**A Kinetic and Metabolic Flux Analysis of the Biphasic Acetone-Butanol Fermentation.**

Muven Naidoo1\*, Siew Tai1, Susan Harrison1

*1 Centre for Bioprocess Engineering Research (CeBER), Department of Chemical Engineering, University of Cape Town, Rondebosch, 7701, South Africa*

*\*Corresponding author:* [*muven.naidoo@uct.ac.za*](mailto:muven.naidoo@uct.ac.za)

**Highlights**

* Two-stage kinetic model constrained by the metabolic network of solventogenic *Clostridia*.
* The model accurately represents the acidogenic phase of the fermentation.
* CO2 and H2 predicted with consistent material balance without experimental analysis

**1. Introduction**

The Acetone-Butanol (AB) fermentation can be characterized as a two-phase process via solventogenic *Clostridia.* In the first phase, growth and acidogenesis occur with the production of acetic and butyric acids. In the second phase, a morphological change is observed. Growth ceases and solventogenesis occurs where the acids are re-assimilated by the organism to form solvents. Throughout the fermentation, hydrogen and carbon dioxide gases are released. Previous kinetic and stoichiometric models for the AB fermentation were relatively simple or were not always consistent in terms of the material balance. Further, several models do not account for the biphasic nature of the *Clostridial* biocatalyst. These models are sufficiently accurate for the modeling of batch fermentations without integrated separation. However, when integrated separation is considered in batch, fed-batch or continuous systems, the previous models do not accurately account for the accumulation of intermediary products and productivities are often inaccurately represented. In this work, we introduce a kinetic two-phase model that is constrained by the metabolic network of the *Clostridial* biocatalyst. This model incorporates the morphological change of the biocatalyst and ensures consistency of the material balance.

**2. Methods**

*C. saccharobutylicum* P262 was used in this study. A spore suspension of the organism was maintained in deionized water at 4 °C. The seed inoculum was prepared in reinforced clostridial medium (Merck) and the main culture fermented in tryptone-yeast extract-acetate (TYA) medium [1] with 60 g L-1 starting glucose concentration. Anaerobic batch cultures were carried out with stirring at 100 rpm at 34 °C in 120 mL serum bottles with an 80 mL working volume. Samples were periodically withdrawn, and fermentations were performed in triplicate.

The metabolic flux analysis (MFA) was based on the metabolic network for *C. acetobutylicum* [2]. The kinetic model was developed using the assumptions listed by [3] except for temperature at 34 °C and pH being uncontrolled. Calculations were carried out on Scilab v. 6.0.1. using the Nelder-Mead simplex method for non-linear regression and the Runge-Kutta method of numerical integration for solving differential equations.

**3. Results and discussion**

To test the modeling approach without the complexity of the two-phase model, the MFA was coupled to the kinetics of a butyric acid fermentation with *C.* tyrobutyricum ATCC 25755. This is metabolically identical to the acidogenic phase of AB fermentations. Data taken from Song et al [4] were regressed and the results are graphically displayed in Figure 1.

**Figure 1.** Butyric acid fermentation profile. Raw data taken from Song et al [4]. 🞵- biomass (exp), + - glucose (exp), ⦁ - butyric acid (exp), ⯅- acetic acid (exp). Solids lines indicate predicted concentrations.

By inspection, there is a good fit of the kinetic model to the experimental results. Using MFA, the yields of hydrogen and carbon dioxide were predicted to be 0.02 g gglucose-1 and 0.40 g gglucose-1 respectively. These results allow for a fundamentally more accurate representation of the fermentation kinetics and material balance.

**4. Conclusions**

The integration of a kinetic model with the constraint of the metabolic network of solventogenic *Clostridia* has been demonstrated for the acidogenic phase. This allows for a fundamentally more accurate representation of the fermentation profile and ensures consistency for the material balance. Future work will demonstrate the effectiveness of the model including the solventogenic phase.

**References**

1. H. Shinto, Y. Tashiro, M. Yamashita, G. Kobayashi, T. Sekiguchi, T. Hanai, Y. Kuriya, M. Okamoto, K. Sonomoto, J. Biotechnol. 131 (2017) 45-56
2. D. T. Jones, D. R. Woods, Microbiol. Rev. 50 (1986) 484-524
3. A. Mulchandani, B. Volesky, Can. J. Chem. Eng. (64) 625 – 631
4. J. Sing, K. Ventura, C. Lee, D. Jahng, Biotechnol. Bioprocess. Eng. 16 (2011) 42 – 49