Environmental Technology & Innovation 14 (2019) 100347

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Biosurfactant produced by the hydrocarbon-degrading bacteria: Characterization, activity and applications in removing TPH from contaminated soil

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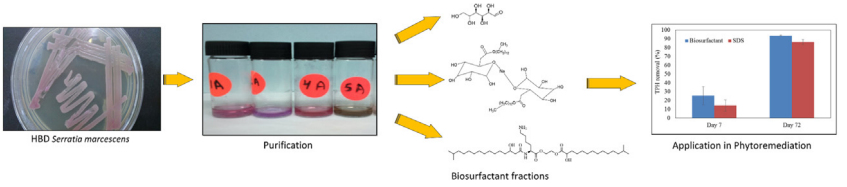
GRAPHICAL ABSTRACT



HIGHLIGHTS

- The stability of the biosurfactant produced by HDB Serratia marcescens was investigated.
- The crude biosurfactant was characterized by TLC, LC–MS and FTIR.
- The biosurfactant had good stability at pH 7, 70 °C and 3% salt concentration.
- The crude biosurfactant contains glucose, 1-tetradecyl D-glucuronate and lipids.
- The addition of biosurfactant enhanced the phytoremediation of TPH.

ARTICLE INFO

Article history:
Received 19 October 2018
Received in revised form 28 February 2019

ABSTRACT

In this study, the activity of the biosurfactant produced by the hydrocarbon-degrading bacteria (HDB) *Serratia marcescens* was investigated at various temperatures (30–100 °C), pH values (pH 4–10) and salt concentrations (1%–9%). The characterization of crude biosurfactant was performed using Fourier transform infrared spectroscopy (FTIR), thin-layer chromatography and tandem mass spectrometry, while purification was conducted

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https://doi.org/10.1016/j.eti.2019.100347

Accepted 1 March 2019
Available online 7 March 2019

Keywords:
Tetradecyl D-glucuronate biosurfactant
Hydrocarbon-degrading bacteria
Serratia marcescens
Contaminated soil
Phytoremediation

by silica gel adsorption chromatography. The biosurfactant displayed good activity at a high temperature of 70 °C, a pH 7 and a salt concentration of 3%. FTIR identified hydroxyl, acetyl, carbonyl and aliphatic chain chemical functional groups. The purification of the crude biosurfactant resulted in three distinct fractions, i.e., glucose, 1-tetradecyl D-glucuronate (novel biosurfactant fraction) and lipids. The crude biosurfactant produced by the HDB *S. marcescens* is a novel, promising class of biosurfactant for the removal total petroleum hydrocarbon from contaminated soil. Based on these results, the biosurfactant may be an attractive choice for the oil industry for the decontamination of polluted sludge.

1. Introduction

Biosurfactants and synthetic surfactants are the main groups of surface-active agents. Biosurfactants are derived from biological processes, whereas synthetic surfactants are produced through organic chemical reactions (Singh et al., 2007). Biosurfactant characteristics include their rather non-toxic nature and biodegradability; thus, biosurfactants are considered environmentally friendly. Recently, their unique structures have attracted attention for potential applications in many aspects of industry, ranging from biotechnology to environmental cleanup (Pornsunthorntawee et al., 2008; Silva et al., 2014; Alvarez et al., 2015; Anjum et al., 2016; Gargouri et al., 2017). Biosurfactants exhibit specific modes of action, widespread applicability, relative ease of preparation, and low toxicity as emulsifiers, foaming and wetting agents, and detergents in petrochemical, petroleum, environmental management, cosmetic, and pharmaceutical industries. They are also used to enhance oil recovery in the petrochemical industry (Onwosi et al., 2012). In addition, biosurfactants have been applied (i) to reduce the cell hydrophobicity, thereby improving cellular attachment to the petroleum hydrocarbon, and (ii) to enhance the petroleum hydrocarbons mass transfer from liquid to bacterial cells (Sponza and Gok, 2011).

Biosurfactants decrease interfacial and surface tension by accumulating at the interface between immiscible fluids due to the presence of amphiphilic molecules, which include a distinguished, hydrophobic tail moieties and hydrophilic head. Moreover, these properties also cause (i) the formation of water interfaces, resulting in emulsifying, foaming, detergency, and other disparate traits, and (ii) different degrees of polarity and hydrogen bonding in air, water and oil (Singh et al., 2007; Cameotra and Makkar, 2010). Biosurfactants comprise a group of molecules with diverse structures that includes simple molecules, such as phospholipids and fatty acids, as well as lipopeptides, glycolipids, and high molecular weight molecules, such as lipopolysaccharides (Darvishi et al., 2011; Keskin et al., 2015; Liu et al., 2016). Low molecular weight biosurfactants represent the most prominent group and consist of mono- or oligosaccharides and more saturated or unsaturated fatty alcohols, fatty acids, or hydroxyl-fatty acids (Yin et al., 2009; Henkel et al., 2012).

Surface-active agents result in hydrocarbon biodegradation by populations of microorganisms; these agents are characterized by their microbial origin and chemical composition. The surface-active agents include phospholipids, glycolipids, polysaccharide–protein complexes, protein-like substances, lipopeptides and lipopolysaccharides (Abbasi et al., 2012). The main hydrocarbon-degrading bacteria (HDB) characteristic is their ability to emulsify hydrocarbons in solution by producing biosurfactants that cause the dispersion of hydrocarbons in water emulsions into micelles or microdroplets (Ibrahim et al., 2013). In industrial applications, these bacteria have been used for oil recovery enhancement (Alvarez et al., 2015; Fernandes et al., 2016), therapeutics (Gudiña et al., 2013), food industry (Anjum et al., 2016), biomedicine (Inès and Dhouha, 2015) and pollutant bioremediation (Anyanwu et al., 2011; Almansoory et al., 2015). The biosurfactant remain stable under extreme conditions such as a wide range of temperature, salinity and pH. The production of biosurfactant is affected by various factors, for example, type of isolate, type of medium, and operating conditions, such as agitation rate, pH and temperature.

The objective of this study was to characterize and determine the activity of the biosurfactant produced by the HDB *Serratia marcescens* under various salinity, pH and temperature conditions for the soil remediation application of removing total petroleum hydrocarbon (TPH). The crude biosurfactant was characterized using thin-layer chromatography (TLC) and tandem mass spectrometry (MS-MS), as well as Fourier transform infrared spectroscopy (FTIR).

2. Methods

2.1. Identification of the hydrocarbon degrading bacteria S. marcescens

The *S. marcescens* was isolated from a hydrocarbon-contaminated site in Melaka, Malaysia. The strain was identified by polymerase chain reaction (PCR). The total genomic DNA was extracted from the HDB *S. marcescens* using a Wizard® Genomic DNA Purification Kit (Promega, USA). The detailed findings of this isolation procedure were presented in our previous report (Almansoory et al., 2014).

2.2. Cultivation medium for biosurfactant production

The 100 mL cultivation medium was simulated using mineral salt medium (MSM), which was prepared in distilled water containing (g L^{-1}): 1.2 KH_2PO_4, 1.8 K_2HPO_4, 0.2 $MgSO_4.7H_2O$, 0.01 $FeSO_4.7H_2O$, 10 NaCl, 0.1% trace elements ((g L^{-1}) 0.1 $MnSO_4.H_2O$, 0.025 $CuCl_2$, 0.025 $(NH_4)_6Mo_7O_{24}$. $4H_2O$, 0.025 $Co(NO_3)_2.6H_2O$, 0.025 $ZnCl_2$, and 0.01 NH_4NO_3), 5% glycerol, and 5 g L^{-1} ammonium sulfate and 4 g L^{-1} peptone as the mixed nitrogen source. The pH of the cultivation medium was adjusted to pH 8.0 using 1 N NaOH or 1 N HCl, and then the medium was autoclaved at 121 °C for 15 min. After sterilization, *S. marcescens* was cultivated at 37 °C and 150 rpm for 5 days.

2.3. Test of the activity of the crude biosurfactant produced by the HDB S. marcescens

2.3.1. Temperature

The effect of temperature on the biosurfactant was investigated by varying the temperature from 30 to 121 °C. As a positive control, the experiment was performed with 1% sodium dodecyl sulfate (SDS). The emulsification index and surface tension were measured to determine the thermal activity of the biosurfactant.

2.3.2. pH

The effect of pH on the biosurfactant activity was determined by varying the pH of the cell-free broth pH 4 to 10 using 1 N NaOH or 1 N HCl.

2.3.3. NaCl concentration

The effect of NaCl concentrations (1 to 9%) on the activity of the cell-free broth was investigated by measuring the emulsification index (E24) and surface tension. Each experiment was conducted in triplicate.

2.4. Crude biosurfactant extraction

The extraction of the crude biosurfactant was conducted after 5 days of incubation in cultivation medium using the methods reported by Anandaraj and Thivakaran (2010) and Vaz et al. (2012), with some modifications. The culture was centrifuged at 8000 rpm for 15 min at 4 °C to remove the bacterial cells. The supernatant was sampled and adjusted to pH 2 using 1 N HCl. Then, the biosurfactant was collected by centrifugation at 12,000 rpm for 15 min at 4 °C. The resulting dried pellet yielded a white sediment. The biosurfactant dry weight was measured in a pre-weighed sterile petri dish. After drying, the dish was weighed. Extracts were concentrated and stored at −20 °C prior to characterization (Thampayak et al., 2008). The biosurfactant dry weight was calculated using Eq. (1):

$$\begin{pmatrix} \text{Dry weight of} \\ \text{biosurfactant} \end{pmatrix} = \begin{pmatrix} \text{Weight of the plate after} \\ \text{drying with biosurfactant} \end{pmatrix} - (\text{Weight of the empty plate}) \quad (1)$$

2.5. Characterization and purification of the biosurfactant

2.5.1. Fourier transform infrared spectroscopy

Identification of the chemical functional groups in the crude biosurfactant was conducted using Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Spectrum BX, USA). Approximately 5 mg of crude biosurfactant were mixed with 80 mg of potassium bromide (KBr). This mixture was compressed into a thin pellet and the measurement was conducted in absorbance mode.

2.5.2. Fractionation of the crude biosurfactant by silica gel adsorption chromatography

A column of silica mesh (Sigma-Aldrich) with a pore size of 60 Å was used to purify the crude biosurfactant. The crude biosurfactant extract was dissolved in 5 mL of chloroform:methanol (2:1) and then filtered through a 0.22 μm syringe filter. Then, the crude biosurfactant was separated on a preparative layer chromatography silica gel column (2.0 cm × 30 cm) and eluted with a mobile phase of chloroform:methanol:5 M ammonia (80:25:4, v/v). A silica gel filter was applied to the column, and the flow-through fraction was withdrawn. Next, 10 mL of the mobile phase were used to wash away impurities. Then, the eluted fractions were pooled and the solvents were evaporated. The concentrations of the crude biosurfactant fractions were measured in mg mL^{-1} (dry weight). The fractions were dried under a nitrogen gas flow and analyzed by liquid chromatography–mass spectrometry (LC–MS) to determine the structure and molecular weight of the biosurfactant produced by the HDB *S. marcescens*.

2.5.3. Thin-layer chromatography analysis

The TLC analysis was conducted by spotting the concentrated residue on pre-coated TLC plates (Merck, Germany) that were developed in a mobile phase of chloroform:methanol:5 M ammonia (80:25:4, v/v) (Suzuki et al., 1974). Chloroform was used to extract the silica from the TLC plate. The extraction yield obtained in the chloroform:methanol:5 M ammonia solvent system exhibited the highest surface activity. The material was detected by air-drying and then spraying the developed plate with a 50% (v/v) sulfuric acid solution of iodine reagent, followed by heating of the plate at 100 °C for 5 min.

Table 1
Infrared spectrum of the purified biosurfactant produced by the HDB *S. marcescens*.

Wavenumber (cm^{-1})	Functional groups
3534	O–H
2969	C–H
1678	C=O
1559	COO
1452	CH_3
1264	C–O
1074	C–O
648	C–H

2.5.4. Tandem mass spectrometry analysis

Fragmentation of the pseudomolecular compound by tandem mass spectrometry (MS/MS) of the parent ion was used to obtain further information about the biosurfactant structure. The isolated fractions eluted from the silica gel column were subjected to mass spectrometry analysis. The experiments were conducted with chloroform on a mass spectrometer using electrospray in positive mode and a scanning mass range of 300–900 Da (Singh et al., 2007).

2.6. Measurements of the emulsification index and surface tension

The emulsifying activity of the biosurfactant was determined using the method reported by Cooper and Goldenberg (1987). About 4 mL of gasoline and 4 mL of crude biosurfactant was homogenized by vortexing for 2 min. The emulsion activity was analyzed after 24 h, and the emulsifying index (E24) was expressed as the percentage of the total height occupied by the emulsion layer calculated using Eq. (2). The results were compared with distilled water as the negative control and the commercial surfactant 1% SDS as the positive control. The surface tension activity of the extracted crude biosurfactant was measured using a du Nouy ring-type tensiometer (KSV-sigma 703D, Finland).

$$E24 = \left(\frac{\text{Height of emulsion layer}}{\text{Total layer height}}\right) \times 100\% \quad (2)$$

3. Results and discussion

3.1. Activity of the crude biosurfactant

3.1.1. Activity at different temperatures

The advantages of biosurfactants compared to synthetic surfactants include biodegradability, lower toxicity and selectivity. The applicability of biosurfactants to some application areas depends on their activity at different temperature, salinity and pH. Biosurfactant activity has been tested at various temperatures from 0 °C (control without heat) to 121 °C (autoclave temperature). It was found that the thermostable biosurfactant was produced by the HDB *S. marcescens*. As shown in Fig. 1(a), an effect of temperature on the change in surface tension was not significantly observed upon heating to 121 °C. The emulsification activity was stable at the temperatures examined (E24 = 64.9%) compared with SDS, which showed a significant loss of emulsification activity beginning at 70 °C (E24 = 50.1%). The emulsification activity and surface tension-reducing activity were stable at a temperature of 121 °C. The extracted biosurfactant maintained its surface properties from 30 °C to 121 °C. The biosurfactant produced by *Candida sphaerica* was thermally stable, with high surface tension-reducing activity (25 mN m^{-1}) in a wide temperature range (5–120 °C) (Luna et al., 2012). Meanwhile, the biosurfactant produced by *Candida lipolytica* exhibited the optimum emulsification activity at a normal temperature, but at a high temperature of 100 °C, the emulsions did not remain stable (Rufino et al., 2007). The thermal activity of the extracted biosurfactant increased the scope of its applications, including under high temperatures conditions for the bioremediation processes.

3.1.2. Activity at various pH values

Studies on the surface tension reduction and emulsification activity by supernatant containing biosurfactant showed that the emulsification activity increased as the pH increased from acidic (pH 4) to neutral pH (pH 7) (Fig. 1b), but the emulsification activity decreased under alkaline conditions. Overall, the surface activity and emulsification activity of the extracted biosurfactant remained stable between pH 4 and 10. The lowest value of surface tension (34.3 mN m^{-1}) was obtained at pH 7, but it increased to 41.6 mN m^{-1} when the emulsification activity decreased at pH 10. The emulsification activity (E24) was 45.6% and 53.3% at pH 6 and 7, respectively, whereas the activity decreased to 20% at pH 10. Lima and Alegre (2009) found that *Saccharomyces lipolytica* CCT-0913 produced biosurfactant with the highest emulsifier activity at pH 7.0 and 60 °C. According to Sarubbo et al. (2006), less surface-active species are transformed into more active emulsifiers at extreme pH values due to increased ionization.

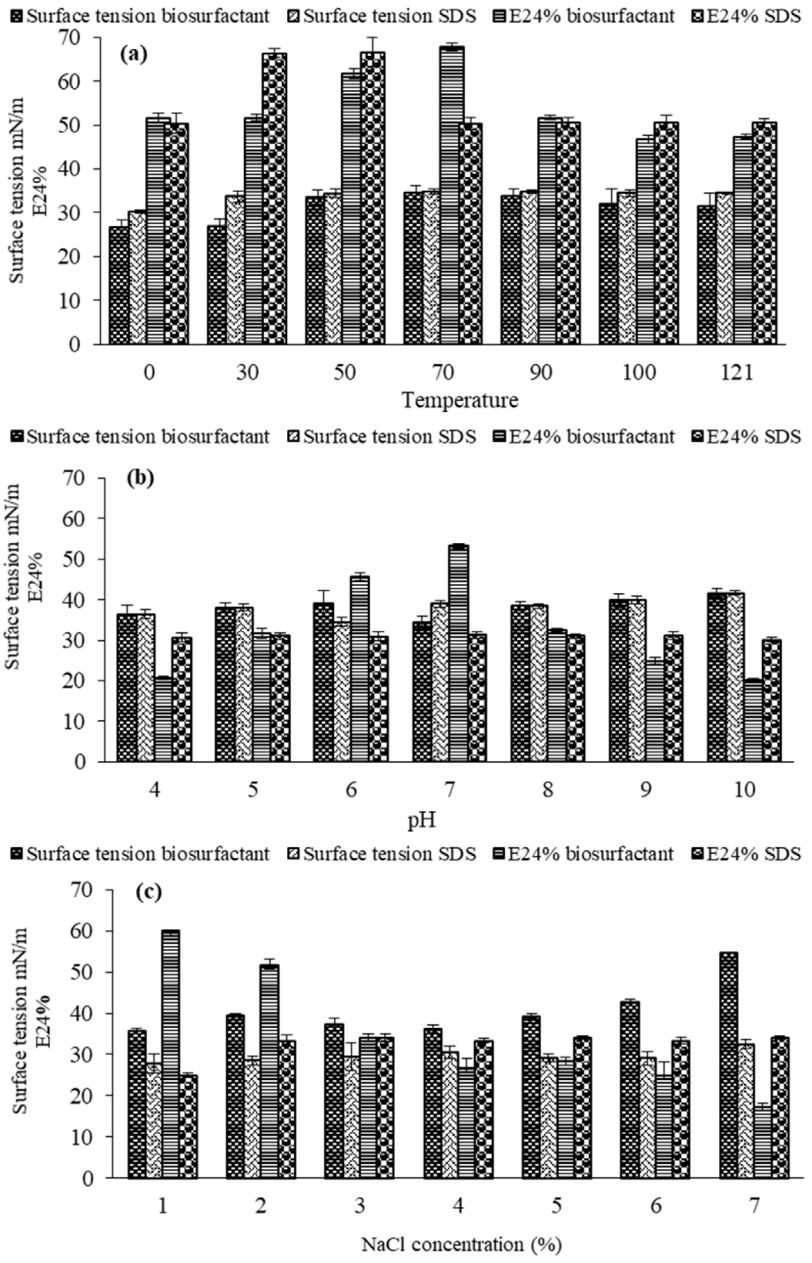

Fig. 1. Effects of different parameters on biosurfactant and SDS activities: (a) temperature, (b) pH and (c) NaCl concentration.

3.1.3. Activity at various salinities

The effect of the NaCl to the cell-free broth on the biosurfactant activity was investigated by varying the NaCl concentration from 1 to 7% (w/v). In the presence of 1% NaCl, the surface tension and emulsification activity were 35.6 mN m^{-1} and 59.9%, respectively (Fig. 1c). Slight changes were observed when the NaCl concentration was increased to 3% (w/v). At higher NaCl concentrations, the biosurfactant retained a surface tension of 54.7 mN m^{-1} and emulsification activity of 17%. Khopade et al. (2012) investigated the activity of the biosurfactant produced from *Nocardiopsis* sp. in the presence of NaCl, and activity was observed with 3% NaCl. The biosurfactant retained 50% of its emulsification activity. This biosurfactant was stable in the presence of high salinity and at an alkaline pH, which contrasts our results showing activity at neutral pH and low salinity. All three tested parameters resulted in comparable superior emulsification activity between the extracted biosurfactant and SDS.

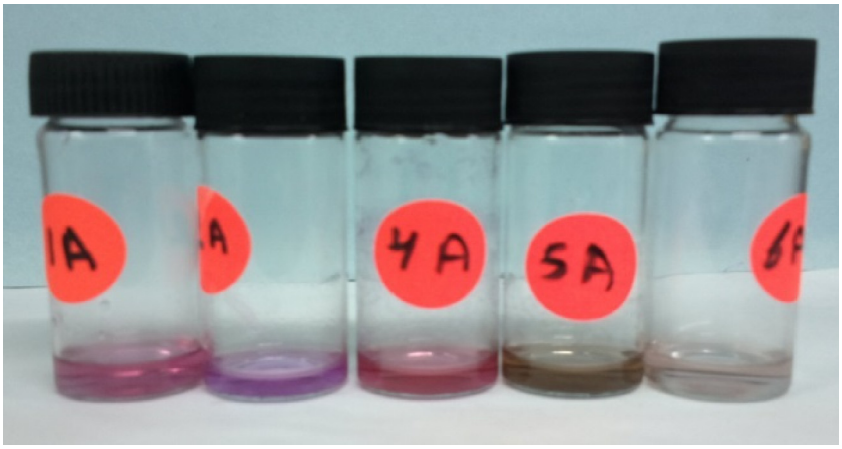

Fig. 2. Fractionation of the crude biosurfactant by silica gel chromatography.

3.2. Characterization and purification of the crude biosurfactant

3.2.1. Fourier transform infrared spectroscopy (FTIR)

The chemical functional groups of the extracted biosurfactant were investigated using FTIR. The absorbance values (Table 1) included a strong band at 3534 cm^{-1}, indicating the presence of a hydroxyl group in the chemical structure of the biosurfactant (Dusane et al., 2011). The acetyl group present in the biosurfactant provides hydrophobicity that enhances the emulsifying activity. The O–H bond vibration was indicated at wavenumber of 2929 cm^{-1}, which was associated with the C–H bond stretching vibration of the constituent sugar residues. The strong peaks present at 2969 and 2853 cm^{-1} are stretching vibrations of the C–H of the hydrocarbon chain positions. The peak sharply observed at 1678 cm^{-1} showed the presence of a carbonyl functionality in the amide moieties or carboxylate of the peptide amines and protein Chandran and Das (2010). Furthermore, the wavenumber at 1074 cm^{-1} (cyclic C–O) indicated the existence of uronic acid (Jain et al., 2012). Moreover, the band at 1452 cm^{-1} was due to the C–H bending vibration mode, indicating the presence of an aliphatic chain Anyanwu et al. (2011). These findings confirm that the extracted biosurfactant contains a peptide-like moiety and aliphatic hydrocarbons.

3.2.2. Fractionation of the crude biosurfactant by silica gel adsorption chromatography

The biosurfactant extracted from the medium of cultures of the HDB *S. marcescens* was purified by adsorption using silica gel chromatography. The bioactive fractions were concentrated, and the details of the biosurfactant structure were analyzed. As shown in Fig. 2, the components of the extracted biosurfactant were divided into four main fractions, i.e., prodigiosin, 1-tetradecyl D-glucuronate, carbohydrate, and lipid.

3.2.3. Analysis of the biosurfactant fractions by TLC

Based on the results of the TLC analysis, the extracted biosurfactant was contained a carbohydrate, 1-tetradecyl D-glucuronate and glycerophosholipids. Carbohydrates were detected by sulfuric acid, revealing the presence of sugars, which appeared as a brown zone on the TLC plate (Fig. 3a). The blue spots on the TLC plate (Fig. 3b) indicated the presence of 1-tetradecyl D-glucuronate. Lipids in the biosurfactant were detected using an iodine reagent and appeared as yellow spots on the TLC plate (Fig. 3c).

3.2.4. Liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) analyses

LC–MS and LC–MS/MS were performed on the crude biosurfactant fractions to confirm the results obtained from silica gel chromatography and TLC. The compositions of the total active fraction with different corresponding molecular weights were analyzed using LC–MS and LC–MS/MS. Biosurfactant-producing bacteria vary in nature and chemical composition regarding carbohydrates, proteins and lipids, which are responsible for the biosurfactant functions. In the present study, the compounds purified by the commercial TLC plate were further analyzed by LC–MS and LC–MS/MS. The LC–MS results showed molecular ions corresponding to the molecular formulas of the expected compounds. LC–MS spectrum of the first fraction identified a species with a molecular weight at mass-to-charge ratio (m/z) of 181.08, which corresponds to the molecular formula of glucose ($C_6H_{12}O_6$).

In addition, the LC–MS spectrum of the second purified fraction displayed a peak at m/z 803, corresponding to a complex ion formed by two ester molecules of 1-tetradecyl D-glucuronate and a sodium ion ($[2M+Na]^+ = [390 + 390 + 23]$

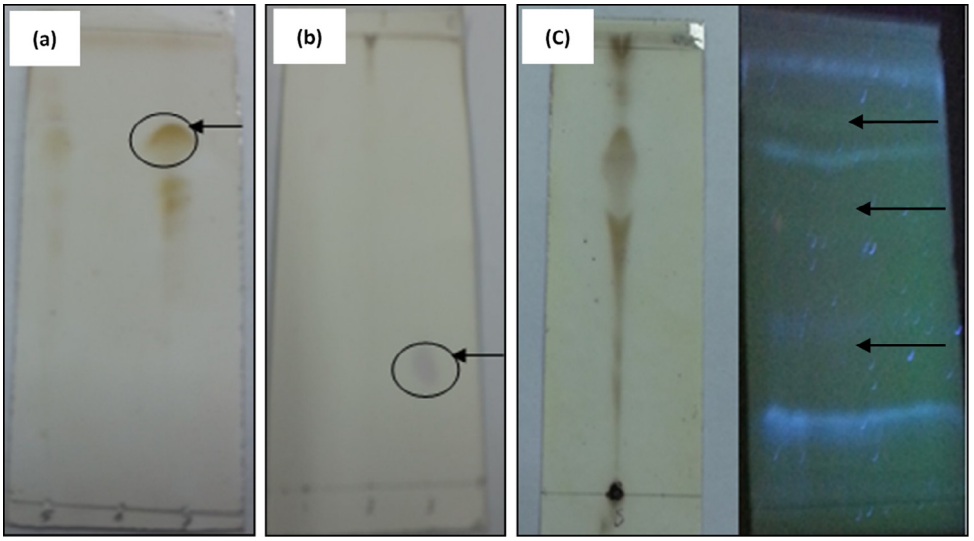

Fig. 3. Thin-layer chromatograms of HDB *S. marcescens* (a) carbohydrate, (b) 1-tetradecyl D-glucuronate and (c) glycerophosholipids . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

= 803), as shown in Fig. 4a, whereas the peak at m/z 413 corresponds to one molecule of 1-tetradecyl D-glucuronate and a sodium ion ($[M+Na]^+ = [390 + 23] = 413$). The LC–MS/MS spectrum showed that the peak at m/z 413 was compatible with the molecular formula of 1-tetradecyl D-glucuronate ($C_{20}H_{38}O_7$, 390.26) plus a sodium ion ($[M+Na]^+ = [390 + 23] = 413$). The possible structure and fragmentation are shown in Fig. 4b, as determined by LC–MS/MS. The peak at m/z 413 represents the dissociation of the compound 1-tetradecyl D-glucuronate into two major fragment ions with a base peak at m/z 301.13, which is typical of a hepta-3,6-dienyl 2,3,4,5,6-(tetrahydroxytetrahydro-2*H*-pyran-2-yl)acetate moiety ($C_{14}H_{21}O_7$), and the fragment ion at m/z 189.01 is associated with a 2,3,4,5,6-(tetrahydroxytetrahydro-2*H*-pyran-2-ylidene) acetaldehyde moiety ($C_7H_{10}O_6$), as shown in Fig. 4b. Sugar-derived fatty esters display good surface properties and non-ionic surfactant activity, including biodegradability. In the present study, the HDB *S. marcescens* produced a biosurfactant containing 1-tetradecyl-D-glucuronate, which is a potential novel class of promising biosurfactants.

Furthermore, the third fraction (lipid) in the biosurfactant showed good surface activity. The LC–MS analysis of this fraction showed three mass-to-charge peaks at m/z 685, 758 and 832, suggesting different types of lipid fractions (Fractions I, II and III) in the biosurfactant. The structures of each lipid fraction corresponded glycerophospholipids, as supported by the electrospray ionization mass spectrometry (MS/MS) and direct infusion mass spectrometry data. As shown in Fig. 5a, the first lipid fraction (I) at m/z 685.03 corresponds to the molecular formula of the glycerophospholipid 6-amino-2-(3-hydroxy-14-methylpentadecanamido)hexanoyl oxy ethyl-2-hydroxy-13-methyl tetradecanoate ($C_{39}H_{76}N_2O_7$). Other peaks detected were at m/z 429.09 and 355.09, representing the molecular formulas of $C_{23}H_{45}N_2O_5$ and $C_{19}H_{33}NO_5$, respectively.

The LC–MS/MS analysis of Fraction II is shown in Fig. 5b. The LC–MS/MS results showed a peak at m/z 758.2 that corresponds to the molecular formula of 2-octadeca-10, 12-dienoyloxy)-3-(palmitoyloxy) propyl-2-(trimethylammonia) ethylphosphate ($C_{42}H_{80}NO_8P$) plus a hydrogen ion ($[M + H]^+ = [757 + 1]$). The peaks from Fraction II at m/z 503.1, 429.09, 355.07 and 299.06 represent the molecular formulas of $C_{26}H_{50}NO_6P$, $C_{22}H_{38}O_6P$, $C_{20}H_{36}O_3P$ and $C_{20}H_{27}O_2$, respectively.

Fig. 5c shows the results of the LC–MS/MS analysis of Fraction III. The LC–MS/MS spectrum of Fraction III showed peak at m/z 832 that corresponds to the molecular formula of 1-heptadecanoyl-2-docosanoyl-glycero-3-phosphocholine ($C_{47}H_{94}NO_8P$). Another peak in the LC–MS/MS spectrum was the base peak at m/z 355.3 that represents the molecular formula of the $C_{23}H_{74}NO_2$ fragment. Other fragmentation peaks at m/z 577.1, 503.1 and 429.2 were assigned to the molecular formulas of $C_{30}H_{60}NO_7P$, $C_{25}H_{46}NO_7P$ and $C_{21}H_{34}O_7P$, respectively.

1-Tetradecyl D-glucuronate and the other lipid compounds provided the medium with surface-active properties. Matsuyama et al. (1987) identified the aminolipid serrawettin in *S. marcescens* cultures, and this lipid is a novel exolipid with a specific function. Meanwhile, the biosurfactant produced by the *S. marcescens* cultures studied by Dusane et al. (2011) consisted of glucose and palmitic acid. The findings of the present study indicated that the biosurfactant product has a structure of sugar-lipid. Therefore, the extracted biosurfactant exhibits various activities, such as solubilizing properties and detergent activity, and can be applied as an interesting and potential compound in various industries.

3.3. Application of the extracted biosurfactant in the phytoremediation of TPH

The extracted crude biosurfactant was tested as an additive in the phytoremediation of TPH using *Ludwigia octovalvis*, and the results are shown in Fig. 6. The addition of the biosurfactant increased the solubilization of gasoline in water,

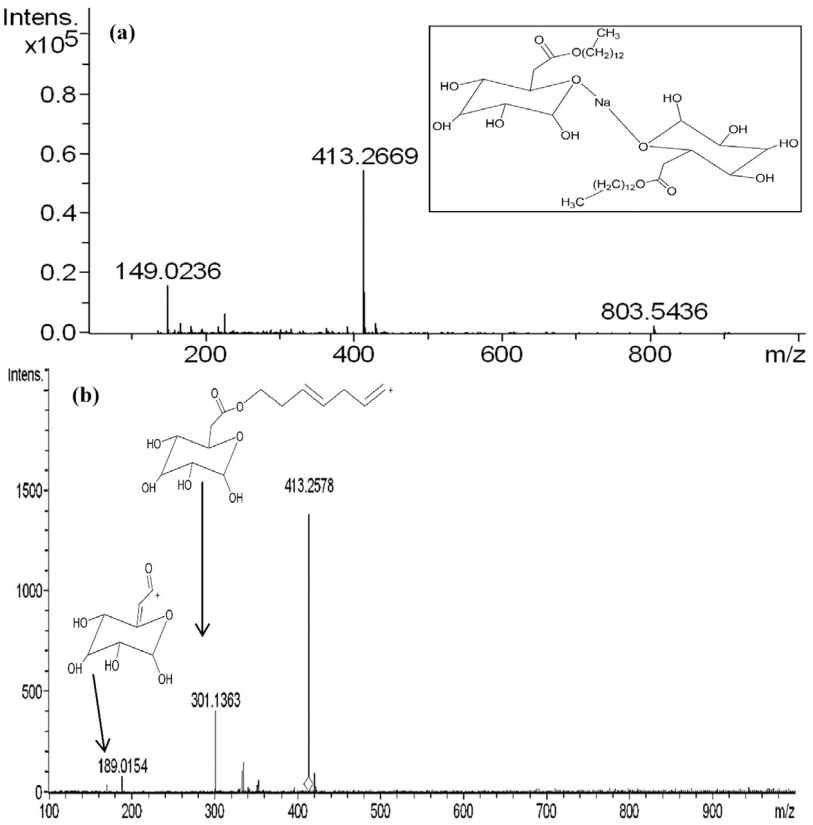

Fig. 4. (a) Positive ion mode LC–MS spectrum of 1-tetradecyl D-glucuronate and (b) positive ion mode LC–MS/MS spectrum of 1-tetradecyl D-glucuronate.

thus enhancing degradation. As shown in Fig. 6, the removal of TPH increased from 23.4% (day 7) to 93.5% (day 72). The performance of the biosurfactant in the phytoremediation application was compared with SDS, and SDS removed only 86.2% of TPH (day 72). A detailed explanation of the application of biosurfactant in phytoremediation was reported in our previous study (Almansoory et al., 2015). Liao et al. (2016) observed the removal of 58% (rhamnolipid) and 62% (soybean lecithin) of TPH after three months following the addition of biosurfactant to phytoremediation using *Zea mays. L* plants. The increased solubilization of TPH in soil by a biosurfactant improves the bioavailability and metabolism of contaminants. In addition, the low environmental toxicity of the biosurfactant favors the bioremediation application. Therefore, the potential application of the biosurfactant produced by the HDB *S. marcescens* for the phytoremediation of gasoline-contaminated soil was confirmed.

4. Conclusions

The biosurfactant produced by the HDB *S. marcescens* was extracted in the present study. A stable biosurfactant was produced at pH 7 and 70 °C that was tolerant to a high salt concentration of 3%. The thermal activity and pH tolerance make this biosurfactant aa a potential alternative to enhances the TPH removal from gasoline-contaminated soil. According to the results of the FTIR analysis, the extracted biosurfactant contains methyl, carboxyl and hydroxyl functional groups, and the presence of these groups was responsible for its anionic nature, which is useful for binding cations. The presence of glucose, 1-tetradecyl D-glucuronate and glycerophospholipids in the crude biosurfactant was confirmed by LC–MS/MS.

Acknowledgments

The authors thank the Universiti Kebangsaan Malaysia (DIP-2018-022), Tasik Chini Research Centre, Malaysia and the Ministry of Education Malaysia for providing financial support. Authors also acknowledge the Iraqi Ministry of Higher Education and Scientific Research for providing a doctoral scholarship to the first author.

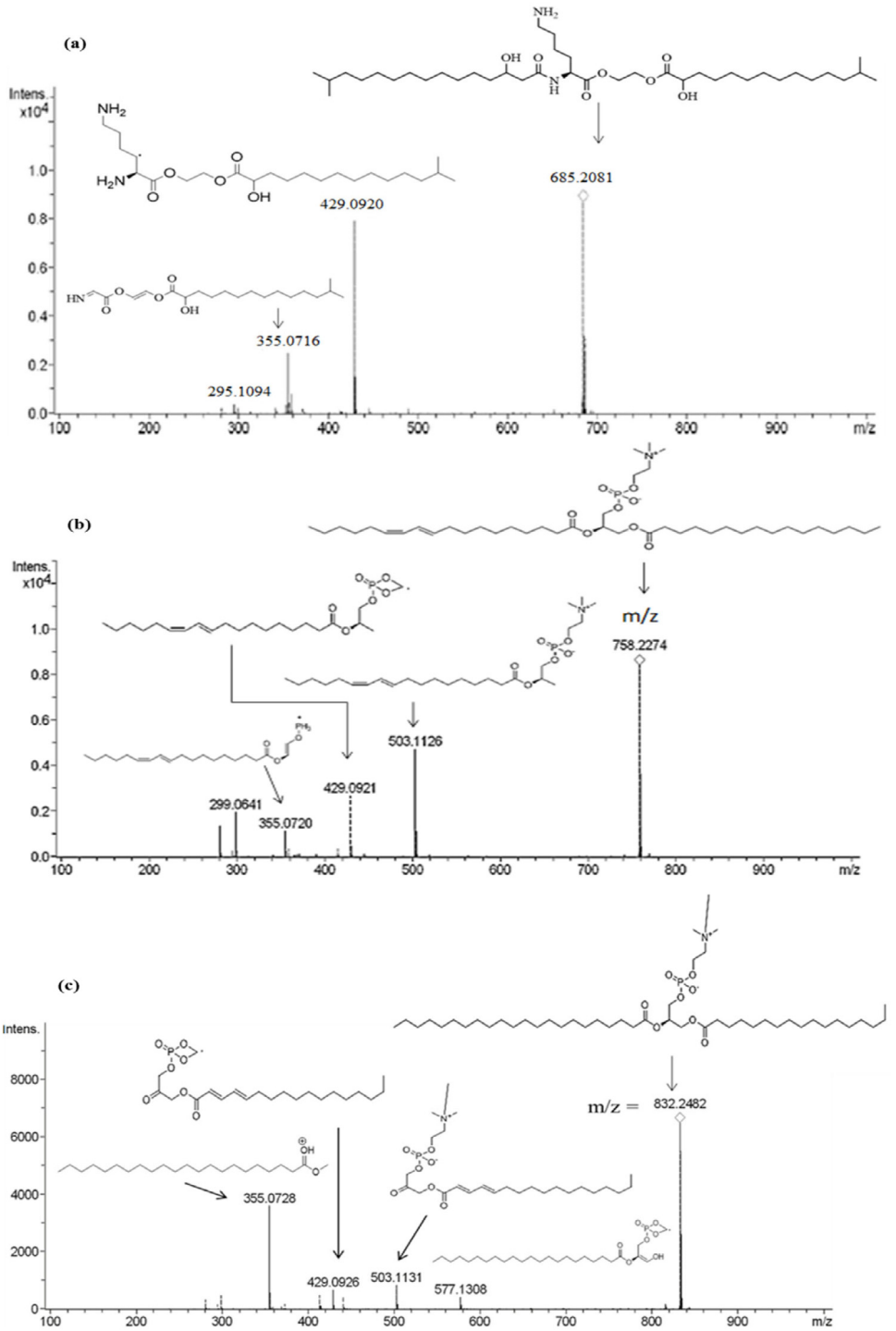

Fig. 5. Tandem MS-MS spectrum of the glycerophospholipids with masses of (a) 685 m/z, (b) 758 m/z and (c) 832 m/z.

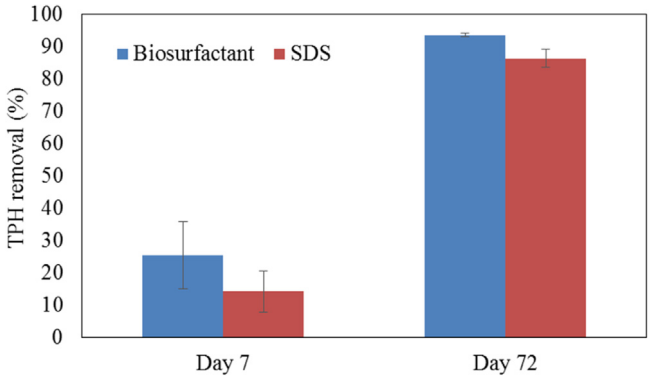

Fig. 6. Removal of TPH by phytoremediation following the addition of the biosurfactant produced by HDB compared with the commercial surfactant SDS.

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