

# 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 levels are very low (<50 ug/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.

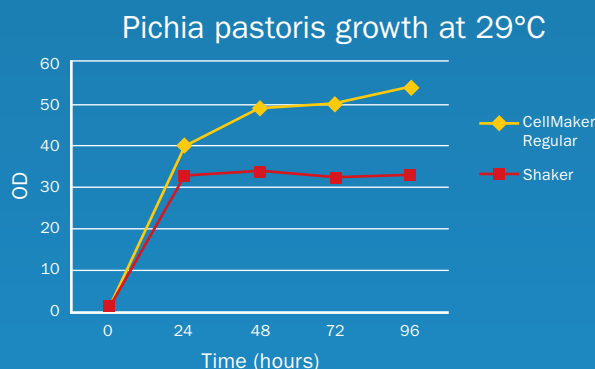
The table (below) outlines the settings and readings from the Cellexus Cellmaker during this experiment:

| TIME (hours) | OD [Shaker] | Set Temp | Process Temp | Gas Mix (Air/O <sub>2</sub> /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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