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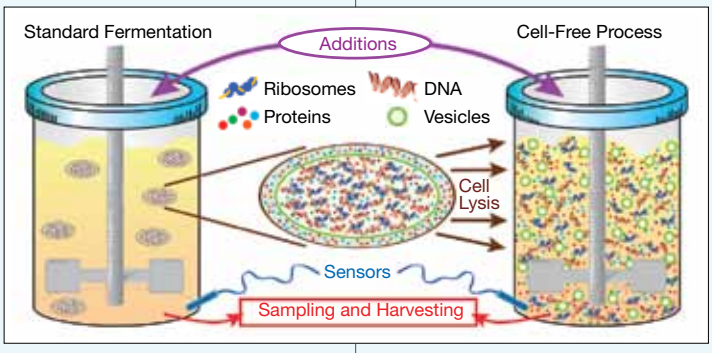
JOURNAL Highlight

Cell-Free Biology Transforms Biochemical Engineering

Modern cell-free biology — *i.e.*, the activation of complex biological processes without using living cells — is a rapidly developing disruptive technology offering game-changing capabilities to chemical engineers. The ability to gain direct access to the intracellular contents, allowing for monitoring and control of the reaction environment, transforms traditional fermentation processes into homogeneous catalysis, says Stanford Univ. professor of chemical engineering and bioengineering James Swartz. In the January *AIChE Journal* Perspective article, "Transforming Biochemical Engineering with Cell-Free Biology," Swartz discusses how this in turn opens the field to the fruits of decades of chemical engineering progress toward producing low-unit-value products at high volumes.

While cell-free biology has been practiced for decades, practitioners were long intimidated by the complexity of crude cell extracts containing hundreds of active biological catalysts. Recently, this black box has been illuminated by numerous examples showing that the reaction networks can be understood, altered, and controlled. These advances have, for example, enabled cell-free pharmaceutical protein production at the 100-L scale with nearly identical performance to that observed in 20-µL experiments.



The figure depicts the basic concepts of cell-free technologies. The source cells are first grown, harvested, and lysed (broken apart). The lysate can then be used directly or centrifuged to remove suspended solids and further processed, if needed. After lysis, hundreds of different enzymes are distributed uniformly throughout the reactor. Now, complex reaction networks can be precisely sensed, sampled, and controlled.

Swartz explains that cell-free reactions contrast sharply with biochemical engineering processes that use living organisms. In the latter, the reactions take place behind a highly selective barrier, the cell wall. Not only are the influential reactions sequestered, but reactants are actively added and expelled by transporters according to an evolved agenda that often contravenes process objectives. The cells also control the concentrations of hundred of catalysts, and these often change dramatically during the course of a batch process. The script dictating these changes has evolved to optimize survival of the individual and the species, objectives that are diametrically opposed to the overproduction of a single product. Thus, it is still virtually impossible to achieve full metabolic control within the cells. Cell-free technologies completely change this scenario.

To date, much of the research has focused on cell-free protein production, and this has resulted in significant cost reduction, improved protein folding, and effective scale-up. Cell-free protein synthesis is a powerful approach for high-throughput protein evolution. Nonetheless, Swartz believes it is especially intriguing to consider the cell-free production of metabolites and other biochemicals. "Our modern chemical industry has its present product portfolio because petroleum was its primary raw material. However, now that petroleum is much more expensive and we are also more aware of the long-term consequences of its conspicuous consumption, we are beginning to turn to biology," he says.

What we find is a much richer source of reactive precursors for modification and for polymerization, *i.e.*, raw materials for a new, much broader and more useful chemical industry. The challenge is that we must quickly develop processes with high conversion yields and high productivities. The flux of the carbon and other atoms must be precisely controlled, and each process must supply the exact reducing equivalent and energy needs of the targeted pathway," he continues. "This is difficult to achieve in a living organism, but much more straightforward with cell-free accessibility."

Looking to the future, Swartz expects cell-free approaches to offer great flexibility for pursuing synthetic biology. For example, he asks, "could we design artificial cells that could circulate in our bloodstream and produce insulin when it was needed? Could we also combine the strengths of organic synthesis with enzymatic catalysis to produce new, more useful molecules that couldn't be produced efficiently with either approach alone? Like any new biotechnology, we must use it responsibly, but the opportunities for serving society with cell-free technologies are very exciting."