AL-FARABI KAZAKH NATIONAL UNIVERSITY FACULTY OF BIOLOGY AND BIOTECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

INTRODUCTION TO BIOLOGICAL ENGINEERING

LECTURE 5.
TOOLS AND TECHNOLOGIES IN BIOLOGICAL ENGINEERING: BIOREACTORS, PROCESS CONTROL, AND SCALING CONCEPTS

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Lecture Plan

1. Bioreactor purpose and types
2. Mass transfer and mixing
3. Sterility and contamination control
4. Sensors and analytical technologies
5. Feedback control
6. Batch, fed-batch and continuous modes
7. Scale-up criteria
8. Scale-down models
9. Process analytical technology
10. Case study: aerobic fermentation

Learning Objectives

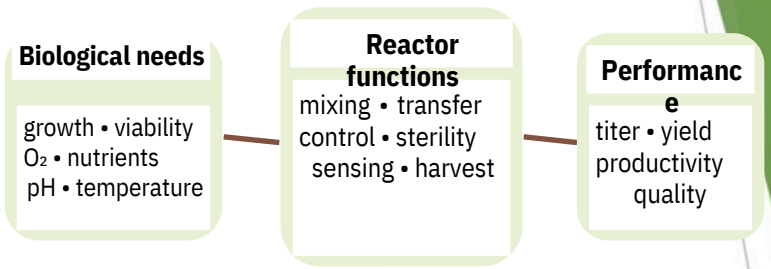
By the end of this lecture, students should be able to:

- Explain the function of a bioreactor and compare common operating modes.
- Describe how mixing and mass transfer affect biological performance.
- Identify major process sensors, manipulated variables and control loops.
- Explain the logic of batch, fed-batch and continuous cultivation.
- Recognize why scale-up changes transport and biological microenvironments.
- Use basic scale-up and control concepts to analyze a bioprocess.

Why a bioreactor?

A bioreactor converts biological potential into reproducible process performance by controlling the environment around cells or enzymes.

- It regulates mixing, gas exchange, temperature, pH, sterility and nutrient delivery rather than simply containing the culture.
- Monitoring and sampling make the biological state observable; actuators allow corrective action during the run.



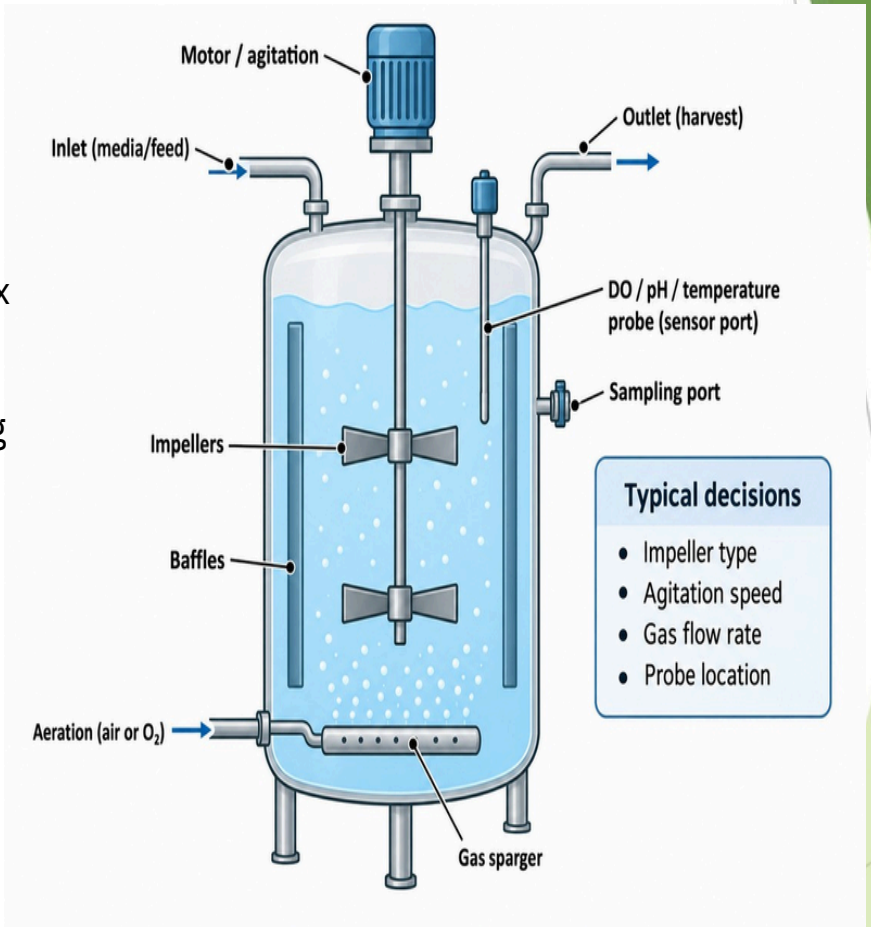
Reactor design must match organism physiology, product requirements, rheology, shear sensitivity and production scale.

- The engineering objective is stable titer, yield, productivity and product quality across the intended operating window.

Stirred-tank reactor

The stirred-tank reactor is the industrial workhorse because one vessel integrates mixing, gas dispersion, sensing, feeding and heat removal.

- Impellers circulate liquid and disperse gas; baffles suppress vortex formation and improve bulk mixing.
- Spargers, probes, feed lines, sampling ports and exhaust handling connect the vessel to the control system.
- Performance depends on agitation, aeration, vessel geometry and broth rheology—not on vessel volume alone.
- Increasing viscosity, biomass concentration or gas hold-up can change mixing and oxygen-transfer performance.



Alternative reactor configurations

Reactor configuration should be selected from the dominant transport, shear, immobilization and manufacturing requirements of the process.

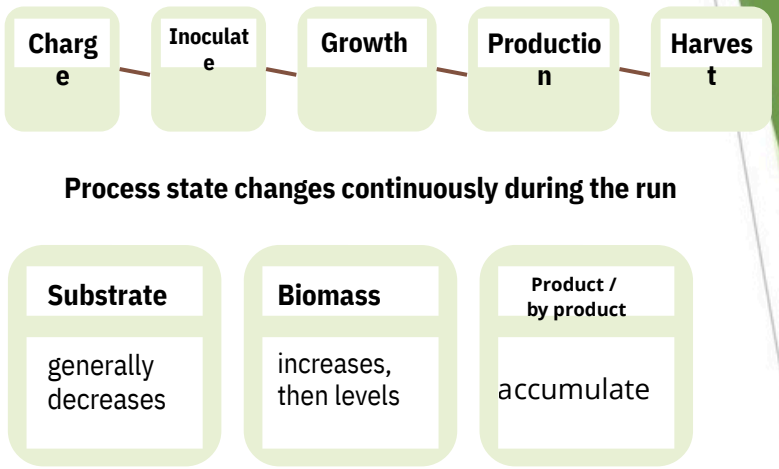
Configuration	Mixing principle	Main strength	Key limitation	Typical use
Stirred tank	Impeller + gas sparging	Flexible control; broad industrial use	Shear / power demand	Microbial & mammalian culture
Airlift	Gas-driven circulation	Low mechanical shear; simpler internals	Lower mixing intensity	Shear-sensitive cultures
Packed / fixed bed	Flow through retained cells or catalyst	High cell density; long residence time	Channeling; gradients	Immobilized cells / enzymes
Wave / single-use	Rocking motion in disposable bag	Fast changeover; low cleaning burden	Scale and mixing limits	Biopharma / multiproduct
Photobioreactor	Light + gas transfer	Controlled photosynthetic production	Light attenuation; heat removal	Algae / cyanobacteria

Batch cultivation

Batch cultivation starts with a defined charge of medium and then allows the culture to evolve without continuous addition of the limiting substrate.

- The operating environment changes continuously as nutrients are consumed and biomass, product and by-products accumulate.
- Batch operation is simple, flexible and relatively easy to segregate between products or campaigns.
- The main productivity penalty is downtime for filling, sterilization, harvest and turnaround.

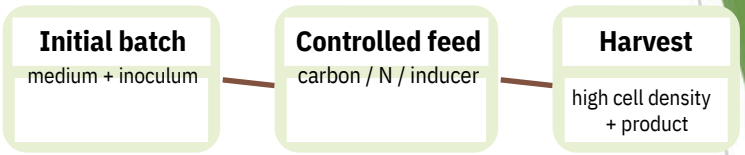
Because cell physiology changes during the run, product quality can depend strongly on harvest time.



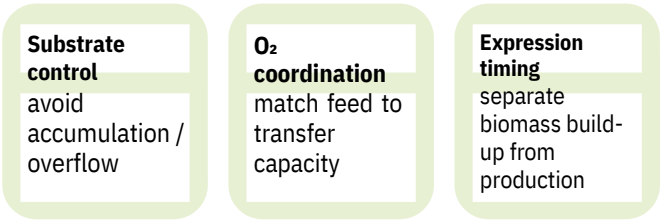
Fed-batch cultivation

Fed-batch adds nutrients during cultivation so that growth and production can be steered rather than allowed to follow the initial medium composition alone.

- Controlled feeding can keep the limiting substrate below concentrations that trigger overflow metabolism or inhibition.
- Feed strategy can shape growth rate, oxygen demand, induction timing and final cell density.
- High titers are possible because biomass and product accumulate while most culture remains in the vessel until harvest.



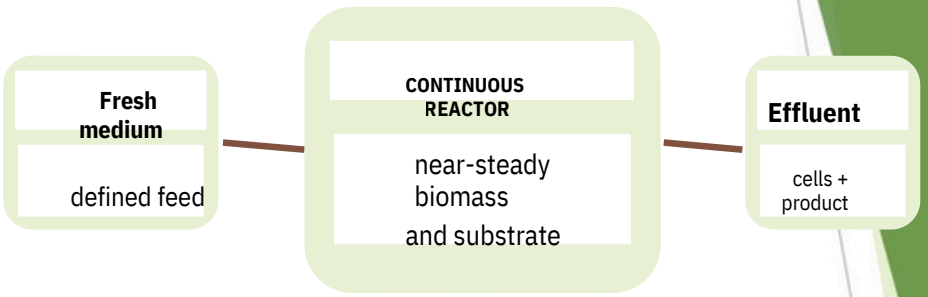
Why feed?



The feed profile must remain compatible with substrate uptake capacity and oxygen-transfer capability.

Continuous cultivation

Continuous cultivation couples fresh-medium inflow to simultaneous culture removal, allowing operation near a defined steady condition when control is successful.



- The dilution setting determines residence time and strongly influences sustainable growth and residual substrate.
- Near-steady operation improves equipment utilization and can reduce batch-to-batch variability.
- Long campaigns increase exposure to contamination, genetic drift and slowly developing equipment or sensor failures.

Aspect	Engineering interpretation
Advantage	Stable operating point; high equipment utilization
Constraint	Long-term contamination / drift / control burden
Decision focus	Residence time, washout margin, product stability

- The process requires tighter monitoring because deviations can propagate continuously rather than being reset at the next batch.

Mixing

Mixing determines how uniformly cells experience nutrients, gases, pH-control agents and temperature— and therefore whether "bulk" measurements represent local conditions.

- Poor mixing creates spatial and temporal gradients that repeatedly expose cells to different microenvironments.
- As scale increases, circulation paths become longer and mixing time often increases unless additional power is supplied.
- More agitation can improve uniformity but may increase shear, foam and energy demand.

- Mixing must therefore be judged by biological consequences, not only by mechanical intensity.



Oxygen transfer

Aerobic performance depends on matching oxygen-transfer capacity to cellular oxygen demand throughout the entire cultivation.

- Oxygen must move from the gas phase into bubbles, dissolve in the liquid, cross local boundary layers and finally reach cells.
- Transfer capacity is influenced by agitation, aeration, pressure, bubble size, viscosity and antifoam.
- Cellular demand rises with biomass concentration and metabolic activity and may change rapidly after feeding or induction.
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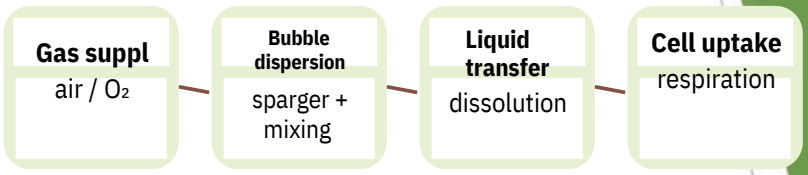
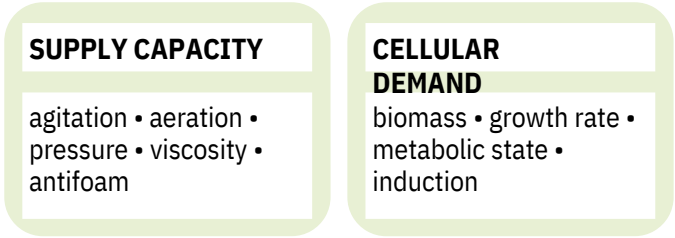
When demand exceeds transfer capacity, dissolved oxygen falls and metabolism, growth or product quality may shift.



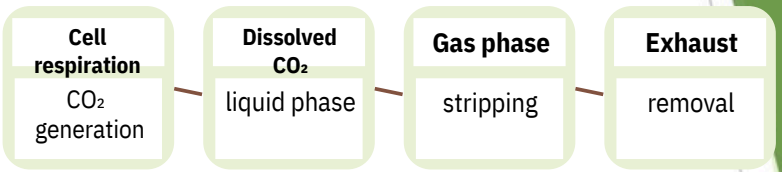


Demand > supply

↓ dissolved oxygen → metabolic shift / productivity or quality risk

Carbon dioxide removal

Carbon dioxide is both a metabolic product and a process variable: it must be removed fast enough to avoid undesirable physiological and product-quality effects.



- Respiration generates CO_2 inside cells; it accumulates in the liquid and must transfer to the gas phase before leaving through the exhaust.
- Elevated dissolved CO_2 can influence intracellular pH, metabolism and product quality, especially in mammalian-cell culture.
- Gas flow, pressure, mixing and gas–liquid transfer conditions determine stripping efficiency.

Intervention	Potential benefit	Trade-off
Increase gas flow	Improves stripping	More foam / evaporation
Reduce back-pressure	Lowers dissolved CO_2	May affect gas delivery
Improve mixing	Reduces local gradients	Higher shear / power
Optimize headspace & exhaust	Improves removal path	Equipment-dependent

- More stripping can increase foam, evaporation and gas consumption, so gas management is a coupled optimization problem.

Heat transfer

Heat generated by metabolism, agitation and gas handling must be removed fast enough to maintain a biologically acceptable temperature throughout the vessel.

- Temperature influences enzyme activity, membrane properties, growth and product stability.
- Cooling jackets, internal coils and external heat exchangers remove heat from the process.
- At larger scale, heat generation grows with vessel volume while heat-transfer area does not increase at the same rate.
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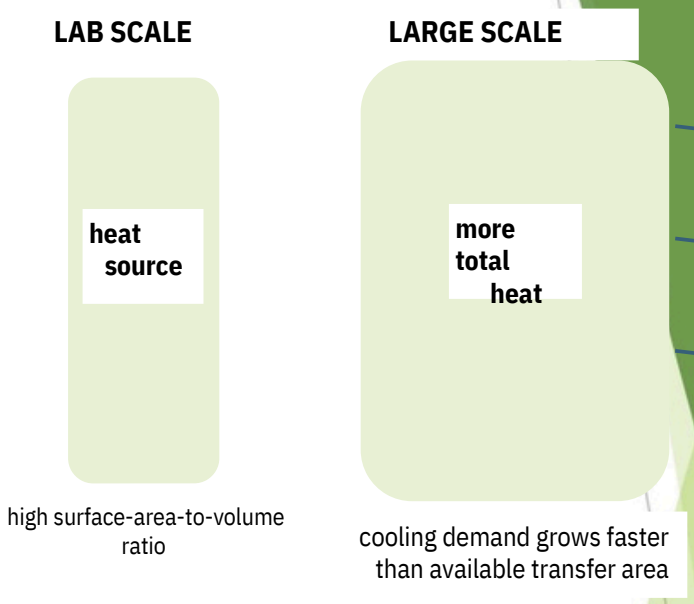
Scale-up can therefore create local temperature gradients or cooling-capacity limits even when laboratory control is straightforward.



pH control

pH control links biological demand, measurement quality, mixing and actuator performance in a closed feedback loop.

- pH affects enzyme activity, membrane transport, nutrient availability and product stability.
- The probe measures local pH; the controller compares it with a target and commands acid/base addition or gas-phase manipulation.
- Slow mixing can create transient high-concentration zones around the addition point even when the bulk pH appears correct.
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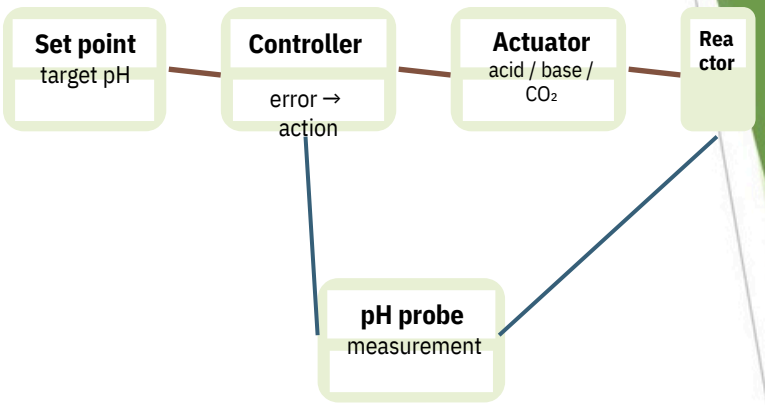
Probe calibration, placement and response time directly influence control quality.



Element	Why it matters
Probe	Accuracy / drift / sterilizability
Addition point	Avoid local pH excursions
Mixing	Distribute corrective reagent quickly
Actuator range	Enough capacity without overshoot

Sterility and contamination control

Sterility is achieved by layered engineering controls that prevent contamination from entering, surviving or spreading through the process.

- Contaminants can compete for substrate, degrade product quality, alter process measurements or create safety risks.
- Validated cleaning and sterilization must reach all product-contact surfaces and dead legs.
- Aseptic feeds, exhaust filtration, sampling and connections are part of the same contamination-control strategy.
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Single-use systems reduce cleaning burden but introduce material, integrity, supply-chain and waste considerations.

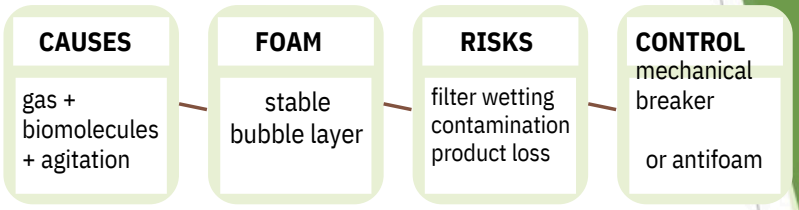
Potential route	Primary engineering barrier
Air / gas	Sterile filtration; integrity testing
Feeds & additions	Sterile connectors; closed transfer
Vessel & piping	CIP/SIP; drainability; dead-leg control
Sampling	Aseptic valve / closed sampling
Exhaust	Hydrophobic filter; condensate management

VALIDATION LOGIC
Control → verify coverage / integrity → monitor → investigate deviations

Foam and antifoam

Foam control is a classic bioprocess trade-off: the intervention that protects containment can also reduce gas-transfer performance.

- Foam forms when gas bubbles are stabilized by proteins, surfactants, cells and agitation at the liquid surface.
- Uncontrolled foam can wet exhaust filters, increase contamination risk and cause loss of culture or product.
- Mechanical breakers avoid chemical additives but add equipment complexity and may be insufficient at high gas rates. •



SECONDARY TRADE-OFF

More antifoam can suppress foam yet reduce oxygen transfer and change downstream behavior.

Antifoam agents are effective but can alter bubble behavior and reduce oxygen-transfer efficiency.

Core sensors

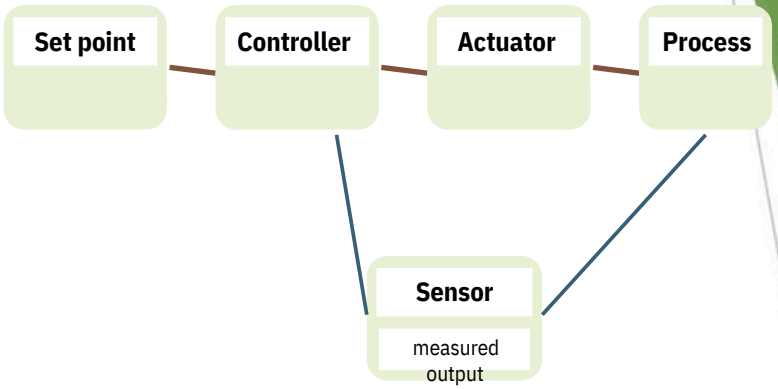
Sensor selection should match the variable, required response time, sterilization conditions and the engineering decision that depends on the measurement.

Measurement	Mode	What it reveals	Key consideration
Temperature	Online	Thermal state	Fast, reliable; placement matters
pH	Online	Acid–base environment	Calibration drift; local gradients
Dissolved O_2	Online	Oxygen availability	Probe dynamics / fouling
Pressure / gas flow	Online	Mechanical & gas-transfer state	Does not directly report cell physiology
Off-gas O_2 / CO_2	Online	Respiration & metabolic trend	Needs gas-flow context
Biomass / metabolites	At-line / offline / spectroscopy	Growth & metabolic state	Sampling delay or model dependence
Product concentration	Offline / at-line	Titer & quality	Often slower than control time scale

Feedback control

Feedback control maintains a process variable near a target by measuring the process, comparing it with the set point and adjusting a manipulated input.

- A controlled variable may be pH, dissolved oxygen, temperature, pressure or another state linked to process performance.
- A manipulated variable may be feed rate, agitation, gas flow, oxygen enrichment, acid/base addition or heating/cooling duty.
- Sensors provide feedback; the controller computes the correction; actuators implement the action

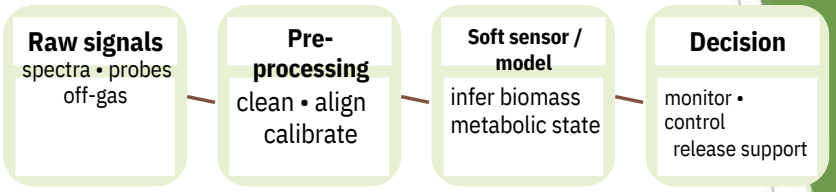


Controlled variable	Typical manipulated variables
DO control	Agitation / airflow / O_2 enrichment
pH control	Acid/base or gas-phase action
Temperature control	Heating / cooling duty

Sensor delay, process nonlinearity and actuator limits must be considered during controller tuning.

Process Analytical Technology

Process Analytical Technology (PAT) combines real-time measurements and models to estimate critical process states and support active process understanding.



- Spectroscopy, off-gas analysis and conventional probes generate high- frequency process data.
- Multivariate models and soft sensors can estimate variables that are difficult or slow to measure directly.
- PAT shifts quality assurance from end-point testing toward continuous understanding of process state and variability.

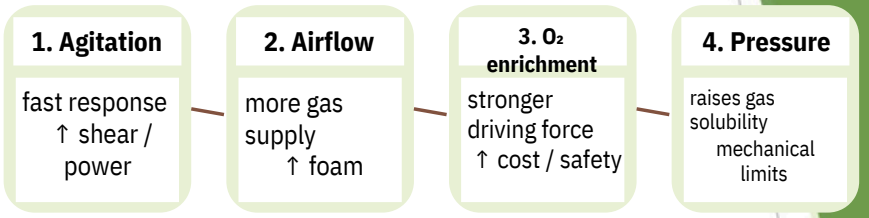
PAT element	Engineering implication
Value	Earlier detection of drift; richer process understanding
Requirement	Representative calibration data and stable data pipeline
Risk	Domain shift, sensor drift, model obsolescence

Long-term reliability depends on calibration maintenance, data quality and model transfer across equipment or products.

Cascade control

Cascade control coordinates several manipulated variables in a prioritized sequence so that simple, low-cost actions are used before stronger or more disruptive interventions.

- Dissolved oxygen is a common example because several actuators can influence oxygen availability.
- A typical sequence increases agitation first, then airflow, then oxygen enrichment and finally pressure if required.
- Each stage is activated only when the previous actuator approaches its operating limit or becomes insufficient.
- The hierarchyshould reflectequipment limits, cost, foam, shear and biological sensitivity.



CONTROL LOGIC

Advance to the next actuator only if DO remains below target and the current actuator is near its allowed limit.

Common scale-up criteria

No single scale-up criterion preserves every physical phenomenon. The criterion should be selected from the dominant biological and transport limitation.

Criterion	What it attempts to preserve	When useful	Main trade-off
Constant power / volume	Mixing intensity and energy dissipation	Useful for bulk mixing	May increase shear or energy demand
Constant tip speed	Impeller peripheral velocity	Useful for shear-sensitive systems	May underperform for mixing / O_2 transfer
Constant oxygen-transfer capacity	Gas–liquid transfer performance	Useful for aerobic high-demand culture	Does not preserve mixing or shear
Constant mixing time	Homogenization time scale	Useful when gradients drive physiology	Can require impractical power at scale
Constant gas velocity / flow regime	Gas residence and dispersion behavior	Useful in gas-sensitive processes	May not preserve transfer efficiency

Dimensionless thinking

Dimensionless groups compare competing physical effects and allow engineers to judge whether two systems operate in physically similar regimes without relying on size alone.

- Reynolds number indicates the relative importance of inertial and viscous forces and therefore helps characterize flow regime.
- Power number connects impeller power demand with geometry and operating speed.
- Froude number becomes important when gravity and free-surface behavior influence the system.
- Weber number is useful when surface tension and bubble or droplet breakup are relevant.

Group	Physical meaning	Typical engineering use
Reynolds	Inertia vs viscosity	Flow regime / mixing similarity
Power	Power demand vs geometry	Agitator scale-up / energy
Froude	Inertia vs gravity	Vortex / free-surface behavior
Weber	Inertia vs surface tension	Bubble / droplet breakup

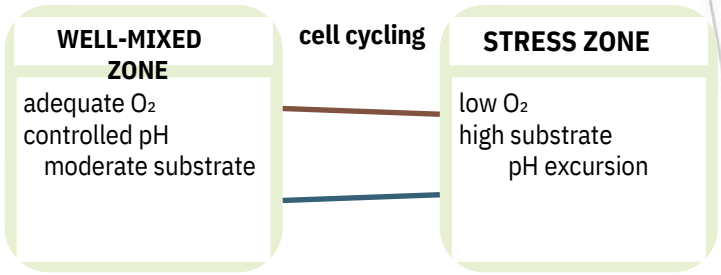
USE WITH CAUTION

Dynamic similarity is meaningful only when the dimensionless groups chosen actually control the phenomenon of interest.

Scale-down models

Scale-down models recreate selected large-scale stresses in a smaller system so that biological responses can be studied under controlled and repeatable conditions.

- Large reactors often expose cells to repeated cycles of high substrate, low oxygen, pH excursions or temperature gradients.
- A useful scale-down model reproduces the stress history and transition frequency—not only the average bulk condition.
- Two-compartment or oscillatory systems can mimic movement between well-mixed and limiting zones.
- Scale-down experiments help define acceptable process windows and identify phenotypes that are robust to manufacturing heterogeneity.



Stress reproduced	Biological question
O_2 oscillation	Can cells tolerate repeated oxygen limitation?
Substrate pulse	Does overflow metabolism or by-product formation increase?
pH excursion	Is product quality or viability affected?

Single-use technologies

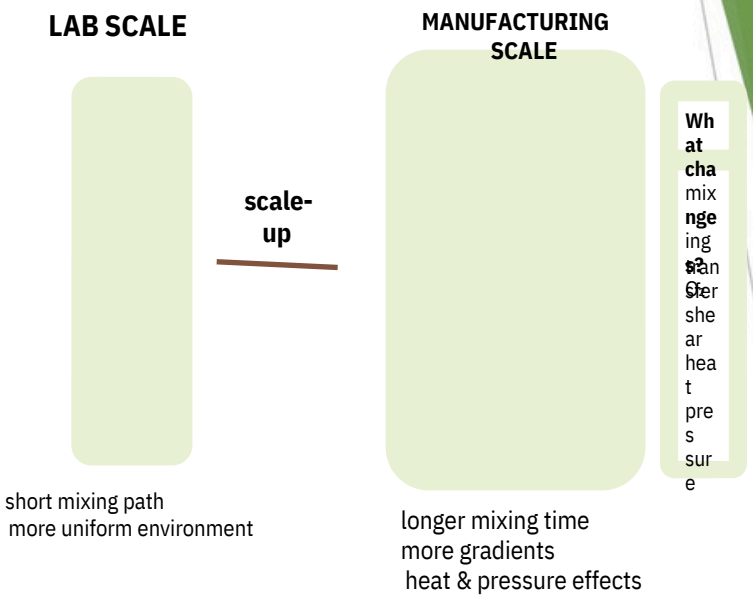
Single-use technologies replace reusable product-contact equipment with disposable bags, tubing, filters and sensors to reduce cleaning burden and accelerate facility changeover.

Design dimension	Potential advantage	Key limitation
Cleaning / sterilization	Minimal cleaning validation; presterilized assemblies	Dependence on supplier integrity
Turnaround	Rapid campaign changeover	More consumables handling
Facility flexibility	Strong for multiproduct manufacturing	Mechanical / scale constraints
Mixing & transfer	Adequate in many applications	Performance may differ from steel tanks
Material risk	No corrosion; closed assemblies	Extractables / leachables
Sustainability	Lower water / steam demand	Plastic waste and end-of-life burden

Scale-up problem

Scale-up changes the physical environment experienced by cells. Geometric enlargement cannot preserve mixing, transfer, shear, heat removal and pressure simultaneously.

- Circulation paths become longer and gradients in substrate, oxygen, pH and temperature become more likely.
- Oxygen transfer, gas hold-up, shear and hydrostatic pressure can shift even when the vessel is geometrically similar. • Heat removal becomes more demanding because generation increases with working volume while transfer area grows more slowly.

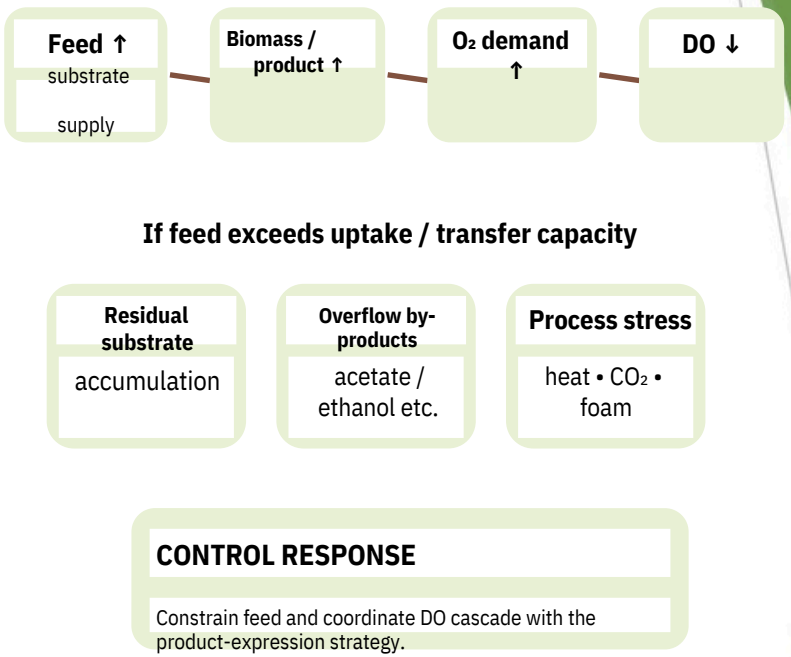


The correct scale-up target is the phenomenon that most strongly controls physiology or product quality.

Case study: aerobic fed-batch

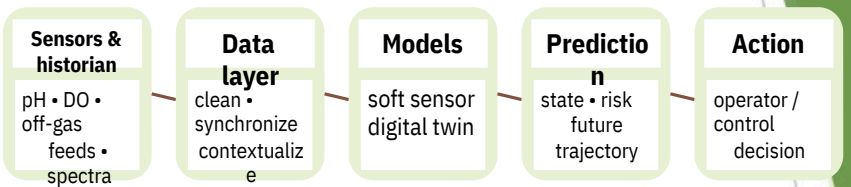
In aerobic fed-batch, feeding, biomass growth and oxygen transfer are coupled: optimizing feed independently can drive the culture beyond the reactor's transfer capacity.

- As biomass increases, oxygen uptake rises and dissolved oxygen becomes progressively harder to maintain.
- Feed rate should be coordinated with oxygen-transfer capacity and the desired growth or expression strategy. • If substrate supply exceeds cellular uptake, residual substrate or overflow by-products may accumulate.
- A robust strategy combines feed control, DO monitoring, off-gas information and a defined response hierarchy.



Digital bioprocessing

Digital bioprocessing combines process data with models so that important states can be estimated, predicted and used for decision support before deviations become obvious.



- Soft sensors estimate difficult-to-measure variables such as biomass, metabolic rate or quality attributes from available process signals.
- Digital twins combine mechanistic knowledge, historical data and the current process state to predict future behavior.
- Predictive control can adjust feeding or operating conditions based on expected trajectories rather than only current error.
- Models must be continuously validated because sensor drift, new raw materials or process changes can cause domain shift.



Student Tasks

- Define the system boundary for one biological engineering example and justify why that boundary is appropriate.
- Draw an input–process–output or conceptual model and label the most important flows and interactions.
- Identify three performance metrics and two important constraints for the selected system.
- Describe one plausible failure mode and propose one engineering control or design improvement.
- Find one recent peer-reviewed article relevant to the lecture topic and summarize the engineering contribution in 3–4 sentences.

Take-home design checklist

Use this checklist to move from biological requirements to an engineering design that can be monitored, controlled and scaled.

1. Biological objective

What cells / enzymes must do; product and quality target

2. Reactor & mode

Batch, fed-batch, continuous; configuration and boundary

3. Transfer capacity

Mixing, O_2 / CO_2, heat and shear across the full run

4. Measurement & control

Which variables are online? Which actuators respond fast enough?

5. Sterility & risk

Contamination routes, foam, materials, failure modes

6. Scale strategy

What criterion protects the limiting biology? What scale-down test is needed?

Selected References and Further Reading

1. Shuler, M.L. & Kargi, F. Bioprocess Engineering: Basic Concepts, 3rd ed. Pearson, 2017.

2. Doran, P.M. Bioprocess Engineering Principles, 2nd ed. Academic Press, 2013.

3. King, M.R. & Walsh, K. Introduction to Bioengineering. Cambridge University Press, 2021.

4. Enderle, J.D. et al. Introduction to Biomedical Engineering. Academic Press, 2019.

5. Garcia-Ochoa, F. & Gomez, E. Bioreactor scale-up and oxygen transfer rate in microbial processes. Biotechnology Advances, 2009.

6. U.S. FDA. PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, 2004.

7. Gurdo, N. et al. Automating the design–build–test–learn cycle towards next-generation bacterial cell factories. Trends in Biotechnology, 2023.

8. Iram, A. et al. Synthetic biology advances towards a bio-based society in the era of artificial intelligence. Current Opinion in Biotechnology, 2024, 87:103143.

9. Karim, A.S. et al. Deconstructing synthetic biology across scales. Nature Communications, 2024.

10. Nature Reviews Bioengineering. Reviews on engineering biology, bioprocessing, biomaterials and translational bioengineering, 2023–2025.

Core course literature follows the approved syllabus; recent reviews complement foundational texts.