RSC Advances

PAPER

View Article Online
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Cite this: *RSC Adv.*, 2023, **13**, 9933

Quantitative analysis of biosurfactants in water samples by a modified oil spreading technique†

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The oil spreading technique relies on biosurfactant to reduce the surface tension of an oil film and form an oil spreading ring in the center, and then judges the content of biosurfactant according to the diameter of the spreading ring. However, the instability and large errors of the traditional oil spreading technique limit its further application. In this paper, we modified the traditional oil spreading technique by optimizing the oily material, image acquisition and calculation method, which improves the accuracy and stability of the quantification of biosurfactant. We screened lipopeptides and glycolipid biosurfactants for rapid and quantitative analysis of biosurfactant concentrations. By selecting areas by color done by the software to modify image acquisition, the results showed that the modified oil spreading technique has a good quantitative effect, reflected in the concentration of biosurfactant being proportional to the diameter of the sample droplet. More importantly, using the pixel ratio method instead of the diameter measurement method to optimize the calculation method, the region selection was more exact, and the accuracy of the data results was high, and the calculation efficiency was improved significantly. Finally, the contents of rhamnolipid and lipopeptide in oilfield water samples were judged by the modified oil spreading technique, the relative errors were analyzed according to the different substances as the standard, and the quantitative measurement and analysis of oilfield water samples (the produced water of Zhan 3-X24 and the injected water of the estuary oil production plant) were realized. The study provides a new perspective on the accuracy and stability of the method in the quantification of biosurfactant, and provided some theoretical and data support for the study of the microbial oil displacement technology mechanism.

Received 6th January 2023
Accepted 28th February 2023

DOI: 10.1039/d3ra00102d

rsc.li/rsc-advances

1. Introduction

Biosurfactants are metabolites with surface activity produced by a variety of microorganisms. They are different sets of compounds such as lipopeptides, lipoproteins, glycolipids, phospholipids and polysaccharide–lipid complexes. They have lower toxicity, higher biodegradability, better environmental compatibility and the ability to be produced using cheaper agricultural materials than chemically synthesized surfactants. The structural feature of biosurfactants is that they contain hydrophobic and hydrophilic groups, so biosurfactants have strong surface activity which can significantly reduce surface and interface tension.[1–6] According to reports, biosurfactants not only have the ability to repair the environment but also have therapeutic and biomedical effects. In addition, biosurfactants in the process of microbial oil recovery can reduce the oil–water interface tension and improve oil displacement efficiency.[7–20] However, the practical applications of biosurfactants are limited by their low yield and high cost of production.[21] The development of rapid and accurate methods for estimating the concentration and yield of biosurfactants is an important factor for selecting strains with high production characteristics and routinely estimating the biosurfactants produced by various strains. In this paper, lipopeptide and glycolipid biosurfactants are used as the research objects for rapid quantitative analysis. Surfactin, which is a representative cyclic lipopeptide, is one of the most powerful microbial biosurfactants and is produced by *Bacillus subtilis*.[22–27]

During the last few decades, researchers have performed much work in screening and quantifying biosurfactants. The drop-collapsing method is a traditional method, and it was demonstrated to be a rapid method for screening bacterial colonies that can produce surfactants.[28] In addition, an improved drip collapse technique is used to quantify the concentration of biosurfactants. Studies have shown that the

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d3ra00102d

surfactant concentration is directly proportional to the diameter of the sample droplets.[29] The turbidometric method is another way to rapidly quantify microbes. This method is based on the theory that the solubility of lipopeptide biosurfactants will decrease at low pH and this method is suitable for the quantitative analysis of high-concentration lipopeptide solutions.[30] High-performance liquid chromatography (HPLC) is also used in the qualitative and quantitative analysis of lipopeptide biosurfactants. It is usually analyzed by ultraviolet detectors, but the ultraviolet absorption of lipopeptides is relatively weak, and it is not suitable for quantitative analysis of lower concentration lipopeptide solutions.[31–33] When lipopeptides were derived from 1-bromoacetylpyrene, they could be analyzed by fluorescence detectors.[34] Although the improved HPLC method overcomes the limitation of the trace detection of lipopeptide solution, the detection limit of lipopeptide is low, but the derivation process is complicated and the accurate quantification range is limited. In addition, a new quantitative method based on visible color shifts for screening surfactin production was reported.[35] It is convenient to screen surfactin-producing strains by the change in color, but the quantitative accuracy of this method is not very good. Other methods or techniques have also been employed to estimate the yields of biosurfactants during screening strains producing biosurfactants through Fourier Transform Infrared (FT-IR) spectroscopy,[36] hemolytic activity[37] or interfacial tension tests. However, these methods are not suitable for rapid and accurate quantification of biosurfactants due to the complexity of the process or inconvenient calibration. The oil spreading technique is a good method for quantitatively analyzing the content of biosurfactants. It relies on biosurfactant to reduce the surface tension of the oil film to form an oil spreading ring in the center of the oil film, and then judges the content of biosurfactant according to the diameter of the spreading ring. However, the instability and large errors of traditional oil spreading technique limit its further application. Different analytical methods have their own characteristics, and the establishment of these analytical methods makes the quantitative analysis of biosurfactants and the screening of surfactant strains increasingly perfected, which also provides a theoretical basis for the development of this paper.

This study aimed to modify the qualitative oil spreading technique described previously[38] so that it could be expediently applied to quantify the concentration of biosurfactants. It includes (1) the modified oil spreading technique was established by optimizing the oily material, image acquisition and calculation method. (2) Complete accurate quantification of 25–300 mg per L rhamnolipid standard solution, 5–200 mg per L lipopeptide standard solution, and rapid quantification of single class of biosurfactant solution. (3) By establishing quantitative standard curves of different biosurfactant standard solutions, the advantages of the improved technology and the traditional technology were compared and analyzed. Finally, the contents of rhamnolipid and lipopeptide in oilfield water samples were judged. The results provided some theoretical and data support for the study of the microbial oil displacement technology mechanism.

2. Materials and methods

2.1. Preparation of sample

Surfactin is one of the approximately 20 families of lipopeptides and most families among the reported lipopeptides are found to be cyclic lipopeptides.[39,40] The surfactin sample used in this study was from the culture broth of *Bacillus subtilis* HSO 121 by the State Key Laboratory of Bioreactor Engineering and Institute of Applied Chemistry, East China University of Science and Technology, and produced surfactin on a sucrose culture medium. The surfactin sample was obtained after separation from cell-free broth, acid precipitation, and extraction with ethyl ether.[41,42] To remove the remaining solvent at room temperature, the surfactin sample was dissolved with methanol. Total ion chromatography (TIC) of the surfactin sample is shown as Fig. 1 and the molecular weights of the different components in the standard lipopeptide solution are shown as Table S1.† The rhamnolipids were obtained from Huzhou Zijin Biotechnology Limited Company, and the sophorolipids were obtained from Ocean University of China. The injection water was selected from the Hekou oil production plant in the Shengli oilfield, and the produced water was selected from the Zhan 3-X24 production well in the Shengli oilfield.

2.2. Oil spreading technique

The oil spreading technique was first proposed to screen the production of surfactant strains.[43] The mechanism of the technology is as follows. The density of polar solvents, such as water, was heavier than that of oily materials; when the solvent or surfactin solutions were delivered into the surface of oily materials, the oily material membrane would break because of the action of gravity.[44] In the absence of biosurfactants, when the polar solvent methanol is added to the surface of the oily material, the polar solvent methanol will be repelled by the hydrophobic oily material and dissolve in the polar solvent water below the oily material, so the center of the area of the oily material cannot be formed. In contrast, if the solvent contains biosurfactant, when the biosurfactant solution is transported to the center of the oily substance film, the oil–water contact angle will change, and the interfacial tension between water and oily substance will decrease, so a clear area can be formed.[45] At the same time, since the red organic dye Sudan Red III is insoluble in water and soluble in oil, fatty substances become orange when they encounter Sudan III. By adding Sudan Red III to the oily substance for dyeing, the small particles dyed orange were observed under an optical microscope. This solves the problem that there is no color difference between the oil phase and the water phase, which can only rely on the naked eye to observe the test results, and the oil drain ring cannot be observed in real-time photo recordings. It is widely used in the screening of surfactant-producing strains because of the intuitiveness of the oil spreading technique. For the same biosurfactant, the size of the formed clear zone is proportional to the content of the biosurfactant. Therefore, the method has also been gradually used to quantify the content of biosurfactants.[43,46] The quantitative analysis of biosurfactants was realized by establishing

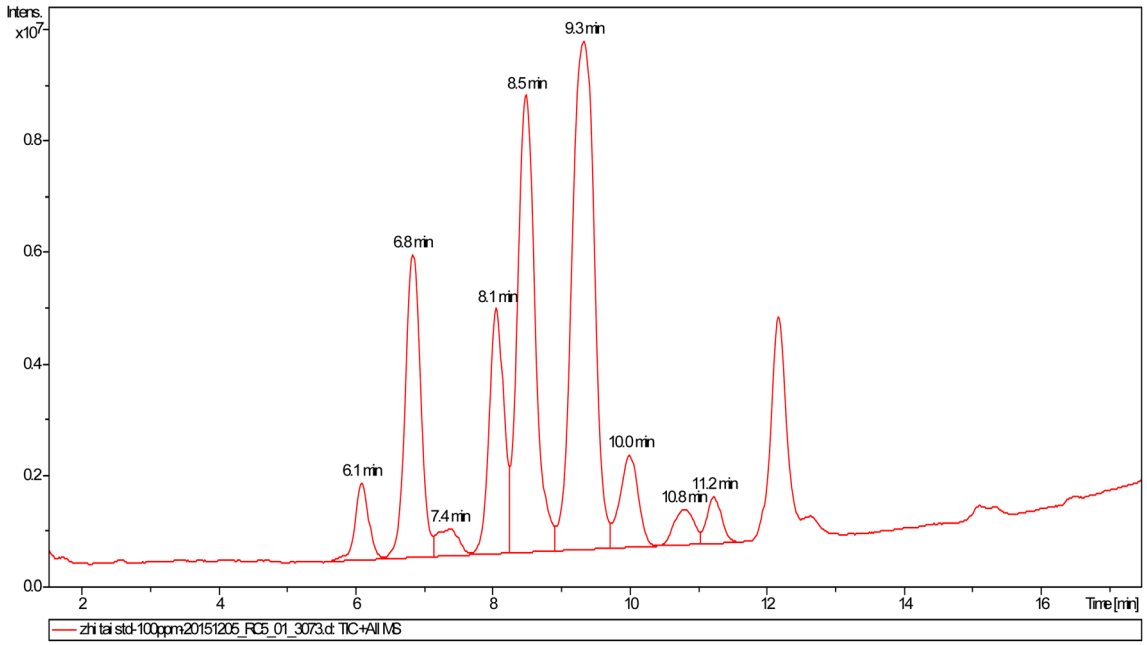

Fig. 1 The TIC of the lipopeptide standard solution.

a standard curve of different concentrations of biosurfactant solution and the diameter or area of corresponding clear zone.

2.3. Quantitative experimental procedure of the improved oil spreading technique

2.3.1. Optimization of oil film materials. During the development of oil diffusion technology, the choice of oil film materials is constantly changing, from crude oil at the beginning to liquid paraffin. However, liquid paraffin still has certain problems when making oil film materials. The main performance is that liquid paraffin is not only slow in spreading speed but also poor in spreading degree and stability. Therefore, we are looking for a substance with a fast spreading speed, better spreading degree and stability as an oily material. When selecting suitable oily materials, we refer to the following criteria: the density of oily substances is lower than that of water, which is the basis of oil spreading technology; the high purity of oily substances is an important factor affecting the stability of the formation of oil layers and the accuracy of quantification of biosurfactant concentration; and the speed and uniformity of oil diffusion on the water surface are also important factors that determine whether the oil is suitable.[47]

In this paper, six oily materials were selected for experimentation, namely, petroleum ether, hexadecane, tetradecane, liquid paraffin, vegetable oil, and silicone oil, to investigate whether there are more suitable oil film materials. Five milliliters of each oil film material was added to the water surface, and the spreading of different oil film materials on the water surface was recorded 5 min and 30 min after dripping. Three kinds of better oily materials were initially selected, and 5 mL of tetradecane, hexadecane and liquid paraffin used in the traditional oil spreading method were dropped onto the water surface. After the oil film was stabilized, 10 μL of 100 mg per L rhamnolipid standard solution was added to the above three oil films. The formation state of the oil drain ring formed in the 3 Petri dishes during the 0–60 min process was recorded separately by the images. By comparing the stability of the blank area formed, a better oil film material is further selected.

2.3.2. Optimization of image recording. In oil diffusion technology, the size of the blank area is used to indicate the level of the measured biosurfactant content. Therefore, high-quality image data are more conducive to the analysis of the size of the blank area. For this reason, this paper designs a set of image acquisition devices for the oil diffusion method. As shown in Fig. S1,† the device is composed of 5 parts, a light-shielding plate, a photographing hole, a photographing device, a base, and a Petri dish. The cabinet structure design can eliminate the interference of surrounding impurity light sources (such as laboratory light, irradiated light, reflected light, *etc.*), it also effectively reduces the interference of various environmental factors on the stability of the oil drain ring method, such as air flow. To ensure that there is no dark area in the base, the selected type of LED light board is four-side light. The Petri dish of $D = 90$ mm was replaced with a Petri dish of $D = 150$ mm. The increase in the area of the Petri dish is conducive to the more adequate spread of the oil film material, and it is also conducive to the stable existence of the blank area formed.

2.3.3. Optimization of the calculation method. In the traditional oil diffusion method, the calculation of the blank area is realized by multiple measurements of the diameter of the formed oil discharge ring. However, sometimes the blank area formed in the oil discharge ring method is not a regular circle. At this time, the method of using the diameter to represent the size of the blank area is not accurate. Therefore, this paper introduces a new calculation method for the

calculation of the blank area size. Photoshop is a widely used image processing software that also used in calculating the area of irregular graphics. The basic theory of this method is to calculate the ratio of the pixels of the same color area to the total pixels of the image in a certain image and correct according to the actual area of the image. Then, the actual area of the selected specific color area can be accurately calculated.

2.3.4. Improved oil diffusion technology to calibrate the concentration of biosurfactants. Because different kinds of biosurfactants have different abilities to form oil spreading, it is necessary to prepare suitable concentration gradient solutions for different biosurfactants. After a series of studies, the concentration gradient of each standard was determined as follows: (solvent is distilled water) lipopeptide, 5, 10, 50, 100, 200 mg L^{-1}, rhamnolipid, 25, 50, 100, 150, 300 mg L^{-1}, sophorolipid: 200, 300, 400, 500, 1000 mg L^{-1}. Five 150 mm Petri dish covers were selected, and the Petri dish lids were first washed with methanol and then with distilled water to ensure that the Petri dish was clean. The Petri dishes were placed on a flat experimental table, and 100 mL of distilled water was added to each Petri dish, followed by 5 mL of tetradecane stained by Sudan III. The Petri dish was then flattened to the center position of the base in the image acquisition device. When the tetradecane was fully spread out (10 min), the standard solution of the biosurfactant was removed with a liquid transfer gun and added dropwise to the center of the spreading tetradecane for 10 μL. After 6 min of dropping the biosurfactant solution, a clear zone was formed at the center of the tetradecane. When the clear zone was basically stable, the photo recording was carried out by using the previously fixed

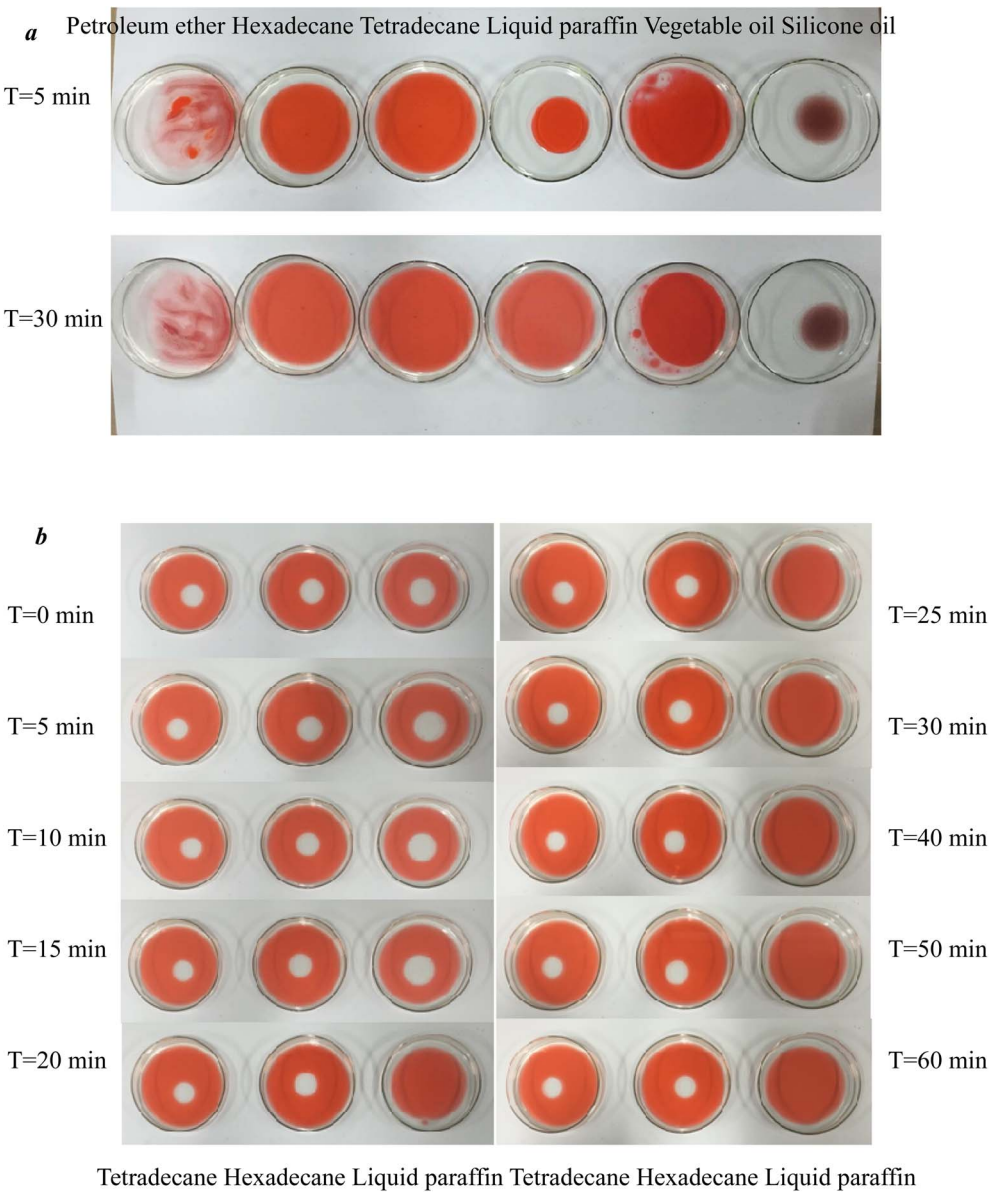

Fig. 2 (a) Diagram of the uniformity of various oily substances spreading on the water surface. (b) Comparison of stabilization time of the clear zone for three different oily materials.

phototaking equipment. The area of the clear zone was obtained by the pixel ratio method, and then the quantitative standard curve of biosurfactant was established.

2.3.5. Improved oil diffusion technology for the quantitative analysis of biosurfactants in oilfield water samples. Quantitative analysis of biosurfactants was carried out on the produced water of Zhan 3-X24 and the injected water of the estuary oil production plant by using the improved oil diffusion method. The basic steps are as described in Section 2.3.4. The produced water and injected water are subjected to 3 sets of parallel experiments. The 100 mg per L rhamnolipid or lipopeptide standard solution was added to the produced water and injected water, and the improved oil diffusion technology was used to determine the biosurfactant in the oilfield water samples after adding the standard.

3. Results and discussion

3.1. Effect of various oily materials on the oil spreading technique

3.1.1. Optimized results of oil film materials. The oil coating effect was tested on the 6 kinds of oil dyed in Sudan III, as shown in Fig. 2a. Petroleum ether is very unstable and basically does not form an oil film on the water surface. Although vegetable oil (soybean oil) spreads quickly on the water surface, the oil film formed is very uneven. The stability of the oil film is also poor, and the phenomenon of aggregation occurs at 30 minutes. Due to its high viscosity, silicone oil does not easily diffuse in water, and Sudan III cannot dye silicone oil, which will result in the inability to observe and calculate the clear area formed by the oiling technique in the later stage. Therefore, silicone oil is not suitable for oily materials. Hexadecane and tetradecane can spread out more quickly after dropping to the water surface and have basically spread out completely at 5 min. It can be seen from the uniformity of the color that the spread of tetradecane and hexadecane on the water surface is very uniform, and the stability of the two oily substances is also very good. It will remain evenly spread on the water surface after 30 minutes. Liquid paraffin can also form a stable oil film on the water surface, but the spreading speed and degree of liquid paraffin are not as good as tetradecane and hexadecane, and after 30 minutes, the oil film formed by liquid paraffin gathers (Liu *et al.*, 2020). Therefore, the spreading speed, spreading degree and spreading state of hexadecane, tetradecane and liquid paraffin are better than those of the other three oils. At the same time, we can see from Fig. 2b that when 10 μL of 100 mg per L rhamnolipid standard solution is dropped into the three oil film materials tetradecane, hexadecane and liquid paraffin, all three oil film materials can form a blank area. Within 15 minutes of forming the blank area, the blank area formed by the three oil film materials can exist stably. However, as time went by, the blank area formed when the liquid paraffin was used as an oil film gradually moved to the edge and quickly ruptured and disappeared after reaching the edge of the oil film materials. When tetradecane and hexadecane were used as oil film materials, the blank area formed and can exist stably for more than 60 minutes. The stable existence of the blank zone is of great significance to the overall stability and accuracy of the oil diffusion method, so tetradecane or hexadecane is a more ideal oil film material than liquid paraffin. However, from an economic point of view, the price of tetradecane is much lower than that of hexadecane. Therefore, after combining various factors, tetradecane was finally selected as the oil film material for the new oil diffusion method.

3.1.2. Optimization result of the calculation method. The comparison of the new and old calculation methods is shown in Fig. 3. Compared with the previous method, the pixel ratio method has the following advantages. First, the area selection is more accurate. The obvious color difference allows the software to quickly and accurately select the same color area. Because the area is selected by color, even irregular blank areas can be accurately selected. Second, the calculation results are highly reliable. For a picture, when the resolution is determined, the pixels of the picture are evenly distributed in each position of the image. Therefore, the ratio of pixels in a certain area to pixels in the total area can accurately represent the proportion of a certain area in the entire area. Third, the calculation efficiency is significantly improved. After the "tolerance value" is determined, the pixel value of a certain color area in the same picture is determined, and there is no need to repeat the

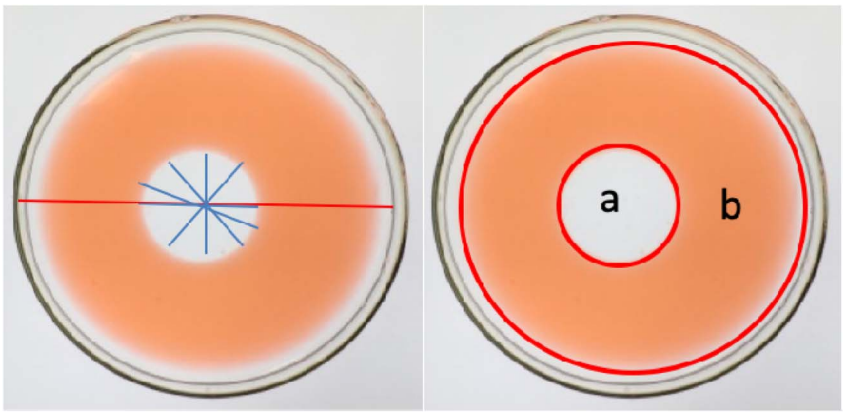

Fig. 3 Comparing two calculation methods of the size of the clear zone.

operation to obtain the value. In addition, the calculation efficiency is greatly improved by introducing the auxiliary calculation of the Excel table.

3.1.3. Optimized result of image recording. The image quality comparison before and after optimization is shown in Fig. S2.† It can be intuitively seen that the optimized oil discharge ring image data are of higher quality, which is mainly manifested in the following three aspects. The first is that the regional division of the image is clearer. The second is to eliminate the interference of various factors, such as shadows and reflections. Because the entire Petri dish is directly placed on the light board the entire Petri dish receives uniform light. The optimized image completely eliminates the unfavorable factors, such as shadows and reflections, that affect data analysis. The third is a more intuitive comparison between different images. After the camera is fixed at a fixed point, the size of each image obtained is consistent when the focal length and camera mode are the same, which not only improves the accuracy of postprocessing data but is also more conducive to intuitive comparison of different sizes of the oil discharge circle formed by different concentrations of biosurfactant.

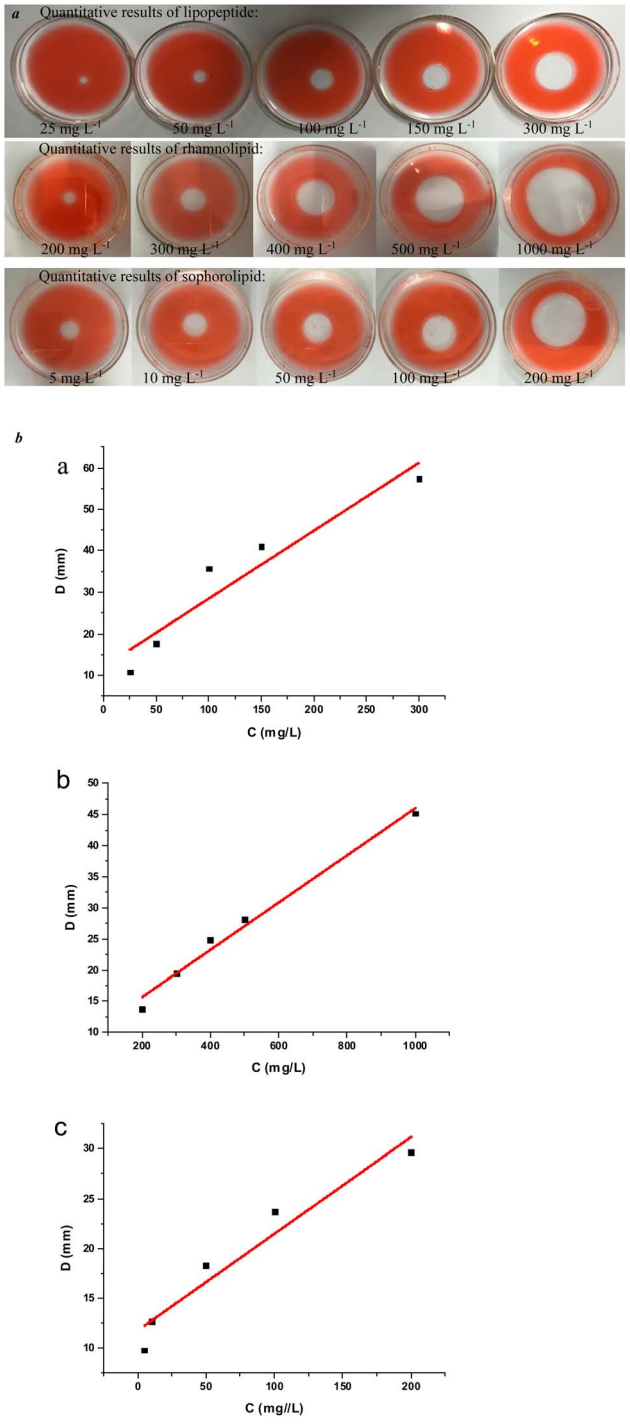

Fig. 4 (a) Oil spreading formed by standard lipopeptide, rhamnolipid and sophorolipid solutions. (b) The standard curve established by standard lipopeptide, rhamnolipid and sophorolipid solutions and oil spreading diameter.

3.2. Biosurfactant concentration calibration by the traditional oil spreading technique

It has been shown that the oil spreading technique could roughly achieve the quantitative analysis of biosurfactants in the lower concentration range, and the concentration of surfactant has a linear relationship with the diameter of the oil spreading.[48] By using the traditional oil spreading technique, we established standard curves with different concentrations of lipopeptide, rhamnolipid and sophorolipid standard solutions, and the corresponding diameters of the oil spreading and normal curve diagrams are shown in Fig. 4.

The quantitative formula of lipopeptide was obtained in formula (1):

$$D = 0.097 \times C + 11.75 \quad (1)$$

The standard curve $R^2 = 0.918$. The linear relationship of the established lipopeptide quantitative standard curve was not ideal, and the accuracy of the accurate quantification of this surfactant was also lacking.

The conversion formula of quantitative rhamnolipid was obtained in formula (2):

$$D = 0.16 \times C + 12.05 \quad (2)$$

The standard curve $R^2 = 0.885$, and the linear relationship was poor in the experimental concentration range, so it was difficult to accurately quantify rhamnolipid.

The quantitative formula of sophorolipid was obtained in formula (3):

$$D = 0.038 \times C + 8.05 \quad (3)$$

The standard curve $R^2 = 0.981$, and the linear relationship of the sophorolipid quantitative standard curve was better in the experimental concentration range.

3.3. Biosurfactant concentration calibration by the improved oil spreading technique

Through the preliminary comparison of several different oily materials, tetradecane and hexadecane can spread evenly on the water surface more quickly, and their stability was better, so

they were ideal oily materials. Because of its more economical use than hexadecane, tetradecane was identified as an oily material. To improve the accuracy of the test results, the oil spreading image acquisition system was optimized. Using the pixel ratio method instead of the diameter measurement method to optimize the calculation method, the region selection was more scientific, and the accuracy of the data results was high, and the calculation efficiency was improved

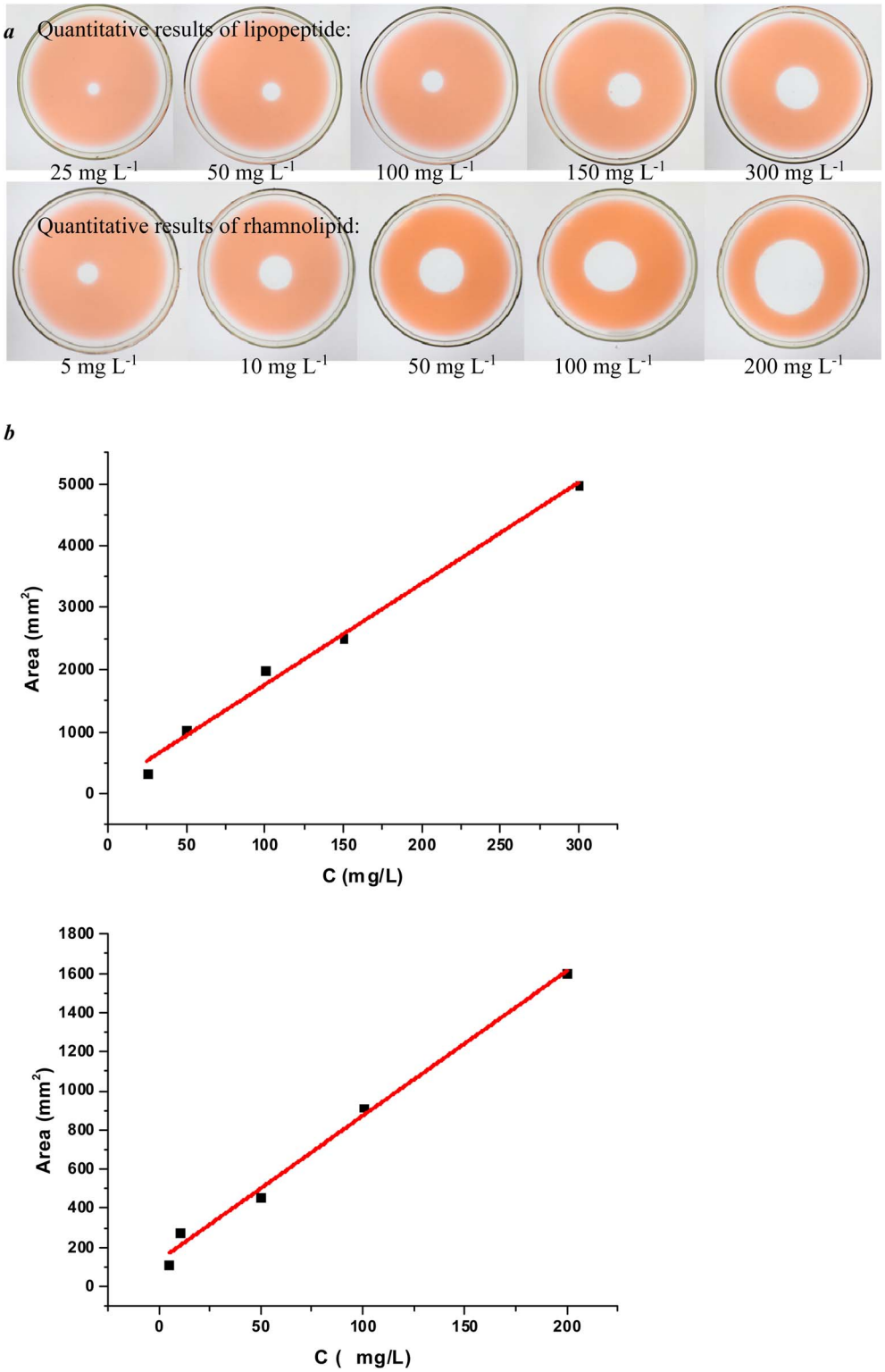

Fig. 5 (a) Oil spreading formed by standard lipopeptide and rhamnolipid solutions. (b) The standard curves established by standard lipopeptide and rhamnolipid solutions and oil spreading area.

significantly. A schematic diagram of the two methods is shown in Fig. 3.

Before improvement, the area of oil spreading was calculated by formula (4):

$$D = 90 \times \frac{d_2}{d_1} \quad (4)$$

where D represents the actual diameter of the oil spreading, d_1 represents the diameter of the Petri dish in the record image, and d_2 represents the diameter of oil spreading in the record image. After improvement, we used formula (5) to calculate:

$$S = \pi r^2 \times \frac{a}{a+b} \quad (5)$$

where S represents the area of oil spreading, a represents the pixel value of oil spreading, b represents the pixel value of the remaining part, and r represents the radius of the Petri dish.

The traditional oiling technique does not give ideal results for the quantitative results of lipopeptides and rhamnolipids but for sophorolipids. Using the improved oil spreading technology, the standard curve of different concentrations of lipopeptides and rhamnolipid standard solutions and the corresponding oil spreading diameters are shown in Fig. 5.

Through the establishment of the standard curve, the quantitative formula of lipopeptide can also be obtained in formula (6):

$$S = 7.40 \times C + 132.58 \quad (6)$$

The standard curve $R^2 = 0.989$, the linear relationship of the established lipopeptide quantitative standard curve was good, and it can basically achieve accurate quantification of lipopeptides in the range of 5–200 mg L^{-1}.

The quantitative formula of rhamnolipid can also be obtained in formula (7):

$$S = 16.33 \times C + 123.78 \quad (7)$$

The standard curve $R^2 = 0.989$, the linear relationship was good, and it can basically achieve accurate quantification of rhamnolipids in the range of 25–300 mg L^{-1}.

Through the above experimental results, it can be concluded that the concentration of the same biosurfactant solution was basically proportional to the diameter of the oil spreading, which also shows that the rapid quantitative analysis of the oil spreading technique for biological surfactants is feasible. However, the linear relationship between the standard curves established by different kinds of biosurfactants varies, which may be related to the respective properties of biosurfactants.

3.4. Validation of the improved oil spreading technique by oilfield samples

Improved oil diffusion technology has been used to achieve the quantitative determination of biosurfactants in oilfield water samples. The comparison of the concentration of biosurfactant in oilfield produced water and oilfield injected water and the data obtained by the internal standard method is shown in the Table S2† and Fig. 6.

Because oilfield water samples are complex and contain a variety of biosurfactants,[49] the quantitative standard curve of rhamnolipids (formula (6)) or lipopeptides (formula (7)) was used to estimate the content of biosurfactants in oilfield water samples. Since the area of the blank area formed by the produced water is too small to be calculated by the formula, the blank area formed by it is compared with the blank area formed by the standard solution of 100 mg per L lipopeptide or rhamnolipid. The content of biosurfactant in the produced water is estimated by the method of calculating the ratio (the calculated concentration is greater than the actual concentration). The calculated results are shown in Table 1.

When rhamnolipids are used as the standard to calculate the biosurfactant content in oilfield water samples, the average concentration of oilfield produced water is 4.3 mg L^{-1}, and the average concentration of oilfield water injection is 5.5 mg L^{-1}.

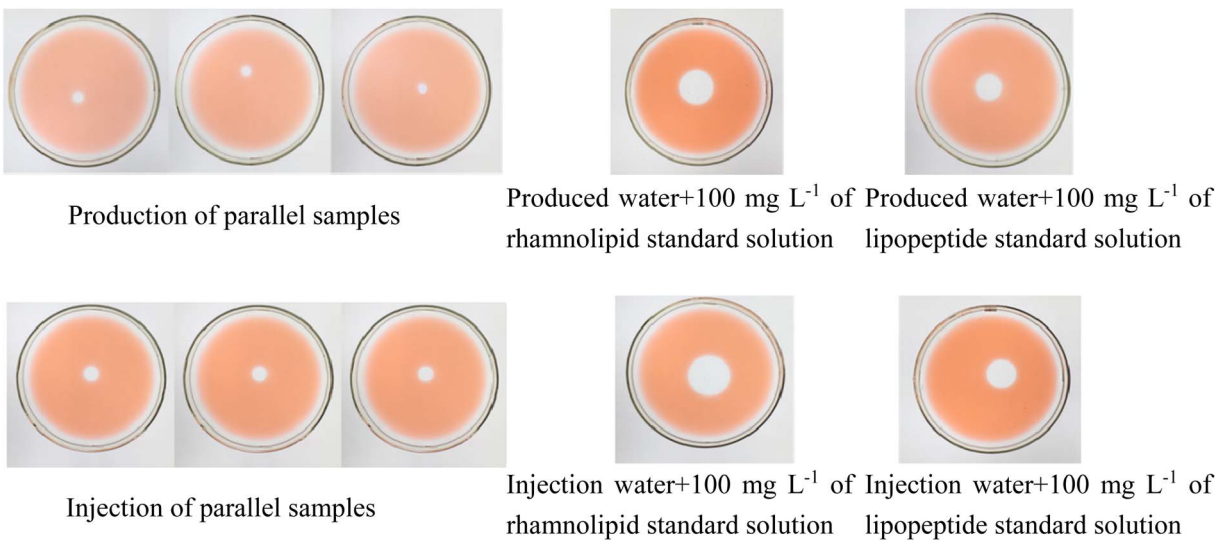

Fig. 6 Determination results of biosurfactants in oilfield water samples.

Table 1 The concentration calculation of biosurfactants in oilfield water samples

Sample content (mg L^{-1})	Average concentration of oilfield produced water	Average concentration of oilfield injection water	Oilfield produced water + 100 mg per L rhamnolipid standard solution	Oilfield produced water + 100 mg per L lipopeptide standard solution	Oilfield injection water + 100 mg per L rhamnolipid standard solution	Oilfield injection water + 100 mg per L lipopeptide standard solution
Calculated with rhamnolipid	4.3	5.5	67.4	—	91.2	—
Calculated with lipopeptide	9.5	11.06	—	81.9	—	119.0
Fractional error	—	—	35.38%	25.21%	12.56%	8.68%

When the peptide is used as a standard for calculation, the average concentration of oilfield-produced water is 9.5 mg L^{-1}, and the average concentration of oilfield water injection is 11.06 mg L^{-1}. When the oilfield water sample plus 100 mg per L rhamnolipid solution is used as the standard to calculate the biosurfactant content in the oilfield water sample, the average concentration of oilfield produced water is 67.4 mg L^{-1}, and the average concentration of oilfield water injection is 91.2 mg L^{-1}. When the oilfield water sample plus 100 mg per L lipopeptide solution is used as the standard to calculate, the average concentration of oilfield produced water is 81.9 mg L^{-1}, and the average concentration of oilfield water injection is 119.0 mg L^{-1}.

The results showed that the content of biosurfactant in oilfield water samples calculated by different standards was quite different, but overall, the error of the measured result of oilfield injection water was smaller than that of oilfield produced water and the error of the calculated result with lipopeptide was smaller than that with rhamnolipid.

3.5. Discussion of the advantages of the improved oil spreading technique *via* rapid *in situ* reaction

The improved oil spreading technique has the advantages of being reasonable, stable and accurate. The stability of the improved oil spreading technique is mainly reflected in two aspects: the stabilized time of formation oil spreading is increased, and the size is basically unchanged. The technique has good repeatability, and it has a small error for three parallel tests. In addition, the linear relationship of the standard curve established by the improved oil spreading technique is very good, which is beneficial to practical applications. However, the content of biosurfactants in oilfield water samples calculated by different standards is quite different. Because the oilfield water samples are complex, there is more than one biosurfactant in water samples, and the ability of different biosurfactants to form oil spreading is different, there is still a large error in the quantity of biosurfactants used in oilfield water samples. From the experimental results of the mixed solution of oilfield water samples with lipopeptide or rhamnolipid standard solution, it can be seen that the interference of extracted water to the quantification of biosurfactant is greater than that of injected water, which may be because the extracted water matrix is more complex and contains more oil phase. The new oil spreading technique is more accurate for the quantification of a single biosurfactant solution, but it is difficult to achieve accurate quantification of a complex multicomponent mixed solution of oilfield water samples. Therefore, the new oil spreading technique can be improved continuously, using its simple and fast characteristics for better application in the production of surfactant strain screening and other aspects.

4. Conclusion

On the basis of the previous research results, the modified oil spreading technique was established by optimizing the oily material, image acquisition and calculation method, and the new technique was applied to complete the accurate quantification of rhamnolipid standard solution in the range of 25–300 mg L^{-1}, lipopeptide standard solution in the concentration range of 5–200 mg L^{-1}, and the rapid quantification of single class biosurfactant solution. We screened lipopeptides and glycolipid biosurfactants for rapid and quantitative analysis of biosurfactant concentrations. By selecting areas by color done by the software to modify image acquisition, the results showed that the modified oil spreading technique has a good quantitative effect, reflected in that the concentration of biosurfactant is proportional to the diameter of the sample droplet. More importantly, using the pixel ratio method instead of the diameter measurement method to optimize the calculation method, the region selection was more scientific, and the accuracy of the data results was high, and the calculation efficiency was improved significantly. Finally, the contents of rhamnolipid and lipopeptide in oilfield water samples were judged, which provided some theoretical and data support for the study of the microbial oil displacement technology mechanism. However, because of the complexity of oilfield water samples, rapid and accurate quantitative analysis of biosurfactants in oilfield water samples cannot be realized completely. In addition, limited by the experimental conditions, time and their own research level, there were still some shortcomings that need to be further solved. Therefore, how to realize the quantitative analysis of mixed solutions of various biosurfactants by using the oil spreading technique needs further study. Alternatively, the new oil spreading technique is constantly being improved, using its simplicity and speed, in other aspects such as in the production of surfactant strain screening to better play its role.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

This work was funded by the National Key Research and Development Program (2016YFC1402301), the Shandong Provincial Natural Science Foundation of China (ZR2018MD017) and the Fundamental Research Funds for the Central Universities (201822009). The Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering. This is MCTL Contribution No. 311.

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