Volume 87(4). Published January, 01, 2022
www.jnsciences.org
E-ISSN 2286-5314

Bioaugmentation of Pentachlorophenol by Pseudomonas mosseli HM627603 and P. putida HM627611in secondary treated wastewater–UVC254 inactivation

RIM WERHENI AMMERI [1,2*], SALMA KLOULA, INES MEHRI[1], WAFA HASSEN[3], ABDENNACEUR HASSEN [1]

[1] Laboratory of Treatment and Valorization of Water Rejects, Center of Research and Technologies of Water (CERTE), Technopole Borj-Cédria, Tunisia, Tunisia.
[2] Eremology and Combating Desertification, Arid Regions Institute of Medenine, Tunisia
[3]Research Unit of Analysis and Process Applied to the Environmental, APAE Higher Institute of Applied Sciences and Technology Mahdia, the University of Monastir, Monastir, Tunisia

***Corresponding author:** rimwerheni@gmail.com

Abstract – The accumulation of organic pollutants, pesticides, fertilizers, etc. causing critical health trouble to humans, animals, many aquatic ecosystems, and plants. This study evaluated the elimination of the pesticides as pentachlorophenol (PCP: 800 mg L^{-1}) by bioaugmentation process in sterile secondary treated wastewater (STWW) and mineral salt medium (MSM). Also, the impact of surfactant addition (sodium dodecyl sulfate 'SDS' and Cetyltrimethylammonium bromide 'CTAB') in PCP elimination phenomena. The PCP rate in different treatments was analyzed by high-performance liquid chromatography (HPLC). The bacteria biomass was measured through a spectroscopy approach at 600 nm optical density (OD_{600}). The chloride rates were determinate through the calorimetric approach. In addition, the STWW disinfection was released through the UV radiation technique. The HPLC analysis confirmed that the strain *P. mosseli* HM627603 and *P. putida* HM627611 were able to tolerate and remove respectively 99.6 and 76.12% of PCP (800 mg. L^{-1}). The chloride rates increased after 7 days in all treatments with a value of 0.64 to 3.12 g L^{-1} for *P. mosseli* HM627603 in MSM. The SDS surfactant induces an increase in the PCP elimination from STWW through *P. putida* HM627611. The bacterial inactivation became quicker within 80 seconds in sterile STWW supplemented with PCP at 800 mg L^{-1} for *P. putida* HM627611 tested in agar medium.

Keywords: Bioaugmentation, Pentachlorophenol, Wastewater, UVC-254 inactivation, Pseudomonas

1. Introduction

The use of wastewater after adequate treatments makes up a potential water resource that could interest several sectors, such as agriculture, especially in countries suffering from a deficiency in water resources (Oppenheimer et al. 1993). Conventional wastewater treatment procedures 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, Aregbesola et al. 2020). On the other side, Tunisia is one country that signed the law of the European Commission that prohibits the use of organochlorines for 80 years (Hassen et al. 2021). Because of its high availability and very favorable price, it has been increasingly used for different purposes in several countries around the world. Its primary use was emphasized in the wood industry as a preservative.

PCP (C_6Cl_5OH) is a highly substituted aromatic compound, prepared by reacting chlorine with phenol in the presence of a catalyst at high temperature with known chemical isomers (Chen et al. 2015, Werheni et al. 2021a). However, PCP is still used in industry as a wood preservative for railroad ties, wharf pilings, and utility poles, but, the sale and use of PCP have been restricted to certified applicators (Kim et al. 2019). Many indigenous microorganisms cannot use PCP as their sole carbon source because of its high toxicity and inhibitory effects, and so it will be found a bio- or -accumulated extensively in the natural environment (Patachia and Croitoru 2016). Several conventional physical and chemical procedures have been used for PCP transformation (Zhang et al. 2008; Ren et al. 2016). Although these techniques are fast, they are very expensive and not environmentally friendly because of the formation of more residual toxic intermediates, requiring further processing for complete mineralization (Olaniran and Igbinosa 2011; Patel and Kumar 2016). But bioremediation offers an effective and eco-friendly method of removing chlorophenols from the environment (Olaniran and Igbinosa 2011). Several bacteria such as *Sphingobium chlorophenolicum* (Takeuchi et al. 2001), *Shingomonas sp.* UG30 (Cassidy et al. 1999), *Acinetobacter sp.* ISTPCP-3 (Sharma et al. 2009), *Pseudomonas stutzeri* CL7 and *Enterobacter sp.* SG1 (Karn et al. 2010), and Burkholderia *cepacia* (Joshi et al. 2015) have been reported as good PCP degraders (Chanama and Chanama 2011; Dai et

al. 2003). Therefore, the availability of pollutants like PCP in contaminated sites could be improved by adding surfactants (Semple et al. 2003), and this surfactant added in contaminated sites could reduce the interfacial tension and enhance the remediation process (Gao et al. 2007; Franzetti et al. 2008). So, surfactant adding may improve the recovery rate of organic pollutants through two important mechanisms. First, through the reduction of the interfacial tension between water and pollutants, thus slowing down the organic component mobility (Cheah et al. 1998).

Besides, disinfection of wastewater by UV-C254 irradiation is a credible alternative to chemical disinfection, because of the absence of toxic residual by-products usually generated and identified during chemical disinfection (Blatchley, 1996; Carrigan and Cairns, 1991; Lindenauer and Darby, 1993; Mark and Gordon, 1994; Oppenheimer et al. 1993). UV disinfection is also characterized by a short contact time and a more efficient antiviral action (Oppenheimer et al. 1993; Masschelein et al. 1989)

The principal aim of the present study was to evaluate the capacity of two strains of Pseudomonas selected from different origins like soils, plants, wastewater…) to tolerate and eliminate the PCP at a high rate of 800 mg. L^{-1}. The effects of SDS and CTAB as surfactants were examined about PCP removal from water. Also, the effects of PCP adding in treated secondary wastewater for the bacterial inactivation assessment through the UV-C254 reactor were monitored.

2. Materiels and methods

2.1. Chemicals and reagents

PCP (98% purity) was 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 sodium dodecyl sulfate (SDS) and Cetyltrimethylammonium bromide (CTAB).

2.2. Physico-chemical characteristics of the water used in this study

The secondary wastewater (STWW) is sampled from the industrial Charguia 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).

2.3. Selected strain

Pseudomonas strain used in this study was isolated from soil and treated macrophyte plant (Table 1), and identified by molecular 16S DNAr sequencing according to Mehri et al. (2011). The process of isolation and selection of these Pseudomonas species was primarily based on their tolerance and PCP removal ability in the mineral salts medium (MSM) growth medium (Sharma et al. 2016). Usually used in phytoremediation process, showing multifunctional important characters (Mehri et al. 2014).

Table1. Identification and description of the strains used in the bio-augmentation process (Mehri et al. 2011)

	Accession number with 16S DNA	Phosphatase production	Bacteriocin production	Biofilm production	Morphotype	Origin
P. mosseli	HM627603	+++	+ (30)	Moderate	Flat non-mucoid	Treated wastewater
P. putida	HM627611	++	+ (10)	Low	Smooth and rigid	Soil

+: Positive test; ++: Important activity; +++: Very important activity

2.4. PCP bioaugmentation experimentation

The PCP removal protocol is achieved by adding 3. 10^8 CFU. mL^{-1} of the two selected strains of *P. putida* HM627611 and *P. mosseli* HM627603 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}: KH_2PO_4, 800; Na_2HPO_4, 800; $MgSO_47H_2O$, 200; $CaCl_2$ $2H_2O$, 10; NH_4Cl, 500 and 1 mL of trace metal solution comprising in (mg. L^{-1}) Fe SO4 $7H_2O$, 5; Zn SO4 H2O, 4; $MnSO_4$ $4H_2O$, 0.2; NaCl $6H_2O$, 0.1; H_3BO_3, 0.1; $CoCl_26H_2$ O, 0.5; $ZnCl_2$, 0.25; EDTA, 2.5. After autoclaving, the PCP solution stock was added in all experimentations at 800 mg L^{-1} and corresponding to 3.04 mM of PCP (Werheni et al., 2021 b). The flasks so prepared will be incubated at 30ºC under constant shaking for 7 days and at 160 rpm. min^{-1} using an incubator shaker (ZHWY-2102 P) as reported by Karn et al. (2010a).

2.5. Chloride dosage

A filtered volume of 50 ml (Whatman filter paper) of different treatments of sterile STWW and MSM (without and with PCP) introduced into a 250 Erlenmeyer flask, will be added successively with 2 to 3 drops

of pure nitric acid, a pinch of 0.2 g of calcium carbonate and 3 drops of 10% potassium chromate. The 0.1 N silver nitrate solution was then poured through a buret until a reddish and persistent colored precipitate appeared. The volume of 0.1 N silver nitrate used for the titration will be noted. The chloride content will be expressed in g. L^{-1} according to the following formula: Cl〗 = (A-B)/V×C×1000×dilution factor; with A: Volume of the titrating solution poured by a volume V ml of sample., B: Volume of the titrating solution poured by a volume V mL of distilled water, and C: Number of mg of chlorides 'equivalent to 1 mL of titrant solution (10 mg Cl-).

2.6. Bacterial growth

Bacterial biomass at the beginning (T0) and end of the bioremediation experiment (TF) within 168 hours of incubation for the different treatments was examined through OD_{600} spectrophotometric techniques (Spectro UVS-2700 Dual Beam Labomed, Inc).

2.7. HPLC analysis

PCP removal change was quantified by using high-performance liquid chromatography (HPLC) through interval sampling of 1 mL of culture within 24 h. HPLC measurement was accomplished by a Perkin Elmer Series YL9100 system filtered on symmetry C18 columns and detector UV at 280 nm. The molecular suspension was centrifuged at 8000 rpm for five minutes, and the supernatant was filtered via a 0.22 mm Cellophane filter. The HPLC parameters were accorded to Karan et al. (2018) study. The percentage of PCP removal was calculated as follows = (PCP awareness T0/PCP awareness Tx) ×100, as reported by Khessairi et al. (2014). All studies have been carried out in triplicates.

2.8. UV-C 254 treatment

Samples of STWW were irradiated using a pilot photoreactor initially described by Hassen et al. (2000) within the Avicenna project 093AVI054. This reactor has a sliding rack having a bench that can receive 6 Petri dishes. At an adjustable height of this bench, a low-pressure mercury-vapor lamp (90 cm length, 13 mm width, variable UV emission power at 254 nm that fluctuated between 10 and 65 Watts) is hung and inserted in a reflector to ensure homogeneous irradiation. The UV intensity emitted by the lamp was measured by Vilbert-Lourmat radiometer a specific UV253.7 detector. The UV dose was expressed in $mW.s.cm^{-2}$ as Dose = UV intensity × Time d′ exposure UV. Bacterial cells were simultaneously or not exposed to UC-C 254 and 800 mg L^{-1} of PCP to assess their survival or resistance in this hostile condition. Bacterial strains were inoculated in 25 mL of three categories of growth medium: Nutrient broth (1), sterile STWW (2), and STWW + PCP (800 mg L^{-1}) (3). Different treatments were incubated at 30°C in a shaker. These samples were poured into five sterile Petri and put into the photoreactor before the photoreactor was closed and samples were taken in sterile tubes (Figure 1). The samples were taken at different successive interval of: 5; 10; 20; 40; 60; 80; 120; 160 seconds. The monitoring of the number of bacteria after UVC-exposure of the different treatments was carried out by bacterial count on a nutrient Agar.

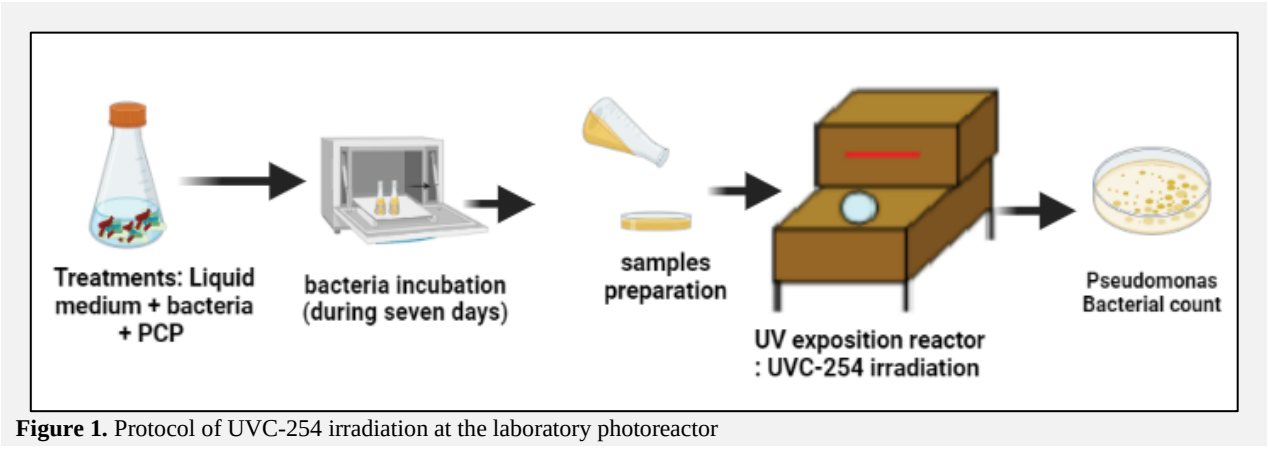


Figure 1. Protocol of UVC-254 irradiation at the laboratory photoreactor

3. Results and discussion

3.1. Physical and chemical characterization of secondary wastewater

The secondary wastewater used in the present study was sampled from the most industrial, municipal sewage plant of Charguia I of Tunis-City. This plant treats on average about 60.000 m^3/day of wastewater (domestic, hospital, rain, and industrial) coming from several areas of the Grand Tunis. The wastewater treatment plant of Charguia I included consecutively four categories of treatment: the pre-

treatment, the primary, the secondary or biological, and the final clarified wastewater. The STWW showed a neutral pH with an average value of 7.35 ± 0.13. The chloride content was on average 3.25 ± 1.76 g. L^{-1} (Table 2). The surfactant content was important and had an average value of 64.75 ± 1.77 mg. L^{-1}.

Table 2. Some physical and chemical analysis for secondary treated wastewater

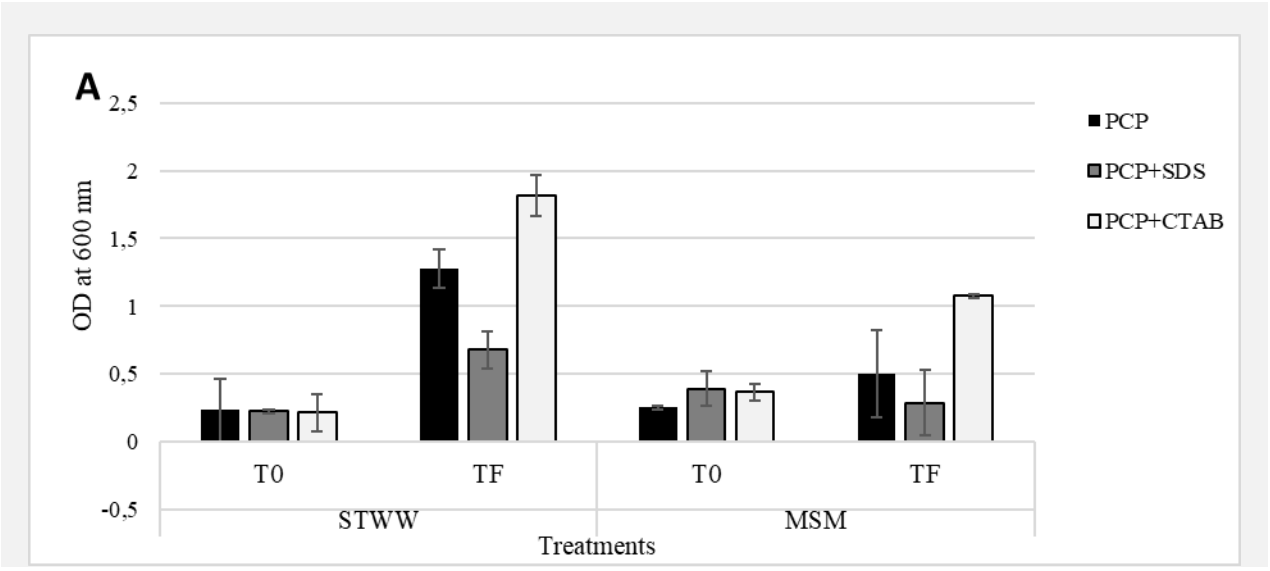
Parameter		value
pH $_{H2O}$		7.35 ± 0.13
Conductivity	µs/cm	1623.07 ± 0.13
COD	mg L^{-1}	40 ± 3.18
C/N		0.30
Chloride	g Cl^{-} L^{-1}	3125 ± 1.76
Dry matter	g L^{-1}	1.46 ± 0.15
SM	g L^{-1}	0.605 ± 0.01
DBO_5	g L^{-1}	0.645
NO_3	mg L^{-1}	14.6 ± 0.28
SUR	mg L^{-1}	64.75 ± 1.77

SUR: Surfactant; $pH_{H2O:}$ Hydrogen potential (1/5); C: Total carbon; N: Nitrogen; $DBO_{5:}$ Biological Oxygen Demand; DCO: Chemical Oxygen Demand; SM: Suspended material; C/N: Carbon/nitrogen ratio.

Effects of PCP on bacterial growth

Bacterial biomass monitoring of the two selected strains *P. mosseli* HM627603 and *P. putida* HM627611 was performed in MSM and STWW medium at 25°C artificially contaminated with PCP at 800 mg. L^{-1} by measuring optical density versus incubation time (Figure 2). Microbial growth showed an increase from T0 to TF in sterile STWW for *P. putida* HM627611 within 7 days of incubation in laboratory-controlled conditions. With STWW treatments, the bacterial growth looked very important with the treatment STWW+ PCP + CTAB and showed an average optical density of about 1.815 nm. Thus, the bacterial growth of *P. putida* HM627611 recorded in MSM looked less important than the one registered in STWW. This bacterial growth with PCP, PCP+SDS, and PCP+CTAB treatments gave an average variable optical density of 0.5, 0.2, and 1.07 respectively.

With *P. mosseli* HM627603, the growth revealed lower than the one obtained for *P. putida* HM627611 in STWW, with values less than 1 except with MSM + PCP treatment where the growth is very important with an OD around 2. The surfactant CTAB adding appeared as a promoter of *P. putida* HM627611 growth. In this research, the selection and choice of *P. putida* strain HM627611 and *P. mosseli* HM627603 are not arbitrary but based essentially on their potential and capacity to tolerate and degrade organic xenobiotic pollutants, a proper character largely recognized in the literature (Karn et al. 2010; Hassen et al. 2018). Werheni et al. (2017) documented that after adding 100 mg L^{-1} of PCP to the growth medium, the species of *P. fuorescens* allowed the removal of around 250 mg. L^{-1} after 96 h of incubation under controlled conditions.



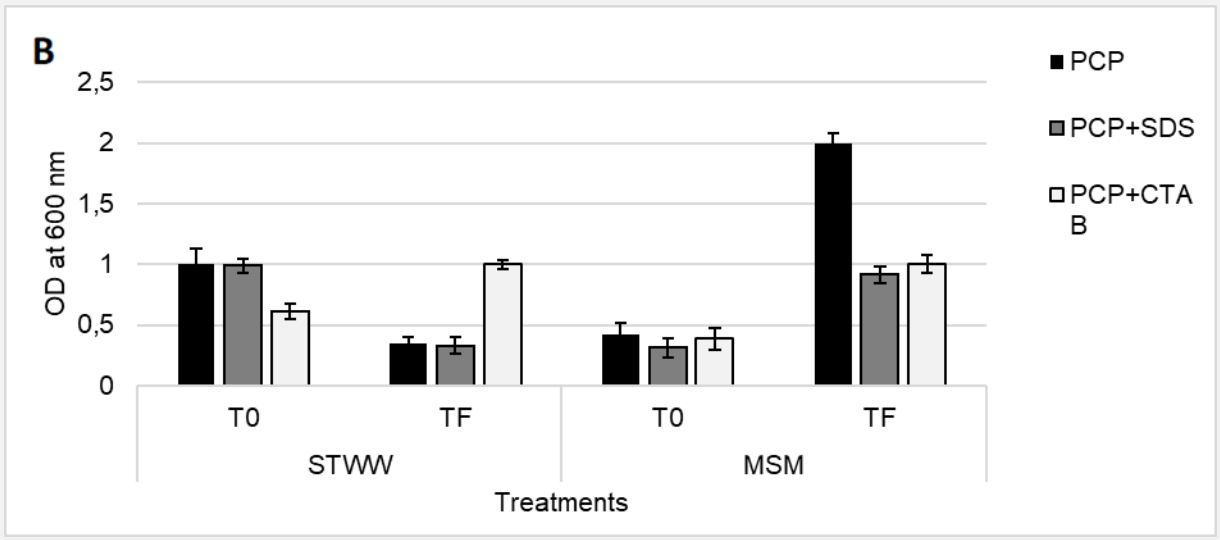
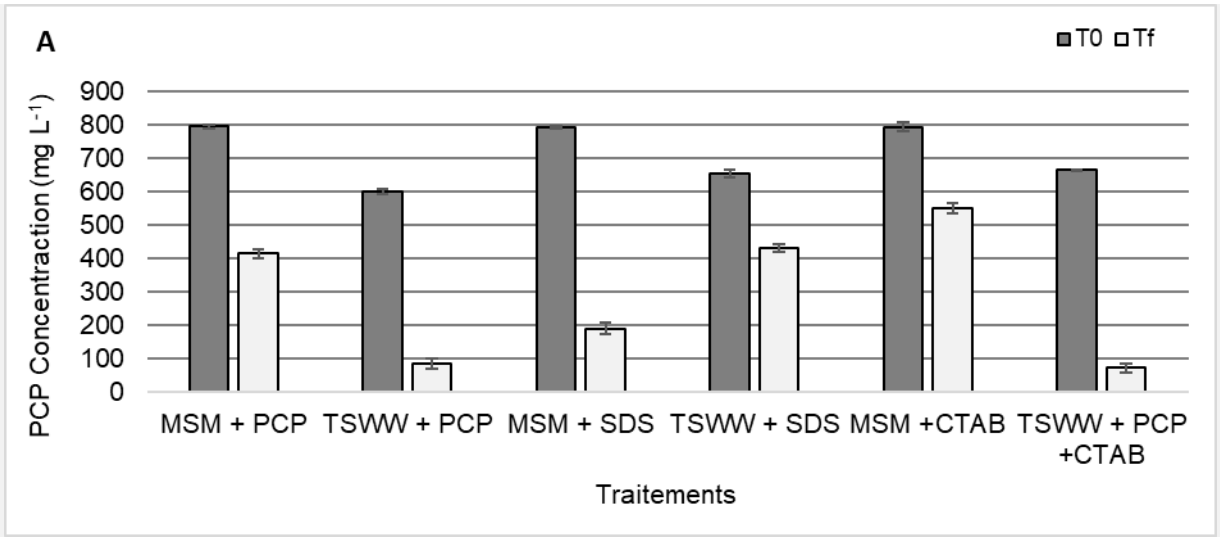


Figure 2. Measurement of bacterial growth of two *P. putida* HM627611 (A) and P. *mosseli* HM627603 (B) in MSM and sterilized STWW artificially contaminated with PCP (800 mg. L^{-1}).
MSM: Mineral salt medium, PCP: Pentachlorophenol, SDS Sodium dodecyl sulfate, CTAB: Cetyltrimethylammonium bromide.

3.2. PCP removal

The monitoring of the PCP content in different bioaugmentation treatments was performed by HPLC analysis were illustrated in Figure 3. The two selected strains were found to have an ample capacity to tolerate and eliminate PCP at 800 mg. L^{-1}. The PCP content in MSM and STWW showed a decrease after seven days, with a value of 415.33 (48 % degradation) and 86.22 mg L^{-1} (89.22 % degradation) for *P. putida* HM627611, respectively. Also, a less important activity has been detected for *P. mosseli* HM627603 after seven days with a value of 327.56 (59.05% removal) and 206.6 mg L^{-1} (74.17 % removal), respectively. The *P. putida* HM627611 showed significant PCP removal in STWW+ PCP+CTAB treatment with an average recorded amount of about 72.89 mg L^{-1} (99.6 % removal). This PCP removal by *P. mosseli* HM627603 looked lower than the one achieved by *P. putida* HM627611. The STWW+PCP+CTAB treatment appeared to be the most efficient with the *P. mosseli* HM627603 by about 191 mg L^{-1} (76.12 % removal). As reported by Lanthier (1999), some known surfactants like SDS, CTAB showed a negative effect on PCP degradation; the study released by Hassen et al. (2021) and Werheni et al. (2021a) confirmed the positive effects of surfactants on PCP removal at a high rate in liquid MSM or STWW.

The solubilization of PCP was evaluated by adding separately these last three synthetic surfactants SDS, CTAB, and Tween 80. For example, the SDS adding in the medium resulted in a speed-up of PCP removal and enhancing the bacterial growth. This improvement registered on the scale of PCP removal and bacterial growth could be explained by the increase of solubility and availability of PCP in the medium following some surfactant positive actions. Our results agreed with those of Hassen et al. (2018) and Mokaberi et al. (2021) who underlined that SDS looked more effective than Tween 80 in enhancing PCP solubilization and bacterial growth.



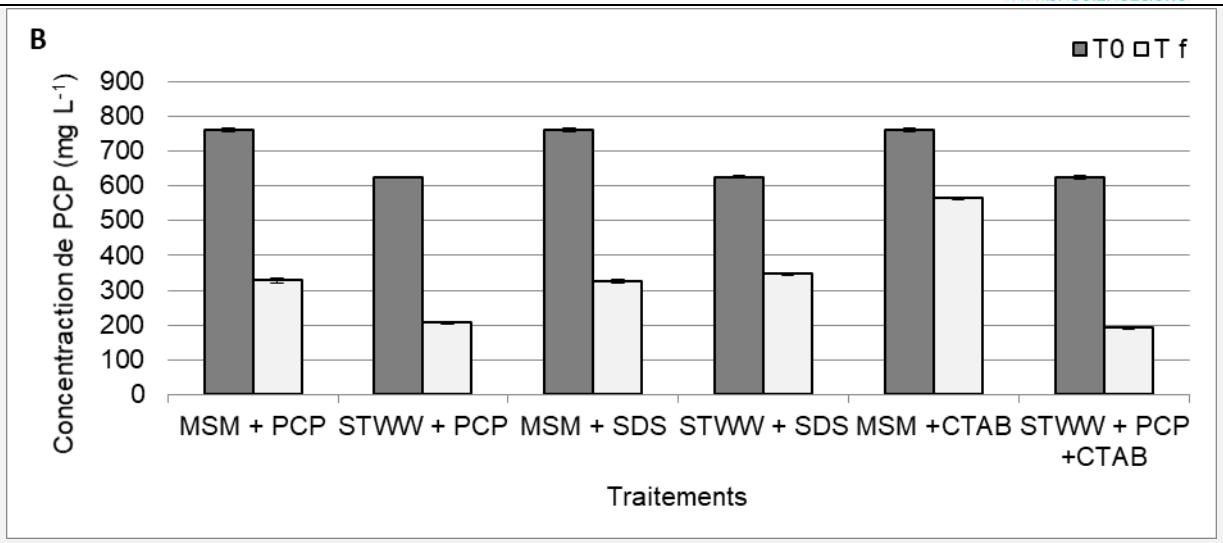


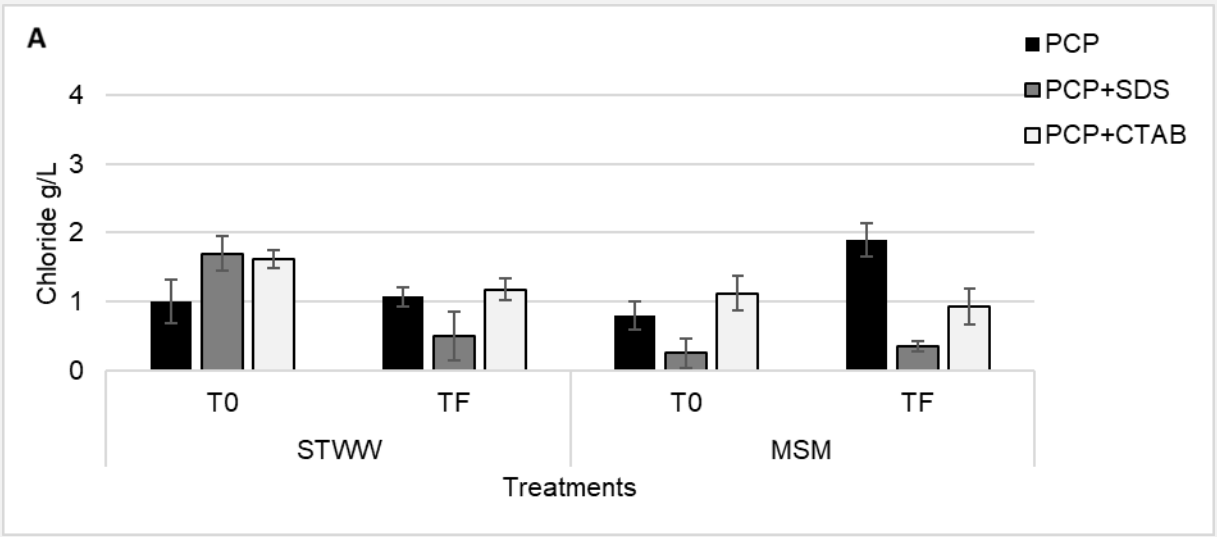
Figure 3. PCP rates were measured in different treatments inoculated with *P. putida* HM627611 (A) and *P. mosseli* HM627603 (B) in MSM and STWW

MSM: Mineral salt medium, PCP: Pentachlorophenol, SDS: Sodium dodecyl sulfate, CTAB: Cetyltrimethylammonium bromide

3.3. Chloride rate variation

The monitoring of chloride rates in the different bioaugmentation treatments processed by the two strains *P. putida* HM627611 and *P. mosseli* HM627603 in the presence of the SDS and CTAB in STWW and MSM were illustrated in Figure 4. The highest concentration of chloride was recorded in the treatment STWW+PCP+CTAB in the presence of *P. mosseli* HM627603 related to the PCP removal. Watts et al. (1990) reported that the increase in chloride content is proportional to the PCP removal increase in wastewater and soil. Besides, Oturan et al. (2001) showed that the increase in chloride is because of the release of CO_2, HCl, and H_2O resulting from the PCP biodegradation process.

These last results agreed with the one reported in STWW that outlined the propositional relationship of chloride variation and PCP removal by *P. putida* strains and synthetic detergents (Werheni et al. 2021 b).



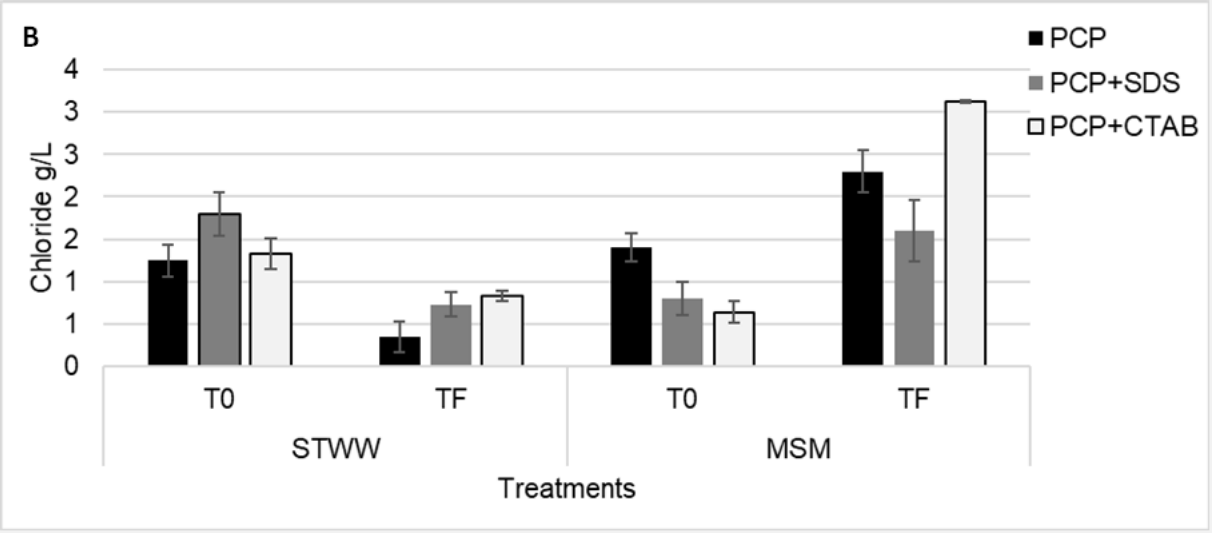


Figure 4. Monitoring of chloride rates in the different bioaugmentation treatments conducted by using the two strains of *P. putida* HM627611 (A) and *P. mosseli* HM627603 (B) in MSM and STWW.
MSM: Mineral salt medium, PCP: Pentachlorophenol, SDS: Sodium dodecyl sulfate, CTAB: Cetyltrimethylammonium bromide.

3.4. UV-C inactivation

The effect of different inactivation treatments of the two Pseudomonas strains was carried out as described by Hassen et al. (2000) in three liquids: the nutriment broth 'NB', STWW, and STWW+ PCP) using the laboratory photoreactor. The rate of inactivation of the two strains of *P. putida* HM627611 and *P. mosseli* HM627603 looked faster in the three liquids added with 100 mg L^{-1} of PCP. The simultaneous effects of PCP and UV facilitate the inactivation of bacterial cells within only 60s of exposure for *P. putida* HM627611 and 120s for *P. mosseli* HM627603 (Table 3). Non-PCP supplemented liquid NB and STWW inactivation was delayed to 140s for *P. mosseli* HM627603. The UV treatment was observed effective in this study for improving the sanitary quality of the water discharged in the natural environment after the bioaugmentation process of STWW contaminated with PCP. The batch study showed that the kinetics of bacterial inactivation varied according to the applied UV dose. The first instance (2 ± 10s), corresponds to the mean doses of 10 and 80 mW. s. cm^{-2} appeared as a determining factor. To verify these UV doses applied, the time needed for the total disinfection of the Pseudomonas was calculated and the results were presented in (table 2). At 253.7 nm, the power of the reactor is between 10 and 65 watts, and the intensity is 10 mW.s.cm-². Intensity= TX × 10 (W.s.cm^{-2}) (Tx: Time needed to totally inactivate the cell bacteria)**.** The doses of disinfection are greater than the one given, which can be explained by the effect of the photoreactor used, the characteristics of the wastewater, and even the environment, which has a significant influence on the dose necessary for total disinfection. We cannot confirm the accuracy of these doses recorded because according to Tormo and Barral (2004), the bactericidal or bacteriostatic effect is changed depending on the bacterial species. The energy (UV doses) at the wavelength of 254 nm to inactivate Pseudomonas is not among the highest, showing their relative sensitivity. The particles suspended in the water will screen the passage of UV RAYS. Thus, we will seek to filter the water to make the treatment much more effective. In this study, the time of inactivation of Pseudomonas (as Gram-negative bacteria) at STWW is revealed shortly as compared to the one registered for bacillus strains (as Gram-positive bacteria), which are about 5 and 10 min (Jiang et al. 2009)

Table 3. Bacteria count of wastewater plus PCP strains after UV (CFU/ml).

		Time (s)									
Strain	**Liquid**	**0**	**5**	**10**	**20**	**40**	**60**	**80**	**120**	**140**	**160**
	NB	$21280\times10^5\pm 123$	$206\times10^5\pm145$	$20\times10^5\pm 136$	$5\times10^5 \pm 169$	$2\times10^5 \pm 168$	$0{,}4\times10^5{}_{\pm}$	20736 ± 321	0	0	0
***P. putida* HM627611 (UFC / mL)**	**STWW**	$336\times10^8 \pm 236$	$4500\times10^5\pm165$	$2540\times10^5 \pm128$	$120\times10^5 \pm 168$	$2\times10^5 \pm 136$	5642 ± 147	432 ± 129	150 ± 12	0	0
	STWW + PCP	$4\times10^{10} \pm 124$	$4521 \times10^5\pm 156$	$523\times10^5 \pm156$	$23\times10^4 \pm 198$	$12\times10^4{}^{\pm} 456$	$6\times10^4 \pm 112$	0	0	0	0
	NB	$28560\times10^5 \pm 165$	$25600\times10^5 \pm 115$	$470\times10^5 \pm 22$	$6\times10^5 \pm 45$	$3\times10^5 \pm 118$	$0{,}19\times10^{5}{}_{\pm 118}$	31680 ± 23	800 ± 116	147 ± 123	0
***P. mosseli* HM627603 (UFC / mL)**	**STWW**	$9\times10^8 \pm121$	$7800 \times10^5 \pm 98$	$6532 \times10^5 \pm 45$	$3562 \times10^5 \pm 63$	$19\times10^5 \pm 224$	$10 \times10^5 \pm 135$	832 ± 38	452 ± 110	38 ± 147	0
	STWW +PCP	$7\times10^8 \pm 136$	$5 \times10^8 \pm 81$	$120\times10^6 \pm 22$	$62\times10^6 \pm 85$	$12\times10^6 \pm 119$	$12\times10^5 \pm 247$	$8\times10^2 \pm 69$	0	0	0

NB: Nutriment broth, STWW: Secondary treated wastewater; PCP: Pentachlorophenol.

4. Conclusion

In this study, we monitored the high ability of two strains of *P. putida* HM627611and *P. mosseli* EU301780 to tolerate and remove the PCP (800 mg L^{-1}). Also, in this work, *P. putida* HM627611showed a better performance of PCP removal than *P. mosseli* EU301780 in artificially contaminated sterile STWW. The monitoring of PCP removal can be related to chloride measurement, which correlated negatively with PCP disappearance. Thus, chloride and PCP rate correlate negatively with bacterial growth. In addition, the bacteria removal was released with UV radiation exposure as a disinfection effect. Besides, the UV inactivation exposure is revealed lower for *P. putida* HM627611than the one *P. mosseli* EU301780 within only 80 seconds. The process of bioaugmentation by the two strains of Pseudomonas was revealed efficient in the treatment of STWW and usually used as a good functioning operator for general clean-up contaminated sites like wastewater; while there are still limitations and drawbacks that need clarifications.

5. References

Aregbesola OA, Mokoena MP, Olaniran AO (2020) Biotransformation of pentachlorophenol by an indigenous Bacillus cereus AOA-CPS1 isolated from wastewater effluent in Durban, South Africa. Biodegrad. doi:10.1007/s10532-020-09915-w.

Blatchley ER (1996) Ultraviolet irradiation and chlorination/dechlorination for municipal wastewater disinfection. Water Environ Res 68, 194-198.

Brookes PC, Kragt JF, Powlson DS, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. *Soil Biol and Biochem 17* (6), 831-835.

Carrigan P, Cairns B (1991) Disinfection application of ultraviolet technology. Water Cond. Purif. 33, 43-48.

Chanama M, Chanama S, Shaw P J, Chitnumsub P, Leartsakulpanich U, Yuthavong Y (2011) Formation of catalytically active cross-species heterodimers of thymidylate synthase from Plasmodium falciparum and Plasmodium vivax. *Molec biol reports*, *38*(2), 1029-1037.

Cheah P, Robbins B (eds) (1998) Cosmopolitics: thinking and feeling beyond the nation, vol 14. U of Minnesota Press, Minneapolis

Chen F, Zhao L, Yu W, Wang Y, Zhang H, Guo LH (2020) Dynamic monitoring and regulation of pentachlorophenol photodegradation process by chemiluminescence and TiO2/PDA. Journal of Hazardous Materials, 399, 123073.

Dai M, Rogers J B, Warner JR, Copley S D (2003) A previously unrecognized step in pentachlorophenol degradation in Sphingobium chlorophenolicum is catalyzed by tetrachlorobenzoquinone reductases (PcpD). J of bact, 185(1), 302-310.

Franzetti A, Di Gennaro P, Bestetti G, Lasagni M, Pitea D, Collina E (2008) Selection of surfactants for enhancing diesel hydrocarbons-contaminated media bioremediation. J Hazard Mat 152(3):1309–1316s

Gao F, He T, Wang H, Yu S, Yi D, Liu W, Cai Z (2007) A promising strategy for the treatment of ischemic heart disease: mesenchymal stem cell-mediated vascular endothelial growth factor gene transfer in rats. Canad J of Card, 23(11), 891-898.

Hassen A, Mahrouk M, Ouzari H, Cherif M, Boudabous A, Damelincourt JJ (2000) UV disinfection of treated wastewater in a large-scale pilot plant and inactivation of selected bacteria in a laboratory UV device. *Bioresource Technology*, *74*(2), 141-150.

Hassen W, Cherif H, Werhani R, Raddadi N, Neifar M, Hassen A, Cherif A (2021) Exhaustion of pentachlorophenol in soil microcosms with three Pseudomonas species as detoxification agents. Arch of Microb, 203(7), 4641-4651.

Hassen W, Cherif H, Werhani R, Raddadi N, Neifar M, Hassen A, Cherif A (2021) Exhaustion of pentachlorophenol in soil microcosms with three Pseudomonas species as detoxification agents. Archives of Microbiology, 203(7), 4641-4651.

Joshi D R, Zhang Y, Gao Y, Liu Y, Yang M (2017) Biotransformation of nitrogen-and sulfur-containing pollutants during coking wastewater treatment: Correspondence of performance to microbial community functional structure. *Water Research*, *121*, 338-348.

Karn SK, Chakrabarty SK, Reddy MS (2010a) Pentachlorophenol degradation by Pseudomonas stutzeri CL7 in the secondary sludge of pulp and paper mill. J Environ Sci 22:1608–1612.

Kim K, Lim SY, Kim J, Lee JU, Lee S, Kim P, … Cheong H (2019) Antiferromagnetic ordering in van der Waals 2D magnetic material MnPS3 probed by Raman spectroscopy. *2D Materials*, *6*(4), 041001.

Jiang C, Sun H, Sun T, Zhang Q, Zhang Y (2009) Immobilization of cadmium in soils by UV-mutated Bacillus subtilis 38 bioaugmentation and NovoGro amendment. *J of hazard mat*, *167*(1-3), 1170-1177.

Lanthier M (1999) *Étude de la biodégradation anaréobie du pentachlorophénol dans le sol par desulfitobacterium frapierie souche PCP-l* (Doctoral dissertation, Université du Québec, Institut national de la recherche scientifique.

Lindenauer KG, Darby J (1993) Evaluation of ultra-violet light disinfection: Signi®cance of photoreactivation. Davis Water Environ. Fed. Plan. Des and Operations of effluent Disinfection Syst. Whippany, NJ, 23±25 May, p. 49

Mark JM, Gordon RF (1994) Will UV be the disinfectant of the 90 s? It others some advantages over other means. Water Technol. 17, 74-78.

Masschelein J, Debacker E, Chebak S (1989) Laboratory investigations on the disinfection of water by UV-light. Rev. Sci. Eau. 2, 29-41.

Mehri I, Turki Y, Chérif H, Hassen A, Meyer JM, Gtari M (2011) Genetic and functional heterogeneities among fluorescent Pseudomonas isolated from environmental samples. The J of Gen and Appl Microb, 57(2), 101-114.

Mokaberi P, Babayan-Mashhadi F, Amiri Tehrani Zadeh Z, Saberi M R, Chamani J (2021) Analysis of the interaction behavior between Nano-Curcumin and two human serum proteins: combining spectroscopy and molecular stimulation to understand protein-protein interaction. Journal of Biomolecular Structure and Dynamics, 39(9), 3358-3377.

Olaniran A O, Igbinosa E O (2011) Chlorophenols and other related derivatives of environmental concern: properties, distribution, and microbial degradation processes. Chemosphere, 83(10), 1297-1306.

Olaniran A O, Igbinosa E O (2011) Chlorophenols and other related derivatives of environmental concern: properties, distribution, and microbial degradation processes. Chemosh, 83(10), 1297-1306.

Oppenheimer JA, Montgomery W, Pasadena CA, Hoagland JE, Laine JM, Jacangelo JG, Bhamrah A (1993) Microbial inactivation and characterization of toxicity and by-products occur in reclaimed wastewater disinfected with UV radiation. Alit Water Environ. Fed. Plan. Des, Operations of effluent Disinfection Syst. Whippany, NJ, 23-25 May, p. 13.

Patachia S, Croitoru C (2016) Biopolymers for wood preservation. In Biopolymers and biotech admixtures for eco-efficient construction materials (pp. 305-332). Woodhead Publishing.

Patel BP, Kumar A (2016) Biodegradation of 2, 4-dichlorophenol by Bacillus endophyticus strain: optimization of experimental parameters using response surface methodology and kinetic study. Desal and Water Treat, 57(34), 15932-15940.

Ren Y, Qiao Z, Niu Q (2016) Topological phases in two-dimensional materials: a review. Reports on Progress in Physics, 79(6), 066501.

Salaudeen SA, Acharya B, Dutta A (2018) CaO-based CO2 sorbents: A review on screening, enhancement, cyclic stability, regeneration and kinetics modeling. *Journal of CO2 Utilization*, *23*, 179-199.

Aregbesola E, Ortega-Beltran A, Falade T, Jonathan G, Hearne S, Bandyopadhyay R (2020) A detached leaf assay to rapidly screen for resistance of maize to Bipolaris maydis, the causal agent of southern corn leaf blight. European Journal of Plant Pathology, 156(1), 133-145.

Semple C, Steel M (2003) Phylogenetics. Oxford University Press on Demand

Sharma A, Thakur S, Kumar V, Kanwar MK, Kesavan AK, Thukral AK, ... Ahmad P (2016) Pre-sowing seed treatment with 24-epibrassinolide ameliorates pesticide stress in Brassica juncea L. through the modulation of stress markers. Front in plant sci, 7, 1569.

Sharma R, Yetton P, Crawford J (2009) Estimating the effect of common method variance: The method—method pair technique with an illustration from TAM Research. Mis Quarterly, 473-490.

Takeuchi M, Hamana K, Hiraishi A (2001) Proposal of the genus Sphingomonas sensu stricto and three new genera, Sphingobium, Novosphingobium, and Sphingopyxis, based on phylogenetic and chemotaxonomic analyses. Int J of Syst and Evolut Microb, 51(4), 1405-1417.

Werheni AR, Hidri Y, Hassen W, Mehri I, Khlif N, Hassen A (2021a) Surfactant efficiency on pentachlorophenol contaminated wastewater enhanced by Pseudomonas putida AJ 785569. Arch Microbiol. https://doi.org/10.1007/s00203-021-02486-1

Werheni AR, Mokni Tlili S, Mehri I, Badi S, Hassen A (2016b) Pentachlorophenol biodegradation by Citrobacter freundii isolated from forest contaminated soil. Wat Air Soil Poll 227, 10:1–12. HTTPS:// doi.org/10.1007/s11270-016-2959-z

Werheni AR, Hassen W, Hidri Y, Simeone GR, Hassen A (2021c) Macrophyte and indigenous bacterial co-remediation process for pentachlorophenol removal from wastewater. Int J Phytoremediation 227:1–12. https://doi.org/10.1080/15226514.2021.1933897

Zhao Y, Yin X, Qin H, Zhu F, Liu H, Yang W, … Deng H (2008) Two supporting factors greatly improve the efficiency of human iPSC generation. *Cell stem cell*, *3*(5), 475-479.

Jiang, C., Sun, H., Sun, T., Zhang, Q., & Zhang, Y. (2009). Immobilization of cadmium in soils by UV-mutated Bacillus subtilis 38 bioaugmentation and NovoGro amendment. *Journal of hazardous materials*, *167*(1-3), 1170-1177.

Karn SK, Chakrabarty SK, Reddy MS (2010) Pentachlorophenol degradation by Pseudomonas stutzeri CL7 in the secondary sludge of pulp and paper mill. J Environ Sci 22:1608–1612

Hassen W, Neifar M, Cherif H, Najjari A, Chouchane H, Driouich RC, Cherif A (2018) Pseudomonas rhizophila S211, a new plant growth-promoting rhizobacterium with potential in pesticide bioremediation. Front Microbiol 9:34.

Werheni RA, Mehri I, Badi S, Hassen W, Hassen A (2017) Pentachlorophenol degradation by Pseudomonas fuorescens. Wat Qual Res J 52(2):99–108