Investigating the effect of oxygen in *Pseudomonas putida*

Supervisors: Sara Moreno-Paz

Contact: sara.morenopaz@wur.nl robert1.smith@wur.nl

Type of thesis: Combination of experimental and computational work

Required competences: basic knowledge of python or R. Either basic knowledge of models of metabolism (for instance BPE-34306 or SSB-50806) or bioreactor operation (such as Bioreactor design BPE-21306/ BPE 36306)

Acquired competences:

- Bioreactor operation
- Omics analysis
- Genome scale metabolic models
- Microbial metabolism

Starting Date: April – May 2021

Description:

The conversion of high-value natural compounds from feedstocks and (waste)streams by microbes plays a major role in biotechnology and is key to the circular, bio-based economy. However, the standardized process for cell factory design presents a clear division between strain design (genetic and metabolic engineering) and bioprocess optimization (media composition and bioreactor operation). This strategy ignores the relations between pathway, metabolism and culture conditions which might lead to delivering suboptimal production strains. Genome scale metabolic models (GEM) are mathematical representations of cell metabolism which in combination with flux balance analysis are useful tools to understand microbial metabolism. Besides, dynamic flux balance analysis allows the simulation of microbial growth in a reactor environment. Unfortunately, predictions from GEM are not always accurate and experimental tests are required to improve them (Shinfuku et al. 2009).

Pseudomonas putida is a strictly aerobic bacterium which growth is highly affected by oxygen availability. However, little is known about how oxygen affects its metabolism. Simulations with GEM suggested that fine-tuning the substrate and oxygen uptakes rates during fermentations can impact the production of pyruvate derived products in engineered *P. putida* strains.

This thesis project starts with the performance of continuous fermentations which allow to control oxygen and substrate uptake rates in order to characterize the effect of oxygen on the metabolism of different *P. putida* mutants. Besides studying possible organic acids production, samples for transcriptomics and proteomics will be taken and analyzed in order to find

differentially expressed genes and pathways under different oxygen availability conditions (Rintal et al. 2009). Finally, data will be integrated with the aid of a GEM to better understand the effect of oxygen on metabolism and suggest strategies to increase the performance of engineered strains (Molina et al. 2019).

References:

Molina, L., Rosa, R. La, Nogales, J., & Rojo, F. (2019). Pseudomonas putida KT2440 metabolism undergoes sequential modifications during exponential growth in a complete medium as compounds are gradually consumed. Environmental Microbiology, 21(7), 2375–2390. https://doi.org/10.1111/1462-2920.14622

Rintala, E., Toivari, M., Pitkänen, J. P., Wiebe, M. G., Ruohonen, L., & Penttilä, M. (2009). Low oxygen levels as a trigger for enhancement of respiratory metabolism in Saccharomyces cerevisiae. BMC Genomics, 10(1), 461. https://doi.org/10.1186/1471-2164-10-461

Shinfuku, Y., Sorpitiporn, N., Sono, M., Furusawa, C., Hirasawa, T., & Shimizu, H. (2009). Development and experimental verification of a genome-scale metabolic model for Corynebacterium glutamicum. Microbial Cell Factories, 8(1), 43. https://doi.org/10.1186/1475-2859-8-43