

## Harnessing Marine Pyoverdine from *Pseudomonas otitidis*: Extraction, Characterization, and Cr (VI) Bioremediation

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### ABSTRACT

This study investigated the extraction and purification of marine pyoverdine from *Pseudomonas otitidis* EGY-NIOF-A1, a fluorescent pigment analyzed for purity using reverse-phase HPLC. Structural characterization through <sup>1</sup>H NMR, <sup>13</sup>C NMR, FT-IR, and XRD revealed aliphatic regions, hydroxyl and amide groups, and a partially crystalline structure, all of which are essential for metal chelation. Cytotoxicity tests confirmed its environmental safety. The potential of pyoverdine for Cr (VI) bioremediation was evaluated through batch experiments, which showed maximum adsorption at pH 1 and a Langmuir isotherm fit, with an adsorption capacity of 63.69mg/ g. These findings position pyoverdine as an eco-friendly, cost-effective agent for chromium remediation, with the potential to enhance water quality in both environmental and aquaculture systems.

### INTRODUCTION

Pyoverdine is a yellow-green, water-soluble fluorescent pigment produced by bacteria of the genus *Pseudomonas* (Meyer, 2000). It plays a pivotal role in iron acquisition, which is essential for bacterial growth and metabolism, particularly under iron-limiting conditions. Pyoverdine contributes to biofilm formation, a protective growth mode that enables bacteria to survive in hostile environments and to resist antimicrobial treatments. Due to its fluorescent properties, pyoverdine serves as a valuable marker in various biological assays to investigate bacterial behavior and iron metabolism. Furthermore, pyoverdine's capacity to chelate iron and other metals has practical applications in bioremediation, aiding in the remediation of heavy metal contamination (Cornelis & Dingemans, 2013).

Chromium ions (VI) represent a major environmental pollutant, commonly found in waste effluents from industries such as leather tanning, electroplating, textiles, petroleum, coal combustion, chromium steel production, fertilizer manufacturing, oil well drilling, dyes, metal plating, paints, pigments, wood preservation, and paper production. Hexavalent chromium [Cr (VI)] is highly toxic, carcinogenic, and associated with various forms of cancer, including pancreatic (**Alguacil *et al.*, 2004**) and respiratory cancers (**Kuo *et al.*, 2006**).

Chromium contamination, particularly in its hexavalent form, poses serious risks to both aquatic ecosystems and human health. Cr (VI) is highly toxic to fish, adversely affecting their gills, liver, and kidneys, and causing oxidative stress that leads to cellular damage and impaired physiological functions. This toxicity can result in decreased swimming activity and feeding behavior, impacting survival and reproduction (**Eisler, 1986**). Furthermore, Cr (VI) bioaccumulates in fish tissues, leading to long-term health effects that render them unsafe for consumption by other animals and humans. Classified as a human carcinogen, Cr (VI) exposure is linked to cancers of the lungs, liver, stomach, and genitourinary system (**Georgaki & Charalambous, 2022**). Ingesting chromium-contaminated water or food can result in systemic toxicity, affecting organs such as the liver, kidneys, and reproductive system (**Hossini *et al.*, 2022**).

Cr (VI) levels in the Mediterranean Sea, particularly Western Harbor of Alexandria, Egypt, can exceed the background levels, with reported concentrations around 0.17 $\mu\text{g}/\text{L}$  for total dissolved chromium. This contamination arises from both natural processes and human activities, including industrial discharges and urban runoff (**Abdallah, 2014**).

**Park *et al.* (2005)** identified that biomass can reduce Cr (VI) to the less toxic Cr (III) through two mechanisms: direct reduction in the aqueous phase via interaction with electron-donor groups and a multi-step process. The latter involves three stages: (1) anionic Cr (VI) ions initially bind to positively charged groups on the biomass surface; (2) these ions are reduced to Cr (III) by nearby electron-donor groups; (3) Cr (III) ions are subsequently released into the aqueous phase due to electronic repulsion with the positively charged groups on the cell surface (**Ahmed *et al.*, 2022**).

Recently, there has been growing interest in the potential of siderophores for metal bioremediation. Siderophores-mediated bioremediation offers an economically and ecologically sustainable alternative to traditional, often hazardous, chemical-based remediation approaches (**Rajkumar *et al.*, 2010**). Siderophores are iron-binding molecules with exceptionally high binding affinity ( $> 10^{30} \text{ M}^{-1}$ ), capable of detoxifying heavy-metal-contaminated soils (**Hesse *et al.*, 2018**) and exhibiting probiotic activities that protect plants from pathogenic infections (**Gu *et al.*, 2020a; Gu *et al.*, 2020b**). Given

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that siderophores are specific to the bacterial species that produce them, there is substantial molecular diversity among these compounds. To date, over 500 chemically distinct siderophores have been identified, with new variants discovered each year (**Hider & Kong, 2010**).

Recent studies highlight the promising application of siderophore-assisted bioremediation in both research and practical settings. The unique dynamics of siderophore-metal complexes significantly influence interactions with organic contaminant by mediating the production of reactive oxygen species (ROS). These ROS facilitates the degradation of toxic organic contaminants, thereby promoting a cleaner and healthier ecosystem.

Siderophore-producing bacteria, especially those resistant to heavy metals, hold great potential as bioinoculants, enhancing plant growth while contributing to soil decontamination and phytoremediation efforts. This dual functionality supports sustainable agriculture and strengthens ecosystem resilience against contamination (**Roskova et al., 2022**)

Among siderophores, pyoverdines, also referred to as fluorescent siderophores, are essential pigments produced by *Pseudomonas* species that play a pivotal role in iron acquisition. These compounds are classified into three main types of pyoverdines, distinguished by variations in their peptide chains and structural composition, which reflect the specific bacterial strain and its adaptation to distinct environmental conditions, with each pyoverdine displaying a high degree of function specificity (**Bonneau et al., 2020**). Pyoverdines, such as those produced by strain PAO1, are iron-chelating chromopeptides composed of 6–14 amino acids and are exclusively produced by fluorescent *Pseudomonas* species. To date, more than 60 unique pyoverdine types have been structurally elucidated (**Meyer et al., 2008**).

Type I pyoverdines are primarily found in strains such as *Pseudomonas aeruginosa*. These pyoverdines have a well-characterized structure comprising a fluorescent chromophore linked to a peptide chain, and they are essential for iron acquisition in many pathogenic strains. This type of pyoverdine is notably significant for the virulence of *P. aeruginosa*, since it enhances the bacterium's ability to thrive in iron-deficient host environments (**Ringel & Brüser, 2018**).

Type II pyoverdines are produced by other *Pseudomonas* species, including *Pseudomonas fluorescens*. They are distinguished by variations in the peptide component of the molecule, which influences their specificity in iron chelation and uptake. The structure flexibility of Type II pyoverdines allows these siderophores to adapt to diverse iron-limited environments, supporting bacterial survival in a range of ecological niches.

Type III pyoverdines are less common and are mainly synthesized by non-pathogenic *Pseudomonas* strains. These pyoverdines exhibit variations in both the chromophore and peptide structures and are predominantly associated with environmental *Pseudomonas* species that inhabit plant roots or soil ecosystems. This type of pyoverdine supports nutrient acquisition in non-pathogenic strains, playing a role in plant-microbe interactions and ecological colonization (Ghssein & Ezzeddine, 2022).

The efficiency of bacterial siderophores, particularly pyoverdines, for bioremediation has been demonstrated in numerous studies. For example, pyoverdines have been shown to extract iron effectively from asbestos waste types, such as chrysotile-gypsum and amosite-gypsum, underscoring their potential for eco-friendly asbestos treatment and reducing associated environmental and health risks (David *et al.*, 2020). Furthermore, coupling microbial bioremediation with phytoremediation has proven successful in enhancing metal remediation efficacy (Newman & Reynolds, 2005). In one study, live *Pseudomonas* sp. cells isolated from Colombian wastewater exhibited significant lead tolerance, with a biosorption efficiency of up to 87% at concentrations exceeding 50mg/ mL (Vélez *et al.*, 2021). Similarly, immobilizing *P. aeruginosa* on Calcium alginate beads increased its resilience to toxic metals and boosted siderophore production in media enriched with Cr, Hg, and Pb (Braud *et al.*, 2006).

Siderophore-mediated bioremediation offers a sustainable and cost-effective alternative to traditional chemical-dependent remediation techniques (Rajkumar *et al.*, 2010). Marine bacteria, with their broad range variability and adaptive potential, are especially promising candidate for Cr (VI) bioremediation, providing eco-friendly solutions to mitigate chromium contamination. Future research will focus on optimizing pyoverdine production using cost effective media for large scale applications and exploring its chelation potential with other heavy metals. Overall, the marine isolate *Pseudomonas otitidis* EGY-NIOF-A1 presents a promising candidate for chromium bioremediation, contributing to enhanced water quality in aquaculture and environmental management.

## MATERIALS AND METHODS

### Pyoverdine extraction and purification

The marine isolate *Pseudomonas otitidis* EGY-NIOF-A1 (Gene accession No: OQ547301) was cultured for 5 days at 37°C with agitation at 120rpm in King's B medium (KB), which selectively enhances the production of pyoverdine over other pigments typically produced by fluorescent pseudomonads (King *et al.*, 1954). After culturing, the cell-free supernatant was obtained by centrifugation, and then treated with chloroform at a ratio of 1:0.6. Upon mixing, a blue-green layer formed, which

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subsequently separated into pink and lemon-yellow phases after acidification with 0.1M HCl at a ratio of 1:0.5. The yellow pyoverdine-rich layer was collected, lyophilized and stored at -20 °C for characterization (Gillani *et al.*, 2023).

### **Reverse-phase high-performance liquid chromatography (HPLC) analysis of marine pyoverdine**

Purified pyoverdine was analyzed by reverse-phase HPLC using a YL9100 vacuum degasser HPLC System (Technolab System Ajman, UAE). Chromatographic separation was achieved with a Hypersil ODS C18 column (Thermo Scientific™, Waltham, Ma, USA) featuring a particle size of 5µm, pore size of 125 Å, and diameter of 4.6mm. The mobile phase consisted of 0.1% trichloroacetic acid (TCA) in water, followed by a linear gradient of 5-65% acetonitrile over a 30-minute period at a flow rate of 1mL/ min. Pyoverdine was detected at 360nm (Gillani *et al.*, 2023).

### **Structural characterization of purified marine pyoverdine**

The structure of purified pyoverdine was characterized spectroscopically using Fourier-transform infrared (FT-IR) spectroscopy (Bruker Tensor 37, USA), X-ray diffraction (XRD) (Bruker D2 Phaser, USA), and nuclear magnetic resonance (NMR) spectroscopy (JEOL JNM-ECZR 500 MHz, Prague).

### **Cytotoxicity assessment of marine pyoverdine**

The cytotoxicity of pyoverdine was evaluated using the African green monkey kidney cell line (Vero) obtained from Nawah Scientific Inc. Egypt. Cell viability was assessed by the sulforhodamine B (SRB) assay, which relies on the binding to proteins of SRB dye to cellular proteins as an indirect measure of cell viability. Absorbance was measured at 540nm using an Infinite F50 microplate reader (TECAN, Switzerland) (Skehan *et al.*, 1990; Allam *et al.*, 2018).

### **Pyoverdine for chromium bioremediation**

#### **Effect of pH**

The pH of the Cr (VI) solution was adjusted in the range of 1 to 8 by adding either 0.1 M HCl or 0.1 M NaOH. A 0.05g sample of Pyoverdine was added to 50ml of Cr (VI) solution (20mg/ L), and the mixture was shaken at 200rpm at room temperature. Residual Cr (VI) concentrations were determined according to ASTM D 1687–02 by measuring the absorbance of the reddish-purple Cr (VI)-1, 5-diphenylcarbohydrazide complex using

a HACH-2800 UV–Vis spectrophotometer at 540nm. The percentage removal (%R) was calculated as follows:

$$\% R = \frac{(C_o - C_e)}{C_o} \times 100 \quad \text{Eq. (2)}$$

Where,  $C_o$  and  $C_e$ (mg/L) are the initial and remaining metal ion concentrations in solution at time  $t$  (min), respectively (**Khalil *et al.*, 2016**).

### Effect of initial Cr (VI) concentration

Cr (VI) solutions of varying initial concentrations ( $C_o$ ) from 0.25 to 30mg/ L were prepared in 50ml aliquots in stoppered 100mL plastic bottles. An equal mass of 0.05g of the adsorbent was added to each solution. The solutions were shaken at 200rpm at room temperature. The adsorption capacity at equilibrium ( $q_t$ , mg/g) was calculated by using the following equation:

$$q_t = \frac{(C_o - C_t) \times V}{m} \quad \text{Eq. (3)}$$

Where,  $q_t$  (mg/g) represents the mass of adsorbed Cr (VI) per gram of adsorbent at time  $t$ ;  $C_o$  and  $C_t$  (mg/L) are the initial and equilibrium Cr (VI) concentrations; respectively,  $m$  (g) is the mass of adsorbent, and  $V$  (L) is the volume of Cr (VI) solution.

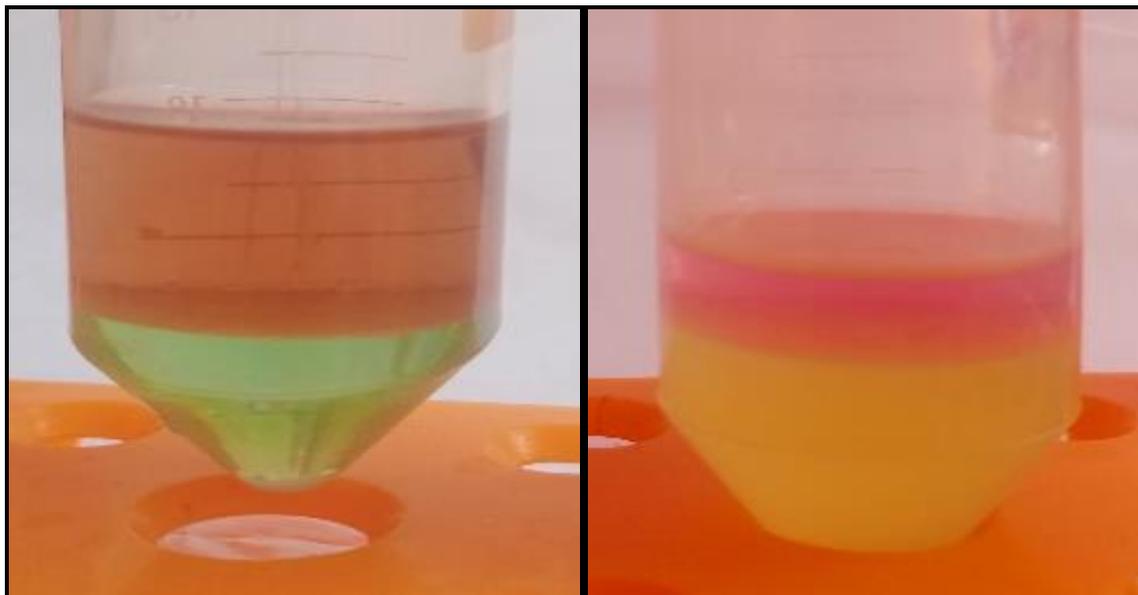
## RESULTS

Reverse-phase high-performance liquid chromatographic analysis of purified marine pyoverdine (HPLC) resulted in a single peak of purified pyoverdine that was distinguished at 360nm (Fig. 3).

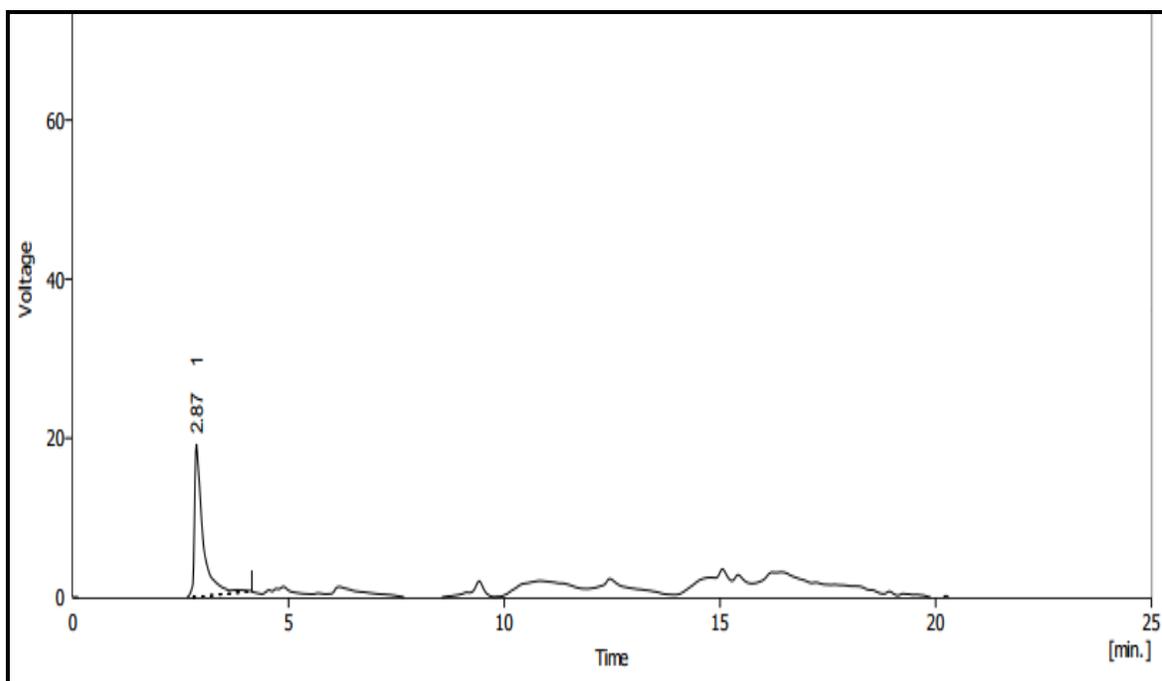


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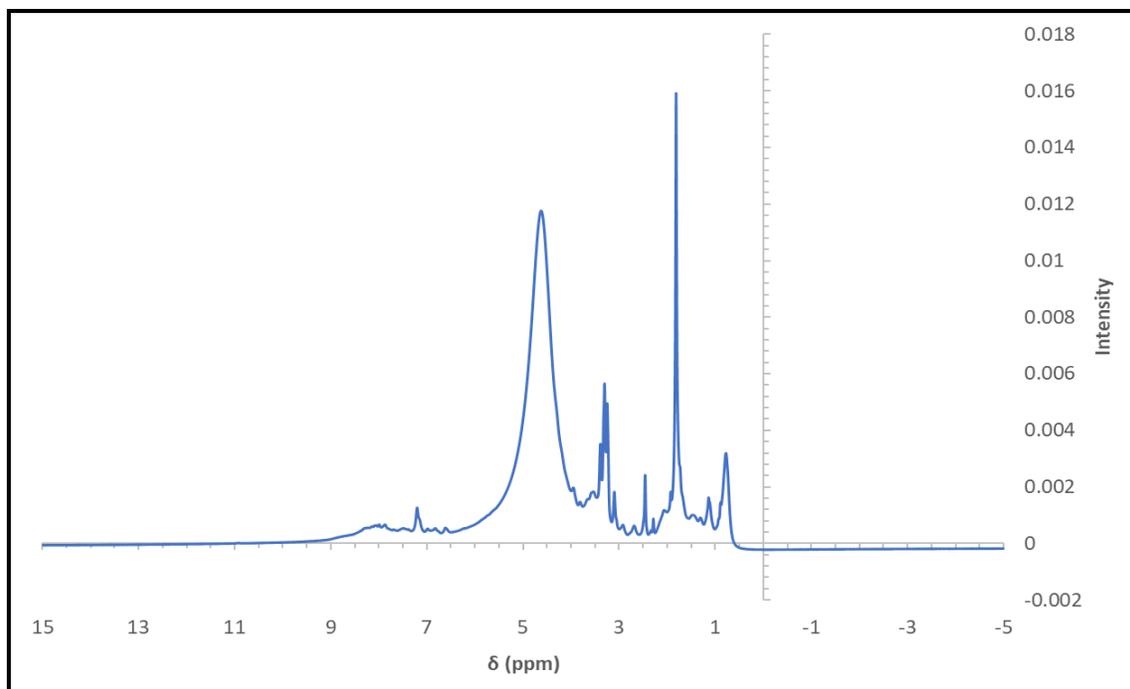
**Fig. 1.** Transformation of culture medium to deep green after 5 days of incubation



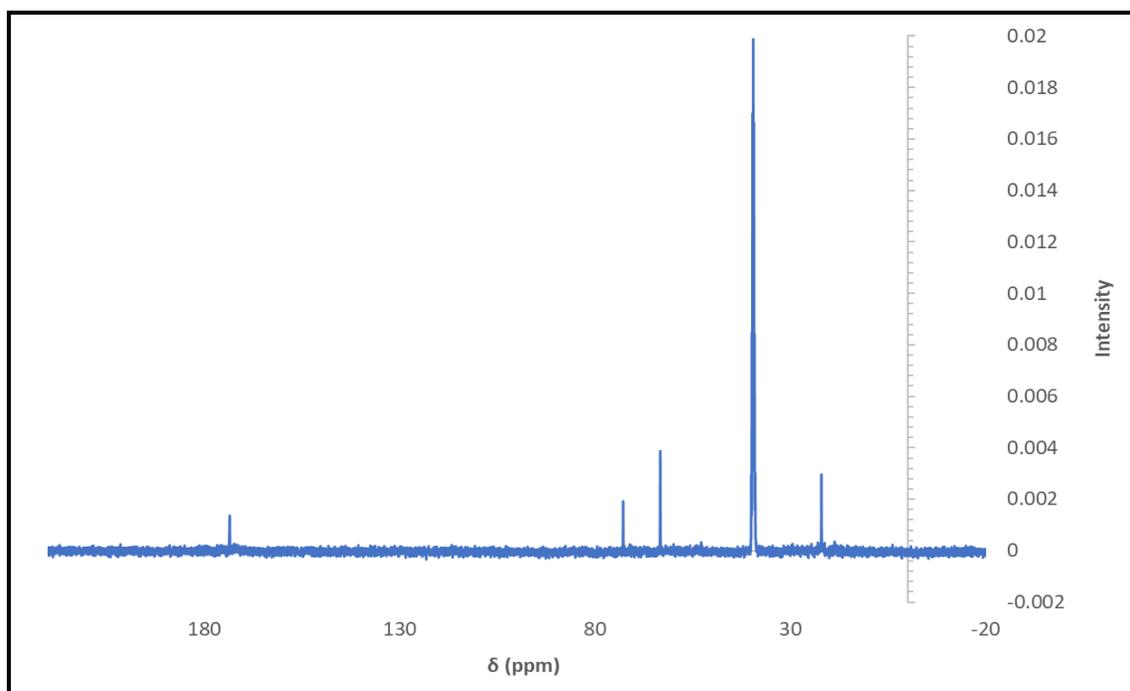
**Fig. 2.** Phase separation of green layer into pink and yellow layers following acidification



**Fig. 3.** Reverse-phase HPLC analysis of purified pyoverdine

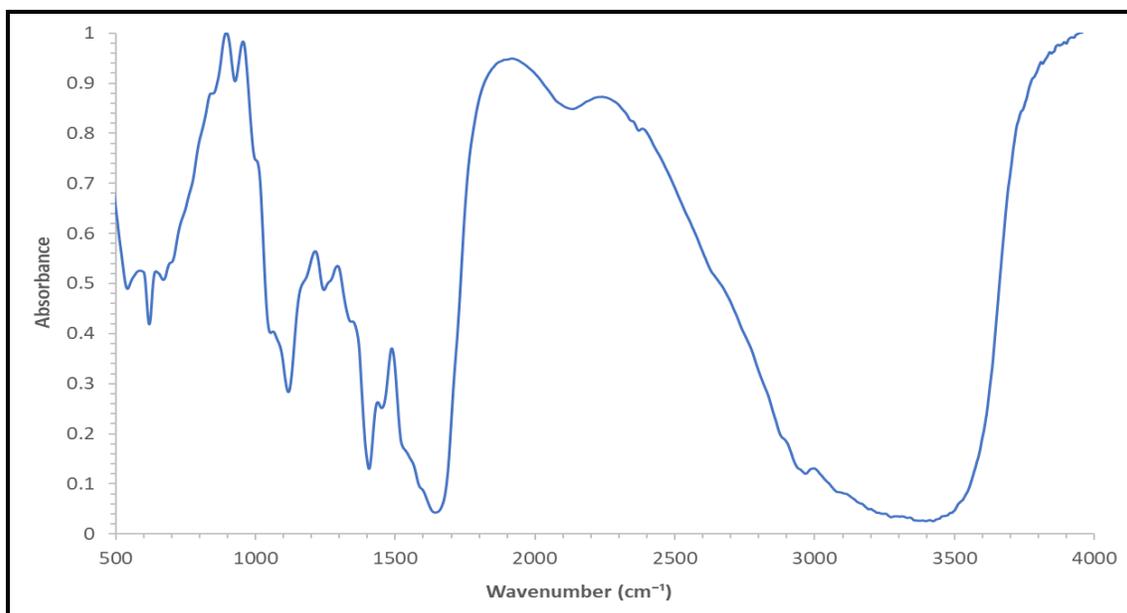


**Fig. 4.**  $^1\text{H}$  NMR spectrum of purified pyoverdine

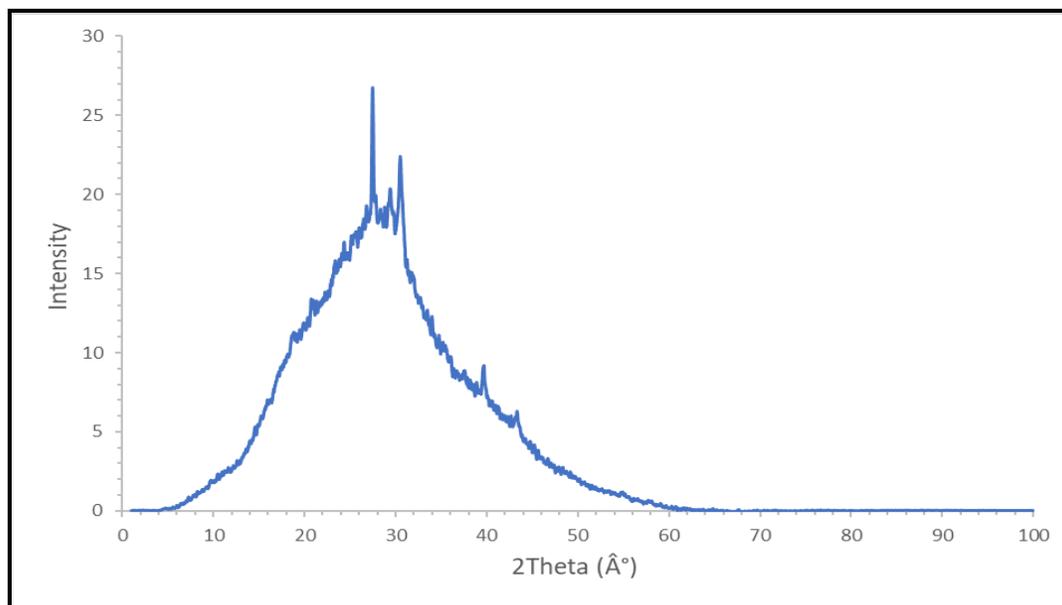


**Fig. 5.**  $^{13}\text{C}$  NMR spectrum of purified pyoverdine

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**Fig. 6.** FT-IR spectrum of purified pyoverdine



**Fig. 7.** XRD pattern of purified pyoverdine

### Cytotoxicity of purified marine pyoverdine

The effect of different doses of the purified pyoverdine on Vero cells utilizing SRB assay showed a neglectable effect on the viability of cells. The value of  $IC_{50} > 100 \mu\text{g/ml}$  confirmed its safety for use in environment, without any toxic effect on living organisms (Figs. 8, 9).

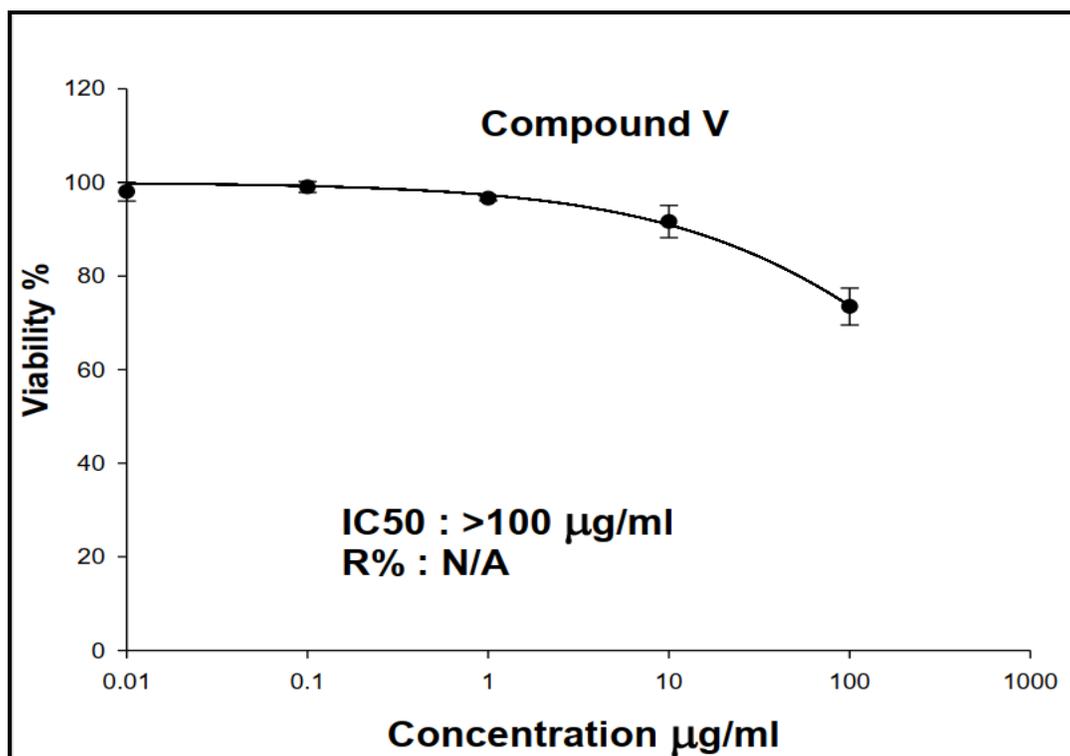
## Bioremediation efficiency of pyoverdine

### *Effect of pH*

The removal of Cr (VI) from aqueous solution is greatly dependent on the pH value and the maximum removal occurs when the pH value is 1.0 and that the removal of Cr (VI) ions decreases with increase in pH values of Cr (VI) solutions from 3.0 to 8.0 (Fig. 10).

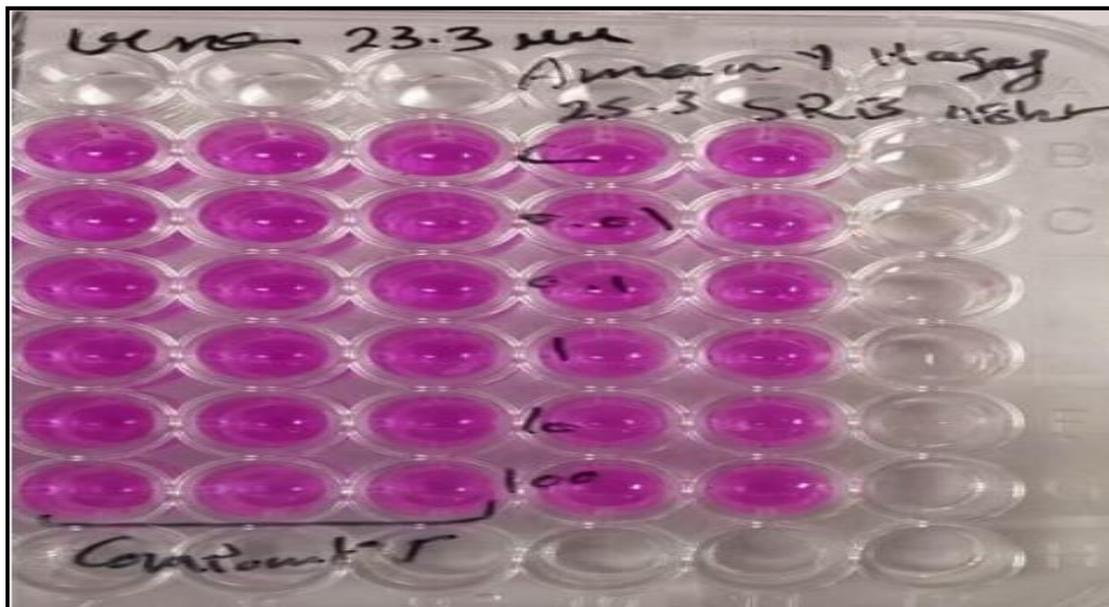
### **Effect of initial concentration**

In a batch mode study, the effect of initial concentration on Cr (VI) ion adsorption was investigated by varying the initial concentration of Cr (VI) solution from 0.25 to 30mg/ L. As shown in Fig. (11), the % removal of Cr (VI) ions decreased with the increase in the initial concentration. When the initial concentration of Cr (VI) solution increased from 0.25 to 30mg/ L the % removal decreased from 100 to 20%.

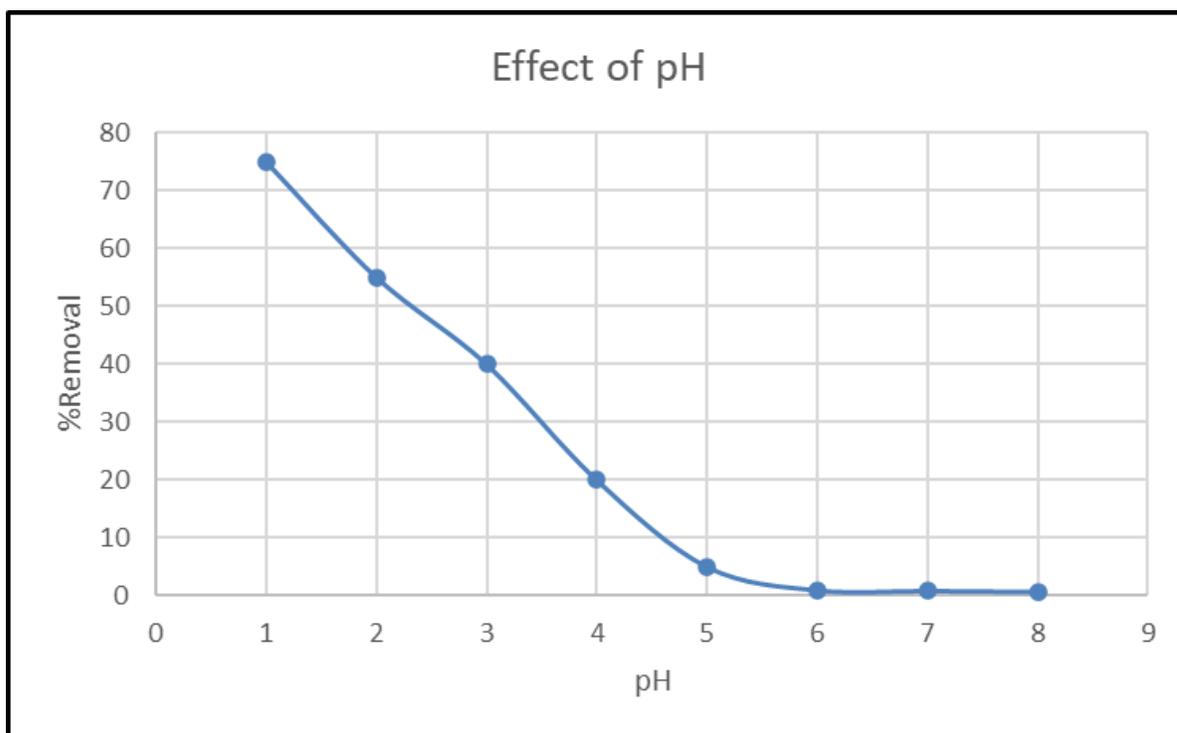


**Fig. 8.** Effect of varying pyoverdine concentrations on cell viability

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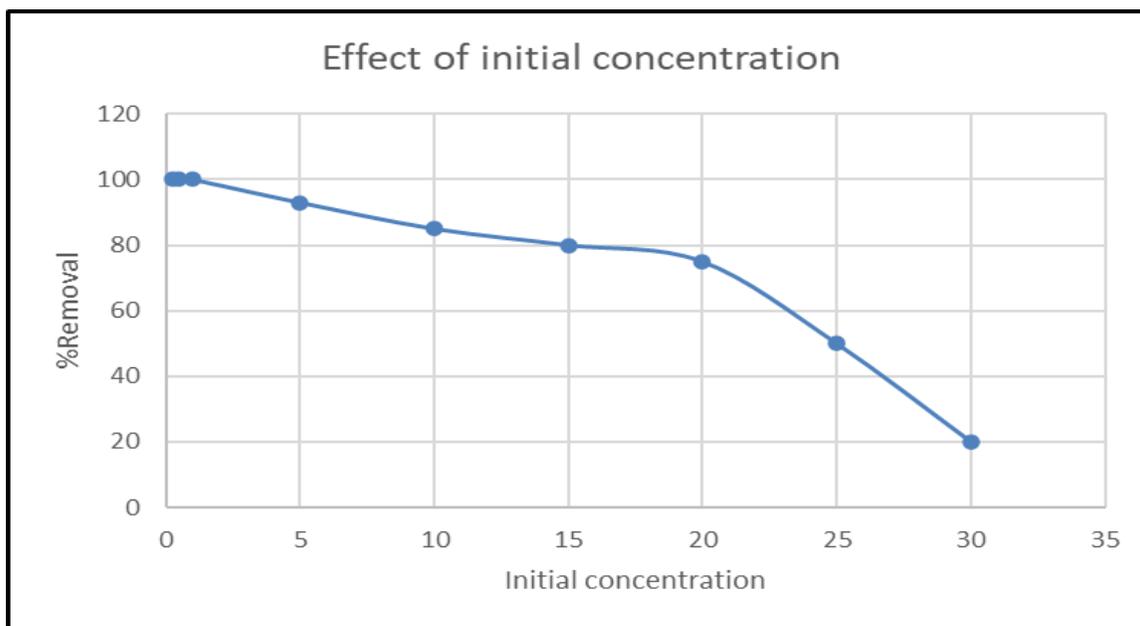
**Fig. 9.** Cell viability assessed via SRB assay following treatment with various concentrations of purified pyoverdine



**Fig. 10.** Effect of pH on Cr (VI) removal efficiency by pyoverdine

## Adsorption isotherm

The equilibrium adsorption isotherms are one of the most important data for understanding the adsorption mechanism. The adsorption isotherm models reflect the adsorbent's surface characteristics, the possibility of adsorbate-adsorbent interaction, and the homogeneity or heterogeneity of the adsorbent's solid surface. It also provides physicochemical information on how adsorption occurs and how the reaction between adsorbate and adsorbent surface occurs. The adsorption isotherm describes the relationship between the amount of a substance adsorbed per unit mass of adsorbent at constant temperature and its concentration in the equilibrium solution. Developing an appropriate isotherm model for adsorption is critical for adsorption process design and optimization. Several isotherm models, such as Langmuir and Freundlich, are commonly used to evaluate the equilibrium adsorption of compounds from solutions. As a result, two isotherm models were applied to analyze the adsorption data of adsorbent.



**Fig. 11.** Effect of initial Cr (VI) concentration on removal efficiency by pyoverdine

## Langmuir isotherm model

The Langmuir isotherm is based on the theoretical principle that only a single adsorption layer exists on an adsorbent and represents the equilibrium distribution of metal ions between the solid and liquid phases. On the other hand, the Langmuir

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isotherm is based on the formation of an adsorbate monolayer on the adsorbent's outer surface, after which no further adsorption occurs (**Al-Anber, 2010**).

The Langmuir isotherm model is the most widely used for practical applications, and it is obtained under the ideal assumption of completely homogeneous adsorption, in which each molecule has constant enthalpies and sorption activation energy (all sites have equal affinity for the adsorbate), and it is represented as follows:

$$\frac{1}{q_e} = \left( \frac{1}{q_{max} \cdot KI} \right) * \left( \frac{1}{C_e} \right) + \left( \frac{1}{q_{max}} \right) \quad (3)$$

Where,  $q_{max}$  (mg/g) is the maximum metal ion uptake per unit mass of adsorbent and  $KI$  (L/mg) is the Langmuir equilibrium constant which determines the direction to which the equilibrium adsorbate-adsorbent moves. High values of  $KI$  indicate that the equilibrium moves to the right side, with the resulting formation of the adsorbate-adsorbent complex (**Bhattacharyya & Gupta, 2011**).

Linear plot of  $1/q_e$  against  $1/C_e$  was used to calculate the parameters of Langmuir isotherm (Fig. 12). The  $KI$  and  $q_{max}$  were obtained from the slope and intercept of the plots, respectively. The isotherm parameters were calculated as shown in Table (1). The adsorption of Cr (VI) onto the adsorbent demonstrated better fit with the Langmuir isotherm ( $R^2=0.9922$ ). It is reasonable to conclude that the process is a monolayer adsorption with chemisorption dominance with  $Q_{max}$  (cal.) of 63.28mg/ g.

### Freundlich isotherm model

The empirical Freundlich equation, which is widely used, is based on adsorption heterogeneous surface. The magnitude of the heat of adsorption decreases as the extent of adsorption increases. If the adsorption heat decline is logarithmic, it means that adsorption sites are distributed exponentially concerning an adsorption energy that varies between groups of adsorption sites. The Freundlich sorption isotherm expresses surface heterogeneity as well as the exponential distribution of active sites and their energies. This isotherm does not predict any saturation of the sorbent by the sorbate thus infinite surface coverage is predicted mathematically, indicating a multilayer sorption of the surface (**Hasany et al., 2002**). The Freundlich isotherm can be expressed in a linear form as follows:

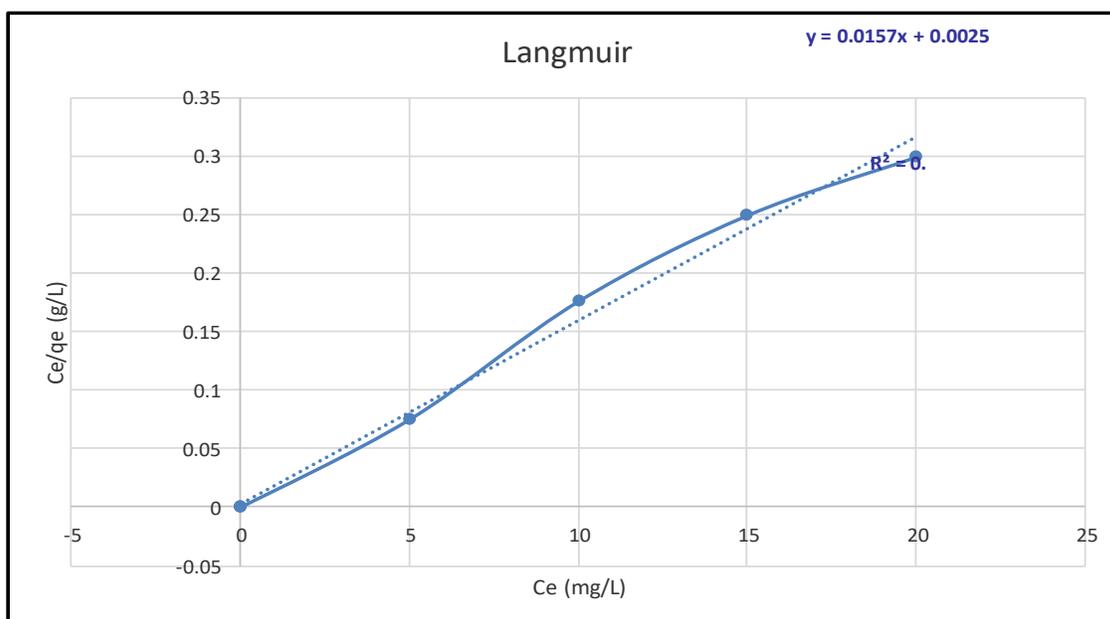
$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad (\text{Afkhami et al., 2010})$$

Where,  $k_f$  (L/g) is the Freundlich constant related to the bonding energy.  $k_f$  can be defined as the adsorption or distribution coefficient and represents the quantity of Cr (VI) adsorbed onto adsorbent for unit equilibrium concentration.  $1/n$  is an empirical constant

indicating the adsorption intensity of the system. The values of  $k_f$  and  $1/n$  can be respectively calculated from the intercept and slope of the linear plot of  $\log q_e$  against  $\log C_e$  (Fig. 13). In the Freundlich model, it is relatively easy for a sorbent to adsorb solute when  $1/n$  is less than 1. In Table (1),  $1/n$  values were less than 1, which indicated that extract was good at adsorbing Cr(VI) ions from aqueous solutions (Mu & Sun, 2019). Based on the  $R^2$  values, the Langmuir isotherm model showed the best fit, with an  $R^2$  value of 0.9922.

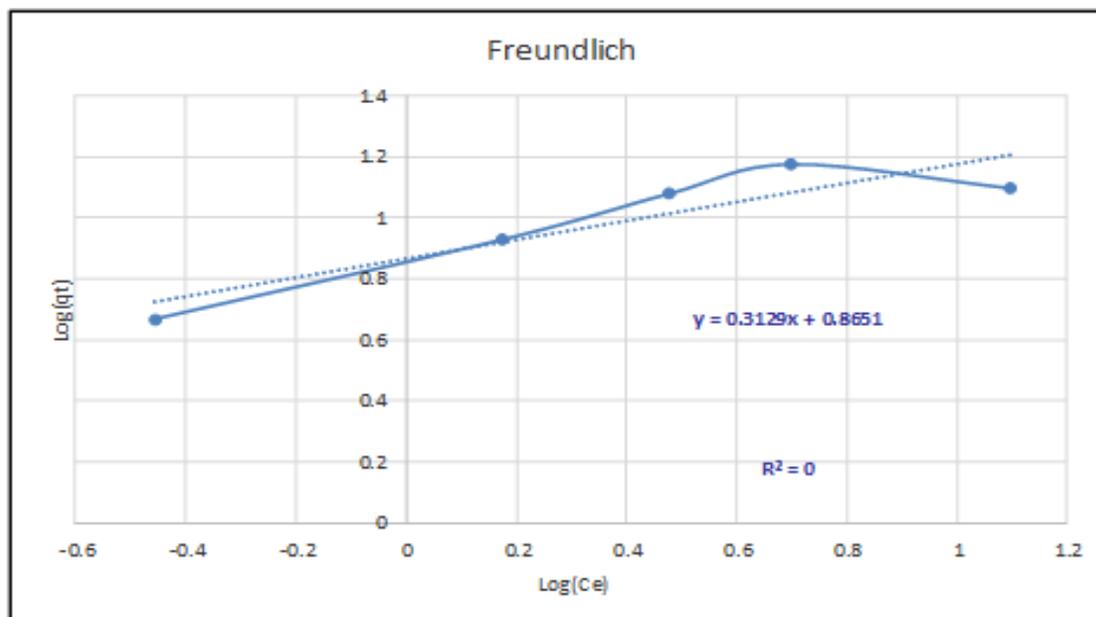
**Table 1.** Calculated adsorption isotherm parameters of the applied isotherm models

Isotherm Model	Parameter	Value	Units
Langmuir Model	$q_m$	63.69	mg/g
	$K_L$	6.28	L/mg
	$R^2$	0.9922	–
Freundlich Model	$K_f$	7.329	mg/(g (mg/L) <sup>1/n</sup> )
	$1/n$	0.3129	–
	$R^2$	0.8254	–



**Fig. 12.** Linearized Langmuir adsorption isotherm for Cr (VI) adsorption onto pyoverdine

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**Fig. 13.** Freundlich adsorption isotherm for Cr (VI) adsorption onto pyoverdine

## DISCUSSION

*Pseudomonas* strains are known for producing various pigments, the most notable being pyocyanin (blue-green), pyoverdine (yellow-green fluorescent), and pyorubin (red). Among these, pyoverdine acts as a siderophore, a molecule that scavenges iron from the environment by binding ferric iron ( $\text{Fe}^{3+}$ ) with high affinity and transporting it back to the microbial cells. Siderophores are categorized based on their chemical structure and iron-chelating mechanisms. Pyoverdine, a mixed-type siderophore, contains both catechol and hydroxamate groups, enabling an efficient iron binding under diverse environmental conditions (Khasheii *et al.*, 2021).

The structural characterization of pyoverdine from *Pseudomonas otitidis* EGY-NIOF-A1, utilizing  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FT-IR, and XRD, provides key insights into its molecular structure and functionality capabilities as a siderophore with potential applications in metal binding, specifically for chromium. Collectively, these techniques reveal structural elements and functional groups essential for pyoverdine's role in metal ions chelation, supporting microbial survival and growth under iron-limited conditions.

The  $^1\text{H}$  NMR spectrum displayed peaks in the 0.6–0.8ppm range, indicative of methyl ( $-\text{CH}_3$ ) and methylene ( $-\text{CH}_2$ ) groups (Fig. 4). These aliphatic side chains, likely

derived from amino acid such as leucine and valine, impart flexibility to pyoverdine, allowing it to conform effectively around including chromium, thereby enhancing binding efficacy. The  $^{13}\text{C}$  NMR spectrum further confirmed the presence of shielded aliphatic carbons at -25ppm (Fig. 5), highlighting the importance of flexible regions within the peptide backbone for stabilizing metal-ligand interactions, particularly under variable environmental conditions relevant to chromium remediation.

Additionally, the absence of downfield signals in the 6–10ppm region, where aromatic or amide protons typically resonate, suggests that the observed NMR signals are primarily derived from aliphatic regions of the molecule. These aliphatic side chains likely contribute to pyoverdine's flexibility, a feature that is essential for the molecule to adopt conformations conducive to metal ion binding.

The FT-IR spectrum provides further insights into the metal-binding functionalities within Pyoverdine (Fig. 6). The broad bands observed between 3000–3500 $\text{cm}^{-1}$  correspond to O-H and N-H stretching, confirming the presence of hydroxyl and amide groups that facilitate coordination through hydrogen bonding or direct metal interactions. The strong C=O stretching bands in the 1600–1700 $\text{cm}^{-1}$  range indicate carbonyl groups associated with hydroxamate functionalities, which are critical for oxygen donation in metal-ligand complexes. This structure enhances pyoverdine's utility in stabilizing metal ions such as chromium, given the complex stabilization needed for effective chelation. Additional absorption bands in the 1000–1500 $\text{cm}^{-1}$  region reflect C-N and C-O stretching, consistent with amide and ether linkages within the peptide backbone, which reinforce the structural framework required for efficient metal coordination and stable binding under variable conditions. Together, these functional groups, particularly hydroxyl, amide, and carbonyl groups, form the core of pyoverdine's metal-binding environment, essential for its biological function.

The XRD analysis reveals a partially crystalline structure, marked by well-defined peaks indicating a periodic arrangement of metal-binding groups (Fig. 7). This ordered molecular configuration optimizes the spatial orientation of hydroxyl, carbonyl, and amide groups, which enhances Pyoverdine's capacity for stable metal coordination. Such a crystalline structure is especially beneficial for chromium binding, as it allows pyoverdine to form stable complexes with chromium and other metal ions, providing resilience in environmental applications, where structural integrity and metal stability are critical.

The structural and functional characterizations of pyoverdine from *Pseudomonas otitidis* EGY-NIOF-A1, based on  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, FT-IR, and XRD analyses, provide a primarily understanding of pyoverdine's structure and its functional role as a siderophore. The aliphatic side chains, identified through NMR, contribute to the

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molecular flexibility necessary for metal binding, while the hydroxyl, amide, and carbonyl groups, detected by FT-IR, are directly involved in metal coordination. The partially crystalline structure observed in XRD underscores the importance of molecular organization in stabilizing metal-ligand interactions. Overall, these findings confirm its classification as a Type II siderophore with a mixed metal-binding profile that enables chelation of heavy metals, particularly chromium. This comprehensive spectroscopic approach reveals the presence of key functional groups and structural features essential for its role in metal acquisition and environmental remediation, aligning with the broader objectives of sustainable remediation in contaminated ecosystems.

Collectively, the NMR, FT-IR, and XRD data (Figs. 4-7) identify pyoverdine from *Pseudomonas otitidis* as a mixed-type siderophore, containing both hydroxamate and catecholate groups. This dual functionality enables versatile binding with metal ions, positioning pyoverdine as an effective agent in both microbial iron acquisition and chromium bioremediation. The presence of hydroxyl, amino, and carbonyl groups provides multiple coordination sites, allowing for the stabilization of chromium and other metal complexes, making pyoverdine a promising candidate for sustainable remediation in metal-contaminated water systems.

For biotechnological applications in aquaculture, the biocompatibility or toxicity of marine pyoverdine should be confirmed safe to the environment and surrounding organisms.

The efficacy of pyoverdine in Cr (VI) bioremediation was further evaluated through batch experiments to assess the impact of pH. Results indicate that Cr (VI) removal from solution is highly pH-dependent, with maximum removal achieved at pH 1.0. At higher pH levels, Cr (VI) removal efficiency declined, particularly as the solution pH increased from 3.0 to 8.0 (Fig. 11). This phenomenon can be attributed to the pH-dependent speciation of Cr (VI), which exists in different oxyanion forms, such as  $\text{HCrO}_4^-$ ,  $\text{CrO}_4^{2-}$ ,  $\text{Cr}_2\text{O}_7^{2-}$ . At pH levels above 1, the predominance of  $\text{H}_2\text{CrO}_4$  in the solution, combined with strong competition for adsorption sites between  $\text{H}_2\text{CrO}_4$  and protons ( $\text{H}^+$ ), leads to a reduction in adsorption capacity.

At pH 1.0, the removal efficiency reaches its maximum due to the protonation of functional groups, including amine and hydroxyl groups, on the surface of pyoverdine. These groups form positively charged active sites ( $\text{NH}_3^+$  and  $\text{OH}_2^+$ ), facilitating electrostatic attraction with negatively charged Cr (VI) ions. However, as the pH increased to 8.0, the adsorption capacity declines. This decrease can be explained by reduced electrostatic attraction between  $\text{CrO}_4^{2-}$  ions and active sites, coupled with competition of  $\text{CrO}_4^{2-}$  and  $\text{OH}^-$  for adsorption (Zia *et al.*, 2019).

The bioremediation experiments showed that the percent removal of Cr (VI) ions decreased with the increase in the initial concentration. When the initial concentration of Cr (VI) solution increased from 0.25 to 30mg/ L, the % removal decreased from 100 to 20%. This decline is due to the continuous reduction in the ratio of available binding sites to remaining adsorbate ions at higher initial concentrations.

## CONCLUSION

This study highlights the potential of pyoverdine as an eco-friendly chelating agent for the sustainable remediation of chromium-contaminated water systems. The findings confirm that the structural and functional features of pyoverdine from *Pseudomonas otitidis* EGY-NIOF-A1 make it a promising candidate for chromium bioremediation. The molecule's architecture is optimized for metal sequestration, aligning well with broader goals in bioremediation and resource recovery in contaminated ecosystems.

The combined presence of hydroxamate and catecholate groups within pyoverdine's structure enables it to effectively chelate chromium ions via multiple coordination sites, stabilizing the ions in complexed forms. Additionally, the identified hydroxyl, amino, and carbonyl groups enhance this capability, providing essential electron-donating sites for binding and stabilizing chromium. The partially crystalline structure observed in the XRD analysis further supports pyoverdine's effectiveness by maintaining an orderly and stable arrangement of metal-binding groups, facilitating the formation of robust chromium complexes even under diverse environmental conditions.

Furthermore, pyoverdine's flexible aliphatic regions, as indicated by the NMR spectra, enable it to adapt to the spatial requirements of different metal ions, adding critical versatility to its metal-binding properties. Altogether, these structural features position pyoverdine as a highly valuable and sustainable agent for chromium remediation in contaminated water systems, offering a biocompatible and environmentally friendly approach to mitigating heavy metal pollution.

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