

Enhanced acetoin production by *Serratia marcescens* H32 with expression of a water-forming NADH oxidase

Jian-An Sun^a, Liao-Yuan Zhang^b, Ben Rao^a, Ya-Ling Shen^{a,*}, Dong-Zhi Wei^{a,*}

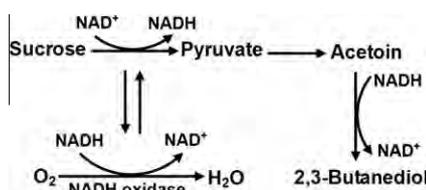
^a State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Meilong Road 130, Shanghai 200237, PR China

^b School of Life Science, Fujian Agriculture and Forestry University, Shangxiadian Road 102, Fuzhou 350002, PR China

HIGHLIGHTS

- ▶ NADH oxidase was expressed to regulate NAD⁺ and NADH concentrations.
- ▶ Expression of NADH oxidase could be self-induced employing the pPbud-nox plasmid.
- ▶ Acetoin production was remarkably enhanced while 2,3-butanediol was decreased.
- ▶ Production and productivity of acetoin were new records on acetoin fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

Cofactor engineering was employed to enhance production of acetoin by *Serratia marcescens* H32. 2,3-Butanediol was a major byproduct of acetoin fermentation by *S. marcescens* H32. In order to decrease 2,3-butanediol formation and achieve a high efficiency of acetoin production, *nox* gene encoding a water-forming NADH oxidase from *Lactobacillus brevis* was expressed. Batch fermentations suggested the expression of the NADH oxidase could increase the intracellular NAD⁺ concentration (1.5-fold) and NAD⁺/NADH ratio (2.9-fold). Meanwhile, 2,3-butanediol was significantly decreased (52%), and the accumulation of acetoin was enhanced (33%) accordingly. By fed-batch culture of the engineered strain, the final acetoin titer up to 75.2 g/l with the productivity of 1.88 g/(l h) was obtained. To the best of our knowledge, these results were new records on acetoin fermentation ever reported.

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1. Introduction

Acetoin is a high value product naturally occurs in wine, honey, cocoa, butter, coffee, strawberry, etc. It can be widely used not only in dairy products, but also in cosmetics, pharmacy and chemical synthesis (Xiao et al., 2010a,b). It was classified as one of the 30 platform chemicals which were given the priority to their develop-

ment and utilization by the U.S. Department of Energy (Werpy and Petersen, 2004). There are several ways to produce acetoin including chemical synthetic methods, enzymatic or whole-cell conversion methods and fermentative technologies (Liu et al., 2011a,b; Xiao et al., 2010a,b; Zhang et al., 2011). Compared to chemical synthetic and enzymatic conversion methods, the microbial fermentation is the most competitive method in acetoin production, because it has advantages of raw materials, mild process conditions, and environmental-friendliness (Xu et al., 2010).

Acetoin could be produced via the mixed acid fermentation pathway by many bacterial species such as *Klebsiella pneumoniae* (Silveira et al., 1998), *Bacillus subtilis* (Xiao et al., 2007), and

* Corresponding authors. Tel.: +86 21 64252981; fax: +86 21 64250068.

E-mail addresses: ylshen@ecust.edu.cn (Y.-L. Shen), dzhwei@ecust.edu.cn (D.-Z. Wei).