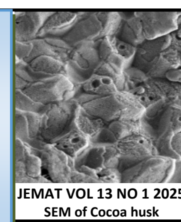
JEMAT 2025, Vol 13, No 1, 9-17
https://doi.org/10.54987/jemat.v13i1.1105

JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEMAT/index



Isolation and Characterization of a Molybdenum-reducing and Phenol-degrading *Pseudomonas* sp. strain Aft-9 in soils from Pakistan

Aftab Khan[1] and Mohd Izuan Effendi Halmi[2]*

[1]Department of Biochemistry & Health Sciences, Hazara University, Garden Campus, 21300 Mansehra, Khyber Pakhtunkhwa, Pakistan.
[2]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Mohd Izuan Effendi Halmi,
Department of Land Management,
Faculty of Agriculture,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor,
Malaysia.
Email: m_izuaneffendi@upm.edu.my

HISTORY

Received: 18th March 2025
Received in revised form: 28th May 2025
Accepted: 15th July 2025

KEYWORDS

Bioremediation
Molybdenum
Molybdenum blue
Bioreduction
Phenol-degrading

ABSTRACT

Bioremediation is a better alternative when other procedures, including physical or chemical ones, don't work to get rid of small levels of dangerous heavy metals and organic contaminants. In this investigation, we found a bacterium that can remove molybdenum from soil that has been polluted. The bacterium could grow on phenolic chemicals such phenol, benzoate, 2-napthol, and catechol. The best rate for the bacterium to change sodium molybdate to molybdenum blue (Mo-blue) is when the pH is between 6.3 and 6.5 and the temperature is between 25 and 30 °C. In that order, glucose, fructose, and galactose were the best electron donors for helping molybdate reduction. It is also important to know the phosphate levels, which should be between 5.0 and 7.5 mM, and the molybdate levels, which should be between 15 and 20 mM. The Mo-blue that came out showed absorption spectra that were extremely similar to those of a phosphomolybdate that had been decreased. At 2 ppm, heavy metals mercury (II), silver (I), copper (II), and chromium (VI) reduced molybdenum reduction by 78.9%, 69.2%, 59.5%, and 40.1%, respectively. Biochemical tests tentatively identified the bacterium as *Pseudomonas* sp. strain Aft-9. This bacteria is useful for bioremediation since it can detoxify molybdenum and grow on poisonous phenolics.

INTRODUCTION

Bioremediation, when applied to contaminants at low concentrations, can ultimately prove to be more cost-effective than alternative approaches like physical or chemical cleanup [1]. One of molybdenum's most common industrial uses is in the production of steel and alloys. More than fifty enzymes rely on molybdenum as a cofactor [2]. New evidence suggests that molybdenum inhibits embryogenesis and spermatogenesis in certain animals, including mice and catfish [3,3–6]. Furthermore, molybdenum is very toxic to ruminants [7,8]. Industries around the world produce scheduled wastes, the most common of which are hydrocarbons (oil, grease, and phenolics) and heavy metals, which are major pollutants [9]. Accidents also contribute to pollution. Damage to the ecosystem was extensive after the 2003 spill of nearly 30,000 tonnes of crude oil by the tanker Tasman Spirit near the Karachi coast, Pakistan [10]. A wide variety of creatures, including humans, are poisoned by phenol and phenolic compounds (**Fig. 1**) [11]. These compounds' vapors are extremely irritating to the respiratory system, skin, eyes, and mucous membranes. Dermatitis and even third-degree burns can result from prolonged skin contact. The kidneys and liver are negatively impacted by prolonged exposure. The formation of phenoxyl radicals and its hydrophobicity contribute to its toxicity [12]. Its global pollution is well known.

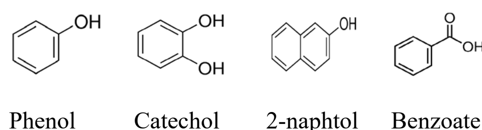


Fig. 1. The structure of some toxic phenolic compounds.

The versatility of certain microbes is highly valued in polluted areas where multiple contaminants are present, and their capacity to break down various xenobiotics and heavy metals all at once is a huge plus. The biodegradation of phenol in conjunction with chromate reduction is one concrete example [13,14]. Molybdenum (Mo), a transition metal and vital micronutrient, occurs in multiple oxidation states, with molybdate (Mo^{6+}) representing the most stable and soluble form in environmental conditions. The versatility of molybdenum makes it an essential ingredient in many different goods. It is a component of electrical

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

and electronic components, paint, pigment, glass, nickel-base alloys, and lubricants (in the form of molybdenum disulfide). The effluents from these businesses account for a considerable amount of the molybdenum contamination [15]. In most naturally occurring waters, Mo concentrations are less than 10 μg/L [16].

The pollution of heavy metals, particularly molybdenum, has worsened in Tokyo Bay and the Black Sea as a result of decades of heavy industry. In comparison to the previously mentioned background level, these levels are substantially greater [15]. Pollution from industrial discharge periods has led to soils in Tyrol, Austria, that ruminants eat having molybdenum levels above 200 parts per million. This has caused scouring in cattle and, finally, its remediation was achieved using bacteria from sewers [17]. Soil molybdenum concentrations ranging from 0.25 to 252 mg/kg was found in a polluted soil. The excess molybdenum content comes from a byproduct of a Chinese light-bulb manufacturing plant that has been operational for decades [18]. The air in Islamabad, Pakistan, contains unusually high concentrations of the elements molybdenum and niobium, according to scientists. The elevated levels of these metals were initially believed to be caused by human activities [19]. Molybdenum concentrations in soil reach 11.7 mg/kg in certain regions of Jordan, where extensive oil shale extraction and processing have occurred. Cows and sheep grazing on grasses in these soils may experience unrecorded diseases due to molybdenum exposure [20].

Some bacteria show the distinctive capability to reduce molybdate to molybdenum blue (Mo-blue), a lower-valence form. This transformation required the enzymatic reduction of molybdate into mixed-valence molybdenum oxide species [21], characterized by a vivid blue hue resulting from charge-transfer transitions between Mo^{5+} and Mo^{6+} oxidation states [22]. Mo-blue production by bacteria serves as a marker for molybdate biotransformation and has been extensively researched in strains of *Pseudomonas*, Enterobacter, Serratia, and Acinetobacter, among others [23]. To date, the process of molybdenum reduction in bacteria remains incompletely elucidated, yet it is thought to involve the electron transfer from intracellular reducing agents, such as NADH or NADPH, to phosphmolybdate through the action of a molybdenum-reducing enzyme. Optimal reduction conditions differ among species but are typically facilitated by the presence of monosaccharides (e.g., glucose), a slightly acidic to neutral pH, and an anaerobic or microaerophilic environment. The resultant Mo-blue is a colloidal and nanoscale molybdenum oxide, generally consisting of molybdenum in +5/+6 oxidation states as mentioned before [24].

In addition to its function in biogeochemical cycling, the biogenic production of Mo-blue holds significant potential in nanotechnology, particularly because of its nanoscale architecture, stability, and redox characteristics. Mo-blue nanoparticles possess distinctive electronic, optical, and catalytic properties that render them suitable for photocatalysis, biosensing, and electrochemical energy storage applications. Their vivid hue and redox properties render them valuable as colorimetric indicators in bioassays and diagnostic kits [25]. Moreover, the biogenic method of Mo-blue synthesis is environmentally sustainable and economically advantageous relative to chemical or physical techniques. An additional emerging application is in environmental remediation, wherein Mo-blue-producing bacteria may be incorporated into biofilters or bioelectrochemical systems. These systems would utilize biotransformation and nanoparticle synthesis to remediate molybdenum-contaminated water while concurrently producing functional nanomaterials [26].

In this study, we test the growth potential of a new molybdenum-reducing bacterium on phenol and other phenolic compounds using soil samples taken from polluted areas. The ability to grow on multiple phenolic compounds isolated from polluted soil is a unique trait of a new molybdenum-reducing bacterium that we describe here. Future bioremediation efforts involving the heavy metal molybdenum and dye, an organic pollutant, might benefit from this bacterium's traits.

MATERIALS AND METHODS

Isolation of molybdenum-reducing bacterium

The presence of blue colonies on a molybdenum minimal salts media indicated the presence of molybdenum-reducing (Mo-reducing) bacteria. Soil samples from the grounds of a contaminated land were taken 5 cm deep from topsoil in the province of Khyber Pakhtunkhwa, Pakistan, in 2013 [27]. A suspension containing one gram of soil sample in sterile tap water was prepared and 0.1 mL was pipetted and spread onto an agar of low phosphate media (LPM) at pH 7.0 with the following: glucose (1%) as the electron donor for reduction, $MgSO_4.7H_2O$ (0.05%), $(NH_4)_2.SO_4$ (0.3%), yeast extract (0.5%), NaCl (0.5%), $Na_2MoO_4.2H_2O$ (0.242 % or 10 mM) and Na_2HPO_4 (0.071% or 5 mM) [28].

The agar plates were taken out after being kept at room temperature for 24 hours. The colony with the most intense blue color was separated and then streaked several times on low phosphate media (LPM) to get a pure culture. A 250 mL shake flask culture with 100 mL of the media stated above was utilized for liquid at room temperature for 48 hours on an orbital shaker set to 120 rpm. Although the reduction medium remained unchanged, the concentration of phosphate was increased to 100 mM. After forming a liquid culture of molybdenum blue (Mo-blue), we spun 1.0 mL of the solution at 10,000 x g for 10 minutes at room temperature to scan its absorption spectra. From 400 to 900 nm, we scanned the supernatant using a Shimadzu 1201 UV-spectrophotometer. The baseline was fixed using the media.

Morphological, physiological and biochemical characterization of the isolated strain

Using Bergey's Manual of Determinative Bacteriology as a guide, we characterized the isolated strain morphologically, physiologically, and biochemically [29]. Interpretation of the results was carried out via the ABIS online system [30].

Preparation of bacterial resting cells

Cells were cultured for 48 hours in a 1-liter dish containing High Phosphate media (HPM) at room temperature, utilizing an orbital shaker set to 150 rpm. The culture was centrifuged at 15,000 x g for 10 minutes at room temperature. Twenty milliliters of low phosphate media (LPM) devoid of glucose was utilized to resuspend the bacterial cell pellets and followed by several washings to remove the residual phosphate. The suspension was diluted using tegh same medium until its absorbance at 600 nm was 1.00. A sterile microplate was utilized in the experiment.

A 180 μL aliquot of the cellular suspension was pipetted into each well. Subsequently, 20 μL of sterile glucose, achieving a final concentration of 1% (w/v), is introduced to each well to initiate the formation of Mo-blue. The Corning® microplate' sterile sealing membrane facilitating gas exchange, was utilized to seal the microtiter plate. The microplate was subsequently incubated at room temperature. Absorbance at 750 nm was quantified using a BioRad Microtiter Plate reader (Model No. 680) at specified intervals. The maximum filter wavelength of the microplate unit was 750 nm, and the specific extinction

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.54987/jemat.v13i1.1105

coefficient at this wavelength, measured at 11.69 $mM^{-1}.cm^{-1}$, was utilized to assess the production of Mo-blue from the media in a microplate format [31]. Seven heavy metals- arsenic (I), arsenic (V), cadmium (II), chromium (VI), copper (II), lead (II), mercury (II), silver sourced from commercial salts or MERCK's Atomic Absorption Spectrometry standard solutions, were incubated with bacterial cells to investigate their impact on molybdenum reduction. Bacteria were exposed to varying concentrations of heavy metals in a microplate format. The quantification of Mo-blue production occurred at a wavelength of 750 nm.

Screening of molybdenum reduction and independent growth using phenolics

The study investigated the efficacy of phenolics as electron donors in the reduction of molybdenum through a microplate experiment as before. This experiment utilized various pehnolic compounds such as 2,4-dinitrophenol, 2-chlorophenol, 2-naphthol, 4-chlorophenol, 4-nonylphenol, benzoate, catechol, p-hydroxybenzoic acid, pentachlorophenol, phenol, salicylic acid as substitutes for glucose in a low phosphate medium. The final concentration of these xenobiotics was 200 mg/L in 50 µL, taking into account their general toxicity and solubility [32]. Fifty microliters of resting cell suspension were combined with 200 microliters of medium in the wells of the microplates. The Mo-blue generation was measured at 750 nm following incubation of the microplate at room temperature. The microplate format was employed to evaluate the capacity of phenolics to promote the growth of this bacteria in the absence of molybdenum reduction. The media utilized was devoid of molybdate. Xenobiotics replaced glucose, achieving a final concentration of 200 mg/L in a volume of 50 µL. Fifty microliters of resting cell suspension were combined with 200 microliters of medium in the wells of the microplates. The components of the Liquid Plant Medium (LPM) are as follows: (NH4)2SO4 (0.3%), NaNO3 (0.2%), MgSO4·7H2O (0.05%), yeast extract (0.01%), NaCl (0.5%), and Na2HPO4 (0.705% or 50 mM). The medium achieved a pH of 7.0. Bacterial growth was observed at 600 nm following three days of incubation at room temperature.

Statistical analysis

For data analyses, we used Graphpad Prism 10.0 trail version from www.graphpad.com. The values include the means plus or minus the standard error of at least three separate experiments. A statistically significant result is $P < 0.05$. We used either an analysis of variance (ANOVA) with Tukey's post hoc analysis or a Student's t-test.

RESULTS AND DISCUSSION

Identification of the phenol-derading and molybdenum reducing bacterium

The bacterium shown a morphology of a flat colony that was 1 to 2 mm wide. It was a Gram-negative bacterium that was shaped like a short rod. We used the ABIS web-based program and looked at the results of cultural, morphological, and biochemical testing (**Table 1**) to determine what genus and species of the bacterium Costin and Ionut 2015). The software proposed three probable bacterial IDs, and *Pseudomonas putida* had the most similarity (81%) and the most accuracy (85%). We tentatively named the bacterium as *Pseudomonas* sp. strain Aft-9 at this time. Past studies have reported several bacteria from this genus that reduces molybdenum to molybdenum blue [27,33–49]. Several Mo-reducing bacterium have been isolated before from Pakistani soils [27,50].

Table 1. Biochemical tests for *Pseudomonas* sp. strain Aft-9.

Test/Feature	Result	Carbohydrate Utilization	Result
Bacterial motility	Positive	L-Arabinose	Positive
Hemolytic activity	Positive	Citrate	Positive
Growth viability at 4 ºC	Negative	Fructose	Positive
Growth viability at 41 ºC	Positive	Glucose	Positive
MacConkey agar tolerance	Negative	meso-Inositol	Negative
Arginine dihydrolase activity	Positive	2-Ketogluconate	Positive
Alkaline phosphatase production	Positive	Mannose	Positive
Indole formation	Negative	Mannitol	Negative
Nitrate reduction capability	Negative	Sorbitol	Negative
Lecithinase activity	Negative	Sucrose	Positive
Lysine decarboxylation	Negative	Trehalose	Negative
Ornithine decarboxylation	Negative	Xylose	Negative
Beta-galactosidase (ONPG) activity	Negative		
Esculin hydrolysis	Negative		
Gelatin hydrolysis	Variable (doubtful/ inconsistent)		
Starch hydrolysis	Negative		
Urease activity	Positive		
Oxidase activity	Positive		

We use a fast and high-throughput screening (HTS) technique using microplate-based assays to speed up the process of characterizing enzymes. This smaller format lets you analyze several samples at once, which cuts down on the amount of reagents needed and the time it takes to process them compared to the old shake-flask method. In this way, it makes experiments more reproducible, allows for statistical validation, and speeds up experimental cycles. Microplate-based HTS has proved notably useful for studying the toxicity of chemicals, how fast enzymes work, and how chemicals break down in the environment [51,52].

Enterobacter cloacae was the first to show that the use of resting bacterial cells may be utilized to reduce molybdenum under static conditions [53]. Resting cells have now become a basic tool for researching how microbes break down heavy metals and harmful chemical compounds because they have stable metabolism and a cytoplasmic milieu rich in enzymes. Some important instances are the lowering of selenate [54], arsenate [55], phenol [56], amides [57], and pentachlorophenol [58]. In biocatalysis frameworks, more recent uses include detoxifying nitroaromatic and organohalide compounds [59]. Resting cell systems are useful not only for studying how pollutants change, but also for optimizing bioprocesses and characterizing microplastics bioremediation [60].

Molybdenum absorbance spectrum

Fig. 2 shows that Mo-blue, a reduced product of *Pseudomonas* sp. strain Aft-9, has an absorption peak in the infra-red range of 860 to 870 nm, with a median around 865 nm, and a shoulder at around 700 nm. The spectra closely resemble those of the phosphate determination method's reduced heteropolymolybdate (phosphomolybdate) [61]. This unique absorption spectra is shown in nearly all Mo-reducing bacteria [28,62–69], which implies that most likely a very similar species of reduced molybdenum is involved.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.54987/jemat.v13i1.1105

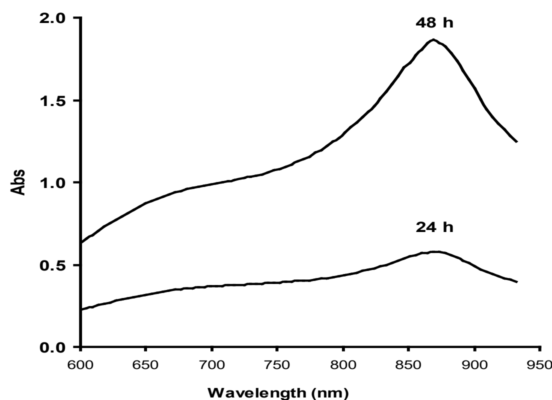


Fig. 2. Scanning absorption spectrum of Mo-blue from *Pseudomonas* sp. strain Aft-9 at different time intervals.

Effect of pH and temperature on molybdate reduction

Using Bis-Tris and Tris.Cl buffers (20 mM), *Pseudomonas* sp. strain Aft-9 was cultured at pH levels ranging from 5.5 to 8.0. The ideal pH range for reduction, according to ANOVA results (**Fig. 3**), was 6.3 to 6.5. **Fig. 4** displays the temperature influence throughout a broad temperature range (20 to 60 °C), with an optimal temperature between 25 and 30 °C.

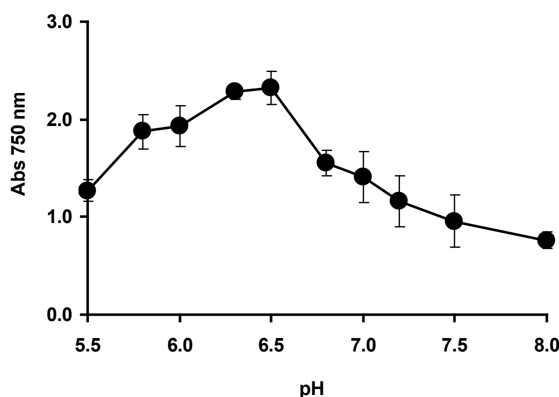


Fig. 3. The reduction of molybdenum by *Pseudomonas* sp. strain Aft-9 as a function of pH. The bacterium's resting cells were cultured in an ideal environment for 72 hours in a microtiter plate. The mean ± standard deviation (n=3) is visually represented as the error bars.

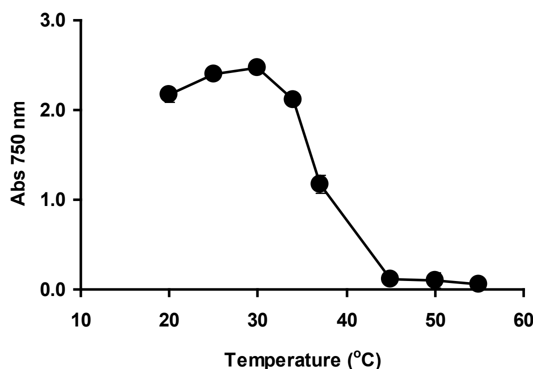


Fig. 4. The reduction of molybdenum by *Pseudomonas* sp. strain Aft-9 as a function of temperature. The bacterium's resting cells were cultured in an ideal environment for 72 hours in a microtiter plate. The mean ± standard deviation (n=3) is visually represented as the error bars.

No significant difference ($p>0.05$) was found among the measured values when ANOVA was performed. The synthesis of Mo-blue from *Pseudomonas* sp. strain Aft-9 was significantly inhibited by temperatures more than 30 °C.As an enzyme-mediated process, molybdenum reduction is sensitive to changes in temperature and pH, which in turn influence protein folding and enzyme activity and hence inhibit molybdenum reduction. Pakistan, a semi-tropical country with an average annual temperature of 10 to 40 ºC, would provide ideal circumstances for bioremediation [70]. Soil bioremediation of molybdenum in this and other tropical and semitropical regions may be possible with this bacterium. The majority of the reducers shows an optimal temperature of between 25 and 37 ºC [28,62,64–68,71–73] because they originate from tropical soils and, according to the one psychrotolerant reducer found in Antarctica, the ideal temperature range for promoting reduction is 15 to 20 ºC [34]. Numerous Mo-reducing bacteria have reported an optimal pH range that is similar to that shown by *Pseudomonas* sp. strain Aft-9 [21,53,62–68,71–73]. Presumably, the formation and stability of phosphomolybdate prior to its reduction to Mo-blue are significantly impacted by acidic pH. Therefore, the ideal reduction happens when the stability of the substrate and the activity of the enzyme are both optimized [74].

Effect of electron donor on molybdate reduction

When we evaluated several electron donors, glucose turned out to be the best substrate for enabling molybdate reduction. Fructose and galactose were next best donors, in that order (**Fig. 5**). Glucose probably works better since it is very metabolically efficient and bacteria can assimilate it quickly. Glucose catabolism produces important reducing equivalents like NADH and NADPH through essential metabolic pathways like glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport chain. Both NADH and NADPH are the main electron donors in the reduction of molybdate. Some molybdenum-reducing bacteria choose sucrose as their main source of carbon, whereas others prefer glucose as their main source. This difference in carbon source choice shows that it depends on the species and strain, and it could be due to changes in how they take up carbohydrates, how much metabolic enzymes they make, or how their regulatory systems work.

Strains that use glucose better frequently have high rates of NAD(P)H production, which makes them better at supporting electron transfer pathways that are important for molybdate reduction. Fructose and galactose are identical to glucose in structure, but they may not be as efficiently metabolized since they need extra enzyme conversions before they can reach central carbon metabolism. The fact that these sugars don't make NADH or NADPH as easily may help explain why they do not assist molybdate-reducing activity as much [75]. Also, the availability and regulation of sugar-specific transporters and kinases can change how quickly these substrates are used in redox metabolism. In general, the preference for glucose shows how important easily digestible carbon supplies are for bioreduction activities. Some strains can thrive on glucose and quickly turn it into energy and reducing power. This makes glucose a good choice for molybdate reduction studies and also useful for biotechnological applications like bioremediation, where efficient and cost-effective carbon sources are needed for field-scale deployment.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.54987/jemat.v13i1.1105

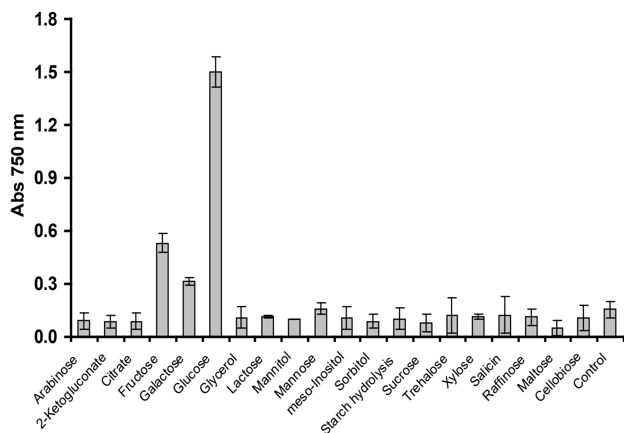


Fig. 5. Effect of different electron donor sources (1% w/v) on molybdenum reduction. The Aft-9 strain of *Pseudomonas* was cultured on a low-phosphate medium that also contained electron donors and 10 mM molybdate. We optimized the settings for 72 hours to incubate the bacterium's resting cells in a microtiter plate. With n = 3, the error bars show the mean ± standard deviation.

Molybdate reduction as a function of phosphate and molybdate concentrations

Determination of the best phosphate and molybdate concentrations is very important for getting the most molybdenum reduction in bacteria. The anion phosphate is an important ingredient of the molybdenum blue complex and can also stop the process if there is too much of it. Experimental results showed that the best phosphate levels for making Mo-blue are between 5.0 and 7.5 mM. Then the activity of reduction reduces dramatically (**Fig. 6**). Higher phosphate levels leads to inhibitory action is probably caused by the instability of phosphomolybdate intermediates, which are important building blocks in the Mo-blue production pathway. The phsophomolybdate complexe is only stable in slightly acidic circumstances, and high phosphate levels may change the balance or stop the complexation process, which would the casue the synthesis of Mo-blue to cease [74].

At the same time, it is just as important to find the right concentration or optimal level of molybdate. This bacterium was able to reduce molybdate levels up to 60 mM, however, at this concentration, production of Mo-blue was inhibited. The best reduction happened in the 15 to 20 mM range (**Fig. 7**), which is also found in other molybdenum-reducing bacterial strains [23]. This drop at high molybdate levels shows that the isolate has a strong tolerance and metabolic flexibility. This could be because it has good detoxifying systems or higher reductase activity.

Being able to remediate high molybdate concentrations has big advantage for the environment polluted with high concentration of molybdenum. Environmental molybdenum contamination can reach levels of up to 2000 ppm (around 20 mM) in places where it has been contaminated, like mine runoff and industrial waste zones [76]. So, a strain that can survive and lower molybdenum levels at these levels is a good candidate for bioremediation. It might turn hazardous soluble molybdate into insoluble Mo-blue, which is easier to remove or immobilize from the environment.

It is also interesting to note that the best molybdate levels for production of Mo-blue are very different for different bacterial strains. This shows that their enzymatic systems and regulatory responses are likely to be different. For example, some bacteria work well when there are only a few molybdate molecules (15–25 mM), whereas others might work well when there are more than 50 mM [77]. The differences shown makes it important to optimize for each strain while designing biotechnological tools for remediation of molybdenum. In general, this variability shows how important it is to choose or design strains based on the amount of molybdenum in the environment and the physical and chemical conditions at the site that needs to be cleaned up.

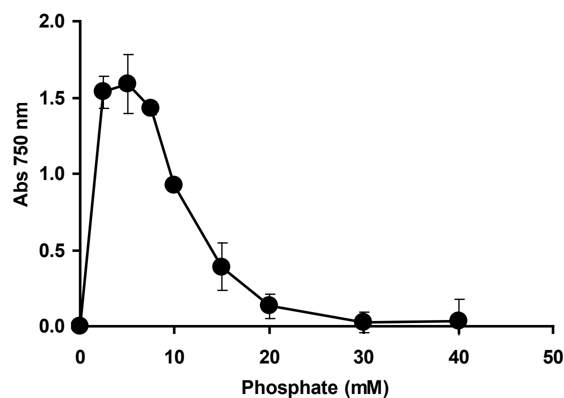


Fig. 6. The reduction of molybdenum by *Pseudomonas* sp. strain Aft-9 as a function of phosphate concentration. The bacterium's resting cells were cultured in an ideal environment for 72 hours in a microtiter plate. The mean ± standard deviation (n = 3) is shown by the error bars.

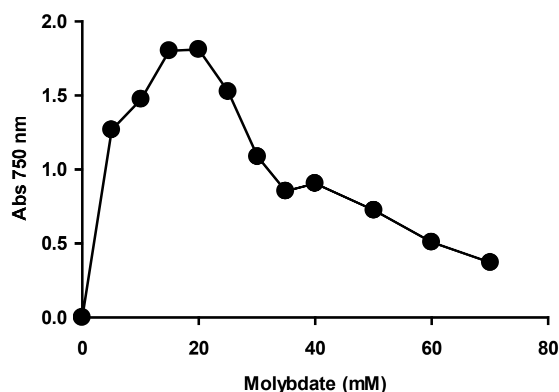


Fig. 7. The reduction of molybdenum by *Pseudomonas* sp. strain Aft-9 as a function of the substrate (molybdate) concentration. The bacterium's resting cells were cultured in an ideal environment for 72 hours in a microtiter plate. The mean ± standard deviation (n = 3) is shown by the error bars.

Effect of heavy metals

The existence of several hazardous metal ions on polluted sites has a big effect on how well bacterial systems can reduce molybdenum. These metal ions are typical environmental co-contaminants in polluted soils and industrial effluents. In this study, mercury (Hg^{2+}), silver (Ag^{+}), copper (Cu^{2+}), and chromium (Cr^{6+}) at 2 ppm caused a strong inhibition of Mo-blue production by 78.9%, 69.2%, 59.5%, and 40.1%, respectively (**Fig. 8**). This amount of inhibition shows how hard it is to use molybdenum-reducing bacteria in real-world bioremediation situations, where the activity of the bacteria may be significantly affected by the toxic combination of several heavy metals. The presence of these cationic metals can disrupt bacterial metabolism by messing with important enzyme systems. One of the postulated mechanisms of toxicity is the binding of these heavy metals to sulfhydryl (-SH) groups in enzymes and proteins. These groups are very important

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.54987/jemat.v13i1.1105

for enzymes to work, and once inhibited, the enzyme will stop working. This is especially true for oxidoreductases, where it is postulate that molybdate converiuson into the Mo-blue form is catalyzed by enzymes from this class. Chromate-reducing bacteria have also been shown to have similar inhibitory effects by metal ions such as mercury and copper. This cross-inhibition between molybdenum and chromate systems, which have similar chemical features, shows how easily bacterial detoxification pathways can be affected by metals [77]. Due to this, it is very important to isolate screen and choose bacterial strains that can not only reduce molybdenum but also resist a wide range of metals.

In places where there are a lot of heavy metals, these strains are more likely to keep working while other strains will cease to work. Mo-reducing isolates are sensitive to a lot of hazardous metal ions (**Table 2**). This suggests that multi-metal resistance is still a problem for bioremediation efforts in the real world. One promising way to get around these problems is to utilize chemical additives that can bind or precipitate dangerous metal ions in the soil. This makes them less available and less harmful to living things. These compounds include phosphate, thiosulfate, elemental sulfur, calcium carbonate, manganese oxide, and magnesium hydroxide [77]. In conjunction to the use of these chemicals at the polluted sites, metal-reducing enzymes from mcriobes can be active and remediate the target compound or metal. This combination chemical-biological method could make molybdenum bioremediation work better, especially in places with a lot of different types of pollution, such mine tailings, industrial sludges, or wastewater treatment areas.

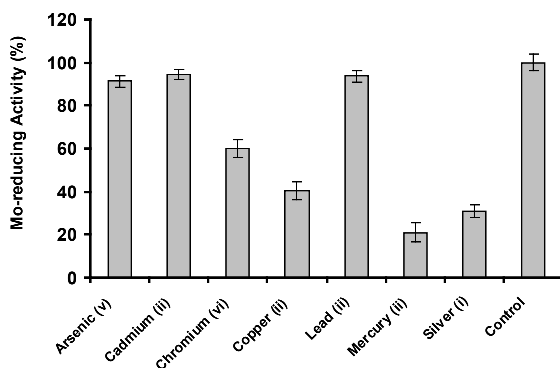


Fig. 8. How metals affect *Pseudomonas* sp. strain Aft-9's ability to produce Mo-blue. The bacterium's resting cells were cultured in an ideal environment for 72 hours in a microtiter plate. The mean ± standard deviation (n = 3) is shown by the error bars.

Table 2. Inhibition of Mo-reducing bacteria by heavy metals

Bacteria	Heavy Metals that inhibit reduction	Author
Bacillus pumilus strain lbna	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6-}, Hg^{2+}, Cu^{2+}	[60]
Bacillus sp. strain A.rzi	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}, Co^{2+},Zn^{2-}	[56]
Serratia sp. strain Dr.Y8	Cr, Cu, Ag, Hg	[52]
S. marcescens strain Dr.Y9	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[16]
Serratia sp. strain Dr.Y5	n.a.	[59]
Pseudomonas sp. strain DRY2	Cr^{6+}, Cu^{2+}, Pb^{2-}, Hg^{2+}	[53]
Pseudomonas sp. strain DRY1	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}	[22]
Enterobacter sp. strain Dr.Y13	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2-}	[51]
Acinetobacter calcoaceticus strain Dr.Y12	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[54]
Serratia marcescens strain DRY6	Cr^{6+}, Cu^{2+}, Hg^{2+}*	[50]
Enterobacter cloacae strain 48	Cr^{6+}, Cu^{2+}	[41]
Escherichia coli K12	Cr^{6+}	[62]
Klebsiella oxytoca strain hkeem	Cu^{2+}, Ag^{+}, Hg^{2+}	[55]

Phenolics as electron donor sources for molybdenum reduction and independent growth

Under the conditions tested, screening for phenolic compounds as electron donors to help reduce molybdate did not work (data not shown). But when the bacteria was grown without molybdate, it could use phenol, benzoate, 2-naphthol, and catechol as the carbon sources, which helped it grow well (**Fig. 9**). This observation demonstrates that the strain can break down and use aromatic chemicals, which suggests that it has important metabolic enzymes like phenol hydroxylase, catechol dioxygenase, and other ring-cleaving enzymes that are necessary for phenolic degradation pathways.

From a bioremediation point of view, bacteria that is able to break down phenolic compounds is very useful because phenol and phenolic compounds are frequent industrial pollutants that can be found in wastewater from the petrochemical, pharmaceutical, dye, and resin sectors [78]. By employing microbial systems to get rid of them is not only cheaper, but it's also better for the environment than employing chemicals. Researchers have been isolating novel phenol-degrading bacteria for a long time because they can use a wide range of enzymes and thrive quickly in polluted areas with a variety of coprocess advantages such as metal, temperature or salinity tolerance [47,79–94].

Most bacteria can either break down organic pollutants or biotransform heavy metals, but not both. The fact that this strain can break down phenol and reduce molybdate at the same time makes it a strong option for bioremediation in places that are polluted with both phenolic chemicals and heavy metals. It is known that in the real world, polluted areas almost never only have one type of pollutant. Sites that have been affected by mining or industrial discharge frequently have a lot of different organic and inorganic poisons mixed together. So, bacterial strains that can break down and detoxify in more than one way are better because they don't need as many microbial consortia or treatment procedures. Using these kinds of strains can make the cleanup process easier, more effective, and less expensive, which makes them very useful for both industrial and environmental purposes.

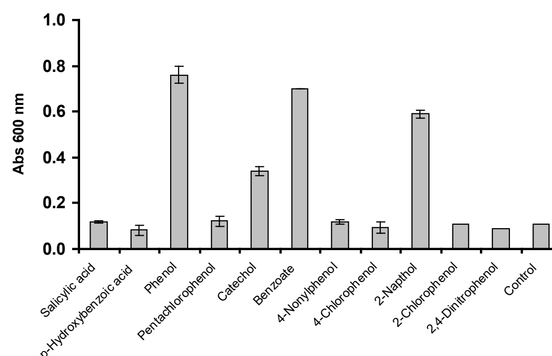


Fig. 9. The Aft-9 strain of *Pseudomonas* sp. grows on xenobiotics without reducing molybdenum levels. The bacterium's resting cells were cultured in an ideal environment for 72 hours in a microtiter plate. The mean ± standard deviation (n = 3) is shown by the error bars.

CONCLUSION

We have discovered a new type of Mo-reducing bacterium that can biodegrade phenol, benzoate, 2-napthol, and catechol. This bacterium is native to Pakistan. At a wide range of pH and temperatures, the bacterium reduces molybdate to Mo-blue

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.54987/jemat.v13i1.1105

optimally. When it came to facilitating molybdate reduction, glucose was the superior electron donor. A phosphate concentration of no more than 7.5 mM is one of the additional prerequisites. The Mo-blue's absorption spectra are comparable to those of earlier Mo-reducing bacteria and, more specifically, to those of a reduced phosphomolybdate, suggesting the presence of a related phosphomolybdate species. Metal ions were toxic and prevented molybdenum reduction. This bacterium is a valuable asset for bioremediation due to its highly desirable ability to detoxify numerous toxicants. Purifying the molybdenum-reducing enzyme from this bacterium is currently in progress, along with further characterization of phenolics biodegradation studies, which will include studies of growth kinetics and confirmation of degradation through spectrophotometric or HPLC assays.

REFERENCES

1. Lim KT, Shukor MY, Wasoh H. Physical, chemical, and biological methods for the removal of arsenic compounds. BioMed Res Int. 2014;2014.
2. Mayr SJ, Mendel RR, Schwarz G. Molybdenum cofactor biology, evolution and deficiency. Biochim Biophys Acta BBA - Mol Cell Res. 2021 Jan 1;1868(1):118883.
3. Bi CM, Zhang YL, Liu FJ, Zhou TZ, Yang ZJ, Gao SY, et al. The effect of molybdenum on the in vitro development of mouse preimplantation embryos. Syst Biol Reprod Med. 2013;59(2):69–73.
4. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, et al. Cadmium, lead, and other metals in relation to semen quality: Human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect. 2008;116(11):1473–9.
5. Zhai XW, Zhang YL, Qi Q, Bai Y, Chen XL, Jin LJ, et al. Effects of molybdenum on sperm quality and testis oxidative stress. Syst Biol Reprod Med. 2013;59(5):251–5.
6. Zhang YL, Liu FJ, Chen XL, Zhang ZQ, Shu RZ, Yu XL, et al. Dual effects of molybdenum on mouse oocyte quality and ovarian oxidative stress. Syst Biol Reprod Med. 2013;59(6):312–8.
7. Underwood EJ. Environmental sources of heavy metals and their toxicity to man and animals. 1979;11(4–5):33–45.
8. Kincaid RL. Toxicity of ammonium molybdate added to drinking water of calves. J Dairy Sci. 1980;63(4):608–10.
9. Achmadi UF. Public health implications of environmental pollution in urban Indonesia. Asia Pac J Clin Nutr. 1996;5(3):141–4.
10. Siddiqi HA, Ansari FA, Munshi AB. Assessment of hydrocarbons concentration in marine fauna due to Tasman Spirit oil spill along the Clifton beach at Karachi coast. Environ Monit Assess. 2009 Jan;148(1–4):139–48.
11. Gami AA, Shukor MY, Khalil KA, Dahalan FA, Khalid A, Ahmad SA. Phenol and its toxicity. J Environ Microbiol Toxicol. 2014;2(1):11–23.
12. Hansch C, McKarns SC, Smith CJ, Doolittle DJ. Comparative QSAR evidence for a free-radical mechanism of phenol-induced toxicity. Chem Biol Interact. 2000;127(1):61–72.
13. Bhattacharya A, Gupta A, Kaur A, Malik D. Efficacy of Acinetobacter sp. B9 for simultaneous removal of phenol and hexavalent chromium from co-contaminated system. Appl Microbiol Biotechnol. 2014;98(23):9829–41.
14. Sun JQ, Xu L, Tang YQ, Chen FM, Liu WQ, Wu XL. Degradation of pyridine by one Rhodococcus strain in the presence of chromium (VI) or phenol. J Hazard Mater. 2011;191(1–3):62–8.
15. Davis GK. Molybdenum. In: Merian E, editor. Metals and their Compounds in the Environment, Occurrence, Analysis and Biological Relevance. VCH Weinheim, New York; 1991. p. 1089–100.
16. Smedley PL, Kinniburgh DG. Molybdenum in natural waters: A review of occurrence, distributions and controls. Appl Geochem. 2017 Sept 1;84:387–432.
17. Neunhäuserer C, Berreck M, Insam H. Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation. Water Air Soil Pollut. 2001;128(1–2):85–96.
18. Geng C, Gao Y, Li D, Jian X, Hu Q. Contamination investigation and risk assessment of molybdenum on an industrial site in China. J Geochem Explor. 2014;144(PB):273–81.
19. Qadir MA, Zaidi JH, Ahmad SA, Gulzar A, Yaseen M, Atta S, et al. Evaluation of trace elemental composition of aerosols in the atmosphere of Rawalpindi and Islamabad using radio analytical methods. Appl Radiat Isot. 2012;70(5):906–10.
20. Ward GM. Molybdenum toxicity and hypocuprosis in ruminants: a review. J Anim Sci. 1978;46(4):1078–85.
21. Campbell AM, Campillo-Campbell AD, Villaret DB. Molybdate reduction by Escherichia coli K-12 and its chl mutants. 1985;82(1):227–31.
22. Sidgwick NV. The chemical elements and their compounds. Oxford, Clarendon Press; 1951.
23. Shukor MY, Syed MA. Microbiological reduction of hexavalent molybdenum to molybdenum blue. In: Mendez-Vilas A, editor. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Badajoz, Spain: Formatex Research Center; 2010. (Microbiology Book Series; vol. 2).
24. Yakasai HM, Rahman MF, Manogaran M, Yasid NA, Syed MA, Shamaan NA, et al. Microbiological Reduction of Molybdenum to Molybdenum Blue as a Sustainable Remediation Tool for Molybdenum: A Comprehensive Review. Int J Environ Res Public Health. 2021 Jan;18(11):5731.
25. Krishnamoorthy K, Veerapandian M, Yun K, Kim SJ. New function of molybdenum trioxide nanoplates: Toxicity towards pathogenic bacteria through membrane stress. Colloids Surf B Biointerfaces. 2013;112:521–4.
26. Nordmeier A, Merwin A, Roeper DF, Chidambaram D. Microbial synthesis of metallic molybdenum nanoparticles. Chemosphere. 2018 July 1;203:521–5.
27. Khan A, Halmi MIE, Shukor MY. Isolation of Mo-reducing bacterium in soils from Pakistan. J Environ Microbiol Toxicol. 2014;2(1):38–41.
28. Yunus SM, Hamim HM, Anas OM, Aripin SN, Arif SM. Mo (VI) reduction to molybdenum blue by Serratia marcescens strain Dr. Y9. Pol J Microbiol. 2009;58(2):141–7.
29. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergeys Man Determinative Bacteriol. 1994;
30. Costin S, Ionut S. ABIS online - bacterial identification software, http://www.tgw1916.net/bacteria_logare.html, database version: Bacillus 022012-2.10, accessed on Mar 2015. 2015.
31. Shukor MY, Lee CH, Omar I, Karim MIA, Syed MA, Shamaan NA. Isolation and characterization of a molybdenum-reducing enzyme in Enterobacter cloacae strain 48. Pertanika J Sci Technol. 2003;11(2):261–72.
32. Arif NM, Ahmad SA, Syed MA, Shukor MY. Isolation and characterization of a phenol-degrading Rhodococcus sp. strain AQ5NOL 2 KCTC 11961BP. J Basic Microbiol. 2013;53(1):9–19.
33. Shukor MY, Ahmad SA, Nadzir MMM, Abdullah MP, Shamaan NA, Syed MA. Molybdate reduction by *Pseudomonas* sp. strain DRY2. J Appl Microbiol. 2010;108(6):2050–8.
34. Ahmad SA, Shukor MY, Shamaan NA, Mac C, Syed MA. Molybdate reduction to molybdenum blue by an Antarctic bacterium. BioMed Res Int. 2013;2013:Article number 871941.
35. Ibrahim Y, Abdel-Mongy M, Shukor MS, Hussein S, Ling APK, Shukor MY. Characterization of a molybdenum-reducing bacterium with the ability to degrade phenol, isolated in soils from Egypt. Biotechnologia. 2015;96(3):234–45.
36. Yakasai MH, Rahman MFA, Rahim MBHA, Khayat ME, Shamaan NA, Shukor MY. Isolation and characterization of a metal-reducing *Pseudomonas* sp. strain 135 with amide-degrading capability. Bioremediation Sci Technol Res. 2017;5(2):32–8.
37. Yakasai HM, Babandi A, Ibrahim S, Sufyan AJ. Characterization of Butachlor degradation by A Molybdenum-Reducing and Aniline-degrading *Pseudomonas* sp. J Env Microbiol Toxicol. 2021;9(2):8–12.
38. Rahman MF, Ahmad SA, MacCormack WP, Ruberto L, Shukor MY. Modelling the Effect of Copper on the Mo-reduction Rate of the Antarctic Bacterium *Pseudomonas* sp. strain DRY1. J Environ Microbiol Toxicol. 2017 July 31;5(1):21–5.
39. Mohamad O, Yakasai HM, Karamba KI, Halmi MIE, Rahman MF, Shukor MY. Reduction of Molybdenum by *Pseudomonas aeruginosa* strain KIK-11 Isolated from a Metal-contaminated Soil

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

with Ability to Grow on Diesel and Sodium Dodecyl Sulphate. J Environ Microbiol Toxicol. 2017 Dec 31;5(2):19–26.
40. Kesavan V, Mansur A, Suhaili Z, Salihan MSR, Rahman MFA, Shukor MY. Isolation and Characterization of a Heavy Metal-reducing *Pseudomonas* sp. strain Dr.Y Kertih with the Ability to Assimilate Phenol and Diesel. Bioremediation Sci Technol Res. 2018 July 31;6(1):14–22.
41. AbdEl-Mongy MA, Aqlima SA, Shukor MS, Hussein S, Ling APK, Shukor MY. A PEG 4000-degrading and hexavalent molybdenum-reducing *Pseudomonas putida* strain Egypt-15. J Natl Sci Found Sri Lanka. 2018 Sept 30;46(3):431–42.
42. Gafasa MA, Ibrahim SS, Babandi A, Abdullahi N, Shehu D, Ya'u M, et al. Characterizing the Molybdenum-reducing Properties of *Pseudomonas* sp. locally isolated from Agricultural soil in Kano Metropolis Nigeria. Bioremediation Sci Technol Res. 2019 July 31;7(1):34–40.
43. Alhassan AY, Babandi A, Uba G, Yakasai HM. Isolation and Characterization of Molybdenum-reducing *Pseudomonas* sp. from Agricultural Land in Northwest-Nigeria. J Biochem Microbiol Biotechnol. 2020 July 31;8(1):23–8.
44. Sufyan AJ, Ibrahim S, Babandi A, Yakasai HM. Characterization of Butachlor Degradation by A Molybdenum-Reducing and Aniline-degrading *Pseudomonas* sp. J Environ Microbiol Toxicol. 2021 Dec 31;9(2):8–12.
45. Tijjani AU, Sufyan AJ, Ibrahim S, Shehu D, Ya'u M, Babagana K, et al. Characterization of Aniline Degradation by A Previously Isolated Molybdenum-reducing *Pseudomonas* sp. Bioremediation Sci Technol Res. 2021 Dec 31;9(2):17–20.
46. Rusnam, Gusmanizar N. Isolation and Characterization of a Molybdenum-reducing and the Congo Red Dye-decolorizing *Pseudomonas* putida strain Neni-3 in soils from West Sumatera, Indonesia. J Biochem Microbiol Biotechnol. 2022 July 31;10(1):17–24.
47. Rusnam, Gusmanizar N, Rahman MF, Yasid NA. Characterization of a Molybdenum-reducing and Phenol-degrading *Pseudomonas* sp. strain Neni-4 from soils in West Sumatera, Indonesia. Bull Environ Sci Sustain Manag. 2022 July 31;6(1):1–8.
48. Aliyu FI, Ibrahim A, Babandi A, Shehu D, Ya'u M, Babagana K, et al. Glyphosate Biodegradation by Molybdenum-Reducing *Pseudomonas* sp. J Environ Microbiol Toxicol. 2022 Dec 31;10(2):42–7.
49. Uba G, Manogaran M, Yakasai HM, Yasid NA, Shukor MY. Response Surface Method for the Optimization of *Pseudomonas* sp. strain DrY135 Growth on Acrylamide as a Nitrogen Source. Bull Environ Sci Sustain Manag. 2022 Dec 31;6(2):23–34.
50. Abd Shukor MS, Aftab K, Norazlina M, Effendi Halmi M, Sheikh A, Shukor M. Isolation of a Novel Molybdenum-reducing and Azo Dye Decolorizing *Enterobacter* sp. Strain Aft-3 from Pakistan. Chiang Mai Univ J Nat Sci. 2016 Jan 1;15:95–114.
51. Shukor MS, Shukor MY. A microplate format for characterizing the growth of molybdenum-reducing bacteria. J Environ Microbiol Toxicol. 2014;2(2):42–4.
52. Iyamu EW, Asakura T, Woods GM. A colorimetric microplate assay method for high-throughput analysis of arginase activity in vitro. Anal Biochem. 2008;383(2):332–4.
53. Ghani B, Takai M, Hisham NZ, Kishimoto N, Ismail AKM, Tano T, et al. Isolation and characterization of a Mo6+-reducing bacterium. 1993;59(4):1176–80.
54. Losi ME, Jr WTF. Reduction of selenium oxyanions by Enterobacter cloacae strain SLD1a-1: Reduction of selenate to selenite. Environ Toxicol Chem. 1997;16(9):1851–8.
55. Simeonova DD, Lièvremont D, Lagarde F, Muller DAE, Groudeva VI, Lett MC. Microplate screening assay for the detection of arsenite-oxidizing and arsenate-reducing bacteria. FEMS Microbiol Lett. 2004 Aug 1;237(2):249–53.
56. Sedighi M, Vahabzadeh F. Kinetic Modeling of cometabolic degradation of ethanethiol and phenol by Ralstonia eutropha. Biotechnol Bioprocess Eng. 2014;19(2):239–49.
57. Raj J, Prasad S, Sharma NN, Bhalla TC. Bioconversion of Acrylonitrile to Acrylamide using Polyacrylamide Entrapped Cells of Rhodococcus rhodochrous PA-34. Folia Microbiol (Praha). 2010;55(5):442–6.
58. Steiert JG, Pignatello JJ, Crawford RL. Degradation of chlorinated phenols by a pentachlorophenol-degrading bacterium. Appl Environ Microbiol. 1987;53(5):907–10.
59. Chan-Hyams JVE. Characterisation and optimisation of nitroreductase-prodrug combinations for bacterial-directed enzyme-prodrug therapy [Internet] [PhD Thesis]. Victoria University of Wellington; 2020 [cited 2025 Aug 7]. Available from: https://openaccess.wgtn.ac.nz/articles/thesis/Characterisation_and_optimisation_of_nitroreductase-prodrug_combinations_for_bacterial-directed_enzyme-prodrug_therapy/17148002?file=31708958
60. Schneier A, Melaugh G, Sadler JC. Engineered plastic-associated bacteria for biodegradation and bioremediation. Biotechnol Environ. 2024 July 15;1(1):7.
61. Sims RPA. Formation of heteropoly blue by some reduction procedures used in the micro-determination of phosphorus. 1961;86(1026):584–90.
62. Shukor MY, Habib SHM, Rahman MFA, Jirangon H, Abdullah MPA, Shamaan NA, et al. Hexavalent molybdenum reduction to molybdenum blue by S. marcescens strain Dr. Y6. Appl Biochem Biotechnol. 2008;149(1):33–43.
63. Shukor MY, Rahman MF, Shamaan NA, Syed MS. Reduction of molybdate to molybdenum blue by Enterobacter sp. strain Dr.Y13. J Basic Microbiol. 2009;49(SUPPL. 1):S43–54.
64. Shukor MY, Rahman MF, Suhaili Z, Shamaan NA, Syed MA. Bacterial reduction of hexavalent molybdenum to molybdenum blue. World J Microbiol Biotechnol. 2009;25(7):1225–34.
65. Shukor MY, Ahmad SA, Nadzir MMM, Abdullah MP, Shamaan NA, Syed MA. Molybdate reduction by *Pseudomonas* sp. strain DRY2. J Appl Microbiol. 2010;108(6):2050–8.
66. Shukor MY, Rahman MF, Suhaili Z, Shamaan NA, Syed MA. Hexavalent molybdenum reduction to Mo-blue by Acinetobacter calcoaceticus. Folia Microbiol (Praha). 2010;55(2):137–43.
67. Lim HK, Syed MA, Shukor MY. Reduction of molybdate to molybdenum blue by Klebsiella sp. strain hkeem. J Basic Microbiol. 2012;52(3):296–305.
68. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H, et al. Kinetics of molybdenum reduction to molybdenum blue by Bacillus sp. strain A.rzi. BioMed Res Int. 2013;2013.
69. Shukor MY, Halmi MIE, Rahman MFA, Shamaan NA, Syed MA. Molybdenum reduction to molybdenum blue in Serratia sp. strain DRY5 is catalyzed by a novel molybdenum-reducing enzyme. BioMed Res Int. 2014;2014.
70. Haider SF, Asif KH, Gilani AH. Weather yield model for the semi tropical region (Pakistan). Adv Atmospheric Sci. 1992;9(3):367–72.
71. Rahman MFA, Shukor MY, Suhaili Z, Mustafa S, Shamaan NA, Syed MA. Reduction of Mo(VI) by the bacterium Serratia sp. strain DRY5. J Environ Biol. 2009;30(1):65–72.
72. Abo-Shakeer LKA, Ahmad SA, Shukor MY, Shamaan NA, Syed MA. Isolation and characterization of a molybdenum-reducing Bacillus pumilus strain lbna. J Environ Microbiol Toxicol. 2013;1(1):9–14.
73. Halmi MIE, Zuhainis SW, Yusof MT, Shaharuddin NA, Helmi W, Shukor Y, et al. Hexavalent molybdenum reduction to Mo-blue by a Sodium-Dodecyl-Sulfate-degrading Klebsiella oxytoca strain DRY14. BioMed Res Int. 2013;2013:e384541.
74. Shukor Y, Adam H, Ithnin K, Yunus I, Shamaan NA, Syed A. Molybdate reduction to molybdenum blue in microbe proceeds via a phosphomolybdate intermediate. 2007;7(8):1448–52.
75. Shukor MY, Halmi MIE, Rahman MFA, Shamaan NA, Syed MA. Molybdenum reduction to molybdenum blue in *Serratia* sp. strain DRY5 is catalyzed by a novel molybdenum-reducing enzyme. BioMed Res Int. 2014;2014 Article ID 853084.
76. Runnells DD, Kaback DS, Thurman EM. Geochemistry and sampling of molybdenum in sediments, soils, and plants in Colorado. In: Chappel WR, Peterson KK, editors. Molybdenum in the environment. New York: Marcel and Dekker, Inc.; 1976.
77. Shukor MYA. Bacterial Reduction of Molybdenum as a Tool for Its Bioremediation. In: Eco-Restoration of Polluted Environment. CRC Press; 2024.
78. Bhattacharya A, Gupta A, Kaur A, Malik D. Remediation of phenol using microorganisms: Sustainable way to tackle the chemical pollution menace. Curr Org Chem. 2018;22(4):370–85.
79. Oh JS a, Han YH b. Isolation and Characterization of Phenol-degrading Rhodococcus sp. DGUM 2011. Korean J Appl Microbiol Biotechnol. 1997;25(5):459–63.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

80. Yoon KP. Construction and characterization of multiple heavy metal-resistant phenol-degrading pseudomonads strains. Vol. 13, Journal of Microbiology and Biotechnology. 2003. p. 1001–7.
81. García MT, Gallego V, Ventosa A, Mellado E. Thalassobacillus devorans gen. nov., sp. nov., a moderately halophilic, phenol-degrading, Gram-positive bacterium. Int J Syst Evol Microbiol. 2005;55(5):1789–95.
82. Yang CF, Lee CM. Enrichment, isolation, and characterization of phenol-degrading *Pseudomonas* resinovorans strain P-1 and Brevibacillus sp. strain P-6. Vol. 59, International Biodeterioration and Biodegradation. 2007. p. 206–10.
83. Xie Q, Yang G, He G. Isolation and characterization of a phenol degrading bacterium PN6-15. Huazhong Keji Daxue Xuebao Ziran Kexue BanJournal Huazhong Univ Sci Technol Nat Sci Ed. 2009;37(8):129–32.
84. Zhan Y, Yan Y, Zhang W, Chen M, Lu W, Ping S, et al. Comparative analysis of the complete genome of an Acinetobacter calcoaceticus strain adapted to a phenol-polluted environment. Res Microbiol. 2012;163(1):36–43.
85. Liu J, Wang Q, Yan J, Qin X, Li L, Xu W, et al. Isolation and characterization of a novel phenol degrading bacterial strain WUST-C1. Ind Eng Chem Res. 2013;52(1):258–65.
86. Tosu P, Luepromchai E, Suttinun O. Activation and immobilization of phenol-degrading bacteria on oil palm residues for enhancing phenols degradation in treated palm oil mill effluent. Environ Eng Res. 2015;20(2):141–8.
87. Wang L, Li Y, Niu L, Dai Y, Wu Y, Wang Q. Isolation and growth kinetics of a novel phenol-degrading bacterium Microbacterium oxydans from the sediment of Taihu Lake (China). Water Sci Technol. 2016;73(8):1882–90.
88. Zin NSMZ, Padrilah SNP, Rahman MFA, Sim HK, Khalid A, Shukor MY. Isolation and characterization of a 2,4-dinitrophenol-degrading bacterium. Bioremediation Sci Technol Res. 2016 Dec 31;4(2):28–33.
89. Aisami A, Yasid NA, Johari WLW, Shukor MY. Estimation of the Q10 value; the temperature coefficient for the growth of *Pseudomonas* sp. aq5-04 on phenol. Bioremediation Sci Technol Res. 2017 July 31;5(1):24–6.
90. Zhao T, Gao Y, Yu T, Zhang Y, Zhang Z, Zhang L, et al. Biodegradation of phenol by a highly tolerant strain *Rhodococcus ruber* C1: Biochemical characterization and comparative genome analysis. Ecotoxicol Environ Saf. 2021 Jan 15;208:111709.
91. Kujur RRA, Das SK. *Pseudomonas* phenolilytica sp. nov., a novel phenol-degrading bacterium. Arch Microbiol. 2022 May 14;204(6):320.
92. Yakasai HM, Zin NSM, Shukor MY. Activation Energy, Temperature Coefficient and Q10 Value Estimations of the Growth of 2,4-dinitrophenol-degrading Bacterium on 2,4-dinitrophenol. J Environ Bioremediation Toxicol. 2022 Aug 5;5(1):38–44.
93. Mortazavi A, Hassanshahian M, Ali E, Fenjan MN, Alawadi A, Alsalamy A. Isolation and Identification of Phenol-Degrading Bacteria from Iranian Soil and Leaf Samples. Front Biosci-Elite. 2023 Dec 12;15(4):29.
94. Huang L, Liang J, Zhang J. Characteristics of a novel acid-resistant phenol-degrading bacterium *Acinetobacter pittii* Hly3: Adaptability, kinetics, degradation pathway and long-term performance. Int Biodeterior Biodegrad. 2024 July 1;192:105825.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).