Bacterial production and the cycling of DOC

Why does DOC accumulate in surface seawater?
The chemical characterization of “reactive” DOC?

How is recalcitrant DOC degraded?

What is bacterial production and how is it measured?

How is bacterial production related to DOC cycling?

What fraction of DOC do bacteria degrade
and where does this DOC come from?

Three classes of reactive organic matter have been recognized through a combination of radiocarbon, inventory, and microbial activity.

Nonreactive DOC = 650 GT C
Reactive DOC = 30-50 GT C
Very reactive DOC = 1-3 GT C
In situ measurements of DOC suggest production/degradation timescales of 1-3 months (seasonal). Can we experimentally show this?

Could this be the result of NUTRIENT LIMITATION? or is the DOM INTRINSICALLY NON-LABILE?

Data from Craig Carlson

Could this be the result of NUTRIENT LIMITATION? or is the DOM INTRINSICALLY NON-LABILE?

What is the chemical composition of DOM?

Data from Craig Carlson
What is “reactive” DOC, why does it accumulate, and what is the rate of gross cycling? (how much carbon goes through the microbial loop?)

Sampling DOC is hard to do... DOC= 0.5-1mg/L C. Salt = 35g/L

Technique transferred from soil and freshwater sciences
Isolation based on chemical properties
Resin affects what is collected
Selects for hydrophobic DOM
5-10% total DOC retained
Very old radiocarbon age
High C/N (40-50)

Cross or tangential flow filtration, Ultra- or nanofiltration
Separation based on size
1 nm pore @ 1 kD
Selects for HMW fraction
about 30-35% TOC (now up to 80%)
C/N = 15
Membrane effects what is collected
Ultrafiltration of high molecular weight DOM (HMWDOM)
>1000 D concentrate; 30% of total DOC

Final product
30% of total DOC
C/N = 15 +/- 2
Nuclear Magnetic Resonance Spectroscopy (NMR)

Can be tuned to different Nuclei of interest (C,N,P…).

Gives information on functional groups which, combined with a knowledge of biochemicals can be used to deduce composition and origin.

Internally quantitative.

\(^{13}\text{C} \text{ Nuclear Magnetic Resonance Spectrum}
\text{ of high molecular weight dissolved organic matter (C/N = 15)}\)
$^{13}$C Nuclear Magnetic Resonance Spectrum
of high molecular weight dissolved organic matter (C/N = 15)
$^{13}$C Nuclear Magnetic Resonance Spectrum
of high molecular weight dissolved organic matter (C/N = 15)

- COOH
- CONH (10%)
- OCO (12%)
- HCOH (55%)
- HCNH (55%)
- CH$_x$ (10%)
## Proximate analysis of algal cells

<table>
<thead>
<tr>
<th>Class</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Lipid</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlorophyceae</strong> (green algae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetraselmis maculata</td>
<td>72</td>
<td>21</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Dunaliella salina</td>
<td>58</td>
<td>33</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td><strong>Chrysophyceae</strong> (golden brown algae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monochrysis lutheri</td>
<td>53</td>
<td>34</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Syracosphaera carterae</td>
<td>70</td>
<td>23</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td><strong>Bacillariophyceae</strong> (brown algae, diatoms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chaetoceros sp.</td>
<td>68</td>
<td>13</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>58</td>
<td>33</td>
<td>10</td>
<td>39</td>
</tr>
<tr>
<td>Coccosidiscus sp.</td>
<td>74</td>
<td>16</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>49</td>
<td>36</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td><strong>Dinophyceae</strong> (dinoflagellates)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphidinium carteri</td>
<td>35</td>
<td>38</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>Exuriella sp.</td>
<td>37</td>
<td>44</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>57</td>
<td>29</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Most POM is protein, and this is probably a large fraction of reactive DOM

Dissolved “free” amino acids have been measured in seawater at 10’s nM

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**HMWDOC, what could it be?**

From our knowledge of cell biochemicals...

C/N….COOH:OCO:HCOH:CHx…

<table>
<thead>
<tr>
<th>Category</th>
<th>C/N</th>
<th>CHx(O):CON</th>
<th>OCO:HCOH:CHx</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteins</strong></td>
<td>C/N = 4,</td>
<td>CHx(O):CON = 3:1</td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td>C maybe N</td>
<td>OCO:HCOH=1:5</td>
<td>CHx:COOH=2:18</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td>C only</td>
<td>CHx:COOH = 2:30</td>
<td>CHx:COH = 2:30</td>
</tr>
</tbody>
</table>
HMWDOC, what could it be?

From our knowledge of cell biochemicals...
C/N...COOH:OCO:HOH:CH₅...

| Proteins | C/N = 4, CH₃(O):CON = 3:1 |
| Lipids   | C only, CH₃:COOH=2:18, CH₅:COH = 2:30 |

unsolved mysteries #1...Carbohydrates are thought to be easily degraded by microbes, so why is the ocean filled with carbohydrates?
unsolved mysteries #2... if the source of DOC is marine plants, and plants change with location, why is the ocean filled with the same type of carbohydrate everywhere?
Carbohydrate 70-90% of surface water HMWDOC

Acid hydrolysis followed by monosaccharide analyses yields 7 major neutral sugars that represent 10-20% of HMWDOC in surface water.

Relative abundance of sugars in HMWDOC

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Woods Hole</th>
<th>Atlantic Bight</th>
<th>Sargasso Sea</th>
<th>Gulf of Mexico</th>
<th>Mikawa Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.25</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.20</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.15</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.10</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

13CNMR spectrum of HMWDOC.
HMWDOM in the deep sea

Sargasso Sea

3 m

2500 m

NPSG

3 m

1800 m

$^{13}$CNMR of XAD and Ultrafiltered DOC in the Pacific Ocean

Surface

4000 m

XAD DOM

UF HMWDOM
A comparison of XAD humic substances and Ultrafiltration HMWDOM in seawater

**XAD Humic substances**
- 5-10% of DOC
- C/N = 40
- lots of aromatic C
- “hydrophobic”
- random assemblage?

**HMWDOM**
- 25-35% of DOC
- C/N = 15
- little aromatic C
- Hydrophilic
- fixed composition?

HMWDOM concentrates from Hawaii
Absorption of light by coastal seawater

- Total water
- DOM (b)
- Particles (c)
Production of LMW highly oxidized DOC with depth in the ocean

DOC + hv \rightarrow LMW photo-oxidation products

Is photochemical degradation the long term sink for recalcitrant DOM?  

DOC + light \rightarrow LMW carbonyls (C=O)
C=O + fluorophore \rightarrow HPLC

Not produced in dark controls, but are produced in sterile controls
Highly oxidized LMW compounds are produced every day in seawater by photo-oxidation. They serve as a substrate for bacteria and therefore a sink for non-reactive DOC.
Some experiments suggest that there is a synergy between photochemistry and microbial utilization of DOM.
Production of very reactive and reactive DOC by phytoplankton and bacteria

Bacterial production is thought to be fueled by very reactive DOC that has a residence time in seawater of hour to days.

The marine food chain circa 1960s (when life was simple)

- CO₂ + Nuts (N, P, Si) → Phyttoplankton (PP) → grazers (g) → Grazers (G)

The arrows represent the carbon flow and nutrient flow.
The marine food web circa 1980
Introduction of the “microbial loop” concept

- **CO₂ + Nuts** (N, P, Si)
- **Phytoplankton** (PP)
- **grazers** (g)
- **Grazers** (G)
- **DOC**
- **Bacteria**
- **grazers** (g)

Carbon flow

Nutrient flow

The marine food web circa >1990s

- **CO₂ + Nuts** (N, P, Si)
- **Phytoplankton** (PP)
- **grazers** (g)
- **Grazers** (G)
- **DOC**
- **Bacteria**
- **grazers** (g)

Carbon flow

Nutrient flow

Viruses

Most DOC accumulation occurs after nutrients are exhausted (bloom crashes). During early log phase growth DOC is being respired by bacteria. Two pools of DOC, reactive and non-reactive (timescale of exp!), are they being produced by two different classes of microbes?

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**Fig. 7.** Stable carbon isotope signal ($\delta^{13}C$) in different carbon pools. Time-courses are shown for DOC (○), DOC (●), POC (●), and extracted bacterial nucleic acids (●) with volume filtered for the extraction of bacterial nucleic acids given beside the symbols. Dashed line indicates the peak of chlorophyll and biomass.

**Fig. 8.** $\delta^{13}C$ plotted as the reciprocal of DOC concentration. Data from determination of DOC with sealed tubes (●) and direct injection (○). Solid line indicates the theoretical mixing curve of no degradation of Woods Hole Harbor DOC occurred during the experiment.
What is bacterial production?

*gross production* = total carbon taken up by bacteria, it is used in the synthesis of new biomass, and includes both the new biomass and carbon respired during biomass production.

*net production* = new bacterial biomass over a set period of time. Bacterial production measurements almost always measure net production.

\[ \text{Gross production} = \text{net production} + \text{respiration} \]

Gross production is also referred to as bacterial carbon demand (BCD) and is related to net production through the growth efficiency or fractional growth yield:

\[ \text{Gross production} = (\text{net production}) \times (\text{growth yield}) \]

How is bacterial production measured?

1) Increase in the number of cells over time
2) \(^3\text{H}-\text{adenine incorporation (DNA synthesis)}\)
3) \(^3\text{H}-\text{thymidine incorporation (DNA synthesis)}\)
4) \(^3\text{H}-\text{leucine incorporation (protein synthesis)}\)

All methods measure net production only (!!!) and require empirically derived conversion factors. Need bacterial growth efficiencies to determine gross production (BCD)
### Measuring the uptake of DFAA using tritiated AA

1. **Seawater (DFAA)**
   - Measure total DFAA (typically 30-50 nM)

2. 1) Add 0.1-1 nM tritiated (*) AA
    2) Incubate 1-4 hr

3. **Particulate *AA**
   - count filter
   - count filtrate
   - count residue (respired AA)

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**Comparison of bacterial and primary production in different ocean basins**

<table>
<thead>
<tr>
<th>Property</th>
<th>N Atlantic</th>
<th>Eq Pac-Spe</th>
<th>Eq Pac-Fal</th>
<th>Sub N Pac</th>
<th>Arabian</th>
<th>Hawaii</th>
<th>Bermuda</th>
<th>Rose Sea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>1000</td>
<td>1200</td>
<td>1407</td>
<td>1142</td>
<td>1441</td>
<td>1900</td>
<td>5117</td>
<td>217</td>
</tr>
<tr>
<td>Bacteria</td>
<td>4050</td>
<td>4050</td>
<td>4050</td>
<td>4050</td>
<td>4050</td>
<td>4050</td>
<td>4050</td>
<td>4050</td>
</tr>
<tr>
<td>Production, mg C m⁻³ d⁻¹</td>
<td>0.4</td>
<td>0.5</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.6</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>2.58</td>
<td>2.63</td>
<td>2.74</td>
<td>2.85</td>
<td>2.92</td>
<td>2.70</td>
<td>2.55</td>
<td>2.55</td>
</tr>
<tr>
<td>Bacteria</td>
<td>0.3</td>
<td>0.13</td>
<td>0.12</td>
<td>0.05</td>
<td>0.18</td>
<td>nd</td>
<td>0.05</td>
<td>0.25</td>
</tr>
</tbody>
</table>

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*All stock ratios based on 20 ng C ml⁻¹. Data may overestimate actual heterotrophic bacterial biomass as a consequence of lower C contents and/or interference by Prochlorococcus and algae. Production estimated from 2000 g C mol⁻¹ biomass respiration.


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**Fig. 1. Location of stations.**

Ferguson and Sunda; L&O 29(2) 258-274 (1984)
Selective uptake and utilization of DOM by different groups of bacteria
Determined using tritiated compounds and MICRO-FISH
**Microbiologists view.....**

If DFAA are about 20-40 nM (80-160 nM C) in the euphotic zone of the open ocean, and turnover times are about 0.5-1 per day; then this will support a bacterial carbon demand of 80-320 nM carbon.

We assume that there are other substrates (glucose, acetic acid, etc.) that are also metabolized very quickly and contribute to the “very reactive” fraction of DOM.

Very reactive DOC supports most bacterial production in the ocean

**But.......**

**Geochemists view....**

Semi-reactive DOC is 25-40 µM carbon, about 100x higher than DFAA. Does semilabile DOC contribute to bacterial carbon demand? If semi-reactive DOC turns over seasonally (every few months) this is 100x or so > than measured AA turnover times and it will support an equal amount of BCD. How do we measure this??

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**FIG. 2.** Component composition and consumption of allochthonous NAA, NOM, and other solutes for the major phyla groups of bacteria isolated in the Rosekoff lab, ascended in MEIOX-2011 (A). Composition of humic/phenolic compounds is not shown as it is unknown. Important: Gut bacteria use protein and polysaccharides (B). Very reactive DOC is shown in gray. DOM turnover times are unknown. We assume that there are other substrates that are also metabolized very quickly and contribute to the “very reactive” fraction of DOM. How do we measure this?..
Humic substances  
Concentration 40 µM  
Inventory = 650 GT C  
$\Delta^{14}C = -400 \text{ to } -600 \%$  
Annual flux = 0.1 GTC

Biopolymers  
(polysaccharides, proteins)*  
Concentration 0-40 µM  
Inventory = 30-50 GT C  
$\Delta^{14}C =$ modern (DIC)  
Annual flux = 10’s GT C?  
* = 80% of cell C, N

Simple biomolecules  
(amino acids, sugars)*  
Concentration 1-2 µM  
Inventory = 1-3 GT C  
$\Delta^{14}C =$ modern (DIC)  
Annual flux = 10’s GT C?  
* = 10-20% of cell C, N

Summary

DOC serves as the substrate for heterotrophic bacterial production in the ocean.

Bacterial (net) production (BP) is measured through the uptake of tritiated substrates or from changes in bacterial cell numbers- large uncertainties (10x) in BP, BCD, GE.

On average gross BP = 15-20% of PP, or about 10-15 GT C yr$^{-1}$. At least this amount of carbon must be processed through the microbial loop.

BP measurements are intimately coupled to protein synthesis, and it is believed that BP production is fueled through the uptake of very reactive DOM (free amino acids, peptides, small sugars, urea, etc.).

Very reactive DOM is introduced into the water column via direct release, grazing, viral lysis, etc.

The role of semi-reactive DOM in BP production is not clear. We still don’t know how quickly it is cycled, how it impacts bacterial diversity, or how it is degraded.
Dissolved organic matter in a changing environment

\[ y = 0.83x + 13916 \quad R^2 = 0.25 \quad (p=0.001) \]

Church et al. L&O 2002