19 October, 2017 (Thursday Morning)

Session: Modelling & System Optimization
Location: Room 306

8:00-8:15	503112 Critical comparison of different model structures for the applied simulation of anaerobic digestion. Jan Liebetrau, DBFZ-Deutsches Biomasse for schungszentrum gemeinn, Germany
8:15-8:30	503587 A new protocol and software tool for standardizing biomethane potential tests: results of an inter-laboratory study. Christof Holliger, EPFL (Ecole Polytechnique Federale Lausanne), Switzerland
8:30-8:45	504094 Calibration and Validation of an Anaerobic Digestion Model for Process Optimization of an Industrial Granular Sludge Reactor. Hannah Feldman, Technical University of Denmark, Denmark
8:45-9:00	515065 Sulfate reduction on acetate and hydrogen alone provides a good compromise on model structure complexity in AD. Wasim Ahmed, Masdar Institute of Science and Technology, United Arab Emirates
9:00-9:15	516003 Improved hydrodynamic characterisation of two-phase flow in gas-mixed anaerobic digester by CFD modelling. Peng Wei, Delft University of Technology, The Netherlands
9:15-9:30	521717 A VFA-based controller for anaerobic digestion of industrial winery wastewater. Gustavo Vargas Morales, Pontificia Universidad Católica de Valparaíso, Chile
9:30-9:45	555007 Modelling the anaerobic treatment of urban wastewater: application to AnMBR technology. Ángel Robles, Universitat de València, Spain
9:45-10:00	521847 Electron bifurcation mechanism and homoacetogenesis explain products yields in mixed culture anaerobic fermentations. Alberte Regueira, Universidade de Santiago de Compostela, Spain
10:00-10:15	523167 Research on Effect of Lipid and NH_4^+-N for Anaerobic Digestion of Food Waste Xing Wang, Biogas Institute of Ministry of Agriculture, China
11:25-11:40	Low cost Laser Raman Gas Analyzer for Anaerobic Fermentation and Bio-methane Process Monitoring. Youhui Xiong, Hubei Cubic-Ruiyi instrument Co., Ltd, China
11:40-11:45	519991 Improved Monitoring of Anaerobic Digestion Processes Using Principal Component Analysis and Control Charts. Wanderli Rogério Moreira Leite, Federal University of Pernambuco, Brazil
11:45-11:50	551626 Introduction of lag phase of microbial growth upon changes, during modeling of the anaerobic digestion process. Merlin Alvarado-Morales, Technical University of Denmark, Denmark
11:50-11:55	553725 Identification of metabolic pathways in a continuously operated methanogenic reactor through δ13C analysis: A simulation study. Manfred Lübken, Ruhr-Universität Bochum, Germany

Electron bifurcation mechanism and homoacetogenesis explain products yields in mixed culture anaerobic fermentations

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Abstract

Anaerobic fermentation of wastes with mixed cultures is a promising avenue to treat residues while producing added-value products. But thus far, the lack of control and prediction of the microbial community behaviour hinders any industrial application. One of the fundamental limitations of the process, is that we are not able to foresee the outcome of the process as its stoichiometry is not fully defined. In this work, we present a new metabolic network of fermentation of glucose by mixed cultures. The metabolic network that defines the stoichiometry of the process includes for the first time Electron Bifurcation and Homoacetogenesis. This novel metabolic network was successfully confronted with experimental data through NADH (i.e. electron) balances. Results show that the inclusion of Electron Bifurcation in the network provides a better description of experimental results and suggest its occurrence in the process. Homoacetogenesis, together with Electron Bifurcation, helps to correctly predict the H2 and CO2 gaseous yields. The new stoichiometry proposed accurately predicts the product yielding, including gaseous products, observed in glucose anaerobic fermentations. This supports the consideration of Electron Bifurcation as an important biochemical mechanism in microbial mixed cultures fermentations and remarks the importance of including Homoacetogenesis when analysing anaerobic fermentations.

Keywords
Anaerobic fermentation, metabolic network, electron bifurcation, homoacetogenesis, VFA production.

INTRODUCTION

Anaerobic treatment of low grade organic wastes using mixed culture fermentations (MCFs) has the potential to treat wastes and recover resources with very low operational costs. These bioprocesses yield a mixture of volatile fatty acids (VFA) and ethanol with value as chemicals or biofuels [1]. MCFs are complex and diverse and this increases the difficulty to set a reliable metabolic network that predicts the output of the operation. As a result, lack of control and robustness are so far slowing the implementation of MCFs in industrial applications [2].

In this work we propose a new metabolic network for MCFs using glucose as substrate with the objective of improving our capacity to predict the observed experimental results reported in literature. After revising the stoichiometry assumed for MCFs [2-4] we included Electron Bifurcation (EB) [5] and Homoacetogenesis (HA) [6] processes in the metabolic network. These metabolic mechanisms have been fully described in literature but their occurrence and importance in MCF has been so far not considered. The new metabolic network is confronted with the experimental data in literature and proves higher accuracy in its predictions than previous networks.

MATERIALS AND METHODS

Electron Bifurcation

EB is a metabolic mechanism whereby cells can drive endergonic reactions by coupling them to sufficiently exergonic reactions. It was confirmed only in *in vitro* fermentations so far but thereis no fundamental impediment to its occurrence in MCFs. EB was first detected in the butyrate pathway linking the exergonic NADH-mediated reduction of crotonyl-CoA with the endergonic Ferredoxin reduction with NADH (Figure 1). As Ferredoxin is automatically regenerated producing H2, if EB is considered the amount of H2 predicted increases, giving thus theoretical support to experiments with higher than previously predicted H2 yields [7]. Moreover, with EB two NADH are consumed in this step (instead of just one), changing thus the stoichiometry. For microorganisms, EB is a useful tool to decrease the electron excess in a fermentative environment and to direct the substrate through pathways that allow them to harvest more energy (acetate).

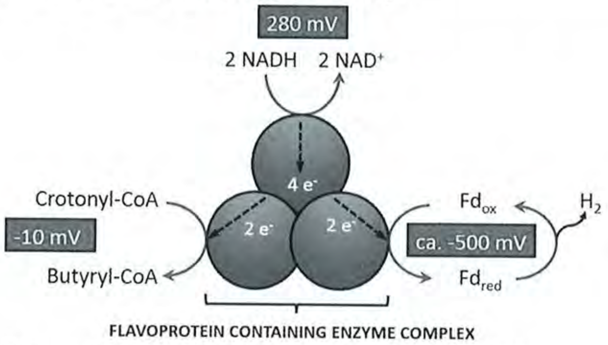

Figure 1. Mechanism and stoichiometry of EB.

Homoacetogenesis

HA is the autotrophic process that consumes H_2 and CO_2 to yield acetate ($4\ H_2 + 2HCO_3 = \text{acetate} + 4H_2O$). This process has not received much attention in previous MCF stoichiometry analyses because methanogens are typically considered the main H_2 consumers. Nevertheless, there is no thermodynamic limitation for its occurrence in MCFs (HA is highly exergonic in typical fermentation conditions). HA presence in MCF is considered to be persistent and the main barrier for H_2 production through dark fermentations [6].

Methodology

To check whether the inclusion of EB in the metabolic network improves the accuracy in predicting the experimental results, we confronted our network (with and without EB) with the experimental measurements of Temudo et al. 2007 [4]. These consist in a series of fermentations at different pH using glucose as substrate. An NADH balance was made for each of the experiments. In a fermentation, this is equivalent to an electron balance since its production and consumption has to be the same to maintain the redox neutrality. The difference between production and consumption was calculated using the experimental product yields and the NADH stoichiometry defined by the metabolic network (with and without EB). In this way, the network that results in a better NADH balance (i.e. closer to 0) will be able to predict more accurately the product yielding in a model.

RESULTS AND DISCUSSION

Metabolic network

The network (Figure 2.a) includes the metabolic pathways of the major products of fermentation from glucose: acetate, ethanol, propionate, lactate and butyrate. All these products derive from pyruvate, which is produced by an initial glycolysis. Propionate and lactate are directly yielded as they are all three-carbon compounds. Acetate, ethanol and butyrate (compounds with an even number of carbons) need first a decarboxylation of pyruvate to yield acetyl-CoA. In this decarboxylation step either formate or an equimolar mixture of H2 and CO2 can be produced. These options are in a thermodynamic equilibrium that is controlled by the pH [3, 4]. At a pH higher than 7, formate is the preferred option. However, for simplicity we consider for the electron balances that only H2 and CO2 are yielded since both options are equivalent as they are at the same state of reduction. EB is considered in the butyrate pathway (crotonyl-CoA reduction) and in the propionate pathway (fumarate reduction step), since both steps are equivalent.

Confronting the network with experimental data

NADH balances are presented in Figure 2.b. Errors are noticeable lower in all cases when EB is included (stripped bars) than when it is not (white bars). But, if EB is considered, it also means that there is an extra H2 yield. In Figure 2.c the experimental H2 yields (grey bars) are always consistently lower than the predicted H2 yields when considering EB in the network (stripped bars). In addition, the ratio between the experimental yields of H2 and CO2 (grey bars) is very close to 1. Both facts seem to contradict the occurrence of EB. However, predicted CO2 yields (Figure 2.c, stripped bars) are as well higher than the experimental yields (Figure 2.c, grey bars) and this difference cannot be explained by the inclusion of EB because EB does not change the CO2 stoichiometry in the network.

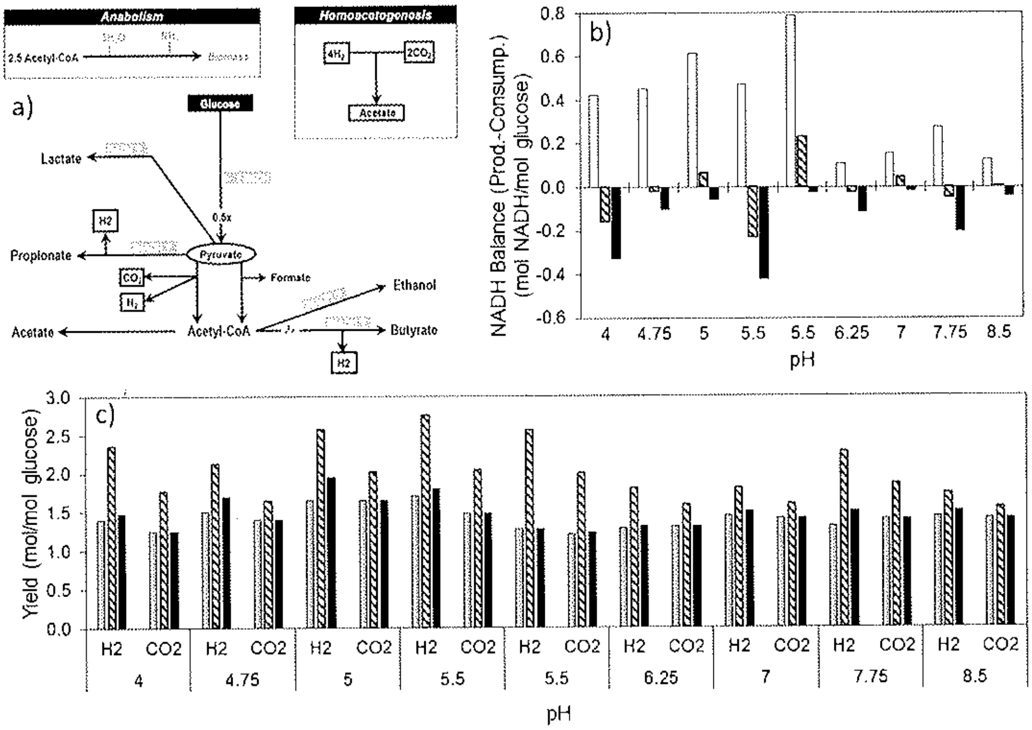

Figure 2. Summary of results: a) Scheme of the metabolic network; b) NADH balance (▯without considering electron bifurcation or homoacetogenesis (▨ only considering electron bifurcation (■considering electron bifurcation and homoacetogenesis; c) H2 and CO2 yields ( ) experimental results ▨ theoretical values only considering electron bifurcation ■ theoretical values considering electron bifurcation and homoacetogenesis

HA presence in the reactor could explain that lower yields than the ones predicted for H2 and CO2 were observed in the experiments (Figure 2.c). Therefore, we included HA in the considered stoichiometry. We calculate that the excess of H2 and CO2 between the predicted yield and the experimental one could be consumed by HA. Black bars in Figure 2.c show that when the assumed stoichiometry is modified to consider both EB and HA it provides a better description of the process as now the gaseous yields fit with the experimental data.

The presence of HA implies as well that part of the acetate yield measured is produced from the H2 and CO2 and not directly from glucose fermentation. Then, NADH balances change for the new stoichiometry. Black bars in Figure 2.b indicate that this correction does not increase significantly the error in the NADH balance except for pH 4 and 5.5 experiments. In this analysis, the only possible source of error is the experimental data. Gaseous products yields have bigger uncertainties due to a possible oversaturation in the liquid phase and this affects the precision of the yields measured [8]. We consider that the deviations found in pH 4 and 5.5 experiments could be explained considering the experimental error of the data. Nevertheless, adding the fact that for each glucose converted to pyruvate 2 NADH molecules are produced, a deviation of 0.4 in the balance means that 20% of the NADH was not consumed. This percentage, although significant, does not invalidate our proposal and is in any case lower than the errors observed when considering a stoichiometry without EB and HA (Figure 2b).

CONCLUSIONS

The proposed metabolic network presented in this work that incorporates new biochemical mechanisms, namely electron bifurcation and homoacetogenesis, provides a superior prediction of the experimental data in MCFs. It is the first time that EB is considered as a mechanism occurring in mixed culture fermentation systems which theoretically explains higher H2 yields in MCFs than the ones previously predicted [3, 4]. Our analysis also confirms that HA may be occurring significantly and must be considered in MCF modelling. With a more accurate stoichiometry of the process, future experimental and modelling approaches on MCF could achieve higher control and robustness of fermentations using complex communities.

ACKNOWLEGEDMENTS

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REFERENCES

1. Agler, M.T., et al., *Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform.* Trends Biotechnol, 2011. **29**(2): p. 70-8.
2. Hoelzle, R.D., B. Virdis, and D.J. Batstone, *Regulation Mechanisms in Mixed and Pure Culture Microbial Fermentation.* Biotechnology and Bioengineering, 2014. **111**(11): p. 2139-2154.
3. González-Cabaleiro, R., J.M. Lema, and J. Rodríguez, *Metabolic Energy-Based Modelling Explains Product Yielding in Anaerobic Mixed Culture Fermentations.* PLoS ONE, 2015. **10**(5): p. e0126739.
4. Temudo, M.F., R. Kleerebezem, and M. van Loosdrecht, *Influence of the pH on (open) mixed culture fermentation of glucose: A chemostat study.* Biotechnology and Bioengineering, 2007. **98**(1): p. 69-79.
5. Buckel, W. and R.K. Thauer, *Energy conservation via electron bifurcating ferredoxin reduction and proton/Na+ translocating ferredoxin oxidation.* Biochimica et Biophysica Acta (BBA) - Bioenergetics, 2013. **1827**(2): p. 94- 113.
6. Saady, N.M.C., *Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: Unresolved challenge.* International Journal of Hydrogen Energy, 2013. **38**(30): p. 13172-13191.
7. Ntaikou, I., G. Antonopoulou, and G. Lyberatos, *Biohydrogen Production from Biomass and Wastes via Dark Fermentation: A Review.* Waste and Biomass Valorization, 2010. **1**(1): p. 21-39.
8. Kraemer, J.T. and D.M. Bagley, *Supersaturation of Dissolved H2 and CO2 During Fermentative Hydrogen Production with N2 Sparging.* Biotechnology Letters, 2006. **28**(18): p. 1485-1491.