399	0.249	0.075	0.075
0.50	Saccharo S. carlsbergensis	0.399	0.249	0.010	0.140
1.00	Dhar Yeast	0.798	0.268	0.360	0.170
1.00	S. carlsbergensis	0.798	0.268	0.310	0.220
2.00	Dhar Yeast	1.596	0.316	0.560	0.720
2.00	S. carlsbergensis	1.596	0.316	0.900	0.380
3.00	Dhar Yeast	2.394	1.934	0.790	0.670
3.00	S. carlsbergensis	2.394	1.934	0.730	0.730
4.00	Dhar Yeast	3.184	2.054	0.530	0.600
4.00	S. carlsbergensis	3.184	2.054	0.430	0.710
5.00	Dhar Yeast	3.980	1.250	1.390	1.340
5.00	S. carlsbergensis	3.980	1.250	1.240	1.490
6.00	Dhar Yeast	4.776	2.526	1.500	1.700
6.00	S. carlsbergensis	4.776	2.526	1.130	1.120

Table II

Acid formation:

% of the ethyl alcohol in beginning	Name of the yeast	gm. eqv. of total acid formed.	gm. eqv. of volatile acid formed.	% of acid formation cal. on the basis of alcohol consumed.
0.50	Dhar yeast	0.0675	0.010	90.00
	S. carlsbergensis	0.0175	0.005	12.50
1.00	Dhar yeast	0.0550	0.030	32.35
	S. carlsbergensis	0.0350	0.005	29.16
2.00	Dhar yeast	0.1100	0.020	19.64
	S. carlsbergensis	0.0500	0.005	13.16
3.00	Dhar yeast	0.2750	0.075	41.34
	S. carlsbergensis	0.2450	0.130	34.56
4.00	Dhar yeast	0.2200	0.030	36.66
	S. carlsbergensis	0.2700	0.035	38.02
5.00	Dhar yeast	0.1850	0.055	13.80
	S. carlsbergensis	0.2750	0.090	18.45
6.00	Dhar yeast	0.1900	0.030	11.21
	S. carlsbergensis	0.3300	0.145	29.46

Table 12

-Yeast growth:

% of ethyl alcohol in the culture	Name of the yeast	gm. of dry yeast produced in the culture.	% of yeast yield cal. on the basis of alcohol consumed.
0.50	Dhar Yeast	0.0740	98.66
"	S. carlsbergensis	0.0760	94.44
1.00	Dhar Yeast	0.0820	48.44
	S. carlsbergensis	0.0998	45.32
2.00	Dhar Yeast	0.1920	28.05
	S. carlsbergensis	0.1606	17.75
3.00	Dhar Yeast	0.2628	39.20
	S. carlsbergensis	0.2586	35.42
4.00	Dhar Yeast	0.3364	56.00
	S. carlsbergensis	0.2990	42.11
5.00	Dhar Yeast	0.3310	24.70
	S. carlsbergensis	0.2830	19.00
6.00	Dhar Yeast	0.2830 ^{0.3056}	17.90
	S. carlsbergensis	0.3300	29.68

Table 13.

Inference: Consumption of ethyl alcohol by Dhar yeast is in general less than that of *Saccharomyces carlsbergensis*. At lower concentration of ethyl alcohol greater acid formation is seen in the case of Dhar yeast. But at higher concentration of alcohol *S. carlsbergensis* produces more acid. At all the concentrations except last, Dhar Yeast produces

more yeast than *S. carlsbergensis*. This greater consumption of ethyl alcohol followed by less yield of yeast cells in the case of *S. carlsbergensis* proves that the rate of respiration of this yeast is greater than that of Dhar yeast.

Summary: Both Dhar yeast and *S. carlsbergensis* can grow in cultures containing only ethyl alcohol as the source of carbon food under non-aerated conditions. The rate of consumption of ethyl alcohol at different concentrations of alcohol is usually less in the case of Dhar yeast and is followed by greater yeast yield as compared to *S. carlsbergensis*. This indicates that the rate of respiration is faster in *S. carlsbergensis*.

At low concentrations of ethyl alcohol in the culture, Dhar yeast produces more acid than *S. carlsbergensis* but at higher concentrations the latter becomes the more acid-producing yeast.

▽
 V A study of the growth of *Rhodotorula gracilis* in cultures containing ethyl alcohol, sucrose, lactose and glucose and a comparison of the growth of this variety of yeast with Dhar yeast in sucrose cultures, under non-aerated conditions.

Experimental: Several cultures each containing 0.25 gm. of magnesium carbonate, 0.2 gm. of calcium carbonate, 0.2 gm. of potassium sulphate, 0.2 gm. of sodium chloride and 0.2 gm. of disodium mono-hydrogen phosphate were prepared. For this, first the

above mineral nutrients were weighed out into flat bottom Pyrex flasks, digested with dilute hydrochloric acid and used. Each culture contained 8 gm. of carbon contributed by the respective carbon source. The total volume of each culture was made to 400 c.c. with distilled water and the pH. was adjusted to 4.5.

The flasks were plugged with surgical cotton and sterilised at 10 lbs. steam pressure for half an hour and after cooling all the cultures except one were seeded with a trace of activated *Rhodotorula gracilis*. The culture left contained sucrose as the source of carbon and it was seeded with a trace of activated Dhar yeast.

The temperature variation during the period of fermentation, which lasted for 40 days was between 28.0° ^{and} 32.0° C.

Observation: The results obtained by the analysis of the above cultures are tabulated below:-

Fermentation data:

Name of the yeast	Name of source of carbon	gm. of total acid produced	gm. of alcohol produced.	gm. of yeast grown
<i>R. gracilis</i>	Alcohol	0.0012	-	0.1712
<i>R. gracilis</i>	sucrose	0.0088	0.60	1.2133
<i>R. gracilis</i>	lactose	0.0001	0.60	0.0084
<i>R. gracilis</i>	glucose	0.0084	0.60	1.2402
Dhar Yeast	sucrose	0.0162	0.91	2.8507

Table 14