Doctoral Program in Chemical Engineering (XXXII cycle)
Candidate: Beatrice Battaglino
Title: Stable production of commodity chemicals in cyanobacterial cell factories

Summary

The sustainable production of commodity chemicals is becoming urgent, given the depletion of petrochemical resources and the climate change effects that we are witnessing. Production based on cyanobacterial cell factories can represent a feasible alternative, since these photosynthetic microorganisms are able to directly convert atmospheric CO_2 into valuable chemical compounds. Upon proper genetic modifications, the range of metabolites produced by cyanobacteria has been broaden, however classic metabolic engineering approaches often introduce a trade-off between biomass growth and product formation. This divergence can lead to genetic instability of such engineered strains, because a positive selection pressure is active towards the cells that accumulate mutations causing the loss of the production trait and a gain in the microbial fitness (retro-mutants). These advantaged mutant cells would easily outcompete the producer cells determining a drastic drop in productivity, which compromises scale-up in real industrial settings.

In this work, a metabolic engineering strategy that aims at aligning microbial fitness and product synthesis has been applied to the production of fumarate and malate in the cyanobacterium *Synechocystis* sp. PCC 6803. Fumarate and malate are two dicarboxylic acids interesting from the biotechnological point of view, since they are widely used in the food and feed industry, as well as in the bio-plastic and polymers sector.

By means of an algorithm analysing the genome scale metabolic model of *Synechocystis* sp. PCC 6803, it was possible to identify the compounds that can be produced in a growth-coupled fashion as sub-products of anabolic reactions, and the genetic modifications necessary to obtain their accumulation. Among these compounds there was present fumarate, which has been chosen as target metabolite in this study. Moreover, the strategy has been extended to malate, a compound that, although not recognized as a strict growth-coupled metabolite, uses as precursor fumarate which is a stoichiometrically growth-coupled compound. Different deletion mutants, able to accumulate fumarate and malate, have been engineered and tested in a laboratory scale photobioreactor in different cultivation modes, including day-night regime. The fumarate and malate producing strains were able to reach a cumulative titer of ∼1.2 m M and 1.0 m M after about 10 and 14 days of cultivation in batch under continuous illumination, respectively. The genetic stability of the most promising strains has been tested through an evolution experiment, which revealed that the mutants were stable for over one month of continuous cultivation, thus demonstrating the effective success of the metabolic engineering strategy adopted.

Finally, a mathematical model that describes the dynamics of retro-mutants into a population of photosynthetic microorganism has been developed. The model has been used to simulate the dynamics of formation of retro mutants in a semi-continuous cultivation setting, where dilutions are performed at certain time-intervals. A sensitivity analysis of different parameters allowed to identify the optimal set of conditions to carry out an evolution experiment. Indeed, the cultivation mode adopted directly influences the time instant at which the retro-mutants can be observed. However, in a generation time-

scale the simulations highlighted that only two parameters (i.e., the production burden and the mutation rate), which are intrinsic genetic features of the strain, determine the appearance of retro-mutants.