**Appendix**

**A.1 Macro-kinetic model of *Escherichia coli***

The mechanistic model of *E. coli* used in this publication is based on the physiological use of glucose, in a glucose partitioning framework as well as the overflow of glucose to acetate through the acetate cycling concept. The two physiological concepts, as given by Neubauer et al. 2000 (Ying Lin & Neubauer, 2000) and Lin et al., (Lin, Mathiszik, Xu, Enfors, & Neubauer, 2001b) and basic growth concepts such as Monod kinetics and acetate inhibition are used to derive simple algebraic equations that describe intracellular pathways of glucose and oxygen usage. Details on the derivation of the model and its subsequent usage in *E. coli* processes can be found in the literature (Anane et al., 2017; Cruz Bournazou et al., 2017; Lin et al., 2001b). The algebraic equations that describe these intracellular activities are as follows:

$q\_{s}=\frac{q\_{smax}S}{S+K\_{s}}.e^{-P\*K\_{ip}}$ (A.1)

$q\_{sox}=\left(q\_{s}-\frac{P\_{Amax}q\_{s}}{q\_{s}+K\_{ap}}\right)∙\frac{DOT}{DOT+K\_{o}}$ (A.2)

$q\_{sof}=q\_{s}-q\_{sox}$ (A.3)

$q\_{PA}=q\_{sof}Y\_{as}$ (A.4)

$q\_{sA}=\frac{q\_{Amax}}{1+\frac{q\_{s}}{K\_{is}}}∙\frac{A}{A+K\_{sa}}$ (A.5)

$q\_{A}=q\_{PA}-q\_{sA}$ (A.6)

$μ=\left(q\_{sox}-q\_{m}\right)Y\_{em}+q\_{sA}Y\_{xa}+\left(q\_{sof}-p\_{A}\right)Y\_{xsof}$ (A.7)

$q\_{o}=\left(q\_{sox}-q\_{m}\right)Y\_{os}+q\_{sA}Y\_{oa}$ (A.8)

$q\_{p}=μY\_{px}$ (A.9)

The algebraic equations are coupled with mass balances for a fed-batch process to yield the full ODE system. The ODE system for the *E. coli* model is derived from mass balances on biomass (X), glucose (substrate, S), acetate (A) and dissolved oxygen measured as the percentage saturation at the operating conditions in the bioreactor (DOT).

$\frac{dX}{dt}=\frac{F}{V}\left(0-X\right)+μX$ (A.10)

$\frac{dS}{dt}=\frac{F}{V}\left(S\_{i}-S\right)-q\_{s}X$ (A.11)

$\frac{dA}{dt}=\frac{F}{V}\left(0-A\right)+q\_{sA}X$ (A.12)

$\frac{dDOT}{dt}=K\_{La}\left(DOT^{\*}-DOT\right)-q\_{o}XH$ (A.13a)

$\frac{dDOT^{m}}{dt}=K\_{p}\left(DOT-DOT^{m}\right)$ (A.14)

$\frac{dP}{dt}= q\_{p}-μP$ (A.15)

There is a significant difference between biological *times* of macroscopic phenomena (growth and cell division, as captured in equation A.7 & A.10) and mass transfer times (Equations A.8 and A.13). The time required for diffusion of oxygen across the gas-liquid interphase into the fermentation broth is several orders of magnitude greater than the cell division time (Lara et al., 2006). The coupling of two differential equations describing a slow process and a very fast process results in a stiff ODE system, which is mathematically difficult to handle. Since oxygen transfer was considered to be very fast, the concentration of dissolved oxygen at any time was considered to be at steady state. Therefore, Equation A.13a was reduced to the algebraic steady-state solution (Equation A.13b), which was used in the mechanistic model instead of Equation A.13a.

$DOT=\frac{K\_{La}DOT^{\*}-q\_{o}XH}{K\_{La}}$ (A.13b)

Additionally, in the presence of a fast changing DOT signal due to the induced gradients (glucose pulses), the response time of the dissolved oxygen probe becomes significant in predicting the DOT profile. This is especially important when the response time of the probe is about 5 or less times slower than the inverse of the KLa ($^{1}/\_{5K\_{La}}>τ$) (Badino, Cândida, Facciotti, & Schmidell, 2000). Since this condition was satisfied for the cultivation system used, a differential equation was added to simulate the measured DOT by the probe (DOTm) which takes into account the probe response time $τ$, where $K\_{p}=^{1}/\_{τ}$. Therefore, the actual dissolved oxygen, which was solved algebraically was equal to the measured DOT, only after the elapse of the response time under equilibrium conditions.

The model (Equations A.1—A.14) was compiled as a single mathematical function (e\_colimodel) and implemented in Matlab® R2016a. The model was integrated with ode15s solver and parameter estimation was done with the *fmincon* optimization routine in Matlab, using the interior-point algorithm.

**A.2 Further Results**

Figure A.1 Growth profile and metabolic response of *E. coli* to the various cultivation conditions induced in the scale-down cultivations in the parallel minibioreactors (See Table 1 for description of the various conditions). Each plot represents the average of the triplicate runs of that condition, whereas error bars represent the standard deviation of the triplicate runs. Dotted lines indicate the start of exponential feed fed-batch (at 5.6 hours) and the point of induction with a switch to constant feed at 9 hours.

Figure A.2 Influence of glucose pulses on the concentration of the non-canonical amino acids norleucine (Nle) and β-Methyl norleucine (β-Mnle): **A**: intracellular soluble fraction (ISF) and purified inclusion bodies (IBs); **B:** incorporation levels in the purified inclusion bodies for all the cultivation conditions presented in Table 1; **C**: Dynamic profile of norvaline incorporation (both 0.5 mM IPTG) as a function of glucose pulse frequency (1xP and 2xP,). Ref: reference cultivation; 2xP—10 min glucose pulse cultivation; 1xP—5 min glucose pulse cultivation; Enp—cultivation with enzymatic glucose release.