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The biosurfactant Surfactin as a kinetic promoter for methane hydrate formation

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Abstract

In the present study, the effect of the biosurfactant Surfactin on methane hydrate formation kinetics was studied. Initially, several marine derived species were screened for the presence of Surfactin. The polymerase chain reaction technique was used as the preliminary screening step for Surfactin which was then followed up by a couple of different assays to provide conclusive evidence of the same. Based on these tests, the D-9 bacterial strain was identified as a producer of Surfactin. Once the presence of Surfactin had been proven, its effect on methane hydrate formation kinetics was investigated upon by carrying out hydrate formation experiments in a stirred tank reactor. The cell free supernatant containing Surfactin was itself used as the hydrate forming solution without any further processing. It was found that the presence of Surfactin in the system greatly enhances hydrate formation kinetics as compared to pure water. In fact the kinetics in presence of Surfactin also surpassed that obtained with 1 wt% SDS, the most commonly used synthetic kinetic hydrate promoter. This basic study can pave the way for more sophisticated research on the use of biosurfactants as kinetic promoters with a view on rapid methane hydrate formation kinetics for applications such as methane separation, storage and transport.

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Keywords: Gas hydrate, biosurfactant, lipopeptide, kinetic promoter, gas uptake

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1. Introduction:

Gas hydrates are ice like crystalline compounds that are formed when small gas (guest) molecules get trapped in hydrogen bonded cages formed by water (host) molecules (Sloan and Koh 2008). Gas hydrates find application in a variety of currently relevant fields such as seawater desalination , gas separation, capture and storage [1], [2], [3], [4].

There are certain technological applications of gas hydrates which require fast hydrate formation and decomposition kinetics. By itself a gradual anad time consuming process, the kinetics of hydrate formation can be increased manifold by the introduction certain additives to the system known as surfactants. The different mechanisms through which these surfactants may enhance hydrate formation kinetics have been extensively discussed in the literature [5]. The surfactants that are used as kinetic promoters for hydrate formation are generally synthetic in nature, example Sodium dodecylsulfate (SDS). Although the use of such additives has proven to be very beneficial for gas hydrate formation, there's a big concern with regards to the toxicity of these compounds and their mode of disposal thereof. This ushers in the need to look for more benign additives which are low on the toxicity front and do not pose any threat to the environment, serious or otherwise.

Biosurfactants, quite simply surfactants of biological origin are one such class of compounds fit the above mentioned criteria perfectly in that being of biological origin, they are essentially green additives. The extreme robustness of biosurfactants (stability at extreme conditions of temperature, salinity and pH) makes the investigation into these compounds as kinetic hydrate promoters all the more worthwhile [6], [7], [8].

Lipopeptides are compounds with cyclic structures generally produced from Bacillus and Pseudomonas species and exhibit diverse properties such as anti-microbial, cytotoxixity and surfactant like behavior. As a result of these various different characteristics, lipopeptides find application in a variety of areas such as food production (as emulsifiesrs), oil recovery from reservoirs, bioremediation etc.

Surfactin, the most popular and widely studied lipopeptide is an excellent biosurfactant and can reduce the surface tension of water from 72 to 27 mN/m. In fact it shows better surface activity than SDS, the surfactant of choice for gas hydrate studies [9]. Surfactin was discovered by Arima et al., 1968 from the culture broth of *bacillussubtilis* in an attempt to discover fibrin clot inhibitor [10].

In the present study, a few different marine derived bacterial species were screened to test for the presence of Surfactin. The Polymerase Chain Reaction (PCR) technique was used for the preliminary screening based on which one of the bacterial isolates D-9 showed the presence of surfactin. The PCR results were followed upon with a few different assays such as oil spread assay and emulsification assay on the isolate D-9 to definitively prove the presence of surfactin. The effect of Surfactin on methane hydrate formation kinetics was then looked into by carrying out hydrate formation experiments in a stirred tank reactor.

2. Materials and Methods:

The marine derived bacterial strains to be screened for the production of Surfactin were field collected. Pure methane gas (purity > 99.5 %) was purchased from Vadilal Gases Pvt. Ltd., India. Peptone, Beef Extract and NaCl were purchased from HiMedia Laboratories, Pvt. Ltd., India. Distilled and deionized water was used for all the experiments performed.

2.1 Procedure followed for the production of Surfactin:

The first step for the production of Surfactin is the preparation of the nutrient broth. The nutrient broth used in the present study consisted of 10 gm Peptone, 10 gm Beef Extract and 5 gm NaCl in 1 litre of

water. Once the nutrient broth had been prepared, the previously isolated marine bacteria was grown in the nutrient borth for 48-72 hours at 30 ^{o}C and the cell free supernatant was obtained through centrifugation at 10,000 rpm for 20 minutes. The supernatant was then subjected to acid precipitation by adjusting the pH to 2.0 with 6M HCl and keeping it overnight at 4^{o}C. The precipitate formed was recovered by centrifugation at 10,000 rpm for 20 minutes at 4^{o}C and then extracted with methanol and concentrated with help of rotary evaporator [11].

2.2 Procedure followed for hydrate formation experiments:

A schematic of the apparatus used and detailed description of the procedure followed for the hydrate formation experiments is given elsewhere in literature [1]. The important thing to note here is that for the hydrate formation experiments, the cell free supernatant containing Surfactin itself was used as the hydrate forming solution. Since this was a basic study performed mainly to gauge whether the presence of Surfactin in the system has any effect on methane hydrate formation kinetics or not, using the crude supernatant sufficed and further processing of the supernatant to exclusively isolate Surfactin was not carried out. The pressure and temperature conditions for hydrate formation were 5.0 MPa and 274.15 K respectively while the stirring speed used for the same was 400 rpm. The volume of hydrate forming solution used was 80 cm^3 while the reactor vessel had a total volume of 250 cm^3.

3. Results and Discussions:

3.1 Screening for the presence of Surfactin:

3.1.1 Preliminary screening using the Polymerase Chain Reaction (PCR) technique:

While the screening of lipopeptide producing bacteria from a large number of isolates using different assays is generally a very arduous task, PCR is widely accepted as a reliable preliminary technique for screening of lipopeptides. Screening of Surfactin using PCR was done by Hsieh et.al, 2004 who found out that PCR was a dependable method for finding out potential good yields of surfactant producing strains [12]. The PCR technique was developed by Karry Mullis in 1984 and consists of three main steps: a) denaturation of the double stranded DNA two single strands at a high temperature of 90-98 ºC, b) annealing of the primers to the single stranded DNA at a lower temperature (50-60 ^{o}C) and c) extension of the bound primers by the addition of nucleotides. A specific SFP gene primer was used for screening of Surfactin gene through PCR and the product was checked using Gel Electrophoresis (0.8% Agarose). Out of all the isolates tested, only the D-9 strain was seen to show a positive marker for the presence of the Surfactin gene using the PCR technique.

The preliminary screening done using PCR was followed up with a couple of other assays to conclusively prove the presence of Surfactin:

3.2 Oil Spreading Assay:

The concept behind the oil spreading assay is relatively simple. The presence of surfactant should ideally displace the oil surface leaving behind a *clearing* zone on the oil surface. The assay was performed according to Morikawa et.al, 2000 [13]. 30 ml of distilled water was taken on a petri dish and 1 ml of oil was placed on the centre of the water layer. 20 µl of cell free supernatant solution was then placed gently on the centre of the oil layer. If the surfactant is present in the supernatant, the oil layer gets gradually displaced and a clearing zone can be observed at the centre of the oil layer. The diameter of displaced oil is measured after 30 seconds which correlates to surfactant activity.

3.3 Emulsification Assay:

Emulsification assay was carried out according to Cooper and Goldenberg, 1987. 6ml of hydrocarbon was taken in a test tube to which 4ml of cell free supernatant was added and vortexed for 2 minutes to ensure homogenous mixing of both the liquids. The emulsification activity was observed after 24 hours and it was calculated by using the formula: total height of emulsion/total height*100 [14]. The D-9 strain was able to emulsify different oil and hydrocarbon (petrol) from 50% to 66% range showing good emulsification property.

3.3 Effect of Surfactin on Methane Hydrate formation kinetics:

The effect of Surfactin on methane hydrate formation kinetics was investigated by using the Surfactin containing cell free supernatant as the hydrate forming solution in a stirred tank reactor. **Fig. 1** given below compares the average gas uptake (mol of gas consumed/ mol of water) obtained using the Surfactin containing supernatant with that for pure water. Hydrate formation kinetics was also recorded using just the nutrient broth as the hydrate forming solution and has been included in **Fig. 1**. Time zero in **Fig. 1** corresponds to the induction time for all the experiments carried out. As can be seen in the figure, hydrate formation kinetics is significantly enhanced in the presence of Surfactin as compared to pure water. The considerable enhancement observed when compared with the kinetics in presence of just the nutrient broth also proves the presence of Surfactin in the supernatant solution used. It also tells us that the biosurfactant Surfactin as an individual has a definite significant promoting effect on methane hydrate formation.

Since the nutrient broth consists of three different components, NaCl, Beef extract and Peptone, it was decided to individually check the effect of these three compounds on methane hydrate formation kinetics. **Fig. 2** given below plots the average rate of gas uptake in presence of these three additives in the system and compares the methane hydrate formation kinetics obtained with that for pure water. The concentrations of the three individual components were kept the same as in the nutrient broth. It can be observed from **Fig. 2** that while Peptone and Beef extract both significantly enhance hydrate formation kinetics, the introduction of NaCl into the system hardly has any effect on the same.

Fig. 3 compares the gas uptake obtained in presence of biosurfactant Surfactin with that obtained in presence of SDS, the most commonly used synthetic kinetic hydrate promoter. The concentration of SDS used was 1 wt% while for Surfactin, the cell free supernatant solution was used. As can be clearly seen in **Fig. 3**, although the initial kinetics is higher with SDS, the overall hydrate formation kinetics is significantly higher for the Surfactin containing supernatant system. There is a considerable jump in the final gas uptake after one hour of hydrate formation for the system containing Surfactin as compared to the 1 wt% SDS system and as hydrate formation has nearly reached saturation at the end of one hour for both systems, the gas uptake at the end of one hour can well be taken as the final gas uptake for hydrate formation for both the systems in consideration here. This result is extremely vital as it shows that the non-toxic and environment friendly biosurfactant Surfactin actually shows better methane hydrate formation kinetics as compared to the commonly used synthetic kinetic hydrate promoter SDS.

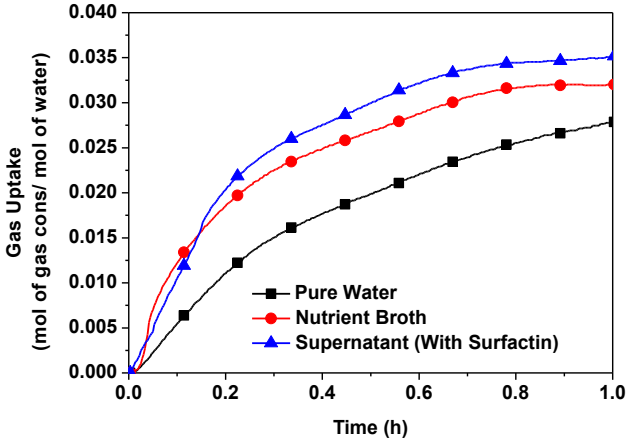

Fig. 1. Comparison of average gas uptake during methane hydrate formation from different hydrate forming solutions: pure water, nutrient broth and cell free Surfactin containing supernatant.

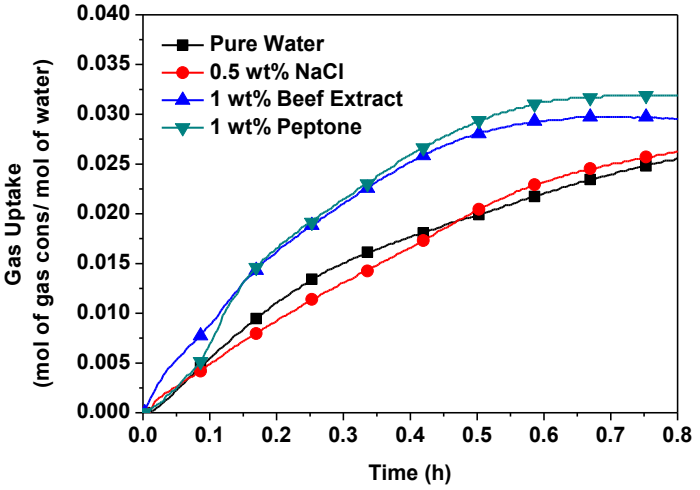

Fig. 2. Comparison of average gas uptake during methane hydrate formation individually with pure water, NaCl, Beef Extract and Peptone (the different components present in the Nutrient Broth).

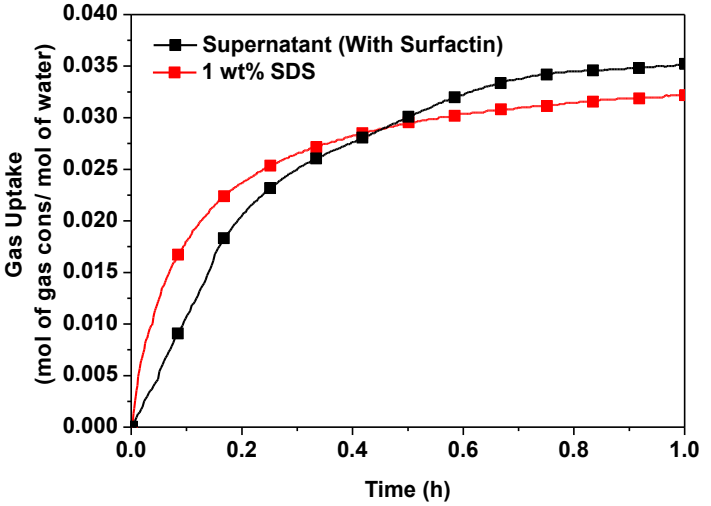

Fig. 3. Comparison of average gas uptake during methane hydrate formation with cell free supernanatant contianing the biosurfactant Surfactin and with 1 wt% SDS.

4. Conclusion

The present study deals with testing the effect of the biosurfactant Surfactin on methane hydrate formation kinetics. Based on preliminary screening using the polymerase chain reaction technique and a couple of other assays, namely the oil spread assay and emulsification assay, the D-9 bacterial strain was concluded to be a producer of Surfactin. Methane hydrate formation experiments were carried out in the presence of Surfactin using a stirred tank reactor. It was found out that the presence of Surfactin favorably affects methane hydrate formation kinetics showing a significant enhancement as compared to pure water. The enhancement in kinetics observed with Surfactin was found to be much greater than that obtained with 1 wt% SDS. The results obtained in this study hold great importance as we look to move away from synthetic additives to benign, environment friendly ones for use as kinetic promoters in gas hydrate based applications such as methane separation, storage and transport.

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