



CASE STUDY

SHORT CULTIVATION TIME AND HIGH TITER

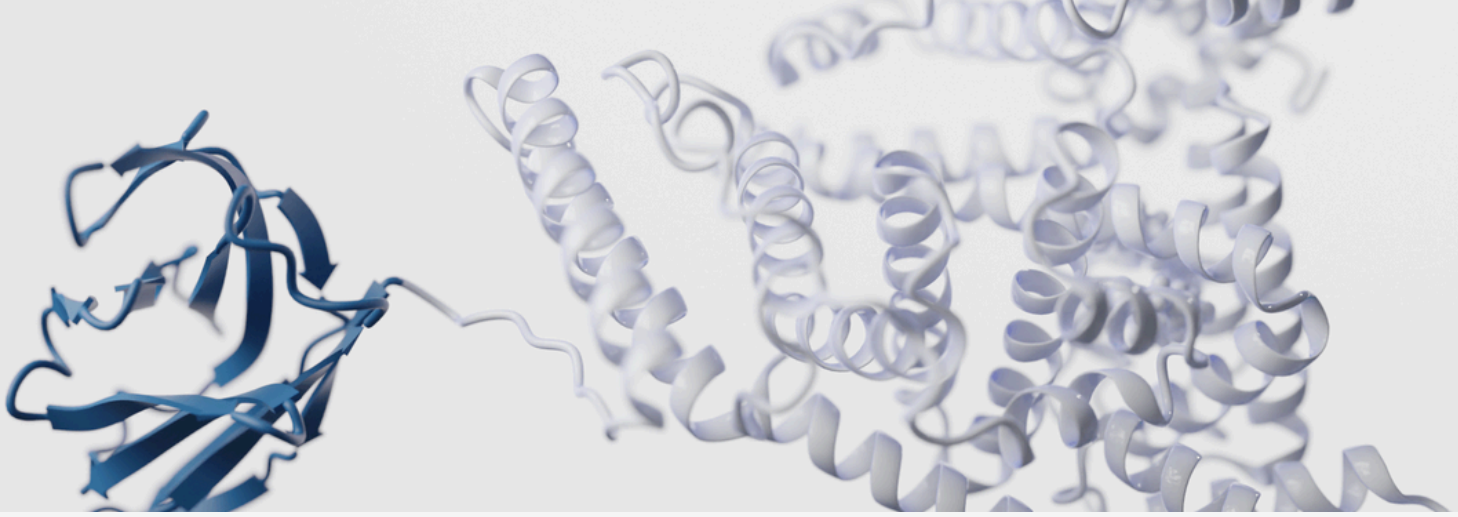
PROTEIN PRODUCTION IN PICHIA WITH MAXIMIZED SPACE-TIME-YIELD

KEY FACTS SUMMARIZING CASE STUDY RESULTS

- 12 g/l of secreted bivalent VHH was produced in a 64 h methanol-free protein production process
- No metabolic burden from switch of carbon source
- By targeted application of VALIDOGEN's UNLOCK PICHIA® toolbox final product titer was increased 5-fold

KEY ADVANTAGES

- 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, no methanol feed is required



VALIDOGEN's ADVANCED STRAIN DEVELOPMENT PROGRAM with manufacturability in mind

Targeted and time-saving generation of high-productivity strains is an integral part of effective bioprocess development. VALIDOGEN is addressing today's protein manufacturing needs by early consideration of production strain and process requirements.

The latest development & research programs (performed within Boehringer Ingelheim's Vienna and VALIDOGEN's joint technology program leveraging expertise from both companies) focus on the targeted development of bioreactor cultivation processes for protein production with *Pichia pastoris* (also known as *Komagataella phaffii*) under methanol-free conditions with an emphasis on maximizing volumetric productivity (space-time yield).

DEVELOPMENT OF SHORT, METHANOL-FREE PROTEIN PRODUCTION PROCESSES FOR MAXIMIZATION OF VOLUMETRIC PRODUCTIVITY

In this case study, a VHH-expressing strain was used to demonstrate that MeOH-free cultivations based on our AOX1 promoter variants can deliver high product yields (>10 g/l of culture supernatant) with considerably shortened cultivation times (~60 h). Additionally, it shows how the targeted application of the UNLOCK PICHIA toolbox helps to incrementally boost productivity by identifying optimum combinations of different elements such as promoter variants, helper factors, secretion signals, and expression strategies. This technology is proofed to be ready for commercial use and large scale manufacturing at both parties.

Please contact us if you are interested to learn more about VALIDOGEN's strain development programs or any other services directly by speaking to our team.

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