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Pentachlorophenol Effect on Auxin Production by *Pseudomonas fluorescens* GU059580 and Its Application in Wastewater Bioremediation

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Abstract

Bioaugmentation by Pseudomonas strains is widely used for the removal of pollutants in wastewater. In this study, we aimed to determine the removal of pentachlorophenol (PCP, 800 mg·L^{-1}) in secondary wastewater by the bioaugmentation process. We determined the effects of using three surfactants, namely sodium dodecyl sulfate (SDS), cetyl-tri-methyl-ammonium bromide (CTAB), and Tween 80 for PCP removal. We determined the effect of the role of PCP surfactant for the biofilm and auxin production of the selected bacterial strain of *P. fluorescens* GU059580. High-performance liquid chromatography and spectroscopic analysis were used to determine PCP removal and bacterial growth, respectively. Biofilm production was determined using 96-well polystyrene plates, and auxin production was determined using spectrophotometric measurement at 535 nm. The results showed the removal of PCP from wastewater by *P. fluorescens* GU059580 is about 90.12%. The PCP

removal from wastewater showed an improvement of about 96.5% after the addition of Tween 80, whereas significant biofilm formation was found in the mineral liquid medium supplemented with PCP and Tween 80, with a value of 3.78. The highest concentration of auxin was found in the presence of PCP without surfactants, showing a value of 1.7 mg·L^{-1}. To conclude, *P. fluorescens* GU059580 can be used in bioreactors or some specific wastewater treatment processes for bioremediation.

Keywords
Pseudomonas fluorescens; wastewater; pentachlorophenol; bioaugmentation; auxin; biofilm

1. Introduction

Water, a valuable resource for all life forms, is continually becoming inaccessible, which is a rapidly growing problem [1]. Water scarcity is due to climate extremes, and improved freshwater is required for municipal, agricultural, and commercial uses [2]. Production wastewater is characterized by its excessive toxicity, disproportionate salt content, pesticide, foul odor, and poor biodegradability [3]. Production wastewater includes polycyclic aromatic hydrocarbons, nitro compounds, and organochlorine pesticides [4]. Owing to their wide availability and low price, some pesticides, such as pentachlorophenol (PCP), have been widely used for different functions and purposes in many countries [5]. PCP is a highly substituted aromatic compound synthesized by reacting chlorine with phenol at a high temperature in the presence of a catalyst [6]. It is a stable organic compound that is slightly soluble in water and highly soluble in organic solvents [7]. Because of its toxicity and carcinogenicity and the large number of known PCP-contaminated sites worldwide, it has been placed on the list of Priority Pollutants worldwide [8].

PCP has been removed through biological processes, such as bioaugmentation [9, 10], phytoremediation [7], biostimulation [11], and other physical processes, such as photo-degradation [12, 13]. In this study, the purification of contaminated wastewater was released by the bioaugmentation process owing to the economic interest and the reliability of this technique. Moreover, PCP can act as a supply of carbon and strength to a few microorganisms, which additionally enables its degradation and bioremediation in contaminated sites. Bioremediation of infected sites can be stronger through inoculation with acknowledged PCP-degrading bacteria, which is known as the bioaugmentation process [14].

PCP, a recalcitrant organic compound, can affect the main bacterial potentiality and functionality, such as biofilm formation, antimicrobial susceptibility, and auxin formation [7]. Auxins are a group of phytohormones that can induce plant growth and reproduce the physiological effects of the naturally occurring indole-3-acetic acid (IAA). The IAA metabolism in higher plants, fungi, and bacteria contains many mechanisms, such as biosynthesis, degradation, catabolism (oxidation and assimilation), conjugation, and hydrolysis of auxin conjugates [15]. Chavez et al. [16] reported that the dynamics of organic elimination of chlorinated aliphatic and polychlorinated hydrocarbons have been accelerated by the use of biofilm reactors. *P. fluorescens* and *P. aeruginosa* isolates can form a biofilm to shield bacteria from PCP [17]. Furthermore, PCP is a version of a natural pollutant intended to be absorbed with biofilm through extracellular polymeric matter [18].

In this study, we aimed to investigate the role of *Pseudomonas fluorescens* GU059580 isolate to resist and use PCP at 800 $mg \cdot L^{-1}$ as a carbon source. The effect of 3 different surfactants, namely anionic, non-anionic, and cationic surfactants, were assessed to determine the PCP utilization by the selected strain and determine its capacity for bacterial biofilm and phytohormones IAA production. All investigations were performed to improve wastewater treatment through common aquatic plant processes.

2. Materials and Methods

2.1 Chemicals and Reagents

PCP of 98% purity was obtained from Sigma–Aldrich, Germany. Chemical surfactants, such as sodium dodecyl sulfate (SDS), cetyl-tri-methyl-ammonium-bromide (CTAB), and tween 80 (TW80), were used for lowering the surface tension between liquid surfaces. All the other chemicals were of the highest commercial purity.

2.2 Physico-chemical Characteristics of the Wastewater Used in This Study

The secondary wastewater (STWW) was sampled from the industrial Charguia I wastewater plant in the northern suburbs of Tunis-city, Tunisia.

The pH of the secondary wastewater–water mix, at a ratio of 1:2.5, was measured with a glass electrode using a low hydrogen electrode pH meter. The total nitrogen content in wastewater was determined by the Kjeldahl method using copper sulfate ($CuSO_4$) and selenium (Se) as catalysts, which was recommended by [19].

2.3 Selection of Pseudomonas Strain

P. fluorescens GU059580 strain was used in this study. The strain was isolated from the macrophyte plant *Typha angustifolia,* which was obtained from the natural lagoon of the secondary wastewater treatment plant (Table 1). The plant is considered an emergent macrophyte and can be found in abundance in the Egyptian Delta lakes and Moroccan mountain lakes. However, owing to the overexploitation of the water resources, its population has declined in the Maghrebian region. Recently, Ramdani et al. [20] and Werheni et al. [21] recommended this plant as a competitive species in aquatic habitats because it effectively functions based on the wetland habitat.

Table 1 Identification and description of the strains used in the bioaugmentation process [22].

	Accession number	Phosphatase production	Bacteriocin production	Biofilm morphotype	Origin
Ps. fluorescens	GU059580	++	+16	Very mucoid	Wastewater

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

Mehri et al. [20] identified the strains of *Pseudomonas* sp., with molecular method, using 16S rRNA sequencing [22]. The 16S rRNA gene sequences are the most common housekeeping genetic

markers to study bacterial phylogeny and taxonomy. These sequences are present in most bacterial species as operons. They have long-lasting genetic evolution stability and are 1,500 bp in length, which makes them sufficiently large to be used for bioinformatic analyses [23].

The process of isolation and selection of these *Pseudomonas* sp. was primarily based on their tolerance and PCP removal ability in the mineral salt medium (MSM) growth medium, which was reported by Sharma et al. [23]. These Pseudomonas sp. have been frequently used in the phytoremediation process owing to their important multifunctional important characteristics [24-26].

2.4 PCP Bioaugmentation Experimentation

The PCP biodegradation process was performed using *P. fluorescens* GU059580, and the inoculum was prepared in an enriched liquid medium for 16 h to obtain around 10^8 CFU·mL^{-1}. This inoculum was then transferred to a 250 mL flask containing 100 mL of MSM or sterilized secondary wastewater. The composition of MSM is in mg·L^{-1}: KH_2PO_4, 800; Na_2HPO_4, 800; $MgSO_4{\cdot}7H_2O$, 200; $CaCl_2{\cdot}2H_2O$, 10; NH_4Cl, 500 and 1 mL of trace metal solution comprising (mg·L^{-1}) $FeSO_4{\cdot}7H_2O$, 5; $ZnSO_4{\cdot}H_2O$, 4; $MnSO_4{\cdot}4H_2O$, 0.2; $NaCl{\cdot}6H_2O$, 0.1; H_3BO_3, 0.1; $CoCl_2{\cdot}6H_2O$, 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}, corresponding to 3.04 mM of PCP [27]. The prepared flasks were incubated at 30 °C under constant shaking for 168 h using an incubator shaker (ZHWY-2102 P) [28].

2.5 Chloride Analysis by Mohr's Method

Whatman filter paper was used to collect 50 mL filtered volumes of different sterile STWW and MSM (with and without PCP). The collected samples were placed into a 250 mL Erlenmeyer flask and 2–3 drops of pure nitric acid, a pinch of 0.2 g calcium carbonate, and 3 drops of 10% potassium chromate were added. The 0.1 N silver nitrate solution was then poured using a burette until a persistent red-colored precipitate appeared. The volume of 0.1 N silver nitrate used for the titration was noted. The chloride content was expressed in g·L^{-1} using the following formula:

$$Cl〗 = (A\text{-}B)/V \times C \times 1000 \times \text{dilution factor}$$

Where A denoted the volume of the titrating solution poured by a volume of V mL of samples, B denoted the volume of the titrating solution poured by a volume of V mL of distilled water, and C denoted the mg of chloride equivalent to 1 mL of the titrant solution (10 mg·Cl^{-1}).

2.6 Bacterial Growth

The bacterial biomass of *P. fluorescens* GU059580 was determined at several instances, within 168 h of incubation at 30 °C, from the beginning (T0) to the end (TF) of the bioremediation experiment. The biomass was measured for different analyses using OD_{600} spectrophotometric examination (Spectro UVS-2700 Dual Beam Labomed, Inc).

2.7 HPLC Analysis

To quantify the PCP removal change, HPLC was used through interval sampling of 1 mL of culture within 24 h. HPLC measurements were obtained using a Perkin Elmer Series YL9100 system filtered on symmetry C18 columns and detector UV at 280 nm. The experiment was performed in triplicates. The molecular suspension was centrifuged at 8000 rpm for 5 min, and the supernatant was filtered using a 0.22 mm Cellophane filter, as described by a previous study [29]. The percentage of PCP removal was calculated as described by Khessairi et al. [30]:

$$\% \text{ PCP removal} = (\text{PCP awareness T0}/\text{PCP awareness Tx}) \times 100$$

2.8 PCP and Surfactant Effects on Biofilm Formation

The bacterial strain of *P. fluorescens* GU059580 was grown on an agar growth medium (King A) and incubated at 30 °C for 48 h, as described by Turki et al. [31]. To determine the effect of PCP on the quantitative biofilm formation, *P. fluorescens* GU059580 was cultured for 24 h at 30 °C in 3 mL of brain infusion broth (BHI) as recommended by Djordjevic et al. [32]and in MSM at PCP concentrations of 100, 500, and 800 mg.L^{-1} as reported by Del Castillo et al. [33]. The cultures were diluted to 1:20 using the same medium, and 200 µL of the final suspension was 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 °C, as reported by Meliani and Ben Soltane [18], the plates were washed three times vigorously with phosphate-buffered saline (PBS; 1×, pH 7.4) to remove the unattached and single bacterial cells. The plate was then stained with 1% crystal violet for 15 min after washing with ethanol–acetone (80:20) for visualization. The biofilms were quantified after adding 200 µL of 95% ethanol, followed by slight manual shaking. The absorbance was measured at 585 nm wavelength (λ), as reported in previous studies [34-37].

2.9 Effects of PCP on IAA Production

Indole acetic acid (IAA) production is a major property of rhizosphere bacteria. IAA is commonly known as plant growth–promoting rhizobacteria (PGPR). IAA is one of the most physiologically active auxins. It is a common product of L-tryptophan metabolism and stimulates and facilitates plant growth. Hall et al [38], reported a method for the characterization of the indole nucleus based on the chemical properties of IAA. The characterization is performed by the formation of a red-colored complex between iron perchloride and the indole nucleus of IAA in the presence of high concentrations of mineral acids ($HClO_4$). The test strains were incubated for 72 h in a minimum medium (MSM), which contained 6.8 g KH_2PO_4, 0.2 g $MgSO_4$, 2 g $(NH_4)SO_4$, 2 g citrate, 0.006 g H_3BO_3, 0.006 g ZnO, 0.0024 g $FeCl_3$, 0.02 g $CaCO_3$, 0.13 mL HCl, 10 g glucose, and 100 µg/mL L-tryptophan per liter of medium. One volume of the medium was added to two volumes of Salkowski's prepared reagent, which contained 1 mL 0.5 M $FeCl_3$ and 50 mL 35% $HClO_4$. The stain developed after waiting for 20–30 min. The optical density (OD) was determined by a spectrophotometer at a λ of 535 nm, and a standard curve was plotted for the observed IAA content, which was recommended by Patten and Glick [39]. The IAA content was expressed in mg·mL^{-1}.

2.10 Statistical Analysis

All experiments were performed in triplicate, and the obtained results were an average of those triplicates. Analysis of Variance (ANOVA) was performed to compare variances across the means of different groups using the SPSS 21.0 statistical program (IBM, SPSS for Windows; SPSS, Inc., Chicago, IL, USA), and the mean values were calculated by the Tukey posthoc test ($p < 0.05$).

3. Results and Discussion

3.1 Physical and Chemical Characteristics of Wastewater

The diiferents samples was collected from the wastewater treatment plant has been operating since 1958. It was rehabilitated in 1978 and 2004 to increase its treatment capacity from 30000 m^3/d to 60000 m^3/day. The treatment plant receives its load for treatment from domestic, rain, and industrial wastewater. The station is sized to handle a load capacity of 400000 equivalent inhabitants (EH). The treatment process used was medium load-activated sludges. The treatment plant has an average daily flow of 60000 m^3/day with a BOD_5 load of 24000 kg·BOD_5/day. The physical and chemical characteristics of the STWW used in this study are described in Table 2. A basic pH of 7.9 and high chemical oxygen demand of around 520 mg·L^{-1} characterized the STWW sample. The STWW chloride content was around 9.6 g·L^{-1}. The nitrogen analysis of the STWW showed an average value of 44.0 mg·L^{-1}, according to Water Agencies and the Ministry of the Environment [40]. The STWW showed a higher chloride concentration than a sample in a study by Werheni et al. [7], and growing *Pseudomonas* in the STWW sample was difficult.

Table 2 Physical and chemical characteristics of the secondary wastewater sample (data are represented as the means ± standard deviation, n = 4).

Parameter	Value
Dry matter, %	33.0 ±1.2
pH	7.9 ±0.4
Conductivity, mS cm^{-1}	2.1 ±0.6
Organic carbon, %	2.1 ±0.1
Nitrogen, mg L^{-1}	44.0 ±0.5
COD, mg L^{-1}	520.0 ±3.2
BOD_5, mg L^{-1}	3.8 ±0.5
Nitrates, mg L^{-1}	2.3 ±0.4
Chlorides, g L^{-1}	9.6 ±0.9

pH: Hydric potential; COD: Chemical Oxygen Demand, BOD_5: Biochemical Oxygen Demand

3.2 Bacterial Strain Selection

Ten *Pseudomonas* strains isolated by Ines Mehri et al. [24] were selected in this study. This selection was based on the capacity of tolerance and transformation of PCP by the *Pseudomonas* isolates. Among the ten *Pseudomonas* isolates, we selected a strain with the highest survival and growth in the mineral salt medium for further experiments. The selected strain isolated from

wastewater was identified as *P. fluorescens* GU059580. This *P. fluorescens* GU059580 strain tolerated different tested PCP concentrations, such as 100 mg·L^{-1}, 500 mg·L^{-1}, 600 mg·L^{-1}, and 800 mg·L^{-1}. This strain removed PCP at 800 mg·L^{-1} (Table 3).

Table 3 The capacity of pentachlorophenol removal by *Pseudomonas fluorescens* GU059580 in the mineral salt medium at 30 °C for 168 h.

		PCP concentrations (mg L^{-1})			
Reference	Strain	100	500	600	800
PsWw128	*Ps. fluorescens* GU059580	+	+	+	+

3.3 PCP Removal

PCP removal at 800 mg·L^{-1} was monitored by determining the PCP elimination percentage by HPLC, and bacterial growth was measured by OD determination at 600 nm (Figure 1). The growth of *P. fluorescens* GU059580 in a liquid medium supplemented with PCP showed three stages as follows: latency, exponential, and toxicity phases. The bacterial activity of this strain was characterized by an extended latency phase of about 96 h with 10% PCP degradation. This phase was followed by a short exponential phase of 24 h with 77% PCP degradation. After 168 h of incubation, the *Pseudomonas* strain could remove 90% of PCP. Previously, Wolski et al. [37] showed that, at 50 mg·L^{-1} and pH 6.3, PCP was degraded rapidly by *Pseudomonas aeruginosa*. Werheni et al. [7] reported the importance of *Pseudomonas* as an efficacious operator and agent for the bioremediation of environmentally contaminated sites. Hassen et al. [9] reported PCP removal in a liquid medium at the rate of 800 mg·L^{-1} by a monoculture or consortia of *Pseudomonas* isolates. PCP bioaccumulation at low and high concentrations explained its removal from the medium [28, 41].

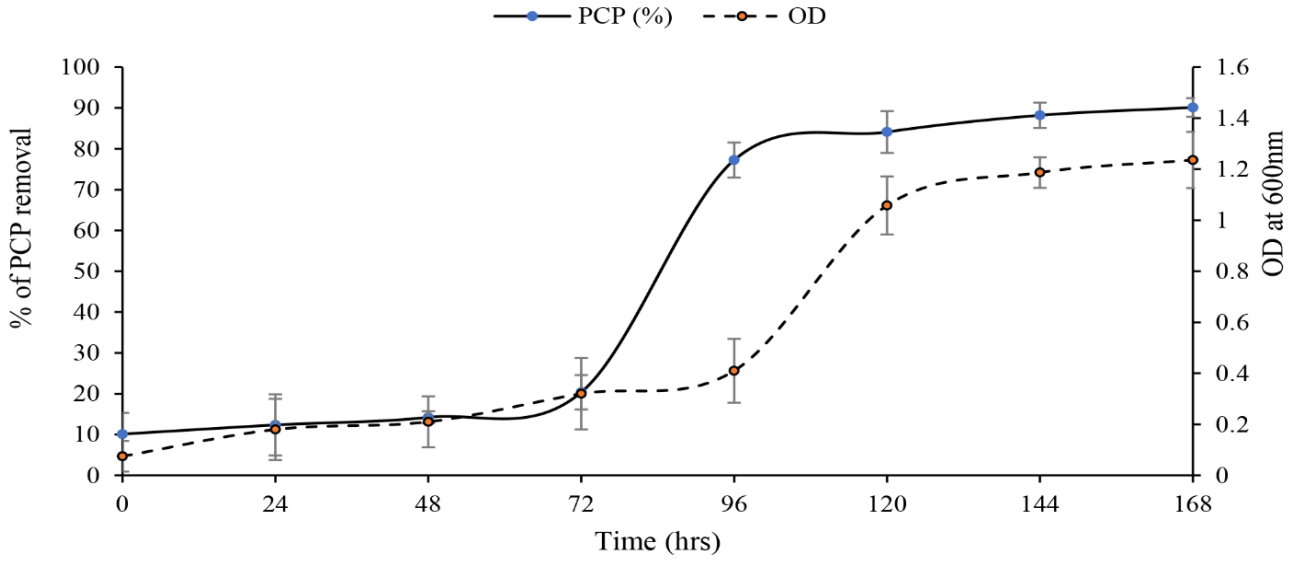

Figure 1 Percentage of pentachlorophenol removal by *Pseudomonas fluorescens* GU059580 and bacterial growth in the mineral salt medium for 168 h and at 30 °C.

3.4 Effects of Surfactants on PCP Removal

PCP contents in bioaugmentation treatments by *P*. *fluorescens* GU059580 in a medium containing these surfactants were determined by HPLC (Figure 2). *P*. *fluorescens* GU059580 tolerated and removed PCP at 800 mg·L^{-1} in the MSM and STWW. After seven days, this PCP amount decreased to 118.6 mg·L^{-1} (79.98% degradation) and 190.24 mg·L^{-1} (76.22% degradation), respectively. Lanthier [42] (1999) reported in a similar study that *P*. *putida HM627611* removed PCP significantly in the STWW + PCP + CTAB (non-anionic) treatment group, with an average concentration 20 mg·L^{-1} (97.5% removal). Furthermore, Lanthier [42] showed that some known surfactants, such as SDS and CTAB, negatively affected PCP degradation. In contrast, Hassen et al. [6] and Werheni et al. [7] confirmed the positive effects of such surfactants on PCP removal at a high rate in the liquid MSM or STWW. PCP solubilization was evaluated by adding the three synthetic surfactants SDS, CTAB, and TW80 separately, which are anionic, non-anionic, and cationic compounds, respectively. SDS added in the medium sped up PCP removal and increased bacterial growth. The present results are consistent with those of Hassen et al. [43] and Mokaberi et al. [30], who underlined that SDS was more effective than TW80 in increasing PCP solubilization and bacterial growth.

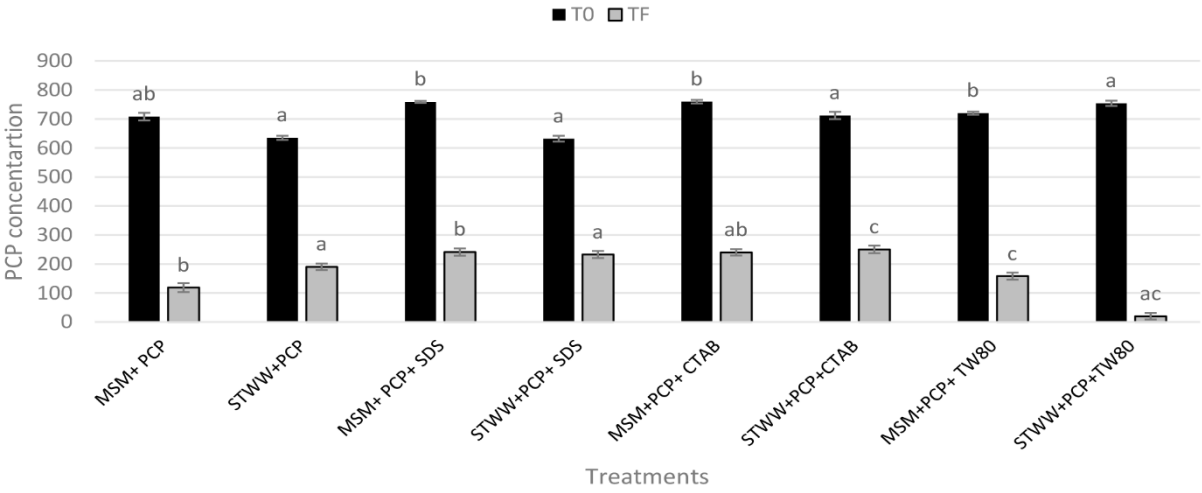

Figure 2 The percentage of pentachlorophenol removal by *Pseudomonas fluorescens* GU059580 in different bioaugmentation treatments in the secondary wastewater and mineral salt medium for 168 h and at 30 °C. SDS: Sodium dodecyl sulfate; CTAB: Cetyltrimethylammonium bromide; TW80: Tween 80.

3.5 Bacterial Biomass Development

The microbial biomass determination of *P*. *fluorescens* GU059580 during bioaugmentation in the presence of PCP is summarized in Figure 3. The different treatments increased microbial growth, which can be explained by PCP tolerance and usage by bacteria as a carbon source. The most significant growth of *P*. *fluorescens* GU059580 was observed in the TW80 treatment group with a value of about 9.21 × 10^8 CFU/mL after seven days of incubation. The other two detergents weakly stimulated bacterial growth. Therefore, detergents can be used in bioaugmentation to improve the performance of bacteria and facilitate their development in wastewater.

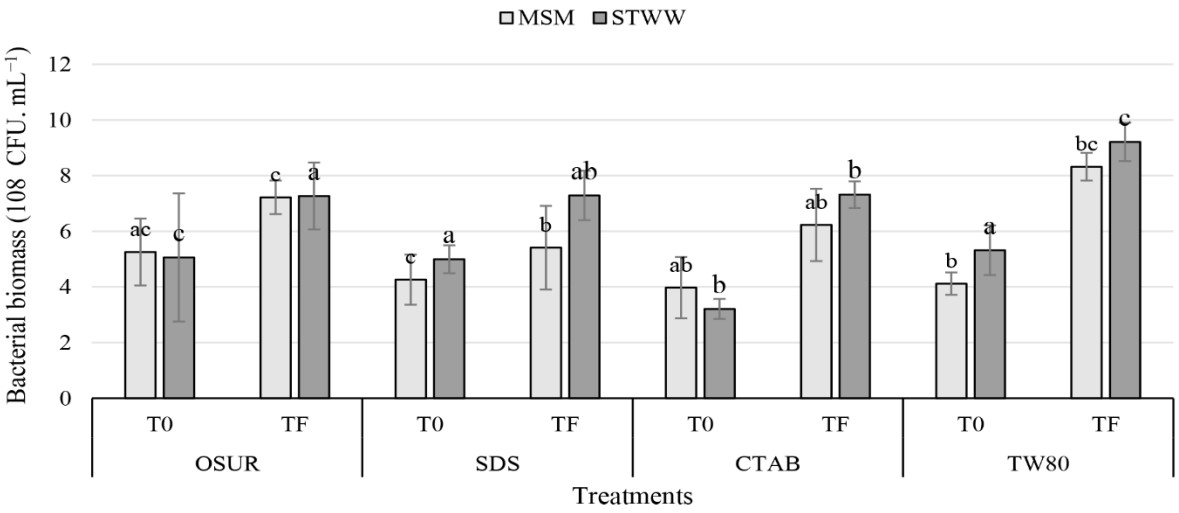

Figure 3 Bacterial biomass of *Pseudomonas fluorescens* GU059580 observed in different bioaugmentation treatments in the secondary wastewater and mineral salt medium for seven days and at 30 °C. SDS: Sodium dodecyl sulfate; CTAB: Cetyl-trimethyl-ammonium bromide; TW80: Tween 80.

Some researchers have shown that different surfactants promote desorption [44-46], solubilization [47], and biodegradation of organic compounds [34]. Cort and Bielefeldt [35] investigated the effects of surfactants on PCP degradation by *Sphingomonas chlorophenolicum* sp., showing no PCP degradation in the presence of cationic surfactants, thus indicating that surfactants were more inhibitory at lower temperatures than at higher temperatures. Moreover, Lanthier [42] reported that surfactants often negatively affect PCP degradation, and bacteria responsible for PCP degradation do not require large amounts of liquid media [48-50].

3.6 Effects of Chloride Content on PCP Removal

The chloride content observed in different bioaugmentation processes via *P*. *fluorescens* GU059580 in the presence of SDS, CTAB, and TW80 in the STWW and MSM samples is shown in Figure 4. In the MSM samples, the chloride content was lower than that in the STWW samples. A high value of 5.36 $g \cdot L^{-1}$ was observed in the MSM + TW80 treatment group. In the STWW samples, a high chloride content was detected in the STWW + TW80 treatment group with a value of 8.6 $g \cdot L^{-1}$. The results were consistent with those of Watts et al. [51], who reported that an increase in the chloride content was proportional to PCP removal from wastewater and soil. In addition, Oturan et al. [52] explained the chloride increase in a medium by releasing CO_2, HCl, and H_2O resulting from PCP biodegradation. These latest results are consistent with the ones reported by Werheni et al. [27] in STWW, which outlined a proportional relationship between chloride variation and PCP removal by *P*. *putida* strains and synthetic detergents.

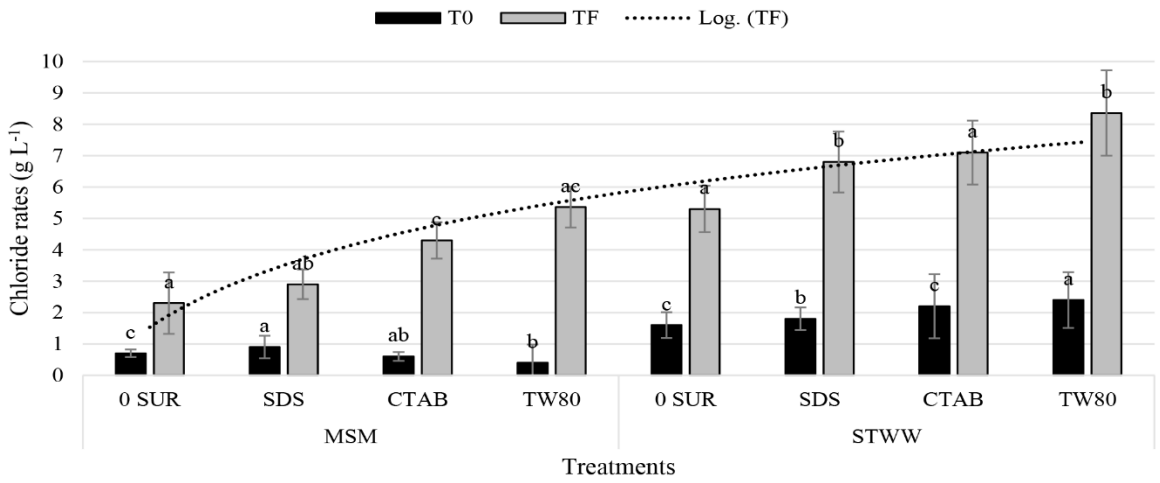

Figure 4 Chloride rate determination in wastewater treated by *Pseudomonas fluorescens* GU059580 bioaugmentation in the liquid mineral salt medium and secondary wastewater for seven days and at 30 °C. SDS: Sodium dodecyl sulfate; CTAB: Cetyltrimethylammonium bromide; TW80: Tween 80.

3.7 Effects of PCP and Surfactants on Biofilm Development

Considering the biofilm production ability of *P*. *fluorescens* GU059580, we performed crystal violet staining, a standard staining method. The results showed that *P*. *fluorescens* GU059580 could form a biofilm in two liquid growth media: BHI and MSM, and the productivity of the biofilm increased with the increasing rate of PCP addition (Table 4). Simultaneously, in this study, we tested the effect of three anionic, non-anionic, and cationic surfactants on the development of a bacterial biofilm of *P*. *fluorescens* GU059580. As per the main results presented in Table 4, SDS (anionic detergent) and CTAB (cationic detergent) treatment showed similar results as that of the treatment of the strain with PCP alone, whereas TW80 (non-anionic detergent) increased the net biofilm production. Figure 5 shows many significant differences between the results of the aforementioned treatments; TW80 showed the highest PCP removal at 100 mg·L^{-1}, 500 mg·L^{-1}, and 800 mg·L^{-1}PCP concentrations, giving OD values of 1.569, 3.365, and 3.78, respectively. CTAB and SDS treatments positively affected biofilm formation by *P*. *fluorescens* GU059580 at the three concentrations mentioned above and showed an average OD of less than 3. However, BHI usually served as a growth medium for bacteria for biofilm formation and was free of surfactants, and PCP showed the lowest biofilm production by *P*. *fluorescens* GU059580 with an OD = 0.54. Moreover, CTAB at 100, 500, and 800 mg·L^{-1} PCP concentrations showed the lowest OD values: 1.36, 1.89, and 2.01, respectively.

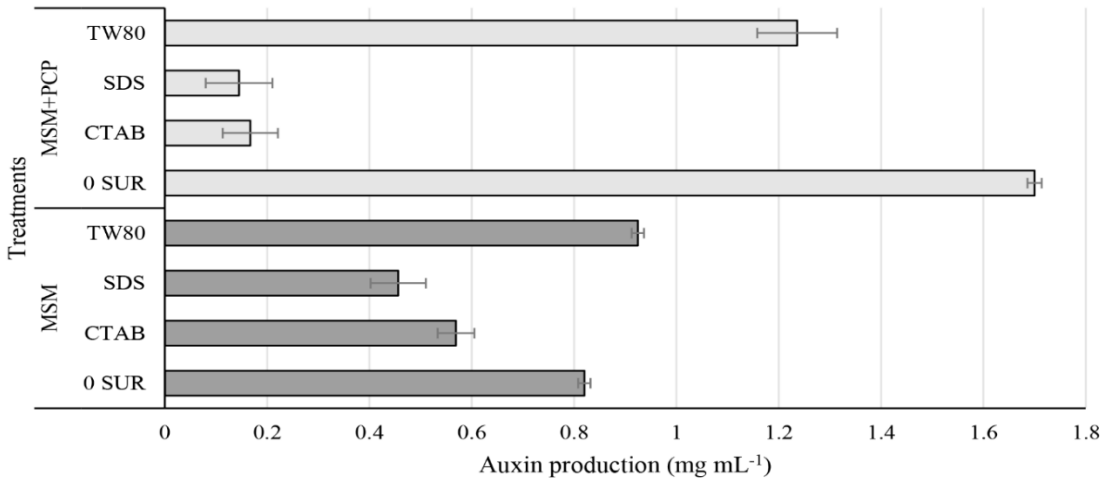

Figure 5 The effects of pentachlorophenol and the surfactants on auxin production for 72 h and at 30 °C. SUR: surfactant; MSM: Mineral salt medium; SDS: Sodium dodecyl sulfate; CTAB: Cetyl-trimethyl-ammonium bromide; TW80: Tween 80.

Table 4 The effects of pentachlorophenol on biofilm development in the MSM and BHI medium with or without the surfactants at 30 °C.

	mg L^{-1}	BHI	MSM + Glu	MSM + PCP No surfactant added	SDS	CTAB	TW80
Ps. fluorescens GU059580	0	0.927 ±0.02a	1.23 ±0.023b	0	0	0	0
	100	1.027 ±0.01ab	0.542 ±0.013a	0.763 ±0.015c	2.61 ±0.01a	1.36 ±0.01a	1.569 ±0.02[a]
	500	1.208 ±0.01b	0.621 ±0.021ab	1.557 ±0.01b	2.8 ±0.02a	1.89 ±0.02ab	3.365 ±0.03[ab]
	800	1.745 ±0.012a	0.864 ±0.036c	1.881 ±0.02ab	2.9 ±0.01b	2.01 ±0.021b	3.78 ±0.012[b]

BHI: Brain infusion broth; MSM: Mineral salt medium; SDS: Sodium dodecyl sulfate; CTAB: Cetyl-trimethyl-ammonium bromide; TW80: Tween 80; n = 4, ±: Deviation standard

One of the major environmental applications of microbial biofilms is wastewater treatment: biofilm reactors, including bacterial filters, biological disks, and fluidized bed biofilm reactors, have been developed both at pilot and large-scale wastewater treatment plants. [53, 54]. Therefore, immobilized microbial cells or biofilm reactors can provide a more stable operating performance, producing various antagonistic compounds [55].

3.8 Effects of PCP on IAA Production

The results showed that the addition of PCP and the three surfactants affected IAA production in the different treatment groups (Figure 5). The highest production of about 1.7 $mg{\cdot}mL^{-1}$ was observed in the MSM treatment group without these surfactants and PCP. The results also showed

that these detergents negatively affected IAA production in the SDS and CTAB treatment groups. TW80 and PCP improved the production of the phytohormone IAA. The release of some common growth substances by fluorescent *Pseudomonas* strains has frequently been observed and reported by Brown [56]. These substances can be absorbed by plant roots, stimulating plant growth. These bacteria are identified as the promoters of plant growth and development (E.g., PGPR). The selected *P*. *fluorescens* GU059580 strain was identified to produce the phytohormone IAA, commonly detected and measured by colorimetry using Salkowski's reagent.

Essentially, saprophytic strains producing this hormone can be used as biofertilizers. Several authors have shown the ability of microbial strains to produce some plant-growth-enhancing substances, leading to important morphological changes in plants [52, 56].

All these earlier studies concluded that stimulating the growth of rhizospheric bacteria enhanced plant growth after a reciprocal exchange of growth substances, including vitamins and saccharides, between these bacteria. This reciprocal biological benefit exchange process is known as symbiosis. Thus, *Pseudomonas sp*. can positively affect plant development directly or indirectly. The of plant growth hormones can act more or less strongly on different stages of growth, depending on the sensitivity of plants [57]. In their presence, bacteria can stimulate the synthesis of these hormones, called plant auxins, released by plant cells themselves [50].

4. Conclusions

STWW treatment by bioaugmentation is a practical and beneficial tool. The results of this study showed the multifunctional properties of the *P*. *fluorescens* isolate GU059580 and its relevance and applicability in treating PCP-contaminated wastewater. Such beneficial microorganisms can be used as bioremediation agents after their inoculation in bioreactors or introduction into natural ponds for wastewater treatment. Using *Pseudomonas* is a promising bioaugmentation approach because it is more economical, faster, easier, and more efficacious. The main results of this study on bioaugmentation showed that adding TW80 as a surfactant positively affected the process, whereas SDS and CTAB led to negative results. The coexistence of TW80 and PCP enhanced bacterial biofilm production, increasing the percentage of PCP removal. Similarly, the coexistence of TW80 and PCP enhanced auxin production, helping macrophyte growth and intensifying biological activities.

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Author Contributions

"RWA analyzed and interpreted all of the data and writing the manuscript, WH, NK and IM contribution in sampling analysis, AH a major contributor in writing and revision of the manuscript. All authors read and approved the final manuscript."

Competing Interests

The authors have declared no conflict of interest.

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