



Increased production of alkaline polygalacturonate lyase in the recombinant *Pichia pastoris* by controlling cell concentration during continuous culture

Huilin Wang^{a,b}, Jianghua Li^c, Long Liu^c, Xiaoman Li^{a,b}, Dongxu Jia^c, Guocheng Du^{c,d,*}, Jian Chen^{c,d,*}, Jiangning Song^{a,b,e,*}

^a National Engineering Laboratory for Industrial Enzymes, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

^b Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

^c Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China

^d State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China

^e Department of Biochemistry and Molecular Biology, Faculty of Medicine, Monash University, Melbourne, VIC 3800, Australia

HIGHLIGHTS

- ▶ Two continuous culture strategies, CCCM and CCDD, were developed in the *Pichia pastoris*.
- ▶ Cell concentration is a key parameter for PGL over-production in the *P. pastoris*.
- ▶ Kinetic parameters for controlling cell density by CCCM and CCDD culture were solved.
- ▶ The CCCM culture exhibited higher PGL productivity and cell viability.
- ▶ Using the continuous mode, protease accumulation was decreased.

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ABSTRACT

Recombinant alkaline polygalacturonate lyase (PGL) production by recombinant *Pichia pastoris* GS115 was selected as a model to study as a continuous culture strategy for enhancing heterologous protein production based on controlling methanol feeding (CCCM culture) or on dual carbon source feeding (CCDD culture). Using the CCCM process with a dry cell weight of 75 g/L regulated by controlling methanol concentration in the induction media, the final PGL activity was 441.9 U/mL. The PGL productivity (Q_p) and the average specific enzyme production rate (Q_x) were 4.65 U mL⁻¹ h⁻¹ and 84.5 U g⁻¹ h⁻¹, an increase of 42.1% and 191.2%, respectively, over what was achieved with traditional fed-batch culture with high cell density. The control strategies also reduced proteolytic degradation by 84.1% in the fermentation broth and increased cell viability by 12.2%.

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1. Introduction

The methylotrophic *Pichia pastoris* is one of the most effective and versatile organisms for the expression of recombinant proteins (Potvin et al., 2012). Methanol metabolism of *P. pastoris* is tightly regulated by a methanol-inducible promoter, which enables to achieve high expression levels of the AOX1 gene and heterologous

proteins (Cregg et al., 1993). Compared to *Escherichia coli*, *P. pastoris* has the advantage of post-translational modifications including disulfide bond formation and glycosylation. Additionally, heterogenous proteins can be readily secreted to the medium, making this organism useful for expression of toxic recombinant proteins. Over 500 recombinant proteins successfully expressed by *P. pastoris* till 2005 (Plantz et al., 2006). The complete genome of *P. pastoris* GS115 strain has been sequenced, facilitating the investigation of *P. pastoris* and genetic manipulation (De Schutter et al., 2009).

A variety of factors to enhance the yield of heterologous proteins have been investigated (Potvin et al., 2012). The expression level of foreign proteins is affected by cultivation conditions, notably methanol concentration, temperature, pH and dissolved oxygen concentration. *P. pastoris* has been grown in fed-batch and continuous culture. In the fed-batch culture, the strategies of

* Corresponding authors. Address: National Engineering Laboratory for Industrial Enzymes, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China. Tel./fax: +86 510 8591 8309 (G. Du and J. Chen), tel./fax: +86 22 2482 8711 (J. Song).

E-mail addresses: gcd@jiangnan.edu.cn (G. Du), jchen@jiangnan.edu.cn (J. Chen), song_jn@tib.cas.cn (J. Song).