EFFECT OF SPECIFIC GROWTH RATE ON FORMATION OF INCLUSION BODIES AND RECOMBINANT PROTEIN. A NOVEL THERMOINDUCED CONTINUOUS CULTURE

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Introduction. Thermoinduction is among the most commonly used induction strategies for production of recombinant proteins as it yields high expression levels, is simple and avoids the introduction of toxic or costly chemical inducers[(1)]. Yet, traditional thermoinduction results in growth cessation due to the high metabolic load caused by heterologous protein production and the stress caused by the heat shock response[(2)]. Additionally, inclusion bodies are commonly formed with this method of induction[(2, 3)].

In this work we will show that a two-compartment chemostat system can be used for continuously producing a recombinant pre-proinsulin by *E. coli*. Such a system has allowed, for the first time, the effect of growth rate on thermoinduction of a recombinant protein production and its percentage of aggregation into inclusion bodies.

Methods. *E.coli* strain BL21 (B F- *dcm ompT hsdS* (r^-_B m^-_B) *gal*: Strategene Cat:200133) was transformed with codon sequence for the pre-proinsulin gene (HPPI) under control of the λ_{PL}-cI857 system. A glucose mineral media was employed with ampicillin as selection pressure. A culture system with two interconected compartments was employed: the first compartments was at 35 °C and 0.7 L and the second was at 42 °C and 0.5 L. The system was maintained at aerobic conditions and constant pH (6.8). The Bradford method and densitometric analysis were employed for protein quantification. Glucose was quantified with a YSI biochemistry analyzer. Organic acids concentrations were determined by HPLC.

Results. The two-compartments system allowed the attained of a stationary state even after induction. Table 1 presents a summary of yields and concentrations of pre-proinsulin obtained under the dilution rates evaluated. It can seen that the maximum concentration and yield were obtained at a dilution rate of 0.07 h^{-1}. Figure 1 shows the effect of dilution rate on percentage of recombinant protein in inclusion bodies during the stationary phase (black columns) and the total protein embedded within inclusion bodies (white columns). Both parameters increases at low dilution rate.

Table 1. Summary of yields and concentration of recombinant protein production at the different dilution rates evaluated

Dilution rate (h^{-1})	Specific yield (mg/g_{DCW})	Concentration (g/L)
D = 0.26 h^{-1}	2.0	0.03
D = 0.22 h^{-1}	8.0	0.11
D = 0.18 h^{-1}	12.1	0.08
D = 0.15 h^{-1}	12.8	0.13
D = 0.11 h^{-1}	12.1	0.13
D = 0.07 h^{-1}	39.33	0.44
D = 0.04 h^{-1}	36.97	0.38
D = 0.01 h^{-1}	16.34	0.16

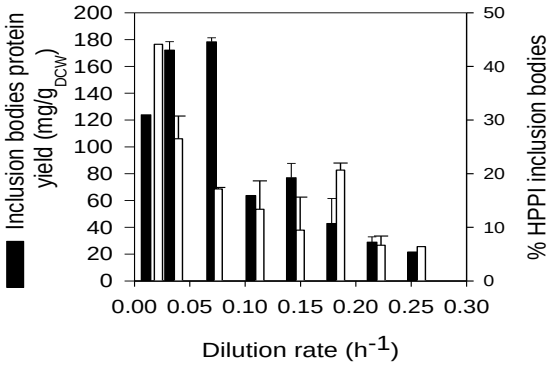

Figure 1. Effect of dilution rate protein yield and percentage of recombinant protein in inclusion bodies

Conclusions. The maximum amount of recombinant protein that formed aggregates increased as dilution rate decreased to 0.04h^{-1}. Additionally, the maximum percentage of pre-proinsulin in inclusion bodies was obtained at lowest dilution rate (0.015 h^{-1}).

In summary, low dilution rates improved the production process of recombinant protein in inclusion bodies.

Acknologedments

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