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Quantitative measurements in single-cell analysis: towards scalability in microbial bioprocess development

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Single-cell analysis in microfluidic cultivation devices bears a great potential for the development and optimization of industrial bioprocesses. High parallelization allows running a large number of cultivation experiments simultaneously even under quick alteration of environmental conditions. For example, the impact of changes in media composition on cell growth during classical batch cultivation can be easily resolved. A missing link for the scalability of microfluidic experiments is, however, their complete characterization via conventional performance indicators such as product titer and productivity. While existing mass spectrometry technology is not yet sufficiently coupled with microfluidics, optical methods like enzymatic assays or fluorescence sensors are promising alternatives but require further improvement to generate quantitative measurements of extracellular metabolites.

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Introduction

Microbial bioprocesses are characterized and validated by specific performance indicators such as growth and substrate uptake rates, product yields and overall productivities. Those are typically determined based on time-resolved measurements of biomass, substrate and product concentrations [1,2]. From the performance indicators, high-performing strains, optimal media compositions as well as robust operating conditions for scale-up and for design-of-experiment approaches can be identified [3]. Furthermore, biochemical network models can be validated and then applied for the prediction of new metabolic engineering targets [4,5].

Nowadays, time is the most limiting factor for a successful bioprocess development and, thus, high throughput screening approaches are urgently required [6]. Since there are many bioprocess variants possible when considering different genotypes, media compositions and operating conditions, miniaturization and automation are mandatory to reduce material costs and manual effort during process development [3]. Next, upscaling from lab-scale to industrial-scale often causes severe problems regarding cell viability and productivity resulting from population heterogeneities and bioreactor inhomogeneities due to non-ideal mixing [7]. In fact, population heterogeneities are thought to be triggered by changes in environmental conditions. Especially under large-scale operation cells constantly face rapidly changing microenvironments and, thus, are under severe stress to adapt their metabolic status accordingly [8–11].

Recently, microfluidic single-cell cultivation devices have attracted increased attention for bioprocess development as dynamic large-scale processes might be emulated in the future [12]. Low volumes and good parallelization possibilities already enable a drastic increase in experimental throughput compared to lab-scale bioreactors. Moreover, with microfluidics fast alterations of environmental conditions can be easily realized, making this technology a promising solution for the major challenges in bioprocess development as mentioned above. By using time-lapse microscopy and fluorescence-based biosensors, specific growth rates of micro-colonies, morphological changes of single cells and cell population heterogeneities can be precisely determined [12,13]. However, with state-of-the-art microfluidic technology not all the important performance indicators mentioned above are currently accessible.

Microfluidic cultivation of organisms encompasses a wide and growing field including several orders of magnitude in size of the cultivation volume. The largest representatives are microtiter plate-based devices working in the

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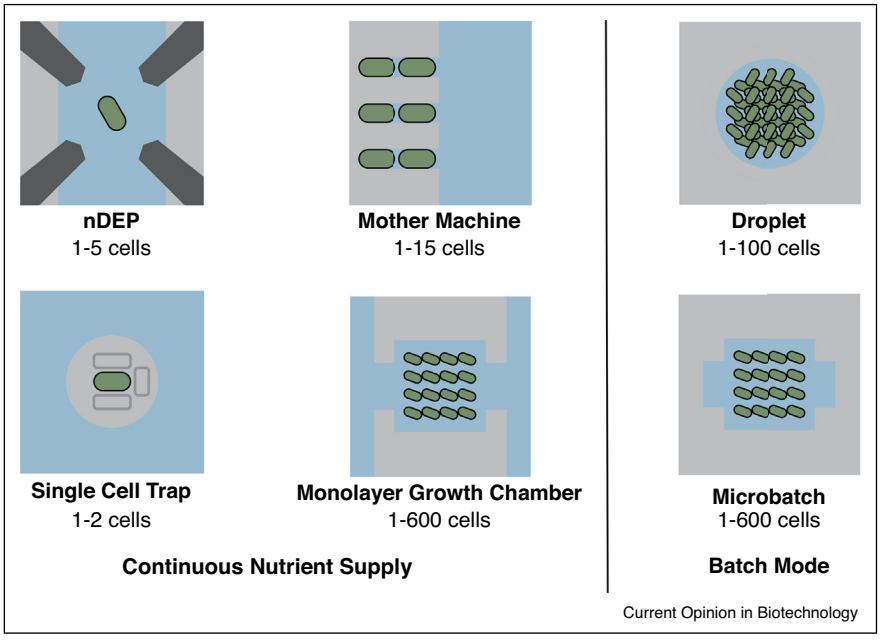
Figure 1



Overview of microfluidic cultivation principles and some of their main characteristics. Different entrapment strategies have been designed for specific cultivation experiments at the single-cell level. Approaches in which the cells are continuously supplied with nutrients (left) are nDEP [22], mother machine [59], single cell trap [60] and monolayer growth chamber [61]; approaches for single-cell batch cultivation are droplets [62] and micro-batches [20•].

microliter-to-milliliter volume range [14•,15,16]. In the present review, microfluidic devices will be defined as devices, which allow a simultaneous observation and discrimination of single cells. Typically, this premise limits the cultivation volume to the picoliter range as otherwise the number of observed cells becomes too high. We focus on the challenges for establishing quantitative analytics in microfluidic single-cell cultivation devices and critically evaluate possible solutions. In contrast to recent reviews focusing on larger eukaryotic cells [17••], the scope lies on the characterization of industrially relevant microorganisms and respective bioprocesses especially via measurements of extracellular metabolites.

Challenges of picoliter fermentation

There are two major approaches to confine growing cells for cultivation and observation: droplets and structures [18]. Due to their inherent properties these often coincide with the two major operating modes of microfluidic cultivations: batch and perfusion or chemostat (Figure 1), and only rare examples for structurally confined droplets [19,20•] and droplet based chemostats do exist [21]. Other contraptions like negative dielectrophoresis (nDEP) will be counted as structural confinement along this review [22]. For a recent review on the field of single-cell technologies refer to Rosenthal *et al.* [23••].

Independent of the chosen cultivation system there are some major challenges in quantitative single-cell analysis, largely arising from the small dimensions, which is why we will distinguish only between the two operating modes. To provide a more concrete example, we estimate the expected ranges for substrate uptake (D-glucose) and product formation (pyruvate) rates for wild-type *Corynebacterium glutamicum* within a microfluidic batch cultivation and a perfusion based cultivation. We did choose pyruvate as example since a theoretical yield of two provides a good upper boundary for most products. Figure 2 shows how volumes and flow rates translate into microscopic dimensions if the volume per cell (1×10^{14} cells/m^3) and the average retention time ($\tau \approx 120$ min for a growth rate of $\mu = 0.5$ h^{-1}) are kept constant.

An exemplary volume of 1 m^3 translates to a single-cell batch of 10 pL. For simplicity we assume the same uptake and production rates as in macroscopic cultivation, so that the concentrations will be equal. By assuming an analyte concentration of 1 mM in a representative microfluidic sample volume of 10 pL, the resulting total number of molecules is only 6×10^6 to start with. Therefore, in a microfluidic batch it might be difficult to resolve the differences between two compared cells since the change in number of analytes can easily fall below the detection limit. Additionally, further analysis and preparation steps