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Short Communication

Fermentatative production of itaconic acid by *Aspergillus terreus* using Jatropha seed cake

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Fermentation process for the production of itaconic acid was carried out using jatropha seed cake. Itaconic acid is commercially produced by the cultivation of *Aspergillus terreus* with molasses. Jatropha seed cake is one of the best carbon sources among various carbohydrates, because it is pure, inexpensive and available in a mass supply. The reaction was carried out at various temperatures, agitations and pH. The samples were collected at 24 h time intervals. Itaconic acid concentration was measured by the rapid spectroscopic method. Jatropha seed cake shows maximum yield of 24.45g/l after 120 h.

Key words: Itaconic acid, fermentations, Jatropha seed cake.

INTRODUCTION

Itaconic acid (methylene butanedioic acid; common synonyms: methylene succinic acid, 3-carboxy-3-butanoic acid, propylenedicarboxylic acid) is one of the promising substances within the group of organic acids. An overview of the properties and possible reactions of itaconic acid was given by Tate (1970). Itaconic acid can be regarded as an α-substituted acrylic or methacrylic acid and is isomeric with citraconic and mesaconic acid (Blatt, 1943). It is stable at acidic, neutral and middle basic conditions at moderate temperatures (Tate, 1981). Due to the two carboxylic groups, rather low monomer contents lead to copolymer with effective acidity and thus better adhesion and increased latex stability (Figure 1).

Itaconic acid was discovered by Baup (1837) as a thermal decomposition product of citric acid. The biosynthesis by fungi from carbohydrates was first reported by Kinoshita (1932), who isolated itaconic acid from the growth medium of an osmophilic fungus, *Aspergillus itaconicus*. Later, other fungal strains, mainly of the species *Aspergillus terreus*, were found to be more suitable. At the Northern Regional Research Laboratory (NRRL) of the U.S. Department of Agriculture in Peoria, Illinois, a screening programme of more than 300 strains identified the most published strain, *A. terreus* NRRL 1960 (Lockwood and Reeves, 1945). Attempts were also made to develop a biotechnical process for itaconic acid production (Nelson et al., 1952; Pfeifer et al., 1952). Later, optimized industrial processes were established providing the limited market with itaconic acid. The prominent developments in itaconic acid production (batch fermentation, free suspended biomass) took place before 1966 (Petuchow et al., 1980). Over the next 15 years, the interest in itaconic acid production declined, as indicated by the few publications during this time. Since the early 1980s, there has been increasing concern regarding sustainability, environmental conservation, renewable resources and rising energy costs. Therefore, the development of new fermentation technologies and more sophisticated bioprocess control has led to renewed inte-

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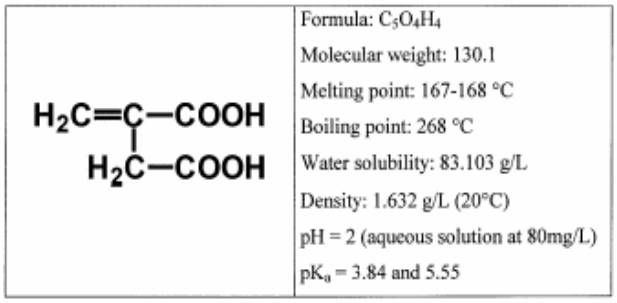

Figure 1. Formula and some properties of itaconic acid.

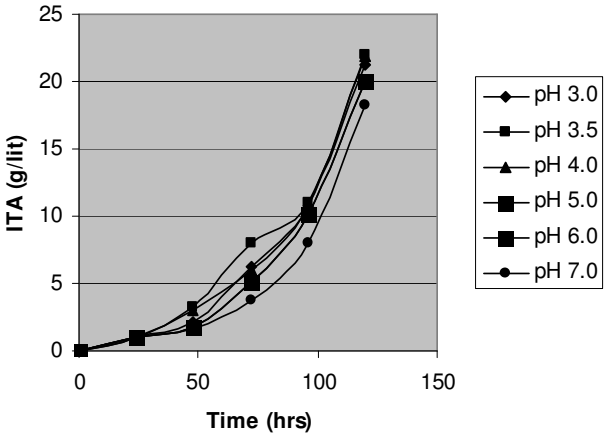

Figure 2. Effect of pH on itaconic acid production.

rest in improving itaconic acid production, novel fed-batch strategies and continuous processes using immobilized cells are now being developed and investigated, Willke and Vorlop (2001).

MATERIAL AND METHODS

Soil isolates of *A. terrius* stock culture was reactivated and cultivated by streaking a loopfull of the culture on Petri dishes containing solidified acidified (with 10% tartaric acid) potato dextrose agar (PDA) and incubated at 25°C for 5 days (Pertruccioli et al., 1989). Spores formed were washed out twice with 10 ml distilled sterilized water each time. Spore suspensions containing about log 8/ml were prepared and used as inoculums for the fermentation process. Surface liquid culture fermentation process was carried out in a 500 ml Erlemyer flask containing 100 ml media. Each flask was inoculated with the given spore suspension and incubated at different temperatures and different time intervals. The effect of Jatropha seed cake on itaconic acid production was investigated by *A. terreus*.

Potato dextrose broth pre-culture medium

2.4 g of potato dextrose broth was mixed in 100 ml distill water. The pH was adjusted to desired value using nitric acid at room temperature. Sterilization was done at 121°C pressure of 15 psi for 20 min in an autoclave.

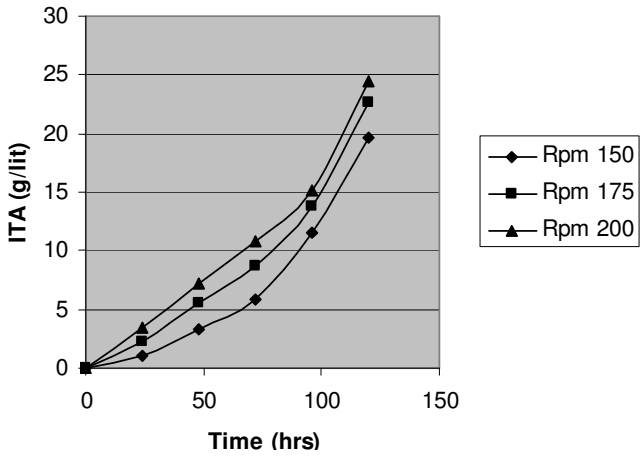

Figure 3. Effect of agitation on itaconic acid production.

Production medium

50 g of processed Jatropha seed cake was mixed in 100 ml of distill water (Bressler and Braun, 2000), sodium nitrate 3.0 g, dipotassium hydrogen potassium phosphate 1 g, magnesium sulfate 0.8 g, ferrous sulfate 0.01 g, potassium chloride 0.5 g. The pH of the solution was adjusted to desired value using nitric acid at room temperature. This medium was sterilized in an autoclave at 121°C at 15 psi for 20 min (Xu et al., 1989; Nubel and Rakajak, 1964). For shake flask fermentation, a different innoculum percentage of *A. terreus* was inoculated to the production medium in a 500 ml shaking flask and cultured on a rotary shaker. The sample was collected at an interval of 24 h (Walinky, 1984). The collected sample was used for the determination of itaconic acid and glucose consumed was estimated. The qualitative analysis of itaconic acid was measured by UV spectrophotometer at 210 nm (Kautola et al., 1985; Welter, 2000).

RESULTS AND DISCUSSION

Jatropha seed cake shows maximum yield 24.46 g/l after 120 h. (Figures 2, 3, 4 and 5). It was concluded that the nature of the substrate, incubation time, temperature and agitation, affect the production of itaconic acid on Jatropha seed cake.

The itaconic acid fermentation process works optimally under phosphate-limited growth conditions (Nubel and Rakajak, 1964). Once the fungal biomass is established-preferably after inoculation from spores, the phosphate level should be kept rather low to prevent growth. Although *A. terreus* is now the mostly frequently used commercial producer of itaconic acid, other microorganisms that are not as sensitive to particular conditions (e.g. substrate impurities) or which have a more favorable product composition have been found. Among the filamentous fungi, some ustilaginales species also produce itaconic acid. The Iwata Corp (Japan) tested different *Ustilago* species including *Ustilago maydis*, which produced 53 g 1A/l within 5 days from glucose (Tabuchi and Nakahara, 1980; Tabuchi, 1991).

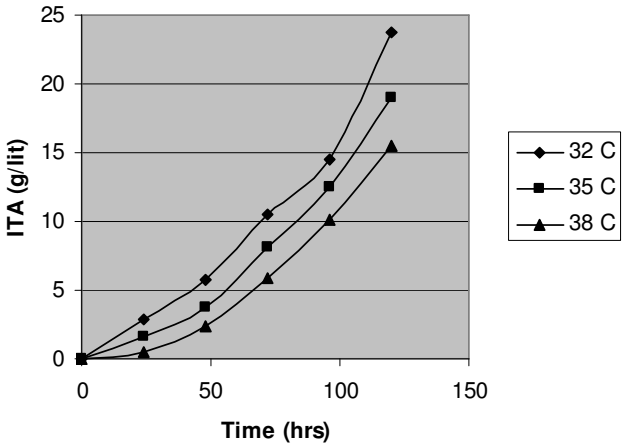

Figure 4. Effect of temperature on itaconic acid production.

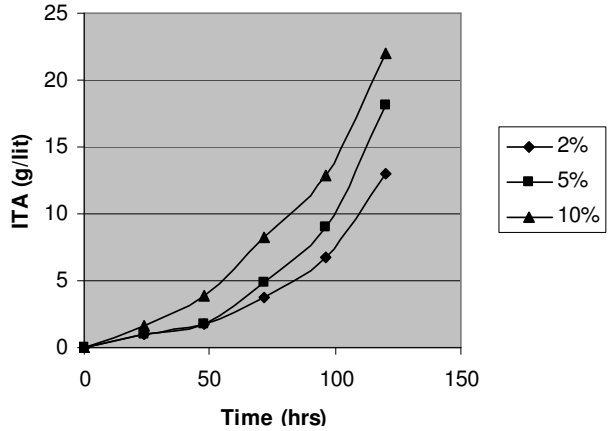

Figure 5. Production of itaconic acid using different amount of inocul

Since growing filamentous fungi may cause particular problems in bioreactor, yeast were also tested for itaconic acid production. Screening and a subsequent mutation yielded a strain, identified as *Candida* mutant, produced up to 42 g lA/l after 5 days (Hashimato et al., 1989), whereas *Rhodotorula* species reached only 15 g 1A/ l from glucose after 7 days (Kawamura et al., 1981). Another way to find better itaconic acid producing strains is by mutagenesis. In Japan, after mutation of A. *terrus*, one strain produced 82 g 1A/l after 7 days (Yahiro et al., 1995). *Jatropha* seed cake shows maximum yield 24.46g/l with 120 h. The present investigation shows that *Jatropha* seed cake can be employed for the fermentation production of itaconic acid.

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