PhD proposal
Doctoral School Interfaces
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Subject: Droplet-based microfluidic platforms for the quantitative analysis of microbial metabolism and the development of predictive bioprocess models

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Droplet-based microfluidic cultures for the quantitative analysis of microbial metabolism and the development of predictive bioprocess models

1. Abstract

Recent advances in droplet-based microfluidics have enabled the miniaturization of microbial cultures into highly controlled microenvironments, where each droplet functions as an independent micro-bioreactor. In a recent study by the host team[1], a cost-effective capillary-based microfluidic platform demonstrated its ability to reproduce growth and GFP expression dynamics of E. coli observed in Erlenmeyer flasks, while reducing reagent consumption by more than 30,000-fold. However, this work revealed that at high glucose concentrations, delayed GFP expression in droplets is governed by oxygen limitation.

To tackle this issue, this Ph.D. project aims to develop and use an improved microfluidic design to quantitatively investigate substrates transfer and microbial metabolism, under controlled conditions. The objective is to use microfluidic to obtain predictive micro-bioreactors capable of linking microscale observations to bioreactor-scale performance.

Innovative microfluidic devices (capillary and PDMS-based) will be used to precisely tune droplet size, spacing, and generation frequency thereby controlling substrate access through both initial stock and boundary-driven transport mechanisms. Combined with real-time fluorescence imaging, microsensors, and image analysis pipelines, the system will generate a comprehensive time-series dataset with constated growth conditions.

The dataset will be used to feed a mechanistic model, which includes the choice of the formulation and parameters identification, enabling the prediction of microbial behavior under varying substrate conditions. The predictive capabilities of such a mechanistic model are intended to be used in bioprocess upscaling[2,3]. Ultimately, this research aims to establish a new paradigm in which microfluidic experiments become predictive tools for industrial biotechnology.

Keywords: Droplet-based microfluidics, oxygen limitation, microbial metabolism, GFP expression, mechanistic modeling, bioprocess optimization.

2. State of the art

Microfluidic technologies have emerged as powerful platforms for studying microorganisms under well-controlled conditions, overcoming the limitations of conventional culture systems. These devices, including droplet-based microfluidics, enable precise control of physicochemical parameters (e.g., nutrient gradients, oxygen availability, temperature) while drastically reducing reagent consumption. Droplet-based systems offer high-throughput screening by compartmentalizing cells in discrete microenvironments, as demonstrated by Dewan et al.[4], Saad et al.[5] for Chlorella vulgaris and Cao et al.[6] for cyanobacteria under varying environmental conditions. More broadly, microfluidic approaches have been applied to bacteria for applications such as antibiotic susceptibility testing and analysis of growth under confined conditions[7,8]. Despite these advances, challenges remain in quantitatively linking microfluidic observations to macroscopic bioprocesses, particularly due to the lack of employing mechanistic modeling in systematic comparisons with conventional cultures and the limited integration of real-time analytical tools and predictive modeling.

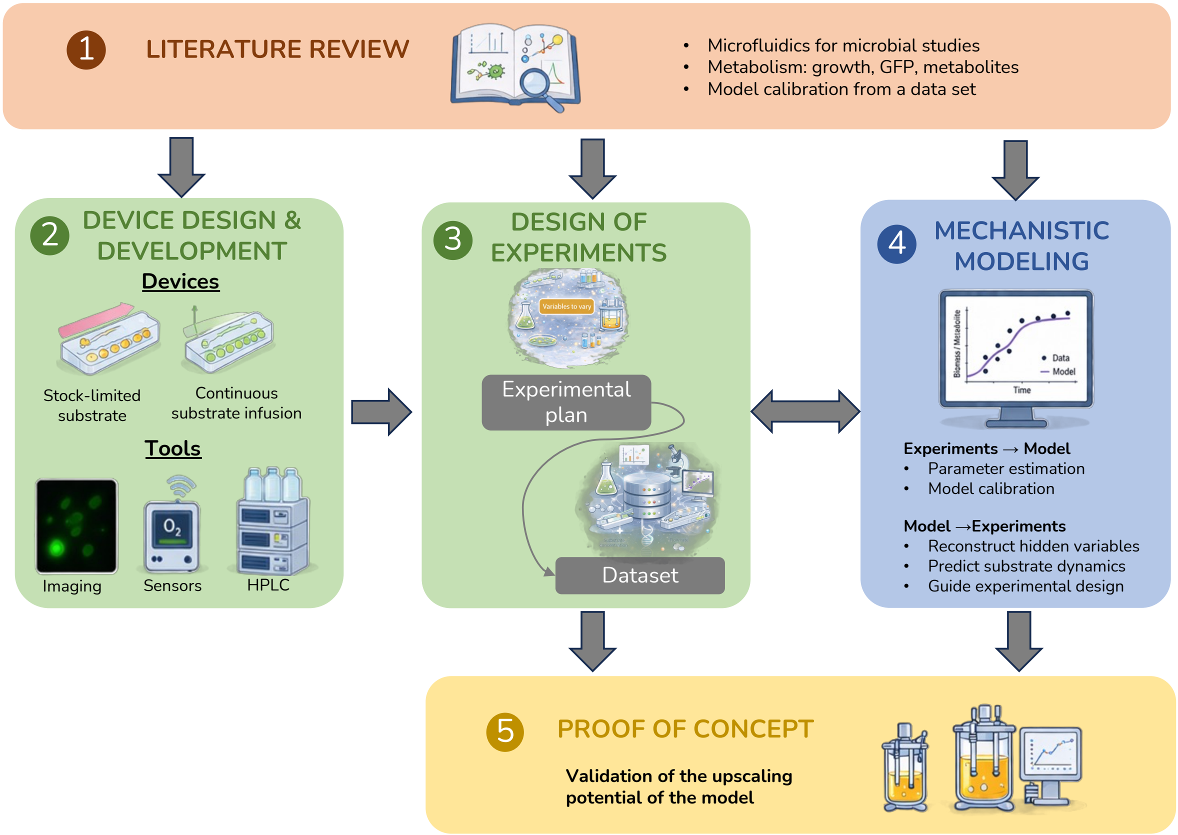

Fig. 1 – Synthetic presentation of the five working packages of the PhD proposal and their interactions.

By generating large, high-resolution datasets in a time- and resource-efficient manner, microfluidic platforms support the construction of mechanistic models capable of predicting microbial growth and biomolecule production under varying conditions[2,9]. In this context, mechanistic modeling acts as a central bridge between experimental data acquisition and process optimization, forming a cornerstone of emerging digital twin approaches in biotechnology[10,11]. However, several challenges remain. First, reliable model development requires that microfluidic data accurately reflect bioreactor conditions, although encouraging consistency between microfluidic and conventional systems has been reported. Second, most current studies rely on observable phenotypic outputs (e.g., growth, fluorescence) without direct access to intracellular states, limiting the depth of mechanistic interpretation. In addition, key transport phenomena such as oxygen transfer, diffusion, and local concentration gradients, critical at the microscale, are often not directly measured and therefore require modeling to reconstruct the actual cellular microenvironment.

Consequently, while microfluidic systems are highly suitable for generating reproducible, high-throughput datasets, they are currently used mainly for offline model calibration and reduced-order validation rather than fully coupled predictive frameworks. This highlights the need for integrated approaches combining microfluidics, mechanistic modeling, and complementary analytical techniques. In this perspective, microfluidics should be leveraged to generate high-quality, high-throughput experimental data, which can be used to train and parameterize mechanistic models, while validation and extrapolation must be performed at larger scales (e.g., bioreactors). Such a strategy is essential to establish a robust and predictive link between microscale observations and macroscale bioprocess performance, paving the way toward truly predictive and scalable biotechnological systems.
In agreement with this need, the PhD project aims to develop predictive models capable of extrapolating droplet-scale observations to conventional bioreactors, thereby linking high-throughput microfluidic approaches to large-scale

bioprocess challenges. To achieve this goal, the work includes two complementary objectives: i) the development and use of microfluidic devices able to control the growth conditions and ii) the use of the data set obtained by this device to formulate and tune a mechanistic model. An engineered strain of *E. Coli* with GFP expression will be used throughout the work as model microorganism.

3. Working plan

To address this challenge, the project is structured by five interconnected working packages, as depicted in figure 1.

WP1 - Literature review: state of the art and methodological framework

The literature review will be the starting point of the work. This review will be done on the three main aspects of the work: microfluidic devices, microbiological cultures and metabolic pathways. These parts will be used receptively in the three main pillars of the project: device development (WP1), biological experiments (WP3) and mechanistic modelling (WP4). This task will define the main hypotheses, guide the experimental strategy. With a focus on this task at the beginning of the PhD, this literature review will remain active throughout the PhD. For this project at the interface between different domains, the student will benefit from the complementary expertise of the supervisory team.

WP2 - Device design and development

The work will focus on the use and development of two microfluidic platforms intended to provide a good knowledge of microbial culture conditions in droplets: (i) a substrate-limited device and (ii) a continuous substrate-controlled device, reflecting two complementary approaches.

In the first approach, culture conditions are governed by the initial stock of substrates (for example glucose or oxygen). An existing capillary-based droplet generator will be used and further developed, where nutrients in the culture medium and dissolved oxygen in the surrounding phase are initially fixed. The droplet formation can then be tuned to vary the volume of the surrounding phase relative to the droplet volume, therefore varying the initial stock. This configuration allows determination of when limitations resulting from depletion occur.

In the second approach, culture conditions are imposed by the system through boundary conditions and controlled diffusion. A PDMS-based microfluidic device will be developed to enable continuous and tunable supply of substrates. In this case, the physicochemical conditions within the droplets are controlled by fluxes occurring at the system interfaces by diffusion. The design will focus on channel geometry and flow configuration to precisely regulate mass transfer and impose well-defined culture conditions.

For both platforms, cultivation data will be collected using by dedicated instrumentation. High-resolution brightfield and fluorescence imaging will be coupled with droplet tracking and image processing pipelines to extract fluorescence signals (like GFP) at the single-droplet level. *In situ* monitoring capabilities will include optical density spectrometry at droplet scale, as well as integrated pH and oxygen sensors. Sampling strategies will enable downstream metabolite quantification by HPLC from large droplet populations.

Design of experiments

Based on literature review and model assumptions, two initial series of experiments will be performed, corresponding to the substrate-limited and the continuous substrate-controlled approaches. In each case, a design of experiment will be established in which operating conditions are systematically varied, including substrate concentration, flow rates, droplet size (and droplet-to-continuous phase ratio), and, for the PDMS device, diffusion-related fluxes through boundary conditions. Time-resolved datasets will be generated by combining *in situ* measurements (OD, fluorescence, pH, relevant physicochemical parameters) with offline metabolite analysis (HPLC), enabling comprehensive data sets to be obtained to be used in WP4. As explained below, the experimental devices will be used for further series of tests to build up a real synergia between WP3 and WP4, as pointed out by the double arrow in figure 1.

WP4 - Mechanistic modeling

The first step will be to identify and formulate the main metabolic pathways. This biological model will be coupled to physics-based models (diffusion, mass transfer, and consumption) for a rigorous analysis of the experimental data. This coupling is required for both devices (stock-based and flux-based devices). These models will explicitly account for microscale transport limitations and their impact on cellular responses.

A strong synergy will be established between experiments (WP3) and modeling (WP4). Experimental datasets will feed model formulation and parameter estimation, while the model will reconstruct otherwise inaccessible variables, such as the temporal evolution of substrate concentrations within droplets. In the substrate-limited approach, the model will enable deduction of concentration dynamics from consumption, whereas in the continuous substrate-controlled approach, it will quantify the conditions imposed by diffusion-driven fluxes. This bidirectional interaction will be implemented iteratively, ensuring continuous completion of the experimental data set and improvement of the prediction potential of the model.

WP5 - Proof of concept of upscaling capabilities

The validated mechanistic model will be used to simulate contrasted operating conditions representative of larger-scale bioprocesses, such as substrate-limited versus non-limited regimes. One or two representative scenarios will be selected and experimentally tested in a 250 mL or 5 L bioreactor.

The objective is to assess the predictive capability of the model and demonstrate that microfluidic experiments, coupled with mechanistic modeling, can reliably inform and predict macroscale bioprocess performance. The synergy between experiments and modeling will be further exploited at the beginning of this work package to guide the selection of relevant operating conditions.

4. Tentative Gantt diagram

	Year 1	Year 2	Year 3
1. Literature review	🟦	▨	▨
2. Device design and development	🟦	▨	
3. Design of experiments	🟦	🟦	
4. Mechanistic modeling	▨	🟦	▨
5. Proof of concept of upscaling capabilities			🟦

🟦 = main focus
▨ = continuous/secondary activity

5. Resources

The Ph.D. student will spend the whole PhD period at LGPM, biotechnology chair of CentraleSupélec. This Ph.D. work will benefit from team experience in microfluidic, scientific instrumentation, microbiology and mechanistic modelling.

LGPM, Chair of Biotechnology, CentraleSupélec conducts a wide range of activities spanning bioprocess engineering, microbial biotechnology, biomass valorization, and the development and characterization of bio-based materials, combining experimental, analytical, and modeling approaches. It has recognized scientific expertise in fermentation processes (sterile hoods, incubators, 5L bioreactors, etc.) as well as in the development, characterization and modeling of biosourced materials. This laboratory hosts high-tech equipment necessary for all parts of the study: Bioreactors, photobioreactors, incubators, cell counts, microplate reader, and microfluidic devices. It also has a very complete imaging platform (confocal laser microscopy, Raman micro-spectroscopy, structured light optical microscopy, environmental scanning electron microscopy, nanotomography). Finally, the laboratory is equipped with a complete analytical platform (HPLC-RI/FLD, UHPLC-UV-MS, Tims-ToF, IC, GC-MS/MS, µ-GC) and a complete Instrumentation & Design platform. In addition, the Chair has strong expertise in multiscale modeling and digital twin development for bioprocesses, enabling the integration of experimental data into predictive frameworks. The students' work will be devoted to the use of all these tools.

6. Profile and required skills

- Engineer or M2 student in biotechnology, chemical engineering, mechanical engineering or a related field.
- Strong interest in microfluidics, bioprocesses, and modelling.
- Motivation to work in a multidisciplinary and collaborative research environment.
- Rigorous, autonomous and dynamic student
- English reading and writing skills
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7. References

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