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Stoichiometric analysis and experimental investigation of glycerol–glucose co-fermentation in *Klebsiella pneumoniae* under microaerobic conditions

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Abstract

The ratio between two substrates is an important parameter in microbial co-fermentation, such as 1,3-propanediol production from glycerol by *Klebsiella pneumoniae* using glucose as the cosubstrate. In this study, the glycerol–glucose cometabolism by *K. pneumoniae* is stoichiometrically analyzed according to energy (ATP), reducing equivalent ($NADH_2$) and product balances. The theoretical analysis reveals that the yield of 1,3-propanediol to glycerol under microaerobic conditions depends not only on the ratio of glucose to glycerol initially added, but also on the molar fraction of reducing equivalent oxidized completely by molecular oxygen in tricarboxylic acid (TCA) cycle (δ) and the molar fraction of TCA cycle in acetyl-CoA metabolism (γ). The maximum ratio of 0.32 mol glucose per mol glycerol is needed to convert glycerol completely to 1,3-propanediol under anaerobic conditions if glycerol neither enters oxidation pathways nor forms biomass. The ratio can be reduced under microaerobic conditions. The experimental results of batch cultures demonstrate that the biomass concentration and yield of 1,3-propanediol on glycerol could be enhanced by using glucose as a co-substrate. The theoretical analysis reveals the relationship between yield of 1,3-propanediol to glycerol, ratio of glucose to glycerol and respiratory quotient (RQ). These results are helpful for the experimental design and control.

Keywords: Stoichiometric analysis; *Klebsiella pneumoniae*; Glycerol; Glucose; 1,3-Propanediol

1. Introduction

The microbial production of 1,3-propanediol (1,3-PD) has recently paid much attention because of its large potential for commercial application, especially as a monomer of polyesters, polyethers or polyurethanes [1,2]. Nowadays, two kinds of fermentation have been developed in order to produce 1,3-PD in high scale. The first one is referred to a single step fermentation by genetically engineered strains, e.g. *Escherichia coli*, which are recombined by introducing genes for both glycerol and 1,3-PD production [3,4]. Glucose is used as a usual carbon source. The second one is referred to glycerol fermentation by natural microorganisms, such as *Klebsiella*, *Enterobacter*, *Citrobacters* and *Clostridia*, which are able to convert glycerol to 1,3-PD [5–10]. Since 1980's, *Klebsiella pneumoniae* has been widely investigated in this type of fermentation due to its high productivity. The maximum yield of 1,3-PD to glycerol is considered to be 0.72 mol mol^{-1} under anaerobic conditions [11] and 0.85 mol mol^{-1} under microaerobic conditions [12]. However, the practical yield of 1,3-PD is usually much lower compared with the theoretical maximum [5,13], because some part of glycerol is taken up to produce biomass and byproducts, e.g. ethanol and acetate. To enhance the yield of 1,3-PD and decrease the cost of production, strategies involving the utilization of raw materials (e. g. industrial-crude glycerol, directly issued from bio-diesel production units) by cells capable of resisting in the impurities of the medium have been utilized [6,7,9,14]. Raw-unpurified glycerol, that does not need further purification, has been used in this type of fermentation. Likewise, a cheap carbon source, such as glucose, is often used as a H-donor substrate instead of the fraction of glycerol to provide both reducing equivalents for 1,3-PD formation

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Nomenclature

C_{Gly}, C_{EtOH}, C_{PD} concentrations of glycerol, ethanol and 1,3-propanediol ($g\,L^{-1}$)
p molar ratio of pyruvic acid produced from glucose to total pyruvic acid ($mol\,mol^{-1}$)
q molar ratio of biomass formed from glucose to total biomass ($mol\,mol^{-1}$)
R stoichiometric ratio of glucose to glycerol ($mol\,mol^{-1}$)
RQ respiratory quotient ($mol\,mol^{-1}$)
$Y_{PD/Gly}$ yield of 1,3-PD on glycerol ($mol\,mol^{-1}$)

Greek letters
α molar ratio of pyruvic acid catalyzed by pyruvate formate lyase to total pyruvic acid
β molar fraction of acetate in total acetyl-CoA metabolism
δ molar fraction of $NADH_2$ oxidized completely by molecular oxygen in TCA cycle
γ molar fraction of tricarboxylic acid cycle in total acetyl-CoA metabolism

Subscripts
EtOH ethanol
Glu glucose
Gly glycerol
HAc acetic acid
PD 1,3-propanediol

and ATP for biomass [15]. A higher yield of 1,3-propanediol to glycerol was obtained by using glucose as co-substrate [16–18], but a high concentration of glucose in the culture medium appears to have a strong inhibition to the enzymes involved in the production of 1,3-PD [19,20]. The ratio of glucose to glycerol, hence seems to be an important parameter for 1,3-PD production. However, it is still not investigated experimentally or theoretically.

K. pneumoniae is a facultative bacterium. The recent experimental results and theoretical analysis show that the microaerobic conditions are favorable for cell growth, 1,3-PD formation and its productivity [21–23]. In this study, the emphasis will focus on the stoichiometric analysis of cometabolism of glycerol and glucose by *K. pneumoniae* under microaerobic conditions according to energy, reducing equivalent and mass balances. Its global object is to determine the ratio of glucose to glycerol and the influence of microaerobic condition.

1.1. Stoichiometric analysis

The pathway of glycerol metabolism with glucose as co-substrate by *K. pneumoniae* is shown in Fig. 1. The formation of biomass from glycerol and glucose can be written as follows [12]:

$$4C_3H_8O_3 + 3NH_3 + 3\frac{MW}{Y_{ATP}}ATP \rightarrow 3C_4H_7O_2N + 4NADH_2 + 6H_2O \quad (1a)$$

$$2C_6H_{12}O_6 + 3NH_3 + \frac{3MW}{Y_{ATP}}ATP \rightarrow 3C_4H_7O_2N + 6H_2O \quad (1b)$$

where $C_4H_7O_2N$ denotes the elemental composition of biomass [12], which corresponds to molecular weight of 101 $g\,mol^{-1}$; Y_{ATP} is the energetic yield of biomass and its value is between 8.4 and 11.5 g biomass per mol ATP in cultures of *K. pneumoniae* [12]. For simplicity, an average Y_{ATP} of 10.1 $g\,mol^{-1}$ is taken here according to the work of Zeng et al. [11]. Thus the Eqs. (1a) and (1b) can be rewritten as

$$4C_3H_8O_3 + 3NH_3 + 30ATP \rightarrow 3C_4H_7O_2N + 4NADH_2 + 6H_2O \quad (2a)$$

$$2C_6H_{12}O_6 + 3NH_3 + 30ATP \rightarrow 3C_4H_7O_2N + 6H_2O \quad (2b)$$

Assuming that q is a molar ratio of biomass formed from glucose to total biomass, therefore $(1-q)$ is the molar ratio of biomass formed from glycerol. Then the total equation of biomass formation can be expressed as follows:

$$4(1-q)C_3H_8O_3 + 2qC_6H_{12}O_6 + 3NH_3 + 30ATP \rightarrow 3C_4H_7O_2N + 4(1-q)NADH_2 + 6H_2O \quad (3)$$