

Bacterial growth efficiency... controls and implications

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

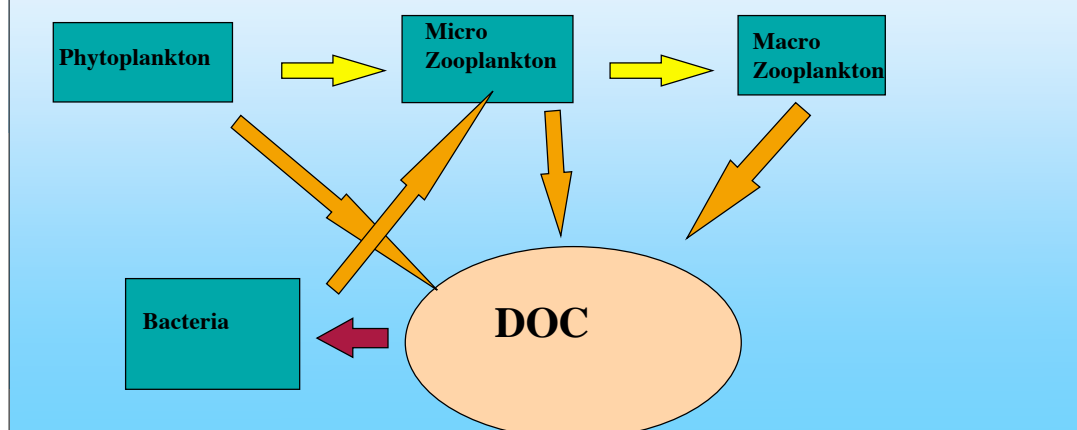
Bacterial community response to varying DOM quality...who's using what?

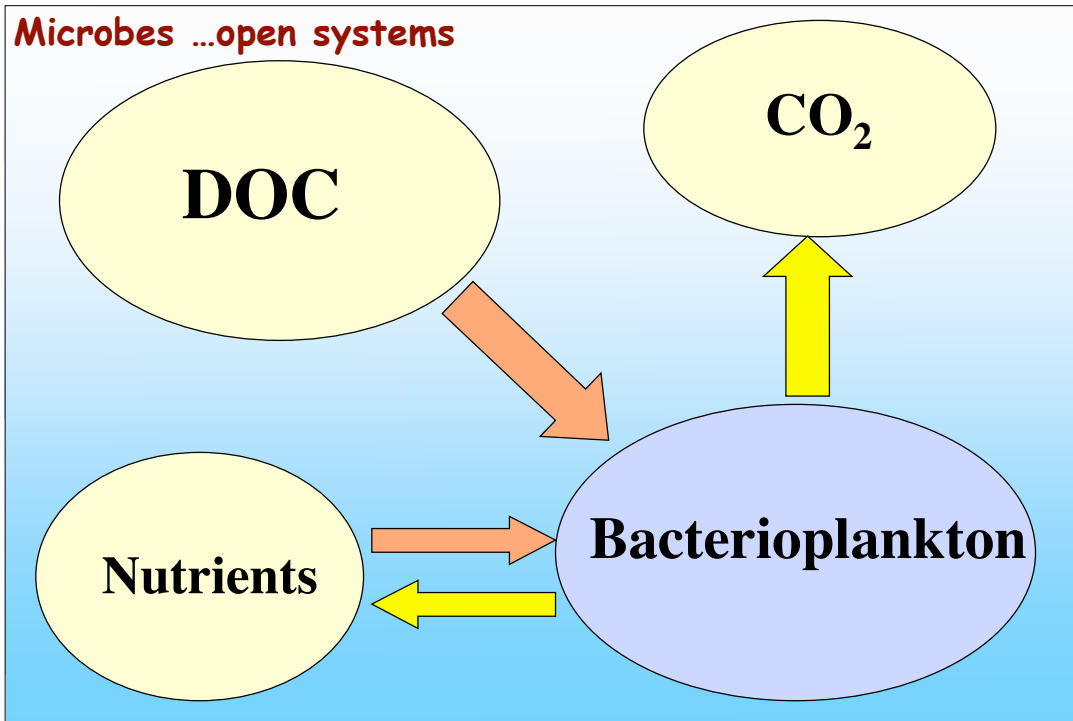
10 µm

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





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

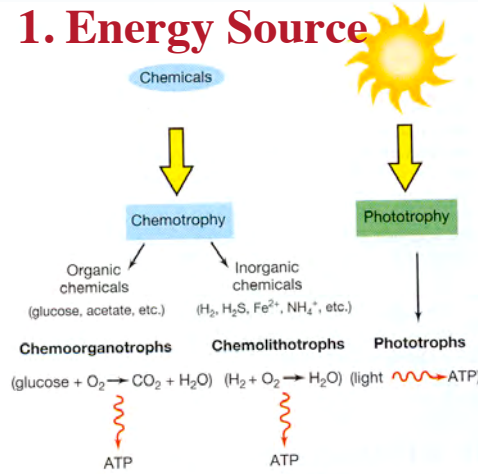


Figure 2.8 Metabolic options for obtaining energy. The organic and inorganic chemicals listed here are just a few of the many different chemicals used by various chemotrophic organisms. Oxidation of the organic or inorganic chemicals yields ATP in chemotrophic organisms while conversion of solar energy to chemical energy (again, in the form of ATP) occurs in phototrophic organisms.

Brock Biology of microorganisms

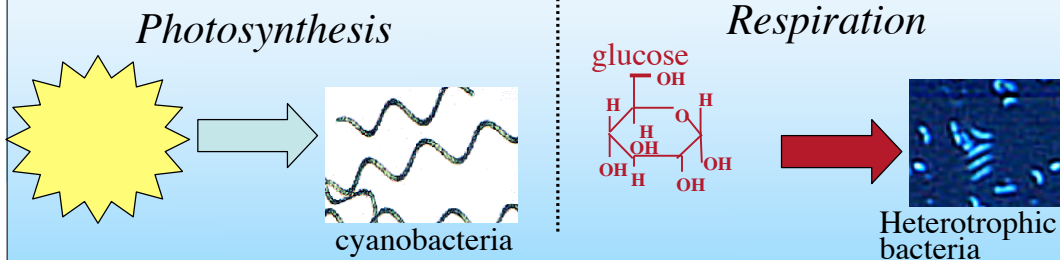
2. Carbon source

CO₂ - autotroph

Organic C - heterotroph

Laws of Thermodynamics

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



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 is not completely oxidized

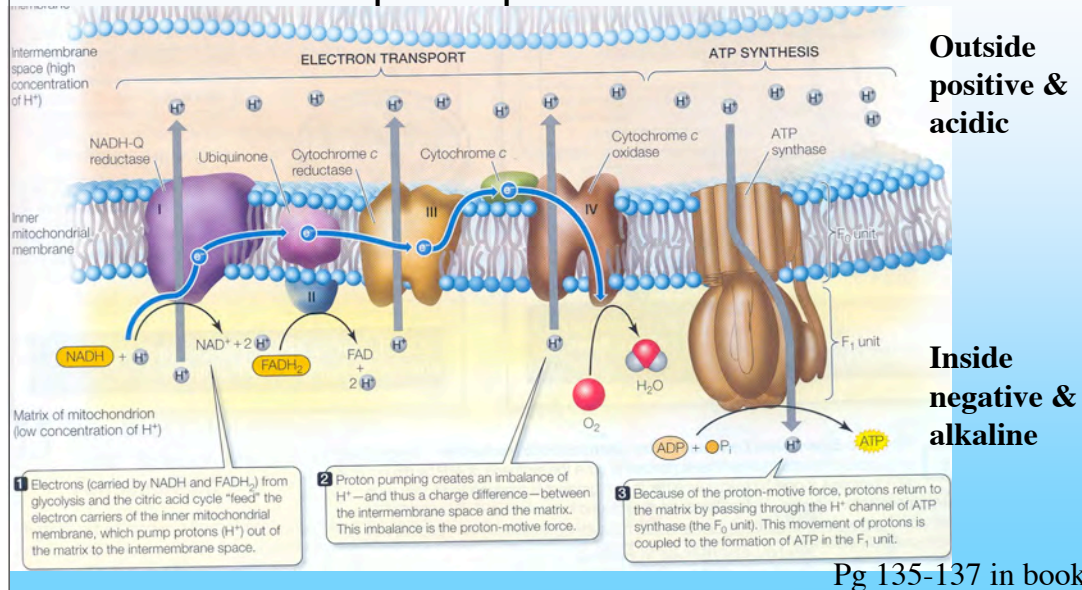
Oxidative Phosphorylation -

- 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



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

$$\text{Bacterial Growth Efficiency} = \text{BP} / (\text{BP} + \text{BR})$$

-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

22% transport of ions and monomers

78% Protein Synthesis
RNA turnover

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:

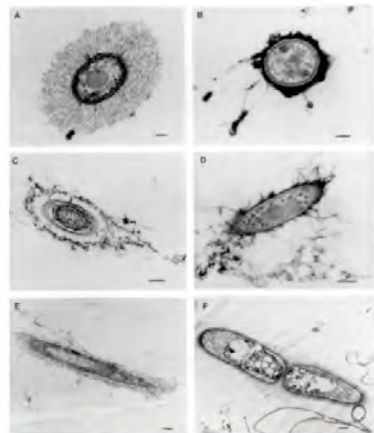


Figure 2. TEM images of the most common types of capsules found in free-living marine bacteria. Note that the morphology of the capsules varies considerably. D and F (a dividing cell) were observed near marine snow particles. Scale bars: A and B—100 nm, C to F—200 nm. TEM pictures adapted from Heissenberger et al. (1996)

• Overflow metabolism

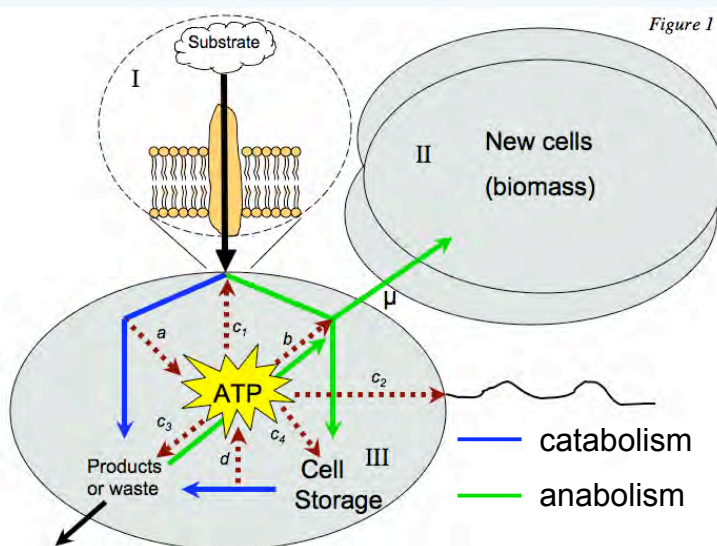
excess energy is released as DOM or large capsule

• 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

↑ CAT: ANAB ↓ BGE

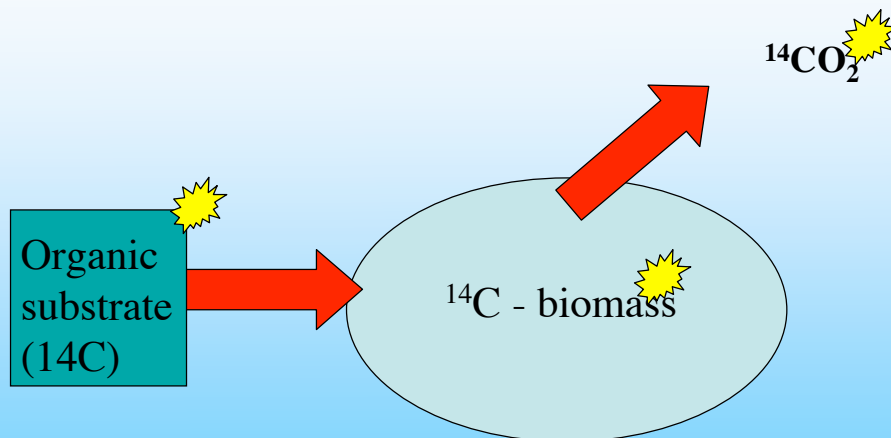
$$BGE = BP/(BP+BR)$$

How do we measure BGE in the field?

Several different approachesbut 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

0.8 filter

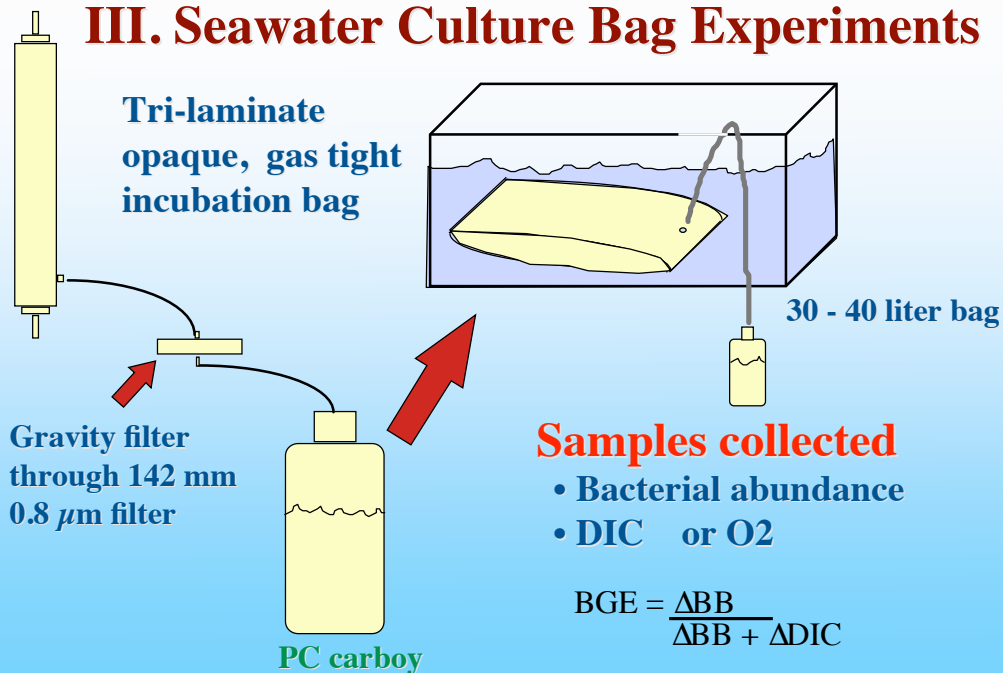
2. Seal replicate filtrates in gas tight bottles



3. sacrifice bottles over time and measure change in gas (O_2 or CO_2) to estimate respiration

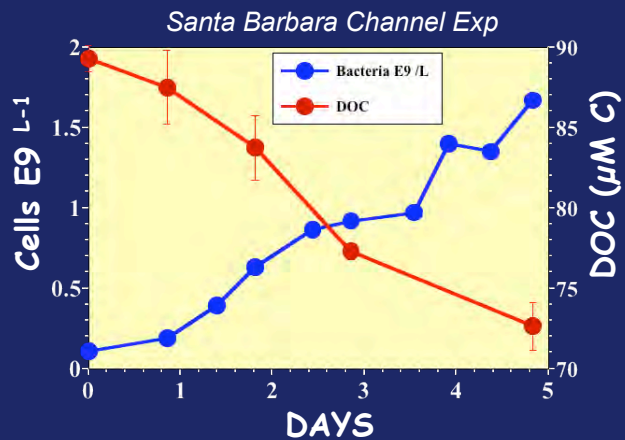
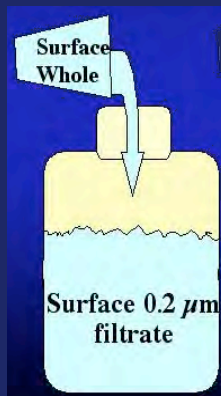
$$BGE = BP / (BP + BR)$$

III. Seawater Culture Bag Experiments



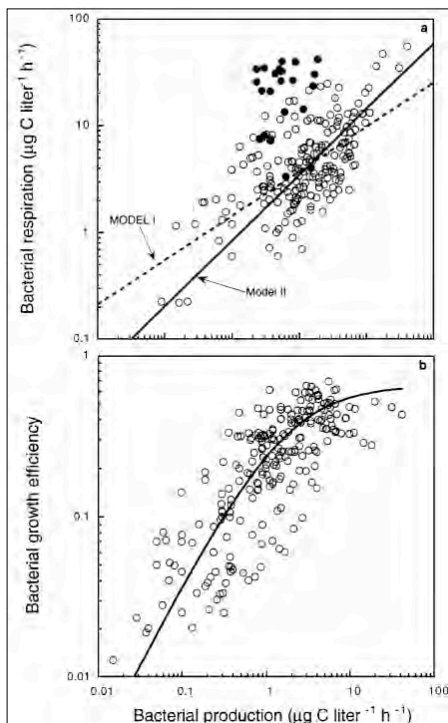
Methods of Estimating BGE:

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



$$\text{BGE} = \text{BP} / (\text{BP} + \text{BR}) \text{ or}$$

$$\text{BGE} = \frac{\Delta \text{BB}}{\Delta \text{DOC}}$$



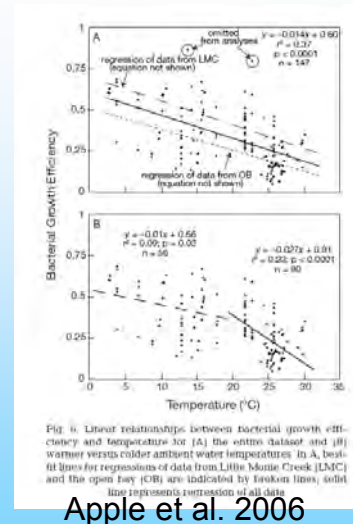
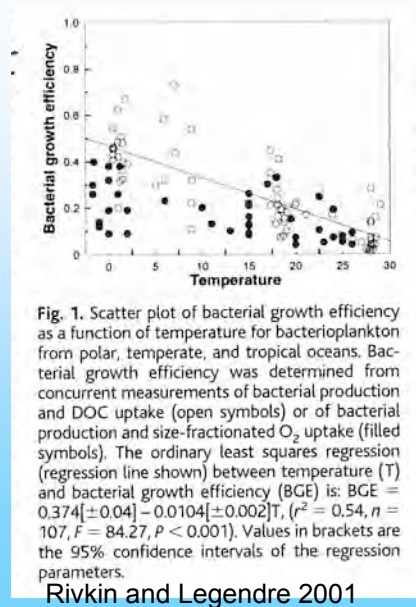
Model constructed from 237 paired observations of BR and BP

$$\text{BGE} = (0.037 + 0.65 \text{ BP}) / (1.8 + \text{BP})$$

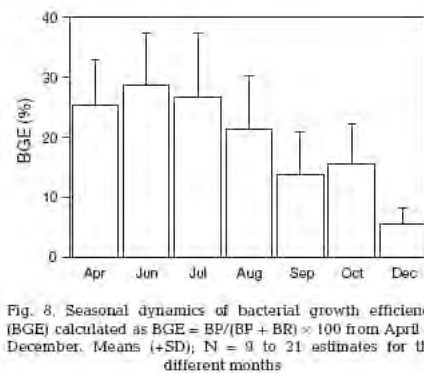
Del Giorgio and Cole, 1998

I. Regulation of BGE... complicated

A. Temperature

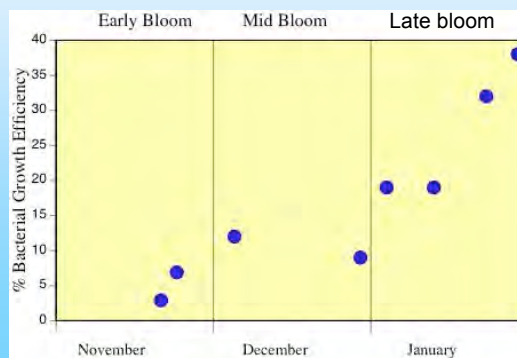


North Sea



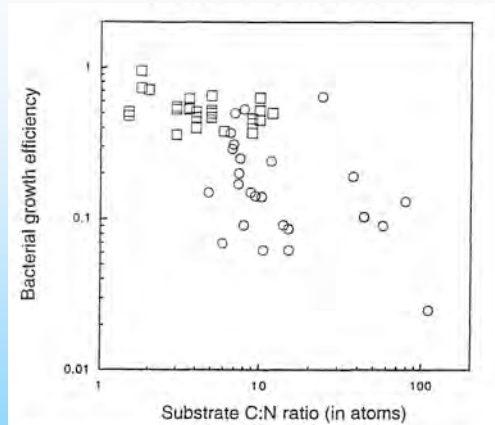
Temporal Variability in BGE

Ross Sea (change in temp $< 4^\circ\text{C}$)



I. Regulation of BGE

B. Substrate Stoichiometry



Del Giorgio and Cole 1998

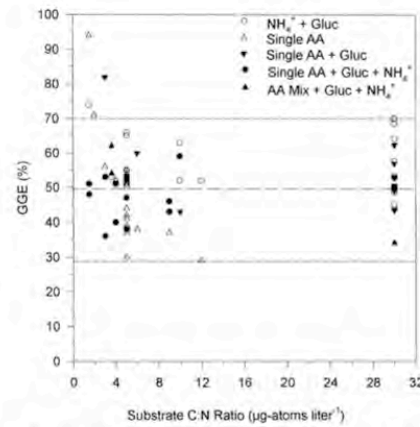


Fig. 10. Summary of GGE data as a function of substrate C:N ratio from current and previous experiments (Goldman and Dennett 1991; Goldman et al. 1987) with natural assemblages of marine bacteria grown with different carbon and nitrogen substrates in batch and continuous cultures. Average GGE from Table 3 is broken line and represents all data encompassed by dashed lines.

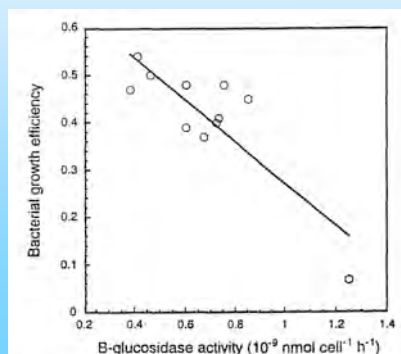
Goldman and Dennett 2000

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)

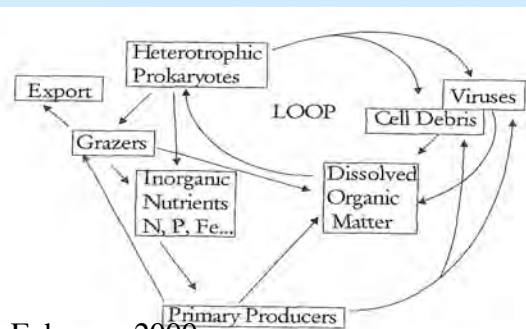
II. Regulation of BGE on the Community Level:

Factors that affect community BGE are:

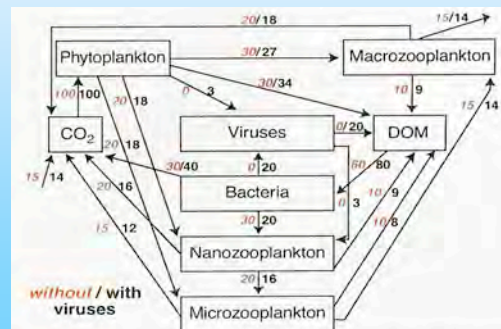
Predation: Selective removal of rapidly growing bacteria may impact mean BGE of assemblage.

- **viral infection**- lytic loop may display low BGE

decrease BGE of non infected cells



Fuhrman 2000



Fuhrman 1999

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

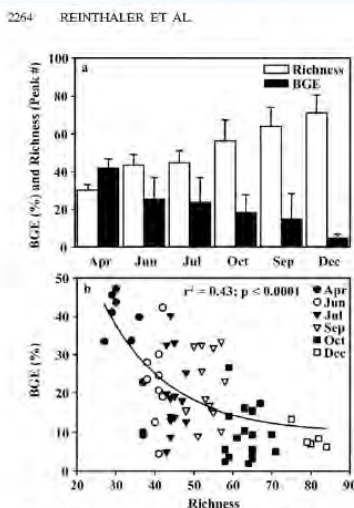


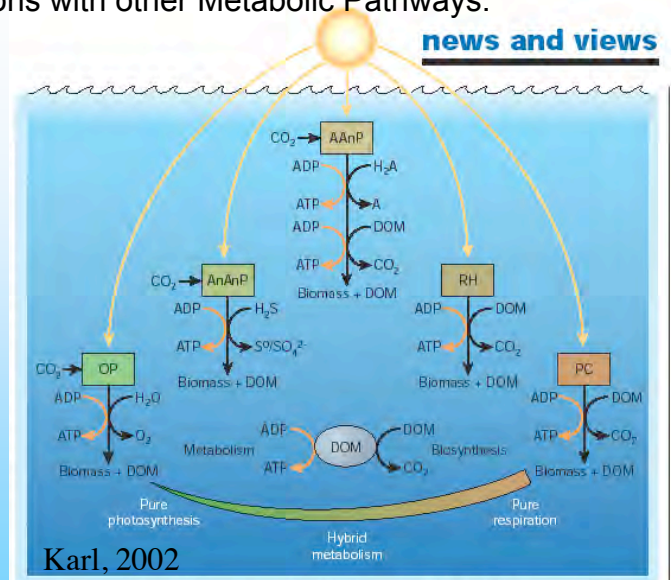
FIG. 5. Dynamics of bacterial growth efficiency (BGE) and richness during the seasonal cycle in the southern North Sea. (a) Monthly averages of BGE and bacterioplankton richness measured by T-RFLP. Error bars indicate standard deviations of the means ($n = 8$ to 19). (b) Relationship between BGE and bacterioplankton richness, with months indicated by different symbols.

Reinthal et al. 2005

Reinthal and colleagues found:

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

Interactions with other Metabolic Pathways:



Light harvesting for photoheterotrophy could have impact on BGE

Proteorhodopsin - impact growth efficiency??

Pelagibacter ubique

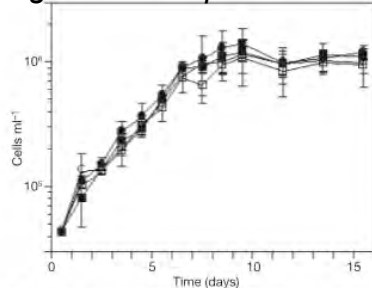
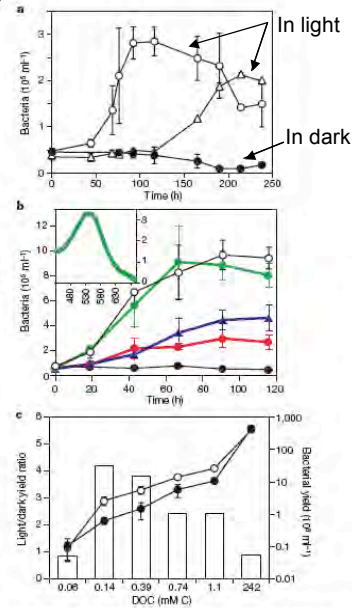


Figure 4 | Growth characteristics of HTCC1062. Bacteria were grown in seawater supplemented with N and P (LNHM) with no added organic carbon, on a diurnal light cycle (open symbols) or in darkness (closed symbols) under high-range light intensity (circles, $680 \mu\text{mol m}^{-2} \text{s}^{-1}$) or middle-range light intensity (squares, $250 \mu\text{mol m}^{-2} \text{s}^{-1}$). Error bars show standard deviation for triplicate experiments. No difference was observed for replicates with and without added retinal (data not shown).

Giovannoni et al. 2005

Marine Flavobacteria



Gomez-Consarnau et al 2007

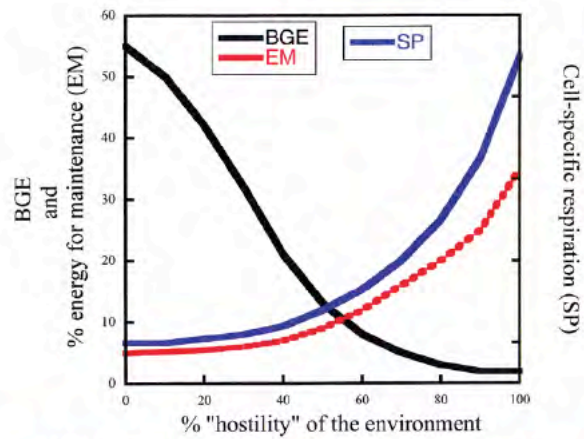
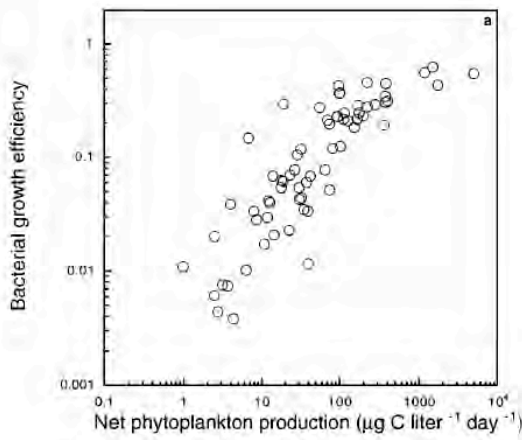


Figure 3. Conceptual diagram demonstrating the relationship between environmental stressors or environmental "hostility" and the partitioning of energy within a bacterial cell, the resulting bacterial growth efficiency (BGE), and cell specific respiration. As environmental hostility increases, more energy 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

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

Relationship between BGE and trophic status



Ducklow and Carlson 1992

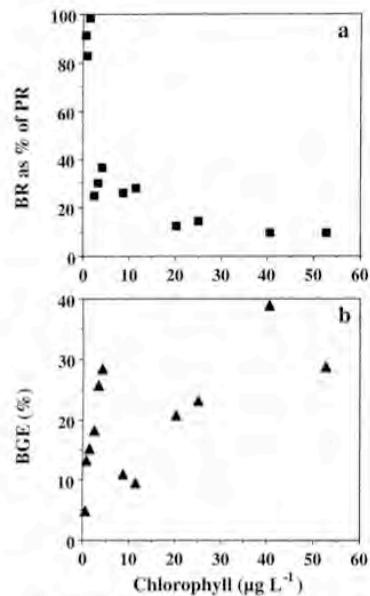


Fig. 3. Relationship of (a) bacterial respiration (BR) as a percent of planktonic respiration (PR) to chlorophyll concentration ($n = 12$, $r^2 = 0.420$, $p < 0.05$) and (b) bacterial growth efficiency (BGE; $n = 12$, $r^2 = 0.414$, $p < 0.05$) to chlorophyll concentration.

Biddanda et al. 2001

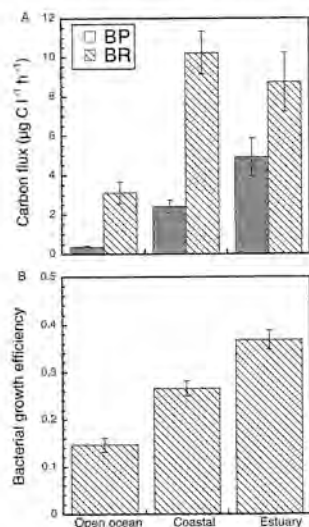


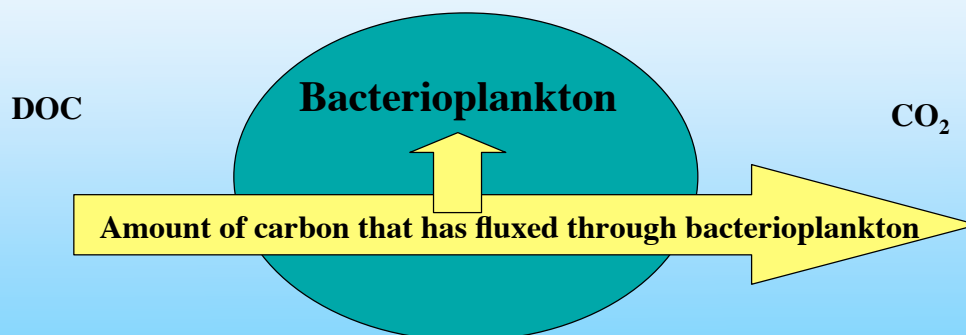
Figure 3. Summary of literature data on direct measurements of bacterial metabolism and growth efficiency in natural aquatic systems, from Table 1. (A) Bacterioplankton production (BP) and respiration (BR) averaged by system (open-ocean, coastal, and estuarine systems). (B) The resulting average bacterial growth efficiency ($BGE = BP / (BP + BR)$) for each system. Bars represent 1 standard error.

Del Giorgio 2000

- 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

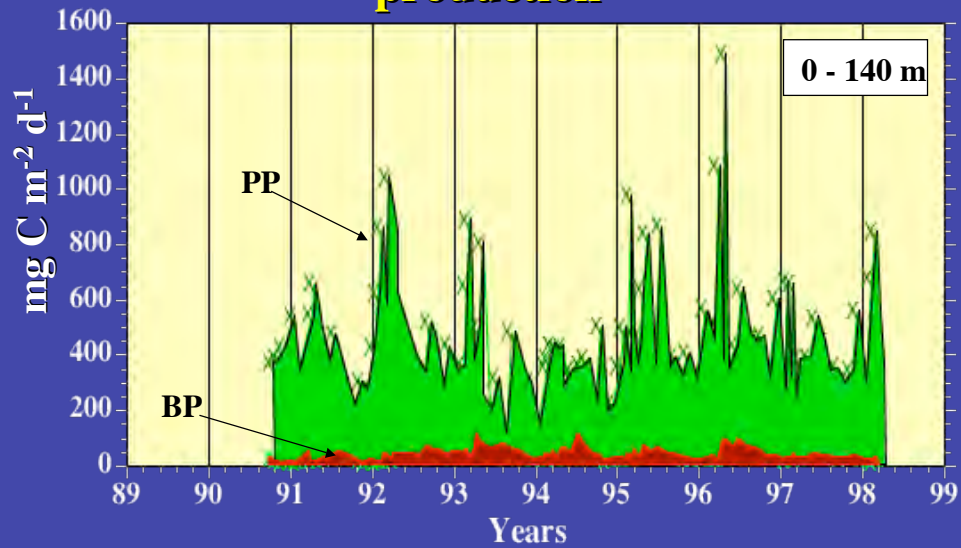
Bacterial Carbon Demand - The amount of carbon processed by bacteria to produce given biomass. **Gross bacterial production**

$$BCD = BP / BGE$$

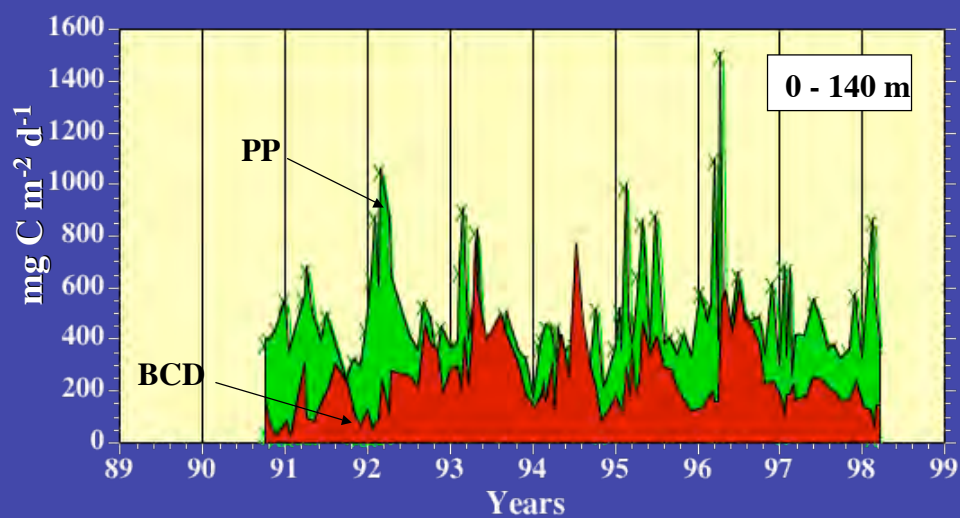


When you calculate BCD you are accounting for the amount of DOC that turns into biomass and the amount of CO₂ produced ...this equals the total amount of DOC consumed

Integrated primary production and net bacterial production



Integrated primary production and bacterial C demand at BATS



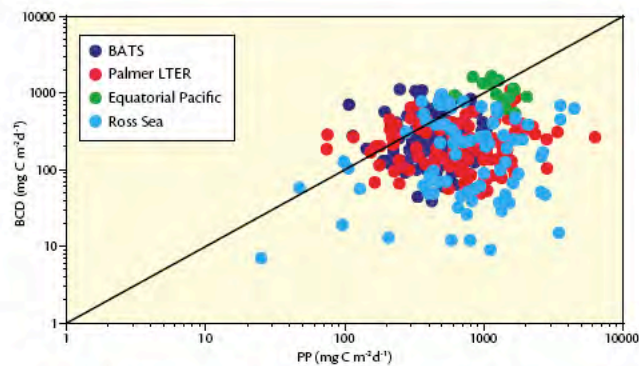


Figure 4. Relationship between integrated bacterial carbon demand (BCD) and primary production (PP) within the euphotic zone of representative ocean sites. All data are derived from paired measurements of bacterial production and PP integrated within the euphotic zone from each study site. A common bacterial growth energy of 0.1 was used to estimate BCD. The black line represents the 1:1 line. Data points that lie above this line indicate that bacterial carbon demand was greater than local primary production at the time of sample collection. The Bermuda Atlantic Time-series Study (BATS) data represent monthly values from 1991–2003 ($n = 155$; see Steinberg et al., 2001 for details; data available at <http://bats.bbr.edu/>). Paired BP and PP from the Equatorial Pacific ($n=16$) and Ross Sea, Antarctica, ($n=77$) were calculated according to Ducklow (1999) (data available at <http://usjgofs.whoi.edu/jgdir/jgofs/>). All data from the Palmer Peninsula, Antarctica, ($n=112$) provided by H. Ducklow and the Palmer LTER program.

II. Regulation of BGE on the Community Level:

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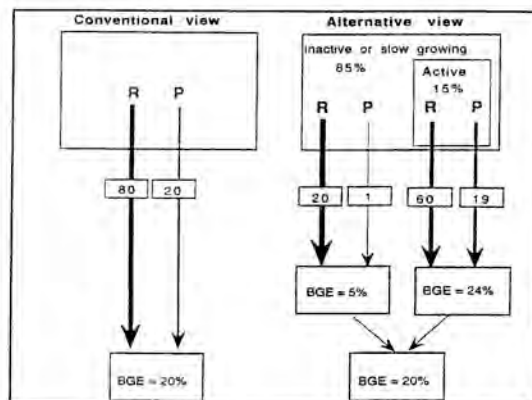


Figure 7 Two alternative depictions of the functions of bacterioplankton assemblages. The left panel assumes that production (P) and respiration (R) are homogeneous among all cells. BGE is related to the average growth rate. The right panel assumes that there are two distinct pools of cells, one highly active and the other relatively inactive. Each pool is characterized by distinct P, R, and BGE, so the resulting 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

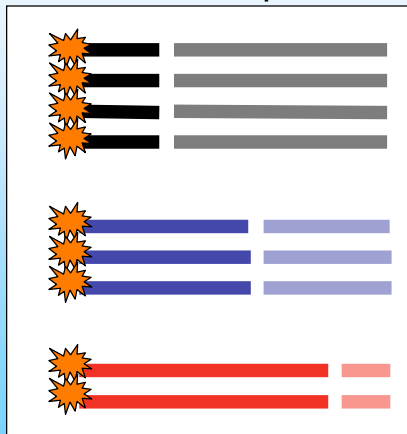
Microbial Communities



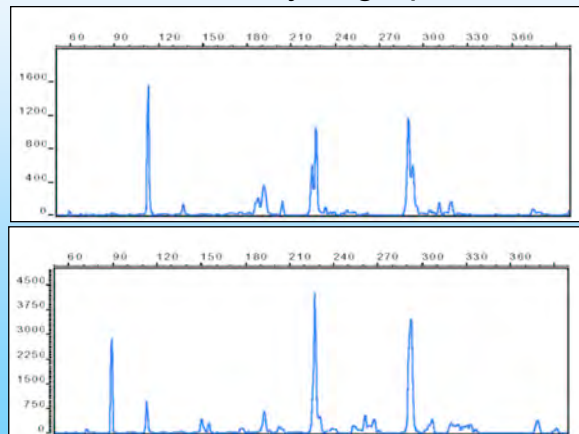
Courtesy of Steve Giovannoni

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

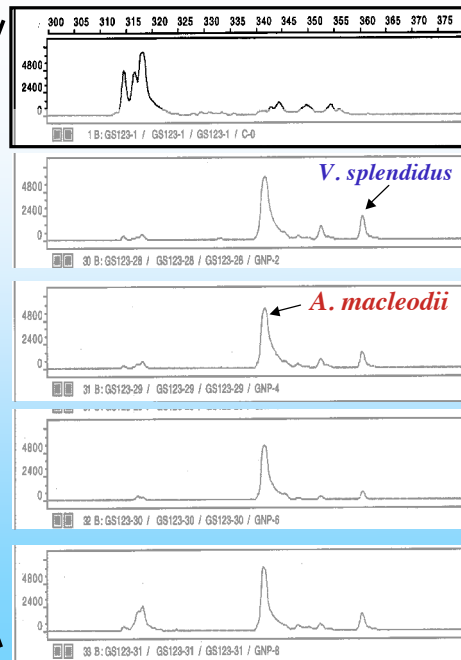
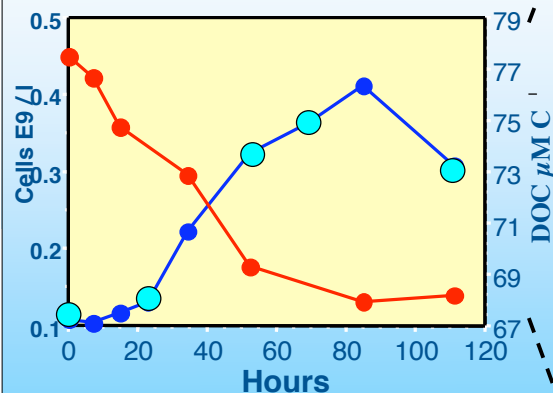
16S rDNA amplicons



Community fingerprint



Example of Community structure shift in seawater culture amended with Gluc, NH₄, PO₄



Using MICRO-FISH to investigate who's using what

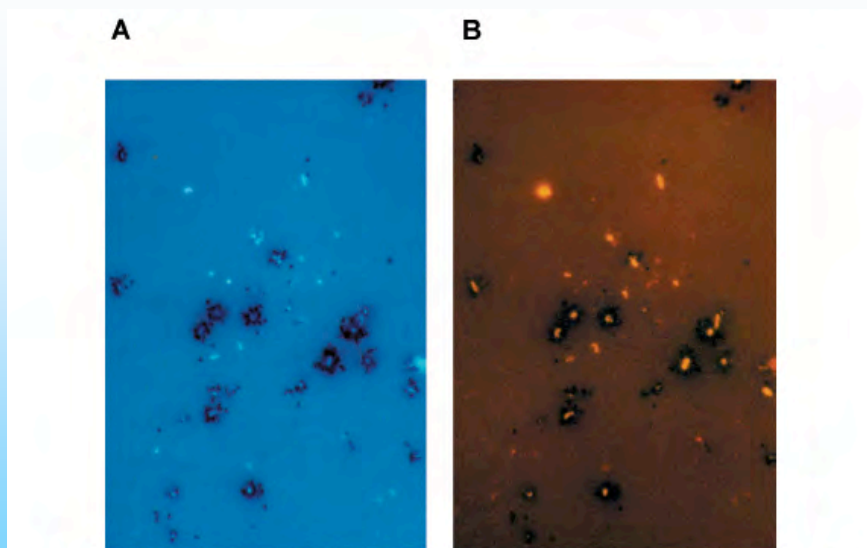


FIG. 1. Micrograph of bacteria assayed by MICRO-FISH. (A) DAPI-stained bacteria (UV excitation). Dark spots surrounding cells are silver grains deposited in photographic emulsion around cells that took up a mixture of tritiated free amino acids. Less than 0.6% of cells in formaldehyde-killed controls had silver grains. (B) Bacteria hybridized with Cy3-labeled oligonucleotide probe Eub338 for eubacteria (green excitation). Cells with bound probe fluoresce yellow. Magnification, $\times 1,350$.

Cottrell and Kirchman 2000

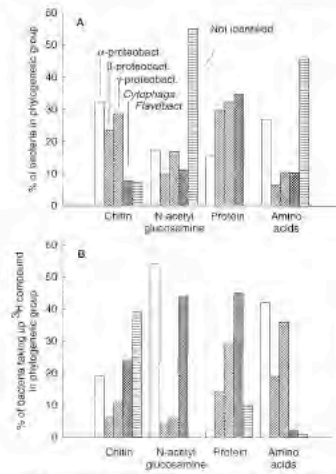


FIG. 2. Community composition and consumption of chitin, NAG, protein, and amino acids by the major phylogenetic groups of bacterioplankton in the Rosebank inlet, assessed by MICRO-FISH. (A) Composition of bacterioplankton communities in incubations containing tritiated compounds. (B) Relative abundance of phylogenetic groups of bacteria consuming various tritiated compounds. Less than 3% of the cells were gram positive. Cells binding none of the group-specific probes are indicated (Not identified). Percentages were calculated relative to total bacteria counted by using DAPI, although the substratum probe (Eub338) detected on average 60% of bacterial abundance: proteobact, proteobact, Flavobact, Flavobact.

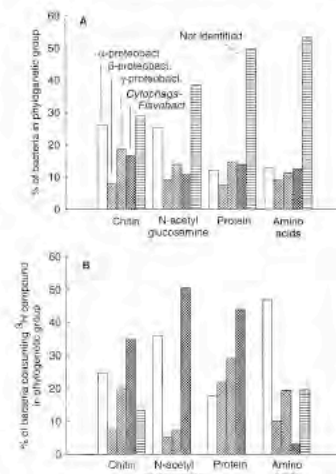


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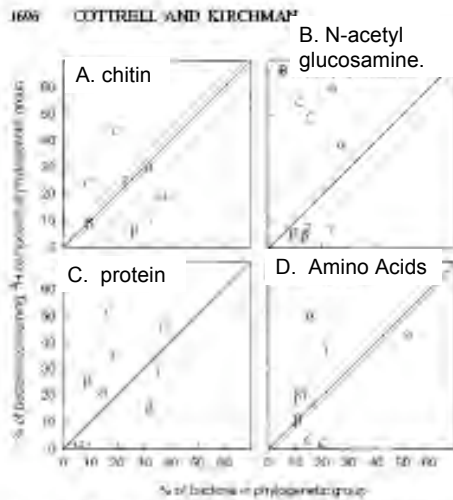


FIG. 4. Relationship among the phylogenetic classification of bacteria consuming chitin (A), NAG (B), protein (C), and amino acids (D) versus phylogenetic classification of cells identified as substrates. Bacteria were classified by using rRNA-binding oligonucleotide probes specific for α-proteobacteria (α), β-proteobacteria (β), γ-proteobacteria (γ), and the Cytophaga-Flavobacter group (C). Data points falling above the 1:1 line indicate phylogenetic groups enriched in the portion of the assemblage consuming the compounds. Results are from control (Fig. 2) and substrate (Fig. 3) incubations. Percentages were calculated relative to the numbers of cells identified as substrates with the Eub338 probe.

Data points failing above the 1:1 line indicate phylogenetic groups enriched in the portion of the assemblage consuming the compounds