

Automated conditional screening of multiple *Escherichia coli* strains in parallel adaptive fed-batch cultivations

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Supplementary Table S1: Experimental measurements

For raw data and further explanations see File: Table_S1_Measurments.xlsx

Supplementary Table S2: Initial parameter values and bounds for the parameter estimation (model calibration):

Parameter	Unit	Lower Bound	initial Value	Upper Bound
K _{ap}	g L ⁻¹	1.00	4.44	10.00
	Monod-type saturation constant, intracellular acetate production			
K _{aq}	g L ⁻¹	0.30	0.40	1.00
	Affinity constant, acetate consumption			
k _{La}	h ⁻¹	200.00	280.00	300.00
	Volume specific mass transfer coefficient			
K _o	g L ⁻¹	1.00	10.00	20.00
	Affinity constant, oxygen consumption			
K _s	g L ⁻¹	0.03	0.05	0.10
	Affinity constant, substrate consumption			
K _{sq}	g L ⁻¹	0.10	1.07	2.00
	Inhibition constant, inhibition of acetate uptake by glucose			
pA _{max}	g g ⁻¹ h ⁻¹	0.50	1.31	1.60
	Maximal specific acetate production rate			
qA _{max}	g g ⁻¹ h ⁻¹	0.50	1.03	1.60
	Maximal specific acetate uptake rate			
q _m	g g ⁻¹ h ⁻¹	0.02	0.04	0.05
	Specific maintenance coefficient			
qS _{max}	g g ⁻¹ h ⁻¹	1.00	1.56	1.60
	Maximal specific glucose uptake rate			
Y _{am}	g g ⁻¹	0.40	0.40	1.00
	Yield of acetate to biomass			
Y _{aof}	g g ⁻¹	0.20	0.46	0.60
	Yield of glucose uptake to overflow metabolism			

Parameter	Unit	Lower Bound	initial Value	Upper Bound
Y_{aresp}	$g\ g^{-1}$ Yield of acetate uptake to oxygen consumption	0.05	0.10	0.15
Y_{em}	$g\ g^{-1}$ Yield of glucose to biomass	0.50	0.56	0.60
Y_{ofm}	$g\ g^{-1}$ Yield of overflow metabolism to biomass	0.20	0.28	0.80
Y_{osresp}	$g\ g^{-1}$ Yield of glucose uptake to oxygen consumption	0.70	1.00	2.00

Supplementary Table S3: Parameter values after each model calibration (MC) for *Escherichia coli* K12 W3110. Parameters marked with * are adjusted in the MC

[illegible]

Supplementary Table S4: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113. Parameters marked with * are adjusted in the MC *

[illegible]

Supplementary Table S5: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113 $\Delta ompT$. Parameters marked with * are adjusted in the MC *

[illegible]

Supplementary Table S6: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113 $\Delta aceA$. Parameters marked with * are adjusted in the MC *

[illegible]

Supplementary Table S7: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113 *ΔfliA*. Parameters marked with * are adjusted in the MC *

[illegible]

Supplementary Table S8: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113 $\Delta gatC$. Parameters marked with * are adjusted in the MC *

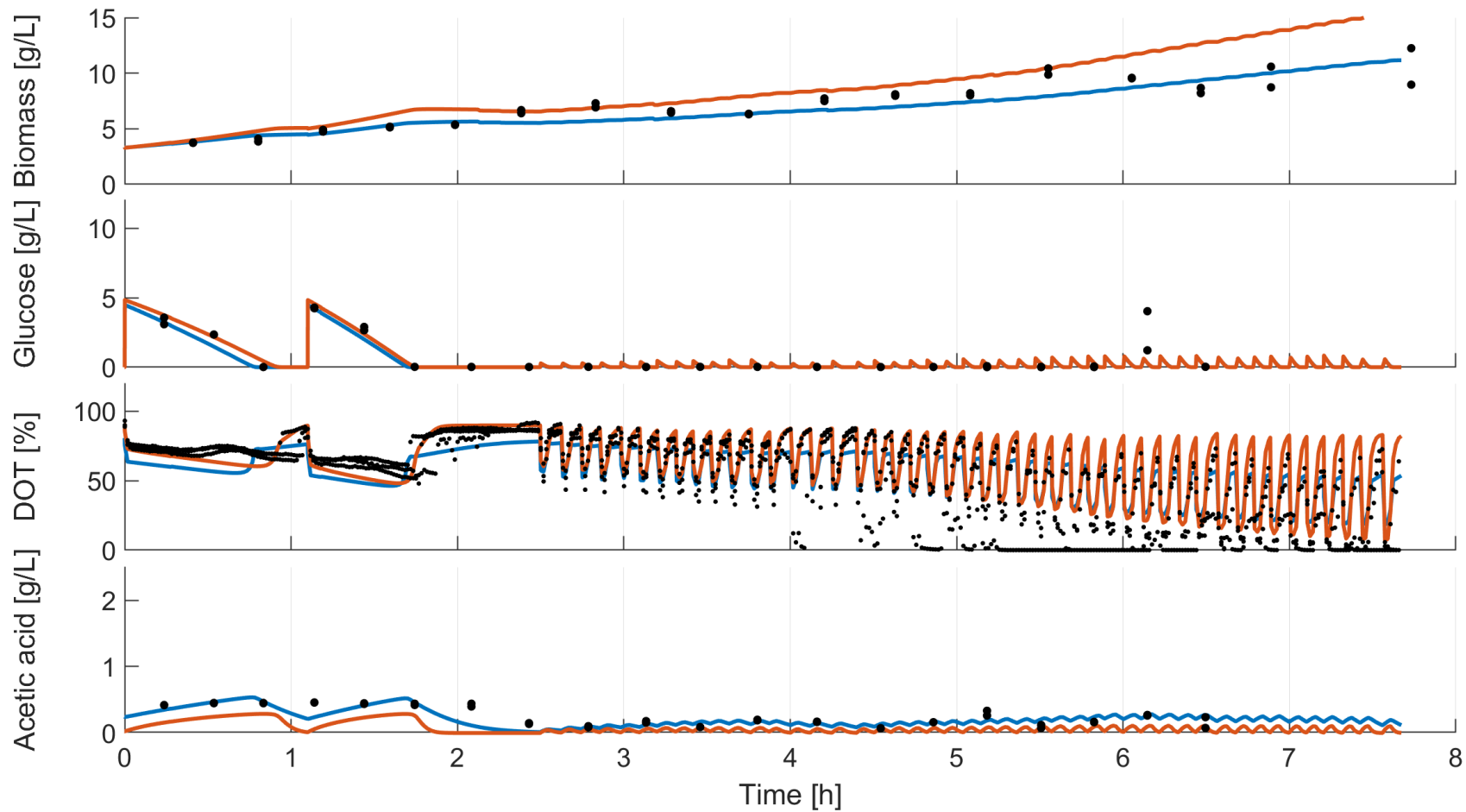
[illegible]

Supplementary Table S9: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113 $\Delta gatZ$. Parameters marked with * are adjusted in the MC *

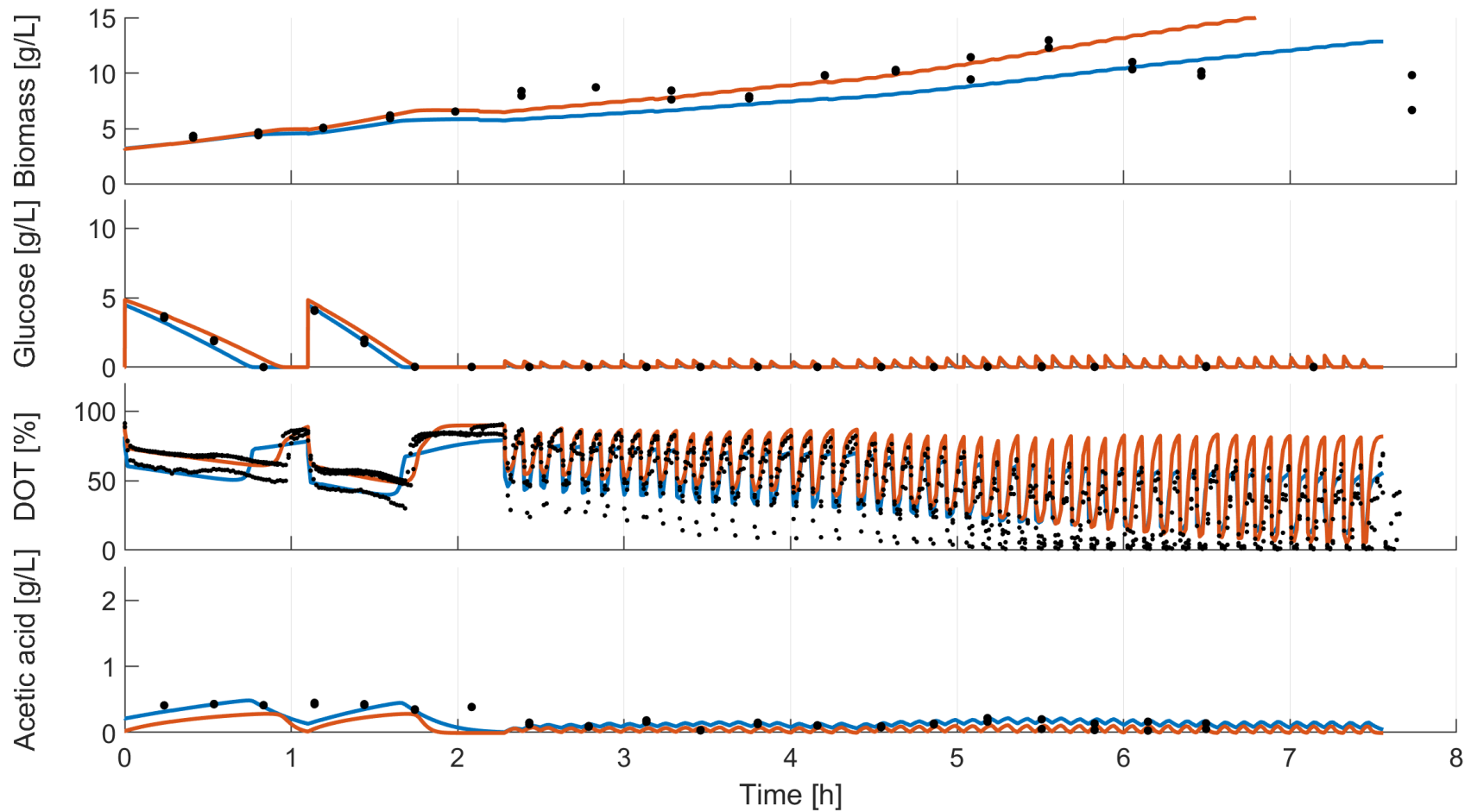
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Supplementary Table S10: Parameter values after each model calibration (MC) for *Escherichia coli* K12 BW25113 Δ glcB. Parameters marked with * are adjusted in the MC *

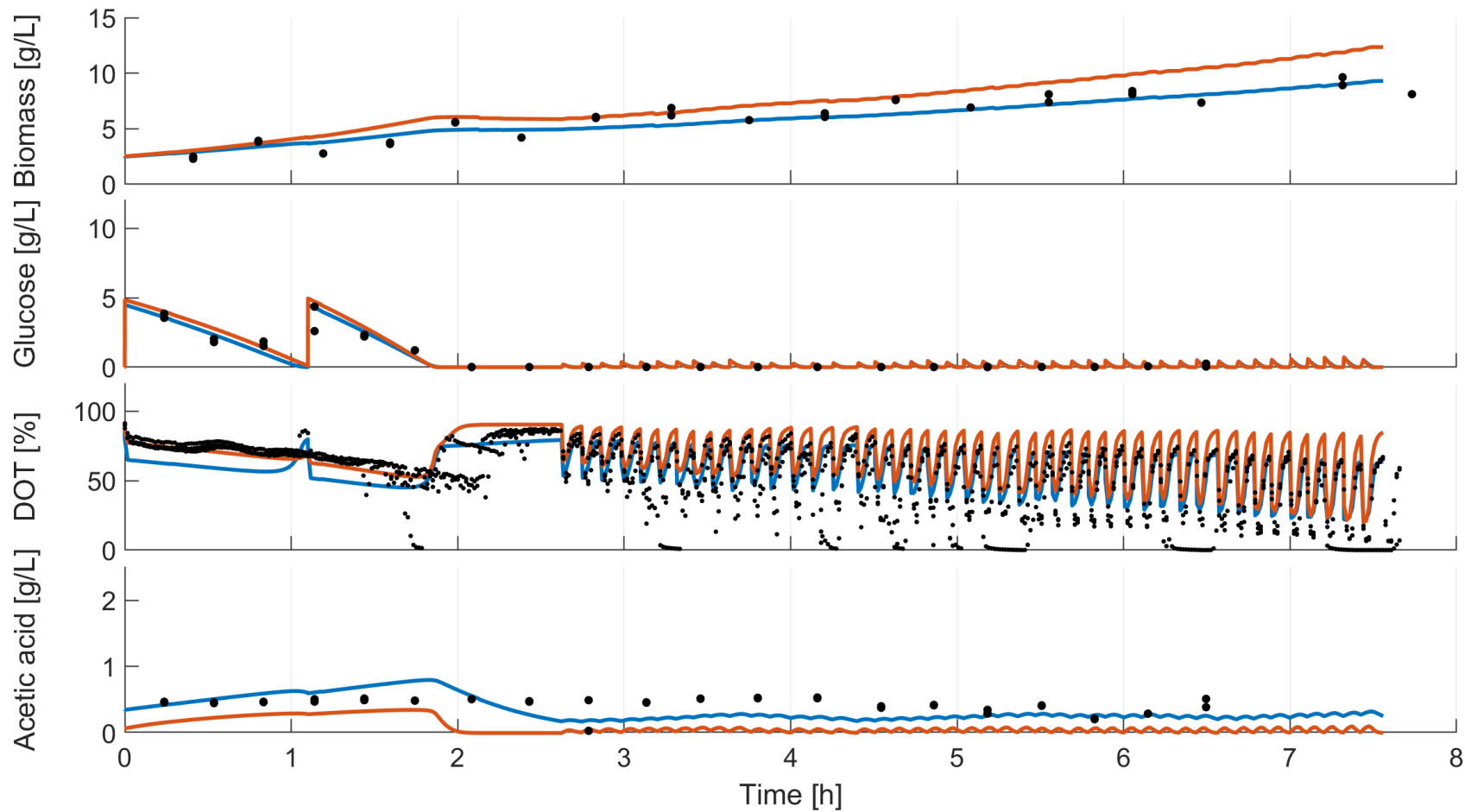
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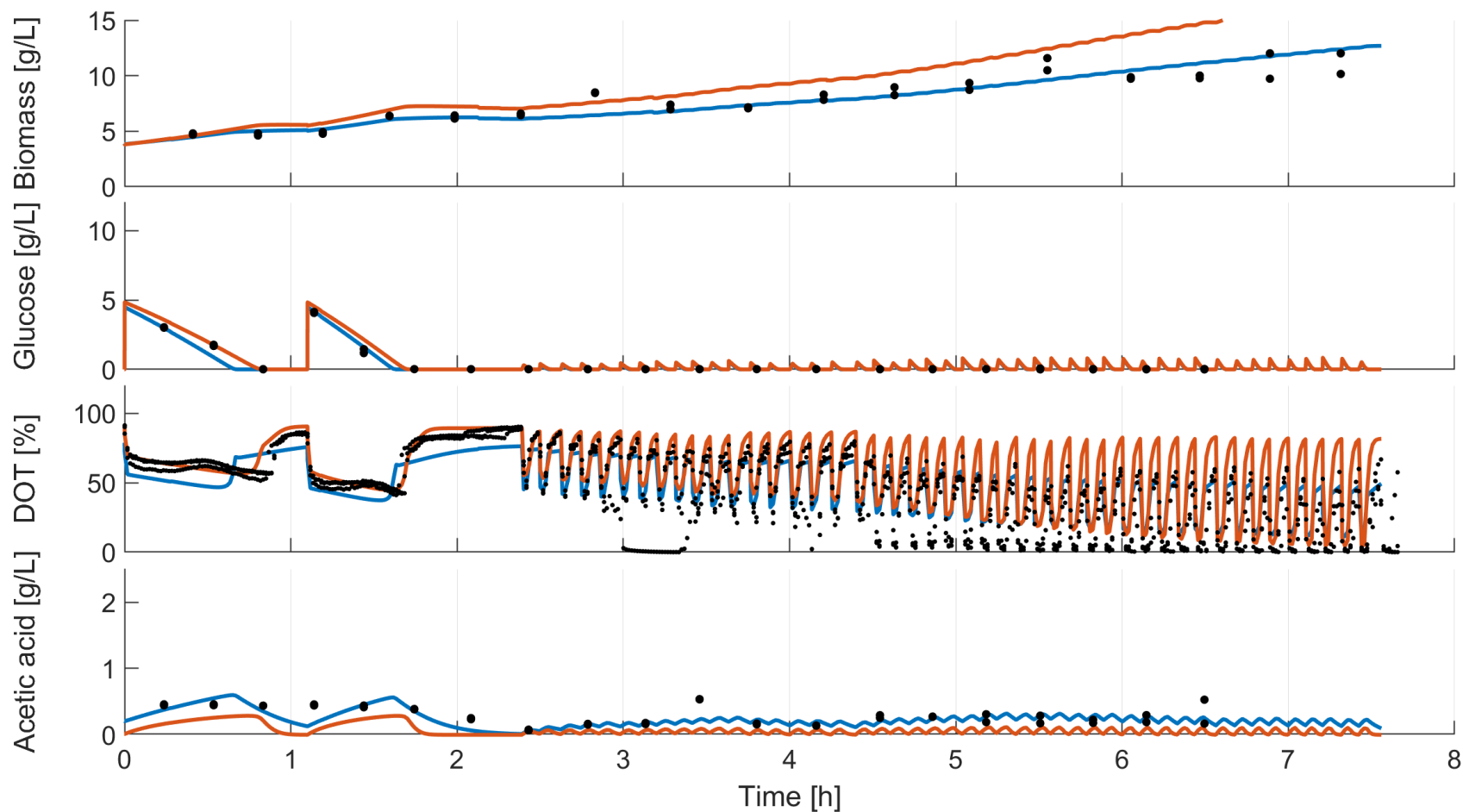
Supplementary Figure 1: Comparison of measurements (black dots) and simulation results of *E. coli* K12 W3110 with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The biomass and acetic acid prediction are improved after the model calibration. Both, the trend of the cultivation and the absolute difference between measurements and simulation is improved. However, the gap between DOT and glucose measurements is slightly higher especially during the batch phase.



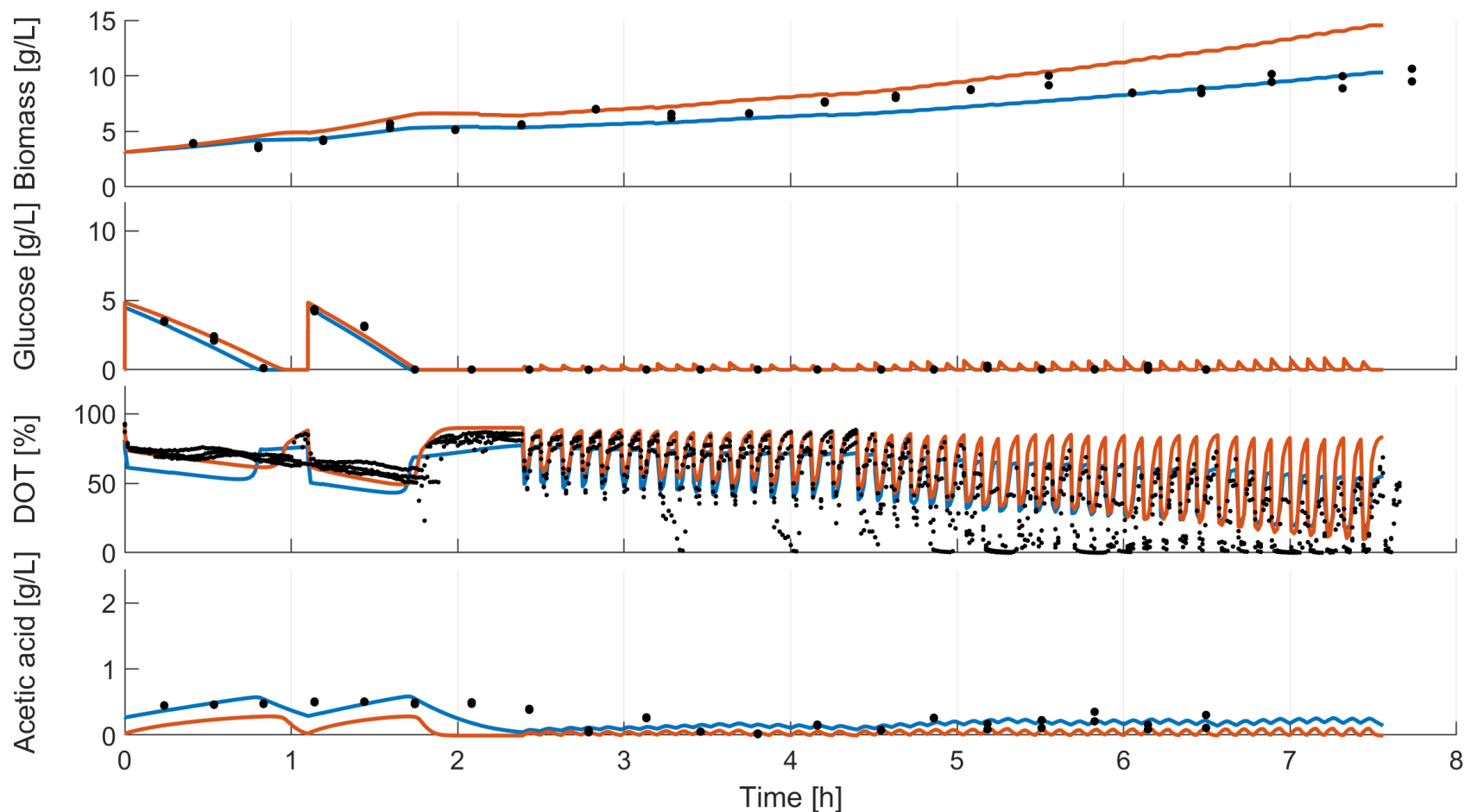
Supplementary Figure 2: Comparison of measurements (black dots) and simulation results of *E. coli* K12 BW25113 with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The acetic acid prediction is slightly improved after the model calibration. However, in general the initial model seems to present the early cultivation better compared to the calibrated simulation. This is due to the difficult cultivation conditions in the last hours of cultivation. Nevertheless, the adaptive process design has been able to prevent glucose overflow and acetate accumulation by automatically reacting to the changed conditions. With a sliding window approach and automated process evaluation to detect outliers and process deviations, this issue can be addressed (see main part discussion)



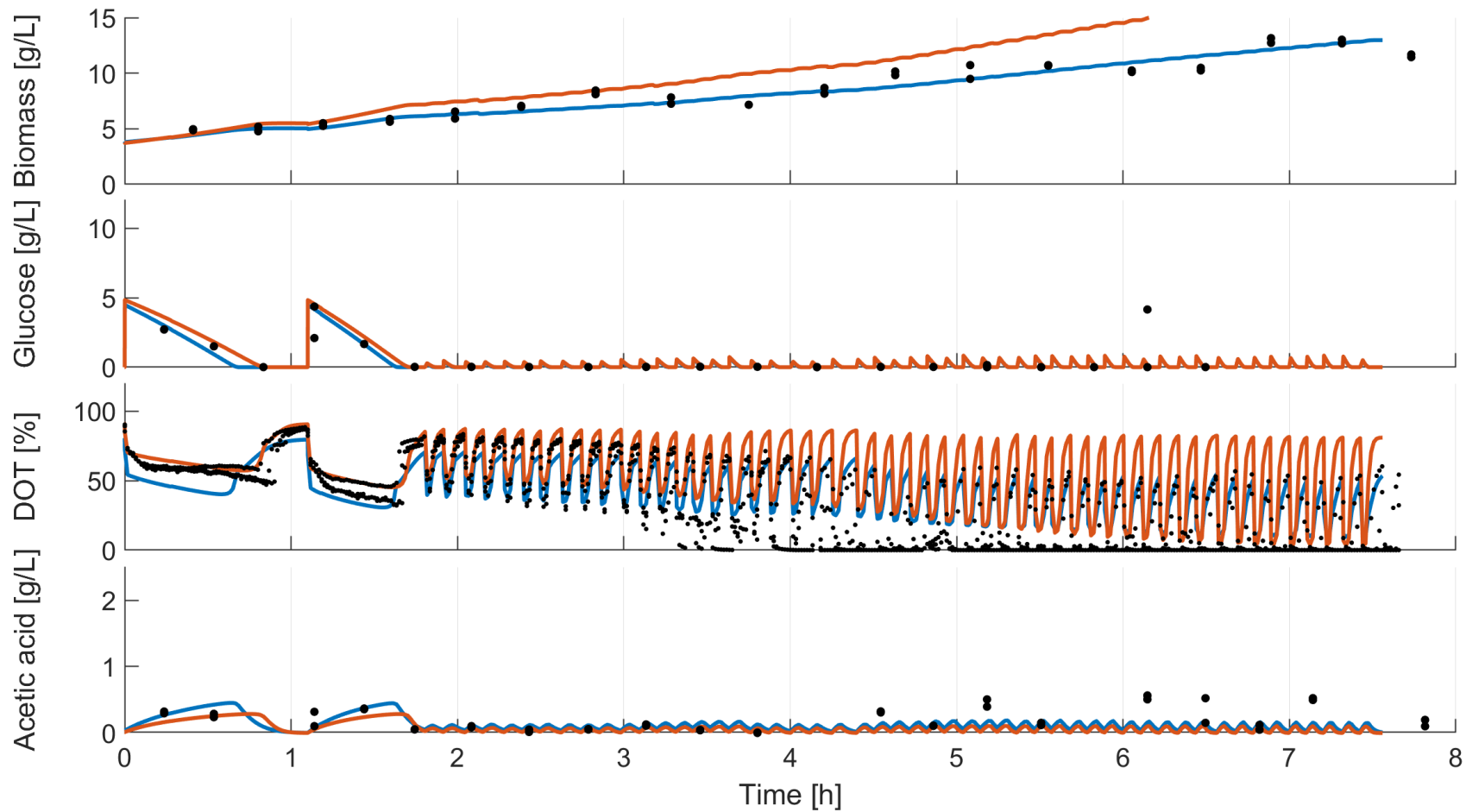
Supplementary Figure 3: Comparison of measurements (black dots) and simulation results of *E. coli* K12 BW25113 $\Delta ompT$ with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The biomass and acetic acid prediction are improved after the model calibration. Both, the trend of the cultivation and the absolute difference between measurements and simulation is improved. The slight difference in the glucose prediction could not visually rated. However, based on the DOT profile the glucose uptake in the batch phase seems to be marginal overpredicted after model calibration, which is the opposed in the fed batch phase.



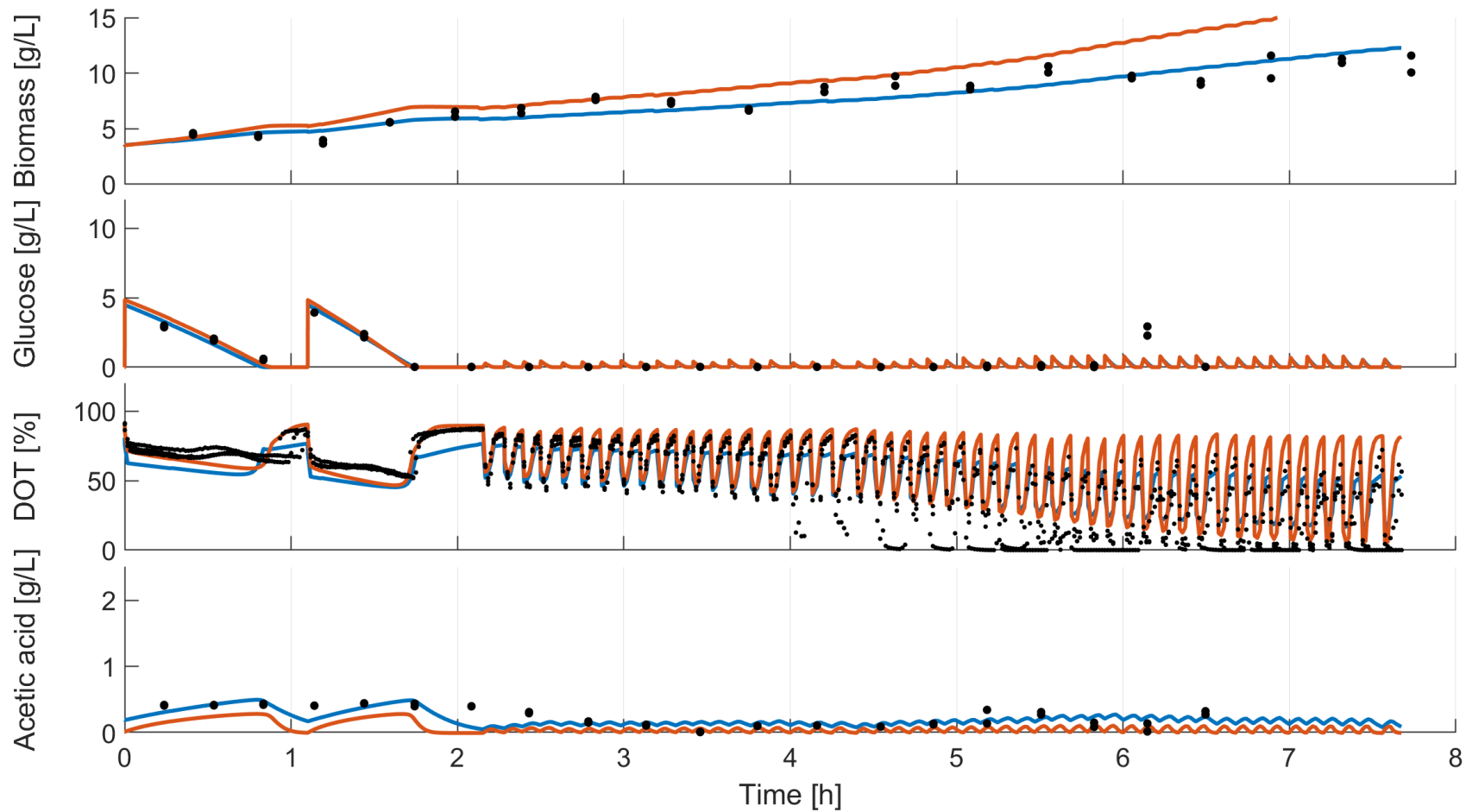
Supplementary Figure 4: Comparison of measurements (black dots) and simulation results of *E. coli* K12 BW25113 $\Delta aceA$ with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The biomass and acetic acid prediction are improved after the model calibration. Both, the trend of the cultivation and the absolute difference between measurements and simulation is improved. However, the gap between DOT and glucose measurements is slightly higher especially during the batch phase.



Supplementary Figure 5: Comparison of measurements (black dots) and simulation results of *E. coli* K12 BW25113 $\Delta fliA$ with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The biomass and acetic acid prediction are improved after the model calibration. Both, the trend of the cultivation and the absolute difference between measurements and simulation is improved. However, the gap between DOT and glucose measurements is slightly higher especially during the batch phase.



Supplementary Figure 6: Comparison of measurements (black dots) and simulation results of *E. coli* K12 BW25113 $\Delta gatC$ with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The biomass is improved after the model calibration in both, the trend of the cultivation and the absolute difference between measurements and simulation. However, the gap between DOT and glucose measurements is slightly higher especially during the batch phase.



Supplementary Figure 7: Comparison of measurements (black dots) and simulation results of *E. coli* K12 BW25113 $\Delta gatZ$ with (orange line) and without model calibration (blue line). The calibrated simulation is based on the parameter set of the last model calibration after 8 hours of cultivation. The biomass and acetic acid prediction are improved after the model calibration. Both, the trend of the cultivation and the absolute difference between measurements and simulation is improved. The slight difference in the glucose prediction cannot be rated visually. The DOT signals followed the same trend in the initial and calibrated model. However, the oxygen uptake is overpredicted in the calibrated model.