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Custom Development & Manufacturing Organization (CDMO)

Scale-up of microbial fermentation processes: Smooth and economical path to large-scale commercial manufacturing

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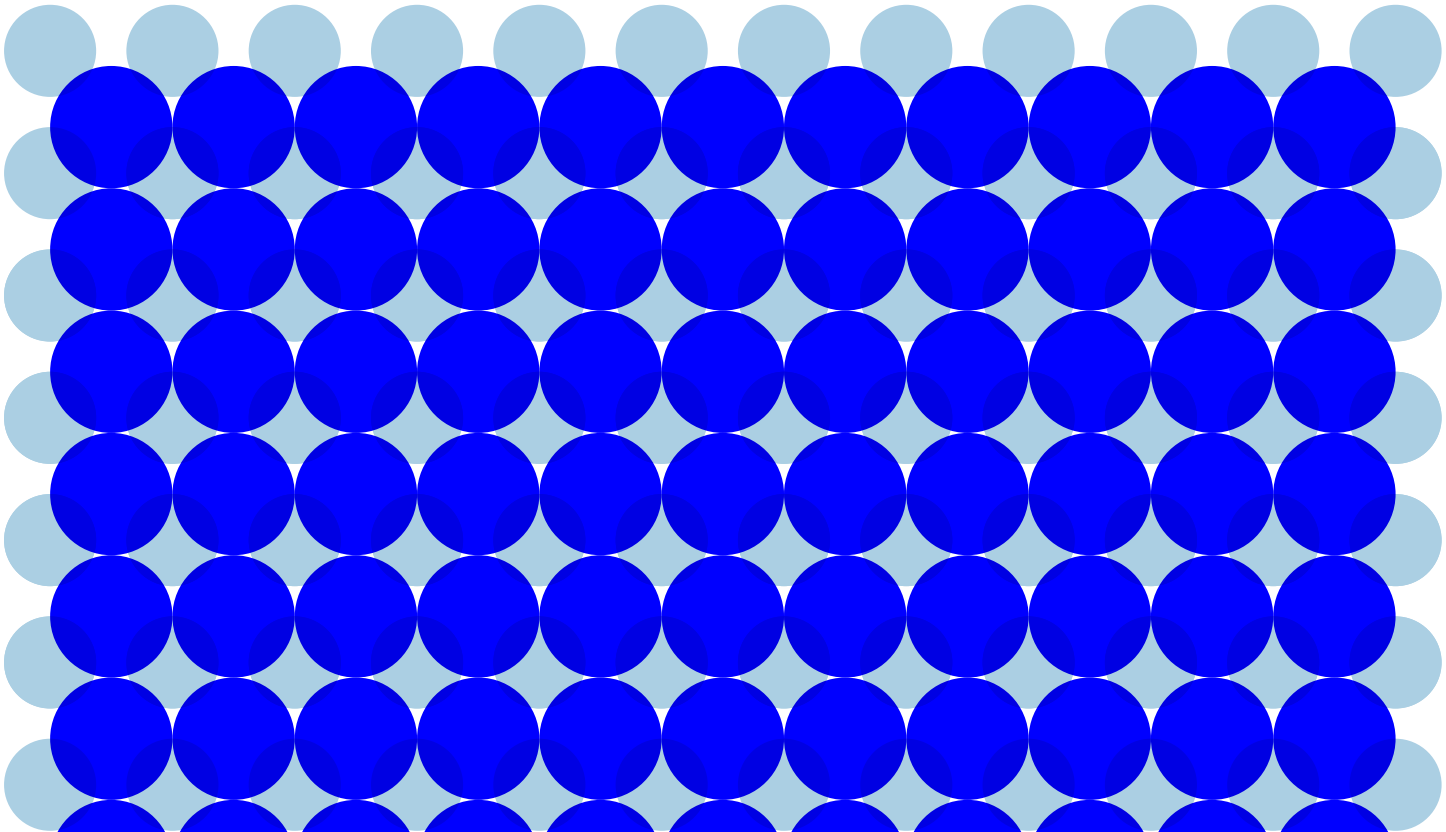
Choosing the right CDMO partner for scale-up of microbial fermentation technologies is an important stage in the biotechnology go-to market journey. Manufacturing experience, a strong level of competence, diligence and reliability are typical criteria for decision making and predestine future commercial success. Arxada's decades-long industrial manufacturing experience ensures that common scale up pitfalls are foreseen and eliminated in early stages of the process transfer, and an adequate risk mitigation plan is in place. Let us dive deeper into how we transfer fermentation processes from the lab to commercial scale and help fulfil or even exceed our customers' expectations.



Scale-up of microbial fermentation processes: Smooth and economical path to large-scale commercial manufacturing

Scaling up industrial microbial fermentation processes towards commercially viable production often requires as much efforts as developing organisms and fermentation processes on a laboratory scale. The microbial fermentation itself is often the first, rather complex, and usually the most valuable step of a bioprocess. If done without adequate preparation it can end-up being costly and perhaps critical to the project's success. Additionally, suboptimal fermentation can impact downstream processing, thus often creating additional cost for the overall manufacturing process.

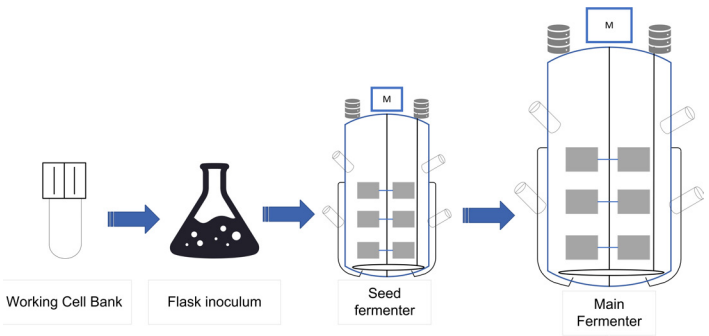
Even for the most reliable and thoroughly optimized lab process, scale-up issues are likely to occur, if not addressed specifically prior to development.

A microbial fermentation process consists of the following stages (Figure 1): a) working cell bank, b) flask inoculum, c) seed fermenter and d) main fermenter. The activities upstream of the main fermenter, the so called "seed train", serve as propagation steps to provide the main fermentation vessel with enough biomass. Accuracy of these propagation steps is as important for the manufacturing process as the main fermentation itself.

Fermentation process documentation should always contain:
- Working cell bank (WCB) information and storage conditions.
- Nutrient media compositions, preparation protocols, and cultivation conditions for both flask inoculum and seed fermenters.
- Main fermenter medium preparation & process control parameters.

Detailed data from already executed (laboratory or pilot scale) fermentations, with a complete set of on-line parameters and off-line analytical results is extremely beneficial as it helps to discover potential process scale-up bottlenecks at the early stage.

Figure 1: Typical stages of the fermentation process

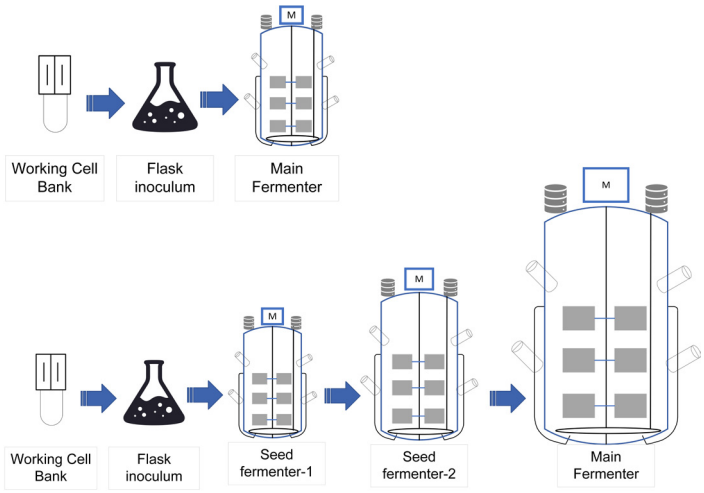


The scope of technology transfer experiments within Arxada's laboratories is defined in accordance with the customer's fermentation process readiness level. In a case where the process has been successfully repeated in fermenters ranging from 100s to 1.000s of liters, the technology transfer can be limited to seed train verification and specific equipment parameter adjustments. Transfer of technology developed at the laboratory scale (single to 10s of liters) requires a different approach. Scale-up of bench scale processes needs a detailed understanding of process parameters and anticipation of their applicability and performance at larger scale. Let us take a closer look at individual tasks that must be addressed during the laboratory fermentation process transfer and related challenges.

Seed train.

The route from a stock culture vial to fermenter is different for a benchtop laboratory bioreactor and an industrial multi-ton vessel. Different microorganisms have different minimal inoculation ratios for optimal growth. Besides, each propagation step may affect the physiological and genetic state of the production strain. Arxada has several manufacturing lines with various sets of seed fermenters and selection of the optimal number of propagation steps and their adaptation to a specific manufacturing line while preserving the strain performance is therefore fundamental. Furthermore, it is important to understand that the microbial strain will undergo significantly more multiplication steps during the process transfer to larger scale than in the laboratory settings (Figure 2). This increases the risk of genetic changes (e.g., undesired mutations, loss of recombinant genes) resulting in poor strain performance. This challenge needs to be explored and addressed already at the strain engineering level.

Figure 2: Seed train – laboratory vs commercial manufacturing scale (up - laboratory scale)



Why is it important?

- Assessment of inoculation ratio of a production strain adapted for manufacturing line seed train.
- Assessment of production strain genetic and physiological stability and robustness.

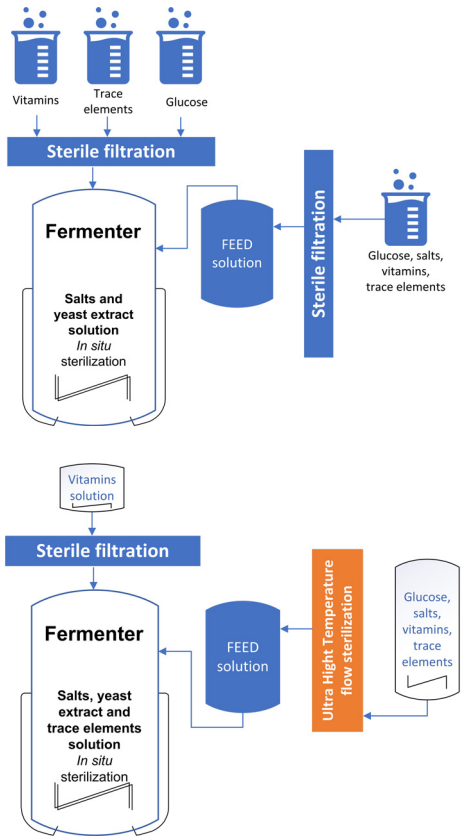
Nutrient medium preparation.
Selection of raw materials makes a significant contribution to the final product cost at commercial scale. Excessive use of expensive medium additives (e.g., IPTG as a common gene expression inducer) may drive COGS unnecessarily high. Use of other additives such as antibiotics (often used to keep recombinant DNA in the production strain) represents an unacceptable burden for wastewater from the environmental and regulatory perspective. Furthermore, raw material grade and purity may influence the process performance. It is often advisable to use the same grade of material intended for larger scale and avoid using reagent grade material.

Nutrient medium preparation is one of the most time and labor consuming parts of the technology. The formulation and preparation of the fermentation medium needs to be straightforward for large scale manufacturing to be cost-effective and to avoid potential CAPEX investments. A high number of fermentation medium components that must be dissolved, pH adjusted and/or sterilized separately is a common challenge (Figure 3). Time needed to prepare the medium, the number of occupied vessels and sterile transfer lines must be reduced at the technology transfer stage leading ultimately to lower OPEX and CAPEX costs. Simplification of the medium composition and preparation protocol comes together with testing of medium heat resistance and robustness. While laboratory scale processes operate with medium heat load of around F0=30 min, industrial scale equipment stresses the medium components with harsh sterilization conditions. This may affect nutrient medium properties, fermentation process performance and subsequent DSP steps. Medium heat load mimicking conditions at larger scale is one of the process robustness tests that we routinely perform during the technology scale up.

For heat liable medium components, sterilization by 0.2 µm filtration is commonly used. However, for industrial operations this requires often complex technical adaptation, use of expensive filters and increased risk of viral or microbial contamination. It is advisable for the initial process design to consider this and avoid heat-sensitive medium components or keep it at the necessary minimum level. Arxada possesses an in-house ultrahigh temperature sterilizing solution for heat-sensitive media that offers the scalability of complex operations.

Frequently, customers' technology requires the use of raw materials which are either incompatible with large-scale equipment or represent an unnecessary challenge from both long-term equipment handling and/or regulatory perspective. Hydrochloric acid used for pH adjustment and tap water for medium preparation are two common examples. Excessive use of organic solvents in fermentation medium may represent a safety risk. Methanol is often used as an inductor and a carbon source for methylotrophic microbes. It is thus present in the fermenter in high concentrations. This requires handling of large amounts of a flammable and toxic chemical, posing unnecessary explosion risk given that alternative solutions are prevalent.

Figure 3: Medium preparation – laboratory vs commercial manufacturing scale (up - laboratory scale)

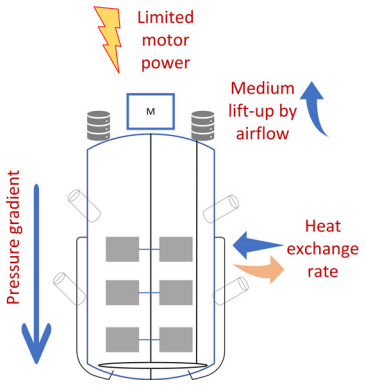


Why is it important?
- Reduction of number of individually prepared solutions saves labor costs, medium preparation and CIP time and CAPEX for additional vessels and piping.
- Reduction of sterile filtrations saves consumables cost (e.g. sterile filters) and minimizes the risk of microbial contamination.
- Ultrahigh temperature sterilizing is a viable option for heat-sensitive medium components.

Fermentation process parameters.
Process parameters are the core of fermentation technology and must be adequately controlled. Only then the maximum biosynthetic potential of the production host can be achieved. Bioreactor's maximal technical capabilities are employed for the fermentation to ensure the best process economy. However, it needs to be understood beforehand that some parameters are not scalable without limitations.
- Heat and mass transfer efficiency is a typical limitation of multi-tone bioreactors. A 50,000-liter fermenter can hardly have similar stirring power and airflow rate per 1 liter of medium, as a benchtop laboratory fermenter. The use of pure oxygen for aeration is not economically viable on a large scale either. This represents a limit to maximum growth rate and metabolic parameters during the large-scale cultivation. Anticipation of these limitations and modifying the process accordingly is an important aspect of the technology scale up.
- An industrial fermenter is a double-digit-meter-tall vessel. Liquid slope pressure and high shear stress become significant parameters affecting dissolution of gases and

overall physiological condition of the microorganism. These parameters impact the process performance and are subject to change during the process scale-up. Thus, choice of a robust microbial strain and rigorous stress-testing is crucial for the successful scale-up outcome.
- Basic parameters robustness is essential for large scale manufacturing. Fermentation technologies with very narrow process parameter limits (such as pH and temperature setpoint ± 0.01 or feed rates with milliliter precision) need to be challenged and modified to guarantee successful scale-up.

Figure 4: Process parameters – laboratory vs commercial manufacturing scale



Why is it important?
- Large scale bioreactors have limited capability of heat and mass transfer. Cultivation process must be tolerant to heat, pH variation in adequate limits and presence of gas and nutrient gradients.
- Dissolved oxygen control at lower airflow, motor power per volume ratio, and unavailability of pure oxygen stream is managed by increased back pressure. Microbial strain robustness to overpressure needs to be addressed.

Summary

Fermentation process is the key for value generation in industrial biotechnology. Its transfer from laboratory conditions to ton-scale bioreactors is accompanied with many challenges impacting the process feasibility and economics. As we outlined above some of the challenges may be solved when selecting/engineering a microbial strain (e.g., genetic stability, cheap inducer of gene expression). Some can be addressed at early process design stage (e.g., use of equipment incompatible medium additives or excessive amounts of flammable solvents for fermentation medium). However, for some biomanufacturing experience is essential to foresee and avoid future failures at large scale. Arxada has encountered and solved many challenges that fermentation process scale-up brings to our biomanufacturing journey. The journey that always starts with end in mind – a fully commercial and economically viable customer's process.

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