Microbial fermentations can produce complex compounds, like medicines and fuels, from renewable raw materials, offering a leap towards bioeconomy. The cells used as industrial hosts have evolved to direct resources towards growth and survival, resulting in inherent resource competition. Achieving industrially feasible production levels requires engineering the cells to divert substantial resources from growth to product synthesis instead. However, compromising cell growth leads to poor production rate. A solution to this is two-stage fermentations, where biomass is accumulated in the first phase, while resources are redirected to production pathways in the second phase.

In her thesis for VTT Technological Research Centre of Finland Ltd, Natalia Kakko implemented, and characterized for targeting three metabolic enzymes, the Tet-ON inducible ClpXP protein degradation system as a synthetic switch in *S. cerevisiae*. By targeting growth essential metabolic enzymes, synthetic growth cessation was achieved. After biomass accumulation, the ClpXP system was induced, recognizing the growth essential enzymes and targeting them for degradation. The cells were characterized for growth, metabolic activity, and proteome states. Natalia showed that cell growth could be synthetically ceased by inducing the proteasome for degrading any of these metabolic enzymes. Continued consumption of the carbon source indicated that the induced cells were metabolically active, though the activity was decreased compared to uninduced cells. Proteomics revealed that the target enzyme amounts were specifically decreased when the ClpXP system was induced.

The characterized inducible ClpXP proteasome offers a solution generalizable across production host species for switching from growth to a production state. The switch separates the growth and production phases of the process, enabling higher yields and production rates than are attainable with conventional microbial fermentation processes. Thus, the outcomes of this thesis work provide an important step towards realizing the potential of microbial fermentations as a sustainable replacement of petrochemical synthesis.

**More information:**

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In her thesis for VTT Technological Research Centre of Finland Ltd, Natalia Kakko developed a synthetic switch for *S. cerevisiae* to enable the repression of growth essential metabolic activity. The developed synthetic switch could allow the maximization of yield and productivity in microbial fermentations.