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Ito, Miu

Department of Earth Resources Engineering, Graduate School of Engineering, Kyushu University

Sugai, Yuichi

Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University

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## Article

# Fundamental Investigation on a Foam-Generating Microorganism and Its Potential for Mobility Reduction in High-Permeability Flow Channels

Miu Ito <sup>1</sup> and Yuichi Sugai <sup>2,\*</sup>

<sup>1</sup> Department of Earth Resources Engineering, Graduate School of Engineering, Kyushu University, Fukuoka 8190395, Japan; ito.miu.422@s.kyushu-u.ac.jp

<sup>2</sup> Department of Earth Resources Engineering, Faculty of Engineering, Kyushu University, Fukuoka 8190395, Japan

\* Correspondence: sugai@mine.kyushu-u.ac.jp; Tel.: +81-92-802-3328

**Abstract:** This study proposed a novel foam EOR technique using *Pseudomonas aeruginosa* to generate the foam and investigated the potential of the microbial foam EOR to modify the permeability of a high-permeability porous system. We investigated oxygen nanobubble, carbon dioxide nanobubble and ferrous sulfate concentrations to discover the optimal levels for activating the foam generation of the microorganism through cultivation experiments. We also clarified the behavior of the microbial foam generation and the bioproducts that contribute to the foam generation. The potential of the foam to decrease the permeability of high-permeability porous systems was evaluated through flooding experiments using sand pack cores. The foam generation became more active with the increase in the number of nanobubbles, while there was an optimal concentration of ferrous sulfate for foam generation. The foam was identified as being induced by the proteins produced by the microorganism, which can be expected to bring about several advantages over surfactant-induced foam. The foam successfully decreased the permeability of high-permeability sand pack cores to half of their initial levels. These results demonstrate that the microbial foam EOR has the potential to decrease the permeability of high-permeability porous systems and improve the permeability heterogeneity in oil reservoirs.

**Keywords:** enhanced oil recovery; *Pseudomonas aeruginosa*; foam; protein; nanobubbles; ferrous sulfate; permeability; flooding experiment; sand pack core; bacterial cell number

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## 1. Introduction

Microbial enhanced oil recovery (MEOR) is an EOR technique involving the injection of microorganisms, which produce materials such as surfactants and polymers that are effective for EOR, into oil reservoirs. *Pseudomonas aeruginosa* is well known as a microorganism that is effective for MEOR because it produces rhamnolipid, which is a strong biosurfactant [1,2], it decreases oil viscosity due to the biodegradation of petroleum hydrocarbons [3,4] and it has been used in several MEOR field pilot trials [5–7]. Although *P. aeruginosa* has been found in and isolated from oil reservoir samples by many researchers [8–10], there is the problem that the production of surfactants and the degradation of petroleum hydrocarbon may not be actively brought by *P. aeruginosa* in poorly oxygenated environments, such as oil reservoirs, because the metabolic activities of *P. aeruginosa* are usually active under aerobic conditions [11–13]. Therefore, we investigated the culture conditions that are required for activating the metabolism of *P. aeruginosa*, even in anaerobic environments. We cultivated *P. aeruginosa* in a culture medium that was supplied with oxygen nanobubbles as the oxygen source under an anaerobic nitrogen atmosphere and found that it actively grew and generated foam on the surface of its culture solution

[14,15]. Then, we conceived a microbial foam EOR that brings about the enhancement of oil recovery through the foam generated in situ by *P. aeruginosa*.

Normal foam EOR is a popular method for improving the mobility in EOR, especially CO<sub>2</sub> EOR [16]. We can also expect it to be applied for the development of shale oil formations derived from that in [17]. The foam can improve sweep efficiency by reducing both viscous fingering and gravity segregation, which are major problems during CO<sub>2</sub> injection [18–25]. Both surfactant solutions and gas, such as CO<sub>2</sub>, must be alternately injected into oil reservoirs during normal foam EOR because the foam formation is usually induced by both surfactants and gas. Therefore, normal foam EOR has several challenges that are similar to those of surfactant EOR. Surfactants are well known as expensive chemicals for EOR. They can only be consumed around the injection site because they may form precipitation due to their chemical reaction with ions that are contained in the reservoir brine and/or adsorption onto the reservoir rock surface. Therefore, surfactants may not be widely spread throughout the reservoir.

The microbial foam EOR that we are proposing has the potential to overcome these challenges. Since only the nutrients for *P. aeruginosa* are required for the microbial foam EOR, it does not require expensive chemicals, such as surfactants, and when the inexpensive nutrients can be prepared, our method can be operated at a low cost. Surfactants may not be provided over a wide region in oil reservoirs during normal foam EOR because surfactants can only be consumed around the injection site just after the injection into the oil reservoir has occurred for the reasons described above. On the other hand, the foam can be provided over a wide region within the reservoir in our method and not only around the injection site because *P. aeruginosa* grows away from the injection site and generates the foam there.

Thus, there are several advantages of using *P. aeruginosa* for microbial foam EOR in oil reservoirs. Our method aimed to reduce the mobility of high-permeability flow channels where oil has been sufficiently recovered and water saturation is high. Therefore, this study determined the suitable cultivation conditions for *P. aeruginosa* to generate the foam. The behavior of the foam generation was examined and the constituent components of the foam were identified. Moreover, this study evaluated the effect of the foam generated by *P. aeruginosa* on the decrease in permeability through flooding experiments using high-permeability sand pack cores.

## 2. Materials and Methods

### 2.1. Culture Medium

*P. aeruginosa* ATCC10145 was inoculated into a culture medium that consisted of 4 g/L of Nutrient Broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), which was sterilized at 121 °C for 20 min and aerobically incubated at 31.5 °C for 3 days for preculture. This preculture solution was used as an inoculum in this study.

A modified Cooper's medium [26] was used for the culture medium of *P. aeruginosa* in this study. The culture medium was prepared by adding 40 g of glucose, 5.7 g of disodium hydrogen phosphate, 4.0 g of potassium dihydrogen phosphate, 4.0 g of ammonium nitrate, 0.1 g of magnesium sulfate, 8.0 mg of calcium chloride, 1.0 mg of ethylenediaminetetraacetic acid, 0.12 mg of ferrous sulfate and 0.2 mg of manganese sulfate to 1 L of tap water.

Our previous studies have shown that both the growth and production of *P. aeruginosa* proteins and gas are activated in culture mediums containing oxygen nanobubbles, carbon dioxide nanobubbles or higher concentrations of ferrous sulfate. In this study, four types of culture media were prepared, including a nanobubble-free medium, an oxygen nanobubbles medium, a carbon dioxide nanobubbles medium and a ferrous sulfate-enriched medium. The number of nanobubbles and the concentration of ferrous sulfate were varied in order to estimate the optimal concentration of each additive for foam generation.

The number of nanobubbles was varied by diluting the nanobubble water with nanobubble-free tap water. The ferrous sulfate-enriched culture medium was prepared by adding ferrous sulfate to the modified Cooper's medium. The culture media containing nanobubbles were prepared using the following procedure.

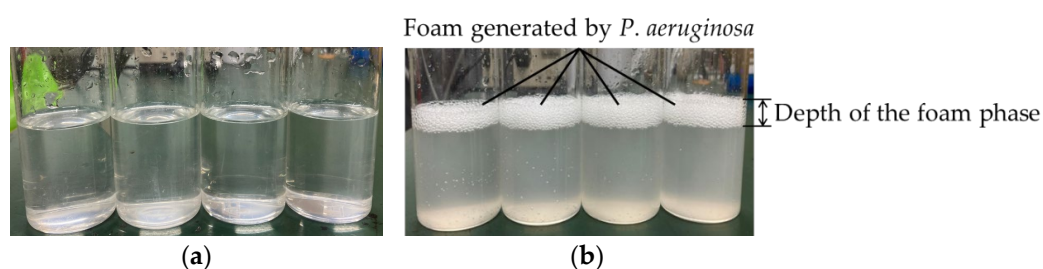
Water containing nanobubbles (hereinafter called "nanobubble water") was prepared using a commercially available nanobubble generator (Art Verre International Co., Ltd., Minoh, Japan). Jadhav et al. (2020) and Yang et al. (2007) [27,28] reported that the number of nanobubbles increased in water containing a small amount of ethanol. Jadhav et al. (2020) [27] also reported that the stability of nanobubbles was increased in water containing a small amount of ethanol. According to their paper, the ethanol molecules strengthened the hydrogen bonds around the nanobubbles. Zimmermann et al. (2011) [29] showed that the strong hydrogen bonds around the nanobubbles dominantly contributed to the stability of the nanobubbles. Moreover, Chen et al. (2021) [30] proposed a process to stabilize the nanobubbles in an aqueous ethanol solution. Their paper concluded that ethanol molecules and water molecules form a stabilizing layer structure around the nanobubbles to promote their stability. Therefore, this study prepared nanobubble water using tap water that was supplemented with 1.1% ethanol in order to increase the number of nanobubbles. The tap water supplemented with 1.1% ethanol was introduced into the nanobubble generator using a submersible pump. The nanobubble water that was discharged through the nanobubble generator was collected in a glass beaker, which was then used for the preparation of the culture media. Since the source of nanobubbles produced by the nanobubble generator is the gas that is dissolved in the original water, the number of nanobubbles can be increased by increasing the amount of gas being dissolved in the original water. The tap water, which had been cooled to 14 °C, was used as the original water in this study in order to increase the amount of gas being dissolved. Moreover, the original water was bubbled with gas in order to increase the amount of gas being dissolved in the original water. Both oxygen nanobubble water and carbon dioxide nanobubble water were prepared and used for the experiments in this study. We conducted preliminary experiments investigating the stability of the nanobubbles by heating the CO<sub>2</sub> nanobubble water to 31.5 °C, which was the cultivation temperature for 20 days. As a result, the scattering intensity of the green laser light emitted through the ethanol–nanobubble water was only slightly decreased, even after 20 days, while that of the ethanol-free nanobubble water was drastically decreased after 7 days and completely unobservable after 20 days.

First out of the components described above, a concentrate was prepared so as to have a concentration of 10 times that of all of the other components apart from glucose, ferrous sulfate and manganese sulfate. To achieve this, 5 mL of the concentrate was poured into 126 mL vials and autoclaved at 121 °C for 20 min. Then, a solution in which glucose, ferrous sulfate and manganese sulfate were added to the ethanol–nanobubble water in concentrations of 1.1 times the amounts described above was subjected to filtration sterilization using a gamma sterilized membrane filter with a pore size of 0.45 µm. Next, 45 mL of this sterilized solution was added to the vial containing the 5 mL of sterile concentrate that was previously described. The median diameter and number of the nanobubbles in the oxygen nanobubble water, carbon dioxide nanobubble water and nanobubble-free water, which were sterilized with the membrane filter, were determined using Zeta View PMX100 (MicrotracBEL Corp., Osaka, Japan). The median diameter and number of oxygen nanobubbles and carbon dioxide nanobubbles in the culture media were 119.6 nm and  $4.0 \times 10^6$  bubbles/mL and 128.6 nm and  $1.0 \times 10^7$  bubbles/mL, respectively. As  $2.2 \times 10^7$  bubbles/mL of nanobubbles were also detected in the nanobubble-free water, the number of nanobubbles in the other solutions was calculated by deducting the concentration of nanobubbles in the nanobubble-free water from that of the two other solutions of nanobubble water.

## 2.2. Culture Experiments

The bacterial cell concentration in the inoculum was counted through the direct counting method using phase contrast microscopy. Then, the inoculum was inoculated into the culture medium so that the initial bacterial cell concentration was  $5.0 \times 10^4$  cells/mL. Thereafter, the vial was sealed with a sterilized butyl rubber cap and an aluminum seal. The gas phase in the vial was completely replaced with pure nitrogen to create an anaerobic condition. The bacteria were stationarily cultivated at 31.5 °C.

First, the optimal concentrations of oxygen nanobubbles, carbon dioxide nanobubbles and ferrous sulfate for foam generation were evaluated through experiments cultivating *P. aeruginosa* in the culture media with different concentrations of each additive. Foam generation was evaluated by measuring the depth of the foam phase in the vial after 72 h of cultivation, as shown in Figure 1.



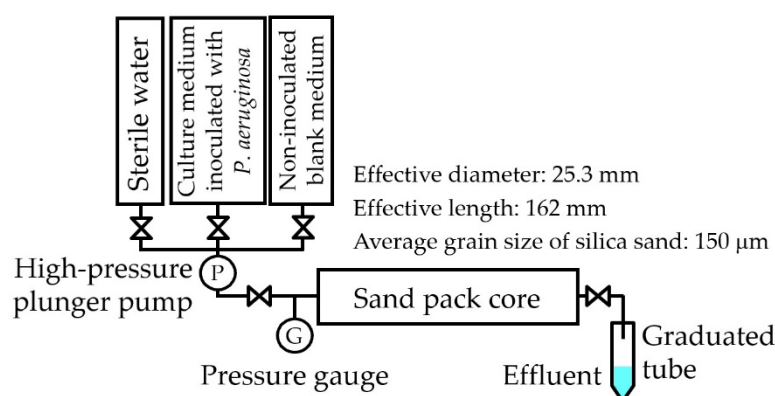
**Figure 1.** Foam generation by *P. aeruginosa*: (a) the original culture medium; (b) the foam generated by *P. aeruginosa* and the depth of the foam phase.

Next, *P. aeruginosa* was cultivated in culture media containing the optimal concentration of each additive in order to understand the behavior of the foam generation by measuring the depth of the foam phase in the vial every 12 h, from 24 h after the start of cultivation. The concentrations of surfactants, polysaccharides and proteins in the culture solutions were also measured in order to identify the bioproducts that were contributing to the foam generation in these experiments. *P. aeruginosa* is well known as a producer of rhamnolipid, which is an anionic biosurfactant. Surfactants are usually used for generating foam in foam EOR because they have the potential to contribute to the foam generation. The concentrations of anionic surfactants in the culture solutions were therefore measured using an HI-97769 anionic surfactant portable photometer (Hanna Instruments, Woonsocket, RI, USA), in accordance with the instruction manual. The culture media contained high concentrations of glucose, which *P. aeruginosa* could convert into polysaccharides. Polysaccharides may contribute to an increase in the stability of the foam, so the concentration of polysaccharides was also measured. First, the total sugar content in each culture solution was measured using the phenol–sulfuric acid method. The concentration of glucose that was not consumed by *P. aeruginosa* and remained in the culture solution was also measured using Reflectoquant® test strips in combination with an RQflex reflectometer (Merck KGaA, Darmstadt, Germany), in accordance with the instruction manual. Then, the concentration of polysaccharides was calculated by deducting the concentration of glucose remaining in the culture solution from that of the total sugar. *P. aeruginosa* is also known as a producer of proteins that contribute to foam generation. The concentrations of proteins in the culture solutions were therefore also measured using a TaKaRa Bradford Protein Assay Kit (Takara Bio Inc., Shiga, Japan), in accordance with the instruction manual.

The cultivation experiments were performed in duplicate with the same cultivation conditions in order to confirm the reproducibility of the results.

### 2.3. Sand Pack Core Flooding Tests

Figure 2 shows the setup that was used for the sand pack core flooding experiments, which evaluated the effect of the foam generated by *P. aeruginosa* on permeability modification. A sand pack core was prepared for each experiment by densely packing silica sand, with an average grain size of 150  $\mu\text{m}$ , into an acrylic pipe. Rubber caps were installed at both ends of the sand pack core and it was then connected to a pump and a pressure gauge. The effective diameter and length of each sand pack core was 25.3 mm and 162 mm, respectively.



**Figure 2.** A schematic image of the setup used for the flooding experiments.

Table 1 shows the initial conditions of the sand pack cores that were used in this study. Four sand pack cores were prepared for the flooding tests, involving an oxygen nanobubble medium, a carbon dioxide nanobubble medium, a ferrous-enriched medium and a non-inoculated blank medium. The absolute permeability of the sand pack cores was measured by injecting five pore volumes of sterile water into the cores with different injection rates between 0.2 and 1.0 mL/min and then calculated using the Darcy equation. The four sand pack cores did not show any differences in porosity but did show differences in absolute permeability, as shown in Table 1. Since the silica sand was artificially packed into the acrylic pipes, it was difficult to maintain the absolute permeability of those cores uniformly. The changes in the absolute permeability of the sand pack cores after in situ cultivation was therefore evaluated based on the relative change of the permeability, as described later.

**Table 1.** The initial conditions of the sand pack cores used in this study.

Culture Medium Injected	Pore Volume, mL	Porosity, %	Absolute Permeability, Darcy
O <sub>2</sub> nanobubble medium	38.6	47.4	1.2
CO <sub>2</sub> nanobubble medium	36.5	44.8	6.0
Ferrous-enriched medium	37.0	45.5	4.8
Non-inoculated blank medium	38.0	46.7	2.7

After the injection of five pore volumes of sterile water, two pore volumes of culture medium that had been inoculated with *P. aeruginosa* were injected into the sand pack core at an injection rate of 0.5 mL/min. Both the injection rate and injection pressure were measured during the injection of the culture medium and the apparent permeability of the sand pack core was calculated using the Darcy equation. Then, the sand pack core was sealed

and placed into an incubator in which the temperature was automatically adjusted to 31.5 °C for cultivating the microorganisms within the sand pack core. The foam generation in the sand pack core was not directly observed; therefore, the culture medium that remained in the vial after injection was also cultivated at the same temperature in order to estimate the foam generation within the sand pack core. The only difference between the culture in the vial and that in the sand pack core was the presence or absence of sand. Although the effect of sand on the foam generation caused by *P. aeruginosa* was not directly considered, the chemical composition of the sand used in this study was mainly 85.0% SiO<sub>2</sub> and 8.6% Al<sub>2</sub>O<sub>3</sub>, which was similar to the chemical composition of the glass bottle used in this study. It was therefore presumed that the effect of sand itself on the foam generation of *P. aeruginosa* was not significant.

When foam is generated by *P. aeruginosa* in the sand pack core, the volume of the culture solution should expand, which should increase the pressure in the sand pack core. The pressure in the sand pack core was measured during the cultivation in order to estimate the foam generation occurring in the sand pack core.

The apparent permeability measurement was performed by injecting post-flush water into the sand pack core at an injection rate of 0.5 mL/min 72 h after foam generation was observed in the vial. The effect of the foam on the permeability modification was evaluated using the value of specific permeability, which was defined as the quotient of the apparent permeability and the apparent permeability that was measured before cultivation. It was calculated using the following equation:

$$k_s = \frac{k_a}{k_b} \quad (1)$$

where  $k_s$  is the specific permeability,  $k_a$  is the apparent permeability that was measured after the cultivation of *P. aeruginosa* in the sand pack core and  $k_b$  is the apparent permeability that was measured before the cultivation. The bacterial cell concentration in the effluent from the sand pack core was also counted every 20 min during the post-flush.

The culture media including nanobubbles or ferrous sulfate with optimal conditions for foam generation were tested in this experiment. A non-inoculated blank medium was also tested as a reference.

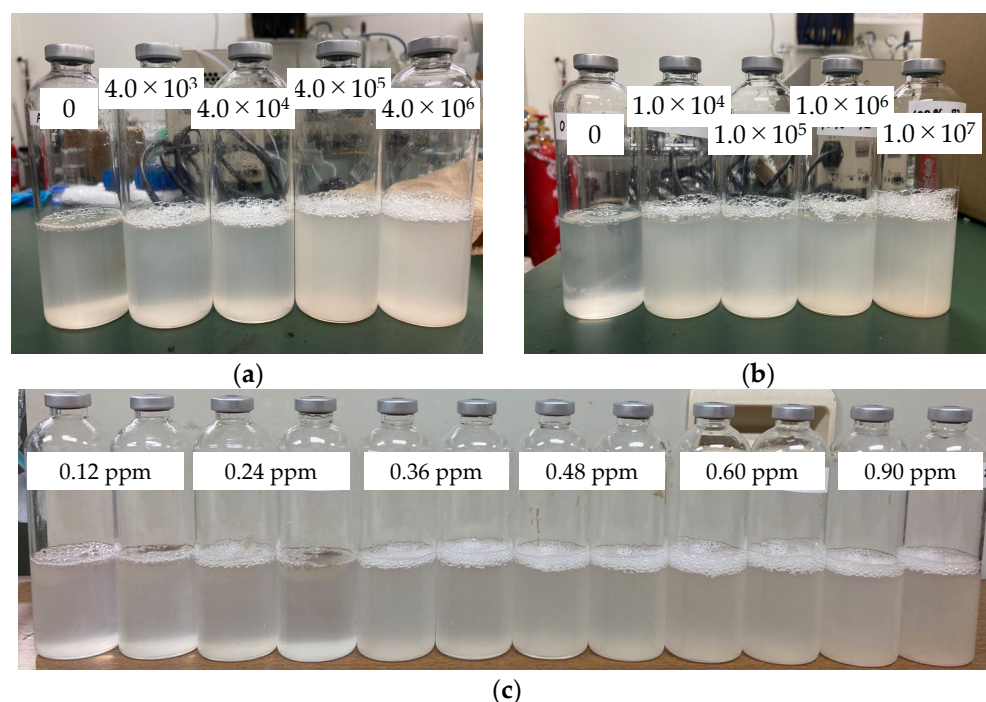
### 3. Results

#### 3.1. Evaluation of the Optimal Concentrations of Additives for Foam Generation

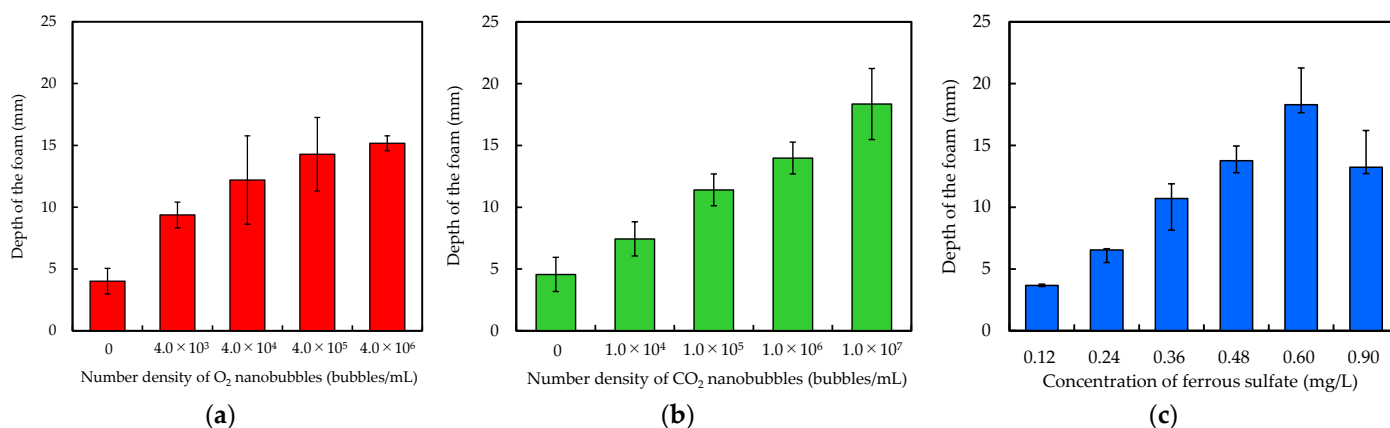
Figure 3 shows the culture solution in which *P. aeruginosa* was cultivated in media with different numbers of nanobubbles and concentrations of ferrous sulfate. Figure 4 shows the depth of the foam phase that was observed in each culture medium after 72 h of *P. aeruginosa* cultivation.

The foam generation became more active as the number of nanobubbles increased in the case of both nanobubble media. Our previous studies have shown that the production of proteins and gas, which are the source of the foam, is activated under the presence of nanobubbles, and foam generation was also activated in this study as the number of nanobubbles increased. There was no significant difference between the foam generation in the oxygen nanobubble medium and the carbon dioxide nanobubble medium in this experiment. Figure 3c shows that foam was generated by *P. aeruginosa* with high reproducibility in media with a ferrous sulfate concentration of 0.36 ppm or more. According to Figure 4, the optimal concentration of ferrous sulfate for foam generation by *P. aeruginosa* was 0.6 ppm.





**Figure 3.** The culture solutions in which *P. aeruginosa* was cultivated in media for 72 h with different numbers of nanobubbles and concentrations of ferrous sulfate: (a) the culture solutions containing different numbers of oxygen nanobubbles; (b) the culture solutions containing different numbers of carbon dioxide nanobubbles; (c) the culture solutions containing different concentrations of ferrous sulfate.



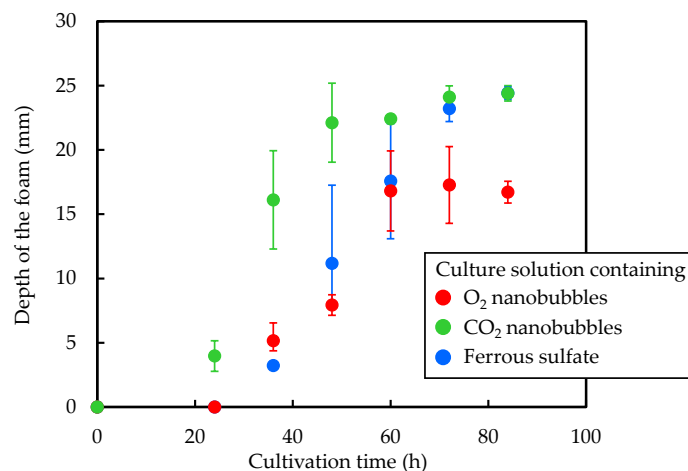
**Figure 4.** The depth of the foam phase observed in each culture medium after 72 h of *P. aeruginosa* cultivation: (a) the oxygen nanobubble medium; (b) the carbon dioxide nanobubble medium; (c) the ferrous sulfate-enriched medium.

### 3.2. Behavior of the Foam Generation in Different Culture Media

Figure 5 shows the temporal changes in the depth of the foam phase in each culture medium. In the culture medium containing carbon dioxide nanobubbles, foam began to be generated 24 h after the start of cultivation, while it began to be generated 36 h after the start of cultivation in the other media. The foam generation rate of the carbon dioxide nanobubble medium was the fastest of the three media. The total amount of foam generated within 84 h was also the largest in that medium. These results suggest that carbon dioxide nanobubbles are the most effective for promoting foam generation by *P. aeruginosa*. Although the foam generation rate in the ferrous sulfate-enriched medium was a little slower than that in the carbon dioxide nanobubble medium, the total amount of the

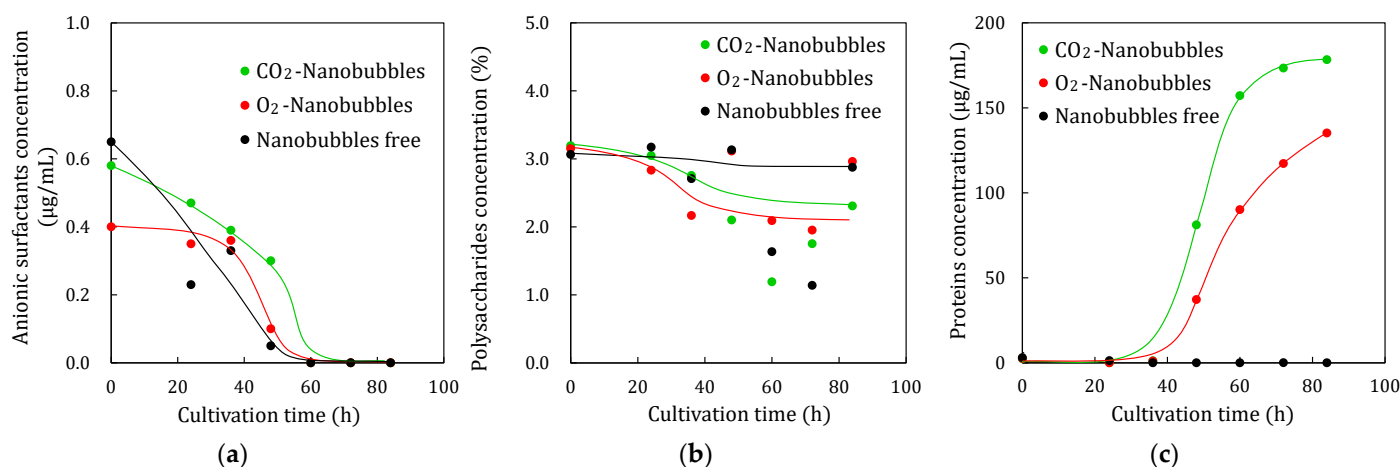


foam generated in the ferrous sulfate-enriched medium was similar to that in the carbon dioxide nanobubble medium. Both the rate and total amount of foam generation in the oxygen nanobubble medium were the smallest of the three media. The foam generation converged within almost 72 h, regardless of the conditions.



**Figure 5.** The temporal changes in the depth of the foam phase in each culture medium.

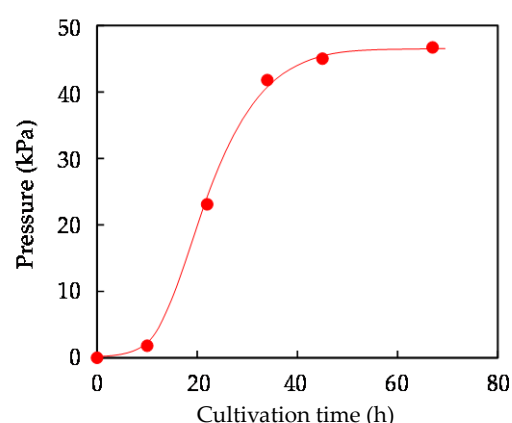
Figure 6a–c shows the temporal changes in the concentrations of surfactants, polysaccharides and proteins in each culture medium, respectively. The concentration of surfactants decreased and reached zero after 60 h, when the depth of foam phase reached its maximum value, in all of the culture solutions. These results were supported by the decrease in the surface tension of the culture solutions never being found (data not shown). The concentration of polysaccharide also decreased during the cultivation experiments. These results indicate that neither surfactants nor polysaccharides were produced by *P. aeruginosa* in this study and that the foam generation was not brought about by these materials. On the other hand, the concentrations of proteins increased following the same trend as the increase in the depth of the foam phase. Moreover, protein production reached a plateau after 60 h, when the foam generation also reached a plateau. The correlation between protein production and foam production was extremely high. These results strongly indicate that the proteins produced by *P. aeruginosa* contributed to the foam generation.



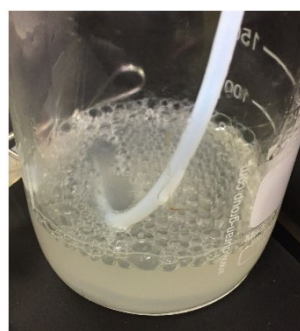
**Figure 6.** The temporal changes in the concentrations of bioproducts in each culture medium: (a) anionic surfactants; (b) polysaccharides; (c) proteins.

### 3.3. Evaluation of Permeability Modification through the Flooding Experiments

Both the increase in pressure during cultivation and the foam generation in the vial were clearly observed, as shown in Figures 7 and 8, respectively. The behavior of the increase in pressure within the sand pack cores was well agreed with the behavior of the foam generation in the vial. This result suggests that the pressure increase in the sand pack cores was indicative of the foam generation in the sand pack cores. This pressure increase was not observed in the sand pack core into which the non-inoculated blank medium was injected. Moreover, because the culture media in the vials and the sand pack cores were originally the same medium and were cultivated at the same temperature, it was presumed that the culture solutions in the sand pack cores were in the same situation as the culture solution in the vials. According to these results, it could be identified that foam was generated by *P. aeruginosa* in the sand pack cores as well as in the vials.



**Figure 7.** The temporal change in the pressure within the sand pack core into which the CO<sub>2</sub> nanobubble medium was injected during the in situ cultivation of *P. aeruginosa*.

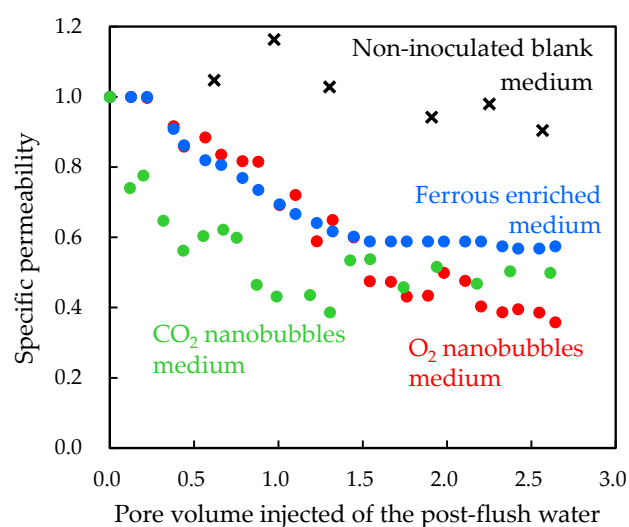


**Figure 8.** The CO<sub>2</sub> nanobubble medium that remained in the vial after the injection and was cultivated for 72 h along with the sand pack core.

Then, the injection of the post-flush water was carried out in order to measure the specific permeability of the cores.

Figure 9 shows the temporal changes in specific permeability during the injection of the post-flush water after the in situ cultivation of *P. aeruginosa*. The specific permeability of the sand pack cores in which *P. aeruginosa* was cultivated decreased to 40–60% of the original permeability, while the core into which the non-inoculated blank medium was injected did not decrease.

The specific permeability of the sand pack core in which *P. aeruginosa* was cultivated in the carbon dioxide nanobubble medium began to decrease immediately after starting the injection of the post-flush water and the decrease in the specific permeability converged to 0.5 after a 0.8 pore volume injection of post-flush water. Thereafter, the specific permeability was maintained at that value.

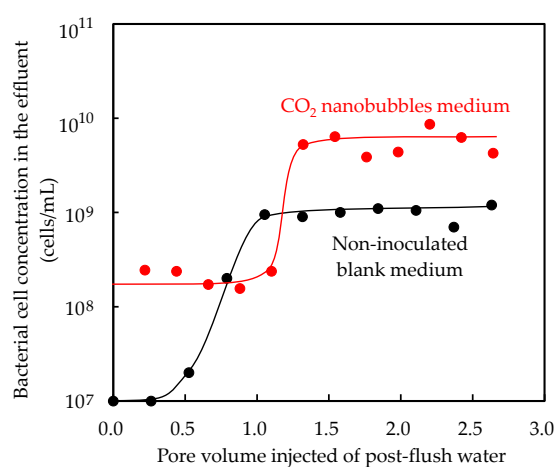


**Figure 9.** The temporal changes in specific permeability during the injection of the post-flush water after the in situ cultivation of *P. aeruginosa*.

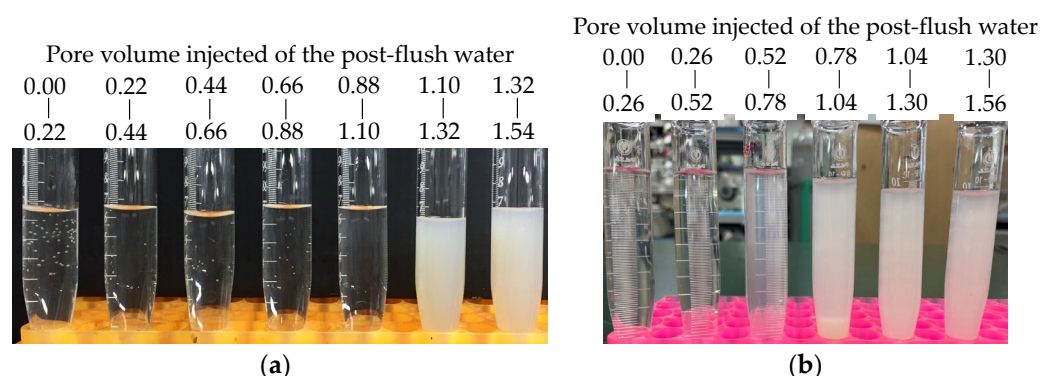
The behavior of the temporal changes in the specific permeability of the sand pack cores in which *P. aeruginosa* was cultivated in the oxygen nanobubble medium and the ferrous sulfate-enriched medium were similar. It began to decrease after a 0.2 pore volume injection of post-flush water. The decrease in the specific permeability of both sand pack cores continued until the volume of the post-flush water injected reached a 1.5 pore volume. The specific permeability of the core in which *P. aeruginosa* was cultivated in the oxygen nanobubble medium eventually converged to 0.4 and then stayed at that value, while that in the ferrous sulfate-enriched medium converged to 0.6 and then stayed at that value.

### 3.4. Analyses of the Effluent from the Sand Pack Cores

Figure 10 shows the temporal changes in the bacterial cell numbers in the effluent collected during the post-flush water injection. The figure shows the bacterial cell numbers in the effluent collected from the cores into which the oxygen nanobubble medium and the non-inoculated blank medium were injected, which were cultivated in situ. The photo images of the effluent are also shown in Figure 11.



**Figure 10.** The temporal changes in the bacterial cell numbers in the effluent collected during the post-flush water injection.



**Figure 11.** The photo images of the effluent collected from the cores during the post-flush water injection: (a) effluent from the core into which *P. aeruginosa* was injected with the carbon dioxide nanobubble medium; (b) effluent from the core into which the non-inoculated blank medium was injected.

Bacterial cells were not only observed in the effluent from the core into which *P. aeruginosa* was injected, but also in the effluent from the core into which the non-inoculated blank medium was injected. It was assumed that there were still some bacteria left in the cores, even after the cores were flooded by sterile water before the injection of the culture medium. Then, those bacteria grew in the sand pack core using the non-inoculated blank medium. It could be assumed that the number of bacterial cells left in the core after the injection of sterile water was smaller than the initial number of *P. aeruginosa* cells that was inoculated in the culture medium. Therefore, *P. aeruginosa* could grow dominantly and generate the foam observed in the cores in this study.

The bacterial cell number in the effluent from the core in which *P. aeruginosa* was cultivated stayed around  $4.0 \times 10^8$  cells/mL for a while after the post-flush water injection started. When more than 1.3 pore volume of the post-flush water was injected into the core, it rapidly increased to  $8.0 \times 10^9$  cells/mL, which was equivalent to 20 times the initial number. On the other hand, the bacterial cell number in the effluent from the core into which the non-inoculated blank medium was injected rapidly increased to  $7.0 \times 10^8$  cells/mL when 0.8 pore volume of the post-flush water was injected. In both cases, the color of the effluent changed from transparent to white at the same time as the bacterial cell number in the effluent rapidly increased, as shown in the Figure 10. This white effluent was the culture solution containing a large number of bacterial cells. These results suggest that the microorganisms grew mainly in the upstream side of the cores. It was assumed that the upstream side was richer in the nutrients required by *P. aeruginosa* than the downstream side because nutritional components in the culture media were concentrated in the upstream side due to filtration and adsorption.

#### 4. Discussion

It was indicated that *P. aeruginosa* generated the foam more actively under the presence of nanobubbles or ferrous sulfate. The foam generation could become more active by increasing the number of nanobubbles. On the other hand, there was an optimal concentration of ferrous sulfate for foam generation. According to our results, 0.6 ppm of ferrous sulfate is the optimal concentration for foam generation; therefore, reservoir brine that contains a little ferrous ion could be used as the base fluid of the culture medium.

The foam started to be generated 24–36 h after the start of cultivation. The foam generation converged within 72 h of cultivation in this study. Although the data are not shown in this paper, the amount of foam reduced when the *P. aeruginosa* was cultivated for a longer time, such as one week. However, foam generation could be continued for a longer time by continuously supplying the *P. aeruginosa* with fresh nutrients. The long-term stability of the foam is a topic for future work.

It was revealed in this study that the foam was induced by the proteins produced by *P. aeruginosa*. Microbial foam EOR with protein-induced foam has the potential to overcome the challenges of conventional foam EOR, which uses surfactants. Unlike the anionic surfactants, the proteins do not react with the cations contained in reservoir brine nor form precipitations. The stability of the foam when the foam coexists with oil is also a challenge in foam EOR. It has been suggested that the stability of surfactant-induced foam becomes low in the presence of oil or high-saline conditions because surfactant micelles become unstable in these conditions [31–34]. On the other hand, the stability of protein-induced foam is reported to be higher than that of surfactant-induced foam in these conditions [35]. Moreover, our previous paper showed that the nitrogen concentration in the gas produced by the *P. aeruginosa* in the culture medium was high, i.e., more than 85% [14,15]. This result showed that the foam was induced by nitrogen in that study. The nitrogen-induced foam is more stable than carbon dioxide-induced foam because nitrogen has lower solubility in water and oil than carbon dioxide [36,37]. Thus, there are several advantages of using EOR with foam induced by nitrogen and the proteins produced by *P. aeruginosa* in situ in oil reservoirs.

The microorganisms that grew mainly in the upstream side of the core flowed out following 1.0 pore volume of post-flush water injection. Such behavior was clearly observed in the case of the non-inoculated blank medium, as shown in Figure 10. In the case of the in situ cultivation of *P. aeruginosa*, the outflow of bacterial cells was delayed and they mainly flowed out from the core after more than 1.3 pore volume of post-flush water had been injected into the core. This result indicated that the fluidity of the *P. aeruginosa* culture solution became extremely low and its efflux from the core was greatly delayed.

The permeability could be decreased by not only the foam, but also the viscosity of the culture solution or the bacterial cells themselves. We therefore hope to consider the possibility of the contribution of these factors to the permeability reduction observed here. The viscosity of the *P. aeruginosa* culture solution and the other bacteria culture solutions was similar, about 1 cP at room temperature; therefore, the viscosity of the culture solution did not contribute to the decrease in permeability. The permeability could be decreased by the pore throat becoming clogged with bacterial cells; therefore, the difference in size between the pore throat and the bacterial cells was considered. The sand grains could be assumed as being filled with the body-centered cubic lattice in the sand pack cores used in this study because the packing factor of the body-centered cubic lattice was 68%, which was similar to the packing factor of the sand pack cores used in this study. The radius of the narrowest gap in the body-centered cubic lattice could be calculated by multiplying 0.155 by the radius of the sand grain. As the diameter of the sand grains was averagely 150  $\mu\text{m}$ , the diameter of the narrowest gap was approximately 23  $\mu\text{m}$ . The volume of the sphere with the narrowest gap radius was calculated to be  $2.1 \times 10^{-9} \text{ cm}^3$ . The number of bacterial cells in a pore space could be roughly calculated by multiplying the volume of the sphere by the bacterial cell concentration in the effluent, which was approximately  $7.0 \times 10^9 \text{ cells/cm}^3$ . As a result of these calculations, it was assumed that there were approximately 15 bacterial cells in a pore space in the sand pack cores. As the longer axis of a bacterial cell is usually 1–2  $\mu\text{m}$ , it could be assumed that all bacterial cells flowed smoothly through even the narrowest gap in the sand pack cores. The contribution of the bacterial cells themselves to the permeability change could therefore be assumed to be little in this study.

These results demonstrate that the permeability of the core in which *P. aeruginosa* was cultivated in situ was decreased by the foam generated by *P. aeruginosa*. The foam had the ability to reduce the permeability of even high-permeability porous systems, such as Darcy order systems. Therefore, microbial foam EOR has the potential to decrease the permeability of high-permeability porous systems and improve the permeability heterogeneity of oil reservoirs.

In addition, because the specific permeability of the cores into which *P. aeruginosa* was injected stayed low after more than 2.5 pore volume injection of post-flush water, the stability of the foam against the fluid flow could be assumed to be high.

The foam was presumed to be generated mainly in the upstream region of the sand pack core, as described in the results section. This was a concern for our method because we were suggesting that an advantage of microbial foam EOR is that the foam can be generated by the microorganisms in wider areas within a reservoir. It was presumed that nutrients could be concentrated in only the upstream region due to adsorption onto the sand and that the foam was generated in only that region by *P. aeruginosa*. It is therefore important to spread the nutrients over wide regions within the reservoir so that the foam can be generated over that wide region. Therefore, we will consider methods for spreading the nutrients over wider regions within reservoirs in future work.

## 5. Conclusions

In this study, we proposed a novel EOR technique using *Pseudomonas aeruginosa* to generate foam and investigated the potential of this microbial foam EOR in modifying the permeability of high-permeability porous systems.

We investigated the optimal concentrations of the oxygen nanobubbles, carbon dioxide nanobubbles and ferrous sulfate for activating the foam generation by the microorganism through cultivation experiments. We also clarified the behavior of the foam generation by that microorganism. Finally, the potential of the foam to decrease the permeability of high-permeability porous systems was evaluated through flooding experiments using sand pack cores. The following conclusions were obtained through this study:

- Foam generation becomes more active with the increase in the number of nanobubbles;
- The optimal concentration of ferrous sulfate for foam generation is 0.6 ppm;
- The foam starts to be generated 24–36 h after the start of cultivation and foam generation converged within 72 h after cultivation in this study;
- The foam decreases the permeability of porous systems whose permeability is quite high, such that the Darcy order can halve;
- Microbial foam EOR has the potential to decrease the permeability of high-permeability porous systems and improve the permeability heterogeneity of oil reservoirs.

**Author Contributions:** M.I. and Y.S. designed the study and M.I. carried out all of the experiments and the sample analyses. Y.S. helped with sample collection and provided valuable discussions. M.I. and Y.S. wrote and completed the manuscript. All authors have read and agreed to the published version of the manuscript.

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## References

1. Chong, H.; Li, Q. Microbial production of rhamnolipids: Opportunities, challenges and strategies. *Microb. Cell. Fact.* **2017**, *16*, 137.
2. Müller, M.M.; Hörmann, B.; Syldatk, C.; Hausmann, R. *Pseudomonas aeruginosa* PAO1 as a model for rhamnolipid production in bioreactor systems. *Appl. Microbiol. Biot.* **2010**, *87*, 167–174.
3. Varjani, J.S.; Upasani, N.V. Biodegradation of petroleum hydrocarbons by oleophilic strain of *Pseudomonas aeruginosa* NCIM 5514. *Bioresour. Technol.* **2016**, *222*, 195–201.
4. Das, K.; Mukherjee, K.A. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresour. Technol.* **2007**, *98*, 1339–1345.
5. Safdel, M.; Anbaz, A.M.; Daryasafar, A.; Jamialahmadi, M. Microbial enhanced oil recovery, a critical review on worldwide implemented field trials in different countries. *Renew. Sust. Energ. Rev.* **2017**, *74*, 159–172.
6. Patel, J.; Borgohain, S.; Kumar, M.; Rangarajan, V.; Somasundaran, P.; Sen, R. Recent developments in microbial enhanced oil recovery. *Renew. Sust. Energ. Rev.* **2015**, *52*, 1539–1558.
7. Bryant, S.R.; Burchfield, E.T. Review of Microbial Technology for Improving Oil Recovery. *SPE Res. Eng.* **1989**, *4*, 151–154.

8. Liu, J.F.; Lu, Y.W.; Liu, X.B.; Li, B.G.; Sun, Y.F.; Zhou, L.; Liu, Y.F.; Yang, S.Z.; Gu, J.D.; Mu, B.Z. Dominance of *Pseudomonas* in bacterial community and inhibition of fumarate addition pathway by injection of nutrients in oil reservoir revealed by functional gene and their transcript analyses. *Int. Biodeterior. Biodegradation*. **2020**, *153*, 105039.
9. Zhao, F.; Li, P.; Guo, C.; Shi, R.-J.; Zhang, Y. Bioaugmentation of oil reservoir indigenous *Pseudomonas aeruginosa* to enhance oil recovery through in-situ biosurfactant production without air injection. *Bioresour. Technol.* **2018**, *251*, 295–302.
10. Hui, L.; Ai, M.; Han, S.; Guo, Y.; Li, N.; Shi, R.; Zeng, X.; Leng, S.; Zhao, J.; Liu, X.; et al. Microbial diversity and functionally distinct groups in produced water from the Daqing Oilfield, China. *Pet. Sci.* **2012**, *9*, 469–484.
11. Zhao, F.; Shi, R.; Cui, Q.; Han, S.; Dong, H.; Zhang, Y. Biosurfactant production under diverse conditions by two kinds of biosurfactant-producing bacteria for microbial enhanced oil recovery. *J. Petrol. Sci. Eng.* **2017**, *157*, 124–130.
12. Abbasian, F.; Lockington, R.; Mallavarapu, M.; Naidu, R. A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. *Appl. Biochem. Biotechnol.* **2015**, *176*, 670–699.
13. Chayabutra, C.; Wu, J.; Ju, L.K. Rhamnolipid production by *Pseudomonas aeruginosa* under denitrification: Effects of limiting nutrients and carbon substrates. *Biotechnol. Bioeng.* **2001**, *72*, 25–33.
14. Ito, M.; Sugai, Y. Nanobubbles activate anaerobic growth and metabolism of *Pseudomonas aeruginosa*. *Sci Rep.* **2021**, *11*, 16858.
15. Ito, M.; Sugai, Y. Study on enhanced oil recovery using microorganism generating foam in presence of nanobubbles. In Proceedings of the SPE/IATMI Asia Pacific Oil and Gas Conference and Exhibition 2021, APOGCE 2021, Online, 12–14 October 2021.
16. Afifi, H.R.; Mohammadi, S.; Derazi, A.M.; Alemi, F.M.; Abad, K.F.H. A comprehensive review on critical affecting parameters on foam stability and recent advancements for foam-based EOR scenario. *J. Mol. Liq.* **2021**, *25*, 116808.
17. Burnside, N.M.; Naylor, M. Review and implications of relative permeability of CO<sub>2</sub>/brine systems and residual trapping of CO<sub>2</sub>. *Int. J. Greenh. Gas Control* **2014**, *23*, 1–11.
18. Lang, L.; Li, H.; Wang, X.; Liu, N. Experimental study and field demonstration of air-foam flooding for heavy oil EOR. *J. Pet. Sci. Eng.* **2020**, *185*, 106659.
19. Talebian, S.; Masoudi, R.; Tan, I.M.; Zitha, P.L.J. Foam assisted CO<sub>2</sub>-EOR: A review of concept, challenges, and future prospects. *J. Pet. Sci. Eng.* **2014**, *120*, 202–215.
20. Farajzadeh, R.; Andrianov, A.; Krastev, R.; Hirasaki, G.J.; Rossen, W.R. Foam–oil interaction in porous media: Implications for foam assisted enhanced oil recovery. *J. Colloid Interface Sci.* **2012**, *183–184*, 1–13.
21. Gauglitz, P.A.; Friedmann, F.; Kam, S.I.; Rossen, W.R. Foam Generation in Porous Media. In Proceedings of the Paper SPE-75177-MS presented at the SPE/DOE Symposium on Improved Oil Recovery 2002, Tulsa, Oklahoma, 13–17 April 2002.
22. Kornev, K.G.; Neimark, A.V.; Rozhkov, A.N. Foam in porous media: Thermodynamic and hydrodynamic peculiarities. *J. Colloid Interface Sci.* **1999**, *82*, 127–187.
23. Fried, A.N. *The Foam-Drive Process for Increasing the Recovery of Oil*; Report of investigation 5866; USBM: Washington, DC, USA, 1961.
24. Yan, W.; Miller, C.A.; Hirasaki, G.J. Foam sweep in fractures for enhanced oil recovery. *Colloids Surf. A Physicochem. Eng. Asp.* **2006**, *282–283*, 348–359.
25. Friedmann, F.; Chen, W.H.; Gauglitz, P.A. Experimental and simulation study of high-temperature foam displacement in porous media. *SPE. Res. Eng.* **1991**, *6*, 37–45.
26. Cooper, G.D.; Macdonald, R.C.; Duff, B.J.S.; Kosaric, N. Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. *Appl. Environ. Microb.* **1981**, *42*, 408–412.
27. Jadhav, A.J.; Mostafa, B. Bulk Nanobubbles or not nanobubbles: That is the question. *Langmuir* **2020**, *36*, 1699–1708.
28. Yang, S.; Dammer, S.M.; Bremond, N.; Zandvliet, H.J.W.; Kooij, E.S.; Lohse, D. Characterization of nanobubbles on hydrophobic surfaces in water. *Langmuir* **2007**, *23*, 7072–7077.
29. Zimmerman, W.B.; Tesař, V.; Bandulasena, H.C.H. Towards energy efficient nanobubble generation with fluidic oscillation. *Curr Opin Colloid Interface Sci.* **2011**, *16*, 350–356.
30. Chen, N.; Wen, Z.; Li, X.; Ye, Z.; Ren, D.; Xu, J.; Chen, Q.; Ma, S. Controllable preparation and formation mechanism of mono-dispersed bulk nanobubbles in dilute ethanol-water solutions. *Colloids Surf. A Physicochem. Eng. Asp.* **2021**, *616*, 126372.
31. Osei-Bonsu, K.; Shokri, N.; Grassia, P. Foam stability in the presence and absence of hydrocarbons: From bubble- to bulk-scale. *Colloids Surf. A Physicochem. Eng. Asp.* **2015**, *481*, 514–526.
32. Behera, M.R.; Varade, S.R.; Ghosh, P.; Paul, P.; Negi, A.S. Foaming in micellar solutions: Effects of surfactant, salt, and oil concentrations. *Ind. Eng. Chem. Res.* **2014**, *53*, 18497–18507.
33. Lee, J.; Nikolov, A.; Wasan, D. Surfactant micelles containing solubilized oil decrease foam film thickness stability. *J. Colloid Interface Sci.* **2014**, *415*, 18–25.
34. Simjoo, M.; Rezaei, T.; Andrianov, A.; Zitha, P.L.J. Foam stability in the presence of oil: Effect of surfactant concentration and oil type. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *438*, 148–158.
35. Samin, A.M.; Manan, M.A.; Idris, A.K.; Said, M.; Alghol, A. Protein foam application for enhanced oil recovery. *J. Dispers Sci. Technol.* **2017**, *38*, 604–609.
36. Wei, P.; Pu, W.; Sun, L.; Wang, B. Research on nitrogen foam for enhancing oil recovery in harsh reservoirs. *J. Pet. Sci. Eng.* **2017**, *157*, 27–38.
37. Sun, L.; Wei, P.; Pu, W.; Wang, B. The oil recovery enhancement by nitrogen foam in high-temperature and high-salinity environments. *J. Pet. Sci. Eng.* **2016**, *147*, 485–494.