Increasing recombinant protein production in Escherichia coli K12 by increasing the biomass yield of the host cell

Hendrik Waegeman[a], M. De Mey[a], and W. Soetaert[a]

[a]*Ghent University/Ghent/Belgium*

hendrik.waegeman@ugent.be

For more than three decades micro-organisms have been employed as hosts for recombinant protein production, with the most popular organisms being *Escherichia coli* and *Saccharomyces cerevisiae* (1). One of the crucial factors to obtain high product yields in recombinant protein bioprocesses is the biomass yield of the host cell. High biomass yields not only result in less carbon loss and higher conversion to recombinant protein due to a potential higher drain of precursors, but are also accompanied by lower conversion to growth inhibiting byproducts, such as acetate (2). Furthermore, acidic byproducts hinder the expression of heterologous proteins (3) and consequently decrease protein yield in a direct and indirect manner.

Many strategies have been tested to decrease the amount of acetate produced, including optimal feeding, choice of other carbon sources and metabolic engineering (4). Fed-batch and continuous feeding strategies result in low residual glucose concentrations and minimize overflow metabolism ('Crabtree effect') (5; 6). Aristidou and coworkers improved biomass yield and protein production by using fructose as a primary carbon source without greatly affecting the fermentation cost (7). A third strategy is to alter the genetic machinery. Knocking out genes coding for acetate producing pathways, i.e. acetate kinase-phosphate acetyltransferase (ackA-pta) and pyruvate oxidase (poxB), decrease acetate yield dramatically, but at the expense of lactate and pyruvate (8).

The objective of this study was to focus on the combined effect of a global and a local regulator to increase biomass yield and hence recombinant protein production using GFP as a biomarker. Deletion of *arcA* reduces the repression on expression of TCA cycle genes (9) while deletion of *iclR* removes the repression on the *aceBAK* operon and opens the glyoxylate pathway (10; 11) in aerobic batch cultivations. This metabolic engineering approach simultaneously decreased the acetate yield with 70% and increased the biomass yield of the host cell with 50%. Due to a lower carbon loss and a lower inhibition of protein production by acetate, the GFP production of the ΔarcAΔiclR double knockout strain increased with 100% as opposed to the wild type *E. coli* K12. Further deletion of genes lon and ompT encoding for non-specific proteases even further increases GFP-production (3 times the wild type value). The effect of a deletion of *arcA* and *iclR* was also evaluated in a *E. coli* BL21 genetic background. However, in this industrial strain the deletion had no effect on protein production.

References:

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