An 'Ammonia'-yeast and some of its Properties

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(*Received* 12 *June* 1945)

An account is given here of the manner of preparing a living yeast in which all the potassium is replaced by ammonia, the process being reversible. The fermenting power, growth and resting oxygen uptake of such yeast are described.

METHODS

Chemical

Ammonia was determined by a microdiffusion method (Conway, 1939).

Potassium was determined as previously described (Boyle, Conway, Kane & O'Reilly, 1941).

Preparation of the 'ammonia'-yeast

Ordinary bakers' yeast (as supplied from the Cork Yeast Factory) was used at the outset; then a pure strain (yeast 'race 12') was investigated and found to behave in a similar manner, but the bakers' yeast was preferred for the investigation as it concentrated K and ammonia better. A pure strain was then grown from the commercial bakers' yeast by plating in agar containing malt extract, and selecting one colony for further growth in Pasteur flasks. This yeast was used in most of the experiments. The growth medium was made up as follows: glucose, 12·5 g.; sucrose, 12·5 g.; KH_2PO_4, 10 g.; $MgSO_4$, 1 g.; yeast extract, 250 ml. The pH was adjusted with NaOH to 5·65 and tap water added to give 1000 ml. The yeast extract was made by boiling 100 g. yeast with 500 ml. water for 1 hr., cooling, filtering and making up to 500 ml.

The process of ammoniating the yeast consists in suspending 1 part of yeast in 50 parts of a medium having the following composition: 363·0 ml. of 1·17% NaCl; 10·0 ml. of 2% $CaCl_2$; 20 ml. of 5% $NaHCO_3$; 10 ml. of 7% glucose; 5 ml. of an equal mixture of 0·02 M-Na_2HPO_4 and 0·02 M-NaH_2PO_4. This was made up to 1 l. with 0·2 N-NH_4Cl. Another suspension of the same yeast was made in a solution similar to the above, but in which KCl replaced NH_4Cl. The yeast was maintained in suspension by bubbling with a gas mixture containing 3% CO_2 and 97% O_2. The solution was at first renewed at intervals of some hours and then left overnight for a 24 hr. period. In later experiments bubbling was suspended in the evening and continued next day. With continued bubbling almost all the K may be removed after 1 day's treatment, but may require 2 days or longer for the traces. Longer periods are also required for the interrupted bubbling.

Examples of the K *removal and reversal*

The sample of bakers' yeast was first washed in Ringer fluid and centrifuged. Analysis of the centrifuged yeast showed 450 mg./100 g. of K and 1 mg./100 g. of ammonia-N. The yeast was then suspended in the fluid described above for the replacement of K by NH_3; two other suspending fluids containing 0·05 N- and 0·01 N-NH_4Cl were also used. The yeast was kept suspended by bubbling with the gas mixture containing 3% CO_2. After 24 hr. the analyses of the centrifuged ammonia-yeast were as follows:

	Normality of NH_4Cl in suspending fluid		
	0·2	0·05	0·01
K content of yeast (mg./100 g.)	0	192	268
Ammonia-N of yeast (mg./100 g.)	400	174	51

Each preparation of yeast was then washed in a similar fluid containing no NH_4Cl but 0·033 N-KCl, and the gas mixture sent through the fluid as before for 24 hr. The analyses of these K-yeasts showed:

	Normality of NH_4Cl in previous suspending fluid		
	0·2	0·05	0·01
K content of yeast after suspension in KCl containing fluid (mg./100 g.)	425	455	436
Ammonia-N content (mg./100 g.)	47	17	7

The following analyses are for two samples of K-yeast and two of ammonia-yeast, the yeast being a pure strain grown from a selected colony after plating bakers' yeast. These yeasts had been suspended for 4 days, with bubbling interrupted overnight. One solution contained 0·2 N-KCl and the other 0·2 N-NH_4Cl.

	K-yeast	Ammonia-yeast
K content (mg./100 g.)	662, 775	2·1, 0·0
Ammonia-N (mg./100 g.)	0·0, 1·1	320, 348
P (mg./100 g.)	265, 313	265, 313

A striking feature of this complete replacement of K by ammonia is that it does not occur in the absence of CO_2, when the suspending fluid is maintained at the same pH. To demonstrate this the bicarbonate in the suspending fluid was replaced by an equivalent amount of NaCl, the gas mixture used for bubbling being pure oxygen. Fig. 1 shows the contrast of entrance of ammonia with and without the bicarbonate-CO_2 system. The upper curve is that of medians from five experiments and the lower curve

is that of means from two, each of which gave almost identical results. The pH for both curves was 7·3.

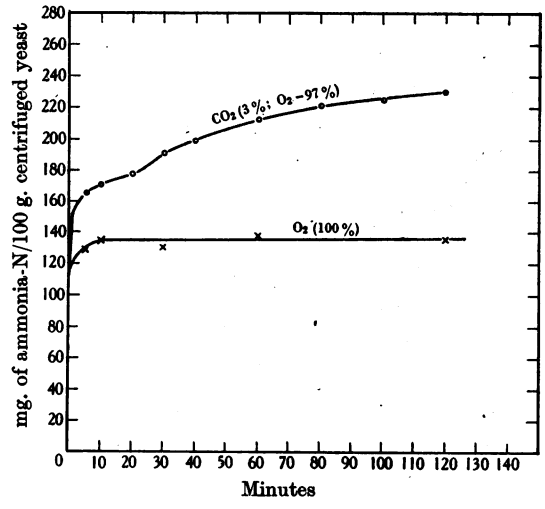

Fig. 1. Ammonia content of centrifuged yeast. The upper curve is for a yeast suspension through which $CO_2 : O_2$ mixture had been passed, in the presence of bicarbonate (pH 7·3). The lower curve is for a similar suspension treated with O_2 without CO_2 or bicarbonate, but at the same pH.

The centrifuged yeast used for the analyses contained a relatively large volume of interspace fluid. As measured by inulin this amounted to 20–30 %, but it will be seen that with the oxygen bubbling the ammonia 'space' is greater than this, though ammonia does not continue to enter. The curve with CO_2 appears to show a marked inflexion at about 20 min.

After 24 hr. of bubbling with 3 % CO_2 in O_2 the yeast suspended in bicarbonate-containing medium had approximately 330 mg. ammonia-N/100 g., while that suspended in bicarbonate-free medium and bubbled with O_2 contained 153 mg. ammonia-N/100 g.—this latter being only slightly higher than the figure for 2 hr.

Some properties of the ammonia-yeast

Fermentation. In the fermentation studies 0·5 ml. of 6 % glucose was introduced into each of a series of Conway units (no. 1) and then 1 ml. of 0·5N-NaOH containing 5 % Universal Indicator (British Drug Houses Ltd.) into the central chambers. As each delivery of the alkali was made a lid smeared with vaseline or liquid paraffin was placed in position. Then 0·5 ml. of the yeast suspension was introduced quickly into the outer chamber, with the lid displaced a little for the purpose, the lid being immediately replaced in position. The contents were mixed by rotation. The yeast suspension consisted of 1 part of yeast in 10 of water. (In some experiments the suspending fluid was 10 ml. of 0·43 % ammonium acid phosphate, or alternatively 0·50 % KH_2PO_4 for the K-yeast, and 0·1 % $MgSO_4$, but these gave essentially the same results.) At varying times after the introduction of the yeast suspension the lids were detached, 0·5 ml. of saturated $BaCl_2$ added to the outer chamber and the titration (to a light green) carried out with N-H_2SO_4 from the Conway burette. In such fermentation the CO_2 absorption very quickly becomes in linear relation with time, and then indicates the rate of fermentation.

A similar series was set up with a 1 in 10 suspension of yeast, which was either K-yeast treated similarly throughout, except that K replaced ammonia, or the ammonia-yeast suspended in a solution containing some added KH_2PO_4, the K concentration being either 23 mg./100 ml. or 93 mg./100 ml. The comparison made in such a way showed throughout a similar relationship between fermentation by the ammonia-yeast and that by the K-yeast or the ammonia-yeast with added potassium. In the absence of K, fermentation proceeded at about 40 % of the rate with K present. The relation is shown in Fig. 2. The curves in Fig. 2 are of interest

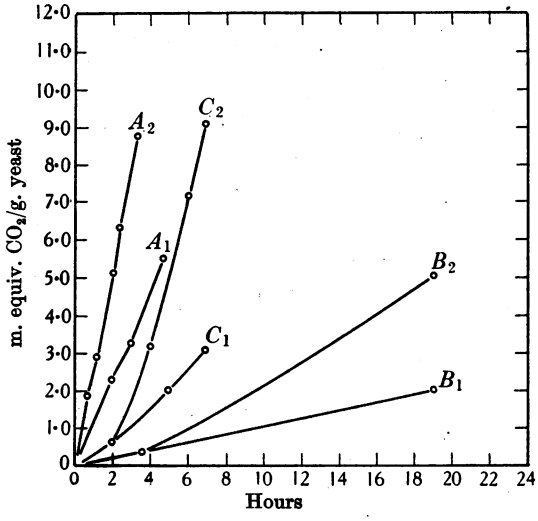

Fig. 2. Fermentation rates of yeast in 1 in 20 suspension, containing 3 % glucose. A_1 and A_2, samples of ammonia-yeast and K-yeast. B_1 and B_2, samples of ammonia-yeast and ammonia-yeast plus K (11·5 mg./100 ml. in the final mixture). C_1 and C_2, for the same ammonia-yeast suspension as B_1 and B_2 but containing 10 % ammonia-yeast extract.

in that fermentation with samples A_1 and A_2 (an ammonia and a similarly treated K-yeast) is comparatively rapid, and with samples B_1 and B_2 (an

ammonia-yeast and the same with K added to the suspension) relatively slow. The ratio of fermentation is, none the less, very similar. The samples C_1 and C_2 are for the same yeast suspensions as B_1 and B_2 but the fermentation rate has been considerably increased by incorporating in 100 vol. of the suspension 20 vol. of yeast extract. Such yeast extract was prepared as under Methods, but from an ammonia-yeast when the fermentation of such was being studied, and from a K-yeast when this was being examined.

In samples B and C there is a noticeable initial lag, but K addition has not removed this lag, while considerably increasing the subsequent rate.

Growth experiments. In the growth experiments the ammonia-yeast was compared with a K-yeast treated similarly throughout with K instead of ammonium ions, or with the same ammonia-yeast in suspension. The result compared with the K-yeast will be first described.

Growth media. For the K-yeast the medium contained: 2·5 g. glucose; 0·50 g. of KH_2PO_4; 0·10 g. of $MgSO_4$ (anhyd.) and 20 ml. of yeast extract (made as described under Methods) to 100 ml. The pH was 5·2, and the mixture was sterilized in the autoclave.

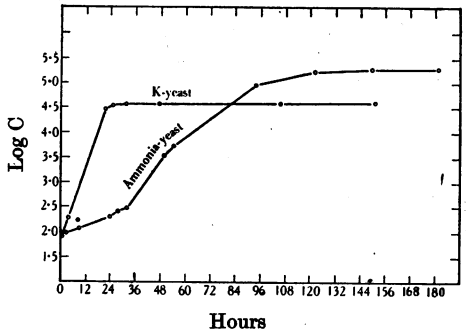

Fig. 3. Growth curves of ammonia-yeast and companion K-yeast. C is the number of yeast cells/cu.mm.

For the ammonia-yeast a similar medium was used, the K salt being substituted by equivalent amounts of the NH_4 salt, and the yeast extract was made from ammonia-yeast prepared as already described.

For seeding the yeasts for growth, 0·1 g. of the K-yeast, for example, was suspended in 10 ml. suspending fluid (containing 0·50 % KH_2PO_4 and 0·1 % $MgSO_4$ as mentioned above under fermentation studies), and 0·1 ml. of this added to the above media in a Pasteur flask, a similar procedure being used for the ammonia-yeast, ammonium ions replacing K ions throughout. A count of yeast cells/cu.mm. was made with the haematocytometer counting slide, and then the flask maintained at 28°, such counts being made at intervals up to about a week.

The results are shown in Fig. 3 in which the logs of the counts/cu.mm. are plotted against the time.

In the first 24 hr. the growth of the ammonia-yeast is only a small fraction of that of the K-yeast. During the second 24 hr. there is a rapid increase and on the third day the number of cells begins to exceed that of the K-yeast. This latter ceases growth after 2 days, the cell count being then about 46,000/cu.mm., whereas the ammonia-yeast continues growing up to the seventh day and ends with cells four times as numerous, but much smaller in size than the K cells.

Effect on growth of small K *additions to the ammonia-yeast suspension.* Comparisons were made with parts of the same ammonia-yeast in similar growth media (as described above), to which small amounts of KH_2PO_4 were added. When such additions were made, equivalent additions of the ammonium salt were made to the suspension containing no K.

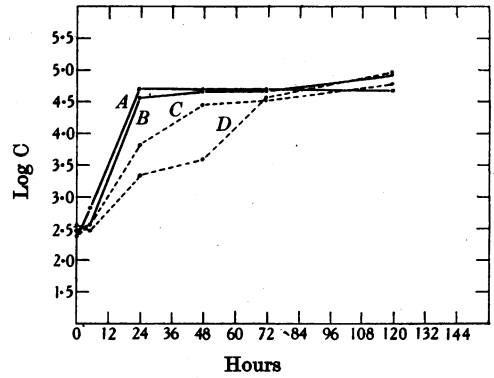

Fig. 4. Growth curves of ammonia-yeast. A and B suspensions contain 11·5 and 46·5 mg. K/100 ml. D and C are for the same ammonia-yeast suspensions without K added, but containing equivalent additions of $(NH_4)H_2PO_4$.

The results are shown in Fig. 4. The curves A and B are for 94 and 23 mg. K/100 ml. suspension, and it will be seen that both are nearly identical and agree closely with the curve for the K-yeast in Fig. 3. Curves D and C are for suspensions in which additions of the ammonium salt equivalent to the K additions of 23 and 94 mg. K/100 ml. were made to the growth media.

It will be seen that comparatively small amounts of K bring the ammonia-yeast to a growth rate and final level similar to the K-yeast, and further that the ammonia preparation behaves again somewhat as in Fig. 3, but that the increased additions of ammonium hydrogen phosphate tend to advance the growth rate to near that of the K preparation.

Oxygen uptake of the resting ammonia- and K-yeasts. 100 mg. of ammonia- or of K-yeast were suspended in 10 ml. of a medium containing 0·095 % Na_2HPO_4, 0·080 % NaH_2PO_4 and 0·60 % NaCl. The pH was 6·7. 3 ml. of this suspension were placed in the Warburg apparatus, the direct method for the estimation of O_2 consumption being used. Any CO_2 produced was absorbed in KOH solution in the central chamber. The temperature was maintained at 30° and the rate of shaking at 120 oscillations/min.

Table 1. *Oxygen uptake by resting ammonia- and K-yeasts*

Ammonia-yeast		K-yeast	
Time (min.)	O_2 uptake (cu.mm.)	Time (min.)	O_2 uptake (cu.mm.)
0	0·0	0	0·0
10	10·7	10	5·0
20	21·5	20	15·1
30	32·2	30	20·1
40	41·1	40	25·1
50	56·5	50	31·9
60	62·6	60	33·5
70	76·9	70	38·6

The amount of dry matter in the samples of ammonia- and of K-yeast was in each case 7·05 mg.

Table 1 illustrates the results obtained. The dry matter in the sample of yeast used was, in each case, 7·05 mg., so that from the curve of O_2 uptake the Q_{O_2} for the K-yeast $= -5·1$, and Q_{O_2} for the ammonia-yeast $= -9·2$. The ammonia-yeast shows a considerably greater oxygen uptake than the K-yeast.

DISCUSSION

In the above experiments it is shown that the K^+ of yeast can be entirely replaced by NH_4^+ and the ammonia-yeast so formed can be again converted to a K yeast. Essential to the interchange of ammonia and K is the presence of CO_2 (which used at a pH of 7·3 necessitates in turn very appreciable bicarbonate concentration). This would seem to indicate that the ammonia may cross the membrane in the form of carbamic acid, ammonium carbamate, or related substance, which dissociates inside the yeast cell.

The experiments show that K^+ is not necessary for fermentation, but that compared with NH_4^+ ions, those of potassium have a pronounced stimulating action on the process. Growth can also occur in the absence of K, but there are striking differences between the rate of growth of the ammonia-yeast as compared with that of the K-yeast. Similar differences exist between the growth of the ammonia-yeast in media containing no K, and one to which some K is added. Little further effect is produced when the suspending fluid contains 23 mg. of K/100 ml.

The increased number of cells produced in a given medium by the ammonia-yeast, and the distortion of shape are likewise of interest.

The fact that the 'resting' ammonia-yeast shows a greater O_2 uptake is perhaps associated with the fact that ammonium salts can be utilized by the cells as their source of nitrogen.

SUMMARY

1. The K of living bakers' yeast can be entirely replaced, under certain conditions, by ammonia. This process can be then reversed, the K being restored.

2. The ammonia-yeast ferments glucose to about 40 % the rate of the K-yeast.

3. It can grow in the absence of K, but the growth rate is much slower in the earlier stages. After some days without change of medium the number of cells produced by the K-yeast reaches a limit which is later much exceeded by the ammonia-yeast. The latter produces smaller cells, which have a tendency to form branching chains.

4. The oxygen consumption of the ammonia-yeast is higher than that of the K-yeast.

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