METABOLIC FLUX ANALYSIS FOR THE HYDROGEN PRODUCTION VIA ANAEROBIC DIGESTION AND PHOTOFERMENTATION

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Introduction. An alternative for the treatment of organic wastes is the anaerobic digestion. This process leads to the production of hydrogen (H_2) and methane, which have been identified as potential biofuels. Due to its high energetic content, several attempts for increasing H_2 production yields in dark fermentation have been done. In recent years the use of photoautotrophic bacteria that are able to produce H_2 has been explored since these microorganisms can use organic acids for growing [1].

The mechanisms and regulation systems for H_2 production are not completely understood due to the complexity of the metabolic networks in dark and photo-fermentation systems. To date, there are some metabolic models which attempt describing the metabolic changes during H_2 production [2,3]. However those models have been developed for specific microorganisms and are so complex that their application becomes complicated. Therefore this work aims the development of condensed metabolic models for the description of the metabolic changes in anaerobic heterotrophic and photoautotrophic mixed cultures during the H_2 production.

Methods. The experimental strategy was performed in three stages. First, information regarding all the biochemical reactions involved in H_2 production was gathered from both dark and photo-fermentation. KEGG and BRENDA databases were the main sources of information. *Clostriduim* and *Rhodobacter* global metabolic maps were considered as the reference points. Then, all the reactions were condensed by applying Mavrouvouniotis' algorithm and a stoichiometric matrix *M* was constructed. Dimensions of matrix *M* are [*m* x *J*], where *m* corresponds to the number of the metabolites and *J* to the number of reactions. Finally, the models were mathematically analyzed to evaluate their feasibility so that the flux distribution for *Clostridium* and *Rhodobacter* could be estimated.

Results. After analyzing the main metabolic pathways involved in H_2 production during the dark-fermentation process, these were simplified for obtaining a reduced metabolic model. The stoichiometric matrix *M* was then constructed. Matrix dimensions were [23 x 18], and it was defined as full-ranked. The system contains 4 degrees of freedom which means that to evaluate the flux distribution 4 values should be known. To evaluate the accuracy of the model, experimental data obtained previously for the research group was employed. Since the number of experimentally measured rates was higher than the number of degrees of freedom required, different combinations were tested for calculating the other production rates (Table 1).

Table 1. Gross Error between Experimental and Calculated Rates. *DF* indicates that the compound was used a degree of freedom.

Metabolite	Measured Rates (mol/h)	Tests (Error, %)				
		T1	T2	T3	T4	T5
Glucose	$-1.34x10^{-3}$	DF	DF	DF	DF	DF
Ethanol	$1.92x10^{-4}$	DF	8	3	99	DF
Butyric Acid	$1.19x10^{-3}$	35	DF	40	DF	DF
H_2	$2.99x10^{-3}$	40	40	40	89	126
Lactic Acid	$8.59x10^{-5}$	DF	DF	DF	100	96
Acetic Acid	$1.35x10^{-4}$	3	4	DF	DF	104
Biomass	$3.10x10^{-5}$	DF	DF	DF	DF	DF

Tests 1, 3, 4 and 5 cannot be accepted since differ noticeably from the measured values. For tests 2, the values obtained with the model presented the lowest errors. Additionally, the same value for H_2 production rate was obtained in tests 1, 2 and 3 ($1.7x10^{-3}$ mol/h). However, this value still presents a gross deviation to experimental one. To overcome these results, an analysis for the over determined system in currently been done.

Conclusions. A simplified metabolic model for H_2 production during dark fermentation was constructed. Although the model follows all the mathematical constrains, results for the hydrogen production rate are still below the experimental value. Additionally, the development of the metabolic model for the phototrophic bacteria is currently being developed.

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References. 1. Kapdan IK, Kargı F. (2006). *Enzym Microb Technol.*38:569-82.

2. Tao Y, Liu X, Zhou Z, Lee JK, Yang, C. (2011). *Journal Bacteriol.* 194(2):274:283

3. Chongcharoenthaweesuk P, Jiangdong Z, Mavituna F. (2011). MFBA for Biological Hydrogen Production by Purple Non-Sulfur Bacteria. *2011 International Conference on Food Engineering and Biotechnology.* CBEES, IEEE. Bangkok, Thailand. 7th-9th May. 125-129