Laboratory Tests to Determine Role of Bioproducts in Seafloor Gas Hydrate Accumulations*

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Abstract

This study investigates possible influences of microbes in prolific gas hydrate occurrences in ocean sediments. Microbial/gas hydrate studies are reported on the laboratory production, separation and hydrate catalysis of a biosurfactant generated from *Bacillus subtilis bacterium* (ATCC 21332), a microbe that has been identified in Gulf of Mexico gas hydrates. *Bacillus subtilis* was cultured in the laboratory from 282 K to 310 K under both aerobic and anaerobic conditions. Biosurfactants were separated from the broth in foam created by bubbling gas through the mixture, and they were recovered from the collapsed-foam distilled-water solution by acid precipitation/dichloromethane extraction. Five surfactin isomers were identified by HPLC spectra. Natural gas hydrates were then created in porous media saturated with the recovered surfactin-water solution. According to gas-hydrate formation rate measurements, the anaerobically-produced biosurfactants catalyzed hydrate formation markedly better compared with that produced under aerobic conditions. Adsorption experiments indicated that surfactin adsorbs on the surfaces of sand, bentonite and nontronite, but not on the surfaces of kaolin. In-situ visual observation of gas hydrate formation in a packing of these minerals saturated with surfactin solution also showed that gas hydrates prefer to form on sand, bentonite and nontronite rather than on kaolin clay. X-ray diffraction spectra indicated that during gas hydrate formation, surfactin adsorbed on the surfaces of bentonite particles rather than in interlayers. The tests suggest that microbial species in ocean sediments of gas hydrate stability zones could promote hydrate formation via biosurfactants produced from metabolic activities; the bioproducts could be distributed by gas flow through the sediments; the bioproducts might collect on specific minerals.

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at San Antonio

Objective

Investigate the roles of microbes in the occurrences of gas hydrates in ocean sediments.

Association of microbes and gas hydrates on seafloor

- Methanogenesis: microbes produce methane through metabolic activities in the sediment and methane forms hydrates

- Anaerobic oxidation of methane (AOM): microbes digest the methane, which is coupled with sulfate reduction (SR), and finally produces carbonates in sediments.

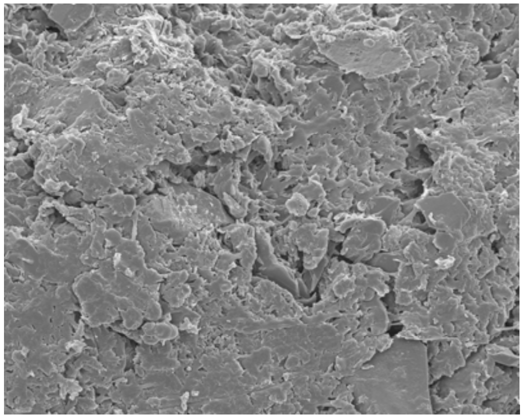
Surfactant effects on hydrate formation

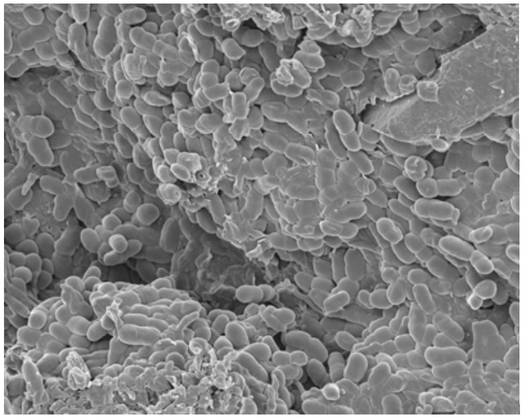
1. Increase formation rates by orders of magnitude.
2. Decrease induction time.
3. Grow hydrate crystals preferentially on specific solid surfaces by adsorption.

Microbes identified in GOM seafloor hydrate sediments

Bacillus subtilis **and** ***Pseudomonas aeruginosa*** **identified in Gulf of Mexico gas hydrate mounds** (*Lanoil, et al., Applied and Envir. Micro., Nov. 2001.)*

These microbes produce surfactin and rhamnolipid, respectively.





Hypothesis:

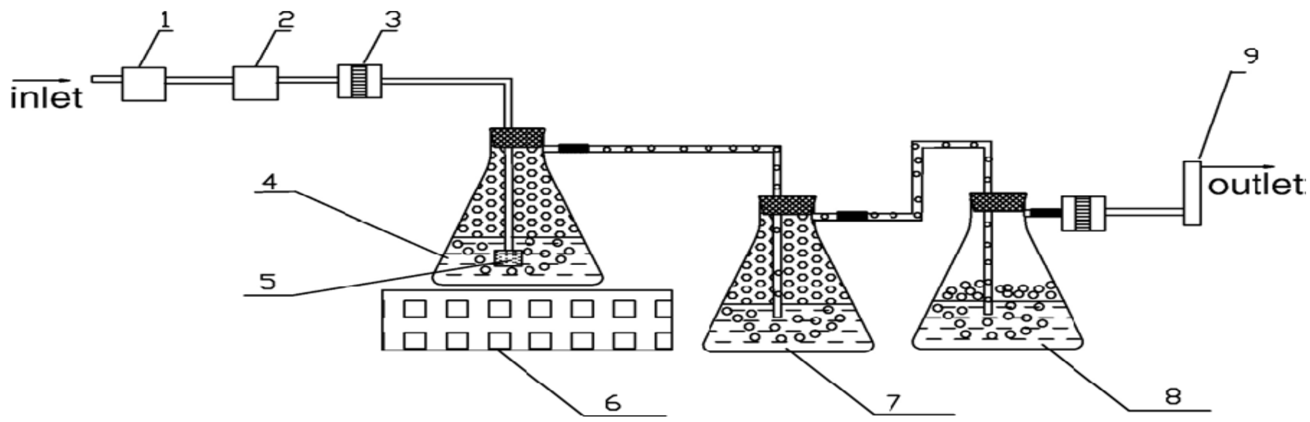
Seafloor microbes promote gas hydrate formation by producing biosurfactants in seafloor gas hydrate stability zone.

Laboratory work to test hypothesis

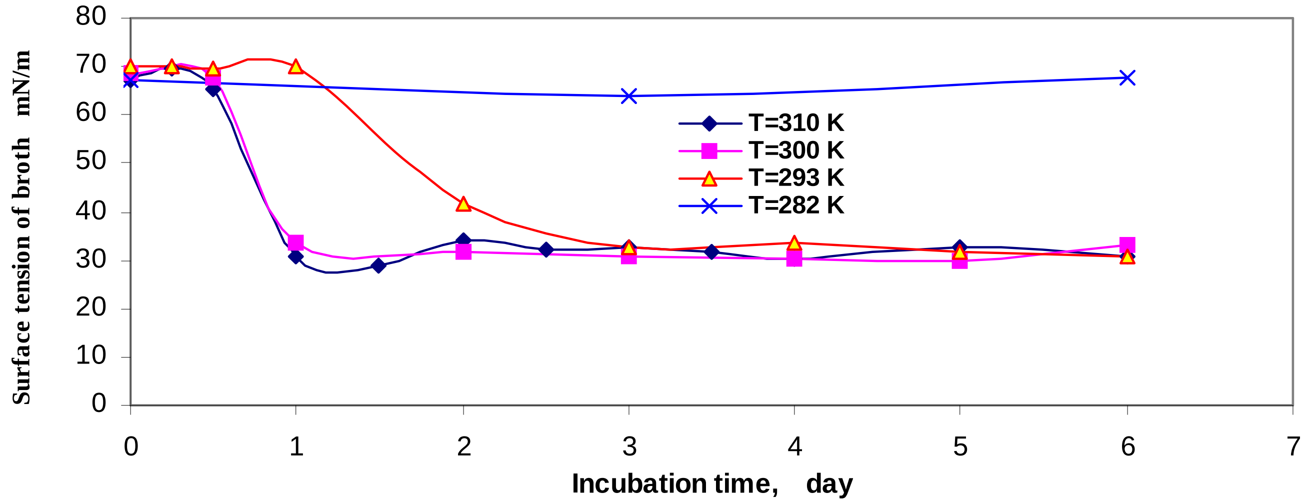
❖Production of biosurfactants under anaerobic condition.

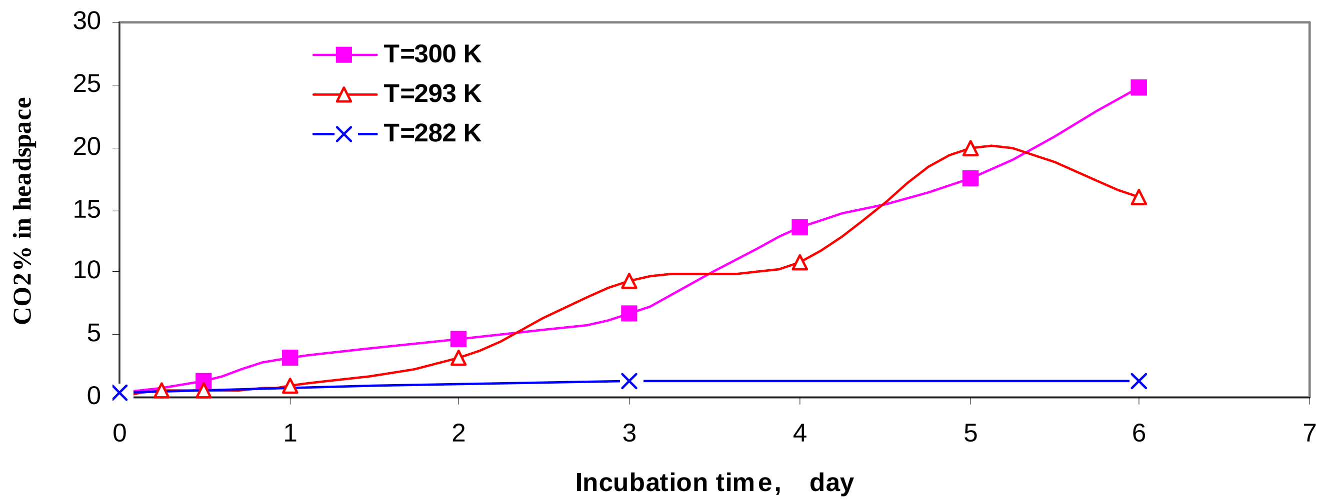
❖Separation of biosurfactants.

❖Test effects of biosurfactants generated on the hydrate formation in porous medium.

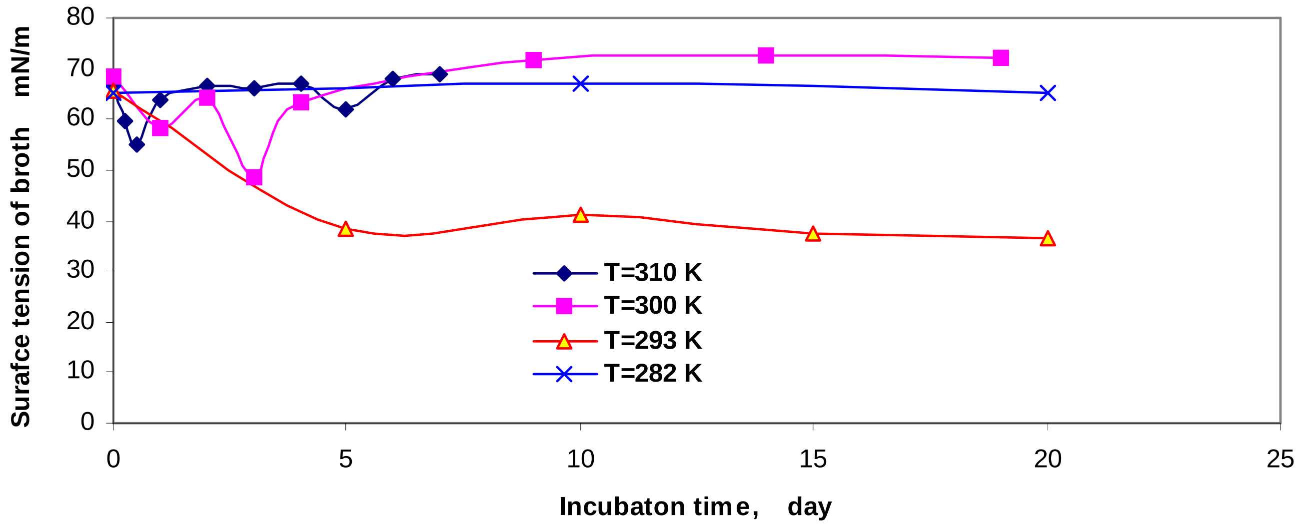


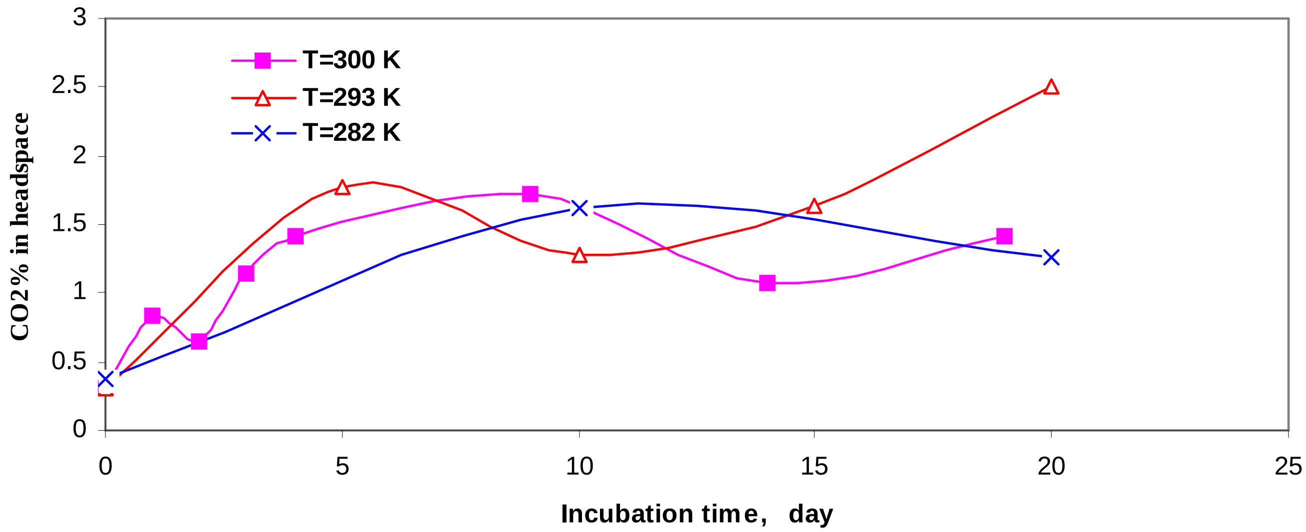
Production of biosurfactants from *Bacillus subtilis* using glucose as sole carbon source



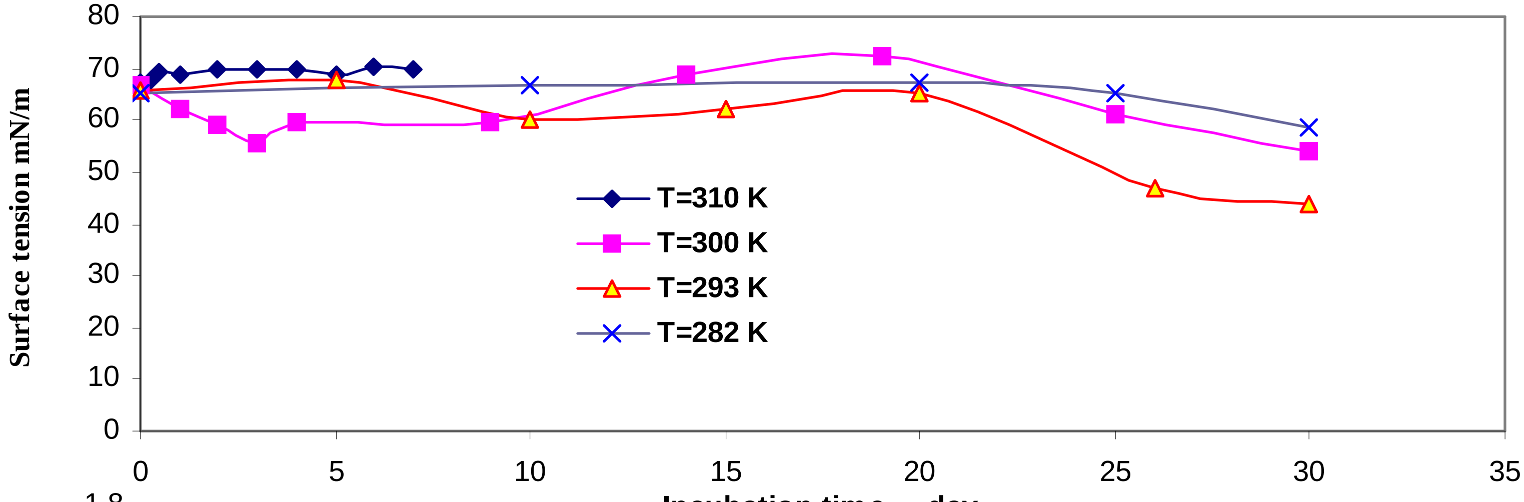


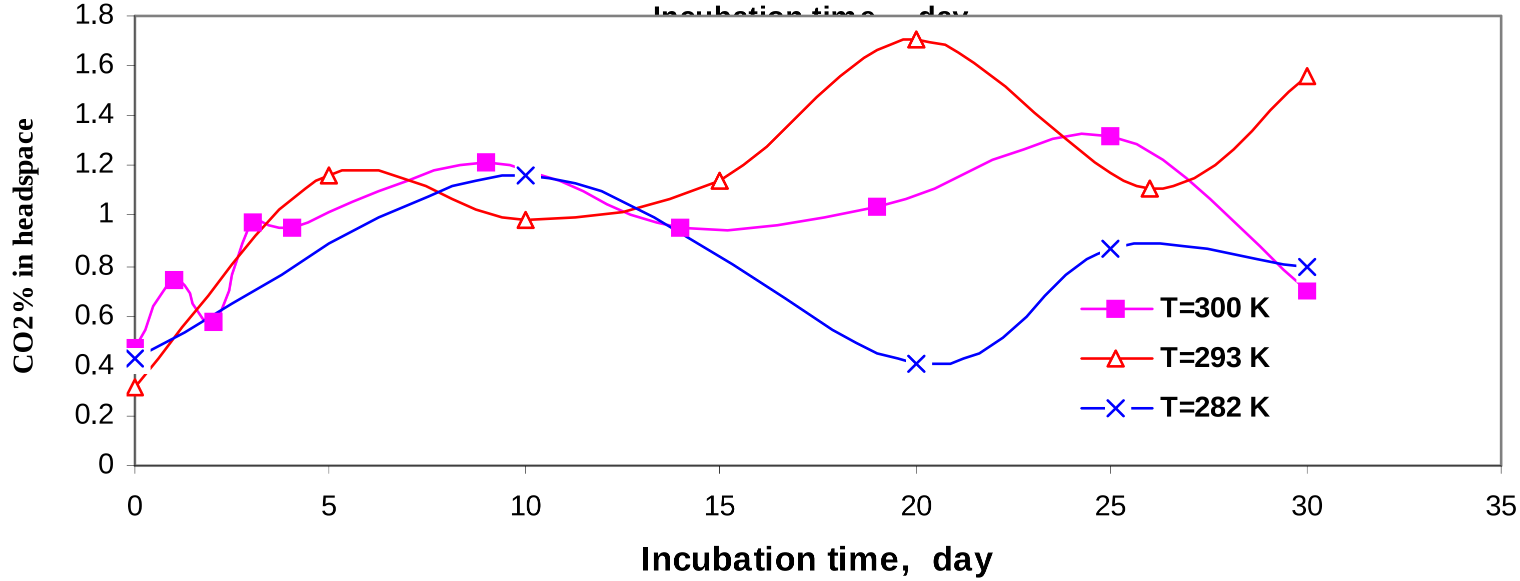
Production of biosurfactants from *Bacillus subtilis* using hexadecane as sole carbon source





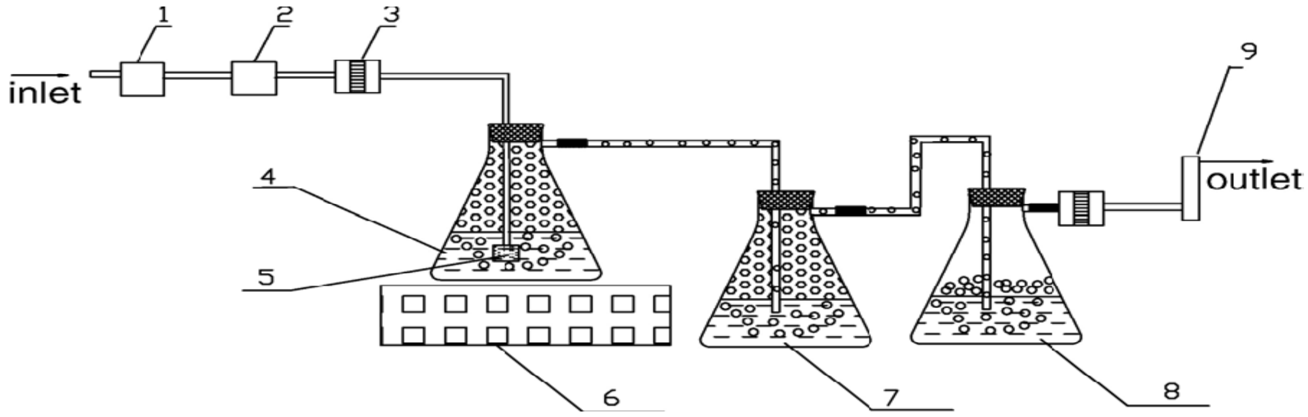
Production of biosurfactants from *Bacillus subtilis* using kerosene as sole carbon source





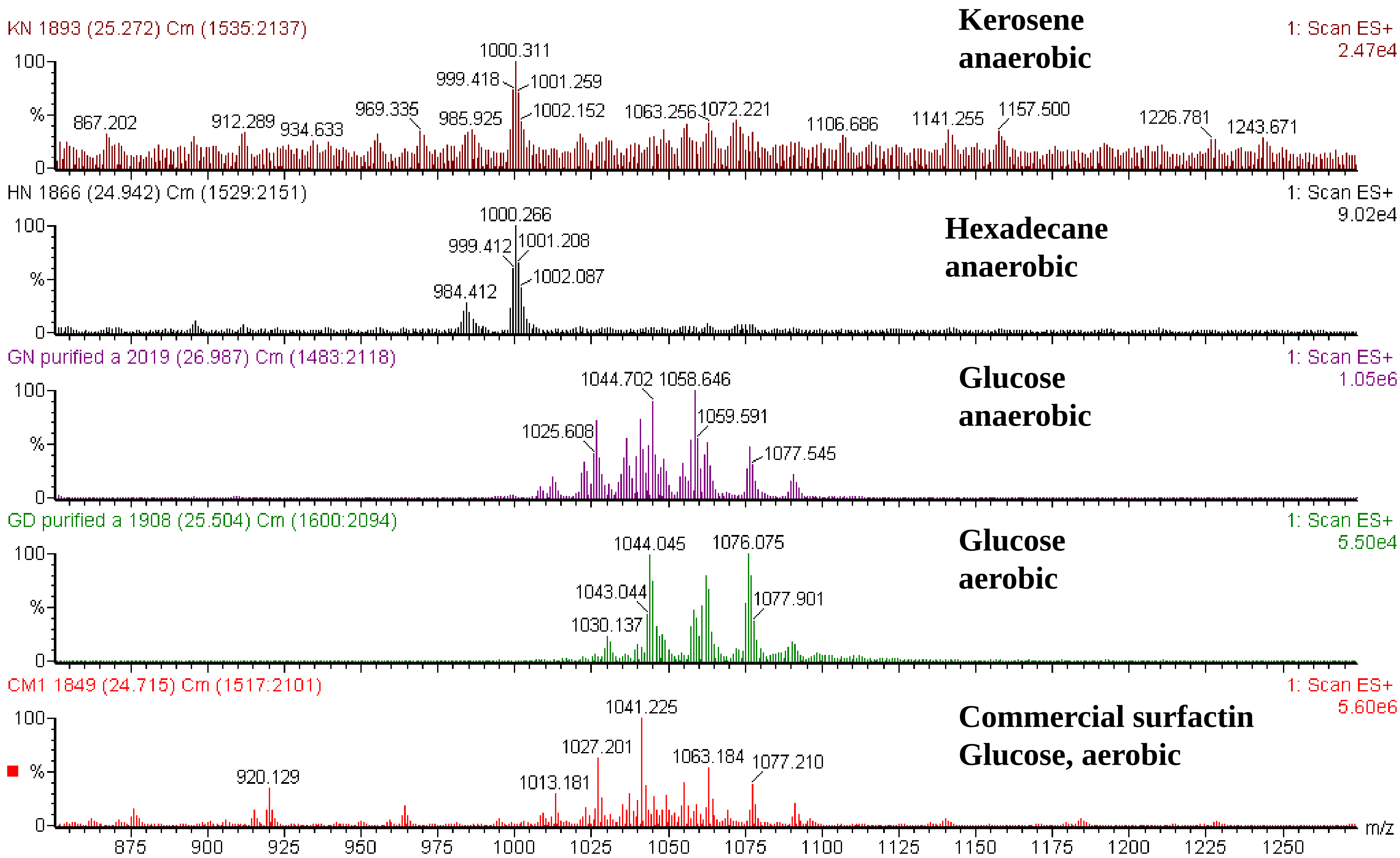
Production of biosurfactants by *Bacillus subtilis* under anaerobic conditions

No	Atmosphere	Carbon Source	Surface Tension of Solution, mN/m		
			Broth before Bubbling	Broth after Bubbling	Water Solution of Collected Biosurfactants
1	95%N_2+5%H_2	Glucose	30.6	68.6	33.1
2	95%N_2+5%H_2	Hexadecane	38.5	66.1	35.5
3	95%N_2+5%H_2	Kerosene	43.8	65.2	36.6



Zhang & Rogers, Marine Chem. 1997

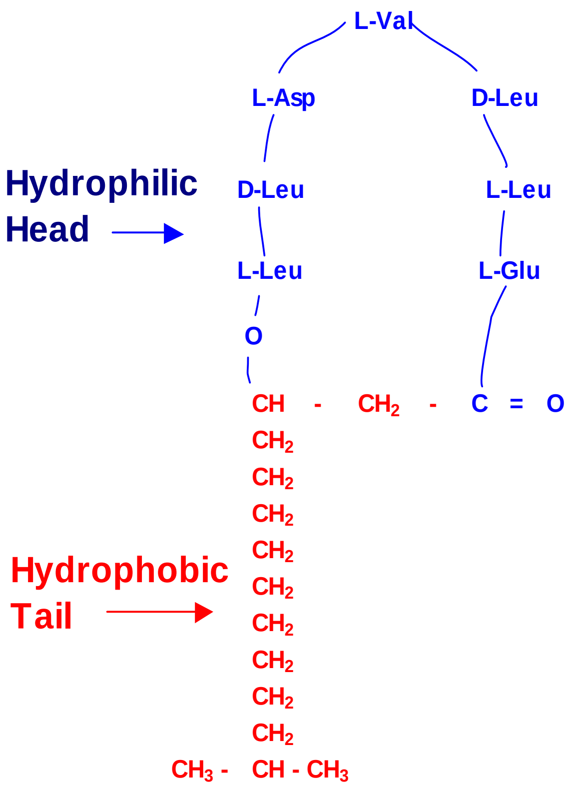
ESI/MS averaged spectra of biosurfactants generated by *B. subtilis*



Surfactin structure

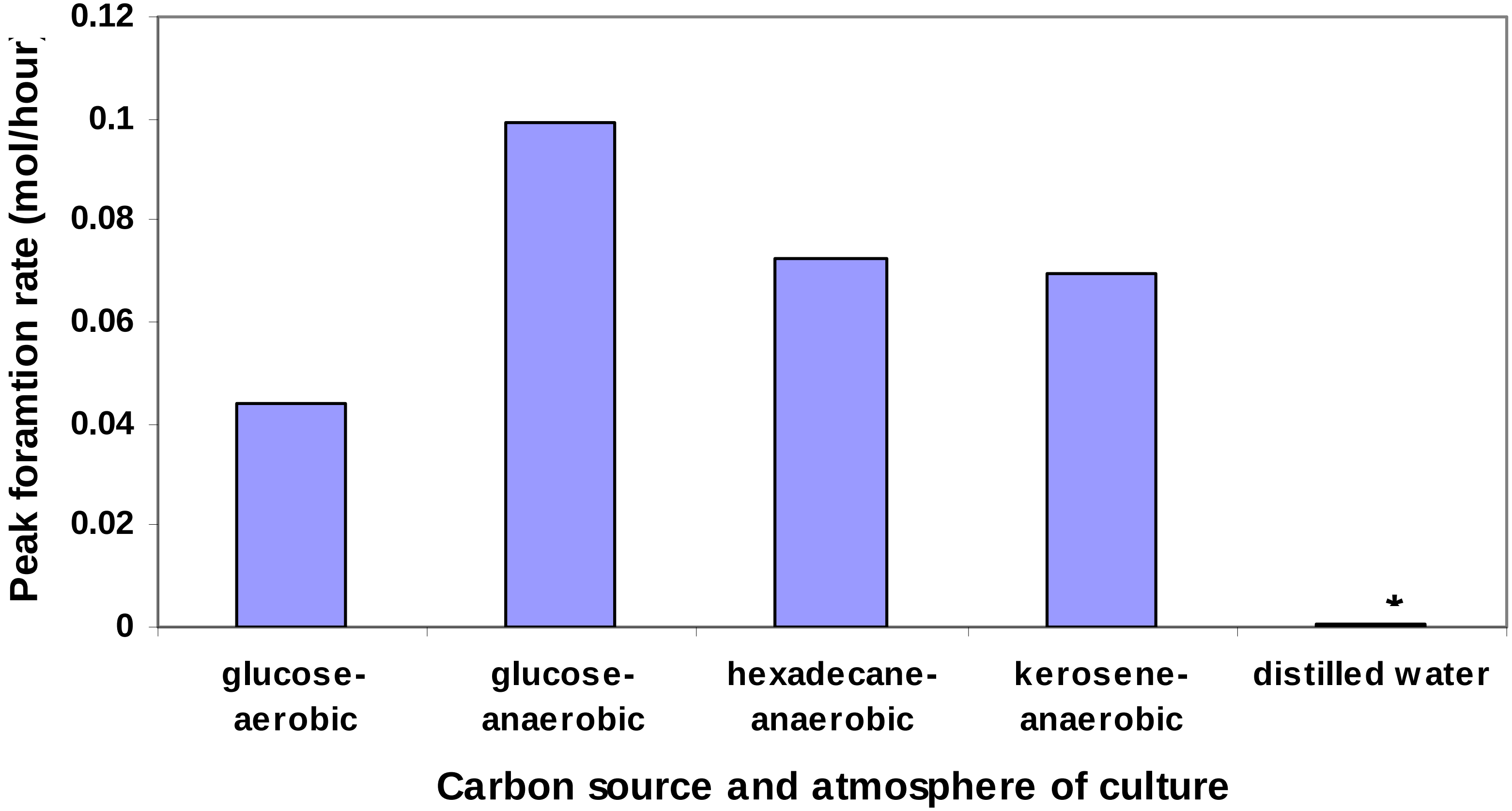
Surfactin $C_{53}H_{93}N_7O_{13}$ Molecular Weight = 1036

Rosenberg, CRC Critical Reviews in Biotechnology

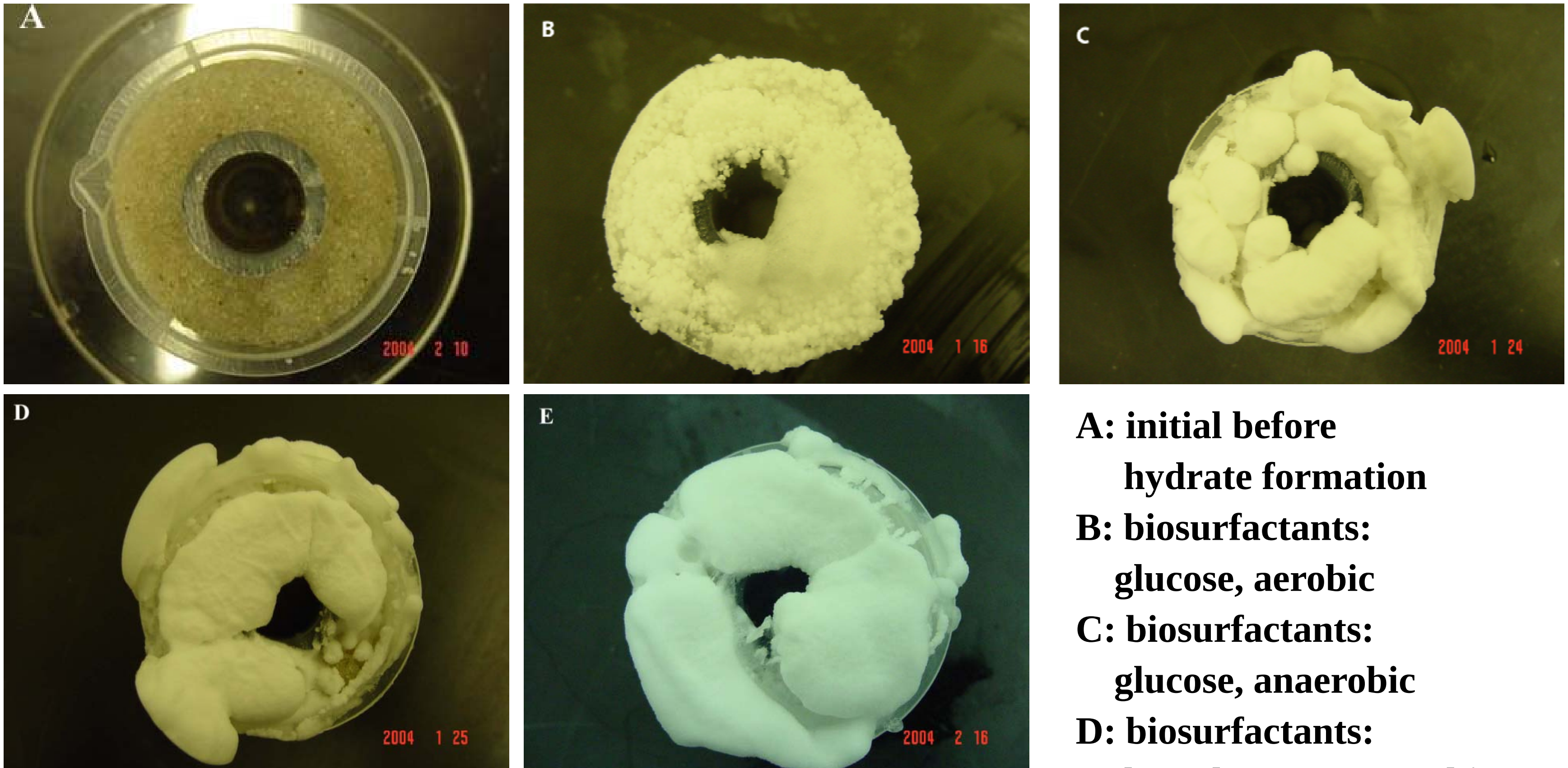


7 Amino Acids, e.g., Aspartic Acid: HOOCCH2CH(NH2)COOH

Effects of biosurfactants on the formation rate of gas hydrates in porous media saturated with biosurfactant solution

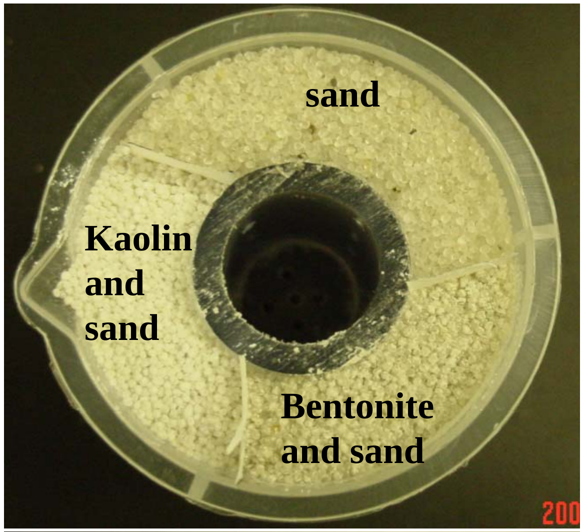


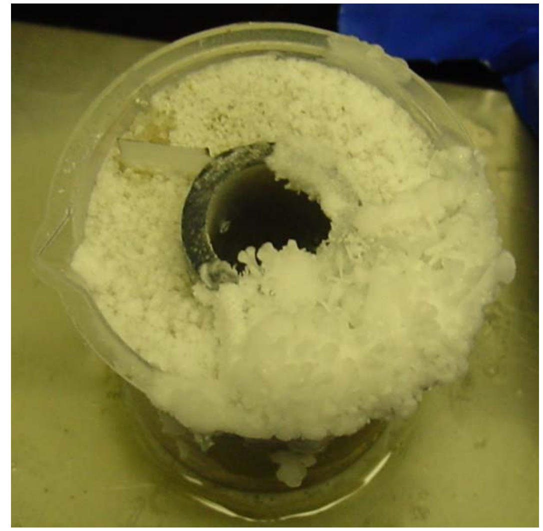
Effect of biosurfactants on the accumulation of gas hydrate in porous media saturated with biosurfactant solution

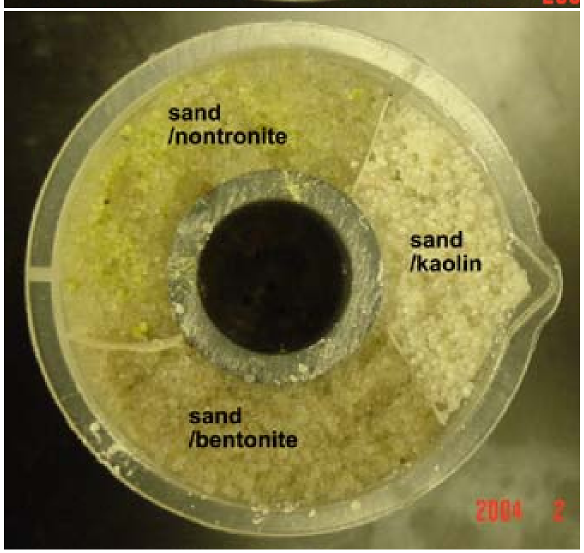


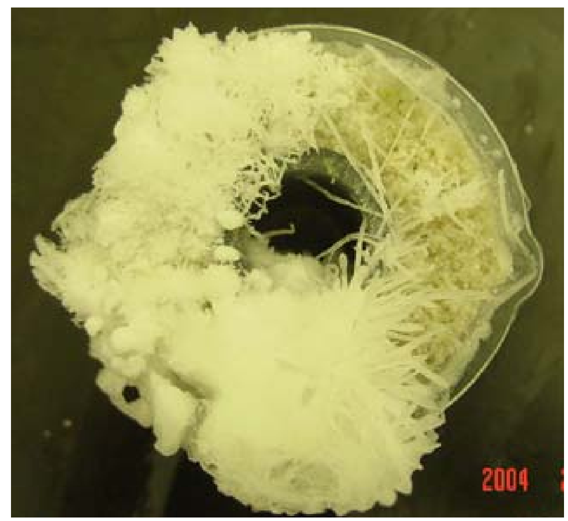
A: initial before hydrate formation
B: biosurfactants: glucose, aerobic
C: biosurfactants: glucose, anaerobic
D: biosurfactants: hexadecane, anaerobic
E: biosurfactants: kerosene, anaerobic

Effect of surfactin on the accumulation of gas hydrate in various porous media saturated with surfactin solution









Summary of laboratory results

- *Bacillus subtilis* is prolific producer of biosurfactants that greatly increases hydrate formation rates in packed porous media.

- Different isoforms influenced formation rate and morphology of gas hydrates.

- Surface properties of porous media have great impacts on hydrate formation and accumulation.

Conclusion:

B. subtilis **and other microbial species in seabed sediments of gas hydrate stability zones produce biosurfactants through anaerobic metabolic activities and enhance gas hydrate formation in the surrounding porous media.**

G. Zhang, R.E. Rogers, et al. Marine Chemistry, (2007), 103, 359-369.

Acknowledgements

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