

Supplementary Information
Applied Microbiology and Biotechnology
Metabolic modeling of *Halomonas campaniensis* improves
polyhydroxybutyrate production under nitrogen limitation

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1 Supplementary Text

1.1 Gap-filling process

Network gaps were automatically filled employing the Gapfill algorithm (Kumar et al., 2007) to generate a functional model using *i*FP764 as reaction database. Consequently, only reactions present in the latter model were considered as candidates for bridging the network gaps that enabled cellular growth *in silico* under reported experimental conditions. This measure avoided inclusion of spurious reactions. The GapFill formulation relies on a binary variable y_j defined as (Equation 1):

$$y_j = \begin{cases} 1 & \text{if reaction } j \text{ from the external database is added to the parent network} \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

Since *i*FP764 is a functional model, a subset of its reactions is likely to produce a functional model. A value of α was defined as the percentage of growth in the *i*FP764 model. An additional constraint was added to the GapFill formulation, considering that the biomass reaction has a value that is a percentage of the template model. This percentage was written as α , with values of 1, 80 and 100% used for the gap-filling process.

The reactions that are present for every α and documented in literature were selected and added to the draft reconstruction using the relevant functions from the COBRA toolbox Heirendt et al., 2019. This step was performed for the five reconstructions built.

1.2 Experimental media definition

A modified LB medium was used for growth and seed culture preparation, containing per liter: NaCl 60, tryptone 10 and yeast extract 5. For PHB production, an MM medium was used, with its composition detailed in tables S3, S4 and S5 adapted from Tan et al., 2011. The pH of the medium was adjusted to 9.0 using 2.5 M NaOH. Trace solutions were filter sterilized and added to the autoclaved media; glucose was autoclaved separately from the rest of the medium components. MM media was modified to obtain a defined media based on observations Quillaguamán et al., 2008 that yeast extract can be substituted with glutamate. To avoid cofactor depletion, the amount of trace solution 1 and 2 were increased to 15 mL/L and 1.5 mL/L respectively. Biotin was added (0.05 mg/L) to account for possible vitamin consumption in absence of yeast extract Strazzullo et al., 2008. Since nitrogen depletion leads to stress in *H. campniensis* the effect of mixed nitrogen sources was studied, and a new defined media was formulated with glutamate and NH_4Cl . To compare across culture conditions, the initial total amount of nitrogen moles was kept fixed. Nitrogen limitation induces a stress response, thus, a mixed nitrogen medium was formulated to assess cellular response. To compare results, cultures were normalized by nitrogen moles, thus mixed nitrogen media contained 1.38 g/L glutamate and 0.5 g/L NH_4Cl .

1.3 Rate formula derivation

The specific secretion and uptake rates for key metabolites were determined from shake flask experiments using the growth rate and biomass yield on the corresponding metabolite S . The derivation of the rate formula starts with the general mass balance in the fermentation under the assumption of perfect mixing:

$$\frac{d(S \cdot V)}{dt} = F_{in}S_{in} - F_{out}S - r_sXV \quad (2)$$

Where S corresponds to the substrate concentration, F to the mass flux, X to the biomass concentration, V to the reaction volume and r_s to the specific substrate rate. The subscripts *in, out* refer to quantities inside or outside of the control volume being analysed. In a shake-flask fermentation, the total volume is constant, thus:

$$V \cdot \frac{dS}{dt} = F_{in}S_{in} - F_{out}S_{out} - r_sXV \quad (3)$$

Since it is a batch fermentation, there is no input or output of flow, thus $F_{in} = F_{out} = 0$, then:

$$V \cdot \frac{dS}{dt} = -r_sXV \quad (4)$$

Dividing by the volume and reorganizing:

$$r_s = -\frac{1}{X} \cdot \frac{dS}{dt} \quad (5)$$

Then, the substrate accumulation and biomass concentration yields the sought uptake rate. For the biomass, the specific growth rate (r_x) corresponds to μ yielding the following balance equation:

$$\frac{dX}{dt} = \mu \cdot X \quad (6)$$

μ is a function of biomass and during the exponential phase is constant. By substituting 6 in 5 we get:

$$r_s = -\frac{dS/dt}{dX/dt} \cdot \mu \quad (7)$$

The observed yield Y_{XS} is then defined as:

$$Y_{XS} = \frac{r_s}{r_x} = -\frac{dS/dt}{dX/dt} \quad (8)$$

The above quantity can be estimated by plotting the substrate versus biomass concentration at the same time points and finding the slope, whereas μ can be calculated as the slope of the natural logarithm of the biomass concentration versus time. Then, the experimental substrate uptake rate can be expressed as:

$$r_s = -Y_{XS} \cdot \mu \quad (9)$$

2 Supplementary Figures

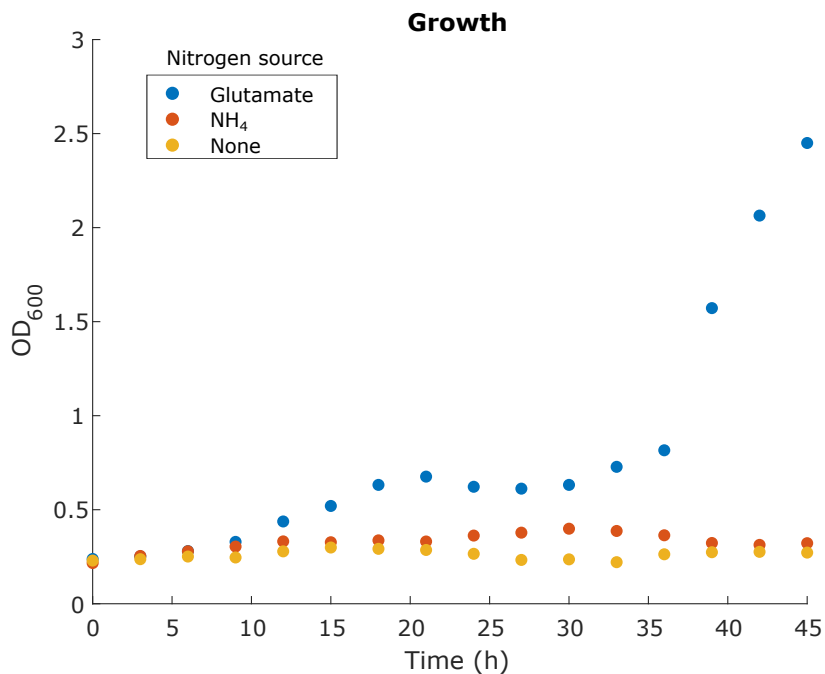


Figure S1: Shake-flasks cultures of *H. campaniensis* in minimal media with glutamate and ammonium as nitrogen sources, both at 18.7 mM. As a control, the base medium did not have any nitrogen sources and was supplemented with 30 g/L glucose as C source. While shake flasks with glutamate exhibited cellular growth, no growth was detected for culture with ammonium. The latter was evidenced when comparing the control and the latter culture.

3 Supplementary Tables

Table S1: GenBank access codes for the strains used in this study.

Genome	GenBank Assembly Accession	Link
<i>H. campaniensis</i> 5AG	GCA_014193375.1	https://www.ncbi.nlm.nih.gov/assembly/GCF_014193375.1
<i>H. boliviensis</i> LC1	GCA_000236035.1	https://www.ncbi.nlm.nih.gov/assembly/GCF_000236035.1
<i>Halomonas</i> sp. ALS9	GCA_001651035.1	https://www.ncbi.nlm.nih.gov/assembly/GCF_001651035.1
<i>Halomonas</i> sp. ISL104	GCA_018612735.1	https://www.ncbi.nlm.nih.gov/assembly/GCF_018612735.1
<i>Halomonas</i> sp. ISL56	GCA_018612805.1	https://www.ncbi.nlm.nih.gov/assembly/GCF_018612805.1

Table S2: Constraints used for qualitative model validation simulating media compositions described in literature (Romano et al., 2005).

Reaction ID	Value	Comments
EX_k_e	-10	Derived from KH_2PO_4
EX_pi_e	-10	Derived from KH_2PO_4
EX_mg2_e	-10	Derived from MgSO_4
EX_so4_e	-10	Derived from MgSO_4 and $(\text{NH}_4)_2\text{SO}_4$
EX_nh4_e	-4	Derived from $(\text{NH}_4)_2\text{SO}_4$
EX_na1_e	-10	Derived from NaCl
EX_cl_e	-10	Derived from NaCl
EX_h_e	-10	Always present in media
EX_h2o_e	-10	Always present in media
EX_o2_e	-15	Simulation in aerobic media
EX_ca2_e	-10	Minerals are unconstrained
EX_fe3_e	-10	Minerals are unconstrained
EX_cu2_e	-10	Minerals are unconstrained
EX_zn2_e	-10	Minerals are unconstrained
EX_mn2_e	-10	Minerals are unconstrained
EX_cobalt2_e	-10	Minerals are unconstrained
EX_ni2_e	-10	Minerals are unconstrained
EX_mobd_e	-10	Minerals are unconstrained
EX_btn_e	-0.1	Biotin is added to the medium

Table S3: Culture media compositions.

Component	MM complex media	Defined media	Mixed nitrogen media
Carbon source	30 g/L	30 g/L	30 g/L
NaCl	60 g/L	60 g/L	60 g/L
Yeast extract	1 g/L	-	-
Glutamate	-	2.75 g/L	1.38 g/L
MgSO ₄	0.2 g/L	0.2 g/L	0.2 g/L
Na ₂ HPO ₄ · 12H ₂ O	9.65 g/L	9.65 g/L	9.65 g/L
KH ₂ PO ₄	1.5 g/L	1.5 g/L	1.5 g/L
NH ₄ Cl	2 g/L	-	0.5 g/L
Trace element solution 1	10 mL/L	15 mL/L	15 mL/L
Trace element solution 2	1 mL/L	1.5 mL/L	1.5 mL/L
Biotin	-	0.5 mg/L	0.5 mg/L

Table S4: Composition of trace elements solution 1

Component	Composition (g/L)
Fe(III)-NH ₄ -citrate	5
CaCl ₂	2
HCl	1 M

Table S5: Composition of trace elements solution 2

Component	Composition (mg/L)
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	100
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	30
H_3BO_3	3300
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	200
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	10
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	20
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	30
HCl	1 M

Table S6: Comparison between available metabolic models for the reconstruction of the metabolic network of *H. campaniensis*.

Criteria	<i>Chromohalobacter salexigens</i>	<i>Halomonas</i> sp. <i>BC1</i>	<i>Halomonas smyrnensis</i>
Number of compounds	1123	309	981
Number of reactions	1528	279	1143
Database identifiers	BiGG	None (arbitrary identifiers)	MetaNetx
Family/Genus	<i>Halomonadaceae</i> / <i>Chromohalobacter</i>	<i>Halomonadaceae</i> / <i>Halomonas</i>	<i>Halomonadaceae</i> / <i>Halomonas</i>
Reference	Piubeli et al., 2018	Carlson et al., 2016	Diken et al., 2015

Table S7: Manually added reactions to HaloGEM.

Reaction ID	Reaction formula	Source
2INSd	1 2ins_c <=>1 h2o_c + 1 dkdi_c	RAVEN
3NUCLE1	1 h2o_c + 1 3amp_c <=>1 pi_c + 1 adn_c	RAVEN
3NUCLE2	1 h2o_c + 1 3ump_c <=>1 pi_c + 1 uri_c	RAVEN
3NUCLE3	1 h2o_c + 1 3cmp_c <=>1 pi_c + 1 cytd_c	RAVEN
3NUCLE4	1 h2o_c + 1 3gmp_c <=>1 pi_c + 1 gsn_c	RAVEN
3OADPCoAT	1 succoa_c + 1 3oxoadp_c <=>1 succ_c + 1 oxadpcoa_c	RAVEN
ACACCT	1 ac_c + 1 aacoa_c <=>1 accoa_c + 1 acac_c	RAVEN
ACKr	1 atp_c + 1 ac_c <=>1 adp_c + 1 actp_c	RAVEN
ADNCYC	1 atp_c <=>1 ppi_c + 1 camp_c	RAVEN
AEPPYRTA	1 pyr_c + 1 2ameph_c <=>1 ala_L_c + 1 Pald_c	RAVEN
ALCD20y	1 nadph_c + 1 h_c + 1 acetone_c <=>1 nadp_c + 1 2ppoh_c	RAVEN
ATHRDHr	1 nadp_c + 1 athr_L_c <=>1 nadph_c + 1 h_c + 1 2aobut_c	RAVEN
CEPA	1 pi_c + 1 cellb_c <=>1 glc_D_c + 1 glp_c	RAVEN
CODSCL8XI	1 codscl8x_c <=>1 cobya_c	RAVEN
CPC3MT	1 amet_c + 1 copre3_c <=>1 ahcys_c + 1 copre4_c	RAVEN
DHPD	1 h2o_c + 1 56dura_c <=>1 cala_c	RAVEN
DURADx	1 nad_c + 1 56dura_c <=>1 nadh_c + 1 h_c + 1 ura_c	RAVEN
FRDx	1 nad_c + 1 succ_c <=>1 nadh_c + 1 h_c + 1 fum_c	RAVEN
GGGABAH	1 h2o_c + 1 gg4abut_c <=>1 glu_L_c + 1 4abut_c	RAVEN
GUACYC	1 gtp_c <=>1 ppi_c + 1 35cgm_p_c	RAVEN
HACD4i	1 nad_c + 1 3hdcoa_c <=>1 nadh_c + 1 h_c + 1 3odcoa_c	RAVEN
HBCO_nadp	1 nadp_c + 1 3hbcoa_c <=>1 nadph_c + 1 h_c + 1 aacoa_c	RAVEN
ICHORS_copy1	1 chor_c <=>1 ichor_c	RAVEN
INS2D	1 nad_c + 1 inost_c <=>1 nadh_c + 1 h_c + 1 2ins_c	RAVEN
IZPN.1	1 h2o_c + 1 4izp_c <=>1 forglu_c	RAVEN
MCDC	1 malcoa_c <=>1 co2_c + 1 accoa_c	RAVEN
MDH	1 nad_c + 1 mal_L_c <=>1 nadh_c + 1 oaa_c + 1 h_c	RAVEN
MSAR	1 nadp_c + 1 3hpp_c <=>1 nadph_c + 1 h_c + 1 msa_c	RAVEN
MTRI	1 5mdr1p_c <=>1 5mdru1p_c	RAVEN
NNDMBRT	1 nicrnt_c + 1 dmbzid_c <=>1 h_c + 1 nac_c + 1 5prdmzb_c	RAVEN
OCOAT1	1 succoa_c + 1 acac_c <=>1 succ_c + 1 aacoa_c	RAVEN
PALDH	1 h2o_c + 1 Pald_c <=>1 pi_c + 1 acald_c	RAVEN
PC20M	1 amet_c + 1 dscl_c <=>1 ahcys_c + 1 h_c + 1 pre3a_c	RAVEN
PC6AR	1 nadp_c + 1 pre6b_c <=>1 nadph_c + 1 h_c + 1 pre6a_c	RAVEN
PRE3BS	1 nadh_c + 1 o2_c + 1 h_c + 1 pre3a_c <=> 1 h2o_c + 1 nad_c + 1 pre3b_c	RAVEN
PTA2	1 pi_c + 1 ppcoa_c <=>1 coa_c + 1 ppap_c	RAVEN
PTAr	1 pi_c + 1 accoa_c <=>1 coa_c + 1 actp_c	RAVEN
PTRCTA	1 ak_g_c + 1 ptrc_c <=>1 glu_L_c + 1 4abutn_c	RAVEN
R00150	1 atp_c + 1 nh3_c + 1 h2co3_c <=> 1 h2o_c + 1 adp_c + 1 cbp_c	RAVEN
R00261	1 glu_L_c <=>1 co2_c + 1 4abut_c	RAVEN
R00282	1 nadph_c + 1 C00028_c + 1 h_c <=>1 nadp_c + 1 C00030_c	RAVEN

Table S7 continued from previous page

R00357	1 h2o_c + 1 o2_c + 1 CE1787_c <=> 1 nh3_c + 1 h2o2_c + 1 oaa_c	RAVEN
R00481	1 o2_c + 1 CE1787_c <=>1 h2o2_c + 1 iasp_c	RAVEN
R00552	1 h2o_c + 1 arg_L_c <=>1 nh3_c + 1 citr_L_c	RAVEN
R00650	1 amet_c + 1 hcys_L_c <=>1 ahcys_c + 1 met_L_c	RAVEN
R00749	1 etha_c <=>1 nh3_c + 1 acald_c	RAVEN
R01168	1 his_L_c <=>1 nh3_c + 1 urcan_c	RAVEN
R01179	1 ac_c + 1 btcoa_c <=>1 accoa_c + 1 C00246_c	RAVEN
R01196	1 co2_c + 1 accoa_c + 2 h_c + 2 fdxrd_c <=> 1 coa_c + 1 pyr_c + 2 fdxo.2.2_c	RAVEN
R01353	1 atp_c + 1 C00163_c <=>1 adp_c + 1 ppap_c	RAVEN
R01360	1 C00356_c <=>1 accoa_c + 1 acac_c	RAVEN
R01365	1 btcoa_c + 1 acac_c <=>1 C00246_c + 1 aacoa_c	RAVEN
R01579	1 h2o_c + 1 C00819_c <=>1 nh3_c + 1 glu_D_c	RAVEN
R01625	1 coa_c + 1 C03688_c <=>1 pap_c + 1 ACP_c	RAVEN
R01812	2 nadp_c + 1 thbpt_c <=>2 nadph_c + 2 h_c + 1 C06313_c	RAVEN
R01959	1 h2o_c + 1 Lfmkynr_c <=>1 for_c + 1 Lkynr_c	RAVEN
R02085	1 C00356_c <=>1 h2o_c + 1 3mgcoa_c	RAVEN
R02328	1 glp_c + 1 dttp_c <=>1 ppi_c + 1 dtdpglu_c	RAVEN
R02466	1 3sala_c <=>1 co2_c + 1 C00519_c	RAVEN
R02487	1 fad_c + 1 glutcoa_c ->1 co2_c + 1 b2coa_c + 1 fadh2_c	RAVEN
R02488	1 glutcoa_c + 1 C04253_c <=> 1 co2_c + 1 b2coa_c + 1 C04570_c	RAVEN
R02662	1 ibcoa_c + 1 C15973_c <=>1 coa_c + 1 C15977_c	RAVEN
R03055	1 h2o_c + 1 C21028_c <=>1 C21029_c	RAVEN
R03174	1 C15973_c + 1 C15980_c <=>1 coa_c + 1 C15979_c	RAVEN
R03546	1 h_c + 1 h2co3_c + 1 cynt_c <=>1 co2_c + 1 cbm_c	RAVEN
R03877	1 h2o_c + 1 atp_c + 1 mg2_c + 1 ppp9_c <=> 1 adp_c + 1 pi_c + 2 h_c + 1 mppp9_c	RAVEN
R04097	1 ivcoa_c + 1 C15973_c <=>1 coa_c + 1 C15975_c	RAVEN
R04143	1 atp_c + 1 5mtr_c <=>1 adp_c + 1 5mdr1p_c	RAVEN
R04383	1 5dh4dglcn_c <=>1 C04349_c	RAVEN
R04435	1 pi_c + 1 C06241_c <=>1 h2o_c + 1 pep_c + 1 acmanap_c	RAVEN
R04448	1 atp_c + 1 4mhetz_c <=>1 adp_c + 1 4mpetz_c	RAVEN
R05062	1 C05986_c <=>2 h_c + 2 cytc_c + 1 C05985_c	RAVEN
R05149	2 amet_c + 1 pre6b_c <=> 1 co2_c + 2 ahcys_c + 1 C06408_c	RAVEN
R05177	1 C06408_c <=>1 hgbyr_c	RAVEN
R05180	1 amet_c + 1 pre3b_c <=>1 ahcys_c + 1 pre4_c	RAVEN
R05181	1 amet_c + 1 pre4_c <=>1 ahcys_c + 1 pre5_c	RAVEN
R05219	1 ahcys_c + 1 ac_c + 1 pre6a_c <=> 1 h2o_c + 1 amet_c + 1 pre5_c	RAVEN
R05221	1 atp_c + 1 adocbi_c <=>1 adp_c + 1 adocbip_c	RAVEN
R05222	1 gtp_c + 1 adocbip_c <=>1 ppi_c + 1 agdpcbi_c	RAVEN
R05223	1 gmp_c + 1 adocbl_c <=>1 rdmbzi_c + 1 agdpcbi_c	RAVEN

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R05225	4 h2o.c + 4 atp.c + 4 gln.L.c + 1 C06506.c <=> 4 adp.c + 4 pi.c + 4 glu.L.c + 1 adcobhex.c	RAVEN
R05227	1 h2o.c + 1 atp.c + 1 cobalt2.c + 1 hgbam.c <=> 1 adp.c + 1 pi.c + 1 h.c + 1 co2dam.c	RAVEN
R05285	1 C06753.c <=> 2 h.c + 2 cytc.c + 1 C06754.c	RAVEN
R05576	1 nadp.c + 1 C05116.c <=> 1 nadph.c + 1 h.c + 1 aacoa.c	RAVEN
R05661	1 atp.c + 1 d5kg.c <=> 1 adp.c + 1 d5kgp.c	RAVEN
R05794	1 chol.c + 1 cdpdag_cho.c <=> 1 cmp.c + 1 pchol_cho.c	RAVEN
R05808	1 amet.c + 1 C11538.c <=> 1 ahcys.c + 1 C17401.c	RAVEN
R05810	1 amet.c + 1 copre4.c <=> 1 ahcys.c + 1 codscl5a.c	RAVEN
R05812	1 nadh.c + 1 h.c + 1 copre6.c <=> 1 nad.c + 1 codhpre6.c	RAVEN
R06529	1 atp.c + 1 applp.c + 1 adcobhex.c <=> 1 adp.c + 1 pi.c + 1 adocbip.c	RAVEN
R06530	1 thrp.c <=> 1 co2.c + 1 applp.c	RAVEN
R06558	1 gtp.c + 1 adocbi.c <=> 1 gdp.c + 1 adocbip.c	RAVEN
R06943	1 fad.c + 1 C14143.c <=> 1 fadh2.c + 1 C14144.c	RAVEN
R07229	1 C00030.c + 1 sel.c -> 1 h2o.c + 1 C00028.c + 1 slnt.c	RAVEN
R07302	1 atp.c + 1 appl.c + 1 adcobhex.c <=> 1 adp.c + 1 pi.c + 1 adocbi.c	RAVEN
R07392	1 5mdru1p.c <=> 1 h2o.c + 1 2h3k5m.c	RAVEN
R07395	1 h2o.c + 1 2h3k5m.c <=> 1 pi.c + 1 dhmtpp.c	RAVEN
R07412	1 h2o.c + 2 C00028.c + 1 hemeO.c <=> 2 C00030.c + 1 hemeA.c	RAVEN
R07599	1 thmpp.c + 1 3mob.c -> 1 co2.c + 1 2mhob.c	RAVEN
R07600	1 C15972.c + 1 2mhob.c -> 1 thmpp.c + 1 C15977.c	RAVEN
R07601	1 thmpp.c + 1 4mop.c -> 1 co2.c + 1 3mhtpp.c	RAVEN
R07602	1 C15972.c + 1 3mhtpp.c -> 1 thmpp.c + 1 C15975.c	RAVEN
R07603	1 thmpp.c + 1 3mop.c -> 1 co2.c + 1 2mhob.c	RAVEN
R07604	1 C15972.c + 1 2mhob.c -> 1 thmpp.c + 1 C15979.c	RAVEN
R07634	1 h2o.c + 1 Lcyst.c <=> 1 nh3.c + 1 pyr.c + 1 so3.c	RAVEN
R07772	1 h2o.c + 1 codscl5a.c <=> 1 acald.c + 1 codscl5b.c	RAVEN
R07774	1 amet.c + 1 codhpre6.c <=> 1 co2.c + 1 ahcys.c + 1 codscl7.c	RAVEN
R07775	1 amet.c + 1 codscl7.c <=> 1 ahcys.c + 1 codscl8x.c	RAVEN
R07832	1 accoa.c + 1 C16272.c <=> 1 ac.c + 1 C16273.c	RAVEN
R08090	1 C04675.c <=> 1 ac.c + 1 C16466.c	RAVEN
R08227	1 h2o.c + 1 56dh5flura.c -> 1 aflburppa.c	RAVEN
R08503	1 C16737.c <=> 1 d5kg.c	RAVEN
R08603	1 h2o.c + 1 dkdi.c <=> 1 C16737.c	RAVEN
R08836	1 o2.c + 1 C00355.c <=> 1 C17758.c	RAVEN
R09083	1 o2.c + 1 fmnh2.c <=> 1 h2o.c + 1 e4p.c + 1 dmbzid.c	RAVEN
R09707	1 C19830.c <=> 1 C19831.c	RAVEN
R09837	1 C20062.c <=> 1 2oxpaccoa.c	RAVEN

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R09951	1 nad_c + 1 inost_c <=> 1 nadh_c + 1 h_c + 1 C20251_c	RAVEN
R09953	1 nad_c + 1 C06153_c <=> 1 nadh_c + 1 h_c + 1 2ins_c	RAVEN
R09993	1 h2o_c + 1 C20267_c <=>1 nh3_c + 1 4ahmmp_c	RAVEN
R10061	2 amet_c + 1 sarcs_c <=>2 ahcys_c + 1 glyb_c	RAVEN
R10074	1 glutcoa_c + 1 C04253_c <=>1 C02411_c + 1 C04570_c	RAVEN
R10150	1 tet_c + 2 C05359_c <=>2 tsul_c	RAVEN
R10151	5 h2o_c + 1 tsul_c <=>2 so4_c + 10 h_c + 8 cytc_c	RAVEN
R10152	1 undefined_148_c + 1 undefined_158_c <=> 1 C19692_c + 1 undefined_159_c	RAVEN
R10757	1 dhna_c + 1 C05847_c <=> 1 co2_c + 1 ppi_c + 1 C19847_c	RAVEN
R10820	1 atp_c + 1 coa_c + 1 3mtp_c <=> 1 ppi_c + 1 amp_c + 1 C20870_c	RAVEN
R10996	1 thmpp_c + 1 2obut_c <=> 1 co2_c + 1 C21017_c	RAVEN
R10997	1 C15972_c + 1 C21017_c <=>1 thmpp_c + 1 C21018_c	RAVEN
R10998	1 ppcoa_c + 1 C15973_c <=>1 coa_c + 1 C21018_c	RAVEN
R11026	1 nad_c + 1 C21028_c <=>1 nadh_c + 1 h_c + 1 thym_c	RAVEN
R11264	1 C21250_c <=>1 2mcacn_c	RAVEN
R11555	1 pe_hs_c + 1 lipa_c ->1 dag_hs_c + 1 C21461_c	RAVEN
R11556	1 pe_hs_c + 1 lipa_c <=>1 dag_hs_c + 1 C21462_c	RAVEN
R11557	1 pe_hs_c + 1 C21461_c ->1 dag_hs_c + 1 C21463_c	RAVEN
R11580	1 amet_c + 1 C00030_c + 1 C17401_c <=> 1 ahcys_c + 1 C00028_c + 1 copre4_c	RAVEN
R12202	1 pe_hs_c + 1 C21994_c <=>1 dag_hs_c + 1 C21995_c	RAVEN
R12644	1 nadp_c + 1 CE2705_c <=> 1 nadph_c + 1 h_c + 1 C06313_c	RAVEN
R12897	1 atp_c + 1 3mbald_c <=>1 adp_c + 1 C21214_c	RAVEN
RZ5PP	1 h2o_c + 1 5prdmzbz_c <=>1 pi_c + 1 rdmbzi_c	RAVEN
SALADC2	1 Lcyst_c <=>1 co2_c + 1 taur_c	RAVEN
TDPDRR	1 nadp_c + 1 dtdprmn_c <=> 1 nadph_c + 1 h_c + 1 dtdp4d6dm_c	RAVEN
TDPGDH	1 dtdpglu_c <=>1 h2o_c + 1 dtdp4d6dg_c	RAVEN
TMN	1 h2o_c + 1 thm_c <=>1 h_c + 1 4ahmmp_c + 1 4mhetz_c	RAVEN
URCN	1 4izp_c <=>1 h2o_c + 1 urcan_c	RAVEN
r0330	1 nad_c + 1 56dthm_c <=>1 nadh_c + 1 h_c + 1 thym_c	RAVEN
ATPM	h2o_c + atp_c ->h_c + pi_c + adp_c	manual
GLCt2pp	glc_D.p + h.p <=>glc_D.c + h.c	manual
F6Pt6_2pp	2 pi_c + f6p.p <=>f6p.c + 2 pi.p	manual
GLYCtpp	glyc_c <=>glyc.p	manual
PYRt2rpp	h.p + pyr.p <=>h.c + pyr.c	manual
FBP	fdp_c + h2o_c <=>f6p_c + pi_c	manual
ACALDtpp	acald.p <=>acald.c	manual

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ACALD	$\text{acald}_c + \text{coa}_c + \text{nad}_c \rightleftharpoons \text{accoa}_c + \text{h}_c + \text{nadh}_c$	manual
ETOHtrpp	$\text{etoh}_p \rightleftharpoons \text{etoh}_c$	manual
PFL	$\text{coa}_c + \text{pyr}_c \rightleftharpoons \text{accoa}_c + \text{for}_c$	manual
FBA3	$\text{s17bp}_c \rightleftharpoons \text{dhap}_c + \text{e4p}_c$	manual
CITt3pp	$\text{h}_p + \text{cit}_p \rightarrow \text{h}_c + \text{cit}_c$	manual
G6Pt6_2pp	$2 \text{ pi}_c + \text{g6p}_p \rightarrow \text{g6p}_c + 2 \text{ pi}_p$	manual
RBK	$\text{atp}_c + \text{rib_D}_c \rightleftharpoons \text{adp}_c + \text{h}_c + \text{r5p}_c$	manual
PPK2	$\text{atp}_c + \text{ppi}_c \rightleftharpoons \text{adp}_c + \text{pppi}_c$	manual
ECTtra	$\text{ect_L}_p \rightleftharpoons \text{ect_L}_c$	manual
AACPS9	$\text{ACP}_c + \text{atp}_c + \text{octa}_c \rightleftharpoons \text{amp}_c + \text{ocACP}_c + \text{ppi}_c$	manual
AACPS8	$\text{ACP}_c + \text{atp}_c + \text{dca}_c \rightleftharpoons \text{amp}_c + \text{dcaACP}_c + \text{ppi}_c$	manual
AACPS7	$\text{ACP}_c + \text{atp}_c + \text{ddca}_c \rightleftharpoons \text{amp}_c + \text{ddcaACP}_c + \text{ppi}_c$	manual
AACPS6	$\text{ACP}_c + \text{atp}_c + \text{ocdca}_c \rightleftharpoons \text{amp}_c + \text{ocdcaACP}_c + \text{ppi}_c$	manual
AACPS3	$\text{ACP}_c + \text{atp}_c + \text{hdca}_c \rightleftharpoons \text{amp}_c + \text{palmACP}_c + \text{ppi}_c$	manual
AACPS1	$\text{ACP}_c + \text{atp}_c + \text{ttdca}_c \rightleftharpoons \text{amp}_c + \text{myrsACP}_c + \text{ppi}_c$	manual
G5SADs	$\text{glu5sa}_c \rightleftharpoons \text{1pyr5c}_c + \text{h}_c + \text{h2o}_c$	manual
MHPGLUT	$\text{hcys_L}_c + \text{mhpglu}_c \rightleftharpoons \text{met_L}_c + \text{hpglu}_c$	manual
FOLR2	$\text{nadph}_c + \text{fol}_c \rightleftharpoons \text{dhf}_c + \text{nadp}_c$	manual
AACOAT	$\text{acac}_c + \text{atp}_c + \text{coa}_c \rightleftharpoons \text{aacoa}_c + \text{amp}_c + \text{ppi}_c$	manual
NDPK(dapd:amp)	$\text{dadp}_c + \text{adp}_c \rightleftharpoons \text{camp}_c + \text{atp}_c + \text{h}_c$	manual
R02088	$\text{dad_2}_c + \text{pi}_c \rightleftharpoons \text{camp}_c + \text{h2o}_c$	manual
R00706	$\text{msa}_c + \text{coa}_c + \text{nad}_c \rightleftharpoons \text{accoa}_c + \text{co2}_c + \text{nadh}_c + \text{h}_c$	manual
FESD1s	$2 \text{ h}_c + \text{h2o2}_c + 2 \text{ 4fe4s}_c \rightarrow 2 \text{ h2o}_c + 2 \text{ fe3}_c + 2 \text{ 3fe4s}_c$	manual
FESR	$\text{fe2}_c + \text{3fe4s}_c \rightarrow \text{4fe4s}_c$	manual
prpB	$2\text{mcaen_T}_c + \text{h2o}_c \rightleftharpoons \text{micit}_c$	manual
R01623	$\text{ACP}_c + \text{h2o}_c \rightleftharpoons \text{pan4p}_c + \text{apoACP}_c$	manual

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BIOMASS_low_salinity	2.6e-05 2fe2s.c + 0.00026 4fe4s.c + 0.0002 5mthf.c + 0.0002 accoa.c + 0.3675 ala.L.c + 0.23851 arg.L.c + 0.07793 asn.L.c + 0.19512 asp.L.c + 59.98 atp.c + 0.0045 ca2.c +0.0045 cl.c + 0.0001 coa.c + 0.003 cobalt2.c + 0.1638 ctp.c + 0.003 cu2.c + 0.03049 cys.L.c + 0.01817 datp.c + 0.032029 dctp.c + 0.03241 dgtp.c + 0.0182 dttp.c + 0.2425 ect.L.c + 0.0002 fad.c + 0.0067 fe2.c + 0.0067 fe3.c + 0.11971 gln.L.c + 0.41808 glu.L.c + 0.25947 gly.c + 0.2086 gtp.c + 45.56 h2o.c + 0.00939 hdict.c + 0.0002 hemeO.c + 0.08145 his.L.c + 0.1459 ile.L.c + 0.1691 k.c + 0.019456 kdo2lipid4.e + 0.3594 leu.L.c + 0.07997 lys.L.c + 0.08023 met.L.c + 0.0075 mg2.c + 0.003 mn2.c + 0.003 mobd.c + 0.013894 murein5px4p.p + 0.263 na1.c + 0.0017 nad.c + 4e-05 nadh.c + 0.0001 nadp.c + 0.0003 nadph.c + 0.0112 nh4.c + 0.0268 pe160.c + 0.045946 pe160.p + 0.0081 pe161.c + 0.02106 pe161.p + 0.10993 phe.L.c + 0.15654 pro.L.c + 0.17139 ser.L.c + 0.0002 sheme.c + 0.0037 so4.c + 9e-05 succoa.c + 0.16669 thr.L.c + 0.04774 trp.L.c + 0.07644 tyr.L.c + 5.5e-05 udcdp.c + 0.126 utp.c + 0.23021 val.L.c + 0.003 zn2.c ->59.81 adp.c + 59.81 h.c + 58.8062 pi.c + 0.7498 ppi.c	manual
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Table S7 continued from previous page

BIOMASS_medium_salinity	2.6e-05 2fe2s_c + 0.00026 4fe4s_c + 0.0002 5mthf_c + 0.0002 accoa_c + 0.333055 ala_L_c + 0.216148 arg_L_c + 0.0706132 asn_L_c + 0.176825 asp_L_c + 56.81 atp_c + 0.0045 ca2_c + 0.0045 cl_c + 0.0001 coa_c + 0.003 cobalt2_c + 0.1612 ctp_c + 0.003 cu2_c + 0.0276294 cys_L_c + 0.0181542 datp_c + 0.0972466 dctp_c + 0.0324077 dgtp_c + 0.0182 dttp_c + 0.338121 ect_L_c + 0.0002 fad_c + 0.0067 fe2_c + 0.0067 fe3_c + 0.349719 glu_L_c + 0.235141 gly_c + 0.214914 gtp_c + 56.81 h2o_c + 0.0926154 hdect_c + 0.0002 hemeO_c + 0.0738118 his_L_c + 0.132208 ile_L_c + 0.19456 kdo2lipid4_e + 0.325711 leu_L_c + 0.0724631 lys_L_c + 0.0727073 met_L_c + 0.0075 mg2_c + 0.003 mn2_c + 0.013894 murein5px4p_p + 0.339268 na1_c + 0.0017 nad_c + 4e-05 nadh_c + 0.0001 nadp_c + 0.0003 nadph_c + 0.0112 nh4_c + 0.0268837 pe160_c + 0.045946 pe160_p + 0.00765868 pe161_c + 0.02106 pe161_p + 0.0991598 phe_L_c + 0.141843 pro_L_c + 0.155319 ser_L_c + 0.0002 sheme_c + 0.0037 so4_c + 9e-05 succoa_c + 0.151061 thr_L_c + 0.0432635 trp_L_c + 0.0692726 tyr_L_c + 5.5e-05 udcpd_c + 0.12309 utp_c + 0.208626 val_L_c + 0.003 zn2_c + 0.108485 gln_L_c ->56.81 adp_c + 56.81 h_c + 56.81 pi_c + 0.745319 ppi_c	manual
BUTt	but_e <=>but_c	manual
IBTt	ibt_e <=>ibt_c	manual
3MBtex	3mb_e <=>3mb_c	manual
PTAt	pta_e <=>pta_c	manual
MALONt	malon_e <=>malon_c	manual
4ABUTt	4abut_e <=>4abut_c	manual
GLUTARt	glutar_e <=>glutar_c	manual
ADPACtd	adpac_e <=>adpac_c	manual
R06944	adpac_c + coa_c + atp_c <=> adpcoa_c + amp_c + ppi_c	manual
R06942	23dhacoa_c + h2o_c <=>3hadpcoa_c	manual
HADPCOAH3	3hadpcoa_c + nad_c <=> h_c + nadh_c + oxadpcoa_c	manual
3OXCOAT	coa_c + oxadpcoa_c <=>accoa_c + succoa_c	manual
OXPTNDH	h2o_c + nad_c + oxptn_c <=> 2 h_c + nadh_c + glutar_c	manual
R12216	glutar_c + akg_c + o2_c <=> 2hglut_c + succ_c + co2_c	manual
APT NAT	akg_c + 5aptn_c <=>glu_L_c + oxptn_c	manual

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R12217	2hglut_c + q8_c <=>akg_c + q8h2_c	manual
ABTA	4abut_c + akc_c <=>glu_L_c + sucsal_c	manual
R04002	atp_c + ibt_c <=>adp_c + 2mpylp_c	manual
EATi4	2mpylp_c + coa_c <=>ibcoa_c + pi_c	manual
ACOAD9m	ibcoa_c + fad_c <=>fadh2_c + 2mp2coa_c	manual
R04095	ivcoa_c + fad_c <=>fadh2_c + 3mb2coa_c	manual
R04138	atp_c + 3mb2coa_c + hco3_c <=> adp_c + pi_c + 3mgcoa_c	manual
IVCS	atp_c + coa_c + 3mb_c <=>amp_c + ppi_c + ivcoa_c	manual
FACOAL50i	atp_c + coa_c + pta_c <=>amp_c + ppi_c + ptcoa_c	manual
MALCT	malon_c + accoa_c <=>ac_c + malcoa_c	manual
CELLBabc	atp_c + h2o_c + cellb_e <=> adp_c + h_c + pi_c + cellb_c	manual
MALT	h2o_c + malt_c <=>2 glc_D_c	manual
MANabcpp	atp_c + h2o_c + man_p <=> adp_c + h_c + man_c + pi_c	manual
HEX4	man_c + atp_c ->h_c + adp_c + man6p_c	manual
R04224	2mp2coa_c + h2o_c <=>3hibutcoa_c	manual
3HBCOHL	3hibutcoa_c + h2o_c <=>coa_c + 3hmp_c	manual
R05066	3hmp_c + nad_c <=>2mop_c + nadh_c + h_c	manual
MMSAD1	coa_c + nad_c + 2mop_c <=> co2_c + nadh_c + ppcoa_c	manual
ASNabcpp	h2o_c + atp_c + asn_L_p -> asn_L_c + h_c + pi_c + adp_c	manual
3HBC3E	3hbcoa_c <=>3hbcoa_R_c	manual
PHB_syn_1	4 3hbcoa_R_c <=>phb_c + 4 coa_c	manual
PHB-transport	phb_c <=>phb_e	manual
EX_phb_e	phb_e <=>	manual
EX_but_e	but_e <=>	manual
EX_ibt_e	ibt_e <=>	manual
EX_pta_e	pta_e <=>	manual
EX_3mb_e	3mb_e <=>	manual
EX_malon_e	malon_e <=>	manual
EX_4abut_e	4abut_e <=>	manual
EX_glutar_e	glutar_e <=>	manual
EX_adpac_e	adpac_e <=>	manual
EX_gal_e	gal_e <=>	manual
EX_arab_D_e	arab_D_e <=>	manual
EX_cellb_e	cellb_e <=>	manual
EX_xyl_D_e	xyl_D_e <=>	manual

Table S8: Qualitative validation of HaloGEM.

Test Condition	Media	Growth in vivo	Growth in sillico	Result	References
Nutrient essentiality	No Peptone	+	+	TP	Romano et al., 2005
	No Oxygen	-	-	TN	Romano et al., 2005
	No Na	-	-	TN	Romano et al., 2005
Carbon sources	Glucose	+	+	TP	This work; Romano et al., 2005; Yue et al., 2014
	Fructose	+	+	TP	This work; Romano et al., 2005; Yue et al., 2014
	Glycerol	+	+	TP	This work; Romano et al., 2005; Yue et al., 2014
	Sucrose	+	+	TP	This work; Romano et al., 2005; Yue et al., 2014
	Maltose	+	+	TP	This work; Romano et al., 2005
	Xylose	-	-	TN	Romano et al., 2005
	Galactose	-	-	TN	Romano et al., 2005; Yue et al., 2014
	Arabinose	-	-	TN	Romano et al., 2005
	Cellobiose	+	+	TP	Romano et al., 2005; Yue et al., 2014
	Mannose	+	+	TP	Romano et al., 2005; Yue et al., 2014
	Sodium acetate	+	+	TP	Romano et al., 2005; Yue et al., 2014; Strazzullo et al., 2008
	Propionic acid	+	+	TP	Strazzullo et al., 2008
	Butyric acid	+	+	TP	Strazzullo et al., 2008
	Iso-butyric acid	+	+	TP	Strazzullo et al., 2008
	Valeric acid	+	-	FN	Strazzullo et al., 2008
	Iso-valeric acid	+	+	TP	Strazzullo et al., 2008
	Malonic acid	+	+	TP	Strazzullo et al., 2008
	Succinic acid	+	+	TP	Strazzullo et al., 2008
	Glutaric acid	+	+	TP	Strazzullo et al., 2008
	Adipic acid	+	+	TP	Strazzullo et al., 2008
	Citric acid	+	+	TP	Strazzullo et al., 2008
	L-glutamine	+	+	TP	Strazzullo et al., 2008
	Aminobutyric acid	+	+	TP	Strazzullo et al., 2008
	L-asparagine	+	+	TP	Strazzullo et al., 2008
	L-arginine	-	-	TN	Strazzullo et al., 2008
	L-glutamic acid	+	+	TP	Strazzullo et al., 2008
	L-aspartic acid	+	+	TP	Strazzullo et al., 2008
	L-tyrosine	+	-	FN	Strazzullo et al., 2008
	L-proline	+	+	TP	Strazzullo et al., 2008
Both carbon and nitrogen sources, supplemented with NH4	L-glutamine	+	+	TP	Strazzullo et al., 2008
	Amino-butyric acid	+	+	TP	Strazzullo et al., 2008
	L-asparagine	+	+	TP	Strazzullo et al., 2008
	L-arginine	-	-	TN	Strazzullo et al., 2008
	L-glutamic acid	+	+	TP	Strazzullo et al., 2008
	L-aspartic acid	+	+	TP	Strazzullo et al., 2008
	L-tyrosine	+	-	FN	Strazzullo et al., 2008
Glucose as a carbon source	L-proline	+	+	TP	Strazzullo et al., 2008
	Ammonium	-	+	FP	This work.

Table S9: Gene essentiality analysis statistics.

Category	Essential	Non-essential	Non-lethal with reduced growth
Amino acid metabolism	64	128	9
Metabolism of cofactors and vitamins	44	87	12
Cell envelope biosynthesis	24	25	0
Nucleotide metabolism	14	26	6
Other	12	86	2
Carbohydrate metabolism	6	105	24
Membrane transport	5	108	4
Biosynthesis of secondary metabolites	4	8	0
Lipid metabolism	4	25	0
Energy metabolism	0	28	28
Exchange	0	0	0
Total	177	626	85

Table S10: Exchange shadow prices in different fermentation phases.

Shadow price	Condition A: Glutamate medium		Condition B: Glutamate and NH ₄ medium	
	Phase I	Phase II	Phase I	Phase II
Glucose	0.0971	0	0.0971	0
Glutamate	0.0671	NA	0.0971	NA
Amonium	NA	0.1174	0	0.1174

Table S11: Nitrogen source ranking. Highlighted rows correspond to tested experimental conditions.

N source 1	N source 2	N source 3	Yield (g DCW/mmol AA)
EX_arg_L.e			0.4910
EX_arg_L.e	EX_gly_e	EX_trp_L.e	0.3493
EX_arg_L.e	EX_lys_L.e	EX_gln_L.e	0.3413
EX_arg_L.e	EX_glu_L.e	EX_phe_L.e	0.3398
EX_arg_L.e	EX_asp_L.e	EX_cys_L.e	0.3398
EX_nh4_e	EX_arg_L.e	EX_ser_L.e	0.3257
EX_arg_L.e	EX_ser_L.e	EX_gln_L.e	0.3108
EX_arg_L.e	EX_his_L.e	EX_asn_L.e	0.2718
EX_arg_L.e	EX_asn_L.e		0.2691
EX_arg_L.e	EX_met_L.e	EX_gln_L.e	0.2665
EX_asn_L.e			0.2455
EX_arg_L.e	EX_asn_L.e	EX_gln_L.e	0.2455
EX_gln_L.e			0.2455
EX_his_L.e	EX_pro_L.e	EX_asn_L.e	0.2219
EX_glu_L.e	EX_met_L.e	EX_asn_L.e	0.2183
EX_lys_L.e	EX_ser_L.e	EX_gln_L.e	0.2179
EX_his_L.e	EX_ser_L.e	EX_asn_L.e	0.2176
EX_glu_L.e	EX_ile_L.e	EX_asn_L.e	0.2169
EX_cys_L.e	EX_gly_e	EX_asn_L.e	0.2159
EX_gly_e	EX_his_L.e	EX_asn_L.e	0.2159
EX_arg_L.e	EX_his_L.e	EX_val_L.e	0.1931
EX_gly_e	EX_pro_L.e	EX_gln_L.e	0.1916
EX_gly_e	EX_val_L.e	EX_gln_L.e	0.1654
EX_nh4_e	EX_cys_L.e	EX_asn_L.e	0.1654
EX_nh4_e	EX_gln_L.e		0.1654
EX_nh4_e	EX_phe_L.e	EX_asn_L.e	0.1654
EX_asp_L.e	EX_pro_L.e	EX_gln_L.e	0.1554
EX_nh4_e	EX_tyr_L.e	EX_gln_L.e	0.1554
EX_asp_L.e	EX_ser_L.e	EX_gln_L.e	0.1554
EX_gly_e	EX_met_L.e	EX_gln_L.e	0.1501
EX_arg_L.e	EX_asp_L.e	EX_his_L.e	0.1444
EX_asp_L.e	EX_his_L.e	EX_gln_L.e	0.1416
EX_nh4_e	EX_his_L.e	EX_gln_L.e	0.1416
EX_arg_L.e	EX_glu_L.e	EX_leu_L.e	0.1414
EX_arg_L.e	EX_glu_L.e	EX_val_L.e	0.1414
EX_arg_L.e	EX_pro_L.e	EX_ser_L.e	0.1334
EX_nh4_e	EX_arg_L.e		0.1334
EX_arg_L.e	EX_ser_L.e		0.1334
EX_nh4_e	EX_his_L.e	EX_pro_L.e	0.1250
EX_his_L.e	EX_pro_L.e	EX_ser_L.e	0.1250
EX_gly_e	EX_his_L.e	EX_pro_L.e	0.1250
EX_asp_L.e	EX_his_L.e	EX_pro_L.e	0.1250
EX_asp_L.e	EX_his_L.e	EX_ser_L.e	0.1250
EX_nh4_e	EX_glu_L.e	EX_lys_L.e	0.1238
EX_asp_L.e	EX_gly_e	EX_lys_L.e	0.1238
EX_pro_L.e	EX_ser_L.e	EX_trp_L.e	0.1234
EX_nh4_e	EX_glu_L.e	EX_phe_L.e	0.1227
EX_asp_L.e	EX_pro_L.e	EX_asn_L.e	0.1227
EX_glu_L.e	EX_ser_L.e	EX_val_L.e	0.1227
EX_gly_e	EX_pro_L.e	EX_ser_L.e	0.1227
EX_asp_L.e			0.1227
EX_asp_L.e	EX_glu_L.e	EX_ser_L.e	0.1227
EX_ser_L.e	EX_val_L.e		0.1227
EX_nh4_e			0.1227
EX_nh4_e	EX_pro_L.e	EX_asn_L.e	0.1227
EX_gly_e			0.1227
EX_asp_L.e	EX_ser_L.e	EX_val_L.e	0.1227
EX_nh4_e	EX_asp_L.e	EX_val_L.e	0.1227
EX_ser_L.e			0.1227

EX_nh4_e	EX_gly_e	EX_pro__L_e	0.1227
EX_asp__L_e	EX_pro__L_e	EX_val__L_e	0.1227
EX_glu__L_e	EX_gly_e		0.1227
EX_val__L_e			0.1227
EX_pro__L_e			0.1227
EX_glu__L_e			0.1227
EX_asp__L_e	EX_cys__L_e	EX_pro__L_e	0.1227

Table S12: Biomass yield on various nitrogen sources using glucose as carbon source.

Nitrogen source	Nitrogen content (mol N/mol N source)	Biomass yield (g DCW/mmol N source)	Biomass yield (g DCW/mmol N)
NH ₄	1	0.1227	0.1227
Arginine	4	0.491	0.1227
Aspartate	1	0.1227	0.1227
Asparagine	2	0.2455	0.1227
Glutamate	1	0.1227	0.1227
Glutamine	2	0.2455	0.1227
Glycine	1	0.1227	0.1227
Proline	1	0.1227	0.1227
Serine	1	0.1227	0.1227

Table S13: Effect of monomer number on PHB composition, molecular weight and maximum theoretical yields calculated with HaloGEM using glucose as carbon source.

Number of monomers	PHB composition	Molecular weight (kDa)	Max. PHB monomer yield (mol/mol)	Max. PHB monomer yield (g/g)
4	$\text{C}_{16}\text{H}_{24}\text{O}_8$	0.344	$3.352 \cdot 10^{-1}$	0.6406
40	$(\text{C}_{16}\text{H}_{24}\text{O}_8)_{10}$	3.44	$3.352 \cdot 10^{-2}$	0.6406
400	$(\text{C}_{16}\text{H}_{24}\text{O}_8)_{100}$	34.4	$3.352 \cdot 10^{-3}$	0.6406
4000	$(\text{C}_{16}\text{H}_{24}\text{O}_8)_{1000}$	344	$3.352 \cdot 10^{-4}$	0.6406
40000	$(\text{C}_{16}\text{H}_{24}\text{O}_8)_{10000}$	3440	$3.352 \cdot 10^{-5}$	0.6406

Table S14: Nitrogen to glucose consumption ratios for different nitrogen sources under optimal growth and minimum glucose uptake.

Nitrogen source(s)	Optimal ratio of nitrogen to glucose uptake (mol/mol)
Arginine	0.885
Arginine and glutamate	1.514
Arginine and asparagine	0.890
Asparagine	0.886
Arginine and glutamine	1.108
Glutamine	1.007
Glutamate	1.514
Glutamate and ammonium	1.514

Table S15: Reported fermentation products in *Halomonas*

Species	Strain	Fermentation products	Reference
<i>H. campaniensis</i>	LC9	cadaverine	Zhao et al., 2022
<i>H. bluephagenesis</i>	TD01	acetate, D-lactate, formate, ethanol	Ren et al., 2018
<i>H. bluephagenesis</i>	TD01	ectoine	Ma et al., 2020
<i>H. bluephagenesis</i>	TD01	L-threonine	Du et al., 2020
<i>H. bluephagenesis</i>	TD01	L-lysine	Zhao et al., 2022
<i>H. boliviensis</i>	DSM 15516	gluconic acid	Bondar et al., 2022
<i>Halomonas sp.</i>	KM1	oxaloacetate, pyruvate	Hannya et al., 2017

Table S16: Fermentation products that cannot be produced by HaloGEM under the studied conditions.

O-Acetyl-L-serine	L-Cysteine	L-Glutamate	lysine	L-Prolinylglycine
3- Methyl butanoic acid	Deoxyguanosine	Glutarate	D-Malate	Pentanoate
4-aminobutanoate	Dihydroxyacetone	Glycine	L-Malate	D-Ribose
Adenine	dIMP	Glycine betaine	Malonate	L-Serine
Adipic acid	Deoxyinosin	Reduced glutathione	Maltose	Succinate
2-Oxoglutarate	Ectoine	GTP	Maltohexaose	D-Mannose 6-phosphate
D-Alanyl-D-alanine	Ethanolamine	Hydrogen sulfide	Maltopentaose	L-Methionine
Allantoin	Formaldehyde	Hexadecanoic acid	Maltotriose	Nitric oxide
N-Acetyl-D-glucosamine N-Acetylmuramic acid	Fe(III)dicitrate	Hexadecanoic-acid (n-C16:1)	Maltotetraose	Nitrite
D-arabinose	Fe(III)hydroxamate	5-hydroxyectoine	myo-Inositol	Nitrate
aerobactin minus Fe3	Ferrichrome	L-Histidine	KDO(2)-lipid IV A	Sucrose
Aerobactin	Glycerophosphoglycerol	L-Homoserine	L-Lactate	Thymine
L-Arginine	sn-Glycero-3-phospho-1-inositol	Hexanoate (n-C6:0)	L-alanine-D-glutamate	Trehalose
L-Asparagine	Glycerophosphoserine	Glycerol	6-diaminoheptanedioate	L- Tryptophan
L-Aspartate	D-galactose	Glycerol 2-phosphate	D-Mannose	tetradecanoate (n-C14:0)
Biotin	D-Galactonate	Glycerol 3-phosphate	Superoxide anion	tetradecenoate (n-C14:1)
Butyrate	D-Glucosamine 6-phosphate	GMP	octadecanoate (n-C18:0)	L-Tyrosine
D-cellobiose	ferroxamine	Guanosine	octadecenoate	Uracil
Decanoate	D-Fructose	Isobutyrate	octanoate (n-C8:0)	L-Valine
Dodecanoate (n-C12:0)	Fumarate	L-Isoleucine	Orotate	Xanthine
dGMP	sn-Glycero-3-phosphocholine	IMP	L-Phenylalanine	Xanthosine 5-phosphate
Cys-Gly	sn-Glycero-3-phosphoethanolamine	Indole	Propionate	Xanthosine
Choline	GDP	L-alanine-D-glutamate	Propanal	D-xylose
coprogen	D-Glucarate	6-diaminoheptanedioate-D-alanine	Phosphonate	
Cytosine	L-Glutamine	L-Leucine	L-Proline	

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