

# A Study of the Growth of Dhar Yeast in Cultures Containing Ethyl Alcohol as Source of Carbon and Different Ammonium Salts as Source of Nitrogen, Under Non-aerated Conditions.

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## Summary

It has been found that certain yeasts, at least (1, 2, 3), may use ethyl alcohol as the sole source of carbon and ammonium sulphate as a source of nitrogen (4, 5, 6, 7, 8, 9, 10). It is known that Dhar Yeast can utilize ethyl alcohol as a source of carbon (11), but there is no evidence as to its ability to use ammonium salts as a source of nitrogen. This paper reports the results of experiments with a variety of ammonium salts on Dhar Yeast grown with ethyl alcohol as the sole source of carbon.

## EXPERIMENTAL

SEVERAL cultures containing each, 0.20 gm. of calcium carbonate, 0.20 gm. of sodium chloride, 0.20 gm. of potassium sulphate, 0.20 gm. of disodium hydrogen phosphate, 0.05 gm. of zinc sulphate, and 0.25 gm. of magnesium carbonate, were made. These minerals were weighed out in 750 c.c. flat bottom pyrex flasks, and 200 c.c. of distilled water were added to each of them and the minerals were digested with dilute hydrochloric acid. To the clear solutions thus obtained, the amounts of ammonium salts, mentioned in the tables, were added and the total volume of each was made to 400 c.c. with distilled water at the same time adjusting the pH to be 4.5.

These flasks were plugged with non-absorbent sterilised cotton, and the cultures were sterilised by heating at 10 lbs pressure for 30 minutes in an autoclave. After cooling, 30 c.c. of absolute ethyl alcohol were added to each culture and then, after gentle shaking by whirling motions, each of them was seeded with a trace of an activated sample of Dhar Yeast.

These cultures were kept together at room temperature during the period of fermentation, which was of 100 days. The temperature variation during this time was between 30° C., and 36° C. The nitrogen content of the filtered solutions and of the dried yeast from each of the experiments shown in the Table II was measured by Kjeldahl Gunning procedure.

To estimate the volatile acids formed during fermentation, 100 c.c. of the clear solution obtained after filtering the yeast was taken in a distilling flask and 80 c.c. of this solution was distilled and made up to 100 c.c. with distilled water, and this solution was titrated with a standard baryta solution using methyl red as indicator.

## RESULTS

Results obtained by the analysis of all the cultures are tabulated below:—

TABLE I.  
Data of Alcohol Consumption and Acid Formation During Fermentation.

Name of the ammonium salt used as source of nitrogen	gm. of salt taken.	gm. of alcohol left.	gm. of alcohol used.	gm. of total acid produced	gm. of volatile acid formed.	% yield of acid on the basis of alcohol used	gm. of dry yeast grown.	% of yeast yield on the basis of alcohol used.
Ammonium sulphate ..	0.9428	0.20	2.12	0.2431	0.0132	11.31	0.9659	45.54
Ammonium carbonate	0.6851	0.40	1.92	0.1157	0.0132	6.24	0.7568	39.41
Ammonium bitartrate	2.1571	0.64	1.68	0.2928	0.0033	17.42	1.1828	70.40
Sodium ammonium hydrogen phosphate %	1.9571	0.00	2.32	0.0000	0.0000	0.00	1.0528	45.38
Ammonium hydrogen phosphate % ..	1.6428	0.40	1.92	0.0000	0.0000	0.00	1.1122	57.86
Ammonium chromate*	1.0856	—	—	—	—	—	—	—
Ammonium oxalate*	0.8856	—	—	—	—	—	—	—

\* Yeast cells died in these cultures

% In presence of these salts cultures become alkaline during fermentation

TABLE II  
Data of Nitrogen Consumption, Nitrogen Content of Yeast Cells and Nitrogen Loss.

Name of the ammonium salt added	gm. of nitrogen in the culture in the beginning.	gm. of nitrogen left in the culture.	gm. of nitrogen consumed.	gm. of nitrogen in the yeast	% of loss of nitrogen on the basis of nitrogen consumed	gm. of yeast yielded by 1 gm. of nitrogen
Ammonium sulphate ..	0.2284	0.1115	0.1169	0.0487	58.30	8.25
Ammonium carbonate	0.1410	0.0420	0.0990	0.0382	57.59	7.63
Ammonium bicarbonate	0.2113	0.0531	0.1581	0.0597	62.23	7.47
Ammonium sodium hydrogen phosphate	0.1542	0.0000	0.1542	0.0531	65.52	6.79
Ammonium hydrogen phosphate ..	0.3769	0.2133	0.1636	0.0561	65.34	6.78

## DISCUSSION

When ammonium oxalate or ammonium chromate is used as the source of nitrogen, in Dhar Yeast cultures containing ethyl alcohol as the source of carbon under non-aerated conditions, no yeast growth is observed. A very low consumption of ethyl alcohol is noted in the cultures containing ammonium tartrate, ammonium carbonate and ammonium hydrogen phosphate. However, this low consumption of alcohol is followed by a very high yield of yeast in the case of ammonium tartrate and very low yeast yield in the case of ammonium carbonate. The consumption of alcohol is maximum in the case of sodium ammonium hydrogen phosphate. The percentage loss of nitrogen is not much influenced by the nature of the ammonium salt in the culture. Percentage of nitrogen in the yeast is maximum when ammonium sulphate is used as source of nitrogen

and least when sodium ammonium hydrogen phosphate is the nitrogen food. Although it has been very well observed that other varieties of yeasts also utilise ammoniacal nitrogen as source of nitrogen, study of the influence of different ammonium salt in their cultures has not been done so far.

It is interesting to note that in the cultures containing sodium ammonium hydrogen phosphate and ammonium hydrogen phosphate, growth of yeast is followed by the production of alkali, whereas in all other cases under study, acid production is seen during the fermentation

### Summary

If different ammonium salts are used as the sources of nitrogen in Dhar Yeast cultures, containing ethyl alcohol as source of carbon, under non-aerated conditions, very little difference, in the loss of nitrogen in these cultures is seen, it being about 60 per cent. in all the cases. Presence of ammonium tartrate as nitrogen food in the cultures produces maximum yeast yield. If ammonium oxalate or ammonium chromate is used as the source of nitrogen no yeast yield is seen. The culture containing sodium ammonium hydrogen phosphate or ammonium hydrogen phosphate as source of nitrogen, shows the formation of alkali during yeast growth which is quite unlike the other cultures where acid production is observed during the yeast growth.

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