

Growth-limiting intracellular metabolites in yeast growing under diverse nutrient limitations – *Supporting Information*

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Supplementary Tables 1 – 3 are provided separately as tab-limited text files

Legend for Supplementary Figures, Tables, and Datasets

Figure S1. Comparison of metabolite yields as a function of the cell harvesting method and composition of the extraction solvent. Two different cell harvesting methods were compared: “Methanol quenching” refers to quenching cells in methanol and then isolating the quenched cells by centrifugation; “Filter” refers to isolating the cells directly by vacuum filtration (see Methods for details). Similarly, two different extraction solvents were compared: “MeOH” refers to 80:20 methanol:water; “ACN” refers to 40:40:20 acetonitrile:methanol:water. For most compounds, yields do not depend substantially on the harvesting method or extraction solvent composition (as exemplified by results for glutamate). For nucleotides and their derivatives, however, the Filter ACN approach dramatically improves yields (as exemplified here by results for ATP). Bars are mean \pm standard error of N = 3 independent cultures. For metabolome-wide data, see Supplementary Table S2.

Figure S2. Variant of Figure 1: Clustered heat map of yeast metabolome variation as a function of growth rate and identity of the limiting nutrient. This figure differs in terms of using a blue-yellow instead of green-red color scheme. In addition, it includes results broken down separately for samples collected by methanol quenching versus vacuum filtration (see Methods and Figure S1 for details). Note that the biological results were the same for both sampling

methods.

Figure S3. Double-reciprocal plot of dilution rate (equivalent to specific growth rate at steady state) as a function of the residual substrate concentration. Growth rate increased monotonically with dilution rate. Most of the results, except those at the slowest dilution rate (top right points), fit well to a Michaelis-Menten relationship between growth rate and substrate concentration, allowing for the calculation of μ_{\max} and K_m . The values at dilution rates of 0.05 h^{-1} are included in the figure, but were not included in the calculation.

Figure S4. Variant of Figure 4: Growth-limiting and overflow metabolites, in this case for the auxotrophic limitations. Scatter plots show growth rate slope versus nutrient mean effect. Compounds plotted are those showing a statistically significant effect ($\text{FDR} < 0.1$) on both dimensions. Within each scatter plot, potentially growth-limiting metabolites fall in the upper left quadrant, and overflow metabolites in the lower right quadrant. (A) Leucine limitation. (B) Uracil limitation.

Figure S5. Variant of Figure 4: Growth-limiting and overflow metabolites, identified here using data from the natural limitations only (i.e., excluding data from uracil and leucine limitation). (A) Nitrogen limitation. (B) Phosphorus limitation. (C) Carbon (glucose) limitation.

Figure S6. Specific glucose consumption and ethanol production rates as a function of dilution rate (equivalent to specific growth rate at steady state) in glucose-limited chemostats.

Figure S7. A. Culture characteristics in nutrient-limited chemostats. Within each limitation, dilution rates varied from left to right from approximately 0.05 to 0.30 h^{-1} . Top panel, cell number; second panel, mean cell volume (mean \pm SD of $N = 4$ independent measurements); third panel, calculated total cellular volume (number of cells times mean cell volume); bottom panel, klett. B. Correlation between klett and culture dry weight. Data points were taken from three different nutrient-limited conditions (nitrogen, phosphorus, and uracil) at different growth rates. Dry weight was determined according to Postma, E., Verduyn, C., Scheffers, W. A., and van Dijken, J. P. (1989) *Appl. Environ. Microbiol.* 55, 468-477.

Supplementary Table S1. Metabolites analyzed and their associated mass spectrometry parameters. In the Excel workbook, the tab "Compounds_included" provides ionization mode, parent ion mass, product ion mass, collision energy, and chromatography retention time for all compounds that were successfully measured. Compounds that were measured unsuccessfully (e.g., did not yield acceptable signal-to-noise from biological samples) are listed separately under the tab "Compounds_excluded."

Supplementary Table S2. Comparison of metabolite yields as a function of the cell harvesting method and composition of the extraction solvent. A two-by-two comparison of cell harvesting method (“MeOH quench” versus “filters”) and extraction solvent composition (“MeOH” versus “ACN”) was performed, using yeast growing exponentially in glucose minimal media (N = 3 independent cultures for each condition). In the Excel workbook, raw data (ion counts) are provided in the first tab, and specific head-to-head comparisons in the subsequent tabs (e.g., the second tab, entitled “Filters-Extract MeOH vs ACN” is a head-to-head comparison, for cells collected by the filtration approach, of the two extraction solvent compositions). The term “MeOH quench” refers to quenching cells in methanol and then isolating the quenched cells by centrifugation; the term “filters” refers to isolating the cells directly by vacuum filtration. The solvent composition “MeOH” refers to 80:20 methanol:water; “ACN” refers to 40:40:20 acetonitrile:methanol:water.

Supplementary Table S3. Chemostat properties: dilution rate, klett, cell count, cell volume, and extracellular nutrient concentrations.

Supplementary Table S4. Media composition.

Dataset 1. Relative metabolite concentration changes across different nutrient limitations and growth rates, expressed as mean-centered \log_2 ratios. Rows are the measured metabolites and columns are the experimental conditions (nutrient limitation and growth rate). The data correspond to that shown in Figure 1.

Dataset 2. Normalized ion counts for each metabolite in every sample. Rows are samples and columns are the measured metabolites. Values of less than 300 normalized ion counts have been set to a floor of 300. To obtain relative concentrations, divide the normalized ion counts in the experimental sample by that in the contemporaneous reference sample.

Growth-limiting intracellular metabolites in yeast under diverse nutrient limitations – *Supplementary Methods*

1. Relationship of Equation 2 to Saturable Growth Function

Assume that growth rate is a saturable function of the concentration of a limiting intracellular metabolite:

$$\mu = \mu_{\max} * [M]^h / ((K_M)^h + [M]^h) \quad (\text{Eqn S1})$$

Within the regime where the metabolite is strongly growth limiting, $[M] < K_M$ and growth rate can be approximated by

$$\mu \approx \mu_{\max} * [M]^h / (K_M)^h \quad (\text{Eqn S2})$$

If the concentration of M is not strongly growth limiting, over any modest range of growth rates, the functional form of Eqn S2 is nevertheless adequate to approximate Eqn S1, although the exponent would need to change (e.g., the value of h would decrease to account for the decreased sensitivity of growth rate to changes in metabolite concentration).

Rearranging terms,

$$[M]^h = \mu / \mu_{\max} (K_M)^h \quad (\text{Eqn S3})$$

Dividing both sides by $\mu_0 [M]_0^h$, where μ_0 is the geometric mean growth rate and $[M]_0$ the geometric mean metabolite concentration, gives

$$([M]/[M]_0)^h / \mu_0 = (\mu/\mu_0) (1/\mu_{\max}) (K_M / [M]_0)^h \quad (\text{Eqn S4})$$

Moving μ_0 to the right hand side and then taking the logarithm gives

$$\log([M]/[M]_0)^h = \log(\mu/\mu_0) + \log((\mu_0 / \mu_{\max}) (K_M / [M]_0)^h) \quad (\text{Eqn S5})$$

which is identical to Eqn 2 of the main text,

$$\log([M]_{n,\mu}/[M]_0) = m_n \log(\mu/\mu_0) + b_n \quad (\text{Eqn 2})$$

with $m_n = h^{-1}$ and $b_n = h^{-1} \log((\mu_0 / \mu_{\max}) (K_M / [M]_0)^h)$.

2. Quantitative Expectations for Overflow Metabolites Based on Feedback Inhibition

Consider an end product P which feedback inhibits its own biosynthesis. A typical Michaelis-Menten type expression for production of P (F_{in}) is

$$F_{in} = V_0 (1 + ([P]/K_p)^h)^{-1} \quad (\text{Eqn S6})$$

where V_0 is the rate production of P in the absence of feedback inhibitor.

During balanced growth, consumption of P (F_{out}) is proportional to growth rate (μ):

$$F_{out} = \alpha \mu \quad (\text{Eqn S7})$$

When growth slows, P accumulates until F_{in} and F_{out} are balanced. The concentration of P at steady-state is

$$[P]_{ss} = K_p (V_0 / (\alpha \mu) - 1)^{1/h} \quad (\text{Eqn S8})$$

Note that P_{ss} varies inversely with μ but is independent of the nature of the limiting nutrient (unless that limitation impacts V_0). For auxotrophic limitations, this leads to the key conclusions that all metabolic end products unrelated to the auxotrophy are expected to accumulate, and that the concentrations of these end products should be a function of growth rate but independent of the nature of the auxotrophy. (Note that these expectations, based on feedback inhibition being the predominant means of metabolic regulation, were not borne out experimentally).

When production of P is substantially feedback inhibited (i.e., $V_0 > \alpha \mu$), then Eqn S8 can be approximated by

$$[P]_{ss} = K_p V_0^{1/h} \alpha^{-1/h} \mu^{-1/h} \quad (\text{Eqn S9})$$

After dividing both sides by $\mu_0^{-1/h} [P]_0$ and taking the logarithm, one gets

$$\log ([P]_{ss}/[P]_0) = -h^{-1} \log(\mu/\mu_0) + \log ((V_0 / (\mu_0 \alpha))^{1/h} (K_p / [P]_0))$$

Note that this again matches the form of Eqn 2 of the main text, in this case with $m_n = -h^{-1}$ and $b_n = \log ((V_0 / (\mu_0 \alpha))^{1/h} (K_p / [P]_0))$.

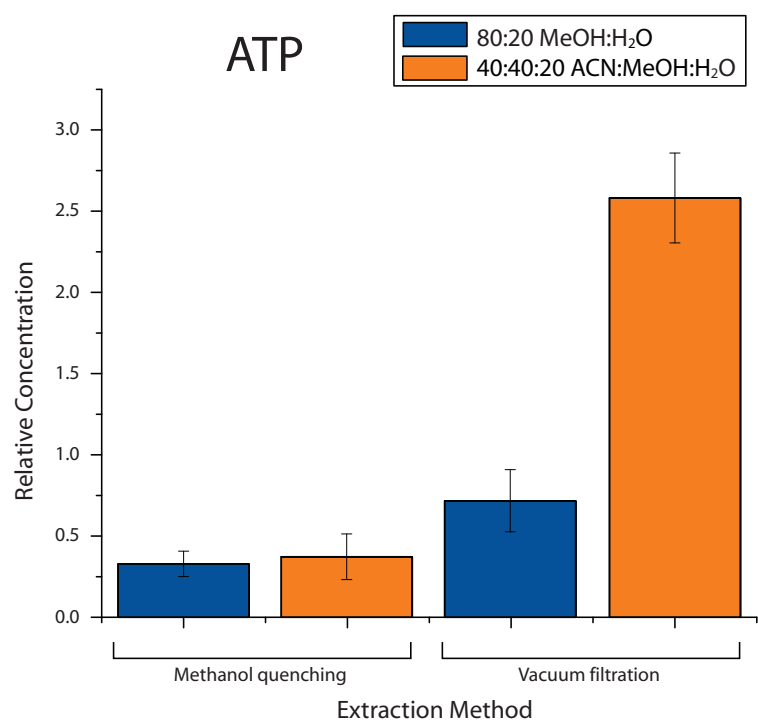
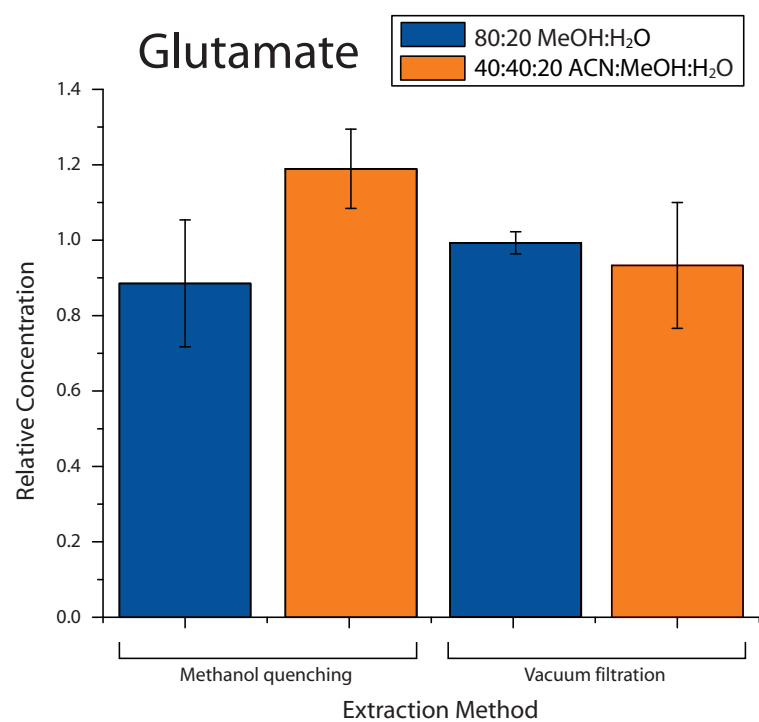


Figure S1

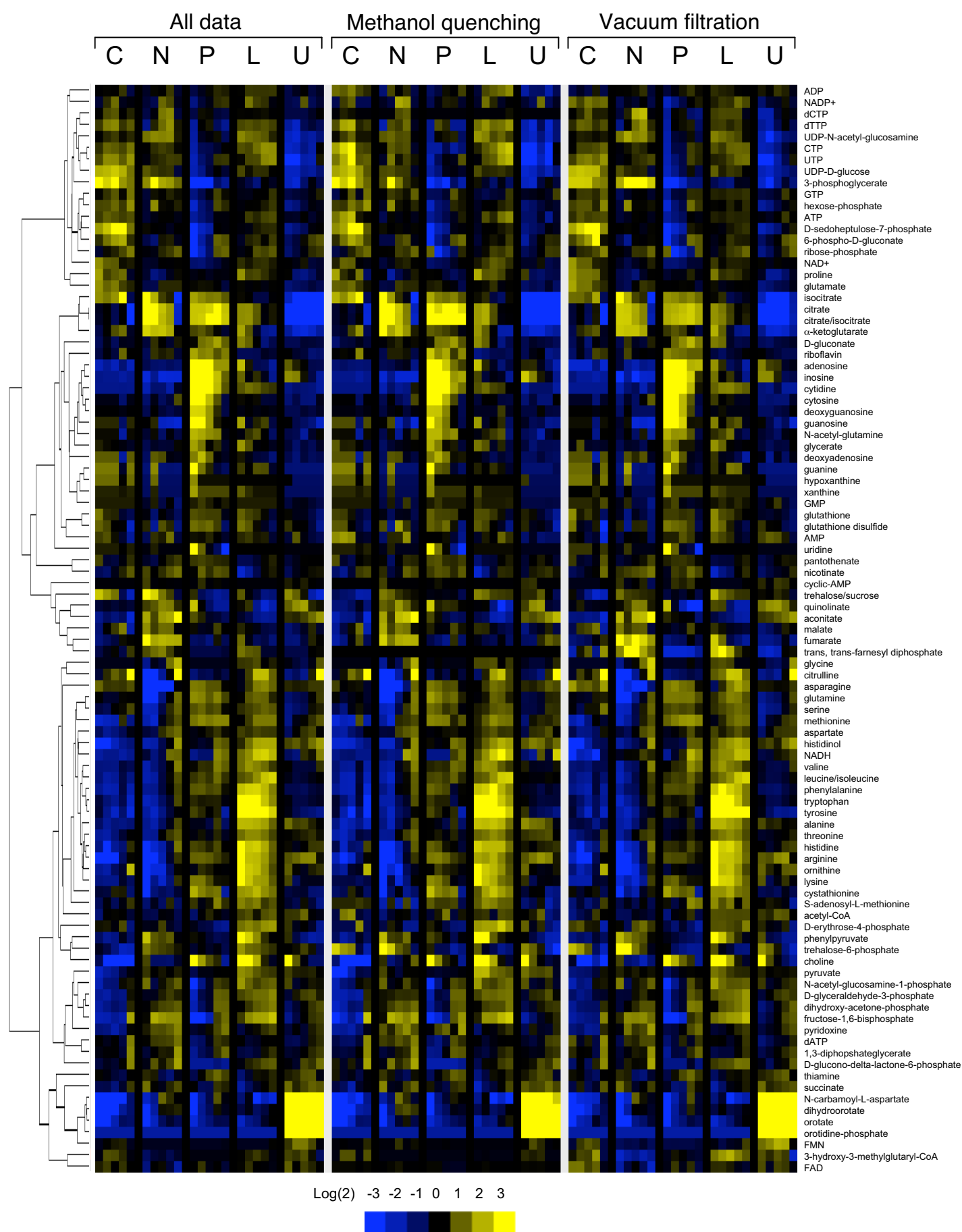


Figure S2

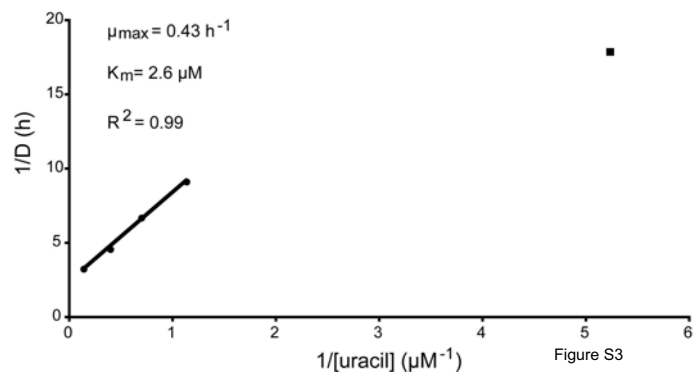
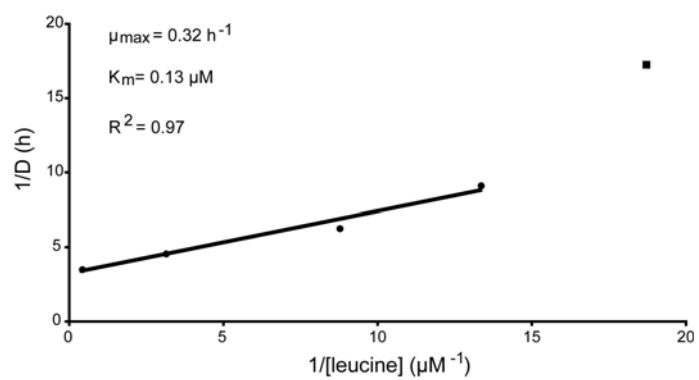
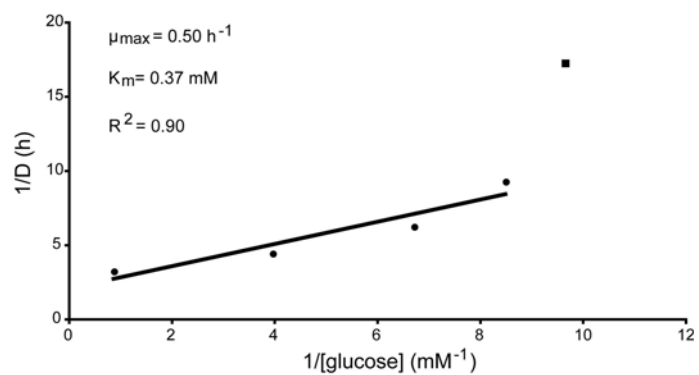
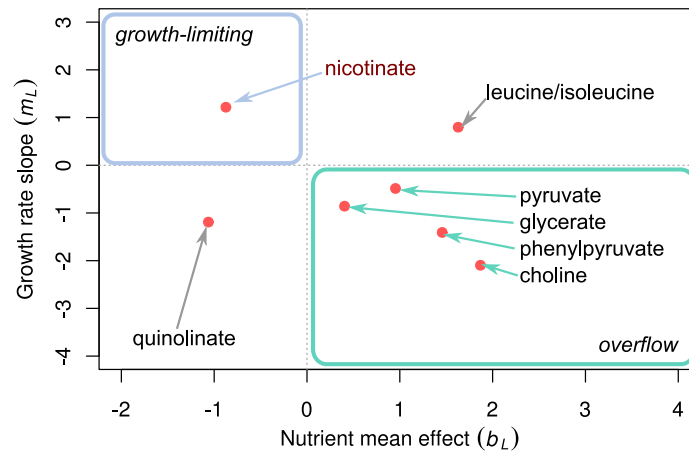


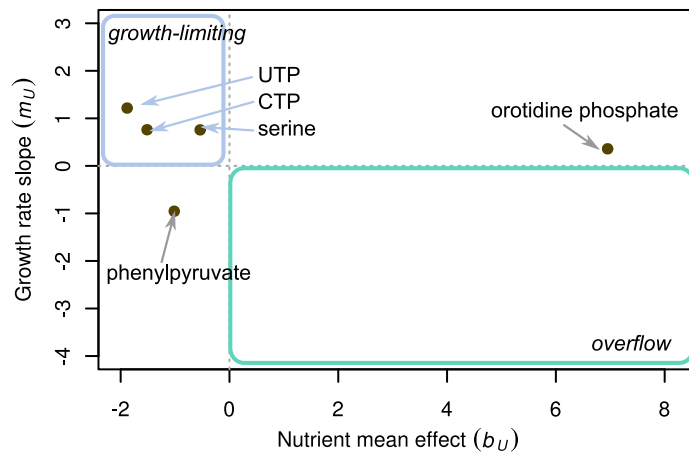
Figure S3

A Leucine limitation

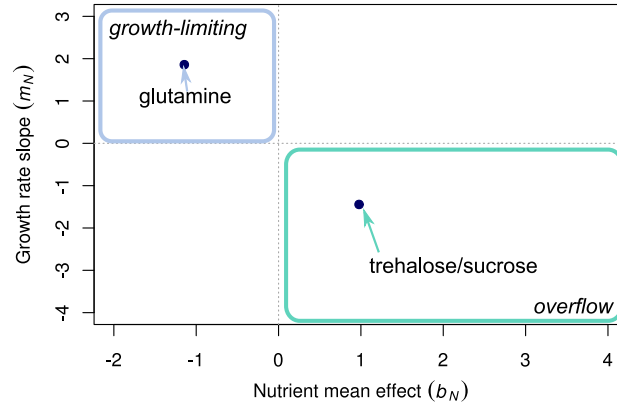
Figure S4



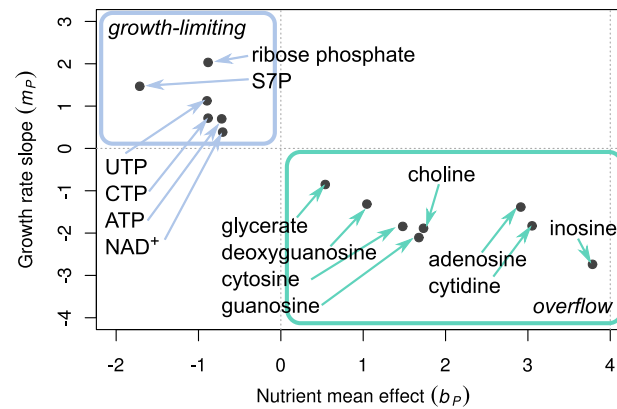
B Uracil limitation



A Nitrogen limitation



B Phosphorus limitation



C Carbon limitation

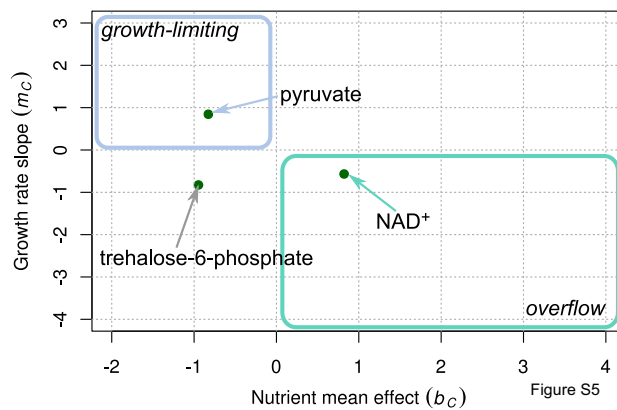


Figure S5

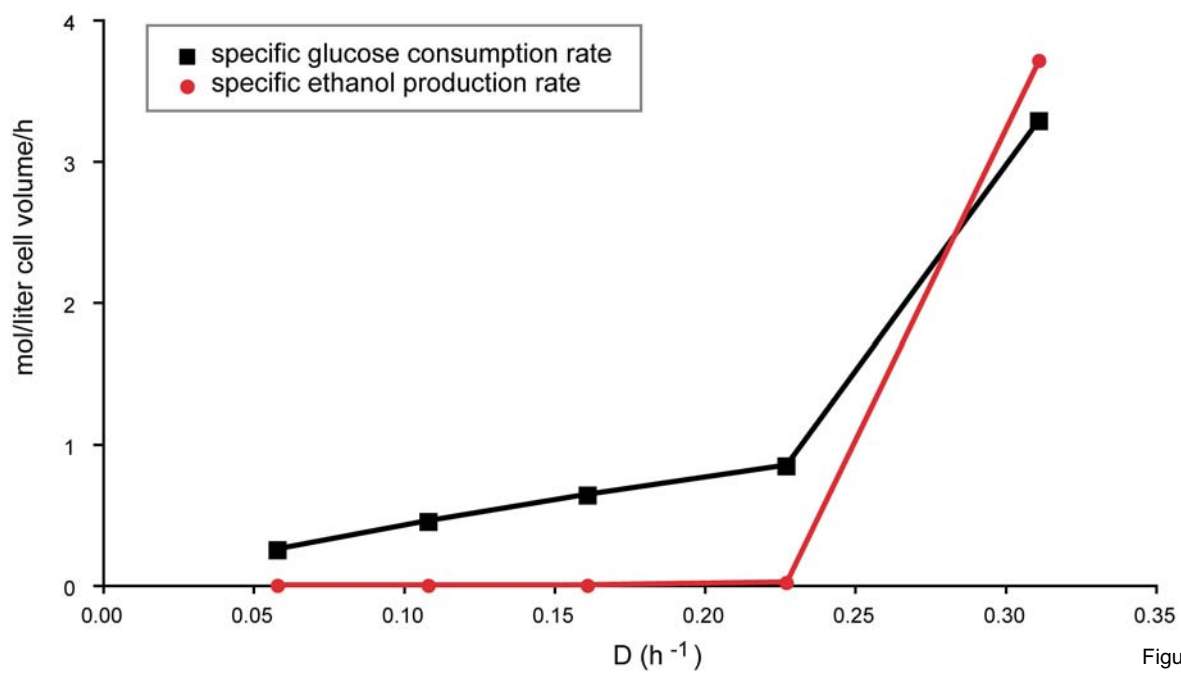


Figure S6

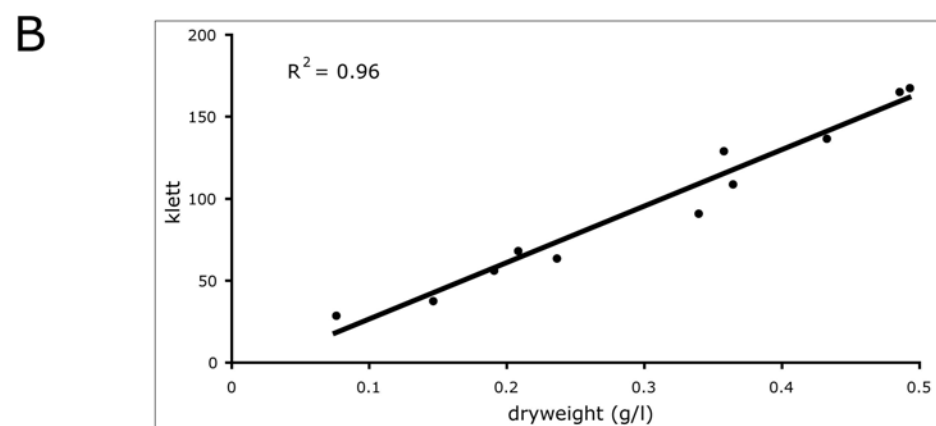
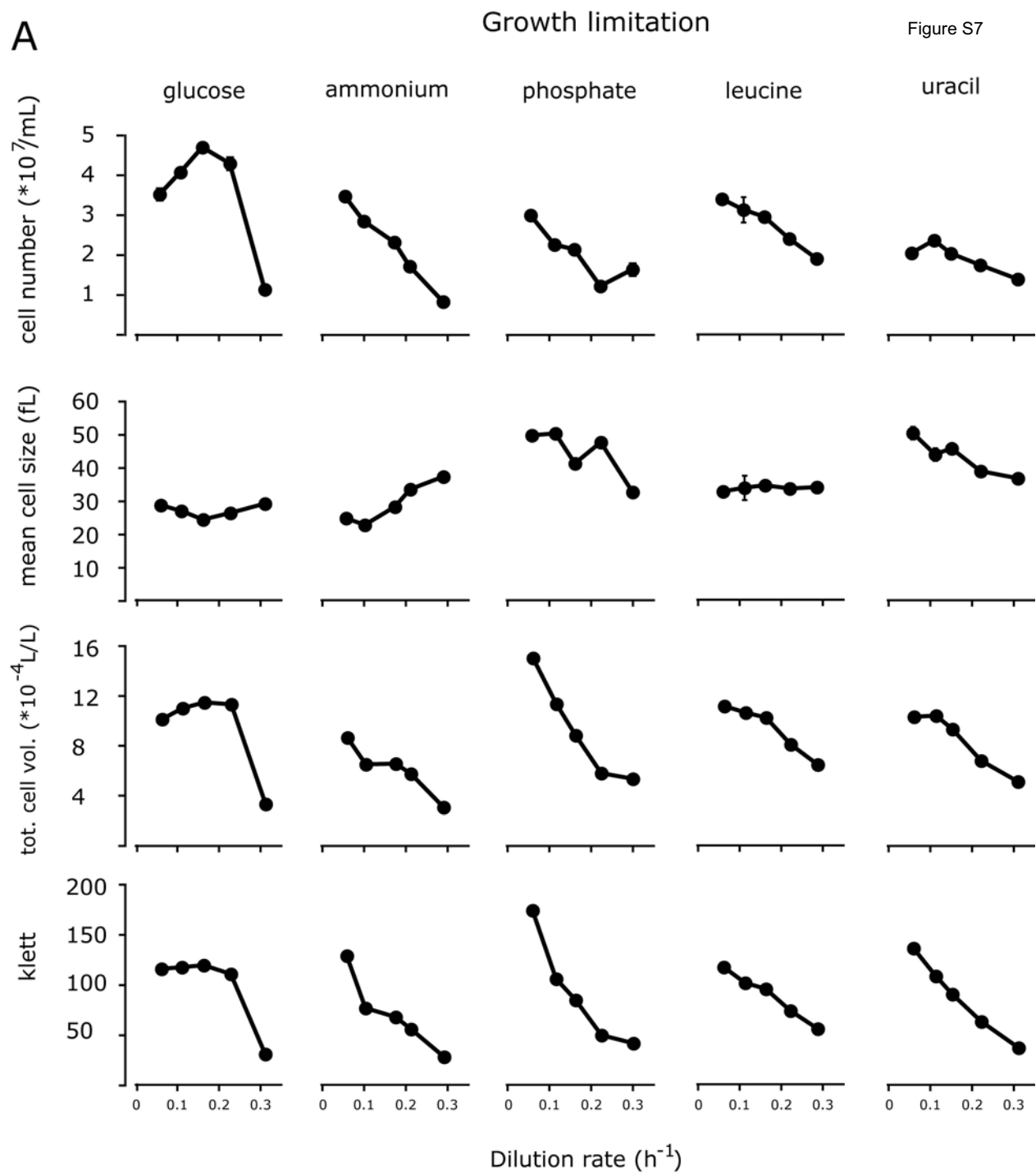


Table S4. Media composition (in grams per liter)

Compound	Limiting nutrient				
	Ammonium	Phosphate	Glucose	Leucine	Uracil
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.10	0.10	0.10	0.10	0.10
NaCl	0.10	0.10	0.10	0.10	0.10
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.50	0.50	0.50	0.50	0.50
KH_2PO_4	1.0	0.010	1.0	1.0	1.0
KCl		1.0			
$(\text{NH}_4)_2\text{SO}_4$	0.050	5.0	5.0	5.0	5.0
Glucose	21	22	0.8	22	22
Leucine				0.011	
Uracil					0.0026
Vitamins and minerals	As described previously (Saldanha et al, 2004)				