Escherichia coli expressing a fluorescence protein as a model system for the optimization of environmental and processing conditions

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Veloso, A. C. A.[1,2], Rocha, I.[1], Carvalho, S.[1], Carneiro, S.[1], Dias, T.[2], Peres, A. M.[2] and Ferreira, E. C.[1]

1. Centro de Engenharia Biológica, Universidade do Minho, Braga
2. Escola Superior Agrária de Bragança, Bragança

E-MAIL : anaveloso@deb.uminho.pt

The ability to cultivate microbial strains, expressing heterologous recombinant proteins, is an increasingly important technique in biotechnology. Among the many systems available *Escherichia coli* remains one of the most attractive due to its ability to grow rapidly and at high densities on inexpensive substrates, its well-characterized genetics and the availability of an increasingly large number of cloning vectors and mutant host strains. However, the recombinant proteins produced with *E. coli* are usually very difficult to measure and so the sampling points along a fermentation run are not sufficient to elucidate some bottlenecks that could exist and are not suitable for process control, modeling and optimization purposes. In this work, a factorial experimental design was used to optimise three variable culture conditions of a model system based on the expression of EYFP protein (Enhanced Yellow Fluorescent Protein): operational temperature, dissolved oxygen and of induction time. During the *E. coli* fermentation, samples were taken every hour for evaluation of standard state variables. Furthermore, the protein concentration was analysed by spectroflorimetry with no sample processing. A western blotting technique was used to validate the fluorimetric measurements and to determine the subcellular localization of EYFP protein. Other tests were also performed for the validation of this methodology, as linearity analysis and effect of the presence of biomass.