Adaptive control of glycerol & methanol feeding in recombinant *Pichia pastoris* cultures: Impact on antibody titre

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Abstract

Pichia pastoris processes for heterolougous protein expression are typically run in three phases: a batch phase, an exponential feeding fed-batch phase and a long oxygen transfer limitation (OTL) phase. The final protein titre is often limited by the oxygen availability in the OTL phase. In this paper, a direct adaptive controller is designed for the OTL phase. The controller was validated in pilot *P. pastoris* cultivations expressing a single chain antibody fragment (ScFv). This work shows that the proposed controller can regulate the dissolved oxygen tension (DOT) at very low levels (5 %) with high stability by manipulating the glycerol feeding rate, thereby enhancing the oxygen transfer at high cell density, which finally leads to a higher antibody titre.

Introduction

Pichia pastoris is currently viewed as a very promising host for heterologous protein expression[1] since it can be easily manipulated at molecular genetic level, it has the ability to express high levels of proteins, either intra or extracellularly and it has the capability to perform eukaryotic post-translational modifications[1,2].
P. pastoris is normally cultivated in fed-batch mode in order to limit the amount of substrate in the reactor, being able to grow at very high cell densities with massive oxygen uptake requirements, turning the oxygen transfer capacity into a critical limitation factor.
Once the maximum oxygen transfer rate is reached, regulation of DOT at very low values by manipulating the carbon source feeding is performed, which has been proposed by several authors[3–5].

Process control feeding strategy

The control of *P. pastoris* cultivation is based on carbon source limitation, based in 3 major steps:

- Cultivation in batch mode with an initial substrate concentration $\sim$ 40g/l – glycerol batch (GB) phase;
- Glycerol feeding according to an exponential profile – glycerol fed-batch (GFB) phase;
- DO control through an adaptive DO-stat feeding controller – oxygen transfer limitation (OTL) phase

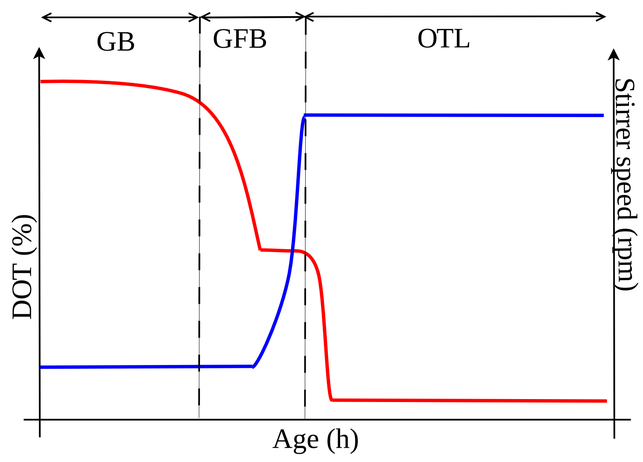


Figure 1: Schematics of the process control (through stirrer speed manipulation) and fermentation phases.

In GB and GFB phases, dissolved oxygen concentration is controlled in cascade mode by:

- Overhead pressure between 80 and 500 mbarg to regulate dissolved oxygen level to 95 % of saturation;
- Agitation rate between 320 and 1000 rpm to regulate the dissolved oxygen level to 50 % of saturation.

At the end of the GFB, the stirrer speed reaches the maximum value of 1000 rpm and DO starts to drop thereby signaling the beginning of OTL phase. In this latter phase, the aforementioned feeding controller was implemented.

Adaptive DO-stat glycerol feeding controller

Material mass balance:

$$\frac{dC_O}{dt} = -\frac{F}{V}S_0 Y_{O/S} + k_L a\left(C_O^* - C_O\right) \rightarrow \frac{dx}{dt} = -a_p x + k_p F$$

Model reference design:

$$\frac{dx}{dt} = \frac{x^* - x}{\tau_c} \longrightarrow \frac{d\hat{x}}{dt} = \frac{x^* - \hat{x}}{\tau_c}$$

Controller equation:

$$F = \theta(t)\,x + K(t)\,x^* \longrightarrow F = \hat{\theta}(t)\,x + \hat{K}(t)\,x^*$$

General considerations:

- Glycerol accumulation is negligible;
- Dynamics of k_P and a_P much lower than $x \longrightarrow$ linear quasi time-invariant system[6];
- k_P and a_P are considered as piecewise time-varying unknown parameters;
- System uniformly stable in the Lyapunov sense.

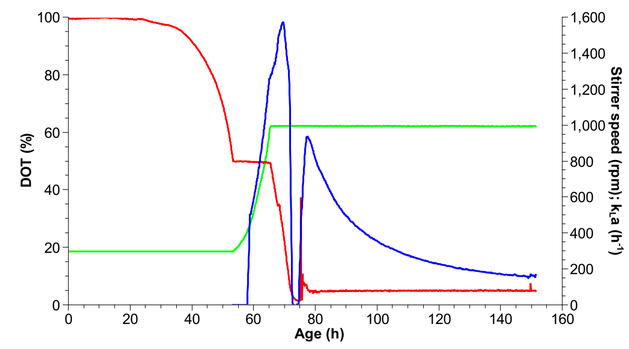
Results

Table 1: The effect of different factors on productivity

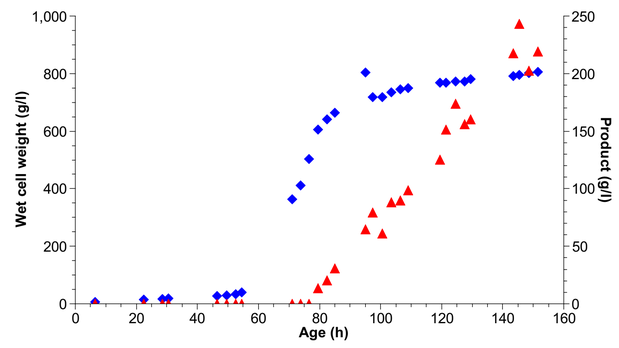
DOT (%)	pH	Medium	Starting biomass (g/l)	Productivity (mg/l)
5	5	Defined	503.8	219.0
5	5		575.8	187.0
5	5	Complex	153.1	238.1
5	4		238.5	325.9
10	5		92.3	440.9
30	5		132.3	252.4

- Decrease in pH $\rightarrow$ increase in productivity (inhibition of proteolysis)[7];
- Decrease in DO-set point $\rightarrow$ increase in productivity;
- Defined medium $\rightarrow$ decrease in productivity (when compared to complex medium);
- Initial biomass concentration $\rightarrow$ no visible correlation on the impact in productivity.

Results (continuation)



(a)



(b)

Figure 2: Fermentation variables' profiles: (a) stirrer speed (green), DOT (red) and k_La (blue); (b) wet cell weight (blue) and product (red).

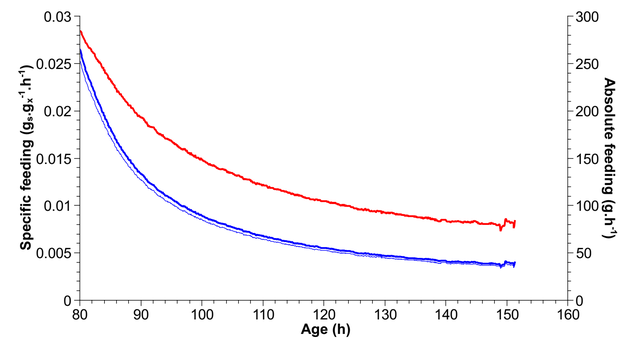


Figure 3: Absolute feeding (red) and specific glycerol feeding for DOT set point of: 5 % (thin blue) and 0 % (blue).

- Specific feeding close to the theoretical maximum (DOT = 0 %) $\rightarrow$ increase in productivity when compared with fermentations without such control

Conclusions

- Adaptive controller derived, proven to regulate DOT at low levels;
- Maximisation of oxygen mass transfer by carbon source limitation;
- Increase of process productivity and final antibody fragment titre.

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