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Studies on the Metabolism of *Aspergillus niger*

III. The Effect of Oxygen Tension on the Formation of Citric and Oxalic Acids in Surface Mould Cultures

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Little information is available concerning the effect of aeration and oxygen concentration on the oxydative fermentation by *Aspergillus niger*. Most of the studies reported so far on the utilization of oxygen by moulds have been conducted with the view of obtaining data on the effect of respiration on metabolism in general rather than on oxidative fermentations (*cf. e. g.*, Tamiya [1]).

This paper presents the results obtained from a study of citric and oxalic acid formations in surface cultures of moulds on employing different oxygen pressures. Since the previous experiments (Erkama *et al.*[2]) showed iron to have an appreciable effect on acid formation and since the effect of iron in some instances at least could be explained by accelerated respiration, the formation of acids in iron-free and iron-containing solutions was also studied.

EXPERIMENTAL

Culture conditions were similar to those used in the previous experiments except that the growth flasks now were plugged with rubber stoppers provided with an inlet tube for admittance of air near the mycelium surface and an outlet tube for taking samples from near the bottom of the flask. Moreover, the flasks were provided with two platinum electrodes and an agar bridge for making potential measurements as well as with a capillary tube for taking gas samples.

A mixture of oxygen and nitrogen was admitted at the rate of about 1.25 litres per hour. In the experiments three different gas mixtures containing 1 %, 9 %, and 71 % oxygen by volume were used. The mould strain employed was HG I. The results given are mean values of two experimental flasks.

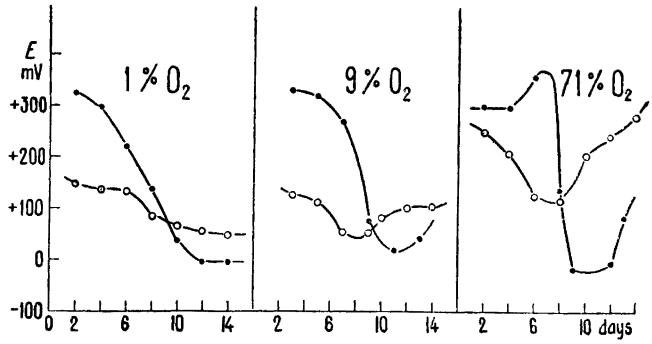

Fig. 1. Potential time curves.

● *Without Fe*
○ *With Fe*

Effect of oxygen content on electrode potential

It was found that when a uniform mould pellicle had developed the potential of the nutrient solution dropped quite abruptly. This drop in potential invariably occurred earlier in iron-free nutrient solutions than in those containing iron, the former developing mould more rapidly in the beginning than the latter. The higher the oxygen content, the more abrupt was the drop in potential (Fig. 1). In iron-containing nutrient solutions the potential always dropped more abruptly than in iron-free solutions.

According to Kusnetzow [3] citric acid cannot be formed in a solution if the potential underneath the mycelium does not drop below rH 17. In the present experiments the rH values corresponding to the potentials measured and referred to the saturated calomel electrode were generally smaller than 17 during intense acid formation; on employing the oxygen content of 71 % a rH-value of 11 was attained in iron-containing solution. On the other hand, on employing the 71 % oxygen concentration the potential did not drop below rH-value 18 in iron-free nutrient solution. Despite this fact the maximum yield of citric acid from the mould was obtained in this experiment.

Effect of oxygen content on acid formation

Examination of the curves in Fig. 2 reveals that when higher oxygen concentrations are employed also the yields of acid obtained are greater. Moreover, the fact that iron has an opposite effect in lower and higher oxygen concentrations deserves attention. In low oxygen concentrations the iron seems

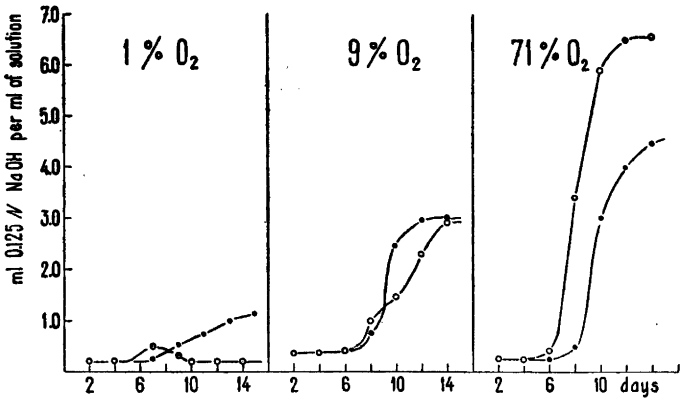

Fig. 2. Titratable acidity time curves.

○ *Without Fe*
● *With Fe*

to speed up acid formation, but in high oxygen concentrations the maximum yields of acid are obtained in iron-free nutrient solutions.

It was demonstrated previously [4] that aeration of the mycelium surface decisively affects the ratio of citric and oxalic acids formed in the nutrient solution, lower yields of citric acid and higher yields of oxalic acid being obtained in aerated cultures than in non-aerated cultures. Although in the investigations referred to above aeration was more intense than in the present experiments the gas in the growth flasks even here was renewed at the rate of about once an hour. Aeration as poor as this even sufficed to decrease markedly the yield of citric acid which in all the oxygen concentrations employed was lower than in non-aerated culture.

Tables 1 and 2 show the ratios between the yields of the two acids as compared with the results of the above-mentioned experiments [2, 4].

Examination of the tables shows that the unfavourable effect of iron on the formation of citric acid becomes evident either in non-aerated cultures or

Table 1. Formation of citric acid in different oxygen concentrations.

	Time of incubation, days	Citric acid formed in nutrient solution, mg/ml			
		unaerated	1 % O_2	9 % O_2	71 % O_2
Without iron	14	31.0	1.5	4.0	18.4
With iron	14	11.8	1.4	5.8	10.6

Table 2. Formation of oxalic acid in different oxygen concentrations.

	Time of incubation, days	Oxalic acid formed in nutrient solution, mg/ml			
		unaerated	1 % O_2	9 % O_2	71 % O_2
Without iron	14	4.6	0	5.0	15.0
With iron	14	6.2	4.0	4.5	9.9

in very high oxygen concentrations only. This observation provides a possible explanation to the disagreement in the reports on the effect of iron on citric acid formation. Aeration affects citric acid formation considerably more than oxalic acid formation. It is very likely that the direct effect of oxygen concentration is much smaller than that caused by the outflow of carbon dioxide through aeration which in turn results in the arrest of the Wood-Werkman reaction (*cf.* Erkama *et al.*[4]). This also partly explains why submerged cultures (*cf. e. g.*, Kluyver and Perquin [5]) never give as great yields of citric acid as surface cultures.

The above explanation is also in agreement with the fact that only in the highest oxygen concentration employed did the yield of oxalic acid equal that obtained on admitting atmospheric air at a rate of approximately 50 times greater than the rate used with oxygen. By using air the yield of oxalic acid increased during 14 days to 15.2 mg per ml while the yield of citric acid was only 2.8 mg per ml (Erkama *et al.*[4]). Consequently, it seems as if the effect of aeration on oxalic acid formation were chiefly due to the effectiveness with which carbon dioxide is blown out and not to the increase in oxygen concentration. This observation is of significance on studying the formation of fruit acids in higher plants.

The effect of iron on formation of oxalic acid in different oxygen concentrations is interesting. As seen from Table 2 iron seems to prevent formation of oxalic acid in high oxygen concentrations, but speeds it up in low oxygen concentrations.

Effect of oxygen content on catalase activity of moulds

It is likely that the respiration system of a surface mould culture differs from that of a submerged mould culture. Tamiya [1] remarks that a surface mould culture is more sensitive to variations in external oxygen pressure than a submerged hyphae which phenomenon he attributed to the fact that the respiration system of a surface mould culture which probably is a flavine

system, has less affinity for oxygen than the relatively more active iron system of a submerged hyphae. According to Tamiya iron respiration even in young surface cultures seems to be most intense underneath the mycelium.

Catalase determinations of moulds cultivated in different oxygen concentrations (Table 3) show that the catalase activity of a mould is greatest in low oxygen concentrations. Likewise the »pseudocatalatic» effect noted in moulds cultivated in iron-free nutrient solution was greatest in the moulds developed in low oxygen concentration.

Table 3. Catalase activity of Aspergillus niger cultivated under different oxygen pressures.

	Kat f/g dry matter		
	1 % O_2	9 % O_2	71 % O_2
Without iron	0.0047	0.0022	0.0015
With iron	0.0960	0.0039	0.0026

The iron porphyrine content of moulds seems to adapt itself as regards catalase at least, smoothly according to external oxygen pressure.

SUMMARY

The formation of citric and oxalic acids was examined in the surface cultures of *Aspergillus niger* under varying oxygen tension and iron concentration. The redox-potential of the nutrient solution as well as the catalase activity of the mould were also considered in these experiments. The effect of these various factors on acid formation is discussed on the basis of the results obtained.

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