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Original Research Article

Pseudomonas aeruginosa Bio-Degradation Chemosate

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Abstract: Since bioremediation of pesticides is environmentally safe, economical, and efficient, it is now the preferred option. The purpose of the study was to assess the Pseudomonas sp. Bioremediation Chemosate at various concentrations (10,20,30) ppm and incubation times. The isolation of the Pseudomonas sp from the soil region in Baghdad grows on Pseudomonas selective at room conditions for (18-48) hours at 35 Ċ. Pseudomonas sp. are the bacteria that are produced when soil samples are diluted using selective media. Because glycerol was added to the culture as a reagent, isolated Pseudomonas aeruginosa produced a strong strain with green-blue. It was then grown on mineral salt media as a source to examine the biodegradation for (10,20,30) ppm during (10,20,30) days to count the bacterial growth. The best results were 20 ppm/0.729, 20 days incubation, while the best Pseudomonas areuginosa degradation rate% was 10 ppm/97.5%, 10 days. The HPLC results showed that 10 ppm at 10 days produced the optimum bio-degradation results.

Keywords: Microbes, Organic compounds, Biodegradation.

INTRODUCTION

Because it is environmentally benign, economically valuable, reduces environmental pollution, and is validated, bioremediation is used as an auxiliary technique (Sviridov AV *et al.,* 2015). An auxiliary technique is a supplementary or supportive method used to improve the effectiveness of a primary technique in various fields. These techniques serve as aids or substitutes for the primary method, helping to clarify actions, enhance performance, overcome limitations, or solve complex problems (Chen J *et al.,* 2023). Pesticides have become an environmental contaminant due to issues with their proper management and disposal (Mali H *et al*., 2023). Regular use of pesticides contaminates the soil and water, lingers in crops and plants, penetrates the food chain, and becomes biomagnified, with dire repercussions. The physicochemical characteristics of the active ingredient, the method of application, and changing environmental factors are the main factors that determine how much pesticide is released into the air, soil, and water (Zhan H *et al.,* 2018). However, in order to increase agricultural productivity, which is responsible for approximately 45% of the annual loss in food production, a wide range of pesticides must be utilized against weeds, poisonous microbial compounds, and effective pests. It improves food security for the world's population, which is continuously. Approximately one-third of agricultural products contain pesticides. Pesticides have improved human health standards by slowing the development of vector-borne illnesses, but long-term, careless pesticide use has had negative effects. Humans, particularly infants and children, are particularly vulnerable to the negative effects of pesticides due to their general nature and indiscriminate application (Chen J *et al.,* 2023). A chemo stat is essentially a bioreactor to which growing medium is continuously fed. The vessel's contents are entirely jumbled. The culture liquid, which comprises substrates, metabolites, and microorganisms, is continually taken from the reactor to maintain a consistent volume (Pashirova T *et al.,* 2024).

Chemosate is an organophosphate, a heterogeneous chemical, and a non-specific herbicide that works by damaging plant leaves (Mousa N *et al.*, 2021). Since the 1960s, organophosphate pesticides have been widely utilized throughout the world (Nepali B *et al.*, 2018). Organophosphate insecticides are less resistant to environmental effects than organochlorine pesticides (Karunya SK & Saranraj P 2014). Additionally, a central phosphate molecular group is included in organophosphate insecticides. The chemosate's high water solubility makes it difficult to determine its physical and chemical properties, making it a difficult herbicide in trace analysis (Upadhyay S *et al.*, 2015).

One of the bioremediation techniques is the capacity of microorganisms to remove contaminants (Mkpuma, DUM & Simeon VOE 2015; Zhu J *et al.*, 2019). Microorganisms require a specific set of chemical and physical conditions to grow, including nutrients such as carbon, nitrogen, phosphorus, and sulfur, as well as trace elements and an energy source (Chandrashekar MA *et al.*, 2017). Physical requirements include a suitable temperature, water (moisture), an appropriate pH level, sufficient osmotic pressure, and specific gases like oxygen for some types. These requirements determine an organism's ability to thrive and vary significantly between different microbial species (Borji A *et al.*, 2014). Pseudomonas species are versatile bioremediation microorganisms that degrade various recalcitrant organic pollutants, including phenolic, hydrocarbons, surfactants, and even certain plastics like poly-phenylene sulfide and polystyrene (Mousa N *et al.*, 2021). They achieve this through diverse metabolic pathways, often enhanced by secreting bio-surfactants to increase substrate bioavailability, making them valuable for environmental cleanup of industrial and agricultural waste (Mattah MM *et al.*, 2015). The goal of the study is to examine bacteria separated by selective media for degradation of chemosate (10,20,30) ppm during (10,20,30) days, then evaluate the residue of it by HPLC.

MATERIALS AND METHODS

Materials

Chemosate supply from the Iraqi Agriculture Ministry, while all equipment, solution, and instruments are available freely at the Center for Environmental, Water, and Renewable Research and Technology, Scientific Research Commission, Al-Jadria, Baghdad.

Pseudomonas Sp. Selective Culture Isolation

From Basmaya City, samples were gathered around (12 cm) in plastic packets (Castro JV *et al.*, 2007; Mousa N *et al.*, 2021). Then 10g of soil was placed in 10 ml of distilled H_2O (D.W.) as a stock solution (Mousa N & Abdul Hassan M 2025). The prepared substrates were poured, utilized dilutions (10^{-2}, 10^{-4},10^{-8}); from the test tube, (0.1) ml solution to the Selective media for *Pseudomonas aeruginosa*, (Table 1), at incubation conditions at (35± 1) °C for (18-48) hrs. (Isenberg HD & Garcia LS 2004).

Table 1: Selective media for *Pseudomonas areuginosa*

Typical Formula	(g/l)	45g from the component solved in 1L D.W., besides 20mL (glycerol). Mix thoroughly and heat gently to then sterilize at 121°C for 15 minutes.
Pepton	16.0	
Magnesium Chloride	10.0	
Potassium Sulfate	10.0	
Irgasan	25 mg	
Agar	13.0	
Glycerol	10.0 ml	
Final pH 7.1 ± 0.2		

Bio- Remediation Assay

The Mineral Salt Media (table 2) promoted with pesticides at different concentrations (10, 20, 30) ppm/l with triply, the falsk of 125ml capacity were supplemented with chemosate (10,20,30) ppm, after that, filling the test tubes with 10 ml of water (D.W), under laminar airflow, and period (10,20,30) days incubation with *Pseudomonas areuginosa*, were examined for the degradation % by UV- spectroscopy (600nm) (Mousa N & Abdul Hassan M 2025).

Table 2: The minerals of the mineral salt media (MSM)

Amount	**Component**	**Amount**	**Component**
0.2	(KH_2PO_4)	0.2	NaCl
0.5	(K_2HPO_4)	0.05	$CaCl_2.2H_2O$
1	$(NH_4)_2SO_4$	0.025	$FeSO_4•7H_2O$
0.2	$MgSO_4.7H_2O$	0.005	$Na_2Mo\ O_4.2H_2O$
All were sterilized (125 ºC /25 min), gathered and added to part A of a 1-liter flask, and adjusted (pH 7.0 ± 0.3)			

By utilizing biodegradation as a key agent, the fate of organic pesticides in the ecosystem can be better understood and the degradation % (Mousa N & Abdul Hassan M 2025) can be measured by equation (1) below:

$P = C1 \times 100 / Cn$ (1)

P= degradation %,
C_1= residue (mg/ l); C_n (10,20,30) mg/l.

The HPLC analysis

The residues from bio-degradation were analysed by high-pressure liquid chromatography technique (HPLC) (Kaczyński P and Łozowicka B 2015; Shweta N *et al.*, 2017; Mousa N & Abdul Hassan M 2025) (Table 3).

Table 3: The HPLC conditions assay

HPLC Condition Analysis	
UV-Vis Detector	254 nm
Manual Injector Equipped	20-µL loop
Column	C-18
Mobile phase	Acetic acid (1%) with CH_3OH (6:4).
Rate Flow (ml/min)	1.0
Temperature	25 °C

By using equation (2), (Mousa N & Abdul Hassan M 2025):

$$The\ Removal\ Efficiency\ (R)\,\% = [C0 - Ct\ /C0]\ x\ 100\,\% \qquad (2)$$

Removal efficiency (R%);
C_0 the (10,20,30) mg/l concentration of pesticide,
C_t= the residues from biodegradation

RESULTS AND DISCUSSION

Pseudomonas Sp. Selective Media

The results from Pseudomonas selective media that distinguish only the *P. aeruginosa* due to glycerol work as a detection based on the pigment formation from the dilution $10^{-2,}$ produce green-blue plates (Isenberg and Garcia, 2004).

Pseudomonas sp. Growth % and Degradation Rate %.

The best growth of *Pseudomonas aeruginosa* was 20 ppm /0.729, 20 days' incubation, (Fig. 1), while the best Pseudomonas *aeruginosa* degradation rate% results were 10 ppm / 97.5%, in 10 days, (Fig. 2).

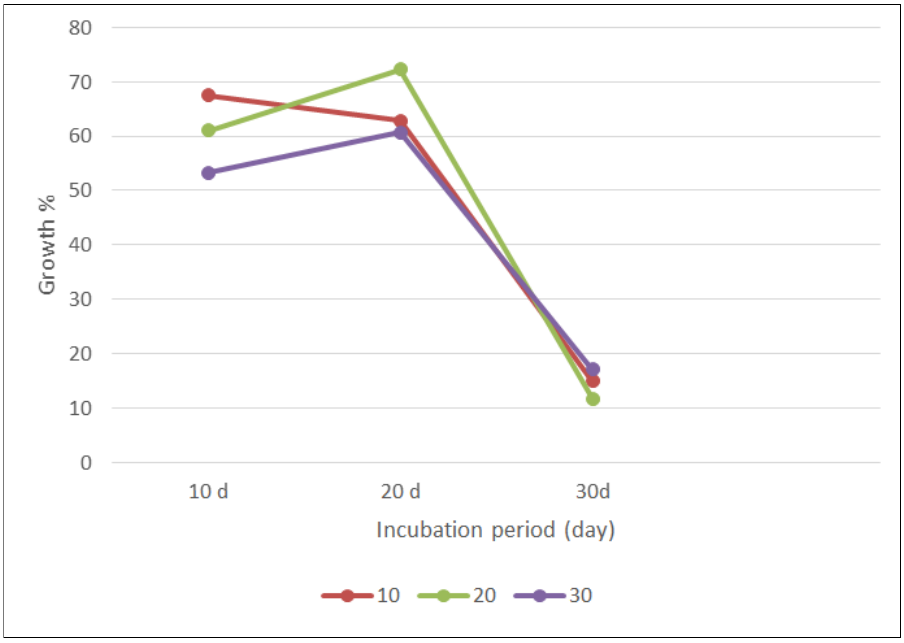


Fig. 1: Chemosate and *Pseudomonas sp.* Growth %(MSM)

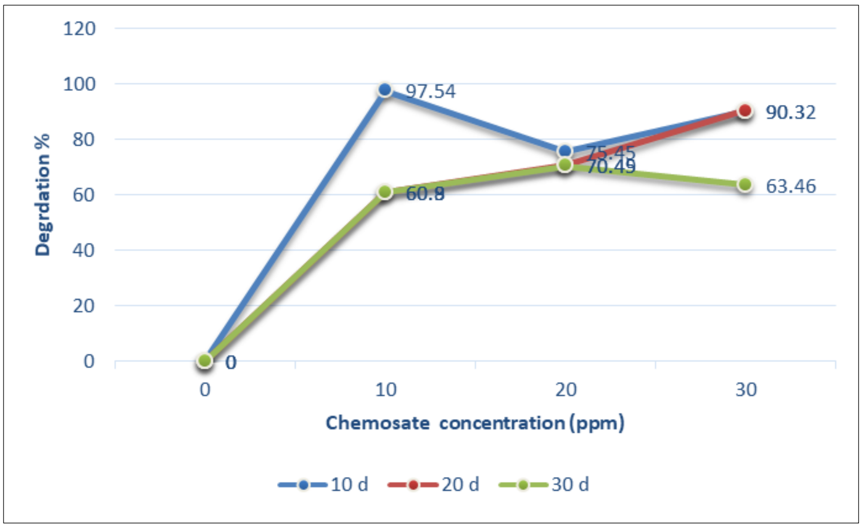


Fig. 2: Chemosate Degradation % in Mineral Salt Media

HPLC Test of the Chemosate Residues

Preparations series concentrations of Chemosate (10,20,30)ppm to create a standard curve calibration (Fig. 3), besides the best results of bio-degradation were for 10 ppm at 10 days (Fig. 4) (Islas G *et al*., 2014).

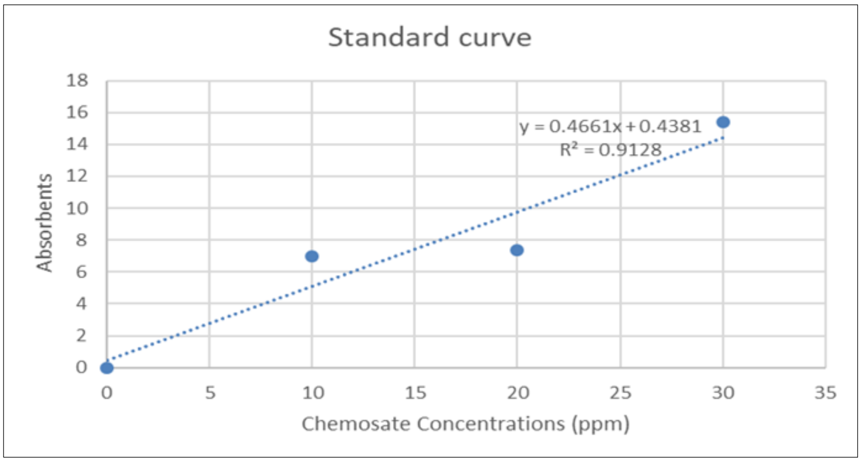
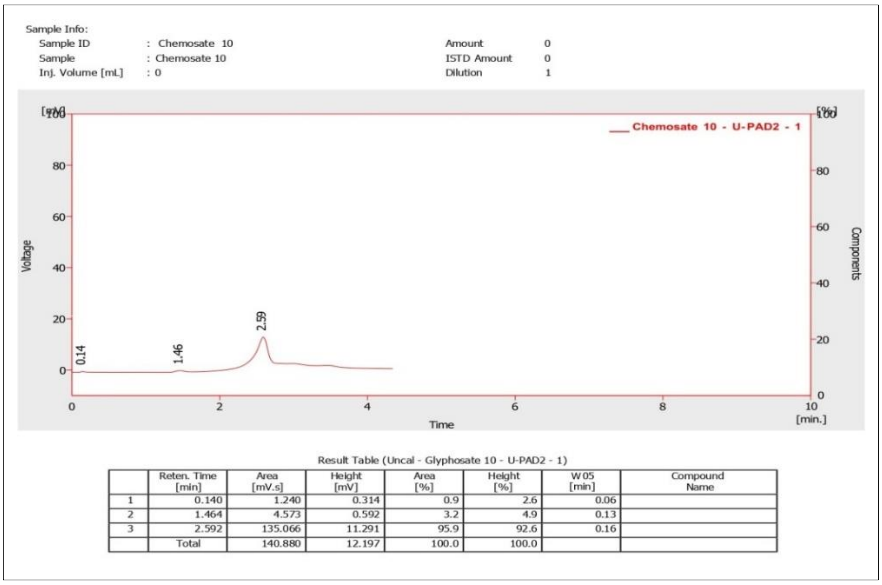


Fig. 3: Standard Curve of Chemosate



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.140	1.240	0.314	0.9	2.6	0.06	
2	1.464	4.573	0.592	3.2	4.9	0.13	
3	2.592	135.066	11.291	95.9	92.6	0.16	
	Total	140.880	12.197	100.0	100.0		

Fig. 4: Chemosate residues at 10 ppm/10 days

Microbes' degradation of harmful components, depending on different methods to promote them as nitrogen, carbon and phosphorus sources that supply them with elements for production and growth metabolism (Pashirova T *et al.*, 2024), also by producing intermediate metabolites as AMPA, sarcosine, glyoxylate, and glycine (Duke SO 2011; Mbanaso FU *et al.*, 2014; Singh S *et al.*, 2020). Tazdaït D *et al.*, (2018) found that glyphosate at initial concentrations of (0.1, 0.5, and 1) g/L was completely degraded within 4, 13, and 18 h of incubation in active sludge. Other microbes like *Bacillus megaterium* and *Azotobacterium* sp., which improved the considerable biodegradation (Mousa N *et al.*, 2021; Mali H *et al.*, 2023). that agreed with *Azotobacter sp*. to utilize Chemosate as nitrogen and carbon sources, also to digest phosphorus in the Media, which increased concentrations, resulting in a significant increase in biodegradations, at 1-2 months incubation periods, digesting from the concentration (25 ppm), reaching (81-79) % (Mousa N *et al.*, 2019). The best residues of glyphosate analysis by PGPB were: *Bacillus megaterium* in comparison to *B. subtilis; Rhizobium sp.* and *Azotobacter sp.* (Mousa N & Abdul Hassan M 2025).

CONCLUSIONS

The bacteria that result from soil samples dilution by the Selective media, Pseudomonas sp. İsolated P. areuginoas gave a strong strain with green-blue due to the glycerol that was added in the culture as a reagent, then its grown on the mineral Salt Media as source for examining the biodegrdation% for (10,20,30) ppm during (10,20,30) days, to count the bacteria growyth% and the best was 20 ppm /0.729, 20 days incubation, while the best *Pseudomonas areuginosa* degradation rate% results were 10 ppm / 97.5%, in 10 days. The HPLC results led to the best results of bio-degradation, which were for 10 ppm at 10 days.

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