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ISOLATION AND CHARACTERIZATION OF A MOLYBDENUM-REDUCING AND AMIDE-DEGRADING *BURKHOLDERIA* SP. STRAIN NENI-11 IN SOILS FROM WEST SUMATERA, INDONESIA

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ABSTRACT

A molybdenum-reducing bacterium isolated from contaminated soil was able to utilize acrylamide as the electron donor source, and was able utilize acrylamide, acetamide and propionamide for growth. Reduction was optimal at pH between 6.0 to 6.3, at temperatures of between 30 and 37 oC, glucose as the electron donor, phosphate at 5.0 mM, and sodium molybdate at 15 mM. The absorption spectrum of the Mo-blue indicates it is a reduced phosphomolybdate. Molybdenum reduction was inhibited by mercury (ii), silver (i) and chromium (vi) at 2 p.p.m. by 91.9, 82.7 and 17.4 %, respectively. Biochemical analysis resulted in a tentative identification of the bacterium as Burkholderia cepacia strain Neni-11. The growth of this bacterium modelled according to the modified Gompertz model. The growth parameters obtained were maximum specific growth rates of 1.241 d-1, 0.971 d-1, 0.85 d-1 for acrylamide, propionamide and acetamide, respectively, while the lag periods of 1.372 d, 1.562 and 1.639 d were observed for acrylamide, propionamide and acetamide, respectively. The ability of this bacterium to detoxify molybdenum and grown on toxic amides makes this bacterium an important tool for bioremediation.

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KEY WORDS

Molybdenum reduction; molybdenum blue; Burkholderia sp.; acrylamide; propionamide; acetamide

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INTRODUCTION

Mining activities are the major source of molybdenum pollution. In Indonesia, copper and gold mining activity from the copper-gold-molybdenum porphyry deposit in Batu Hijau, Sumbawa has steadily contaminated surrounding coastal regions. The mine deposits nearly several million tonnes of waste tailings to the sea annually. This has led to decreased fish population and water quality [1,2]. A similar situation is seen in a molybdenum mine in western Liaoning China, where the molybdenum mine tailings have polluted the Nver River. The river water and sediment contain molybdenum at levels far exceeding the statutory limits [3]. In Armenia, numerous copper-molybdenum mines and a copper-molybdenum metallurgical plant in Alaverdi, the latter operating without a proper filtration system since 1996 have polluted nearly 300 square kilometres of land [4]. In the Miduk Copper Complex in Iran, in which molybdenum is a valuable by-product, the complex tailings dam has triggered a high concentration of molybdenum found in the borehole near a drinking water source. Metal seepage and infiltration towards the surrounding surface and groundwater from the metal tailings dam is frequently inevitable, causing the observed pollution [5]. In Malaysia, the Mamut copper mine in Ranau Sabah, produced gold and molybdenum as

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by-products. Episodic ruptures of the pipes carrying metal-rich wastes have caused the contamination of the surrounding agricultural areas and the Ranau River [6,7].

Substantial nutritional consumption of molybdenum brings about a secondary copper deficit. The symptoms, mainly documented in ruminants are observed globally. Cattle and sheep are ten times more prone compared to non-ruminants. The sulfur-rich conditionss in the rumen favour formation of thiomolybdate compounds. As these compounds chelate copper, the bioavailability of copper diminished and copper deficiency symptoms such as weight loss, anemia, diarrhea kidney damage, and osteoporosis occur [8]. Spermatogenesis in several organisms is also negatively affected by molybdenum. Molybdenum supplementation in the fruit fly drosophila severely disturbed spermatogenesis [9]. Rats are also affected. Molybdenum (ammonium molybdate) supplementation to the adult male Wistar rats diet leads to histopathological and histomorphometric with a substantial weight reduction of the testes [10]. Spermatogenesis in the testicular organ culture of the Japanese eel induced by the compound 11-ketotestosterone (11KT) is also inhibited by a combination of heavy metals including molybdenum. A synergistic effect of molybdenum was observed in this study [11].

Aside from heavy metals, organic pollutants or manmade chemicals (xenobiotics) such as phenol, acrylamide, nicotinamide, acetamide, iodoacetamide, propionamide, acetamide, sodium dodecyl sulfate (SDS) and diesel are major global pollutants [12–14]. Amides such as acrylamide, acetamide and propionamide are produced in the order of millions of tonnes per year [15]. Acrylamide is chiefly used to synthesize the polymer polyacrylamides [16]. Acetamide is used as a plasticizer and as an industrial solvent while propionamide is used as an ingredient in many different organic processes to form other useful compounds. Amongst these amides, acrylamide is very toxic. The acrylonitrile-acrylamide industries are known sources acrylamide pollution with levels as high as 1 g/L have been reported [17]. Another non-documented source of acrylamide comes from glyphosate application in agriculture areas. The formulation of this pesticide uses 20-30% polyacrylamide as a dispersing agent [18], and this could be a substantial source of acrylamide pollution in soils and run-offs.

Removal of soluble molybdenum through bacterial reduction is a promising bioremediation strategy [19]. In bacterial reduction of molybdenum to the colloidal molybdenum blue, the Mo-blue aggregates with bacterial biomass can aid in its removal [20]. Since it was first discovered in 1896, [21] many more Mo-reducing bacteria have been isolated [19, 22, 23]. Some microbes are able to degrade a variety of xenobiotics including acrylamide [24] and detoxify heavy metals at the same time including the reduction of chromate coupled with the biodegradation of phenol [25].

In this work, we successfully isolated a novel molybdenum reducing bacterium showing the capacity to grow on various amide and nitrile compounds. The novel characteristics of this bacterium will make the bacterium suitable for the bioremediation of polluted sites having these pollutants in the future.

MATERIALS AND METHODS

Molybdenum-reducing bacterium growth and maintenance

Soil samples were taken (5 cm deep from topsoil) from the grounds of a garbage-contaminated land in the province of Pariaman, Sumatera, Indonesia in January 2009. Isolation of molybdenum-reducing bacteria utilized a minimal salts media (MSM) with the phosphate concentration set at 5 mM. The MSM was also supplemented with sodium molybdate at 10 mM. Preparation of soil bacterial suspension was carried out by adding soil (1.0 gram) to 10 ml of deionized water. The soil suspension was thoroughly mixed, and 0.1 mL of the soil suspension was then spread onto a petri dish containing agar of a media (w/v) as follows: yeast extract (0.5%), $MgSO_4 \cdot 7H_2O$ (0.05%), $Na_2MoO_4.2H_2O$ (0.242 % or 10 mM), glucose (1%), $(NH_4)_2 \cdot SO_4$ (0.3%), NaCl (0.5%), agar (1.5%), and Na_2HPO_4 (0.071% or 5 mM). The pH of the media was adjusted to pH 6.5 [23]. This media is known as a low phosphate molybdate media or LPM. After 48 hours of incubation at room temperature, several white and ten blue colonies appeared on the plate. The ten isolates were then restreaked on the LPM agar several times in order to get pure culture. Mo-blue production from these bacteria was then quantified in 100 mL liquid culture (LPM) to select the best isolate. Mo-blue production was quantified at 865 nm utilizing the extinction coefficient of 16.3 $mM.^{-1}.cm^{-1}$ to choose the best isolate. Characterization of the molybdenum blue produced was carried out by scanning the absorption spectrum of the blue supernatant from the liquid culture from 400 to 900 nm (UV-spectrophotometer, Shimadzu 1201) with low phosphate media minus bacterium as the baseline correction. Briefly, the culture supernatant was centrifuged at 10,000 x g for 10 minutes at room temperature to remove bacterial aggregates. The bacterium was identified via biochemical and phenotypical methods [23] in accordance to the Bergey's Manual of Determinative Bacteriology [26], and the results plugged into the ABIS online system [27].

Bacterial resting cells preparation

The characterizations of molybdenum reduction including the effects of carbon sources, heavy metals, concentrations of phosphate, molybdate, pH and temperature were carried out utilizing resting cells in a microtiter format as before, but with slight modifications [28]. Briefly, bacterial cells were grown aerobically in several 250 mL shake flasks with shaking at 120 rpm on an

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orbital shaker (Yihder, Taiwan) in a volume of 1 L. Incubation was carried out at room temperature. The media utilized was the High Phosphate media (HPM) with the only difference to the LPM was the phosphate concentration set at 100 mM. This was carried out to prevent bacterial aggregations to molybdenum blue, which leads to cellular harvesting complications. Cells were centrifuged at 15,000 x g at 4 ^{o}C for 10 minutes. The bacterial pellets were then rinsed with deionized water twice. The pellets were then resuspended in 20 μL of LPM with glucose omitted. Appropriate alterations in the LPM were carried out to meet the needs of modifications in the carbon sources, phosphate, molybdate and pH conditions during the characterization works. About 180 μL of the appropriately modified LPM was sterically transferred into the wells of a sterile microplate. This was followed by the addition of 20 mL of sterile glucose or other carbon sources from a stock solution to the final concentration of 1.0 % (w/v). The total volume was 200 μL. The microplates were then sealed (Corning® microplate), and incubated at room temperature. Readings at 750 nm were periodically taken using a BioRad Microtiter Plate reader (Model No. 680, Richmond, CA). This wavelength is the maximum filter available for the microplate unit [28]. Quantification of the Mo-blue produced was carried out utilizing the extinction coefficient of 11.69 $mM.^{-1}.cm^{-1}$ at 750 nm was utilized to quantify Mo-blue production. The effect of several heavy metals was studied utilizing Atomic Absorption Spectrometry calibration standard solutions from MERCK.

Test of amides and nitriles as sources of electron donor or growth

The capacity of various amides and nitriles to support molybdenum reduction as electron donors was tested using the microplate format above by replacing glucose from the low phosphate medium with nicotinamide, acetamide, iodoacetamide, acrylamide, propionamide, acetamide, acetonitrile, acrylonitrile 2-chloroacetamide, and benzonitrile to the final concentration of 2,000 mg/L [29]. Glucose was the positive control, and was added to the final concentration of 2,000 mg/L. Then 200 μL of the media was added into the microplate wells with 50 μL of resting cells suspension. The microplate was incubated at room temperature for three days and the amount of Mo-blue production was measured at 750 nm as before. The ability of the compounds above to support the growth of this bacterium independent of molybdenum-reduction was tested using the microplate format above using the media below minus molybdate, and replacing glucose with the xenobiotics at the final concentration of 2,000 mg/L in a volume of 50 μL. The ingredients of the growth media (LPM) were as follows: $(NH_4)_2$•SO_4 (0.3%), $NaNO_3$ (0.2%), $MgSO_4$•$7H_2O$ (0.05%), yeast extract (0.01%), NaCl (0.5%) and Na_2HPO_4 (0.705% or 50 mM). Then 200 μL of the media was added into the microplate wells and mixed with 50 μL of resting cells suspension. The media was adjusted to pH 7.0. The increase of bacterial growth was measured at 600 nm after three days of incubation at room temperature.

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Mathematical modelling of bacterial growth on amides

Bacterial growth on these xenobiotics was modeled using the modified Gompertz model (Eqn. **1**), having three parameters to be solved as this model is frequently used to model microbial growth [16].where A=bacterial growth at lower asymptote; μ_m= maximum specific bacterial growth rate, λ=lag time, e = exponent (2.718281828) and t = sampling time.

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\} \quad (1)$$

RESULTS

Isolation of Mo-reducing bacteria

The ten Mo-reducing bacterial isolates were quantified for their capacity to produce Mo-blue by monitoring production at 865 nm. The best isolate was 6a **[Table–1]**, and was chosen for further studies.

Table: 1. Mo-blue production by bacterial isolates.

Isolate	nmole Mo-blue
1a	0.23
2a	1.87
3a	1.03
4a	0.45
5a	2.19
6a	15.02
7a	3.42
8a	2.13
9a	7.02
10a	3.04

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Identification of bacterium

Isolate 6a was a short rod-shaped, motile, Gram-negative bacterium. Identification of the bacterium was carried out by computing the results of cultural, morphological and various biochemical tests **[Table–2]** into the ABIS online software. Analysis using the software indicated that the bacterial identity giving the highest homology (73%) and accuracy at 91% as *Burkholderia cepacia*. Despite this, molecular identification technique through comparison of the 16srRNA gene is needed to identify this species further. The bacterium is tentatively identified as *Burkholderia*sp. strain Neni-11 in honor of the late Dr. Neni Gusmanizar. The bacterium exhibited optimum pH for reduction of between 6.0 and 6.3, and an optimum temperature ranging from 30 °C to 37°C (Data not shown).

Table: 2. Morphological and biochemical tests of *Burkholderia* sp. strain Neni-11.

Test		Acid production from	
Gram staining	–	:	
Motility	+	L-Arabinose	+
Growth at 4 ºC	–	Citrate	+
Growth at 41 ºC	+	Fructose	+
Growth on MacConkey agar	–	Glucose	+
Arginine dihydrolase (ADH)	–	meso-Inositol	+
Alkaline phosphatase (PAL)	–	2-Ketogluconate	+
H2S production	+	Mannose	+
Indole production	+	Mannitol	+
Nitrates reduction	–	Sorbitol	+
Lecithinase	–	Sucrose	+
Lysine decarboxylase (LDC)	+	Trehalose	+
Ornithine decarboxylase (ODC)	–	Xylose	+
ONPG (beta-galactosidase)	–	Glycogen	–
Esculin hydrolysis	+	Methyl-mannoside	–
Gelatin hydrolysis	–	D-Melezitose	–
Starch hydrolysis	–	Inulin	–
Urea hydrolysis	–	Starch	–
Oxidase reaction	+	D-Turanose	–

Note: + positive result, − negative result

Molybdenum absorbance spectrum

Through the entire progress of molybdate reduction to Mo-blue, scanning of the supernatants of the culture media from 400 to 1000 nm demonstrated that the bacterium showed an exceptional Mo-blue spectrum having a maximum peak at 865 nm and a shoulder at 700 nm. This unique profile was noticed to be conserved through the entire incubation period **[Figure– 1]**.

Effect of electron donor on molybdate reduction

The best electron donor for supporting molybdate reduction was glucose with an optimal concentration at 1% (w/v) (data not shown). This is followed by sorbitol, fructose, 2-ketogluconate, mannose, sucrose, l-arabinose, mannitol, xylose, meso-inositol, trehalose and citrate in descending order **[Figure– 2]**.

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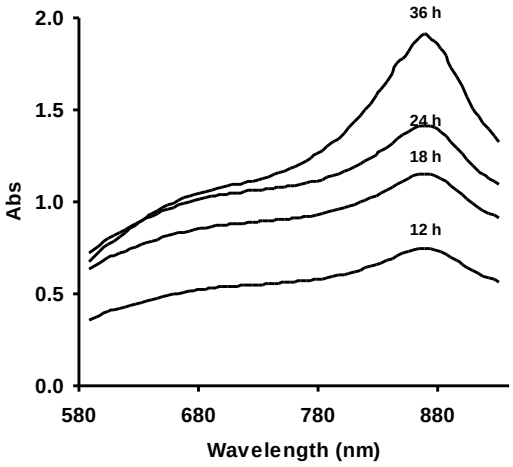

Fig: 1. Scanning absorption spectrum of Mo-blue from *Burkholderia*sp. strain Neni-11 at different time intervals.

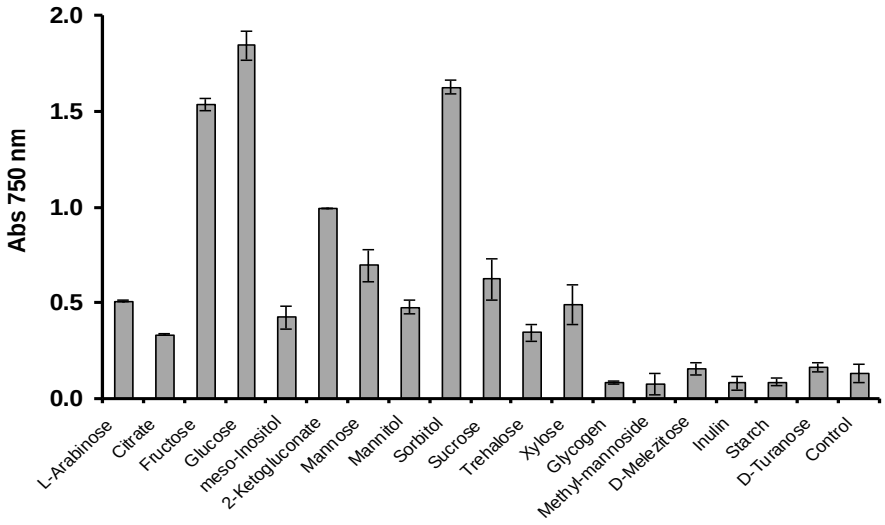

Fig: 2. Mo-blue production utilizing various electron donor sources (1% w/v). The error bars indicate mean ± standard deviation of three replicates.

Molybdate reduction under various concentrations of phosphate and molybdate

The optimum concentration of phosphate supporting molybdenum reduction occurred between 5.0 and 7.5 mM with higher concentrations were strongly inhibitory to reduction **[Figure– 3A]**. Maximum amount of Mo-blue produced was seen at concentrations of molybdate at 15 mM, and after an incubation period of 52 hours approximately **[Figure– 3B]**. A lag period of about 10 hours was observed

Effect of heavy metals

Molybdenum reduction was inhibited by mercury (ii), silver (i) and chromium (vi) at 2 p.p.m. by 91.9, 82.7 and 17.4 %, respectively. The heavy metals arsenic, cadmium, copper and lead did not exhibit inhibition to molybdenum reduction **[Figure– 4]**.

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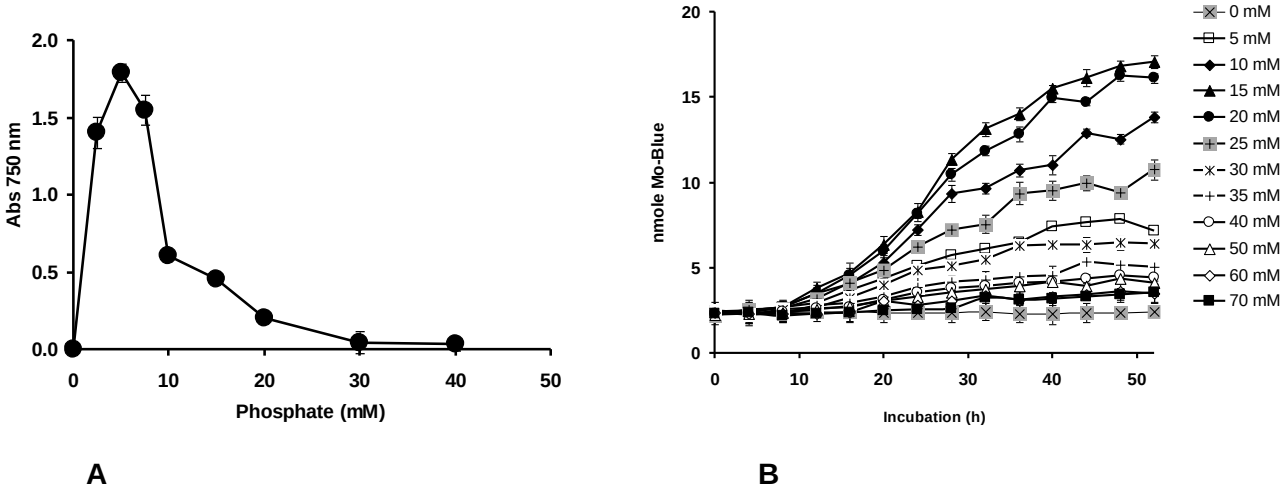

Fig: 3. The effect of phosphate (A) and molybdate (B) concentrations on molybdenum reduction by *Burkholderia*sp. strain Neni-11. The error bars indicate mean ± standard deviation of three replicates.

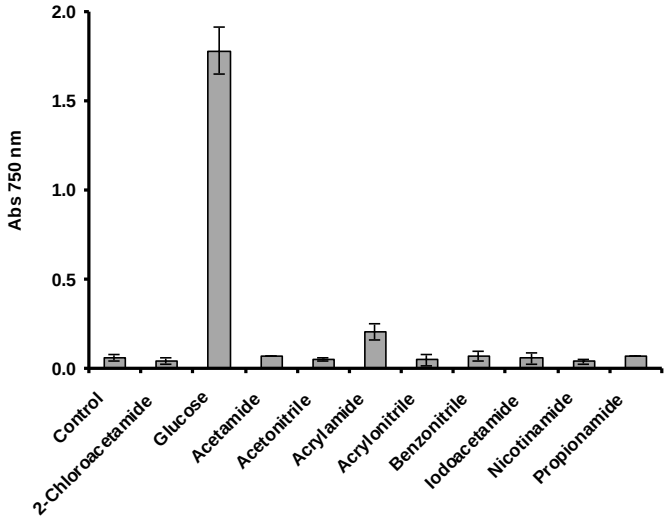

Fig: 4. Mo-blue reduction by xenobiotics at 10 mM in low phosphate media. Glucose was the positive control. The error bars indicate mean ± standard deviation of three replicates.

Amides and nitriles as electron donors for reduction and growth

The ability of these amides and nitriles to act as electron donor for molybdenum reduction was studied. Only acrylamide was shown to support molybdenum reduction but at a lower efficiency than glucose **[Figure–5A]**. The amides acrylamide, acetamide and propionamide supported the growth of this bacterium independently of molybdenum reduction **[Figure–5B]**. The growth of this bacterium on these amides was modelled according to the modified Gompertz model **[Figure**–6]. The absorbance values at 600 nm were first converted to natural logarithm. The correlation coefficients obtained for the model at 0.99, 0.98 and 0.98 for acrylamide, propionamide and acetamide, respectively, indicated good agreement between predicted and observed values. The growth parameters obtained were maximum specific growth rates of 1.241, 0.971 and 0.85 d^{-1} for acrylamide, propionamide and acetamide, respectively, while the lag periods of 1.372, 1.562 and 1.639 days were observed for acrylamide, propionamide and acetamide, respectively.

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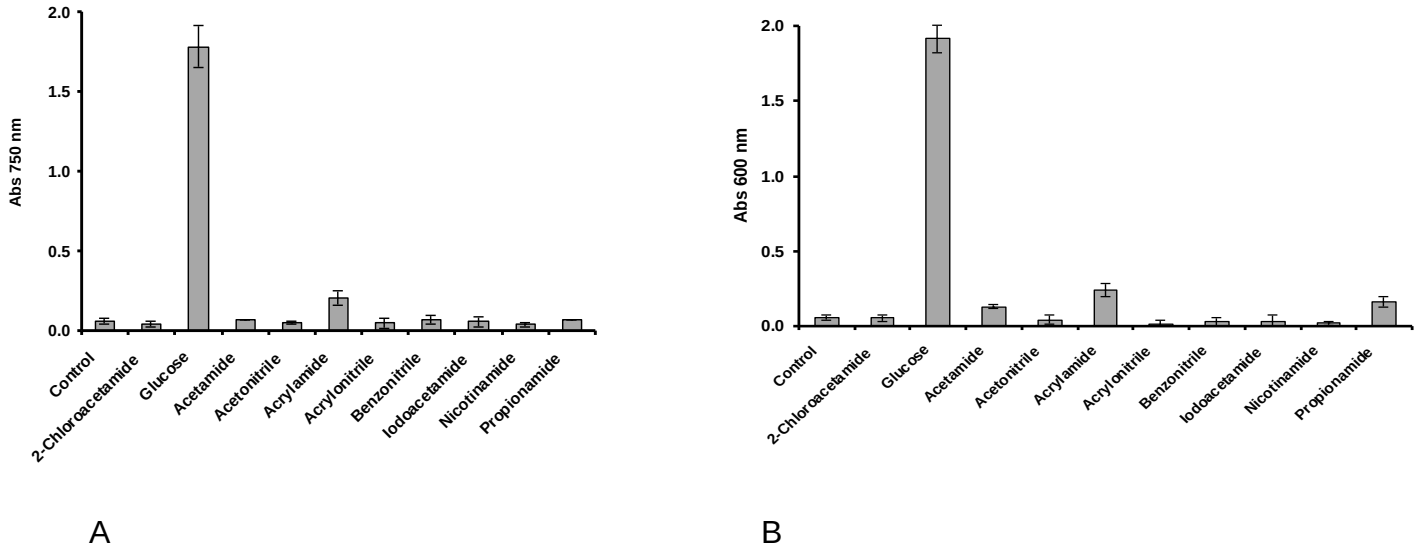

Fig: 5. Mo-blue reduction (A) measured at 750 nm and growth (B) measured at 600 nm by xenobiotics at 10 mM in low phosphate media. Glucose was the positive control. The error bars indicate mean ± standard deviation of three replicates.

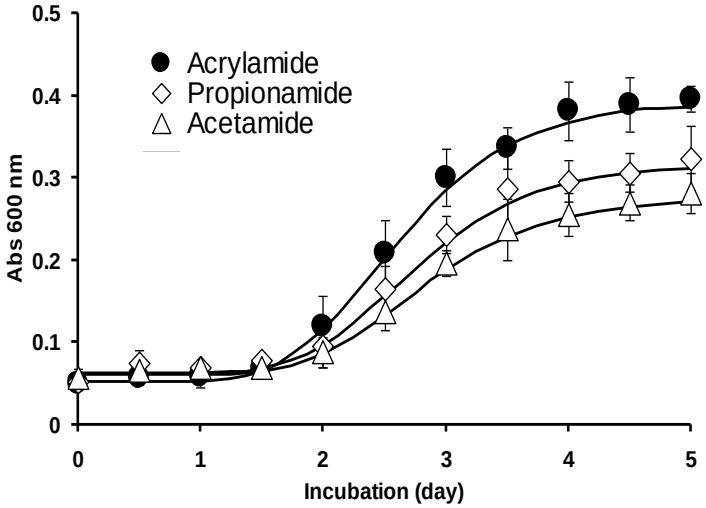

Fig: 6. Growth of *Burkholderia*sp. strain Neni-11 on acrylamide, propionamide and acetamide as modelled using the modified Gompertz model (solid lines). Bacterium was incubated at room temperature in a microtiter plate. The error bars indicate mean ± standard deviation of three replicates.

DISCUSSION

The phenomenon of molybdenum blue formation from bacterial reduction of molybdenum was first reported in *E. coli* about more than one hundred years ago in 1896 [21]. This is followed in the last century, in 1939 [30]. It was reported again in 1985 after a long absence in *E. coli*K12 [31], and in 1993 in *Enterobacter cloacae* strain 48 [32]. The potential of this phenomenon to be used in the bioremediation of molybdenum is first realized by Ghani et al. [32]. Since then, numerous Mo-reducing bacteria have been isolated **[Table–3]**, including two bacteria that can degrade SDS [23,33] as well as a psychrophilic Mo-reducing bacterium isolated from Antarctica. As the latter is a *Pseudomonas* species, we screened a similar bacterium initially isolated for diesel-degrading capacity to reduce molybdenum to molybdenum blue. This is a second bacterium isolated from Antarctica showing molybdenum-

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reducing property.The microtiter plate format utilizing resting cells allows a high throughput characterization format [23,28,34]. The utilization of resting cells or whole cells was first initiated in the bacterium *Enterobacter cloacae* strain 48 [32]. Several bacterial characterizations work such as in selenate [35], chromate [36] and vanadate [37] reductions also utilize resting cells. Furthermore, biodegradation of xenobiotics for example SDS [38,39] and diesel [40] also takes advantage of resting cells. The use of resting cells bypasses the initial stage of the growth process that is normally affected by toxic xenobiotics.

In the past, Mo-blue production by Mo-reducing bacteria has been hypothesized to commence initially by an enzymatic reduction from the Mo^{6+} to the Mo^{5}+ oxidation state. This is eventually accompanied by the add-on of phosphate ions from the surroundings producing Mo-blue [32]. However, this mechanism has some issues. In reality, Mo^{6+} ion does not exist in liquid solution. At neutral pH, molybdate appears as $[MoO_4]^{2-}$ or molybdate anions with its protonated form, either $HMoO_4^-$ or H_2MoO^4. Molybdate concentrations of above 1 mM and at acidic pHs, molybdate ions instantly formed polyions among others $H_2Mo_7O_{24}^{4-}$, $HMo_7O_{24}^{5-}$, $Mo_7O_{24}^{6-}$, and $Mo_{12}O_{37}^{2-}$. At very acidic pHs (<2.0), species such as $Mo_8O_{26}^{4-}$ and $Mo_{36}O_{112}(H_2O)_{16}^{8-}$ started to form with even complex species forming with further acidification. These forms of molybdenum are called polyoxomolybdates. The structure can incorporate heteroatoms such as silicate or phosphate, in the latter forming heteropolyoxomolybdates [51]. Enzymes for examples aldehyde oxidase and xantine oxidase can reduce these compounds into Mo-blue, which is an intense colloidal product with fractional oxidation state [52]. Nearly all bacterially produced Mo-blue show spectra with close similarity to the phosphate determination method [23,53], the latter is a reduced phosphomolybdate having a characteristics shoulder of from 700 to 720 nm, and a peak maximum from 870 nm to 890 nm [52,54]. We put forward a new hypothesis on Mo-blue production in bacteria based on molybdenum chemistry and Mo-blue spectral analysis that a phoshomolybdate intermediate is formed during the reduction of sodium molybdate to Mo-blue in bacteria [53]. The presence of an intermediate species during heavy metal reduction has been reported in chromate reduction (6+ to 3+) at least in the bacteria *Pseudomonas ambigua*[55] and *Shewanella putrefaciens* (now known as *S. oneidensis*) [56], where spectroscopic and paramagnetic resonance works have confirmed the presence of the intermediate species Cr^{5+}. Spectroscopic analysis employed in in this work is regarded as a simple method for distinguishing between the existing heteropolymolybdates, which include silicomolybdate, phosphomolybdate, and sulfomolybdate. On the other hand, additional investigations making use of nuclear magnetic resonance and electron spin resonance are essential for in depth identification of the precise lacunary species of phosphomolybdate associated with bacterial reduction of molybdenum [52, 57].

The majority of the molybdenum reducers prefer either glucose or sucrose as the best carbon source **Table– 3.** One of the reasons is the easily assimilable characteristics of these carbohydrates. With generic metabolic pathways, the reducing equivalents NADH and NADPH can be generated easily using these carbon sources. Both of these compounds are electron-donating substrates for the molybdenum reducing-enzyme [58,59]. Despite sucrose and glucose being excellent electron donor, a cheaper carbon source for example molasses can be utilized especially in actual bioremediation, since molasses can be obtained economically and in large quantity from the sugar cane industry in Malaysia [60]. Molasses has been utilized as electron donor in the reduction of hexavalent chromate by the bacterium *Flexivirga alba*[61] and selenate reduction by five bacterial isolates [62]. The possible utilization of molasses as a carbon source is currently being evaluated.

The presence of this lag period is probably because the conversion of molybdate to the intermediate phosphomolybdate needs to reach a critical value before reduction can take place as discussed previously [19]. It has been reported that bacterial molybdenum reduction is inhibited by phosphate at concentrations higher than 2.9 mM [32]. In general, concentrations higher than 5 mM are inhibitory to many Mo-reducing bacteria [**Table–3**]. Phosphomolybdate is rapidly oxidized at neutral pHs, and its stability requires acidic pH [63]. At concentrations of 20 mM and higher, phosphate maintains the environment at neutrality. This rapidly destabilizes phosphomolybdate. Phosphate itself can destabilize the phosphomolybdate complex as a study has demonstrated that an acidified phosphate solution destabilizes an ascorbate-reduced phosphomolybdate [64].

The concentrations of molybdate supporting optimal Mo-blue production in bacteria range between 5 and 80 mM [**Table**–3]. In contrast to cationic heavy metals, bacteria can tolerate and reduce high concentrations of anionic heavy metals. For instance, the most tolerant microorganism can tolerate and reduce arsenate at 30 mM in *Desulfomicrobium strain* Ben-RB [65], chromate at 30 mM in *Pseudomonas putida*[66], selenate at 20 mM in *Bacillus* sp. [67], and vanadate at 50 mM in *Pseudomonas isachenkovii*[68]. These bacteria can be employed to cleanup molybdenum-polluted areas with high concentrations of molybdenum. A number of areas are stated to be polluted with high concentrations of molybdenum. In Colorado, contaminated sites from a discontinued uranium mine show molybdenum concentration as much as 6,500 mg/Kg in soils and 900 mg/L in water [69]. For efficient

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reduction to take place, the phosphate concentrations should not exceed 20 mM. It is fortunate that most sites do not contain phosphate at concentrations exceeding this value [70].

Table: 3. Characterization of Mo-reducing bacteria isolated to date.

Bacteria	Optimal C source	Optimal Molybdate (mM)	Optimal Phosphate (mM)	Heavy metals inhibition	Author
Klebsiella oxytoca strain Aft-7	glucose	5-20	5-7.5	Cu^{2+}, Ag^{+}, Hg^{2+}	[23]
Bacillus pumilus strain Ibna	glucose	40	2.5-5	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}	[41]
Bacillus sp. strain A.rzi	glucose	50	4	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}, Co^{2+},Zn^{2+}	[42]
Serratia sp. strain Dr.Y8	sucrose	50	5	Cr, Cu, Ag, Hg	[43]
S. marcescens strain Dr.Y9	sucrose	20	5	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[44]
Serratia sp. strain Dr.Y5	glucose	30	5	n.a.	[45]
Pseudomonas sp.strainDRY2	glucose	15-20	5	Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[46]
Pseudomonas sp. strain DRY1	glucose	30-50	5	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}	[22]
Enterobacter sp. strain Dr.Y13	glucose	25-50	5	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[47]
Acinetobacter calcoaceticus strain Dr.Y12	glucose	20	5	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[48]
Serratia marcescens strain DRY6	sucrose	15-25	5	Cr^{6+}, Cu^{2+}, Hg^{2+}	[49]
Enterobacter cloacae strain 48	sucrose	20	2.9	Cr^{6+}, Cu^{2+}	[32]
Escherichia coli K12	glucose	80	5	Cr^{6+}	[31]
Klebsiella oxytoca strain hkeem	fructose	80	4.5	Cu^{2+}, Ag^{+}, Hg^{2+}	[50]

Two of the heavy metals tested that exhibit strong inhibitory response to molybdenum reduction, mercury and chromium, inhibit many of the Mo-reducing bacteria isolate to date [**Table**– 3]. Mercury is a strong inhibitor to bacterial chromate reduction from Cr^{6+} to Cr^{3+} in *Bacillus* sp. with the target site of inhibition is proposed as the sulfhydryl group [71]. Chromate inhibits the enzyme glucose oxidase [72] and nitrogen metabolism enzymes [73]. The addition of certain metal-sequestering or chelating substances such as calcium carbonate, manganese oxide, phosphate, and magnesium hydroxide to bioremediation sites may overcome the problem of mercury inhibition [74], and allowing molybdenum remediation to proceed. Another alternative to reduce the toxicity of mercury and copper is to immobilize the molybdenum-reducing bacterium in membrane or dialysis tubing [20].

The growth rate obtained indicates that growth on acrylamide was faster than either acetamide or propionamide, while the lag period observed also indicates that the bacteria could grow on acrylamide faster with a lower lag period than acetamide and propionamide. The presence of lag periods indicates that the bacterial cells spend energy to tolerate and activate metabolic pathways needed for amide assimilation. The ability of acrylamide to support Mo-reduction is novel. In the reduction of chromate, the xenobiotic phenol could be used as electron donor [75]. However, these two phenomena are very rare as most of the time simple carbohydrates such as lactate, sucrose or glucose are preferred donors [19]. The amides acrylamide, acetamide and propionamide are manufactured in the millions of tons annually. As the pollution of these amides is increasingly being reported, ways to remediate them are being sought. To date, several microorganisms have been isolated that can use these amides as carbon or nitrogen sources for growth. These microorganisms are potential bioremediation candidates [15,16,24,76–84]. Nonetheless, hardly any bacteria have been mentioned capable of degrading amide and detoxify heavy metals. Thus, the potential of this bacterium to accomplish the two functions shows that this bacterium can be beneficial as a bioremediation agent in contaminated sites co-contaminated with amides and heavy metals.

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CONCLUSION

A molybdenum-reducing bacterium showing the novel ability to use acrylamide as a source of electron donor for reduction is reported. In addition, the amides acrylamide, propionamide, and acetamide can be utilized as the growth of this bacterium. Characterization of molybdenum reduction including screening of potential xenobiotics acting as electron donor or carbon sources for growth was carried out utilizing resting cells in a microplate format allowing a potentially high throughput process. Glucose was the best electron donor for supporting reduction, while a critical phosphate concentration of 5.0 mM was optimal. Higher concentrations of phosphate were strongly inhibitory. The identity of the molybdenum blue produced indicated that it is a reduced phoshomolybdate based on scanning absorption spectrum. A modified Gompertz model was successfully used in modelling the growth of this bacterium on these amides. Nonetheless, hardly any bacteria have been mentioned capable of degrading amide and detoxify heavy metals. Thus, the potential of this bacterium to accomplish the two functions shows that this bacterium can be beneficial as a bioremediation agent in contaminated sites co-contaminated with amides and heavy metals.

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CONFLICT OF INTEREST

The author declares having no competing interests.

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FINANCIAL DISCLOSURE

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