© 2019 Journal of Pharmacy & Pharmacognosy Research, 7 (6), 413-420, 2019
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Optimizing the immobilization of biosurfactant-producing *Pseudomonas aeruginosa* in alginate beads

[Optimización de la inmovilización de *Pseudomonas aeruginosa* productora de biosurfactantes en perlas de alginato]

Gholamreza Dehghannoudeh[1,2], Kioomars Kiani[1], Mohammad Hassan Moshafi[1], Negar Dehghannoudeh[3], Majid Rajaee[1], Soodeh Salarpour[1], Mandana Ohadi[2]*

[1]Department of Pharmaceutics, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.
[2]Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.
[3]Faculty of Arts and Science, University of Toronto, 100 St George St, M5S3G3, Toronto, Ontario, Canada.
*E-mail: mandanaohady@gmail.com, m.ohadi@kmu.ac.ir

Abstract

Context: Biosurfactants are amphipathic molecules that reduce surface tension. The major reasons to economically production of the biosurfactant are their health, safety, environmental management, and promising applications.

Aims: To optimize the immobilization of *Pseudomonas aeruginosa* as a biosurfactant producer in alginate beads.

Methods: Biosurfactant production by *P. aeruginosa* was confirmed through the hemolysis test, emulsification index, and surface activity measurement. Calcium alginate encapsulation technique was used in order to entrap the *P. aeruginosa* cells. Full factorial design was employed to optimize bead preparation. Furthermore, the morphology and the stability of beads were evaluated.

Results: It was proved that immobilized cells can be preserved the viability and biosurfactant production. Application of full factorial design indicated that the values of three parameters sodium alginate 3%, $CaCl_2$ 1% (w/v), and hardening time of 35 min, was found to be too stable for minimum surface tension and stable alginate gel beads.

Conclusions: The alginate gel beads showed stability during the growing process and the immobilized cells efficiently were viable. Alginate source, hardening time, and the interaction between $CaCl_2$ concentration and hardening time influenced on the bead preparation.

Keywords: alginate beads; biosurfactants; immobilization; optimizing; *Pseudomonas aeruginosa*.

Resumen

Contexto: Los biosurfactantes son moléculas anfipáticas que reducen la tensión superficial. Las principales razones para la producción económica del biosurfactante son la salud, seguridad, gestión ambiental y aplicaciones prometedoras.

Objetivos: Optimizar la inmovilización de *Pseudomonas aeruginosa* como productora de biosurfactantes en perlas de alginato.

Métodos: La producción de biosurfactantes por *P. aeruginosa* se confirmó mediante la prueba de hemólisis, el índice de emulsión y la medición de la actividad de la superficie. Se utilizó la técnica de encapsulación de alginato de calcio para atrapar las células de *P. aeruginosa*. Se empleó un diseño factorial completo para optimizar la preparación de las perlas de alginato. Además, se evaluó la morfología y la estabilidad de las perlas.

Resultados: Se demostró que las células inmovilizadas pueden preservar la viabilidad y la producción de biosurfactantes. La aplicación del diseño factorial completo indicó que los valores de tres parámetros de alginato de sodio al 3%, $CaCl_2$ al 1% (p/v), y un tiempo de endurecimiento de 35 min, resultaron ser estables para una tensión superficial mínima y perlas de gel de alginato estables.

Conclusiones: Las perlas de gel de alginato mostraron estabilidad durante el proceso de crecimiento y las células inmovilizadas fueron viables de manera eficiente. La fuente de alginato, el tiempo de endurecimiento y la interacción entre la concentración de $CaCl_2$ y el tiempo de endurecimiento influyeron en la preparación de las perlas.

Palabras Clave: biosurfactantes; inmovilización; optimización; perlas de alginato; *Pseudomonas aeruginosa*.

ARTICLE INFO
Received: April 15, 2019.
Received in revised form: September 14, 2019.
Accepted: September 18, 2019.
Available Online: September 22, 2019.
Declaration of interests: The authors declare no conflict of interest.
Funding: The authors confirm that the project has not funding or grants.

INTRODUCTION

Biosurfactants are amphipathic molecules that reduce surface tension. They are produced by a wide variety of microorganisms (Banat et al., 2014). Biosurfactants are classified e.g. lipopeptide, lipoproteins, glycolipids, phospholipids, and polymeric ones according to their origin and nature of chemical structures (Abouseoud et al., 2008, Ohadi et al., 2018). Compared to chemo-synthetic surfactants, biosurfactants have many advantages. They are environmentally friendly, biodegradable, less toxic, non-hazardous, and have stability in extreme pH, salinity, and temperature (Chen et al., 2018). They have the potential to be used as surfactants and emulsifiers in the pharmaceutical (drug delivery), cosmetics (foaming agent), and food industries (emulsifier and stabilizer) (Ohadi et al., 2017a;b). Despite all these advantages, the greatest limiting for using biosurfactant in large industrial is the high cost of their production (Rita de Cássia et al., 2017). The most economical production method of the biosurfactants is cell immobilization that showed some advantages e.g. no need to restock microorganism cells, absence of cell washout, high stability, extended reaction times, and high density of cells (Lotfabad et al., 2017). Inanition, immobilized bacteria are alive for a long time, and resistance in the hard conditions, which makes it a promising alternative tool in flow bioreactors (Ferhat et al., 2017). One of the immobilization methods is the entrapment of cells in insoluble calcium alginate beads. This method, which is commonly used to produce biosurfactants is rapid, nontoxic, and economic (Ohadi et al., 2014, Darah et al., 2015). Recently, biological materials such as calcium alginate are used as an encapsulation agent to immobilize prokaryote and eukaryote cells (Koch et al., 2003, Heidari et al., 2016). For instance, they are biodegradable, biocompatible, and they have low toxicity and low cost (Gonzalez-Pujana et al., 2018). However, there are some complications in cell entrapment such as a decrease in the activity of a microorganism and diffusive problems between substrates and product due to the process of matrix formation (Bayramoglu et al., 2002). For these reasons, microorganism immobilization by bioencapsulation should be optimized. Randomization, replication, and blocking as three steps of the design expert are using to improve the efficiency of the experimentation (Ekpenyong et al., 2017). Application of statistical experimental design for the immobilization method results in improved product yield, further validation of the response to the desired product, reduction in the time, and total cost (Sharifi et al., 2018). In the present study, we evaluated the biosurfactant production by *P. aeruginosa* followed by statistical optimization of entrapment of *P. aeruginosa* in calcium alginate beads.

MATERIAL AND METHODS

Microorganism and chemicals

P. aeruginosa (PTCC 1557) was obtained from the Persian Type Culture Collection, Tehran, Iran. All materials were provided from Merck Company (Darmstadt, Germany).

Evaluation of biosurfactant production

The biosurfactant production by *P. aeruginosa* were evaluated using following methods, which are as follows:

Hemolysis activity

The blood agar plates containing (g/L); tryptic case (10), beef extract (3), NaCl (5), and agar (15) supplemented by sterile sheep blood (5%, v/v) were used to seed *P. aeruginosa,* incubated at 37℃ for 48 h (Carrillo et al., 1996). Then the plates were visually inspected for zones of clearing around the bacteria colonies, indicative of biosurfactant production.

Media and cultivation conditions

Cultures of *P. aeruginosa* were prepared in 50 mL nutrient broth (NB) medium and overnighted. Next day, 100 mL NB was inoculated by 1% of the prepared bacterial (OD_{600} = 0.8) and incubated for 72 h. Samples were taken intervallic and analyzed for optical density (OD_{600}) by spectrophotometer (UV-1800, Shimadzu CO, USA), followed by de-

termination of surface tension and emulsification index (Ohadi et al., 2017a; b).

Surface activity measurement

The surface tension of the culture supernatant (at room temperature) was estimated by measuring the surface tension for different concentrations with a Whilmhelmy tensiometer (KRUSS-K100, Germany). A volume of 20 mL of the obtained supernatant was put into a glass beaker (50 mL) and placed onto the tensiometer platform. A platinum plate was slowly touched the liquid-air interface, to measure the surface tension (mN/m). Between each measurement, the platinum plate was rinsed three times with water, three times with acetone, and was then allowed to dry (Dehghan-Noudeh et al., 2005).

Emulsification index

Emulsifier activity was measured by method that was described perilously (Ohadi et al., 2017a). Mineral oil (5 mL) was added to equal volume (5 mL) of supernatant and vortexing at high speed (300 rpm) for 2 min. The emulsion stability was determined after 24 (E24), 48 (E48), and 72 (E72) hours. Then emulsification index percent was determined by using the following equation [1] (Ohadi et al., 2017a):

$$\text{Emulsification index \%} = \frac{\text{Emulsion layer (height)}}{\text{Solution (height)}} \times 100 \quad [1]$$

Where height was measured in mm.

Beads preparation

Beads were obtained according to the method described previously by (Heidari et al., 2016). Briefly, sodium alginate solution was prepared in hot distilled water and then alginate solution (100 mL) was inoculated by 1% of bacterial inoculum (OD_{600}= 0.8). Then resulting mixture was taken into a sterile syringe and added drop wise to $CaCl_2$ solution from 5 cm height. All process has been done in sterile condition.

Statistical optimization of encapsulation by experimental design

Full factorial design was employed to identify the most important various parameters that has a potential effect on immobilization of *P. aeruginosa* in calcium alginate beads. Experimental parameters were alginate, $CaCl_2$ concentration, and hardening times of the beads as described in Table 1 (Lotfipour et al., 2012). Based on the full factorial design, 11 runs were performed include one replicate and three center points (2^3). Thereafter, analysis of variance (ANOVA) of the obtained responses was carried out using Design Expert 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA) (Ohadi et al., 2017a). Thereafter, the mathematical relation between variables was estimated by the two-factor interaction (2FI) model as the following equation [2]:

$$y = \beta_0 + \Sigma_i^3{}_{=1} \beta_i X_i + \Sigma_i^3{}_{=1} \Sigma_j^3{}_{=1} \beta_{ijxixj} \quad [2]$$

In this formula, the predicted response is y, the coded levels of the independent variables represented as X, β_0 is the model constant, β_i is the linear coefficient, and β_{ij} is the cross-product coefficient.

Morphological characteristics of beads

Formed beads were transformed to NB culture medium and kept at 37°C for 72 h. The shape and stability of the beads were evaluated visually at definite time intervals (every 24 h).

Table 1. The parameters and their levels applied for optimizing bead production in the full factorial design.

Variables	Symbol	Unit	Levels		
			-1	0	+1
Alginate concentration	(A)	(%w/v)	1	2	3
$CaCl_2$ concentration	(B)	(%w/v)	1	2.5	4
Hardening time	(C)	(min)	15	25	35

RESULTS AND DISCUSSION

Hemolytic activity

The present of the clear zone around the bacterial colonies indicates as surfactant production, which is applied as a primary method to screen biosurfactant production (Carrillo et al., 1996). Hemolytic activity measurement showed that similar to synthetic surfactants, it was able to rupture erythrocyte and therefore could be used as absorption enhancer (De et al., 2015). Similar result was reported by Carrillo et al. (1996) who have found an association between blood hemolysis and biosurfactants production.

Biosurfactant production profile

Time course study of bacterial growth (OD_{600}), surface tension, and emulsification index% determinations are shown in Fig. 1. During exponential growth phase, maximum emulsifying activity and minimal surface tension were reached. So, it can be supposed that the biosurfactant production by *P. aeruginosa* occurred during the exponential growth phase. Growth-associated biosurfactant production has been described in previous studies (Liu et al., 2012, Ferhat et al., 2017, Ohadi et al., 2017a). They suggested that the biosurfactant was produced as primary metabolite accompanying cellular biomass formation.

Optimization of bead preparation

In this study, two-level full factorial design was employed to recognize the most effective parameters (alginate and $CaCl_2$ concentration, hardening time) on bead preparation (Lotfipour et al., 2012). The effects of these parameters on the surface tension reduction were examined (Table 2).

Response was calculated according to the following equation [3]:

$$ST = +41.68 - 0.88\,(B) - 3.71\,(C) + 0.56\,(A \times B) + 1.60\,(B \times C) + 0.51(A \times B \times C) \quad [3]$$

Where: ST is surface tension (mN/m); A, B, and C are coded values pertaining to alginate concentration, $CaCl_2$ concentration and hardening time, respectively.

According to Table 3 an ANOVA of the surface tension was performed. The model was statistically significant ($p \leq 0.002$,) also the curvature ($p \geq 0.044$) and the lack of fit ($p \geq 0.808$) was non-significant. These results confirmed that this model has a high degree of precision of 16.258, and the reliability of the experimental values were in a low value of the coefficient of variation (CV = 2.20%). So, the prediction effect of the parameters on the response could be done according to the suggested model. Predicted R-squared (Pred R^2) = 0.894 was in a reasonable agreement with the Adjusted R-squared (Adj R^2) of 0.948 that suggests the significant correlation between the predicted values and the experimental results (R-Squared = 0.977) (Fig. 2).

A reduction in the surface tension value (36.40 mN/m) in the predicted optimal conditions (alginate 3%, calcium chloride 1% and hardening time 35 min) was validated by the prediction point CI (99%) by the software. The effect of parameters on the ST are shown in the three-dimensional surface and contour plots (Fig. 3).

Equation [3] represents the parameters and their effective contribution (AB, BC and ABC) on the decrease of ST (Response). According to equation [3] and Table 3, the contribution of hardening time and calcium chloride concentration was significant, and they have a positive effect on the response. Increasing the concentration of Ca^{++} cations lead to a greater degree of cross-linking and more stability of bead structure. As previous studies, the strength of calcium alginate structure can entrap a higher number of bacteria in the alginate network that leads to a higher biosurfactant production (Lotfipour et al., 2012). Hardening time affected the stability of beads (Table 3). The beads hardened for 35 min, which were stable and resulted in a further decrease of ST. Increase of hardening time affected the diffusion of calcium to the inner of the beads, causing a higher degree of cross-linking in bead structures which resulted in surface roughness and porosity (Ellaiah et al.,

2004, Sankalia et al., 2005). According to equation [3], contribution of alginate concentration and two other parameters affected the ST reduction. Although alginate concentration had not entered in equation directly, its contribution to calcium chloride and hardening time had affected the response. The increased viscosity of the polymer solution was caused by increasing the alginate concentration, which resulted in the reduction and retardation diffusion of calcium to the internal of the bead and consequently decreased cross-linking by calcium. Therefore, the prolongation of the hardening time could increase calcium penetration to the alginate network; hence, lead to an increase in cross-linking and an increase in the porosity of beads (Sankalia et al., 2005, Goh et al., 2012).

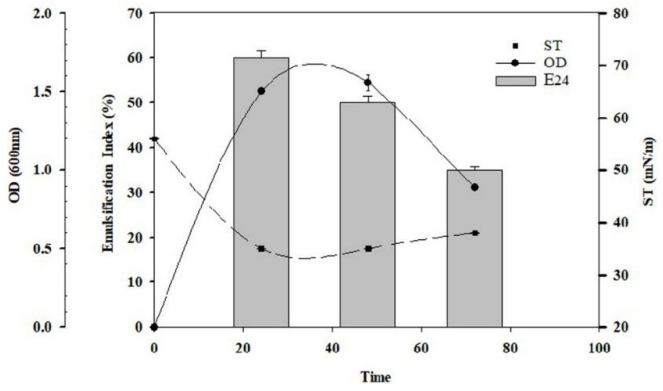

Figure 1. Time course profile of the growth (OD600), %emulsification index (E_{24}), and surface tension (ST) reduction of *P. aeroginosa* during 96 h cultivation.

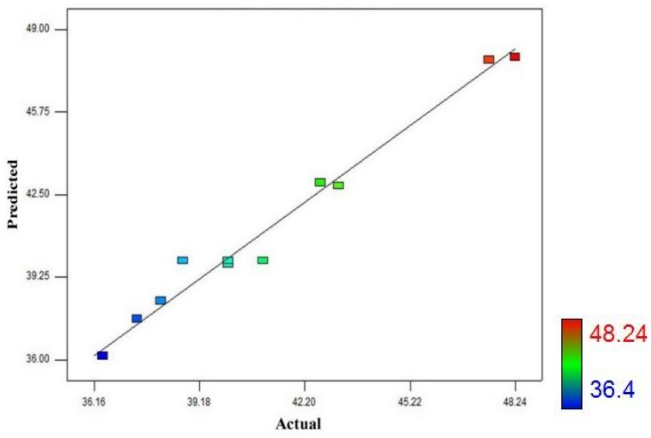

Figure 2. The related plot of predicted *vs.* actual response of experimental run (surface tension) derived from design expert software. Color points associated with the surface tension that it was increased from blue to red.

Table 2. Experimental design and results of the full factorial design.

Run	Coded value of independent factor			Surface tension (mN/m)	
	A	B	C	Experimental	Predicted
1	-1	-1	-1	48.24 ± 0.09	47.92
2	1	-1	-1	47.49 ± 0.09	47.80
3	-1	1	-1	43.17 ± 0.06	42.85
4	1	1	-1	42.65 ± 0.08	42.96
5	-1	-1	1	38.07 ± 0.074	38.30
6	-1	1	1	37.38 ± 0.08	36.16
7	1	-1	1	36.41 ± 0.09	38.83
8	1	1	1	40.27 ± 0.074	38.60
9	0	0	0	38.71 ± 0.046	41.67
10	0	0	0	38.71 ± 0.046	41.67
11	0	0	0	38.71 ± 0.046	41.67

Data are expressed as mean ± SD

Table 3. Analysis of variance (ANOVA) for full factorial design.

Source	Sum of Squares	Degree of freedom	Mean square	F-value	P-value Prob > F
Model	141.54	5	28.31	34.43	0.0022
B-Ca	6.13	1	6.13	7.45	0.0525
C-Ht	110.26	1	110.26	134.10	0.0003
AB	2.55	1	2.55	3.11	0.1528
BC	20.54	1	20.54	24.99	0.0075
ABC	2.06	1	2.06	2.51	0.1886
Curvature	6.87	1	6.87	8.36	0.0445
Residual	3.29	4	0.82		
Lack of Fit	0.63	2	0.31	0.24	0.8088
Pure Error	2.66	2	1.33		
Corrected total	151.71	10			

R^2 = 0.977; Adj R^2 = 0.948; Pred R^2 = 0.894; Adeq precision =16.258; A: Alginate concentration; B: $CaCl_2$ concentration; C: Hardening time (Ht).

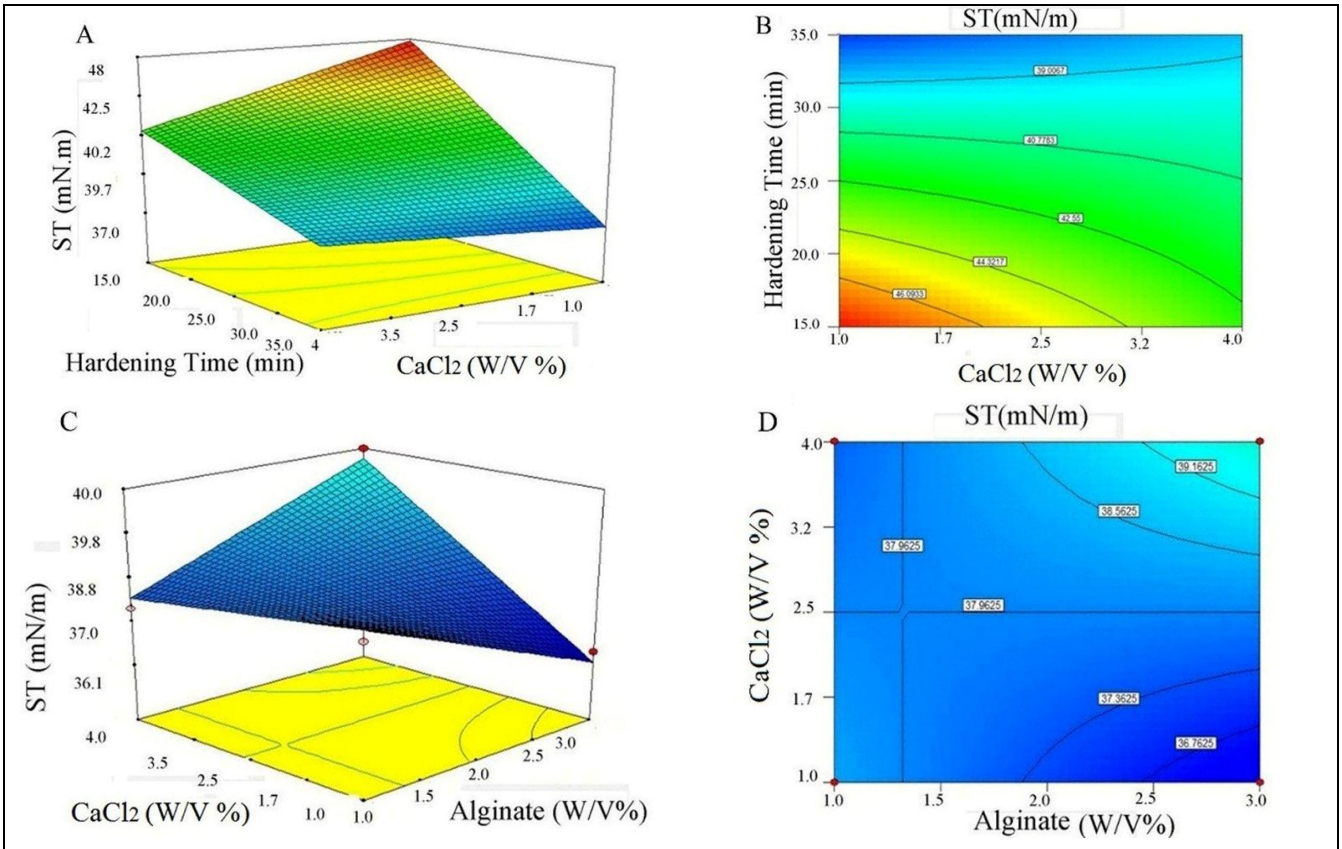

Figure 3. The related 3D surface **(A)** and contour **(B)** plots of $CaCl_2$ concentration and hardening time versus surface tension and 3D surface **(C)** and contour **(D)** plots of $CaCl_2$ concentration and alginate concentration versus surface tension (ST) drawn by Design Expert software.

Figure 4. Morphology of prepared beads.

Morphology and stability of prepared beads

Between 11 runs suggested by the software, run 7 exhibited the best stability of spherical shaped beads (Fig. 4). Also, according to Table 2, maximum surface tension reduction was observed in this run.

CONCLUSIONS

It could be concluded that the immobilized bacteria could preserve their viability and biosurfactant production capability during the storage and successive fermentation. The result of experimental design showed that alginate concentration, hardening time and the interaction between $CaCl_2$ concentration, and hardening time influenced on the bead preparation. Therefore, immobilization methods may be considered as an economic strategy in the biosurfactants production.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank Kerman University of Medical Sciences (KMU) for facilitating research into the conclusions of this study. The authors declare that the present research study was performed without any funding from any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Abouseoud M, Yataghene A, Amrane A, Maachi R (2008) Biosurfactant production by free and alginate entrapped cells of *Pseudomonas fluorescens*. J Ind Microbiol Biotechnol 35(11): 1303–1308.

Banat IM, Satpute SK, Cameotra SS, Patil R, Nyayanit NV (2014) Cost effective technologies and renewable substrates for biosurfactants' production. Front Microbiol 5: 697.

Bayramoglu G, Denizli A, Bektas S, Arica MY (2002) Entrapment of *Lentinus sajor-caju* into Ca-alginate gel beads for removal of Cd (II) ions from aqueous solution: preparation and biosorption kinetics analysis. Microchem J 72(1): 63–76.

Carrillo P, Mardaraz C, Pitta-Alvarez S, Giulietti A (1996) Isolation and selection of biosurfactant-producing bacteria. World J Microbiol Biotechnol 12(1): 82–84.

Chen C, Sun N, Li D, Long S, Tang X, Xiao G, Wang L (2018) Optimization and characterization of biosurfactant production from kitchen waste oil using *Pseudomonas aeruginosa*. Environ Sci Pollut Res Int: 1–10.

Darah I, Nisha M, Lim S-H (2015) Polygalacturonase production by calcium alginate immobilized *Enterobacter aerogenes* NBO2 cells. Appl Biochem Biotechnol 175(5): 2629–2636.

De S, Malik S, Ghosh A, Saha R, Saha B (2015) A review on natural surfactants. RSC advances 5(81): 65757–65767.

Dehghan-Noudeh G, Housaindokht M, Bazzaz BSF (2005) Isolation, characterization, and investigation of surface and hemolytic activities of a lipopeptide biosurfactant produced by *Bacillus subtilis* ATCC 6633. J Microbiol-Seoul 43(3): 272.

Ekpenyong M, Antai S, Asitok A, Ekpo B (2017) Response surface modeling and optimization of major medium variables for glycolipopeptide production. Biocatal Agric Biotechnol. 10: 113–121.

Ellaiah P, Prabhakar T, Ramakrishna B, Taleb AT, Adinarayana K (2004) Production of lipase by immobilized cells of *Aspergillus niger*. Proc Biochem 39(5): 525–528.

Ferhat S, Alouaoui R, Badis A, Moulai-Mostefa N (2017) Production and characterization of biosurfactant by free and immobilized cells from *Ochrobactrum intermedium* isolated from the soil of southern Algeria with a view to environmental application. Biotechnol Biotec Eq 31(4): 733–742.

Goh CH, Heng PWS, Chan LW (2012) Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohyd Polym 88(1): 1–12.

Gonzalez-Pujana A, Orive G, Pedraz JL, Santos-Vizcaino E, Hernandez RM (2018). Alginate microcapsules for drug delivery. Alginates and Their Biomedical Applications, Springer, p. 67–100.

Heidari BA, Badinloo S, Ohadi M, Noudeh GD (2016) Bioencapsulation of biosurfactant-producing *Bacillus subtilis* (PTCC 1023) in alginate beads. Jundishapur Nat Pharm Prod 11(4): e33935.

Koch S, Schwinger C, Kressler J, Heinzen C, Rainov N (2003) Alginate encapsulation of genetically engineered mammalian cells: comparison of production devices, methods and microcapsule characteristics. J Microencaps 20(3): 303–316.

Liu X, Chen X, Li Y, Wang X, Peng X, Zhu W (2012) Preparation of superparamagnetic Fe3O4@ alginate/ chitosan nanospheres for *Candida rugosa* lipase immobilization and utilization of layer-by-layer assembly to enhance the stability of immobilized lipase. ACS Appl Mater Interfaces 4(10): 5169–5178.

Lotfabad TB, Ebadipour N, Roostaazad R, Partovi M, Bahmaei M (2017) Two schemes for production of biosurfactant from *Pseudomonas aeruginosa* MR01: Applying residues from soybean oil industry and silica sol–gel immobilized cells. Colloids Surf B 152: 159–168.

Lotfipour F, Mirzaeei S, Maghsoodi M (2012) Evaluation of the effect of $CaCl_2$ and alginate concentrations and hardening time on the characteristics of *Lactobacillus acidophilus* loaded alginate beads using response surface analysis. Adv Pharm Bull 2(1): 71.

Ohadi M, Amir-Heidari B, Moshafi MH, Mirparizi A, Basir M, Dehghan-Noudeh G (2014) Encapsulation of biosurfactant-producing *Bacillus licheniformis* (PTCC 1320) in alginate beads. Biotechnology 13(5): 239–244.

Ohadi M, Dehghannoudeh G, Forootanfar H, Shakibaie M, Rajaee M (2018) Investigation of the structural, physicochemical properties, and aggregation behavior of lipopeptide biosurfactant produced by *Acinetobacter junii* B6. Int J Biol Macromol 112: 712–719.

Ohadi M, Dehghannoudeh G, Shakibaie M, Banat IM, Pournamdari M, Forootanfar H (2017a) Isolation, characterization, and optimization of biosurfactant production by an oil-degrading *Acinetobacter junii* B6 isolated from an Iranian oil excavation site. Biocatal Agric Biotechnol 12: 1–9.

Ohadi M, Forootanfar H, Rahimi HR, Jafari E, Shakibaie M, Eslaminejad T, Dehghannoudeh G (2017b) Antioxidant potential and wound healing activity of biosurfactant produced by *Acinetobacter junii* B6. Curr Pharm Biotechnol 18(11): 900–908.

Rita de Cássia F, Almeida DG, Meira HM, Silva EJ, Farias CB, Rufino RD, Luna JM, Sarubbo LA (2017) Production and characterization of a new biosurfactant from *Pseudomonas cepacia* grown in low-cost fermentative medium and its application in the oil industry. Biocatal Agric Biotechnol. 12: 206–215.

Sankalia MG, Mashru RC, Sankalia JM, Sutariya VB (2005) Papain entrapment in alginate beads for stability improvement and site-specific delivery: physicochemical characterization and factorial optimization using neural network modeling. AAPS Pharmscitech 6(2): E209–E222.

Sharifi H, Zabihzadeh SM, Ghorbani M (2018) The application of response surface methodology on the synthesis of conductive polyaniline/cellulosic fiber nanocomposites. Carbohydr Polym 194: 384–394.

AUTHOR CONTRIBUTION:

Contribution	Dehghannoudeh G	Kiani K	Moshafi MH	Dehghannoudeh N	Rajaee M	Salarpour S	Ohadi M
Concepts or ideas	x						x
Design	x						x
Definition of intellectual content	x						x
Literature search	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x	x
Data analysis	x	x	x	x	x	x	x
Statistical analysis	x	x	x	x	x	x	x
Manuscript preparation	x	x	x	x	x	x	x
Manuscript editing	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x

Citation Format: Dehghannoudeh G, Kiani K, Moshafi MH, Dehghannoudeh N, Rajaee M, Salarpour S, Ohadi M (2019) Optimizing the immobilization of biosurfactant-producing *Pseudomonas aeruginosa* in alginate beads. J Pharm Pharmacogn Res 7(6): 413–420.