

CellMaker Regular Application Note:



Increasing protein expression yields of a 44kDa His-tagged protein in *Pichia pastoris* in a single-use bioreactor (CellMaker Regular) rapid, proof-of-concept experiment.

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Introduction

Pichia pastoris is a strain of yeast that is very often preferred to *Saccharomyces cerevisiae*, other yeast strains, and *E. coli* because it generates more human-like glycosylation patterns/structures within recombinant proteins. As a result it has become a major method of recombinant human protein expression. Expression is driven by the tightly regulated AOX1 promoter, which is induced by methanol. Secretion of recombinant protein (directed by a signal sequence) into the media simplifies the downstream protein purification process.

The CellMaker Regular is a single-use bioreactor offering considerable ease-of-use and is designed to give high protein yield in oxygen-hungry bacterial and yeast expression systems. The system is available in both 8 litre and 50 litre formats. The following results were generated using the smaller system.

Experimental details

The target protein (44kDa) contains a C-terminal His-tag. It is secreted into the supernatant using alpha-factor signal sequence. The expression level are very low (<50 µg/L) when produced in shaker flasks. A modified BMMY media was used and supplemented by the addition of 1% MeOH and 0.1% glucose every 24 hours.

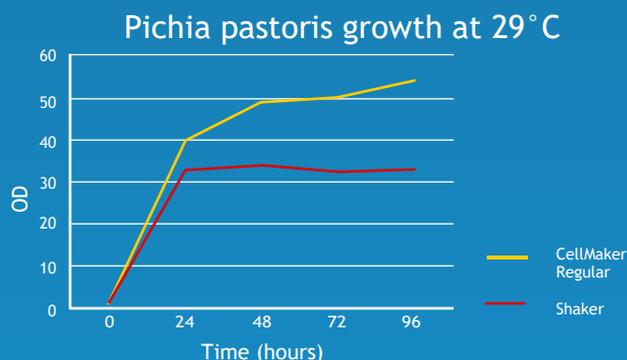
The table (below) outlines the settings and readings from the Cellexus CellMaker during this experiment.

TIME (hours)	OD [Shaker]	Set Temp	Process Temp	Gas Mix (Air/O ₂ /Headspace) (all L/min)	Pressure (mBar)
0	0.94 [0.94]	29	29	4/4/1	40
24	40 [33]	29	29	2/4/1	50
48	49 [33.8]	29	29	2/4/1	50
72	50 [32.5]	29	29	2/4/1	50
96	54 [33]	29	29	2/4/1	50

Results

The graph below shows the increased OD and relates to the yield of biomass compared to shaker flasks prior to harvesting from a 6 litre fermentation.

In this sample enhanced aeration was achieved using an oxygen enriched gas supply (from the built-in oxygen generator) at 4l/min oxygen and 2l/min of air. Higher ODs resulted in 1.5-fold increase in biomass and 2-fold increase in protein yield (as determined by western blot analysis). Protein was found to be glycosylated correctly and was secreted appropriately into the medium prior to purification.



Conclusions

The data generated here shows that the 2-fold increase in expression levels of protein can be quickly achieved by switching from shaker flasks to a single-use bioreactor in a proof-of-concept of this type. With additional optimisation using this bioreactor it is likely that the yield of protein will be further enhanced. Scaling from the 8 litre unit to the identically designed 50 litre unit may offer a further 6-fold increase in yield.

Data kindly provided by Dr. Maxim Rossmann, Laboratory of Molecular Biology, Cambridge, UK.

For further details, or to request a quotation, contact us now.

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