

***P. pastoris* fed-batch fermentation monitored with Online HPLC**

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Short description of the experiment: A recombinant enzyme was produced with *P. pastoris* in a fed-batch process using mixed substrates of glucose and methanol. The process consisted of a batch phase with glucose followed by fed-batch with mixed substrates. The fed-batch phase continued 40 hours while total fermentation time was 63 hours. Glucose and methanol levels were monitored using Online HPLC during fermentation. The Online HPLC was attached to the fermentor through a septum, and the sample size was 6 mL. Online HPLC was equipped with ICsep ICE-ION-310 Column (Transgenomic, USA), and a RI detector (Knauer, Germany). The mobile phase was 0.005 M H₂SO₄ with a constant flow rate of 0.4 mL min⁻¹. Temperature of the column was kept at 50 °C. Cell dry weight (CDW) and enzyme activity were measured offline. De-repression of *P. pastoris* methanol metabolism pathway can be seen (Figure 1.). Pre-programmed fed-batch phase with mixed substrates was initiated directly after the batch glucose was consumed. It can be seen that methanol accumulated in the reactor during the first hours of fed-batch while all the glucose that was fed in was consumed. During the last 25 hours of fed-batch phase both glucose and methanol were at limiting level. Cell growth and product accumulation were smooth throughout the fed-batch phase. It can be concluded that Online HPLC is an applicable tool for monitoring the substrate levels during a fermentation and with additional tools can give valuable insight into process dynamics.

ONLINE HPLC

P.pastoris fed-batch

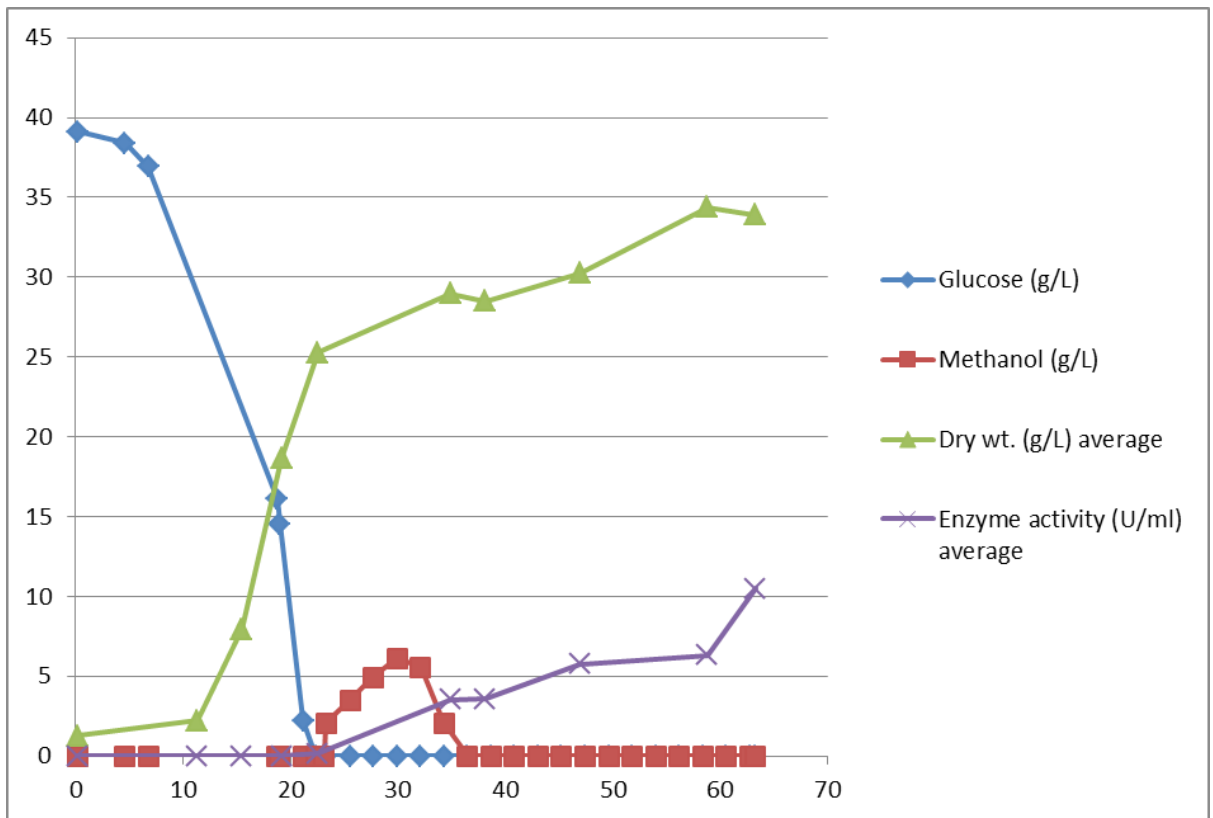


Figure 1. Online HPLC measurements (glucose and methanol) from *P. pastoris* fermentation. Cell dry weight (CDW) and product concentrations are included in the figure as well