Biotechnology Advances 32 (2014) 1328–1335

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

From a co-production design to an integrated single-cell biorefinery

Quanfeng Liang, Qingsheng Qi *

State Key Laboratory of Microbial Technology, School of Life Sciences, Shandong University, Jinan 250100, China

ARTICLE INFO

Article history:
Received 13 January 2014
Received in revised form 23 July 2014
Accepted 17 August 2014
Available online 27 August 2014

Keywords:
Co-production
Single-cell biorefinery
Metabolic engineering
Microorganism

ABSTRACT

Engineering microorganisms capable of accumulating multiple products are sometimes attractive because they yield several advantages in balancing the in vivo metabolic flux and restoring the optimal cell physiology. With the development of metabolic engineering and synthetic biology, numerous strategies for minimizing the substrate waste, optimizing the product portfolios, and maximizing the product yield in co-production systems have been designed and applied. This paper reviewed the recent developments in this field and discussed the challenges that may be encountered during the scaling up of the co-production systems. Finally, the importance of product portfolios and biorefinery strategy of single-cell in co-production processes was proposed.

Contents

Introduction

Metabolites accumulated from natural microorganisms are generally a mixture of various compounds. In most situations, researchers select one of these compounds as the targeted product to generate a high production yield and facilitate efficient downstream recovery. Therefore, screening microorganisms with desired metabolite phenotypes has been an unavoidable task for researchers. Moreover, numerous advanced biological technologies have been developed to minimize the accumulation of byproducts and maximize the yield of targeted products (Kang et al., 2009; Otero and Nielsen, 2010; Park et al., 2008). One well-known example is the fermentation of *Clostridium acetobutylicum*, which naturally accumulates a mixture of acetone, butanol, and ethanol at a

* Corresponding author at: State Key Laboratory of Microbial Technology, School of Life Sciences, Shandong University, 27 Shanda Nanlu, Jinan 250100, China. Tel./fax: +86 531 88365628.
E-mail address: qiqingsheng@sdu.edu.cn (Q. Qi).

http://dx.doi.org/10.1016/j.biotechadv.2014.08.004
0734-9750/

ratio of 3:6:1 (ABE fermentation). Although acetone, butanol, and ethanol are crucial biofuels or solvents, the existence of a second or third metabolite in this production system affects the cost of the fermentation and recovery processing (Jones and Woods, 1986; Tracy et al., 2012). Therefore, researchers have used several methods, including metabolic engineering, to improve the selectivity of this fermentation system, specifically by converting ABE fermentation into one-product-only fermentation (Cooksley et al., 2012).

However, in certain situations, 2 or more metabolites that have accumulated in natural microorganisms can be simultaneously collected and applied without separation; for example, 2 bacteriocins with complementary antimicrobial spectra can coexist in one preparation (Cleveland et al., 2001). Compared with an isolated pure bacteriocin, mixtures of bacteriocins with complementary functions are more economical and efficient in application (Bravo et al., 2009; Cintas et al., 1998, 2000; Quadri et al., 1994). Meanwhile, the genetic manipulations of microorganisms used for accumulating targeted products often disturb the redox balance and carbon flux in vivo (Lee et al., 2008, 2012). The simultaneous accumulation of 2 or more targeted products in engineered microorganisms can balance the metabolism and restore the optimal cell physiology. In the meantime, it may also improve the economics of the fermentation process. However, methods for designing and engineering a coproduction system in microorganisms have not been summarized. This review summarizes the strategies that have been applied in designing and engineering coproduction systems in microorganisms (Table 1) and discusses the major challenges and implications of these strategies. Based on the summary, we proposed that suitable product portfolios and single-cell biorefinery strategy (Fig. 1) played an important role in co-production processes.

Co-production in a single cell

In vivo metabolic balance as the first consideration in coproduction design

Co-production in a single cell to maintain carbon flux balance

Excess accumulation of the targeted metabolites also causes an imbalance in the carbon flux in vivo and the inhibition of cell growth. Researchers have generally resolved this imbalance in metabolism by overexpressing or inactivating the genes involved in the metabolic network (Chemler et al., 2010; Ma et al., 2011; Martínez et al., 2008). Introducing a heterologous biosynthesis pathway is another strategy used to balance the metabolic flux caused by the over accumulation of the targeted product.

In our previous study, we observed that pyruvate accumulated in a succinate-producing engineered *Escherichia coli* because of the deletion of the succinate dehydrogenase gene *sdhA*. To remove this imbalanced in metabolism, we introduced a PHB biosynthesis pathway into the succinate-producing strain. Because the pyruvate derivative acetyl-CoA is a precursor of PHB synthesis, PHB accumulation not only reduced pyruvate and acetate accumulation but also improved the succinate production yield from 20.3 g/L to 24.6 g/L. Consequently, this engineered strain accumulated 4.95 g/L of PHB, corresponding to a PHB content of 41.3% of the cell dry weight (Kang et al., 2010). Intracellular NADPH/$NADP^+$ analysis revealed that the redox ratios in both the PHB-accumulating and non-PHB-accumulating *E. coli* strains were nearly the same, indicating that the consumed NADPH was replenished through increased carbon flow to the tricarboxylic acid cycle (TCA cycle). The consumption of NADPH through PHB biosynthesis also accelerated the TCA cycle and benefitted succinate accumulation. Recently, recombinant *E. coli* expressed hydrogenase 3 (*hycBCDEFGHI*) and the PHB biosynthesis pathway, confirming that recombinant *E. coli* can produce hydrogen and PHB simultaneously. The simultaneous formation of 2 products prevented the accumulation of 2 toxic compounds, formate and acetate (Wang et al., 2012a).

PHB biosynthesis not only restores metabolic balance by diverting the flux to polymer accumulation but also directly exerts metabolic control over carbon flux distribution. Quantitative real-time PCR analysis revealed that the transcription of tryptophan operon genes in an engineered tryptophan-producing *E. coli* strain increased by 1.9 to 4.3 times when the PHB biosynthesis pathway was introduced (Gu et al., 2013). The positive effect of PHB synthesis on the accumulation of other metabolic products (such as hyaluronic acid and L-glutamate) has been reported in *Streptococcus zooepidemicus* (Zhang et al., 2006) and *Corynebacterium glutamicum* (Liu et al., 2007).

Co-production in a single cell to maintain redox balance

Redox balancing is a critical consideration in metabolic engineering as well as in fermentation control (Charusanti et al., 2010; Ida et al., 2013; Wang et al., 2012c). The simultaneous accumulation of 2 or more targeted products with different redox statuses in engineered microorganisms can balance the metabolism and eliminate redox perturbation. For example, the reductive compound 1,3-propnediol (1,3-PDO) is accumulated from glycerol in *Klebsiella pneumoniae*

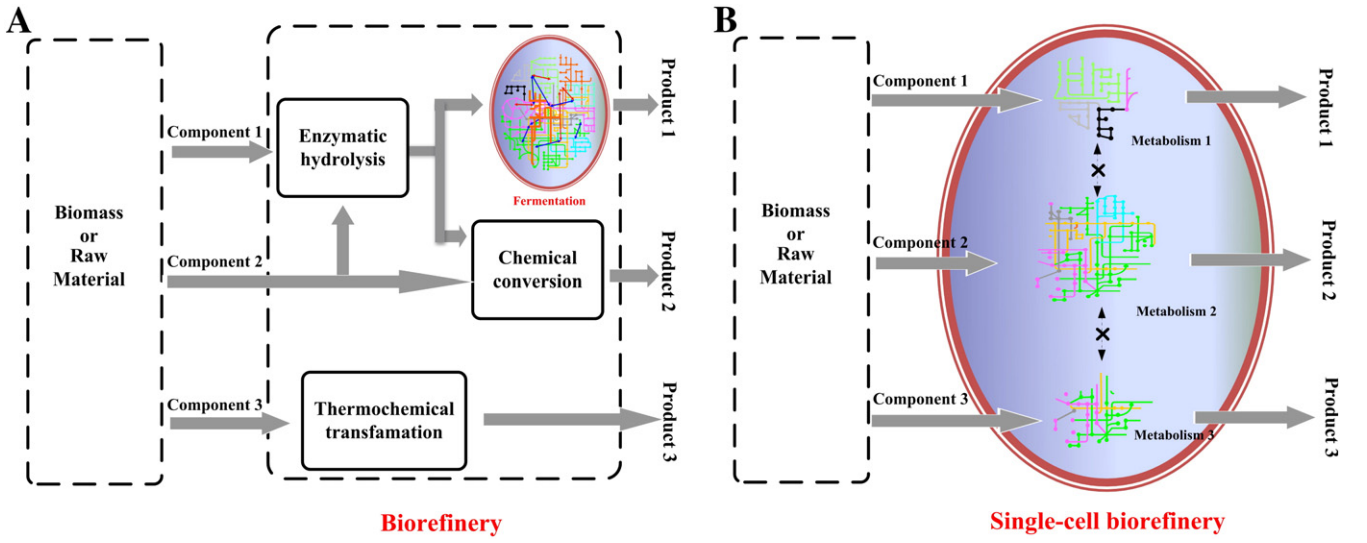

Fig. 1. Biorefinery and single-cell biorefinery. (A) Biorefinery: A facility that integrates biomass conversion processes and equipment to produce fuel, power, heat, and value-added chemicals from biomass. (B) Single-cell biorefinery: An integrated single-cell factory that utilizes various components in biomass or waste material and coproduces multiple value-added compounds.