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Short Communication

Flux balance analysis of different carbon source fermentation with hydrogen producing *Clostridium butyricum* using Cell Net Analyzer

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HIGHLIGHTS

- A metabolic network model was developed for H_2 production using six carbon sources.
- H_2 yields and growth ability estimations were well correlated with experiments results.
- Sucrose and trehalose supported the maximum growth and H_2 yields.

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ABSTRACT

A metabolic network model for *Clostridium butyricum* was developed using six different carbon sources (sucrose, fructose, galactose, mannose, trehalose and ribose) to study the fermentative H_2 production. The model was used for investigation of H_2 production and the ability of growth on different substrates to predict the maximum abilities for fermentative H_2 production that each substrate can support. NADH fluxes were calculated by the model as an important cofactor affecting on H_2 production. Butyrate and acetate production were used as model assumptions and biomass formation was chosen as the objective function for flux analysis calculations. Among the substrates selected, sucrose and trehalose supported the maximum growth and H_2 yields. The Cell Net Analyzer metabolic network model developed for H_2 estimation revealed good correlation with experimental data and could be further used to study the effect of environmental conditions and substrates concentration on H_2 yield.

1. Introduction

Dark fermentation using *Clostridium* species is a promising process for hydrogen production among other chemical and biological methods as it guarantees higher product yields and lower process costs (Mathews and Wang, 2009).In addition, other valuable byproducts such as acetic acid and butyric acid can be produced during H_2 production. As a renewable energy, H_2 can be a promising source which can decrease the air pollution caused by fossil fuels. ForH_2 production through dark fermentation which is the most convenient method for this aim, *Clostridium butyricum* is a high producer among some other *Clostridium* sp. (Hawkes et al., 2002).

Different methods are utilized for optimization of fermentation processes including experimental design, strain, improvement and mathematical modeling. Constraint-based metabolic models can be utilized to analyze the cellular functions in different conditions. These models are profitable to offer metabolic engineering strategies for increasing desired metabolites production. Metabolic flux analysis is a useful method for modeling of microbial processes in and predicts optimal fluxes during growth and metabolites production. Furthermore, it could be used to study the effect of environmental changes (pH, temperature, etc.) on productivity and cell growth. Since all these predictions can be done in silico, the cost of the industrial operations could be decreased compared to other methods for improving product yield. In addition, due to elimination of unfruitful experiments less time would be needed for optimization process.

Although many studies have investigated effect of different carbon sources on H_2 yields, there are only a very few studies which have studied *Clostridium* sp. fermentation using different

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substrates and most of them are focused on mixed microbial cultures (Junghare et al., 2012; Li et al., 2008; Ren et al., 2008; Chong et al., 2009; Cheng et al., 2013). Most of the studies on mathematical modeling of microorganisms are focused on using pure carbohydrate cultures and to our knowledge there is no comprehensive report about metabolic modeling of *C. butyricum* and flux distribution using different monosaccharide and disaccharides. However, several studies have been done on flux analysis for H_2 production using glucose as the carbon substrate for *Clostridium* sp. and mixed cultures (Chaganti et al., 2011; Lalman et al., 2013; Cai et al., 2010; Cheng et al., 2013).

In the present study, metabolic flux distribution through metabolic pathways of *C. butyricum* has been studied for monosaccharide and disaccharides. The main objective of this study is to develop a metabolic model which could predict the ability of different carbon substrate for H_2 production and show the flux distribution.

2. Methods

2.1. Model Construction and verification

Clostridium butyricum is one of the best strains for H_2 production because of high product yields and its ability to grow on different simple and complex substrates. Metabolic modeling is an increasingly widespread method which can be used for prediction of metabolic fluxes and maximum product yields with stoichiometric information of metabolic reactions. Metabolic models have been previously used for *Clostridium* sp. by other researchers for investigation of fermentative hydrogen production using glucose as the carbon substrate (Cai et al., 2010; Junghare et al., 2012; Cheng et al., 2013). In the present study, metabolic pathways and reactions associated with different sugars degradation were extracted from KEGG and BioCyc databases and also a genome based metabolic model (Senger and papoutsakis, 2008) to be included in the model. In the case of biomass formation equation, the data was extracted from the work by Cai et al. (2010). A list of reactions used in the model is given in Appendix B. The analysis for determination of metabolic fluxes was carried out by Cell Net Analyzer (CNA) which is a comprehensive toolbox for MATLAB (Mathworks Inc.) and Linear Programming method was used for flux optimization.

In this study maximization of growth was chosen as the objective function because it is the most used objective function for optimization of metabolic fluxes (Varma and Palsson, 1994). Since the number of reactions exceeds the number of metabolites in most metabolic networks, some of the fluxes should be determined experimentally and constraints for fluxes should be applied. Acetate and butyrate are the major soluble products in fermentation of *C. butyricum*, however lactate and ethanol are likely to be produced in some cases (Junghare et al., 2012; Plangklang et al., 2012). In addition, for irreversible reactions the lower constraint for metabolic fluxes was set to be zero.

2.2. Effect of different substrates on growth and H_2 production

In order to compare the effect of different substrates on growth and H_2 yield, Flux analysis was performed using Cell Net Analyzer. For verification of the model some of the exchange fluxes should be defined and used as the constraint for the metabolic model. Four different monosaccharide (fructose, ribose, galactose and mannose) and two disaccharides (trehalose and sucrose) were chosen as carbon substrates. In this study the data reported by Junghare et al. (2012) including substrate concentration and soluble metabolites production (acetate and butyrate) was used as model assumptions. H_2 yields obtained by the model were compared with reported yields. In order to simulate the experimental conditions to assess the model accuracy in prediction the H_2 yields, the concentration of each carbon source was assumed to be 10 g L^{-1} (Junghare et al., 2012). The stoichiometric matrix for sucrose is shown in Fig. 1.

3. Results and discussion

3.1. Flux distribution for different substrates

Fig. 2 shows the metabolic pathways of *C. butyricum* for 6 different carbon substrates. Possible byproducts have been shown, though in this study, acetate and butyrate are the major soluble metabolites. Table 1 summarizes the H_2 yields obtained by the model and those measured ones. The difference between the predicted and measured values is due to biomass formation equation which was assumed to be similar to *Clostridium acetobutylicum* because no equation could be found in the literature for *C. butyricum*. As it can be seen, the lowest biomass was obtained for ribose (0.05 g mol^{-1} ribose) and followed by galactose (0.07 g mol^{-1} galactose) and mannose (0.1 g mol^{-1} mannose). In comparison with monosaccharides, disaccharides (trehalose and sucrose) support higher biomass production (0.21 and 0.3 g mol^{-1} substrate respectively). The lower biomass yields for monosaccharides compared to

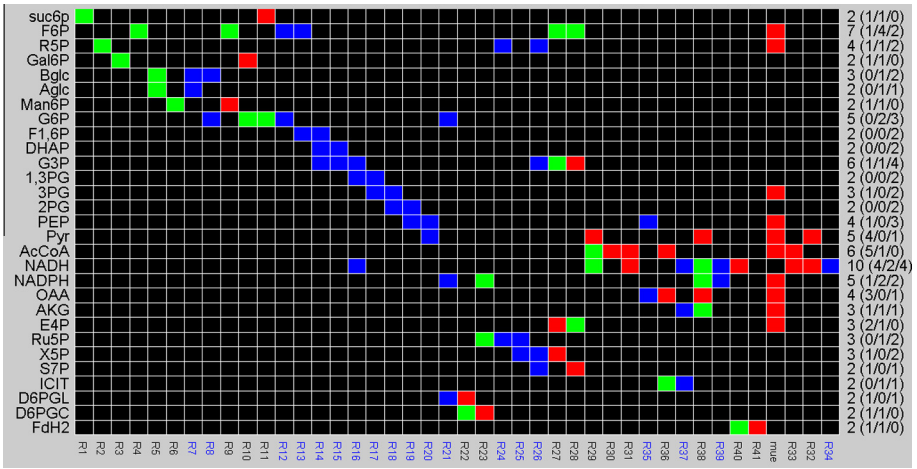

Fig. 1. Stoichiometric matrix for sucrose as carbon substrate (red, green and blue colors indicate substrate, product and intermediates respectively. For right side number first number indicates the compound involved in total number of reactions. Number in the brackets indicates input, output and intermediate respectively.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)