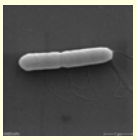
pH-Dependent Modelling of ABE Fermentation in *Clostridium acetobutylicum*

Sylvia Haus*, Thomas Millat, Olaf Wolkenhauer

University of Rostock, Germany

www.sbi.uni-rostock.de

Motivation



Clostridium acetobutylicum is a commercially valuable bacterium, first isolated from corn in 1912 by Chaim Weizmann. It is a Gram-positive, sporulating, obligate anaerobe organism.

The metabolism of *C. acetobutylicum* is characterised by the Acetone-Butanol-Ethanol (ABE) fermentation:

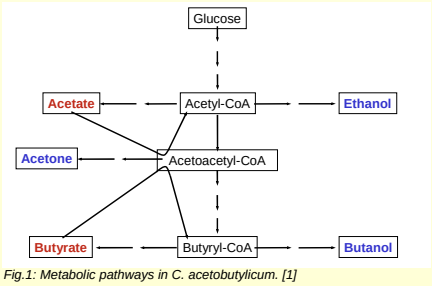


Fig.1: Metabolic pathways in C. acetobutylicum. [1]

Exponentially growing cells mainly produce the organic acids **acetate** and **butyrate**. During the transition phase *C. acetobutylicum* switches towards the generation of the solvents **butanol** and **acetone** as dominant fermentation products, a process called solventogenic shift. The details of the metabolic switch are not well understood:

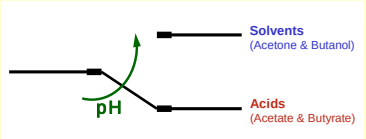


Fig.2: The ph-dependent metabolic swtich of C. acetobutylicum.

Facts & Knowledge

C. acetobutylicum shifts its metabolism as a function of the external pH. The cells produce predominantly acetate and butyrate when grown at a pH of 5.7 and acetone and butanol when grown at a pH of 4.5. [2] (See Figure 3)

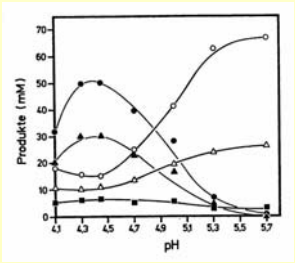


Fig.3: ● acetone, ▲butanol, ■ethanol, △acetate, ○ butyrate

C. acetobutylicum seems to be unable to maintain the internal pH at a more or less constant level above that of the external medium, when it produces weak acids (acetic & butyric acids). These bacteria maintain a limited, but more or less constant pH gradient across the membrane. The internal pH is approximately one unit higher than the external one. [4]

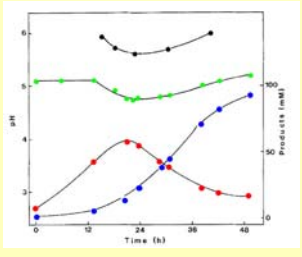


Fig.4: The internal pH (black) follows the changes of external pH (green) such that the delta pH is almost constant. (Blue: butanol, red: butyrate)

The shift in metabolic activity is accompanied by a corresponding shift in the activity of the enzymes involved in the acid and solvent producing pathways. (See Figure 5 and Figure 6) Certain proteines are only present in solvent producing cells. [3]

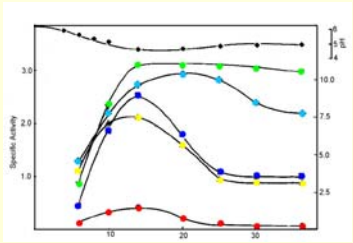


Fig.5: Specific activity of enzymes involved in acidogenesis [3]: phosphotransacetylase (red), acetate kinase (dark blue), phosphotrans-butyrylase (green), butyrate kinase (yellow)

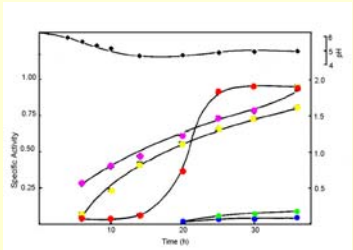


Fig.6: Specific activity of enzymes involved in solventogenesis [3]: acetoacetyl-CoA: acetate coenzyme A-transferase (magenta), acetoacetyl-CoA: butyrate coenzyme A-trans-ferase (yellow), acetoacetate decarboxylase (red), butyraldehyde dehydrogenase (green), butanol dehydrogenase (blue)

Furthermore it is known that the following parameters can have an effect of the metabolism of *C. acetobutylicum*

- Temperature
- Dilution rate
- Phostphate limitation

At the moment it is not clear if there is an effect of the pH value on the transcriptome level. Preliminary results show no indication for this phenomenon.

Standardised Experimental Design

For our system we use a standardised experimental setup with

- Strain ATCC 824
- Chemostat
- Phosphate limitation
- Cells were harvested at steady state

And constant parameters:

- 4% glucose in medium
- Dilution rate 0.1$[h^{-1}]$
- Temperature 37 °C

In our system we change only one parameter – the external pH! It is adjusted to constant values:

- Acids: 5.7 pH
- Solvents: 4.5 pH

Moreover there were defined standard operating procedures (SOP) for extracting and handling of different types of samples.

References

[1] Jones, D.T. & Woods, D., *Mol. Biol. Rev.*, 1986, 50(4), 484-524
[2] Bahl, H. et al., *Appl. Microbiol. Biotechnol.*,1982, 14, 17-20
[3] Andersch, W. et al., *Appl. Microbiol. Biotechnol.*, 1983, 18, 327-332
[4] Gottwald, M. et al., *Archives of Microbiology*, 1985, 143, 42-46
[5] Shinto, H. et al., *J. Biotechnol.*, 2007, 131, 45-56
[6] Desai, R.P., *Metab. Eng.*, 1999, 1, 206-213
[7] Papoutsakis, E.T., *Biotechnol. Bioeng.*, 1984, 26, 174-187
[8] Hüsemann, M. et al., *Appl. Microbiol.*, 1989, 30, 585-595

Shinto's Model

Shinto *et al.* [5] developed kinetic simulation models to describe the dynamic behavior of the metabolites in the ABE fermentation:

- *C. saccharoperbutylacetonicum* N1-4 ATCC13564 in batch culture with synthetic medium
- Wide range of different initial glucose concentration

Modelling and simulation:

- Considering butanol inhibition to cell growth
- Combining substrate inhibition by glucose, uncompetative inhibition by butanol and using specific activation by butyrate
- Glucose dependent on-off mechanism

Conclusion: 5% increase in reverse reaction of butyrate production and 5% decrease in reaction of CoA transferase for butyrate results a higher production of butanol.

This model neglects the known pH dependent behaviour of *C. acetobutylicum* !

Our Modelling Approach

Based on the model of Shinto *et al.* we developed an advanced model by

- Chemostat instead of batch culture
- Including the stoichiometry of the glycolysis
- Ignoring the energetic and Clostridia specific improbable reactions [1]
- Splitting up the reaction for acetate and butyrate production
- Constant biomass, no cell death
- **Focusing on the pH dependency and adding a pH dependent switching factor instead of a glucose dependent on-off factor**

Current Status and Future Work

With our first model we confirmed the knowledge about the metabolic pathway in batch culture. [8]

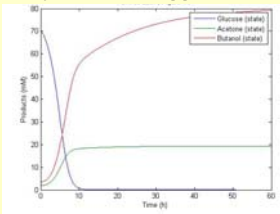


Fig.7: Simulation results of metabolites acetone & butanol in solventogenic phase.

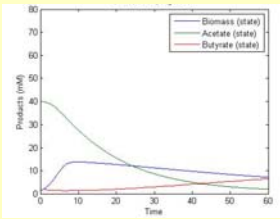


Fig.8: Simulation results of metabolites acetate & butyrate in acidogenic phase.

We currently designing the experiments for continuous culture:

- Generating time course data to determine and estimate model parameters for continuous culture
- Studying sensitivity and robustness & simulation studies in MATLAB® & SBToolbox²
- Explore different modelling approaches (**metabolic control analysis**, dynamic, stoichiometric, …)
- Analysing pH-dependency of enzyme activity and its consequences for dynamic modelling
- Prediction of changing environmental conditions & optimisation of the (bio)butanol production

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* Email: sylvia.haus@uni-rostock.de

Systems Biology & Bioinformatic Group
Department of Computer Science
University of Rostock
Albert-Einstein-Straße 21
18051 Rostock, Germany