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Influence of Cobalt, Nickel and Zinc on Itaconic Acid Production by a Local Isolate *Aspergillus terreus* IMI28243

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Abstract: The effect of cobalt, nickel and zinc in the production of itaconic acid was investigated in submerged culture batch fermentation by *Aspergillus terreus* IMI28243 using glucose as substrate. A higher concentration of itaconic acid was achieved when the fungus was grown in the presence of cobalt, nickel and zinc compared with experiments that excluded the above metals in the medium. Medium that contained concentrations of 1. 25 g/l cobalt chloride, 1.25 g/l nickel chloride and 0.02 g/l zinc sulphate resulted in increased yield of itaconic acid by 12.45, 2.18 and 12.54 folds, respectively. The presence of metals was also found to accelerate the production of itaconic acid. In the presence of cobalt, nickel and zinc, the highest producti on (24.18 mg/lh for cobalt medium, 2.68 mg/lh for nickel medium and 48.86 mg/l.h for zinc medium) of itaconic acid was achieved in 48, 72 and 24 hours respectively, whereas the highest production of itaconic acid (1.05 mg/lh) in medium without above metals were achieved only after 96 hours of fermentation. Cultures containing zinc resulted in the highest production of itaconic acid followed by cultures containing cobalt and cultures containing nickel.

Key words: Metal addition, itaconic acid, batch fermentation

Introduction

Oue to the extensive usage of itaconic acid in industry, soeveral researchers have directed their efforts to find methods to enhance the production of itaconic acid. Important aspects in the production of itaconic acid that have been reported includes the mechanism of itaconic acid dormation by *Aspergillus terreus* by Eimhjellen and Larsen (1955) with references to effect of acidity. They reported that most of the glucose carbon were converted to itaconic acid at pH 2.1 whilst at higher pH, glucose was converted to L-malic, succinic acid and fumaric acids, carbon dioxide and mycelium. Another important factor in the production bf itaconic acid is aeration. According to Gyamerah (1995), aeration is very important in determining the success of itaconic acid production. Shortage of oxygen will cause a rapid destruction of the itaconic acid producing mechanism. Research concerning the effect of trace elements on the production of itaconic acid also received attention. Magnesium is one of the many metals that are essential in itaconic production. It improves culture acid tolerance and hence increases the production. Lockwood and Reeves (1945) reported toxicity effect by aluminium. Calcium and copper was reported to inhibit itaconic acid oxidase in the production of itaconic acid (Kautola *et al.,* 1991). From literature search, reports on the effects of cobalt, nickel and zinc on itaconic acid production are varied. Cochrane (1958) reported that cobalt enhance growth of the fungus but did not improve itaconic acid production. Lockwood and Reeves (1945) reported that all the three metals of inter est (cobalt and nickel) did not enhance the growth of the fungus or the production of itaconic acid. However there is a strong indication that cobalt, nickel and zinc may have the same positive effect on itaconic acid production as copper, as reported by Kautola *et al.* (1991). This paper reports the influence of cobalt, nickel and zinc on the produc tion of itaconic acid.

Materials and Methods

Microorganism, mediums and preparation of spore suspension: *Aspergillus terreus* IMI28243 was maintained on potato dextrose agar (oxoid), incubated at 3°C for 7 days and kept in the freezer at 4°C. Subculturing was done every 1 month. Two mediums were employed throughout the fermentation, namely growth medium and production medium. The growth medium consisted of 6% (w/v) D-glucose, 4 g/l ammonium nitrate, 0.95 g/l magnesium sulphate, 0.004 g/l cupric sulphate and 0.088 g/l potassium dihydrogen sulphate. The production medium consisted of 15% (w/w) D-glucose, 4 g/l ammonium nitrate, 3 g/l magnesium sulphate, 0.022 g/l cupric sulphate, 6. g/l calcium chloride, (0.02, 0.2, 1.25 and 5.0 g/l) cobalt chloride, (0.02, 0.2, 1.25 and 5.0 g/l) nickel chloride and (0.02, 0.2, 1.25 and 5.0 g/l) zinc sulphate. Spores for inoculum was harvested by using sterile distilled water and were filtered using 4 layers of muslin cloth. The concentration of spores was estimated to 10^6-10^7 spores per ml using haemasitometer.

Batch fermentation: Batch fermentation was carried out in 0.1 litre flask with 0.04 litre of growth medium, agitated at 150 rpm and at temperatures of 30°C. After 3 days in growth medium, the culture was added to 0.360 litre of production medium in a 1.0 litre flask. Fermentation in production medium was carried out for 4 days with the same agitation rate and temperature.

Itaconic acid, glucose and biomas dry weight determinations: Concentration of itaconic acid was determined by Waters 600E HPLC system (Waters Corporation, Milford, Massachusetts, USA) with a 7.8 × 0.150 m “Fast Fruit Juice" column at 27°C. 0.01% (v/v) of phosphoric acid as a mobile phase at a flow rate of 1.0 ml/min. The UV detector (Waters 484) was set at 240 nm. Concentration of glucose was determined according to the method of Smogyi (1952) and measured using spectrophotometer at 520 nm. The cultures were filtered using filtration paper and dried in the oven at 80°C until no changes in weight was observed.

Results and Discussion

Figure 1, 2 and 3 show the proflie of itaconic acid concentration at different times and at various concentrations of cobalt, nickel and zinc in the medium. Addition of certain concentration of cobalt, nickel and zinc was found to increase the concentration of itaconic acid at the different times. The highest concentration was obtained when the concentration of 1.25 g/l cobalt, 1.25 g/l nickel and 0.02 g/l zinc was incorporated in the medium. The use of cobalt at 1.25 g/l resulted in 1160.6 mg/l itaconic acid within 48 hours, whilst the use of nickel at 1.25 g/l resulted in only 192.9 mg/l itaconic acid but took longer hours (72 h). Meanwhile, the use of medium containing zinc at 0.02 g/l resulted in 1174.9 mg/l within 24 hours. As a comparison, the maximum itaconic acid concentration (100.8 mg/l) recorded when none of the above metals was employed in the medium was achieved only after 96 hours. Results in Fig. 4 further supported the idea that the productivity of itaconic acid can be increased significantly when specific concentration of a particular metal are employed in the production medium. The maximum itaconic acid concentration when cobalt was employed was achieved using 1.25 g/l of cobalt just as when nickel was employed. But for zinc, the peak of itaconic acid production is at much lower concentration, that is, 0.020. Results in Fig. 5 also show that the presence of these metals in the appropriate concentration increased the dry weight of biomass. Concentration of 0.20 g/l cobalt and 1.25 g/l nickel resulted in the highest dry weight data. This is in agreement with Cochrane (1958) reported that the use of cobalt could enhance growth. Our results also show that zinc increased the dry weight of biomass when used at 5.00 g/l concentration. Addition of zinc that resulted in increased growth of the fungus and also the glucose consumption rates was also reported by Suppiah (1998). Addition of certain concentration of cobalt, nickel and zinc was found to increase the concentration of itaconic acid as shown in Fig. 1, 2 and 3. The results shown in Fig. 4 did not agree with earlier reports in which the presence of zinc, nickel and cobalt did not enhance the production of itaconic acid (Cochrane, 1958). This differences in results may be due to the fact that the metals was treated as trace elements and therefore the usual concentration employed was small and approximately up to 10^{-5} g/l. In our experiments the metals was treated as a major component in the medium and the concentrations employed was up to 10 g/l. Therefore the metals employed in our experiment no longer behaves like micronutrients but may behaves like macronutrients. The peak of itaconic acid concentration in Fig. 1, 2 and 3 was achieved faster when cobalt, nickel or zinc was employed in the medium lends further support to the idea that addition of metals increased the metabolism of itaconic acid from glucose. These metals may have functioned as an enhancer in the mechanism to produce itaconic acid and may have enhanced the activity of the cis-aconitate decarboxylase or created the positive pressure to produce itaconic acid, something like a metal-active site complex. The behaviour of zinc as an activator or substructure in the enzyme involved in itaconic acid production was reported. Also according to Dixon and Webb (1979), there is a bonding site for zinc in the carboxilase enzyme. When zinc is expelled from the enzyme structure, the enzyme activity is reduced. The enzyme activity improved only when zinc was added back to the enzyme. These reports support the results from our experiments where the highest concentration of itaconic acid is when zinc is added to the medium. In addition, there is also a report on the metabolic pattern when certain concentration

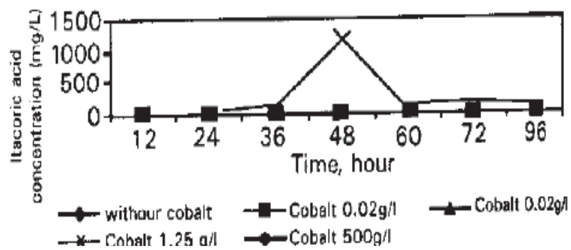

Fig. 1: Effect of cobalt in itaconic acid production

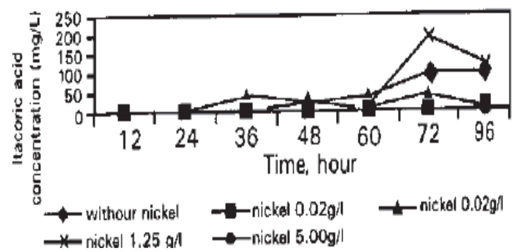

Fig. 2: Effect of nickel in itaconic acid production

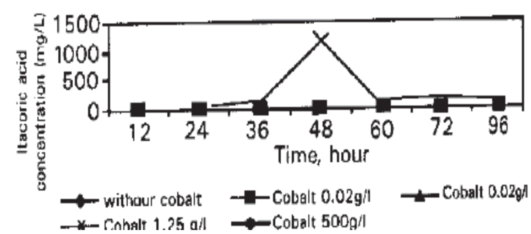

Fig. 3: Effect of zinc in itaconic acid production

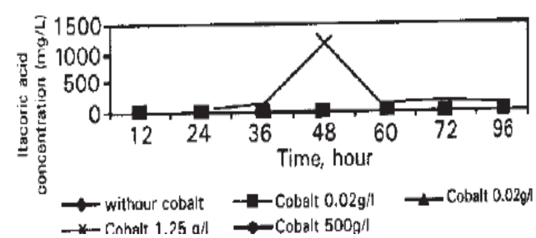

Fig. 4: Effect of cobalt, nickel and zinc on production of itaconic acid (g/L)

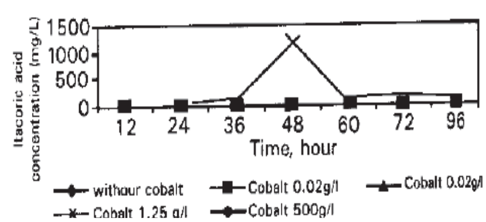

Fig. 5: Effect of cobalt, nickel and zinc in dry weight of biomes (g/L)

of zinc is added in aerobic and anaerobic fermentation (Eimhjellen and Larsen, 1955). This strengthens our data that zinc enhanced the production of itaconic acid and that the rate of metabolism for itaconic acid production was enhanced by a high concentration of metals in the medium. The increased in concentration of itaconic acid in the presence of cobalt, nickel and zinc compared to when no metals were employed are 1.16, 0.20 and 1.17% respectively. In conclusion, the production of itaconic acid can be increased significantly by the addition of cobalt, nickel and zinc at the appropriate concentration in the medium.

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