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Methanol Independent Expression by *Pichia Pastoris* Employing Derepression Technologies

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Abstract:

Methanol is a well-established carbon source and inducer for efficient protein production employing *Pichia pastoris* (*P. pastoris*) as a host on micro-, lab and industrial scale. However, due to its toxicity and flammability, there is a desire to avoid methanol while maintaining the high productivity of *P. pastoris*. Small scale bioreactor cultivations (0.5 – 5 L working volume) are commonly used to evaluate a strain and its protein production characteristics since microscale cultivation in deep well plates can be hardly controlled or relies on expensive equipment. Furthermore, traditional protocols for the cultivation and induction of *P. pastoris* were established for constitutive expression or methanol induction and so far, no reliable protocols were described to screen *P. pastoris* expression strains with derepressible promoters in (controlled and monitored) parallel cultivations. To simplify such initial cultivations to characterize and compare new protein production strains, we established a simple shake flask cultivation system for methanol free expression that simulates bioreactor conditions including a constant slow glycerol feed and online monitoring, thereby coming closer to the real conditions in bioreactors compared to mostly applied small scale batch cultivations. To drive recombinant protein expression in *P. pastoris*, the carbon source repressed promoters P_{DC} and P_{DF} were applied. Polymer discs with embedded carbon source, releasing a constant amount of glycerol, assured a feed rate delivering the necessary energy for maintaining the promoters active while keeping the biomass generation low.

Keywords: *Pichia pastoris*, *Komagataella phaffii*, promoter, derepression, feed control, shake flasks, cultivation, bioengineering, Issue 143