Surfactant efficiency on pentachlorophenol-contaminated wastewater enhanced by *Pseudomonas putida* AJ 785569

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Abstract
This study aims to evaluate the effect of three surfactants on the removal of PCP (800 mg L⁻¹) from Secondary Treated Wastewater (STWW) by *Pseudomonas putida* AJ 785569. The effect of surfactants [sodium lauryl sulfate (SDS) as anionic, Tween 80 (TW80) as non-anionic and cetyltrimethylammonium bromide (CTAB) as cationic] is tested about the following aspects: (1) bacterial growth, (2) bacterial biofilm formation or development and (3) PCP rate removal. The results showed that strain *P. putida* AJ 785569 could adsorb around 30 mg L⁻¹ and remove 600 mg L⁻¹ of PCP within 168 h of incubation. The SDS developed the growth of bacteria and the removal of PCP. This PCP removal in mineral salt medium (MSM) is around 760 mg L⁻¹ (95% degradation) higher than the ones registered with CTAB and TW80 with a value 506.75 (63% degradation) and 364.1 mg L⁻¹ (45% degradation), respectively. The obtained results of chloride concentration showed an important relation with PCP removal during incubation with an important value. Monitoring the development of bacterial biofilm, in MSM medium added with PCP (100 mg L⁻¹) by strain *P. putida* AJ 785569, showed a significant increase in the optical density value from 0.9 to 4 at λ = 595 nm, a modification of strain *P. putida* AJ 785569’s morphotype, density and color colonies.

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Introduction

The exponential growth of the human population and the resulting demand for water require careful planning to manage the water resources (Suresh and Nagesh 2015). Nowadays, water shortage and access to clean water have become serious problems worldwide (Yakamercan and Aygun 2020). Conventional wastewater treatment methods do not allow some micro-pollutant removal, such as pentachlorophenol (PCP), which is known as a biocide, herbicide, fungicide, and wood preservation industry (Salaudeen et al. 2018). PCP concentration at contaminated sites has been reported between 100 and 500 mg kg\(^{-1}\) in soil and 10–1000 mg L\(^{-1}\) in groundwater (Cort 2020). The PCP (C\(_6\)Cl\(_5\)OH) is an aromatic hydrocarbon of the chlorophenol family characterized by high relative volatility (Werheni et al. 2021), low solubility, and recalcitrant for degradation (United Nations Environment Program 1991). For a long time, PCP and various residual by-products have been highly refractory compounds in the natural environment (Monfort et al. 2020), especially suspected of being carcinogens, teratogens and highly embryotoxic compounds (Li et al. 2019; Crosby 1981). These compounds are listed among the “Pollutant Priority List” by the US EPA (1988). However, the biological treatment process as bioaugmentation is revealed as effective for PCP removal (Grandclement et al. 2017). Various microorganisms could eliminate many pesticides via their metabolic activities and their exceptional ability to adapt to harsh environments. These microbes could use several organic pollutants as a source of carbon and energy for their growth (Muraldo et al. 2003; Fahmida and Fakhruddin 2012). For example, certain oxygenic bacteria such as *Pseudomonas sp* might exhibit different rates and degrees of biodegradation and biotransformation of polychlorinated pesticides (Kelly et al. 1996; Werheni et al. 2016). After extensive research, *Pseudomonas genii* is revealed as important bacteria involved in many various phenolic products elimination (Ali 2005). These bacteria could show an important biofilm development on the interface: solid/liquid and/or the liquid/liquid during their growth (Whitely 2001). Besides, the bioaugmentation process is always negatively affected by the low water solubility of certain pollutants. This phenomenon could be primarily related to protein–protein interactions that could occur in living cells, such as signal transduction, enzyme activity regulation, immune response and as cellular components assemble (Mokaberi et al. 2020). Therefore, the bioavailability of pollutants could be improved by adding surfactants (Semple et al. 2003) and surfactant adding in
Selected bacterial strains used in the study: the *Pseudomonas* strain used in this study is isolated from seawater and identified with molecular 16S DNA sequence as *P. putida* AJ 785569 by Mehri et al. (2011). This species of *Pseudomonas* is usually used in phytoremediation process showing multifunctional important characters, like phosphate solubilization, indole-3-acetic production, antagonistic activity against pathogenic and phytopathogenic *Pseudomonas* (Mehri et al. 2014). The selection of this bacterium in this study was primarily based on its PCP tolerance and removal ability, its tolerance to various surfactants, rather than its non-pathogenic character, and its high salt tolerance considering its environmental seawater origin. This strain *P. putida* AJ 785569 used in the experiments of bioremediation is cultured in the MSM until the exponential stage. Then, all cells are collected after centrifugation at 5000 g for 5 min at +4 °C, washed twice with sterilized MSM and adjusted to approximately 310⁸ CFU mL⁻¹ for earlier experimentation as recommended by Sharma et al. (2016).

**PCP removal study in microcosm experiments**

The PCP removal is performed by injecting 1% of 310⁸ CFU mL⁻¹ of *P. putida* AJ 785569 as inoculum into a 250 mL flask containing 100 mL of MSM or sterile secondary wastewater. The composition of MSM is in mg L⁻¹: KH₂PO₄, 800; Na₂HPO₄, 800; MgSO₄7H₂O, 200; CaCl₂ 2H₂O, 10; NH₄Cl, 500 and 1 mL of metal solution comprising in (mg L⁻¹): Fe SO₄ 7H₂O, 5; Zn SO₄ H₂O. 4; MnSO₄ 4H₂O, 0.2; NiCl 6H₂O, 0.1; H₂BO₃, 0.1; CoCl₂6H₂O, 0.5; ZnCl₂, 0.25; EDTA, 2.5. After autoclaving, the solution stock of PCP is added in all experiments at 800 mg L⁻¹ and corresponding to 3.04 mM of PCP. The flasks will be incubated at 30 °C under constant shaking for seven days (Karn et al. 2010a) and at 160 rpm. min⁻¹ using an incubator shaker (ZHWH-2102 P). In bioaugmentation experiments, the *P. putida* AJ 785569 selected for this study is inoculated in MSM medium and sterilized secondary wastewater, separately added with SDS TW80 and CTAB at 20 mg L⁻¹ and PCP at 800 mg L⁻¹. All bioaugmentation experiments are conducted in darkness under shaking for 7 days and at 30 °C (ZHWH-2102 P, shaker). The bacterial cell growth is determined by measuring the optical density (OD) at 600 nm. The PCP contents from each treatment are determined by HPLC, as described by Yang et al. (2006). The cell suspension is centrifuged to separate the biomass at 8000 rpm for 5 min, and therefore the supernatant is filtrated through 0.22 µm cellophane filters as recommended by Karn et al. (2010a, b) and Sharif-Barfeh et al. (2019). The supernatant is analyzed by HPLC (Perkin Elmer Series YL9100 system) fitted on Symmetry C18 columns and UV detectors at 280 nm. The column is eluted in an isocratic mode employing a mobile phase (acetonitrile/orthophosphoric acid) at a flow of 1 mL.

**Methods and materials**

**Common conditions of the different bioremediation experiments**

Chemicals and reagents: PCP (98% purity) is obtained from Sigma—Aldrich, Germany. All other chemicals used are of the highest commercial purity. Surfactants used are known as chemically synthetic surfactants and they are SDS, Cetyltrimethylammonium bromide (CTAB), and Tween 80 (TW80).

Water used in different bioremediation experiments and physico-chemical characteristics: in this study, the secondary wastewater (STWW) is sampled from the Chargua wastewater plant in the northern suburbs of Tunis-city, Tunisia. The pH of the sample is determined using a low hydrogen electrode pH meter at 1:2.5 of secondary wastewater to water. The total nitrogen is determined by the Kjeldahl method, as recommended by Brookes et al. (1985). The total carbon is determined by the Walkley–Black chromic acid wet oxidation method according to Brookes et al. (1985). Wastewater sterilization is assured by three consecutive autoclaving processes at 120 °C and for 30 min. Sterilized wastewater will be artificially polluted by 800 mg L⁻¹ of PCP from a pre-prepared and sterilized PCP stock of 10 g L⁻¹, as a stock solution and as shown by Karn et al. (2010a).
min⁻¹. Bacterial pellets found after centrifugation are also treated with 1 ml of methanol to extract adsorbed PCP. As reported by Wattanaphon et al. (2008), the addition of surfactants could enhance the solubility of PCP in the liquid media, and since various surfactants are always present in domestic or industrial wastewater as a detergent or other various similar products, we are interested in studying the effect of PCP removal in the presence of three kinds of usual surfactants, SDS as anionic surfactant, TW80 as non-ionic surfactant, and at last CTAB as cationic surfactant. All these experiments are conducted as specified in Table 1. The dose of PCP tested is 800 mg L⁻¹ in 100 mL of MSM or sterile wastewater, while, surfactants are always tested at 20 mg L⁻¹.

**Surfactants and PCP effects in bacterial biofilm formation**

The effect of PCP on the qualitative biofilm formation and development is made in Congo red agar (CRA) medium supplemented with PCP at 100 mg L⁻¹. The surfactant SDS, CTAB, and TW80 are used to evaluate its influence on the biofilm formation, and the phenotypic release of exopolysaccharides as shown by the National Committee for Clinical Laboratory Standards (2008). The bacterial strain is streaked in triplicates and incubated at 30 ºC for 48 h (Turki et al. 2014). For PCP effect on the quantitative biofilm formation, *P. putida* AJ 785569 is cultured for 24 h at 30 ºC in 3 mL of Brain infusion broth (BHI) (Djordjevic et al. 2002), MSM + PCP (100, 500 and 800 mg L⁻¹) (Del Castillo et al. 2012). The cultures are diluted 1:20 within the same medium, and 200 µL of the ultimate suspension is added to every well of a 96-well tissue culture-treated polystyrene plate (Becton Dickinson, Franklin Lakes, NJ). After 24 h of growth at 30 ºC as reported by Meliani and Bensoltane (2014), the plates are washed vigorously three times with phosphate-buffered saline or PBS (1X, pH 7.4) to get rid of unattached and single bacterial cells, and visualized by staining with 1% crystal violet for 15 min after washing with ethanol acetone (80:20). The biofilm is quantified in quadruplicate essays, after adding 200 µL of 95% ethanol and absorbance is measured at λ = 585 nm with shaking as reported by Arena et al. (2010), Kaczorek and Olszanowski (2011) and Turki et al. (2014a, b).

**Results**

**Physico-chemical characteristics of wastewater**

A summary of the physico-chemical STWW characteristics is reported in Table 1. The STWW is neutral with a pH 7.06 ± 0.12, COD with 0.4 g L⁻¹ ± 0.08, conductivity 1827.07 µS ± 0.13, and 1.035 mg L⁻¹ total organic carbon content. According to Table 1, all these parameters are conformed to the Tunisian standard of discharges NT106.02. Some other parameters determined in this study, like suspended solids (SS), nitrates and surfactants are presented in Table 2.

**PCP removal examination**

These results of PCP removal showed the capacity of strain *P. putida* AJ 785569 to tolerate the PCP as toxic compounds at rates of 800 mg L⁻¹ in batch liquid medium MSM and sterile STWW. The PCP removal progress during 168 h presented three respective distinct phases: lag, exponential and stationary phase (Fig. 1A). A long lag phase during 0–72 h,
which could be explained by the effect of PCP present in the microcosm, and the need of *P. putida* AJ 785569 to an adaptation period. After 144 h of incubation, the strain *P. putida* AJ 785569 reached the value of 120. 10^7 UFC mL\(^{-1}\), and in parallel, 675 mg L\(^{-1}\) of PCP is found removed in MSM (Fig. 1). HPLC analysis revealed a PCP adsorption on the bacterial cells (Fig. 1, A and B), and 30 mg L\(^{-1}\) of PCP is extracted from bacterial cells after 96 h of incubation. Also, after 168 h of incubation, the PCP extracted from the bacterial cell pellet increased to 1.0235 mg L\(^{-1}\) (Fig. 1). This result allowed to show that the PCP could be attached to the cell surface and would be removed during the exponential growth phase. So, PCP could be fixed and adsorbed to the cell surface and of suspension material in each microcosm. This adsorption phenomenon would heavily depend on the concentration of PCP and the complex suspension matrix of the media.

**Surfactant effects on the PCP removal**

**PCP rates variation:** The main results of the bioaugmentation process conducted under strain *P. putida* AJ 785569 in liquid MSM and STWW added each by one surfactant as anionic (SDS), or non-anionic (TW80), or cationic (CTAB) and PCP are presented in Fig. 2. The results showed a remarkable increase in PCP elimination rate in all the treatments in sterile STWW (T\(_1\): + PCP; T\(_3\): PCP + CTAB; T\(_4\): PCP + SDS; T\(_5\): PCP + TW80) and MSM (T\(_2\): + PCP; T\(_6\): PCP + CTAB; T\(_7\): PCP + SDS; T\(_8\): PCP + TW80). Analysis of the PCP variation histogram in all treatments showed a significant PCP decrease in MSM and STWW after seven days, with a value of 459 and 380 mg L\(^{-1}\), respectively (Fig. 2). Besides, this difference in PCP elimination results in MSM and STWW could be explained by the fixation of PCP to the varied wastewater material or the pH effect. Also, the surfactant addition enhanced PCP elimination in MSM and STWW. This result seemed related to the bioavailability and adsorption phenomena of PCP in the liquid medium. In addition, PCP rates showed that SDS influenced the PCP elimination than TW80 and CTAB in STWW and MSM. At all, remarkable PCP rate removal was observed in medium added with different surfactants: SDS at 577.99 mg L\(^{-1}\) (MSM) and 676.66 mg L\(^{-1}\) (STWW); TW80 with a value of 506.75 mg L\(^{-1}\) (MSM) and 364.1 mg L\(^{-1}\) (STWW) and CTAB with a value of 236.52 mg L\(^{-1}\) (MSM) and of 413.76 mg L\(^{-1}\) (STWW).

**Bacterial growth** The growth of strain *P. putida* AJ 785569 is monitored in STWW and MSM supplemented by surfactants (SDS, TW80 and GTAB) and contaminated with 800 mg L\(^{-1}\) of PCP (Fig. 3). The results showed that the latency phase is slow and takes 24 h. The number of bacteria does not exceed 30 10^7 CFU. mL\(^{-1}\) in SDS treatment. During this phase, the bacteria resisted adaptation to this hostile environment and exploit pesticide as a source of carbon. Thus, the cell would have the possibility of new enzymes synthesis, and the cell size increased getting ready for a new cell division. The duration of this phase would depend on the bacterial inoculum and the composition of the growth medium used. With strain *P. putida* AJ 785569 in sterile STWW, this phase does not appear to be influenced.

**Fig. 1** PCP (800 mg L\(^{-1}\)) removal by *Pseudomonas putida* AJ 785569 (A) in MSM in the supernatant (a) and adsorbed (a), HPLC Chromatograph example at 30 °C at 1 and 7 days B of bioaugmentation treatment in MSM.
Fig. 2 Evaluation of the bioaugmentation process of the *Pseudomonas* strain in the MSM and STWW by monitoring the concentration of PCP (800 mg L⁻¹) after 168 h at 30 °C. SDS sodium dodecyl sulfate; CTAB hexadecyltrimethyl ammonium bromide; TW80 Tween 80; PCP pentachlorophenol.

Fig. 3 Monitoring of microbial growth in MSM (A) and wastewater (B) of exogenous Pseudomonas strains on the five bioaugmentation treatments at 30 °C and for 7 days at 150 rpm. SDS sodium dodecyl sulfate; CTAB cetyltrimethylammonium bromide; TW80: Tween 80; PCP pentachlorophenol.
by the increase of PCP rates in the liquid medium MSM or STWW. After this phase, growth would resume again until a maximum reached indifferent time at 48, 72, 72 and 24 h in STWW: with a value 80.74 $10^7$ UFC. mL$^{-1}$ (without surfactants), 122.094 $10^7$ UFC. mL$^{-1}$ (SDS), 72.05.2 $10^7$ UFC. mL$^{-1}$ (CTAB), and 82.98 UFC. mL$^{-1}$ (TW80) (Fig. 3). In MSM, the maximum growth without surfactants is 107.34 UFC. mL$^{-1}$. During this exponential phase, once the bacterial division has started and the bacteria are in full activity, the degradation of PCP increases until it reaches its maximum value. It is also possible that a smaller amount of liquid medium promotes contact between the bacteria and the contaminated medium. Thus speeding up the degradation process. SDS binds to polysaccharides (carbohydrates) when the salt concentration is high, thus eliminating the polysaccharides consumed by the strain from the solution, which slows degradation. The declining part of all curves after 144 h under different treatments represents the dead phase. At this stage, the bacteria lose the ability to divide, and the number of dead cells exceeds the number of living cells. Similarly, at this stage, bacteria lack nutrients.

Free chloride variation In this study, the variation of free chloride rates in the medium is carried out to treat the process of bioaugmentation every 24 h and during 168 h of incubation at 30 °C and under shaking at 150 rpm (Fig. 4). The results shown in Fig. 4 exhibited that the evolution of chloride is proportional to the bacterial biomass and the PCP elimination. The highest value obtained in chloride rates is around 3.25 mg L$^{-1}$ with STWW + SDS + PCP. The lowest value is in the treatment of STWW + SDS with a value of 1 mg L$^{-1}$. So, it is noticed that the bacterial biomass increased in parallel with the free chloride content in the microcosm experiment. The decrease in chloride at the end of each incubation experiment and the slowdown in the degradation of PCP could probably be related to the death of bacteria following the worst new conditions of salinity ruling in the field. We recognize that wastewater contains a higher chlorine level, and particularly with MSM mineral experiments. The strain *P. putida* AJ 785569 showed a high potential for PCP degradation in the presence of a high level of residual free chlorine. In addition, SDS surfactant at 20 mg L$^{-1}$ rate affects and speeds up the PCP degradation.

Principal component analysis about surfactant effect in bioaugmentation of PCP contaminated wastewater in supplementary material 1 allowed to show positive correlations between bacterial biomass, chloride at 168 h growth and PCP rates. So, the increase of bacterial biomass in the growth medium could show a removal PCP process.
Surfactants and PCP effects on biofilm formation

To show the capacity of biofilm development by the strain *P. putida* AJ 785569, two methods are adopted; the standard method of staining with Violet Crystal (CV) and the method of culture on Rouge Congo (RCA) agar added with 100 mg L⁻¹ PCP. Rouge Congo agar is a very suitable medium for the detection of biofilm-producing strains as recommended by Chaieb et al. (2005). Strain *P. putida* AJ 785569 presented on BHI medium a morphotype with a large colony, mucoid and brown. Under the effect of the toxic effect of PCP, the size of the bacterial colony becomes tiny. In this study, the effect of three surfactants (anionic, non-anionic, and cationic) about the formation of bacterial biofilm is monitored. The two detergent CTAB (cationic) and TW 80 (non-anionic) presented the same results as the treatment with PCP alone, besides adding SDS (anionic detergent) allowed an increase of the size of the colony that remained large with the color orange change (Table 3, supplementary material 2). On the other side, these results lead to saying that strain *P. putida* AJ 785569 could form biofilm in two liquid growth mediums: BHI and MSM, and the productivity of biofilm increased with the rate of PCP. The results obtained in Fig. 5 showed many significant differences between the different treatments; the most important PCP removal is registered with SDS treatments (with 100, 500, and 800 mg L⁻¹ PCP treatments respectively giving 3.8, 3.7, and 4 OD). Treatments with the two detergents CTAB and TW80 affected positively as well the biofilm formation by the strain *P. putida* AJ 785569 for the three rates of PCP tested and showing a means OD values around 1.4. However, the BHI usually served as a growth medium for bacteria in like biofilm investigation and free of surfactants, and PCP showed the lowest biofilm production by this species of Pseudomonas with an OD = 0.54; beside the Tween 80 asses at 100 or

| Treatment | BHI | MSM + PCP | MSM + SDS + PCP | MSM + CTAB + PCP | Tween 80 |
|-----------|-----|-----------|-----------------|-----------------|----------|
| BHI       | kldh| aldhi     | dhij            | ef              | a        |
| MSM + PCP | ijbmc| ef        | eg              | f               | ab       |
| MSM + SDS + PCP | bcd | eg        | f               | d               |
| MSM + CTAB + PCP | abed| ef        | f               | cd              |
| Tween 80 | a   | ab        | d               |                 |

SDS sodium dodecyl sulfate, CTAB cetyltrimethylammonium bromide, TW 80 Tween 80, PCP pentachlorophenol, BHI brain infusion broth
500 of mg L⁻¹ PCP gave the lowest OD of 0.24 and 0.58, respectively.

Discussion

Our results showed that the strain *P. putida* AJ 785569 showed the ability to survive in some hostile environment represented by wastewater formerly contaminated with PCP as a large toxic element, and to degrade this compound added in wastewater at 800 mg L⁻¹ PCP. We could explain this result by being isolated from seawater as a hostile environment and salt content and can withstand and degrade PCP despite a high concentration of 800 mg L⁻¹ of PCP in soil at 160 mg Kg⁻¹ could degrade around 100% of PCP. *P. aeruginosa* strain RA2 selected from PCP contaminated value 6.3. In addition, Chang et al. (1995) described that the *P. aeruginosa* could rapidly eliminate around 50 mg L⁻¹ of PCP at pH conditions.

In addition, previous work showed that some Pseudomonas species usually grow rapidly and are known for their ability to metabolize large amounts of substrates, including toxic organic chemicals, such as PCP molecules. For example, *Pseudomonas sp.* (Bu34) could degrade about 4000 mg L⁻¹ of PCP, as reported by Lee et al. (1998). Karn et al. (2010a) showed that *P. stutzeri* CL7 is ready to grow up to 600 mg L⁻¹ of PCP. Werheni et al. (2017) documented that after adding 100 mg L⁻¹ of PCP to the culture growth medium, the species of *P. fluorescens* allowed the removal of around 250 mg L⁻¹ after 96 h of incubation at controlled conditions.

Wolski et al. (2006) reported that *Pseudomonas aeruginosa* could rapidly eliminate around 50 mg L⁻¹ of PCP at pH value 6.3. In addition, Chang et al. (1995) described that the *P. aeruginosa* strain RA2 selected from PCP contaminated soil at 160 mg Kg⁻¹ could degrade around 100% of PCP after 16 days of incubation. In our work, we studied the strain *P. putida* AJ 785569 ability to remove and solubilize PCP in two growths medium, STWW and MSM added by three surfactants, Tween 80, SDS and CTAB. The potential solubilization of PCP is evaluated by adding separately these last three synthetic surfactants. Adding the “SDS” in the medium resulted in a speed-up removal of PCP and enhancing the growth of this bacterium. This improvement registered about PCP removal and bacterial growth increase could be explained by the solubility increase and the great bioavailability of PCP in wastewater following some surfactant positive actions. Our results agreed with Hassen et al. (2018) in the point that SDS is more effective than Tween 80 in enhancing PCP solubilization. The role of Tween 80 in this experiment is to solubilize pesticides and later to enhance PCP removal by strain *P. putida* AJ 785569. Similarly, the results of Bustamante et al. (2012) showed that the critical value of micellar concentration produced by the TW80 is around 0.01 mM, value much lower than the one recorded for SDS with a value around 8.10 mM. We could anticipate according to Bustamante et al. (2012) that surfactant addition to the contaminated environment allowed an interfacial tension reduction, increasing the mass transfer of pollutants and improving the desorption, solubilization, and biodegradation of organic compounds. The improved biomass production of bacteria is often explained by an intensification of some important protein phenomena. When protein–protein interactions occurred in living cells, such as signal transduction, regulation of enzyme activity, immune response, and assembly of cellular components, surfactants could improve the activity of biomass enzyme (Mokaber et al. 2021). The bioaugmentation process is performed in vitro (MSM) and compared with the one in vivo conditions (STWW), and since physical and chemical parameters prevailing in the proper milieu largely affect the molecular interactions, and principally, the interaction between enzymes and some residual compounds like PCP. It is well established that pH affects seriously the whole biological activities and especially those related to proteins and to enzyme activity. PCP is a hydrophobic pesticide, and surfactants allowed an increase in their solubility in STWW (Chamani and Heshmati 2008).

In the same investigations, Chojnacka (2010) indicated that PCP as a contaminant could be adsorbed to different surface materials through the process of bioaccumulation in the presence of surfactants. Therefore, biodegradation is a kinetic process that affects the sensitivity of living organisms to chemical substances (Ashauer et al. 2012), and the absorption of pollutants is higher than its loss rate, the process will be profitable (Chojnacka 2010).

Therefore, various contaminants and by-products available in several environments could be kept by diversified living cells, like bacteria and fungi where they accumulate and reacted with some special compounds. Some studies in this field have shown that the availability of some surfactants in the milieu could allow or enhance the desorption of these compounds (Aronstein et al. 1991; Mata Sandoval et al. 2002), following complex solubilization processes and some organic compounds biotransformation activities (Garon et al. 2002). The study of Cort and Bielefeldt (2000) reported the consequences of various kinds of surfactant availability regarding the PCP degradation by *Sphingomonas chlorophenolicum* sp. strain RA2 and revealed no PCP degradation effect in the presence of cationic surfactants. The study of Cort and Bielefeldt (2000) agreed with our results about the surfactant effects and its availability in the medium. Lanthier (1999) reported that surfactants usually showed a negative effect on PCP degradation, and the bacteria that cause PCP
degradation require little liquid culture medium for PCP degradation. Besides, the previous study of Riviere et al. (2001) showed that simulated solvents and/or surfactant mixtures could affect the PCP absorption at a well-insulated cell bacterium. Therefore, surfactants or surface-active agents are substances that altered the prevailing conditions of the adsorption surface, resulting in a decrease in the surface tension between liquids or between liquids and solids (Reznick et al. 2010a, b).

To summarize the changes observed in the bio-adsorption-bioaugmentation treatment of PCP contaminated secondary wastewater during the incubation period, the different treatments are illustrated by the principal component analysis (PCA) program (supplementary material 1). PCA graphically differentiated diverse wastewater sample treatments, like MSM, MSM + SDS, MSM + CTAB; MSM + TW80: STWW, STWW + SDS, STWW + CTAB, STWW + TW80, based on the sampling time, T0 start of treatment and TF end of treatment.

The two main components account 61.17% of the total variance. STWW + SDS and MSM + SDS wastewater treatment are positively correlated with the two parameters, chloride content and bacteria in number. The formation of bacterial biofilm might be a point in favor that accentuated the efficiency degree of the bioremediation process and the bioaugmentation of wastewater polluted by PCP (Werheni et al. 2017). Regarding various electrostatic and hydrophobic interactions and adsorption/desorption phenomena, biofilm systems have been shown to contribute effectively for toxic trace compounds removal in wastewater (Rakmi and Anuar 2009). To evaluate the role of bacterial biofilm about PCP removal, we tested the qualitative and quantitative biofilm development of our strain P. putida AJ 785569. Results showed an accelerated biofilm formation and a changed morphotype according to the liquid medium used. During an antecedent study, mucous colony morphotype is registered with the RCA method and mainly characterized by their width, roughness, convexity, and smoothness (Touati 2013).

The result is similar to the one obtained for our strain P.putida AJ 785569 cultured in free PCP medium, and this morphology is found affected and changed after PCP was added in the culturing medium. Also, our results showed a clear improvement of the biofilm development succeeding the SDS adding in the medium. Thus, the enhancement of biofilm formation may be related to the exopolysaccharide (EPS) matrix embedding bacterial cells (Mokeberi et al. 2021). This exopolysaccharide matrix plays a major role in the protection of agglomerated bacteria and its survival related to its structural stability, besides its role in some nutrient delivery and genetic transfer (Kumar and Digamber 2018). Therefore, many studies have been conducted on the bioremediation of some aromatic pollutants in biofilm principle reactors (Baraldi 2008, and Farhadian et al. 2008).

At last, the strain P. putida AJ 785569 selected for this study might be a potential candidate for satisfactory PCP removal when monitored at adequately controlled conditions, promoting bacterial biofilm development and PCP removal.

Conclusion

Because the bioremediation aimed to decrease the organic pollutants at undetectable levels or lower than the limits established by standards as safe or tolerable, several criteria must be filled and seen to be a practical method for treatment. Bioremediation may restore contaminated water through the broad biodegradable capabilities developed by microorganisms toward undesirable organic compounds. The selected strain P.putida AJ 785569 isolated from seawater showed a clear aptitude for PCP degradation. How completely and how efficiently this occurs depending on the microorganisms and some environmental conditions. This bacterium could remove 800 mg L−1 of PCP in wastewater bio-augmentation process. This process could be accelerated with synthetic surfactant adding like SDS, since surfactants contained both hydrophilic and hydrophobic groups, therefore reducing surface and interfacial tensions of immiscible fluids, and led increase the solubility and sorption of hydrophobic organic and inorganic compounds. Also, the strain P. putida AJ 785569 could develop an important biofilm. The biofilm, as cited in many works, could be an important factor to stimulate PCP biodegradation in wastewater. This study showed the effects of surfactants as a stimulating product for PCP removal in treated wastewater. These results are only preliminary and require consolidation by more in-depth analyzes to better understand the PCP degradation and its residual metabolites and by-products generated in wastewater.

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