A Study of the Growth of Pichia Membranaefaciens Utilising the Energy Obtained by the Oxidation of Different Metallic Hydroxides by Aeration

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Introduction

Winogradsky observed long ago that micro-organisms can utilise the energy obtained by the oxidation of mineral substances for their life activity. This statement got confirmation from the findings of Temple and Colmer[1] who could grow *Thiobacillus ferroxidans* by means of energy obtained from the oxidation of ferrous ions.

I have succeeded in growing *Pichia membranaefaciens*[2] in cultures containing only one of such energy sources. In this paper, I am giving the results obtained by growing *this yeast* in cultures containing some metallic hydroxides which by slow aeration liberate energy required for growth. The hydroxides under study were manganese hydroxide, cobalt hydroxide, ferrous hydroxide, stannous hydroxide and cerous hydroxide.

Experimental

Several cultures, each containing 0.30 g. of sodium nitrate, 0.08 g. of potassium hydrogen phosphate, 0.006 g. of calcium carbonate and 0.009 g. of magnesium sulfate, were prepared. These minerals were put into 750 cc. flat bottom pyrex flasks and digested with dilute hydrochloric acid. The total volume of each culture was made to 500 cc. and the *p*H adjusted to 4.5. One gram of the freshly precipitated and dried hydroxide under investigation was added to each culture. The flasks were then cotton-plugged and sterilised by boiling at 15 lbs. pressure for 30 minutes in an autoclave. After cooling, these cultures were seeded with 0.2 cc. of an activated sample of *Pichia membranaefaciens*. This sample contained about 600 cells per cc.

Sterilised air was passed through each culture and the cell-count of the cultures was taken by the method described below. The temperature variation during the period of study was between 29.8°C and 33.0°C.

To take the cell-count of the cultures, first a culture solution was made by the following method. 0.20 g. of calcium carbonate, 0.25 g. of magnesium carbonate, 0.20 g. of sodium chloride, 0.20 g. of potassium sulfate, 0.20 g. of disodium hydrogen phosphate, 10 g. of sucrose and 2.5 g. of ammonium sulfate were weighed out into a flask. These were digested with dilute hydrochloric acid and the total volume of the culture was made up to one litre, adjusting the *p*H to 4.5. Twenty grams of agar-agar were added to it and the solution was sterilised by boiling at 10 lbs. pressure for 30 minutes. On cooling, it solidified. This solution was warmed by keeping it in a water bath. Five cc. of this melted solution were put into a sterilised Petri dish with the help of a sterilised pipette. The solution to be tested was shaken well and 1 cc. of this was added to the agar-agar culture in the Petri dish before it solidified. The dish was closed with the upper lid and kept in an incubator for 24 hours. A small star-like yeast colony developed around each cell. By counting these stars, the number of yeast cells was known. Ten of such Petri dishes were prepared for each culture whose cells were to be counted and the average of these ten countings is shown in the tables.

One culture was kept as control, in which all the other substances were present in the same amount except the source of energy. This control culture was also seeded with the same amount of yeast and aerated similarly. Its cell-count after 2 days and onwards was always zero.

Results

The results obtained by counting the cells in each culture are tabulated below:

No. of days after seeding	Average cell-count					
	Control	Manganese hydroxide	Cobalt hydroxide	Ferrous hydroxide	Stannous hydroxide	Cerous hydroxide
1	0.2	1.8	11.0	1.5	0.5	0.0
3	0.0	3.5	15.0	2.0	1.0	0.0
6	0.0	2.5	19.0	1.0	0.8	0.0
8	0.0	1.5	20.0	0.5	0.1	0.0
14	0.0	0.0	8.0	0.0	0.0	0.0
22	0.0	0.0	2.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0

1) K. L. Temple and A. R. Colmer, *J. Bact.*, **62** 605-11 (1951).

2) According to the classification scheme of Lodder and Kreger-van Rij.

Discussion

The culture containing cobalt hydroxide as the source of energy material shows good growth of yeast; moreover this growth continues for a fairly long time. The death of yeast in the control culture is due to the fact that it did not contain any source of carbon. Cerous hydroxide is poisonous for the growth of yeast and no yeast cell is seen at any stage. The other hydroxides favour yeast growth in the following order: manganese hydroxide, ferrous hydroxide and stannous hydroxide.

In these cultures, the energy needed for the growth and life activity of the yeast is supplied by the slow oxidation of these hydroxides by aeration. The carbon needed for making the body of the yeast is obtained by the fixation of atmospheric carbon dioxide. Probably the energy needed in this fixation is supplied by the oxidation of the hydroxide.

In these cultures, it is seen that the yeast stops growing after some time and later on, dies out completely as the aeration continues. This is perhaps due to this fact, that the carbon compound synthesised by the fixation of atmospheric carbon dioxide is in a form which can only partially satisfy the carbon need of the organism. It may be that yeast needs carbon food of some other type which cannot be synthesised by this method. Therefore the growth of yeast stops and further aeration in the absence of the essential carbon food proves fatal to the yeast.

Summary

When in *Pichia membranaefaciens* cultures, all other nutrients are present except the carbon source and the hydroxide of some metal is added and the culture aerated, a growth of yeast is observed. Maximum growth is observed in the case of cobalt hydroxide. Cerous hydroxide is poisonous and does not favour any growth.

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