Summary of microbial implications on formation/dissociation of seafloor hydrates

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University of South Carolina
Synthetic surfactants found to catalyze gas hydrate formation in gas-storage project (nucleate and collect on metal surfaces)

No surfactant… Film forms

Synthetic surfactant: 1 hour

No surfactant: 10 days hydrate growth

Synthetic surfactant: 2 hours
Surfactant lab process scaled up: 5,300 scf gas storage demonstrated

1...chiller
2...feed gas
3...formation tank
4...glycol surge
5...burner/boiler
Would bio-surfactants in seafloor act similarly?

Surfactin $C_{53}H_{93}N_7O_{13}$ Molecular Weight = 1036
From: Rosenberg, CRC Critical Reviews in Biotechnology

Surfactin from Bacillus subtilis

Synthetic Surfactant
What kind of bio-surfactants can microbes produce?

Biosurfactant Classifications
(Kosaric, 1992; Fujii, 1998)

<table>
<thead>
<tr>
<th>Biosurfactant Classifications</th>
<th>Microbe</th>
<th>Biosurfactants Evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxylated and Crosslinked Fatty Acids</td>
<td>Corynebacterium lepus</td>
<td>DL-A-Hydroxystearic acid*</td>
</tr>
<tr>
<td>Polysaccharide-lipid-complexes</td>
<td>1. Pseudomonas syringae</td>
<td>1. Snomax</td>
</tr>
<tr>
<td></td>
<td>2. Acinetobacter calcoaceticus</td>
<td>2. Emulsan</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>Pseudomonas aeruginosa</td>
<td>Rhamnose lipid</td>
</tr>
<tr>
<td>Lipoprotein-lipopeptides</td>
<td>Bacillus subtilis</td>
<td>Surfactin</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>1. Thiobacillus species</td>
<td>DMPC *</td>
</tr>
<tr>
<td></td>
<td>2. Corynebacterium species</td>
<td>DPPS *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>POPC *</td>
</tr>
</tbody>
</table>

Have any of these been found in seafloor sediments?

Answer: Surfactin, rhamnolipid

What concentrations of surfactants necessary to catalyze hydrate formation?

**Synthetic surfactant**

**Bio-surfactant**

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[Graphs showing the effect of surfactant concentration on hydrate induction time for synthetic and bio-surfactants.]
(1) Do bio-surfactants catalyze hydrates in seafloor?
(2) If so, what mineral surfaces collect them?

**Answers:**

(1) Hydrate formation rate in porous media can be increased order(s) of magnitude with ppm of bio-surfactants.

(2) In photos below hydrates preferentially collect on smectite clays.

Rhamnolipid

Emulsan

Can microbes at seafloor conditions produce enough surfactants fast enough to influence hydrate formation? Can the surfactants be dispersed throughout sediment?

Bio-surfactants **promote** hydrates. Why? How?

**Answers:**

**Why?** To access the vast amount of carbon that the hydrates will sequester.

**How?** By providing nucleating sites for hydrate crystallization and by facilitating hydrate collection on mineral surfaces.

**Our latest research results develop mechanisms and further involvement of the microbes...**
But microbial cells retard hydrates!!

Why?

How?

- Cell wall polymers, peptidoglycan (PGN)
- And teichoic acid (TA) are kinetic and thermodynamic inhibitors
Inhibition experiments using MC-118 indigenous microbes

Treat cell mass with 1% and 5% NaCl hypertonic solutions:
- Geometrical irregularities created with PGN and TA polymer linkages, each of which contains dipoles that associate with water.
## Biopolymer inhibition results

<table>
<thead>
<tr>
<th></th>
<th>Cell Mass</th>
<th>Induction Time (h)</th>
<th>Maximum Formation Rate (mmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC+S+NM-0.36</td>
<td>0.36</td>
<td>18.38</td>
<td>2.15</td>
</tr>
<tr>
<td>MC+S+NM-0.387</td>
<td>0.387</td>
<td>174.83</td>
<td>1.62</td>
</tr>
<tr>
<td>MC+S+NM-0.368</td>
<td>0.368</td>
<td>151.97</td>
<td>1.58</td>
</tr>
<tr>
<td>NMZ+S+W Broth</td>
<td>-</td>
<td>8.28</td>
<td>1.53</td>
</tr>
<tr>
<td>Hypertonic-1%</td>
<td>0.35</td>
<td>80.50</td>
<td>1.38</td>
</tr>
<tr>
<td>Hypertonic-5%</td>
<td>0.35</td>
<td>&gt;235</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Can *B. subtilis* be used to predict effects of indigenous microbes on hydrate formation/dissociation?

<table>
<thead>
<tr>
<th>Experiment ID</th>
<th>Cell Mass (mg/mL)</th>
<th>Maximum Formation Rate (mmol/min)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>B+S+NM</td>
<td>0.391</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td>B+S+NM</td>
<td>0.405</td>
<td>2.69</td>
<td></td>
</tr>
<tr>
<td>B+S+NM</td>
<td>0.415</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>MC+S+NM</td>
<td>0.36</td>
<td>2.15</td>
<td>2.61</td>
</tr>
<tr>
<td>MC+S+NM</td>
<td>0.368</td>
<td>1.62</td>
<td>1.78</td>
</tr>
<tr>
<td>MC+S+NM</td>
<td>0.387</td>
<td>1.57</td>
<td></td>
</tr>
</tbody>
</table>

Same trend on hydrate formation rate with cell mass content.
Do indigenous microbes on MC-118 seafloor surface promote hydrates uniformly?

Lag phase and proximity

J. Radich, Laboratory and theoretical investigations of direct and indirect microbial influences on seafloor gas hydrates, M.S. thesis, Mississippi State University, 2009.
Are the MC-118 indigenous microbes producing effective biosurfactant?

- MC-118 seawater medium containing indigenous microbes were cultured

- Biosurfactant centrifuged out of seawater medium of culture

- Biosurfactant concentrated and used in hydrate formation tests

- Raw saturated sediment SWBC 1110-01 used for comparison for hydrate formation
# Hydrate formation tests using MC-118 biosurfactants in saturated porous media

<table>
<thead>
<tr>
<th>Experiment ID</th>
<th>Surface Tension (mN/m)</th>
<th>Po</th>
<th>Pf</th>
<th>dP</th>
<th>Te (h)</th>
<th>Ti (h)</th>
<th>Td (min)</th>
<th>Maximum Formation Rate (mmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW Only</td>
<td>71.5</td>
<td>402.1</td>
<td>350.7</td>
<td>51.4</td>
<td>72.0</td>
<td>15.7</td>
<td>112.0</td>
<td>1.6</td>
</tr>
<tr>
<td>SW Only</td>
<td>71.4</td>
<td>400.8</td>
<td>352.8</td>
<td>48</td>
<td>99.1</td>
<td>37.0</td>
<td>114.5</td>
<td>1.52</td>
</tr>
<tr>
<td>SW + 25% BC</td>
<td>69.3</td>
<td>401.1</td>
<td>350.4</td>
<td>50.7</td>
<td>47.9</td>
<td>15.3</td>
<td>64.5</td>
<td>1.81</td>
</tr>
<tr>
<td>SW + 50% BC</td>
<td>47.7</td>
<td>401.4</td>
<td>329.5</td>
<td>71.9</td>
<td>38.1</td>
<td>0.4</td>
<td>94.0</td>
<td>3.21</td>
</tr>
<tr>
<td>SWBC 1110-01</td>
<td>57.2</td>
<td>402.6</td>
<td>346.3</td>
<td>56.3</td>
<td>15.6</td>
<td>4.5</td>
<td>-</td>
<td>1.35</td>
</tr>
</tbody>
</table>

SW – seawater; BC – biosurfactant concentrate; SWBC 1110-01 – MC-118
Te – elapsed time; Ti – induction time; Td – dissociation time
Comparing MC-118 and *B. subtilis*: Gas hydrate formation extent

![Bar chart comparing the pressure drop (psi) for MC-118 and B. subtilis cultures. The chart shows that MC-118 culture has a lower pressure drop compared to B. subtilis culture.](chart.png)
When bentonite is present, the bad effects of microbial cell wall material on hydrate formation are negated!!!!

![Bar graph showing induction time with and without bentonite](image)

- **WITHOUT Bentonite**
  - B+S+NM: 42.3
  - S+NM: 0.79

- **WITH Bentonite**
  - B+S+NM+Ben: 0.8
  - S+NM+Ben: 1.0
In bentonite presence, more hydrates form with microbes present than without microbes—despite retarding effect of microbes. Why?

So, how do the microbes overcome this retardation of hydrate formation by the materials in the cell walls?
Proposed mechanism to explain how microbes protect themselves from heat of hydrate formation, yet obtain access to the vast carbon reservoir within massive hydrates.

1st Step.

*Not to scale

Illustrated mechanism for microbial entrance to gas hydrate macrostructures.

Step 2. Hydrates then form on biosurfactant/clay surrounding microbes—placing microbes within hydrate.

MC-118 gas hydrate melt substantiates mechanism hypothesis?

SEM Micrographs:
J. Dearman
Hypothesis: Microbes consume hydrocarbons and metabolize other substrates from within gas hydrate interstitial and capillary spaces.

In massive hydrate accumulations, such as seafloor mounds, capillaries provide a tortuous path by which nutrients from the surroundings, gases generated within the mass, or even microbes could diffuse into the interior where microbes are active.

What are the diameters of these capillaries?

Answer: See following slide and refer to our article published in:

Determining hydrate capillary sizes
(Emulsan/nontronite associate & act as hydrate nuclei)

Note: Nontronite is smectite clay. Emulsan is anionic biopolymer.

Analyses based on Dynamic Laser Light Scattering, x-ray diffraction & Scanning Electron Microscope:

Hydrate capillary diameters found to be:
Smallest: 100 – 200 nm
Largest: 1500 nm (approx)


These particles diffused through capillaries of hydrates formed in lab porous media.

Nontronite-Emulsan Particle

X45,000 magnification
THEORETICAL IMPLICATIONS OF MICROBIAL ACTIVITY WITHIN GAS HYDRATE MOUNDS ON SEAFLOOR

- Microbial metabolism, diffusion rates, pH, and nutrient/hydrogen availability are directly responsible for the long-term fate of seafloor gas hydrates.

- Methanotrophs sequester hydrocarbon gases to solid carbonates.

- Methanogens enhance methane hydrate stability.
We believe that microbial activity within gas hydrate mounds on the seafloor and within massive hydrate accumulations are major determinants of mound growths, dissociations, and longevities.

To help predict these effects, James Radich has developed the first mathematical model that considers internal microbial activities on the fate of hydrate accumulations in the seafloor. See the following publication:

Recommended future work: Extend microbial research to deep hydrate zone.

Comprehensively study microbial effects on hydrate formation/dissociation in deep gas hydrate zone—extend these findings of seafloor surface to the bottom of the gas hydrate zone.