Bacterial growth efficiency... controls and implications

- Bacterial Growth Efficiency
- Regulation of BGE on cell level
- Regulation of BGE on community level
- bacterial carbon demand

Bacterial community response to varying DOM quality... who's using what?

The Microbial Loop (Pomeroy 1974; Azam et al. 1983)
- salvage pathway in which bacterioplankton repackage and reincorporate DOC back into the aquatic food web

Classical Food Chain with the Microbial Loop
**Microbes ...open systems**

**DOC** → **Nutrients** → **Bacterioplankton** → **CO₂**

**Link:** At high bacterial growth efficiencies a significant amount of carbon can be passed on to higher trophic level

**Sink:** At low bacterial growth efficiencies a significant amount of carbon is respired and little is available to higher trophic levels

Ducklow et al 1986
Nutritional requirements for anabolism:

1. **Energy Source**

   - **Chemicals**
     - Organic chemicals (glucose, acetate, etc.)
     - Inorganic chemicals (H₂, H₂S, Fe²⁺, NH₄⁺, etc.)

   - **Chemolithotrophs**
     - Photosynthesis (glucose + O₂ → CO₂ + H₂O + ATP)
     - Respiration (H₂ + O₂ → H₂O (light) → ATP)

   - **Chemooorganotrophs**

2. **Carbon source**

   - CO₂ - autotroph
   - Organic C - heterotroph

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**Laws of Thermodynamics**

1. *Energy can neither be created nor destroyed in the universe.*

   - **Photosynthesis**
     - cyanobacteria

   - **Respiration**
     - Heterotrophic bacteria

   Energy conservation --- formation of ATP
Energy Conservation: All organisms on this planet generate their ATP using one of three processes.

Substrate level Phosphorylation or Fermentation-
- Anaerobic
- e acceptor is an organic molecule
- compound it not completely oxidized

Oxidative Phosphorylation -
- oxidation with external e acceptor
- substrate is completely oxidized to CO2
- uses Electron transport system and proton motive force

Photophosphorylation -
- light energy generates proton gradient and proton motive force

Proton motive force: e⁻ carriers are oriented in membrane that separate protons from electrons

Outside positive & acidic

Inside negative & alkaline

Pg 135-137 in book
2. In all processes or reactions, some of the energy involved irreversibly loses its ability to do work.

Growth efficiency (yield) - is the quantity of biomass synthesized per unit of substrate assimilated

Bacterial Growth Efficiency = BP / (BP+BR)

- BP easy measure to make (accuracy ???)
- --BR more important but hard measure to make

Theoretical maximal Growth Efficiency
Maximal BGE = 88% which corresponds to 1 mmol ATP used = 32 mg of dry biomass produced

Partitioning of ATP use

<table>
<thead>
<tr>
<th>22% transport of ions and monomers</th>
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<td>78% Protein Synthesis RNATurnover</td>
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In the Real world

- Growth efficiency extremely variable
- always substantially lower
Anabolism and catabolism are decoupled (del Giorgio and Cole)

Cells will expend energy in ways that are independent from biomass production:

- **Overflow metabolism**
  - excess energy is released as DOM or large capsule

- **Maintenance** - allocation of energy to non growth reactions
  - fixes things that are broken
  - maintains cellular & functional integrity

**Heissenberger 1996**

**Bacterial Growth Efficiency (BGE) - is an integration of all the anabolic and catabolic processes needed to meet the cells energy budget**

Partitioning of E for biosynthesis is variable

E demand for maintenance remains constant

**BGE = BP/(BP+BR)**
How do we measure BGE in the field?

Several different approaches ....but all have their own set of pros and cons

Methods of Estimating BGE:

I. Early studies used $^{14}$C radioactive tracers

\[ \text{BGE} = \frac{\Delta^{14}\text{C biomass}}{\Delta^{14}\text{C biomass} + \Delta^{14}\text{CO}_2} \]

Led to BGE estimate of 40-60%
**Methods of Estimating BGE:**

II. Measure respiratory gasses produced over time and relate to estimate of BP

1. Measure short BP via radioisotope incorporation

2. Seal replicate filtrates in gas tight bottles

3. Sacrifice bottles over time and measure change in gas (O$_2$ or CO$_2$) to estimate respiration

\[
BGE = \frac{BP}{BP + BR}
\]

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**III. Seawater Culture Bag Experiments**

- Tri-laminate opaque, gas tight incubation bag
- Gravity filter through 142 mm 0.8 µm filter
- 30 - 40 liter bag
- Samples collected
  - Bacterial abundance
  - DIC or O$_2$

\[
BGE = \frac{\Delta BB}{\Delta BB + \Delta DIC}
\]
Methods of Estimating BGE:

III. Measure change in DOC and bacterial biomass over time.

\[ \text{BGE} = \frac{\Delta \text{BB}}{\Delta \text{DOC}} \]

or

\[ \text{BGE} = \frac{0.037 + 0.65 \times \text{BP}}{1.8 + \text{BP}} \]

Del Giorgio and Cole, 1998

Model constructed from 237 paired observations of BR and BP

\[ \text{BGE} = \frac{(0.037 + 0.65 \times \text{BP})}{(1.8 + \text{BP})} \]

Del Giorgio and Cole, 1998
I. Regulation of BGE... complicated

A. Temperature

Fig. 1. Scatter plot of bacterial growth efficiency as a function of temperature for bacterioplankton from polar, temperate, and tropical oceans. Bacterial growth efficiency was determined from concurrent measurements of bacterial production and DOC uptake (open symbols) or of bacterial production and size-fractionated O\textsubscript{2} uptake (filled symbols). The ordinary least squares regression (regression line shown) between temperature \(T\) and bacterial growth efficiency (BGE) is BGE = 0.37\(T\) + 0.04\(T\) - 0.0010\(T\) + 0.002\(T\) \(r^2 = 0.54, n = 107, F = 84.27, P < 0.001\). Values in brackets are the \(95\%\) confidence intervals of the regression parameters.

Rivkin and Legendre 2001

North Sea

Fig. 8. Seasonal dynamics of bacterial growth efficiency (BGE) calculated as BGE = BPP \(\times\) BPP / BDI \(\times\) 100 from April to December. Means (+SD, N = 9 to 21 estimations for the different months).

Reinthaler and Herndl 2005

Temporal Variability in BGE

Ross Sea (change in temp < 4°C)

Fig. 13. Variations in bacterial growth efficiency (BGE) with temperature in the Ross Sea (changes in temperature < 4°C) in A. bounded (closed squares) and B. unbounded (open squares) areas. The open squares indicate values indicated by different years, while the closed squares indicate values from the same year. The error bars represent the standard deviation.

Carlson and Hansell 2003
I. Regulation of BGE

B. Substrate Stoichiometry

Del Giorgio and Cole 1998

Goldman and Dennett 2000

Multiple C&N sources -- relationship falls apart

D. Supply vs nature of organic matter

E. Energetic Cost of:

• Uptake and Transport - at low concentration of substrate may scavenge other substrates

• Enzymatic breakdown: Most DOC is polymeric and needs to be broken down

Middleboe and Sondergaard (1993)
II. Regulation of BGE on the Community Level:

Factors that affect community BGE are:

**Predation:** Selective removal of rapidly growing bacteria may impact mean BGE of assemblage.

- **viral infection:** Lytic loop may display low BGE decrease BGE of non infected cells

*Reinthaler et al. 2005*

- **Phylogenetic composition:** Little known at this time

Reinthaler and colleagues found:

- BP decreased with bacterioplankton richness
- BR was variable along richness gradients
- This resulted in an inverse relationship between BGE and richness

*Reinthaler et al. 2005*
Interactions with other Metabolic Pathways:

Light harvesting for photoheterotrophy could have impact on BGE

Proteorhodopsin - impact growth efficiency??

Pelagibacter ubique

Marine Flavobacteria

Giovannoni et al. 2005

Gomez-Consarnau et al 2007
Allocation of carbon and energy in marine bacteria depends on many factors... difficult if not impossible to place variation of BGE on a single variable.

Ducklow and Carlson 1992
Biddanda et al. 2001

**Figure 3.** Conceptual diagram demonstrating the relationship between environmental stressors or environmental "hostility" and the partitioning of energy within a bacterial cell, the resulting bacterial growth efficiency (BGE), and cell-specific respiration. As environmental hostility increases, more energy is partitioned into maintenance energy (EM). Thus, bacterial growth efficiency decreases and cell-specific respiration (SP) increases. Some combination of both physical (temperature, pH, salinity) and chemical (toxins, substrate availability) factors contribute to environmental hostility.

**Figure 3.** Relationship between BGE and trophic status.

Ducklow and Carlson 1992
Biddanda et al. 2001
• BGE in marine systems < 0.4
• BGE increases with increasing BP
• decrease in BGE from coast to open ocean and is likely related to overall system productivity

Bacterial Carbon Demand - The amount of carbon processed by bacteria to produce given biomass. .... Gross bacterial production

\[ \text{BCD} = \frac{\text{BP}}{\text{BGE}} \]

When you calculate BCD you are accounting for the amount of DOC that turns into biomass and the amount of CO₂ produced …this equals the total amount of DOC consumed
Integrated primary production and net bacterial production

Integrated primary production and bacterial C demand at BATS
II. Regulation of BGE on the Community Level:

**Figure 7** Two alternative descriptions of the functions of bacterioplankton assemblages. The left panel assumes that production (P) and respiration (R) are homogeneous among all cells. BGES is related to the average growth rate. The right panel assumes that there are two distinct pools of cells: one highly active and the other relatively inactive. Each pool is characterized by distinct P and R, and BGES, so the respiration growth efficiency of the assemblage is dependent on the relative size of the active and the inactive pools. Numbers are for purposes of example only.

del Giorgio and Cole 1998
Microbial Communities

![Microbial Communities Image](image)

Courtesy of Steve Giovannoni

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

<table>
<thead>
<tr>
<th>16S rDNA amplicons</th>
<th>Community fingerprint</th>
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<td>![Amplicon Image]</td>
<td>![Community Fingerprint Image]</td>
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Example of Community structure shift in seawater culture amended with Gluc, NH4, PO4

Using MICRO-FISH to investigate who’s using what

FIG. 1: Micrograph of bacteria covered by MICRO-FISH (A) DAPI stained bacteria (UV emission). Dark spots surrounding cells are silver grains deposited in photographic emulsion around cells that took up a mixture of stained fish mucus nuclei. Less than 40% of cells in the sample were killed controls (not shown). (B) Bacteria hybridized with Cy3 labeled oligonucleotide probe 262/289 for eubacteria (green emission). Cells with bound probe fluoresce yellow. Magnification, X400. Cottrell and Kirchman 2000
Data points failing above the 1:1 line indicate phylogenetic groups enriched in the portion of the assemblage consuming the compounds.