

Fumaric acid production through valorization of biodiesel and sugar cane industries by-products

Aikaterini Papadaki^a, Nikolaos Kopsahelis^{a,b}, Maria Patsalou^c, Michalis Koutinas^c,
Seraphim Papanikolaou^a, Apostolis A. Koutinas^a

^a Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece

^b Department of Food Technology, Technological Educational Institute (TEI) of Ionian Islands, Argostoli 28100, Kefalonia, Greece.

^c Department of Environmental Science & Technology, Cyprus University of Technology, 30 Archbishop Kyprianou Str., 3036 Limassol, Cyprus

Abstract

The present study focused on the valorization of renewable resources for the biotechnological production of fumaric acid, which is considered as one of the top building-block chemicals.

Microbial production of fumaric acid was achieved through valorization of biodiesel industry waste stream, namely soybean cake, and utilization of sugar cane mill by-products, such as molasses. More specifically, soybean cake (SBC) was enzymatically processed in order to produce a nitrogen- and nutrient-rich hydrolysate. Initially, solid state fermentations using the fungal strains *Aspergillus oryzae* and *Rhizopus arrhizus* NRRL 2582 were performed and the obtained crude enzyme extract was utilised for the hydrolysis of 50 g/L SBC. A co-substrate consisting of SBC hydrolysate and sugarcane or molasses, was further utilized for the production of fumaric acid, through submerged fermentations by *Rhizopus arrhizus* NRRL 2582.

The effect of different initial FAN concentrations (100, 200 and 400 mg/L) on fumaric acid production was initially evaluated during fed-batch fermentations, using sugarcane and SBC hydrolysate (which was derived from the crude enzyme extract of *Rhizopus arrhizus* NRRL 2582). Fumaric acid concentration reached 27.3 g/L with a yield of 0.71 g/g, at 200 mg/L initial FAN concentration.

The SBC hydrolysate, which was derived using crude enzymes from *Aspergillus oryzae*, was also evaluated for fumaric acid production, in fed batch mode and at the optimum initial FAN concentration (200 mg/L). The results showed that fumaric acid production was enhanced, reaching 40 g/L with a yield of 0.86 g/g of consumed sugars. Under these conditions, fermentations were also conducted using molasses instead of sugarcane. Nevertheless, fumaric acid concentration and production yield were significantly lower. This might be attributed to alterations of the fermentation medium which affects *Rhizopus arrhizus* NRRL 2582 metabolism. As a matter of fact, ICP-MS analysis showed that the molasses-based fermentation media, contained significantly higher amounts of Mg and Ca, when compared to the sugarcane-based medium.