Bioresource Technology 118 (2012) 257–264

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Development of an industrial medium and a novel fed-batch strategy for high-level expression of recombinant β-mananase by *Pichia pastoris*

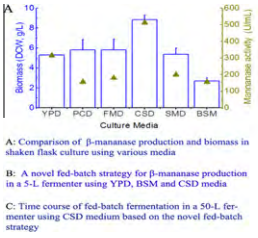
Jia Zheng [a], Wei Zhao [a,b], Ning Guo [a], Fulai Lin [a], Jian Tian [a], Lishuang Wu [a], Hongbo Zhou [a,b,*]

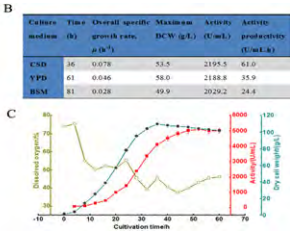
[a] School of Minerals Processing and Bioengineering, Central South University, Changsha 410083, China
[b] Key Laboratory of Biometallurgy of Ministry of Education, Central South University, Changsha 410083, China

## HIGHLIGHTS

- A novel industrial medium was developed for high-density fermentation of *Pichia pastoris*.
- A fed-batch strategy combining the real-time exponential feed and DO-stat feed mode was developed.
- The high-level expression of β-mananase was obtained in a 50-L fermenter.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 2 March 2012
Received in revised form 13 May 2012
Accepted 14 May 2012
Available online 23 May 2012

*Keywords:*
*Pichia pastoris*
Mannanase
Fed-batch cultivation
pGAP

## ABSTRACT

An industrial medium, Corn Steep Liquor Powder Dextrose (CSD medium) was developed for constitutive expression of recombinant β-mananase by *Pichia pastoris*. The β-mananase activity (513 U/mL) with CSD medium was 1.64- and 2.5-fold higher than with YPD and BSM in shaken flasks. The β-mananase productivity with CSD medium was 61.0 U/mL h, which was 1.7- and 2.5-fold higher than with YPD and BSM in a 5-L fermenter based on a novel fed-batch strategy combining the real-time exponential feed mode with the DO-stat feed mode. The β-mananase activity, dry cell weight and the recombinant enzyme reached up to 5132 U/mL, 110.0 g/L and 4.50 g/L after 50 h cultivation in a 50-L fermenter. The high efficient expression of recombinant β-mananase by *P. pastoris* indicated that CSD medium and the novel fed-batch strategy have great potential for the production of recombinant β-mananase in industrial fermentation.



## 1. Introduction

Mannans, widely distributed in wood, tubers, plant seeds, beans and cell walls of certain marine algae (Gübitz et al., 2001), are a major component of hemicelluloses (Petkowicz et al., 2001). Endo-1,4-β-D-mannanase (β-mannanase, EC 3.2.1.78) randomly hydrolyzes (1→4)-beta-D-mannosidic linkages in mannans, galactomannans and glucomannans (http://www.expasy.ch/enzyme/3.2.1.78). It has been applied in papermaking, food, animal feed, drilling industries and the production of second generation biofuels (Dhawan and Kaur, 2007; Moreira and Filho, 2008), and many mannanase genes have been cloned and expressed in *Pichia pastoris* (Bien-Cuong et al., 2009; Luo et al., 2009).

The methylotrophic yeast *P. pastoris* has been widely used for the heterologous protein expression. In particular, the high-level expression of heterologous protein by *P. pastoris* has been obtained using pAOX1 expression system (Luo et al., 2009; Schenk et al., 2007). However, the fermentation process in the AOX1-based expression system is difficult to control because the excessive accumulation of methanol inhibits the cell growth and reduces the total yield of heterologous protein (Pal et al., 2006). Furthermore, there is a fire hazard using methanol as an inducer and it is inappropriate for the production of foods and drugs (Cereghino and Cregg, 1999). Recently, some researchers employed constitutive glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter for heterologous protein expression in *P. pastoris* and it also led to the high-level expression of target proteins (Goodrick et al., 2001;

* Corresponding author at: School of Minerals Processing and Bioengineering, Central South University, South Lushan Road 932, Changsha, Hunan, People's Republic of China. Tel.: +86 731 88877216; fax: +86 731 88710804.
*E-mail address:* zhouhb@mail.csu.edu.cn (H. Zhou).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.05.065