Supplements to: Design of cell expansion processes for adherent-growing cells with mDoE-workflow

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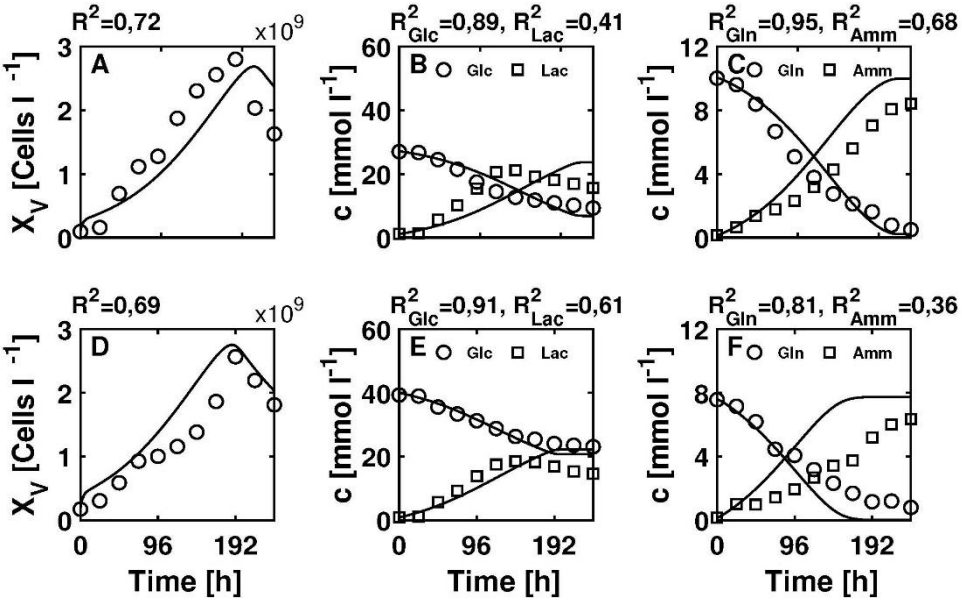
Abbreviations: Amm, Ammonia; **ATMP**, Advanced Therapy Medicinal Products; **BBD**, Box-Behnken Design; **CCD**, Central Composite Design; **CHO**, Chinese Hamster Ovary; **DAPI** 4',6-diamidine-2-phenylindole; **DMEM**, Dulbecco's Modified Eagle Medium; **DoE,** Design of Experiments; **EDTA,** Ethylenediaminetetraacetic acid; **FBS**, Fetal Bovine Serum; **Glc**, Glucose; **Gln**, Glutamine; **Lac**, Lactate; **LHSD**, Latin Hypercube sample design; **LS**, Limiting substrate; **MC**, Microcarrier; **mDoE** model-assisted Design of Experiments; **PBS,** Phosphate buffered saline; **PD**, Population doublings; **SG**, SYBR Green; **VF**, Multiplication factor

Supplementary Table 1 Implemented boundary condition of the mathematical process model to consider the influence of MC concentration.

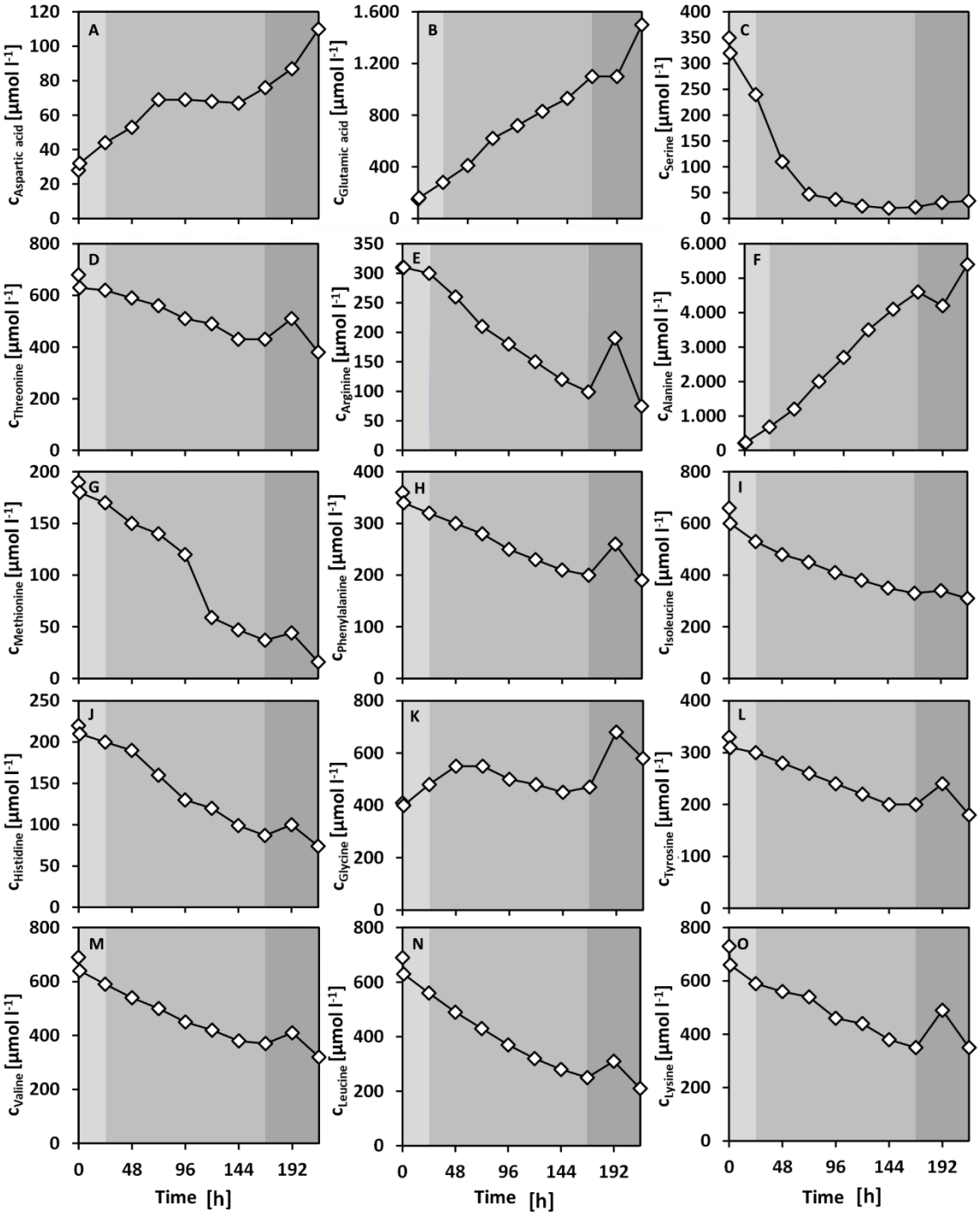
c_{MC} [g l^{-1}]	Boundary condition
Experiments for modelling	
20	$\mu = 0.6x\mu_{max}$
	$q_{ls,max} = 0.3xq_{ls,max}$
	$Y_{X,Glc} = 1.4xY_{X,Glc}$
5	$Y_{X,Glc} = 0.5xY_{X,Glc}$
	$Y_{X,Gln} = 0.5xY_{X,Gln}$
Verification of mDoE	
3	$q_{LS,max}=0.5\ q_{LS,max}$
	$Y_{X,Glc}=0.5\ Y_{X,Glc}$
	$Y_{X,Gln}=0.5\ Y_{X,Gln}$
1	$\mu_{max}=1.2\ \mu_{max}$
	$Y_{X,Glc}=0.5\ Y_{X,Glc}$
	$Y_{X,Gln}=0.5\ Y_{X,Gln}$

Supplementary Table 2 Parameters of the mathematical process model as well as the associated start values and resulting values.

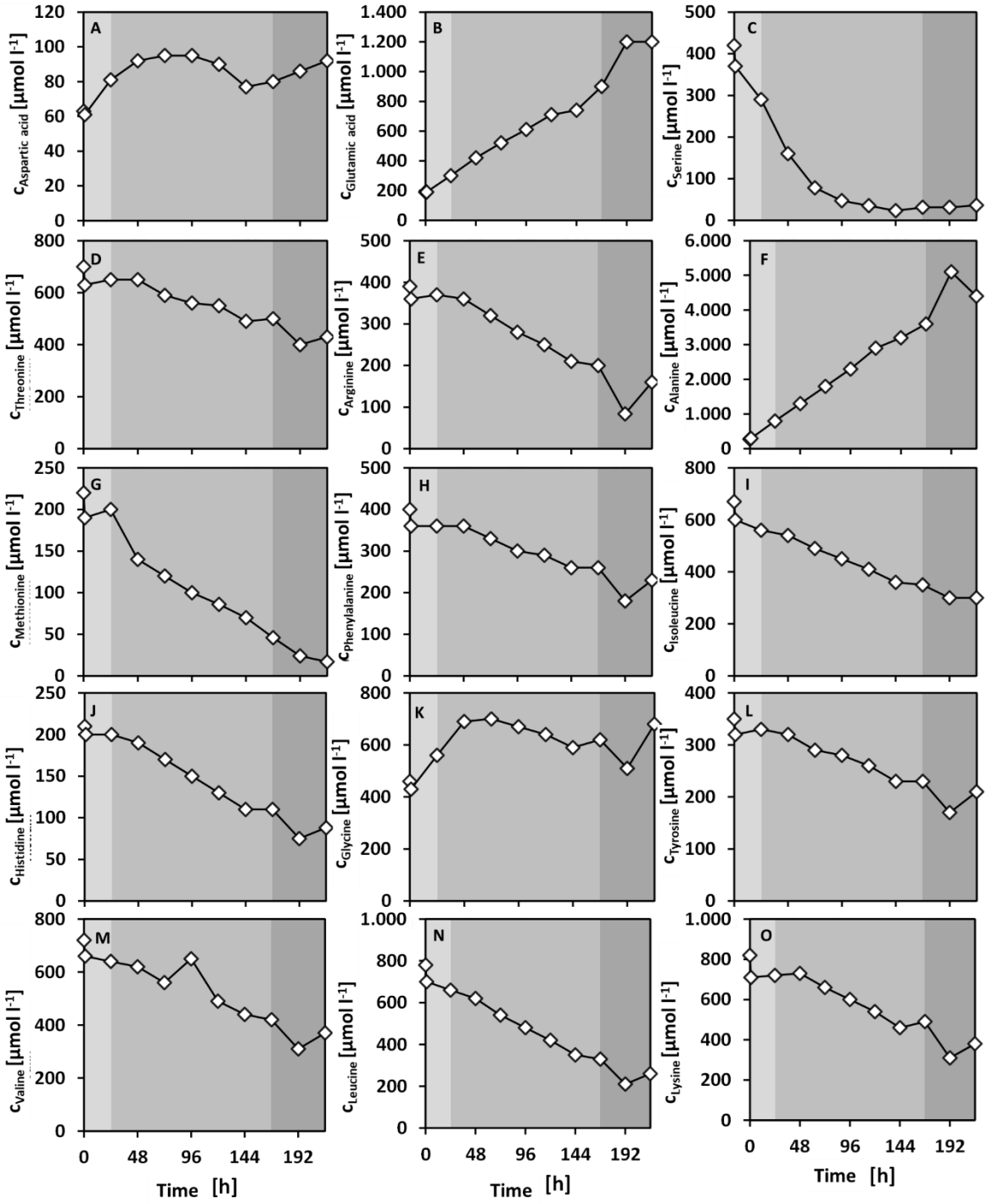
Parameter	Unit	Starting value	Adapted value
$\mu_{d,max}$	h^{-1}	0.02	0.0064
$\mu_{d,min}$	h^{-1}	0.003	0.0002
μ_{max}	h^{-1}	0.02	0.026
$K_{att,max}$	h^{-1}	0.03	0.4
$K_{d,LS}$	mmol l^{-1}	0.005	0.065
K_{Glc}	mmol l^{-1}	0.19	0.03
K_{Gln}	mmol l^{-1}	2.5	0.61
$k_{s,LS}$	mmol l^{-1}	0.01	0.002
k_{LS}	mmol l^{-1}	ß.1	0.035
$Y_{X/Glc}$	10^7 cells l^{-1} $mmol^{-1}$	7.2	7.2
$Y_{X/Gln}$	10^8 cells l^{-1} $mmol^{-1}$	1.7	1.7
$q_{LS,max}$	10^{-11} mmol $cell^{-1}$ h^{-1}	1.4	1.4
$Y_{Lac/Glc}$	-	1.5	1.1
$Y_{Amm/Gln}$	-	0.6	1



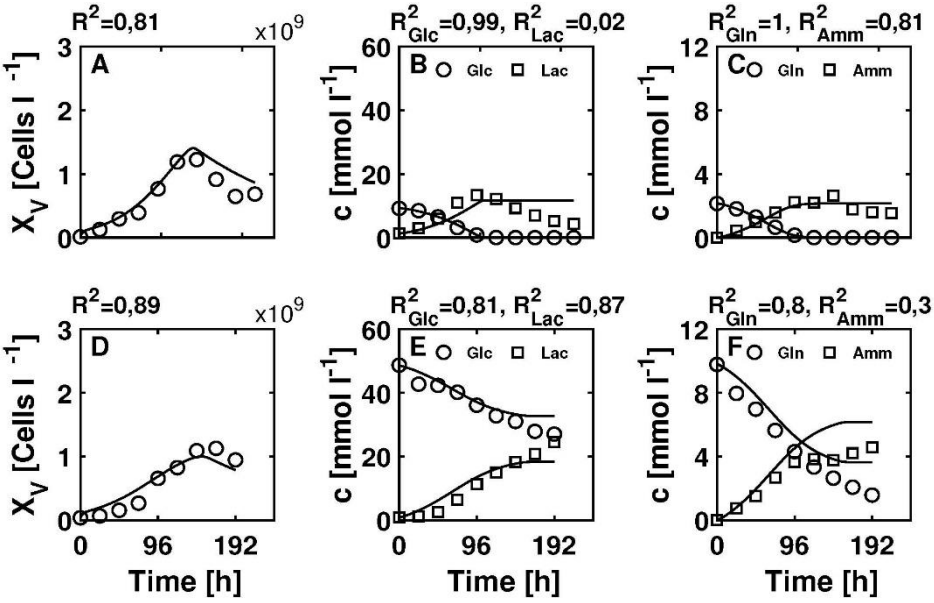
Supplementary Figure 1 Growth curves of the cultivation in the shake flask (symbols) compared to the simulated growth curve (line). Plotted are cell numbers, substrates and metabolites against cultivation time. The MC concentration was 20 g l^{-1}. Data in graphs A, B and C are from experiment 1, Table 1, while D, E and F are from experiment 2, Table 1.



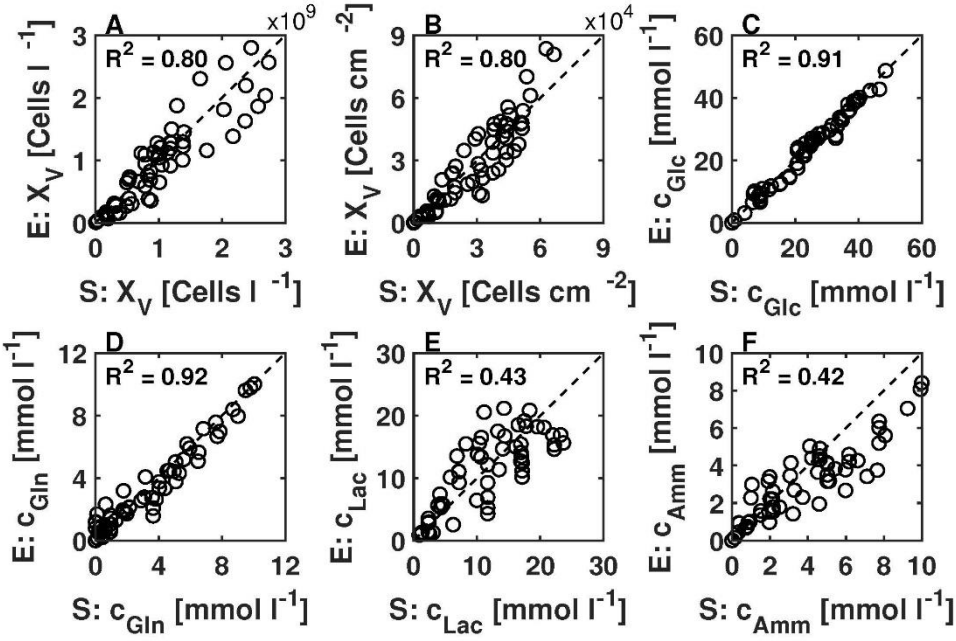
Supplementary Figure 2 Diagrams of the 15 evaluated amino acids of experiment 1, Table 1. The colored background illustrates the attachment, the exponential phase and the death phase.



Supplementary Figure 3 Diagrams of the 15 evaluated amino acids of experiment 2, Table 1. The colored background illustrates the attachment, the exponential phase and the death phase.



Supplementary Figure 4 Growth curves of the cultivation in the shake flasks (symbols) compared to the simulated growth curve (line). Plotted are cell numbers, substrates and metabolites against cultivation time. The MC concentration was 10 g l^{-1} (experiment 3, Table 1) and 5 g l^{-1} (experiment 6, Table 1). Data in graphs A, B and C are from experiment 3, Table 1, while D, E and F are from experiment 6, Table 1.



Supplementary Figure 5 Growth curves of the cultivation in the shake flask in comparison to the simulated growth curve. The time course of the cell numbers and the time course of the substrate and metabolite concentrations (Glc, Gln, Lac, Amm) are shown.