2-STAGE CONTINUOUS GROWTH-DECOUPLED BIOMOLECULES PRODUCTION USING ESCHERICHIA COLI – TOWARDS MICROBIAL SMALL-FOOTPRINT MANUFACTURING

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Continuous manufacturing using microbial expression systems, like *Escherichia coli (E. coli)*, is troubled by process instabilities and an overall low productivity. The main reason for this is the high mutation rate and the fast growth of bacteria inevitable stalling every chemostat production process.

We have developed a unique *E. coli* host cell line (see Figure 1/A), that enables growth-decoupled recombinant protein or biomolecules production (DNA, RNA, small molecules). This technology, named enGenes-X-press, is based on a synthetic biology approach which improves the capacity of the bacterial host to express a protein-of-interest by the co-expression of the phage-derived Gp2 protein during the production phase. This non-DNA-binding transcription factor binds the *E. coli* RNA polymerase and therefore prevents σ-factor 70 mediated formation of transcriptional qualified open promoter complexes. Thereby, the transcription of host genes is inhibited, cell division is stopped, and metabolic resources can be exclusively utilized for synthesis of the POI, which significantly improves recombinant protein production under industrial relevant process conditions.
When this cell line is operated in a continuous 2-stage chemostat approach (see Figure 1/B) the productivity can be preserved over a long period of time by decoupling production form cell growth and stopping cell division in the 2nd stage chemostat. Here, we present details on our enGenes-X-press technology, our proprietary 2-stage chemostat production process and showcase the latest findings in continuous upstream- and downstream-processing using the extracellularly secreted Protein A (from *S. aureus*) and will discuss potential fields of application in the light of small-footprint manufacturing.

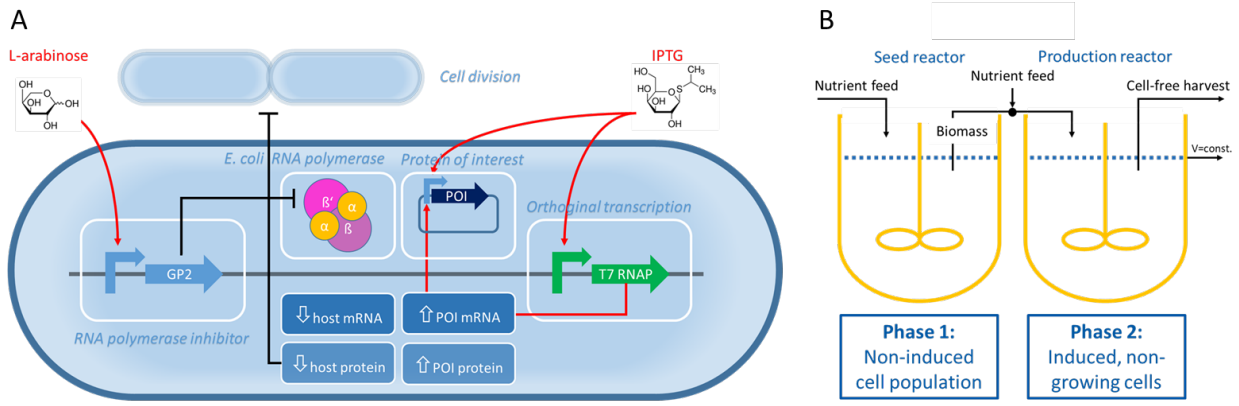

Figure 1 Schematic representation of the enGenes-X-press cell line (A) and 2-phase continuous bioprocess (B). A) Upon induction Gp2 inhibits host mRNA production by binding to the ß' jaw domain of host RNAP, consequently leading to a stop of cell division and decoupling of protein production and cell 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..