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RESOLVING CULTIVATION BOTTLENECKS: THE BIOPROCESSING JOURNEY

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Do You Want to Know More About Scale-Up?

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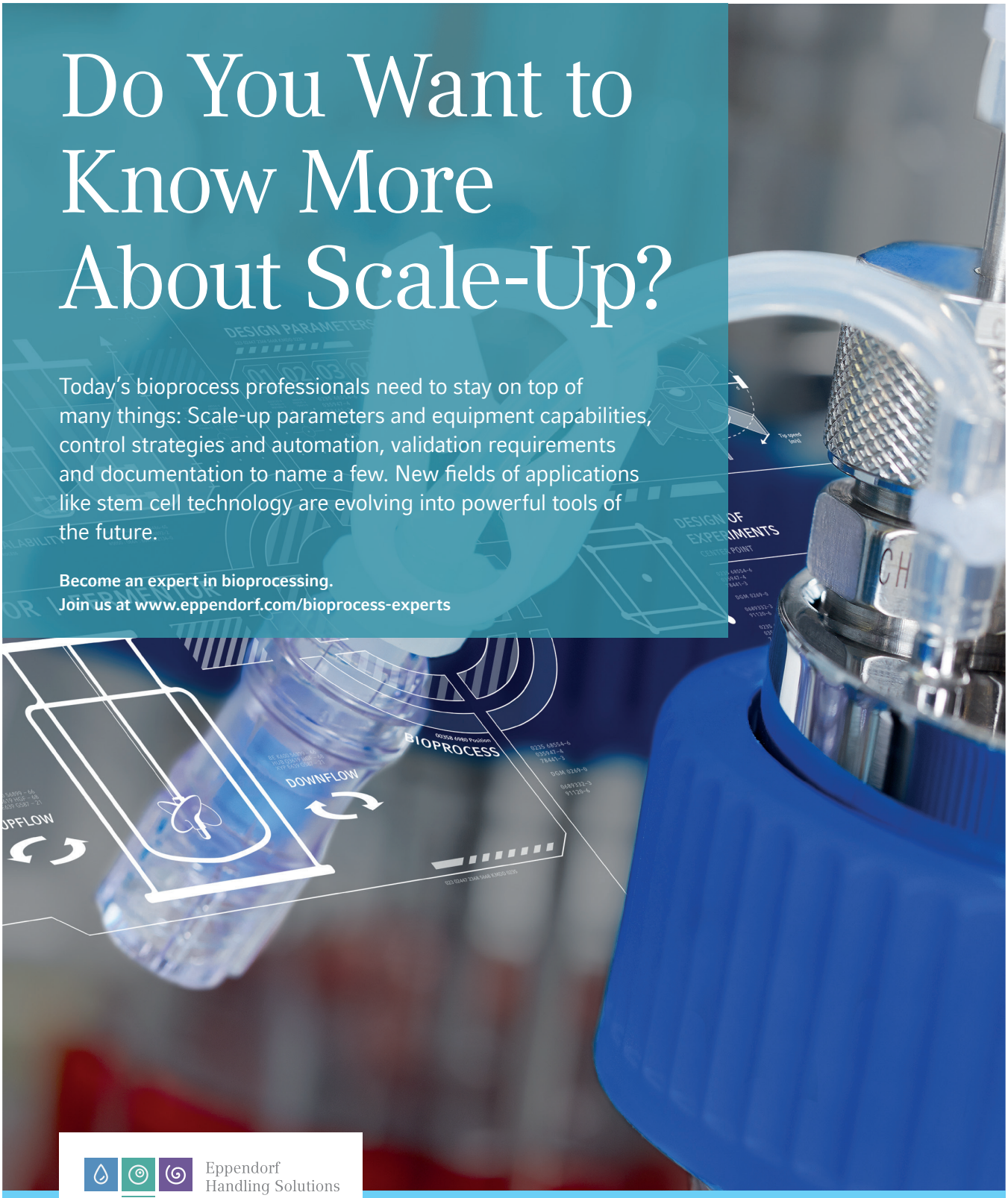

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The Next Level: Why Move to a Bioreactor?

Bioprocessing relies on living cells or their components to create pharmaceutical, biofuel, or nutrition products. Whether bacterial, fungal, plant, or mammalian, cells used for these processes need unique conditions for optimal growth and product formation. Important parameters include temperature, pH, and the concentrations of nutrients and oxygen in the culture medium.

When cultivating cells and microbes in flasks or plates for large-scale production, scientists often need to combine material from multiple culture vessels. This creates a substantial manual work load and may be a source for variability. Alternatively, they may use bioreactors and fermentors*, which simplify the process of increasing biomass and product formation, boost efficiency while lowering costs, and help generate reproducible results.

Beginning the Bioprocessing Journey

Bioprocessing often begins with cultivating cells in flasks or on plates, strategies that are inexpensive and do not require a high level of technical skill. Growing small volumes in simple containers provides useful data for initial studies, but the weaknesses in this approach become apparent when scaling up production, producing a product repeatedly, or performing long-term experiments.

Moving beyond the incubator and shaker to bioreactors and fermentors allows scientists to create and monitor ideal environments for cellular reproduction and product formation, increasing experimental yields and enhancing reproducibility. Bioreactors contain sensors that monitor culture media conditions, such as pH, dissolved oxygen, and temperature, and then convey that data to software for online review. With this technology, scientists can program bioreactors to automatically respond to changing conditions by actively tempering the system, modifying agitation, adjusting the pH, or changing other parameters to control growth conditions. Some of this, like heating the culture, is also done in shakers and incubators. However, bioreactors and fermentors offer additional possibilities to control critical process parameters that can be key to reach higher cell densities and product yields. For example, in addition to heating the culture medium, bioreactors and fermentors also facilitate cooling. Fast-growing microbial cultures produce heat, which, if not dissipated, may limit growth to high cell densities. Bioreactors and fermentors are often equipped with spargers to introduce air or pure oxygen to the culture. This is more efficient than oxygen transfer from the surrounding air in cultures incubated in shakers or incubators; therefore, oxygen does not become growth-limiting.[1]

Over time, nutrients become depleted, hindering cell growth and limiting product formation. Experiments often must be repeated to accumulate sufficient cell numbers or product concentrations, increasing the amount of work and introducing variability in production. Unlike in flasks and plates, feed solutions can be automatically added to bioreactors via a system's integrated pumps, raising efficiency of cultivation.

Level-Up to Large Dimensions

Large bioreactor vessels decrease the need for monitoring small, individual flasks and repeating experiments to enhance product concentrations. Growing cultures in large bioreactors or fermentors saves time and money. Additionally, bioreactors have enhanced surface area to volume ratios compared to typical flasks, boosting biomass formation.[2] Scaling-up experiments can be tricky as the shape and dimensions of the reaction vessel can impact parameters like nutrient and oxygen distribution. Bioreactors and fermentors come in a variety of sizes to meet the needs of every workflow, often with consistent geometries that allow for predictable experiment scale-up. Cultivating large amounts of cells in an ideal environment ensures the consistency of the products formed.

For references, please refer to page 7

** The term fermentor is typically used when describing the cultivation of microorganisms. The term bioreactor often refers to the cultivation of mammalian cells but is also generically used.*

How Do Bioreactors Boost Reproducibility?

Whether repeating experiments for publication or producing compounds in an industrial lab, reproducibility is key. Differences in cultures from batch to batch often arise due to variability in flask size, flask geometry, shake speed, and shake orbit.[1] Therefore, combining cells or products from individual small cultures should be avoided when uniformity is essential.

One Size Does Not Fit All

Vessels of various sizes work best for different applications, from running initial processes to obtaining maximum biomass and product concentrations. A range of bioreactors are available, from large capacities that can produce thousands of liters of culture to mini reactors that can run reactions in parallel. Running pilot studies in mini bioreactors with the same geometry as their larger counterparts makes scaling-up process conditions simple and reproducible. Additionally, experiments in mini reactors can be run and monitored in parallel, allowing scientists to compare the effects of many protocol tweaks side-by-side. When it's time to increase production, performing one large experiment in a single bioreactor saves time and money compared to combining multiple small experiments, with the added benefit of uniformity of the cells and products formed.

Dynamic Control

Bioreactors are not merely inert vessels for cell growth; they closely monitor and dynamically adjust biological and mechanical process conditions automatically. Bioreactors have inputs for feeding cultures as nutrients become depleted and, eventually, for removing spent media while retaining the cells. Additionally, impellers control agitation, uniformly mixing medium components. Throughout the growth of the culture, sensors in bioreactors measure important cell growth parameters such as pH, temperature, dissolved oxygen, oxygen transfer rate, biomass and more. Software that tracks changes and engages pumps and motors alleviates variations in these conditions in real time.

Temperature changes greatly affect growth conditions. Cells producing exothermic reactions can increase the temperature within flasks beyond the desired incubation temperature. Temperature control elements, like thermal jackets and cooling fingers, make adjustments when necessary, heating or cooling the liquid inside.

Fermentation reactions form acidic products, such as acetic and lactic acid. As these growth byproducts acidify the culture medium, growth rate often declines.[2] Bioreactors usually connect to software applications for online monitoring of pH, which alleviates the need for a heavily buffered medium that can slow cell growth and may not buffer optimally at physiological pH. Instead, a pH sensor in the medium transfers readings to bioprocess control software that can activate pumps to inject acids or bases as needed to return the culture to the desired pH.

Controlling amounts of dissolved oxygen in growth vessels is essential for predictable metabolic processes and avoiding cell death.[3] While both flasks and bioreactors can have baffles (insertions in the vessels that promote agitation) to increase oxygen transfer rates in cultures, bioreactor spargers, vessel components that bubble gases through liquid, add additional air or pure oxygen into the medium, increasing the amount of available oxygen. Impellers, or paddles, in bioreactors control agitation of the medium and can be adjusted to reduce foaming, control transfer rates, and increase homogeneity of other parameters, such as pH and temperature. There is a wide range of options when it comes to impeller types, baffles, vessel dimensions, and sparger types to best meet a researcher's needs.

Seeing the Big Picture

Options for vessel size, control sensors, and pumps in modern bioreactors make them ideal growth containers for a variety of cell types. Controlling reaction environments using software enables better control and data collection throughout the growth phase. In addition, software coupled with devices such as spectrometers (and mass spectrometers), HPLC, or NMR, allow scientists to obtain detailed information about product formation in the medium. By generating this data in parallel experiments, scientists can easily detect key growth parameters and apply them to future workflows, reproducing optimal environments.

For references, please refer to page 7

A Balanced Diet: Feeding Bioreactor Cultures

When culturing cells, scientists need to account for the cost of supplies, process runtime, desired product yield and quality, and the organism being cultivated when designing appropriate feeding strategies. Understanding the strengths and weaknesses of each feeding strategy aids in designing efficient experiments with optimal results.

They Grow up so Fast

Cells in a closed system grow in predictable stages. After inoculation, cells initially enter a lag phase where little division occurs. During this stage, cells adapt to the conditions of their new environment, changing their metabolic processes to accommodate the nutrients present and synthesizing appropriate RNA and enzymes. Once the cells are ready to use the available nutrients, exponential cell growth begins via binary fission and the culture enters logarithmic (log) phase. Researchers typically study cellular functions during this phase because cells rapidly proliferate and are in their most active state. During late log phase, cells may be passaged into new media to avoid overcrowding or apoptosis. As nutrients become limited and waste products accrue, causing stress, cell growth plateaus into a stationary phase. In this phase, cell quantities remain constant as the number of cells dividing equals that of those dying; living cells remain metabolically active. Ultimately, nutrient depletion and waste accumulation will create conditions where life is unsustainable, and all cells enter death phase. The timing of each stage depends on factors such as cell species or phenotype, temperature, and nutrient availability.

Too Much or Too Little of a Good Thing

Balancing nutrient levels throughout the growth cycle optimizes cell reproduction and product output. Starvation results in decreased protein synthesis, elevated apoptosis, and potential metabolic dormancy, resulting in decreased cell counts and protein production.[1,2,3] Surprisingly, nutrient excess can also negatively influence cell populations. Changes in metabolism due to surplus nutrients may generate reactive oxygen species that inactivate proteins and mutate DNA, impacting cellular production.[4]

Which Process Is Right?

For bioprocessing, there are three main feeding strategies to consider: batch, fed-batch, and continuous cultures.[5] Researchers maintain batch cultures in shake flasks or bioreactors at constant working volumes without adding or removing anything in the culture. While bioreactor sensors measure parameters within a culture, balancing nutrients and waste build-up often cannot take place, slowing growth, limiting cell density, and leading to death phase. Batch growth strategies are easy to operate and have a low risk of contamination; however, the comparatively low cell densities obtained in these uncontrolled environments often require repeat experiments in fresh batches. Long downtimes may be required between batches as equipment is cleaned, sterilized, and reassembled. Batch cultures are often used to test conditions in early experiment design stages.

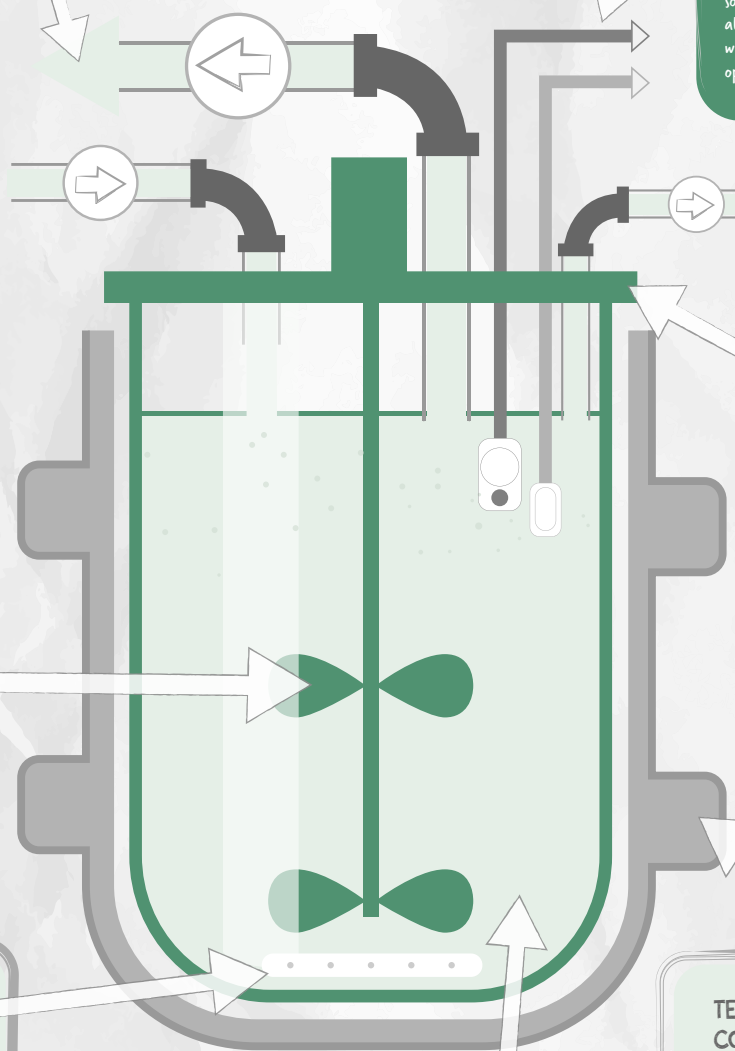
Fed-batch cultures increase biomass through an extended exponential phase, resulting in proportional product yield with limited waste product accumulation. This process starts with the same conditions as batch cultures, but additional nutrients are added in proportion to cell growth rates (exponential feeding) or at a constant rate (constant feeding). Constant feeding gradually decreases nutrition availability as culture density increases. The addition of fresh nutrients should be closely monitored to avoid issues associated with overfeeding.

Using a continuous culture strategy allows scientists to harvest products at a constant rate. Once a culture reaches stationary phase, the bioreactor expels spent medium containing toxic metabolites and adds new media with fresh nutrients. Because the rates of removal and addition are equivalent, the culture remains in a steady state with a consistent working volume. Products can be harvested from the waste medium continuously, from days to even months, while the biomass within the vessel remains constant. A perfusion strategy with a cell retention device connected to the bioreactor exhaust enables higher cell density cultures. Long cultivation times challenge sterility within a continuous culture, but continuous production and product uniformity offer benefits to balance this risk.

For references, please refer to page 7

The Basic Components of a Bioreactor.

Bioreactors and fermentors offer a variety of options for applications ranging from research and development at a small scale to large cultures with optimal product formation. Bioreactor control devices help form ideal environments for cell proliferation and product generation, enhancing uniformity and reproducibility.



DIP TUBE

Removal of waste products in spent medium prolongs cell growth and product formation. Taking samples allows for offline analysis of cells and medium composition.

PARAMETER SENSORS

Process parameters, such as pH, temperature, and oxygen levels, are monitored by a variety of sensors that communicate with bioprocess software. Parameters can be automatically altered to adjust to the changing conditions within the vessel throughout cell culture growth, optimizing yield and improving reproducibility.

FEED LINES

Feed lines allow for the addition of fresh medium and other nutrients, extending cell division compared to batch culture.

EXHAUST GAS

Sensors for exhaust components facilitate insights into critical culture parameters, like biomass development and substrate consumption.

HEADPLATE

The headplate provides ports for multiple inputs, outputs, sensors, and pumps, which enable tightly controlled growth parameters and real-time product analysis.

AGITATION SYSTEM

Proper mixing ensures that cells stay in suspension and increases homogeneity of important variables (pH, temperature, concentrations) throughout the culture. A variety of impeller shapes influence medium flow and mechanical stresses like foaming.

AERATION SYSTEM

Dissolved oxygen (DO) is a critical parameter for optimal cell growth and production. Spargers that input air or pure oxygen, agitation by impellers, vessel geometry, and baffles all impact DO.

BIOREACTOR VESSEL

Bioreactor vessels come in a variety of sizes, from mini reactors for pilot studies to containers for large scale production.

TEMPERATURE CONTROL ELEMENTS

In combination with temperature sensors, water jackets, cooling fingers, and heat blankets control temperature changes from exo- or endothermic reactions.

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