Deepwater biodegradation of oil

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Deepwater biodegradation of oil

- General considerations related to deepwater microbiology
- Literature studies of deepwater biodegradation of oil
- Deepwater Horizon incident - Results and experiences so far from

"Deepwater" conditions

- Deepwater – the world's largest ecosystem
- Mesopelagic ("twilight") zone
  - Insufficient light for photosynthesis
  - Some temperature variation in the upper part (thermocline)
  - Lower part – constant temperature close to 4°C
  - High nutrient levels (N- and P-sources) due to particle transport ("marine snow" or upwelling)
  - Some oxygen depletion in parts of the mesopelagic (400-1000 m) due to high respiration and limited water circulation
- Bathypelagic ("midnight") zone
  - No light – no primary production
  - Temperature constant (÷1 to 5°C)
  - Oxygen saturation increases with pressure, but may be restricted due to lack of inflowing O_2-rich water
  - Limitations of nutrients (N and P)
- Abyssopelagic ("deep midnight") and hadopelagic (trench) zones
  - Continuously cold (÷1 to 5°C)
  - Lack of nutrients

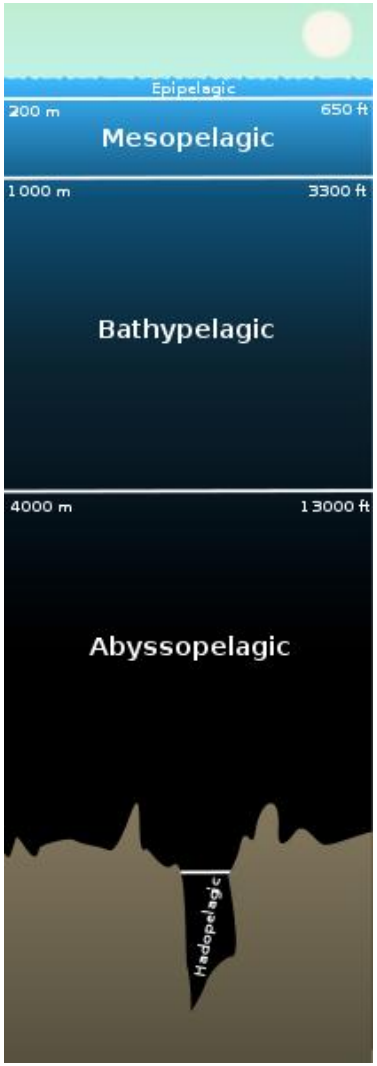


Illustration: Wikipedia

Deepwater conditions and microbiology

- Temperature
  - Constant low water temperature (≤ 5°C) results in an environment enriched with *psychrophilic* (psychrophilic = "cold"-loving) or *psychrotolerant* microbes
    - Psychrophilic microbes require low temperature (< 10°C) for optimal metabolic activities
  - Several psychrophilic microbes known to be involved in oil biodegradation

- Pressure
  - Increasing pressure from 40-200 bar (400-2000 m depth) results in an environment enriched with *piezophilic* (piezophilic = "pressure"-loving) and *piezotolerant* microbes
    - Piezophilic microbes require high pressure (> 1 bar) for optimal metabolic activities
  - Little is known about the potential for oil biodegradation by piezophilic microbes
    - High pressure requirement – challenging to isolate and grow these microbes
    - Metabolic activities reduced by decompression after sampling

Deepwater microbiology

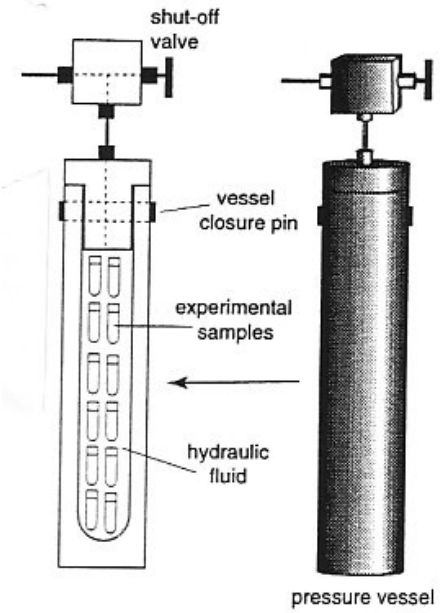
Psychropiezophilic microbes

- Psychropiezophiles – requiring both low water temperature and high pressure for metabolic activity

- Psychropiezophilic microbes restricted to very few genera: *Shewanella, Photobacterium, Colwellia, Moritella, Psychromonas*
  - No psychropiezophilic isolates have been tested for hydrocarbon biodegradation potential to our knowledge

Sampling and handling of deep sea microbial samples

- Some experiences recorded (review by Deming, 1995)
  - Avoid sample warming – dormant shallow-water microbes may become predominant
  - Marine prokaryotes live in pressure equilibrium with their fluid surroundings – will not implode upon decompression
  - Most deep-sea microbes will survive brief periods of decompression
  - If measurement of true metabolic activity is the goal communities should be protected against changes in temperature and pressure
- *In-situ* sampling may be performed by oil/hydrocarbon-baited Biotraps to enrich deepwater biofilms of oil-degrading microbes



Ill.: Deming, 1995

Ill.: Microbial Insight, Inc.

Deepwater microorganisms associated with hydrocarbon biodegradation

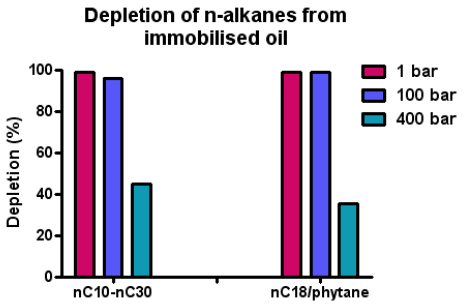
Microbes and source				Hydrocarbon degradation	Pressure (Bar)	Temp. (°C)	References
Class	**Genus**	**Source**	**Depth (m)**				
Alphaprot.	*Thalassospira*	Sed: Atlantic	3962	PAH	1	25	Shao, 2010
		Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
	Roseovarius	Sed: Pacific	2682	PAH	1	18	Wang , 2008
		Sed: Atlantic	3962	PAH	1	25	Shao, 2010
Gammaprot.	*Alcanivorax*	Sed: Atlantic	3962	PAH	1	25	Shao, 2010
		Sed: Mediterranean	2400	n-alkanes	1	20	Tapilatu, 2010
		Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
		Sed: Pacific	2682	PAH	1	18	Wang, 2008
	Marinobacter	Sed: Atlantic	3962	PAH	1	25	Shao, 2010
		SW: Mediterranean	3475	n-C16 alkane	1 and 350	22	Grossi, 2010
		Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
	Cycloclasticus	Sed: Atlantic	3962	PAH	1	25	Shao, 2010
		Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
		Sed: Pacific	2682	PAH	1	18	Wang, 2008
	Halomonas	Sed: Atlantic	3962	PAH	1	25	Shao, 2010
		Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
		Sed: Pacific	2682	PAH	1	18	Wang, 2008
	Pseudomonas	Sagami Bay, Japan	1168	PAH	1	30	Abe, 1995
	Pseudoalteromonas	Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
	Alteromonas	Sed: Atlantic	3542	Crude oil, PAH	1	25	Cul, 2008
Actinobacteria	*Rhodococcus*	Sed: Mediterranean	2400	n-alkanes	1	20	Tapilatu, 2010
Flavobacteria	*Flavobacterium*	Sed: Pacific	2682	PAH	1	18	Wang, 2008
Flavobacteria	*Flavobacterium*	Suruga Bay, Japan	1945	nC7-nC16 alkanes	1	25	Moriya, 1993

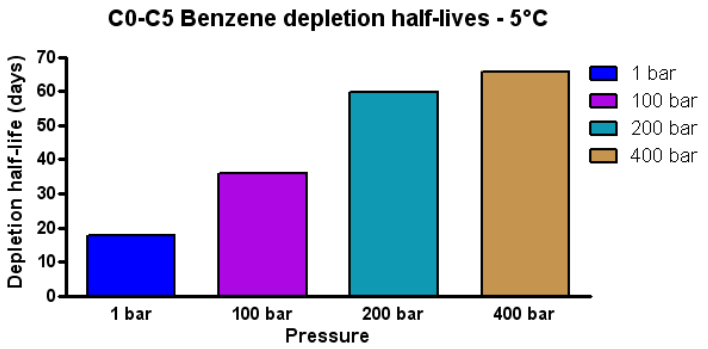
Biodegradation of oil in seawater at low temperature and high pressure

- Project sponsored by the *Norwegian Deepwater Programme* (NDP) 2004-2007

- Collaboration between the International Research Institute of Stavanger (IRIS) and SINTEF (pressure experiments at IRIS)

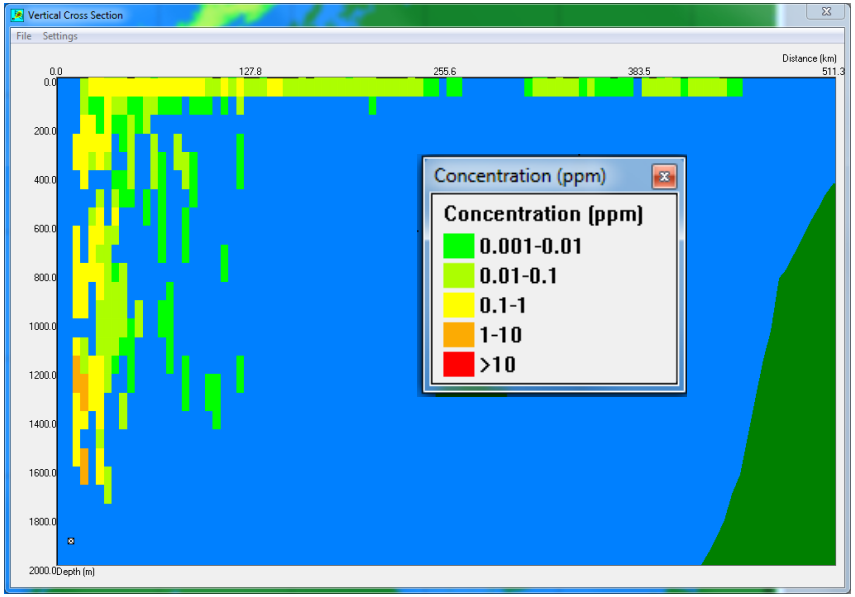
- Experiments
  - Seawater collected from shallow water (80-90 m depth) used for oil biodegradation experiments at elevated pressures (100, 200 and 400 bar) and low temperature (4-5°C) for 2 months
  - Biodegradation of alkanes and monoaromatics (BTEX) determined
- Results
  - Biodegradation of n-alkanes and aromatics appeared at all pressures, indicating piezotolerant oil-degrading microbes in the seawater

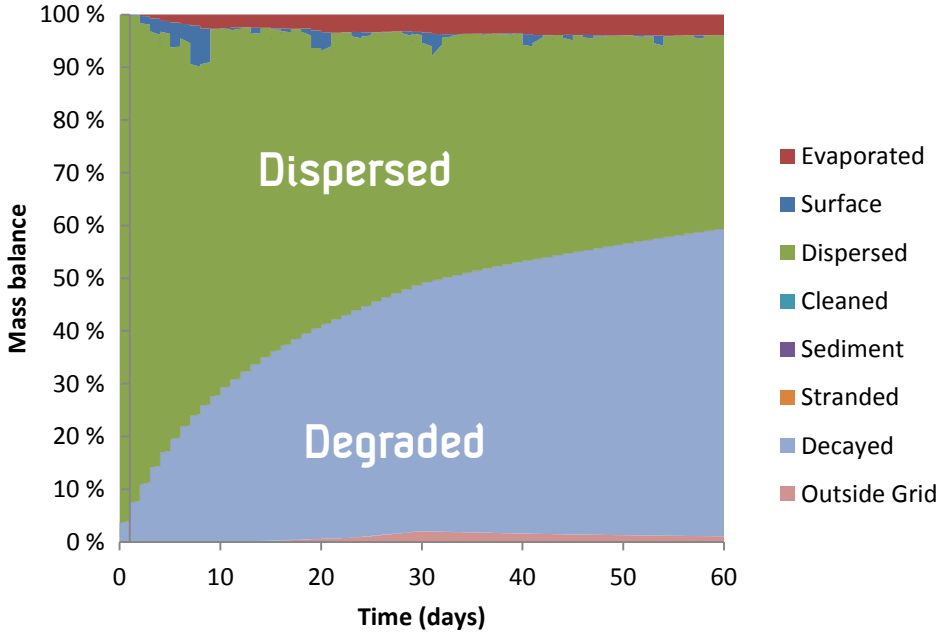




Simulation of a deepwater release outside the Norwegian coast

- Use of OSCAR-Model
  - A Multi-Component 3-Dimensional Oil Spill Contingency And Response
  - Use of 25 oil pseudo-component groups
- Simulation conditions
  - Norwegian paraffinic oil
  - 1600 m depth and 60 days blowout period
  - Simulation of release outside the Norwegian coast
- Mass balance after 60 days
  - Low degree of evaporation
  - Biodegradation accounts for 60 % of the depletion after 60 days blowout period





Deepwater Horizon spill and oil biodegradation

- DWH spill conditions (reviewed by Atlas and Hazen, 2011)
  - 779 mill L estimate of Light Lousiana oil (API 35.2) – Macondo 252
  - 84 days of release period
  - Discharge 1500 m depth; 77 km offshore
  - Large amounts of natural gas (methane) released
- Offshore cleanup
  - Injection of dispersant (Corexit 9500) at the wellhead
  - Sea surface cleanup operation: skimming and surface dispersant application
- DWH Oil behaviour
  - Deep-sea cloud of fine droplets (900-1300 m depth) – droplet size 10-60 µm (SW temperature appr. 5°C)
    - *Oil concentrations less than 10 ppm total petroleum hydrocarbons (TPH)*
    - *Small droplets – large surface-to-volume ratio: Good potential for oil biodegradation*

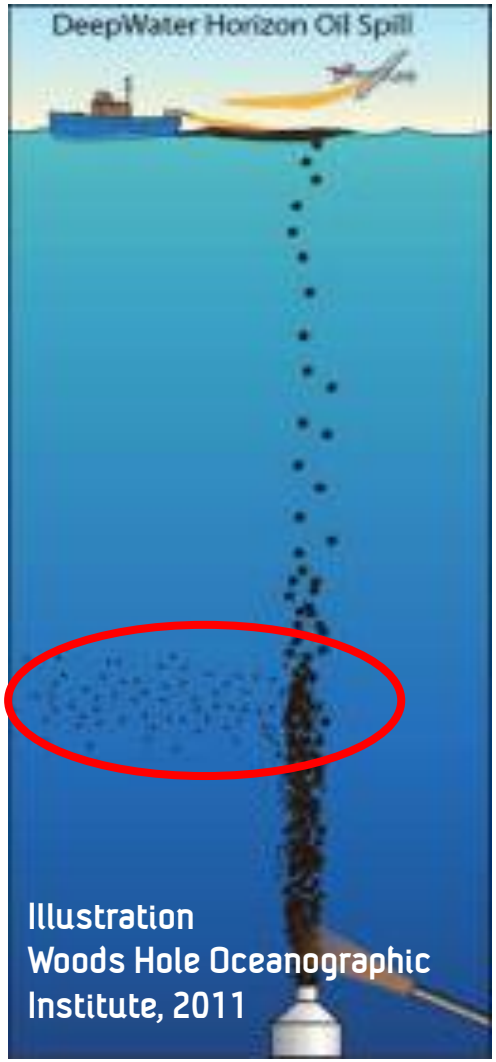


Illustration
Woods Hole Oceanographic Institute, 2011

DWH and deepwater oil biodegradation
Field studies

- Demonstration of deepsea biodegradation (Hazen et al., 2010)
  - Microbial respiration in plume area (800-1200 m depth)
    - Reduced O_2, PO_4 and NO_3; increased NH_4
  - Increased cell density: 2.7 x 10^4 cells/ml outside plume to 5.5 x 10^4 cell/ml inside plume
  - Enrichment of bacteria groups associated with hydrocarbon biodegradation

Glass/group	Order/family/genus	Degradation Function	References
Alphaproteobacteria	*Rhodobacterales*	Oil	Redmond, 2011
Betaproteobacteria	*Methylococcaceae*	Methane oxidation	Redmond, 2011
Betaproteobacteria	*Methylophilaceae*	C1-assimilation	Redmond, 2011
Gammaproteobacteria	*Oceanospirillales*	Alkane and metabolites	Hazen, 2010; Redmond, 2011
Gammaproteobacteria	*Cycloclasticus*	Aromatic hydrocarbons, ethane, propane	Valentine, 2010; Redmond, 2011
Gammaproteobacteria	*Colwellia*	Ethane, propane, metabolites	Valentine, 2010; Redmond, 2011
Gammaproteobacteria	*Methylophaga*	C1-assimilation	Redmond, 2011
Flavobacteria	*Polaribacter*	Metabolites	Redmond, 2011
Flavobacteria	*Owenweeksia*	Metabolites	Redmond, 2011

DWH and oil biodegradation
Laboratory studies

- The MC252 oil is very dispersable – advantageous for oil biodegradation due to the generation of small oil droplets
- Laboratory studies at SINTEF (GoM surface conditions) showed that chemically dispersed MC252 oil biodegraded more rapidly than physically dispersed oil (Brakstad et al., 2011)
- nC14 to nC26 alkane biodegradation half-time at 5°C – 1 – 6 days (Hazen et al., 2010)
- Crude oil biodegradation at 4°C was predominated by members of the genus *Colwellia* (Redmond and Valentine, 2011)
- Stable isotope analyses ($^{13}\delta$C-) of biodegradation at 4.7°C with labelled methane, ethane and propane showed (Valentine et al., 2010) -
  - *Bacterial propane and ethane consumption, but not methane consumption*
  - *Bacterial respiration of propane+ethane expected to account for 70 % of O_2-reduction in the plume*
  - *Rapid propane consumption expected to jump-start response of hydrocarbon-degrading bacteria in the plume (Valentine et al., 2010)*

DWH and deepwater oil biodegradation

Model for biodegradation in the deepwater plume

- Sequencial and pulsed propagation of bacteria specific for different hydrocarbons (Valentine et al., 2011)
- Suggested Interactions between bacterial successions and degradation pattern

Pre-spill microbial community	→ *Oceanospirillales* *Colwellia* *Cycloclasticus*	→ *Colwellia* *Methylophaga* *Methylophilaceae* *Methylococcaceae*
Pre-spill situation No biodegradation	→ n-Alkane biodegradation Aromatic HC biodegradation Propane biodegradation Metabolite biodegradation	→ Ethane biodegradation C1-assimilation Methane oxidation

Water parcels may come in contract with the hydrocarbon source at multiple occasions

↓

Repeated pulses of blooming and respiration
Increasingly faster blooms and respiration rates

Deepwater biodegradation – Some conclusions

- Hydrocarbon-degrading bacteria are "*everywhere*"
- Oil characteristics are important for biodegradation both at surface and deepwater conditions
- Gases and volatile hydrocarbons from subsea releases are also important substrates for microbial metabolism (biodegradation and assimilation)
- Oil biodegradation studies with seawater from meso- and bathypelagic zones (800-4000 m depths) show the presence of piezotolerant and psychrophilic/phychrotolerant hydrocarbon-degrading bacteria
- At "moderate" water depths (e.g. DWH incident) low temperature may be more important than high pressure for hydrocarbon biodegradation
- We may expect that the contribution of piezophilic microbes will increase by depth, but nothing is known about piezophilic oil-degrading microbes

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