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Fermentation strategy for enhancing phytase production under D-mannitol co-induction in *Pichia pastoris*

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The addition of D-mannitol with methanol as a co-substrate at the induction phase during fermentation by *Pichia pastoris* (Mut+) is a new beneficial technology for recombinant protein production. The major challenges as heat generation and high oxygen demand are increasing during induction phases with methanol at large scale and this causes high oxygen demand during cultivation. One possible way to reduce the oxygen demand for getting more protein productivity is the addition of D-mannitol along with methanol during induction time. The promoter of alcohol oxidase I (*AOX1*) gene in *P. pastoris* is exclusively matched for the controlled expression of foreign genes and the high levels of foreign proteins can be expressed, even if they are lethal to the cell. The co-feeding strategy was optimized to produce phytase activity of 10280 U/ml compared to methanol fed alone and which could be used as food additives for non-ruminant animals and deep understanding of the regulation of *AOX1* promoter and the physiology of the cells which are being used to govern protein.

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