ACTA CHEMICA SCANDINAVICA 3 (1949) 862—866

Studies on the Metabolism of *Aspergillus niger*

II. Effect of Iron on the Production of Citric and Oxalic Acids

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The effect of iron on the production of citric acid by *Aspergillus niger* continues to be a subject of controversy. Bernhauer[1] observed no notable effect on the addition of 0.005 % ferric chloride. Porgas[2] as also Chrzaszcz and Peyros[3] ascertained the accelerative effect of iron on the citric acid formation by certain mould strains. On the contrary, Knobloch and Sellmann[4] as well as Giordani[5] and Perquin[6] are of the opinion that iron in most cases has an unfavourable effect on the production of citric acid. This unfavourable effect is contrary to the effect of iron on mould development which, according to various investigators, is promoted by iron, (*cf. e. g.* Bernhauer and Knobloch[7]). Perlman *et al.*[8] have studied the effect of iron on the production of citric acid by several mould strains and found that different mould strains vary greatly in their behaviour.

Characteristic of all the above mentioned investigations is the fact that the nutrient solutions of the control experiments were not free of iron. Thus, for instance, Knobloch and Sellmann used in their investigations tap water and Perlman *et al.* employed nutrient solutions having an iron content of 162 to 102 γ per liter, as determined spectrographically. In most instances no detailed information was given as regards the culture conditions employed; nor was the continuous observation of acid production mentioned.

The present experiments were made with nutrient solutions having an accurately determined iron content, and the nutrient solutions of the control experiments were freed of iron. During the experiments the acid production was under continuous observation.

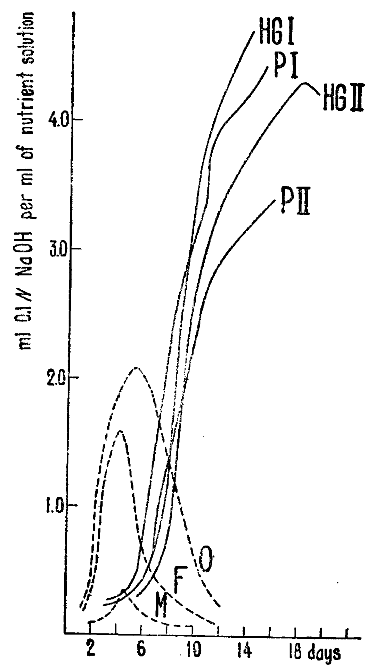

Fig. 1. Acid forming potency of various *Aspergillus strains.*

——— *fair acid production*
– – – – *poor acid production*

EXPERIMENTAL

The nutrient solution used in the experiments was similar to that previously employed by Erkama *et al.*[9]. Prior to the addition of magnesium sulphate and the heavy metals the concentrated nutrient solution was, according to Waring and Werkman [10], purified of iron by treating it with a chloroform solution of 8-hydroxyquinoline at pH 6.2. The magnesium sulphate was purified of the heavy metals according to Steinberg by treating with calcium carbonate in an autoclave [11]. In all the experiments the same iron content, *i. e.* 10 mg Fe per liter, was employed. The fermentations were carried out in 1 liter Erlenmeyer flasks. 100 ml of medium were added to each flask, and the flasks were plugged with cotton wool. All the results given below are mean values of two test flasks.

Determination of citric acid was made according to Pucher *et al.*[12]. The previously used method of Kometiani [13] gave unsatisfactory results. The oxalic acid was precipitated at pH 5 as calcium salt, then dissolved in sulphuric acid and titrated with potassium permanganate. The sugar was determined according to Bertrand.

Of the various mould strains isolated, generally the poor acid producers developed more rapidly than the fair ones. As a result of rapid growth the poor acid producers consumed more sugar and consequently, the fermentation products disappeared quickly from the solutions in the absence of sugar. Fig. 1 illustrates some typical examples of acid production by fair and poor acid producer strains.

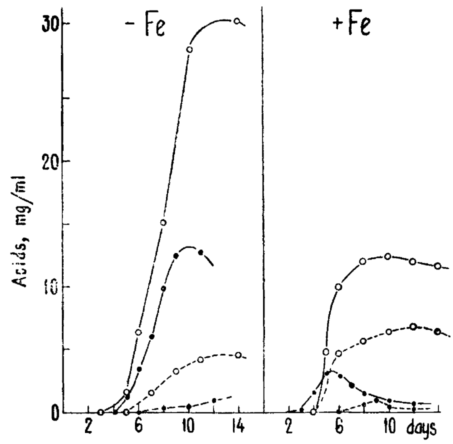

Fig. 2. Effect of iron on the citric and oxalic acid formation by different A s p a r g i l l u s *strains.*

——— *citric acid*
– – – – *oxalic acid*
○ *strain HGI*
● *strain O*

The effect of iron on the production of acids is evident from fig. 2. In these experiments the strains HG I and O were used, the former producing about 50 % acid from the fermented sugar and the latter about 10 %.

Greater yields of citric acid are obtained in iron-free solution than in iron-containing solution. On the other hand addition of iron promotes oxalic acid production to some extent provided there is enough sugar in the solution. In comparing acid production and sugar consumption (Fig. 3) with each other it is seen that the iron addition does not appreciably affect the sugar consumption of fair acid producers but greatly increases the sugar consumption of poor acid producers. In poor acid-producer strains this fact leads to cessation of acid production and relatively rapid disappearance of acids from iron-containing solution.

DISCUSSION

In the present experiments the strains of *Aspergillus* were observed to differ in the acid production ability in that the oxalic acid formation was induced by the poor acid-producing strains 2 to 4 days later than the citric acid formation, whereas the fair acid producers started the oxalic acid production almost simultaneously with the formation of citric acid, especially in the presence of iron, or only 1 to 2 days later. Moreover the formation of oxalic acid by poor acid producers was very slight, but in some instances increased, whereas the percentage of citric acid decreased in the absence of sugar.

It is generally known that mould thrives on acids it has produced in the nutrient solution after complete consumption of the carbohydrate nutrition

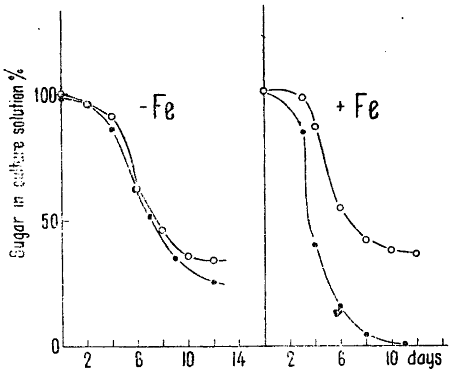

Fig. 3. Effect of iron on the sugar consumption by different Aspargillus strains.

○ *strain HGI*
● *strain O*

proper. Consequently, citric acid and oxalic acid may be regarded as constituting a reserve set aside by the organism when carbohydrate nutrient is promptly on hand. During carbohydrate starvation conditions the reserve acids are oxidized.

In the different experiments comparison of sugar concentrations corresponding to the maximum yields of citric acid reveals that said concentrations are then approximately on the same level: with fair acid producers the level is about 35 % and with poor acid producers about 25 to 30 % of the initial sugar concentration irrespective of the iron content of the nutrient solution. In many instances on employing poor acid producers the formation of oxalic acid begins only in sugar concentrations below 25 per cent. This observation is confirmed by the investigations of Kusnetzow [14] which shows that sugar concentration determines in which direction fermentation will proceed, *i. e.*, whether citric or oxalic acid is formed. On the basis of the investigations of Kusnetzow it seems that oxalic acid is formed directly from the sugar. Butkewisch [15], again, has shown that oxalic acid can be formed from citric acid. According to the present experiments oxalic acid formation by poor acid-producer strains appears chiefly to be effected by oxidation of citric acid but fair acid-producer strains form oxalic acid directly from sugar simultaneously with citric acid formation.

Iron greatly accelerates growth of poor acid producers whose mycelium dry weight in iron-containing solution is almost regularly greater than in iron-free solution. The effect of iron on the development of fair acid producers is not as distinct although the mycelium dry weight in iron-containing solution is generally greater than in ironfree solution. In the light of our experiments it seems that the effect of iron on the acid formation of poor acid-producer strains is indirect in that it accelerates the growth greatly and thus leads to the rapid disappearance of sugar from the nutrient solution. Possibly there may be a question of great acceleration of iron respiration, the mechanism for regulating the relationship between respiration and fermentation being more poorly developed than in strains forming acid abundantly.

The sugar content in nutrient solutions of fair acid-producers never dropped during the experiment as low as to cause starvation conditions. Nor did the iron have an appreciable effect on the consumption of sugar. Possibly the effect of iron on acid formation by strains producing acid abundantly is of a more primary nature than on that by poor acid producers.

SUMMARY

The effect of iron on the production of citric acid and oxalic acid was studied by comparing the fair and poor acid-forming strains of *Aspergillus niger*. 10 mg of iron added to a liter of medium containing sucrose and the usual salts decreased citric acid production by about 60 per cent when strains forming acid abundantly were employed and by about 75 per cent when strains forming acid weakly were employed. The oxalic acid production was increased by about 45 per cent on addition of iron when strains forming acids abundantly were employed.

The iron appreciably increased the sugar consumption of strains forming acids weakly.

We are indebted to Mrs. Inkeri Heikkinen for assistance in some of the experiments.

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Received May 21, 1949.