Small Metabolites Are Growth Inhibitors

Landwall and Homes (1977) reported small metabolites, which are accumulated in the media, are byproducts from the cells, and prevent cells from growing more than 30-40 g/l in the feed-batch mode. The small metabolites were confirmed to be lactate, pyruvate, succinate, propionate isobutyrate, and acetate by gas chromatography. They can be removed from the media by dialysis method, and by doing so, cell biomass increased to 140–150 g/l.

Specific growth rate smaller than the critical specific growth rate ($\mu < \mu_{\text{crit}}$) prevents accumulation of the growth inhibitory metabolites.

Paalme et al. observed that there is a critical specific growth rate ($\mu_{\text{crit}}$) causing the accumulation of byproducts in the media (different values in different cells). Since then, numerous trials were attempted to achieve the simplest and the most robust way of controlling the speed of cell growth. Korz et al. used a simple feed-batch technique utilizing a ‘pre-determined feeding rate of liquid medium’ to achieve a specific growth rate smaller than the critical specific growth rate ($\mu < \mu_{\text{crit}}$). The fermentation started with a simple batch with the initial media, followed by a fed-batch with an additional carbon source (glucose) and supplemented with micronutrients at a set specific growth rate ($\mu_{\text{set}}$) which was tested to be smaller than the $\mu_{\text{crit}}$.

$$m(t) = F(t)S_{f}(t)$$

$$F = \frac{\mu_{\text{set}}}{y_{X/S} + \mu_{\text{set}}}V_{f}X_{f}e^{\mu_{\text{set}}(t-t_{p})}$$

$m$: the mass flow of substrate (g h$^{-1}$)

$F$: the volumetric feeding rate (l h$^{-1}$)

$S_{f}$: the concentration of the substrate in the feeding solution (g l$^{-1}$)

$\mu$: the specific growth rate (h$^{-1}$)

$y_{X/S}$: the biomass/substrate yield coefficient (g g$^{-1}$)

$\mu_{\text{set}}$: the specific maintenance coefficient (g g$^{-1}$ h$^{-1}$)

$X$: the biomass concentration (g l$^{-1}$)

$V$: the cultivation volume (l)

![Fermentation profile of a Fed-Batch conducted in the CBR Fermentation Facility.](image)

The UBC CBR Fermentation Facility is equipped with bio-controllers which are capable of increasing the feed rates exponentially (Figure 1). The flow rates of variable pumps (0-700 ml/h) are controlled automatically by software programmed to determine the flow rate using Korz’s algorithm listed on the bottom left.

Result: The duration of the total fermentation was 28 hours. Final biomass was 157 g/L. In general, batch fermentations produce 8-12 g/L in LB and 30-40 g/L in SB.

Important Note

The high cell density fermentation requires chemically defined media. If the cells were grown in other media (such as LB or SB etc), it is highly recommend to prepare the glycerol stocks and starter cultures in the chemically define media. The CBR Fermentation Facility will provide the media upon request. The cost of the media will be added to the fermentation service fee.

Contact Information

If you have further questions/interests on this service, please contact Dr. Grieco at sungbye.grieco@ubc.ca.

References