

Exploiting non-growing microbes for biosynthesis of useful fuels and chemicals is a promising approach with distinct economic advantages. The vast majority of current biosynthetic products are growth-coupled, meaning cells must be maintained in a growth phase, and if they stop growing they drastically reduce their productivity. Given that cells can only grow to a finite density, this puts a hard constraint on bioprocess performance. If highly-productive, non-growing cells could be engineered, bioreactors could be run for extended amounts of time, resulting in superior yields and productivities compared to growth-coupled processes. Recent advances in Synthetic Biology allow the dynamic control of genetic programs in microbes, which account for the unique phases of a bioreactor. By growing cells with the production program 'off' and turning the program 'on' in high density, non-growing cultures has many advantages. The cells (i.e. catalyst) growth can be stopped, so there is no additional loss in carbon yield due to growing more cells. As well, engineering strategies that are toxic or detrimental to cell growth can be readily used if no more cell growth is required. Examples include: knockout of essential enzymes, overexpression of toxic genes, and draining a metabolic pool that is otherwise needed for growth. There are also advantages to having the biosynthesis program 'off' during growth. Cell growth is shortened (and with it, batch time), because there is not a burden for making product. As well, contaminating microbes have a diminished opportunity to overtake the culture, because uninduced strains grow quickly.

Our lab has recently addressed two challenges with exploiting non-growth metabolism: (a) turning the production program 'on' at high cell density must be done on the industrial scale making typical lab-scale techniques prohibitively costly, and (b) metabolic activity is typically suppressed when cells stop growing, which is detrimental to productivity. To address the need for high cell density induction, we have developed a glucose starvation toggle genetic circuit. After a cell has experienced an induction signal, the response remains active, even after the induction signal is removed. We have engineered a switch to use glucose starvation as an induction signal. From a bioprocessing standpoint, this enables growth on glucose to a defined density (based on the initial glucose concentration). After glucose is exhausted, the switch is toggled and the biosynthesis program is activated. Once the switch has toggled, glucose can be reintroduced and consumed by the cells to produce the desired product.

To address low metabolic activity in non-growing cells, we have identified essential gene knockouts that maintain high metabolic activity for different parts of metabolism when growth is stopped. The strategy would be to allow expression of these essential enzymes to grow cells, but repress them once an adequate cell density is reached. Using metabolic modeling, we have identified thirty gene knockouts that are essential on minimal media and characterized their metabolic capabilities. Several mutants maintained high glucose uptake rates and secreted a variety of byproducts once limited by the essentiality. I will discuss our characterization of these strains for both uptake/secretion fluxes, as well as intracellular metabolomics. In total, we have developed tools to enable facile switching to production phase at industrial scale and identified metabolic operating conditions that should enable high productivity in non-growing cells. This work should be enabling for a number of industrial biotechnology processes.