The Future of Metabolic Engineering: Dynamic Metabolic Network Minimization

In the field of metabolic engineering, researchers have historically found it extremely difficult to predict the behavior of complex biological systems *in vivo* from simplified models and basic *in vitro* biochemical principles. In many cases, it has proven much more challenging than expected to integrate a well-characterized production pathway into a living host and balance the complex requirements of biomass growth and production.

The difficulty lies in the interconnected nature of metabolic networks, wherein each metabolite and/or enzyme can interact with endless others. This combinatorial complexity creates a huge potential design space, which is intractable to the kinds of systematic experimentation required for the development of standardized design principles. The global challenges in addressing such a large biological design space have persisted despite the dramatic advances in, and decreased costs of, reading and writing DNA, high-throughput DNA assembly, and microbial strain construction.

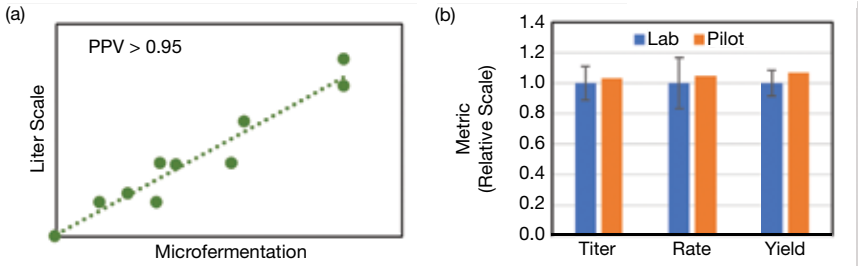
With funding from the National Science Foundation, DMC Biotechnologies is deploying its technology platform for the production of biobased chemicals. The platform technology, which bridges the gap between current *in vivo* and *in vitro* bioproduction approaches, relies on the dynamic minimization of the active metabolic network. Dynamic metabolic network minimization allows researchers to construct a less-complex design space and create robust strains. Robust strains enable predictable scalability from high-throughput, small-scale screens or micro-fermentations to fully instrumented bioreactors.

The DMC technology is a scalable, high-throughput metabolic engineering platform that enables the rapid development of microbial production strains. The patented Dynamic Metabolic Control technology combines a standardized, two-stage fermentation process (that decouples biomass growth from product formation) with gene silencing and targeted proteolysis to engineer metabolic fluxes. The end result is a growth phase with maximum theoretical rate and yield followed by a production phase with near-theoretical yield and unprecedented production rate.

The technology dramatically reduces the cost and risk currently associated with the production of biobased products, enabling ultra-low-cost product development. This significant reduction in development cost enables companies to produce numerous specialty products that would otherwise not have an acceptable financial return on development capital. With the deployment of this platform, DMC envisions democratizing metabolic engineering and bioprocess design efforts to the point where creating designer biocatalyst strains becomes as facile as ordering DNA oligos today.

DMC's lead products are specialty amino acids, including L-alanine, valine, isoleucine, and leucine. These amino acids can be used in human nutritional, wellness, and personal care products, as well as in animal feed. In particular, the animal feed market is constrained by the price of branched-chain amino acids (BCAA). DMC has demonstrated commercial key performance metrics for fermentation and downstream purification (DSP) at the lab scale, and has achieved costs of production well below the current selling prices for each amino acid product.

The first amino acid product has recently been produced at pilot scale (*i.e.,* 4,000-L fermentation volume) using a third-party facility. This effort has validated two key technology features — predictable performance across several scales and robustness of the strain to the process environment — and has generated material for customer acceptance testing. DMC is providing kilogram-sized samples to potential customers to drive business development efforts. Contracting with third-party tolling providers (approaching the 100,000 L fermentation scale) is currently in progress with a forecasted first commercial launch in 2020. CEP



▲ (a) DMC Biotechnologies is the first in the field to demonstrate predictive performance from microfermentation to liter scale. Data are for dozens of unique products and multiple variants of each product with a positive predictive value (PPV) > 0.95. (b) Process performance for DMC's lead product (L-alanine) was demonstrated at pilot scale (4,000-L fermentation) with results matching lab scale (1 L and 6 L).

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