Improving phytase production in Pichia pastoris fermentations through de-repression and methanol induction optimisation

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Abstract

Pichia pastoris (*Komagataella phaffii*) is a fast-growing methylotrophic yeast with the ability to assimilate several carbon sources such as methanol, glucose, or glycerol. It has been shown to have outstanding secretion capability with a variety of heterologous proteins. In previous studies, we engineered *P. pastoris* to co-express *E. coli* AppA phytase and the HAC1 transcriptional activator using a bidirectional promoter. Phytase production was characterised in shake flasks and did not reflect industrial conditions. In the present study, phytase expression was explored and optimised using instrumented fermenters in continuous and fed-batch modes. First, the production of phytase was investigated under glucose de-repression in continuous culture at three dilution factors, 0.5 d^{-1}, 1 d^{-1}, and 1.5 d^{-1}. The fermenter parameters of these cultures were used to inform a kinetic model in batch and fed-batch modes for growth and phytase production. The kinetic model developed aided to design the glucose feeding profile of a fed-batch culture. Kinetic model simulations under glucose de-repression and fed-batch conditions identified an optimal phytase productivity at the specific growth rate of 0.041 h^{-1}. Validation of the model simulation with experimental data confirmed the feasibility of the model to predict phytase production in our newly engineered strain. Methanol was used only to induce the expression of phytase at high cell densities. Our results showed that high phytase production required two stages, the first stage used glucose under de-repression conditions to generate biomass while expressing phytase, and stage two used methanol to induce phytase expression. The production of phytase was improved 3.5-fold by methanol induction compared to the expression with glucose alone under de-repression conditions to a final phytase activity of 12.65 MU/L. This final volumetric phytase production represented an approximate 36-fold change compared to the flask fermentations. This two-phase strategy allowed to optimise phytase productivity by producing phytase during both growth and methanol induction phases. Finally, the phytase protein produced was assayed to confirm its molecular weight, and pH and temperature profiles. This study highlights the importance of optimising protein production in *P. pastoris* when using novel promoters and presents a general approach to performing bioprocess optimisation in this important production host.

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