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Identifying the shared metabolic objectives of glycerol bioconversion in *Klebsiella pneumoniae* under different culture conditions

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ABSTRACT

This paper addresses the problem of identifying the shared metabolic objectives of glycerol bioconversion in *Klebsiella pneumoniae* for production of 1,3-propanediol (1,3-PD) under different culture conditions. To achieve this goal, we propose a multi-level programming model. This model includes three optimization problems, where the constraint region of the first level problem is implicitly determined by the other two optimization problems. The optimized objectives of the first and second level problems are to minimize the set of fluxes that are of major importance to glycerol metabolism and the difference between the observed fluxes and those computed by the model, respectively. The third level problem in the proposed multi-level programming simultaneously solves a set of flux balance analysis (FBA) models. A method is proposed to solve efficiently the presented multi-level programming problem. In this method, we first transform the proposed multi-level problem into a bi-level problem by applying the dual theory of linear programming to the FBA models of the third level. Next, the optimal solution of the above bi-level problem is obtained by iteratively solving a sequence of mixed integer programming problems. Optimization results reveal that the proposed method can identify the shared metabolic objectives of glycerol bioconversion in *Klebsiella pneumoniae* under three groups of experimental data.

1. Introduction

1,3-propanediol (1,3-PD) has a wide range of potential applications on a large commercial scale (Biebl et al., 1999). Among the types of microbial production of 1,3-PD, bioconversion of glycerol to 1,3-PD has been studied extensively since 1980s due to its relatively high yield and productivity (Xiu et al., 2004). Much research has been directed toward the quantitative description (Silva et al., 2015; Xiu et al., 1998; Zeng and Biebl, 2002; Zeng and Deckwer, 1995), mathematical modelling (Rodriguez et al., 2017; Sun et al., 2008), parameter identification (Yin et al., 2016; Yuan et al., 2014), robust control (Xu, 2010; Xu et al., 2015; Zhu et al., 2014) and multi-objective optimization (Xu et al., 2016) for the bioconversion of glycerol to 1,3-PD. Some metabolic engineering studies address improvements in the production efficiency of 1,3-PD through utilization of cell physiology and metabolic regulation (Celińska, 2010, 2012; Chen et al., 2011; Hirokawa et al., 2016; Kumar et al., 2016; Wischral et al., 2016; Zhang et al., 2008).

In recent years, considerable research has sought to improve bioprocesses by metabolic engineering (Bailey, 1991; Burgard and Maranas, 2003; Maranas and Zomorrodi, 2016; Palsson, 2015; Stephanopoulos et al., 1998; Uygun et al., 2007). In these studies, computational approaches play a key role in analyzing and understanding the complex biochemical interactions within cells. One successful method widely used in the computational estimation of metabolic fluxes is the flux balance analysis (FBA) (Kauffman et al., 2003; Maranas and Zomorrodi, 2016; Orth et al., 2010; Stephanopoulos et al., 1998), currently one of the most important techniques for quantitative analysis of metabolic networks (García Sánchez and Torres Sáez, 2014). In the FBA approach, an optimization criterion is utilized to select a metabolic flux distribution from the feasible flux space. This optimization criterion is mathematically described as an objective function representing what an organism strives for. Knowing such an objective function is a fundamental problem in the field of biology (Feist and Palsson, 2016). Some objective functions have been used in the FBA of metabolic networks, including the maximization of biomass yield (Edwards and Palsson, 2000; Van Gulik and Heijnen, 1995), maximization of ATP yield (Ramakrishna et al., 2001; Van Gulik and Heijnen, 1995), minimization of the overall intracellular flux (Blank et al., 2005; Bonarius et al., 1996), minimization of a linear combination of reaction fluxes (Burgard and Maranas, 2003; Maranas and Zomorrodi, 2016), *etc.* An objective function can also be identified from experimentally determined metabolic fluxes (Burgard and Maranas, 2003; Gianchandani et al., 2008; Zhao et al., 2016). This approach integrates the FBA model with the observed

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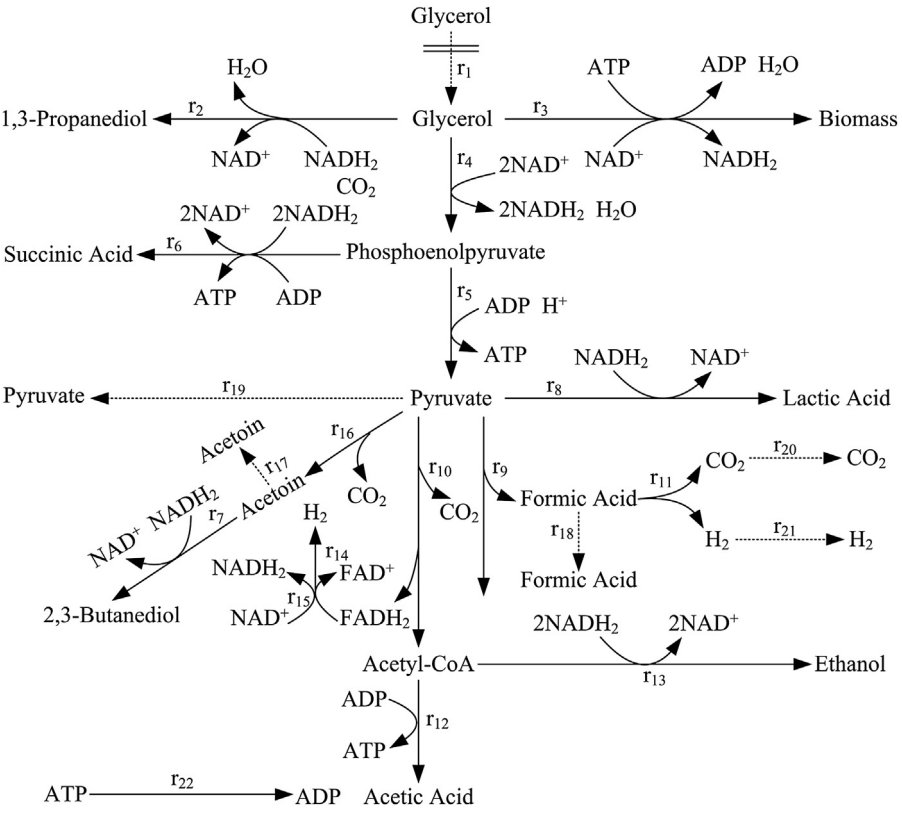

Fig. 1. Metabolic network of anaerobic glycerol metabolism in *Klebsiella pneumoniae* for 1,3-PD production.

fluxes to determine an objective function of a metabolic network through optimization methods. An optimization-based computational method has been proposed to infer the objective function of glycerol metabolism in *Klebsiella pneumoniae* for 1,3-PD production (Gong et al., 2009). This approach was then applied to three groups of experimental flux data, respectively. However, the flux distributions attained by the Gong et al. (2009) approach seriously violate the metabolite balancing Eqs. (1)–(11), as shown in Table 7 of Section 3. This observation concludes that the Gong et al. (2009) approach obtains the flux distributions without good feasibility or improved prediction performance. To address this drawback, we propose a new method for inferring the objective function of glycerol metabolism. In addition, we address the problem of whether the shared metabolic objectives of glycerol bioconversion in *Klebsiella pneumoniae* to produce 1,3-PD under different culture conditions can be identified from experimentally determined metabolic fluxes.

For these two objectives, we first propose a multi-level programming model to address the problem of identifying the shared metabolic objectives of glycerol bioconversion in *Klebsiella pneumoniae* to produce 1,3-PD under different culture conditions. Next, the proposed multi-level programming model is globally solved by proposing an efficient method. In Section 3, we use the proposed method to identify the shared metabolic objective of glycerol bioconversion in *Klebsiella pneumoniae* for 1,3-PD production under three groups of experimental data. In this section, the attained optimization results are presented and discussed. Finally, a summary and conclusions of the present work are presented.

2. Materials and methods

In this section, we propose a multi-level programming model to identify the shared metabolic objectives of anaerobic glycerol bioconversion in *Klebsiella pneumoniae* for the production of 1,3-PD under different culture conditions.

2.1. Optimization models

2.1.1. Metabolic reaction network

For identifying the shared metabolic objectives of anaerobic bio-dissimilation of glycerol to 1,3-PD by *Klebsiella pneumoniae* under different culture conditions, the metabolic network model developed by Zhang et al. (2006) and Gong et al. (2009) is used. As depicted in Fig. 1, this metabolic network model includes 22 irreversible reactions (r_j, $j = 1, 2, \cdots, 22$) and 11 metabolites (Glycerol, Phosphoenolpyruvate, Pyruvate, Acetyl-CoA, Acetoin, Formic Acid, CO_2, H_2, $NADH_2$, ATP, $FADH_2$). We assume that the metabolic system as shown in Fig. 1 have reached a quasi or pseudo steady-state, where metabolite concentrations do not change. Thus, the steady-state mass balances on the intracellular metabolites can be described by the following 11 linear equations:

$$v_1 - v_2 - v_3 - v_4 = 0 \quad (1)$$

$$v_4 - v_5 - v_6 = 0 \quad (2)$$

$$v_5 - v_8 - v_9 - v_{10} - v_{16} - v_{19} = 0 \quad (3)$$

$$v_9 + v_{10} - v_{12} - v_{13} = 0 \quad (4)$$

$$0.5v_{16} - v_{17} - v_7 = 0 \quad (5)$$

$$v_9 - v_{11} - v_{18} = 0 \quad (6)$$

$$v_{10} + v_{11} + v_{16} - v_6 - v_{20} = 0 \quad (7)$$

$$v_{14} + v_{11} - v_{21} = 0 \quad (8)$$

$$v_3 - v_2 + 2v_4 - 2v_6 - v_8 - v_7 + v_{15} - 2v_{13} = 0 \quad (9)$$

$$-7.5v_3 + v_5 + v_6 + v_{12} - v_{22} = 0 \quad (10)$$