

72 70.

Study of the course of yeast growth in two per cent ethyl alcohol culture by volume ~~water~~ <sup>under</sup> aerated condition -

A culture was prepared containing the 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.
Water	—	500 c.c.

These substances are taken in a 700 c.c. flask and digested with dil. hydrochloric acid in 200 c.c. of water till the precipitates just dissolved. The ~~and~~ whole volume was made up to 500 c.c. and pH adjusted near 4.5. The flask, after being cotton plugged, was sterilised at 10 lb. of steam pressure for 20 min. After ~~the~~ it cooled 10 c.c. of absolute ethyl alcohol was added ~~to~~ the culture and the culture seeded with pure yeast.

The culture was kept in aerated condition by sterilised air obtained by bubbling air <sup>first</sup> through con. sulphuric acid and then ~~distilled~~ distilled water. 100 c.c. of this culture was taken out just after seeding and analysed for standard in the results. For each analysis on <sup>other</sup> subsequent days similar 100 c.c. ~~portion~~ of the culture was taken and analysed till ~~the~~ the whole alcohol of the culture was consumed. Following results were obtained.

Growth of yeast in 2% ethyl alcohol by volume under aerated condition -

pH. 4.5-

Temp. 27.4°C to 30.8°C

SN.	number of days passed after fermentation started.	gm. mol. of total acids produced per 100 c.c. of the culture	gm. mol. of Volatile acids produced per 100 c.c. of the culture	gm. mol. of non-volatile acids quantified in 100 c.c. of culture.	Relative Density of the distillate	gms. of alcohol distill present in 100 c.c. of the culture	gm. of alcohol used up in fermentation.	gms. of yeast produced in 100 c.c. of the culture.	percentage of yield of yeast.
1.	On the day of seeding	nil	nil	nil	0.9967	1.77	nil	Only negligible amount of seed yeast.	nil.
2	10	0.3047	0.0002	0.3045	0.9984	0.83	0.72	0.2154	<del>30</del> 30.55
3	16	0.4267	0.0004	0.4263	0.9997	0.15	1.65	0.6500	39.39
4	22	0.4370	0.0005	0.4370	0.9999	0.01	1.76	0.5308	30.12

8 Study of the growth of yeast in ethyl alcohol cultures

Containing 0.2, 0.4, 0.6, 0.8 and 1.0 per cent of Carbon  
of <sup>ethyl</sup> alcohol ~~under~~ <sup>in</sup> aerated condition.

Following cultures were prepared.

1) Culture containing 0.2 per cent of ethyl alcohol carbon contained the following substances.

Potassium dihydrogen phosphate	-	0.0944 gm.
Potassium sulphate	-	0.0252 gm.
Sodium chloride	-	0.0061 gm.
Calcium <del>chloride</del> carbonate	-	0.0120 gm.
Magnesium sulphate	-	0.0240 gm.
Zinc sulphate	-	0.0125 gm.
Ammonium nitrate	-	0.5750 gm.
Water	-	500 c.c.

Above substances were taken in a 700 c.c. flask and digested in 200 c.c. of water with little dil. hydrochloric acid till the precipitates just dissolved. The volume was made to 500 c.c. and pH adjusted near 4.5. The culture was then cotton plugged and sterilized at 10 lb. steam pressure for 20 min. After cooling 1.90 gms. of absolute ethyl alcohol was added in the culture.

(2) The culture which contained 0.4 per cent of ethyl alcohol Carbon ~~cont~~ was prepared containing the following substances.

Potassium dihydrogen phosphate	-	0.1888 gm.
Potassium sulphate	-	0.0504 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0240 gm.
Mg Magnesium sulphate	-	0.0480 gm.
Zinc sulphate	-	0.0250 gm.
Ammonium nitrate	-	1.1500 gms.
Water	-	500 c.c.

All the above substances were taken in <sup>a</sup> 700 c.c. Capacity flask and ~~and~~ digested in 200 c.c. of water with dil. hydrochloric acid and till the precipitates just disappeared. The volume was made up to 500 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 3.80 gms of ethyl alcohol was added in the culture.

(3) Following substances were added in the culture containing 0.6 per cent of ~~carbon~~ ethyl alcohol carbon. in the

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

These substances were weighted in 700 c.c. flask and digested in 200 c.c. of water with dil. hydrochloric acid till the precipitate just dissolved. Final volume of the culture was made to 500 c.c. and pH adjusted near 4.5. The flask was then cotton plugged and sterilized at 10 lb steam pressure for 20 min. After cooling 5.75 gms of absolute ethyl alcohol was added in this culture.

(4) The culture which had ~~carbon~~ 0.8 per cent concentration of ethyl alcohol carbon ~~was~~ contained the following mineral substances.

Potassium dihydrogen <del>sof</del> phosphate	-	0.2835 gm.
Potassium sulphate	-	0.0856 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0181 gm.

Magnesium sulphate	-	0.0360 gm.
Ammonium nitrate	-	0.8700 gm.
Zinc sulphate	-	0.0375 gm.
Water	-	500.

The above substances were taken in a 700 c.c. capacity flask and digested with dil. hydrochloric acid in 200 c.c. of water till the precipitates just dissolved. The final volume of the culture was made up to 500 c.c. and pH adjusted to 4.5. The flask was cotton plugged and sterilised at 10 lb. steam pressure for 20 min. After cooling 7.65 gms. of absolute ethyl alcohol was added in the culture.

(4) The ~~flask~~ <sup>culture</sup> which contained 1.0% per cent of ethyl alcohol carbon ~~contained~~ had following substances in it.

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 gms.
Zinc sulphate	-	0.0250 gm.
Water	-	500 c.c.

All the above substances were taken in a 700 c.c. flask and digested with a dil. hydrochloric acid ~~dil~~ till the whole precipitate just dissolved. The final volume of the culture was made up to 500 by adding more water and flask <sup>was</sup> cotton plugged. This culture was sterilised ~~by~~ by 10 lb. steam pressure ~~under~~ for 20 min. After cooling 9.50 gms of absolute ethyl alcohol was added in the culture.

All the above cultures were seeded with pure yeast and aerated with sterilised air obtained by bubbling it through con. sulphuric acid and distilled water. After the fermentation completed cultures were analysed and following results obtained. Temperature variation during the experiment was 24.2°C to 28.6°C.

Growth of yeast at various carbon concentration of ethyl alcohol under aerated condition -

Temp. 24.2°C to 28.6°C

S.N.	percentage of carbon in the culture before the fermentation started.	percentage of alcohol in the culture for seeding in gms.	gms. of alcohol present at the time of analysis in the culture	gms. mol. of total acid produced in the culture	gms. mol. of volatile acid generated in the culture.	gms. mol. of non-volatile acid in the culture.	gms. of yeast produced in the culture	percentage of the yield of yeast calculated on alcohol used up.	gms. of carbon taken up by yeast.	efficiency.
1.	0.20	0.38	0.00	0.2040	0.0008	0.2032	0.2460	64.73	0.0536	28.10
2	0.40	0.76	0.01	0.2672	0.0012	0.2660	0.3420	45.00	0.0781	19.42 <del>45.00</del>
3	0.60	1.15	0.02	0.2836	0.0014	0.2822	0.3844 <del>0.4448</del>	33.38	0.0878	14.99
4	0.80	1.53	0.03	0.3104	0.0018	0.3086	0.4448	29.07	0.1016	12.70
5	1.00	1.92	0.08	0.3508	0.0102	0.3406	0.4820	25.10	0.1102	11.02

Study of the course and efficiency of process of conversion of the ethyl alcohol carbon in the carbon of yeast at <sup>2.0</sup> ~~0.2~~ per cent of concentration of carbon of ethyl alcohol <sup>under</sup> ~~at~~ aerated condition.

A culture was prepared containing the following substances.

Potassium dihydrogen phosphate	—	0.7558 gm.
Potassium sulphate	—	0.0216 gm.
Sodium chloride	—	0.0122 gm.
Calcium carbonate	—	0.0482 gm.
Magnesium carbonate	—	0.0880 gm.
Ammonium nitrate	—	2.30 gms.
Zinc sulphate	—	0.0500 gm.
Water	—	500 c.c.

All the above substances were weighed and taken in a 700 c.c. flask and digested with dil. hydrochloric acid in 200 c.c. of water. The volume till the precipitates just dissolved. Total volume of the culture was made to 500 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 19.20 gms. of absolute alcohol was introduced in the flask and the culture was seeded with pure yeast.

The culture was kept continuously aerated by sterilised air obtained by bubbling air through con. sulphuric acid and distilled water. 100 c.c. of the culture was taken out after ~~at~~ <sup>every</sup> 48 hours and analysed for its yeast, alcohol and acid contents. Following results ~~were~~ were thus obtained.

Study of the efficiency of yeast growth in 2.0 per cent of ethyl alcohol Carbon.

Temp. 24.2°C to 27°C.

S.N.	per cent of ethyl alcohol Carbon in the culture	grs. of alcohol in 100 c.c. of the culture.	number of hours passed after seeding.	grs. of alcohol consumed per 100 c.c. of the culture.	gr. mol. of total acid in 100 c.c. of the culture.	gr. mol. of Volatile acid produced in 100 c.c.	gr. mol. of non-volatile acid produced in 100 c.c. of culture.	gr. of yeast formed in 100 c.c. of culture.	% of yeast formed calculated on the basis of alcohol consumed.	grs. of C formed in the alcohol fermented.	grs. of Carbon in the yeast formed.	efficiency.
1.	2.00	3.84	48	0.09	0.0486	0.0006	0.0480	0.0218	24.22	0.0469	0.0050	10.82
2.	2.00	3.84	96	0.22	0.1002	0.0014	0.0988	0.0554	25.04	0.1147	0.0126	11.02
3.	2.00	3.84	144	0.52	0.2034	0.0038	0.1196	0.1339	25.75	0.2713	0.0306	11.28
4.	2.00	3.84	192	0.65	0.1218	0.0040	0.1178	0.1724	26.52	0.3390	0.0394	11.62

Study of the efficiency of the process of yeast conversion of the carbon of ~~the~~ culture of 1.0 per cent concentration of carbon of ethyl alcohol into the carbon of yeast.

A culture containing the following substances was prepared.

Potassium dihydrogen phosphate	-	0.3779 gm.
Potassium sulphate	-	<del>0.4008</del> 0.0108 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0241 gm.
Magnesium carbonate	-	<del>0.0448</del> 0.0440 gm.
Ammonium nitrate	-	1.1500 gms.
Zinc sulphate	-	0.0250 gm.
Water	-	<del>10</del> 500 c.c.

All these substances were taken in a 700 c.c. flask and digested with dil. hydrochloric acid with ~~40~~ 200 c.c. of water till the whole precipitate just dissolved. The total volume of the culture was made up to 500 c.c. and pH adjusted near 4.5.

Flask was cotton plugged and sterilised ~~to~~ at 10 lb. steam pressure for 20 min.. After cooling 9.60 gms of absolute ethyl alcohol was ~~added~~ introduced in the culture.

This culture was seeded with little pure yeast and kept aerated by ~~so~~ passing slow stream of ~~sterilised~~ sterilised air obtained by first bubbling it through con. sulphuric acid and then through distilled water. At regular intervals of 48 hours 100 c.c. of culture was taken out and analysed. The following results were obtained.

Study of the efficiency of yeast growth in 1.0 per cent ethyl alcohol carbon solution :-

Temp.  $23.8^{\circ}\text{C}$  to  $27.0^{\circ}\text{C}$ .

	per cent of alcohol carbon originally present.	gms. of ethyl alcohol originally present in 100 c.c. of culture.	number of hours passed after seeding.	gms. of alcohol consumed in 100 c.c. of culture.	gms. of total acids in 100 c.c. of the culture.	gms. of volatile acids in 100 c.c. of the culture.	gms. of non-volatile acids in 100 c.c. of culture.	gms. of yeast formed in 100 c.c. of culture.	per cent of yeast or alcohol consumed.	gms. of yeast present in alcohol consumed.	gms. of yeast present in the yeast found.	efficiency.
1	1.00	1.92	48	0.12	0.0410	0.0011	0.0399	0.0324	27.00	0.0625	0.0074	11.82
2	1.00	1.92	96	0.28	0.0988	0.0023	0.0965	0.0765	30.60	0.1460	0.0175	12.00
3	1.00	1.92	144	0.56	0.1108	0.0030	0.1078	0.1602	30.82	0.2921	0.0356	12.20
4	1.00	1.92	192	0.70	0.1285	0.0041	0.1244	0.2205	31.5	0.3652	0.0456	12.80

Study of the efficiency of growth of yeast in 0.5 per cent concentration of ethyl alcohol carbon <sup>in</sup> water aerated condition.

A culture was prepared which contained following substances.

Potassium dihydrogen phosphate	-	0.1889 gm.
Potassium sulphate	-	0.0304 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0120 gm.
Magnesium carbonate	-	0.0240 gm.
Zinc sulphate	-	<del>0.5750 gm.</del> 0.0125 gm.
Ammonium nitrate	-	0.5750 gm.
Water	-	500 c.c.

These substances were taken in a 700 c.c. flask and digested with dil hydrochloric acid ~~till~~ in 20 c.c. of water till the precipitates just dissolved. Total volume of the culture was made to 500 c.c. and pH adjusted to 4.5. Flask was cotton plugged and sterilized at 10 lb. pressure for 20 min. After cooling 4.80 gms. of absolute ethyl alcohol was added in the culture.

The culture was seeded with <sup>pure</sup> yeast and kept aerated with sterilized air obtained by passing air through con. sulphuric acid and distilled water. 100 c.c. of this culture was taken out after ~~each~~ <sup>every</sup> 48 hours and analysed for its yeast, alcohol and acids contents. Thus following results were obtained.

## Study of the efficiency of yeast growth in 0.5 percent carbon culture of ethyl alcohol

Temp. - 23.8°C to 27.2°C.

S. N.	Percent of Carbon of ethyl alcohol in the culture in begining.	gms. of ethyl alcohol originally present in 100 c.c. of culture.	number of hours passed after reading of the culture.	gms. of ethyl alcohol consumed for 100 c.c. of the culture.	gms. of yeast produced for 100 c.c. of the culture.	gms. int. of lactic acid in 100 c.c. of the culture.	gms. int. of volatile acids generated in 100 c.c. culture.	gms. int. of non-volatile acids produced in 100 c.c. culture.	Percent of yeast cell to alcohol consumed.	gms. of Carbon present in the alcohol consumed.	gms. of Carbon present in the yeast formed.	efficiency.
1	0.50	0.96	48	0.10	0.0369	0.0462	0.0012	0.0450	36.90	0.0521	0.0084	16.10
2	0.50	0.96	96	0.25	0.0962	0.1020	0.0016	0.1018	38.52	0.1305	0.0220	16.84
3	0.50	0.96	144	0.60	0.2302	0.1646	0.0024	0.1622	38.60	0.3130	0.0536	17.12
4	0.50	0.96	192	0.88	0.3855	0.1973	0.0029	0.1944	41.50	0.4512	0.0871	17.42

Study of the efficiency of yeast growth in a culture containing 0.2 per cent of ethyl alcohol in carbon in it <sup>under</sup> ~~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 carbonate	-	0.0220 gm.
Zinc sulphate	-	0.0125 gm.
Ammonium nitrate	-	0.5750 gm.
Water	-	500 c.c.

Above substances were taken in a 700 c.c. capacity of flask and digested with dil. hydrochloric acid till the precipitates just dissolved. Final volume of the culture was made up to 500 c.c. by water and pH adjusted near 4.5. Flask was lightly cotton plugged and sterilised at 10 lb. pressure of steam for 20 min. After cooling 1.90 gms of absolute ethyl alcohol was introduced in the ~~culture~~ to it. Culture was seeded with pure yeast and kept under continuously aerated condition by sterilised air obtained by passing it through con. sulphuric acid and distilled water. 100 c.c. of this culture was taken out after each 48 hours and analysed. Following results were thus obtained.

Study of the efficiency of yeast growth in 0.2 per cent ethyl alcohol carbon culture.

Temp. - 22.8°C to 26.4°C

S.N.	Per cent of ethyl alcohol present in the culture.	Per cent of ethyl alcohol originally present in the culture.	Number of hours passed after seeding of the culture.	gr. wt. of total acids produced in 100 cc of the culture.	gr. wt. of volatile acids produced in 100 cc. of the culture.	gr. wt. of the non-volatile acids produced in 100 cc. of the culture.	gr. of alcohol consumed by this amount of ferment when in 100 cc. of the culture.	grs. of yeast formed in 100 cc. of the culture.	Per cent of the yeast yield calculated on the basis of alcohol consumed.	grs. of carbon present in the alcohol consumed.	grs. of carbon present in the yeast formed.	Efficiency
1	0.20	0.38	48	0.0620	0.0010	0.0610	0.07	0.0463	66.14	0.0364	0.0106	29.05
2	0.20	0.38	96	0.1365	0.0020	0.1345	0.15	0.1064	70.93	0.0782	0.0243	31.02
3	0.20	0.38	144	0.1542	0.0025	0.1517	0.38	0.3015	79.34	0.2000	0.0684	34.46
4	0.20	0.38	192	0.1137	0.0018	0.1119	0.38	0.2721	71.60	0.2000	0.0622	31.10

Q Study of the growth of yeast <sup>using</sup> ethyl alcohol ~~using~~ as the carbon source at concentrations of 24%, 20%, 10%, 8%, 4% and 2% of ethyl alcohol by volume <sup>under</sup> ~~in~~ non-aerated ~~condense~~ conditions.

(1) The culture containing 24 per cent of ethyl alcohol by volume contained the following ~~are~~ substances.

Potassium dihydrogen phosphate	-	1.1447 gms.
Potassium sulphate	-	0.0324 gm.
Sodium chloride	-	0.0183 gm.
Calcium carbonate	-	0.0723 gm.
Magnesium carbonate	-	0.1320 gm.
Zinc sulphate	-	0.0500 gm.
Ammonium nitrate	-	3.45 gms.
Water	-	residue. 76 c.c.

Above substances were taken in 200 c.c. flask and digested with dil. hydrochloric acid ~~to~~ in 50 c.c. of water till the precipitates just dissolved. Total volume of the culture was made up to ~~170~~ 76 c.c. and ~~flask~~ was The flask was cotton plugged and sterilized at 10 lb. steam pressure for 20 min.. ~~cult~~ After cooling 24 c.c. of absolute ethyl alcohol was introduced in the culture and after making it homogenous it was seeded with little pure yeast. After 150 days 50 c.c. of the culture was analysed. The temperature variation during this period was 21.8°C to 34.6°C.

(2) The culture containing 20 per cent of ethyl alcohol contained following minerals in it.

Potassium <del>dihyph</del> 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	-	1.500 gms.	2.30 gms.
Zinc sulphate	-	0.250 gm.	0.0500 gm.
Water	-	100 c.c.	80 c.c.

Above substances were digested in 200 c.c. flask with 50 c.c. of water ~~and~~ dil. hydrochloric acid like the precipitates just dissolved. Total volume of the culture was made up to ~~100~~ 80 c.c. and pH adjusted near 4.5. Flask was then cotton plugged and sterilised at 10 lb. of steam pressure under for 20 min.. After cooling 20 c.c. of absolute ~~ethyl~~ ethyl alcohol was introduced in ~~the~~ <sup>the</sup> culture and ~~seeded~~ <sup>culture was</sup> seeded ~~was~~ with little pure yeast. The culture was kept at a temperature between 22.8°C to 27.5°C. It was analysed after 150 days. ~~and~~

(3) The culture containing 10 per cent of ethyl alcohol contained the following mineral substances -

Potassium dihydrogen phosphate	-	0.3779 gm.
Potassium sulphate	-	0.100 0.0546 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0241 gm.
Magnesium carbonate	-	0.0440 gm.
Ammonium nitrate	-	1.1500 gms.
Zinc sulphate	-	0.0500 0.0250 gm.
Water	-	<del>100</del> 90 c.c.

All the above mineral salts were taken in a flask of 200 c.c. capacity and digested with dil. hydrochloric acid ~~in~~ <sup>in</sup> 50 c.c. of water like the whole precipitates dissolved. Final volume of the culture was made up to 90 c.c. by adding more water and the flask <sup>was</sup> cotton plugged. Culture was sterilised at 10 lb. of steam pressure for 20 min.. After cooling the culture 10 c.c. of absolute <sup>ethyl</sup> alcohol was introduced in the culture and the culture seeded with pure yeast. ~~After~~ the

(88)

Culture was kept at a temperature <sup>variation</sup> between  $22.8^{\circ}\text{C}$  to  $28^{\circ}\text{C}$ .  
It was analysed after 150 days. ~~and gave the following~~

(4) In the culture containing 8 per cent of ethyl alcohol ~~also the~~ <sup>mineral</sup> substances as in the third culture ~~are~~ following substances were present.

Potassium dihydrogen phosphate	- <del>0.7558 gm</del> 0.6043 gm.
Potassium sulphate	- 0.0437 gm.
Sodium chloride	- 0.0097 gm.
Calcium carbonate	- 0.0385 gm.
Magnesium carbonate	- 0.0704 gm.
Ammonium nitrate	- 1.8400 gms.
Zinc sulphate	- 0.0400 gm.
Water	- 92 c.c.

All the above substances ~~are~~ <sup>were</sup> taken in a 200 c.c. flask and digested in 50 c.c. of water with dil. ~~the~~ hydrochloric acid till the whole precipitates <sup>just</sup> disappeared. Total volume of the culture was made up to 92 c.c. The flask was cotton plugged and sterilised at 10 lb. steam pressure for 20 min. After cooling the culture ~~to~~ 8 c.c. of absolute ethyl alcohol was introduced in it and ~~later~~ <sup>after</sup> it the ~~was~~ culture was seeded with pure yeast. It was kept at a temperature difference between  $23.4^{\circ}\text{C}$  to  $30.8^{\circ}\text{C}$ . and After 150 days the culture was examined and analysed.

(5) & The culture which had 4 per cent of ethyl alcohol by volume contained the following substances.

Potassium dihydrogen phosphate	- 0.3024 gm. <del>0.6043 gm.</del>
Potassium sulphate	- 0.0218 gm. <del>0.0437 gm.</del>
Sodium chloride	- 0.0048 gm. <del>0.0097 gm.</del>
Calcium carbonate	- 0.0197 gm. <del>0.0385 gm.</del>
Magnesium carbonate	- 0.0352 gm. <del>0.0704 gm.</del>
Ammonium chloride nitrate	- 0.9200 gm. <del>1.8400 gms.</del>
Zinc sulphate	- 0.0200 gm.
Water	- <del>450 c.c.</del> 96 c.c.

All the above substances were taken ~~to~~ with 50 c.c. of distilled water in a 200 c.c. flask and digested with dil. hydrochloric acid till the whole precipitates just dissolved. Total volume of the culture was then made up to 96 c.c. and pH adjusted near 4.5. The flask was ~~to~~ cotton plugged and sterilised at ~~a~~ 10 lb. steam pressure for 20 min. After cooling 4 c.c. of absolute ethyl alcohol was added in the culture and it was seeded ~~after~~ with little pure yeast. After 130 days culture was analysed. The temperature variation of the culture was between 20.8°C to 26.5°C.

(6) The culture, containing 2 per cent of ethyl alcohol, contained the following substances.

Potassium dihydrogen phosphate	-	0.1512 gm.
Potassium sulphate	-	0.0109 gm.
Sodium chloride	-	0.0048 gm.
Calcium carbonate	-	0.0197 0.0098 gm.
Magnesium carbonate	-	0.0176 gm.
Zinc sulphate	-	0.0100 gm.
Ammonium nitrate	-	0.9200 gm.
Water	-	98 c.c.

Above substances were taken in a 200 c.c. flask and digested in 50 c.c. water with dil. Hydrochloric acid till the precipitates ~~to~~ just dissolved. Total volume of the culture was made up to 98 c.c. and the flask was cotton-plugged. It was sterilised at 10 lb. pressure for 20 min. and after cooling ~~seeded with~~ it was seeded with pure yeast. The culture was kept at a temperature variation between 22.8°C to 28.4°C and analysed after 150 days.

Growth of yeast at various concentrations of ethyl alcohol cultures under non-aerated condition.

~~Standard No. 10<sup>th</sup> Nov. 1947 and analysed on the 3<sup>rd</sup> March 1947.~~

Analyses carried out after 150 days.

	Concentration of alcohol by volume present originally in the culture.	Temperature variation during the experiment.	gm. mol. of total acids present in 50 c.c. of the culture.	gm. mol. of Volatile acids present in 50 c.c. of the culture.	gm. mol. of non-volatile acids present in 50 c.c. of the culture.	gms. of yeast produced in 50 c.c. of the culture.	Percentage of yield of yeast calculated on the basis of volume of absolute ethyl alcohol in c.c. originally present in the culture.
1	24	21.8°C - 34.6°C.	0.3201	0.0014	0.3197	0.0825	0.69
2	20	22.8°C - 27.5°C.	0.2918	0.0017	0.2901	0.29 0.0880	0.88
3	10	22.8°C - 28.0°C.	0.2824	0.0004	0.2820	0.0788	1.58 <del>1.76</del>
4	8	23.4°C - 30.8°C.	0.2015	0.0002	0.2013	0.0670	1.68
5	4	20.8°C - 26.5°C.	0.1224	0.0004	0.1220	0.0476	2.38
6	2	22.8°C - 28.4°C	0.0812	0.0005	0.0807	0.0524	5.24

(10)

Study of the growth of yeast in cultures containing various concentration of glycerine as carbon ~~source~~ source of the organism in <sup>undecarated</sup> aerated condition.

3 solutions of Glycerine concentrations of 5.11 percent, 2.55%, 1.28% and 0.51% were tried, <sup>while</sup> the carbon concentrations of 2%, 1%, 0.5% and 0.2% respectively. In all these cultures glycerine is used as carbon source of the yeast.

(1) This ~~culture~~ culture contained 5.11% of glycerine by weight. It was prepared with the following substances.

Potassium dihydrogen phosphate -	0.7558 gm.
Potassium sulphate -	0.0580 gm.
Sodium chloride -	0.0122 gm.
Calcium chloride carbonate -	0.0482 gm.
Magnesium carbonate -	0.0880 gm.
Zinc sulphate -	0.0500 gm.
Ammonium sulphate -	2.53 gm.
Water -	Base. 400 c.c.
Glycerine -	20.44 gms.

Above mineral substances were first taken in 700 c.c. flask and digested with dil. hydrochloric acid in 200 c.c. was water till the precipitates just dissolved. Total volume of the culture <sup>was</sup> then made up to 400 c.c. and its pH <sup>was</sup> adjusted near 4.5. 20.44 gms. of glycerine <sup>was</sup> then introduced in the culture and it ~~is~~ was cotton plugged and sterilised at 10 lb. steam pressure ~~at~~ for 20 min. After cooling it was seeded with yeast. ~~Culture~~ This culture was aerated ~~at~~ by sterilised air obtained by passing air through first con. sulphuric acid and then distilled water. The temperature variation during the experiment was between 20.4°C to 24.8°C. 100 c.c. of the culture were ~~examined~~ <sup>analysed</sup> after ~~each~~ <sup>every</sup> fourth day.

~~and~~ (2) The culture which contained 2.55 per cent of glycerine by weight contained the following substances.

Potassium dihydrogen phosphate -	0.3779 gm.
Potassium sulphate -	0.0290 gm.
Sodium chloride -	0.0062 gm.

Calcium carbonate	-	0.0241 gm.	(22)
Magnesium carbonate	-	0.0440 gm.	
Zinc sulphate	-	0.0250 gm.	
Ammonium sulphate	-	1.2660 gm.	
Glycerine	-	12.75 gm.	
Water	-	500 c.c.	

All the above substances were taken in a 700 c.c. capacity flask and digested with dil. hydrochloric acid in 200 c.c. of water till the whole precipitates just dissolved. Total volume of the culture was made up to 500 c.c. and pH maintained 4.5. 12.75 gm. of glycerine is added in the culture and ~~it~~<sup>the culture</sup> was sterilised at 10 lb. steam pressure for 20 min. and after cooling it was seeded. The culture was aerated by sterilised air obtained by passing it through con. sulphuric acid and distilled water. The culture was at a temperature variation of 20.4 °C to 24.8 °C. After every fourth day 100 c.c. of the culture was analysed.

(3) The culture which contained 1.28 per cent of glycerine by weight contained following substances.

Potassium dihydrogen sulphate	-	0.1889 gm.
Potassium sulphate	-	0.0504 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0120 gm.
Magnesium carbonate	-	0.0241 gm.
Zinc sulphate	-	0.0220 gm.
Ammonium sulphate	-	0.0125 gm.
Water	-	500 c.c.
Glycerine	-	7.40 gm.

Above substances were taken in 700 c.c. capacity flask and digested with dil. hydrochloric acid till the precipitates just dissolved. The total volume of the culture was made up to 500 c.c. and <sup>its</sup> pH ~~of~~ adjusted as near 4.5 as possible. Flask was cotton plugged and sterilised at 10 lb. steam pressure for 20 min. After ~~cool~~ cooling it was seeded with yeast and kept at a temperature ranging 20.4 °C to 24.8 °C in aerated condition, by passing sterilised

(150)

air through it obtained by bubbling it through con. sulphuric acid and distilled water. 100 c.c. of this culture was analysed after every fourth day.

(4) Culture containing 0.51 per cent of glycerine as carbon food for the yeast contained the following substances.

Potassium dihydrogen <del>sulfate</del> phosphate	—	0.0944 gm.
Potassium sulphate	—	0.0202 gm.
Sodium chloride	—	0.0061 gm.
Calcium carbonate	—	0.0060 gm.
Magnesium <del>sulfate</del> carbonate	—	0.0110 gm.
Ammonium sulphate	—	0.3165 gm.
Zinc sulphate	—	0.0063 gm.
Water	—	500 c.c. <del>2.5 gm.</del>
Glycerine	—	2.55 gm.

Above <sup>mineral</sup> substances were taken <sup>with</sup> 200 c.c. of water in a 700 c.c. capacity flask and digested with ~~just~~ dil. hydrochloric acid till the precipitates just dissolved. Total volume of the culture was made up to 500 c.c. and pH. adjusted near 4.5. The flask was cotton plugged and sterilised at a pressure of 10 lb. for 20 min. and after cooling seeded with yeast. It was aerated with sterilised air obtained by bubbling it through con. sulphuric acid and distilled water. The temperature variation during the experiment was between 20.4°C to 24.8°C. 100 c.c. of this culture was analysed after every fourth day.

Following ~~table~~ <sup>data</sup> was obtained as the result of analysis of the above ~~one~~ cultures:—

101

Growth of yeast <sup>using</sup> glycerine as source of carbon in the culture -

pH - 4.5.

Temperature - 20.4 °C to 29.8 °C.

S. No.	number of days passed after seeding the culture.	percentage of glycerine carbon in the culture.	gm. of glycerine in 100 c.c. of the culture.	gm. mol. of total acids produced after fermentation in 100 c.c. of the culture.	gm. mol. of D-lactate acids produced in 100 c.c. of the culture.	gm. mol. of non-volatile acids generated in 100 c.c. of the culture.	gm. of yeast produced in 100 c.c. culture.	yield of yeast in % calculated on the basis of glycerine present in culture.
1	4	2.00	5.11	0.0960	0.0007	0.0953	0.1562	3.05
2	4	1.00	2.55	0.0880	0.0005	0.0875	0.0970	3.80
3	4	0.50	1.28	0.0125	0.0029	0.0096	0.0536	4.18
4	4	0.20	0.51	0.0064	0.0003	0.0061	0.0500	9.86.
1	8	2.00	5.11	0.1040	0.0007	0.1033	0.2116	4.14
2	8	1.00	2.55	0.0960	0.0008	0.0952	0.1196	4.70
3	8	0.50	1.28	0.0360	0.0036	0.0334	0.0636	4.96
4	8	0.20	0.51	0.0464	0.0014	0.0450	0.0896	17.53.
1	12	2.00	5.11	0.1408	0.0012	0.1396	0.2197	4.30
2	12	1.00	2.55	0.1102	0.0010	0.1092	0.1448	5.87
3	12	0.50	1.28	0.2000	0.0042	0.1958	0.1528	11.93
4	12	0.20	0.51	0.0621	0.0017	0.0604	0.0520	10.01.

Study of the effect of oxidising salts as ~~potassium~~ potassium  
 dichromate ~~and~~, potassium permanganate / on the growth of yeast in  
 sucrose cultures of 2 per cent concentration <sup>under</sup> non-aerated condition.

In the preceding experiments with aerated cultures it was noted that in all cases aeration of the cultures helped in yeast formation. ~~These~~ <sup>These</sup> experiments were performed with a view to see if ~~some~~ oxidising substances have also any ~~beneficial~~ <sup>beneficial</sup> ~~result~~ <sup>effect</sup> on yeast growth. To study this, following ~~these~~ <sup>five</sup> cultures were prepared which contained the following substances.

- Potassium dihydrogen phosphate . . . . . 0.3779 gms.
- ~~Potassium~~ Calcium carbonate - . . . . . 0.0241 gm
- Magnesium carbonate - . . . . . 0.0440 gm
- Sodium chloride - . . . . . 0.0061 gm
- Ammonium sulphate - . . . . . 1.2660 gms.
- zinc sulphate - . . . . . 0.0250 gm.
- water - . . . . . 500 c.c.
- sucrose - . . . . . 10 gms.

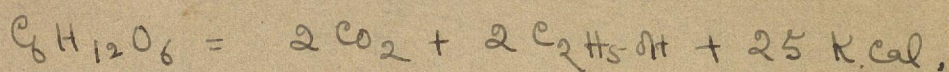
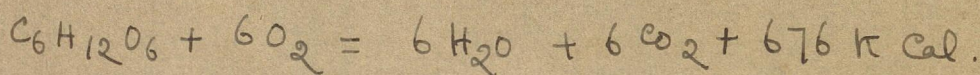
Above substances were weighed in each <sup>700 c.c.</sup> flask. In one flask 2.50 gms. of potassium dichromate was introduced in the second flask 2.50 gms of potassium permanganate was added in the third 2.50 gms. of ferrrous oxide ~~and~~ was put and in the fourth 2.50 gms of manganese dioxide was taken. Fifth culture was kept as such to serve the purpose of a control experiment.

All the flasks were cotton plugged and sterilised as usual and <sup>after cooling</sup> seeded with pure yeast. ~~But~~ The cultures were kept at a temperature varying between 24° to 30.4°C. After 30 days the cultures were examined under microscope and by cell counting showed that equal number of cells had grown in the cultures containing ferrrous sulphate oxide ~~and~~ <sup>and control experiment</sup> manganese oxide <sup>of yeast</sup> but there ~~no~~ <sup>no</sup> significant difference in the cultures containing potassium dichromate and potassium permanganate. ~~A~~

(103)

Increased amount of yeast growth in the preceding experiments can not be achieved by introducing oxidising agents in it because perhaps they are either too strong or too ~~more~~ mild to ~~be~~ act beneficially for yeast growth.

By the following reactions:-



We see that if glucose is oxidised to carbon dioxide and water 676 K. Cal. of heat is liberated but if it is ~~not~~ oxidised to carbon dioxide and alcohol 25 K. Cal. of heat is liberated. Yeasts can grow without oxygen and in this case they convert sugar to alcohol and utilise this 25 K. Cal. of energy for its activity. If some how the first reaction in which 676 K. Cal. of heat is evolved, is allowed to take place in the culture then perhaps no alcohol will be produced because then there will be large amount of energy ~~at~~ available to maintain the activities of yeasts.

We have done many experiment in which alcohol, starch, ~~sugar~~ <sup>glycerine</sup> and starch were oxidised by passing air in them and simultaneously yeast was grown in the culture. We have observed that if necessary ~~amount~~ amount of energy for the growth and activity of yeast cells are supplied by oxidations then yeast can grow ~~at~~ even in alcohol. We have in preceding experiments tried to grow yeast in  $\frac{1}{2}$  0.38 per cent by weight <sup>ethyl</sup> alcohol culture in which air was passed and have obtained

(104)

as much as 79.34 per cent of yield.

Similarly although yeast do not possess the property of hydrolysing starch to glucose and so can not utilise ~~it~~ <sup>starch</sup> for its growth, if air is passed in weak solutions of starch, with the help of the energy liberated by the oxidation of the ~~starch~~ starch, yeasts are able to maintain their life activity in such culture and grow in it. We have ~~but~~ tried to grow yeast in dil. salt solutions of starch and have obtained good results.

Growth of yeast in cultures containing n-propyl and n-butyl <sup>alcohols</sup> as the source of carbon in the cultures is under aerated and non-aerated condition.

Two cultures were prepared which contained 0.5 per cent carbon of n-propyl alcohol as the source of carbon food for yeast and two cultures contained 0.5 per cent carbon of n-butyl alcohol as the source of carbon food. All the above cultures contained following mineral substances.

Potassium dihydrogen phosphate	-	0.1889 gm.
Potassium sulphate	-	0.0504 gm.
Sodium chloride	-	0.0061 gm.
Calcium carbonate	-	0.0120 gm.
Magnesium <del>so</del> carbonate	-	0.0220 gm.
Zinc sulphate	-	0.0125 gm.
Water	-	500 c.c.
Ammonium sulphate	-	0.6325 gm.

Above substances were taken in each 700 c.c. flask and digested with dil. hydrochloric acid in 200 c.c. of water till the precipitate just dissolved. Total volumes of the cultures was made up to 500 c.c. each and pH adjusted at 4.5. Flasks were cotton plugged and sterilised at 10 lb. steam pressure for 20 min. In two ~~flasks~~ flasks 4.15 gms of n-propyl alcohol was introduced and in the other two flasks 3.85 gm of n-butyl alcohol was added. The cultures were seeded with yeast and one culture of n-propyl and n-butyl alcohol <sup>each</sup> was kept at ~~some~~<sup>a</sup> temperature varying between 22.8°C to 31.5°C. ~~The other set of~~ <sup>In the other</sup> ~~Another~~ set of these alcoholic cultures a slow current of ~~the~~ sterilised air was passed through. ~~This was done~~ All the above cultures were ~~examined~~ All the

All the above cultures were ~~so~~ examined under ~~main~~ microscope after 15 days but no sign of yeast cell was seen in any of these cultures. The temperature

variation during this period was between 22.8°C to 28.8°C.

Above cultures were again examined after one month but again no sign of yeast cell was observed under microscope and after this aeration experiments were stopped.

However after ~~the~~ nearly six months the non-aerated culture of n-propyl alcohol showed a little growth of yeasts. The yeasts were isolated and filtered out and 0.0529 gms. of yeasts ~~was~~ <sup>were</sup> obtained. This brings the yield to 1.28 per cent of the original amount of n-propyl alcohol <sup>present</sup> in the culture.

151

Method of measuring yeast growth in culture containing  
P very small amount of yeast <sup>This method is</sup> specially employed in the cultures  
in which only inorganic materials are used as  
the carbon and nitrogen source of food:—

One drop is taken on a clean slide of a  
microscope. ~~This is~~ <sup>And examined under a</sup> a D.R.P. Leitz Wetzlar microscope.  
The slide was examined ~~is~~ <sup>with</sup> a ~~oil immersion~~ <sup>oil immersion</sup>  
objective. ~~of~~ <sup>Object is</sup>  $\frac{1}{12}$  oil immersion, Ernst Leitz  
Wetzlar, of apert. 1.30, 100x. The eyepiece ~~of~~ of the  
microscope is ~~Ernst~~ Ernst Leitz Wetzlar Periplan O.K.

10x.

For finding the average number of cells per  
slide cell counting is done in 100 slides of the culture  
and then the average is calculated.

It has been established ~~is~~ by me that after  
<sup>making</sup> ~~taking~~ 10,000 cell countings ~~is~~ of 24 different cultures that  
if <sup>one</sup>  $\frac{1}{2}$  cell is seen per view of the ~~at~~ slide the culture  
contains approx.  $5.8 \times 10^{-6}$  gms. of yeasts per c.c. of ~~that~~

~~The~~ In the ~~succeeding~~ experiments, of growing  
yeasts in ~~in~~ purely mineral medium, where the  
growth of yeast is very small and is of only  
classical interest this <sup>cell</sup> counting method is employed  
to measure the growth of yeasts.

B Study of the growth of yeast in culture containing urea as a source of carbon and nitrogen for yeast under aerated and non-aerated culture conditions.

Two cultures were prepared containing the following substances

- Potassium dihydrogen phosphate - 0.0096 gm.
- Calcium ~~sulfate~~ carbonate - 0.0012 gm.
- Magnesium ~~carbonate~~ sulphate - 0.0024 gm.
- Urea - 0.5000 gm.
- Water - 500 c.c.

Cultures contained all the above substances in each. They were digested with very dil. hydrochloric acid till the precipitates just dissolved and cultures were cotton plugged and sterilised as usual. After <sup>cooling</sup> seeding, they were seeded with very little yeast. One culture was aerated with sterilised air and other culture was kept in ~~non-~~ non-aerated condition. Temperature variation was between 26°C to 30.8°C. The cultures were seeded on the 9<sup>th</sup> Feb. 1948.

13<sup>th</sup> Feb. 1948 —

The non-aerated culture of urea showed very few decaying yeasts. Cells were <sup>transparent,</sup> ~~small~~ very small and dormant.

The aerated cultures showed healthy yeast. An average of 2 cells was ~~obs~~ per view ~~was~~ were seen. ~~Cells~~ They appeared swollen and were budding.

14<sup>th</sup> Feb. 1948 — #

The non aerated cultures showed decaying cells. They had reduced in size and ~~was~~ no bud was seen. An average ~~no~~ of one cell was seen per view of the microscope under ~~brother~~ ~~to~~ 1200 magnification of oil immersion lens.

The aerated culture showed an average of 4 healthy, swollen and budding cells per view in the microscope.

16<sup>th</sup> Feb. 1948 -

The non-aerated cultures did not show any yeast cell.

Aerated cultures showed ~~between~~ healthy cells at an average of 5 <sup>yeast</sup> cells per view. ~~all~~ They were healthy, swollen and budding.

24<sup>th</sup> Feb 1948 -

In aerated cultures an average of 6.5 <sup>yeast</sup> cells per view of the microscope was ~~at~~ observed. Cells were healthy and budding.

The non-aerated culture did not show any yeast.

29<sup>th</sup> Feb 1948 -

The ~~at~~ yeast cells in aerated culture were healthy swollen. Swollen but they were quite dormant. An average of 7 yeast cells per ~~view~~ view of the microscope was obtained.

6<sup>th</sup> March 1948 -

Aerated culture contained an average of 6.0 ~~at~~ yeast cells per view of the microscope. Cells were healthy but smaller in size and no bud was seen.

10<sup>th</sup> March 1948 -

Aerated culture showed decaying yeasts. ~~an~~ An average of 3.5 <sup>yeast</sup> cells was obtained. Cells had reduced in size.

11<sup>th</sup> March 1948 -

No yeast cell was seen in the culture.

Thus we see that urea ~~is~~ can be utilised as carbon and nitrogen food of yeast in aerated condition. But however the growth of yeast stops and ultimately yeasts die out in this culture after a month. The yield of yeast is exceedingly small.

Study of the effect of Vitamins B complex and C on the growth of yeast, alcohol formation and acid production under aerated and non-aerated conditions. Ethyl alcohol is used as the ~~several~~ carbon source of the yeast.

Several cultures were prepared in 100 c.c. flasks containing the following substances:-

$K_2H_2PO_4$ -	0.7558 gm.
NaCl -	0.0122 gm.
$K_2SO_4$ -	0.0504 gm.
$CaCO_3$ -	0.0482 gm.
$MgCO_3$ -	0.0880 gm.
$5NH_4NO_3$ -	2.3000 gm.
Water -	400 c.c.
Ethyl alcohol (absolute) -	20 c.c.

The cultures were sterilised and after cooling above 20 c.c. of absolute ethyl alcohol was added. After ~~to~~ <sup>two</sup> ~~three~~ <sup>four</sup> one set of such flasks following Vitamins of the B complex group, were added.

Vitamin B <sub>1</sub> -	3 mgm.
Vitamin B <sub>2</sub> -	1 mgm.
Nicotinic acid -	25 mgm.
Vitamin B <sub>6</sub> -	0.5 mgm.
Pantothenic acid -	3.0 mgm.

In another set of ~~three~~ <sup>two</sup> flasks containing above mineral substances 50 mg. of l-ascorbic acid was added. Similarly in the other set of ~~three~~ <sup>two</sup> flasks all the above Vitamin B Complex in the above proportion and 50 mg l-ascorbic acid was added. One ~~see~~ <sup>two</sup> set of ~~three~~ <sup>two</sup> flasks was kept without any Vitamin as control culture.

All the above cultures were seeded by a very little quantity of ~~see~~ yeast (approx .0002 gm.) and kept at a temperature of 25°C - 30°C.

After 46 days the yeast formations

One set of the above culture was kept as such

to in the another set slow stream of sterilised air was passed.

The growth of yeast was in the aerated cultures completed after 30 days and in the case of non-aerated cultures it took 46 days. After complete fermentation the cultures on analyses gave the following results:-  
non-aerated cultures:-

Vitamins	gm. of yeast produced	gm. of alcohol left in the culture	Total gm. mol of acid produced.	gm. mol. of Volatile acid.	gm. mol. of non-volatile acid.	yield of yeast in percentage.
B+C	5.8804	0	0.0584	0.0052	0.0532	29.40
B	5.2990	0	0.0512	0.0050	0.0462	26.45
C	4.7110	0	0.0582	0.0061	0.0521	23.50
non-vitaminised culture	3.8050	0	0.0522	0.0042	0.0480	19.00

Aerated Cultures:-

Vitamins	gm. of yeast produced	gm. of alcohol left in the culture.	Total gm. mol of acid produced.	gm. mol. of Volatile acid	gm. mol. of non-volatile acid	yield of yeast in %.
B+C	6.4320	0	0.0856	0.0056	0.0800	32.15
B	5.9241	0	0.0852	0.0054	0.0798	29.60
C	5.1214	0	0.0848	0.0071	0.0777	25.60
non-vitaminised culture	4.2010	0	0.0802	0.0051	0.0751	21.00

Conclusion:-

Thus we see that if ethyl alcohol is used as source of food energy material Vitamin C has little beneficial effect in comparison with Vitamin B complex. But the combination of Vitamin B complex and C is most beneficial for the yeast growth. These vitamins have no marked effect on the acid productive property of yeast. In all cases the aerated cultures gave more yeast than the non-aerated cultures.

Studies in the preparation of yeast from molasses in presence of Vitamin B and C under aerated and non-aerated conditions.

Culture was prepared in 750 c.c. flasks containing the following mineral matters

$KH_2PO_4$	-	0.3879 gm.
NaCl	-	0.0061 gm.
$K_2SO_4$	-	0.0252 gm.
$CaCO_3$	-	0.0241 gm.
$NH_4NO_3$	-	1.1500 gm.
Water	-	400 c.c.

Total amount of sugars present in the molasses added in each flask = 5.342 gm.

Four sets of two flasks each were separated. To one ~~set~~ set of flasks following vitamins were added.

- Vitamin B<sub>1</sub> - 3 mg.
- Vitamin B<sub>2</sub> - 1 mg.
- Nicotinic acid - 25 mg.
- Vitamin B<sub>6</sub> - 0.5 mg.
- Pantothenic acid - 3.0 mg.

In the another set of two flasks l-ascorbic acid - 50 mg was added.

In the third set following Vitamins were introduced.

- Vitamin B<sub>1</sub> - 3 mg.
- Vitamin B<sub>2</sub> - 1 mg.
- Nicotinic acid - 25 mg.
- Vitamin B<sub>6</sub> - 0.5 mg.
- Pantothenic acid - 3.0 mg.
- l-ascorbic acid - 50 mg.

The fourth set of two flasks was kept as such without any Vitamin.

All the flasks were properly cotton plugged and sterilised.

After cooling the cultures were seeded with little amount of yeast and one set was ~~kept~~ kept as such and ~~the~~ to the other sterilised air was passed through. The temperature variation during the experiments was between 24°C to 29°C.

The <sup>aerated</sup> Cultures showed no sign of presence of sugar after 11 days. On analyses after 11 days they gave the following results.

Aerated Cultures.

Temp - 24°C to 29°C.  
gm. of sugar consumed - 5.342 gm.

Vitamins	gm. of yeast produced.	gm. of alcoh. produced.	gm. mol. of Total acid produced.	gm. mol. of Volatile acid produced.	gm. mol. of non-volatile acid prod.	Yeast in per cent of sugar consumed.
B+C	2.0850	0.44	0.0504	0.0062	0.0442	38.65
B	1.9000	0.45	0.0500 <del>0.0504</del>	0.0058 <del>0.0058</del>	0.0442 <del>0.0463</del>	35.57
C	1.3730	0.48	0.0508	0.0060	0.0448	24.01
non-vitaminised cultures.	1.2050	0.51	0.0501	0.0061	0.0440	22.55

The growth of yeast completed.

In the non-aerated cultures the growth of yeast completed after 15 days and on analyses the cultures gave following results:-

non-aerated Cultures.

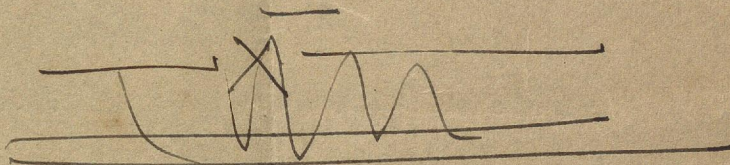
Temp - 24°C to 29°C.  
Total gm. of sugar consumed - 5.342 gm.

Vitamins	gm. of yeast produced.	gm. of alcoh. formed	gm. mol. of Total Acid generated	gm. mol. of Volatile acid produced.	gm. mol. of non-volatile acid.	per cent of yeast produced.
BC.	1.3662	0.86	0.0648	0.0072	0.0546	25.57
B	1.3662	0.87	0.0640	0.0070	0.0570	25.57
C	1.1622	0.89	0.0642	0.0080	0.0562	21.75
non-vitaminised cultures	1.1406	0.86	0.0821	0.0084	0.0737	21.35

## Conclusion —

A combination of Vitamins B and C have very beneficial effect on the growth of yeast in sugar solution under aerated condition. Vitamin B ~~to~~ has more growth promoting effect on yeast under these conditions than Vitamin C. & Vitamin C is very ~~a~~ little beneficial.

~~The~~ In non-aerated cultures, if the energy <sup>vitamins</sup> material is sugar the effect of B and C combined is as much as B alone and C has very little use.



of sucrose in culture

Study of ~~the~~ effect of the concentration on the growth  
 yeast, alcohol production and acid generation.

Following ~~kind of~~ ~~media~~  
 Cultures containing the following substances were

prepared.

- (a)
- $KH_2PO_4$  — 2.2674 gms.
  - $K_2SO_4$  — 0.1512 gms.
  - $NaCl$  — 0.0366 gms.
  - $CaCO_3$  — 0.1452 gms.
  - $ZnSO_4$  — 0.1000 gms.
  - $MgCO_3$  — 0.2640 gms.
  - $NH_4NO_3$  — ~~2.30~~ 6.60 gms.
  - Sucrose Sugar — ~~50.0~~ 50.0 gms.
  - Water — ~~500.00~~ 400 c.c.

- (b)
- $KH_2PO_4$  — 0.7558 gms.
  - $K_2SO_4$  — 0.2016 gms.
  - $NaCl$  — 0.0122 gms.
  - $CaCO_3$  — 0.0482 gms.
  - $MgCO_3$  — 0.0880 gms.
  - $ZnSO_4$  — 0.1000 gm.
  - $NH_4NO_3$  — 2.30 gms.
  - Sucrose Sugar — 20 gms.
  - Water — 400 c.c.

- (c)
- $KH_2PO_4$  — 0.3779 gms.
  - $K_2SO_4$  — 0.1008 gms.
  - $NaCl$  — 0.0061 gm.
  - $CaCO_3$  — 0.0241 gms.
  - $ZnSO_4$  — 0.1000 gm.
  - $MgCO_3$  — 0.0440 gm.
  - $NH_4NO_3$  — ~~1.0~~ 1.1500 gm.
  - Sugar Sucrose — 10 gms.
  - Water — 400 c.c.

- (d)
- ~~0.1~~  $KH_2PO_4$  — 0.1889 gms.
  - $K_2SO_4$  — 0.0504 gms.
  - $NaCl$  — 0.0030 gm.
  - $ZnSO_4$  — 0.1000 gms.
  - $CaCO_3$  — 0.0720 gm.
  - $MgCO_3$  — 0.0220 gm.
  - $NH_4NO_3$  — 0.525 gm.
  - Sucrose — 2.0 gm.
  - Water — 400 c.c.

Method of preparing Cultures -

First all the substances to be added in a culture were taken in 100 cc. flask and then 100 c.c. of water was added in it and the substances were digested with acid till the ppt. just dissolved. The pH. of the cultures were <sup>was</sup> adjusted to 4.5. Then total volume was made 400 cc. Thus in the flasks containing the (a) substances had a sucrose concentration of 10, (b) 5, (c) 2.5, and (d) 1 per cent. These flasks were carefully cotton-plugged and sterilised at 15 lb. pressure for 20 min. After cooling they were carefully inoculated with pure yeast and kept at 28°C to 30.5°C. As soon as the fermentation stopped, which was judged by seeing the formation of yeast on the surface of the culture and presence of sugar in the culture they were analysed and following results were obtained.

pH - 4.5

Temp - 28°C to 30.5°C

S.No.	% of sugar in the culture	Total amount of sugar in the culture in gms.	days needed for complete fermentation	gms. mol. of total acid produced.	gms. mol. of volatile acid produced.	gms. mol. of non-volatile acid produced.	gms. of alcohol produced.	% of alcohol production	gms. of yeast produced.	wt. of yeast
1	12.5	50	<del>50</del> 62	4.0121	0.8412	3.1719	20.00	40.00	12.2600	29.52
2	5.0	20	50	0.9736	0.0211	0.9525	3.40	17.00	5.506	21.50
3	2.5	10	20	0.3214	0.0621	0.2593	1.12	11.20	2.90	29.21
4	1.0	4	8	0.0986	0.0022	0.0964	0.43	10.75	1.2	30.25

~~Conclusion~~ As the concentration of sucrose decreases in the culture the percentage yield of yeast calculated on the sucrose fermented is <sup>increases</sup> less and alcohol formation decreased but as the concentration increases in the culture the yeast yield is reduced but the alcohol formation