Maximum entropy and population heterogeneity in continuous cell cultures meet experimental data, preliminary results

September 22, 2019

1 Materials and Method

1.1 Experimental data

Experimental data was taken from *Rath*, in this work the author performed 6 continuous cultures, (A, B, C, D, E, F), with the cell line AGE1.HN.AA1, which parental line AGE1.HN was established by the company ProBioGen (ProBioGen AG, Berlin, Germany) from a tissue sample of a human brain. All cultures feed mediums was based on the standard 42-Max-UB-medium, which is serum-free and was especially developed for the AGE1.HN cell line $Table1$. The experiments was run under various conditions, differing mainly in the dilution rate (D) and the feed medium composition of glucose (GLC), glutamine (GLN) and galactose (GAL) $Table2$.
For each experiment, a steady state condition was reached, (A, B, C, D, E, $F01$), and several observables was reported $Tables3-4$. Particularly relevant for this work was the growth rate (μ), D, the viable cell density (Xv) and the medium concentration (s) and derived uptake rate (u) for a set of metabolites (GLC, lactose (LAC), GLN, ammonium ($NH4$), GAL, pyruvate (PYR), glutamate (GLU), alanine (ALA), asparagine (ASP)). Unit conversion was required for make experimental data and models compatible. For this propose the only external data needed was the cell mass density. It was used 0.25 pgDW/ μm^3 $Niklas$.

1.2 Preparing GEMs

In order to evaluate the capacity of the ...

1.2.1 Recon3d

Recon3DModel_301.mat was downloaded from http://vmh.life [2], the biomass equation was modified to adjust the biomass demand reported by [1] for the

Substance	Value	Dimension	Analytical method
Pluronic	1.0	g/L	as stated by Xell
NaHCO3	2.1	g/L	as stated by Xell
Osmolality	290.0	$^{mOsm}/_{kg}$	FPDO[a]
pH value	7.4	-	pH meter
GALC	0.5	g/L	AEC[b]
GLC	5.5	g/L	Bioprofile
AMM	0.3	mM	Bioprofile
LAC	0.0	g/L	Bioprofile
PYR	2.9	mM	AEC
GLU	636.9	µM	AEC
ALA	437.1	µM	AEC
ARG	1588.2	µM	AEC
ASN	920.4	µM	AEC
ASP	2197.9	µM	AEC
CYS	963.1	µM	AEC
GLY	1196.0	µM	AEC
HIS	642.7	µM	AEC
ILE	1744.9	µM	AEC
LEU	1893.2	µM	AEC
LYS	1256.0	µM	AEC
MET	601.3	µM	AEC
PHE	1039.4	µM	AEC
PRO	1040.5	µM	AEC
SER	3027.4	µM	AEC
THR	1502.4	µM	AEC
TRP	383.8	µM	AEC
TYR	1109.7	µM	AEC
VAL	1811.9	µM	AEC

a: freezing point depression osmometer (FPDO); b: anion exchanger chromatography (AEC);

Table 1: Measured medium composition of the 42-MAX-UB standard medium. Extracted from *Rath*

Exp. ID	*DR* ($^1/_h$)	Preculture (passage no.)	GLC (mM)	GLN (mM)	GALC (mM)
A	0.0140	7	10	5	3
B	0.0120	7	10	5	3
C	0.0100	5	10	5	3
D	0.0150	8	10	2	3
E	0.0133	4	8	2	3
F01	0.0150	10	10	5	0

Table 2: The dilution rates, preculture ages and the 42-Max-UB-medium modified components concentrations used in *Rath* for the 6 steady states. Table adapted from *Rath*

Variable	Exp. A		Exp. B		Exp. C		Exp. D	
	Average ± SD	Rel. SD (%)	Average ± SD	Rel. SD (%)	Average ± SD	Rel. SD (%)	Average ± SD	Rel. SD (%)
Setpoints:								
DR (1/h)	0.0140	-	0.0120	-	0.0100	-	0.0150	-
GLC feed conc. (mM)	10.0	-	10.0	-	10.0	-	10.0	-
GLN feed conc. (mM)	5.0	-	5.0	-	5.0	-	2.0	-
GALC feed conc. (mM)	3.0	-	3.0	-	3.0	-	3.0	-
GLC/GLN ratio (mol/mol)	2	-	2	-	2	-	5	-
X_V (E6 cells/mL)	2.490 ± 0.100	4.0	2.745 ± 0.118	4.3	2.735 ± 0.056	2.0	1.489 ± 0.043	2.9
X_D (E6 cells/mL)	0.210 ± 0.032	15.2	0.291 ± 0.021	7.4	0.378 ± 0.047	12.5	0.086 ± 0.006	6.5
CV_v (μL/mL)	6.56 ± 0.48	7.4	7.19 ± 0.67	9.4	6.23 ± 0.21	3.4	4.33 ± 0.27	6.3
μ (1/h)	0.0152 ± 0.0002	1.2	0.0133 ± 0.0001	1.1	0.0114 ± 0.0002	1.4	0.0159 ± 0.0001	0.6
CD (μm)	17.1 ± 0.25	1.5	17.2 ± 0.19	1.1	16.3 ± 0.24	1.5	17.7 ± 0.30	1.5
GLC (mM)	0.0 ± 0.00	-	0.0 ± 0.00	-	0.0 ± 0.00	-	0.0 ± 0.00	-
LAC (mM)	2.1 ± 1.52	-	0.0 ± 0.00	-	0.0 ± 0.00	-	12.5 ± 0.60	5.0
GLN (mM)	0.8 ± 0.08	10.0	0.5 ± 0.02	3.5	0.6 ± 0.04	7.3	0.5 ± 0.10	11.1
AMM (mM)	3.4 ± 0.22	6.7	5.5 ± 0.19	3.5	5.9 ± 0.09	1.5	1.5 ± 0.03	2.1
GALC (mM)	1.9 ± 0.08	4.5	1.9 ± 0.06	2.9	1.7 ± 0.02	0.9	2.1 ± 0.10	2.5
PYR (mM)	0.2 ± 0.00	1.8	0.1 ± 0.00	3.2	0.1 ± 0.00	3.2	0.3 ± 0.04	12.6
GLU (mM)	1.2 ± 0.08	6.2	1.3 ± 0.01	0.5	1.0 ± 0.05	5.0	0.7 ± 0.10	12.9
ALA (mM)	1.4 ± 0.09	6.4	0.2 ± 0.03	13.8	0.1 ± 0.01	14.2	0.3 ± 0.02	8.9
ASP (mM)	1.6 ± 0.06	3.9	1.5 ± 0.05	3.2	1.3 ± 0.02	1.2	2.4 ± 0.17	7.1
A1AT (mg/L)	57.9 ± 3.03	5.2	81.0 ± 9.21	11.4	72.1 ± 1.09	1.5	50.9 ± 1.50	2.9
$Y_{CVv/glc}$ (μL/mmol)	711 ± 47	6.6	827 ± 77	9.4	753 ± 17	2.3	482 ± 30	6.3
$Y_{CVv/gln}$ (μL/mmol)	1679 ± 140	8.3	1770 ± 145	8.2	1601 ± 52	3.3	3617 ± 215	6.0
$Y_{lac/glc}$ (mol/mol)	0.2 ± 0.15	-	0.0 ± 0.01	-	0.0 ± 0.00	-	1.2 ± 0.10	5.3
$Y_{amm/gln}$ (mol/mol)	0.8 ± 0.05	6.6	1.2 ± 0.04	3.6	1.3 ± 0.03	1.9	1.0 ± 0.05	4.9
$Y_{ala/gln}$ (mol/mol)	0.2 ± 0.02	9.5	0.0 ± 0.00	-	0.0 ± 0.00	-	0.0 ± 0.00	-
q_{GLC} (nmol/μL h)[a]	21.43 ± 1.56	7.3	16.17 ± 1.76	10.9	15.13 ± 0.51	3.4	33.05 ± 2.17	6.6
q_{LAC} (nmol/μL h)[a]	-4.43 ± 3.05	-	0.00 ± 0.00	-	0.00 ± 0.00	-	-41.23 ± 4.13	10.0
q_{GLN} (nmol/μL h)[a]	9.10 ± 0.82	9.0	7.55 ± 0.72	9.5	7.12 ± 0.31	4.3	4.40 ± 0.28	6.4
q_{AMM} (nmol/μL h)[a]	-7.19 ± 0.67	9.3	-9.21 ± 0.97	10.5	-9.55 ± 0.30	3.2	-4.48 ± 0.35	7.8
q_{GALAC} (nmol/μL h)[a]	1.94 ± 0.21	10.8	1.55 ± 0.22	14.3	2.46 ± 1.26	51.1	1.92 ± 0.24	12.3
q_{PYR} (nmol/μL h)[a]	5.82 ± 0.52	8.9	4.70 ± 0.57	12.1	4.48 ± 0.11	2.4	9.10 ± 0.64	7.1
q_{GLU} (nmol/μL h)[a]	-1.31 ± 0.18	14.0	-1.05 ± 0.10	9.4	-0.52 ± 0.09	17.9	-0.71 ± 0.31	43.5
q_{ALA} (nmol/μL h)[a]	-2.14 ± 0.27	12.8	0.39 ± 0.03	6.7	0.54 ± 0.03	5.9	0.10 ± 0.12	127.8
q_{ASP} (nmol/μL h)[a]	1.19 ± 0.19	15.9	1.20 ± 0.10	8.1	1.49 ± 0.05	3.5	-0.41 ± 0.62	150.7
q_{A1AT} (pg/cell d)[a]	-7.87 ± 0.19	2.4	-8.54 ± 1.34	15.7	-6.33 ± 0.19	3.0	-12.31 ± 0.60	4.9

a: Substrate uptake is indicated by a positive rate, whereas a negative value indicates a production rate.

Table 3: The dilution rates, preculture ages and the 42-Max-UB-medium modified components concentrations used in *Rath* for the 6 steady states. Table adapted from *Rath*

cell line AGE1_HN. All the others original demands from recon3d were deactivated. An extra demand representing the maintenance demand of atp was set according to [5]. All the fluxes representing exchangeable metabolites (external reactions) were set as reversible (lb and ub set to a big number), so the only effective constraints (for FBA and EP) is the one produced by the chemostat consideration[4]. Additionally, to include the molecular crowding constraints we map [7] enzymatic costs, initially defined for recon1, to recon3d.
The external metabolites concentrations was set according 42_MAX_UB feed medium [6] for each of the 6 continuous cultivation conditions. A set of extra external metabolites, not specified in the medium, was also added. Particularly, Recon3d was unable to growth without pe_hs[e], Phosphatidylethanolamine, (or similar) lipid in the feeding medium. Later we will discuss the impact of this metabolite in the medium.

1.2.2 CHO

CHO bla bla... buscar como Cossio setea CHO en primer lugar. The external metabolites concentrations was set similar to Recon3d for each experiment. The only different was that CHO is able to growth without pe_hs[e], named pe_cho_e.

2 Results

2.1 Low concentrations of phosphatidylethanolamine

Here are shown the results for the experiments with 0.1 mM for Recon3D and 0 mM for CHO of phosphatidylethanolamine in the feed medium.

2.2 High concentrations of phosphatidylethanolamine

3 Bibliography

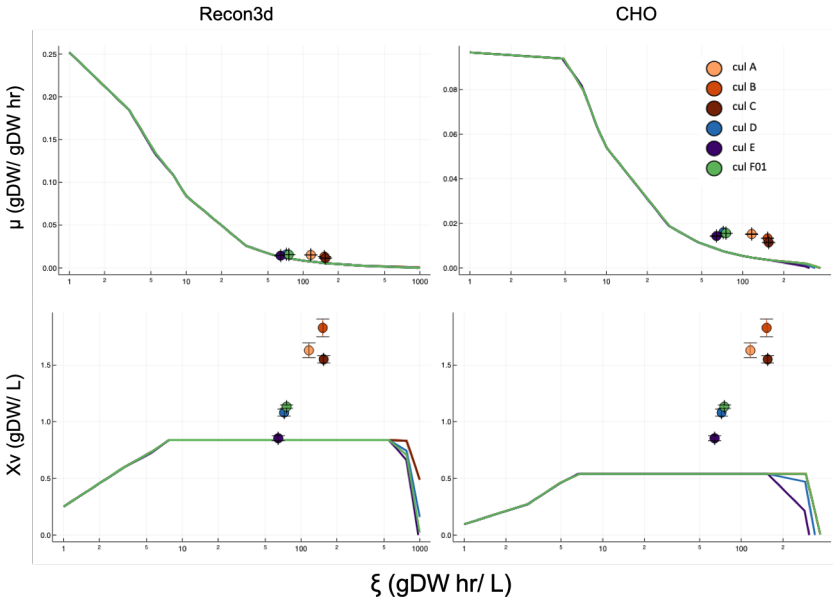

Figure 1: FBA results showing the growth rate and the viable cell density dependence of ξ for the six culture conditions. The solid lines represents the model predictions and the color points shows the experimental results. The data was obtained for feed mediums with low concentrations of phosphatidylethanolamine

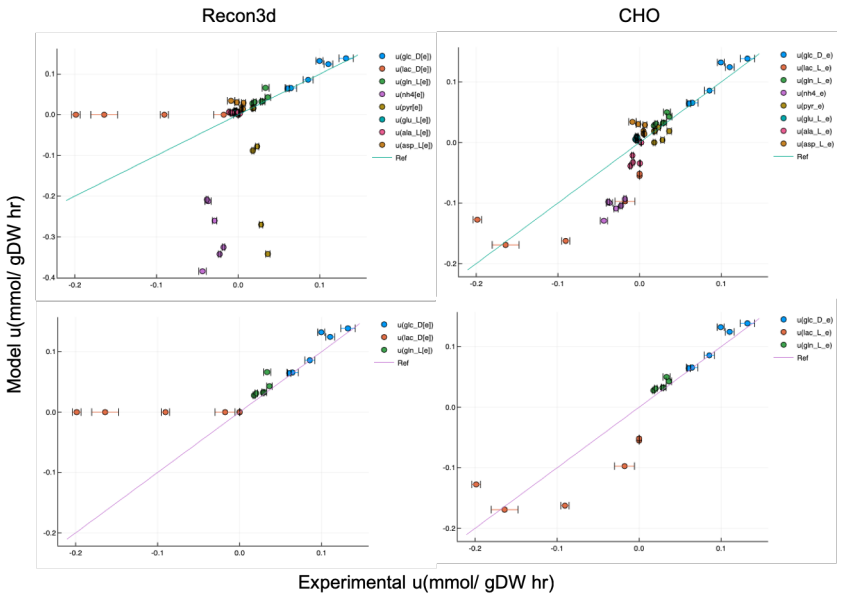

Figure 2: Correlations of all the experimental uptakes, upper graphs, and a selected subset, inferior graphs, respect to the predicted value from for the experiments with low concentration of phosphatidylethanolamine.

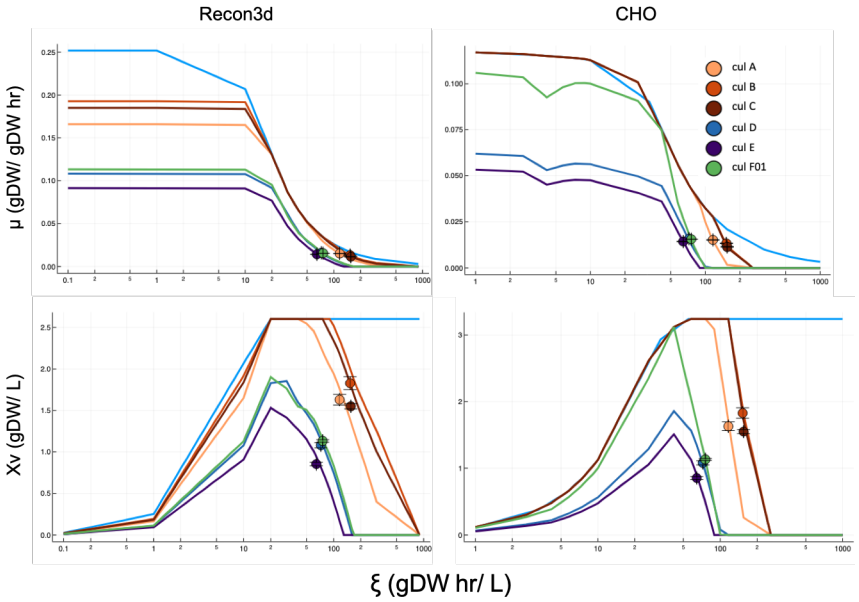

Figure 3: EP and FBA results showing the growth rate and the viable cell density dependence of ξ for the six culture conditions. The solid lines represents the model predictions and the color points shows the experimental results. FBA results are shown as the solid light blue line.

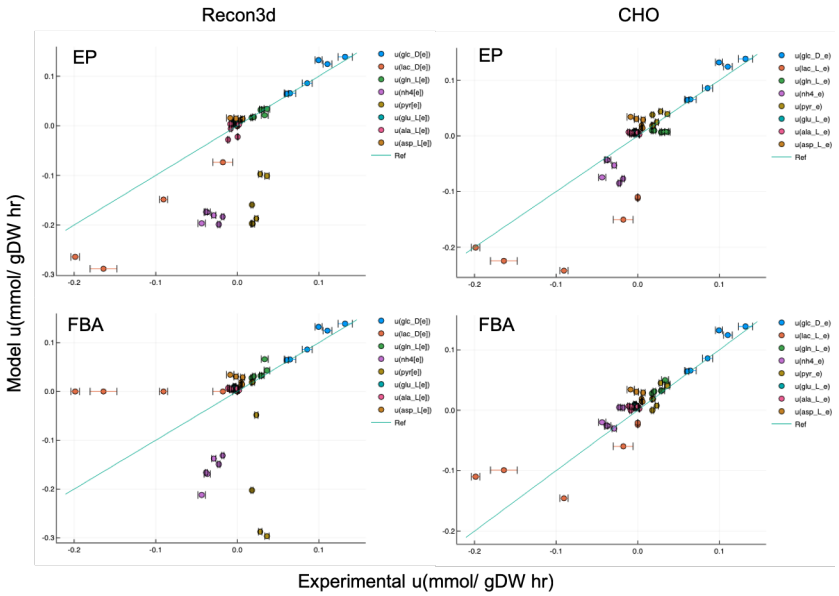

Figure 4: Correlations of all the experimental uptakes compared with the predicted value from EP and FBA.

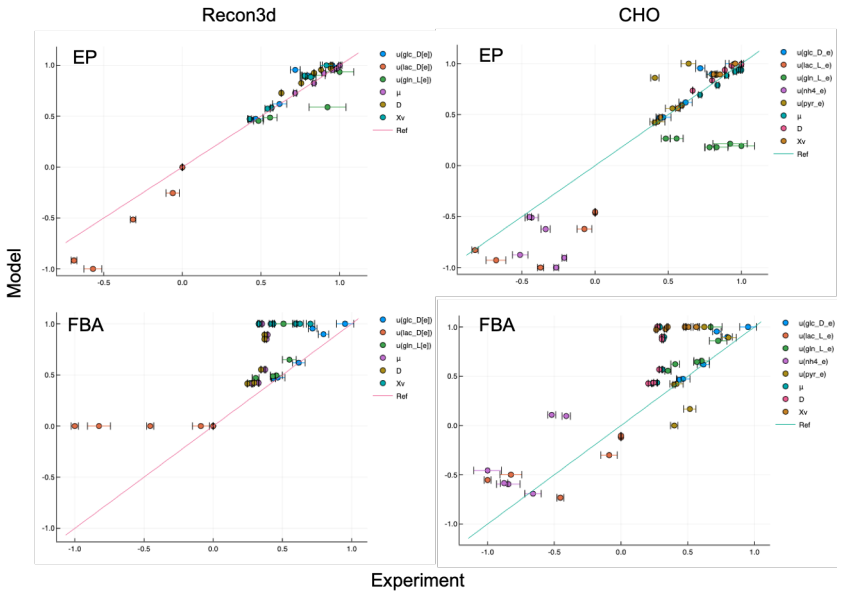

Figure 5: Normalized correlations of some experimental uptakes, growth rate, dilution rate and viable cell density compared with the predicted value from EP and FBA