Bacterial Production Lab

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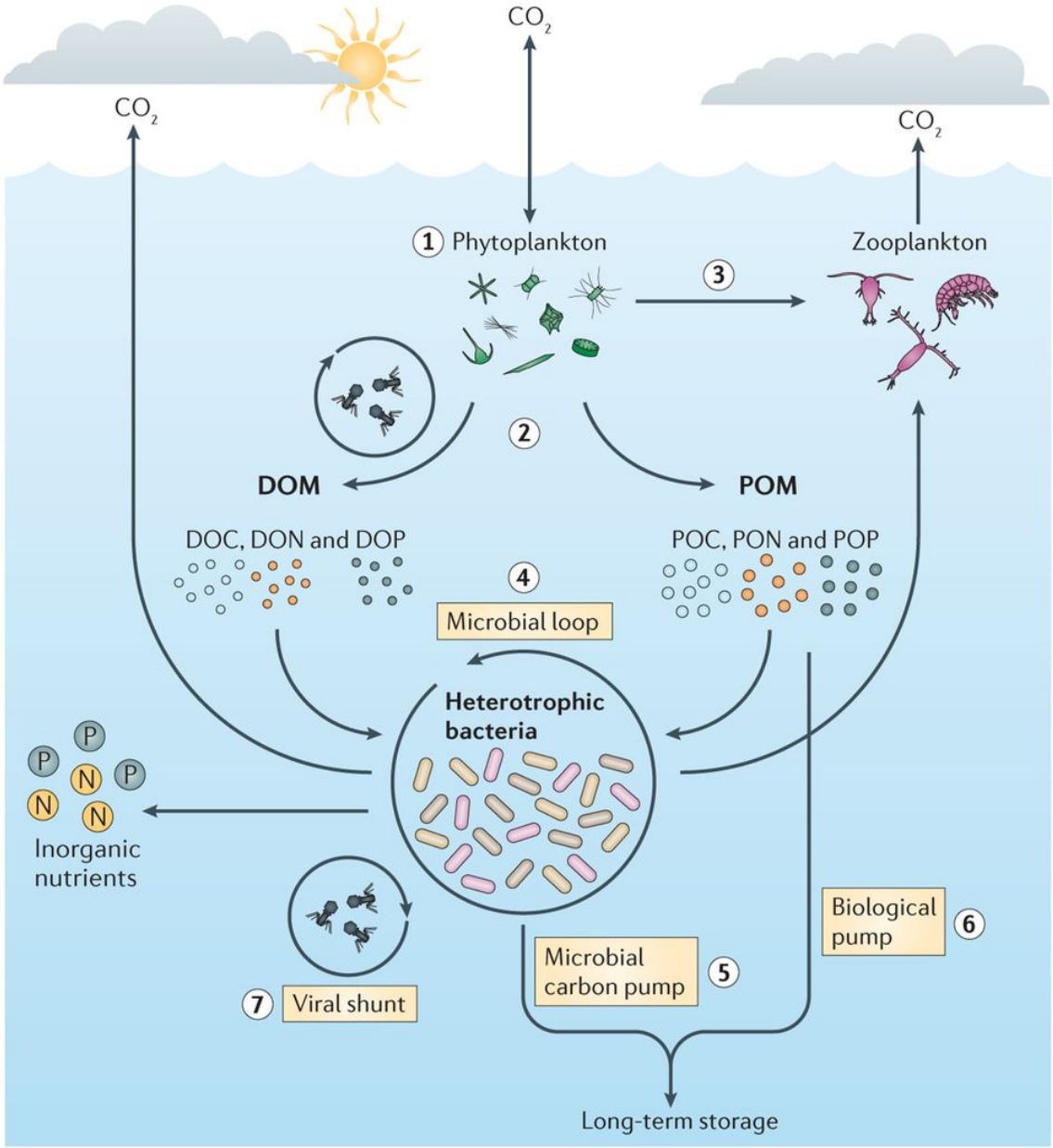


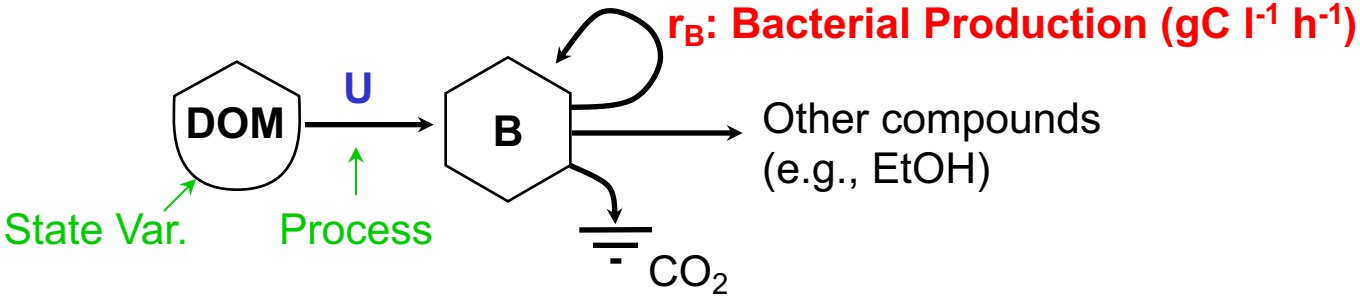
https://www.nature.com/articles/nrmicro3326

Nature Reviews | Microbiology

Bacterial Production Lab

Additional reading: Simon and Azam (1989)

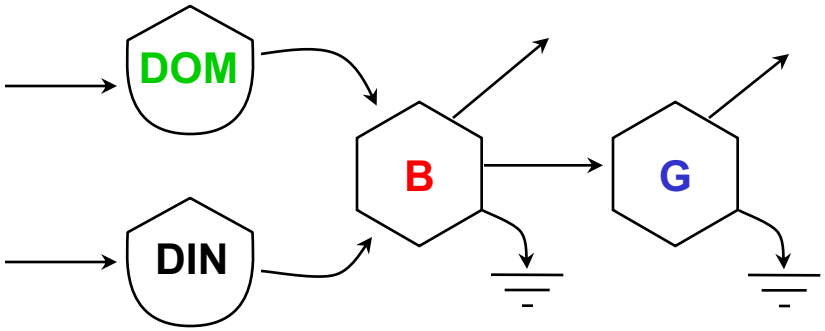
State variables and processes

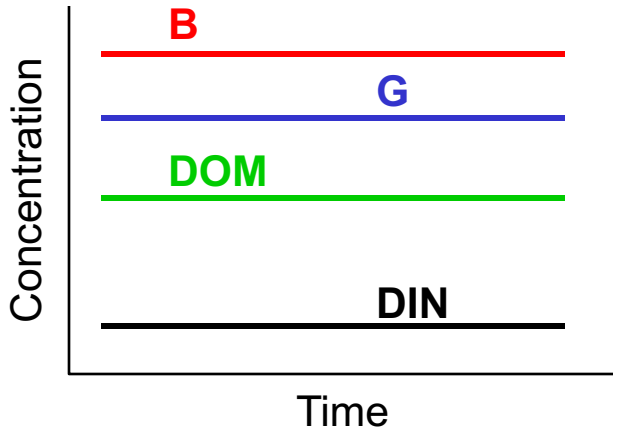


Objective: Measure bacterial growth rate (also called bacterial production)

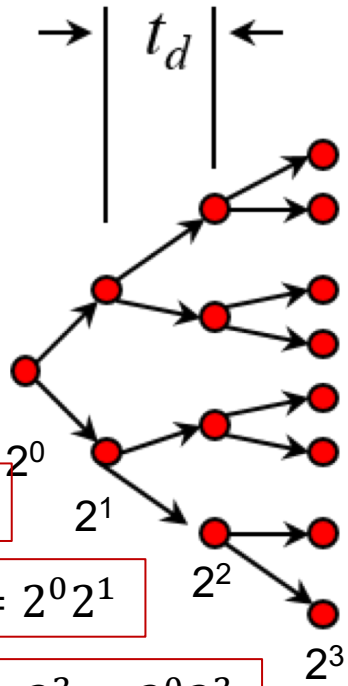
Why do we want to measure processes?

Turnover: [B]/r_B





Growth Equation



$2^0 = 2^0 2^0$

$2^1 = 2^0 2^1$

$2^2 = 2^0 2^2$

$2^3 = 2^0 2^3$

$X_n = X_0 2^n$

n: *Generations*

to include the doubling time (t_d):

$$x(t) = x(t_0) 2^{\frac{t-t_0}{t_d}}$$

t_d: *doubling time*

$\frac{t-t_0}{t_d}$: *how often did cell divide during the time passed?*

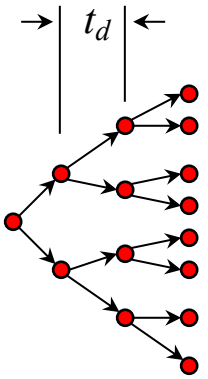
e.g. after 24 hours: t-t_0 = 24
And a doubling time t_d of 2 hours
Time for 12 generations

Where:

t_d	Doubling time of population.
$x(t)$	Number or mass of cells per unit volume at time t.
t_0	Time at start (usually set to 0).

Note, cell mass or numbers are easily converted if we assume cells are all the same size: $x(t) = \varphi n(t)$, where φ is the mass per cell and n is the number of cells per unit volume and x(t) is the mass of cells per unit volume.

Growth Equation



Specific growth rate constant, μ

$$x(t) = x(t_0)2^{\frac{t-t_0}{t_d}}$$

For a doubling of x: $\frac{x(t)}{x(t_0)} = 2$

$$x(t) = x(0)e^{\mu t_d}$$

$$\frac{x(t)}{x(0)} = e^{\mu t_d}$$

$$2 = e^{\mu t_d}$$

$$\mu = \frac{\ln(2)}{t_d}$$

$$\frac{dx}{dt} = \mu x$$

For exponential growth!

$$\frac{dx}{x} = \mu dt$$

$$\int_{x_0}^{x} \frac{dx}{x} = \mu \int_{0}^{t} dt$$

$$\ln(x) - \ln(x_0) = \mu t$$

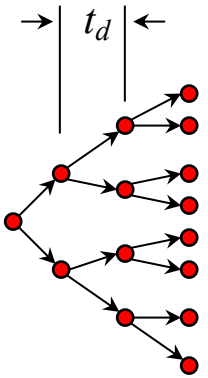
$$\ln(x) = \mu t + \ln(x_0)$$

$$e^{\ln(x)} = e^{\mu t + \ln(x_0)}$$

$$e^{\ln(x)} = e^{\mu t} e^{\ln(x_0)}$$

$$x = e^{\mu t} x_0 \rightarrow x(t) = x(0)e^{\mu t}$$

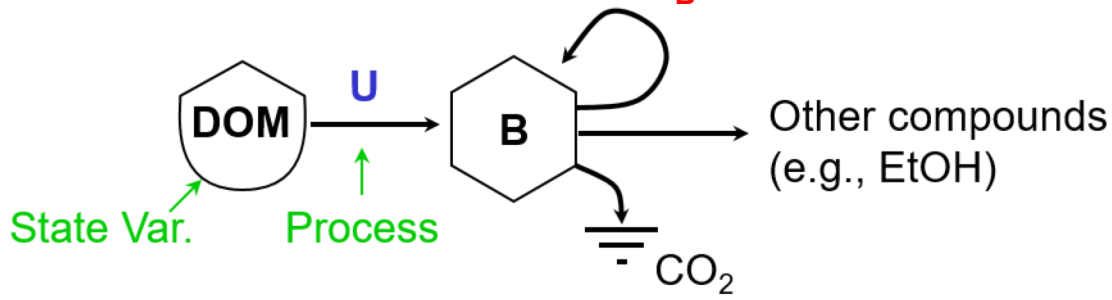
Bacterial Production



$$x(t) = x(t_0)2^{\frac{t-t_0}{t_d}}$$

$$x(t) = x(0)e^{\mu t}$$

r_B: Bacterial Production (gC l^{-1} h^{-1})



$$\frac{dx}{dt} = \mu x = r_B$$

$$\frac{dx}{dt}\frac{1}{x} = \mu = \frac{1}{x}r_B$$

$$r_B = x\mu$$

Size and Growth Rate

Plankton: Net movement dependent on flow field.

Nekton: Move independent of flow field.

Phyto: Autotrophic

Zoo: Heterotrophic

Femtoplankton: 0.02 - 0.2 μm
- Mostly viruses

Picoplankton: 0.2 - 2 μm
- Bacteria, cyanobacteria

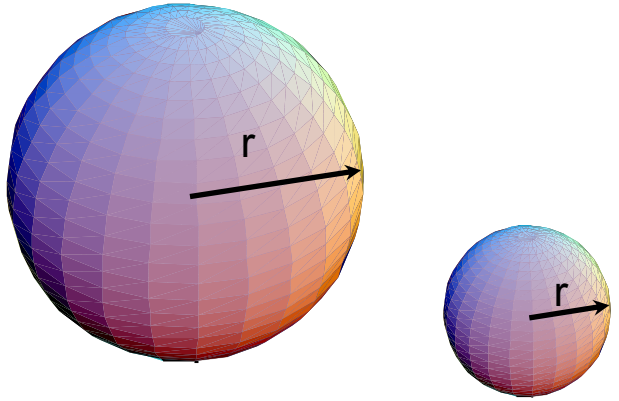
Nanoplankton: 2 - 20 μm
- Flagellates, dinoflagellates

Microplankton 20 - 200 μm
- Diatoms, ciliates.

Mesoplankton > 200 μm
- Zooplankton (copepods)

Bacteria: 0.2 μm - 1000 μm (1 mm)
- Typically 1 - 2 μm culture, or < 1 μm natural environments.

Surface area to volume



Surface area: $4 \pi r^2$

Volume: $4/3 \pi r^3$

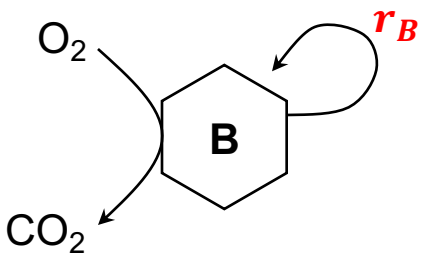
SA/V = $3/r$

$\mu \propto SA/V$

Consequently, smaller cells will have a higher specific growth rate

How are growth rates measured?

Accumulation or Loss Rates

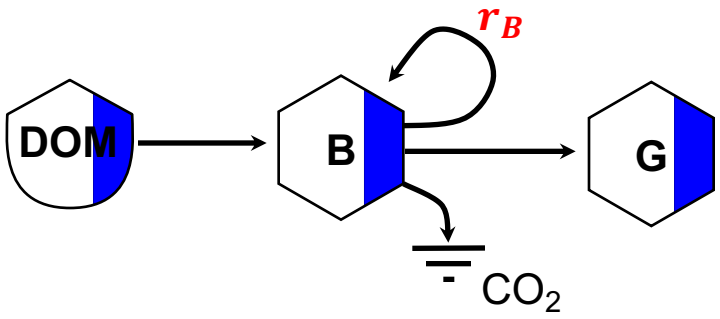


Isolate bacteria (How?), then measure:

$$r_B = \frac{dx(t)}{dt} \propto \frac{dCO_2}{dt} \propto -\frac{dO_2}{dt}$$

What is main problem with this technique?

Use a Tracer



$$r_{Blue} = \frac{[Blue(t)] - [Blue(0)]}{\Delta t}; \quad r_B = \frac{r_{Blue}}{f}$$

where r_{Blue} is the rate of "blue" accumulation and f is the fraction of dissolved organic matter (DOM) that is labeled "blue".

Tracer Requirements

- Should not change environment
- Not preferentially consumed.
- Bacteria must utilize for growth
- Must be able to measure at low concentrations. Low detection limits reduce incubation times.
- Need some measure of f

Radio-isotope Tracers

Radionuclides typically used in biology:

	Half Life	Type
Tritium (^{3}H)	12.26 y	β
Carbon-14 (^{14}C)	5730 y	β
Sulfur-35 (^{35}S)	87.2 d	β
Chlorine-36 (^{36}Cl)	300,000 y	β
Phosphorus-32 (^{32}P)	14.3 d	β
Iodine-131 (^{131}I)	8.06 d	β, γ
Iodine-125 (^{125}I)	60 d	γ

Source of He

Types

α	Helium nuclei
β	Electron
γ	Gamma ray (and x-ray)
n	Neutron

For bacterial production, ^{3}H and ^{14}C used.

Note, ^{3}H and ^{14}C are weak β emitters, so shielding is not required.

Units: Curie, Ci: 2.2×10^{12} disintegrations per min (DPM)
(activity of 1 gram of radium-226)

SI Units: Becquerel, Bq: 1 DPS = 60 DPM = 2.7×10^{-11} Ci

Specific activity (SA): Ci $mmol^{-1}$

Concentration: Ci ml^{-1}

Radioactivity measurements:
- Geiger counter
- Scintillation counter (method we will use)

Measurements are given in counts per min. (CPM)
Due to some losses, CPM < DPM

Levels of detection

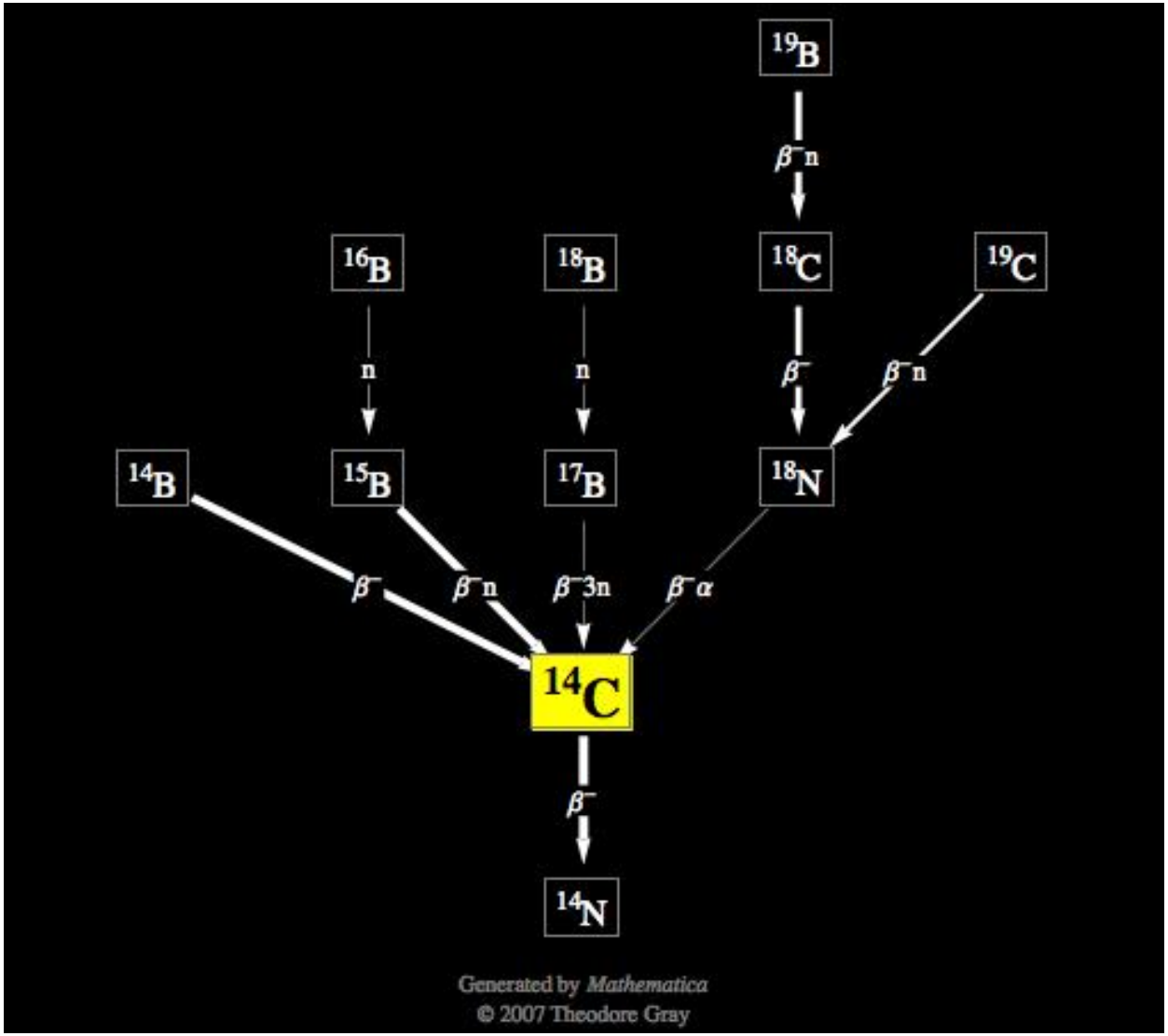
SA: 371 mCi (mmol $^{14}C)^{-1}$

Measure: 10 CPM~10 DPM

Detect: 1×10^{-14} mol
10 fmol

Annual Limit on ^{14}C Ingestion: 2 mCi

^{14}C formation and decay path



Radiation Exposure Limits and Comparisons (UC Davis)

rem: Roentgen equivalent man. Sievert (Sv) = 100 rem

Dose Equivalent Limits (Monitored Radiation Workers)

Target Tissue	Regulatory Limit	UC Davis Guideline
Whole Body	5000 mrem/year	2500 mrem/year
Extremities	50000 mrem/year	25000 mrem/year
Skin of the Whole body	50000 mrem/year	25000 mrem/year
Fetus	500 mrem/gestational period	50 mrem/month

Common Radiation Exposures (Natural Sources and Human Made)

One Coast to Coast Flight	3 mrem
Natural Background Radiation in the U.S.	150 - 300 mrem/year
Chest Radiograph, A/P view	15 - 25 mrem/view
Chest Radiograph, Lateral view	50 - 65 mrem/view
Screening Mammography (film/screen combination)	60 - 135 mrem/view
Computer Aided Tomography (CAT) scan of Body (20 slices)	3000 - 6000 mrem

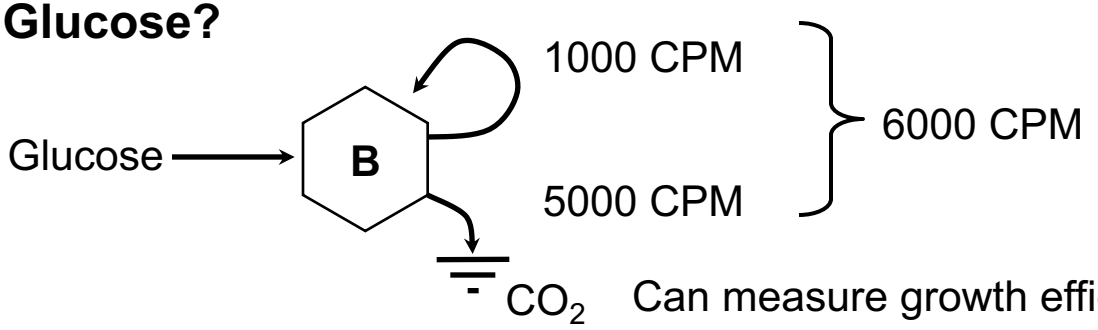
Biologically Significant Radiation Exposures (Absorbed/Acute Exposure)

Risk of contracting cancer increased 0.09%	1000 mrem
Temporary blood count change	25000 mrem
Permanent sterilization in men	100000 mrem
Permanent sterilization in women	250000 mrem
Skin Erythema	300000 mrem
Cataract formation	600000 mrem

What Compounds to Label?

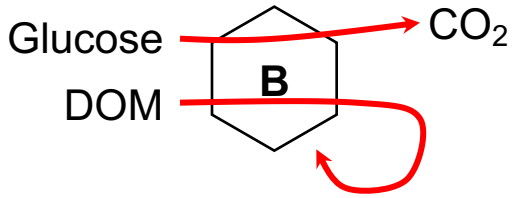
Can't ^{14}C-label all DOM, so label only certain compounds

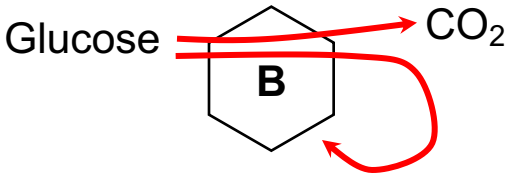
Glucose?

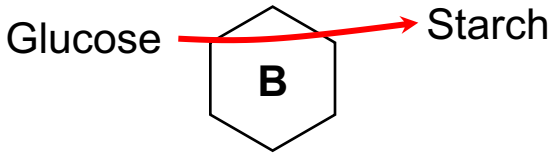


Can measure growth efficiency if CO_2 is captured.

What fraction of the bacterial cell is produced from glucose?







Problem: it is difficult to know what fraction of bacterial synthesis comes from glucose.

Label macromolecules instead using appropriate monomer:

	Monomer	**% CDW**	
Protein	Amino Acids	55.0	Cultured *E. coli*
RNA	A, G, C, U	20.5	
DNA	A, G, C, T	3.1	

Bacterial Production from ^{14}C-Leucine Uptake

Use ^{14}C-leucine to measure the rate of bacterial protein synthesis. Calculate bacterial production rate using the following "pseudo constants":

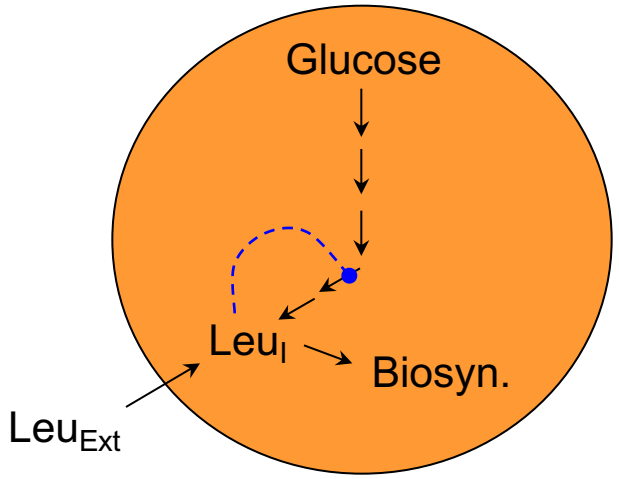
Pseudo constants:

Leucine content in protein	7.3 mol %
Protein Ave MW	131.9
Protein	63 % CDW
Cell dry weight (CDW)	54 % Carbon

Isotope Dilution Problem

Occurs when radioisotope is mixed with non-radioisotope.

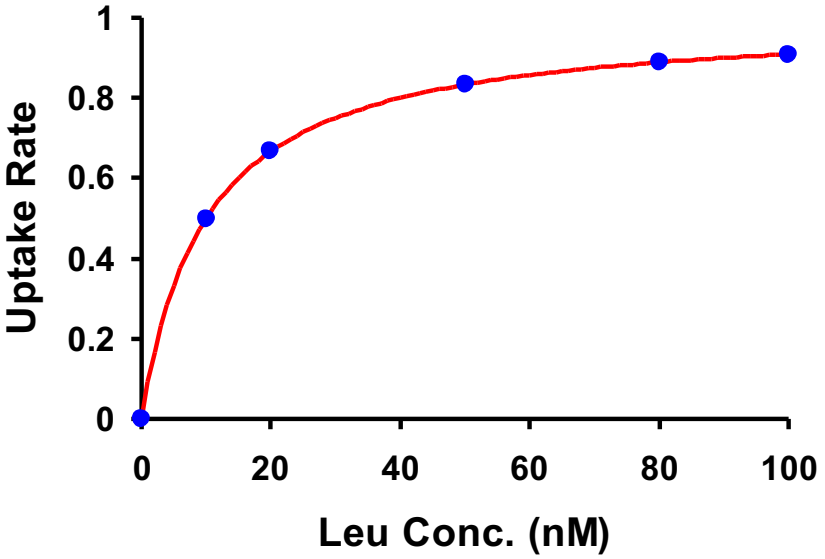
- Extracellular
  - Caused by presence of Leu in solution.
  - Leu Concentration is small (< 1 nM), so add >10 nM Leu and ignore extracellular dilution.
- Intracellular
  - Caused by de novo Leu synthesis.
  - Assume negligible, or measure.



Assessing Isotope Dilution

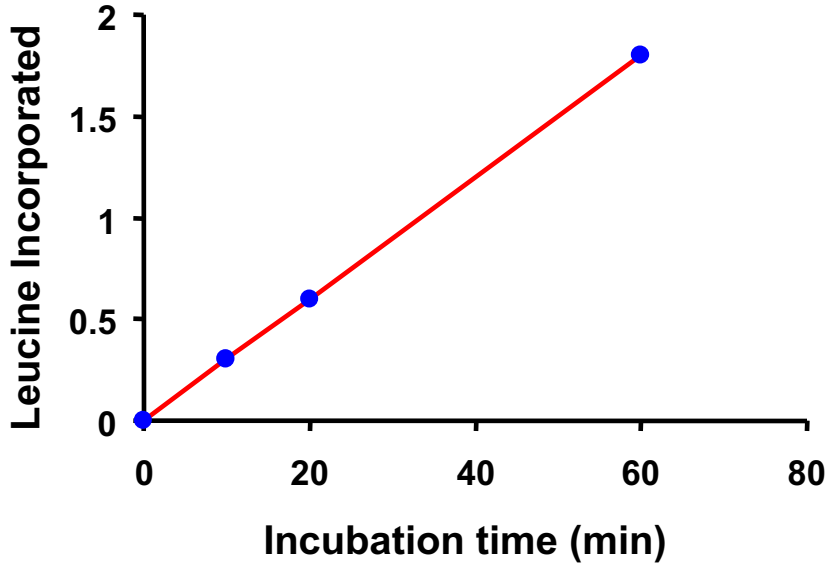
Extracellular dilution and Incubation Time:

- Measure background leucine concentration.
- Construct kinetic curve (top right fig).
- Construct time course curve (bottom left fig).



Intracellular dilution:

- Measure Sp. activity of Leu in protein.
- Measure actual protein synthesis rate and compare isotope-measured value.
- Often, intracellular dilution is assumed not to be significant.



Example Calculations

Experimental Setup

SA Leu: 100 Ci mmol^{-1}

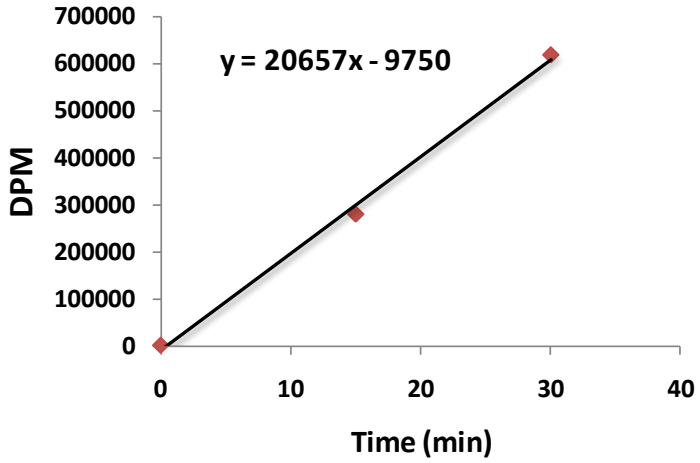
Incubations: 15 and 30 min

Volume 1.5 ml

Measure activity after incubation

20,657 DPM min^{-1} (from slope)

Note, CPM ≠ DPM



$$Leu = 20657\,\frac{DPM}{min} \times \frac{1}{2.2\times10^{12}}\,\frac{Ci}{DPM} \times \frac{1}{100}\,\frac{mmol\ Leu}{Ci} \times \frac{1}{1000}\,\frac{mol\ Leu}{mmol\ Leu} = 9.39\times10^{-14}\,\frac{mol\ Leu}{min}$$

$$Cells = 9.39\times10^{-14}\,\frac{mol\ Leu}{min} \times \frac{1}{0.073}\,\frac{mol\ Protein}{mol\ Leu} \times 132\,\frac{g\ Protein}{mol\ Protein} \times \frac{1}{0.63}\,\frac{g\ DCW}{g\ Protein} \times 0.54\,\frac{g\ C}{g\ DCW}$$

$$= 1.46\times10^{-10}\,\frac{g\ C}{min}$$

$$BP = 1.46\times10^{-10}\,\frac{g\ C}{min} \times 1440\,\frac{min}{d} \times \frac{1}{1.5\ mL} \times 1000\,\frac{mL}{L} = 1.40\times10^{-4}\,\frac{g\ C}{L\ d}$$

$$= 1.40\times10^{-4}\,\frac{g\ C}{L\ d} \times 10^{6}\,\frac{\mu g\ C}{g\ C} \times \frac{1}{12}\,\frac{\mu mol\ C}{\mu g\ C} = 11.7\,\frac{\mu mol\ C}{L\ d}$$

Notes

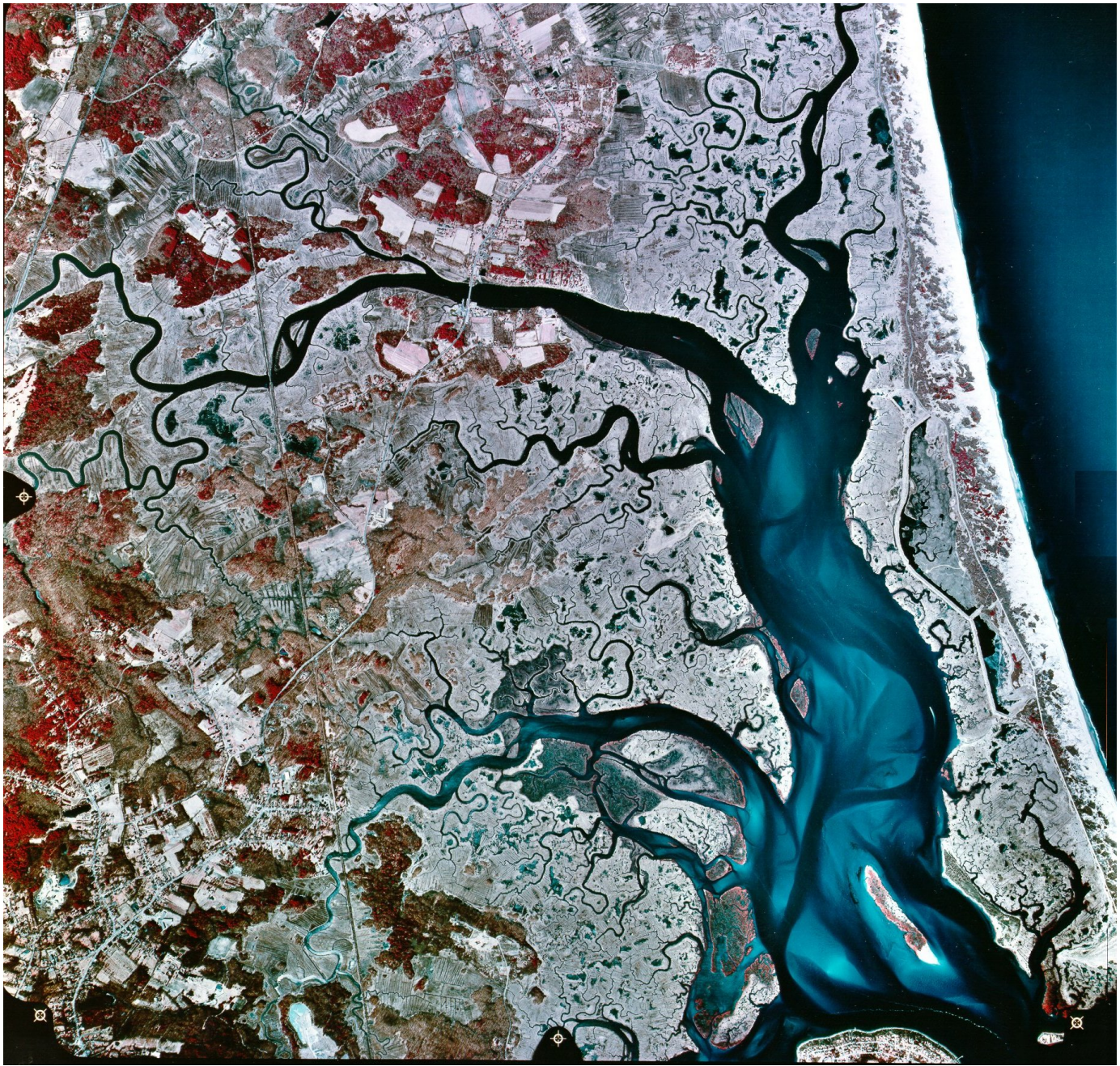
- Similar procedure can be done using thymidine incorporation into DNA.
- Centrifugation plus rinsing (or filtration plus washing) is used to separate added Leu from bacterial incorporated Leu.
- A killed control is run under identical conditions to account for abiotic adsorption of Leu onto particulate matter.
- Isotope dilution due to extracellular matrix may not be insignificant in eutrophic environments.
- Conversion factors are dependent on cellular conditions, and values reported are controversial. Often, only Leu incorporation is reported (i.e., not converted into cell biomass).

Safety Notes

- Wear gloves had lab coats at all times
- No open-top shoes
- Wear safety glasses (TCA is an acid)
- Keep all radioactive materials on your trays and conduct lab work on trays
- Eject pipette tips onto kimwipes on your tray
- All liquid waste must be pored into jugs labels for ^{14}C waste
- All solids that come into contact with ^{14}C must be disposed of in ^{14}C solid waste bins
- If you spill a sample containing ^{14}C, lets us know so it can be properly cleaned.

Plum Island Estuary

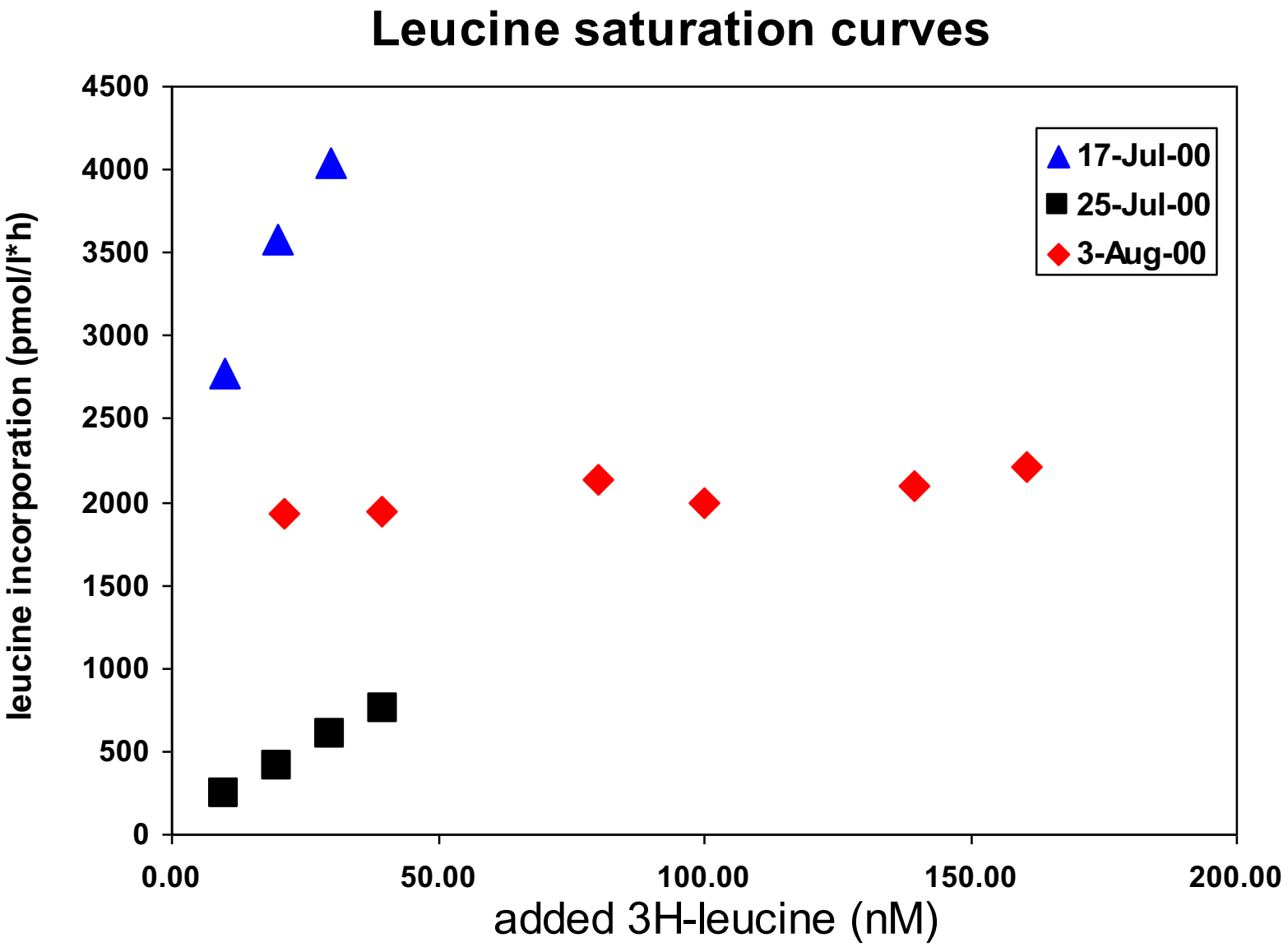
Land Use Change



Sea Level Rise

Example: Isotope Dilution

Byron Crump



Example: Plum Island Estuary Survey

(Byron Crump)