EFFECT OF HEATING RATE IN THERMO-INDUCED PRODUCTION OF pDNA IN HIGH CELL-DENSITY CULTIVATIONS of *E. coli* DH5α

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Introduction. Plasmid DNA (pDNA) is currently produced in high-cell density cultivations (HCDC) of *E. coli.* Several strategies have been directed towards boosting pDNA specific yields (Y_{px}), with the aim of developing a robust manufacturing platform for pDNA biopharmaceuticals production. One that stands out for its effectiveness and simplicity, is thermo-induction (1). There are several reports concerning the application of this inexpensive strategy in pDNA production at lab scale, but such reports almost often neglect process conditions of productive-scale reactors (0-1-100 m^3).

In order to reproduce large-scale heating conditions in lab-scale reactors (2), the effect of heating rate (q) on Y_{px}, productivity (Q_p) and supercoiled content (SCF) of a prophylactic plasmid (pHN) produced in thermo-induced HCDC of *E. coli* DH5α, was investigated.

Methods. *E. coli* DH5α cells harboring a 6.1 kb pUC plasmid containing the mumps virus antigen, were grown in exponentially fed-batch bioreactors in mineral media, at a specific growth rate of 0.15 h^{-1}; temperature was increased linearly from 35 to 42 °C, at q values of 0, 0.025, 0.05, 0.10 and 0.25 °C/min, that prevail during heating in productive-scale reactors. All conditions were evaluated twice. Biomass, glucose and acetate concentration were quantified as described previously (3). pDNA was isolated using Qiagen´s miniprep kit and quantified by UV-spectrophotometry. pDNA topologies were resolved by agarose gel electrophoresis and SCF was obtained by image densitometry.

Results. Biomass yield on glucose (Y_{xs}) and ammonia (Y_{xNH3}) are presented in Figure 1a. The time at which acetate and glucose began accumulating in the culture broth after cultivation temperature reached 42 °C, is shown if Fig 1b. pDNA concentration and Y_{px} are presented in Fig 1c, and Q_P and SCF are depicted in Fig 1d.

In constrast to Y_{xNH3}, Y_{xs} was lower in heated than in constant temperature cultivations and remained without important changes at heat rates above 0.025 °C/min. The onset of acetate production as well as glucose accumulation, was not affected importantly by heat rate. However, heat rates below 0.1 °C/min increased pDNA volumetric yield, as well as Y_{px} and Q_p. Interestingly, SCF did not show noticeable changes with heat rate and was 80 ± 5 %.

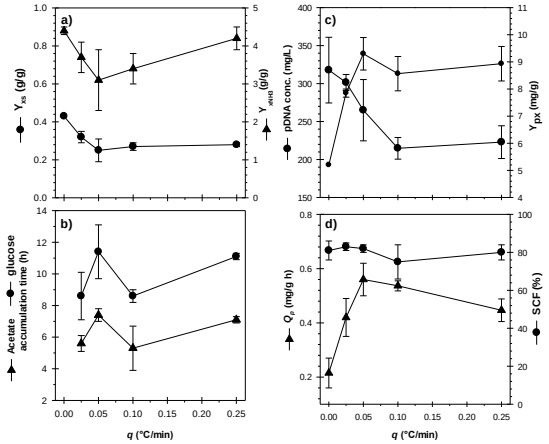

Fig.1 Effect of q on *E. coli* DH5α pHN growth performance and pDNA production.

Conclusions. Heating rate did not delay acetate excretion and the subsequent loss of viability. Both parameters are useful as thermal stress indicators. As q increased from 0 to 0.05 °C/min, Y_{px} and Q_P increased up to 79 and 250 %, respectively. Under all conditions tested, SCF was maintained at satisfactory levels for pDNA vaccine production purposes.

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