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A Systematic Comparison of Biosurfactant Effects on Physicochemical Properties and Growth Rates of *P. aeruginosa* MM1011 and TMU56: A Bioremediation Perspective

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Abstract: The main objective of this research was to focus on enhancing the substrate uptake rate of *P. aeruginosa* using various biosurfactants as well as carbon sources in the medium culture. While hexadecane and Polychlorinated Biphenyls (PCBs) were chosen as hydrophobic carbon sources, the effects of glucose on two strains of *P. aeruginosa,* MM1011 and TMU56, were evaluated. Two kinds of biosurfactants, including surfactin and rhamnolipid at higher and lower than the critical micelle concentrations were added into the medium. After that, the response of bacterium based on cell surface hydrophobicity (CSH) was measured through the BATH assay. General full factorial technique was used to organize the experiments and analyze the effects of input factors on CSH. Although the both strains showed similar growth trend under conditions of different carbon sources, the order of affinity between the various substrates and the specific growth rates was as PCBs> glucose> nutrient broth> hexadecane. The analysis of variance showed that both type of carbon source and the biosurfactant had a significant effect on the CSH of *P. aeruginosa* TMU56. However, the *P. aeruginosa* MM1011 strain had no meaningful reaction in the presence of biosurfactant. High value of coefficient of determination (R^2=0.95) indicated a good agreement between experimental data and predicted values by models. Moreover, SDS-PAGE analysis demonstrated that the variation in hydrophobicity was a result of fluctuation in the amount of major proteins on the bacteria cell wall. The significant effect of biosurfactant on the *P. aeruginosa* TMU56 at concentration under critical micelle point was related to the release of more outer membrane proteins (OMPs).

Keywords: Biosurfactant, Cell Surface Hydrophobicity, Factorial Design, Statistical Analysis

1. Introduction

Petroleum oil polluted soil and water have been considered as crucial environmental challenges, and the pollutants scattered in a vast scale escalates this problem [1]. Bioremediation processes have recently received widespread attention in biological waste treatment to address the devastating consequences [2, 3]. Thus, there is press need to conduct many researches in this area [4, 5]. One of the efficient ways to get high degree of remediation is identifying potential breeds of microorganism for especial pollutant and then, evaluating their feasibility in treatment processes [4, 6]. Moreover, improving the process performance by adding external biochemical substances to pollution treating environment is another promising alternative. From this point of view, biosurfactants have many industrial applications, particularly in environmental area [4].

Biosurfactants are the main group of components that can

meet this demand to some extent by changing the cell surface characteristic, permeability, and hydrophobicity of bacterium membrane [7, 8]. Therefore, biosurfactants have been recognized as potential remedy to enhance biodegradation of hydrocarbons. The low amount of bioavailable hydrocarbons and hydrophobic compounds for microorganisms is main controlling factor to biodegrade the oily contamination in the environment [9, 10]. Biosurfactant improves cell surface hydrophobicity (CSH) so that hydrocarbon droplets can contact the cell directly. Several benefits are enumerated for biosurfactant including biodegradability, low toxicity, and structural diversity [9]. A study performed by Kaczorek *et al*. showed that rhamnolipids can cause a decrease of hydrophobicity in liquid cultures compared with saponins; however, adding diesel oil results in the dramatic rise of the cell surface hydrophobicity [11]. In another research by the same authors, the effect of Glucopon 215 was evaluated on *Pseudomonas stutzeri* cell surface properties [12]. Results showed that CSH depends on the kind of surfactant and its concentration; however, the trend was upward in presence of diesel oil as well as surface-active agent in liquid culture. In addition, Sotirova *et al*. [13] studied the effect of rhamnolipid on the cell surface of *P. aeruginosa* NBIMCC 1390. They found that the rhamnolipid, at concentration above critical micelle concentration (CMC), caused a decrease in the lipo-polysaccharide (LPS) content and at concentration below CMC, changed the OMP composition. Some studies revealed that mutations or exposing of gram-negative bacteria to chemical agents modify bacterial outer membrane [14-16]. Cell surface characteristic depends on the unique structure of the cell surface, and based on the criterion, bacteria has been divided into two main groups, gram-negative and gram-positive. While peptidoglycan constitutes the great segment of the cell wall in gram-positive bacteria, just a thin peptidoglycan layer is involved in the cell wall in gram-negative strains [17]. In addition to peptidoglycan layer, cell wall of gram-negative bacteria such as *P. aeruginosa* contains an asymmetric bilayer structure named outer membrane. Porins and efflux pump are embedded within the outer membrane. Particularly, in *P. aeruginosa*, four major (OprF, OprP, OprB, OprD) and two minor (OprC, OprE) outer membrane proteins (OMPs) can perform porin role [17].

Despite several publications about enhancing CSH, there is no systematic research indicating insight into the process along with its main, interaction effects, and relative importance on the target factors. Therefore, to address the current drawback, part of the work is allocated to design of experiment technique. Statistical analysis is a pertinent option where an accurate evaluation of the impact of each operating parameter on target factor is concern [18]. To exploit an approximation model that can consists interactions among the input variables as well as each main factors, a full factorial approach can be helpful to consider all probable combinations. This technique clearly showed its potential in modelling of complex systems over conventional methods such as one factor at a time [19, 20].

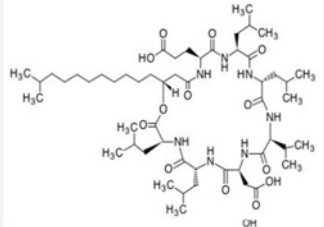
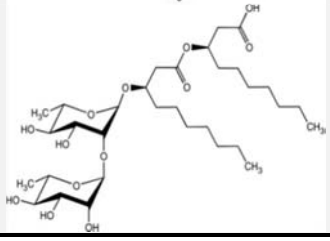
In this research, the effects of surfactin-biosurfactant and rhamnolipid-biosurfactant on the cell surface of *P. aeruginosa* MM1011 (native microorganism) and *P. aeruginosa* TMU56 were studied at two levels. The CSH % and the OMP profiles of microorganisms were evaluated during the growth period where two types of hydrophobic (hexadecane and PCBs) and hydrophilic (glucose) substrates were used. For the first time, CSH % of native microorganisms such as *P. aeruginosa* MM1011 was measured in the presence of biosurfactants for bioremediation application. SDS-PAGE analysis was employed to indicate the majority of the proteins on outer membrane. By using general full factorial design along with analysis of variance (ANOVA), the process performance and effects of main variables were examined.

2. Materials and Methods

2.1. Biosurfactants

P. aeruginosa MM1011 and *Bacillus subtilis* ATCC 21332 produced the applied rhamnolipid and surfactin, respectively. Purification of the biosurfactants was caried out by magnetic nano-particles, which were synthesized at Department of Life Science Engineering, University of Tehran. At the first stage, to extract the crude rhamnolipid from *P. aeruginosa* MM1011, the produced supernatant was centrifuged to separate the cellular remnant from the solution. At the second stage, the pH was set in 2.0 by using HCl, then, the solution was centrifuged again. However, in this time, to have an efficient separation a chloroform/ethanol solution with the ratio of 2:1 was used. The organic solvent was evaporated and a yellowish viscose substance obtained. To form the yellowish powder, the product was dried by using freeze dryer, and finally it was purified by nano-magnetite materials. The produced surfactin by *Bacillus subtilis* ATCC 663 was purified to 50%, and then added to the medium culture. The CMC of each biosurfactant was determined by measuring the surface tension of solution at several concentrations and drawing the corresponding graph [21]. The chemical structures and characteristics of the both biosurfactants are presented in Table 1.

Table 1. *Characteristics of surfactin and rhamnolipid biosurfactants.*

Characteristics	Chemical structure	Molar mass
Surfactin		1036.3
Rhamnolipid		650.8

The CMC for surfactin was measured as 23 mg/L, whereas this value for rhamnolipid was calculated to be 31 mg/L. 20 mg/L was considered as below the CMC for the both biosurfactants, and the above points for surfactin and rhamnolipid were set at 30 and 40 mg/L, respectively.

2.2. Microorganisms and Growth Conditions

Two *P. aeruginosa* strains, including *P. aeruginosa* MM1011 and *P. aeruginosa* TMU56, used in this study were isolated from Iranian oil wells. The both strains were maintained at 4°C on nutrient agar (Merck) slants and transferred monthly. They were grown in a mineral salts medium (MSM) containing 0.6% (w/v) carbon source (glucose, hexadecane, or PCBs), 5 g yeast extract, 3.4 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 4.4 g K_2HPO_4, 1.1 g NaCl, 1.1 g KCl, and 15 g $NaNO_3$ in 1 L of distilled water. The pH of the culture was set at 7.2 and the trace elements solution was consisted of 0.29 g $ZnSO_4.7H_2O$, 0.24 g $CaCl_2.2H_2O$, 0.25 g $CuSO_4.7H_2O$, 0.17 g $MnSO_4.7H_2O$, and 0.00028 g $FeSO_4.7H_2O$ in 1 L of the solution; 5 mL of it was added to the 1 L of medium. All the chemicals used in this study were in analytical grade and purchased from Merck company. To make inoculum, the cells which had been grown in the nutrient agar slant were added to the nutrient broth flask. Then, the agitation rate of cultivated medium was set at 200 rpm and the temperature was kept at its optimum degree to meet the maximum growth rate of each strain (37°C for the *P. aeruginosa* MM1011 and 30°C for the *P. aeruginosa* TMU56) for 17 h. The inoculum was washed twice and then, suspended in MSM with 0.6% carbon source. The 25 mL experimental cultures were inoculated in 250 mL flasks with 2% (v/v) of inoculum. Experimental cultures were grown to late exponential phase. When MSM was amended with 0.6% glucose, hexadecane, and PCBs, the calculated times to get the stationary phase were 10, 25, and 7 hours for the *P. aeruginosa* MM1011 and 7, 6, and 6 hours for the *P. aeruginosa* TMU56, respectively. The situations of growth environment were set in accordance with the inoculum, and the microorganism growth was traced by determining the maximum medium absorption at 610 and 615 nm for the *P. aeruginosa* MM1011 and *P. aeruginosa* TMU56, respectively.

2.3. Assay of CSH and OMPs

The CSH of bacteria was determined by bacterial adherence to hexadecane (BATH-assay) [17]. The samples were withdrawn at different growth stages to determine the relative hydrophobicity of the cells. Since BATH-assay does not indicate the exact amount of CSH, the results are comparative and can be used to compare CSH under various conditions. According to the protocol of BATH-assay, the cells were washed twice with PUM buffer at pH 7.1 (22.2 g $K_2HPO_4.3H_2O$, 7.26 g KH_2PO_4, 1.8 g urea, 0.2 g $MgSO_4.7H_2O$, and 1000 mL distilled water). Then, the cells were re-suspended in the PUM buffer in order to measure the initial absorbance of the cell suspension at 610 nm (for *P. aeruginosa* MM1011) and 615 nm (for *P. aeruginosa* TMU56). Hexadecane (0.2 mL) was added to cell suspension (1.2 mL) in a test tube, mixed for 2 min, and allowed to equilibrate and separate for 1 hour. Afterwards, the optical density (OD) of the medium was recorded. The factor of hydrophobicity, which is a criterion to show the percentage of the cells adhered to the hydrocarbon was expressed based on Eq. 1 as follows:

$$\text{Hydrophobicity}=(1-\frac{\text{OD of the aqueous phase}}{\text{OD of the initial cell suspension}})\times 100 \quad (1)$$

While experimental cultures were grown to late exponential phase, the cells were collected, centrifuged at 7000 g for 10 min at 4°C and then, suspended in 50 mM Tris-HCl (pH 8.0). Lysis buffer was added to the pellet to analyze the cell wall (50 mM Tris-HCl, 1mM EDTA, 0.5 Mm NaCl, pH 7.5). Then, the mixture was shaken for 30 min, followed by 15 min sonication. The membranes were pelleted by centrifugation at 13000 g for 45 min at 4°C and suspended in 50 mM Tris-HCl. Triton X-100 was added to the suspension at an ultimate concentration of 1%, then samples were incubated for 1 hour at 30°C. Subsequently the samples were treated by 25% glycerol (v/v), 5% SDS (w/v), 5% 2-mercaptoethanol (v/v), and 1% bromophenol blue at 96°C for 5 min. Eventually, outer membrane sections were evaluated by SDS-PAGE with 12% (w/v) acrylamide at a constant current of 30 mA.

2.4. Experimental Design

General full factorial design was the base strategy to introduce the conditions and number of experiments. To evaluate the influence of the operating parameters on CSH, two categorical factors at various levels were used for the process promotion. Two independent variables, including types of carbon sources and biosurfactant were chosen as input parameters, whereas CSH % was considered as a response. The input variables and their levels in the experiment are presented in Table 2. 30 experiments were conducted in this study, i.e., 15 runs for each strain of bacteria, comprising combination of all levels of other factors (5×3=15). All the factorial designs were randomly performed to minimize experimental errors. The confidence interval of 95% and coefficient of determination (R^2) were considered as criteria to realize the significance of terms and the fitting quality of proposed model, respectively.

Table 2. *Levels and the corresponding factors for the tests performed in the experimental design.*

Operating variables	Levels				
	1	2	3	4	5
Condition of medium	Control	Surfactin below CMC	Surfactin above CMC	Rhamnolipid below CMC	Rhamnolipid above CMC
C-Source	Glucose	Hexadecane	PCBs	-	-

The obtained experimental data based on the full factorial design were applied as input information for Minitab 17 software.

3. Results and Discussion

3.1. Experimental Design and Data Analysis

By using ANOVA technique, an attempt was performed to assess the impact of the main factors and their interactions on CSH %. The F-test, mean square, sum of squares, and P-value of each component are presented in Table 3.

Table 3. *Analysis of variance for the performances of two kinds of bacteria with 0.6% of C-source.*

		***P. aeruginosa* MM1011**			
Source	**Degrees of freedom**	**Sum of squares**	**Mean square**	**F_0**	**P-value**
Model	6	1734	289	24.5	0.000
Condition of medium	4	59.6	14.9	1.26	0.359
C-Source	2	1674.6	837.3	71	0.000
Residual error	8	94.4	11.8	-	-
Total	14	1828.6	-	-	-
Standard deviation = 3.4	R^2 = 94.8%		R^2 (adjusted) = 91%		
		***P. aeruginosa* TMU56**			
Model	6	5245	874	27.8	0.000
Condition of medium	4	2883	721	22.9	0.000
C-Source	2	2362	1181	37.5	0.000
Residual error	8	251.8	31.48	-	-
Total	14	5497.4	-	-	-
Standard deviation = 5.6	R^2 = 95.4%		R^2 (adjusted) = 92%		

P-values were considered as a means of assessing the significance of each input parameter, and for the values less than the chosen level (i.e., 0.05 in this research), the relationship between the predictor and response is statistically significant. The P-value less than or equal to 0.05 is a proper criterion to determine whether or not the observed data are statistically significant, and the values closer to zero indicate greater significance [18]. According to the obtained F-ratio and P-value from ANOVA, the effects of main variables, type of carbon source, and presence of biosurfactant were statistically significant for the *P. aeruginosa* TMU56 strains, while based on this analysis, *P. aeruginosa* MM1011 was only affected by carbon source as the P-value for the condition of medium was obtained 0.359 (more than 0.05). Figure 1 depicts the main effects plot of three different carbon sources. As demonstrated, the mean CSH % of *P. aeruginosa* TMU56 strain is higher than *P. aeruginosa* MM1011 at the similar experimental conditions.

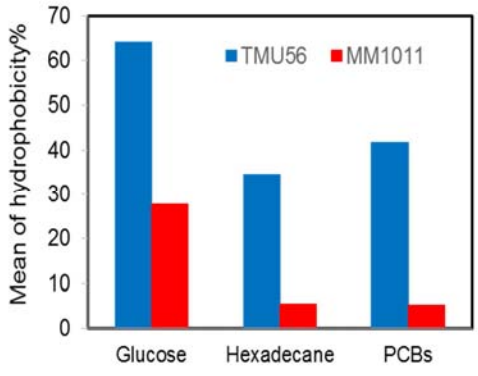


Figure 1. *Main effects plot for hydrophobicity in terms of different C-Sources.*

Experiments showed that when glucose was used as main carbon source for the both strains, the hydrophobicity conspicuously increased compared to the other two hydrophobic carbon sources. Although there were no meaningful differences in the performance of *P. aeruginosa* MM1011 when two types of hydrophobic carbon sources were used, PCBs was demonstrated to be more influential than hexadecane in case of *P. aeruginosa* TMU56 with an 8% improvement of CSH %. Regular sampling from the culture revealed that the *P. aeruginosa* TMU56 had a shorter exponential phase than the *P. aeruginosa* MM1011, which was quickly shifted to stationary phase. In fact, it was demonstrated that the existence of biosurfactants in the medium had neutral effect on the *P. aeruginosa* TMU56 growth. However, the bacterium division rate was constant at its maximum growth, and the growth rate was increasing in this stage (figure 2).

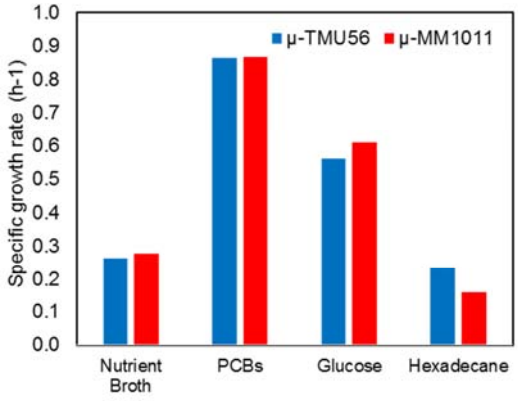


Figure 2. *Measurement of specific bacterial growth under different conditions of carbon source.*

Based on the experimental data, biosurfactants had no effects on this period; for example, the measured time to reach the stationary phase with all 5 different conditions of biosurfactants was remained 12 hours where the carbon source was hexadecane. According to the obtained results, two practical relationships between CSH % and the input parameters were proposed in form of the following polynomial equations (Eq. (2-3)):

$$\text{Hydrophobicity}_{TMU56}\ \% = 46.73 - 24.39\ \text{Control} + 11.71\ \text{Surfactin below CMC} - 1.13\ \text{Surfactin above CMC} + 15.04\ \text{Rhamnolipid below CMC} - 1.23\ \text{Rhamnolipid above CMC} + 17.27\ \text{Glucose} - 12.16\ \text{Hexadecane} - 5.11\ PCB_S \quad (2)$$

$$\text{Hydrophobicity}_{MM1011}\ \% = 12.799 + 1.00\ \text{Control} - 1.27\ \text{Surfactin below CMC} + 2.57\ \text{Surfactin above CMC} - 3.16\ \text{Rhamnolipid below CMC} + 0.85\ \text{Rhamnolipid above CMC} + 14.94\ \text{Glucose} - 7.31\ \text{Hexadecane} - 7.63\ PCB_S \quad (3)$$

Output results from the model indicated good agreement between the experimental and predicted values of CSH %. The statistical significance of the model equation for the *P. aeruginosa* MM1011 was assessed by the F-test, which showed that the regression is statistically significant at P-value less than 5%. According to the Table 3 approximately 95% of the variability in the response can be elucidated by model with satisfactory coefficient of determination (R^2=0.948). A brief information about the proposed models is presented in Table 3, this includes coefficients, and testing the significance of the regression based on the ANOVA for the *P. aeruginosa* TMU56 strain. As shown, more than 95% of the data (R^2 =0.954) can be well predicted by the model for the *P. aeruginosa* TMU56, signifying that the expressions considered in the proposed model play a substantial role in the process.

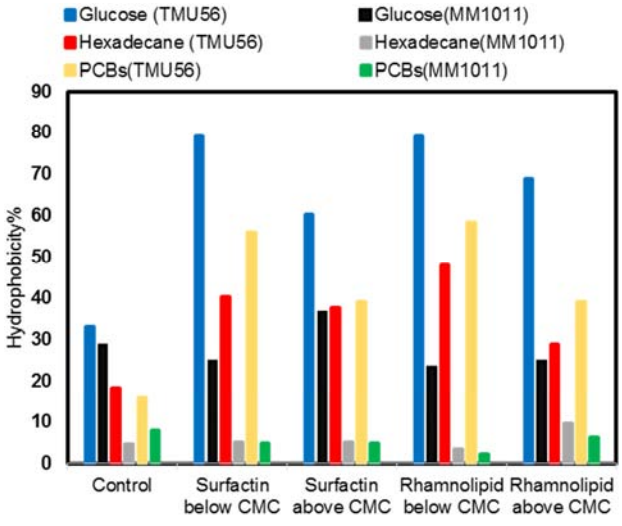


Figure 3. *Interaction between the C-sources and medium condition at different concentrations of biosurfactant.*

The statistical significance of input parameters in full factorial design were confirmed by the F-test (defined as the ratio of the respective mean square effect to the MSE) and the analysis were visualized over the two figures (1 and 3) named main effects and interactions.

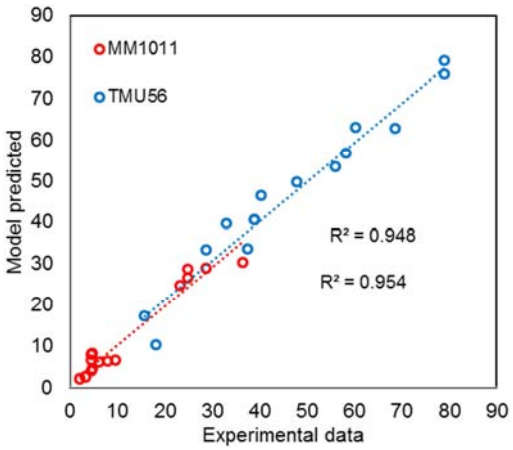


Figure 4. *The experimental data versus the model predicted values for CSH %.*

These illustrations are helpful to indicate how a dependable factor alters according to the corresponded independent factor(s).

Interaction plot is another helpful figure that demonstrates possible interactions for each pair of factors in experimental design where the effect of one factor depends on the level of other factors. The significant interactions of glucose and surfactin above CMC were shown in figure 3. This means that CSH % can be enhanced when the pair of glucose as carbon source and surfactin above CMC is used. The experimental and predicted CSH % for the both strains are displayed in figure 4. Experimental data are the measured data over the experiments, and the predicted values are the calculated data based on the proposed models. High coefficient of determination for the both models maintained a satisfactory fitness to the experimental data.

3.2. Outer Membrane Proteins Variation and the Mechanism

To investigate the relationship between the increase of CSH % and the cell structure, OMPs of the *P. aeruginosa* TMU56 were analysed by SDS-PAGE. With respect to the figure 5, the variation of CSH is attributed to the biosurfactant adsorption on the cell surface, as SDS-PAGE analysis shows the amount of released proteins from outer membrane. The inconspicuous band in fifth and sixth lines of figure 5 belong to the concentration of the both biosurfactants (surfactin and rhamnolipid) below the CMC, which are clear evidence of carrying a lower amount of OMPs rather than in third and fourth lines. Al-tahhan *et al.* reported that rhamnolipid caused the release of lipopolysaccharide from the cell surface of *Pseudomonas spp*. and an improvement in CSH % [9]. Decreasing of lipopolysaccharide content and changing in the OMPs as a result of the presence of rhamnolipid in the medium was observed by Sitrova *et al* [17] as well. Since adsorption of biosurfactant onto the cell surfaces depends on its free concentration in a medium, most studies focused on the

performance of biosurfactants at the concentrations above CMC [17, 22]. However, employing high concentration of surfactant carries several drawbacks, including toxicity effect on microorganisms in some cases, reducing the adhesion of bacteria to hydrophobic surfaces, additional cost, and the negative environmental impacts.

Modifications of cell surface properties by biosurfactant is another potential pathway to enhance the CSH. Biosurfactant can accomplish its role in bioremediation of hydrophobic compounds through the two main mechanisms: (i) interaction between biosurfactant and hydrophobic compound (contaminant), and (ii) interaction between biosurfactant and cell surface of bacteria.

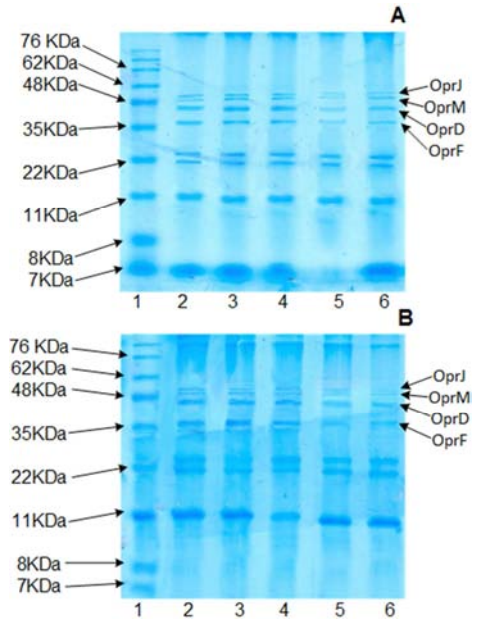


Figure 5. *SDS-PAGE of the outer membrane proteins of the P. aeruginosa TMU56 on A) hexadecane B) PCBs. Specification: Line 1, protein markers; Line 2, OMP_S of the cells grown in the control condition; Line 3, OMP_S of the cells grown in presence of surfactin above CMC; Line 4, OMP_S of the cells grown in the presence of rhamnolipid above CMC; Line 5, OMP_S of the cells grown in the presence of surfactin below CMC; Line 6, OMP_S of the cells grown in the presence of rhamnolipid below CMC.*

The results of this study suggested that the second pathway is more likely to happen and the effects of biosurfactant can be induced by an alteration in bacterial cell surface properties. The surfactin and rhamnolipid (purified by magnetic nanoparticles) at concentrations below CMC affect the OMPs composition of the *P. aeruginosa* TMU56 outer membrane. Analysis of the OMP profiles indicated that the OMPs bands of cells grown in the presence of biosurfactants at concentration below CMC were less intense than those observed in the control condition. It implies that the amount of significant proteins with molecular weights of 54 KDa (OprJ), 50 KDa (OprM), 45 KDa (OprD), and 38 KDa (OprF) decreased from the outer membrane. This process made possible alterations in physicochemical properties of *P. aeruginosa* TMU56 cell surface. The observed changes in the membrane structure may arise from binding of biosurfactant monomers to the outer membrane. Therefore, a reduction in the number of proteins, which are responsible for the transportation of materials within the cell membrane, can lead to a reduction in the cell surface molecular congestion and solidity. This phenomenon can be followed by a smoother, indistinct, and thinner membrane along with disruptions of certain zones within outer membrane so that typical hydrophobic substrates can pass through the membrane easily. Moreover, this pathway of mechanism is consistently confirmed by transmission electron microscopy analysis that was represented by Sotirova *et al.* [13, 17].

The obtained results from the SDS-PAGE of OMPs support the data presented in figure 3, which shows higher values of CSH % at the concentration of the biosurfactant below CMC. The discriminated line and band intensity in figures 5(A) and 5(B) showed that hexadecane and PCBs as carbon sources, respectively, had relatively different effects on majority of the released proteins. In addition, different band thicknesses in each line obviously demonstrated that concentration of biosurfactants was an influential factor in reduction of OMPs.

Table 4. *Comparison of some literature data reported maximum improvement in the CSH % regardless of surfactant level.*

Strain	C-Source	Surfactant	CSH %	Ref
P. aeruginosa NBIMCC 1390	Maltose	Rhamnolipid	10	[9]
P. aeruginosa ATCC 27853	Hexadecane	Rhamnolipid	50	[8]
Bacillus cereus KE	Diesel oil	Rhamnolipid	30	[11]
P. fluorescens SM	Diesel oil	Rhamnolipid	10	[11]
P. aeruginosa TMU56	PCBs	Rhamnolipid	45	This study

Variations in the amount of OMPs and CSH may decrease the thickness of the outer membrane and increase the direct contact between substrate and cell surface of bacteria. Further, loss of outer membrane components led to more hydrophobic zones on the surface and consequently higher CSH. Thus, uptake of hydrophobic compounds such as PCBs and hexadecane increased, and as a result, such compounds can also be easily biodegraded. Table 4 makes a comparison among some experimental conditions reported in the literatures in terms of bacterium strains, types of carbon sources, and the percentage of CSH improvement [9, 17, 23].

4. Conclusion

In the bioremediation process, introducing appropriate breed of bacteria with the high efficiency at toxic reduction is inevitable. In the present study, capability of two strains of bacteria, including *P. aeruginosa* MM1011 and *P. aeruginosa*

TMU56, was evaluated in terms of CSH %. All the experiments were conducted based on the experimental design. CSHs of 15.8-79% and 2.2-36.5%, disregarding any particular condition, were achieved for the *P. aeruginosa* TMU56 and *P. aeruginosa* MM1011, respectively. When glucose was used as the carbon source along with the presence of biosurfactant below CMC, the maximum CSH % of 79% was obtained for the *P. aeruginosa* TMU56. The data analysis based on the 95% confidence interval showed the importance of both input parameters of carbon source and the presence of biosurfactant for the *P. aeruginosa* TMU56 strain. This is while only the types of used carbon sources were important for the *P. aeruginosa* MM1011. Moreover, the results were consistently validated through the SDS-PAGE analysis. According to this analysis, rhamnolipid and surfactin changed the properties of the cell surface of *P. aeruginosa* TMU56 that led to loss of outer membrane component and consequently, facilitated bioavailability of hydrophobic compounds via direct contact between the cells and oil pollution.

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