SPORULATION OF *BACILLUS* SP. 83 IN FEDBATCH CULTURES DOES NOT ONLY DEPEND ON STARVATION

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Introduction. *Bacillus* sp. 83 spores production is important for the development and formulation of biological control agents [1]. Fedbatch cultures (FBC) are an alternative for increasing the production of spores, in which high cell densities can be reached. In most of the cases, nutrient limitation was selected as the main stimulus for spore formation; however, no attention has been given to culture physiological changes and cellular differentiation that may limit cell growth and can induce sporulation in early stages of FBC.
The aim of this of this work was to analyze culture morphology during growth of *Bacillus* sp. 83 in FBC and to find out how sporulation proceeds along the culture.

Methods. *Bacillus* sp. 83 was grown in mineral medium supplemented with glucose. FBC were developed in 10-l and 30-l stirred-tank fermenters, operated at 30°C, constant air flow rate of 1 vvm and increasing the impeller speed from 230 to 800 rpm, in order to keep dissolved oxygen concentration above 10% of air saturation. After an initial batch phase of 4 h, the feeding operation was started. Feeding solution containing glucose 500 g/l and ammonium sulfate 85 g/l, was added evaluating different feeding profiles (exponential, linear, constant or mixed).

Results. Only selected results are presented in this abstract. Typical time courses of colony forming units (CFU), heat-resistant spores and glucose concentration of *Bacillus* sp. 83 FBC are summarized in figure 1. Exponential growth of planktonic cells (0-8 h) was followed by a “non growing” phase and onset of sporulation (8–18 h). Afterwards, a mixed population of vegetative cells and spores increased their concentration in the culture, consuming residual glucose slower than in the exponential phase. Figure 2 shows micrographs of samples from the culture, that demonstrate that aggregates appear in the “non growing” phase, even without glucose depletion. We speculate that, inside aggregates, the vegetative cells could be submitted to local nutrient limitations [2] and/or to an enhanced cell-to-cell communication phenomenon (via *quorum sensing* molecules) that could trigger cellular differentiation and sporulation [3]. This behavior limits the maximum cell densities achieved by *Bacillus* FBC comparing with other bacteria using FBC (v.g. at least 5 times higher for *E.coli* FBC [4]).

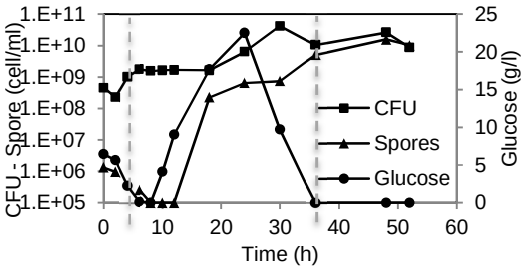

Fig 1. *Bacillus* sp. 83 growth in FBC. Dotted lines indicates beginning and end of glucose and ammonium sulfate feeding.

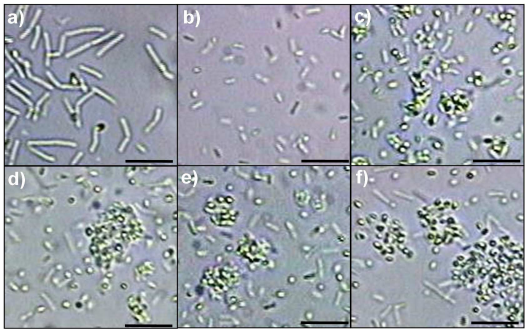

Fig 2. Fedbatch micrographs showing aggregates: a) 0 h; b) 8 h; c) 18 h; d) 24 h; e) 30 h; f) 52 h. From b) to f) culture dilution 1:10 are shown. Bars: 10 µm.

Conclusions. *Bacillus* sp. 83 sporulation was not only induced by starvation. The achieved cell and spore concentrations were lower in comparison to those obtained with other bacteria [4], due to aggregate formation in early stages of the culture that may cause local mass transfer limitations and/or due to possible enhancement of bacteria *quorum sensing* responses, which prompt sporulation.

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References.

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