Rhamnolipids Sustain Unchanged Surface Activities during Decomposition in Alkaline Solutions

Shuai Kong, Chong Shen, Yizeng Li, and Qin Meng*

Cite This: ACS Omega 2021, 6, 15750−15755

ABSTRACT: Biosurfactant rhamnolipids (RLs) have gained global interests owing to their fully green properties, potentially wide applications in diverse fields, as well as high stabilities under various harsh conditions. Nevertheless, we doubted the reputed stability of RLs in considering their natural structure of carbohydrate heads and lipid tails. This study, for the first time, systematically investigated the stability of RLs at varying temperatures and pH. As found, the concentration of RLs in an aqueous solution was significantly reduced when the pH was over 11 at room temperature, and this was much more severe with the increase in temperature and preservation time. According to the high-performance liquid chromatography−mass spectrometry (HPLC−MS) analysis, degradation yielded other RL congeners, 3-hydroxy fatty acids, rhamnose, methyl furfural, and organic acids. The newly generated RL congeners and fatty acids still possessed equivalent surface activities in reducing the surface tension of the aqueous solution, well explaining the previously claimed high stability of RLs. The finding will be greatly valued for commercially developing the industrial applications of RLs and other biosurfactants.

1. INTRODUCTION

Rhamnolipids (RLs) are anionic biosurfactants containing one or two rhamnose molecules and one or two 3-hydroxy fatty acid molecules. They can effectively reduce the surface tension of water from 72 to 30 mN/m at low concentrations and decrease the interfacial tension in water/oil systems from 43 to below 1 mN/m.1,2 Beyond these general criteria for an efficient anionic surfactant, fully biobased RLs possess many spectacular green properties that are “eco/health-friendly” because of the fact that they are manufactured via microbial fermentation from renewable agroresources (vegetable oil, oleic acid, and carbohydrates) and could be rapidly biodegraded in the environment, and they are also amiable to the human skin.

Thanks to their natural origin and green properties, RLs are superior to other chemically synthesized surfactants and have aroused the current global interest.3 In this respect, they have established a great prospect of applications, including formulations in many products varying from cosmetics to environmental control.4 For example, RLs have potential uses as an active ingredient for treating wrinkles,5 autoimmune/dermatological diseases,6 and for bakery purposes.7 Moreover, RLs have been employed as agrochemicals against plant fungal diseases,8 in improving drought conditions,9 or in dissolving persistent pesticides.10 In petroleum oil fields, RLs have been used as a superior oil displacement agent in enhanced oil recovery (EOR)1,11−13 and could recover over 98% of crude oil from refractory waste crude oil as an efficient biodemulsifier.14

As RLs are highly supposed in the near future to partly replace industrial petrochemical surfactants, their applications could enter almost each sector of the modern industry.

Naturally, some applications of RLs are carried out under harsh conditions. Generally, as RLs are preferred to be dissolved in alkaline solutions, high pH is more frequently encountered during applications.15 For instance, a pH of 10 is common for RLs in treating sediments16 and refractory waste oil,14 while a higher pH of even around 13 is necessary for enhanced oil recovery.17 Meanwhile, a relatively high temperature of more than 70 °C is preferred for RLs in waste oil treating14 or tertiary oil recovery.17 It seems that alkaline conditions or high temperatures are frequently encountered in RL applications. In addition to these diverse applications, high temperature is indispensable in manufacture because of autoclaving at 121 °C at the end of fermentation for inactivating the opportunistic pathogen of Pseudomonas aeruginosa, the major RL producer.

Received: March 1, 2021
Accepted: May 21, 2021
Published: June 8, 2021

https://doi.org/10.1021/acsomega.1c01099
ACS Omega 2021, 6, 15750−15755
As harsh conditions are generally involved in manufacturing, preservation, and applications, the stability of RLs is crucial for industrialization as well as commercialization. So far, RLs have gained high reputation for their high stability at a wide range of pH (4−10) and temperature (4−100 °C).3,18 The high stability has also been claimed in autoclaving whereas RLs were subjected to alkaline environments with pH 12 under a high temperature of 121 °C for 30 min.19,20 However, RLs are of particular structure that one or two rhamnose sugar molecules are connected to β-hydroxy fatty acids. Such glycosidic/ester bonds are generally unstable and susceptible to hydrolysis. For this reason, RLs are highly susceptible to exhibiting the desired stability, with exposure to harsh conditions. We have noticed that the abovementioned high stability of RLs are all drawn because of the unchanging properties under harsh conditions on reducing surface tension19 and sustaining emulsification,20 but these are only superficial phenomena and are deceptive.

This study has re-investigated the stability of RLs by detecting residual Rs after exposure to varying pH and temperatures. The findings will be critically important for the future wide applications and storage of RLs because stability is very important for the development of commercial products.

2. EXPERIMENTAL

2.1. Materials. Both amorphous mono-RLs and crystal di-RLs were at a purity of over 95% and offered by Huzhou Gemking Biotechnology Co., Ltd. (Huzhou, China); acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Also, all solutions were filtered with a 0.22 μm filter (Sterivex-GV 0.22 μm, Millipore, Bedford, MA, USA) before experiments to avoid the influence of microorganisms.

2.2. Evaluation of RL Stability. The RL solutions were prepared by individually dissolving either mono-RLs or di-RLs into an alkaline solution at pH 9, and their pH was adjusted by adding 1 N HCl or 1 N NaOH. All the aqueous solutions were stored in a water bath at a fixed temperature. The sample of each solution was taken and stored at 4 °C until analysis. To examine the stability in autoclaving, di-RLs in an aqueous solution with a fixed pH was sterilized in an autoclave under 121 °C for 30 min. Before analysis, the pH of all samples was adjusted to 7 by adding HCl or NaOH solution, and the resulting concentration change was eliminated by multiplying with the volume change rate.

2.3. Quantification of RLs by HPLC. All the concentrations of RLs were determined by HPLC equipped with a C18 column (Varian, California, USA) and a Waters 2487 refractive index detector (Waters, Milford, MA, USA). The analysis was performed at 50 °C under a flow rate of 1 mL/min using a mobile phase of acetone/acetonitrile (30: 70 v/v). The injected sample volume was 20 μL.

2.4. Detection of the Surface Activity of RLs. The surface tension of the di-RL solution was measured using a spinning drop interfacial tensiometer (Model TXS00, CNG USA). Briefly, 1 mL of solution was injected into a glass tube. After the tube was spun at up to 5000 rpm, an air bubble was created in the middle of the tube. The width of the bubble was measured by computer camera and used for calculating the surface tension.

2.5. Thin-Layer Chromatography of RLs. The RL solution was analyzed by thin-layer chromatography.
Briefly, 2 μL of each sample was applied to the TLC plate and dried. Then, the TLC plate was placed in a paper-lined rectangular glass chamber which was freshly filled with the developing reagent (chloroform–methanol–acetic acid, 40:9:1, v/v) for 25 min, followed by hot-air drying. For visualizing, the plates were sprayed with the reagent of sulfuric acid–ethanol (1:1, v/v) and heated at 120 °C for 2 min.

2.6. Analysis by LC–MS. RLs and their degradants were analyzed by HPLC–MS (Agilent 1100/6460 Triple Quad, CA, USA) equipped with an electrospray ionization (ESI) ion source operated in the negative-ion mode. Each sample at 20 μL was introduced into the HPLC system equipped with a Varian C18 (150 mm × 4.6 mm, 5 μm) reverse-phase column. The mobile phases consisted of solution A (acetonitrile) and solution B (2 mM ammonium acetate aqueous solution). Gradient elution was adopted starting with 40% solution A for 4 min and subsequently 90% solution A for 31 min. The HPLC flow rate was 0.25 mL/min, and the eluates were directly introduced into a mass spectrometer. The MS conditions were as follows: gas flow pressure, 40 psi; gas flow rate, 8 mL/min; drying gas temperature, 365 °C; and capillary, 3.5 kV. The scanning mass range was from 150 to 800 Da. The quantification of major components was performed by the integration of pseudomolecular ions.

3. RESULTS AND DISCUSSION

3.1. Decomposition of RLs in Alkaline Solutions without Impairing Surface Activities. RLs in manufacture, storage, as well as applications could most possibly meet alkaline/acidic environments varying in temperature.14–17 Hence, the impact of pH and temperature on RL concentrations was first addressed. When the RL aqueous solutions with different pH was left at room temperature for 12 h, their concentrations maintained the initial level at pH 4–10 but significantly decreased at a pH of over 11 (Figure 1A). More severe degradation was observed when pH was 12, whereas di-RL was reduced by 39% while mono-RLs decreased by 29%. Also, such degradation increased with temperature (Figure 1B). Under 80 °C and pH 12, more than 94% of di-RLs disappeared upon overnight storage, while this value was 90% for mono-RLs. It seems that temperature elicited much more critical impact in destroying RLs in the alkali solution.

The influence of exposure time was inspected by examining the residual concentration of RL under pH 12 at 65 °C. As seen in Figure 1C, RLs degraded quickly within the first 4 h and then entered a relatively stable state that could have resulted from their low residual concentration. After 12 h, di-RLs were reduced by almost 75% while mono-RLs decreased by 39%.

Because RLs were largely manufactured by the opportunistic microorganism Pseudomonas aeruginosa, the culture broth must be sterilized to inactivate the bacteria thoroughly before performing separation and purification.22 Hence, the stability of di-RLs was examined upon exposure to autoclaving. As the pH of the final fermentation broth may be slightly alkaline according to our observation in industrial manufacture, pH was chosen to be within a range of 8–11. As shown in Figure 1D, the loss of di-RLs was slight at pH 8–9 and it became more apparent at pH 10–11 with a degradation rate of 8–19%. Mono-RL exhibited similar loss during autoclaving (Figure S1).

The alternation on surface activity was represented by detecting the surface tension of the RL solution after degradation. As shown in Figure 2, after exposure to pH 12 and 65 °C for 12 h, the RL solution sustained its initial surface activity in reducing the surface tension of water. The RL solution with or without degradation exhibited the same CMC values. Moreover, the RL solution postdegradation sustained the surface activities on emulsification (Table S1), wetting, and permeation (Table S2). Hence, the preservation of RLs in alkaline solutions does not impair their surface activities, helping in understanding the previously claimed high stability.19,23

3.2. Visualization of RL Decomposition. The degradation of RLs, although not changing their surface activities, elicited a critical change on its chemical composition. As shown in Figure 2A, it can be seen that after exposure to hot (65 °C) and alkali surroundings (pH 12) for 12 h, the di-RL solution turned bright yellow in color from the initial light one, indicative of the generation of new components. Moreover, the pH decreased to around pH 10. Hence, the color change along with the decrease in pH served as a strong support for the decomposition of RLs.

Furthermore, the analysis by TLC more directly reflected the decomposition of RL. As shown in Figure 3B, the decomposition of di-RLs produced new hydrophobic components (shown in black arrows) with a higher Rf and hydrophilic smear (shown in red arrows) with a much lower Rf.

3.3. Determination of RL Degradants by HPLC–MS. To investigate the relevant chemical reactions involved in RL degradations, the degradant solution abovementioned was analyzed by HPLC–MS, in comparison with the starting solution. Two HPLC columns, C18 HPLC column and Carbomix H-NP column, were used for the separation of hydrophobic and hydrophilic components, respectively.

The analysis using the C18 HPLC column confirmed that the starting solution was largely composed of di-RLs containing two lipid tails, among which Rha-Rha-C10-C10 was the dominant component, taking a percentage of about 60. As shown in Figure 4, the degradation reduced the amount of all the di-RLs containing two fatty acids (in black color), among which Rha-Rha-C10-C10 was most significantly destroyed. In contrast, the degradation increased the amounts of some compounds (marked in red color, Figure 4), among which 3-hydroxydecanoic acid and Rha-Rha-C10 are the two most distinguished accumulated ones. It is naturally understood that the major component (Rha-Rha-C10-C10) could break into fragments.
novel di-RL (Rha-Rha-C\(_{10}\)) and 3-hydroxydecanoic acid (C\(_{10}\)) via the hydrolysis of the ester bond. The quantification of the major substances that were produced or reduced apparently during decomposition is shown in Table 1. As seen, except for the appearance of di-RLs containing one fatty acid (C\(_{10}\) or C\(_{12}\)), two mono-RLs, Rha-C\(_{10}-C_{10}\) (m/z 503) and Rha-C\(_{12}-\), were largely produced after degradation. These two products should be resulted from the hydrolysis of glycosidic bonds from di-RLs.

Diverse hydrophilic compounds were identified. As the hydrophilic components were much complicated, only di-rhamnose, methyl furfural, oxalic acid, and acetic acid were defined, and they are listed in Table S3. Di-rhamnose found in the degradation solution could be resulted from the hydrolysis of the \(\beta\)-glycosidic bond between the rhamnose unit and fatty acid train, while methyl furfural should be the dehydration product of rhamnose. Generally, methyl furfural, the common degradants of sugar derivatives, usually presents a deep color in aqueous solutions, well interpreting the color change from light to bright yellow postdegradation (Figure 3A). The detected degradants of organic acids, formic acid, and oxalic acid were most possibly caused by the oxidation of rhamnose,\(^{24}\) resulting in the decrease of pH after degradation. As noticed, rhamnose, if stored overnight at pH 9–12 under 65 °C, shows a much more severe concentration reduction and a more significant appearance of bright yellow color with the increase of pH (Figure S2), exhibiting a remarkable similarity of decomposition as RLs. This could help demonstrate the involvement of rhamnose in the decomposition of RLs.

With the HPLC–MS analysis, it is clear that new amphiphilic RLs were produced and could compensate for the loss of degraded di-RLs, thereby sustaining the initial surface activities (Figure 2). Rha-Rha-C\(_{10}\) the most accumulated amphiphilic congener, was supposed to locate at the lower position than the starting Rha-Rha-C\(_{10}\) in the TLC plate (indicated by black arrow in Figure 3B). In contrast, C\(_{12}\), the most accumulated hydrophobic degradants, was assumed to locate at the top position in the TLC plate (indicated by red arrow in Figure 3B). It seems that all the analysis by surface tension measurements, HPLC/TLC detection, and so forth systematically illustrated that RLs could well manipulate the paradoxicity between the decomposition and sustenance of functional surface activities.

### 3.4. Proposed Schematic of RL Degradation and Its Significance on Industrial Applications

According to the results above, Rha-Rha-C\(_{10}-C_{10}\) which represents di-RLs containing two fatty acid tails, could possibly go through degradation in the three pathways, as shown in Figure 5. The dominated pathway via the hydrolysis of the ester bond was supposed to break Rha-Rha-C\(_{10}-C_{10}\) into Rha-Rha-C\(_{10}\) and 3-hydroxydecanoic acid. In the second pathway, Rha-Rha-C\(_{10}-C_{10}\) could release one rhamnose and Rha-C\(_{12}-C_{10}\) via the hydrolytic breakage of O-glycosidic bonds. In the third pathway, Rha-Rha-C\(_{10}-C_{10}\) could further decompose to form methyl furfural, followed by forming organic acids such as formic acid, acetic acid, and oxalic acid.\(^{24}\) The specific degradation pathways involved in each RL may depend on its particular molecular structures. For example, mono-RLs with two lipid tails could degrade, following the first and the third pathways.

Normally, the confirmed instability of RLs should abate their potential marketing in considering that stability is a key factor in developing a commercial product. Surprisingly, RLs, under harsh environments, could peel off either the rhamnose unit or the 3-hydroxyl acid unit, generating new amphipathic congeners such as Rha-Rha-C\(_{10}\), Rha-C\(_{10}-C_{10}\), and Rha-C\(_{10}-C_{12}\) (Figure 5). This compensates for the loss of

---

**Table 1.** Dominant Molecules and Content in RLs with or without Degradation

| Retention time (min) | m/z | Structure | Content (%) before | Content (%) after | Alternation |
|----------------------|-----|-----------|--------------------|------------------|-------------|
|                      |     |           | before             | after            | alternation |
| 6.76                 | 479 | Rha-Rha-C\(_{10}\) | 0.8               | 8.8              | 8.0         |
| 9.20                 | 507 | Rha-Rha-C\(_{12}\) | 0.2               | 1.0              | 0.8         |
| 23.48                | 649 | Rha-Rha-C\(_{10}-C_{10}\) | 59.6             | 42.8             | 16.8        |
| 27.13                | 677 | Rha-Rha-C\(_{10}-C_{12}\) | 9.8              | 9.0              | 0.8         |
| 27.92                | 705 | Rha-C\(_{12}-C_{10}\) | 0.0               | 2.7              | 0.0         |
| 30.24                | 531 | Rha-C\(_{12}-C_{12}\) | 16.8             | 14.6             | 2.2         |
| 33.66                | 187 | C\(_{10}\) | 0.0               | 6.9              | 6.9         |

*The molecules were listed according to the assays by HPLC–MS (C18 column). Hydroxy fatty acids are abbreviated as C\(_{n}\), whereas \(n\) represents the number of carbon atoms.*

---

**Figure 3.** (A) Color appearance of the di-RL solution with or without decomposition. (B) TLC plate of RLs and their degradants. BF: di-RL solution before decomposition at 65 °C, and pH 12 for 12 h. Spots were stained with sulfuric acid–ethanol (1:1, v/v). Black arrows: hydrophobic degradants; red arrows: hydrophilic degradants.

**Figure 4.** LC–MS spectrum of di-RL before and after degradation with a C18 column. Degradation occurred at 65 °C and pH 12 for 12 h.
biosurfactants and thus sustains the initial surface activities. Hence, the good sustenance of the surface activities in decomposition (Figure 2) well interpreted the successful application of RLs in tertiary oil recovery\textsuperscript{17} and demulsification of waste crude oil\textsuperscript{14} under hot and alkali surroundings. Such functional maintenance in decomposition has been rarely reported in petrochemical surfactants. Nevertheless, RLs, if applied for biological functions and are unrelated to the surface activities, should not be exposed to alkali aqueous solutions, particularly under high temperatures, in the whole process from the initial manufacture to the final application. The results in this study may be helpful for understanding the similarly claimed high stability of other biosurfactants, including surfactin\textsuperscript{25} or other glycolipids.\textsuperscript{26}

4. CONCLUSIONS

RLs, previously well known for high stability, could readily degrade in alkaline aqueous solutions. The degradation is much more severe with the increase of pH, temperature, and time. The identified degradants include other RL congeners, 3-hydroxy fatty acids, rhamnose, methyl furfural, organic acids, and so forth. Interestingly, the decomposition of RLs does not impair their functional activities as surfactants, thus showing no impact on the applications under harsh conditions. The well-sustained functional stability makes RLs promising for applications under harsh environments.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c01099.

Emulsification properties of di-RLs, wetting and permeation properties of di-RLs, stability of rhamnose at different pH, and HPLC–MS analysis of hydrophilic components (PDF)

AUTHOR INFORMATION

Corresponding Author
Qin Meng – College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China;
orcid.org/0000-0002-8017-6852; Email: mengq@zju.edu.cn
Shuai Kong — College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China; orcid.org/0000-0001-5518-4642
Chong Shen — College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China; orcid.org/0000-0002-0606-6989
Yizeng Li — College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c01099

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
We gratefully acknowledge the financial support of this study by NSFC (National Natural Science Foundation of China No. 21776242) and National Key R&D Plan (No. 2016YFD0400801).

REFERENCES
(1) Amani, H.; Mehrnia, M. R.; Sarrafzadeh, M. H.; Haghighi, M.; Soudi, M. R. Scale up and application of biosurfactant from Bacillus subtilis in enhanced oil recovery. Appl. Biochem. Biotechnol. 2010, 162, 510–523.
(2) Hörmann, B.; Müller, M. M.; Syldatk, C.; Hausmann, R. Rhamnolipid production by Burkholderia plantarii DSM 9509T. Eur. J. Lipid Sci. Technol. 2010, 112, 674–680.
(3) Sekhon Randhawa, K. K.; Rahman, P. K. Rhamnolipid biosurfactants-past, present, and future scenario of global market. Front. Microbiol. 2014, 5, 1–7.
(4) Kourmentza, C.; Costa, J.; Azevedo, Z.; Servin, C.; Grandfils, C.; De Freitas, V.; Reis, M. A. M. Burkholderia thailandensis as a microbial cell factory for the bioconversion of used cooking oil to polyhydroxyalkanoates and rhamnolipids. Bioresour. Technol. 2018, 247, 829–837.
(5) Piljac, T.; Piljac, G. Use of rhamnolipids as cosmetics. Int. J. Cosmet. Sci. 2007, 29, 370–379.
(6) Piljac, G.; Piljac, V. Immunological activity of rhamnolipids. J. Microb. Meth. 1995, 24, 1–10.
(7) Vanzeveren, I. V. H. Rhamnolipids in bakery products. 20 060 233 935, 2006.
(8) Meng, Q.; Zhang, G. Application of rhamnolipid as biological pesticide and biological insecticide. 103 202 295, 2013.
(9) Renfro, T. D.; Xie, W.; Yang, G.; Chen, G. Rhamnolipid surface thermodynamic properties and transport in agricultural soil. Colloids Surf., B: Biointerfaces 2014, 115, 317–322.
(10) Gaur, V. K.; Bajaj, A.; Regar, R. K.; Kamthan, M.; Jha, R. R.; Srivastava, J. K.; Manickam, N. J. B. T. Rhamnolipid from a Lysinibacillus sphaericus strain ITFR51 and its potential application for dissolution of hydrophobic pesticides. Bioresour. Technol. 2019, 272, 19–25.
(11) Sen, S. Biotechnology in petroleum recovery: The microbial EOR. Prog. Energy Combust. Sci. 2008, 34, 714–724.
(12) Yousef, N.; Simpson, D. R.; Mcinerney, M. J.; Duncan, K. E. In-situ lipopeptide biosurfactant production by Bacillus strains correlates with improved oil recovery in two oil wells approaching their economic limit of production. Int. Biodeterior. Biodegrad. 2013, 81, 127–132.
(13) Banat, I. M.; Makkar, R. S.; Cameotra, S. S. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biotechnol. 2000, 53, 495–508.
(14) Long, X.; Zhang, G.; Shen, C.; Sun, G.; Wang, R.; Yin, L.; Qin, M. Application of rhamnolipid as a novel biodemulsifier for destabilizing waste crude oil. Bioresour. Technol. 2013, 131, 1–5.
(15) Shin, K. H.; Kim, K. W.; Kim, J. Y.; Lee, K. E.; Han, S. S. Rhamnolipid morphology and phenanthrene solubility at different pH values. J. Environ. Qual. 2008, 37, 509–514.
(16) Wu, W.; Hu, Y.; Guo, Q.; Yan, J.; Chen, Y.; Cheng, J. Sorption/desorption behavior of triclosan in sediment-water-rhamnolipid systems: Effects of pH, ionic strength, and DOM. J. Hazard. Mater. 2015, 297, 59–65.
(17) Guo, H.; Li, Y.; Wang, F.; Yu, Z.; Chen, Z.; Wang, Y.; Gao, X. ASP Flooding: Theory and Practice Progress in China. J. Chem. 2017, 2017, 1–18.
(18) Varjani, S. J.; Upasani, V. N. Core Flood study for Enhanced Oil Recovery through ex-situ bioaugmentation with thermo- and halotolerant rhamnolipid produced by Pseudomonas aeruginosa NCIM 5514. Bioresour. Technol. 2016, 220, 175–182.
(19) Sathi, R. K.; Yahya, K. M.; Archana, K.; Gopal, R. M.; Hameeda, B. Utilization of mango kernel oil for the rhamnolipid production by Pseudomonas aeruginosa DRI towards its application as biocontrol agent. Bioresour. Technol. 2016, 221, 291–299.
(20) Meganathan, V.; Baskaran, A.; Kumar, S. D.; Sureka, I.; Sathiyanurthy, K. Screening, stability and antibacterial potential of rhamnolipids from Pseudomonas sp., isolated from hydrocarbon contaminated soil. J. Appl. Pharm. Sci. 2015, 5, 26–33.
(21) Keboubache-Gana, S.; Gana, M. L.; Khemili, S.; Fazouane-Naimi, F.; Bouanane, N. A.; Pennincks, M.; Hacene, H. Isolation and characterization of halophilic Archaea able to produce biosurfactants. J. Ind. Microbiol. Biotechnol. 2009, 36, 727–738.
(22) de Bentzmann, S.; Plesiat, P. The Pseudomonas aeruginosa opportunistic pathogen and human infections. Environ. Microbiol. 2011, 13, 1655–1665.
(23) Zhao, F.; Yuan, M.; Lei, L.; Li, C.; Xu, X. Enhanced production of mono-rhamnolipid in Pseudomonas aeruginosa and application potential in agriculture and petroleum industry. Bioresour. Technol. 2021, 323, No. 124605.
(24) Gleason, W. B.; Barker, R. Oxidation of Pentoses in Alkaline Solution. Can. J. Chem. 1971, 49, 1425–1432.
(25) Araújo, H. W. C.; Andrade, R. F. S.; Montero-Rodriguez, D.; Rubio-Ribeaux, D.; Alves da Silva, C. A.; Campos-Takaki, G. M. Sustainable biosurfactant produced by Serratia marcescens UCP 1549 and its suitability for agricultural and marine bioremediation applications. Microb. Cell Fact. 2019, 18, 1–13.
(26) Sen, S.; Borah, S. N.; Bora, A.; Deka, S. Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3. Microb. Cell Fact. 2017, 16, 1–14.