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# BACTERIOPHAGE INSPIRED GROWTH-DECOUPLED RECOMBINANT PROTEIN PRODUCTION IN ESCHERICHIA COLI

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In conventional microbial expression systems recombinant protein production (RPP) is typically growth associated and therefore, metabolic resources are diverted into the formation of biomass rather than heterologous product formation. Consequently, the expression of the protein of interest (POI) is always in direct competition with the simultaneous production of host proteins by the transcription and translation machinery of the cell. Most of the time this circumstance is unfavorable for achieving high yields of correctly folded recombinant proteins and triggers adverse reactions of the host cell caused by a metabolic overburden of the cell factory.

Inspired by phage derived regulation of the bacterial transcription machinery, we have engineered a phage T7-based protein expression system for *E. coli* in which RPP is non-growth associated. This technology is based on synthetic biology approach which improves the capacity of the bacterial host to express a POI by the partial expression of a T7-derived Gp2 protein during the RPP phase. This non-DNA-binding transcription factor binds the *E. coli* RNA polymerase and therefore prevents  $\sigma$ -factor 70 mediated formation of transcriptional qualified open promoter complexes. Thereby, the transcription of host genes is inhibited, and metabolic resources can be exclusively utilized for synthesis of the POI, which significantly improves RPP in industrial relevant process conditions. We demonstrate that this expression technology, called enGenes-X-press, allows for extraordinary high product yields and especially high specific amounts of correctly folded soluble recombinant protein in standard fed-batch cultivations. Further we present our continuous 2-stage chemostat, driven by growth decoupled protein production concept, that facilitates production of >100 g recombinant protein in 12 days with benchtop equipment (1 L scale bioreactor).

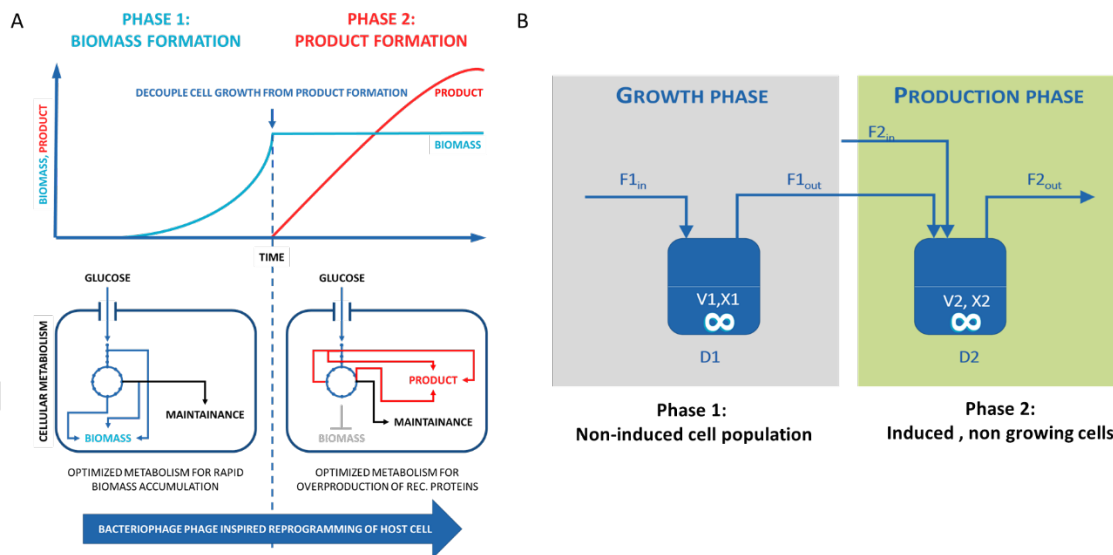


Figure 1 Growth decoupled recombinant protein production in fed-batch (A) and 2-phase continuous bioprocess (B). Upon induction Gp2 inhibits host mRNA production by binding to the  $\beta'$  jaw domain of host RNAP, consequently leading to a stop of cell division and decoupling of protein production and cell growth. Overexpression of the gene of interest is initiated by addition of IPTG and driven by the orthogonal T7 RNAP. This approach allows clear separation of a RPP process into two phases to re-organize cellular metabolism to become optimal for RPP. B) The growth decoupled protein production approach can be used in continuous fermentation environment by establishing a 2-phase continuous bioprocess during RPP.