

Recycled Medium Meta-analysis Data Extraction Protocol

Cross-study analysis of factors affecting algae cultivation in recycled medium for biofuel production

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Terms

Recycled medium: growth medium (or, water) that has previously been used to grow microalgae and is being used again to grow microalgae, either in continuous or batch cultures. May or may not have nutrients added back or be diluted with fresh medium.

Fresh medium: growth medium that has not been used previously to grow microalgae, and has the full nutrient concentrations as specified in the recipe used.

Treatment: An experimental test in which microalgae are grown in recycled medium.

Control: An experimental test in which microalgae are grown in fresh medium, for comparison to a treatment.

Source: resource from which experimental data were extracted. These are publications by majority, but include gray literature such as theses and conference presentations. In the context of recycled medium itself, “source” can also refer to the algae culture from which recycled medium was created by removing the algae.

Criteria for Relevant Sources

- 1) There is an experimental treatment in which microalgae are grown in recycled medium
- 2) The source reports data for microalgae grown in both fresh and recycled media
- 3) The algae strains are not strains associated with harmful algal blooms, and are not *Prochlorococcus* (both of which are not candidates for biofuel production)

Construct & Operational Definitions

Effects sizes are based on the construct of growth. Growth is defined here as:

- 1) specific growth rate in exponential phase with units day^{-1} (for batch cultures, and for semicontinuous cultures that have full growth curves);
- 2) biomass productivity with units g/L/day (for continuous cultures, and for semicontinuous cultures effectively operated as continuous cultures); and
- 3) biomass concentration with variable units, such as g/L or cells/mL (for all cultures).

Further details on these variables are described in the subsequent section “Definitions of Variables/Study Descriptors.” These growth definitions represent three different response variables and can be measured by cell concentrations (microscope, Coulter counter), optical density, or dry weight.

Guidelines for Data Collection

In addition to the following variables that were recorded/extracted from each source, the following were recorded:

- How the source was found (Table S1 in Supp Info A: Supplementary Tables and Figures).
- How the data were extracted from the source; where the data came from within a source, e.g., text, figure number, table number; which treatments were chosen and which were not, following the same inclusion criteria outlined above for relevant sources (not published).

- Raw data directly extracted from sources via Webplot Digitizer (Rohatgi), and any calculations done with that data (not published). Final response variable data and effect sizes are available in Supp Info C: Recycled Medium Database.

Definition of Variables/Study Descriptors

The following variables/study descriptors (in **bold**) were recorded for each study according to the definitions/protocols described below. These variables refer to those in the Recycled Medium Database (provided as Supplementary Information C). Variables referred to within definitions are in *italics*.

source_num

Number assigned to the source based on the chronological order in which data were extracted.

year

Year the source was published.

journal

Journal in which the source was found. For theses, use the university name. For conferences, use the conference name.

eco_ind

“eco” if source’s main purpose was to answer ecological research questions (i.e., to understand algal ecology)

“ind” if source was mainly for an industrial or biotechnological purpose (i.e., application of algae cultivation for bioproducts)

treatment_num

Number assigned to each treatment within a source. Relevant treatments are numbered starting from 1 and incremented by 1 up to the total number of relevant treatments per study. Include all relevant treatments (even if they have the same experimental conditions or minor changes in conditions). Each medium reuse in batch or semicontinuous cultures counts as a separate treatment because the *times_reused* variable has changed (this does not apply for continuous cultures, or semicontinuous cultures effectively treated as continuous cultures, if growth measurements are averaged together over the course of the experiment).

harvest

Method by which microalgae were removed from the source culture to create recycled medium. One of the following, or a combination of the following connected by “_”.

- “filtration” (any pore size)
- “centrifugation”
- “activated_carbon_filtration”
- “flocculation” (use of a chemical, bioflocculant, electricity, or other mechanism to either settle or float the algae cells for removal)
- “dialysis”
- “unknown” if source does not report how microalgae were removed from the growth medium

sterilization

Method of sterilization of recycled medium.

- “0” if source does not report sterilizing the recycled medium
- “autoclave”
- “ozonation”
- “filtration”, only if 0.2 μm or smaller pore size; also include filtration as a harvesting method if 0.2 μm filter was used
- “unknown” if source reports sterilizing the recycled medium, but does not specify how

nuts_measured

“1” if nitrogen (furthermore “N”) and phosphate (furthermore “P”) nutrients were measured in the recycled medium

“0” if N and P were not measured in the recycled medium. If a source does not state whether nutrients were measured, assume nutrients were not measured.

N_added

“1” if N was resupplied to the recycled medium

“0” if not resupplied. If source does not specify whether N was resupplied, assume it was not.

P_added

“1” if P was resupplied to the recycled medium

“0” if not resupplied. If source does not specify whether P was resupplied, assume it was not.

other_nuts_added

“1” if other nutrients were resupplied that were originally in the fresh medium, such as trace metals, vitamins, and silicate

“0” if not resupplied. If source does not specify whether other nutrients were resupplied, assume they were not.

fraction_fresh

Continuous variable between 0 and 1 (1 would mean completely fresh medium). Percentage of either clean water (i.e., not previously used to grow microalgae; does not contain added nutrients) or fresh medium added to the treatment's recycled medium, based on volume. If source restored volume with water from evaporative losses or sampling, but does not state volume or percentage, assume 0.05 as *fraction_fresh*. Inoculum was not counted as fresh medium.

control_N

Initial μM concentration of N in the control. If the source only provides the common name of the growth medium (e.g., BG-11, f/2, Bold's Basal Medium), the concentration from the medium's recipe was used (Table B1).

“unknown” if not reported.

Table B1: N and P concentrations in common algae growth media. This table was used for all sources that named a common growth medium but did not report the concentration.

Growth medium	N source(s)	g/L N source(s)	μM N	P source(s)	g/L P source(s)	μM PO ₄	Reference
BG-11, Modified	NaNO ₃	1.5	17648	K ₂ HPO ₄ 3H ₂ O	0.04	175	Algae Culturing Techniques (Andersen 2005)
Bold's Basal	NaNO ₃	0.25	2941	K ₂ HPO ₄ KH ₂ PO ₄	0.075 0.175	431 + 1286 = 1717	Algae Culturing Techniques (Andersen 2005)
F/2	NaNO ₃	0.075	882	NaH ₂ PO ₄ H ₂ O	0.005	36.2	Algae Culturing Techniques (Andersen 2005)
LC Oligo	Ca(NO ₃) ₂ 4H ₂ O KNO ₃	0.04 0.1	340 + 1000 = 1340	K ₂ HPO ₄	0.04	230	(Chia et al. 2013)
WC Medium	NaNO ₃	0.08501	1000	K ₂ HPO ₄	0.00871	50	Algae Culturing Techniques (Andersen 2005)
Zarrouk's	NaNO ₃	2.5	29414	K ₂ HPO ₄	0.5	2870	(Tarko et al. 2012)

exp_N

Initial μM concentration of N in the recycled medium, if known exactly.

If *nuts_measured* = "1", and source reported that N was resupplied back to the original concentration, record the same value as *control_N*.

"lower" if *N_added* = "0", and no concentration in the recycled medium was given.

"higher" if *N_added* = "1", and no concentration in the recycled medium was given. It was assumed that less than 100% of the initial N concentration was consumed in the source culture used to make the recycled medium.

exp_N_group

Categorical variable of N nutrient addition.

"higher" if *exp_N* = higher, or if *exp_N* is a value that is at least 20% greater than *control_N*.

"lower" if *exp_N* = lower, or if *exp_N* is a value that is at least 20% lower than *control_N*.

"same" if *exp_N* is a value within 20% of the *control_N* value.

N_source

Nutrient added containing N, such as one of the following N sources. If multiple N sources were added, they are both listed and separated by "_". The molecular weights used for conversion to μM are listed below.

- NaNO₃ (84.9947 g/mol)
- urea (60.06 g/mol; 2 mol N per 1 mol urea)
- NH₄Cl (53.491 g/mol)
- KNO₃ (101.1032 g/mol)
- Ca(NO₃)₂ 4H₂O (236.1489 g/mol)
- Ca(NO₃)₂ (164.0878 g/mol)
- NH₄HCO₃ (79.0553 g/mol)

"unknown" if N nutrient source was not reported.

control_P

Initial μM concentration of P in the control. If the source only provides the common name of the growth medium (e.g., BG-11, f/2, Bold's Basal Medium), the concentration in the medium's recipe was used (Table B1).
"unknown" if not reported.

exp_P

Initial μM concentration of P in the recycled medium, if known exactly.

If *nuts_measured* = "1", and source reported that P was resupplied back to the original concentration, record the same value as *control_P*.

"lower" if *P_added* = "0", and no concentration in the recycled medium was given.

"higher" if *P_added* = "1", and no concentration in the recycled medium was given. It was assumed that less than 100% of the initial P concentration was consumed in the source culture used to make the recycled medium.

exp_P_group

Categorical variable of P nutrient addition.

"higher" if *exp_P* = higher, or if *exp_P* is a value that is at least 20% greater than *control_P*.

"lower" if *exp_P* = lower, or if *exp_P* is a value that is at least 20% lower than *control_P*.

"same" if *exp_P* is a value within 20% of the *control_P* value.

P source

Nutrient added containing P. Choose from the following P nutrient sources, or a combination thereof separated by "_". The molecular weights used for conversion to μM are listed below. Note: If sources do not report P as a hydrate (i.e., no "n H₂O" in the chemical formula), we assumed the P nutrient was not a hydrate, and this affects the molar concentration conversion. However, if a source reports the P nutrient without hydration yet also reports using a common growth medium (e.g., in Table B1) in which the recipe calls for a hydrated P nutrient, we assumed the hydrated version was actually used.

- KH₂PO₄ (136.086 g/mol)
- K₂HPO₄ (174.2 g/mol)
- NaH₂PO₄ 2H₂O (156.0076 g/mol)
- Na₂HPO₄ (141.9588 g/mol)
- K₂HPO₄ 3H₂O (228.2217 g/mol)

"unknown" if P nutrient source not reported.

NP_ratio_control

N/P ratio in the fresh medium control. Equal to *control_N* divided by *control_P*.

"NA" if cannot calculate due to unknown exact concentrations.

NP_ratio_exp

N/P ratio in the experimental treatment. Equal to *exp_N* divided by *exp_P*.

"NA" if cannot calculate due to unknown exact concentrations.

vol

Volume in liters of the cultures in both the control and experimental treatments.

"unknown" if not reported.

aeration

Gas the culture was aerated with.

"none" indicates there was no aeration or the source does not report aeration, in which case it was assumed aeration did not occur.

"X_CO2" indicates cultures were aerated with X% CO₂

“air” indicates the culture was aerated with air, of atmospheric composition.
If multiple gases were used to aerate the cultures, they are both listed with “_” to separate them.

aerate_rate

Rate in volume/volume/min in which the culture was aerated. If source does not report the flow rate in vvm, divide the aeration rate by the culture volume and convert time units to minutes.

“unknown” if the source indicates that aeration occurred but does not specify a flow rate.

“NA” if *aeration* is “none”.

aerate_time

Hours per day the culture was aerated. If the source only reports that the culture was aerated at day or at night, estimate the hours from the photoperiod given; if no photoperiod is given, record 12 hours.

If source does not indicate the hours per day the culture was aerated, assume it was aerated continuously, and record 24 hours.

“pH” if the aeration only turned on in order to maintain a culture pH.

“unknown” if it was ambiguous how long the culture was aerated for per day.

“NA” if the *aeration* is “none.”

If multiple times are relevant (e.g, culture was sparged with 5% CO₂ only to maintain pH but also aerated with air 24 hours per day), they are both listed and separated with “_”.

axenic

“1” if source reports that the cultures were axenic.

“0” if source reports the cultures were xenic or does not report that they were axenic, in which case it was assumed the cultures were xenic.

pH_control

pH of the control culture. If pH was not maintained by aeration, this is solely the initial pH.

“unknown” if source does not report pH.

pH_adjust

“1” if the source adjusted the pH of the recycled medium at least initially.

“0” if the source did not adjust the recycled medium pH; assume recycled medium pH was not adjusted if the source does not report doing this.

pH_exp

pH of the recycled medium culture. If pH was not maintained by aeration, this is solely the initial pH.

“unknown” if source does not report pH.

light_level

Number indicating irradiance (or illuminance, in some cases) used to incubate cultures during the photoperiod.

Used number directly reported in source. Did not estimate light level if source only reported that cultures were lighted by certain bulbs. If a figure of light versus time was given for an outdoor culture, the figure was digitized and the average light level over the course of the experiment was reported.

“unknown” if source does not report light level.

light_unit

Units used to report light level in the source.

“NA” if *light_level* is “unknown”

“umol” for $\mu\text{mol photons/m}^2/\text{s}$ or $\mu\text{E/m}^2/\text{s}$

“W_m2” for Watts/m^2

“lux” (note that this is illuminance, not irradiance; foot-candle units were converted to lux)

light_time

Hours per day that the culture was exposed to light.

Record 12 hours if natural light/solar cycle was used and source does not report exact hours.

“unknown” if source does not report the photoperiod.

“NA” if *light_level* is “unknown” and source does not report the photoperiod.

temp

Temperature in degrees Celsius that the culture was incubated at or exposed to.

If it was an outdoor culture, use the average outdoor temperature during the experiment. If culture was outdoors but no temperature data were given, record as “unknown”.

“unknown” if temperature was not reported.

batch_cont

How the cultures were operated in the experiment.

“batch” if cultures were operated as batches, which were inoculated at the start of the experiment and sacrificed at the end of each experiment/batch.

“semicontinuous” if the culture was harvested at discrete time points (e.g., every 5 days) and a portion of the remaining culture was used to start the next culture (used as inoculum).

“continuous” if medium was continuously being supplied to the culture and the culture was continuously being harvested.

“semicontinuous_continuous” if the culture was effectively operated as a continuous culture but was harvested discretely (i.e., harvested once per day and kept in exponential phase).

times_reused

Number of times the recycled medium was previously used to grow microalgae.

If *fraction_fresh* = 0:

- For batch cultures, *times_reused* = the number of batches the medium was previously used in.
- For continuous cultures, *times_reused* = the experiment duration divided by the retention time of medium in the reactor. Retention time is equal to the volume of reactor divided by the flow rate of medium into reactor (which should be same as flow rate of culture out of the reactor).
- For semicontinuous cultures, treat as batch cultures and count each time that the medium was previously used.
- If algae growth data were reported as an average over multiple times of medium reuse, report the total number of medium reuses.

If *fraction_fresh* > 0:

- For semicontinuous and batch cultures, need to account for *fraction_fresh* by calculating *times_reused* with a weighted average method, where:
 - $times_reused = n \cdot (1 - fraction_fresh)^n + (fraction_fresh) \cdot [(n-1)(1 - fraction_fresh)^{(n-1)} + (n-2)(1 - fraction_fresh)^{(n-2)} + \dots + (n-x)(1 - fraction_fresh)^{(n-x)}]$ continue on as long as $n-x > 0$
 - where n is the reported number of times reused (e.g., number of rounds/cycles/batches)
 - Example 1: If medium was previously used 3 times in a semicontinuous culture, and the clean water addition is 10% in each cycle, then the effective number of reuses would be:
 - $times_reused = (3 \text{ reuses} \cdot (1 - 0.1)^3) + 0.1 \cdot [2 \text{ reuses} \cdot (1 - 0.1)^2 + 1 \text{ reuse} \cdot (1 - 0.1)^1] = 2.439 \text{ reuses.}$
 - Example 2: if a batch culture of recycled medium was previously used once and had 25% fresh medium added, then $times_reused = 1 \text{ reuse} \cdot (1 - 0.25)^1 = 0.75$.
- For truly continuous cultures, it is impractical to calculate the exact number of reuses when a fresh source is added to the recycled medium, since the input would need to be discretized in order to calculate the number of harvests. Instead, *times_reused* was calculated by $(1 - fraction_fresh) \cdot \text{duration} \cdot \text{flow rate} / \text{reactor volume}$.

- If algae growth data were reported as an average over multiple medium reuses, report the total effective number of medium reuses, using the equations above.

Note: Inoculum, or any culture left in the culture vessel to start subsequent cultures, does not count towards *fraction_fresh*, and is considered recycled medium for the purposes of the *times_reused* variable.

“unknown” if number of reuses was not specified in the source and cannot be calculated from information provided.

marine_fresh

“marine” if algae strains are marine/growing in salt water medium.

“fresh” if algae strains are freshwater/not growing in salt water medium.

rotated

“0” if the algae strain grown in the recycled medium (*strain_1* below) was the same as that previously grown in the source culture(s) used to create the recycled medium (*strain_2* below).

“1” if the strain growing in the recycled medium (*strain_1* below) was not the same as the strain that was previously grown in the source culture(s) used to create the recycled medium (*strain_2* below).

strain_1

Full name of the algae strain growing in the recycled medium, as reported by the source.

“polyculture” if a mixed culture of many strains was used.

strain_2

Full name of the source algae strain (strain that was previously grown in the recycled medium), as reported by the source.

“polyculture” if a mixed culture of many strains was used.

genus_1

Genus of the growing algae strain.

“Polyculture” if a mixed culture of many strains was used.

genus_2

Genus of the source strain (strain previously grown in the recycled medium).

“Polyculture” if a mixed culture of many strains was used.

common_name_1

Broad name for the growing algae strain. Examples below.

“Chlorophytes” for all green algae (*Chlorella*, *Scenedesmus*, *Dunaliella*, *Chlorococcum*, *Tetraselmis*, *Hormotila*, *Chlamydomonas*, *Haematococcus*, *Nannochloris*, *Pandorina*, *Botryococcus*, *Oocystis*)

“Bacillariophyta” for diatoms (*Skeletonema*, *Phaeodactylum*, *Ditylum*, *Asterionella*, *Nitzschia*, *Thalassiosira*, *Chaetoceros*, *Fragilariopsis*, *Cyclotella*)

“Haptophyte” for *Emiliana*, *Isochrysis*

“Prasinophyte” for *Micromonas*

“Cryptophyte” for *Cryptomonas*, *Rhodomonas*

“Eustigmatophyte” for *Monodus*, *Nannochloropsis*

“Chrysophyte” for *Ochromonas*

“Cyanobacteria” for *Arthrospira*, *Synechocystis*, *Synechococcus*, *Anabaena*

“Xanthophyte” for *Botrydium*

“polyculture” if a mixed culture of many strains was used; but, if most of the strains in the mixed culture belonged to a certain common group, record that common group’s name.

common_name_2

Broad name for the algae strain previously grown in the recycled medium. See *common_name_1* for examples.

phase_inoc

Growth phase of the inoculum for the control and experimental treatments.

If *batch_cont* = "continuous", use "exponential" unless shown or stated otherwise in the source.

If *batch_cont* = "semicontinuous", use the growth phase at which algae were harvested from a portion of the culture (the last day of the growth curve before being transferred), since the inoculum is the remaining portion of the culture.

Otherwise, use the growth phase reported in the text, in any. If growth phase was not reported but a growth curve figure of the inoculum culture was provided, determine the growth phase based on the point on the curve at which it was used as inoculum:

- "exponential" for point in growth curve when there is a steep upward curve (or for any "continuous" cultures or "semicontinuous_continuous" cultures kept in exponential phase)

- "late exponential" for linear portion of growth curve

- "early stationary" for point in growth curve when biomass is just starting to level off

- "stationary" for point in growth curve when biomass has leveled off

- "late stationary" for point in growth curve when it has started to drop from the stationary phase. Note: if a source reported "decay phase," this was recorded as "late stationary."

"unknown" if the source does not provide any indication of the growth phase of algae used as inoculum.

phase_harvest

Growth phase of algae in the source culture when they were harvested to create the recycled medium. If only the number of days are given for how long the algae grew before being harvested, estimate the growth phase from a growth curve of the source culture if available, or from the growth curve of the experimental treatment (if the same strain was being grown in the recycled medium):

- "exponential" for point in growth curve when there is a steep upward curve (or for any continuous cultures or semicontinuous_continuous cultures kept in exponential phase)

- "late exponential" for linear portion of growth curve

- "early stationary" for point in growth curve when biomass is just starting to level off

- "stationary" for point in growth curve when biomass has leveled off

- "late stationary" for point in growth curve when it has started to drop from the stationary phase. Note: if a source reported "decay phase," this was recorded as "late stationary."

"unknown" if the source does not report what growth phase or the number of days the algae grew in the medium before being harvested.

inoc_biomass

Initial concentration of biomass in the experimental treatment after inoculation.

Report as given in the source (units will be recorded in next variable). If a growth curve was provided, digitize the first point in a growth curve to determine this.

"NA" if *batch_cont* = "continuous".

"unknown" if not reported or discernible from data.

inoc_biomass_unit

Unit of measurement used to report *inoc_biomass*.

"AFDW" for g/L ash-free dry weight

"cells" for 10^6 cells per mL

"DW" for g/L DW

"OD" for optical density

"PVC" for packed cell volume

"NA" if *inoc_biomass* is "unknown" or "NA"

bio_measurement

Method used to measure algae biomass during the experiment. If multiple methods were used, they are both recorded with a “_” in between them.

“AFDW” for ash-free dry weight

“C_uptake” for overall long-term uptake of radio-labelled carbon in g/L

“Coulter” for cell counting by Coulter counter

“DW” for dry weight

“microscope” for cell counting by microscopy

“ODXXX” for optical density at XXX wavelength, even if the OD was converted to dry weight afterwards using a conversion equation

“PCV” for packed cell volume

“unknown” if source does not report how biomass was measured

rep_control

Number of replicates (individual cultures) used for the control.

Estimate the number of replicates if enough information is available in the source to reasonably do so.

“unknown” if source does not specify how many replicates were used and it cannot be discerned from the data.

“0” if there was no separate control culture in a continuous culture experiment (i.e., the control culture became the recycled medium culture); in this case the 1st measurement of an experimental treatment was used as the control’s growth data.

rep_exp

Number of replicates (individual cultures) used for the experimental treatment.

Estimate number of replicates if enough information is available in the source to reasonably do so.

“unknown” if source does not specify how many replicates were used and it cannot be discerned from the data.

notes

Any other descriptions to distinguish treatments from one another.

Specific Growth Rate (μ) variables

General notes about these variables:

- Specific growth rate is the growth rate, in units of day^{-1} , during exponential phase.
- We either took the growth rate reported in a source directly or calculated it from a growth curve figure if provided, whose data was digitized using WebPlot Digitizer (Rohatgi).
 - If a growth curve figure was available, we calculated the specific growth rate whether or not the growth rate was reported elsewhere in the source (figure, table, text). If growth rate was not reported in the source, we recorded the calculated value. If there was a growth rate reported in the source, we compared it to our calculated growth rate and determined which one to use. If the calculated values and the reported values were considerably different ($>20\%$ difference) (e.g., the authors may have used data points outside of the exponential growth stage, or only used 2 data points), we recorded the calculated values for consistency across sources. If the calculated and reported values were not considerably different, the reported values were recorded.
 - Calculation: From digitized growth curve data, specific growth rate was calculated as the slope of the natural log of biomass versus time, using only the exponential growth phase where there is a linear relationship between these variables (Wood et al. 2005). Linearity was checked by comparing the R^2 values of $\ln(\text{biomass})$ versus time and choosing points that yielded the highest R^2 value, as well as observing the original figures and those with log-transformation of biomass (y-axis). A minimum of 3 points in exponential phase was required for specific growth rate calculation.
 - If an R^2 of at least 0.8 was unobtainable from the digitized data of log-transformed biomass versus time, the growth rate was reported as “NA.”
 - We only used portions of the growth curve figures where data were available from both the control and experimental treatment.
- Specific growth rate is relevant for batch and semicontinuous cultures with full growth curves only. It is not relevant for continuous cultures because the dilution rate determines the specific growth rate.

u_control

The average specific growth rate of the control culture(s) in units of 1/day.

If data were reported as a ratio of the recycled medium growth rate to the control growth rate, record the control growth rate as “1.”

“unknown” if *batch_cont* = “batch” or “semicontinuous” but the growth rate was not reported and cannot be calculated from provided data/figures.

“NA” if *batch_cont* = “continuous”.

u_control_stdev

Standard deviation of *u_control*. Can be retrieved directly from text or table, or by digitizing standard deviation bar distance in a bar graph of growth rates.

“NA” if there was only 1 replicate; if *u_control* was “unknown” or “NA”; or if growth rate standard deviation cannot be calculated from available data (e.g., if *u_control* was calculated from a single averaged biomass growth curve, from which growth rates of individual replicates cannot be calculated and therefore standard deviation cannot be calculated).

u_exp

The average specific growth rate of the experimental treatments in units of 1/day.

“unknown” if *batch_cont* = “batch” or “semicontinuous” but the growth rate was not reported and cannot be calculated from provided data/figures.

“NA” if *batch_cont* = “continuous”.

u_exp_stdev

Standard deviation of u_{exp} . Can be retrieved directly from text or table, or by digitizing standard deviation bar distance in a bar graph of growth rates.

“NA” if there was only 1 replicate; if u_{exp} was “unknown” or “NA”; or if growth rate standard deviation cannot be calculated from available data (e.g., if u_{exp} was calculated from a single averaged biomass growth curve, from which growth rates of individual replicates cannot be calculated and therefore standard deviation cannot be calculated).

u_digitized

“1” if data were obtained by digitizing a figure. This includes digitizing the growth curve (and calculating growth rates from that), and digitizing figures that report growth rates directly (e.g., bar graphs).

“0” if data were obtained directly from text or a table.

“NA” if growth rates are “unknown” or “NA”.

u_calc

“1” if growth rates were calculated from growth curve data.

“0” if growth rates were not calculated from growth curve data (i.e., either used a value directly from the text, or used from a figure that reported growth rates directly).

“NA” if growth rates are “unknown” or “NA”.

u_sig

“1” if significance of the difference in specific growth rate between the control and experimental treatment is reported.

“0” if significance is not reported.

“NA” if growth rates are “unknown” or “NA”.

u_sig_outcome

“0” if $u_{sig} = 1$ and the difference between the growth rates was not significant.

“positive” if $u_{sig} = 1$ and the recycled medium treatment growth rate was higher than the control’s growth rate, and significant as defined by the study.

“negative” if $u_{sig} = 1$ and the recycled medium treatment growth rate was lower than the control’s growth rate, and significant as defined by the study.

“NA” if $u_{sig} = “0”$ or “NA”

u_RR

Specific growth rate response ratio. Calculated as $u_{exp}/u_{control}$.

“NA” if $u_{control}$ and/or $u_{exp} = “unknown”$ or “NA”

u_effectsize

Specific growth rate effect size (log-transformed response ratio). Natural log of u_{RR} ($= \ln(u_{RR})$).

“NA” if u_{RR} is “NA”

Biomass productivity variables

General notes about these variables:

- Biomass productivity units are g/L/day. This variable is relevant for continuous cultures and in some cases semicontinuous cultures if harvested at short time-scales (*batch_cont* = "semicontinuous_continuous"). If the cultures were operated in batch mode yet the source reports values for biomass productivity, these were also recorded.
- Productivity was either retrieved directly from a source (e.g., from the text, a table, or a figure that plotted productivity) or calculated from a 'biomass concentration versus time' figure if provided, whose data were digitized using WebPlot Digitizer (Rohatgi).
 - In cases where multiple productivity values were available for the duration of a single experiment (i.e., multiple values of productivity from a single culture or multiple values of average productivity from replicates), productivity was averaged over time.

prod_control

Average biomass productivity of the control culture(s) in g/L/day. See above notes for how average productivity was obtained.

"unknown" if *batch_cont* = "continuous" but productivity was not reported or able to be calculated from the available data or figures.

"NA" if productivity is not a relevant growth definition, such as in batch cultures.

prod_control_stdev

Standard deviation of the biomass productivity in control cultures.

Can be retrieved directly from the text or a table, measured by digitizing the standard deviation bar distance in a bar graph of productivity, or calculated from available productivity values (see notes above about how productivity values are obtained). In the latter case, it can be either the standard deviation of single values of the control replicates' productivities, OR the standard deviation of all available productivities over the experimental period (from a single control culture or from replicates averaged at each time point).

"NA" if *prod_control* = "unknown" or "NA," or if there were not sufficient data or replicates to calculate the standard deviation.

prod_exp

Average biomass productivity of the experimental treatment culture(s) in g/L/day. See above notes for how average productivity was obtained.

"unknown" if *batch_cont* = "continuous" but productivity was not reported or able to be calculated from the available data or figures.

"NA" if productivity is not a relevant growth definition, such as in batch cultures.

prod_exp_stdev

Standard deviation of the biomass productivity in experimental treatment cultures.

Can be retrieved directly from the text or a table, measured by digitizing the standard deviation bar distance in a bar graph of productivity, or calculated from available productivity values (see notes above about how productivity values are obtained). In the latter case, it can be either the standard deviation of single values of the experimental treatment replicates' productivities, OR the standard deviation of all available productivities over the experimental period (from a single experimental treatment culture or from replicates averaged at each time point).

"NA" if *prod_exp* = "unknown" or "NA," or if there were not sufficient data or replicates to calculate the standard deviation.

prod_digitized

“1” if data were obtained by digitization of a figure. Includes digitizing a ‘biomass versus time’ graph and calculating productivities, as well as digitizing figures that report productivities directly (e.g., bar graphs).

“0” if data were obtained directly from text or a table.

“NA” if productivities are “unknown” or “NA.”

prod_calc

“1” if average productivities were calculated from digitized data.

“0” if productivities were not calculated (instead were retrieved directly from the text or from a figure that reported productivity directly).

“NA” if productivities are “unknown” or “NA.”

prod_sig

“1” if significance of the difference between control and experimental biomass productivity was reported.

“0” if significance was not reported.

“NA” if productivities were “unknown” or “NA.”

prod_sig_outcome

“0” if *prod_sig* = 1 and the difference between the productivities of controls and experimental treatments was not significant.

“positive” if *prod_sig* = 1 and the recycled medium treatment productivity was higher than the control’s productivity, and significant as defined by the study.

“negative” if *prod_sig* = 1 and the recycled medium treatment productivity was lower than the control’s productivity, and significant as defined by the study.

“NA” if *prod_sig* = “0” or “NA”

prod_RR

Biomass productivity response ratio. Calculated as *prod_exp/prod_control*.

“NA” if *prod_control* and/or *prod_exp* = “unknown” or “NA”

prod_effectsize

Effect size of biomass productivity (log-transformed response ratio). Natural log of *prod_RR* (= $\ln(\text{prod_RR})$).

“NA” if *prod_RR* is “NA”

Biomass concentration variables

General notes about these variables:

Biomass concentration can be measured by a variety of methods, and units are recorded.

- For batch and semicontinuous cultures with a full growth curve available, the maximum biomass concentration over the course of the experiment was recorded, if available. Can be taken from digitized data by finding the maximum biomass point over the growth curve. If a growth curve was not available, the final biomass concentration was recorded as reported either in the text, a table, or directly in a figure such as a bar graph of total or final biomass.
- For continuous cultures, the biomass concentration values were recorded as reported. If digitized data were used from a figure of biomass versus time, the average of the biomass concentrations over time were calculated (from a single culture or from replicates averaged at each time point).
- For semicontinuous cultures effectively operated as continuous cultures (*batch_cont* = "semicontinuous_continuous") (e.g., 50% harvested per day and replaced), the average of only the peak biomass concentrations over time were calculated (from a single culture or from replicates averaged at each peak time point).
- We only used portions of the growth curve or growth period where data were available for both the control and experimental treatment.

bio_control

Average biomass concentration of the control cultures in whichever unit was provided (see 'notes' above for how biomass concentration was obtained).

If data were reported as a ratio of the recycled medium biomass concentration to the control biomass concentration, record the control biomass as "1".

In cases where a control was not performed separately in a continuous culture (i.e., the control was the period before medium recycling began), we used the initial biomass concentration of the culture as the fresh medium control.

"unknown" if biomass was not reported or able to be calculated from the given figures.

bio_control_stdev

Standard deviation of the biomass concentration in control cultures.

Can be retrieved directly from the text or a table, measured by digitizing standard deviation bar distance in a bar graph of final or maximum biomass concentration, or calculated from multiple biomass concentration values. In the latter case, this can be the standard deviation of multiple control replicates if *batch_cont* = "batch" or "semicontinuous" OR the standard deviation of all available biomass concentrations over the experimental period (from a single control culture or from replicates averaged at each time point) if *batch_cont* = "continuous" or "semicontinuous_continuous".

"NA" if *bio_control* is "unknown" or if standard deviation was not reported and cannot be calculated.

bio_exp

Average biomass concentration of the experimental treatments in whichever unit was provided (see 'notes' above for how biomass concentration was obtained).

"unknown" if biomass was not reported or able to be calculated from the given figures.

bio_exp_stdev

Standard deviation of the biomass concentration in experimental treatment cultures.

Can be retrieved directly from the text or a table, measured by digitizing standard deviation bar distance in a bar graph of final or maximum biomass concentration, or calculated from multiple biomass concentration values. In the latter case, this can be the standard deviation of multiple control replicates if *batch_cont* = "batch" or "semicontinuous" OR the standard deviation of all available biomass concentrations over the experimental period

(from a single control culture or from replicates averaged at each time point) if *batch_cont* = "continuous" or "semicontinuous_continuous".

"NA" if *bio_exp* is "unknown" or if standard deviation was not reported and cannot be calculated.

bio_unit

Unit of biomass concentration recorded.

- "ODXXX" for optical density at wavelength XXX
- "DW" for dry weight in g/L, even if it was originally measured as OD, but was reported as DW
- "AFDW" for ash-free dry weight in g/L
- "cells" for 10⁶ cells/mL
- "PCV" for packed-cell volume
- "none" if the data were reported as a ratio, so are unitless. Growth measurement method is still recorded under *bio_measurement*.
- "NA" if biomass is "unknown"

bio_digitized

"1" if data were obtained by data digitization of a figure. This includes both digitizing the 'biomass concentration versus time' graph and calculating maximum biomass from that data, as well as digitizing figures that report final or maximum biomass directly (e.g., bar graphs).

"0" if data were obtained directly from the text or a table.

"NA" if biomass is "unknown".

bio_calc

"1" if biomass concentrations were calculated from digitized data or averaged from multiple points over time.

"0" if biomass concentrations were taken directly from the text, or digitized from a figure that reported maximum or final biomass directly.

"NA" if biomass is "unknown".

bio_sig

"1" if significance between the difference in control and experimental biomass concentration was reported.

"0" if significance was not reported.

"NA" if biomass is "unknown".

bio_sig_outcome

"0" if *bio_sig* = 1 and the difference between the biomasses of controls and experimental treatments was not significant.

"positive" if *bio_sig* = 1 and the recycled medium treatment biomass concentration was higher than the control's biomass, and significant as defined by the study.

"negative" if *bio_sig* = 1 and the recycled medium treatment biomass concentration was lower than the control's biomass, and significant as defined by the study.

"NA" if *bio_sig* is "NA" or "0"

bio_RR

The biomass concentration response ratio. Calculated as *bio_exp/bio_control*.

"NA" if *bio_control* and/or *bio_exp* were "unknown".

bio_effectsize

Effect size of biomass concentration (log-transformed response ratio). Natural log of *bio_RR* (= $\ln(\text{bio_RR})$).

"NA" if *bio_RR* is "NA".

share_control

Variable indicating whether the experimental treatment was compared to a control that was also used for comparison to another treatment from the same data source (i.e., the same control was used in more than 1 effect size calculations).

“0” if the experimental treatment does not share a control culture with another treatment from the same source.

“1” if the experimental treatment shares a control culture with another treatment from the same source.

other_dep

Variable indicating if the culture used to produce the recycled medium for the experimental treatment was exclusive to that treatment.

“0” if the culture used to produce the recycled medium for the experimental treatment was not used for other treatments in the same source. This was the default value if it could not be discerned from the study whether treatments shared a source of recycled medium.

“1” if the culture used to produce the recycled medium for the experimental treatment was aliquoted and divided among multiple treatments (so that these treatments are not independent). This still applies if the original culture was aliquoted and then different harvesting methods were applied to create different recycled medium treatments, since the source of recycled medium was still shared among these treatments and therefore they are not independent. Treatments are also given a “1” if they are in a successive reuse experiment, and the same culture medium was being reused over and over again in discrete treatments.

which_dep

If *other_dep* = 1, this is a list of treatments (*treatment_num*; separated by commas if more than one) that are from the same data source as the given experimental treatment and share the same source of recycled medium (i.e., the recycled medium used for these treatments originated from the same original culture(s)).

“NA” if *other_dep* = 0

weight

The weight assigned to a treatment to calculate weighted mean effect sizes in analysis.

“1” if *other_dep* = 0

If *other_dep* = 1, $\text{weight} = 1/n$, where n is the total number of treatments sharing the same culture(s) used to create their recycled medium. Therefore $n = 1 + \text{number of treatments listed in } \textit{which_dep}$.

reference

Abbreviated reference of the study (data source), including first author last name, year of publication, journal name, volume, and pages (where available).

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