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Custom Development & Manufacturing Organization (CDMO)

Scale-up of biotechnology downstream processes: How to deliver required bioproduct quality at commercial manufacturing scale

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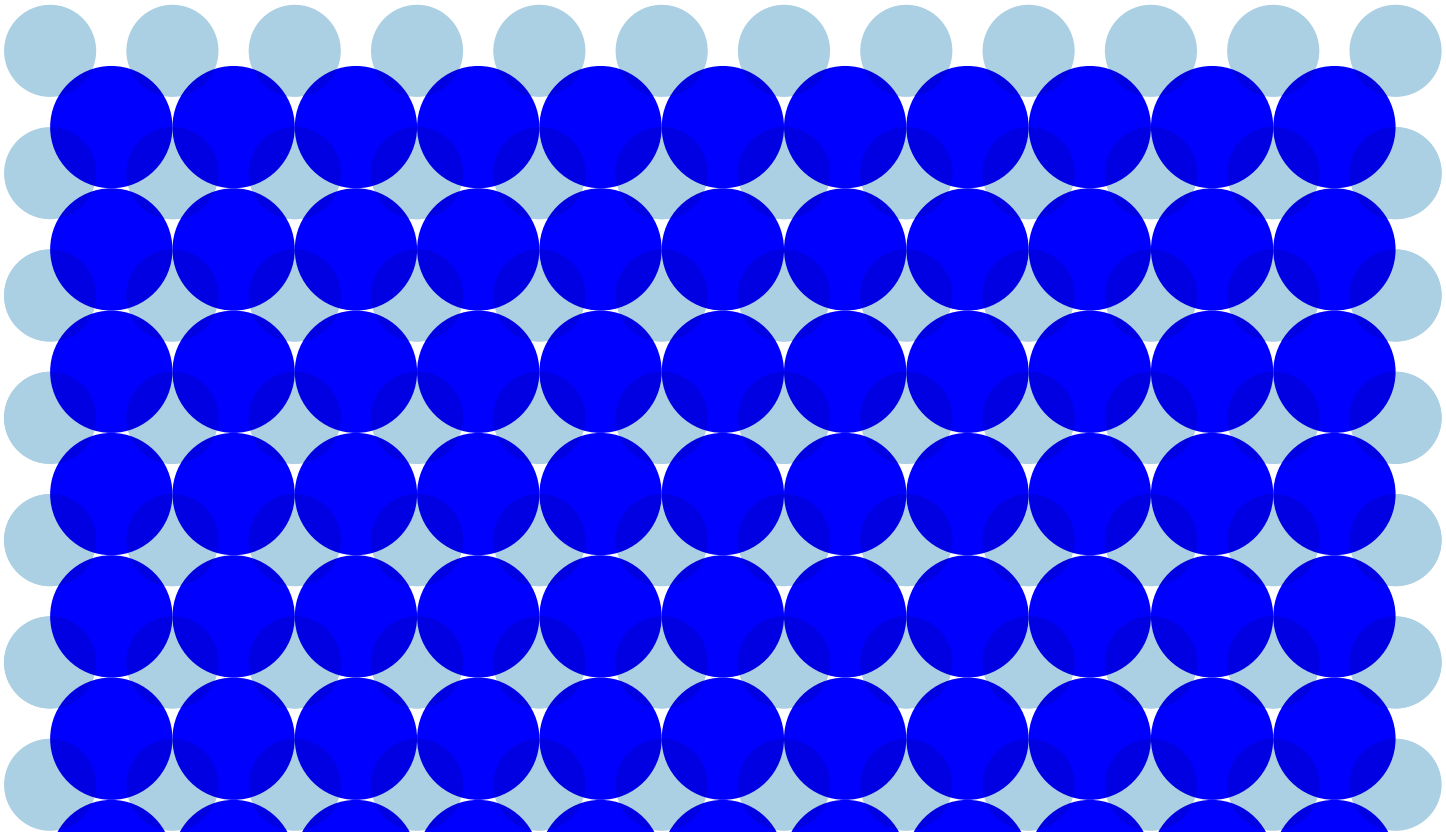
Recovery and purification of a bioproduct known as downstream processing (DSP) can account for up to 70% of the total costs of industrial bioprocesses. As unit operations and equipment parameters may substantially differ comparing laboratory and industrial scale, the DSP scale-up does not come without challenges. Process development, optimization and large-scale fit can save not only operational but also capital costs and thus predestine future commercial success. At Arxada we focus on solving DSP challenges that are hard to foresee without operational experience early enough in the process development stage. This helps us find the right DSP setup to obtain the product of required quality and desired process economics.



Scale-up of biotechnology downstream processes: How to deliver required bioproduct quality at commercial manufacturing scale

Michal Norek, Marek Jiricek, Vratislav Stovicek

Downstream processing is an inseparable part of industrial biotechnology processes. It refers to various stages of the bioprocess that occur after the completion of the fermentation or bioconversion step. The stages and unit operation steps may greatly vary depending on the final product characteristics, its formulation, specification and intended application. The goal is always to deliver a product with desired quality while minimizing product losses. In contrast to fermentation bioprocess stage, the different chemical nature of bioproducts and wide range of their applications require high level of flexibility when it comes to DSP equipment. Also, thorough process design flow considerations and knowledge of equipment parameters is essential to avoid potential bottlenecks and ensure successful scale-up transfer.

DSP is usually divided into three basic sections covering different stages of the process flow (Figure 1):

1. primary recovery,
2. purification/concentration and
3. product polishing and formulation

Each stage consists of several process steps and unit operations. Their employment depends on the product's characteristics and specifications. Arxada's DSP capabilities allow for manufacturing of wide range of products from small organic molecules over peptides, proteins, and enzymes to active biomass.

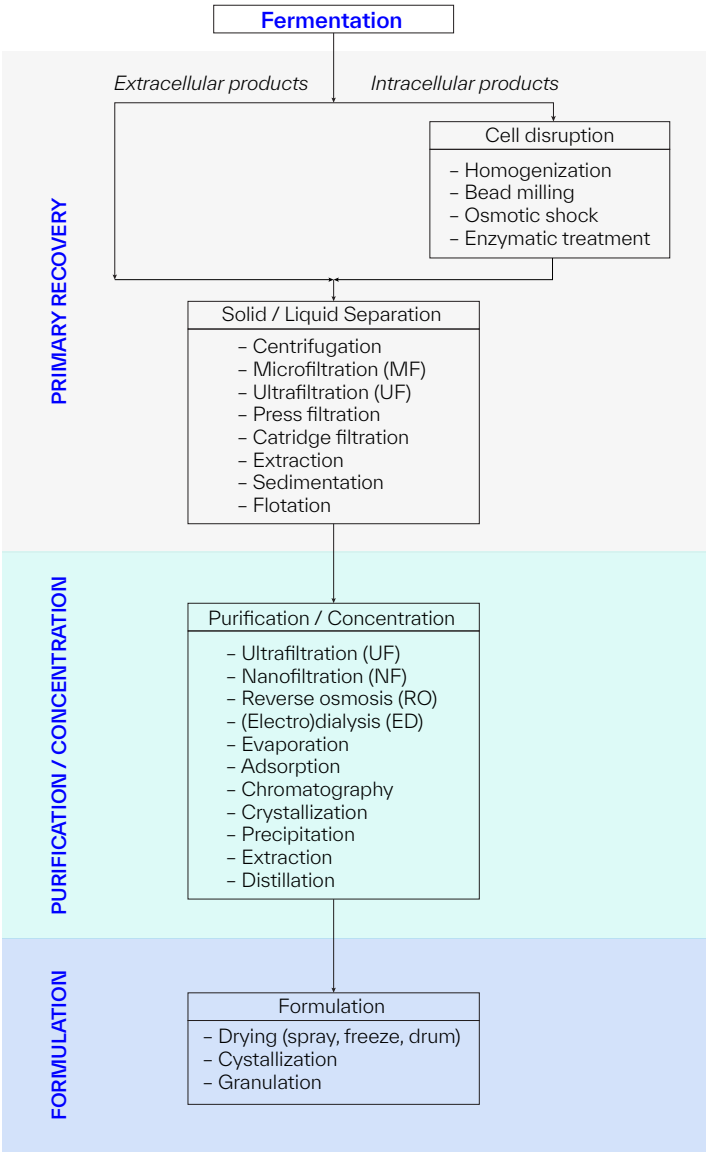


Figure 1: Schematic illustration of DSP and associated unit operations

Arxada CDMO can offer most of listed isolation, purification, and formulation technologies such as:

- High pressure cell homogenizers (Pressure up to 1 200 bars; Flow rate 900-3000 L/h)
- Continuous disc stack centrifuges (centrifuge with intermittent discharge, nozzle centrifuge, liquid-liquid centrifuge)
- Tangential flow filtration skids for MF, UF, NF and RO (ceramic, plate and frame, spiral and hollow fiber configurations)
- Various set of housings for cartridge filtration (depth filtration, bioburden reduction filtration, sterile filtration)
- Thin film vacuum evaporators (water evaporation rate 1000-3000 kg/h)
- Cricket filters for powder activated carbon treatment (4.5-6.5 m² filter cloth area)

- Columns for ion exchange chromatography (400-1000 L)
- Crystallizers (500-15 000 L) with filter cloth centrifuges for crystal separation
- Additional storage capacities for liquid intermediates/ products (1-100 m³ storage tanks)
- Double cone dryer, Agitated spherical dryer, Spray dryer with rotating atomizer, Lyophilizer
- Conical Nautamixer blenders for product blending/ homogenization
- Filling and bulk packaging lines in clean rooms or protected clean room area

DSP technology transfer and development

As in case of fermentation technology scale-up, a thorough understanding of the DSP process at small scale is necessary before trying a scale-up. This could begin with a detailed analysis of the customer's process and its verification in the laboratory during the "familiarization" phase (Figure 2). As an alternative, the DSP process can be created from scratch if it has not already been developed considering the specifications, product attributes, and client needs. Physico-chemical characteristics of the target molecule (proteins, small molecules, active cells) determine the technique that can be used for its isolation and purification and significantly influence the design of the whole downstream process. Besides this product quality requirements (pharma, food, feed, cosmetics), process yield, cycle time, raw material, energy demands and waste generation are among the key considerations relevant for DSP development.

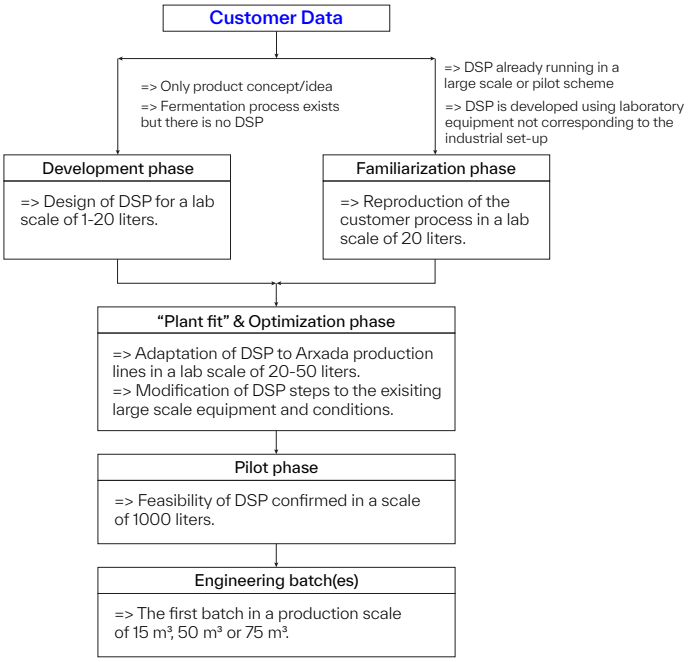


Figure 2: DSP technology transfer at Arxada

Screening various membrane types, resins, or adsorbents, testing the stability of the product (pH, temperature), etc., are typical investigations during the development phase.

DSP familiarization, optimization and plant fit

The familiarization phase is carried out in case that the customer process has been developed and tested in large, pilot or at least laboratory scale. This phase's primary goals are to fully understand the process, replicate it using the equipment configuration that best suits the customer, and verify that the process is transferable, repeatable, and similar to the process data provided. An important task of the familiarization phase is to define all known parameters of each unit operation employed during the process (see e.g., Table 1 - centrifugation). These ones are more specific during the actual tests and might already be included in the process documentation provided by the customer.

The familiarization phase helps foresee and avoid technology scale up challenges such as:
- Too many DSP steps leading to lower yields and longer cycle times
- DSP steps arranged in an inappropriate sequence leading to higher operational costs
- Parameters do not correspond to the industrial ones (very low membrane load; too high resin capacity related to input material)
- Low product stability (pH, temperature, biological)
- Utilization of raw materials not available in bulk quantities or not compliant from the regulatory perspective

Table 1: Example of DSP unit operation parameters - centrifugation

Centrifugation
Supplier of the centrifuge
Type of the centrifuge (bucket, disc stack)
Bowl type (solid wall, intermittent, nozzle)
Nozzle type (mm)
Number of nozzles
Bowl volume (L)
Solids handling space (L)
Σ factor (m²)
Rotation speed (RPM)
Relative centrifugal force (G)
Feed rate (L/h)
Back pressure (bar)
Centrifugation temperature (°C)
Suspension packed cell volume (%)
Suspension OD_{600}
Supernatant turbidity (NTU) or OD_{600}
Dilution and wash buffer composition
Dilution factor

DSP “plant-fit” and optimization

The bottlenecks identified in the familiarization phase are addressed in ”plant fit“ and optimization phase.

Simplifying the downstream process and modifying it to best fit the plant's production lines is the primary objective here. The ultimate result is the development of a scalable, cost-effective process that improves overall yield and produces the desired output within the parameters specified. Arxada's project team conducts a process design review reflecting the capabilities of the suitable manufacturing line to ensure that the appropriate scale-up parameters are tested.
The following topics are typically addressed:

- **Raw material and consumables, e.g.,** replacement of lab-gradeless efficient or non- compliant (from regulatory perspective) raw materials used in the familiarization phase (buffer components, activated carbon, chromatography resins, antisolvents etc.), replacement of TFF membranes for more efficient and/or cost- effective alternatives or replacement of equipment parts due to specific equipment limitations (e.g., a hollow fiber module change for spiral or plate & frame configuration)
- **Equipment replacement:** e.g., the replacement disc stack centrifuge with intermittent discharge with nozzle disc stack centrifuge due to the high content of suspended solids in the feed
- **Process step change:** e.g., the replacement of centrifugation step with microfiltration step to get higher yields and stream sufficiently free of particles for subsequent ultrafiltration, carbon filtration or chromatography step.
- **Reduction the number of DSP steps:** e.g., the reduction of centrifugation, microfiltration and ultrafiltration sequence to only one direct ultrafiltration step to increase the process yield and decrease the batch/cycle time
- **Process robustness testing:** e.g., defining the maximum resin capacity, finding the optimal concentration of activated carbon, testing of different concentration and diafiltration factors, holding time and stability testing (pH, temperature, biological)

DSP in the laboratory and large-scale settings

The equipment design used in the downstream process directly impacts the scale-up success. Therefore, it is important to ensure that the equipment mimics the conditions at production scale as much as possible. However, there are often situations where the laboratory equipment does not match the large-scale equipment parameters. For instance, laboratory glass Rotavapor with water bath can never reflect the conditions of large scale two-stage film evaporator and vacuum drying oven will never provide the same bulk density or particle size distribution as large-scale rotary vacuum dryer. Here, pilot batches may be performed to test the process concept and to demonstrate that the process can be successfully reproduced using the equipment parameters closer to the intended manufacturing line equipment. Complex parameters such as heat/mass transfer rates or mixing dynamics can be hard to simulate in laboratory conditions and can vary significantly between small scale and large scale due to a different surface area, vessel geometry or impeller size/shape. Such parameters are usually fine-tuned during the engineering batch directly using the large-scale equipment (see the different parameters of laboratory, pilot, and large-scale equipment in Table 2).

Table 2: Examples of Arxada CDMO devices used in the different phases of DSP tech transfer

Lab scale (20-75 L)	Pilot scale (1 m³)	Production scale (50-75 m³)
Homogenization		
High pressure homogenizer GEA NIRO Soavi Ariette NS3006H	High pressure homogenizer GEA Panther NS3006L	High pressure homogenizer GEA NIRO Soavi Ariette NS5180H
Maximum pressure 1200 bar	Maximum pressure 1200 bar	Maximum pressure 1200 bar
Flow rate 100 L/h	Flow rate 120 L/h	Flow rate 900-3000 L/h
Centrifugation		
Continuous disc stack centrifuge Alfa Laval LAPX 404-SGP-31C with intermittent discharge	Continuous disc stack centrifuge GEA Easyscale 10 with intermittent discharge	Continuous disc stack centrifuge GEA Westfalia CSE 170-06-477 with intermittent discharge
Clarification area 5230 m²	Clarification area ~10 000 m²	Clarification area ~200 852 m²
Rotation speed 9 500 RPM	Rotation speed 12 000 RPM	Rotation speed 5900 RPM
Rel. centrifugal force 11 130 G	Rel. centrifugal force 11 270 G	Rel. centrifugal force 10 623 G

Lab scale (20-75 L)	Pilot scale (1 m³)	Production scale (50-75 m³)
Flow rate 50-100 L/h	Flow rate 150-300 L/h	Flow rate 2 500-10 000 L/h
Microfiltration		
Tangential flow filtration skid Alfa Laval LabStak M20	Tangential flow filtration skid Alfa Laval PilotUnit Multi	Tangential flow filtration skid Alfa Laval
0.2 um PES membrane	0.2 um PES membrane	0.2 um PES membrane
1x Spiral module 2517	2x Spiral module 3838	24x Spiral module 8338
Membrane area 1x 0.65 m²	Membrane area 2x 3.4 m²	Membrane area 24x 19.3 m²
Powder Activated Carbon Treatment		
TSD pocket filter DrM	AMAfilter cricket filter	AMAfilter cricket filter
Filter volume 1-2 L	Filter volume 315 L	Filter volume 680 L
Filter cloth area 120-240 cm²	Filter cloth area 2.3 m²	Filter cloth area 6.4 m²
Vacuum Evaporation		
Glass Büchi Rotavapor R-215 with water bath	Glass Büchi Rotavapor R-153 with water bath	Vogelbusch two stage thin film evaporator; 1° Falling film 43 m²; 2° Wiped film 25 m²
Capacity up to 3 L	Capacity up to 20 L	Water evaporation capacity 2 500-3 000 kg/h
Crystallization		
HWS glass jacketed reactor vessels	VSK stainless steel jacketed reactor vessel	Penta stainless steel jacketed reactor vessel
Reactor volume 0.25-10 L	Reactor volume 50 L	Reactor volume 12.5 m³
Spray Drying		
Büchi mini spray dryer B-290	APV Anhydro LAB S1 spray dryer	Anhydro CSD 70 spray dryer
Nozzle	Nozzle or rotating atomizer	Rotating atomizer
Max. input temperature 220°C	Max. input temperature 200°C	Max. input temperature 180°C
Water evaporation rate 1 L/h	Water evaporation rate 4 L/h	Water evaporation rate 150 L/h

Summary

Scale-up of a downstream process is a complex task requiring accurate planning and execution. Detailed process characterization helps to identify potential bottlenecks and sets the right scale-up strategy. As the bioprocess moves from lab to production scale, certain effects that can impact the product yield and quality (e.g., mixing efficiency, shear forces and heat and mass transfer) become more pronounced. It is critical to define how these scale-dependent parameters might affect the process. The successful DSP scale up delivering desired quality and economical metrics comes with choosing the right equipment, process design and strict control of process parameters. Let us not forget the need for experienced personnel ready to take on new challenges.

Our offer

- **One stop shop CDMO services in the field of industrial biotechnology**
- **Engagement at any stage of product/process development stage**
- **Dedicated team for technical evaluation and consulting for your project stage**
- **Facility registered as food manufacturing site at FDA. Holding additional certification cosmetic manufacturing (EFfCI cGMP) and has certificates for ISO 9001:2015, FSSC 22000/HACCP, FAMI QS, Halal, Kosher and EFfCI**
- **Long lasting experience with high quality, speed, and strong focus on continuous process improvement**
- ***Focus on what matters to you***

Acknowledgments

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