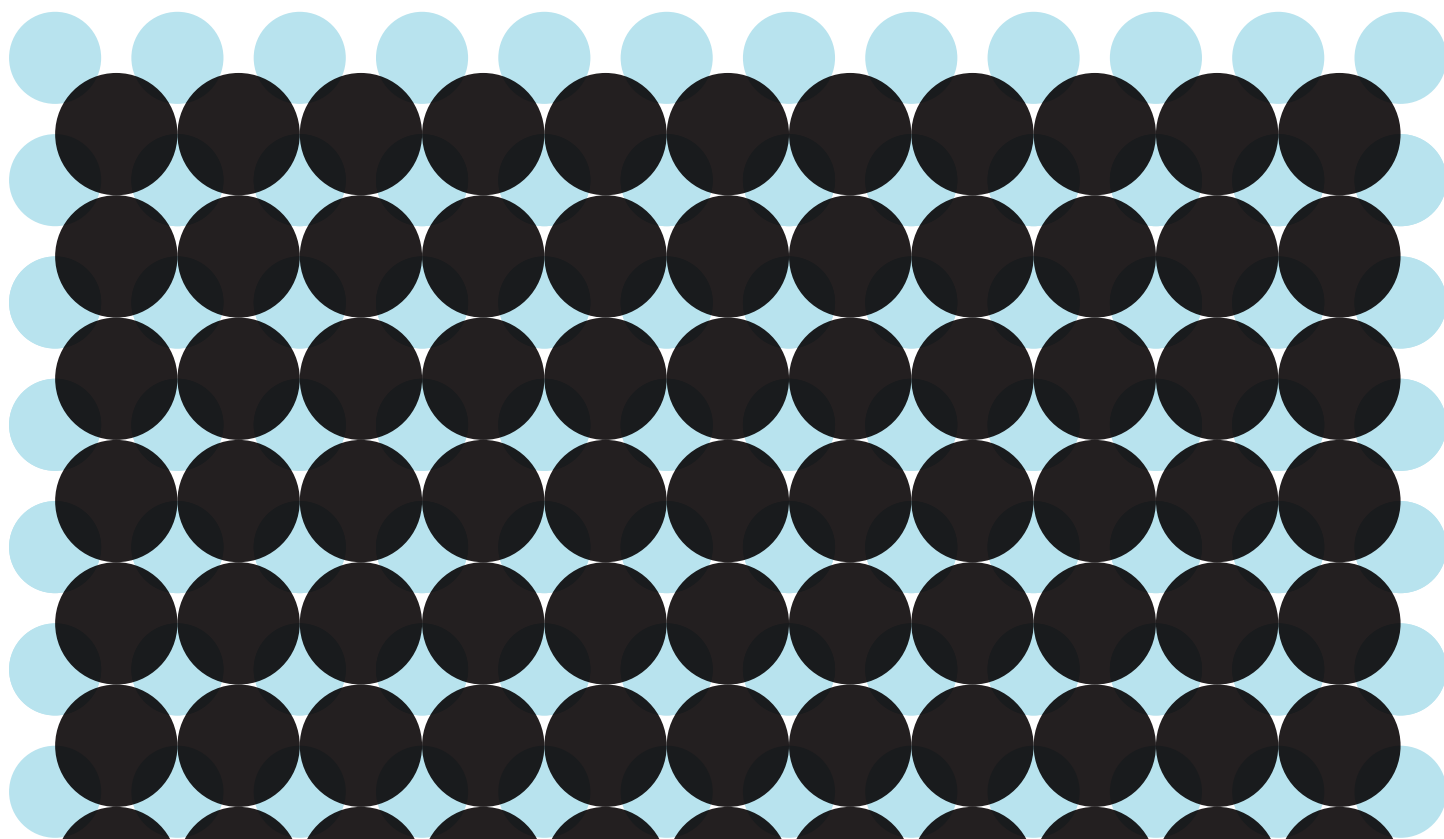


From fermentation to final product: small molecule manufacturing – a case study

Martin Venzhofer and Vratislav Stovicek

Biotechnology complements chemical synthesis in manufacturing of small organic molecules, often providing improved functionality and higher product purity. How does a biotechnology manufacturing process for small organics look like? What is the typical process flow and unit operations at large scale? Let us unpack a commercial campaign and highlight relevant trends, bioprocess metrics and challenges.



From fermentation to final product: small molecule manufacturing – a case study

Small molecules produced via microbial fermentation play a vital role in pharmaceutical, nutraceutical, and specialty chemical markets, representing a rapidly growing multibillion-dollar segment. While chemical synthesis remains dominant, biotechnology offers advantages such as desired molecule chirality, higher purity and sustainable production. This white paper provides a comprehensive overview of the manufacturing process for production of a small organic molecule, combining upstream (USP) and downstream processing (DSP), and shares operational insights from an industrial scale campaign.

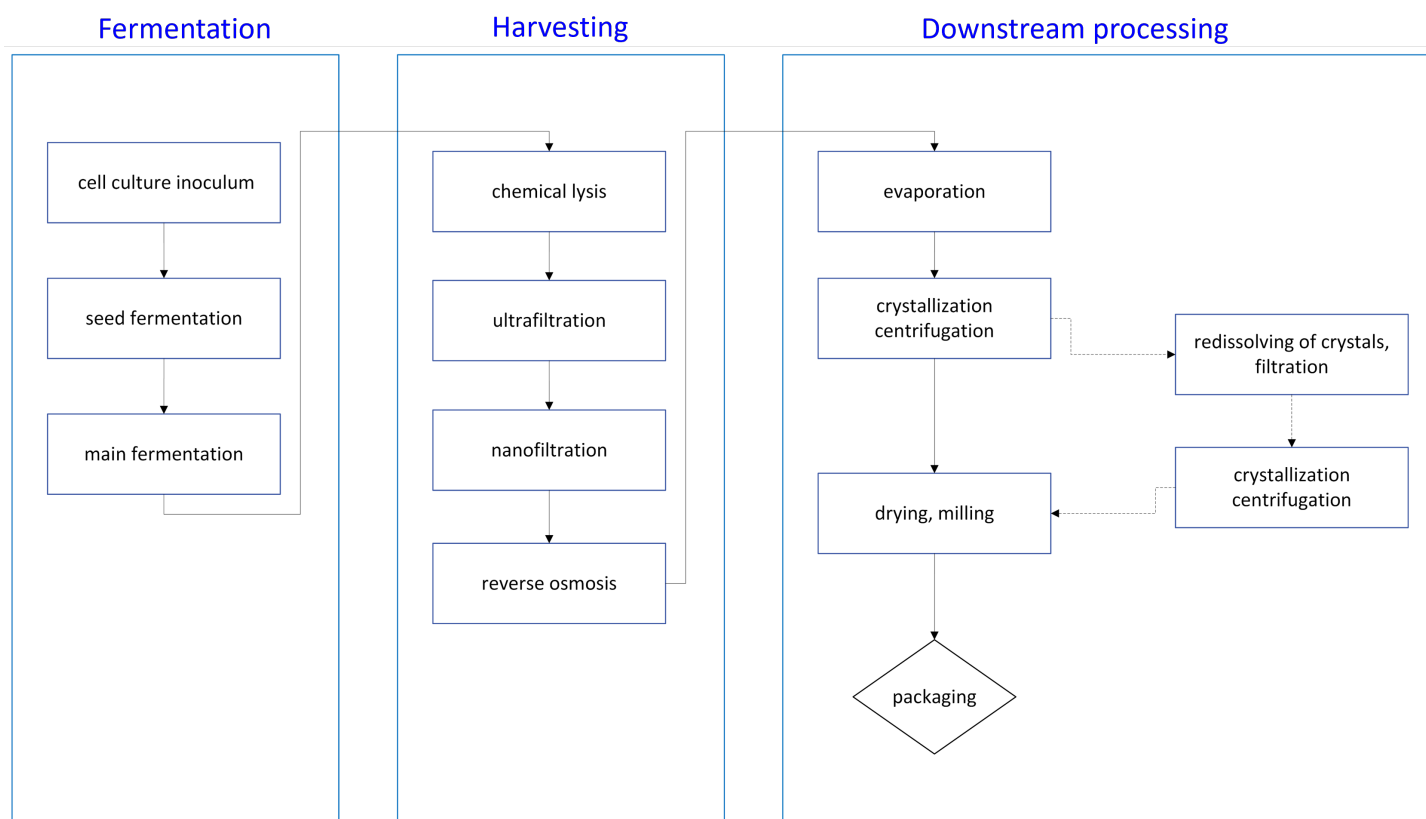
Small organic molecules are low-molecular-weight compounds (<1 kDa) that play an important role as building blocks and regulators of biological processes. They include amino acids, sugars, organic acids, lipids, and fatty acids, and are distinct from macromolecules such as proteins, nucleic acids, and polysaccharides. Microbial fermentation has long been used to produce primary metabolites like amino acids, organic acids, and vitamins. Active pharmaceutical ingredients (APIs) and their intermediates are also small molecules, typically classified as secondary metabolites.

Beyond well-known antibiotics, bio-manufactured drugs now target a wide range of (even non-infectious) diseases by interacting with specific biological targets. Advances in strain and metabolic engineering have significantly improved bioprocess productivity, enabling commercialization of additional secondary metabolites, often plant-derived, such as flavonoids for nutraceutical and personal care applications.

Process Overview

Any bioprocess starts with cultivation of either native or engineered microbial strain in stainless steel bioreactors with controlled conditions favorable for the product accumulation, followed by cell disruption and biomass debris removal (alternatively, the cell disruption step may be skipped in case of extracellular production), broth concentration and product purification to obtain the final product. Downstream processing and its employed unit operations largely depend on the nature and properties of the isolated molecule. Nevertheless, the steps illustrated in Figure 1 serve as an example of the manufacturing process for a water-soluble small molecule.

Figure 1: Block flow diagram of the manufacturing process.



Upstream processing

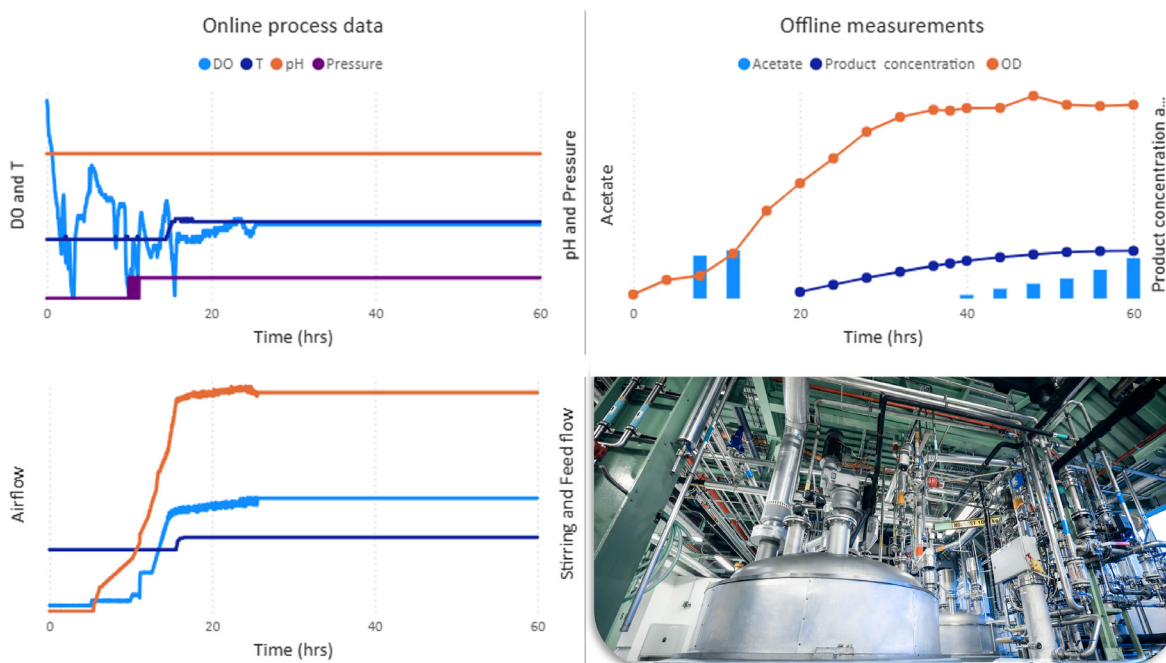
Upstream processing begins with inoculum preparation from a working cell bank (WCB). In this example, a genetically engineered bacterial strain was employed, leveraging metabolic engineering strategies such as targeted gene deletions, pathway overexpression, and cofactor balancing to enhance biosynthetic efficiency. The culture was expanded stepwise through a seed train, transferring from small inoculum volumes to progressively larger seed fermenters until reaching production scale. Main fermentation was operated in fed-batch mode, with feeding initiated upon depletion of initial carbon sources, indicated by a dissolved oxygen (DO) spike. The feeding strategy combined exponential feeding for rapid biomass growth with linear and later constant feeding to promote product formation. The production phase was triggered by a one-time temperature shift, otherwise temperature as well as pH were maintained constant, with pH controlled via automated ammonium hydroxide dosing. DO was regulated primarily through dynamic aeration, with only minor adjustments to agitation and pressure. Optimized feeding and DO control were critical to prevent accumulation of overflow metabolites such as acetate, which can significantly reduce process yield.

Figure 2: USP process profile. The charts illustrate the relative trends of bioprocess control parameters (dissolved oxygen - DO, temperature - T, pH, pressure, airflow, feed flow, stirring). On the right-hand side, product, biomass, and by-product (acetate) formation throughout the fermentation is displayed. Values and scale have been intentionally omitted to emphasize trend visualization and comparative patterns rather than precise figures. The picture displays the upper floor of the upstream manufacturing asset.

Harvesting and downstream processing (DSP)

After completion of the fermentation stage, the broth was subjected to cell lysis using a chemical agent. Cellular debris and high-molecular-weight impurities were removed through a series of filtration steps. The clarified broth was then concentrated via reverse osmosis followed by evaporation. The resulting concentrate underwent crystallization, and crystals were separated from the mother liquor by centrifugation. If the impurity profile did not meet specifications, the crystals were redissolved, filtered, and the crystallization-centrifugation cycle repeated. The process concluded with vacuum drying of the crystals and milling-sieving to achieve a uniform particle size in the final product (Figure 1).

Analytical control was maintained throughout the process, including in-process measurements, impurity profiling, and final product quality assessment using advanced instrumentation such as HPLC, FTIR, NIR, UV-VIS spectrophotometry, and moisture analyzers



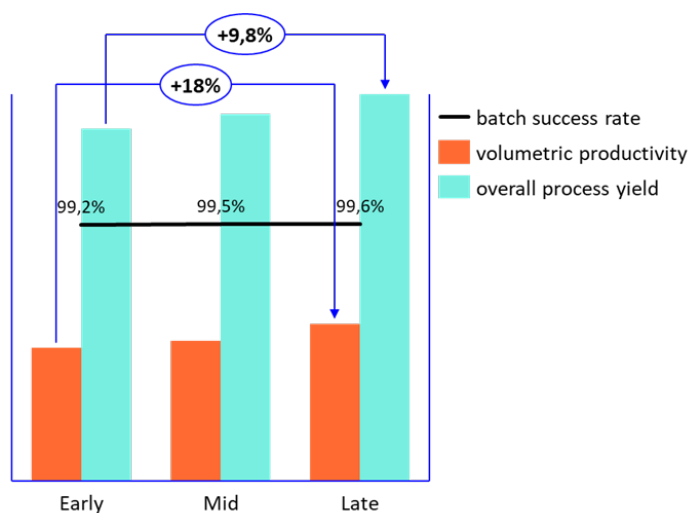
Manufacturing performance and challenges

Even mature manufacturing processes can present operational challenges. During the showcased campaign, key issues included microbial contamination, oxygen transfer limitations leading to productivity deviations during fermentation, membrane fouling during harvesting, and extended drying times coupled with crystal handling difficulties in downstream processing (DSP).

These challenges were addressed through enhanced sterilization and cleaning protocols, optimized feeding strategies with dynamic feed control, adjustments to aeration and pressure, and equipment upgrades in case of dryer and advanced CIP (clean-in-place) systems for crystallizers.

The implemented improvements delivered an 18% increase in volumetric productivity during fermentation compared to the initial campaign stage. Overall process yield improved by 10% (Figure 3). A slight yield reduction in harvesting and DSP was observed, primarily due to stricter cleaning protocols introduced to ensure quality and maintain a success rate above 99% (Figure 3).

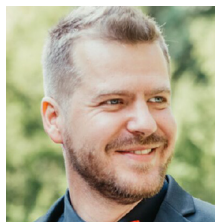
Figure 3: Manufacturing campaign performance. Values and scale have been intentionally omitted to emphasize trend visualization and percentual changes rather than precise figures.



Summary

Biotechnological production of small molecules offers a sustainable alternative to chemical synthesis, delivering high purity and stability. Optimized fed-batch fermentation, combined with robust downstream processing, can achieve high yields and consistent quality. Trade-offs between yield and quality are inevitable and should be managed proactively. Continuous improvement, through dynamic process control with real-time analytics, preventive maintenance, enhanced cleaning protocols, and equipment upgrades, is critical to remain competitive in a rapidly evolving market.

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