

# Pichia protein production

**BIOPROCESS DEVELOPMENT** Recombinant protein production in *Pichia* typically relies on AOX1 promoter-driven expression using methanol for induction. However, some companies avoid methanol due to safety concerns or operational demands. VALIDOGEN's unique AOX1 promoter variants enable methanol-free protein production at high space-time yields, offering advantages beyond safety. Latest case studies underscore the strength of this technology.

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Methanol-induced protein expression in *Pichia pastoris*, leveraging the strong AOX1 promoter, is a highly efficient system for producing recombinant proteins. This approach is well-established and scalable, making it suitable for both laboratory and industrial-scale bioreactors. However, due to safety concerns and the operational challenges associated with methanol handling, recent advancements have focused on developing methanol-free alternatives.

## Methanol-free expression

VALIDOGEN develops high-performance MeOH-induced and MeOH-free *Pichia* protein production strains for various industries. Their MeOH-free system utilizes a specialized subset of their library of AOX1 promoter variants, eliminating the need for methanol while maintaining the beneficial characteristics of the original AOX1 promoter.

Unlike constitutive promoters, this system enables strong, time-controlled initiation of protein expression while reducing the metabolic burden from carbon source switching, as well as minimizing oxygen consumption and heat evolution. This technology is already in commercial use in bioreactors up to 100,000L.

## Process Intensification

In collaboration with Boehringer Ingelheim, VALIDOGEN has developed op-

timised MeOH-free bioreactor cultivation processes. A recent collaborative case study highlighted two of these approaches: one process in bacterial-like fermentation time (64 hours), and one in VALIDOGEN standard process time of 111 hours, both applied for secreting a bivalent VHH.

The optimised MeOH-free short cultivation process achieved high product yields of 12 g/L and significantly increased space-time yield. The op-

timised 111-hour process, applying a specialised feeding strategy to maximise product titer, achieved a yield of 19 g/L, while maintaining high space-time yield.

VALIDOGEN's methanol-free processes provide a safe, efficient, and flexible alternative to conventional methanol-based or constitutive *Pichia* expression systems, offering enhanced safety and streamlined efficiency without compromising performance. ■



**UNLOCK PICHIA® protein production strain generation and process development at VALIDOGEN: high-yield expression strains and intensified processes considering safety, economic efficiency, and regulatory requirements. Serving industries including biopharma, food and feed, industrial biotechnology, and diagnostics**