Single-cell level based approach to investigate bacterial metabolism during batch industrial fermentation

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Single-cell level based approach to investigate bacterial metabolism during batch industrial fermentation

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Introduction

Most of the data from *Escherichia coli* fermentations are based on the average measurement of the whole population, which can mask the distribution of the activity on the sub-population level. It is known that a population of genetically identical cells can exhibit different phenotypes under specific environmental conditions, however, studies concerning the segregation of starting populations into metabolically diversified sub-populations are scarce.

Acetate is a product of *E.coli* overflow metabolism when cells are grown under aerobic conditions in the presence of excess glucose. Minimizing the accumulation of acetate is critical in batch fermentation processes as this undesirable by-product has a negative affect on the growth, physiology, and performance of *E.coli*.

Monitoring the fate of glucose and acetate on the single-cell level will provide valuable insight into bacterial metabolism in the fermentation process; shedding more light on the differentiation of isogenic populations into sub-populations that exhibit different metabolic profiles.

Objectives

- To observe and quantify the *in situ* metabolism of glucose and acetate by *E.coli* during batch fermentation at the single cell level
- To check if bacterial sub-populations exist, that exhibit different metabolic strategies towards the investigated substrates
- To find out when the acetate uptake starts in the batch fermentation process and if all cells equally contribute to its consumption

Methods

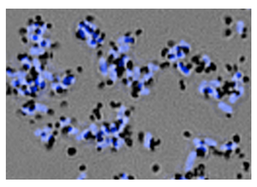
A pure culture of *E.coli* MG1655 was used to investigate *in situ* glucose and acetate metabolism at the single-cell level.

Fermentation 1

Batch fermentations (MOPS defined mineral media + 2.5 g/L glucose) were performed in order to examine bacterial metabolism on the consecutive stages of the fermentation process.

Samples for metabolic activity determination were taken at different time points during the fermentation process.

Substrate uptake determination 2



Incubation with ^{3}H-acetate / ^{3}H-glucose

The uptake of acetate/glucose at the single cell level was investigated *in situ* by means of **Microautoradiography (MAR)**.

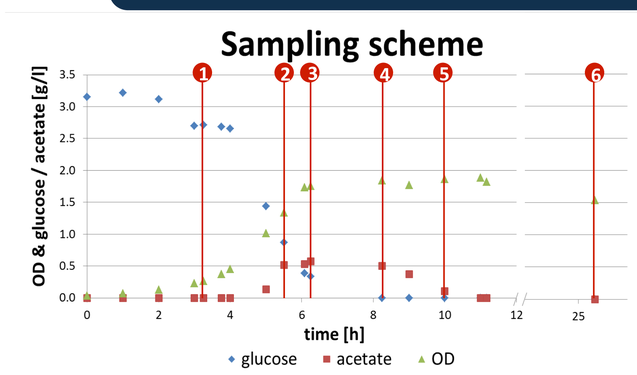
Microscopy & Image Analysis 3

Cells were stained with fluorescent dye (DAPI or PI) and observed under the microscope.
MAR signal was quantified with the help of freeware ImageJ.
Substrate uptake was evaluated by enumerating the number of silver grains within the defined boundary around individual *E.coli* cells.

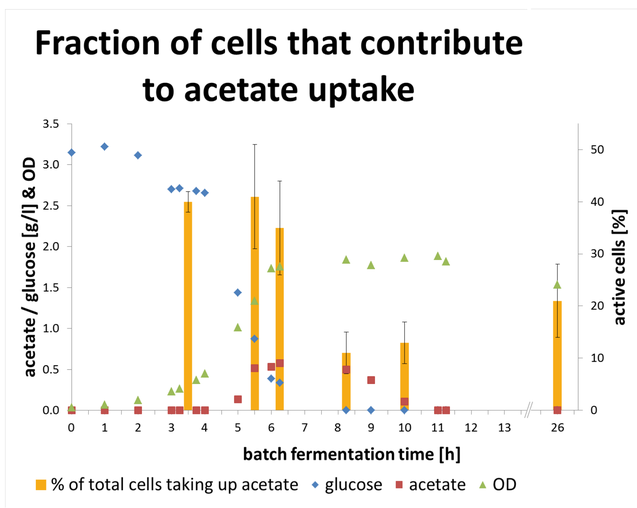
Conclusions

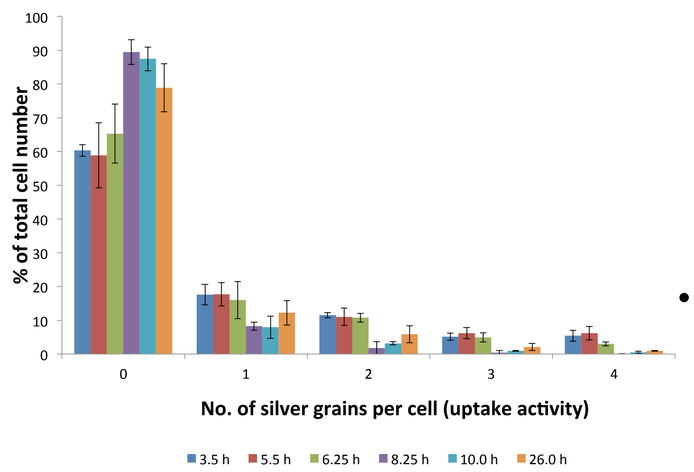
- **Heterogeneity in the uptake of both glucose and acetate exists in batch industrial fermentations and is present at each stage of the process**
- **Fermenting *E.coli* populations differentiate into sub-populations of cells exhibiting different metabolic strategies and different levels of metabolic activity**
- **The consumption of acetate during batch fermentation was shown to start surprisingly early in the exponential phase. This phenomenon has not been demonstrated before as previous studies, based on population average measurements, indicated that acetate uptake starts closer to glucose depletion.**

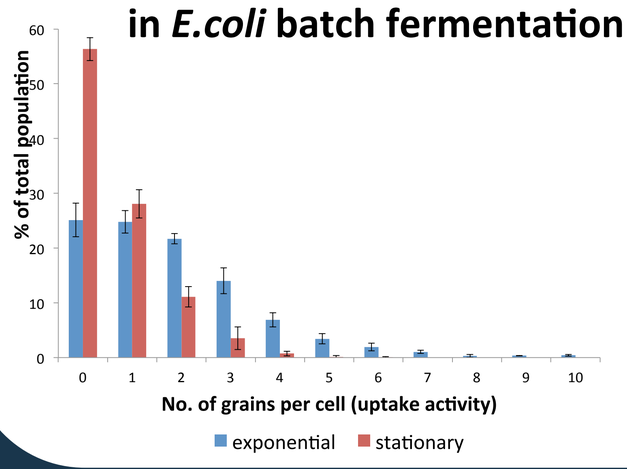
Results



Sample no.	Sample description
1	exponential glucose uptake
2	cease of exponential growth
3	glucose exhaustion
4	beginning of acetate uptake
5	advanced acetate consumption
6	starvation







- Acetate consumption starts early in the course of batch fermentation
- Only a sub-population of *E.coli* cells are able to take up acetate
- The number of cells taking up acetate during fermentation changes depending on the stage of the process
- As expected, the cells in exponential phase exhibit much higher metabolic activity than the cells in stationary phase
- A significant number of *E.coli* cells retain metabolic activity during the stationary phase
- There are a number of cells in exponential phase, which are thought to be active, that do not show substrate uptake