Revista Internacional de Investigación e Innovación Tecnológica

Página principal: www.riiit.com.mx

Biodegradation of organochlorine compounds with the *Pseudomonas chlororaphis* strain in liquid medium as the only carbon source

Biodegradación de compuestos organoclorados por *Pseudomonas chlororaphis* en medio líquido como única fuente de carbono

Bautista-Bautista, O.[a], Sánchez-Minutti, L.[a], Ortega-Sánchez, E.[b], Tellez-Girón, J.D.[c], García-Dávila, J.[a]*

[a] Universidad Politécnica de Tlaxcala. Avenida Universidad Politécnica, No.1, San Pedro Xalcaltzinco, C.P. 90180 Tlaxcala, México (https://orcid.org/0009-0009-8614-7743, https://orcid.org/0000-0003-4739-5196, https://orcid.org/0000-0002-8013-4177).

[b] Instituto Tecnológico de Tepic, División de estudios de posgrado Av. Instituto Tecnológico No. 2595, Col Lagos del Country, C.P. 63175. Nayarit, México (https://orcid.org/0000-0002-9283-0778).

[c] Facility Manager S.A. de C.V., Polonia 1430-3 Col Las Hadas Mundial 86, C.P. 72070. Puebla, México (https://orcid.org/0000-0001-5805-531X).

oscar.bautista@uptlax.edu.mx; lilia.sanchez@uptlax.edu.mx; biotecnocios@gmail.com; jabeldtg77@yahoo.com.mx; jorge.garcia@uptlax.edu.mx*

Technological innovation: Bioremediation of organochlorine compounds.
Application area: Environmental biotechnology.

Received: July 24th, 2025
Accepted: December 17th, 2025

Resumen

Actualmente los suelos presentan alteraciones debido a la presencia de contaminantes de diferentes orígenes. La biorremediación es una de las áreas de mayor investigación para mitigar el efecto de los desechos orgánicos, entre los que destacan los compuestos organoclorados, conocidos por su alta resistencia a los procesos naturales de biodegradación.

Las cepas del género *Pseudomonas spp.* han sido ampliamente estudiadas por su capacidad de crecer en ambientes con presencia de compuestos organoclorados y degradarlos. En este trabajo se utilizó la cepa *Pseudomona chlororaphis* CA-1 aislada de un sitio de confinamiento con presencia de compuesto organoclorados. Se estudio la cinética de crecimiento en medio tripticaseína de soya y en un medio mínimo en sales con presencia del sustrato organoclorado (Diclorobenceno 84% (Triclorobenceno 5%, Tetraclorobenceno 10.5%, y Pentaclorobenceno 0.5%) como fuente de carbono. El crecimiento microbiano se cuantificó mediante, densidad óptica y peso seco. El cultivo fue incubado por tres días a 30 °C, y posteriormente se validó la morfología característica de *Pseudomonas* mediante un frotis bacteriano. Se inoculó la cepa en dos concentraciones del sustrato

organoclorado como única fuente de carbono, para evaluar si su metabolismo puede asimilar estos compuestos tóxicos. Los resultados demuestran una mayor producción de biomasa de $3.49x10^{-3}$ µg/mL. a una concentración de 100 ppm de sustrato organoclorado, con una remoción del 83.60%; por otra parte, a concentración de 1000 ppm se obtuvo una producción de biomasa de $1.48x10^{-3}$ µg/mL con un porcentaje de remoción del sustrato de 89.5%. Lo que demuestra que *P. chlororaphis* CA-1 es capaz de utilizar a los compuestos organoclorados como fuente de carbono.

Palabras clave: Biodegradación, Cinética de crecimiento, Sustrato organoclorado, *Pseudomonas chlororaphis*.

Abstract

Soils are currently altered due to the presence of pollutants from various sources. Bioremediation is one of the most researched approaches to mitigate the effects of organic waste, especially organochlorine compounds, known for their high resistance to natural biodegradation processes.

Strains of the genus *Pseudomonas spp.* have been widely studied for their ability to survive in environments containing organochlorine compounds and to degrade them. In this study, the *Pseudomonas chlororaphis* CA-1 strain, isolated from a confinement site containing organochlorine compounds, was used. Growth kinetics were studied in Trypticase soy broth (TSB) and mineral salts medium (MSM) in the presence of the organochlorine substrate (dichlorobenzene 84%, trichlorobenzene 5%, tetrachlorobenzene 10.5%, and pentachlorobenzene 0.5%) as a carbon source. Microbial growth was quantified by optical density and dry weight. The culture was incubated for three days at 30°C, and the characteristic morphology of *Pseudomonas* was validated using a bacterial smear.

The strain was inoculated at two concentrations of the organochlorine substrate as the sole carbon source to evaluate whether its metabolism could assimilate these toxic compounds. The results demonstrate biomass production of $3.49x10^{-3}$ µg/mL at 100 ppm, with an organochlorine-substrate removal percentage of 83.6%. At 1000 ppm, a substrate removal percentage of 89.5% was obtained, with biomass production of $1.48x10^{-3}$ µg/mL. This demonstrates that *P. chlororaphis* CA-1 is capable of using organochlorine compounds as a carbon source.

Key words: Biodegradation, Growth kinetics, Organochlorine substrate, *Pseudomonas chlororaphis*.

I. Introduction

Ecosystems have been altered by the uncontrolled disposal of inorganic and organic agents. Among them, pesticides, due to their excessive use, have accumulated in the environment, showing high persistence thanks to their physicochemical properties such as their low solubility, which favors their bioaccumulation in living organisms (Sierra-Cortés, *et al*., 2019).

Among the most persistent chemical compounds in the environment are organochlorine compounds (Pimviriyakul, P. *et al*., 2020), which inhibit the growth of microorganisms, hindering their biodegradation (Kopytko, *et al*., 2017). Organochlorine compounds are widely used as solvents, odorants, pesticides, and flame retardants, and are highly resistant to

microbial mineralization due to their recalcitrant haloaromatic structure (Saibu, *et al*., 2020).

There is a wide variety of bacteria capable of degrading organochlorine compounds (Otiniano-García, *et al*., 2013; Contreras *et al*., 2020), among which those of the genus *Pseudomonas spp*. stand out. These are Gram-negative aerobic bacilli, with a remarkable ability to live in environments contaminated with petroleum derivatives and aromatic organic compounds (Saibu, *et al*., 2020; Kahlon., 2016).

The genus *Pseudomonas sp.* can degrade organochlorine compounds in liquid or solid systems (Luján, D., 2019); however, most studies have focused on liquid cultures due to their diversity of applications in environmental biotechnology (Huang, *et al.,* 2019). However, biodegradation in liquid medium presents certain difficulties (Cui., *et al*., 2017), since compounds such as dichlorobenzene, trichlorobenzene, and tetrachlorobenzenes are highly insoluble in water, which makes it difficult to prepare suitable solutions for their bioavailability (Lu, *et al*., 2017). This low solubility limits the ability of microorganisms to use organochlorine compounds as a source of carbon and energy for their growth (Gilani, *et al*., 2016; Ibáñez-Moreno *et al*., 2020).

In the present study, a strain of the genus *Pseudomonas chlororaphis* CA-1 was used to study growth kinetics in the absence and presence of organochlorine compounds to demonstrate its tolerance and capacity to degrade these compounds as the sole carbon source in liquid-state fermentation using a mineral salts medium (MSM).

II. Materials and Methods

2.1 Microorganism studied

The *Pseudomonas chlororaphis* CA-1 strain with registration code CDBB 91 (ATCC 9447) was donated by the company Clorobencenos S.A. de C.V. in the state of Tlaxcala. This strain was selected for its ability to grow in environments containing toxic compounds derived from the company's waste. It was subsequently preserved by lyophilization using 10% glycerol as a cryoprotectant in TSB medium.

For reactivation of the microorganism, a liquid culture medium of Trypticase soy broth (TSB) was used, adjusted to pH 7.1 ± 0.2 and sterilized at 120 °C. Subsequently, the microorganism was inoculated. For its growth, a King B culture medium was prepared with the following formulation: Sabouraud agar (65 g/L), potassium phosphate (1.5 g/L), magnesium sulfate (1.5 g/L), yeast extract (20 g/L), and glycerin (10 mL/L). The medium was sterilized for 15 minutes at 120 °C.

2.2 Growth kinetics

Twenty-five 125 mL Erlenmeyer flasks were prepared with 30 mL of sterile TSB culture medium adjusted to pH 7.1 ± 0.2. Twenty-four flasks were inoculated with 4 mL of the primary culture, and one flask served as a blank containing only TSB medium. The flasks were incubated at 30°C. Aliquots were taken every hour for seven days to measure the optical density in a Thermo Scientific UV–Vis spectrophotometer (Genesys 10S model).

Biomass was quantified by the gravimetric method by measuring the weight per unit volume (Gómez-Reyes *et al*., 2017). For this, cells were separated from the liquid medium by centrifugation at 13,000 rpm for 5 minutes (Thermo Scientific centrifuge). The supernatant was removed, and the pellet was

dried in an oven (model HS-60, Prendo) at 50 °C for 10 minutes. Subsequently, the dried pellet was weighed on an Ohaus Pioneer analytical balance.

The cell counting procedure was performed using a Neubauer chamber to determine the concentration of cells in the medium. A 10 µL aliquot of the diluted cell sample (1:2) was taken and loaded into the counting chamber, which has a depth of 0.1 mm. The chamber was allowed to stand for one minute to stabilize the cell distribution before reading. The count was performed using an optical microscope equipped with a 40x objective lens. The number of cells in each square was recorded, and the average cell count per square was calculated. To determine the final cell concentration in the suspension, the following formula was applied:

Cell concentration (cells/mL)=(Average number of cells per square×Dilution factor×10^4)/1

2.3 Degradation kinetics

The organochlorine liquid substrate used for this analysis was obtained from the waste of the Clorobencenos S. A. de C. V. plant in the municipality of El Carmen Tequexquitla in the state of Tlaxcala. Due to the nature of the substrate, it cannot be sterilized. The composition of the organochlorine substrate was analyzed by gas chromatography in the company's quality control laboratory using its own analytical methods (Table 1).

The degradation kinetics of the organochlorine substrate by *Pseudomonas chlororaphis* CA-1 was evaluated by liquid fermentation, using a mineral salts medium (MSM). With the following composition: calcium nitrate (60 mg/L), sodium bicarbonate (125 mg/L), potassium nitrate (70 mg/L), monobasic potassium phosphate (100 mg/L), iron sulfate (10 mg/L), zinc sulfate (1.5 mg/L) and agar 15 (g/L). The medium was inoculated with a bacterial concentration of approximately 2.48 x10^6 CFU/mL. Incubation was carried out at 30 ± 2 °C on an orbital shaker at 130 rpm, with pH 7.2 ± 0.2 at the beginning of fermentation.

Five one-liter Erlenmeyer flasks were prepared in duplicate, each with 500 mL of MSM medium. Of these, two flasks were loaded with a concentration of 100 ppm of organochlorine substrate, two more with 1000 ppm of the same substrate, and the fifth flask was used as a control. Over a period of 7 days, two 1 mL samples were taken from each flask every 2 hours to evaluate microbial growth, to maintain the homogeneity of the medium, it was kept in constant agitation at 200 rpm.

Table 1. Concentrations of compounds present in the organochlorine substrate.

Composition of the liquid organochlorine substrate	
According to a chromatographic analysis performed	
% Concentration	Chemical compound
84 ± 0.3%	Dichlorobenzene
5% ± 0.2%	Trichlorobenzene
10.5% ± 0.3%	Tetrachlorobenzene
0.5% ± 0.2%	Pentachlorobenzene

The kinetic parameters were determined using a modified version of the logistic model proposed by Zwietering *et al*. (1990), based on the evolution of bacterial density over time.

2.4 Determination of the percentage of organochlorine substrate removal by GC/MSD

Over a period of seven days, one 1 mL sample was taken daily from each flask to evaluate the percentage removal of the organochlorine substrate. The sample was extracted with 2 mL of hexane, and sodium sulfate was added to the organic layer for drying. Samples were analyzed on an Agilent 7890A gas chromatograph coupled with a mass

spectrometer (triple-axis detector 5975C). Three microliters (3 µL) of the sample were injected in splitless mode (50:1) and carried through an HP 5-MS capillary column (30 m × 320 µm × 0.25 µm). The injector temperature was 300 °C, and helium carrier gas was set at a constant flow rate of 1 mL min^{-1}. The oven program was set to 90 °C for 5 min, then ramped to 300 °C at 20 °C min^{-1} and held for 2 min. The mass detector was operated with an ion source temperature of 230 °C and a quadrupole temperature of 150 °C. Ion acquisition was performed in scan mode (m/z 25 to 550 amu) in selective ion mode (SIM) using the NIST 2.0 library.

The experiment was performed in duplicate using two generations of the *Pseudomonas chlororaphis* CA-1 strain, which were tested at different concentrations of the organochlorine substrate.

III. Results

3.1 Growth kinetics

In the analysis of the growth *of Pseudomonas chlororaphis* CA-1 in TSB culture medium, a specific growth rate of 0.15 h^{-1} was obtained; the exponential phase was recorded during the first 12 hours of fermentation.

Figure 1 shows the correlation between cell dry weight and optical density (OD) during the first 12 hours of culture of *Pseudomonas chlororaphis* CA-1 in two different media, TSB and MSM, with organochlorine substrate. This correlation between both techniques allows evaluation of bacterial growth as a function of the medium and the available carbon source.

For the analysis of *Pseudomonas chlororaphis* CA-1 growth, the specific growth rate was determined. According to Student's t-test, a value of t = 0.048 was obtained, which was much smaller than the tabulated value for a significance level of α = 0.05, indicating that there was no significant difference between the dry weight and optical density measurements.

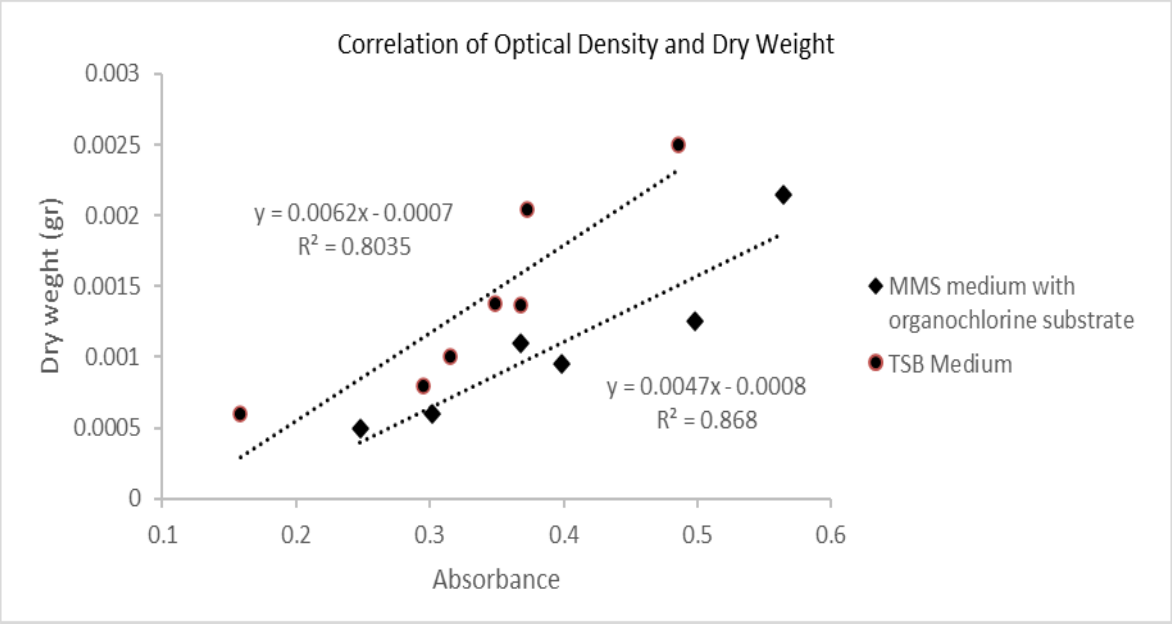

Figure 1. Linear correlation between dry weight and optical density (OD) for *Pseudomonas chlororaphis* cultured in TSB and MSM media with organochlorine substrate at 100 ppm.

3.2 Growth kinetics with and without organochlorine substrate

Figure 2 shows the complete growth kinetics of *Pseudomonas chlororaphis* CA-1 in the presence and absence of organochlorine substrate. Similar growth was observed in both treatments, although with a slight decrease in biomass production when the organochlorine substrate was present. This suggests that *Pseudomonas chlororaphis* CA-1 is capable of growing and using said substrate as a carbon source.

Significant cell growth was observed at a concentration of 100 ppm, similar to that reported by Mohanty *et al*. (2017). The authors pointed out that microorganisms of the genus *Pseudomonas* maintain growth, although it is affected when a toxic compound is used as the sole carbon source. Mohanty reported a maximum biomass production of 0.079 mg/mL, while in this study a biomass of 25 mg/mL was obtained after 14 hours of fermentation in TSB medium (Saibu *et al*., 2020). These results indicate that the presence of the organochlorine substrate affects cell viability after 30 hours of fermentation compared to experiments without the organochlorine substrate.

The difference in means analysis was performed using the Student t-test. The results showed an observed difference greater than the expected random variability, indicating a significant difference between growth kinetics with and without the presence of the organochlorine substrate. This confirms that the organochlorine substrate affects biomass production, although it does not alter the growth profile of *Pseudomonas chlororaphis* (Nelson, J., *et al*., 2014).

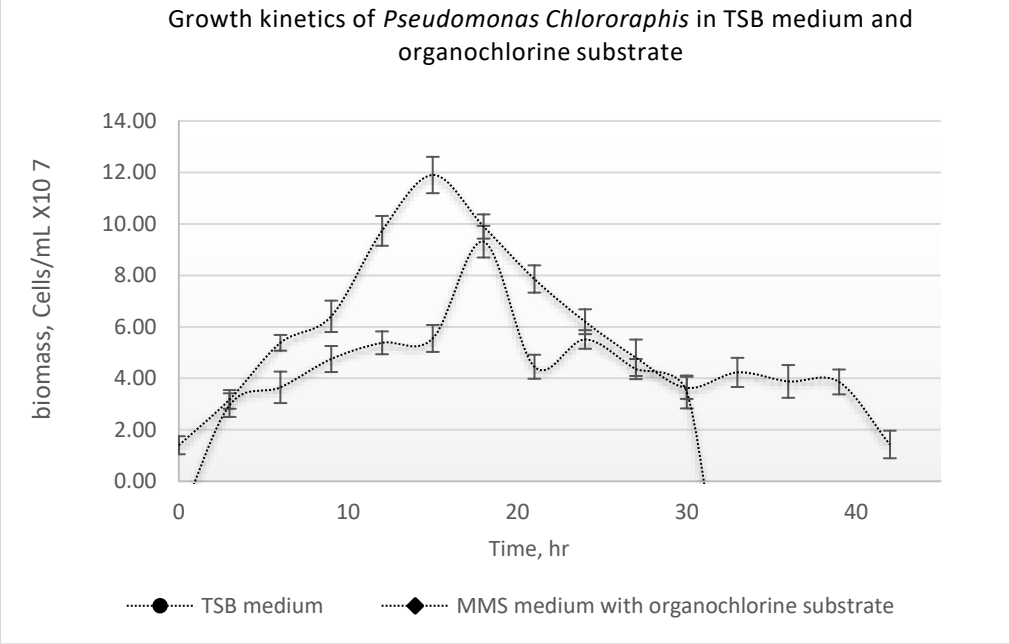

Figure 2. Growth kinetics of *Pseudomona chlororaphis* with organochlorine substrate ♦ at 100 ppm, and ● TSB medium.

These results are consistent with those reported by Aswathi (2019), who documented the use of *Pseudomonas* for the biodegradation of chlorpyrifos, observing growth kinetics similar to those in this work, with a removal of 97%. However, it should be considered that chlorpyrifos has a similar degree of recalcitrance because it contains

chlorine atoms in its structure, like the organochlorine substrate evaluated in this research.

From the growth kinetics in the presence of the organochlorine substrate, a modified version of the logistic model (Zwietering *et al*., 1990) was used to obtain kinetic growth parameters for *Pseudomonas chlororaphis* CA-1, yielding a growth rate of $\mu = 0.16176$ cells/mL·h and an $R^2 > 0.94$, which indicates that the model fits the experimental data. This is similar to what was observed by Aswathi *et al*. (2019), who reported *Pseudomonas* in the degradation of chlorpyrifos, an organophosphorus pesticide. In turn, Gilani *et al*. (2016) reported 92% removal of chlorpyrifos at concentrations of 50 ppm at 720 hours. On the other hand, Kong, J. *et al*. (2017) studied the degradation of phenanthrene by a strain *of Pseudomonas stutzeri* JP1, showing removal results of 33.23% in the first 24 hours at a concentration of 20 mg/L.

Figure 3 shows the early stages of biomass production in MSM at two different concentrations of the organochlorine substrate. In both growth curves, the adaptation and initial exponential phases are observed. Biomass production was affected by the concentration of the organochlorine substrate in the medium, resulting in a higher biomass yield of 3.49×10^{-3} µg/mL at 100 ppm compared to 1.48×10^{-3} µg/mL at 1000 ppm.

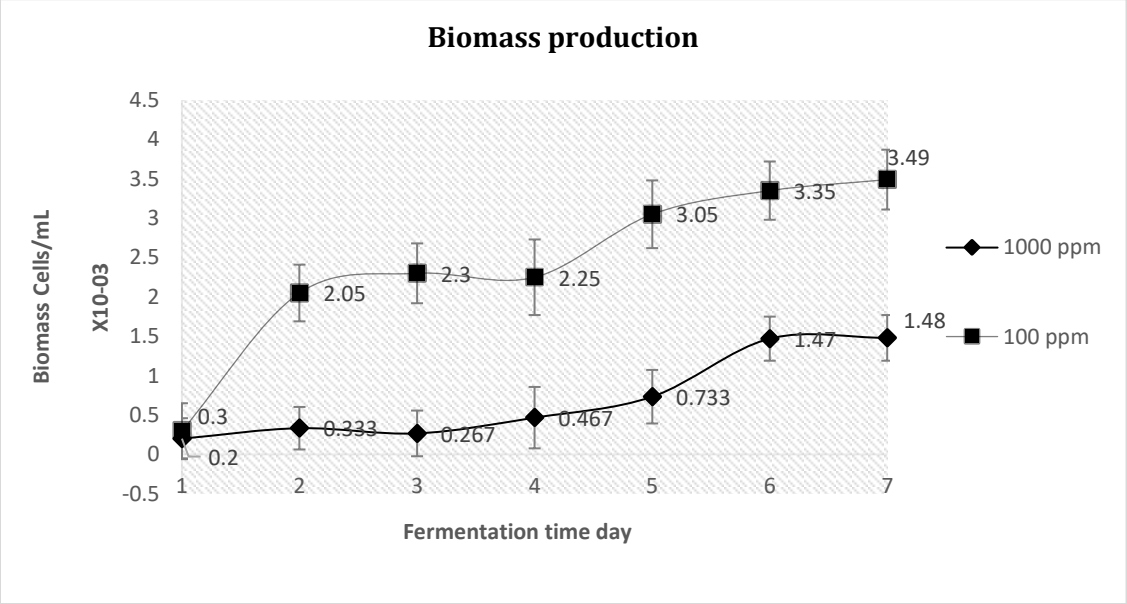

Figure 3. Growth of *P. chlororaphis* CA-1 in MSM, ■ 100 ppm organochlorine and ♦ 1000 ppm organochlorine.

Similar growth was observed in both treatments, although with a slight decrease in biomass production at 1000 ppm of organochlorine substrate. This suggests that *Pseudomonas chlororaphis* CA-1 is capable of growing and using this substrate as a carbon source, but at 1000 ppm, it inhibits biomass production by up to 50%.

3.3. Degradation of the organochlorine substrate

The percentage removal of the organochlorine substrate was determined. The data are presented in Table 2, where the percentage decrease for each compound present in the organochlorine substrate and the total area corresponding to the original substrate can be observed (Figure 4).

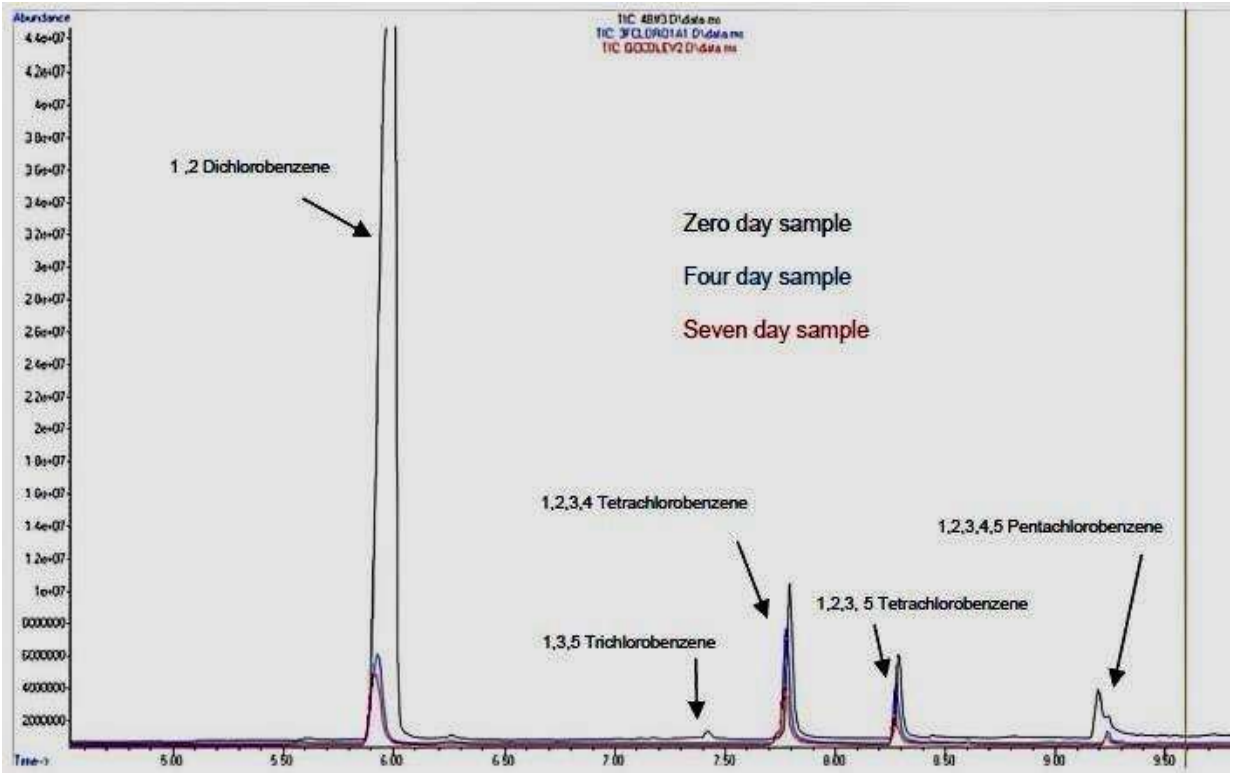

Figure 4. Overlay chromatograms at different incubation times showing the area reduction in peaks representative of the organochlorine substrate.

The data presented in Table 2 indicate the percentage of degradation by *P. chlororaphis*, as well as the ppm corresponding to the degradation. This percentage removal of the organochlorine substrate can be explained by its low solubility, which limits its bioavailability for cells (Otiniano-García, *et al*., 2013).

The data show greater removal of 1,2-dichlorobenzene (91%), while removal of tetrachlorobenzene is 33%. These data coincide with those reported by Sander *et al.* (1991), who demonstrated that a strain of *Pseudomonas* is capable of dechlorinating chlorobenzene molecules, including 1,2,3-trichlorobenzene and 1,2,3,4-tetrachlorobenzene, to obtain monochlorobenzene. Their work indicates that molecules with a higher number of chlorine atoms are less susceptible to losing chlorine atoms anchored in the aromatic ring, demonstrating that the higher the number of chlorine atoms, the lower the ability of the Pseudomonas strain to degrade them.

Potrawfke *et al*. (1998) studied the degradation of haloaromatics by *Pseudomonas chlororaphis* RW71 and demonstrated that the greater the number of chlorine atoms (e.g., penta- and hexachlorobenzene), the more resistant the compounds are to bacterial attack, showing greater degradation of molecules such as 1,3-dichlorobenzene, 1,4-dichlorobenzene, and 1,2,4-trichlorobenzene.

These studies demonstrate that *Pseudomonas* CA-1 has a greater affinity for degrading molecules with fewer chlorine atoms. This effect is observed in the greater removal of 1,2-dichlorobenzene (91%) compared to other organochlorine compounds, which range from 66% to 33%.

The data in Table 3 show that the percentage of degradation of each peak of the initial organochlorine substrate was quantified. It can be observed that Pseudomonas more easily degrades compounds with fewer chlorine atoms. Table 3 summarizes degradation of the organochlorine substrate at an initial concentration of 1000 ppm by *P. chlororaphis*. When comparing these results with those obtained at 100 ppm, a higher percentage of removal is observed, despite the low biomass production.

Table 2. Degradation percent of organochlorine substrate at 100 ppm.

Initial concentration 100 ppm	**7 days fermentation**		**Degradation percentage**
Compound	Initial percent	Final percent	
Dichlorobenzene	83.76	37.28	91.66
Trichlorobenzene	5.44	1.73	66.11
Tetrachlorobenzene	10.46	46.66	33.68
Total			83.60

There are no previous reports indicating the use of an organochlorine mixture similar to the one used in this study as the sole carbon source. Furthermore, bacterial tolerance at the concentrations applied in this study has not been documented (Molano *et al*., 2016).

Table 3. Degradation percent of organochlorine substrate at 1000 ppm.

Initial concentration 1000 ppm	**7 days fermentation**		**Degradation percentage**
Compound	Initial percent	Final percent	
Dichlorobenzene	83.76	50.36	93.72
Trichlorobenzene	5.44	1.53	63.86
Tetrachlorobenzene	10.46	42.86	57.23
Total			89.55

The data obtained were compared with a previous study in which endosulfan was used as the sole substrate at a concentration of 4 ppm, achieving 99% removal. However, that study showed an inhibitory effect attributed to the presence of the organochlorine compound. Contreras *et al*. (2020) reported that concentrations of 800 ppm cause total inhibition of bacterial growth. In contrast, the results of the present study demonstrate that *Pseudomonas chlororaphis* CA-1 exhibits greater tolerance to the presence of organochlorine compounds, even achieving removal at higher concentrations.

Vanitha *et al*. (2023) investigated the decomposition of 2,4-dichlorophenoxyacetic acid, a chlorinated herbicide, at maximum concentrations of 100 ppm during 12 days of incubation, achieving a maximum biodegradation of 90%. They used a microbial consortium that included A*rthrobacter sp.*, *Sphingomonas sp*., and *Stenotrophomonas sp*.

Simultaneously, they used a microbial consortium containing strains of *Bacillus sp*. and *Pseudomonas sp*., achieving nearly 100%

removal in an incubation time of 4 days, with an inoculum volume of 10%.

A similar study was carried out by Samiappan and Ravichandran (2023), who worked on the elimination of organochlorine compounds related to chlorpyrifos using two strains, *P. aeruginosa* and *P. fluorescens*. These strains were the only ones capable of tolerating the toxic compound, despite increasing the concentration of the organochlorine compound fivefold. The removal percentages ranged from 14 to 25% at high concentrations and approached 100% at low concentrations of chlorpyrifos during a five-day incubation period.

This phenomenon has also been observed in previous studies. Maymó-Gatell, *et al*. (1999) used a microbial consortium where slow biomass growth but high enzymatic activity was observed, using the chlorinated compound as an electron acceptor, providing little energy available for cell growth.

Similarly, Nzila, A. (2013) demonstrated that *Pseudomonas* performs cometabolism due to the high presence of a cosubstrate, showing effective degradation without an increase in biomass induced by the contaminant present. This phenomenon is considered to be occurring in our experiments with the increase in organochlorine substrate concentration. *Pseudomonas chlororaphis* CA-1 uses the organochlorine substrate as a cosubstrate to induce enzymes to transform it. Cometabolism allows the chlorinated substrate to be degraded without it contributing to significant biomass growth.

These studies support the idea that *Pseudomonas sp*. strains have a superior ability to biodegrade chlorinated compounds. This assertion is reinforced by studies conducted by Muhammad *et al*. (2025), who studied the biodegradation of butachlor in soil using a strain of *Pseudomonas aeruginosa*, demonstrating a 90% removal at concentrations of 100 ppm after 5 days of fermentation. This indicates that strains of the genus *Pseudomonas sp*. are more tolerant to high concentrations of chlorinated compounds and can use them as an energy source, achieving higher removal rates in a shorter period of time compared to strains of other genera.

Rodríguez-Orozco *et al.* (2025) studied strains of *Bacillus cereus* and *Paenibacillus lautus* for the removal of organochlorine and organophosphorus compounds, showing removal percentages approximately from 50 to 80% during 12 days of incubation at maximum concentrations of 80 ppm.

These studies show that the *Pseudomonas chlororaphis* CA-1 strain has a higher tolerance to the presence of organochlorine compounds at high concentrations and achieves higher biodegradation percentages, as reported in these studies.

IV. Conclusions

The *P. chlororaphis* CA-1 strain tolerates high concentrations of organochlorine compounds and uses them as a carbon and energy source to produce biomass. The results showed higher biomass production, reaching $3.49x10^{-3}$ µg/mL at a concentration of 100 ppm, while at 1000 ppm biomass production of $1.48x10^{-3}$ µg/mL was obtained.

This difference is due to the recalcitrant nature of organochlorine compounds at high concentrations in the medium. However, the *P. chlororaphis* strain also tolerates high concentrations of haloaromatic compounds, achieving removal of 83.6% at 100 ppm and 89.5% at 1000 ppm. This phenomenon has previously been reported as cometabolism, where the chlorinated substrate is eliminated by high enzymatic activity despite low biomass production, since the microorganism

does not use it as a source of carbon and energy.

Future research will focus on this particular topic, seeking to understand the process of cometabolism activation in *Pseudomonas CA-1* and modulate enzymatic activity in this type of degradation.

V. References

1. Sierra-Cortés, J. C., Vega y León, S., Gutiérrez-Tolentino, R., Ortis-Salinas, R., Pérez-González, J. J., and Escobar-Medina, A. C. (2019). Plaguicidas organoclorados en agua de la laguna negra de puerto marqués, acapulco, guerrero, México. *Rev. Int. Contam. Ambiente*, 35(2), 397-406. https://doi.org/10.20937/rica.2019.35.02.11

2. Pimviriyakul, P., Wongnate, T., Tinikul, R., and Chaiyen, P. (2020). Microbial degradation of halogenated aromatics: molecular mechanisms and enzymatic reactions. *Microbial Biotechnology*, *13*(1), 67-86. https://doi.org/10.1111/1751-7915.13|8.

3. Kopytko, M., Correa-Torres, S. N., and Estévez- Gómez, M. J. (2017). Biodegradación estimulada de los suelos contaminados con pesticidas organoclorados. *Revista De Investigación Agraria Y Ambiental*, 8 (1), 119-130. https://doi.org/10.22490/21456453.1843.

4. Saibu, S., Adebusoye, S. A., Oyetibo, G. O., and Rodrigues, D. F. (2020). Aerobic degradation of dichlorinated dibenzo-p-dioxin and dichlorinated dibenzofuran by bacteria strains obtained from tropical contaminated soil. *Biodegradation*, 31(1), 123-137. https://doi.org/10.1007/s10532-020-09898-8.

5. Otiniano-García, N. M. (2013). Efecto de la fuente nitrogenada sobre la capacidad de degradación del petróleo diesel 2 por *Pseudomonas sp*. octubre 10-11: Cuenca Ecuador. 867-881 Universidad Politécnica Salesiana, Memorias del II Congreso Binacional de investigación, Ciencia y tecnología de las Universidades. http://dspace.ups.edu.ec/handle/123456789/11201.

6. Sander, P., Wittich, R. M., Fortnagel, P., Wilkes, H., and Francke, W. (1991). Degradation of 1, 2, 4-trichloro-and 1, 2, 4, 5-tetrachlorobenzene by *Pseudomonas* strains. *Applied and Environmental Microbiology*, *57*(5), 1430-1440. https://doi.org/10.1128/aem.57.5.1430-1440.1991.

7. Potrawfke, T., Timmis, K. N., & Wittich, R. M. (1998). Degradation of 1, 2, 3, 4-tetrachlorobenzene by *Pseudomonas chlororaphis* RW71. *Applied and environmental microbiology*, *64*(10), 3798-3806. https://doi.org/10.1128/AEM.64.10.3798-3806.1998.

8. Contreras, F. P. V., Alvarado, K. D. C. C., Rosalba, E., Escalante, M., Valencia, L. H. Á., Tenorio, L. M. D., and Palacios, D. S. (2020) Evaluación de la eliminación del endosulfán en un sistema hibrido y su efecto sobre el consorcio microbiano presente. *Revista Latinoamericana el Ambiente y las Ciencias*, 11 (28), 252-256. (rlac.buap.mx).

9. Molano Niño, J. E., and Flórez Pachón, M. D. P. (2016). Prueba piloto para la determinación de la tolerabilidad del carbofurano usando *pseudomonas sp* proveniente del suelo. https://ciencia.lasalle.edu.co/ing_ambiental_sanitaria/1

10. Kahlon, R. S. (Ed.). (2016). *Pseudomonas: molecular and applied biology*. Editorial Berlin, Germany:Springer. DOI 10.1007/978-3-319-31198-2.

11. Cui, G., Chien, M. F., Suto, K., and Inoue, C. (2017). Analysis of stable 1, 2-dichlorobenzene-degrading enrichments and two newly isolated degrading strains, *Acidovorax sp*. sk40 and *Ralstonia sp*. sk41. *Applied microbiology and biotechnology*, 101(17), 6821-6828. https://doi.org/10.1007/s00253-017-8406-2.

12. Luján, D. (2019). Uso de *Pseudomonas aeruginosa* en biorremediación. *Bio Tecnología*, *23*(1), 32-42. https://www.researchgate.net/publication/335871095.

13. Huang, L., Wang, W., Shah, S. B., Hu, H., Xu, P., and Tang, H. (2019). The HBCDs biodegradation using a *Pseudomonas* strain and its application in soil phytoremediation. *Journal of Hazardous Materials*, 380, 120833. https://doi.org/10.1016/j.jhazmat.2019.12083.

14. Lu, Y., Ramiro-Garcia, J., Vandermeeren, P., Herrmann, S., Cichocka, D., Springael, D., and Smidt, H. (2017). Dechlorination of three tetrachlorobenzene isomers by contaminated harbor sludge-derived enrichment cultures follows thermodynamically favorable reactions. *Applied Microbiology and Biotechnology*, 101(6), 2589-2601. https://doi.org/10.1007/s00253-016-8004-8.

15. Gilani, R. A., Rafique, M., Rehman, A., Munis, M. F. H., Rehman, S. U.,and Chaudhary, H. J. (2016). Biodegradation of chlorpyrifos by bacterial genus Pseudomonas. *Journal of basic microbiology*, 56(2), 105-119. https://doi.org/10.1002/jobm.201500336.

16. Moreno, W. X. I., Logroño, J. P. A., & Zambrano, P. V. M. (2020). Degradación de pesticidas organoclorados mediante la aplicación de nanopartículas de TiO_2 y sus posibles aplicaciones. *dominio de las ciencias*, *6*(2), 803–834. https://Doi.org/10.23857/dc.v6i2.1195.

17. Zwietering, M. H., Rombouts, F. M. and Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Appl. Environ. Microbiol*. 56 (6), 1875-1881. DOI: https://doi.org/10.1128/aem.56.6.1875-1881.1990.

18. Gómez-Reyes, R., Medina-Moreno, S. A., Jiménez-González, A., and Lizardi-Jiménez, M. A. (2017). Aislamiento y análisis cualitativo de biomasa microbiana fúngica degradadora de hidrocarburos de un cenote de Quintana Roo. *Revista Internacional de Contaminación Ambiental* 33,53-61.

https://doi.org/10.20937/RICA.2017.33.esp01.05

19. Mohanty, S. S., and Jena, H. M. (2017). Biodegradation of phenol by free and immobilized cells of a novel Pseudomonas sp. NBM11. *Brazilian Journal of Chemical Engineering*, 34(1), 75-84. https://doi.org/10.1590/0104-6632.20170341s20150388.

20. Nelson, J. L., Jiang, J., and Zinder, S. H. (2014). Dehalogenation of chlorobenzenes, dichlorotoluenes, and tetrachloroethene by three *Dehalobacter spp*. *Environmental science & technology*, *48*(7), 3776-3782. https://doi.org/10.1021/es4044769.

21. Aswathi, A., Pandey, A., and Sukumaran, R. K. (2019). Rapid degradation of the organophosphate pesticide–Chlorpyrifos by a novel strain of *Pseudomonas nitroreducens* AR-3. *Bioresource technology*, 292, 122025. DOI: 10.1016/j.biortech.2019.122025.

22. Kong, J., Wang, H., Liang, L., Li, L., Xiong, G., and Hu, Z. (2017). Phenanthrene degradation by the bacterium *Pseudomonas stutzeri* JP1 under low oxygen condition. *International Biodeterioration & Biodegradation*, *123*, 121-126. https://doi.org/10.1016/j.ibiod.2017.06.001.

23. Vanitha, T. K., Suresh, G., Bhandi, M. M., Mudiam, M. K. R., and Mohan, S. V. (2023). Microbial degradation of organochlorine pesticide: 2, 4-Dichlorophenoxyacetic acid by axenic and mixed consortium. *Bioresource technology*, 382, 129031. https://doi.org/10.1016/j.biortech.2023.129031.

24. Samiappan, S.C., Ravichandran, M. (2023). Characterization of Chlorpyrifos Degrading *Pseudomonas sp*. Isolated from Contaminated Soil. In *Sustainable and Cleaner Technologies for Environmental Remediation: Anenuesin Nano and Biotechnology*. 35-47, Cham Springer International Publishing. https://doi.org/10.1007/978-3-031-29597-3_4.

25. Maymó-Gatell, X., Chien, Y. T., Gossett, J. M., and Zinder, S. H. (1999). Reductive dechlorination of tetrachloroethene to ethene by a microbial consortium. *Environmental Science & Technology*, 33(3), 479–484. https://pubmed.ncbi.nlm.nih.gov/10329282/

26. Nzila, A. (2013). Update on the cometabolism of organic pollutants by bacteria. *Environmental pollution*, 178, 474-482. https://doi.org/10.1016/j.envpol.2013.03.042.

27. Muhammad, B., Bukar, A., and Umar, S. (2025). Biodegradation of Butachlor by *Pseudomonas aeruginosa* Strain B2 Isolated From Agricultural Soil. *Journal of Environmental Microbiology and Toxicology*. 13 (1), 18-22. https://doi.org/10.54987/jemat.v13i1.1106.

28. Rodríguez-Orozco, V. A., Duarte-Restrepo, E., and Jaramillo-Colorado, B. E. (2025). Isolation of Bacteria from Agricultural Soils and

Evaluation of Their Degradative Capacity for Organochlorine and Organophosphorus Pesticides. *Agronomy*, 15(4). https://doi.org/10.3390/agronomy15040814.