***In line* particle and single cell measurements in microbial bioprocesses**

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**Highlights**

* Single cell measurement for intracellular concentration and co-culture monitoring
* *In situ* microscopy identifies budding and non-budding yeast for viability analysis
* Chain length distribution of lactic acid bacteria is related to metabolic activity
* Focus beam backreflection measurement for *on-line* optimization of feedstock pretreatment

**1. Introduction**

Monitoring tools for the liquid phase of bioprocesses are often restricted to a very few parameters. This might be insufficient if data from the single-cell level is required. While several *off line* tools have been commercialized, not many were adjusted and applied *in line* in bioprocess development and control.

This presentation aims to show the importance of single-cell based measurements and population heterogeneity for scale up and scale down of bioprocesses; recent advances achieved [1]; the potential of such methods to recognize and quantify the impact of unfavorable cultivation conditions in several microbial bioprocesses; and finally several examples of successful application for process optimization, including feedstock pre-treatment and determination of intracellular components on the single-cell level. Examples of long chain fatty acid accumulation in heterotrophic algae, budding in yeast, chain length determination in lactic acid bacteria, optimization of feedstock pretreatment for anaerobic digestion and the application in co-cultures are presented.

**2. Methods**

Among the techniques that are able to capture the morphological characteristics of cells in real time, automated imaging technologies are promising, because they provide additional information about cellular structures, shape and cell aggregation beyond size. Photo-optical *in situ* microscopy (ISM) and 3-dimensional holographic microscopy (DHM) were used in this study to measure the morphological dynamics in eukaryotic cultures on a single-cell basis, with heterotrophic algae and yeast as examples [2,3]. ISM was used further to monitor the chain length distribution of lactic acid bacteria (LAB). Both, yeast and LAB, were monitored under homogeneous and heterogeneous conditions in a scale down multi-compartment approach. The relation of morphological changes and metabolic activity and growth vitality in relation to scale up effects is assessed for both cases.

A second technique (focus beam backreflection) describes the monitoring of the particle size distribution in culture broth of anaerobic digestion. This method was successfully used to evaluate the efficiency of pre-treatment methods for hydrolysis based on the particle size, while distinguishing between non-living feedstock particles and active cells.

**3. Results and discussion**

Based on the morphological and optical cell features, the intracellular accumulation of the polyunsaturated fatty acid docosahexaenoic acid (DHA) was monitored in real-time on a single cell level in cultivations of the heterotrophic algae *C. cohnii* [2]. This methods allows to quantify the product yield of a process instead of time consuming *off line* gas chromatography analyses. It further allows to optimize process conditions under consideration of population heterogeneity, as productive cells can be distinguished from weak cells fractions.

Multi-compartment scale-down reactors were used to investigate the influence of gradients, as they occur in large scale, on the morphological heterogeneity in *Saccharomyces cerevisiae* cultures and relate this to process performance [3]. Budding of yeast was monitored *in line* in batch cultivations with ISM [4]. The ratio of budding and total cells was successfully applied to differentiate between the different cultivation stages and to predict growth. Vitality and viability of such cultures can be measured with ISM.

In large-scale lactic acid bacteria production, the main concern is the uneven distribution of the pH value, mainly due to the external addition of base to compensate the acid produced by cells. In order to study the impact of these gradients on the population heterogeneity in various scales, different scale-down approaches were applied, especially multi-compartment systems and pulse-based parallel experiments. A relation was found between gradient formation in large scale cultivations and the development of the chain length formation of *Streptococcus thermophilus*, which allows for an *on line* estimation of the viability. A growth rate prediction based on the cell size distribution was feasible.

The last example shows how feedstock pre-treatment, in particular with ultrasound, can be optimized by particle size measurement with laserlight focus beam back reflection. The same technology allows to distinguish between circular and spiral-shaped organisms, which seems to be a promising feature for the investigation and finally control of co-cultures up to the large scale.

**4. Conclusions**

The information, which is gained by ISM and laserlight focus beam back reflection can be coupled with population balance models, which enable a model-based prediction of growth and production performance. Coupled with automated image analysis, real-time monitoring and the concept of “population control” [1] becomes possible for a wide range of bioprocesses.

**References**

1. F. Delvigne et al. FEMS Microbiol Lett. 365 (2018) 22.
2. A.M. Marbà-Ardébol et al. Process Biochem. (2017) 52 pp. 223–32.
3. A.M. Marbà-Ardébol et al. Yeast. (2018) 2 pp. 213–23.
4. A.M. Marbà-Ardébol et al. Microb. Cell Fact. (2018) 17 (1).