

This highlight refers to the PhD Thesis by Kartik Totlani (2021)

Droplet based microfluidic platforms for bioprocess engineering

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Challenges in bioprocess development

A critical challenge during the initial stages of bioprocess development is that tools used to screen microorganisms and optimize cultivation conditions do not represent the environment imposed at the industrial scale. Inside an industrial-scale bioreactor, microorganisms are often cultivated under fed-batch conditions, where nutrients are supplied during the culture. Additionally, microorganisms continuously keep crossing zones with low and high concentrations of substrate and dissolved oxygen. However, during the initial stages of bioprocess development, growth and productivity of microorganisms are evaluated under batch conditions due to the difficulty of dynamically controlling nutrient and dissolved oxygen concentrations in screening equipment such as micotiter plates (Totlani 2021, Totlani et al. 2021a). This inconsistency in cultivation conditions often leads to selection of strains that fail to perform at industrial scale. Droplet microfluidics holds the potential to address this inconsistency with fidelity by offering high-throughput experimentation and excellent control over the culture microenvironment. Herein, we present the design and development of droplet-based microfluidic technology that enables studying of microorganisms under dynamically controlled cultivation conditions.

Droplet on demand generator

The first type of tool developed in the Ph.D. project is a droplet-based nanobioreactor that facilitated nutrient-controlled cultivation in fed-batch mode. Since its operation needed a reliable method for supplying nutrient droplets to cell-containing droplets, a strategy for nutrient droplet generation was initially designed. As a first goal, a scalable microfluidic droplet on-demand (DoD) generator was developed for producing monodisperse water in oil microdroplets, where the droplet volume was primarily dictated by the generator geometry and was independent of operating conditions (Figure 1) (Totlani et al. 2020 [5]). The DoD generator was characterized for a range of operating conditions and flow parameters while generating droplets with a high monodispersity. By decoupling droplet formation from its transport, a reliable scale-out was achieved for the sequential generation of droplets on-demand at multiple DoD junctions in the chip (Totlani et al. 2020 [5]).

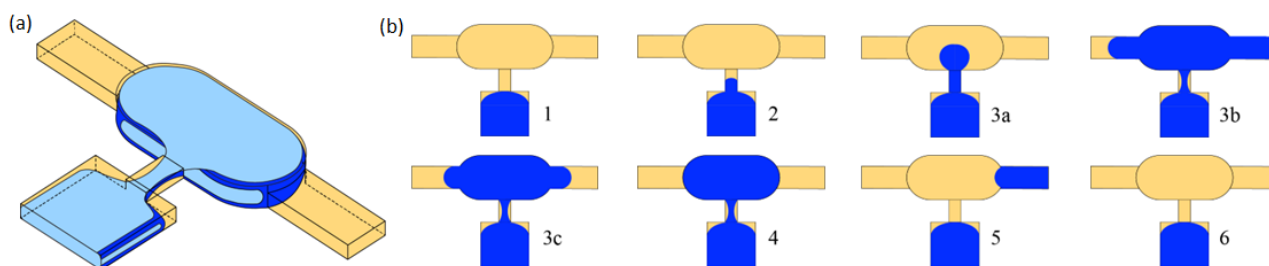


Figure 1: (a) Three-dimensional schematic and (b) working principle of microfluidic droplet on demand (DoD) generator, showing the dispersed phase steadily pressed against the nozzle (1), the dispersed phase filling the chamber (2-3c), the interfaces of the dispersed phase steadily pressed against the entrance of the main channel (4), the release of a droplet with a volume similar to the volume of the chamber into the main channel (5), after which the new interface is steadily pressed against the nozzle (6) ready for a new DoD cycle. [2][5]. Yellow depicts continuous oil phase and blue depicts dispersed aqueous phase.

Fed-batch droplet nanobioreactor

This DoD methodology was used to develop a microfluidic tool that enabled studying microorganisms under nutrient-controlled fed-batch conditions. The droplet-based fed-batch nanobioreactor comprised of two separate DoD generators, where the first one was used for creating droplets encapsulated with microorganisms and the second one for making nutrient droplets at the desired frequency throughout the cultivation. The nutrient droplets were chemically coalesced to the cell-containing droplet, immobilized inside a trap (Figure 2) (Totlani et al. 2020, 2021b), by temporarily destabilizing the droplet-droplet interface through the flow of a poor solvent around it, thereby establishing a fed-batch process. The yeast *Cyberlindnera (Pichia) Jadinii* was used as the model organism. Nutrient-controlled cell growth experiments as illustrated in Figure 3a were performed by varying the glucose concentration inside the nutrient droplets and by varying the frequency of droplet generation.

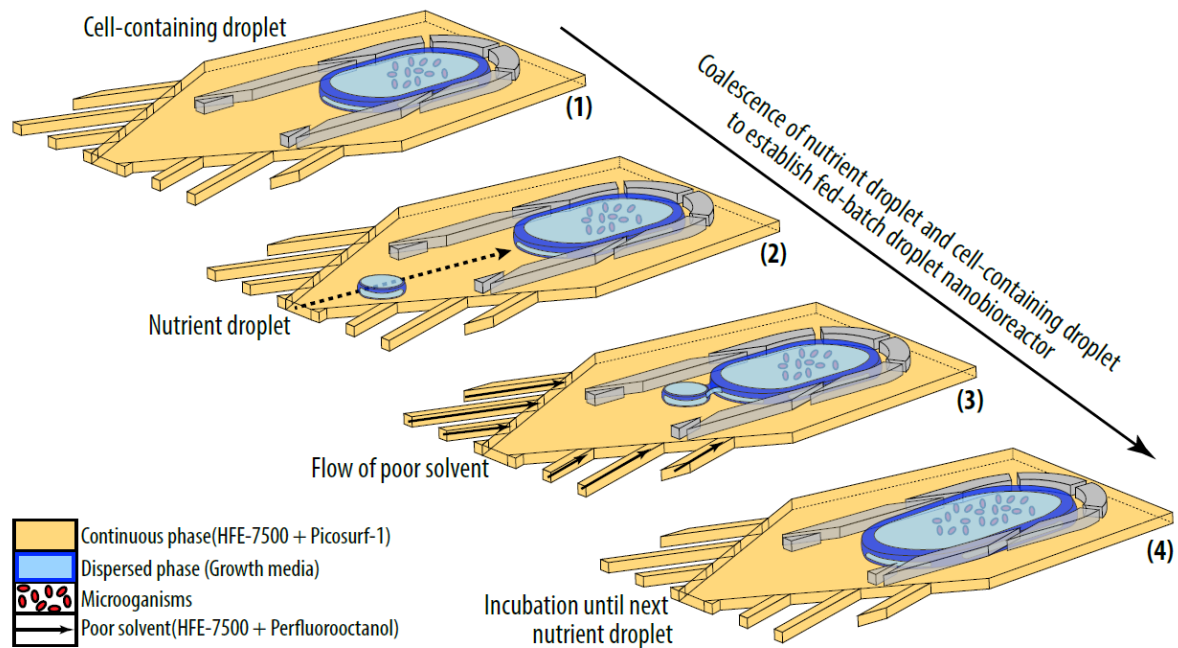


Figure 2: Microdroplet based fed-batch process-on-a-chip illustrating the controlled supply of nutrients to a cell-containing droplet. Cell-containing droplet immobilized inside a cup-shaped trap (1). On-demand supply of a nutrient-containing droplet (2). Coalescence of the surfactant-stabilized interfaces induced by temporarily injecting a solvent in which the surfactant is less soluble through the fork-like structures (3). Incubation until the next nutrient supply (4) (Totlani et al. 2020, 2021b).

Nutrient controlled growth inside the droplets was established by demonstrating different cell growth rates with different glucose concentrations inside nutrient droplets. The growth behavior of the microorganisms for a different set of glucose concentrations agreed well with a simple kinetic growth model (Figure 3b) (Totlani 2021, Totlani et al. 2021b).

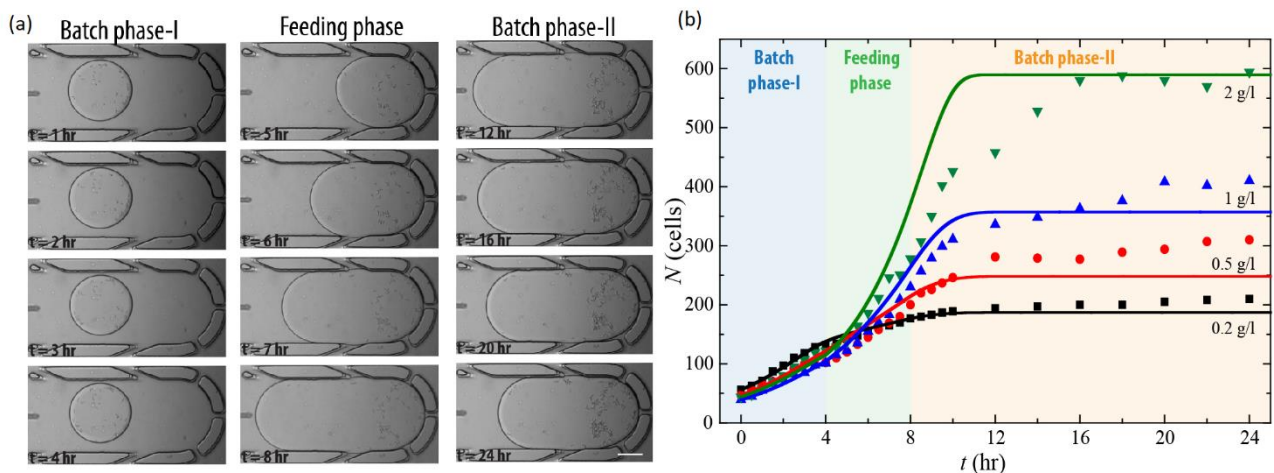


Figure 3: (a) Time lapse of grey scale images depicting nutrient controlled fed-batch growth of *Cyberlindnera (Pichia) jadinii* inside a fed-batch droplet nanobioreactor. (b) Growth curves for nutrient-controlled growth in the fed-batch droplet nanobioreactor for different concentrations of glucose concentration in nutrient droplets. The effect of controlled nutrient supply is seen from the difference in the growth rates in the feeding phase (Totlani 2021, Totlani et al. 2021b).

Microdroplet static array for dissolved oxygen

The second type of microfluidic tool developed in this work enabled the cultivation of yeast inside microdroplets with the main supply of oxygen to the cells coming from the fluorinated oil flowing around the droplets (Figure 4a,b) (Totlani 2021, Totlani et al. 2021c). Batch growth of *Cyberlindnera (Pichia) Jadinii* was performed inside droplets under two limiting cases. For the first case, oil saturated with oxygen was flown around the droplets containing microorganisms, which showed exponential cell growth. In contrast, negligible growth was observed in the second case where the oil was saturated with nitrogen and flown around the droplets.

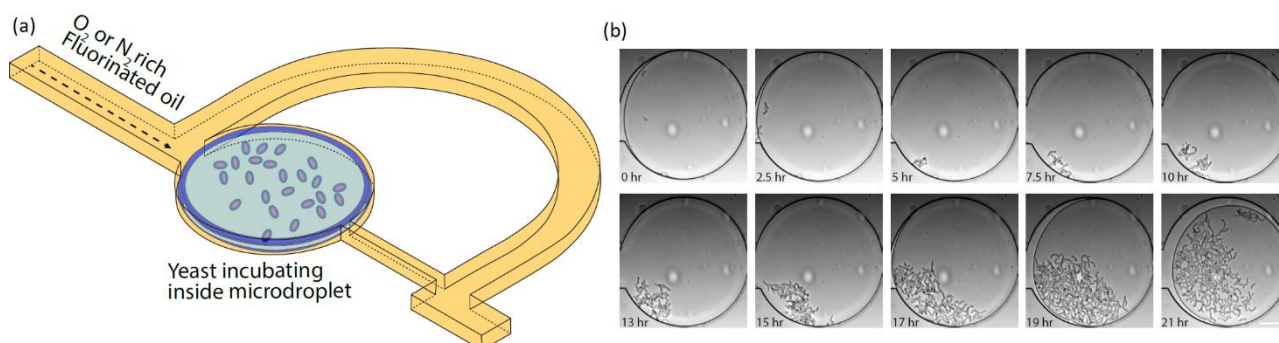


Figure 4: (a) 3D schematic of a single trap with a cell-containing droplet incubated in a growth chamber, while the supply of dissolved oxygen to the cells is controlled through the amount of oxygen dissolved into the oil phase that continuously flows around the droplet through the bypass. (b) Time lapse of grey scale images illustrating the growth of *C. Jadinii* inside a single microdroplet. Scale bar is 100 μm . (Totlani 2021, Totlani et al. 2021c).

The results from this work form a proof-of-concept of long-term and nutrient-controlled growth of microorganisms inside microdroplets through a controlled supply of nutrients. Diverting away from continuous droplet microfluidics which require sophisticated workflows and integration of multiple devices, a strategy that facilitated simple operation and fabrication of devices from standard procedures was developed. The results from this work form a foundation step towards narrowing the gap between screening and industrial-scale use, with an eye to keeping the technology sufficiently simple to be adopted by the biotechnology and bioengineering community.

References

- Totlani, K., Kreutzer, M. T., van Gulik, W., & van Steijn, V., 2021a. Microbioreactors for nutrient-controlled microbial cultures: Bridging the gap between early bioprocess development and industrial scale use. Manuscript in preparation.
- Totlani, K., Hurkmans, J., van Gulik, W., Kreutzer, M. T., & van Steijn, V., 2020. Scalable microfluidic droplet on-demand generator for non-steady operation of droplet-based assays. *Lab on a Chip*, 20(8), 1398-1409.
- Totlani, K., Wang, Y., Bisschops, M.M., Riese T., Kreutzer, M. T., van Gulik, W., & van Steijn, V., 2021b. Fed-batch droplet nanobioreactor for controlled growth of *Cyberlindnera (Pichia) Jadinii*: A proof-of-concept demonstration. *Advanced Materials Technologies*, 6(9), 2100083.
- Totlani, K., Katakwar, P., du Pree, N., Kreutzer, M. T., van Gulik, W., & van Steijn, V., 2021c. Microfluidic droplet array to study microbial growth under different levels of dissolved oxygen. Manuscript in preparation.
- Totlani, K., 2021. Droplet microfluidics for bioprocess engineering, PhD Thesis, Delft University of Technology.