Journal of Hazardous Materials 285 (2015) 383–388

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Effect of low-concentration rhamnolipid on adsorption of *Pseudomonas aeruginosa* ATCC 9027 on hydrophilic and hydrophobic surfaces

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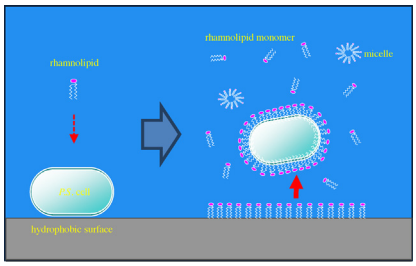
HIGHLIGHTS

- Adsorption of cells to glass beads fits Freundlich model better than Langmuir model.
- Hydrophobic interaction favors cell adsorption to glass bead surface.
- Rhamnolipid weakens bacterial adsorption by reducing cell surface hydrophobicity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 August 2014
Received in revised form 14 October 2014
Accepted 8 November 2014
Available online 5 December 2014

Keywords:
Rhamnolipid
Adsorption
Freundlich
Bacteria
Cell surface hydrophobicity

ABSTRACT

The effects of low-concentration monorhamnolipid (monoRL) on the adsorption of *Pseudomonas aeruginosa* ATCC 9027 grown on glucose or hexadecane to glass beads with hydrophobic or hydrophilic surfaces was investigated using batch adsorption experiments. Results showed that adsorption isotherms of the cells on both types of glass beads fitted the Freundlich equation better than the Langmuir equation. The K_f of the Freundlich equation for adsorption of hexadecane-grown cell to glass beads with hydrophobic surface was remarkably higher than that for adsorption of hexadecane-grown cell to glass beads with hydrophilic surface, or glucose-grown cell to glass beads with either hydrophilic or hydrophobic surface. Furthermore, it decreased with the increasing monoRL concentration. For both groups of cells, the zeta potential was close to each other and stable with the increase of monoRL concentration. The surface hydrophobicity of hexadecane-grown cells, however, was significantly higher than that of the glucose-grown cells and it decreased with the increase of monoRL concentration. The results indicate the importance of hydrophobic interaction on adsorption of bacterial cells to surfaces and monoRL plays a role in reducing the bacterial adsorption by affecting cell surface hydrophobicity.

1. Introduction

Soil and groundwater contamination by petroleum-based organic compounds [1], pesticides [2] and heavy metals [3] has become a global environmental problem, giving rise to a con-

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http://dx.doi.org/10.1016/j.jhazmat.2014.11.050
0304-3894/

cern in developing innovative and sound remediation technologies [4,5]. Biodegradation of hydrocarbons by microorganisms has been considered as an effective remediation technology for its safety, economy and high ecological compatibility. However, the adsorption of those hydrocarbon-degrading bacteria on subsurface porous media makes it difficult for these bacteria to reach the contaminated areas in in-situ bioremediation practice, for the soil can act as an efficient filter and reduce the concentration of suspended bacteria by several orders-of-magnitude within 10–100 cm of the well [6]. Successful bioremediation processes require minimal bacterial adhesion to soil particles in order to maintain significant cell transport throughout the aquifer [7].

Recently, biosurfactants with application in bioremediation of subsurface contamination have attracted increasing attention, because biosurfactants may not only enhance the apparent solubility and bioavailability of the organic contaminants [8], but also were found to reduce adsorption of bacteria to surfaces which may potentially enhance transportation of the bacteria in soil porous media [9]. Chen et al. [10] studied the function of rhamnolipid on two typical gram-positive bacterial desorption in water-saturated silica sand. They found that rhamnolipid weakened interaction between bacteria and the sand surface by decreasing the negative zeta potential of both bacterial strains and the sand, resulting in less bacteria retention. Sodagari et al. [11] examined the effects of rhamnolipid on the initial attachment of cells of three gram-negative strains and two gram-positive strains to glass and octadecyltrichlorosilane (OTS)-modified glass, and results showed that rhamnolipids significantly reduced the initial attachments of the gram-negative *Escherichia coli, Pseudomonas putida*, *Pseudomonas aeruginosa* and the gram-positive *Bacillus subtilis* on both the hydrophilic and OTS-modified hydrophobic glass surfaces. Zeraik and Nitschke studied the anti-adhesive activity of surfactin and rhamnolipid on adhesion of *Staphylococcus aureus*, *Listeria monocytogenes* and *Micrococcus luteus* to polystyrene surfaces [12], and the data showed that surfactin inhibited the adhesion of all the bacteria studied, however, the effect of rhamnolipid depended on type of bacteria. Fu et al. [13] found that the adsorption of rhamnolipid on the granular compost solids with high content of organic matter weakened the interaction between the solids and bacterial cells and reduced the adsorption.

The potential mechanism for biosurfactant to influence attachment of bacteria to abiotic surfaces in soil porous media is that biosurfactant affects the bacterial cell surface properties, the surface properties of porous media solids or the constituents dissolved in aqueous phase [14]. For example, biosurfactants are effective, even at low concentrations, in altering the cell surface zeta potential (CSZP) [4,15] and cell surface hydrophobicity [16–18]. Bai et al. [9] and Chen et al. [10] reported that zeta potentials of silica sand as the porous medium decreased with increasing rhamnolipid biosurfactant concentrations. Real soil may always contain both solids with hydrophilic surfaces such as silica sand or particles, and constituents with hydrophobic surfaces such as organic matter. For insight to the role of biosurfactant in modifying bacterial adsorption to soil porous media and to some extent makes it controllable, the examination on the influence of biosurfactant on bacterial adsorption isotherms for hydrophilic or hydrophobic surfaces and on the adsorption model parameters may give more convincing results. To our knowledge, however, such kind of researches are scarce. Another concern about application of biosurfactant is that the high cost for rhamnolipid production, which in turn results in high market price of the biosurfactant, e.g. the price of commercially available rhamnolipid ranges from approximately $100 per kilogram to $300 per gram depending on the purity. However, based on the results of our prior studies biosurfactants such as rhamnolipid may change bacterial cell surface properties at concentrations within critical micelle concentration range [16,17], thus the low-concentration biosurfactant could be potentially effective in affecting cell adsorption to surfaces. Taking advantage of this feature of biosurfactants is of potential importance for economic application of them in bioremediation of hydrocarbon-contaminated soil.

In this study, adsorption behavior of *P. aeruginosa* ATCC 9027 cells to silica glass beads with different surface hydrophobicities in the presence of monoRL biosurfactant with concentration close to critical micelle concentration (CMC) was investigated. The Langmuir and the Freundlich adsorption equations were evaluated and compared in characterizing the adsorption.

2. Materials and methods

2.1. Microorganisms, biosurfactant and chemicals

The gram-negative strain *P. aeruginosa* ATCC 9027 used in this study was obtained from the American Type Culture Collection (Rockville, Md.). The bacterium was maintained on 4 °C peptone agar slants, and transferred every month until use.

Cell concentration was monitored by measuring the absorbance of the cell suspension at the wavelength of 600 nm (OD_{600}) using an ultra-variable wavelength UV-2550 spectrometer (Shimadzu, Japan). The linear correlation between cell suspension OD_{600} and cell concentration is $C = 1.65 \times OD_{600} \times 10^8$ CFU/ml obtained by testing colony formation of diluted cell suspension on agar plate. In the following experiments, OD_{600} of cell suspension was measured to obtain concentration of the cells.

The pure monoRL biosurfactant (99%) was purchased from Huzhou Zijin Biotechnology Co., Ltd. (Zhejiang, China). The analyses of monoRL components were performed by high-performance liquid chromatography–mass spectroscopy (HPLC–MS) (Agilent Technologies, 6460 Triple Quad LC/MS). A modified method described by Zhong et al. [17] was used. Briefly, 0.1 mg/mL monoRL was prepared in CH_3CN solution and filtered through a 0.22 μm membrane (Millipore, Billerica, MA, USA). 5 μL sample was introduced into the Agilent 1290HPLC using an acetonitrile/water (v/v) gradient phase (40:60, for 5 min; from 40:60 to 90:10, for 35 min; 90:10, for 10 min) at the flow rate of 0.2 mL/min on a Zorbax Eclipse C18 (2.1 × 50 mm, 1.8 μm) column. The eluate of HPLC was directly introduced into the mass spectrometer. Negative-ion mode was employed and scanning was done at 100–1000 *m*/*z* range. The relative mole abundance of each species in the rhamnolipid was calculated by assuming that the molar ratio of the species is equal to the ratio of peak area of the species in the HPCL–MS chromatograph.

To obtain the adsorbents with homogeneous hydrophobic or hydrophilic surfaces, glass beads ($\Phi = 0.2 \sim 0.4$ mm) were utilized and prepared by the method described by Yuan et al. [19]. Hexadecane was obtained from Sigma–Aldrich (Germany). All the other chemicals were obtained from Sinoharm Chemical Reagent Co., Ltd. (Shanghai, China) and used as received.

Table 1
Structure and relative abundances of rhamnolipid homologues.

Congener[a]	Molecular weight	Pseudomolecular ion (*m*/*z*)	Relative molar abundance (%)
Rha–C_{10}–C_8	476	475.3	7.8
Rha–C_{10}–$C_{10:1}$	502	501.3	0.9
Rha–C_{10}–C_{10}	504	503.3	75.5
Rha–C_{10}–$C_{12:1}$	530	529.4	3.1
Rha–C_{10}–C_{12}	532	531.4	12.7

[a] The abbreviation Rha–C*x*–C*y*:*z*) designates the individual component with *x* and *y* as the carbon atom number of each aliphatic chain in the lipid moieties, and *z* as the number of unsaturated bonds in lipid moieties.

2.2. Preparation of cells for adsorption

P. aeruginosa ATCC 9027 inoculum from peptone agar slant were transferred to 50 mL mineral salt medium (MSM) with 0.5 g/L yeast extract in a 250 mL Erlenmeyer flask and enriched at 37 °C on a gyratory shaker at 200 rpm for 24 h. Then 5 mL of the enriched cell suspension was further transferred to 100 mL MSM in 500 mL Erlenmeyer flask containing 20 g/L glucose or 20 g/L hexadecane as the carbon source. This culture was incubated at 37 °C and 200 rpm on a gyratory shaker. Cells were harvested after growing to stationary phase because in this stage the physiological status of cells is comparatively stable. The growth period is 36 h with glucose or 28 d with hexadecane according to the growth curves of the bacterium on these two substrates (Fig. S1). Composition of MSM was as follows: NH_4Cl (5.0 g/L), $MgSO_4.7H_2O$ (0.5 g/L), Na_2HPO_4 (5.0 g/L), K_2HPO_4 (2.5 g/L), and pH adjusted to 6.8. The cells were collected by centrifugation at 4000 × *g* for 10 min. The precipitated cells were washed twice with the sterilized artificial groundwater (AGW) (ingredients per liter: 0.006 g NaCl, 0.012 g $CaSO_4$, 0.012 g $NaHCO_3$, 0.002 g KNO_3, 0.035 g $MgSO_4{\cdot}7H_2O$ [9]), re-suspended in the AGW solution with rhamnolipid of different concentrations (0, 20, 40 or 80 mg/L). After 1 h, the cell suspension was used for cell surface Zeta potential (CSZP) and cell surface hydrophobicity measurement, and for cell adsorption experiment. CSZP were measured with a ZEN 3600 Zetasizer Nano (Malvern Instruments, Malvern, UK). The cell suspensions in AGW with OD of 0.6 at 400 nm were loaded to the DTS1060 folded capillary cell, and the electrophoretic mobilities of the cells were measured at 30 °C under automatic voltage using a laser doppler velocimetry with M3-PALS technique to avoid electroosimosis [4,17]. The cell surface hydrophobicity with AGW as the background solution was measured by the bacterial-adhesion-to-hydrocarbon (BATH) method as described by Zhong et al. [17].

2.3. Rhamnolipid-mediated adsorption of cells on glass bead **s**

One hour is long enough for the adsorption of rhamnolipid on the cells to reach adsorption equilibrium [19], which guaranteed full interaction between rhamnolipid and cells. Then every 6 mL of the cell suspension was added to 25 mL Erlenmeyer flasks with 2 g of glass beads each. The flasks were incubated at 30 °C, 100 rpm on a reciprocal water-bath shaker for 1.5 h. Time-course adsorption test showed that the adsorption of glucose-fed cells on hydrophobic glass beads reached adsorption equilibrium within 1.5 h (Fig. S2). Blanks were also run to determine the adsorption quantity of bacteria on the flask walls. 3 ml of cell suspension was then removed for optical density measurement. The cell quantity adsorbed on glass beads was calculated by the differences of concentrations of cells in the bulk solution before and after adsorption and corrected by blank adsorption data. The adsorption tests were run in triplicate and data were processed with ORIGIN (version 8.0) for Windows (Origin Lab USA).

3. Results and discussion

3.1. HPLC–MS characterization of the monoRL

An HPLC–MS chromatogram of the monoRL showed that five monoRL fractions isolated at 5.649–6.424, 8.592–8.920, 11.126–11.864, 15.456–15.926 and 18.363–18.832 min, yielding a mass spectrum with the most intense signals at *m*/*z* 475.3, 501.3, 503.3, 529.4 and 531.4 $[M–H]^-$, respectively (Fig. S3). Based on the known molecule structures of rhamnolipid family, the monoRL species were further determined to be Rha–C_{10}–C_8, Rha–C_{10}–$C_{10:1}$, Rha–C_{10}–C_{10}, Rha–C_{10}–$C_{12:1}$, Rha–C_{10}–C_{12} (Table 1). This result is similar to that of our previous HPLC analysis on monoRL constitutes

Table 2
Regressed Langmuir constants for *P. aeruginosa* ATCC 9027 on glass beads.

	Glucose-fed *P.aeruginosa*						Hexadecane-fed *P. aeruginosa*					
C^a	Hydrophilic surface			Hydrophobic surface			Hydrophilic surface			Hydrophobic surface		
	q_{max}	b	R^2	q_{max}	b	R^2	q_{max}	b	R^2	q_{max}	b	R^2
0	2.58	0.27	0.44	1.33	1.17	0.73	1.76	0.87	0.79	9.36	0.85	0.52
20	1.75	1.18	0.53	2.45	0.51	0.78	2.31	1.53	0.92	3.20	4.83	0.99
40	8.64	0.11	0.21	1.36	0.72	0.46	1.01	3.97	0.9	2.74	3.56	0.91
80	1.54	1.65	0.89	2.08	2.45	0.97	1.37	6.74	0.98	1.68	2.66	0.86

[a] *C*, the monoRL concentration, mg/L.

Table 3
Regressed Freundlich constants for *P. aeruginosa* ATCC 9027 on glass beads.

	Glucose-fed *P. aeruginosa*						Hexadecane-fed *P. aeruginosa*					
C^a	Hydrophilic surface			Hydrophobic surface			Hydrophilic surface			Hydrophobic surface		
	K_f	$1/n$	R^2	K_f	$1/n$	R^2	K_f	$1/n$	R^2	K_f	$1/n$	R^2
0	1.41	0.88	0.97	1.46	0.57	0.96	1.15	0.57	0.94	5.24	0.86	0.97
20	1.07	0.76	0.88	1.18	0.83	0.99	1.52	0.67	0.97	3.78	0.6	0.99
40	1.15	0.96	0.99	1.71	0.76	0.93	1.18	0.35	0.91	2.69	0.6	0.99
80	1.01	0.6	0.98	1.56	0.54	0.99	1.33	0.41	0.92	1.32	0.51	0.97

[a] *C*, the monoRL concentration, mg/L.

Table 4
Surface properties of *P.aeruginosa* ATCC 9027 for adsorption with the presence of rhamnolipid.

	Glucose-fed *P. aeruginosa*		Hexadecane-fed *P. aeruginosa*	
C^a	Zeta potential(mV)	BATH	Zeta potential(mV)	BATH
0	−22.1	0.294	−21.9	0.608
20	−23.6	0.231	−21.6	0.544
40	−23.2	0.103	−22.1	0.528
80	−25.7	0.287	−22.9	0.489

[a] *C*, the monoRL concentration, mg/L.

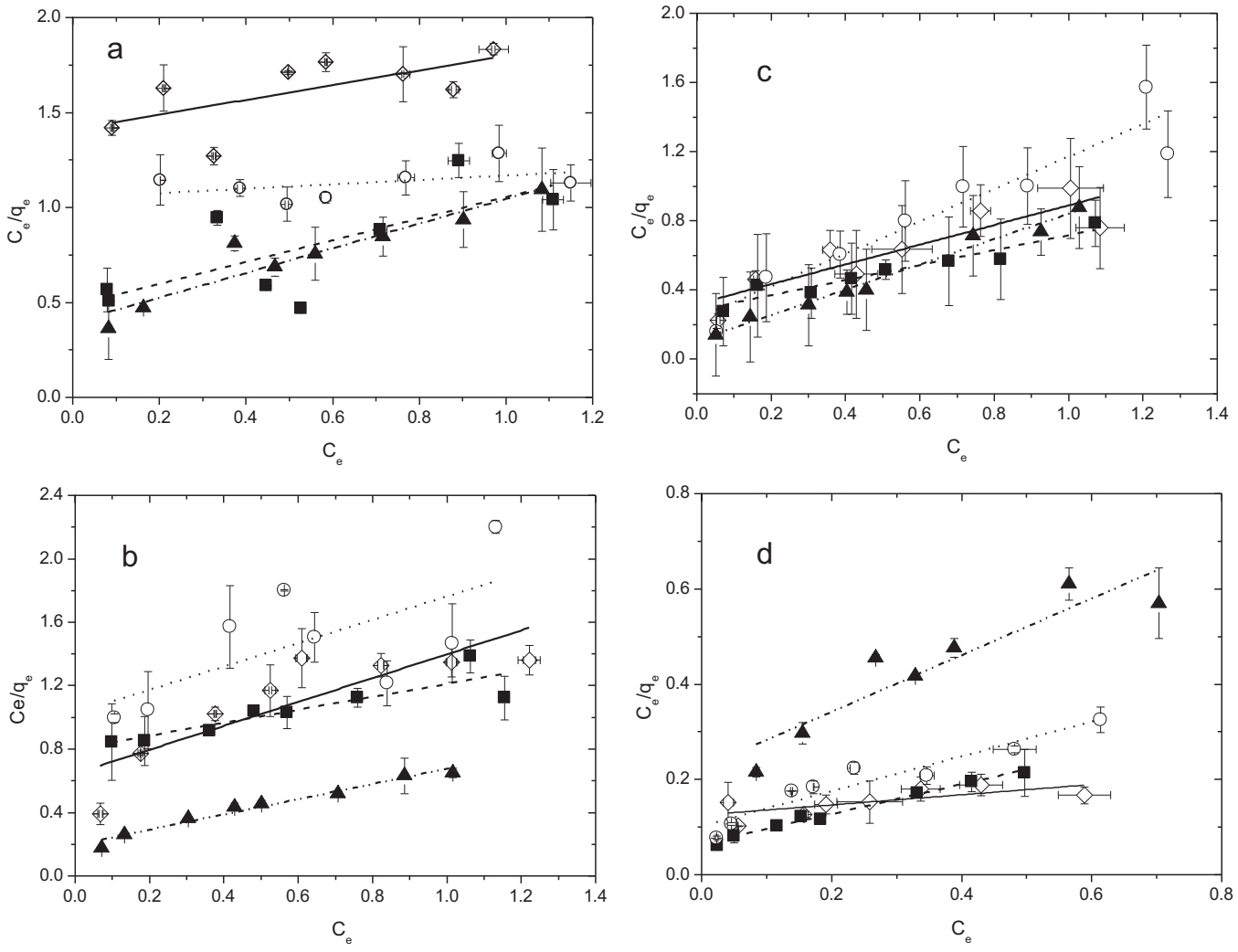

Fig. 1. Langmuir adsorption isotherms of *P. aeruginosa* cells on glass beads in the presence of monoRL at concentrations of 0 mg/L (opened diamonds), 20 mg/L (filled squares), 40 mg/L (opened circles), and 80 mg/L (filled triangles). (A) Glucose-grown cells and hydrophilic surface; (B) Glucose-grown cells and hydrophobic surface; (C) Hexadecane-grown cells and hydrophilic surface; (D) Hexadecane-grown cells and hydrophobic surface. Symbols and error bars represent mean ± SD of triplicate experiments.

produced by *P. aeruginosa* [16] and showed that Rha–C_{10}–C_{10} with the relative molar abundance of 75.5% was the major component in the monoRL used.

3.2. Adsorption isotherms

Langmuir and Freundlich equations were used to characterize the adsorption thermodynamics. Comparison of parameters in the equations obtained at varied rhamnolipid concentrations was assumed to provide information regarding the role of rhamnolipid played in cell adsorption. The Langmuir adsorption equation is:

$$q_e = q_{max}\frac{bC_e}{1+bC_e} \quad (1)$$

Or

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{b \times q_{max}} \quad (2)$$

where C_e (10^8 CFU/mL) is equilibrium cell concentration in bulk cell suspension, q_e (10^8 CFU/g) is equilibrium adsorption quantity of cells on glass beads, q_{max} (10^8 CFU/g) is the maximum adsorption capacity, and b (mL/10^8 CFU) is the correlated characteristic constant.

The Freundlich adsorption equation is:

$$q_e = K_f C_e^{1/n} \quad (3)$$

Or

$$\ln q_e = \ln K_f + \frac{1}{n}\ln C_e \quad (4)$$

where K_f [$mL^{1/n}(10^8$ CFU$)^{1-1/n}$/g] and $1/n$ are constants [15,20]. q_e was obtained using the equation:

$$q_e = \frac{C_0 - C_e}{W} \quad (5)$$

where W (g/mL) is the quantity of the adsorbents in per unit volume of aqueous solution.

Eqs. (2) and (4) were used to fit the experimental data. The adsorption isotherms are showed in Fig. 1 and Fig. 2, respectively. The retrieved adsorption equation parameters are showed in Table 2 and Table 3, respectively. Adsorptions of *P. aeruginosa* ATCC 9027 on glass beads fit Freundlich equation better, as indicated by the higher correlation coefficients R^2 (Tables 2 and 3).

The Langmuir model is based on the assumptions including (i) adsorbates form a mono layer on adsorbent surfaces containing finite number of identical sorption sites; (ii) adsorption site and adsorbate hold a one-to-one rule of interaction; (iii) dimension of

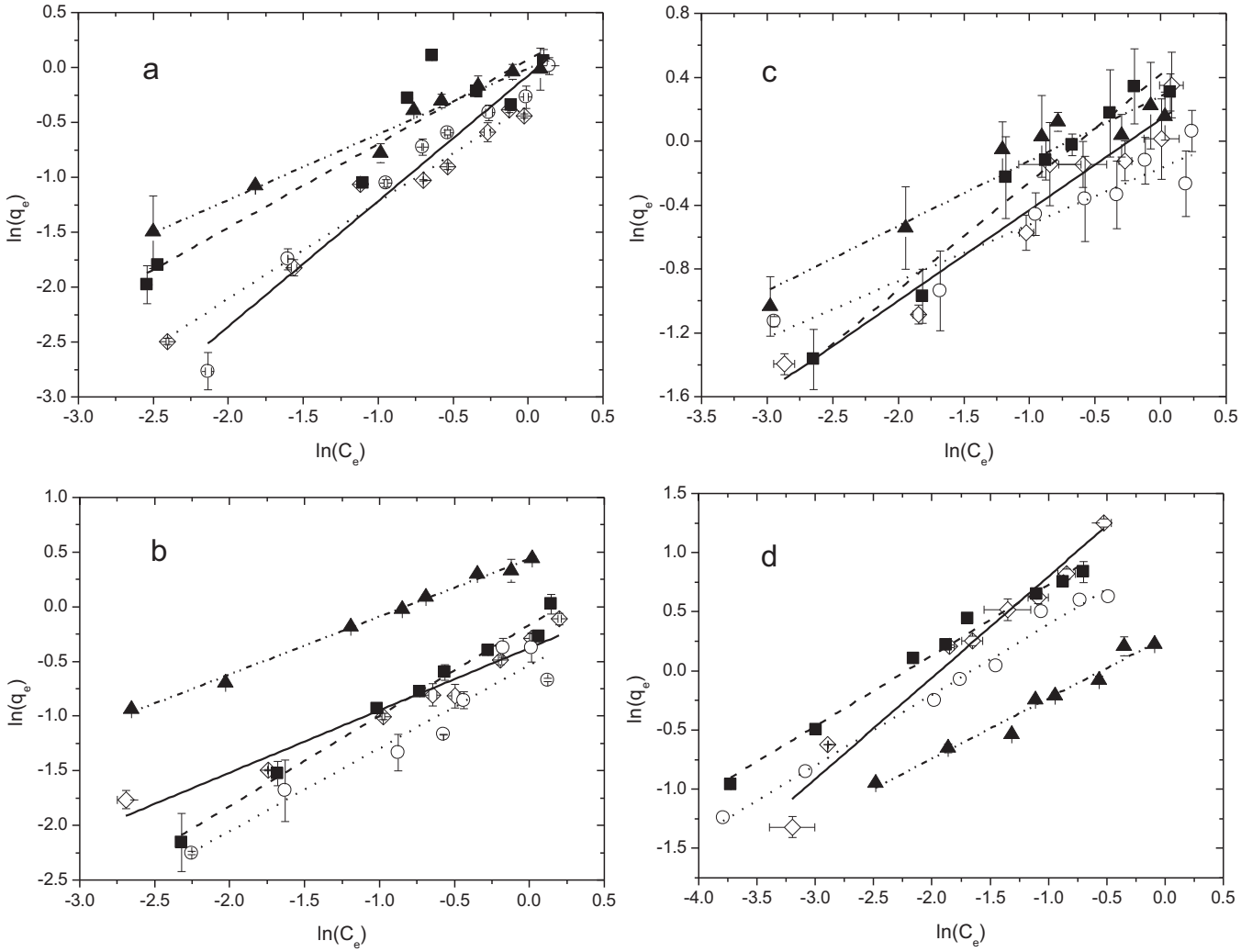

Fig. 2. Freundlich adsorption isotherms of *P. aeruginosa* cells on two kinds of glass beads in the presence of different monoRL concentrations, 0 mg/L (opened diamonds), 20 mg/L (filled squares), 40 mg/L (opened circles), 80 mg/L (filled triangles). (A) Glucose-grown cells and hydrophilic surface; (B) Glucose-grown cells and hydrophobic surface; (C) Hexadecane-grown cells and hydrophilic surface; (D) Hexadecane-grown cells and hydrophobic surface. Symbols and error bars represent mean ± SD of triplicate experiments.

the site-to-site distance is large compared to the size of adsorbate and interaction between adsorbed adsorbates is negligible [21,22]. The Freundlich equation is purely empirical based on the assumption of a heterogeneous surface [23] and it is not restricted to the monolayer adsorption [21,24]. The bacterial cells are large in size and the interaction between adsorbed cells may not be negligible. Thus, the Langmuir sorption assumption may not be valid for bacterial cells, and the Langmuir model is not as good as the Freundlich one in describing the cell adsorption.

In Freundlich adsorption equation, K_f is related to both the intensity of adsorption interaction and the relative adsorption capacity of adsorbent for the adsorbate. $1/n$ is related to the adsorption intensity. Based on $1/n$ the adsorption isotherm can be classified into S-, L-, and C-type. When $1/n < 1$, the adsorption isotherm is the L-type, suggesting a relatively high affinity between the adsorbate and adsorbent. When $1/n = 1$, the adsorption isotherm is C-type or linear, suggesting moderate adsorption force and the adsorption sites are uniform. When $1/n > 1$, the adsorption isotherm is S-type, suggesting physical adsorption and the adsorbate–adsorbate interaction is stronger than the adsorbate–adsorbent interaction [8,19,25,26].

As shown in Table 3, for all the cell and glass bead combinations, $1/n$ is lower than 1, indicating a relatively strong affinity between cells and glass bead surface under experiment conditions [27]. K_f values of the adsorption of glucose-grown cells to hydrophilic or hydrophobic surface, or hexadecane-grown cells to hydrophilic surface, were close (1.01–1.71) and relatively stable with increasing rhamnolipid concentration (Table 3). The K_f for adsorption of hexadecane-grown cell to glass beads with hydrophobic surface, however, was much larger than that for any other combinations of cell and surface type (Table 3), indicating even stronger attraction between hexadecane-grown cells and the hydrophobic surface. Both K_f and $1/n$ (K_f in particular) for adsorption of hexadecane-grown cells on the hydrophobic surface decreased as rhamnolipid concentration increased, showing that monoRL reduced both the intensity and the capacity of adsorption.

3.3. Cell surface properties

As the two factors that may play a crucial role in bacterial adhesion to a solid surface [28], zeta potential and surface hydrophobicity of the cells were examined. The zeta potential of glucose-grown cells and hexadecane-grown cells were close around −22 mV (Table 4). Bacterial cells exhibit negative zeta potential, due to the presence of anionic surface groups such as carboxyl and phosphate on cell surface [19]. For both groups of cells,

the zeta potentials were stable with the increase of rhamnolipid concentration. This result was consistent with the results of our prior study that the zeta potential of another *P. aeruginosa* strain, CCTCC AB93072, was inert to dirhamnolipid adsorption and ranged from −20 to −25 mV [17]. This consistency further confirmed that rhamnolipid does not have significant effect on *P. aeruginosa* cell surface charge. The surface hydrophobicity of hexadecane-grown cells was significantly higher than that of the glucose-grown cells. For hexadecane-grown cells, the surface hydrophobicity decreased with the increase of monoRL concentration from 0 to 80 mg/L (Table 4).

The results of cell zeta potential and cell surface hydrophobicity indicate that rhamnolipid plays a role in the adsorption by affecting cell surface hydrophobicity. Hydrophobic interaction facilitates the elimination of water present between the interaction surfaces and favors the close approach of microbial cells to solid surface [12,29]. Therefore, when both the cell surface and the solid surface are hydrophobic, the adsorption could be stronger, which is the case for adsorption of hexadecane-grown cells to glass beads with hydrophobic surface in this study. Rhamnolipid reduced the surface hydrophobicity of hexadecane-grown cells probably due to orientation preference of rhamnolipid molecule on the cell surface with polar moiety exposed to bulk solution [17]. Adsorption of rhamnolipid molecule on glass bead with hydrophobic surface may also reduce the hydrophobicity of the surface. As a result the rhamnolipid weakened the hydrophobic interaction and hence the cell adsorption to the surface.

4. Conclusion

A novel method by comparing between the parameters in adsorption equations was used to illuminate how low-concentration rhamnolipid affects *P. aeruginosa* cells to interfaces. The importance of hydrophobicity interaction on the adsorption was demonstrated by the remarkably large K_f in the Freundlich equation for adsorption of cell with high cell hydrophobicity to glass beads with hydrophobic surface. Application of monorhamnolipid resulted in decrease of the K_f and $1/n$ in Freundlich equation, and at the same time decrease of cell surface hydrophobicity. Such a correlation indicates monorhamnolpid could reduce bacterial adsorption to interface by affecting cell surface hydrophobicity, even at a concentration less than 100 mg/L. This study adds insight to understanding the mechanism of rhamnolipid affecting bacterial adsorption on silica porous media, which is of importance for economic application of this biosurfactant in bioremediation of hydrocarbon-contaminated soils.

Acknowledgements

This project was supported by grants from the National Natural Science Foundation of China (No. 51378192, 50908081, 51378190, 51039001 and 51308200).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2014.11.050.

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