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

INTRODUCTION TO BIOLOGICAL ENGINEERING

LECTURE 8.

ENGINEERING ANALYSIS OF BIOLOGICAL PROCESSES: INPUT-PROCESS-OUTPUT MODELS

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Lecture Plan

1. Analysis workflow
2. IPO models
3. Balances and efficiencies
4. Dynamic behavior
5. Limiting steps
6. Performance metrics
7. Scenario analysis
8. Sensitivity
9. Optimization logic
10. Bioprocess examples

Learning Outcomes

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

LO1 Analyze a simple biological process using an input-process-output model.

LO2 Translate the model into material flows and performance metrics.

LO3 Identify limiting steps and major sources of loss.

LO4 Interpret dynamic changes in batch and fed-batch systems.

LO5 Use scenario and sensitivity analysis to compare engineering alternatives.

LO6 Propose improvements based on evidence rather than descriptive biology alone.

From description to analysis

Description lists what happens; engineering analysis asks how much, how fast, under what constraints and with what performance.

The first step is to define the **purpose of the analysis**: what should be predicted, compared, optimized or controlled?

An **input–process–output framework** organizes the biological system into measurable inputs, transformations and outputs.

Each block is then associated with **units, rates, capacities and operating conditions**, converting qualitative descriptions into quantitative variables.

Engineering analysis distinguishes between **controllable variables, disturbances and constraints**, because they play different roles in process performance.

Performance should be evaluated using explicit metrics such as **yield, productivity, conversion, recovery, selectivity, energy use or cost**.

Once the system is quantified, alternative designs can be compared and **bottlenecks, trade-offs and limiting steps** can be identified

PURPOSE

What do we need to decide?

→

INPUTS

amount • concentration • flow

→

TRANSFORMATION

rate • capacity • efficiency

→

OUTPUTS

product • biomass • waste

→

PERFORMANCE METRICS

yield • productivity • recovery

Step 1: define purpose and boundary

State the product or function and the context of use. Start by stating the **product, biological function or engineering objective** and the context in which performance will be evaluated.

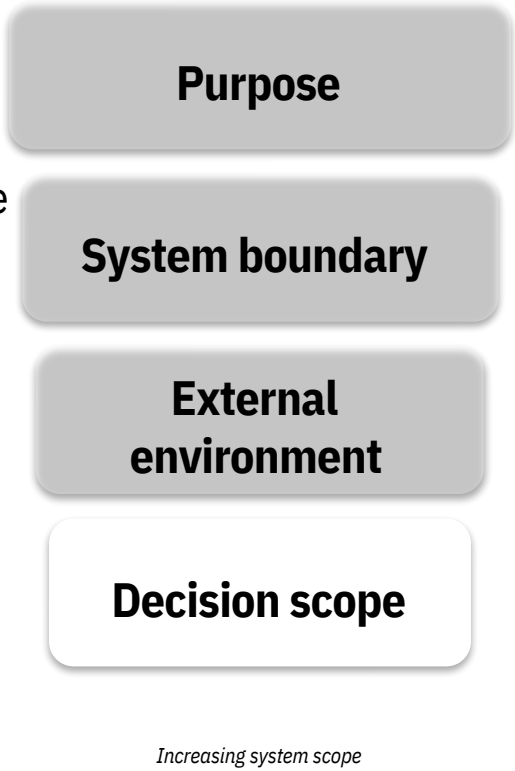
Define the **system boundary** so that it includes the mechanisms and variables necessary to explain the selected performance metric.

Clearly distinguish between what is **inside the model**, what crosses the boundary as an input or output, and what remains part of the external environment.

Specify the relevant **time horizon and operating scale**, for example seconds, batch duration, continuous operation or the full product life cycle.

Identify the main **decision variable and performance metric** before introducing detailed equations or mechanistic complexity.

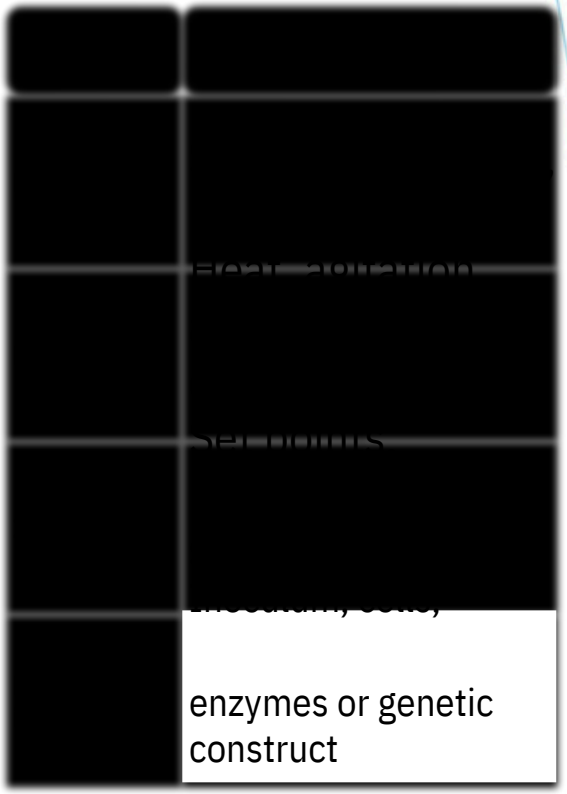
A useful boundary should be broad enough to capture the dominant constraints, but not so broad that unnecessary detail obscures the engineering question.



Step 2: inventory inputs

Inputs can include substrates, gases, cells, water, energy, media components, information and operator actions.

- Classify each input by amount, concentration, rate and controllability.
- Input-process-output analysis is most useful when the categories are quantified.
- Record variability and quality attributes of raw materials.
- A process cannot be optimized if major resource inputs are not quantified.
- Inputs should be defined by amount or rate, transformations by capacity and efficiency, and outputs by both desired products and losses.



Step 3: define transformations

Transformations may be biochemical reactions, growth, separation, transport, heating, mixing or signal conversion.

Transformations may include **transport, biochemical reactions, metabolism, cell growth, product formation, mixing, heating, separation or signal conversion**.

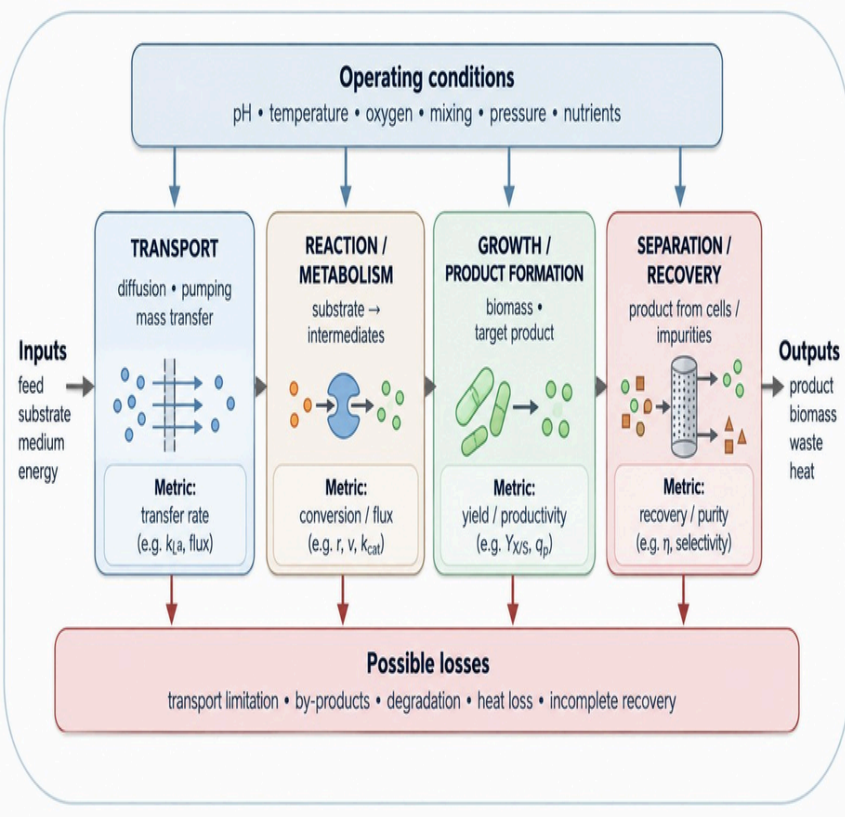
Each transformation converts defined inputs into outputs and may also generate **by-products, heat, waste or other losses**.

Biological transformations are often **adaptive and condition-dependent**, whereas physical unit operations are usually governed by transport, equipment capacity and operating conditions.

Important engineering descriptors include **reaction rate, conversion, yield, transfer capacity, recovery and selectivity**.

The process chain should identify where **bottlenecks or irreversible losses** occur, because a single inefficient step can limit overall system performance.

Transformations should be represented at the level of detail necessary to explain the selected performance metric and support the engineering decision.



Step 4: characterize outputs

Outputs should be defined not only by how much material leaves the system, but also by its composition, quality, value and environmental or economic impact.

Основной текст слева

Process outputs may include the **target product, biomass, by-products, waste streams, exhaust gases, heat and process data**.

The desired product should be characterized using both **quantity and quality attributes**, such as concentration, purity, activity, stability or functionality.

Biomass may represent either a valuable product or a process intermediate, and should be quantified by **cell concentration, dry mass, viability or productivity**.

By-products and waste streams are important because they reduce material efficiency and may increase purification cost, treatment requirements and environmental burden.

Off-gas and heat outputs can reveal important process behaviour, including **metabolic activity, oxygen demand and energy losses**.

Unmeasured outputs can create apparent gaps in **mass, carbon or energy balances**, so significant losses should be identified explicitly.

Process efficiency

Process efficiency describes how effectively a biological process converts limiting resources into useful product while minimizing material, energy and product losses.

Conversion describes how much of the supplied substrate or reactant is consumed during the process.

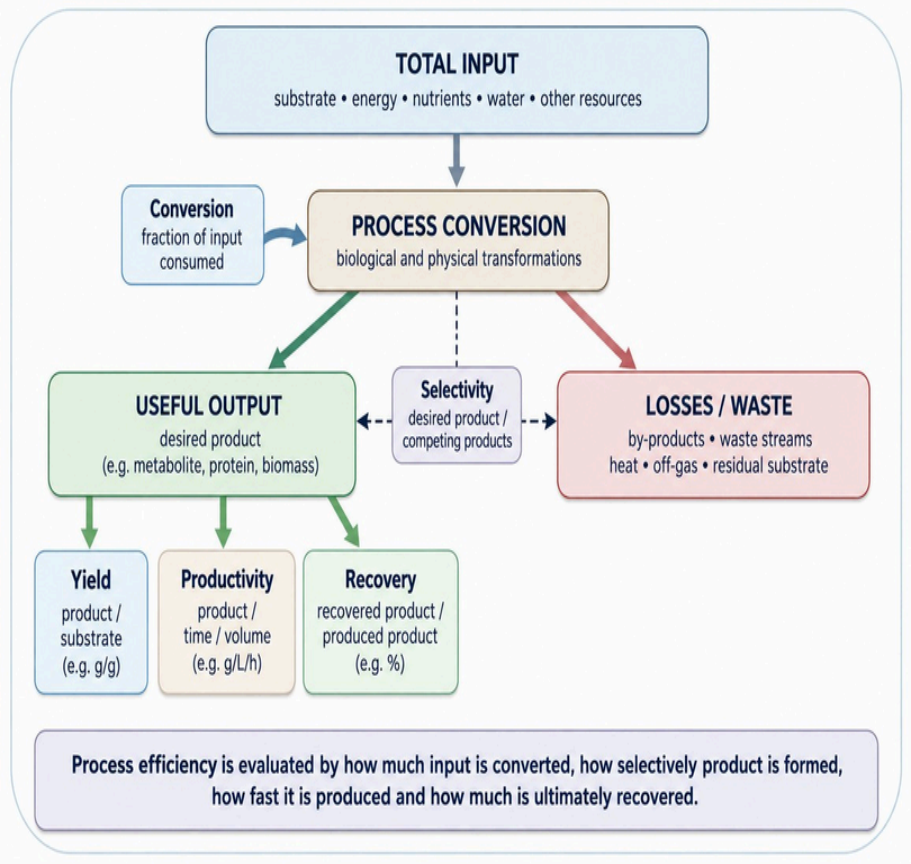
Yield relates useful product or biomass formation to the amount of substrate consumed and therefore reflects material efficiency.

Productivity measures how rapidly useful product is generated, often expressed per unit time and reactor volume.

Recovery describes how much of the product produced upstream remains after harvesting, purification and other downstream operations.

Selectivity indicates how strongly the process favors the desired product over competing products or by-products.

High performance requires considering these metrics together: a process may have high conversion but poor yield, or high productivity but large downstream losses.



Loss mapping

Losses may arise from incomplete conversion, by-product formation, degradation, cell death, adsorption, filtration or purification.

Losses can occur through **incomplete conversion, competing by-product formation, degradation, cell death, adsorption, filtration losses and incomplete purification recovery**.

A process should be analysed stage by stage to determine how much material enters, how much useful product leaves and how much is lost at each operation. **Sankey-style material-flow diagrams** are especially useful because stream width can represent the amount of material or product retained and lost through the process.

Small losses at several consecutive stages can accumulate into a large reduction in final product recovery.

Improvement efforts should therefore focus on the **dominant loss or bottleneck**, rather than optimizing a step that contributes little to total process performance.

Loss mapping can be combined with **yield, recovery, selectivity and cost data** to identify the highest-value engineering intervention.

RAW MATERIAL
100%
→ **CONVERSION**
Product retained: **82%**
Loss: **18%**
unreacted substrate + by-products
→ **RECOVERY**
Product retained: **74%**
Loss: **8%**
cell/debris separation
→ **PURIFICATION**
Product retained: **61%**
Loss: **13%**
adsorption • filtration • degradation
→
FINAL PRODUCT
61% overall recovery

Steady-state versus dynamic analysis

Main statement

Steady-state analysis describes a system when key variables remain approximately constant over time, whereas dynamic analysis captures how those variables change during startup, disturbances, batch operation or control actions.

In **steady state**, there is no net accumulation of the modelled quantities, so inputs, outputs and internal generation or consumption remain balanced over time.

This approximation is useful for **continuous processes operating near a stable condition**, especially when average performance is more important than transient behaviour.

In **dynamic systems**, variables such as biomass, substrate, product concentration, dissolved oxygen or volume change with time.

Batch and fed-batch processes are inherently dynamic because **cell growth, nutrient consumption and product formation evolve continuously during operation**.

Dynamic models are essential when analysing **startup, shutdown, disturbances, feedback control, oscillations or time-dependent responses**.

A steady-state approximation may still be useful locally when changes are slow compared with the time scale of interest.

Dynamic balance concept

For any species: rate of accumulation = rate in - rate out + rate formed - rate consumed.

For any component, the rate of accumulation depends on four contributions: **inflow, outflow, internal generation and internal consumption**.

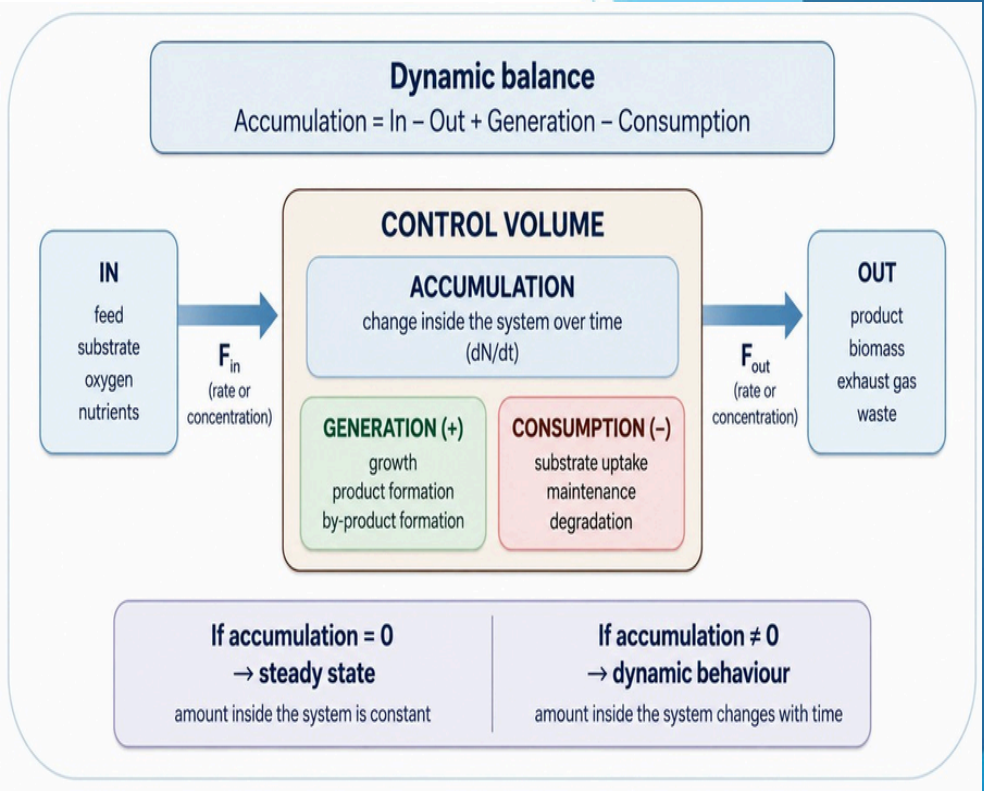
In bioprocesses, **feed and harvest streams** determine external flow terms, while biological kinetics determine internal formation and consumption.

If inflow and generation exceed outflow and consumption, the component **accumulates**; if the opposite occurs, its amount decreases.

Dynamic balances are therefore essential for describing time-dependent changes in **biomass, substrate, product, oxygen and metabolites**.

At steady state, net accumulation becomes approximately zero, whereas in batch and fed-batch systems accumulation is generally non-zero.

Combining balance relationships with kinetic expressions converts a conceptual process model into a **dynamic quantitative model**.



Limiting-substrate analysis

A substrate is limiting when its availability constrains the rate of growth or product formation.

- Kinetics describe how fast biological transformation occurs and how rate changes with state variables such as substrate, biomass or product concentration.
- Increasing a non-limiting nutrient will not improve performance and may create waste.
- In fed-batch processes, deliberate substrate limitation can prevent overflow metabolism.
- Limitation must be distinguished from inhibition by excess substrate or product.
- Rate expressions are useful only within the range in which their assumptions are valid and should be interpreted together with transport limitations.

Transport / supply versus biological demand



limitin g step

Performance depends on the slower side

Oxygen as a coupled limitation

Main statement

Oxygen limitation emerges from the interaction between biological demand and reactor-scale mass transfer: cells consume oxygen according to their metabolic activity, while the reactor can supply oxygen only at a finite transfer rate.

Cellular oxygen demand increases with **biomass concentration, metabolic activity and substrate availability**.

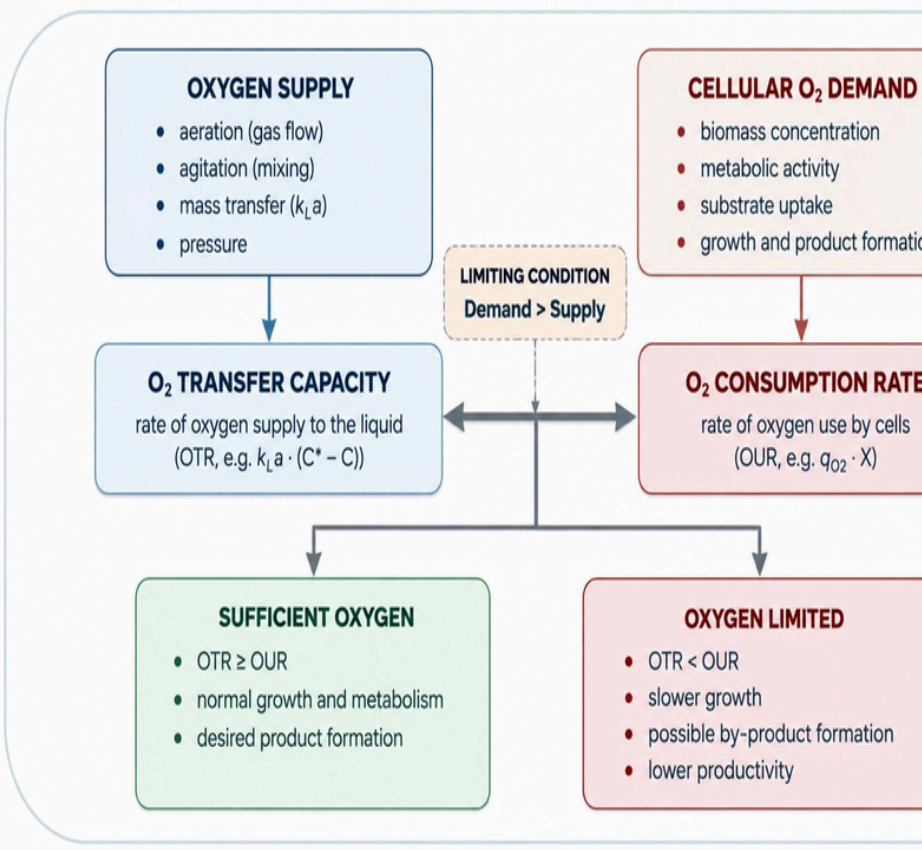
Oxygen supply depends on **gas–liquid mass transfer**, which is influenced by agitation, aeration rate, bubble size, pressure and reactor geometry.

As biomass increases, a reactor that initially provides excess oxygen can gradually shift into an **oxygen-limited regime**.

Increasing agitation or aeration can improve oxygen transfer, but only until **power input, shear stress, foaming or gas-flow constraints** become limiting.

Feed rate should therefore be coordinated with oxygen-transfer capacity, especially in **high-cell-density and fed-batch cultures**.

When oxygen transfer cannot meet cellular demand, growth and product formation may decrease and metabolism may shift toward **undesired by-products**.



Bottleneck versus constraint

A bottleneck is the step currently limiting throughput; a constraint is a hard or soft boundary that restricts possible operation.

A **bottleneck** is the stage with the lowest effective capacity relative to process demand, so it limits the rate at which the entire system can operate.

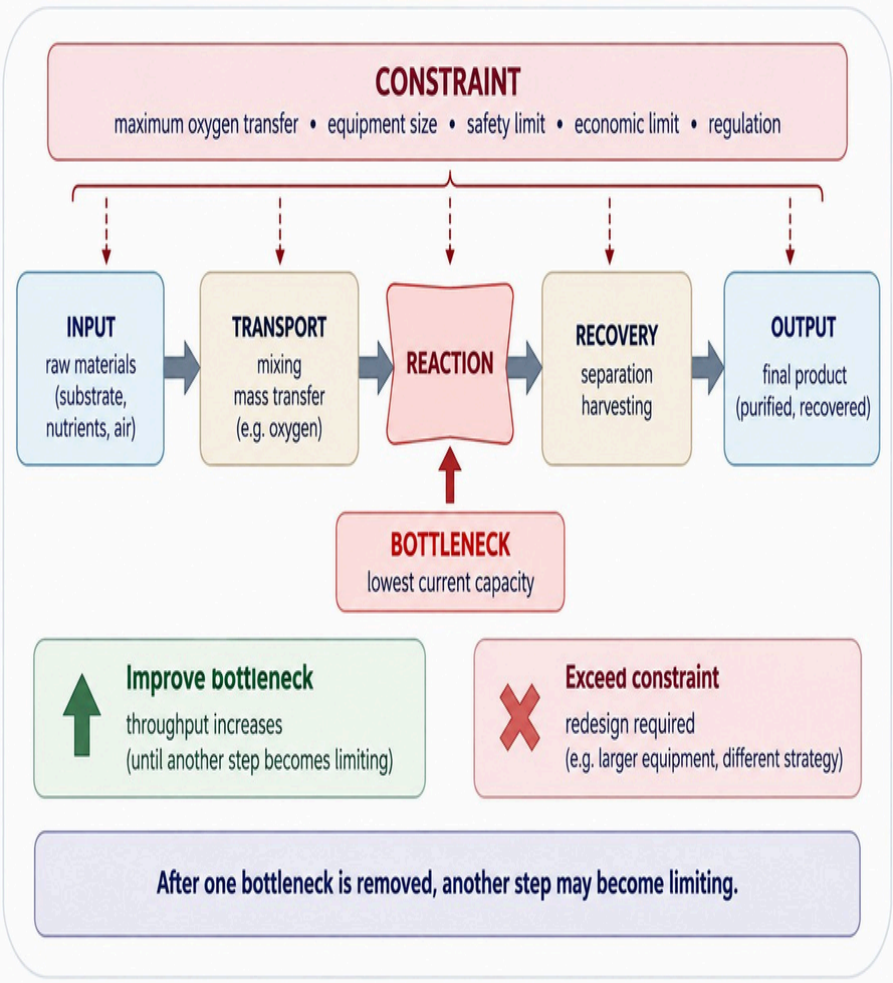
Removing or improving one bottleneck can increase performance, but it may immediately expose a **new bottleneck at another stage**.

A **constraint** is a boundary that cannot be exceeded without changing the process design, equipment, operating strategy or regulatory conditions.

Constraints may be **biological, physical, equipment-based, economic, safety-related or regulatory**.

A bottleneck can often move as operating conditions change, whereas some constraints remain fixed unless the system itself is redesigned.

Distinguishing between them helps engineers decide whether to **optimize an existing step or redesign the system boundary or equipment**.



Performance dashboards

A small set of metrics should cover quantity, quality, time, resource use and reliability.

For bioprocesses, key performance indicators often include **titer, yield, productivity, product quality, recovery and resource consumption**.

Titer shows how much product is obtained per unit volume, whereas **yield** relates product formation to the amount of substrate or another limiting resource consumed.

Productivity describes how quickly product is generated and is therefore important for reactor utilization and manufacturing capacity.

Quality metrics may include **purity, activity, stability or consistency**, depending on the biological product.

Additional indicators such as **oxygen demand, by-product formation, batch success rate, energy use or cost** help identify hidden trade-offs.

Dashboards are most useful when metrics are interpreted together, because improving one KPI may reduce another—for example, higher productivity may come with lower yield or poorer product quality.

TITER

g/L

How much product is present?

YIELD

g product / g substrate

How efficiently are resources converted?

PRODUCTIVITY

g/L/h

How fast is product generated?

QUALITY

purity • activity • stability

Normalization and fair comparison

Processes should be compared on a consistent basis such as per liter, per gram substrate, per batch or per unit product.

Normalization expresses a performance metric relative to a common denominator, allowing processes of different scale or duration to be compared fairly.

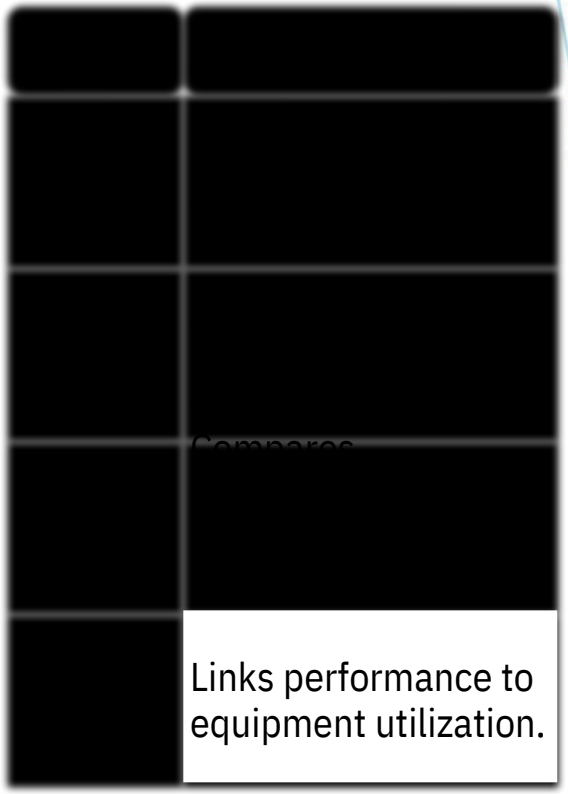
Per unit biomass separates cellular performance from differences in total cell concentration.

Per unit substrate is useful for comparing conversion efficiency and product yield between conditions.

Per unit reactor volume and time reflects volumetric productivity and equipment utilization.

Per unit product is useful for comparing resource use, energy demand, waste generation or cost across alternative processes.

Different normalization bases answer different engineering questions, so the **denominator must always be reported together with the metric**.



Scenario analysis

Scenario analysis asks "What if?" under plausible alternative conditions.

A scenario changes one or more important assumptions, inputs or operating conditions and examines the resulting effect on system performance. Typical scenarios may include **lower feed concentration, higher biomass, reduced oxygen-transfer capacity, shorter cycle time or increased raw-material variability**.

A **base case** represents the expected operating condition and provides the reference for comparison. **Best- and worst-case scenarios** explore favorable and unfavorable combinations of conditions and reveal the range of possible outcomes.

Alternative-design scenarios compare different equipment, control strategies or process configurations before costly experiments or scale-up decisions are made.

Scenario analysis is especially useful when data are limited because it can identify **which assumptions most strongly affect yield, productivity, quality, cost or process reliability**.

BASE CASE

reference operating condition

→ **BEST CASE**

higher transfer • favorable feed • high recovery

→ **WORST CASE**

lower transfer • feed variability • product loss

→ **ALTERNATIVE DESIGN**

different reactor • control strategy • process configuration

Sensitivity analysis

Feed rate Uncertainty should be propagated rather than hidden.

It helps identify which variables have the **greatest influence on process performance** and which have relatively little effect.

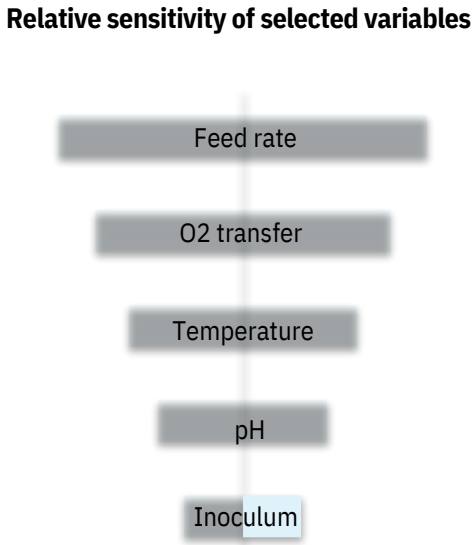
High-sensitivity parameters require **accurate measurement, careful control and reliable parameter estimation**, because small errors can strongly affect predictions.

Low-sensitivity parameters may often be simplified or measured less frequently without significantly changing the engineering conclusion.

Local sensitivity analysis examines small changes around a reference operating point, whereas **global sensitivity analysis** explores variation across a wider parameter range.

Sensitivity results help prioritize **experiments, sensor placement, process-control effort and model refinement**.

In a bioprocess, variables such as **feed rate, oxygen-transfer capacity, temperature, pH and inoculum size** may influence yield, productivity or product quality to different degrees.



illustrative ranking - not experimental data

Optimization logic

Optimization requires an objective, decision variables and constraints.

The first step is to define the **objective**, such as maximizing productivity or yield, minimizing cost, reducing waste, or improving product quality.

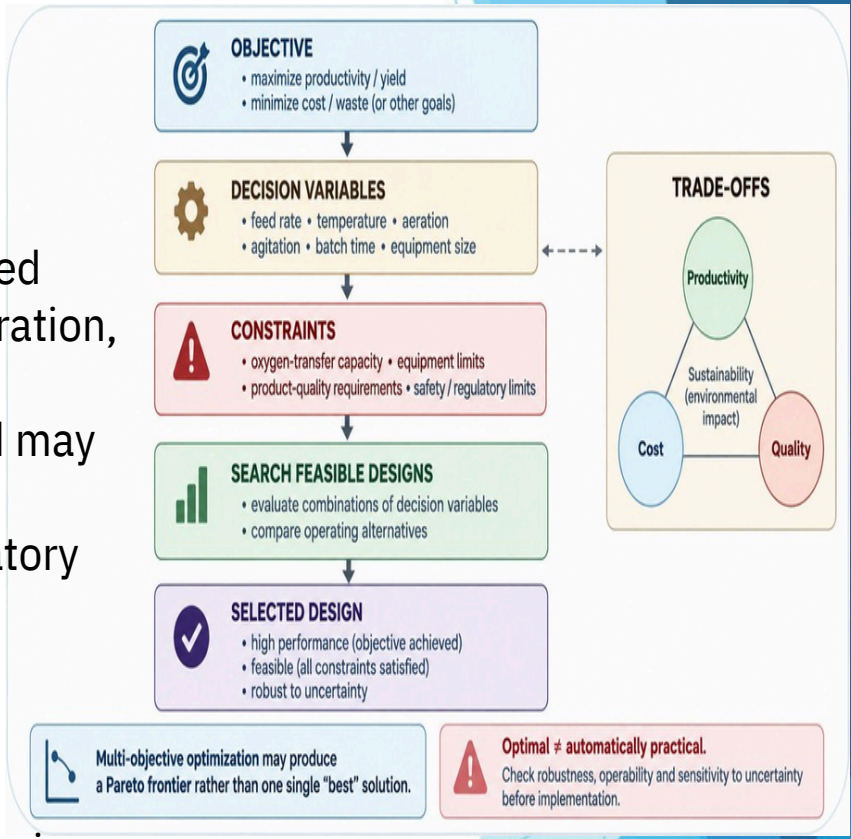
Decision variables are quantities that can be changed deliberately, for example feed rate, temperature, aeration, agitation, batch time or equipment size.

Constraints define the feasible operating region and may include oxygen-transfer capacity, equipment limits, product-quality requirements, safety limits or regulatory conditions.

The optimization procedure evaluates alternative combinations of decision variables and searches for the **best feasible operating point or design**.

In real bioprocesses, objectives often conflict: increasing productivity may increase cost, energy demand or by-product formation.

For this reason, a mathematically optimal solution should also be checked for **robustness, operability and sensitivity to uncertainty** before implementation.



Case: ethanol fermentation

Inputs: sugar, water, nutrients, yeast and process energy.

Main inputs include **fermentable sugar, water, nutrients, yeast inoculum and process energy**.

During fermentation, yeast takes up sugar and converts it through **glycolysis and alcoholic fermentation** into ethanol and CO_2, while part of the substrate is also used for biomass formation and cellular maintenance.

Important operating variables include **temperature, pH, sugar concentration, inoculum level and fermentation time**.

Ethanol concentration cannot increase indefinitely because high ethanol levels can inhibit yeast growth and metabolic activity.

Main outputs include **ethanol, CO_2, biomass, residual sugar, heat and minor metabolic by-products**.

Overall process performance should be evaluated using **ethanol yield, final titer, productivity, residual substrate and recovery after downstream separation**.

SUGAR FEED

glucose • nutrients • water

→

YEAST FERMENTATION

glycolysis • ethanol formation • growth

↘

CO_2 + heat

→

FERMENTATION BROTH

ethanol • biomass • residual sugar

→

RECOVERY

distillation / separation

→

ETHANOL PRODUCT

Case: recombinant protein production

The biological process creates protein, but system performance also depends on cell harvest, lysis or secretion, capture and purification.
The process begins with an engineered **host–vector system** that produces the target recombinant protein during cultivation.
Upstream performance depends on **cell growth, expression level, oxygen transfer, nutrient supply and induction conditions**.
Very high expression can increase **metabolic burden, protein misfolding, aggregation or inclusion-body formation**, reducing the amount of functional protein that can ultimately be recovered.
Product location determines downstream strategy: **secreted proteins** are recovered from the culture medium, whereas intracellular proteins require **cell harvest and lysis**.
During clarification, capture and purification, product can be lost through **adsorption, degradation, incomplete recovery or removal with impurities**.
Overall process performance should therefore be evaluated using **final recovery, purity, activity and product quality**, not upstream titer alone.

Case: wastewater bioprocess

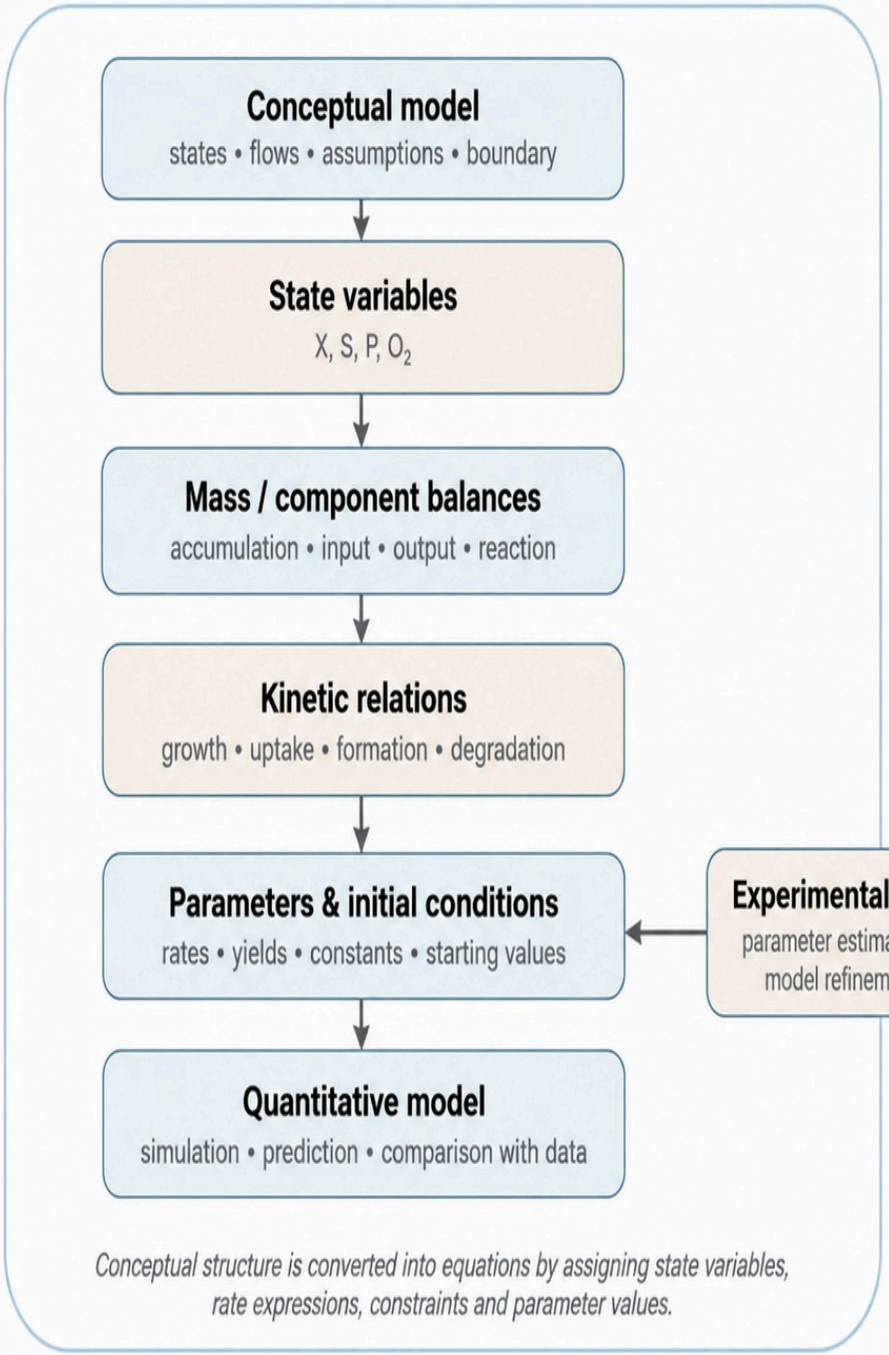
The useful output is pollutant removal to a required water-quality standard.

• A case study should be reduced to an engineering structure: objective, boundary, controllable variables, constraints, measurable outputs and acceptance criteria. • Inputs include influent load, aeration energy and chemicals; outputs include treated water, sludge and greenhouse-gas emissions.

• Residence time and oxygen supply affect both removal efficiency and energy use.

• System analysis exposes trade-offs between treatment performance and operational cost.

• The value of the case is not memorizing the example but learning how the same reasoning transfers to a new system.



Case: microbial biosensor

Input concentration is transformed into a biological signal and then into a measured output.

The process begins with **sample handling and analyte transport**, which determine whether the target molecule reaches the engineered microbial sensor.

A biological recognition element, such as a **receptor, transcription factor or regulatory promoter**, detects the analyte and activates the sensing circuit.

The intracellular circuit converts recognition into a measurable **reporter signal**, for example fluorescence, luminescence or pigment production.

Biosensor performance depends on more than sensitivity: **response time, selectivity, dynamic range, background noise and signal stability** are also critical. Environmental conditions such as **temperature, pH, inhibitors and sample composition** can alter both cellular activity and reporter output.

The final engineering decision is based on **calibrated signal interpretation**, for example whether the analyte is present or whether its concentration exceeds a defined threshold.

SAMPLE / ANALYTE
target concentration
→
BIOLOGICAL RECOGNITION
receptor • transcription factor
→
CELLULAR SIGNAL PROCESSING
genetic circuit • amplification
→
REPORTER OUTPUT
fluorescence • luminescence
→
SIGNAL ACQUISITION
measurement • calibration
→
DECISION
detected / not detected
or
concentration estimate

Case: cell therapy manufacturing

Inputs include patient cells, reagents, vectors, media and labor.

Major inputs include **starting cells, culture media, cytokines, genetic vectors or editing reagents, consumables and skilled labor**.

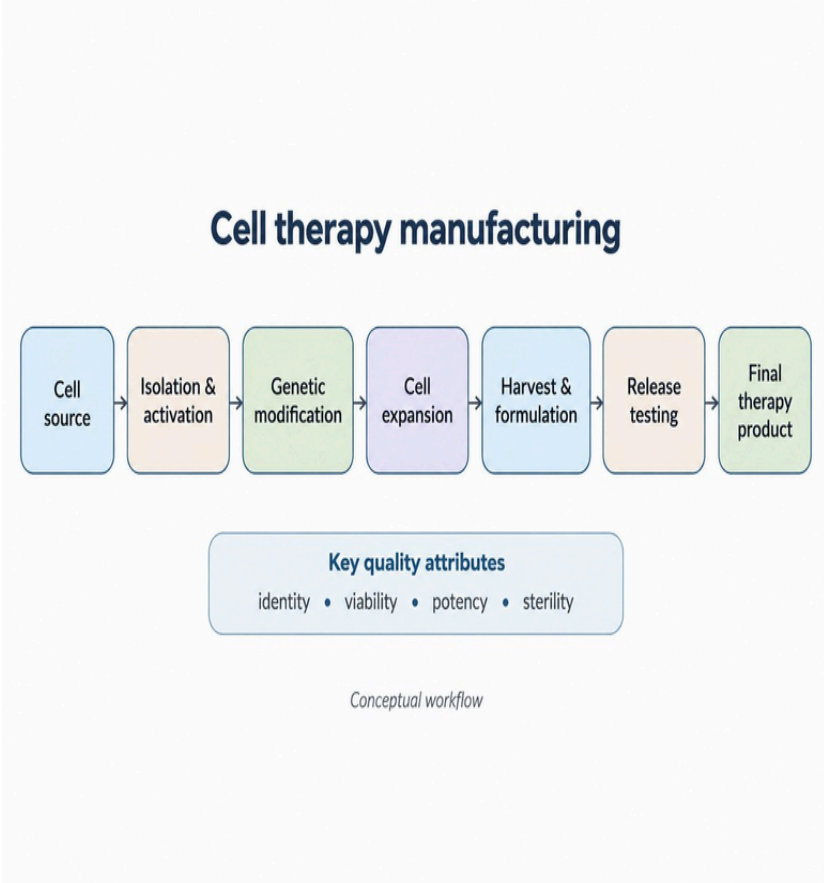
Manufacturing may involve **cell isolation, activation, genetic modification, expansion, harvest, formulation and cryopreservation**.

Because the product consists of living cells, process conditions can change **cell phenotype, viability, differentiation state and therapeutic potency**.

Critical process variables include **cell density, culture duration, nutrient supply, temperature, activation conditions and expansion strategy**.

Quality control must confirm **identity, purity, viability, potency, sterility and genetic or phenotypic stability** before product release.

Manufacturing performance should also consider **turnaround time, batch failure rate, scalability, chain of identity and consistency between patients or manufacturing runs**.



Improvement hierarchy

First eliminate gross losses and unstable steps; then optimize high-leverage variables.

The first priority is to eliminate **major losses, failures and unstable process steps** that strongly reduce yield, quality or throughput.

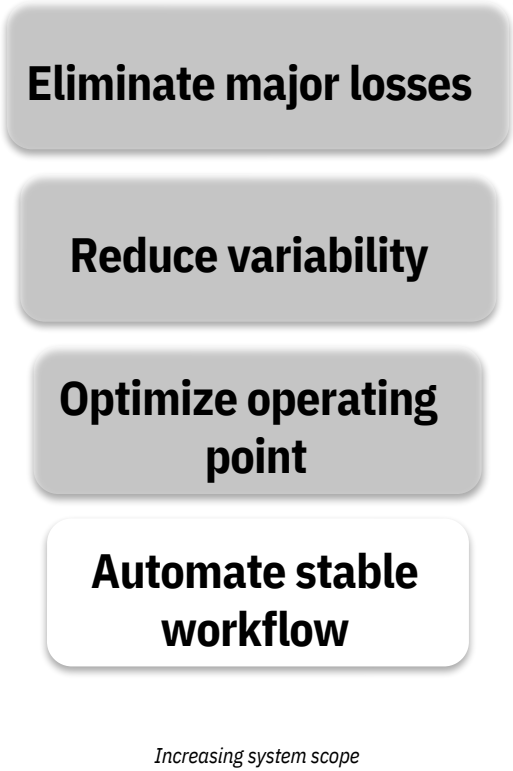
Once gross losses are controlled, attention should shift to **reducing process variability** and improving reproducibility between runs.

Only after the process is stable should engineers optimize high-impact variables such as **feed rate, temperature, aeration, residence time or recovery conditions**.

Optimization should focus on variables that are actually **limiting performance**, rather than on parameters that are easy to adjust but have little system-level effect.

After each major intervention, the process should be re-evaluated because the **bottleneck may move to another stage**.

Automation is most effective when applied to a process that is already **stable, understood and measurable**, rather than being used to automate an unstable workflow.



Communicating analysis

Use diagrams to show system structure, tables for assumptions and units, and plots for dynamic or comparative results.

Engineering analysis is valuable only when the reasoning, assumptions, evidence, uncertainty and decision are communicated clearly enough for another person to understand and verify.

Start with the **engineering question** and state exactly what decision or comparison the analysis is intended to support.

Present the supporting **evidence and data sources**, including experimental measurements, literature values and important assumptions.

Use the appropriate format for each type of information: **diagrams for system structure, tables for parameters and assumptions, and plots for trends, dynamics and comparisons**.

Report **uncertainty, operating limits and data gaps** rather than presenting one numerical result as perfectly certain.

Clearly separate **measured data, model predictions and engineering interpretation**, so the reader can trace how the conclusion was reached.

Finish with the **decision, its justification and the next measurement or experiment** that would most reduce remaining uncertainty.

Take-home IPO analysis template

1. Define the purpose and boundary. State what the system should achieve, what is included in the analysis and which decision the model should support.

2. Characterize the inputs. Quantify material, energy, biological and information inputs using appropriate amounts, concentrations or flow rates.

3. Map the process transformations. Identify the main biological and physical operations and describe their **capacity, efficiency and possible losses**.

4. Characterize the outputs. Include the desired product as well as biomass, by-products, waste, heat and other relevant outputs.

5. Add performance metrics. Evaluate the system using **yield, productivity, recovery, quality, resource use and reliability**.

6. Identify bottlenecks, constraints and uncertainty. Use scenario and sensitivity analysis to determine where improvement will have the greatest system-level effect.

7. Propose and validate an improvement. Compare alternatives, verify feasibility and reassess the system after the intervention.

Student Tasks and Discussion

1. Define one system boundary relevant to this lecture and explain what is deliberately excluded from the analysis.

2. Identify the three most important measurable variables for judging performance; state their units and why they matter.

3. Draw one conceptual diagram (process, causal loop, balance, or decision map) using the notation introduced in the lecture.

4. Describe one realistic failure mode or limitation and propose an engineering control, design change or monitoring strategy.

5. Locate one peer-reviewed article from the last five years that extends the lecture topic and summarize its engineering contribution, evidence and remaining limitation.

Selected References and Further Reading

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