INCLUSION BODIES FORMATION OF A RECOMBINANT PROTEIN IS AFFECTED BY THE pH IN *E. coli* SUBMERGED CULTURES.

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Introduction. The production of recombinant proteins (RP) has become an area of interest in biotechnology since there is a great demand for this type of molecules. Usually, RP are produced in *E. coli*, which can form aggregates of RP under high levels of expression. The aggregates of RP are called inclusion bodies (IB) and due to the assumption that proteins in this aggregates fold in an inactive conformation, represented a major drawback in the bioprocess (1). However, it recently has been demonstrated that inside the IBs proteins may be active due to a portion of partially well folded RP (2). This finding has lead to research about the application of IBs in different areas such as nanotechnology, immunology and others (3). Therefore, the definition of parameters that could control the IBs morphology, size and physical or chemical characteristics is valuable (4). The aim of this work is evaluate the pH effect of culture conditions in the morphology, size and number of the IBs contend in *E. coli* producer of a recombinant phospholipase A2 (rPLA2).

Methods. Four different conditions of pH (6.5, 7.5, 8.5 and without pH control) in 1L bioreactor cultures were evaluated; using the *E. coli* strain origami producer of rPLA2 from *Micrurus laticollaris* under IPTG (0.1 mM) induction at the end of exponential grow. Cultures were performed at 37 C, 30% dissolve oxygen by agitation cascade in LB medium. Samples were collected at the end of the culture. Total cells were fixed and observed by Transmission Electron Microscopy. IBs also were analyzed by SDS-PAGE to quantify RP.

Results. The biomass produced in all condition tested were similar, except for the pH 8.5, which was only 50% of the maximum (table 1). The average biomasses produced by the others conditions were 2 g/L. The specific growth rate (μ) for the cultures at pH 6.5, 7,5 and without pH control was similar (0.64 h^{-1}). While cultures at pH 8.5 present a reduction of 15% of μ max. By analyzing the IBs produced in each pH condition, it was observed important differences in number, shape and size (table 2).

Table 1. Kinetic parameters and biomass concentration of different culture conditions.

pH condition	μ (h^{-1})	Biomass (g/L)
6.5	0.65±0.02	2.2±0.2
7.5	0.64±0.03	1.9±0.1
8.5	0.55±0.01	0.9±0.1
WO	0.64±0.03	1.8±0.2

Table 2. Morphological characteristics of IBs under different pH.

pH condition	Number of IB per cell	Size (μm)	Shape
6.5	Up to 5	0.3-0.1	Spherical
7.5	Up to 2	0.4-0.2	Spherical
8.5	Up to 4	0.5-0.1	Amorphous
WO	Up to 1	1-0.8	Spherical

Conclusions. The pH culture condition modified the size, shape and number of IBs produced in the strain *E. coli* origami. In cultures without pH control, largest IB were found been only one per cell. This suggests that a gradient in this variable promotes an exclusive point of aggregation or the coalescence of different smalls IBs. In a constant, under controlled pH conditions the formation of more than one point of nucleation is promoted without the coalescences of these small IBs.

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