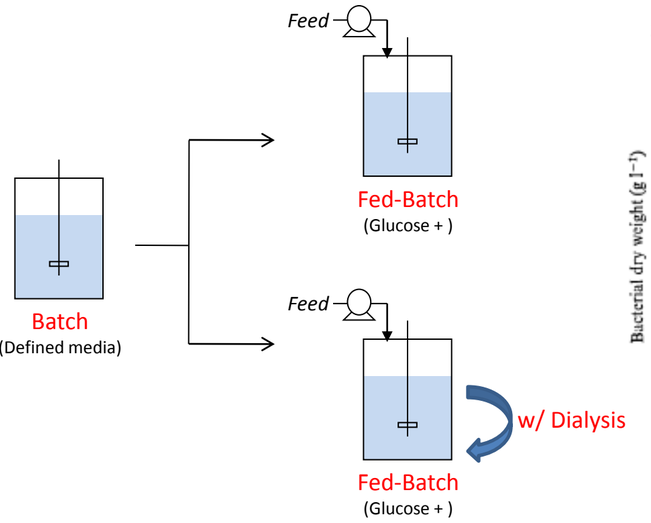
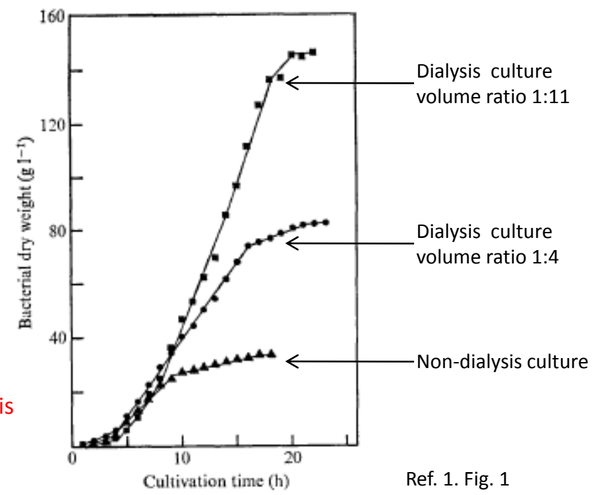
Posted by UBC Centre for Blood Research Fermentation Facility in January, 2012. Rm4303 (Office), Rm4332 (Lab) 2350 Health Sciences Mall, Vancouver, BC, V6T 1Z3, Canada Website: www.cbr.ubc.ca/fermenter.htm

High Cell Density Fermentation for *bacterial* culture: How to increase biomass to > WCW 100 g/l (OD 200)

Small Metabolites Are Growth Inhibitors

Landwall and Homes (1977) [1,2] 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 fed-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.





Ref. 1. Fig 1

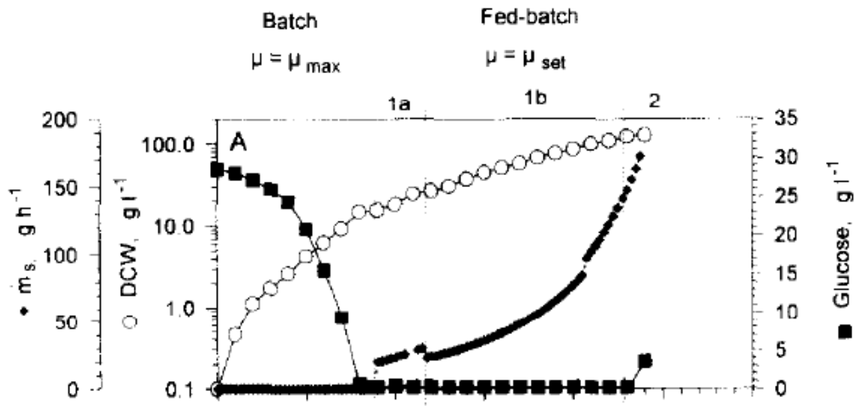
Specific growth rate smaller than the critical specific growth rate ($\mu < \mu_{crit}$) prevents accumulation of the growth inhibitory metabolites.

Paalme *et al.*[3] observed that there is a *critical* specific growth rate (μ_{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.*[4] used a simple fed-batch technique utilizing a 'pre-determined feeding rate of liquid medium' to achieve a specific growth rate smaller than the critical specific growth rate ($\mu < \mu_{crit}$). 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 (μ_{set}) which was tested to be smaller than the μ_{crit}.

$$m_S(t) = F(t)S_F(t)$$

$$= \left(\frac{\mu_{set}}{y_{X/S}} + m\right) V_{t_F} X_{t_F} e^{\mu_{set}(t-t_F)}$$

m_s: 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})
μ: the specific growth rate (h^{-1})
$y_{x/s}$: the biomass/substrate yield coefficient (g g^{-1})
m: the specific maintenance coefficient (g g^{-1} h^{-1})
X: the biomass concentration (g l^{-1})
V: the cultivation volume (l)



Ref. 4. Fig 1A.

See Our Examples (CBR Fermentation Facility)

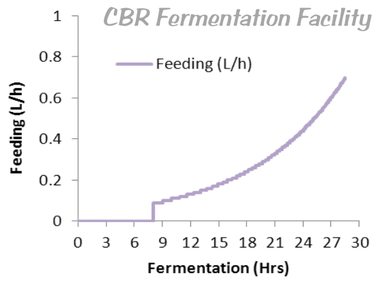


Figure 1. An example of the exponential feeding profile of a variable pump. The tested condition was μ_{set} = 0.10 h^{-1}, x = 132 g/l, V_0= 15 L. Feeding was set to started at 8 fermentation hrs.

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.

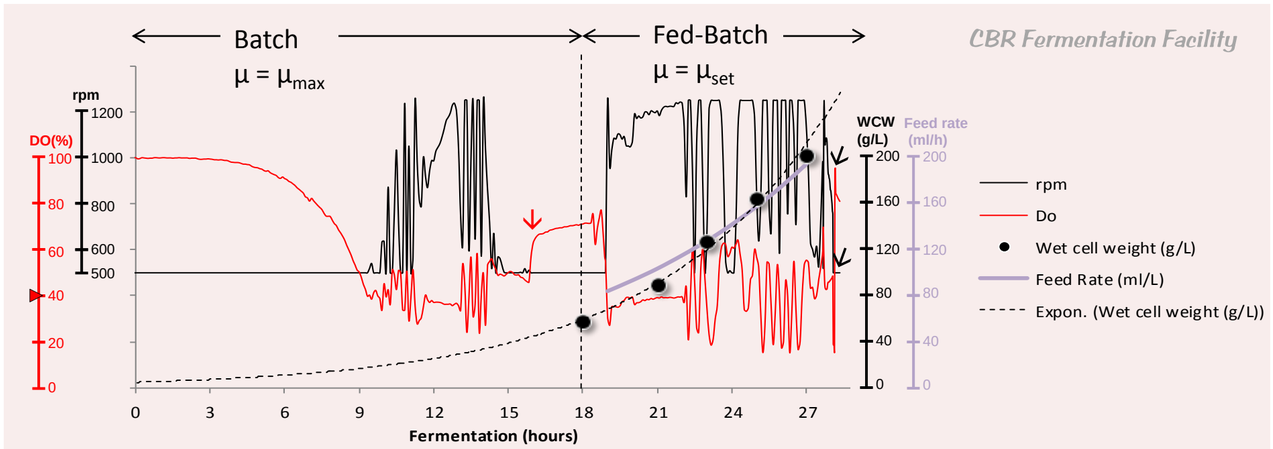


Figure 2. Fermentation profile of a Fed-Batch conducted in the CBR Fermentation Facility.

One example of high cell density fermentation conducted in our facility is shown in Figure 2. The fermentation condition was from Korz[4] and Seeger[5] with minor modifications. ***The batch fermentation*** lasted about sixteen hours. At the end of the batch fermentation, the DO level increased above the set point due to the depletion of the initial carbon source (red arrow, Fig. 2). ***The fed-batch fermentation*** was followed with the feeding of glucose and micronutrients, which was calculated at μ_{set} = 0.12 h^{-1}. It is crucial to grow cells dependent on a key nutrient (growth-limiting condition). This can be observed by a sharp increase of the DO level and instant decrease of the rpm to the minimal level upon the completion of feeding (black arrows).

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 sunghye.grieco@ubc.ca.

References

1. Landwall and Holme. Removal of inhibitors of bacterial growth by dialysis culture. (1977) J. General Microbiology 103, 345 – 352.
2. Landwall and Holmes. Influence of glucose and dissolved oxygen concentrations on yields of *Escherichia coli* B in dialysis culture. (1977) J. General Microbiology 103, 353 – 358.
3. Paalme *et al.* Glucose-limited fed-batch cultivation of *Escherichia coli* with computer-controlled fixed growth rate. (1989) Biotechnology and Bioengineering. 35, 312 – 319.
4. Korz *et al.* Simple fed-batch technique for high cell density cultivation of *Escherichia coli.* (1995) J. Biotechnology. 39, 59-65.
5. Seeger *et al.* Comparison of temperature- and isopropyl-β-D-thiogalacto-pyranoside-induced synthesis of basic fibroblast growth factor in high-cell-density cultures of recombinant *Escherichia coli*. (1995) Enzyme and Microbial Technology. 17. 947 – 953.