IMMOBILIZATION OF *Actinobacillus succinogenes* FOR PRODUCTION OF SUCCINIC ACID IN BATCH CULTURE

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Introduction. Succinic acid is a versatile compound which can be used in the preparation of antiseptics, sutures, cosmetics, pharmaceuticals, biodegradable polymers, solvents, and growth promoting in plants and animals (1). Traditionally, succinic acid is made from petroleum; however, in recent years its microbial production has been successfully attempted. It is known that *Actinobacillus succinogenes* produces succinate as main product of fermentation (2). To increase productivity biomass concentration should be increased in the bioreactor, for this purpose cell immobilization using κ-carrageenan, or alginate, has been attempted in the recent years; however, swelling and poor mechanical stability, were big disadvantages (3).

In this study, the production of succinic acid in a batch culture with *Actinobacillus succinogenes* cells, immobilized in agar, was evaluated.

Methods. Fermentation was carried out with immobilized cells. The initial glucose concentrations were 20, 40, 60 and 80 g/L, in a medium containing yeast extract (10 g/L), sodium bicarbonate (10 g/L), sodium chloride (1 g/L), magnesium chloride (0.05 g/L), dibasic potassium phosphate (15.4 g/L) and monobasic sodium phosphate (6.4 g/L).

Results. All fermentations were performed using the immobilized cells from the previous fermentation (every time the cells were carefully washed). Production of succinic acid with immobilized cells is shown in Figure 1; as expected, increasing the initial glucose concentration produces larger amounts of succinic acid. In comparison with the production of succinic acid with free cells, the immobilization of cells helped to reduce the time to reach maximum concentration of succinic acid for all initial glucose concentrations. The highest final concentration reported for *Actinobacillus succinogenes*, in fermentations without separation processes in situ, was 40.4 g/L of succinic acid.

Excluding the fermentation for 20 g/L of initial glucose, production of formic acid was 6 g/L. These data indicate that the production of this acid is probably a metabolic path deviation in favor of the formation of succinic acid.

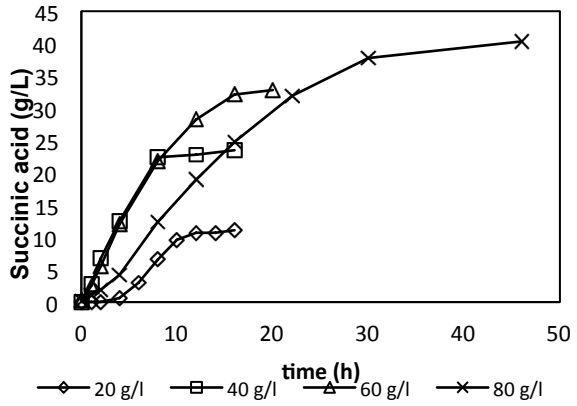

Fig.1 Effect of initial glucose concentration on succinic acid production in batch fermentations of cell *A. succinogenes* immobilized in agar.

It is possible that cells immobilization could induce a regulatory mechanism to deviate carbon flow toward succinic acid formation. This probably happens since cells immobilizations enables the greater local cell concentration within the support, and/or provide a microenvironment characterized by pH gradients.

Conclusions. In comparison with the production of succinic acid with free cells, the immobilization of cells helped to reduce the time to reach maximum concentration of succinic acid for all initial glucose concentrations.

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References.

1. Zeikus JG, Jain MK, and Elankovan P (1999). Biotechnology of succinic acid production and markets for derived industrial products. *Appl Microbiol Biotechnol.* 51: 545-552.

2. Corona-González R.I, Bories A, González Álvarez V, Pelayo-Ortiz C. (2008). Kinetic study of succinic acid production by *Actinobacillus succinogenes* ZT-130. *Process Biochemistry*.43:1047-1053.

3. Urbance S, Pometto A, DiSpirito A, Denli Y, (2004). Evaluation of succinic acid continuous and repeat-batch biofilm fermentation by *Actinobacillus succinogenes* using plastic composite support bioreactors.