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Research article

# Accurate investigation of the mechanism of rhamnolipid biosurfactant effects on food waste composting: A comparison of in-situ and ex-situ techniques

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ABSTRACT

The long process time and low product quality are major challenges in the composting process. To overcome the above challenges, the effects of produced biosurfactants on composting were investigated as a biological model. *Pseudomonas aeruginosa* IBRC-M 11180 inoculum and its supernatant were used as in-situ and ex-situ treatments in the composting process, respectively. The results showed that the presence of rhamnolipid biosurfactants in the composting process could improve many parameters such as maximum temperature, electrical conductivity (EC), cation exchange capacity (CEC), C/N, and germination index (GI). The GI value above 80% was observed for in-situ and ex-situ reactors on 12th day, while for the control was observed on 18th day, which indicates the significant effects of rhamnolipids on process time reduction. The C/N ratios of final compost for ex-situ, in-situ, and control reactors were 12.83, 13.27, and 17.05, respectively, which indicates the rhamnolipids also improves the quality of the final product. To better understand the performance of the rhamnolipids in the composting, wettability changes of the compost surface were evaluated. Our results show that the produced rhamnolipids altered the waste wettability from intermediate wet ($\theta = 85^\circ$) to water-wet ($\theta = 40^\circ$). It can be concluded that the presence of biosurfactants in composting leads to an increase in the contact surface area of microorganisms with nutrient sources and consequently improves the composting process. Furthermore, comparative studies showed that the in-situ treatment has better effects on composting, thus it can be an economically significant achievement because of the high cost of ex-situ treatment.

## 1. Introduction

Due to the growing population and urban development, collecting, transporting, and processing of solid urban waste have become a pervasive problem. The development of consumer culture has led to a significant increase in agricultural and food waste. As a result, people and international organizations are putting pressure on governments to make appropriate waste management decisions. Generally, landfill, anaerobic digestion, incineration, and composting are common waste treatment methods (Sohoo et al., 2021). Due to the large volume of organic waste, use of composting has received much attention. In the composting method, which is a thermophilic biological process, organic matter is transformed into a more stable material which forms the basis of humus (Füleky and Benedek, 2010). Adding compost enriches the soil with substances such as phosphorus, nitrogen, and potassium, but the most important advantage of compost is that it strengthens the soil structure and increases the plant's ability to absorb nutrients (Cayuela et al., 2010; Tavali, 2021).

Parameters such as carbon to nitrogen ratio, aeration rate, particle size, temperature, and additives have long been considered by researchers to optimize the composting process (Van Fan et al., 2018), but recently, studies on the effect of inoculation of microorganisms on composting to improve the quality of the final product and reduce process time are increasing. For example, Ohtaki et al. (1998) reported that commercial inoculum including *thermophilic actinomycetes* and *mesophilic fungi* could significantly increase the biodegradation of polycaprolactone in the composting process. Ke et al. (2010) showed that the inoculation of *thermotolerant lipolytic actinomyces* on composting can reduce maturity time significantly. The study of Heidarzadeh et al. (2019) about the effect of inoculation of old compost and *Aspergillus*

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*niger* into OFMSW composting showed a decrease in C/N (63.37%, 59.6%, and 46%) for *Aspergillus niger*, old compost, and control treatments, respectively.

Also, biochemical reactions occur in a film layer between the microorganisms and organic particles' surface, therefore the physical and chemical conditions of this layer can be important in the quality of the composting process (Xi et al., 2005b). Surface active agents such as surfactants are suitable materials for obtaining good wettability and achieving equal distribution of organic matter in the film layer (Banat et al., 2000). Zhang et al. (2011) investigated the effect of adding alkyl polyglycoside surfactant on the composting process. According to their work, the temperature and rate of carbon decomposition increased. Also based on Jahanshah et al. (2013) a reduction in composting time occurred in the composting using whey-grown biosurfactant-producing bacteria. According to Zeng et al. (2006) it was found that surfactants such as Tween 80 and biosurfactants such as rhamnolipid can accelerate the decomposition of cellulose and hemicellulose from agricultural wastes.

Due to the benefits that biosurfactants have, such as biodegradability and biocompatibility, compared to their chemical and synthetic surfactants, they can be a suitable option for studying the improvement of the composting process. Although several studies have been conducted on the effect of inoculation on the composting process, there is a gap in the in-depth study about the mechanism of biosurfactants performance and the microorganisms that produce it on composting. This research work tries to fill the gap. In this study, in-situ treatment was applied by injecting biosurfactant producing microorganisms into the composting reactor, whereas for ex-situ treatment, the biosurfactant was produced outside the process and injected directly into the composting reactor. Additionally, the effect of final compost in each treatment as a soil modifier on plant growth was evaluated. To achieve the final goal, the efficiency of in-situ and ex-situ treatments was evaluated by *Pseudomonas aeruginosa* IBRC-M 11180 as a producer of rhamnolipids on organic fraction of municipal solid waste (OFSWM) composting as a model.

## 2. Material and methods

### 2.1. Batch production of biosurfactant

In this study, *Pseudomonas aeruginosa* IBRC-M 11180 (Iranian Biological Resource Center) was used to produce rhamnolipid. First, for cultivation, Lysogeny broth (LB) was autoclaved at 121 °C for 15 min and used as a pre-culture and then after cooling, a loop of *Pseudomonas aeruginosa* was added to the pre-culture. The pre-culture was incubated at 37 °C for 24 h in a shaker incubator (MehrTajhiz, Iran ISH 554D) at 120 rpm. Then 5% by volume of the pre-culture medium was added to the main culture medium including minerals, carbon source, and trace elements. The main culture medium containing 125 g/L of sunflower oil, 0.05 g/L MgSO4 × 7 H2O, 0.1 g/L KCl, 1.5 g/L NaNO3, 0.79 g/L Na2HPO4.2H2O, 1.16 g/L NaH2PO4. H2O and 0.1 mL/L trace elements (Amani et al., 2013). The main culture was incubated at 37 °C for one week in a shaker incubator at 120 rpm. For ex-situ treatment, 500 ml of culture suspension was centrifuged (4600 rpm; 30 min) for the separation of cells and the aqueous phase. Also, the obtained supernatant (aqueous phase) for characterization of the produced biosurfactant was analyzed. For in-situ treatment, the main culture after 24 h cultivation was added to a composting reactor directly. The purified biosurfactant was obtained according to Amani et al. (2013) and then FTIR spectroscopy (Bruker Tensor 27 IR) in ATR (attenuated total reflectance) mode was used to identify the functional groups of the produced biosurfactant.

### 2.2. Preparation of compost materials

Mixed household waste was collected from the Amol (Iran, Mazandaran) garbage transfer station as a case study, where garbage from all parts of the city is transferred. The organic fraction of municipal solid waste was separated and shredded into 20–30 mm lengths using a mechanical shredder (KHD 18.19). Mixed waste was tested for pH, conductivity, moisture content, total carbon, and total nitrogen (TKN).

### 2.3. Laboratory-scale OFMSW aerobic composting reactors

In this research work, three identical closed thermally insulated cylindrical polyethylene reactors with an approximate volume of 30 L were made (Fig. 1). The height of the reactors was 40 cm, which was filled to the height of 7 cm from the floor of the reactor by a bed of washed stone, and a mesh plate with a size of 0.5 cm holes was installed on the bed. All around the reactor was insulated with polyurethane foam. A 5 rpm Synchronous motor (49TYD-500 Synchronous AC Motor HZType) was used to mix the material inside the reactor and a centrifuge blower (JXS Small Centrifugal) was used for aeration. The air inlet was located at the bottom of the reactor and the reactor was operated at an airflow rate of 1.1 dm$^3$ min-1 kg-1 (dry mass basis). First, 10 kg of the homogeneous shredded waste was added to the three reactors and then produced Rhamnolipid from *Pseudomonas aeruginosa* (Ex-situ) and *Pseudomonas aeruginosa* inoculum (In-situ) were added to the two of them. One of them without any additives was used as a control.

### 2.4. Sample analysis

Compost samples were collected daily from different parts of the reactors and mixed well. Some parameters such as surface tension, temperature, C and N were measured daily. Parameters such as germination index (GI), Electrical conductivity (EC), pH, cation exchange capacity (CEC) and microbial counts were measured once every three days. Temperatures were measured by a digital thermometer with cable sensor probe (TFA LT-102 30.1034). The organic carbon (TOC) and nitrogen content of the samples was measured by the Walkley and Black method and modified Kajeldal method (Chemists and Chemists, 1980; Walkley and Black, 1934). The laboratory pH/EC meter (PHOE_2016) was used to measure the EC and pH of the compost extract (1:10 water: compost). CEC of compost was measured by the saturation-exchange method (Saharinen, 1996). For bacterial counts, the dilution plate method was used (Ben-David and Davidson, 2014). A mixture of 10 g of the compost sample and 90 ml of distilled water was centrifuged for 30 min at 4000 rpm and the supernatant solution was used to measure surface tension with a tensiometer (Xi et al., 2005b). Drop shape analysis (DSA 100, KRUSS, Germany) was used to measure the contact angle of the biosurfactant solution on the compacted waste surface at ambient

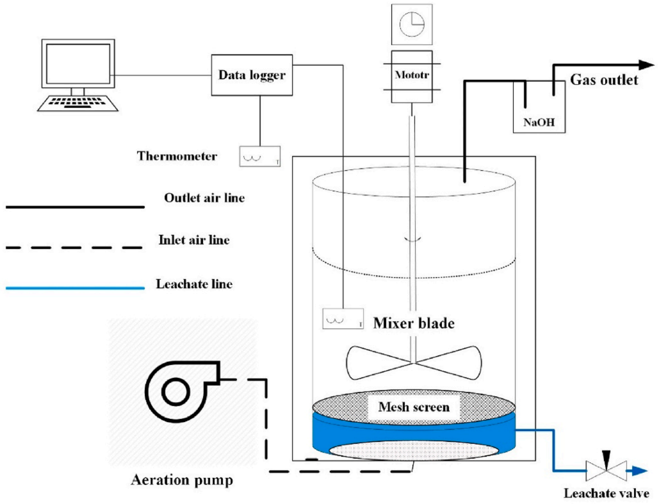

**Fig. 1.** Schematic diagram of composting reactor.

pressure and temperature. To create a homogeneous and smooth surface, the crushed waste was created by the press in the form of blocks with dimensions of 2 * 3 cm. Drop conditions on the surface of the waste were investigated by imaging according to Quetzeri-Santiago et al. (2020). In this research work, all analyses were performed in triplicate.

### 2.5. *Compost maturation testing methods*

Immature and unstable compost inhibits plant growth and has negative effects on the soil (Tang et al., 2004). In this work, to investigate the negative effects of compost on plant growth, the germination index (GI) and crop growth rate (CGR) were measured. In this study, the number of germinated Cress (*Lepidium sativum*) was counted and root length was measured based on Selim et al. (2012). The Cress seeds that were grown in Petri dishes containing a mixture of soil and composts were collected at different times (every 4 days) and weighed after drying for 3 days at 80 °C.

## 3. Results and discussion

### 3.1. *Investigation of biosurfactant production*

Batch production of biosurfactant performed at 37 °C for a one week in a shaker incubator at 120 rpm. Maximum production of biosurfactant after purification obtained about 1.5 g/L. For accurate identification and proof of the produced biosurfactant, FTIR analysis was performed in the spectral range of 4000–400 cm-1. As shown in Fig. 2, due to the C–H stretching of -CH2 and -CH3 groups, absorbance bands formed at 2926, 2856, 724 cm-1, and 840 cm-1. Also, C–O stretching bands from ester and carboxylic groups were found at 1172 cm-1. Further bands observed at 1740 cm-1 points to the occurrence of C═O stretching group. Similar results have been reported by Rikalovic et al. (2012).

### 3.2. *Investigation of temperature profile during composting*

Accurate temperature monitoring during composting is a simple way that can help us to understand the speed and quality of the process. As shown in Fig. 3, at the beginning of the composting, an increase in temperature was observed in the thermophilic phase, which occurs due to the rapid decomposition and breakdown of compounds (Haug, 2018). The initial temperature increase occurred in all reactors so that the highest temperature of ex-situ, in-situ, and control reactors reached 54, 52, and 48 °C, respectively. In addition, as shown in Fig. 3, in the initial four days, a sharp rise in temperature due to the decomposition of simple decomposable materials in all three reactors with a similar trend can be seen. After the fourth day and the completion of the simple decomposing material, the temperature rise slope in the reactors changes. In terms of time, the maximum temperature in the ex-situ reactor occurs one day earlier than other reactors on the fifth day, which indicates that the addition of biosurfactant accelerates the process. For In-situ reactors, the *Pseudomonas aeruginosa* IBRC-M 11180 didn't have enough time to grow, so that they have not been able to produce enough rhamnolipid yet. For this reason, the maximum temperature for in-situ reactor was lower than that of the ex-situ reactor and was occurred one day later on the sixth day. It is interesting that in the study of rhamnolipid production in solid-state fermentation by El-Housseiny et al. (2019), it was found that the concentration of rhamnolipid reaches a maximum on the sixth day. Gong et al. (2020) also investigated the optimization and scale-up of rhamnolipid production in the presence of polyurethane foam as inert support and found that between the 5th to the 7th days, the highest efficiency of rhamnolipid production occurs. After the sixth day, the temperature profile in all reactors had been declining. However, until the seventeenth day, (when the reactor temperature reached ambient temperature) the temperature of the in-situ reactor was higher than that of the other reactors. The high temperature of the in-situ reactor can be due to the consumption of biosurfactants in the ex-situ reactor and the continuous production of biosurfactants in the in-situ reactor. Further decomposition of organic matter in the presence of *Pseudomonas aeruginosa* IBRC-M 11180 in the in-situ reactor is also the cause. Fu et al. (2007) also showed that in the presence of biosurfactants in organic environments, water retention and organic matter dispersion increase. However, a significant difference was observed in the maximum temperature of ex-situ and in-situ reactors than that of the control reactor. This indicates that the addition of ex-situ production of rhamnolipid and in-situ production of rhamnolipid leads to further process acceleration. Similar results also have been reported by Zeng et al. (2006). One of the interesting and innovative researches that can be considered in future studies for composting process is keeping the temperature constant in the reactors (in-vessel) and comparing its performance with conventional methods, but it should also be noted that keeping the temperature constant in windrow composting is very difficult and expensive.

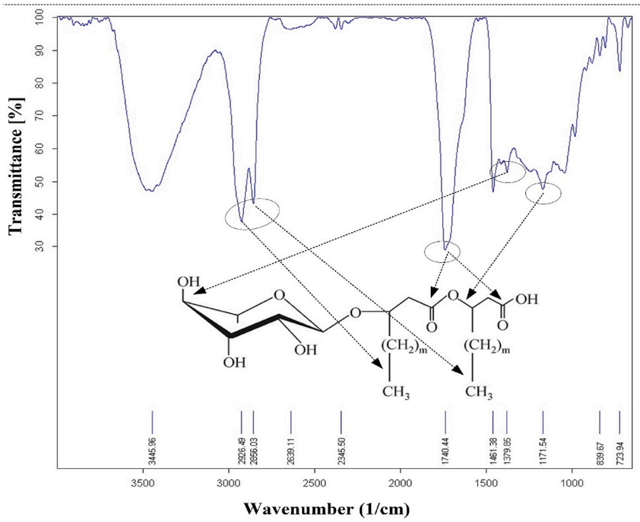

**Fig. 2.** FTIR spectrum of rhamnolipid extracted from *P. aeruginosa* IBRC-M 11180.

### 3.3. *Investigation of surface tension, CEC, pH and EC profile during composting*

The surface tension profile during the process is shown in Fig. 4 (a). The surface tension of ex-situ compost decreased to its minimum value (44.7 mN/m) on the first day, while the minimum surface tension of in-situ compost (44.81 mN/m) was observed on the sixth day. Also, the surface tension of the control compost was 56.59 mN/m on the third day of the process. The surface tension of control, in-situ, and ex-situ compost reached 65.37, 56.85, and 62.66 mN/m on the 20th day of the composting, respectively. As shown in Fig. 4 (a), a significant reduction of surface tension was observed for the in-situ and ex-situ composting than that for control composting. This can be due to the presence of biosurfactants in the compost production environment. It can be also seen in Fig. 4 (a) that the profile of surface tension for in-situ compost is significantly lower than that of ex-situ compost. This result could be due to the production of rhamnolipid by *Pseudomonas aeruginosa* IBRC-M 11180 throughout the composting period (20 days). In other words, for ex-situ composting, it seems rhamnolipid is consumed as a carbon source by native microorganisms. These results are in line with the temperature profile in the previous section. Xi et al. (2005) showed that the reduction of surface tension between a liquid and solid by inoculation of biosurfactant producing bacteria causes the substance easily to be extracted from the inherent of solid waste. According to Fig. 4 (b), during composting, CEC increased substantially from 61.4 meq/100g to 94.2, and 97.5, 103.4 meq/100g in the control, ex-situ, and

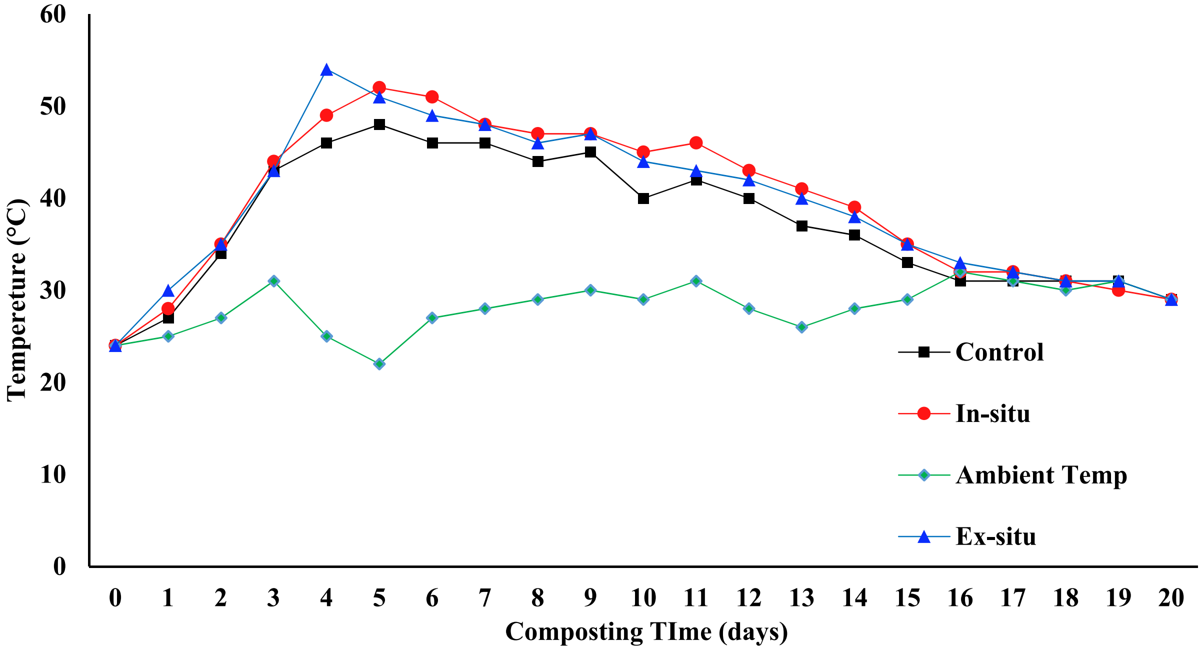

**Fig. 3.** Temperature profile during composting period.

in-situ reactors, respectively. In this work as shown Fig. 4 (b), CEC values for all reactors during composting were more than 60 meq/100g. According to Harada and Inoko (1980) the CEC of the final product should be > 60 meq/100g to be considered mature compost, therefore it could be said that the produced composts in all reactors are considered as mature compost. From Fig. 4 (b), it is clearly seen that during composting, CEC profiles for in-situ and ex-situ reactors are above the control CEC profile. These results could be due to the effect of biosurfactants in a composting environment. In addition, as seen in Fig. 4 (b), until the sixth day, the ex-situ CEC profile was higher than the in-situ CEC profile. As previously discussed in the temperature profile, there was not enough time to produce biosurfactant in the in-situ reactor yet. But after sixth day, in-situ CEC profile was higher than that of ex-situ CEC profile. These results also are in line with the previous discussion about temperature profile that biosurfactants are consumed as a carbon source by native microorganisms in ex-situ reactor. Since the CEC of the final product indicates the level of humification, therefore in our work, it could be concluded that the produced compost using the in-situ method has higher quality than the other compost. In addition, the surface tension results in the previous section (Fig. 4 (a)) also had been shown that the surface tension values in in-situ composting were lower than the surface tension values in ex-situ composting. However, from the obtained results, it can be said that further reduction of surface tension can significantly play a role in increasing product quality.

The EC and pH changes are depicted in Fig. 4(c and d). As seen in this Fig. 4, the EC values increased gradually from 1.25 mS.cm−1 to about 1.9 mS.cm−1 after 9 days for all reactors. This increase occurs due to the release of large amounts of mineral salts resulting from the rapid decomposition of organic matter in the early days of the process. Most researchers also observed this increase in their work (Grgić et al., 2019; Jahanshah et al., 2013). The EC in the control reactor decreased after the ninth day and reached 1.58 on the fifteenth day. Also from Fig. 4 (c), it can be seen that EC values between the ninth and twentieth days for both in-situ and ex-situ reactors were higher than that of the control reactor. This can be due to the presence of rhamnolipid in the composting and consequently reduction of surface tension and increase in the solubility of mineral salts such as Na +, SO42-, K +, NO3−, Cl−, and NH4+ in the samples. Although the final EC values in the in-situ and ex-situ reactors were 8.4% and 12% higher than that of the control reactor, respectively but they were in the acceptable rang according to US EPA(Walker, 1996). Meanwhile, no significant difference was observed for EC values in in-situ and ex-situ reactors. Based on Qasim et al. (2019) EC value greater than 2 in a compost reduces the plant's ability to absorb nutrients and the amount of water available. Based on the results of this section it could be said that the produced compost from our work can be used as an acceptable compost in agriculture.

As seen in Fig. 4 (d), no significant change was observed in the pH profile in all reactors. For all reactors, a rapid increase from the initial pH to about 8.5 was observed on the sixth day of composting and then a decrease occurred until the initial pH value. At the beginning of the process, the release of large amounts of ammonia from the rapid decomposition of organic nitrogen and consumption of organic acids raised the pH about 8.5. After the sixth day, the rate of decomposition and subsequently NH3 formation reduced which led to a decrease in pH. Several researchers have reported similar results (Fersi et al., 2019; Qasim et al., 2018; Tangour et al., 2019). According to Leitermann et al. (2010) due to the terminal carboxylic group, rhamnolipids are weak acids, therefore, the low pH of the ex-situ reactor can be explained in the early days of composting. Furthermore in the final days, due to the consumption of rhamnolipids as a carbon source in the ex-situ reactor by native bacteria and the production of more rhamnolipids by *Pseudomonas aeruginosa* IBRC-M in the in-situ reactor, the pH values of the ex-situ reactor were higher than that of the in-situ reactor. However, the studies of surface tension, CEC, EC, and pH, showed that the use of the produced rhamnolipid can increase the composting rate and subsequently the final product quality with the preference for in-situ product.

### *3.4. Bacterial populations profile during composting period*

The bacterial population profile during the composting period was investigated. As shown in Fig. 5, bacterial populations in all reactors increased rapidly until the 3rd day so the highest bacterial population was observed in ex-situ, in-situ, and control reactors, respectively. Further focus on Fig. 5, shows that the bacterial population decreased in the ex-situ and control reactors after the third day, but, the bacterial population increased again between the sixth and ninth days. As described in Figs. 3 and 4, it can be due to the consumption of biosurfactants in the ex-situ reactor by the native microorganisms and the

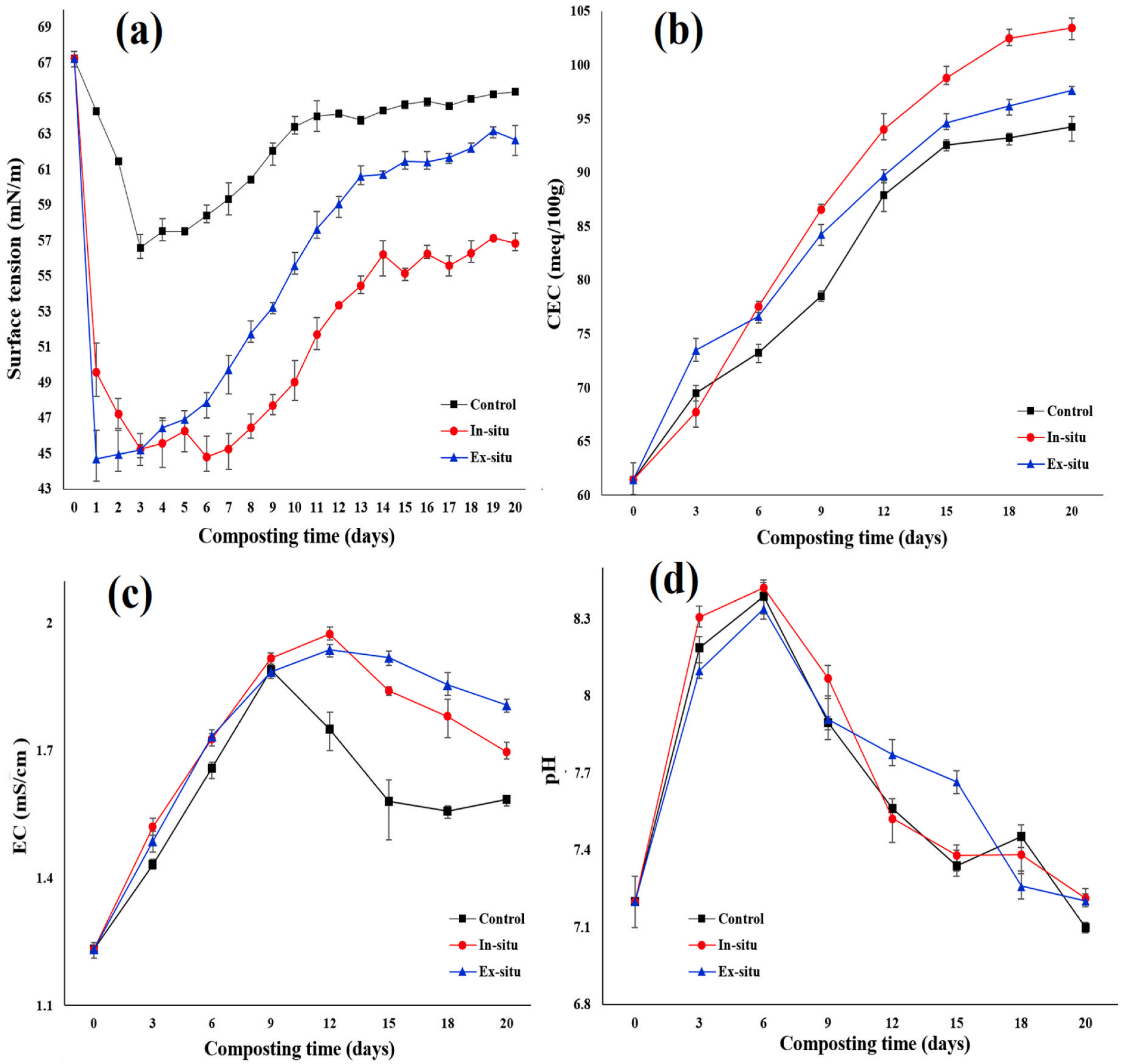

**Fig. 4.** a) Surface tension, b) CEC, c) EC, d) pH profiles during composting period.

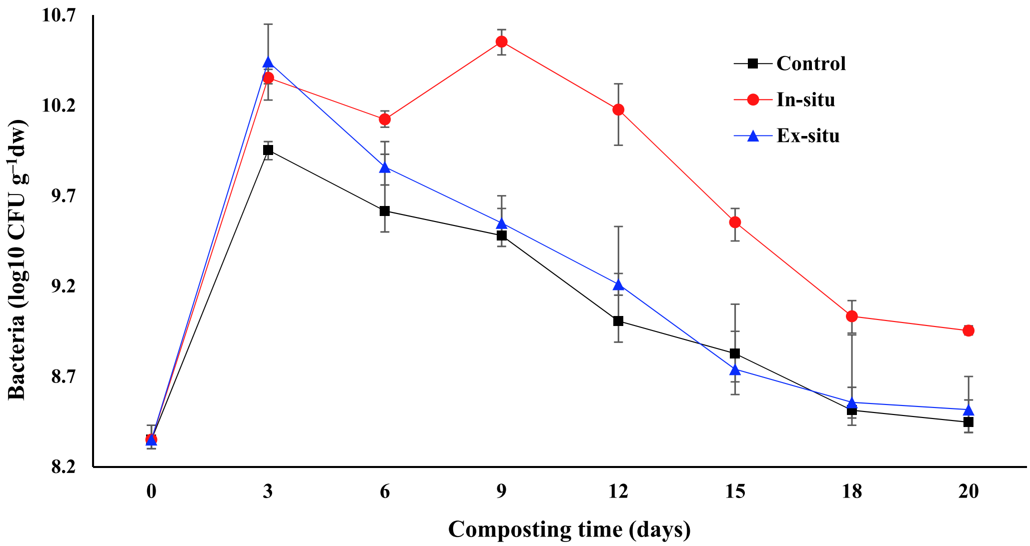

**Fig. 5.** Bacterial populations during composting period.

continuous production of biosurfactants in the in-situ reactor by *Pseudomonas aeruginosa* IBRC-M 11180. These findings are consistent with the previous results. In general, it can be said that reducing surface tension increases the transfer of organic matter as a nutrient for bacteria from solid waste to the aquatic phase and subsequently their further growth.

### 3.5. *Investigation of C/N profile during composting*

Changes in TOC, TKN, and C/N ratio can indicate the rate of decomposition of organic matter in the composting, thus, in this section, these parameters for all the reactors were investigated. The results are shown in Fig. 6. According to our experiments, TOC values were reduced in during composting (20 days) for all reactors, so that TOC variations for ex-situ, in-situ, and control reactors were 40.48%, 39.54%, and 32.65%, respectively. Also, the TKN values in control, ex-situ, and in-situ reactors increased from 1.52% to 2.27, 2.25, and 2.35, respectively, so there was no significant difference in nitrogen changes in all reactors. Since that C/N always plays an important role in the composting process and the final product quality, therefore, the C/N values investigated in this study. As seen in Fig. 6, a decrease in the C/N was also observed in all reactors, so that C/N ratios for in-situ and ex-situ reactors during the process were lower than those of the control reactor, indicating a significant effect of the biosurfactant on the process speed. However, no significant changes in C/N ratios were observed in ex-situ and in-situ reactors. The C/N ratios of final compost in ex-situ, in-situ, and control reactors were 12.83, 13.27, and 17.05, respectively. Also from our results, the C/N of the final compost in all reactors is $< 20$, which represents the produced composts are stabilized (Karnchanawong and Suriyanon, 2011).

### 3.6. *Biological assay (GI and CGR investigation)*

Immature compost will contain salt and possibly other phytotoxins. Seed germination experiments can show the toxic effects of compost on plant growth (Zucconi, 1987). According to Lasaridi et al. (2006) compost with GI values above 80% can be considered a mature and non-toxic compost. The GI values of final compost in control, in-situ, and ex-situ reactors were 98%, 118%, and 108%, respectively. Fig. 7 (a) shows that the GI value above 80% was observed for in-situ and ex-situ reactors on the 12th day, while for control reactor, it was observed on 18th day. The fact that in the presence of biosurfactants, the reduction of compost toxicity occurs faster, can lead to a significant reduction in operating and fixed costs of compost production units on an industrial scale. The results reported in Fig. 7 (a) show that overall changes in the GI have been on the rise: but a slight decrease was observed between the 3rd and 6th days in the ex-situ and control reactors. Wang et al. (2016) reported that at the beginning of the process, during the active decomposition of carbon compounds, many phytotoxic compounds, such as; ammonium ions, fatty acids, and low molecular weight phenolic acids produced that may lead to a decrease in the GI. Subsequently, by decomposition of phytotoxic compounds, reduction of compost toxicity and increase of GI occurred.

In this research work, CGR also was investigated during composting. As shown in Fig. 7 (b), the CGR in soil with ex-situ and in-situ treatments compost was more than a control process, it could be said that the produced biosurfactant can increase the quality of compost and reduce the inhibition effect on plant growth. Real images of the biosurfactant effect on the growth of *Lepidium sativum* during the composting process are also shown in Fig. 7 (b). The important point in this section is the higher CGR for a product of the in-situ reactor. It seems that the continuous production of biosurfactants in the in-situ reactor by *Pseudomonas aeruginosa* IBRC-M 11180 has led to this result. Meanwhile as seen in Fig. 7 (b), CGR values for all reactors increased until the 8th days and then began to decline.

According to kinds of literatures, at the beginning of plant growth, with increasing leaf area, as a result of better use of light, the amount of plant body production per unit area increases, and subsequently, the CGR increases(Weraduwage et al., 2015). In addition, less surface tension of the final product causes the plant roots to withstand fewer forces to absorb water and materials from the soil (Jahanshah et al., 2013). Based on Major (1980), due to the shading of the upper limbs on the lower leaves at the final growth level (8th day), the reduction of photosynthetic power of the plant, leaf fall, and subsequently the CGR decreases.

### 3.7. *Investigation of wettability alteration during composting*

According to the obtained good results from previous sections such as improving product quality and reducing process time, to better understand the performance of biosurfactants in the composting process, wettability changes of the compost surface were evaluated by drop shape analysis. The results of wettability analysis along with the schematic model are shown in Fig. 8. As shown in Fig. 8 a and 8 b, the

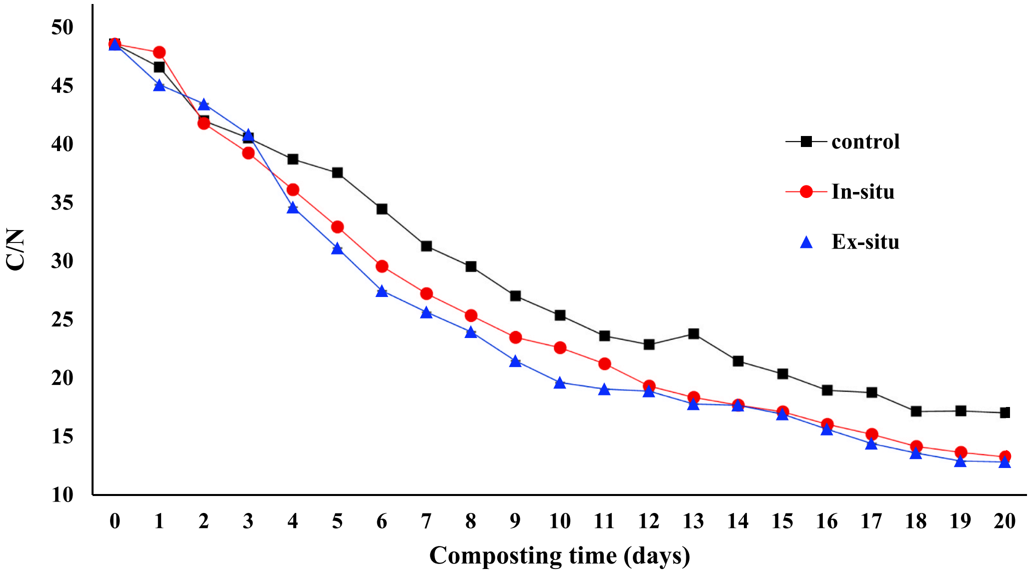

**Fig. 6.** C/N profiles during composting period.

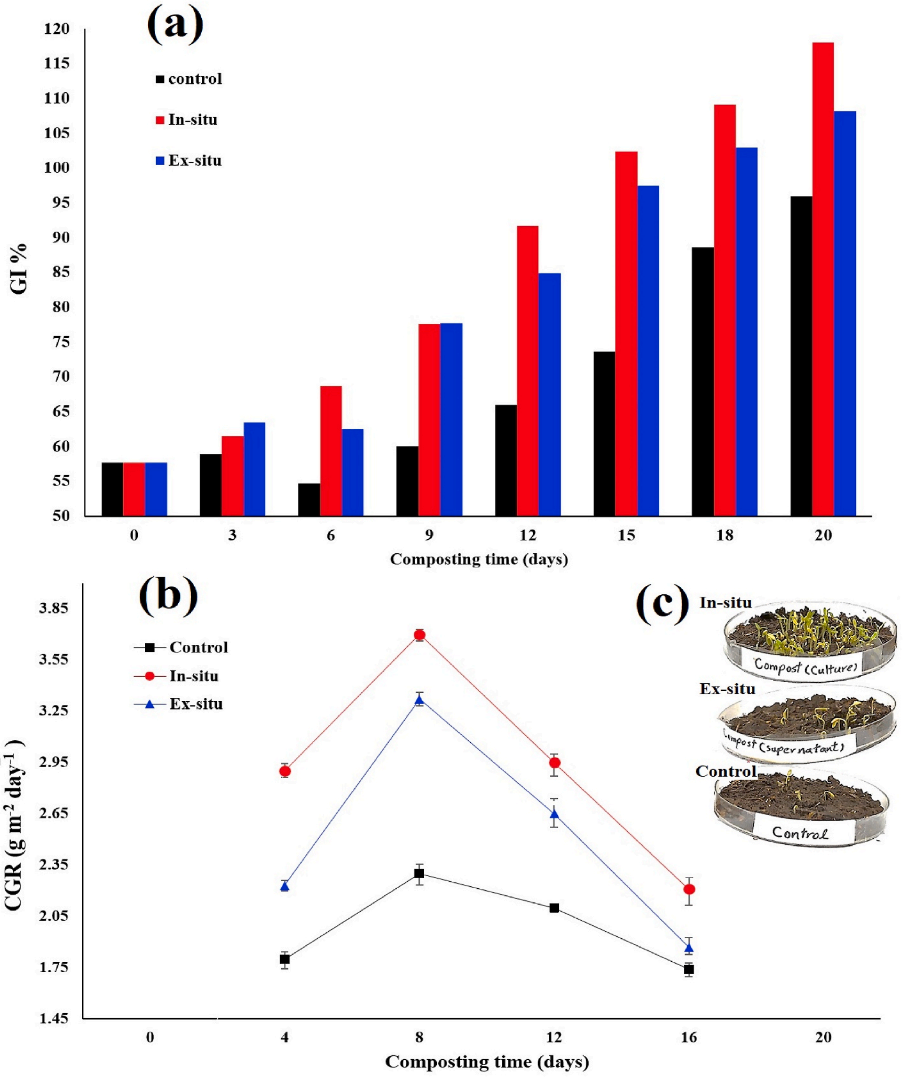

**Fig. 7.** a) GI and b) CGR profile during cress seed planting, c) Real picture of seedling growth during CGR measurement.

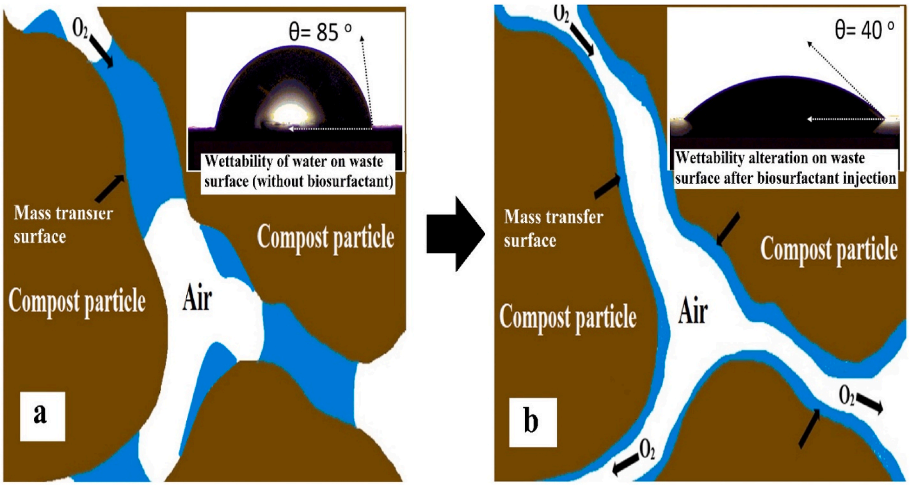

**Fig. 8.** Schematic representation of the effects of produced rhamnolipid on the composting process. a) Wettability of water on waste surface without biosurfactant (control) and b) wettability alteration on waste surface after biosurfactant injection.

addition of the biosurfactant increases the hydrophilicity of the compost surface so that produced biosurfactant altered the compact mixed waste wettability from intermediate wet (θ = 85°) toward water-wet (θ = 40°). The presence of produced rhamnolipid leads to droplet adhesion (wettability alteration) and the formation of a thin film layer of water on the surface of the waste particles, which increases the contact surface and thus better access of microorganisms to nutrient sources. Also, along with the formation of water film on waste particle, air flow occurs better through the pores of the waste, which leads to better access of microorganisms to oxygen. It can be concluded that better access of microorganisms to nutrient sources and oxygen can increase the growth of microorganisms and subsequently faster decomposition of waste particles. Anyway, according to the results obtained from this research work, it was observed that the presence of biosurfactant significantly improves the composting process compared to the control. Meanwhile, since the experiments were repeated three times, the obtained results could be also statistically reliable. Thus, the proposed techniques in this paper have considerable potential as a useful model for improvement of large scale composting.

## 4. Conclusions

The long process time and low product quality are major challenges in the composting process. To overcome these challenges, in-situ and ex-situ biological treatments were investigated using produced rhamnolipids by *Pseudomonas aeruginosa* IBRC-M 11180 because of their high biodegradability, low toxicity, biocompatibility, and availability of resources. The results showed that the presence of rhamnolipids in the composting process could improve many parameters such as maximum temperature, EC, CEC, C/N, and GI. Also, a significant reduction of 30% in composting process time was observed using these treatments, which is an economic achievement. Furthermore, the produced rhamnolipids altered the waste wettability from intermediate wet (θ = 85°) toward water-wet (θ = 40°). This result leads to an increase in the contact surface area of microorganisms with nutrient sources and consequently improves the composting process. Based on our results, the in-situ treatment due to low cost compared to ex-situ treatment has considerable potential as a useful model for improvement of large scale composting.

## Credit author statement

Mohammad Hossein Heidarzadeh carried out the research and wrote the first draft of the manuscript, Hossein Amani conceived and designed the study, Ghasem Najafpour Darzi analyzed the data and approved the final draft of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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