ELECTRIC FIELD AND HEXADECANE PROMOTED EMULSIFYING PROTEINS PRODUCTION BY *ASPERGILLUS NIGER*

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Introduction. The uptake of hydrophobic substrates (HS) as hydrocarbons is limited by their low solubility [1]. A mechanism of microbial uptake of HS is mediated by biosurfactant production, which modifies cell surface hydrophobicity or improve emulsification of HS enhancing their bioavailability [2]; the filamentous fungi can produce small proteins known as hydrophobins which are able to emulsify HS [3]. In a previous work, we observed that *Aspergillus niger*, enhanced its hexadecane (HXD) uptake capacity after the exposure to an electric field (EF) at the same time produced soluble emulsifying protein.
In this work the inducing effect of an EF as pretreatment of *A. niger* and the presence of HXD on the production of soluble protein in an 1L air-lift bioreactor were evaluated.

Methods. The biomass of *A. niger* was produced in a 0.5 L cylindrical solid culture bioreactor provided with electrochemical devices. Perlite was used as inert support; it was impregnated with HXD (180 mg g^{-1}) as sole carbon and energy source. An electric field (6V) was applied during 24 h after germination of spores (4.5 d of culture) [4]. A control without EF was used to compare with. In both cases, after the solid culture (12 d), the entrapped biomass was passed to an air-lift bioreactor (5 g L^{-1}) containing mineral medium (1 L) and HXD (1.3 g L^{-1}). Soluble protein production was evaluated by Lowry's method during 48 h (30°C; Ug, 6 m s^{-1}); a control (without HXD) was also used. All determinations were carried out in triplicate and data were then evaluated by analysis of variance (ANOVA), $\alpha < 0.05$.

Results. Soluble protein production was observed just in presence of HXD (Fig. 1). Biomass without EF pretreatment produced the higher soluble protein concentration at 36 h (0.33 g L^{-1}). Biomass with EF achieved the highest concentration at 18 h (0.51 g L^{-1}), and it was maintained until 36 h (Fig.1.A). The rate of soluble protein production with EF pretreatment was 3.1-fold higher than without. In both cases the concentration of soluble protein decreased after 36 h. When HXD was not added into the liquid medium in air-lift bioreactor, the soluble protein concentration did not significantly change (0.018g L^{-1}).

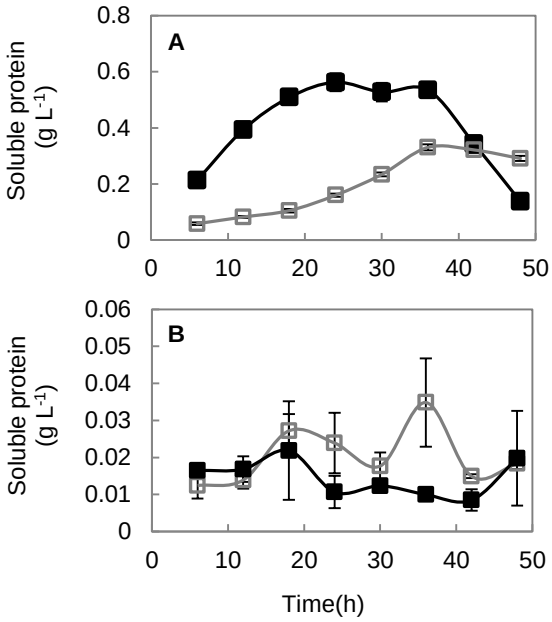

Fig.1 Production of soluble protein with and without HXD (A, B respectively), by *A. niger* with (—■—) and without (—□—) EF pretreatment.

Conclusions. In presence of HXD the rate of soluble protein production was 3.1 fold higher due to EF; EF pretreatment and HXD are necessary to produce the soluble protein. However, the presence of a hydrophobic substrate as HXD was essential.

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