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EFFECT OF LIPIDS ON CITRIC ACID PRODUCTION BY ASPERGILLUS NIGER

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Abstract. Addition of any of the lipids such as peanut oil, soyabean oil, olive oil and almond oil at about 2–10% to a sucrose + NH_4NO_3 + salts medium stimulated citric acid production by *Aspergillus niger* in shake flasks. Of all oils, soyabean oil gave maximum stimulation. The growth process of *Aspergillus niger* was sensitive to lipids added during exponential growth phase. The mould morphology was modified to the form of separate small and round pellets with improved aeration and agitation of the culture.

In continuation of work on citric acid fermentation in the laboratory,[1,2] effect of lipids was studied on the yield of citric acid by *Aspergillus niger*. Trumphy and Millis[6] reported that addition of lipids to the cultures stimulate citric acid production. The actual mechanism of increasing citric acid production by lipids still needs thorough studies.

Methods

The strain of *Aspergillus niger* WRL-6 was used in the present work. The preparation of the spore inoculum, the conditions for shake flask fermentations, the methods for the determination of sugar, citric acid and dry weight of mycelium respectively, were as described by Qadeer *et al.*[3] Briefly, a simple synthetic sucrose-salt medium consisting of (g/l): Sucrose 150; NH_4NO_3 2.5; KH_2PO_4 2.5; $MgSO_4.7H_2O$ 0.25; and trace metals (mg/l): Fe^{+3} ($FeCl_3$) 1.3; Cu^{+2}($CuSO_4$) 0.06 and Zn^{+2} ($ZnSO_4$) 0.25 was employed. The initial pH of the medium was 3.5. All media unless otherwise stated were autoclaved at 121°C for 15 min. The oils were sterilized separately and were added to the fermentation medium aseptically in desired concentrations. The culture temperature was always 30°C. All fermentations, in duplicate, were carried out in 300 ml conical flasks containing 25 ml fermentation medium. The rotary shaker (designed and fabricated in the workshop of the PCSIR Laboratories, Lahore) was rotated at 125 rev/min with 1½ inch amplitude throw. Mycelial weight was determined by filtering 25 ml of culture through weighed Whatman paper no. 41 and washed 3–4 times with tap water. The mycelium was dried at 105°C overnight, before weighing, citric acid was estimated colorimetrically by the method of Marrier and Boulet[5] and sugar by ferricyanide reduction method, a modification of Fujita and Iwatake.[4]

Results

The amount of citric acid produced in control culture, 168 hrs after inoculation with spores, was 10–16 g/l and sugar consumption was 65–75 g/l. The mycelial dry weight was 23–28 g/l. The mould growth was in the form of large filamentous and gelatinuous pellets with the results that both aeration and agitation of the cultures as observed visually were greatly affected.

Effect of Concentration of Lipids. The data of Table 1 shows the effect of the addition of soyabean oil on citric acid production. The oil was added to the culture medium at the time of spore inoculation. The citric acid production was increased by adding oil but the stimulatory effect was lesser than with ferrocyanide or alcohols reported earlier.[1,2] The optimum level of soyabean oil, was generally about 2% and maximum citric acid was 39 g/l. Mycelial dry weight was little affected by the addition of oil to the culture medium. The mould morphology, however, was modified to the form of smooth, round and separate pellets. The size of the pellets was greater than that obtained when the cultures were grown containing ferrocyanide or alcohols.

The addition of peanut oil, olive oil or almond oil (2–10%) to shake flask culture was also investigated. These oils increased the citric acid formation as compared with control cultures but their stimulatory effect was lesser than that of soyabean oil. The optimum level of oils in general was 2% and the amount of citric acid produced varied from 25–30% g/l. The mycelial dry weight was little affected and the mould morphology within the pellets form.

Table 1. Effect of Soyabean Oil on Citric Acid Production, Sugar Consumption and Mycelial Dry Weight by *Aspergillus niger*, WRL—06.

Oil conc (v/v%)	Mycelial (dry wt g/l)	Sugar used (g/l)	Citric acid (g/l)
0	23.82	75	16.00
1	22.75	92	27.60
2	22.32	100.5	39.80
3	22.56	95.5	31.20
4	24.21	53.0	26.80
10	24.80	99.5	34.80

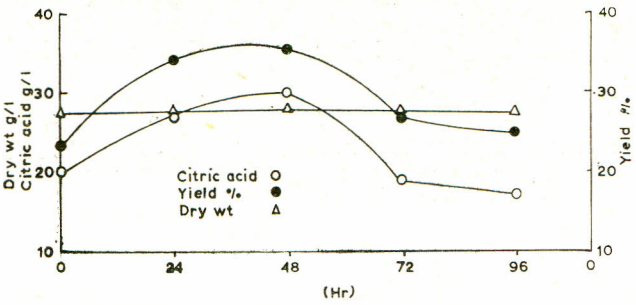

Fig. 1. Effect of olive oil 2% v/v added at different intervals after inoculation on citric acid production sugar consumption and mycelial dry wt by *A. niger* W.R.L.6.

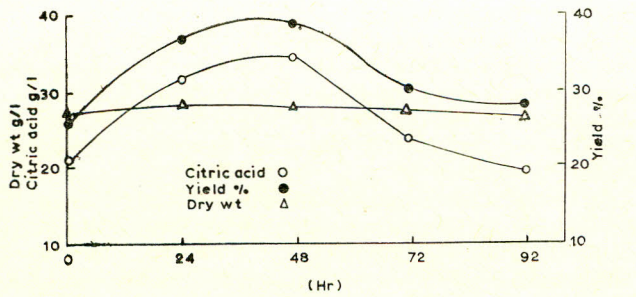

Fig. 2. Effect of peanut oil 2% v/v added at different intervals after inoculation on citric acid production sugar consumption and mycelial dry wt by *A. niger* W.R.L.6.

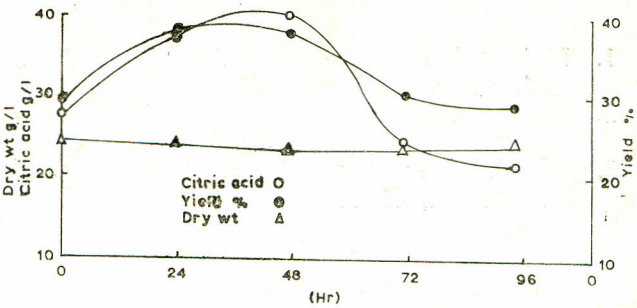

Fig. 3. Effect of soyabean oil 2% v/v added at different intervals after inoculation on citric acid production sugar consumption and mycelial dry wt by *A. niger* W.R.L.6.

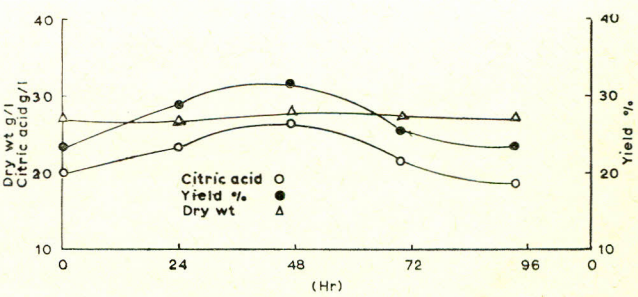

Fig. 4. Effect of almond oil 2% v/v added at different intervals after inoculation on citric acid production sugar consumption and mycelial dry wt by *A. niger* W.R.L.6.

Effect of Time Addition. Figures 1–4 indicate the effect of peanut oil, soyabean oil and olive oils and almonld oil on the production of citric acid, sugar consumption and mycelial dry weight. The addition of oils to the cultures was made at different times after spore inoculation. The object was to determine whether stimulatory effect of oils on the biosynthesis of citric acid was due to their effect on the growth process of the mould, i.e. exponential phase, or its metabolic system can be interrupted any time during fermentation. The mould morphology was modified to the form of separate, smooth and round pellets by addition oils at 0–48 hr after spore inoculation and citric acid synthesis by mould was increased. The addition of oils, however, when made at 72–96 hr after inoculation, had no effect on the mould morphology and citric acid formation remained unchanged. Mycelial dry weight and sugar consumption were little affected, by adding oils at different intervals after inoculation.

Discussion

The growth process of *A. niger* was sensitive to lipids during the exponential growth phase. The mould morphology, in the presence of lipids, was modified to the form of separate, small and round pellets instead of large filamentous and gelatinuous pellets in control cultures. In cultures with pellet type growth, agitation and aeration (or oxygen supply) was greatly improved in comparison with control cultures. The lipids, however, had no effect on both the mould growth citric acid synthesis when their addition was made in the stationary phase, i.e. 72–96 hr after spore inoculation. Citric acid production was increased by the addition of lipids but the yield was maximum in the presence of soyabean oils. The stimulatory effect of lipids may be due to certain changes brought about in the enzyme make up of mould. The lipids used in the present study were oils with a higher content of unsaturated fatty acids or unsaturated oleic acid. That may be acting as alternate hydrogen acceptor to oxygen during fermentation as suggested by Millis *et al.*[6] thus improving the yield of citric acid. The lipids are generally added to the culture medium in stirred fermentators to change surface tention and also act as antifoaming agents, these physical effects, therefore, seem to be not of great significance in increasing citric acid production in shake flasks. The size of the pellets production in submerged culture also plays an important role in citric acid syntheses. The pellets produced in the presence of ferrocyanide reported earlier[7] was smaller, with large surface area, than in the presence of lipids. Hence, citric acid formation was greater in the presence of ferrocyanide than that of lipids.

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