HIBISCUS PUBLISHER

BIOREMEDIATION SCIENCE AND TECHNOLOGY RESEARCH

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

Isolation and Characterization of a Heavy Metal-reducing *Pseudomonas* sp. strain Dr.Y Kertih with the Ability to Assimilate Phenol and Diesel

Veeraswamy Kesavan[1], Abdulrasheed Mansur[1,2], Suhaili, Zarizal[3], Mohd Syahmi Ramzi Salihan[1], Rahman, M. F[1], and Shukor, M.Y.[1]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2]Department of Microbiology, Gombe State University, P.M.B 127, Gombe, Nigeria.
[3]Department Of Animal Science, Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Gong Badak, 21300 Kuala Terengganu, Terengganu, Malaysia.

*Corresponding author:
Mohd. Yunus Shukor
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400, Serdang,
Selangor, Malaysia.
Email: mohdyunus@upm.edu.my / yunus.upm@gmail.com

HISTORY

Received: 7th March 2018
Received in revised form: 15th April 2018
Accepted: 5th of June 2018

KEYWORDS

Pseudomonas sp. strain Dr.Y Kertih
molybdenum reduction
molybdenum blue
phenol
diesel

ABSTRACT

The indiscriminate released of heavy metals and xenobiotics into soils and aquatic bodies severely alter soil organisms and the ecosystem. The isolation of xenobiotics degrading microorganisms is cost-effective and naturally pleasant approach. Lately, the toxicological effect of molybdenum to the spermatogenesis of several organisms has been record. This present study is aimed at the isolation and characterization of a bacterium capable of converting molybdenum to the colloidal molybdenum blue. Bacteria characterization was performed in a microplate format using resting cells. Thus, the reduction process can be employed as a device for molybdenum bioremediation. The results of the study revealed an optimum reduction at pH between 6.0 and 6.3 and temperatures of between 25 and 40 °C. Similarly, it was also observed that a phosphate concentration not greater than 5.0 mM and a sodium molybdate concentration at 20 mM was required for reduction. Glucose was observed as the best carbon source to support reduction. Following the scanning of molybdenum blue, it revealed an absorption spectrum indicating the characteristics of molybdenum blue as a reduced phosphomolybdate. Molybdenum reduction is inhibited by heavy metals like silver, lead, arsenic and mercury. Furthermore, the ability of the bacterium (*Pseudomonas* sp. strain Dr.Y Kertih) to utilize several organic xenobiotics such as phenol, acrylamide, nicotinamide, acetamide, iodoacetamide, propionamide, acetamide, sodium dodecyl sulfate (SDS) and diesel as electron donor sources for aiding reduction or as carbon sources for growth was also examined. Finding showed that none was capable of aiding molybdenum reduction, however the bacterium was capable of growing on both diesel and phenol as carbon sources. GC analysis was used to confirmed diesel degradation.

INTRODUCTION

The continuous discharge of heavy metals, organic and inorganic pollutants or artificial chemicals (xenobiotics) such as phenol, acrylamide, nicotinamide, acetamide, iodoacetamide, propionamide, acetamide, pentachlorophenol, sodium dodecyl sulfate (SDS) into the environment are considered as the main global problem [1, 2, 3]. These chemicals are produced in millions of tonnes yearly with a momentous quantity found contaminating the environment.

Anthropogenic activities such as mining are the major cause of molybdenum contamination. The releases molybdenum into the red river by the Molycorp molybdenum mine in new Mexico causes the devastation of river ecosystem [4]. Also in western Liaoning, China, mining activities have resulted in contaminating the river with molybdenum far beyond the standards [5]. The

contamination of nearly 300 square kilometres of land by Alaverdi copper molybdenum miners in Armenia has been reported [6]. Similarly, occurrence of molybdenum levels ranging from 900 to 2,000 mg/l in both water bodies and soils in southern Colorado, have been recorded [7]. Molybdenum is not produce in huge quantity in Malaysia; it is form as a by-product of copper excavation in Ranau sabah. However, due to depletion of mineral the mining activity has long stopped functioning. Reports of environmental pollution due to damage of mine tailings waste-carrying pipes has been recorded [8]. In Batu hijau, Sumbawa, Indonesia, copper and gold mining activity from a copper-gold-molybdenum porphyry deposit is slowly polluting the surrounding coastal areas with heavy metals from mine tailings dumped into the sea [9].

Malaysia is known as one of the major producers of oil and gas globally. Conversely, the transportation of these hydrocarbons via sea or land results in polluting the straits of Malacca, which is considered as one of the busiest waterways worldwide. Nearly 44,900 tons of hydrocarbon crude oil polluted the straits of Malacca as a result of tanker collision [10]. In addition soil contamination with 15 tons of diesel was recorded in 2001 at Seremban due to overturned lorry tanker [11]. Phenol vapours are toxic to both mucous membranes, and the respiratory tract. Toxicity is predominantly based on the formation of phenoxyl radicals [12]. Malaysian department of environment (doe) categorized both phenolic and hydrocarbon compounds as one of the topmost listed wastes [13,14]. Studies showed that several groundwater, wells, and landfills in Malaysia contain hydrocarbon and phenolic constituents beyond the maximum acceptable range. As such various remediation approaches are being conducted locally to clean up these contaminants [15].

The bacterial reduction of molybdate anions into colloidal molybdenum blue [16] and insoluble molybdenum disulphide [17] have long been proposed as a method for bioremediation [18,19,20]. Several molybdenum reducing bacteria with enhanced tolerance to molybdenum have been isolated *Enterobacter* [19, 21], *Acinetobacter calcoaceticus* [22], *E. coli* k12 [23], and bacteria from the genera of *Serratia* [24, 25, 26, 27]. *Pseudomonas* [28, 29], *Klebsiella* [30,31,32] and *Bacillus* [33, 34]. Few bacteria capable of degrading molybdenum employ sodium dodecyl sulfate (SDS) as sole source of carbon for growth [31,32]. This thus exhibit the probability of the bacteria using xenobiotics as either electron donors for Mo- reduction or as sources of carbon for growth.

This work is therefore, aimed at proving the novel ability of a molybdenum-reducing bacterium to use diesel and phenol as carbon sources, even though these substrates did not support molybdenum reduction.

MATERIALS AND METHODS

Isolation of Mo-reducing bacterium

Contaminated soil samples were collected from hydrocarbon storage unit in Kertih, state of Terengganu, Malaysia, in January 2006. About 5 to 10 cm soil samples were collected from topsoil. A low phosphate molybdate media comprising 10 mM sodium molybdate and 5 mM phosphate was used as molybdenum-reducing media. The composition of the media is as follows; glucose anhydrous (Sigma-Aldrich, United Kingdom) (1%), yeast extract (Oxoid Ltd., Hants, United Kingdom) (0.5%), $Na_2MoO_4.2H_2O$ (Sigma-Aldrich, United Kingdom) (0.242 % or 10 mM), $MgSO_4.7H_2O$ (Sigma-Aldrich, United Kingdom) (0.05%), $(NH_4)_2.SO_4$ (Sigma-Aldrich, United Kingdom) (0.3%), NaCl (Merck, Poole, Dorset, United Kingdom) (0.5%), and Na_2HPO_4 (Merck, Poole, Dorset, United Kingdom) (0.071% or 5 mM) [32]. Soil bacterial suspension was prepared by adding 1.0 gram of soil to 10 ml of deionized water. This is follow by mixing the soil suspension thoroughly, then 0.1 ml of these suspension was spread onto an agar plate (1.5 % w/v) containing the above LPM medium, and adjusting pH to 6.5. Following an incubation for 48 hours at room temperature, several blue and white colonies were observed on the plate. Colony observed with the strongest blue intensity was restreaked onto LPM agar obtain pure culture. The molybdenum blue produced was characterized by centrifuging the liquid culture at 10,000 × g for about 10 minutes at room temperature to remove bacterial aggregates and then scanned at 400 to 900 to obtain the absorption spectrum (UV-Spectrophotometer, Shimadzu 1201).

16s rDNA gene sequencing

DNA was extracted from bacterial colony grown at 48-hour using alkaline lysis procedure. The extraction was performed following manufactures instruction. The extracted DNA was amplified using 16s rDNA reverse primer (5'-aaggaggtgatccagccgca-3') and forward primer (5'-agagtttgatcctggctcag-3') (First Base Sdn Bhd., Malaysia) [35]. The bacterial genomic PCR amplification was performed on a thermal cycler (Biometra, Gottingen, Germany). Using the following PCR reaction mixture DNA 50 µl, 200 µm of each deoxynucleotide triphosphate, 1 x reaction buffer, 0.5 pm of each primer and 2.5 u of taq DNA polymerase (Promega). Initiation of PCR was started with an initial denaturation for 3 minutes at 94 °C. This is followed by 25 cycles of 94 °C for 1 minute, 50 °C for 1 minute, 72 °C for 2 minutes, and a final extension lasting for 10 minutes at 72 °C. A cycle sequencing operation using the big dye terminator kit was used to sequence the PCR product (Perkin-Elmer applied Biosystems). The sequenced data was edited and recorded applying the Chromas software (version 1.45), and then blasted through the Genbank database (http://www.ncbi.nlm.nih.gov/blast/). The resultant 16s rDNA gene sequence was deposited in the Genbank database with the accession number **EF121820**.

Identification of Mo-reducing bacterium

The bacterium was identified based on biochemical and molecular methods. Biochemical identification was performed used the Biolog GN Gram-negative bacteria (GN microplate) Biolog, Hayward, CA, USA). While molecular analysis was performed using program Clustal_w by multiple aligning of the closely matched 16s rDNA gene sequences which are retrieved from Genbank [36], and the output format was PHYLIP. Before further processing gene sequence, proofreading and manual curation were carried out. Phylogenetic tree was constructed out using the Phylip suits program (J. Q. Felsenstein, http://evolution.genetics.washington.edu/phylip.html, PHYLIP, Department of Genetics, University of Washington, Seattle, WA., v. 3.573.)].

While the phylogenetic relationship was computed using the straightforward and computationally economic distance-based technique over the computationally demanding maximum parsimony and maximum likelihood techniques. Evolutionary distance matrices were computed in accordance with the Neighbour-joining/UPGMA method making use of the DNADIST algorithm, and also the output file of the distance matrix was evaluated using the jukes and cantor model of nucleotide substitution [38]. Inference of the phylogenetic tree applied the Neighbour-joining procedure of Saitou and Nei [39]. A 1000 (sampling with replacement) bootstraps repetition was performed using SEQBOOT program rather than the jackknife (sampling without replacement), and the method was used for

each algorithm. This procedure evaluates the confidence levels of the individual branches in the tree. A majority rule consensus trees (50%) were constructed in the form of Newick standard format from a series of trees for the topologies found using the CONSENSE Program. Finally, the tree was viewed using Treeview program [40].

Preparation of resting cells

Bacterium resting cells were set on the microtiter format as previously stated, though with minor modification of the LPM composition by increased in phosphate concentration to 100 mM. This is however the high phosphate Molybdate media or HPM [32, 41]. Growing in LPM triggered the formation of Mo-blue aggregates with bacterial cells, therefore increasing phosphate concentrations becomes necessary. This is to circumvent bacterial aggregations to molybdenum blue which may results into cellular harvesting difficulties. Bacterial cells were grown aerobically at room temperature at 48 hours of incubation with shaking at 120 rpm on an orbital shaker. Bacterial cells were then centrifuged at 10,000 × g for 10 minutes. Following rinsing the cellular pellets with deionized water twice, the pellets were then suspended in 20 ml of LPM media ignoring glucose.

While alternate set of cellular pellets were resuspended in 20 ml of LPM media containing glucose and neglecting molybdate. Then 180 µl of the bacterial cell suspension from the first set was pipetted into the wells of a sterile microplate. In order to initiate Mo-blue production, 20 µl sterile glucose from a 10% (w/v) stock solution was added to the cell suspension. The plates were then covered with a sterile sealing tape (Corning® Microplate), and incubated at room temperature. The production of Mo-blue was determined at 750 nm (Model No. 680 spectrophotometer, Biorad, Richmond, Ca). The effect of heavy metals on mo-blue production was studied using the atomic absorption spectrometry (AAS) calibration solutions (Merck Chemical Co., Germany).

The ability of phenol, acrylamide, nicotinamide, acetamide, iodoacetamide, Propionamide, Sodium dodecyl Sulfate (SDS) and diesel to support molybdenum reduction was verified at 500 mg/l, but in a volume of 50 µl [42], by omitting glucose in the experiment. Concurrently, the ability of the bacterium to grow on these xenobiotics separate from molybdenum-reduction was carried out using the second set of bacterial cellular suspension with a similar volume of xenobiotics utilized. Bacterial growth increase after 72 hours of incubation at room temperature was assessed at 600 nm.

GC analysis of diesel degradation

The ability of *pseudomonas* sp. Strain dr.y kertih to degrade diesel was performed using 100 ml high phosphate media augmented with 1,000 mg/l. Biodegradation of diesel by the bacterium *pseudomonas* sp. Strain Dr.Y Kertih was assessed using gas chromatographic- mass spectrometry analysis as previously described [43] with minor modifications. Briefly, the bacterial culture was aerobically incubated at room temperature with shaking at 120 rpm on an orbital shaker for ten days. Following incubation period, biodegradation of diesel was determined by extracting 5 ml culture media with two volumes of 20 ml of n-hexane (solvent) with the aid of separating funnel to exclude cellular materials.

The filtrates were then moved into a tarred vial, also n-hexane was further added to adjust the volume of each extract to 100 ml. The vials were preserved at 4 °C pending gas chromatographic analysis. To monitor loss in abiotic substrate, corresponding inoculated control was also incubated concurrently with the inoculated media. The effect of diesel biodegradation by assessed by GC-MS (Thermo GC- trace ultra ver: 5.0, Thermo MS DSQ ii), that was furnished with a DB 35-MS capillary standard non-polar column (30 m × 0.25 mm × 0.25 µm). 1 microliter of the organic stage was evaluated by GC-MC. The gas chromatograph prepared with a split–split less injector (split ratios of 50:1) was used for the GC-MS analysis. The oven temperature was originally at 40°C before preset to 270°C at 8°C/min rate for 5 min. Both injector temperature, transfer line and ionization source were kept at 250°C. The electron control ionization was regulated at 70 ev, with helium as carrier gas at average linear velocity of 1.0 ml/min.

RESULTS

Identification of Mo-reducing bacterial isolate

Mo-reducing bacterial isolates was enumerated for its ability to produce Mo-blue via checking production at 865 nm. The isolate was used for further studies. Bacterium identification was performed by comparing both molecular identification method using 16srrna gene and results of cultural morphology and that of confirmatory biochemical tests (Biolog GN). Analysis based on sequence analysis of the 16s rRNA gene showed a low bootstrap (29.4%) on aligning strain Kertih to the clade harboring several *Pseudomonas* species such as *Pseudomonas* sp. and *Pseudomonas stutzerii.* This indicates that the isolates Kertih cannot be aligned with any particular *pseudomonas* species (**fig. 1**). However, analysis by biolog GN provide the neighboring identification to the *Pseudomonas* genus with a low possibility. Thus, the bacterium is tentatively identified as *Pseudomonas* sp. Strain Dr.Y Kkertih. The bacterium demonstrated optimum pH for reduction of between 6.0 and 6.25, and an optimum temperature ranging from 25 °C to 34°C (data not shown).

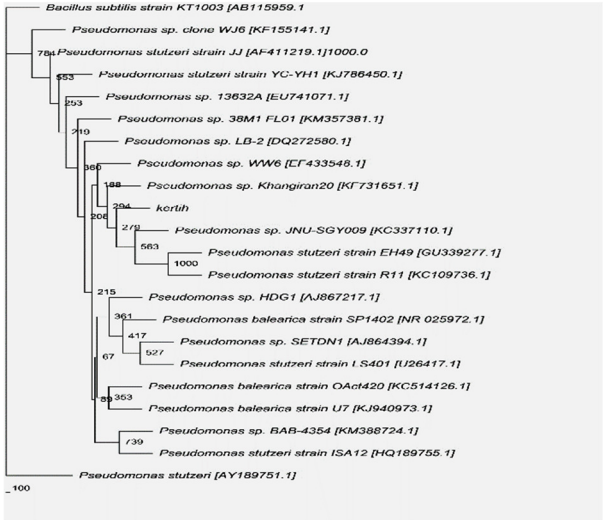

Fig. 1. A neighbour-joining method phylogram indicating the genetic relationship between strain Kertih and other microorganisms based on sequence analysis of the 16S rRNA gene. Bootstrap values are indicated at the branching points or nodes. The values are based on 1000 samplings with replacement. The accession numbers of their 16s rRNA sequences follows species names. The scale bar represented 100 nucleotide substitutions. The outgroup is *B. subtilis*.

Molybdenum absorbance spectrum

Over the complete progression of the Molybdate reduction to Mo-blue, the result of the scanned culture media supernatants from 400 to 1000 nm revealed that the bacterium exhibited a remarkable Mo-blue spectrum having an optimum peak at 865

nm and a shoulder at 710 nm. This exceptional profile was observed to be preserved through the whole cultivation period (**Fig. 2**).

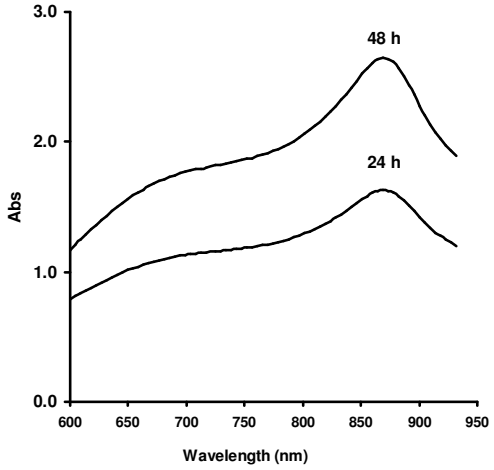

Fig. 2. Molybdenum blue scanning absorption spectrum for *pseudomonas* sp. Strain Dr.Y_Kertih at different time intervals.

Effect of electron donor on molybdate reduction

Glucose with an optimal concentration at 1% (w/v) was observed as the utmost electron donor supporting molybdate reduction (data not shown). This is followed by fructose, galactose, citrate and lactose in descending order **(Fig. 3)**.

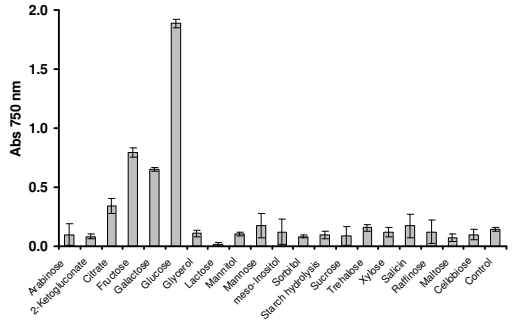

Fig. 3. The effect of various carbon sources on bacterial reduction of molybdenum. Error bars represent mean ± standard deviation (n = 3).

Molybdate reduction under various concentrations of Phosphate and Sodium molybdate

Both phosphate and molybdate anions affect Mo-blue production. Yet, phosphate strongly affect reduction than molybdate. At 5.0 mM concentration phosphate best supports molybdenum reduction, however at higher concentrations molybdenum reduction is strongly inhibited **(Fig. 4)**. Similarly, at 15 mM sodium molybdate concentrations maximum amount of Mo-blue produced was observed, inhibition of reduction occurs at concentrations higher than 20 mM **(Fig. 5)**

Effect of heavy metals

At 2 ppm, 91.9, 82.7 and 17.4 %, molybdenum reduction was inhibited by silver, lead, arsenic and mercury respectively. The heavy metals arsenic, cadmium, copper and lead did not exhibit inhibition to molybdenum reduction **(Fig. 6)**.

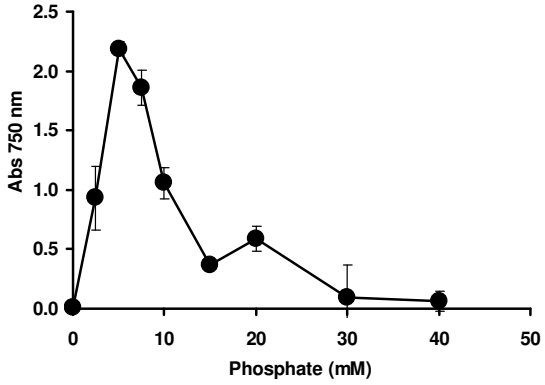

Fig. 4. The effect of phosphate (Na_2HPO_4) concentrations on bacterial reduction of molybdenum. Error bars represent mean ± standard deviation (n = 3).

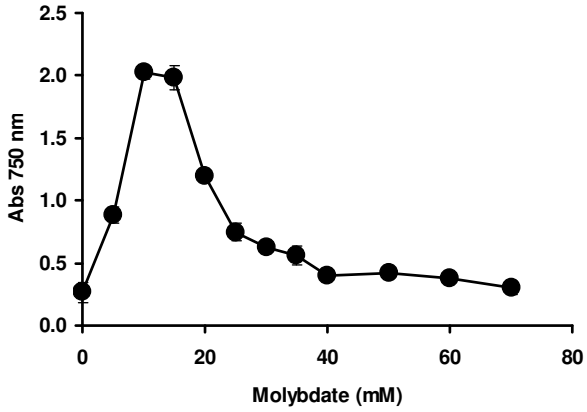

Fig. 5. The effect of sodium molybdate concentrations on bacterial reduction of Molybdenum. Error bars represent mean ± standard deviation (n = 3).

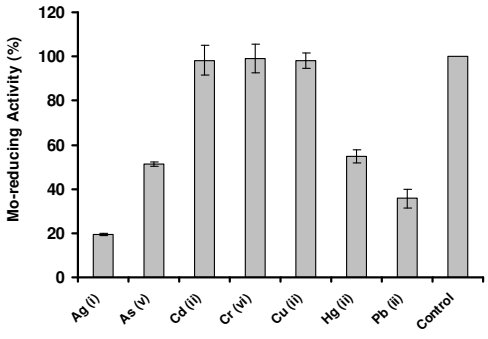

Fig. 6. The effect of selected heavy metals on bacterial reduction of molybdenum. Error bars represent mean ± standard deviation (n = 3).

Effect of various organic compounds on molybdenum reduction and bacterial growth

The capability of these organic compounds to function as an electron donor for molybdenum reduction was examined. Results revealed that no organic compound have the ability to support molybdenum reduction. However, it was observed the bacterium have the ability to utilize only diesel and phenol were utilized as its sole source of carbon for growth (**Fig. 7**). GC analysis was used to ascertain diesel biodegradation, thus a reduction in aliphatic carbon peaks specifically at holding time of 14 minutes and above was observed (**Fig. 8**).

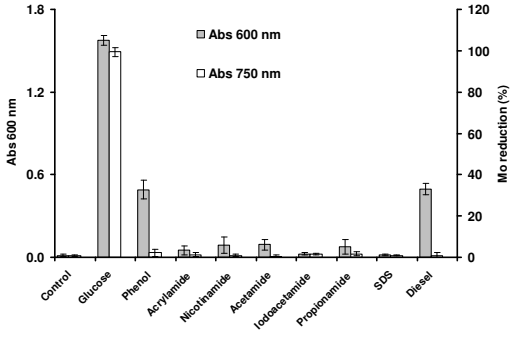

Fig. 7. Mo-blue production (abs 750 nm) and growth (Abs 600 nm) of *Pseudomonas* sp. Strain Dr.Y_Kertih on xenobiotics independent of molybdenum reduction. Error bars represent mean ± standard deviation (n = 3).

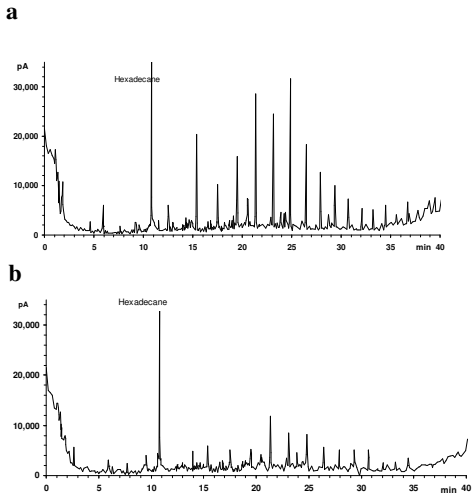

Fig. 8. Diesel biodegradation by *Pseudomonas* sp. strain Dr.Y_Kertih at the start of incubation (a), and after 10 days of incubation at room temperature (b), monitored through GC-FID (flame ionization detector). Hexadecane is the internal standard.

DISCUSSIONS

To date various bacteria capable of degrading Molybdenum have been isolated and characterize (**Table 1**). Previously, two Molybdenum degrading bacteria from the genus; *Pseudomona*s sp. strain DRY2 [28] and psychrophilic Antarctic bacterium *Pseudomon*as sp. strain DRY1 [29] have been documented. Interestingly, the optimum ranges stated in this study are comparable to the optimal conditions recorded for other Mo-reducing bacteria (**Table 1**). The microtiter plate-based resting cells has been fully utilized in the characterization of Mo-reducing bacterium *Enterobacter cloacae* strain 48 [19]. This method permits a high throughput characterization format [44, 41]. In addition resting cells methods are employed in several reduction studies such as chromate, vanadate [45], and Selenate [46]. Likewise, xenobiotics degradation for instance SDS and diesel too take advantage of resting cells. The usage of resting cells circumvents the early phase of the development process that is typically affected by lethal xenobiotics.

Table 1. Characteristics of various Mo-reducing bacteria isolated to date.

Bacteria	Optimal Tempe-rature ºC	Optimal pH	Optimal C source	Optimal MoO_4 (mM)	Optimal PO_4 (mM)	Heavy metals inhibit	Ref.
Klebsiella oxytoca strain aft-7	25 and 34	5.8-6.3	glucose	5-20	5-7.5	Cu^{2+}, Ag^{+}, Hg^{2+}	[32]
Bacillus pumilus strain lbna	37	7.0-8.0	glucose	40	2.5-5	As^{3+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}	[33]
Bacillus sp. Strain a.rzi	28-30	7.3	glucose	50	4	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}, Co^{2+},Zn^{2+}	[34]
Serratia sp. Strain dr.y8	37	6.0	sucrose	50	5	Cr, Cu, Ag, Hg	[26]
S. Marcescens strain dr.y9	37	7.0	sucrose	20	5	Cr^{6+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[27]
Serratia sp. Strain dr.y5	37	7.0	glucose	30	5	N.A.	[25]
Pseudomonas sp. Strain dry2	40	6.0	glucose	15-20	5	Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[28]
Pseudomonas sp. Strain dry1	15-20	6.5-7.5	glucose	30-50	5	Cd^{2+}, Cr^{6+}, Cu^{2+},Ag^{+}, Pb^{2+}, Hg^{2+}	[29]
Enterobacter sp. Strain dr.y13	37	6.5	glucose	25-50	5	Cr^{6+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}	[26]
Acinetobacter calcoaceticus strain dr.y12	37	6.5	glucose	20	5	Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, Hg^{2+}	[28]
Serratia marcescens strain dry6	35	7.0	sucrose	15-25	5	Cr^{6+}, Cu^{2+}, Hg^{2+*}	[24]
Enterobacter cloacae strain 48	30	7.0	sucrose	20	2.9	Cr^{6+}, Cu^{2+}	[19]
Escherichia coli k12	30-36	7.0	glucose	80	5	Cr^{6+}	[23]
Klebsiella oxytoca strain hkeem	30	7.3	fructose	80	4.5	Cu^{2+}, ag^{+}, hg^{2+}	(30]

Furthermore, the bacterium spectrum observed in this study was almost equal to that of other Mo-reducing bacteria isolated [30, 32, 34, 59]. Virtually all Mo-blue producing bacterial display spectra with closeness to the phosphate determination method. The phosphomolybdate in the latter is changed to Mo-blue using ascorbate as the reducing agent. This reduced phosphomolybdate exhibit a characterized shoulder of from 700 to 720 nm, plus a maximum peak of about 890 nm [48,49,50]. This findings in in agreement to that of [23], who also recorded similar result. Based on molybdenum chemistry and Mo-blue spectral analysis, a new postulate was proposed that during reduction of sodium molybdate to Mo-blue by Mo-blue production bacteria a phoshomolybdate transition is formed [51]. The formation of a transitional species during heavy metal reduction such as chromate reduction (6+ to 3+) has been slightest shown in the bacteria *Pseudomonas ambigua* and *Shewanella putrefaciens* (now known as *S. oneidensis*), existence of the intermediate species Cr5+ were ascertain via spectroscopic and paramagnetic resonance works.

Spectroscopic analysis used in this study is considered as the easiest technique for differentiating among the existing heteropolymolybdates, which include silicomolybdate, phosphomolybdate, and sulfomolybdate [52,53]. The actual electron acceptor substrate for molybdenum reduction uses sodium molybdate. We suggest phoshomolybdate as an alternative of molybdate as the Mo-reducing enzyme substrate. The reformed in substrate allows enzyme purification for the first time [24,55]. The reduced phosphomolybdate or Mo-blue consist of a complex structure, with a fractional oxidation state between 6+ and 5+. It structure is thus Keggin in nature with a general

molecular formula of $[XM_{12}O_{40}]^{n-}$, the X are heteroatoms such as Si^{4+}, P^{5+}, or B^{3+} and M as the addenda atom for examples molybdenum and tungsten [57,56,48]. While Mo-blue displayed a maximum wavelength at 865 nm. In this study Mo-blue was monitored at 750 nm, monitoring at such wavelength was aimed at facilitating microplate method. However, the Monitored wavelength is relatively below 865 nm with nearly 30%. And yet 750 nm wavelength is greater than bacterial cellular absorption, which is normally ranged amid 600 and 620 nm. Thus, monitoring at 600 and 620 nm wavelength is suitable for quantifying Mo-blue production [41]. In addition, the wavelengths such as 820 nm [23] and 710 nm [19] have been employed to monitor Mo-blue production.

Earlier studies revealed that most of the molybdenum-reducing bacteria prefer either glucose or sucrose as the utmost electron donor (Table 1). This choice may be due to the simply assimilable characteristics of these carbohydrates. Using either glucose or sucrose as source of carbon NADH and NADPH reduced equivalent can be easily produce. These compounds functions as electron donor substrate for Mo-reducing enzymes [55]. The reducing equivalents are formed through broad metabolic pathways such as glycolysis, Kreb's cycle. Regardless of sucrose and glucose being tremendous electron donor, an inexpensive carbon source like molasses can be employed particularly in real bioremediation, as molasses can be acquired economically and in huge amount from the sugar cane industry in Malaysia. Also it has been utilized by the bacterium *Flexivirga alba* as electron donor in the reduction of hexavalent chromate [49] The promising utilization of molasses as a carbon source is presently being assessed. It has been reported that phosphate at concentrations higher than 2.9 mM inhibit bacterial molybdenum reduction [19].

Generally, several Mo-reducing bacteria are inhibited at concentrations higher than 5 mM (**Table 1**). At a neutral pH Phosphomolybdate is speedily oxidized, this is because acidic pH is required for its stability [58, 59]. Phosphate retains neutral environment at concentrations of 20 mM and higher, this rapidly destabilizes phosphomolybdate. Also phosphate itself can disrupt phosphomolybdate complex as a study has revealed that an acidified phosphate solution disrupts an ascorbate-reduced phosphomolybdate [59]. An optimum Mo-blue production is observed in bacteria at molybdate concentration between 5 and 80 mM (**Table** 1). In divergence to cationic heavy metals, bacteria can tolerate and decrease high concentrations of anionic heavy metals. For example, the most tolerant bacteria can tolerate and reduce arsenate at 30 mM as observed in *Desulfomicrobium strain* Ben-RB [60] chromate at 30 mM in *Pseudomonas putida* [61], selenate at 20 mM in *Bacillus* sp. [62], and vanadate at 50 mM in *Pseudomonas isachenkovii* [63]. These bacteria can be used for bio-remediating molybdenum-contaminated areas.

Mercury and Copper were observed to display resilient inhibitory response to molybdenum reduction among the heavy metals tested and are to date inhibit several of the Mo-reducing bacteria isolate (**Table 1**). These two-heavy metal (mercury and copper) are also known to demonstrate a strong inhibitor in *Bacillus* sp. to chromate reduction from Cr^{6+} to Cr^{3+}. through the sulfhydryl group as the targeted inhibition site [64], and *Enterobacter cloacae* strain H01 [52]. Enzyme glucose oxidase [65] and nitrogen metabolism enzymes are often inhibits by chromate [66]. The threat of mercury inhibition can be thus prevented through use of certain metal-sequestering or chelating supplements such as calcium carbonate, manganese oxide, phosphate, and magnesium hydroxide at the sites of bioremediation [68,67], and allowing remediation of molybdenum to progress. In addition, the toxicity of mercury and copper can also be reduced alternatively by use of immobilized molybdenum-reducing bacterium in dialysis tubing or suitable membrane [69]. The growth rate observed shows that growth on diesel and phenol was rapidly than either acrylamide, acetamide or propionamide, whereas the lag phase also revealed that the bacteria could grow on both diesel and phenol faster with a lower lag phase than acetamide and propionamide. The occurrence of lag phase shows that for bacterial cells to tolerate and start metabolic pathways required for assimilation extra energy is required. The xenobiotic phenol could be serve as electron donor in the reduction of chromate, [70]. Nonetheless, mostly the use of simple carbohydrates such as lactate, sucrose or glucose as donor are more preferred, as such it is a very rare phenomena for these two.

The ability of carbon sources other than carbohydrates to support Mo-reduction in bacterium is new. Diesel is lethal to microorganisms at a high concentration, owing to the solvent effect that injure bacterial cell membrane [26]. Several bacteria genus have been described to utilizes hydrocarbon; among are *Pseudomonas, Bacillus, Proteus, Aeruginosa, Klebsiella, Aeromonas, Micrococcus, Serratia, Acinetobacter, Staphylococcus,* and *Flavobacterium* [86, 71, 11, 72, 73, 74, 75, 76,77,79,78]. Phenol-degrading bacteria are suitable for remediation of phenol contamination due to cost-effective. Phenol degradation by microbes has been a persistence study worldwide. Thus phenol degrading bacteria include *Pseudomonas* species [80,81,82], *Bacillus brevis* [83], *Alcaligenes* sp. [84], *Ochrobactrum* sp. [85], *Acinetobacter* sp. [86] and *Rhodococcus* species [42]. Conversely, barely any bacteria capable of degrading xenobiotic and detoxify heavy metals have been documented. Presently, study are ongoing to purify the molybdenum-reducing enzyme from this bacterium and to wholly typify the xenobiotic-degrading property. Thus, the prospective of this bacterium to achieve the two functions demonstrate that this bacterium can be advantageous as a bioremediation agent in polluted sites co-polluted with xenobiotic and heavy metals.

Conclusively, the characterization of molybdenum reduction couple with screening of potential xenobiotics acting as electron donor or carbon sources for growth was performed utilizing resting cells in a microplate format allowing a theoretically high throughput process. Glucose was observed to be the best electron donor for supporting reduction, whereas a serious phosphate concentration of 5.0 mM was optimal. Higher concentrations of phosphate were strongly inhibitory. The identity of the molybdenum blue produced showed that it is a reduced phoshomolybdate based on scanning absorption spectrum

ACKNOWLEDGMENT

This project was supported by the Research University fund from Universiti Putra Malaysia (05-01-09-0750RU).

REFERENCES

1. Al-baldawi IAW, Sheikh Abdullah SR, Abu Hasan H, Suja F, Anuar N, Mushrifah i. Optimized conditions for phytoremediation of diesel by *scirpus grossus* in horizontal subsurface flow constructed wetlands (hsfcws) using response surface methodology. *J Environ Manage*. 2014;140:152–9.
2. Daneshfozoun S, Abdullah B, Abdullah MA. Heavy metal removal by oil palm empty fruit bunches (OPEFB) biosorbent. Appl Mech Mater. 2014;625:889–92.

3. Khudhair Ab, Hadibarata T, Yusoff ARM, Teh ZC, Adnan LA, Kamyab H. Pyrene metabolism by new species isolated from soil *Rhizoctonia zeae* SOL3. *Water Air Soil Pollut*. 2015;226(6).
4. Jacobs JA, Lehr JH, Testa SM. Acid mine drainage, rock drainage, and acid sulfate soils: causes, assessment, prediction, prevention, and remediation. Hoboken, New Jersey: John Wiley & sons, inc.; 2014.
5. Yu C, Xu S, Gang M, Chen G, Zhou l. Molybdenum pollution and speciation in nver river sediments impacted with mo mining activities in western Liaoning, Northeast China. *Int J Environ Res*. 2011;5(1):205–12.
6. Simeonov LI, Kochubovski MV., Simeonova BG. Environmental heavy metal pollution and effects on child mental development. Dordrecht: Springer Netherlands; 2011. (Nato Science for Peace and Security Series C: Environmental Security; vol. 1).
7. Legendre GR, Runnells DD. Removal of dissolved molybdenum from wastewaters by precipitates of ferric iron. *Environ Sci Technol*. 1975;9(8):744–9.
8. Yong FS. MamutCcopper Mine - the untold story. The national seminar on the malaysian minerals industry. Miner underpinning yesterday's needs, today's dev tomorrow's growth. 2000;
9. Angel BM, Simpson SL, Jarolimek CV, Jung R, Waworuntu J, Batterham G. Trace metals associated with deep-sea tailings placement at the Batu Hijau Copper-gold mine, Sumbawa, Indonesia. *Mar pollut bull*. 2013;73(1):306–13.
10. Etkin DS. Historical review of oil spills from all sources. 1999 int oil spill conf. 1999;1097–102.
11. Dahalan FA, Yunus I, Johari WLW, Shukor MY, Halmi MIE, Shamaan NA. Growth kinetics of a diesel-degrading bacterial strain from petroleum-contaminated soil. *J Environ Biol*. 2014;35(2):399–406.
12. 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.
13. Lazim ZM, Hadibarata T, Puteh MH, Yusop Z, Wirasnita R, Mohd Nor N. Utilization of durian peel as potential adsorbent for bisphenol a removal in aquoeus solution. *J Teknol*. 2015;74(11):109–15.
14. Yadzir ZHM, Shukor MY, Ahmad A, Nazir MS, Shah SMU, Abdullah MA. Phenol removal by newly isolated *Acinetobacter baumannii* strain serdang 1 in a packed-bed column reactor. *Desalin Wat Treat*. 2015;
15. Sing NN, Zulkharnain A, Roslan HA, Assim Z, Husaini A. Bioremediation of PCP by *Trichoderma* and *Cunninghamella* strains isolated from sawdust. *Brazilian Arch Biol Technol.* 2014;57(6):811–20.
16. Capaldi A, Proskauer B. Beiträge ZUR kenntniss DER säurebildung BEI *typhus-bacillen* und *bacterium coli* - eine differential-diagnostische studie. *Zeitschrift Für Hyg und Infect.* 1896;23(3):452–74.
17. Tucker MD., Barton LL, Thomson BM. Reduction and immobilization of molybdenum by *desulfovibrio Desulfuricans*. *J Environ Qual*. 1997;26(4):1146–52.
18. Bautista EM, Alexander M. Reduction of inorganic compounds by soil microorganisms. *Soil Sci Soc Am Proc.* 1972;36:918–20.
19. Ghani B, Takai M, Hisham NZ, Kishimoto N, Ismail AKM, Tano T. Isolation and characterization of a mo6+-reducing bacterium. *Appl Environ Microbiol.* 1993;59(4):1176–80.
20. Wan Ahmad WH, Chyan JB, Zakaria ZA, Ahmad WA. Sugarcane bagasse as nutrient and support material for cr(vi)-reducing biofilm. *Int Biodeterior Biodegrad.* 2015;102:3–10.
21. 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.
22. Shukor MY, Rahman MF, Suhaili Z, Shamaan NA, Syed MA. Hexavalent molybdenum reduction to mo-blue by A*cinetobacter calcoaceticus*. Folia Microbiol (praha). 2010;55(2):137–43.
23. Campbell AM, Del Campillo-Campbell A, Villaret DB. Molybdate reduction by *escherichia coli* k-12 and its chl mutants. Proc natl acad sci u s a. 1985;82(1):227–31.
24. Shukor MY, Habib SHM, Rahman MFA, Jirangon H, Abdullah MPA, Shamaan NA, Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain DRY6. *Appl Biochem Biotechnol*. 2008;149(1):33–43.
25. 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.
26. 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.
27. Yunus SM, Hamim HM, Anas OM, Aripin SN, Arif SM. Mo (vi) reduction to molybdenum blue by *serratia marcescens* strain DRY9. Polish *J Microbiol.* 2009;58(2):141–7.
28. 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.
29. Ahmad SA, Shukor MY, Shamaan NA, Mac Cormack WP, Syed MA. Molybdate reduction to molybdenum blue by an antarctic bacterium. *Biomed Res Int*. 2013;2013.
30. 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.
31. Halmi MIE, Zuhainis SW, Yusof MT, Shaharuddin NA, Helmi W, Shukor Y. Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate- degrading *Klebsiella oxytoca* strain dry14. *Biomed Res Int*. 2013;2013.
32. Masdor N, Abd Shukor MS, Khan A, Bin Halmi MIE, Abdullah SRS, Shamaan NA. Isolation and characterization of a molybdenum-reducing and SDS-degrading *Klebsiella oxytoca* strain aft-7 and its bioremediation application in the environment. *Biodiversitas*. 2015;16(2):238–46.
33. 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.
34. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. Strain a.rzi. *Biomed Res Int.* 2013;2013.
35. Devereux R, Willis SG. Amplification of ribosomal RNA sequences. In: Akkermans ADL, Elsas jd v., Bruijn FJD, Editors. Springer Netherlands; 1995. P. 277–87. (Molecular Microbial Ecology Manual).

36. Thompson JD, Higgins DG, Gibson TJ. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. 1994;22(22):4673–80.
37. Felsenstein J. Estimating effective population size from samples of sequences: a bootstrap monte carlo integration method. 1992;60(3):209–20.
38. Jukes TH, Cantor CR. Evolution of protein molecules. Mamm protein metab. 1969;21–132.
39. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol.* 1987;4(4):406–25.
40. D.M.page r. Treeview: an application to display phylogenetic trees on personal computers. 1996;12(4):357–8.
41. Shukor MS, Shukor MY. A microplate format for characterizing the growth of molybdenum-reducing bacteria. *J Environ Microbiol Toxicol.* 2014;2(2):42–4.
42. 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.
43. Ahmad SA, Ahamad Knek, Johari WLW, Halmi MIE, Shukor MY, Yusof MT. Kinetics of diesel degradation by an acrylamide-degrading bacterium. Rend Lincei. 2014;25(4):505–12.
44. 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.
45. Chaturvedi V, Kumar A. Diversity of culturable sodium dodecyl sulfate (SDS) degrading bacteria isolated from detergent contaminated ponds situated in Varanasi City, India. *Int Biodeterior Biodegrad*. 2011;65(7):961–71.
46. 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.
47. Shukor MY, Syed, M.A. Microbiological reduction of hexavalent molybdenum to molybdenum blue. In: vilas am, editor. Microbiology book series number 2: “current research, technology and education topics in Applied Microbiology and Microbial Biotechnology. 2010.
48. Sims RPA. Formation of heteropoly blue by some reduction procedures used in the micro-determination of phosphorus. 86(1026):584–90.
49. Hori T, Sugiyama M, Himeno S. Direct spectrophotometric determination of sulphate ion based on the formation of a blue molybdosulphate complex. Analyst. 1988;113(11):1639–42.
50. Clesceri LS, Greenberg AE, Trussell RR. Standard methods for the examination of water and wastewater. Port City Press, Baltimore; 1989.
51. Shukor Y, Adam H, Ithnin K, Yunus I, Shamaan NA, Syed A. Molybdate reduction to molybdenum blue in microbe proceeds via a phosphomolybdate intermediate. *J Biol Sci*. 2007;7(8):1448–52.
52. Rege MA, Petersen JN, Johnstone DI, Turick CE, Yonge DR, Apel WA. Bacterial reduction of hexavalent chromium by *Enterobacter cloacae* strain H01 grown on sucrose. *Biotechnol lett*. 1997;19(7):691–4.
53. Elangovan R, Philip I, Chandraraj K. Hexavalent chromium reduction by free and immobilized cell-free extract of *Arthrobacter rhombi*-re. *Appl Biochem Biotechnol.* 2010;160(1):81–97.
54. Shukor MY, Rahman MFA, Shamaan NA, Lee CH, Karim MIA, Syed MA. An improved enzyme assay for molybdenum-reducing activity in bacteria. *Appl Biochem Biotechnol*. 2008;144(3):293–300.
55. 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.
56. Sidgwick NV. The chemical elements and their compounds. Oxford, Clarendon Press; 1951.
57. Kazansky LP, Fedotov MA. Phosphorus-31 and oxygen-17 n.m.r. Evidence of trapped electrons in reduced 18-molybdodiphosphate(v), p2mo18o62 8-. *J Chem Soc Chem Commun.* 1980;(14):644–6.
58. Glenn J, Crane F. Studies on metalloflavoproteins. V. The action of silicomolybdate in the reduction of cytochrome c by aldehyde oxidase. *Biochim Biophys Acta*. 1956;22:111–5.
59. Shukor, MA, syed MA, Lee M., Karim MI, Shamaan N. A method to distinguish between chemical and enzymatic reduction of molybdenum in enterobacter cloacae strain 48, *Malaysia. J Biochem.* 2002;7:71–2.
60. Macy JM, ClennMJj, Steve, JM, Peter, BV, O'Neill, AJ, Sly L. Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. *Remediat contam*. 2000;173:49–57.
61. Keyhan M, Ackerley dD, Matin A. Targets of improvement in bacterial chromate bioremediation. *Remediat contam.* 2003;
62. Fujita M, Ike M, Nishimoto S, Takahash IK, Kashiwa M. Isolation and characterization of a novel selenate-reducing bacterium, bacillus sp. SF-1. *J Ferment Bioeng*. 1997;83:517–22.
63. Antipov A, lyalikova N, khijniak T, l’vov N. Vanadate reduction by molybdenum-free dissimilatory nitrate reductases from vanadate-reducing bacteria. IUBMB life. 2004;84:761–74.
64. Elangovan R, Abhipsa S, Rohit B, Ligy P, Chandraraj K. Reduction of cr(vi) by a *bacillus* sp. *Biotechnol Lett*. 2006;28(4):247–52.
65. Zeng G-M, Tang L, Shen G-L, huang G-H, Niu G-C. Determination of trace chromium (VI) by an inhibition-based enzyme biosensor incorporating an electropolymerized aniline membrane and ferrocene as electron transfer mediator. *Int J Env Analchem*. 2004;84:761–74.
66. Sangwan P, Kumar V JU. Effect of chromium (vi) toxicity on enzymes of nitrogen metabolism in clusterbean (cyamopsis tetragonoloba l.). *Enzym Res*,. 2014;7(8):36–40.
67. Hettiarachchi GM, Pierzynski GM, Ransom MD. In situ stabilization of soil lead using phosphorus and manganese oxide. *Environ Sci Technol.* 2000;34(21):4614–9.
68. Deeb B El, Altalhi AD. Degradative plasmid and heavy metal resistance plasmid naturally coexist in phenol and cyanide assimilating bacteria. *Am J Biochem Biotechnol*. 2009;5(2):84–93.
69. Halmi MIE, Wasoh H, Sukor S, Ahmad SA, Yusof MT, Shukor MY. Bioremoval of molybdenum from aqueous solution. *Int J Agric Biol*. 2014;16(4):848–50.
70. Anu M, Salom Gnana TV, Reshma JK. Simultaneous Phenol degradation and Chromium (vi) reduction by bacterial isolates. *Res J Biotechnol*. 2010;5(1):46–9.

71. Bicca FC, Fleck LC, Ayub MAZ. Production of biosurfactant by hydrocarbon degrading *Rhodococcus ruber* and *Rhodococcus erythropolis*. *Rev Microbiol.* 1999;30(3):231–6.
72. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Modeling the growth curves of *Acinetobacter* sp. Strain Dr.Y12 grown on diesel. *J Environ Bioremediation Toxicol*. 2014;2(1):33–7.
73. Hong JH, Kim J, Choi OK, Cho K-S, Ryu HW. Characterization of a diesel-degrading bacterium, *Pseudomonas aeruginosa* IU5, isolated from oil-contaminated soil in Korea. *World J Microbiol Biotechnol*. 2005;21(3):381–4.
74. Ku Ahamad KE, Halmi MIE, Shukor MY, Wasoh H, Amir S, Abdul Rachman AR, et al. Isolation and characterization of a heavy metal-tolerant diesel oil - degrading strain. *Bull Environ Sci Manag*. 2013;1(1):14–9.
75. Lee M, Kim MK, Kwon M-J, Park BD, Kim MH, Goodfellow M. Effect of the synthesized mycolic acid on the biodegradation of diesel oil by *Gordonia nitida* strain LE31. *J Biosci Bioeng*. 2005;100(4):429–36.
76. Lee M, Woo S-G, Ten LN. Characterization of novel diesel-degrading strains *Acinetobacter haemolyticus* MJ01 and *Acinetobacter johnsonii* MJ4 isolated from oil-contaminated soil. *World J Microbiol Biotechnol*. 2012;28(5):2057–67.
77. Mukherji S, Jagadevan S, Mohapatra G, Vijay A. Biodegradation of diesel oil by an arabian sea sediment culture isolated from the vicinity of an oil field. *Bioresour Technol.* 2004;95(3):281–6.
78. Rajasekar A, Babu TG, Pandian STK, Maruthamuthu S, Palaniswamy N, Rajendran A. Role of *Serratia marcescens* ACE2 on diesel degradation and its influence on corrosion. *J Ind Microbiol Biotechnol*. 2007;34(9):589–98.
79. Rajasekar A, Balasubramanian R, Kuma JV. Role of hydrocarbon degrading bacteria *Serratia marcescens* ACE2 and *Bacillus cereus* ACE4 on corrosion of carbon steel API 5LX. *Ind Eng Chem Res*. 2011;50(17):10041–6.
80. Aravindhan R, Sreeram KJ, Rao JR, Nair BU. Biological removal of carcinogenic chromium (vi) using mixed *Pseudomonas* strains. *J Gen Appl Microbiol*. 2007;53(2):71–9.
81. Bakhshi Z, Najafpour G, Kariminezhad E, Pishgar R, Mousavi N, Taghizade T. Growth kinetic models for phenol biodegradation in a batch culture of *Pseudomonas putida*. *Environ Technol*. 2011;32(16):1835–41.
82. Hasan SA, Jabeen S. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. *Biotechnol Biotechnol Equip*. 2015;29(1):45–53.
83. Arutchelvan V, Kanakasabai V, Elangovan R, Nagarajan S, Muralikrishnan V. Kinetics of high strength phenol degradation using *Bacillus brevis*. *J Hazard Mater.* 2006;129(1–3):216–22.
84. Bai J, Wen JP, Li HM, Jiang Y. Kinetic modeling of growth and biodegradation of phenol and m-cresol using *Alcaligenes faecalis*. *Process Biochem*. 2007;42(4):510–7.
85. Kiliç NK. Enhancement of phenol biodegradation by *Ochrobactrum* sp. Isolated from industrial wastewaters. *Int Biodeterior Biodegrad.* 2009;63(6):778–81.
86. Ahmad SA, Shamaan NA, Arif NM, Koon GB, Shukor MYA, syed MA. Enhanced phenol degradation by immobilized *acinetobacter* sp. Strain AQ5NOL 1. *World* J *Microbiol Biotechnol*. 2012;28(1):347–52.