

Effects of Biosurfactants on Gas Hydrates

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Abstract

The increasing demand of fossil fuels resources and their continuous consumption has created a necessity for exploring the alternative resources of energy like gas hydrates. Gas hydrates exist under oceans and in permafrost regions. They are also known as methane hydrates or methane clathrates. These are stable at high pressure and low temperature. These are formed when methane gas comes in contact with sediments saturated with water. *Pseudomonas aeruginosa* and *Bacillus subtilis* were found in gas hydrates samples investigations from Gulf of Mexico and these microbes generated biosurfactants like Rhamnolipid and Surfactin. The current paper reports the effects of biosurfactants such as Rhamnolipid, Surfactin, Snomax, Emulsan, Phospholipids, Hydroxystearic acid etc. on Gas Hydrate formation.

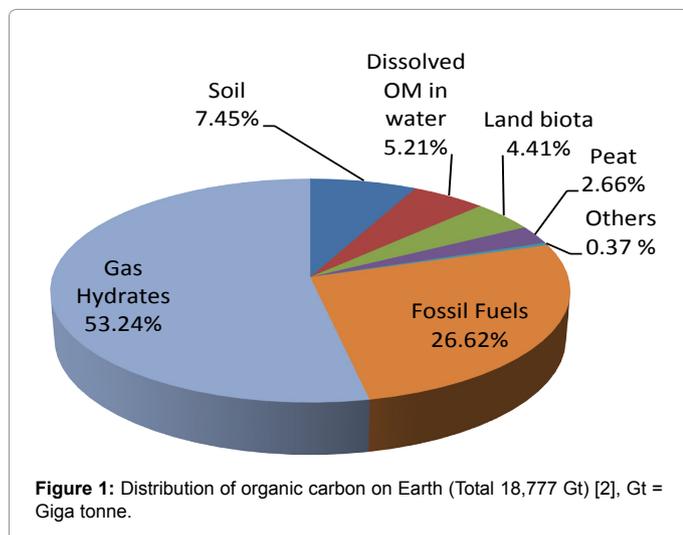
Keywords: Gas hydrates; Rhamnolipid; Surfactin; Snomax; Emulsan; Hydroxystearic acid; Phospholipid

Introduction

Gas hydrates belong to a class of inclusion compounds and are known as clathrates. In these class of compounds host molecules like water encapsulates the guest molecules like methane, ethane, propane, CO₂ etc. and forms a molecular cage. In these types of compounds only intermingling of molecules takes place and there is no chemical reaction occurs as such. Water molecules form hydrogen bonding and forms a polyhedral cavity and the guest molecules occupies the void.

The CO₂ sequestration can help in releasing the methane captured in gas hydrates below the sea floors. The amount of energy released when CO₂ gas hydrates gets formed is more than the amount of energy required to dissociate methane gas hydrates. So, CO₂ sequestration in gas hydrates can achieve two objectives at one time i.e. it can reduce the global warming and give the energy [1].

The gas hydrate reserves around the globe have organic carbon contained in them almost double than the amount contained in all fossil fuels on Earth as shown in Figure 1.



Molecular Structure

The common structure formed by gas hydrates is structure I which is formed by gases like methane and molecules like propane, natural gas form structure II.

The Structure I consist of 2 small cages made up by 12 pentagonal surfaces called “5¹²” and 6 larger cages of again 12 pentagonal and two hexagonal surfaces called 5¹² 6² [3].

The unit cell consists of water molecules = (12x5¹² + 2x5¹² 6² = 46) [4].

Structure II consist of 16 small cages made up by 12 pentagonal surfaces called “5¹²” and 8 larger cages of again 12 pentagonal and four hexagonal surfaces called 5¹² 6⁴ [3].

The unit cell consists of water molecules = (16x5¹² + 8x5¹² 6⁴ = 136) [4].

The hydrate cavities of structure I and structure II as shown in Figure 2.

For methane hydrate in structure I complete filling of both the large (5¹²6²) and small (5¹²) cavities by methane molecules will give hydrate i.e. CH₄•5.75H₂O. However complete filling of occupancy is not possible which leads to a hydrate ratio of around 1:6 (i.e. CH₄•6H₂O), which is equivalent to 96% cage occupancy [5,6], based on this 1 m³ of methane gas hydrate can occupy 167 m³ of methane at STP.

Physical Properties of Gas Hydrates

Gas Hydrates are non-stoichiometric compounds Gas Hydrates

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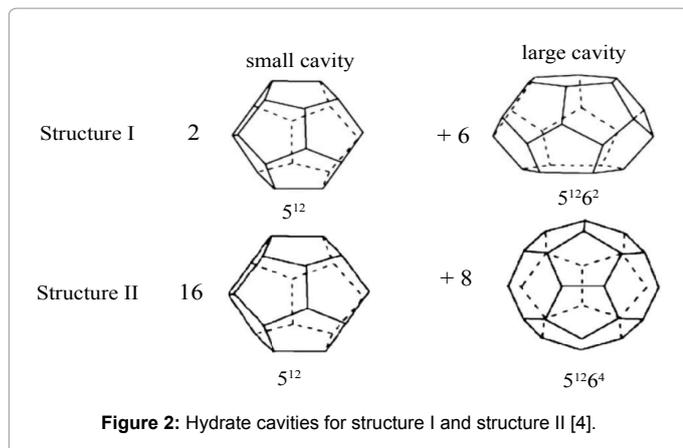


Figure 2: Hydrate cavities for structure I and structure II [4].

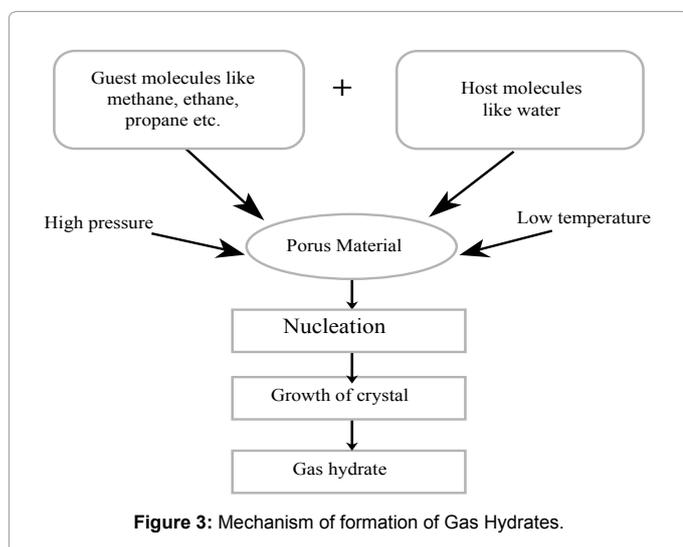


Figure 3: Mechanism of formation of Gas Hydrates.

are also known as crystalline form of Natural Gas. Physically natural Gas Hydrate is like white crystalline substance which can burn hence it is also known as burning ice. These types of molecules are formed under natural conditions of low temperature and high pressure. In gas hydrates the guest molecules are not physically bonded with the water lattice instead they are held through weak Van der Waals interactions.

Occurrence of gas hydrates

Global scenario

The occurrence of Gas Hydrate has been known since the mid of 1960's when they were discovered in Russia. They have been also discovered in permafrost regions, but mostly they occur in oceanic sediments below the sea floor where the depths are greater than about 500 m. Gas Hydrates are detected at lesser depths also. These are present in oceanic sediments along continental margins as well as in polar continental settings [7]. Gas Hydrates are scattered from South Eastern coast of United States on the Black Ridge, in the Gulf of Mexico, western and eastern margins of Japan, the Middle America Trench and in the Cascadian Basin near Oregon, Peru. The worldwide occurrence of Gas Hydrate is shown in Figure 4.

Indian scenario

The gas hydrates are present in the K-G basin, Mahanadi Offshore

Basin and Andaman regions. Though gas hydrates have not been recovered from the drill site in the KK basin, various geo-scientific investigations indicate gas hydrates may be present in the Saurashtra and KK basins in western India margins [9]. The richest gas hydrate deposits (~130 meter thick with ~70% saturation and 60% porous fractured shale) are in the K-G basin and the thickest (260-600 meters) in Andaman sea (Figure 5).

Energy Potential of Gas Hydrates

Among all renewable (Solar, Wind, Wave, Nuclear, Hydro, Geothermal, Bioprocess etc.) and non-conventional (Gas Shale, Coal Bed Methane, Basin Centred Gas, Tight Gas Shale, Gas Hydrates etc.) energy resources, Gas Hydrates are regarded as one of the most suitable candidates for cleaner energy resources in this century. As per the USA department of energy, if only 1% of the methane stored in these

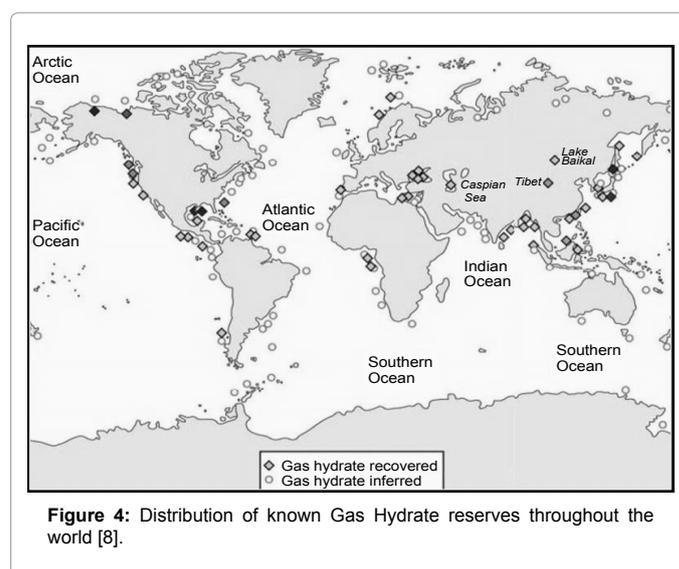


Figure 4: Distribution of known Gas Hydrate reserves throughout the world [8].

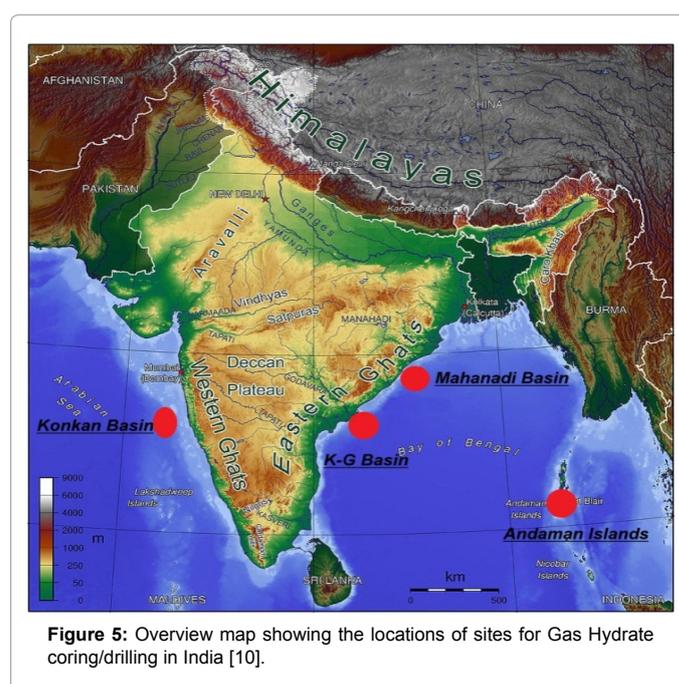


Figure 5: Overview map showing the locations of sites for Gas Hydrate coring/drilling in India [10].

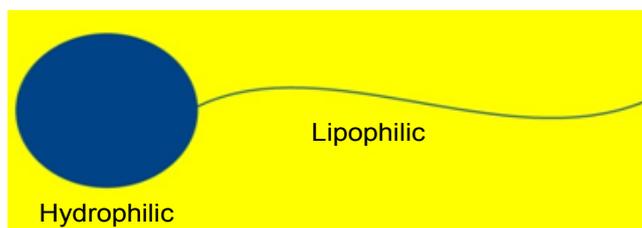


Figure 6: Structure of Biosurfactant.

| S.No. | Biosurfactant Type | Biosurfactant | Microorganism | Reference |
|-------|-------------------------------|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1. | Glycolipids | Rhamnolipids | <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas chlororaphis</i> | [21] |
| | | Diglycosyl diglycerides | <i>Lactobacillus fermentum</i> | [22] |
| | | Trehalose mycolates | <i>Rhodococcus erythropolis</i> , <i>Arthrobacter paraffineu</i> , <i>Mycobacterium phlei</i> , <i>Nocardia erythropolis</i> | [23] |
| | | Sophorolipids | <i>Candida bombicola</i> , <i>C. antarctica</i> , <i>Torulopsis petrophilum</i> <i>C. botistae</i> , <i>C. apicola</i> , <i>C. riodecensis</i> , <i>C. stellata</i> , <i>C. bogoriensis</i> | [24] |
| | | Aminoacids lipids | <i>Bacillus sp.</i> | [25] |
| 2. | Phospholipid and Fatty acids | Fatty acids/neutral lipids | <i>Clavibacter michiganensis</i> subsp. <i>Insidiosus</i> | [26] |
| | | Phospholipids | <i>Acinetobacter sp.</i> | [27] |
| 3. | Lipopeptides and lipoproteins | Peptide lipids | <i>Bacillus licheniformis</i> | [28] |
| | | Serrawettin | <i>Serratia marcescens</i> | [29] |
| | | Ornithine lipid | <i>Pseudomonas sp.</i> , <i>Thiobacillust hiooxidans</i> | [20] |
| | | Viscosin | <i>Pseudomonas fluorescens</i> , <i>Leuconostoc mesenteriods</i> | [30] |
| | | Surfactin | <i>Bacillus subtilis</i> | [31] |
| | | Subtilisin | <i>Bacillus subtilis</i> | [32] |
| | | Lichenysin | <i>Bacillus licheniformis</i> , <i>Bacillus subtilis</i> | [33] |
| 4. | Polymeric Surfactant | Protein PA | <i>Pseudomonas aeruginosa</i> | [34] |
| | | Liposan | <i>Candida lipolytica</i> | [35] |
| | | Lipoheteropolysaccharide (Emulsan) | <i>Acinetobacter calcoaceticus</i> RAG-1, <i>Arthrobacter calcoaceticus</i> | [36] |
| | | Polysaccharide-lipid complex (Snomax) | <i>Pseudomonas syringae</i> | [37] |
| 5. | Particulate biosurfactants | Alasan | <i>A. radio resistens</i> | [38] |
| | | Whole cells | <i>cyanobacteria</i> | [39] |
| | | Vesicles | <i>Acinetobacter sp.</i> | [20] |

Table 1: Various types of Biosurfactants produced by Microorganisms.

Hydrates could be recovered, it would be more than double the current domestic supply of Natural Gas [11,12].

Role of Microbes in Gas Hydrates

Actually high porosity of Methane Gas Hydrate with pore sizes of 100-400 nm and pore volumes of 25-40% [13] allows potential substrate eg. sulphate to enter and products of microbial metabolism eg. sulphite to exit the gas hydrate structure without difficulty. The microbe community is active with the Gas Hydrates.

Bacillus subtilis and *Pseudomonas aeruginosa* are identified in Gulf of Mexico gas hydrate mounds [14]. These microbes produce Surfactin and Rhamnolipid biosurfactants respectively.

The above generated biosurfactants i.e. Surfactin and Rhamnolipid reduce the surface tension of water and produce carbon alkyl group chains which form spherical micelles that solubilize hydrocarbon gases [15]. Water associates around the periphery of the micelles in close proximity to the solubilized gas. Thus micelles act as nucleation site for hydrate crystals to grow.

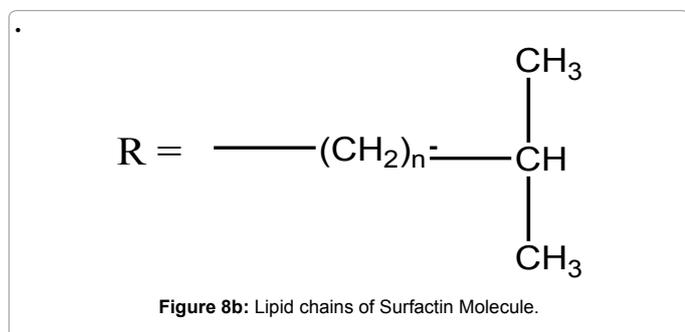
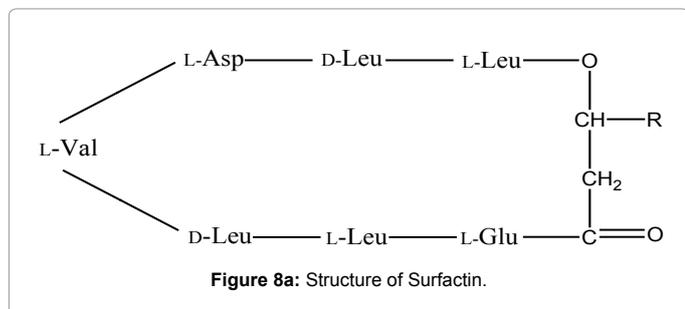
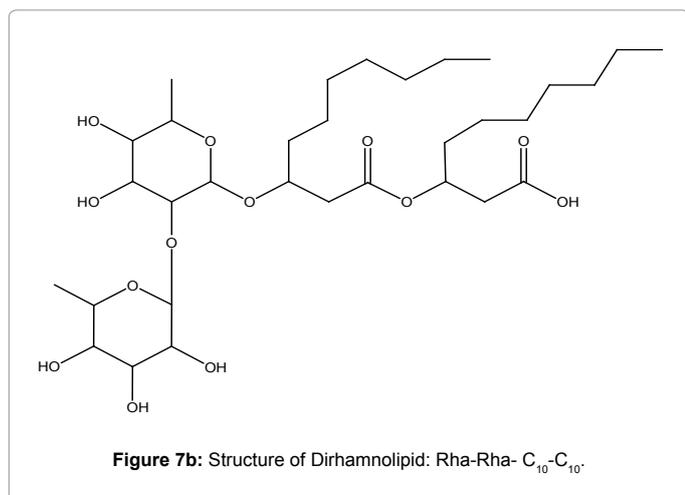
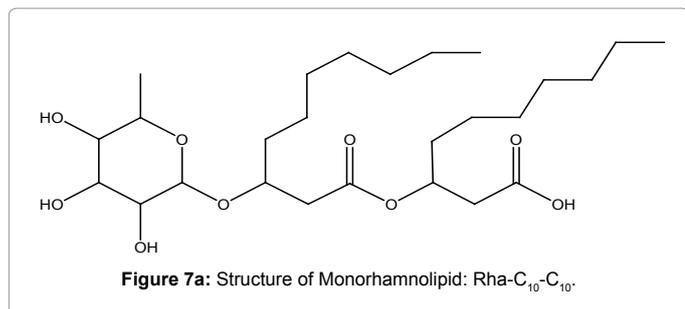
Biosurfactants

Biosurfactants are amphiphilic molecules (water loving and fat loving) consisting of a hydrophilic and a hydrophobic domain. Usually the hydrophobic domain is a hydrocarbon whereas the hydrophilic domain can be non-ionic positively or negatively charged or amphoteric [16,17] which is shown in Figure 6.

Biosurfactants have a unique property of lowering the surface and interfacial tension and Critical Micelles Concentration (CMC) in both aqueous solution and hydrocarbon mixtures [18,19]. CMC is the threshold concentration of a surfactant to form micelles. A good surfactant can lower the surface tension of water from 72 to 35 mN/m. Generally biosurfactants are more efficient and their CMC is about 10-40 times lower than that of chemical surfactants, i.e. less surfactant is necessary to get a maximum decrease in surface tension [20].

Microorganisms producing biosurfactants

Biosurfactants produced by microorganisms mainly bacteria, fungi and yeasts are diverse in chemical composition. The nature



and the amount of biosurfactant produced depend on the type of microorganism producing it. Biosurfactants can be classified as: (1) Glycolipids, (2) Phospholipid and Fatty acids. (3) Lipopeptides and lipoproteins, (4) Polymeric surfactant and (5) Particulate biosurfactants the various biosurfactants along with the micro-organisms producing them is as shown in table 1.

Types of Biosurfactants

Glycolipids

Rhamnolipid: Rhamnolipids are produced by *Pseudomonas aeruginosa*. They have a glycosyl head group, a Rhamnose moiety, and a 3-(hydroxyalkanoyloxy) alkanic acid (HAA) fatty acid tail. Mono-rhamnolipids and di-Rhamnolipids are two main classes of rhamnolipids; consisting of one or two Rhamnose groups respectively. Rhamnolipids are also heterogeneous in the length and degree of branching of the HAA moiety which varies with the growth media used and the environmental conditions. Rhamnolipids have much lower CMC than even Sodium dodecyl Sulphate [26,40,41]; and they can be adsorbed on sediment particle surfaces [18,26]. Micelles can migrate through porous media if micelle diameter is less than about 50 nm [26,42]. Rhamnolipid micelles is of the order of 5 nm diameter [43]; whereas SDS micelles are more accurately reported as spheres with 33.4 nm diameter [15], hence it can easily migrate through porous media. In the analysis of sand associated with the gas hydrates from Gulf of Mexico [14], the *Pseudomonas aeruginosa* bacterium was identified which generated Rhamnolipid. The structure of Rhamnolipid is shown in Figure 7a and 7b.

Lipopeptides and lipoproteins

Surfactin: Surfactin is lipopeptide formed from *Bacillus subtilis* bacterium. It is a lipopeptide that is composed of one β -hydroxy fatty acid, which has a long fatty acid moiety, and seven amino acids. It can lower the surface tension of water from 72 to 27 mN/m. [44]. It is named surfactin due to its exceptional surfactant activity [45].

Bacillus subtilis bacterium was identified in the analysis of sand associated with gas hydrates in the Gulf of Mexico [14], which generated surfactin.

The Structure of Surfactin is shown in Figure 8a and 8b

(where 'n' can vary for various Surfactin Molecules as 7,8,9,10)

Various types of amino acids present in a Surfactin is as given in Table 2.

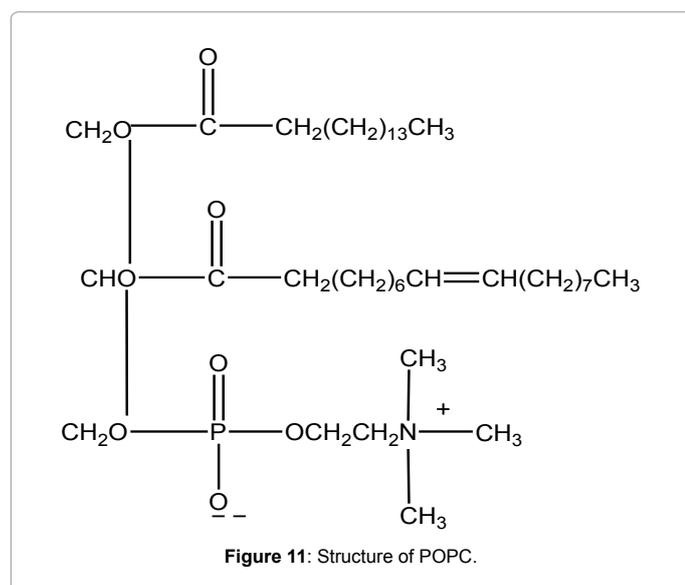
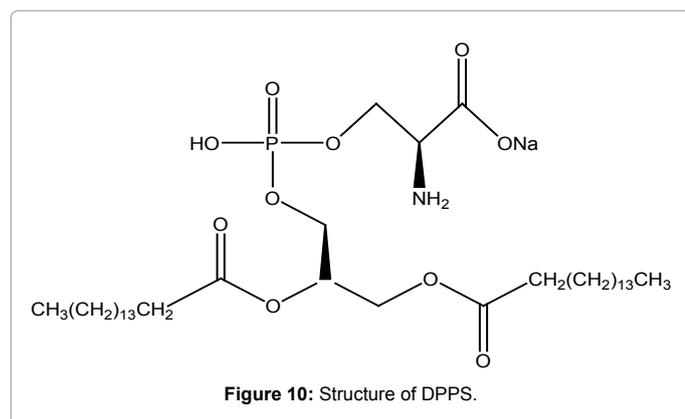
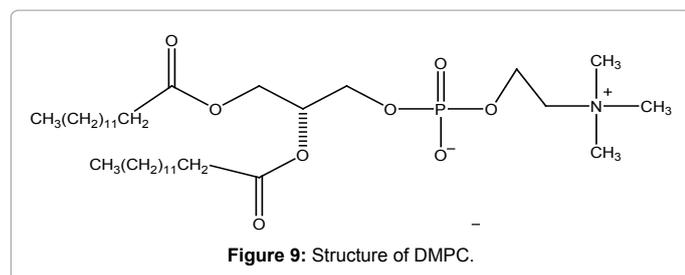
Phospholipid and fatty acids

Phospholipids: Phospholipids are a class of lipids and can form lipid bilayers, they are a major component of all cell membranes. Phospholipids generally contain a phosphate group, diglyceride, and a simple organic molecule such as choline. The structure of the phospholipid molecule consists of a hydrophilic head and a hydrophobic tails. Phospholipids provide membrane fluidity and mechanical strength in eukaryotic cells. There are three types of phospholipids that are synthetically derived as described below.

Phosphocholines (DMPC): DMPC has a chemical name as 1,2-Dimyristoyl-sn-glycero-3-phosphocholine. Its empirical formula is C₃₆H₇₂NO₈P and has a molecular weight 677.93. The structure of DMPC is as shown in Figure 9.

| S.No | Letter | Name of Amino Acid | Molecular Formula | Mass (g/mol) |
|------|--------|--------------------|------------------------------------------------|--------------|
| 1. | Val | Valine | C ₅ H ₉ NO ₂ | 117.151 |
| 2. | Asp | Aspartic acid | C ₄ H ₇ NO ₄ | 133.11 |
| 3. | Leu | Leucine | C ₆ H ₁₃ NO ₂ | 131.17 |
| 4. | Glu | Glutamic acid | C ₅ H ₉ NO ₄ | 147.13 |

Table 2: Amino Acids found in Surfactin.



Phosphoserines (DPPS): DPPS has a chemical name as 1, 2 Dipalmitoyl-sn-glycero-3-phospho-L-serine sodium salt. Its empirical formula is $C_{38}H_{73}NO_{10}PNa$ and has a molecular weight 757.95 The structure of DPPS is as shown in Figure 10.

Mixed Chain Phospholipids (POPC): POPC has a chemical name as 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. It is available commercially and synthetically. It is also naturally present in eukaryotic cell membranes. Its empirical formula is $C_{42}H_{82}NO_8P$ and has a molecular weight 760.08. The structure of POPC is as shown in Figure 11.

Polysaccharide-lipid complex

Snomax: Snomax is produced by *Pseudomonas syringae* bacterium.

It has ice nucleation protein. It is a type of polysaccharide-lipid complex [46]. It contains 34% protein, 15% mono and disaccharides, and 11% nucleic acids [47].

Emulsan: Emulsan is a type of polysaccharide-lipid complex produced by *Acinetobacter calcoaceticus* bacterium. The molecular weight of Emulsan is 89,000 [48]. Emulsan does not form micelles; however, its hydrophobic-hydrophilic moieties could serve to unite the water and the host hydrocarbon to promote hydrate formation.

Hydroxylated fatty acid

DL- α -Hydroxystearic acid: It is a synthetic fatty acid and has chemical name as DL- α -Hydroxystearic acid (2-Hydroxyoctadecanoic Acid) $C_{18}H_{36}O_3$, has a molecular weight 300.48. DL- α -Hydroxystearic Acid is a mixture of D and L- α -hydroxystearic Acid (2-Hydroxyoctadecanoic Acid) enantiomers. It has surface properties similar to corynocylic fatty acids [49]. The structure of Hydroxystearic acid is as shown in Figure 12.

Relationship between Gas Hydrates and Biosurfactants

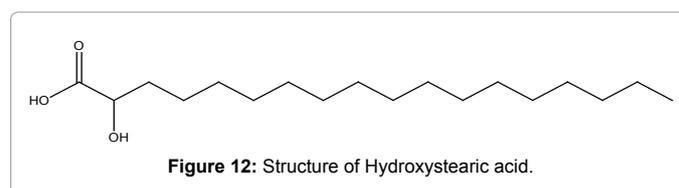
The Biosurfactants influence the induction time and rate of formation of Gas Hydrate. The induction time is the time elapsed till the appearance of a detectable volume of hydrate phase or until the consumption of a detectable number of moles of hydrate former gas. The induction time is also termed the hydrate nucleation time.

The kinetics of gas hydrate formation has been studied with biosurfactants in laboratory. It is reported that Rhamnolipids has much lower CMC than SDS (sodium dodecyl sulphate) [41,42]. Biosurfactant increase the rate of formation of gas hydrates above the CMC of the surfactant [50]. The surfactin is a powerful biosurfactant which can reduce the surface tension of water to as low as 27 mN/m. [42]. Biosurfactants catalyze gas hydrate formation in sea beds by following ways: (1) They reduce induction time of gas hydrate formation, (2) They increase formation rate of gas hydrate, (3) They solubilize guest gases in micelles that migrate through sands to a hydrate zone. The effects of Biosurfactants on Gas Hydrates kinetics is as shown in Table 3.

As it is evident from the above Table 3, that the biosurfactants increase the Hydrate formation rate and decrease the hydrate induction time. Surfactin has increased the hydrate formation rate by 288 %, which is the highest amongst the all biosurfactant. Reduction in Hydrate induction rate was also found maximum in surfactin. DL- α -Hydroxy-stearic acid ** is the only biosurfactant which decreased the hydrate formation rate instead of increasing. This could be due to fact that the fatty acid solution must have covered the wetted porous media-gas interface which could have resulted in low permeability for the diffusion of gas which resulted into the decrease in hydrate formation rate, which is exception amongst all.

Production technologies

The natural gas from gas hydrate can be produced via Depressurization [55], Thermal stimulation [56], Chemical Inhibitor Injection [57], and



| S.No | Micro-organism | Biosurfactant Produced | Induction Time in minute | Increase in Hydrate formation rate | Reduction in hydrate Induction time (approx.) | Reference |
|------|---------------------------------------------------------------|---------------------------|--------------------------|------------------------------------|-----------------------------------------------|---------------|
| | | | (approx.) | | | |
| 1 | <i>Pseudomonas aeruginosa</i> | Rhamnolipid | 53 | 96% to 107% | 58% | [21,51-53] |
| 2 | <i>Bacillus subtilis</i> | Surfactin | 35 | 288% | 71% | [31,49,51-53] |
| 3 | <i>Thiobacillus species</i> <i>Corynebacterium species</i> | Phospholipid | 1. DPPS | 1. 67 | 96% to 107% | 1. 47% |
| | | | 2. POPC | 2. 90 | | 2. 28% |
| | | | 3. DMPC | 3. 100 | | 3. 20% |
| 4 | <i>Pseudomonas syringae</i> | Snomax | 87 | 135% | 30% | [37,46,51-53] |
| 5 | <i>Corynebacterium lepous</i> | DL-A-Hydroxy-stearic acid | 56 | 49% ** | 58% | [49,51-53] |
| 6 | <i>Acinetobacter calcoaceti-cus</i> | Emulsan | 75 | 135% | 40% | [36,51-53,54] |

Table 3: Effects of various Biosurfactants on Gas Hydrate formation.

CO₂ sequestration [58]. Among these, Depressurization and Thermal stimulation have been considered to be the most economical, though other methods are under investigation. Due to less energy input for Depressurization, this method has been studied more than the Thermal Stimulation. Chemical Inhibitor Injection is not economical as it includes the cost of chemicals and their pumping. The efficiency of CO₂ sequestration is also less and investigations are going on at laboratory scale to enhance the efficiency of this method as it is greener than any other method.

Conclusion

A huge amount of methane is captured in gas hydrates even a small percentage of this can meet the energy demands of today's world for centuries. If we are able to exploit this resource of energy then these could be substitute to the fossil fuel and will put an advantage over them as cleaner fuels. Even these can be exploited by using CO₂ Sequestration which can give us the future energy resource by reducing the global warming. It was evident from the NGHP (National Gas Hydrate Program) expedition that the gas hydrates are distributed in the Krishna-Godavari, Mahanadi and Andaman off shores in Indian context. The *Pseudomonas aeruginosa* and *Bacillus subtilis* were found in the gas hydrate analyzed in the Gulf of Mexico which generated Rhamnolipid and Surfactin respectively. Hence there is a role of biosurfactants in the formation of gas hydrates in natural conditions. Long term production tests are planned in USA and Japan to establish the viability of efficient and long term production technologies. It is expected that the dream of taking energy from fire in the ice will be a reality very soon.

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