**Supplementary Information**

**Contextualized genome-scale model unveils high-order metabolic effects of the specific growth rate and oxygenation level in recombinant *Pichia pastoris***

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Supplementary Text

Text S1. Plasmids construction and *Pichia pastoris* transformation

Plasmid construction

We constructed two plasmids using the commercial recombinant vectors pGAPZB (Invitrogen, Carlsbad, CA, USA): one for integrating the thaumatin gene (pGAPZB-TAU) (see Table 1) and the other to revert the auxotrophy of the strain *P. pastoris* GS115 (PGAPZB-HIS4) (Invitrogen, Carlsbad, CA, USA).

PCR amplification of the *his4* fragment was performed using genomic DNA from strain *P. pastoris* X-33 (Invitrogen, Carlsbad, CA, USA). The genomic DNA was extracted using Wizard Genomic DNA purification kitaccording to the manufacturer’s instructions (Promega, Madison, WI, USA). PCR amplification of the DNA fragments was performed by Gibson assembly in 35 PCR cycles using Phusion High-Fidelity DNA Polymerase (ThermoFisher, Waltham, MA, USA). All PCR products were treated with DpnI enzyme to eliminate original vector residues and purified by gel extraction using the Qiaquick Gel Extraction kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. The purified genes fragments and vectors were mixed based on their molar ratios in a final volume of 5 μL containing 100 ng of total DNA. This DNA mix was added to 15 μL of 1.33X master mix (5X isothermal mix buffer, T5 exonuclease 1 U μL-1, Phusion DNA polymerase 2U/μL, Taq DNA ligase 40 U μL-1 and milli-Q purified water) and the reaction mixture was incubated at 50 °C for 1 h. Finally, 10 μL of the reaction mix were used directly to transform chemically competent *E. coli TOP 10* cells. The cells were grown at 37 °C in low salt-LB medium containing 25 μg mL−1 zeocin for selection of clones transformed with both vectors.

Transformation of P. pastoris

*P. pastoris* GS115 wild type strain was used as host. Transformation was performed in two steps. The first transformation was performed using the vector pGAPZB-TAU and the AvrII restriction enzyme to linearize and introduce the latter vector into the cells by electroporation ([Gasser et al., 2006](#_ENREF_7)). In this case, zeocin (100 μg mL−1) was used for the selection of positively transformed clones. Once positive clones were obtained, the second transformation was carried out using the empty vector PGAPZB-HIS4 (primers in Table below). In this case, the XbaI restriction enzyme was used to linearize the vector. Zeocin-supplemented YNB plates (100 μg mL−1 zeocin) were employed for the selection of positively transformed clones. All transformations were verified by genomic sequencing (Macrogen Inc., Seoul, Korea).

**Table.** Primers used in this study

|  |  |
| --- | --- |
| Primer | Sequence |
| HIS-pGAPZB-F | CACACCATAGCTTCAAAATGTTCTTAATGTCTTCCCCAATCACT |
| HIS- pGAPZB-R  | GAGTAAAAAAGGAGTAGAAAGGGGTTAGACCTTATTTATTATGTTCAG |
| pGAPZB-HIS-F | ATTGGGGAAGACATTAAGAACATTTTGAAGCTATGGTGTGTG |
| pGAPZB-HIS-R | AATAAATAAGGTCTAACCCCTTTCTACTCCTTTTTTACTCTTCCAGA |

Text S2. Bioreactor set-up and culture medium

The strain was cultivated in glucose‐limited chemostat cultures at different dilution rates and dissolved oxygen. Overall, seven different experimental conditions were evaluated following a Doehrlet experimental design. Continuous cultures were performed at a working volume of 1 L in 2-L benchtop bioreactors (Sartorius AG, Göttingen, Germany) using peristaltic pumps (Ismatec, IDEX Health & Science, Germany) to control the feeding and at 1.2 bar total pressure. Once the batch phase was concluded, the continuous phase was initiated at the appropriate specific growth rate and dissolved oxygen (DO) level. DO was maintained using a mixture of three gases; air, pure nitrogen and pure oxygen depending on the DO set point. In all experimental conditions, the stirring rate was set to 700 rpm, aeration to 1 VVM (1 L min-1), and the temperature and pH were respectively kept at 25 °C and 5.0. Off‐gases were cooled in a condenser at 4 °C and desiccated in two silica gel columns. CO2 and O2 abundances were analyzed through BCP‐CO2 and BCP‐O2 Sensors (BlueSens gas sensor GmbH, Herten, Germany). Each dilution rate was kept for at least five residence times.

Batch medium was composed of (per liter): 39.9 g glycerol, 1.8 g citric acid, 12.6 g (NH4)2HPO4, 0.022 g CaCl2·H2O, 0.9 g KCl, 0.5 g MgSO4·7H2O, 2 mL biotin (0.2 g L−1), 4 mL PTM1 trace salts stock solution. Chemostat medium composition was composed of (per liter): 50 g glucose, 0.9 g citric acid, 3.45 g (NH4)2HPO4, 0.01 g CaCl2·H2O, 1.7 g KCl, 0.65 g MgSO4·7H2O, 1 mL biotin (0.2 g L−1), 1.6 mL PTM1 trace salts stock solution, and 0.2 mL of antifoam Glanapon 2000 (Bussetti & Co GmbH, Vienna, Austria). HCl 25% was used to reach pH 5.0.

Text S3. Contextualization and refinement of the *Pichia pastoris* GSMM

GSMM contextualization for describing aerobic growth on glucose

The *i*MT1026 v3.0 genome-scale metabolic reconstruction from Tomàs-Gamisans et al. ([2018](#_ENREF_13)) was employed to describe the metabolism *Pichia pastoris*. In order to accurately represent the central carbon metabolism behavior of this yeast, we systematically adapted the reconstruction to represent and capture known metabolic features. This process was composed of two main steps: 1) Blocking and modification of reaction directionalities based on published data (e.g., transcriptomics, proteomics, thermodynamics, etc.), and 2) Modification of reaction directionalities based on topological considerations, i.e., removal of thermodynamically infeasible internal loops ([Saa and Nielsen, 2016](#_ENREF_11)). The details of each step are detailed below.

The first step leveraged the abundant experimental omics data available for this yeast under glucose-limited conditions ([Clasquin et al., 2011](#_ENREF_5); [Gasser et al., 2007](#_ENREF_8); [Krivoruchko et al., 2015](#_ENREF_9); [Rußmayer et al., 2015](#_ENREF_10); [Zhang et al., 2017](#_ENREF_15)). Application of this data greatly constrained the genome-scale metabolic model (see Table S1 for the applied constraints), significantly impacting the flux distribution predictions (see Fig. S1 for main impacts). The second step involved the modification of reaction directionalities based on flux simulations imposing the loopless flux condition. To this task, the Fast-SNP algorithm ([Saa and Nielsen, 2016](#_ENREF_11)) was used using experimental training data (i.e., fluxomic constraints) under glucose-limited conditions. Briefly, three flux scenarios were simulated: 1) aerobic growth in glucose with *q*S values between 0.025 to 0.275 mmol gDWC-1 h-1 and conventional exchange directions, 2) 11 aerobic growth conditions on glucose without secondary product formation (i.e., arabitol and ethanol) ([Adelantado et al., 2017](#_ENREF_1); [Baumann et al., 2010](#_ENREF_2); [Carnicer et al., 2012](#_ENREF_4); [Garcia-Ortega et al., 2017](#_ENREF_6)), and 3) 15 aerobic growth conditions on glucose with formation of secondary products ([Adelantado et al., 2017](#_ENREF_1); [Baumann et al., 2010](#_ENREF_2); [Carnicer et al., 2012](#_ENREF_4); [Garcia-Ortega et al., 2017](#_ENREF_6)) (Table S2). Reactions that had the same directionality across all the conditions were set to irreversible. The definitive list with the constraints on the GSMM is shown in Table S3.

Flux simulations

Simulations were performed using the Constraint-Based Reconstruction and Analysis (COBRA) toolbox (Schellenberger et al., 2011) in MATLAB 2013 (Mathworks, Natick, MA, USA). Flux predictions were performed using parsimonious Flux Balance Analysis (pFBA). Topological modification of the reaction directionalities was performed using Fast-SNP algorithm ([Saa and Nielsen, 2016](#_ENREF_11)). Finally, Gurobi 2016 was employed for the optimizations.

Supplementary Figures



Figure S1. Main impacts of the contextualization of the *i*MT1026 v3.0 GSMM for describing aerobic growth using glucose as sole carbon source.

Changes due to reactions inactivation and directionalities modifications are shown in black squares. The effects of the loopless flux condition are displayed in red squares.



**A)** Prediction of intracellular fluxes (cytoplasm)

**B)** Prediction of intracellular fluxes (mitochondria)

Figure S2. Evaluation of the contextualized *P. pastoris* GSMM for metabolic flux prediction under glucose-limited conditions correcting by the estimated ashes content.

**A)** Comparison of the predicted intracellular flux through cytoplasmic reactions of the GSMM considering (slope = 1.033, *R*2 = 0.970) against experimental data under appropriate growth conditions. **B)** Comparison of the predicted intracellular flux through mitochondrial reactions of the GSMM (slope = 0.787, *R*2 = 0.853) against experimental data under the same conditions used in **A)**. For more information about the employed experimental conditions refer to Tables S3 and S4.

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**A)** Flux variability of intracellular flux predictions (cytoplasm)

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**B)** Flux variability of intracellular flux predictions (mitochondria)

Figure S3. Evaluation of the flux variability of the *P. pastoris* GSMM for metabolic flux prediction under glucose-limited conditions for the model with and without contextualization.

**Supplementary Tables**

Table S1. Summary of reaction modifications based on experimental and literature data

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Reaction ID | Stoichiometric equation | Gene | Comment | Action |
| Exchange reactions |
| Ex\_glyc | gly => |  | Not supplied in the medium | Blocked |
| Ex\_etoh | etoh => |  | Not supplied in the medium | Blocked |
| Ex\_abt | abt => |  | Not detected in the medium | Blocked |
| Ex\_fab | fab => |  | Protein not expressed | Blocked |
| Ex\_pyr | pyr => |  | Present in the medium | Open |
| Ex\_cit | cit => |  | Present in the medium | Open |
| Cytoplasm |
| GLUK | glc + atp => h + adp + g6p | PAS\_chr4\_0624 | Duplicated reaction | Blocked |
| MDH | mal + nad + h <=> nadh + oaa | PAS\_chr4\_0815 | Part of the glyoxylate cycle (not needed for glucose growth) (Rußmayer et al., 2015b) | Reversibility constrained |
| FBA3 | s17bp <=> dhap+e4p | PAS\_chr1-1\_0072 | Used in the direction S17BP in ribogenesis (Clasquin et al., 2011; Rußmayer et al., 2015b) | Reversibility constrained |
| SULR | 3 h2o + h2s + 3 nadp <=> 5 h + 3 nadph + so3  | (PAS\_chr4\_0369 or PAS\_chr3\_1084) | Thermodynamically favorable in the reverse direction (dG=103 KJ/mol)  | Reversibility constrained |
| MMSAD3 | nad + coa + msa => co2 + nadh + accoa |  | Not considered for lipid synthesis (Krivoruchko et al., 2014) | Blocked |
| Mitochondrion |
| MDHm | mal + nad + h <=> nadh + oaa | PAS\_chr2-1\_0238 | Part of the glyoxylate cycle (not needed for glucose growth) (Rußmayer et al., 2015b)  | Reversibility constrained |
| ME1m | nad + mal => pyr + co2 + nadh | PAS\_chr3\_0181 | Inactive during glucose growth (Zhang et al., 2017) | Blocked |
| ME2m | nadp + mal => pyr + co2 + nadph | PAS\_chr3\_0181 | Inactive during glucose growth (Zhang et al., 2017) | Blocked |
| Peroxisome |
| AOD | meoh + o2 => h2o2 + fald | (PAS\_chr4\_0821 or PAS\_chr4\_0152) | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| DAS | fald + xu5p => g3p + dha | (PAS\_chr3\_0832 or PAS\_chr3\_0834) | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked  |
| DHAKx | atp + dha => h + adp + dhap | PAS\_chr3\_0841  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| FBAx | fdp => g3p + dhap | (PAS\_chr1-1\_0072 or PAS\_chr1-1\_0319) | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| FBPx | h2o + fdp => pi + f6p | PAS\_chr3\_0868  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| SHBPH | h2o + s17bp => pi + s7p | PAS\_chr2-2\_0177  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| CATp | 2 h2o2 => o2 + h2o | PAS\_chr2-2\_0131 | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| FALDH2 | nad + hmgth => ndh + sfglutth | PAS\_chr3\_1028  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| SFGTH | h2o + sfglutth => h + gthrd + for | PAS\_chr3\_0867 | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| FDH | nad + h + for => co2 + nadh | PAS\_chr3\_0932  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| CSp | h2o + accoa + oaa => coa + h + cit | PAS\_chr1-1\_0475  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| ICLx | icit => glx + succ | PAS\_chr1-4\_0338  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| MDHp | nad + mal => oaa + h + nad | PAS\_chr4\_0815  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| ASPTAp | ak + asp <=> oaa + glu | PAS\_chr4\_0974 | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| TKT1x | xu5p + r5p => g3p + s7p | (PAS\_chr1-4\_0150 or PAS\_chr3\_0834 or PAS\_chr3\_0832) | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| CATp | 2 h2o2 => o2 + h2o | PAS\_chr2-2\_0131 | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |
| RPIx | r5p => ru5p | PAS\_chr4\_0212  | Absent in peroxisomal fraction during glucose growth (Rußmayer et al., 2015b) | Blocked |

## Table S2. Constraints used to simulate the different scenarios for the modification of reaction directionalities

|  |  |  |  |
| --- | --- | --- | --- |
| Conditions | Flux bounds | Exchanges | References |
| Glucose | Arabitol | Ethanol | CO2 | O2 | Biomass |
| **Scenario 1** | 1 | LB | -0.025 | 0 | 0 | 0 | -1000 | 0 |   |
| UB | -0.025 | 0 | 0 | 1000 | 0 | 1000 |  |
| 2 | LB | -0.05 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.05 | 0 | 0 | 1000 | 0 | 1000 |  |
| 3 | LB | -0.075 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.075 | 0 | 0 | 1000 | 0 | 1000 |  |
| 4 | LB | -0.1 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.1 | 0 | 0 | 1000 | 0 | 1000 |  |
| 5 | LB | -0.125 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.125 | 0 | 0 | 1000 | 0 | 1000 |  |
| 6 | LB | -0.15 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.15 | 0 | 0 | 1000 | 0 | 1000 |  |
| 7 | LB | -0.175 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.175 | 0 | 0 | 1000 | 0 | 1000 |  |
| 8 | LB | -0.2 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.2 | 0 | 0 | 1000 | 0 | 1000 |  |
| 9 | LB | -0.225 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.225 | 0 | 0 | 1000 | 0 | 1000 |  |
| 10 | LB | -0.25 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.25 | 0 | 0 | 1000 | 0 | 1000 |  |
| 11 | LB | -0.275 | 0 | 0 | 0 | -1000 | 0 |  |
| UB | -0.275 | 0 | 0 | 1000 | 0 | 1000 |   |
| **Scenario 2** | 12 | LB | -0.97 | 0 | 0 | 2.73 | -2.84 | 0.1 | ([Carnicer et al., 2012](#_ENREF_4)) |
| UB | -0.97 | 0 | 0 | 3.01 | -2.57 | 0.1 |
| 13 | LB | -1.04 | 0 | 0 | 2.29 | -2.47 | 0.095 | ([Tomàs-Gamisans et al., 2016](#_ENREF_14)) |
| UB | -0.96 | 0 | 0 | 2.55 | -2.23 | 0.105 |
| 14 | LB | -1.05 | 0 | 0 | 2.38 | -2.58 | 0.095 | ([Tomàs-Gamisans et al., 2016](#_ENREF_14)) |
| UB | -0.97 | 0 | 0 | 2.66 | -2.3 | 0.105 |
| 15 | LB | -1.55 | 0 | 0 | 3.02 | -2.87 | 0.16 | ([Sola et al., 2004](#_ENREF_12)) |
| UB | -1.55 | 0 | 0 | 3.34 | -2.59 | 0.16 |
| 16 | LB | -0.94 | 0 | 0 | 2.3 | -2.26 | 0.1 | ([Baumann et al., 2010](#_ENREF_2)) |
| UB | -0.94 | 0 | 0 | 2.38 | -2.2 | 0.1 |
| 17 | LB | -1.04 | 0 | 0 | 2.18 | -2.29 | 0.1 | ([Carnicer et al., 2009](#_ENREF_3)) |
| UB | -0.94 | 0 | 0 | 2.56 | -1.95 | 0.1 |
| 18 | LB | -0.957 | 0 | 0 | 1.99 | -2.05 | 0.1 | ([Adelantado et al., 2017](#_ENREF_1)) |
| UB | -0.957 | 0 | 0 | 2.01 | -2.03 | 0.1 |
| 19 | LB | -1.11 | 0 | 0 | 2.52 | -2.3 | 0.1 | ([Adelantado et al., 2017](#_ENREF_1)) |
| UB | -1.11 | 0 | 0 | 2.62 | -2.2 | 0.1 |
| 20 | LB | -1.2 | 0 | 0 | 2.22 | -2.81 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -1.2 | 0 | 0 | 2.46 | -2.55 | 0.1 |
| 21 | LB | -1.2 | 0 | 0 | 2.06 | -2.98 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -1.2 | 0 | 0 | 2.29 | -2.7 | 0.1 |
| 22 | LB | -1.2 | 0 | 0 | 2.04 | -2.97 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -1.2 | 0 | 0 | 2.26 | -2.69 | 0.1 |
| **Scenario 3** | 23 | LB | -1.31 | 0.02 | 0.069 | 2.41 | -2.42 | 0.1 | ([Baumann et al., 2010](#_ENREF_2)) |
| UB | -1.31 | 0.033 | 0.099 | 2.73 | -2.1 | 0.1 |
| 24 | LB | -1.34 | 0.11 | 0.27 | 2.43 | -2.67 | 0.095 | ([Tomàs-Gamisans et al., 2016](#_ENREF_14)) |
| UB | -1.22 | 0.15 | 0.35 | 2.67 | -2.43 | 0.105 |
| 25 | LB | -1.82 | 0.31 | 0.72 | 2.93 | -2.15 | 0.095 | ([Tomàs-Gamisans et al., 2016](#_ENREF_14)) |
| UB | -1.62 | 0.35 | 0.96 | 3.49 | -1.87 | 0.105 |
| 26 | LB | -1.43 | 0.17 | 0.35 | 2.54 | -2.15 | 0.095 | ([Tomàs-Gamisans et al., 2016](#_ENREF_14)) |
| UB | -1.31 | 0.21 | 0.47 | 2.82 | -1.83 | 0.105 |
| 27 | LB | -1.64 | 0.18 | 0.71 | 2.7 | -2.07 | 0.095 | ([Tomàs-Gamisans et al., 2016](#_ENREF_14)) |
| UB | -1.48 | 0.26 | 0.95 | 3.18 | -1.55 | 0.105 |
| 28 | LB | -1.69 | 0.13 | 1.1 | 5.3 | -4.35 | 0.1 | ([Baumann et al., 2010](#_ENREF_2)) |
| UB | -1.69 | 0.167 | 1.22 | 5.94 | -3.93 | 0.1 |
| 29 | LB | -1.4 | 0.2 | 0.31 | 1.93 | -1.65 | 0.1 | ([Baumann et al., 2010](#_ENREF_2)) |
| UB | -1.26 | 0.22 | 0.35 | 2.13 | -1.49 | 0.1 |
| 30 | LB | -1.83 | 0.4 | 0.95 | 1.57 | -0.64 | 0.1 | ([Baumann et al., 2010](#_ENREF_2)) |
| UB | -1.65 | 0.44 | 1.05 | 1.73 | -0.44 | 0.1 |
| 31 | LB | -1.32 | 0.035 | 0.38 | 2.55 | -1.94 | 0.1 | ([Adelantado et al., 2017](#_ENREF_1)) |
| UB | -1.32 | 0.061 | 0.49 | 2.65 | -1.84 | 0.1 |
| 32 | LB | -1.98 | 0.45 | 1.164 | 5.5 | -3.81 | 0.1 | ([Adelantado et al., 2017](#_ENREF_1)) |
| UB | -1.98 | 0.47 | 1.198 | 5.7 | -3.67 | 0.1 |
| 33 | LB | -1.28 | 0.0095 | 0.0665 | 2.06 | -3.08 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -1.28 | 0.0105 | 0.0735 | 2.28 | -2.78 | 0.1 |
| 34 | LB | -1.6 | 0.026 | 0.665 | 2.49 | -2.99 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -1.6 | 0.028 | 0.735 | 2.75 | -2.71 | 0.1 |
| 35 | LB | -2 | 0.038 | 1.33 | 3.06 | -2.84 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -2 | 0.042 | 1.47 | 3.38 | -2.57 | 0.1 |
| 36 | LB | -2.4 | 0.05 | 1.995 | 3.53 | -2.89 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -2.4 | 0.056 | 2.205 | 3.91 | -2.61 | 0.1 |
| 37 | LB | -3.5 | 0.252 | 3.325 | 4.42 | -2.81 | 0.1 | ([Garcia-Ortega et al., 2017](#_ENREF_6)) |
| UB | -3.5 | 0.278 | 3.675 | 4.88 | -2.55 | 0.1 |

Table S3. List of reaction constraints for oxygen-sufficient, glucose-limited *P.* *pastoris* growth simulation

|  |  |  |
| --- | --- | --- |
| Reaction ID | LB | UB |
| Ex\_glyc | 0 | 0 |
| Ex\_fab | 0 | 0 |
| Ex\_rol | 0 | 0 |
| BIOMASS\_glyc | 0 | 0 |
| Ex\_pyr | 0 | 1000 |
| Ex\_cit | -1000 | 1000 |
| BIOMASS | 0 | 1000 |
| Ex\_thau | 0 | 1000 |
| thaut | 0 | 1000 |
| pThau | 0 | 1000 |
| thauAA | 0 | 1000 |
| thauRNA | 0 | 1000 |
| thauDNA | 0 | 1000 |
| GLUK | 0 | 0 |
| MDHm | 0 | 1000 |
| MDH | 0 | 1000 |
| SULR | -1000 | 0 |
| AOD | 0 | 0 |
| DAS | 0 | 0 |
| DHAKx | 0 | 0 |
| FBAx | 0 | 0 |
| FBPx | 0 | 0 |
| SHBPH | 0 | 0 |
| RPIx | 0 | 0 |
| CATp | 0 | 0 |
| FALDH2 | 0 | 0 |
| SFGTH | 0 | 0 |
| FDH | 0 | 0 |
| TKT1x | 0 | 0 |
| CSp | 0 | 0 |
| ICLx | 0 | 0 |
| MDHp | 0 | 0 |
| ASPTAp | 0 | 0 |
| ME1m | 0 | 0 |
| ME2m | 0 | 0 |
| PFK\_3 | -1000 | 0 |
| Ex\_pyr | 0 | 1000 |
| MMSAD3 | 0 | 0 |
| Ex\_h2o | 0 | 1000 |
| H2Ot | -1000 | 0 |
| O2t | 0 | 1000 |
| GLCter | -1000 | 0 |
| M4MPDOLter | 0 | 1000 |
| M7MASNBterg | 0 | 1000 |
| HCO3DH | -1000 | 0 |
| FAcoaRavge | 0 | 1000 |
| HXDCEALR | 0 | 1000 |
| OGLYCOStg | 0 | 1000 |
| CHITINtg | 0 | 1000 |
| MANNANtg | -1000 | 0 |
| FARCOAtm | 0 | 1000 |
| FACOAtm | -1000 | 0 |
| CLPNtm | 0 | 1000 |
| NADHter | 0 | 1000 |
| NADter | -1000 | 0 |
| NADPHter | 0 | 1000 |
| NADPter | -1000 | 0 |
| 12DGRter | -1000 | 0 |
| PAter | 0 | 1000 |
| DOLter | -1000 | 0 |
| 34HPPt2p | 0 | 1000 |
| 3C4MOPtm | 0 | 1000 |
| 4ABUTNtm | 0 | 1000 |
| 4ABUTtm | -1000 | 0 |
| 4H2OGLTtm | -1000 | 0 |
| 4H2OGLTtp | -1000 | 0 |
| 4HPRO\_LTtm | 0 | 1000 |
| ACtp | 0 | 1000 |
| AKGtp | -1000 | 0 |
| CBPtn | -1000 | 0 |
| CITtap | -1000 | 0 |
| CITtcp | 0 | 1000 |
| CYSTtp | 0 | 1000 |
| E4HGLUtm | -1000 | 0 |
| E4HGLUtp | 0 | 1000 |
| E4Ptm | 0 | 1000 |
| GLNt2n | 0 | 1000 |
| GLUt2n | -1000 | 0 |
| GLXtp | 0 | 1000 |
| HCYSt2p | -1000 | 0 |
| MANNANter | -1000 | 0 |
| PAN4Ptm | -1000 | 0 |
| PAPtm | -1000 | 0 |
| PHCHGS | 0 | 1000 |
| PIt2n | -1000 | 0 |
| PNP | -1000 | 0 |
| PTD1INOtn\_SC | 0 | 1000 |
| PYRt2p | -1000 | 0 |
| SHSL4r | -1000 | 0 |
| MPK | -1000 | 0 |
| TYRt2m | -1000 | 0 |
| ASPt5n | -1000 | 0 |
| PI4Ptn | -1000 | 0 |
| 2DDA7Ptm | -1000 | 0 |
| 4HGLSDm | 0 | 1000 |
| ADK1 | 0 | 1000 |
| ADK3m | 0 | 1000 |
| AKGDam | 0 | 1000 |
| ALATA\_Lm | -1000 | 0 |
| ASNt2r | -1000 | 0 |
| ASPt2n | -1000 | 0 |
| ASPt2r | 0 | 1000 |
| CITtbm | -1000 | 0 |
| ERGSTter | 0 | 1000 |
| ERGTETROLter | 0 | 1000 |
| FBA2 | -1000 | 0 |
| FBA3 | -1000 | 0 |
| GCC2am | 0 | 1000 |
| GCC2cm | 0 | 1000 |
| GK2 | 0 | 1000 |
| GLCt1 | 0 | 1000 |
| HCO3E | 0 | 1000 |
| HCO3Em | 0 | 1000 |
| HICITDm | 0 | 1000 |
| HMGCOASm | -1000 | 0 |
| ILETAm | -1000 | 0 |
| NDPK9 | 0 | 1000 |
| O2ter | 0 | 1000 |
| OXAGm | 0 | 1000 |
| PANTtm | -1000 | 0 |
| PAtm\_PP | 0 | 1000 |
| PDX5PO | 0 | 1000 |
| PUNP7 | 0 | 1000 |
| SQ23EPXter | 0 | 1000 |
| SQLter | 0 | 1000 |
| DASYNm\_PP | 0 | 1000 |
| THIORDXm | 0 | 1000 |
| AACOAT | 0 | 1000 |
| ACONTx | 0 | 1000 |
| ALCD19 | 0 | 1000 |
| COAtp | -1000 | 0 |
| FRDO | -1000 | 0 |
| FTCD | 0 | 1000 |
| GluForTx | -1000 | 0 |
| HACD9m | 0 | 1000 |
| HIBDm | 0 | 1000 |
| MGCHrm | 0 | 1000 |
| MI14PP | 0 | 1000 |
| MI4PP | 0 | 1000 |
| MICITDm | -1000 | 0 |
| MTRK | 0 | 1000 |
| NP1 | -1000 | 0 |
| PPItx | 0 | 1000 |
| PROAKGOX1r | 0 | 1000 |
| SBTPD | 0 | 1000 |
| TRPS3r | 0 | 1000 |
| ASNtm | -1000 | 0 |
| GLCISO | 0 | 1000 |
| DHORDm | 0 | 1000 |
| OLIGOPK | 0 | 1000 |
| FOLR2m | 0 | 1000 |
| GGLUCTC | 0 | 1000 |
| ALLTNISOR | -1000 | 0 |
| OHCUREIMDZLNCAR | 0 | 1000 |
| OGLYCOS | 0 | 1000 |
| G15LACH | 0 | 1000 |
| PIt2er | 0 | 1000 |
| ATPter | 0 | 1000 |
| ADPter | -1000 | 0 |
| ATPter\_H | 0 | 1000 |
| H2Stm | -1000 | 0 |
| OROTtm | -1000 | 0 |
| CYStm | -1000 | 0 |
| ACSERtm | 0 | 1000 |
| ACACtm | 0 | 1000 |
| DHORtm | 0 | 1000 |
| CITtp | -1000 | 0 |
| AKGMALtp | 0 | 1000 |
| DHAtv | 0 | 1000 |
| GTPGDPtv | -1000 | 0 |
| CO2tg | -1000 | 0 |
| DHFtm | 0 | 1000 |
| NH4tp | -1000 | 0 |
| PEtg\_SC | -1000 | 0 |
| PROtm | 0 | 1000 |
| PStg\_SC | 0 | 1000 |
| PStv\_PP | 0 | 1000 |
| PEtv\_PP | -1000 | 0 |
| CDPDGPm\_PP | 0 | 1000 |
| 2DHPtm | 0 | 1000 |
| 3C3HMPtm | -1000 | 0 |
| 3MOBtm | -1000 | 0 |
| 5AOPtm | -1000 | 0 |
| AATA | 0 | 1000 |
| ACALDtm | -1000 | 0 |
| ACCOACr | 0 | 1000 |
| ACGAM6PS | 0 | 1000 |
| ACGAMPM | 0 | 1000 |
| ACOATA | 0 | 1000 |
| ACONT | 0 | 1000 |
| ACONTm | 0 | 1000 |
| ADSL1r | 0 | 1000 |
| ADSL2r | 0 | 1000 |
| AHCYStm | -1000 | 0 |
| AICART | 0 | 1000 |
| AIRCr | 0 | 1000 |
| ALCD2x | 0 | 1000 |
| ALLTAHr | 0 | 1000 |
| ALLTNr | 0 | 1000 |
| AMETtm | 0 | 1000 |
| AP4AHr | 0 | 1000 |
| ARGSL | 0 | 1000 |
| ARGSSr | 0 | 1000 |
| CO2t | -1000 | 0 |
| CO2tm | -1000 | 0 |
| CO2tv | -1000 | 0 |
| CSNATm | 0 | 1000 |
| CSNATp | 0 | 1000 |
| DADK | -1000 | 0 |
| DHORTS | -1000 | 0 |
| DTMPK | 0 | 1000 |
| DURIPP | -1000 | 0 |
| ETHAPT\_PP | 0 | 1000 |
| ETOHtm | -1000 | 0 |
| G5SADr | 0 | 1000 |
| GALU | 0 | 1000 |
| GCCam | 0 | 1000 |
| GCCcm | 0 | 1000 |
| GLCtv | -1000 | 0 |
| H2Ot | -1000 | 0 |
| H2Otp | 0 | 1000 |
| HACNHm | 0 | 1000 |
| HMGCOAR | -1000 | 0 |
| HMGCOAS | -1000 | 0 |
| HMGCOAtm | -1000 | 0 |
| GLYCLTDy | -1000 | 0 |
| IMPC | -1000 | 0 |
| IPDDI | 0 | 1000 |
| IPPMIa | -1000 | 0 |
| IPPMIb | -1000 | 0 |
| LEUTA | -1000 | 0 |
| MAN6PI | -1000 | 0 |
| MCITDm | 0 | 1000 |
| MCOATA | 0 | 1000 |
| MTRI | 0 | 1000 |
| NDPK1 | 0 | 1000 |
| NDPK2 | 0 | 1000 |
| NDPK4 | 0 | 1000 |
| NH4t | 0 | 1000 |
| NH4tm | -1000 | 0 |
| NNAMr | 0 | 1000 |
| O2t | 0 | 1000 |
| O2tm | 0 | 1000 |
| ORPT | -1000 | 0 |
| PGMT | -1000 | 0 |
| PMANM | -1000 | 0 |
| PRAGSr | 0 | 1000 |
| PRASCS | 0 | 1000 |
| PRPPS | 0 | 1000 |
| PUNP4 | 0 | 1000 |
| PUNP6 | 0 | 1000 |
| SACCD1 | 0 | 1000 |
| SACCD2 | 0 | 1000 |
| TREt2v | 0 | 1000 |
| UDPACGLP | 0 | 1000 |
| UGLYCHr | 0 | 1000 |
| UMPK | 0 | 1000 |
| UREASE | 0 | 1000 |
| DASYN\_PP | 0 | 1000 |
| PEtm\_PP | -1000 | 0 |
| Ex\_h2o | 0 | 1000 |
| Ex\_o2 | -1000 | 0 |
| Ex\_co2 | 0 | 1000 |
| RBK\_D | -1000 | 0 |
| Htr | 0 | 1000 |
| PIt2m | 0 | 1000 |
| H2Otm | -1000 | 0 |
| RPI | -1000 | 0 |
| ETOHt | -1000 | 0 |
| ETOHtm | -1000 | 0 |
| FBA | 0 | 1000 |
| GAPD | 0 | 1000 |
| PFK | 0 | 1000 |
| PGK | -1000 | 0 |
| Ex\_etoh | 0 | 1000 |
| D\_ABTt | 0 | 1000 |

## Table S4. Macromolecular composition of *P. pastoris* GS115

|  |  |  |
| --- | --- | --- |
| Component | Experimental composition (g gDCW-1)a | Estimated composition without ashes (g/gDCW-1)b |
| Proteins | 0.3700 | 0.3983 |
| Carbohydrates | 0.3690 | 0.3972 |
| Lipids | 0.0620 | 0.0667 |
| RNA | 0.0660 | 0.0710 |
| DNA | 0.0013 | 0.0014 |
| a Data taken from Carnicer et al., ([2009](#_ENREF_3)) |
| b Assuming 7.1% ashes content based on Carnicer et al., ([2012](#_ENREF_4)) |

**Table S5.** **Raw *P. pastoris* chemostat data under different μ and DO conditions measured in this study**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Condition |  (h-1) | DOsat | *Y*S,X (gDCW g-1) | *q*S (mmol gDCW-1h-1) | *q*CO2 (mmol gDCW-1h-1) | *q*O2 (mmol gDCW-1h-1) | RQ (-) |
| Meana | S.E.b | (%) | Meana | S.E.b | Meana | S.E.b | Meana | S.E.b | Meana | S.E.b | Meana | S.E.b |
| 1.1 | 6.86 E-02 | - | 4 | 5.09 E-01 | 9.71 E-03 | 7.49 E-01 | 2.25 E-05 | 1.74 E+00 | 5.70 E-05 | 1.74 E+00 | 9.12 E-05 | 9.95 E-01 | 3.31 E-02 |
| 1.2 | 7.50 E-02 | - | 4 | 5.05 E-01 | 9.22 E-03 | 8.25 E-01 | 4.09 E-05 | 1.94 E+00 | 4.86 E-05 | 1.94 E+00 | 4.86 E-05 | 1.01 E+00 | 5.88 E-03 |
| 1c | 7.18 E-02 | 3.16 E-03 | 4 | 5.07 E-01 | 1.87 E-03 | 7.87 E-01 | 3.76 E-02 | 1.84 E+00 | 1.01 E-01 | 1.84 E+00 | 9.70 E-02 | 1.00 E+00 | 5.50 E-03 |
| 2.1 | 1.14 E-01 | - | 4 | 5.19 E-01 | 1.02 E-02 | 1.22 E+00 | 4.20 E-05 | 2.78 E+00 | 9.32 E-05 | 2.80 E+00 | 9.28 E-05 | 9.94 E-01 | 4.10 E-03 |
| 2.2 | 1.25 E-01 | - | 4 | 5.42 E-01 | 1.28 E-02 | 1.28 E+00 | 4.04 E-05 | 2.75 E+00 | 5.10 E-05 | 2.78 E+00 | 4.91 E-05 | 9.88 E-01 | 4.56 E-03 |
| 2c | 1.19 E-01 | 5.51 E-03 | 4 | 5.31 E-01 | 1.13 E-02 | 1.25 E+00 | 3.10 E-02 | 2.77 E+00 | 1.68 E-02 | 2.79 E+00 | 3.17 E-01 | 9.91 E-01 | 2.97 E-03 |
| 3.1 | 4.54 E-02 | - | 30 | 5.59 E-01 | 1.15 E-02 | 4.50 E-01 | 1.63 E-05 | 9.78 E-01 | 7.33 E-05 | 9.84 E-01 | 6.38 E-05 | 9.93 E-01 | 6.00 E-02 |
| 3.2 | 4.98 E-02 | - | 30 | 5.43 E-01 | 8.70 E-03 | 5.09 E-01 | 3.45 E-05 | 1.08 E+00 | 2.59 E-05 | 1.12 E+00 | 2.67 E-05 | 9.64 E-01 | 8.28 E-03 |
| 3c | 4.76 E-02 | 2.24 E-03 | 30 | 5.51 E-01 | 7.88 E-03 | 4.80 E-01 | 2.94 E-02 | 1.03 E+00 | 5.06 E-02 | 1.05 E+00 | 6.77 E-02 | 9.78 E-01 | 1.48 E-02 |
| 4.1 | 9.92 E-02 | - | 30 | 5.65 E-01 | 1.04 E-02 | 9.75 E-01 | 2.77 E-05 | 1.97 E+00 | 4.98 E-05 | 2.03 E+00 | 5.13 E-05 | 9.72 E-01 | 2.29 E-16 |
| 4.2 | 1.02 E-01 | - | 30 | 5.64 E-01 | 1.46 E-02 | 1.00 E+00 | 6.00 E-05 | 1.95 E+00 | 9.50 E-05 | 1.84 E+00 | 3.52 E-04 | 1.06 E+00 | 1.39 E-01 |
| 4.3 | 9.99 E-02 | - | 30 | 5.69 E-01 | 1.20 E-02 | 9.75 E-01 | 3.43 E-05 | 2.01 E+00 | 6.38 E-05 | 2.10 E+00 | 6.66 E-05 | 9.56 E-01 | 3.97 E-03 |
| 4c | 1.01 E-01 | 8.20 E-04 | 30 | 5.66 E-01 | 1.58 E-03 | 9.89 E-01 | 9.43 E-03 | 1.98 E+00 | 1.61 E-02 | 1.97 E+00 | 7.64 E-02 | 1.01 E+00 | 3.22 E-02 |
| 5.1 | 1.53 E-01 | - | 30 | 5.57 E-01 | 8.04 E-03 | 1.53 E+00 | 3.22 E-05 | 3.12 E+00 | 6.07 E-05 | 3.13 E+00 | 6.08 E-05 | 9.98 E-01 | 2.29 E-16 |
| 5.2 | 1.52 E-01 | - | 30 | 5.61 E-01 | 4.41 E-03 | 1.51 E+00 | 6.44 E-05 | 2.99 E+00 | 3.18 E-05 | 2.96 E+00 | 3.58 E-05 | 1.01 E+00 | 3.89 E-03 |
| 5.3 | 1.45 E-01 | - | 30 | 5.55 E-01 | 1.78 E-02 | 1.45 E+00 | 7.00 E-05 | 2.94 E+00 | 2.21 E-04 | 2.78 E+00 | 2.01 E-04 | 1.06 E+00 | 5.76 E-02 |
| 5c | 1.50 E-01 | 2.44 E-03 | 30 | 5.58 E-01 | 1.77 E-03 | 1.50 E+00 | 2.10 E-02 | 3.02 E+00 | 5.24 E-02 | 2.96 E+00 | 9.90 E-02 | 1.02 E+00 | 1.85 E-02 |
| 6.1 | 7.19 E-02 | - | 56 | 5.89 E-01 | 1.11 E-02 | 6.78 E-01 | 5.58 E-05 | 1.22 E+00 | 3.39 E-05 | 1.29 E+00 | 3.30 E-05 | 9.40 E-01 | 7.52 E-03 |
| 6.2 | 7.17 E-02 | - | 56 | 5.90 E-01 | 1.76 E-02 | 6.75 E-01 | 2.58 E-05 | 1.21 E+00 | 3.72 E-05 | 1.30 E+00 | 3.99 E-05 | 9.27 E-01 | 3.54 E-03 |
| 6.3 | 7.18 E-02 | - | 56 | 5.79 E-01 | 8.74 E-03 | 6.89 E-01 | 4.42 E-05 | 1.28 E+00 | 3.66 E-05 | 1.29 E+00 | 5.05 E-05 | 9.98 E-01 | 2.43 E-02 |
| 6c | 7.18 E-02 | 6.62 E-05 | 56 | 5.89 E-01 | 4.12 E-03 | 6.82 E-01 | 6.79 E-03 | 1.21 E+00 | 4.19 E-03 | 1.30 E+00 | 4.59 E-03 | 9.63 E-01 | 3.53 E-02 |
| 7.1 | 1.22 E-01 | - | 56 | 5.79 E-01 | 3.61 E-03 | 1.17 E+00 | 5.60 E-05 | 2.33 E+00 | 2.50 E-05 | 2.44 E+00 | 2.18 E-05 | 9.55 E-01 | 4.64 E-03 |
| 7.2 | 1.19 E-01 | - | 56 | 5.87 E-01 | 2.08 E-02 | 1.13 E+00 | 7.55 E-05 | 2.04 E+00 | 9.02 E-05 | 2.18 E+00 | 9.78 E-05 | 9.34 E-01 | 5.00 E-03 |
| 7.3 | 1.19 E-01 | - | 56 | 5.69 E-01 | 8.47 E-03 | 1.16 E+00 | 3.02 E-05 | 2.27 E+00 | 6.46 E-05 | 2.40 E+00 | 5.46 E-05 | 9.46 E-01 | 1.16 E-02 |
| 7c | 1.20 E-01 | 1.04 E-03 | 56 | 5.83 E-01 | 3.94 E-03 | 1.14 E+00 | 1.41 E-02 | 2.18 E+00 | 1.47 E-01 | 2.31 E+00 | 1.29 E-01 | 9.40 E-01 | 6.31 E-03 |

a,b Measured mean and standard error calculated for 3 technical replicates.

c Mean and standard error calculated for 2 or 3 biological replicates.

**Table S5. Raw P. pastoris chemostat data under different μ and DO conditions measured in this study (continuation)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Condition | Carbon Balance (-) | Electronic balance (-) | *C*X (gDCW) | *Y*S,P (mg g-1) | *q*P (mg gDCW-1·h-1) |
| Meana | S.E.b | Meana | S.E.b | Meana | S.E.b | Meana | S.E.b | Meana | S.E.b |
| 1.1 | 9.46 E-01 | 1.07 E-02 | 9.59 E-01 | 1.09 E-02 | 2.52 E+01 | 4.82 E-01 | 3.09 E-01 | 1.91 E-02 | 4.16 E-02 | 2.70 E-03 |
| 1.2 | 9.48 E-01 | 1.02 E-02 | 9.57 E-01 | 1.04 E-02 | 2.50 E+01 | 4.57 E-01 | 2.25 E-01 | 3.66 E-03 | 5.25 E-02 | 1.35 E-06 |
| 1c | 9.47 E-01 | 7.60 E-04 | 9.58 E-01 | 1.42 E-03 | 2.51 E+01 | 9.34 E-02 | 2.67 E-01 | 4.16 E-02 | 4.70 E-02 | 5.41 E-03 |
| 2.1 | 9.54 E-01 | 1.13 E-02 | 9.67 E-01 | 1.15 E-02 | 2.58 E+01 | 5.07 E-01 | 1.75 E-01 | 1.36 E-02 | 3.82 E-02 | 3.12 E-03 |
| 2.2 | 9.56 E-01 | 1.41 E-02 | 9.72 E-01 | 1.43 E-02 | 2.69 E+01 | 6.33 E-01 | 1.43 E-01 | 4.24 E-03 | 3.29 E-02 | 1.08 E-03 |
| 2c | 9.55 E-01 | 1.04 E-03 | 9.70 E-01 | 2.31 E-03 | 2.63 E+01 | 5.62 E-01 | 1.59 E-01 | 1.58 E-02 | 3.56 E-02 | 2.66 E-03 |
| 3.1 | 9.78 E-01 | 1.27 E-02 | 9.92 E-01 | 1.29 E-02 | 2.83 E+01 | 5.81 E-01 | 4.18 E-01 | 1.17 E-02 | 3.39 E-02 | 1.18 E-03 |
| 3.2 | 9.52 E-01 | 9.58 E-03 | 9.77 E-01 | 9.77 E-03 | 2.70 E+01 | 4.31 E-01 | 3.81 E-01 | 5.56 E-03 | 3.49 E-02 | 7.65 E-04 |
| 3c | 9.65 E-01 | 1.30 E-02 | 9.85 E-01 | 7.76 E-03 | 2.76 E+01 | 6.71 E-01 | 3.99 E-01 | 1.87 E-02 | 3.44 E-02 | 5.03 E-04 |
| 4.1 | 9.58 E-01 | 1.15 E-02 | 9.81 E-01 | 1.17 E-02 | 2.80 E+01 | 5.18 E-01 | 1.83 E-01 | 7.75 E-03 | 3.22 E-02 | 1.49 E-03 |
| 4.2 | 9.46 E-01 | 1.61 E-02 | 9.40 E-01 | 1.64 E-02 | 2.76 E+01 | 7.15 E-01 | 1.87 E-01 | 4.94 E-03 | 3.38 E-02 | 1.09 E-03 |
| 4.3 | 9.70 E-01 | 1.32 E-02 | 9.98 E-01 | 1.35 E-02 | 2.85 E+01 | 5.99 E-01 | 1.85 E-01 | 4.60 E-03 | 3.25 E-02 | 1.11 E-03 |
| 4c | 9.58 E-01 | 7.00 E-03 | 9.69 E-01 | 1.73 E-02 | 2.81 E+01 | 2.44 E-01 | 1.86 E-01 | 1.19 E-03 | 3.32 E-02 | 5.13 E-04 |
| 5.1 | 9.54 E-01 | 8.85 E-03 | 9.68 E-01 | 9.03 E-03 | 2.76 E+01 | 3.99 E-01 | 1.34 E-01 | 3.19 E-03 | 3.67 E-02 | 1.02 E-03 |
| 5.2 | 9.48 E-01 | 4.86 E-03 | 9.57 E-01 | 4.96 E-03 | 2.82 E+01 | 2.22 E-01 | 1.25 E-01 | 5.28 E-03 | 3.39 E-02 | 1.46 E-03 |
| 5.3 | 9.48 E-01 | 1.96 E-02 | 9.42 E-01 | 2.00 E-02 | 2.78 E+01 | 8.91 E-01 | 1.29 E-01 | 3.68 E-03 | 3.38 E-02 | 1.52 E-03 |
| 5c | 9.50 E-01 | 2.07 E-03 | 9.56 E-01 | 7.45 E-03 | 2.79 E+01 | 1.61 E-01 | 1.29 E-01 | 2.47 E-03 | 3.48 E-02 | 9.56 E-04 |
| 6.1 | 9.48 E-01 | 1.22 E-02 | 9.80 E-01 | 1.25 E-02 | 2.95 E+01 | 5.56 E-01 | - | - | - | - |
| 6.2 | 9.48 E-01 | 1.94 E-02 | 9.84 E-01 | 1.98 E-02 | 2.93 E+01 | 8.75 E-01 | 2.49 E-01 | 1.75 E-02 | 3.03 E-02 | 2.22 E-03 |
| 6.3 | 9.49 E-01 | 9.63 E-03 | 9.62 E-01 | 9.82 E-03 | 2.91 E+01 | 4.40 E-01 | 2.63 E-01 | 6.40 E-03 | 3.26 E-02 | 9.53 E-04 |
| 6c | 9.48 E-01 | 3.87 E-04 | 9.73 E-01 | 1.11 E-02 | 2.92 E+01 | 6.01 E-02 | 2.56 E-01 | 6.85 E-03 | 3.14 E-02 | 1.15 E-03 |
| 7.1 | 9.69 E-01 | 3.98 E-03 | 9.97 E-01 | 4.06 E-03 | 2.89 E+01 | 1.81 E-01 | 1.85 E-01 | 1.20 E-02 | 3.89 E-02 | 2.53 E-03 |
| 7.2 | 9.46 E-01 | 2.30 E-02 | 9.81 E-01 | 2.34 E-02 | 2.95 E+01 | 1.05 E+00 | 1.66 E-01 | 1.12 E-02 | 3.37 E-02 | 2.52 E-03 |
| 7.3 | 9.53 E-01 | 9.33 E-03 | 9.84 E-01 | 9.52 E-03 | 2.85 E+01 | 4.24 E-01 | 2.61 E-01 | 4.02 E-03 | 3.10 E-02 | 1.50 E-03 |
| 7c | 9.50 E-01 | 3.29 E-03 | 9.82 E-01 | 1.69 E-03 | 2.90 E+01 | 5.01 E-01 | 1.75 E-01 | 9.49 E-03 | 3.23 E-02 | 1.36 E-03 |

a,b Measured mean and standard error calculated for 3 technical replicates.

c Mean and standard error calculated for 2 or 3 biological replicates.

Table S6. Regression analysis and effects estimation for different macroscopic culture parameters

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Response coefficients | *Y*S,X (gDCW g-1) | *q*S (mmol gDCW-1h-1) | *q*CO2 (mmol gDCW-1h-1) | *q*O2 (mmol gDCW-1h-1) | *q*P (mg gDCW-1h-1) | *Y*S,P (mg g-1) |
| value | *p*-value | value | *p*-value | value | *p*-value | value | *p*-value | value | *p*-value | value | *p*-value |
|   |   |   |   |   | Regression Analysis |   |   |   |   |   |
| Response mean | *b*0 | 0.565 | - | 0.984 | - | 1.983 | - | 1.993 | - | 0.033 | - | 0.186 | - |
| μ | *b*1 | 0.005 | 0.1311 | 0.492 | 0 | 0.975 | 0 | 0.963 | 0 | -0.001 | 0.5509 | -0.127 | 0 |
| DO | *b*2 | 0.036 | 0 | -0.062 | 0 | -0.348 | 0 | -0.292 | 0 | -0.004 | 0.0256 | -0.004 | 0.625 |
| μ2 | *b*11 | -0.01 | 0.0753 | 0.015 | 0.1513 | 0.06 | 0.2717 | 0.022 | 0.7724 | 0.002 | 0.5222 | 0.08 | 0.0004 |
| μ·DO | *b*12 | -0.02 | 0.0071 | 0.004 | 0.713 | 0.032 | 0.5938 | 0.07 | 0.4176 | 0.008 | 0.0205 | 0.011 | 0.52 |
| DO2 | *b*22 | -0.017 | 0.0153 | 0.032 | 0.0143 | 0.149 | 0.0265 | 0.207 | 0.0298 | 0.005 | 0.125 | 0.006 | 0.7349 |
| *R*2 | - | 0.948 | - | 0.938 | - | 0.944 | - | 0.942 | - | 0.683 | - | 0.959 | - |
| Effects |  |  |  |  |  | Effects estimation |  |  |  |  |  |
| value | std | value | std | value | std | value | std | value | std | value | std |
| Average | 0.565 | 0.005 | 0.984 | 0.008 | 1.983 | 0.043 | 1.994 | 0.061 | 0.033 | 0.002 | 0.186 | 0.0127 |
| μ | 0.009 | 0.006 | 0.985 | 0.011 | 1.95 | 0.056 | 1.926 | 0.081 | -0.002 | 0.003 | -0.254 | 0.0164 |
| DO | 0.072 | 0.006 | -0.124 | 0.011 | -0.696 | 0.06 | -0.583 | 0.085 | -0.008 | 0.003 | -0.008 | 0.0164 |
| μ2 | -0.021 | 0.01 | 0.03 | 0.019 | 0.121 | 0.104 | 0.044 | 0.148 | 0.003 | 0.005 | 0.16 | 0.0324 |
| μ·DO | -0.039 | 0.012 | 0.008 | 0.022 | 0.064 | 0.116 | 0.14 | 0.165 | 0.016 | 0.006 | 0.022 | 0.0324 |
| DO2 | -0.034 | 0.012 | 0.063 | 0.021 | 0.298 | 0.115 | 0.414 | 0.164 | 0.01 | 0.006 | 0.011 | 0.0324 |

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