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Strategy for characterizing microbial physiology across scales in fermentation processes

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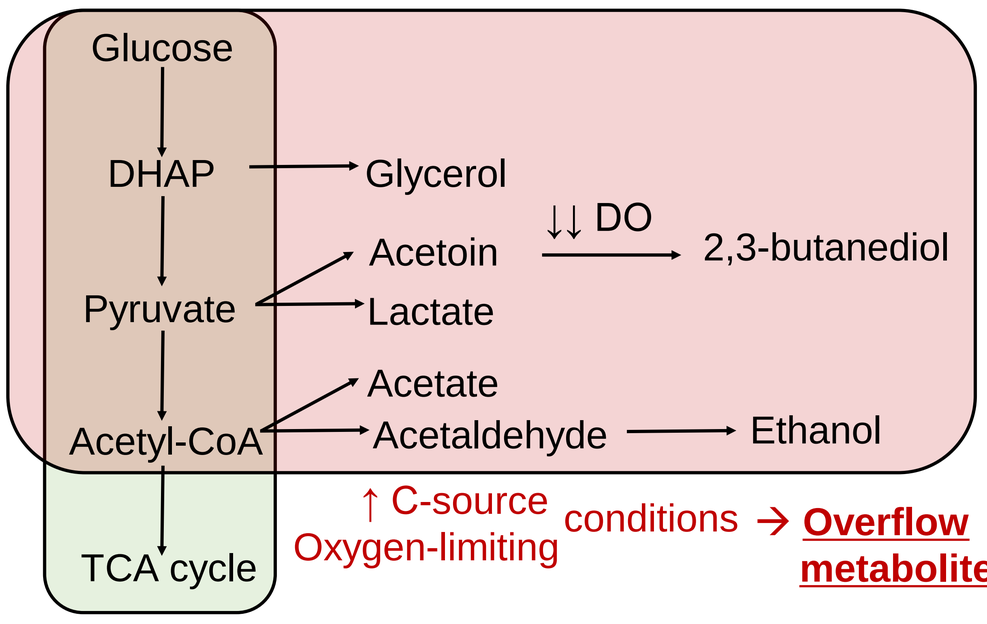
Introduction

Industrial fermentation processes are **heterogeneous** because of limitations in the mixing and the mass transfer capabilities of the system. Thus, gradients in relevant reactor parameters such as **C-source and nutrient concentrations, pH, dissolved oxygen (DO) and dissolved carbon dioxide concentration** are likely to occur in large-scale [1]. As cells transition through the various zones of the reactor, they are constantly exposed to oscillatory conditions. Such oscillations can **affect cell physiology** at several levels. For instance, inducing stress responses, reducing yields and product quality [2] and causing changes in the cell metabolism and in the physiological properties of the cell [3]. Seeing the importance of physiology in the correct development of fermentation processes and its influence on productivity, this work presents a strategy to characterize gradients in different scales from a physiological point of view.

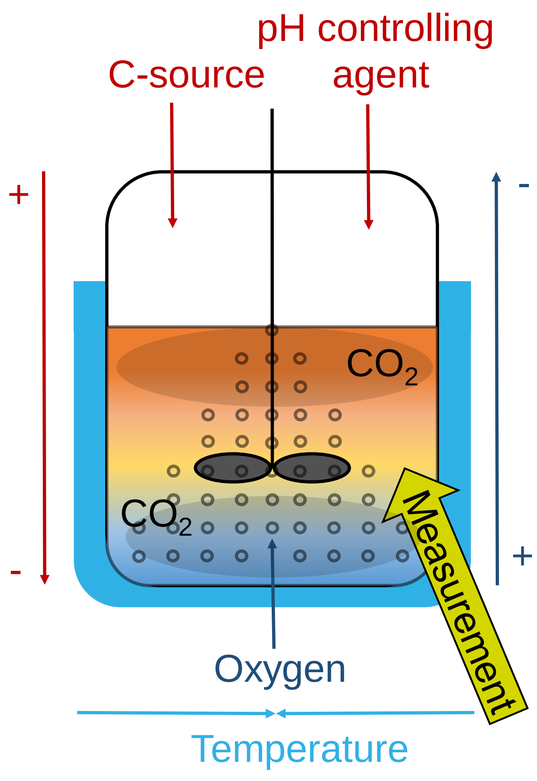
Bacillus licheniformis as model organism

- ✓ Industrially relevant organism
- ✓ Sensitive to gradients – rapid metabolism
- ✓ Varied overflow metabolism in response to exceeding oxidative fluxes

Normal conditions



Types of gradients



- Glucose and oxygen gradients are likely to occur in opposite directions
- DO is closely connected to the consumption of the C-source:

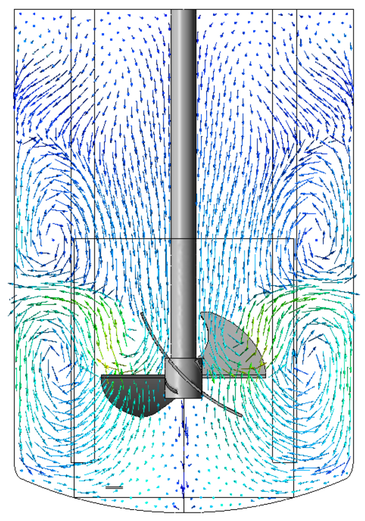
C-source + $\mathbf{O_2}$ → Energy

(ATP) + H_2O + CO_2

- If ↑↑[C-source] → O_2 supply can become **insufficient**
- Interest in studying the **combination of both gradients** and their implications in cell physiology

Case study: glucose and oxygen gradients in an industrial fed-batch fermentation process

1. Design of scale-down experiments using CFD



Geometry and mesh from [4]

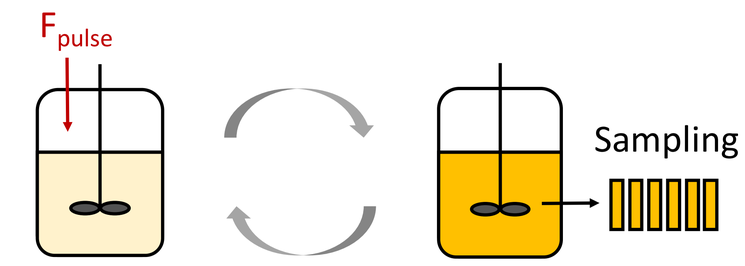
Simulation of:
- ✓ Flow pattern
- ✓ Glucose and oxygen distributions
- ✓ Kinetics of *B. licheniformis*

Outcome:
- ✓ Heterogeneous profile of substrate and product concentrations at several time points
- ✓ Starting point for scale-down experiments

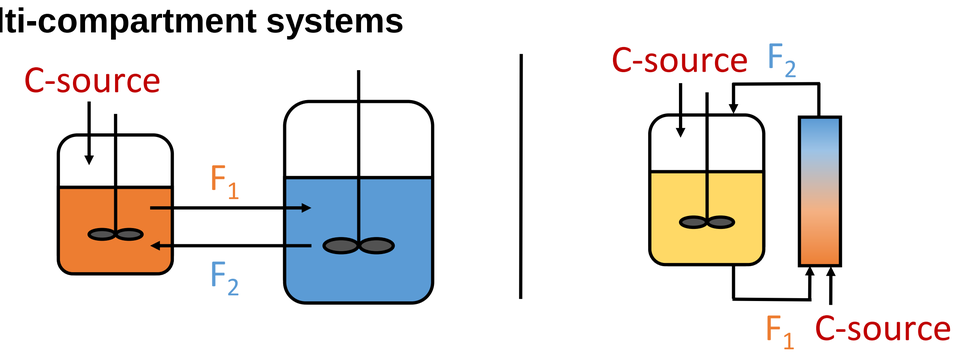
2. Performance of scale-down experiments

Lab-scale systems in which microorganisms are subjected to temporal variations in given process parameters [5].

- **Pulsed addition**



- **Multi-compartment systems**

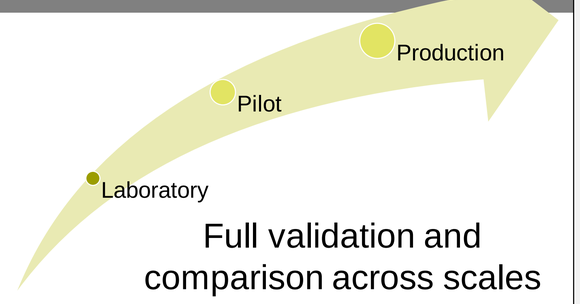


Seeing the results of the CFD model, one type or a combination of scale-down systems will be used.

3. Performance of fermentations at larger scales

Parameters to compare:

- ✓Metabolic markers
- ✓Productivity and product quality
- ✓Cell physiological state



Full validation and comparison across scales

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Acknowledgments

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