



## Influence of oxygen levels on a genome reduced *Pseudomonas putida* strain

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last years, strong efforts have been focused on finding novel expression systems, since they are crucial for the bioprocess performance.

In this work, a study of a methanol-free expression system based on a heat-shock gene promoter (PDH) was conducted using glycerol as sole carbon source. Using this promoter, the recombinant expression is strongly induced in carbon-starving conditions. The classical PGAP was used as reference expression system, taking for both strains the lipase B from *C. antarctica* (CalB) as model protein. PDH expressing CalB exceeded PGAP in shake-flask cultivations when using a slow-release feeding technology, confirming that PDH was induced in conditions of no growth.

This increase was confirmed in fed-batch cultivations. Three optimization rounds were carried out for PDH under different feeding strategies. In all of them the PDH outperformed the CalB titer achieved by PGAP. The single biomass production and induction phase at a very low constant feeding rate was the best approach, reaching 2.8-fold higher specific productivity than PGAP fed-batch at low  $\mu$  (0.025 h<sup>-1</sup>). Unless fed-batch at high  $\mu$  (0.15 h<sup>-1</sup>) with PGAP strain presented 36.2 % higher specific productivity, the best PDH fed-batch strategy had 2.6-fold more titer. To summarize, PDH is an inducible promoter that exhibited a higher efficiency, consuming less substrate and oxygen than the benchmark. Thus, this novel system emerges as a potential alternative for *P. pastoris* RPP bioprocess.

### P05: Influence of oxygen levels on a genome reduced *Pseudomonas putida* strain

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The bacterium *Pseudomonas putida* is becoming a cell factory of choice due to its versatile metabolism and high stress tolerance, the latter being of prime importance when considering production at large scale and bioconversion of potentially toxic chemicals. These phenotypic features have led to the development of *P. putida* as a platform organism including the development of genome reduced chassis strains like the SEM10 [1]. However, thorough characterization of the genome-reduced strain under industrially relevant conditions is still lacking. In order to compare the wild type KT2440 with the genome reduced SEM10 we cultivated both strains under varied oxygen levels. The oxygen levels were varied by changing the partial pressure of oxygen in the cultivation gas, thereby reducing the influence of shear. SEM10 showed better growth properties compared to the wild type during cultivation with the pO<sub>2</sub> equal to ambient air. The wild type KT2440 strain accumulated 3.84±0.06 g/L CDW compared to 4.42±0.23 g/L CDW by SEM10 under conditions of ambient air. When cultivated under a partial pressure of oxygen equal to 25% of ambient air, SEM10 accumulated 4.56±0.23 g/L CDW compared to 3.64±0.28 g/L CDW by KT2440 within the same time span. The ability of SEM10 to accumulate more biomass during conditions of both ambient and low partial pressure of oxygen suggests that the genome reduced strain is able to retain its superior growth properties even at poor oxygen availability.

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**P06: withdrawn**

### P07: Parallel scale-down tool to accelerate fermenter-ophile selection

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Successful scale-up of fermentation processes relies on the selection of fermenterphiles, i.e. robust production strains that maintain optimal performance under industrial conditions [1]. Screening and selection of strains with fermenterophile phenotype require suitable scale-down simulators that mimic relevant industrial conditions [2]. Commonly used scale-down simulators often have a large footprint and low throughput [3], hence they are used at later stages of bioprocess development, whereas including high-throughput scale-down systems at the early stages of bioprocess development would improve the chances of finding fermenterphiles [4]. In this study, we present a parallel scale-down tool based on the Sartorius Ambr<sup>®</sup> 250 high-throughput system to accelerate fermenterophile research in the early stage of bioprocess development. For the scale-down cultivation, we designed an intermittent feeding regime scale-down strategy to simulate the impact of substrate gradients in large bioreactors on *Escherichia coli*. In our design, intermittent feeding occurs in cycles of 1-2 minutes to impose repeated glucose starvation, however, the intermittent feeding regime can be adjusted freely to simulate a wide range of substrate gradients and liquid mixing times expected in industrial-scale bioreactors. With the liquid handling solutions of Ambr<sup>®</sup> 250, metabolome and transcriptome analysis can be performed to elucidate the response of the strains to industrially relevant conditions. We postulate that the presented scale-down tool can be used to efficiently characterize the performance of microbial production strains in industrial fermentation processes and accelerate fermenterophile selection. This would allow the development of fermenterophile strains to be implemented at an early stage of bioprocess development.

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### P08: Controlling *Aspergillus niger* morphology in a rocking motion bioreactor

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The filamentous fungus *Aspergillus niger* has been widely utilized in the production of organic acids, proteins, and secondary metabolites. Its productivity, however, is highly affected by the unpredictable morphological form that it develops in a bioprocess. In particular, shear force regimes as they appear in stirred tank bioreactors have a huge impact on the macro-morphology and finally the product selectivity and yield. The mechanical stress imposed by the stirrers lead to either fragmented hyphae or pellets of varying density. The zones of different shear forces in large scale reactors also lead to different macromorphologies than in lab scale stirred tank reactors (STR).