



CASE STUDY I & II

METHANOL-FREE BIVALENT VHH PRODUCTION IN PICHIA MAXIMIZING PRODUCT TITER AND SPACE-TIME YIELD



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.

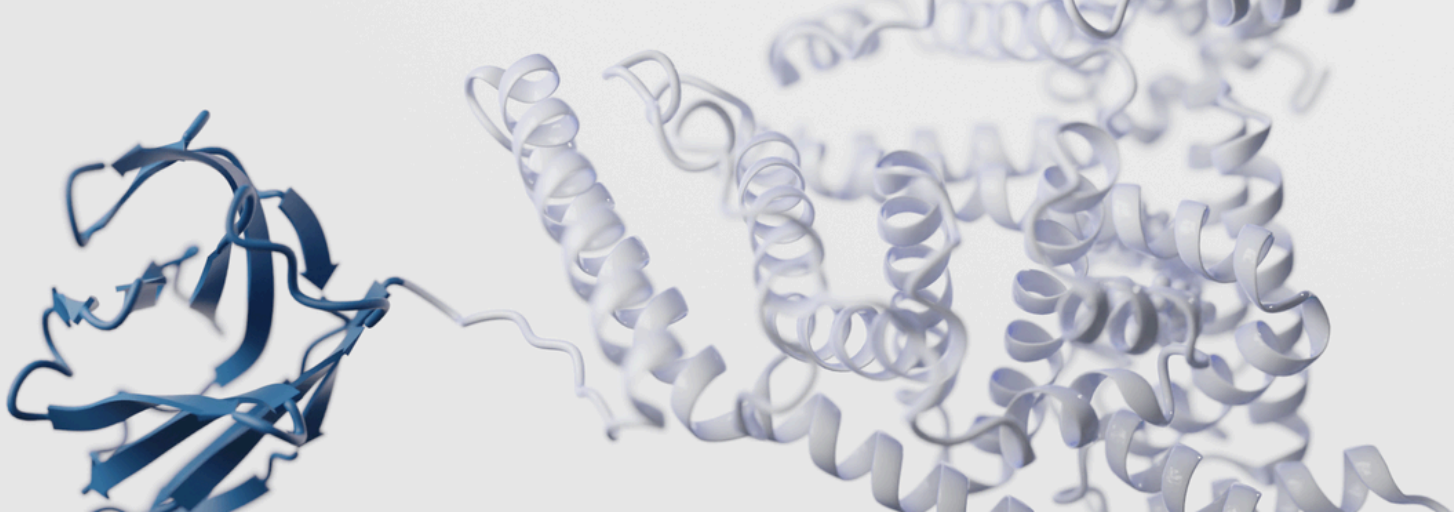
- 12 g/L of secreted VHH in culture supernatant
- 64-hours process time
- High space-time yield of 96 mg/L/h



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

Please contact us if you are interested to learn more about VALIDOGEN's methanol-free technology or our services directly by speaking to our team.

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