**Supplementary materials - Process Engineering of the Acetone-Ethanol-Butanol (ABE) Fermentation in a Linear and Feedback Loop Cascade of Continuous Stirred Tank Reactors: Experiments, Modeling and Optimization**

**Katja Karstens 1, Sergej Trippel 1 and Peter Götz 1,\***

1 Department of Bioprocess Engineering, Beuth University of Applied Sciences Berlin, Seestraße 64, 13347 Berlin, Germany

\* Correspondence: goetz@beuth-hochschule.de

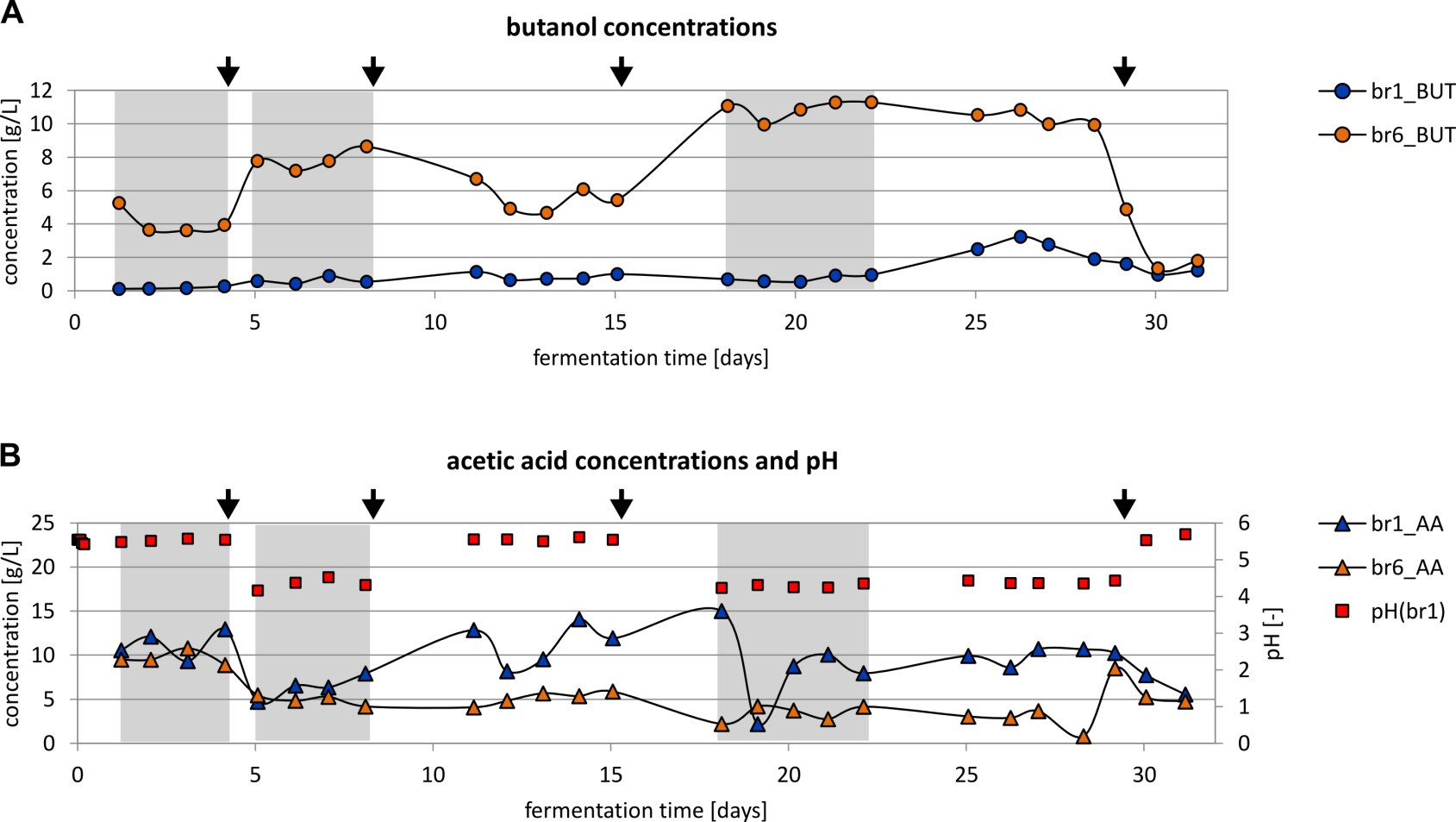


Figure SIa - Concentration and pH profiles during an exemplary fermentation. Shown are the butanol (A) and acetic acid (B) concentrations in bioreactor 1 and 6 of fermentation F18 (Dtotal = 0.092 h‑1 during the complete fermentation time). Arrows indicate the change in the set point pH of bioreactor1. High-lighted periods were considered as steady states.

Table SIa – Variables used in the mathematical model

| **variable** | **description** | **unit** |
| --- | --- | --- |
|  | *concentration of the metabolite M in bioreactor stage k at time point t* | g L-1 |
|  | *concentration of the metabolite M in bioreactor stage k at next time point t+dt* | g L-1 |
|  | *concentration of the metabolite M in the preceding bioreactor stage k-1 at time point t* | g L-1 |
| *dt* | *time increment of the simulation, default value used for all simulations was dt = 0.05 h-1* | h-1 |
|  | *feeding rate to the bioreactor stage* | L h-1 |
|  | *outflow rate from the bioreactor stage; Fout was equal to the feeding rate Fin* | L h-1 |
|  | *volume of the bioreactor stage, for all simulations Vbr was 0.4 L* | L |
|  | *specific conversion rate of the metabolite M in bioreactor stage k at time point t as result of the total biomass activity* | gM gX-1 h-1 |
|  | *proportion of the subpopulation P on the total biomass in bioreactor stage k at time point t* | - |
|  | *number of cells in the metabolic state P in bioreactor stage k at time point t* | - |
|  | *total number of cells in bioreactor stage k at time point t* | - |
|  | *number of cells in the metabolic state P in bioreactor stage k at next time point t+dt* | - |
|  | *number of cells in the metabolic state P in the preceding bioreactor stage k-1 at time point t* | - |
|  | *specific growth rate of the subpopulation P in bioreactor stage k at time point t (including cell lysis)* | h-1 |
|  | *specific gross growth rate of the subpopulation P in bioreactor stage k at time point t not considering cell lysis* | h-1 |
|  | *specific differentiation rate from subpopulation P to I (for P=A) or to S (for P=I) in bioreactor stage k at time point t* | h-1 |
|  | *specific differentiation rate from A (if P=I) or I (if P=S) to subpopulation P in bioreactor stage k at time point t* | h-1 |
|  | *number of cells in the metabolic state P\*(P\*=A for P=I and P\*=I for P=S) in bioreactor stage k at time point t* | - |
|  | *specific conversion rate of the metabolite M in bioreactor stage k at time point t as result of the activity of subpopulation P* | gM gX-1 h-1 |
| *Dbr1-1* | *residence time in one bioreactor stage; Dbr-1=1/6 Dtotal* | h |
|  | *weighted residual square sum overall Metabolites M’’ and all bioreactor stages k* | - |
|  | *experimentally observed steady state concentration of metabolite M’ in bioreactor stage k* | g L-1 |
|  | *simulated steady state concentration of metabolite M’ in bioreactor stage k* | g L-1 |
|  | *experimentally observed variance in the concentration of metabolite M’ in bioreactor stage k; maximum of standard derivations of individual or averaged data sets and 0.1* | g L-1 |
| (t) | *concentration of undissociated acetic and butyric acid in bioreactor stage k at time point t* | g L-1 |
| *pHk* | *pH in bioreactor stage k* | - |

Table SIb – Variable kinetic model parameters optimized on the basis of the experimental data

| **parameter** | **low boundary** | **upper**  **boundary** | **initial value\*** | **optimized value (OPT10e)** | **description** | **unit** |
| --- | --- | --- | --- | --- | --- | --- |
| mu\_max\_(A) | 0.65 | 1.5 | 1.16 | **0.7273** | *maximal growth rate of acidogenic cells (A)* | h-1 |
| mu\_max\_(I) | 0 | 0.5 | 0 | **0.4641** | *maximal growth rate of intermediate cells (I)* | h-1 |
| mu\_max\_(S) | 0 | 0.5 | 0 | **0.4204** | *maximal growth rate of solventogenic cells (S)* | h-1 |
| Y\_aax \_(A) | 0.5 | 3 | 1.7 | **0.5224** | *growth-dependent acetic acid per biomass yield of A* | gAA gX-1 |
| Y\_aax\_(I) | 0 | 0.5 | 0 | **0.5** | *growth-dependent acetic acid per biomass yield of I* | gAA gX-1 |
| r\_aa\_max \_(A) | 0.5 | 3 | 1.9 | **0.5097** | *growth-independent acetic acid production rate of A* | gAA gX-1 h-1 |
| r\_aa\_max\_(I) | 0 | 0.5 | 0 | **0.0296** | *growth-independent acetic acid production rate of I* | gAA gX-1 h-1 |
| k\_AA\_up\_(I) | 0 | 0.5 | 0 | **0.4224** | *growth-independent acetic acid uptake rate of I* | gAA gX-1 h-1 |
| k\_AA\_up\_(S) | 0.5 | 3 | 1.1 | **0.9485** | *growth-independent acetic acid uptake rate of S* | gAA gX-1 h-1 |
| Y\_bax\_(A) | 0.5 | 3 | 1 | **0.5** | *growth-dependent butyric acid per biomass yield of A* | gBA gX-1 |
| Y\_bax\_(I) | 0 | 0.5 | 0 | **0** | *growth-dependent butyric acid per biomass yield of I* | gBA gX-1 |
| r\_ba\_max\_(A) | 0.5 | 3 | 1.2 | **0.5248** | *growth-independent butyric acid production rate of A* | gBA gX-1 h-1 |
| r\_ba\_max\_(I) | 0 | 0.5 | 0 | **0** | *growth-independent butyric acid production rate of I* | gBA gX-1 h-1 |
| k\_BA\_up\_(I) | 0 | 0.5 | 0.25 | **0.5** | *growth-independent butyric acid uptake rate of I* | gBA gX-1 h-1 |
| k\_BA\_up\_(S) | 0.5 | 3 | 0.5 | **1.8594** | *growth-independent butyric acid uptake rate of S* | gBA gX-1 h-1 |
| r\_eth\_max\_(A) | 0 | 0.5 | 0.01 | **0** | *growth-independent ethanol production rate of A* | gETH gX-1 h-1 |
| r\_eth\_max\_(I) | 0 | 0.5 | 0.03 | **0** | *growth-independent ethanol production rate of I* | gETH gX-1 h-1 |
| r\_eth\_max\_(S) | 0 | 0.5 | 0.1 | **0.2458** | *growth-independent ethanol production rate of S* | gETH gX-1 h-1 |
| r\_act\_max\_(I) | 0 | 0.5 | 0.2 | **0.0866** | *growth-independent acetone production rate of I* | gACT gX-1 h-1 |
| r\_act\_max\_(S) | 0.3 | 1.5 | 0.32 | **0.6213** | *growth-independent acetone production rate of S* | gACT gX-1 h-1 |
| r\_but\_max\_(I) | 0 | 1 | 0.7 | **0.0203** | *growth-independent butanol production rate of I* | gBUT gX-1 h-1 |
| r\_but\_max\_(S) | 0.5 | 2.5 | 0.8 | **2.4485** | *growth-independent butanol production rate of I* | gBUT gX-1 h-1 |
| sk | 0.7 | 1.3 | 1 | **1.1020** | *C-balance adaptation coefficient* | molC molGLU-1 |
| r\_CO2\_(A) | 0 | 2.5 | 0.4 | **0.0019** | *carbon lost as CO2 in A* | molC molGLU-1 |
| r\_CO2\_(I) | 0 | 2.5 | 0.8 | **0** | *carbon lost as CO2 in I* | molC molGLU-1 |
| r\_CO2\_(S) | 0 | 2.5 | 0 | **2.4904** | *carbon lost as CO2 in S* | molC molGLU-1 |
| mu\_d\_(A) | 0 | 0.95 | 0.4 | **0.1681** | *differentiation rate of A to I* | h-1 |
| mu\_d\_(I) | 0 | 0.95 | 0.85 | **0.1681** | *differentiation rate of I to S* | h-1 |
| K\_UDA\_(A) | 0.9 | 5 | 1.5 | **3.0203** | *concentration of undissociated organic acids inducing half maximal differentiation from A to I* | g L-1 |
| n\_iUDA\_(A) | 1 | 5 | 3 | **4.3175** | *coefficient varying the steepness of the stepwise induction of differentiation at the threshold concentration of undissociated acids* | - |

\* *Initial values were taken from Karstens et al. (2016) doi.org/10.3303/CET1649046, except for k\_BA\_up\_(I); A - acidogenic cells, I – intermediate cells, S – solventogenic cells*

Table SIc– Fixed kinetic model parameters derived from literature, experimental studies or estimations

| **parameter** | **value** | **description** | **reference** | **unit** |
| --- | --- | --- | --- | --- |
| KsGLU | **6.5** | *affinity constant for glucose* | *(Srivastava & Volesky, 1990) x 0.198 g mmol-1* | gGLU L-1 |
| KsPO4 | **0.005** | *affinity constant for phosphate* | *estimated from experimental data* | gKH2PO4 L-1 |
| KiB | **5** | *concentration of butanol inducing half maximal growth inhibition* | *estimated from experimental data* | gBUT L-1 |
| niB | **3** | *coefficient varying the steepness of the stepwise growth inhibition at the threshold butanol concentration* | *estimated from experimental data* | - |
| Y\_xp | **29** | *biomass per KH2PO4 yield* | *calculated from data set D=0.042 h-1/ pHbr1 4.3* | gX gKH2PO4-1 |
| Y\_aax\_(S) | **0** | *growth-dependent acetic acid per biomass yield of S* | *per definition* | gAA gX-1 |
| r\_aa\_max\_(S) | **0** | *growth-independent acetic acid production rate of S* | *per definition* | gAA gX-1 h-1 |
| k\_AA\_up\_(A) | **0** | *growth-independent acetic acid uptake rate of A* | *per definition* | gAA gX-1 h-1 |
| Y\_bax\_(A) | **0** | *growth-dependent butyric acid per biomass yield of S* | *per definition* | gBA gX-1 |
| r\_ba\_max\_(I) | **0** | *growth-independent butyric acid production rate of S* | *per definition* | gBA gX-1 h-1 |
| k\_BA\_up\_(I) | **0** | *growth-independent butyric acid uptake rate of A* | *per definition* | gBA gX-1 h-1 |
| KsAA | **0.6** | *affinity constant for acetic acid* | *(Srivastava & Volesky, 1990) x 0.068 g mmol-1* | gAA L-1 |
| KsBA | **0.734** | *affinity constant for butyric acid* | *(Srivastava & Volesky, 1990) x 0.088 g mmol-1* | gBA L-1 |
| r\_act\_max\_(A) | **0** | *growth-independent acetone production rate of A* | *per definition* | gACT gX-1 h-1 |
| r\_but\_max\_(A) | **0** | *growth-independent butanol production rate of A* | *per definition* | gBUT gX-1 h-1 |
| Y\_xs | **0.475** | *biomass per glucose yield* | *typical value from literature and own results* | gX gGLU-1 |
| Y\_aas | **0.9091** | *acetic acid per glucose yield* | *calculated for stoichiometric conversion* | gAA gGLU-1 |
| Y\_bas | **0.6667** | *butyric acid per glucose yield* | *calculated for stoichiometric conversion* | gBA gGLU-1 |
| Y\_eths | **0.6970** | *ethanol per glucose yield* | *calculated for stoichiometric conversion* | gETH gGLU-1 |
| Y\_acts | **0.5859** | *acetone per glucose yield* | *calculated for stoichiometric conversion* | gACT gGLU-1 |
| Y\_buts | **0.5606** | *butanol per glucose yield* | *calculated for stoichiometric conversion* | gBUT gGLU-1 |
| Kd | **0.02** | *butanol dependent cell lysing rate* | *calculated from data set D=0.042 h-1/ pHbr1 4.3* | L gBUT-1 h-1 |
| KdGLU | **1.0353** | *concentration of glucose inducing half maximal cell lysis* | *optimized based on experimental data sets and a preliminarily variable parameter set* | gGLU L-1 |
| n\_dGLU | **1.7332** | *coefficient varying the steepness of the stepwise induction of cell lysis at the threshold glucose concentration* | *optimized based on experimental data sets and a preliminarily variable parameter set* | - |
| mu\_d\_(S) | **0** | *differentiation rate of A* | *per definition* | h-1 |

*A - acidogenic cells, I – intermediate cells, S – solventogenic cells; (Srivastava & Volesky, 1990, doi.org/10.1007/BF01088196)*