



VHH EXPRESSION CASE STUDY



METHANOL-FREE & METHANOL-INDUCED BIVALENT VHH PRODUCTION IN PICHIA PASTORIS



Case Study I: Development of a short, methanol-free Pichia protein production process for maximization of volumetric productivity

A *Pichia pastoris* strain expressing a bivalent VHH furnished high yields of 12 g/L in culture supernatant under methanol-free conditions, with significantly reduced cultivation time of just 64 hours. This technology is ready for commercial use and is transferable for large scale manufacturing.

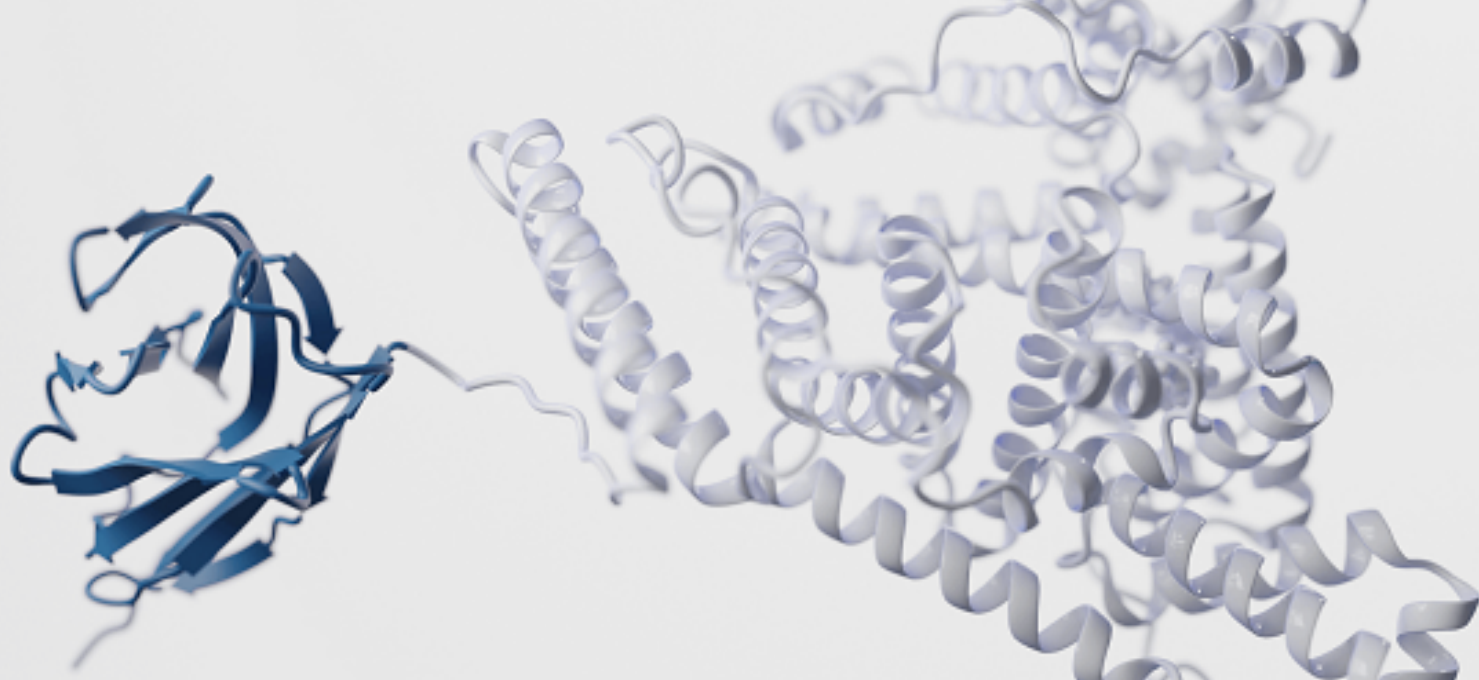
- 12 g/L of secreted VHH in culture supernatant
- 64-hours process time
- High space-time yield of 96 mg/L/h



Case Study II: Development of a methanol-free Pichia protein production process for maximization of product titer and specific productivity

Based on applying VALIDOGEN's standard fermentation protocol spanning 111 hours, the development of an optimized feeding strategy to maximize product titer, resulted in a yield of 19 g/L of secreted bivalent VHH, while maintaining a high space-time yield.

- 19 g/L of secreted VHH in culture supernatant
- 111-hours process time
- High space-time yield of 95 mg/L/h & increase in specific productivity by 14%
- In comparison to non-optimized VALIDOGEN standard process, product titer was increased by 36%



Maximizing space-time yield for protein production with *Pichia pastoris* in short (64h) and VALIDOGEN standard cultivation times (111h)

VALIDOGEN's MeOH-free AOX1 promotor variants from our [UNLOCK PICHIA® toolbox](#) present a unique solution to achieve high protein expression levels in the absence of methanol. These promoters, along with other toolbox elements such as platform strains, helper factors, secretion signals, and expression strategies, work synergistically to enhance strain productivity when applied in optimized combinations. The case studies, which were carried out in collaboration with Boehringer Ingelheim, highlight the potential of our methanol-free processes, offering high yields and exceptional volumetric productivity in short batch cultivations, ideal for scenarios with downstream limitations.

For maximizing product titer, our novel feeding strategy enables significant increases within standard cultivation times. VALIDOGEN's methanol-free processes are unmatched and tailored to meet specific goals such as optimizing titer, reducing process time, or aligning with downstream capacity.

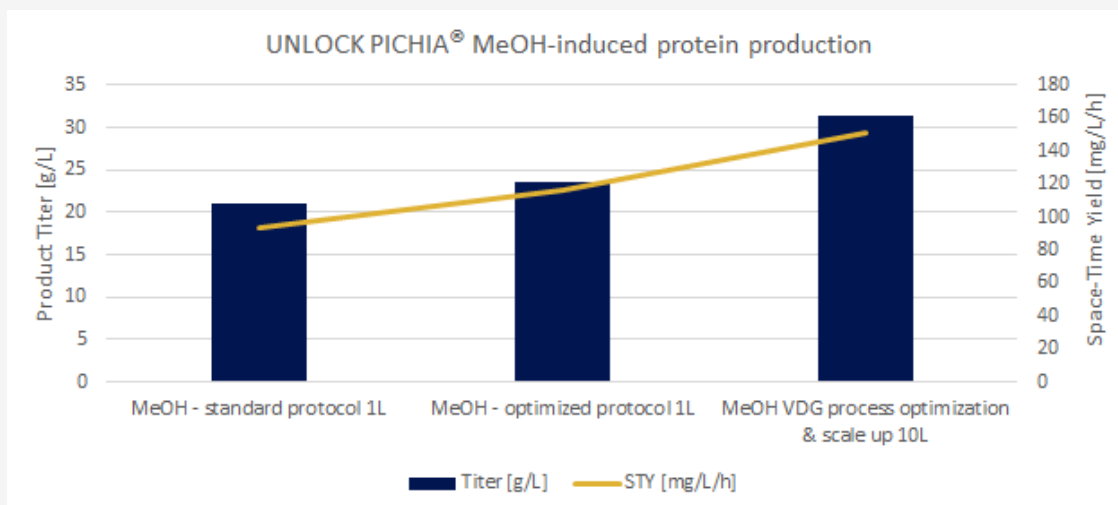
Key advantages UNLOCK PICHIA® MEOH-free processing

- VALIDOGEN's UNLOCK PICHIA® technology provides effective methanol-free protein production processes with maximized space-time yield
- UNLOCK PICHIA® methanol-free processes are based on AOX1 promoter variants, but use glycerol throughout the process (glucose is also possible)
- No metabolic burden from switch of carbon source
- High titer achieved in an exceptionally short cultivation time (64h)
- Optimized standard cultivation strategies (111h) maximize product titer and specific productivity



Case Study III: Development of a methanol-induced *Pichia* protein production process for maximization of product titer and specific productivity

- 21 g/L of bivalent VHH applying one of our generic standard protocols
- 31 g/L in just 111 hours cultivation though advanced process optimization
- Increase in specific productivity by 80%



This case study aimed to develop a high-performance methanol-inducible production process for the expression of a bivalent VHH

Using a VALIDOGEN generic standard protocol 21 g/L of bivalent VHH was secreted in 1 L bioreactors. In an initial development phase existing VALIDOGEN optimization protocols were applied to this strain in small-scale trials. This resulted in an increase of yield to 24 g/L, and a notable improvement in specific productivity.

By subsequently applying a targeted process development and optimization strategy at the 10 L bioreactor scale we achieved an impressive titer of 31 g/L, alongside a remarkable ~80% improvement in specific productivity compared to the standard methanol process. A customized methanol feeding strategy was developed specifically for this strain, enabling both a successful scale-up and further enhancement of productivity.

These results demonstrate the exceptional potential of VALIDOGEN's UNLOCK PICHIA® platform and *Pichia* expert know-how to develop high-performance protein production processes for commercial application.

Our platform provides extensive customization options, including both methanol-induced and methanol-free expression strategies. This flexibility, combined with our expertise in Pichia process development & optimization, enables us to develop robust, scalable, and economically viable processes that significantly reduce the cost of goods for protein manufacturing.

Please contact us if you are interested to learn more about VALIDOGEN's UNLOCK PICHIA® technology or our services directly by speaking to our team.

Iskandar Dib, Principal Scientist & BD Manager, iskandar.dib@validogen.com

Rosie Maddock, BD Manager, rosie.maddock@validogen.com