TEACHING AN OLD PET NEW TRICKS – EXPRESSION TUNING IN *E. COLI* BL21(DE3)

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INTRODUCTION

Escherichia coli is the most widely used host organism for recombinant protein production due to its well-studied genome, the existence of numerous cloning vectors and engineered strains, as well as the possibility of cheap and straight-forward cultivation to high cell densities yielding high product titers [1,2]. Strong induction of recombinant protein production in *E. coli* can lead to agglomeration of inactive product, so called inclusion bodies (IBs), and also imposes a high metabolic burden which can result in cell death [3].

CHALLENGE

To reduce metabolic burden and increase product quality it is important to tailor the induction level of recombinant protein expression. Within this project the goal was to tune recombinant protein expression by supplying different limiting amounts of the inducer lactose. A prerequisite thereof is to characterize the strain regarding the maximum specific uptake rate of lactose ($q_{s,lac}$) to know the possible feeding ranges and prevent sugar accumulation as this can lead to osmotic stress [4].

RESULTS AND DISCUSSION

First the correlation between the lactose and the glucose uptake were evaluated by conducting several experiments and fitting the obtained data by a mechanistic model [5,6] (open circles and black line in Figure 1). Afterwards we investigated the impact of $q_{s,lac}$ on the formation of soluble protein and inclusion bodies. We decoupled growth from recombinant protein expression in a series of controlled bioreactor cultivations of *E. coli* expressing the model protein enhanced green fluorescent protein (eGFP) at a constant $q_{s,glu}$ and varied $q_{s,lac}$ (Figure 1). Furthermore we performed induction with the very commonly used inducer IPTG.

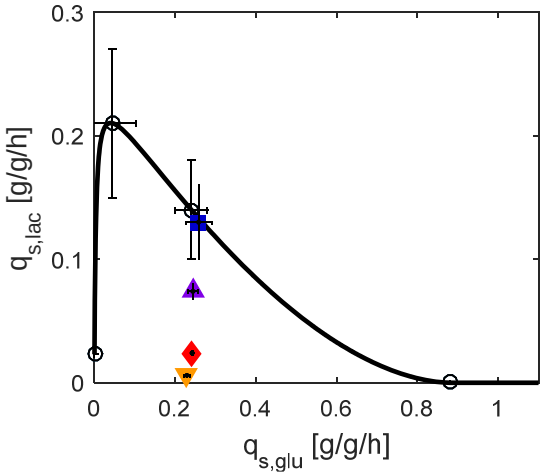

Figure 1: Black line indicates maximum specific uptake rate of lactose ($q_{s,lac}$) as a function of specific uptake rate of glucose ($q_{s,glu}$) for *Escherichia coli* BL21(DE3) strain producing enhanced green fluorescent protein (eGFP). Data points (open circles) were obtained from several batch and fed-batch cultivations and fitted by the mechanistic model according to our previous study[10]. Filled symbols indicate performed experiments

We found that by varying the specific uptake rate of the inducer lactose we were able to tune the induction level. Both soluble protein and inclusion bodies formation were strongly impacted by the induction conditions.

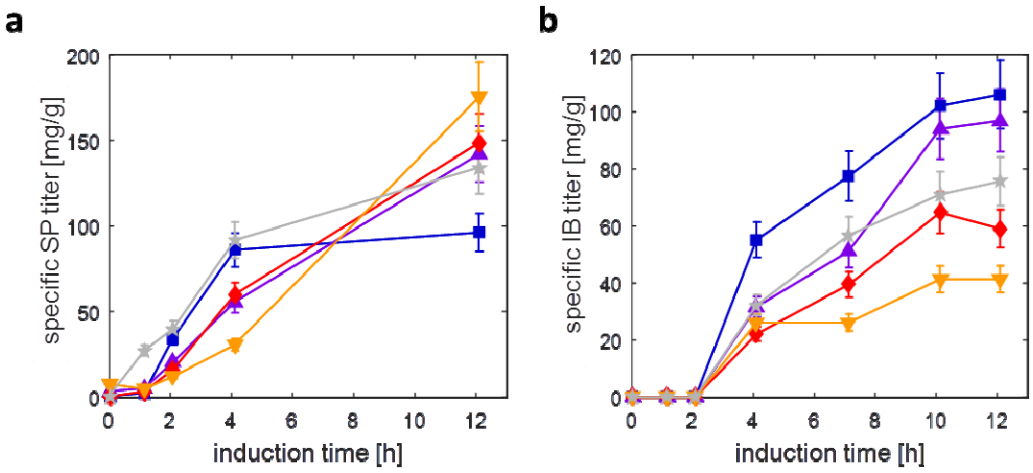

Figure 2: Specific titers of soluble product (SP; Figure 2a) and inclusion bodies (IB; Figure 2b) in the different cultivations over time.

CONCLUSION

We found that induction at high $q_{s,lac}$ and IPTG gave a high specific titer of soluble product in the early phases of induction, but for prolonged production times induction a low $q_{s,lac}$ is favourable and leads to higher product titers. For the inclusion body formation rate we saw a clear correlation between specific uptake rate of the inducer lactose and the specific inclusion body titer throughout the whole induction phase and we were able to obtain higher production rates with the inducer lactose compared to IPTG.

Our developed method of feeding limiting amounts of the inducer lactose allows tuning the recombinant protein expression rate. This might pave the way for obtaining “difficult-to-expressed proteins” and to increase product titers as well as product quality.

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