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Response surface-based optimization of a novel molybdenum-reducing and cyanide-degrading *Serratia* sp. strain HMY1

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ABSTRACT

The success of microbial-based remediation processes relies on finding the suitable microorganism that can efficiently tolerate and detoxify contaminants in polluted sites. In this work, a bacterium that reduces as high as 100 mM sodium molybdate to molybdenum blue (Mo-blue) and can grow on 100 mg/L of potassium cyanide as a nitrogen source is reported for the first time. The isolate was tentatively identified as *Serratia* sp. strain HMY1 based on partial 16S rDNA molecular phylogeny. Molybdate reduction in this strain was supported (in descending order of efficiency) by electron donor sources like sucrose, galactose, fructose, glucose, mannitol, xylitol and sorbitol, whereas ammonium sulfate, nicotinamide, cysteine, aspartate, phenylalanine, asparagine, acrylamide and glutamate were the nitrogen sources that supported Mo-blue production. Optimization of molybdate reduction was carried out via one-factor-at-a time (OFAT) and Response Surface Method (RSM). A preliminary screening experiment using the Plackett-Burman design indicated the factors molybdenum, phosphate and incubation time were significant out of seven factors screened. Response Surface Method was then utilized to further optimize molybdenum reduction using the Central Composite Design (CCD). The optimum predicted conditions via RSM were molybdenum at 55 mM, phosphate at 3.95 mM and incubation time of 48 h. Compared to OFAT, RSM optimization showed a remarkable increase from an absorbance value of 8.06–13.91, respectively. The maximum tolerable cyanide concentration for molybdenum reduction was 25 mg/L. The reduction characteristics and cyanide tolerance of strain HMY1 suggest that it would be useful in future bioremediation of polluted sites and treatment of water bodies contaminated with both molybdenum and cyanide.

Keywords: Molybdenum; Mo-blue; cyanide-degrading; Bioremediation, *Serratia*

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1. Introduction

Metals and their compounds have been long recognized as toxic agents, causing a range of acute to chronic toxicity cases in occupational and environmental high-exposure settings. Heavy metals are elements that occur naturally in the environment. Presently, the increased industrialization and population growth have led to a considerable and indiscriminate release of pollutants into the environment [1,2]. When heavy metal levels exceed the so-called critical loads, they exert harmful effects to the human health and biota [3]. Additionally, heavy metals are known to be toxic in their elemental and combinatorial forms [4], are non-degradable [5] and accumulate in the food chain [6].

Molybdenum is an essential trace element and a micronutrient required as a cofactor to more than 50 enzymes involved in sulfur, nitrogen and carbon cycles [7,8]. It promotes cellular function by catalyzing a variety of hydroxylation and redox transfer reactions thus playing important roles in the physiology of animals and plants [9]. Earlier studies have shown that molybdenum is an endocrine disruptor; nevertheless, it is ubiquitously found in a number of food and water sources [7]. Today, the wide distribution of molybdenum in the industrial manufacture of ceramics, glass and contact lens solution, metallurgical processes, lubricants, pigment, catalyst, electronic products and as color additives in cosmetics has increased the risk of human exposure to its toxicity [7,9]. In Malaysia, molybdenum pollution comes from the indiscriminate dumping of molybdenum disulphide-based lubricant into the soils [7]. Molybdenum leaching into the soils from these illegal dumped sites can enter water sources. Animals exposed to molybdenum in drinking water or while foraging plants are likely to reflect symptoms of hypocuprosis or suffer from molybdenosis that can lead to death [7,10].

Bioremediation is a cost-efficient technology that provides an alternative to the existing physicochemical methods for heavy metals removal. A number of physicochemical techniques such as evaporation, chemical precipitation, filtration, ion exchange, membrane technology, reverse osmosis and electrochemical treatment have been used to treat industrial wastes. However, these methods are often laborious and expensive [11]. Bacterial-based remediation of environmental toxicants is a promising innovative technology that has attracted great interest over the years [12]. It uses the catabolic property of microbes to eliminate, attenuate or transform pollutants into less hazardous products, carbon dioxide, water, inorganic salts and microbial biomass [13]. Inorganic toxicants such as heavy metals cannot be degraded by microbes; thus, their bioremediation depends on mechanisms such as bioreduction, bioprecipitation, bioaccumulation or sequestration, efflux pumping and biosorption to detoxify and immobilize metal ions such as molybdenum, chromium, copper and mercury [14–17]. Interestingly, microbial molybdate (Mo^{6+}) reduction to Mo-blue was reported over a century ago in *E. coli* by Capaldi and Proskauer [18], even though the details on the reduction phenomenon came up only during the last three decades by Campbell et al. in *E. coli* K12 [19]. The first documented report on molybdenum bioremediation was in Tyrol, Austria, where a plant-microbial consortium was used to clean up large pasture areas contaminated with molybdenum, reaching as high as 200 ppm and causing scouring in ruminants [10].

Previous studies on bacterial molybdenum reduction are centered toward isolating better molybdenum-reducers with higher Mo-blue production capacity as a tool for bioremediation. However, since most polluted sites contain mixed contaminants of both organic and inorganic origin, it is, therefore, necessary to find better candidates that can efficiently detoxify mixed-contaminants. Thus, attention has now shifted from isolating better molybdenum-reducers to finding a multi-reducer/degrader microbe with potentials to remediate co-contaminated areas. To date, about eight molybdenum-reducers with the potential to degrade other organic contaminants have been isolated (Table 1). This paper represents for the first time, the isolation and characterization of a molybdenum-reducing bacterium that can degrade cyanide.

2. Materials and methods

All chemicals used in this work are of analytical grade. Metal ions such as arsenic (H_3AsO_4), copper $Cu(NO_3)_2$, cadmium $Cd(NO_3)_2$, chromium $Cr(NO_3)_3$, lead $Pb(NO_3)_2$, mercury $Hg(NO_3)_2$, nickel $Ni(NO_3)_2$, and silver ($AgNO_3$), potassium cyanide (KCN) were purchased from Merck (Darmstadt, Germany).

2.1. Isolation and screening of molybdenum-reducing and cyanide-degrading bacterium

Seven cyanide-degrading bacteria previously isolated from various sites in Malaysia [28,29] were screened for their molybdenum-reducing property. The bacteria were streaked onto low phosphate (5 mM phosphate) agar media (pH 7.0) containing (w/v) glucose (1.0%), $(NH_4)_2SO_4$ (0.3%), $MgSO_4$·7H2O (0.05%), NaCl (0.5%), yeast extract (0.05%), $Na_2MoO_4 \cdot 2H_2O$ (0.242%) and $Na_2HPO_4 \cdot 2H_2O$ (0.071%). Glucose was separately autoclaved [30]. The best isolate; HMY1 was chosen for further studies.

2.2. 16S rDNA gene sequencing and multiple alignments

A single colony of isolateHMY1 grown on nutrient agar (Oxoid) was suspended in 1 mL of physiological saline, and the genomic DNA was extracted by alkaline lysis using commercially prepared genomic DNA purification kit (GeneJET, Thermo Scientific). The forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse (5′-AAGGAGGTGATCCAGCCGCA-3′) primers were used to amplify the 16S rRNA. A mixture containing 0.5 pM of each primer, 200 µM of each deoxynucleotide triphosphate, 1× reaction buffer and 2.5 U of TaqDNA polymerase (Vivantis) was prepared to achieve a final volume of 50 µL [31]. PCR amplification was performed by initial denaturation at 94°C for 3 min, 25 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, then a final extension at 72°C for 10 min using Gradient touch thermo-cycler (Hercuvan Lab System Inc., USA). Big Dye terminator kit (Perkin-Elmer Applied Biosystems) was subsequently used for cycle sequencing according to manufacturer's recommendation. CHROMAS (Version 1.45S) was subsequently used to edit the sequenced data. The resul-

Table 1
Characteristics of previously isolated molybdenum-reducers that degrade other contaminants

Bacteria	Place	Contaminants degraded	Reference
Bacillus sp. strain khayat	Malaysia	Diesel	[20]
Burkholderia sp. strain neni-11	Indonesia	Amide	[21]
Enterobacter sp. strain Aft-3	Pakistan	Azo dye	[22]
Klebsiella oxytoca strain saw-5	Malaysia	Glyphosate	[23]
P. aeruginosa strain Amr-11	Egypt	Phenol	[24]
Klebsiella oxytoca strain Aft-7	Pakistan	SDS	[25]
Enterobacter sp. strain Zeid-6	Sudan	Orange G	[26]
Pseudomonas putida strain Amr-12	Egypt	Phenol and catechol	[27]
Klebsiella oxytoca strain DRY14	Malaysia	SDS	[14]

tant 1,482 bases were blast using the NCBI server (http://www.ncbi.nlm.nih.gov/BLAST/) and then compare with the GenBank database. This analysis revealed the sequence to be related to from Enterobacteriaceae. The 16S ribosomal RNA gene sequence of this isolate was deposited in the GenBank under the accession number KY608077.

2.3. Phylogenetic analysis

The phylogenetic analysis was performed using clustalW [32], by multiple alignments of twenty retrieved 16S rRNA gene sequences (from GenBank) that closely matched strain HMY1 through PHYLIP output option. All possible missed-alignments were manually checked, and gaps were excluded from the computation. The phylogenetic tree was constructed using PHYLIP version 3.573 (http://evolution.genetics.washington.edu/phylip), with *E. coli* as the outgroup in the phylogram. The evolutionary distance matrices for the neighbour-joining/ UPGMA method of [33] were computed through DNADIST algorithm program, while nucleotide substitution was performed using [34] model. The confidence levels of individual tree branches were checked by repeating the PHYLIP analysis with 1,000 bootstraps [35]. Majority rule (50%) consensus trees were constructed for the topologies found using a family of consensus tree methods called the ML methods [36] using the CONSENSE program and the tree was viewed using TreeView [37].

2.4. Effect of electron donor sources

A sterile stock solution (10% w/v) of electron donor sources such as glucose, fructose, sucrose, galactose, lactose, mannitol, xylitol, sorbitol and arabitol (Sigma, St Loius, USA) were produced while preparing starch solution 1% (w/v) into a 50-mL conical flask containing low phosphate media (LPM). A graded concentration of the electron donors was added to the final concentration of 0.2% (w/v). Aliquot (2% v/v) of *Serratia* sp. strain HMY1 grown with 100 mL of high phosphate media (HPM) in a 250 mL conical flask at 30°C for 24 h on an orbital shaker (150 rpm) was inoculated into each of the 50ml conical flask containing 20 mL low phosphate media and electron donor sources. The increase in absorbance at 865 nm was measured following a static growth at 30°C after24 h of incubation.

2.5. Effect of nitrogen sources on molybdate reduction

The effects of nitrogen sources on molybdate reduction were studied using ammonium sulfate, ammonium acetate, acrylamide, nicotinamide, aspartate, glutamate, phenylalanine asparagine, cysteine, cyanide and urea. A graded concentration from 0.3 to 6.0% ammonium sulfate was used to determine the effect of nitrogen source on strain HMY1.

2.6. Growth medium for cyanide degradation

The growth of the bacterium at 100 mg/L cyanide was carried out in a medium (1 L) consisting of 3.5 g of K_2HPO_4, 7.2 g of KH_2PO_4, 0.5 g of yeast extract, 5 g of glucose and 10 mL of a trace metal solution (180 mg/L of $MgCl_2{\cdot}6H_2O$, 300 mg/L of $FeSO_4{\cdot}7H_20$, 40 mg/L of $ZnSO_4$, 130 mg/L of $Co(NO_3)_2{\cdot}6H_2O$, 40 mg/L of $CaCl_2$ and 20 mg/L of MoO_3). The pH of the medium was adjusted to 7.0. Glucose was autoclaved separately, and cyanide was added using filter sterilization (Millipore nylon 0.2 mm filter membrane). The bacterium was incubated on an orbital shaker at 150 rpm at room temperature (28–30°C) for five days [28].

2.7. Assay for cyanide degradation

Residual cyanide was determined using the ϒ-picoline and barbituric acid method. The reaction of cyanide with chloramine T produces a soluble violet blue compound that is measured at 605 nm. The cyanide reagent was prepared by dissolving 13.6 g of potassium dihydrogen phosphate (KH_2PO_4) and 0.28 g of disodium hydrogen phosphate (Na_2HPO_4) in 1 L of deionised water. The pH of the solution was adjusted to pH 5.2. This is reagent A. In a 50 mL volumetric flask containing 20 mL of deionised water, three grams of barbituric acid was added with constant stirring followed by the addition of 15 mL of ϒ-picoline (4-methyl pyridine) into the solution. Then three mL of conc. hydrochloric acid was added to the mixture. The mixture was continuously stirred during this process. The flask was topped up to 50 mL after the mixture cools down. After cooling down, the mixture was topped up to 50 mL with deionised water. To assay for residual cyanide, a suitable aliquot of the growth media was first centrifuged at 10,000 g for 10 min at room temperature and the supernatant taken out after suitable dilution for cyanide analysis. In a dried centrifuge

tube, 10 mL of cyanide standards or growth media aliquot was mixed with 5 mL of reagent A. Then, 0.25 mL of 1% w/v chloramine T solution was added to the mixture and vortexed briefly. The mixture was incubated for 2 min at room temperature. Finally, 3 mL of the barbituric and ϒ-picoline reagent was added to the mixture and further incubated for 5 min at 25°C. Cyanide was determined at 605 nm with the reagent blank utilized as a control [38].

2.8. Statistical analysis

All data are presented as mean ± SD of triplicate determination and were analyzed using InStat GraphPad version 3.05. Comparison between groups was performed using ANOVA or t–test with Tukey's post-hoc test. $P < 0.05$ is considered statistically significant.

3. Results and discussion

3.1. Identification of the isolate

The strain HMY1 revealed a low bootstrap value of less than 50% similarity to the genus *Serratia* spp, in particular to *Serratia* sp. and *Serratia mercescens* (Fig. 1), indicating that the phylogenetic relationship of this isolate to a particular species is difficult to be tied up. Together with the Biolog identification system that gave the closest ID to *Serratia* sp. with a low probability, the isolate was assigned tentatively as *Serratia* sp. strain HMY1. This bacterium was the sixth isolated molybdenum-reducer from the genus *Serratia* so far, signifying its importance in molybdate reduction. Earlier in 1939, Jan reported the first bacterium from this genus while *Serratia marcescens* strain Dr. Y6 [30], *Serratia* sp. strain Dr. Y5 (2009), *Serratia* sp. strain Dr. Y8 (2009) and *Serratia marcescens* strain Dr. Y9 (2009) were the second, third, fourth and fifth, respectively.

3.2. Optimization of molybdenum reduction by Serratia sp. strain HMY1

Optimization through one-factor-at-a-time (OFAT) of molybdenum reduction by *Serratia* sp. strain HMY1 involves the effects of temperature, pH, electron donors, nitrogen sources, phosphate and molybdate (Fig. 2).

ANOVA analysis showed that sucrose was the best electron donor source followed by galactose, fructose and glucose ($P < 0.05$) (Fig. 2a). The concentration of sucrose supporting optimum Mo-blue production in this strain was 1.5% (w/v). A number of electron donors have been previously reported to support microbial molybdate reduction. To date, 75% of isolated Mo-reducers require glucose as the best electron donor for optimum Mo-blue production [14,16,17,20,23–27,39–44]; 20% of the reducers require sucrose [30,45–47], while only one report shows a preferential requirement for fructose as the best source [48]. The requirement for easy assimilable carbon source by most Mo-reducers justifies that molybdenum reduction is a growth-associated process. Additionally, both sucrose and glucose are easily metabolized via glycolysis, TCA cycle, pentose phosphate pathways and the electron transport chain, producing reducing equivalents (NADH and NADPH) that serve as substrates for the molybdenum-reducing enzyme [45,47].

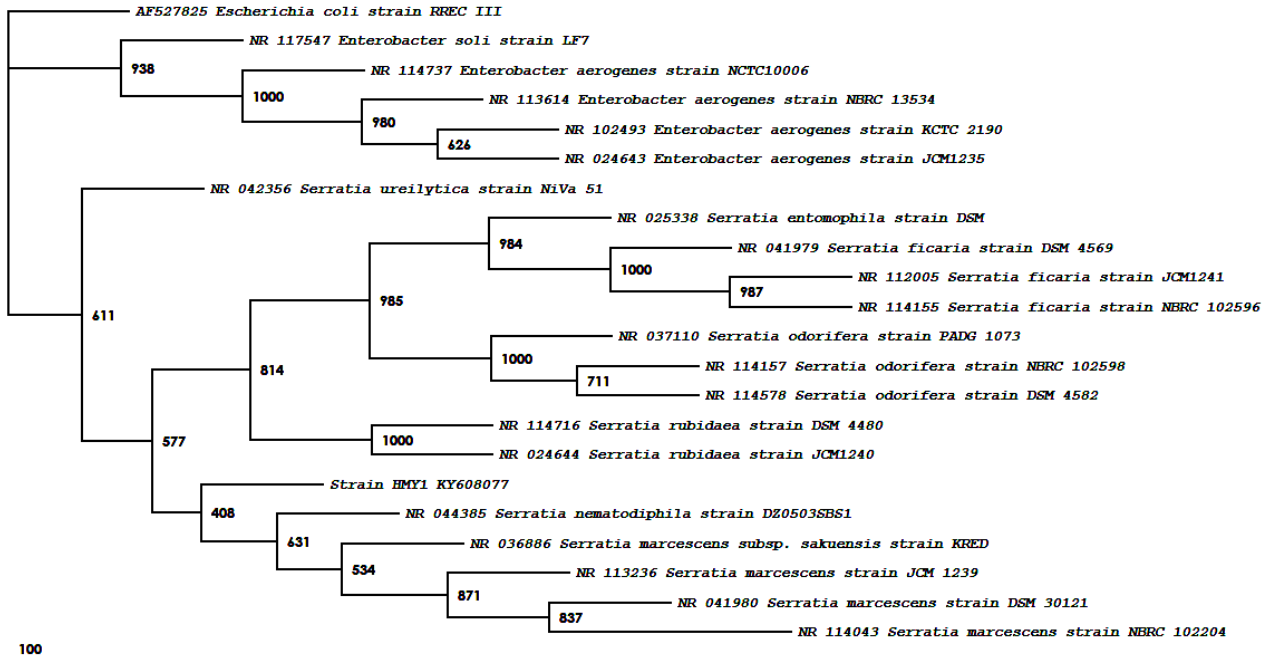

Fig. 1. Phylogram (neighbour-joining method) indicating the genetic relationship between strain HMY1 and referenced related microorganisms based on the 16S rRNA gene sequence analysis. Species names are accompanied by the accession numbers of their 16S rRNA sequences. The numbers at branching points or nodes refer to bootstrap values, based on 1000 re-samplings. Scale bar represents 100 nucleotide substitutions. *E. coli* is the out-group.

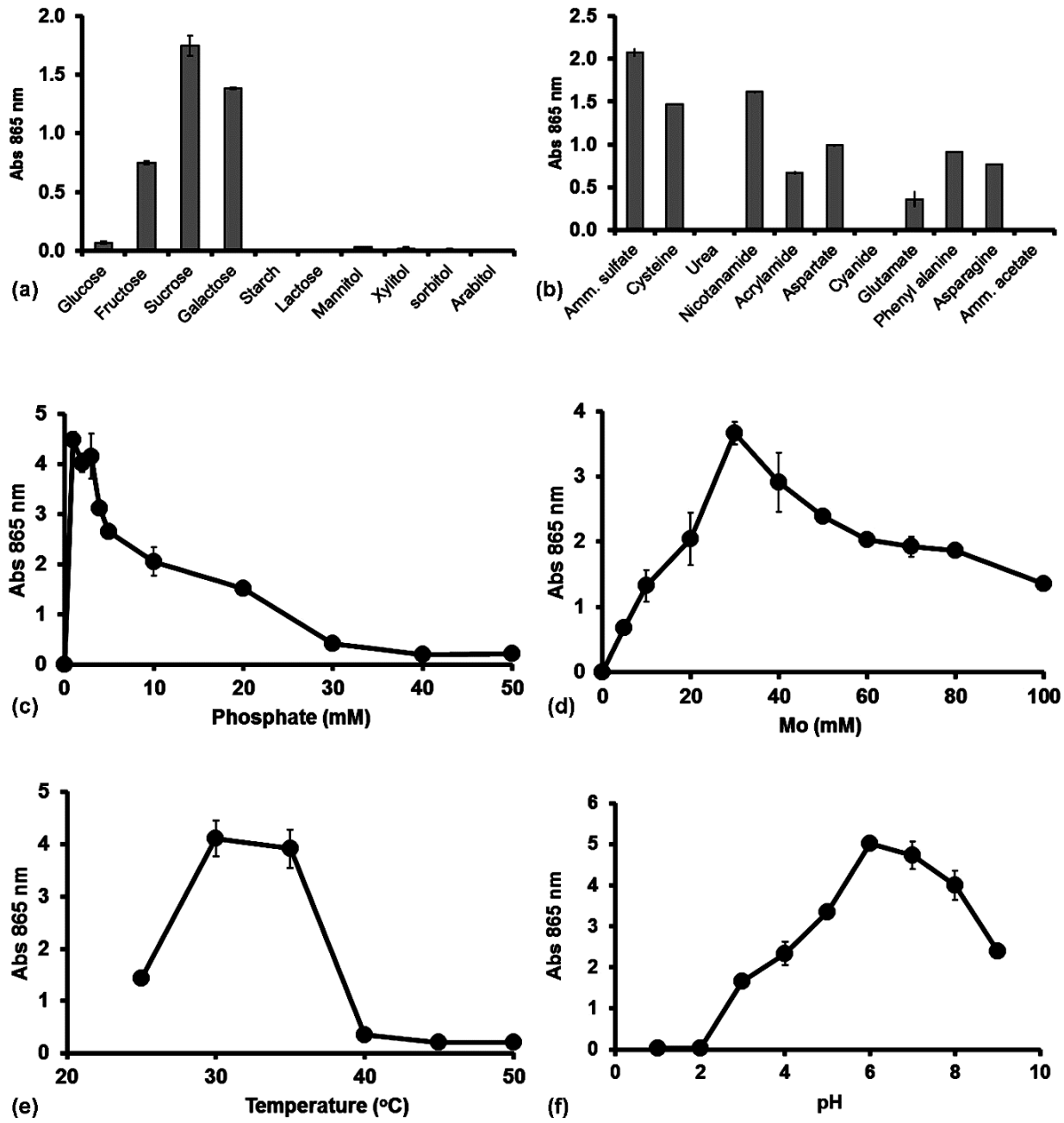

Fig. 2. Optimization of the parameters (a) carbon source, (b) nitrogen source, (c) phosphate, (d) molybdate, (e) temperature and (f) pH on molybdenum reduction measured as Mo-blue production by *Serratia* sp. strain HMY1 through OFAT. Data are presented as mean ± SD of triplicate determinations.

3.3. Effect of nitrogen sources on molybdate reduction

All nitrogen sources used in this study are able to support molybdate reduction except urea, cyanide and ammonium acetate (Fig. 2b). Since molybdate reduction in heterotrophic bacteria has been earlier reported to be growth-associated [19,49], factors that stimulate growth would also promote molybdate reduction. Ammonium sulfate was found to be the best nitrogen source supporting optimum Mo-blue production (Fig. 2b) with an optimum at 1% (w/v) following a 24 h static incubation. The fact that urea did not support molybdate reduction in this strain was similarly reported in *Serratia marcescens* strain Dr. Y6 [30]. However, contrary to strain Dr. Y6, asparagine, aspartate, cysteine and nicotinamide supported molybdate reduction in this strain. Previously, the amino acids valine and cysteine were also reported to support molybdate reduction in *Serratia* sp. strain Dr. Y5 [42], while cysteine, nitrate and nitrite were reported to inhibit the molybdate reduction in *E. coli* K12 [19].

3.4. The effect of temperature and pH on molybdate reduction

Molybdate reduction in this strain was found to be optimum between 30 and 35°C and Mo-blue production was abolished at temperatures higher than this (Fig. 2e). To date, approximately 80% of the isolated molybdenum reducers exhibit optimal temperatures of between 30 and 37°C while several have optimal temperatures of between 25 and 30°C [14,26] and one; a psychrophilic bacterium exhibited an optimum temperature of between 15 and 20°C [41]. An optimal pH of between 6.5 and 7.0 was required for molybdenum reduction (Fig. 2f), which is within the range of nearly all of the isolated molybdate reducers to date [50].

3.5. Effect of molybdate and phosphate concentrations on molybdate reduction

The effect of molybdate and phosphate concentrations on molybdenum reduction showed an optimum reduction at five mM phosphate and 30 mM sodium molybdate with higher concentrations dramatically inhibiting reduction (Figs. 2c, d). The inhibitory effect of phosphate has been discussed in previous works while the optimal concentration of molybdate supporting reduction is within the 10–30 mM range for many molybdenum reducers [50,51] with very few reducers exhibiting higher concentrations for reduction at molybdenum concentration as high as 80 mM [19,48]. More efficient reducers are in demand especially to remediate mining sites as these sites usually contain very high metal content in the soil. For instance, in Colorado, contaminated sites from a discontinued uranium mine show molybdenum concentration as much as 6,500 mg/kg or nearly 70 mM in the surrounding soil.

3.6. Optimization of molybdate reduction using Response Surface Methodology (RSM)

In this study, optimization of the reduction process by this bacterium was further evaluated using response surface method (RSM) with a pre-screening exercise using the Plackett Burman factorial design. The RSM has a number of advantages over the usual OFAT, including that RSM reduces the number of experimental trials needed to evaluate multiple variables and the discovery of parameter interactions that can lead to higher optimal results or response than OFAT. Therefore, it is less laborious and time-consuming [29,52,53]. Most previous studies on bacterial molybdenum reduction are carried out using one factor at a time. Halmi et al. [53] were the first to optimize molybdenum reduction using RSM as a novel approach. Therefore, optimization of molybdate reduction by strain HMY1 was performed in two stages: Firstly, a screening for significant parameters using the Plackett-Burman design was carried out, in which seven parameters were considered. Secondly, the optimum values of significant parameters were determined using RSM. Before which, a preliminary analysis using first-order factorial design is required for the selection of experimental design, where screening of the significant variables is preferred when a number of variables are large [29].

3.7. Screening for significant parameters using Plackett-Burman design

Experiment using the Plackett-Burman design was performed to identify the factors that are most significant in facilitating molybdate reduction. A total of 7 parameters, pH, temperature, sucrose, ammonium sulfate, molybdate, phosphate and incubation time were considered in 12 experimental runs with the absorbance of Mo-blue intensity at 865 nm as a response (Table 2). Based on a regression analysis, parameters with p values of less than 0.05 ($p < 0.05$) are considered to have significant impacts on molybdate reduction to Mo-blue. These parameters will be then further optimized through RSM.

Statistical analysis using a Plackett–Burman design indicated that molybdate (A), phosphate (B) and incubation time (G) were the significant ($P < 0.05$) parameters influ-

Table 2
Plackett–Burman design to evaluate significant parameters influencing Mo-blue production in strain HMY1

Run	Factor A	Factor B	Factor C	Factor D	Factor E	Factor F	Factor G	Response
	Molybdate (mM)	Phosphate (mM)	pH	Temperature (°C)	Sucrose (g/L)	Ammonium sulfate (g/L)	Incubation time (h)	A 865 nm
1	50.00	2.90	6.50	25.00	10.00	5.00	24.00	6.83
2	60.00	2.90	7.50	35.00	10.00	10.00	24.00	8.88
3	50.00	5.00	7.50	35.00	10.00	10.00	48.00	11.76
4	60.00	5.00	6.50	35.00	10.00	5.00	24.00	9.93
5	60.00	5.00	7.50	25.00	20.00	10.00	24.00	11.28
6	60.00	2.90	6.50	25.00	20.00	10.00	48.00	12.38
7	50.00	5.00	6.50	25.00	10.00	10.00	48.00	9.88
8	50.00	2.90	7.50	35.00	20.00	5.00	48.00	9.72
9	50.00	2.90	6.50	35.00	20.00	10.00	24.00	6.83
10	50.00	5.00	7.50	25.00	20.00	5.00	24.00	8.03
11	60.00	5.00	6.50	35.00	20.00	5.00	48.00	12.7
12	60.00	2.90	7.50	25.00	10.00	5.00	48.00	11.75

encing Mo-blue production in strain HMY1. Analysis of the relationship between significant independent variables and the response could not be done using the first-order equation, indicating the inappropriateness of the model to predict the response. Thus, a second-order model using RSM was employed to further investigate the relationship.

Statistical analysis for the responses was performed as depicted in Supplementary Table S1. The model F value of 12.91 with probability values > F less than 0.05 indicate that the model terms are significant [29]. The "Adeq Precision" ratio of 11.40 obtained in this study, indicates an adequate signal. Hence, the model can be used to navigate the design space.

The model R^2 value was 0.957 and the "Pred R-Squared" of 0.883 is in reasonable agreement with the "Adj R-Squared" of 0.688. Regression analysis was performed, and a first-order polynomial equation was derived, representing absorbance at 865 nm for Mo-blue production as a function of the independent variables:

3.8. Final equation in terms of coded factors

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as –1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

$$A_{865} = +10.00 + 1.16A + 0.5992B + 0.2392C - 0.0275D + 0.1592E + 0.1708F + 1.37G$$

3.9. Optimization of molybdate reduction using RSM

Response surface method (RSM) as a statistical technique comprising of stages to select an appropriate experimental design, determine the effective levels/optimum points of various independent parameters, predict and verify model equations, generate response surfaces and contour plots [29]. Recently, RSM has been successfully applied to optimize bioremediation processes, like caffeine degradation [52], cyanide degradation [29], molybdenum reduction [53] and hexavalent chromium reduction to a less toxic form [54]. The response surface equation could be optimized for maximum yield in the range of process, with the variables determined using mathematical software like Design Expert® or MATLAB®. The optimal response could be seen on the contour plots, which give the effects of the levels of two parameters and their interactions by setting other parameters at their optimal concentrations [29].

The present analysis focused on studying the combined effect of significant parameters from previous experiments molybdate, phosphate and time on molybdate reduction, and optimize the process variables for maximum Mo-blue production using a central composite design in a 20 experimental runs. The results obtained were analyzed using Design Expert 6.0 software (Stat-Ease Inc., Minneapolis, USA). The experimental parameters and responses (actual and predicted) from RSM are presented in Table 3. The maximum and minimum Mo-blue production were observed at runs 4 and 19, respectively.

Table 3
Central composite matrix for experimental design and predicted response using RSM

Run	Factor 1	Factor 2	Factor 3	Response 1
	A: Molybdate Concentration	B: Phosphate Concentration	C: Time	Absorbance
	mM	mM	h	nm
1	55	3.95	48	13.801
2	55	3.95	88	7.021
3	60	5	72	7.032
4	55	3.95	48	13.911
5	50	2.9	24	7.02
6	55	3.95	48	11.998
7	46	3.95	48	5.995
8	55	3.95	8	3.201
9	55	3.95	48	13.113
10	63	3.95	48	11.996
11	55	2	48	4.296
12	55	6	48	4.096
13	55	3.95	48	13.304
14	50	5	72	7.997
15	60	5	24	7.03
16	60	2.9	72	3.998
17	60	2.9	24	10.04
18	55	3.95	48	13.307
19	50	5	24	1.995
20	50	2.9	72	6.07

3.10. Final equation in terms of coded factors

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as –1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The quadratic regression model for molybdate reduction is depicted as follows:

$$A_{865nm} = +13.26 + 0.9804A - 0.1142B + 0.3968C + 0.3903AB - 1.39AC + 1.62BC - 1.47A^2 - 2.48B^2 - 2.93C^2$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as –1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Analysis of variance (ANOVA) of the fitted quadratic model for optimization of molybdate reduction in strain HMY1 is presented in Supplementary Table S2. The result shows that the F-value of the model was 28.23 with a low probability value ($F < 0.0001$) indicating that the model is significant for molybdate reduction. In this case A, AC, BC, A^2, B^2, C^2 were the significant model terms. Values of P of less than 0.05 indicate that the model terms are significant

[29]. The Predicted R^2 of 0.7675 is in reasonable agreement with the Adjusted R^2 of 0.9285. The "adequate precision" ratio for the model's signal was 15.62. The "PRESS" (prediction error sum of the square) value of 69.77 is a measure of how well the experimental model is likely to predict the responses in a new experiment [29]. The lack of fit F-value (3.87) was not statistically significant, implying that the model fits well with the data. The use of RSM in optimizing molybdenum reduction has been carried out before with good results [53].

3.11. Determination and validation of optimum conditions

The conditions (parameter levels) necessary for optimal molybdate reduction to Mo-blue in strain HMY1 were ascertained by RSM. The optimum predicted condition was molybdenum at 55 mM, phosphate at 3.95 mM and incubation time of 48 h, with the overall Mo-blue production of 13.26 (95% confidence interval between 12.29 and14.23). In order to validate this, a set of experiments were performed based on the optimum condition predicted, to compare the experimental results with the predicted values of the responses using the model equation. The experimental value for molybdate reduction was an absorbance value of 13.91 indicating that the suitability of the developed quadratic models. However, it is worthy to note that the optimal values are valid within a specified range of process parameters.

Molybdenum reduction before optimization using RSM showed molybdenum reduction starting at the absorbance value of 8.06 at 865 nm going up to an absorbance value above 13.91 after RSM. In a previous study of molybdenum reduction and its optimization using RSM, a significant improvement of molybdenum blue production measured at 865 nm from 10 to 20.87 was reported [53], indicating an excellent optimization results through RSM. A number of reports have shown that after optimization using RSM, the response obtained increase up to several folds compared to an OFAT approach [29]. Based on this study, it is proven that RSM is a robust optimization tool compared to the classical OFAT method. Molybdenum reduction using strain HMY1 was successfully optimized and maximized using RSM.

3.12. Effect of process variables using response surface plot

The RSM was visualized using 3D surfaces to analyze the interactive effect of two parameters at a time by holding the value of the other parameter. The contours represent the optimal value of the parameter that shows the maximum Mo-blue production (response). Normally, the elliptical shape of the contour obtained suggests an interaction between two parameters [29]. Otherwise, the round or circular shape of the contour indicates that there is no interaction between the parameters at a time.

3.13. Molybdate concentration vs. phosphate concentration

The 3D response surface showed the effect of molybdate and phosphate concentrations on Mo-blue production (Fig. 3). The result reveals that, as molybdate and phosphate concentrations increase, Mo-blue production increases until the optimum condition was attained. Both surface plots show that molybdate and phosphate concentrations were optimum at 55 mM and 3.95 mM, respectively. These parameters are necessary for the formation of Mo-blue intermediate, phosphomolybdate as suggested previously [55,56].

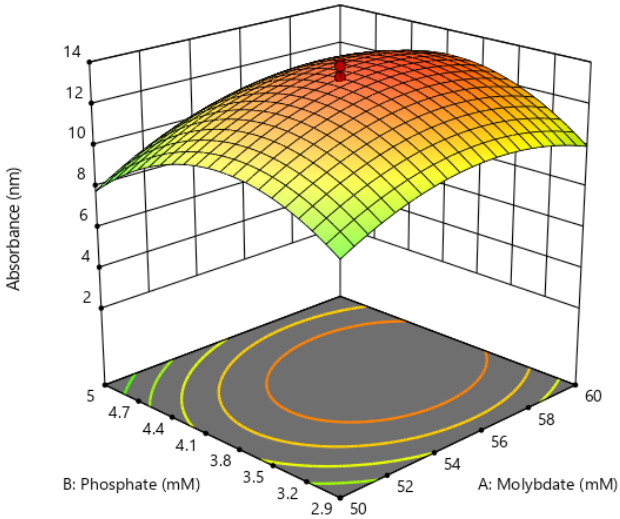

Fig. 3. 3D plot showing the effect of molybdate and phosphate concentrations on Mo-blue production by strain HMY1.

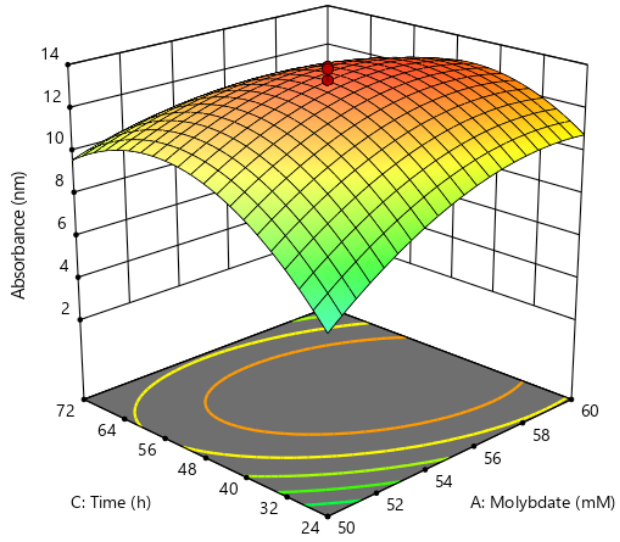

Fig. 4. 3D plot showing the effect of molybdate concentration and time on Mo-blue production by strain HMY1.

3.14. Molybdate concentration vs time

The interactive effect of molybdate and time on Mo-blue production reveals that, as molybdate concentration increases with time, Mo-blue production increases until the optimum conditions were attained (Fig. 6). Both surface plots showed that molybdate concentration and time were

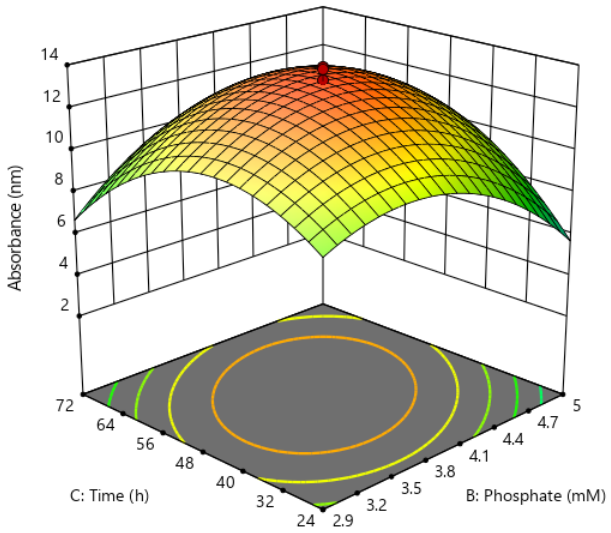

Fig. 5. 3D plot showing the effect of phosphate concentration and time on Mo-blue production by strain HMY1.

optimum at 55 mM and 48 h, respectively. The elliptical plot obtained indicated an interaction between the two parameters. A previous response surface-based optimization work on molybdenum reduction to molybdenum blue by *Serratia* sp. MIE2 shows a much lower optimal molybdate concentration of 20 mM [53].

3.15. Phosphate concentration vs time

The interactive effect of phosphate and time on Mo-blue production reveals that, as phosphate concentration increases with time, Mo-blue production increases until the optimum conditions were attained at 3.95 mM and 48 h, respectively (Fig. 5).

3.16. Growth and degradation of cyanide by Serratia sp. strain HMY1

Serratia sp. strain HMY1 utilized cyanide as a nitrogen source with glucose as a carbon source with about 80% of cyanide degraded after 72 h of incubation producing nearly one log CFU/mL of growth over this incubation time (Fig. 6). This bacterium shows a lower cyanide degrading capacity in comparison to *Serratia marcescens* strain AQ07; another cyanide-degrading bacterium from our previous work where it can completely degrade 200 mg/L of cyanide after 72 h of incubation period [29]. *Serratia* sp. strain HMY1 shows comparable performance over a cyanide-degrading bacterial consortium operating under a rotating biological contactor (RBC) where 99.9% removal of 52 mg/L cyanide was achieved within 15 h using sugarcane molasses as a carbon source [57]. Another local cyanide-degrading strain; *Rhodococcus* UKMP-5M exhibit a better degrading performance than *Serratia* sp. strain HMY1 with the former able to degrade nearly 40% of 390 mg/L of cyanide as a nitrogen source after 10 hours of incubation [58] but a resting cells preparation was utilized instead of the free cells used in this work. However, to date, this is the first report of a cyanide-degrading bacterium with the molybdenum-reducing property.

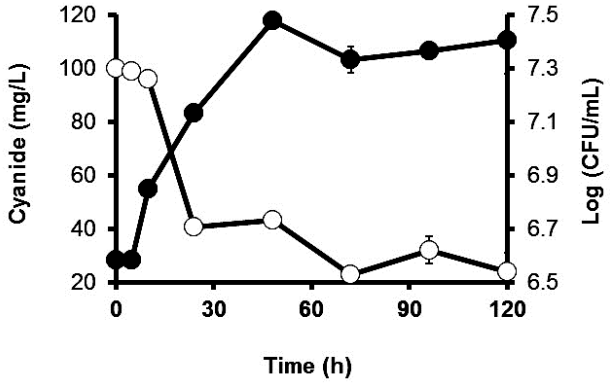

Fig. 6. *Serratia* sp. strain HMY1 growth (•) on 100 mg/L cyanide and its degradation (○). Data presented as mean ± SD of triplicate determinations.

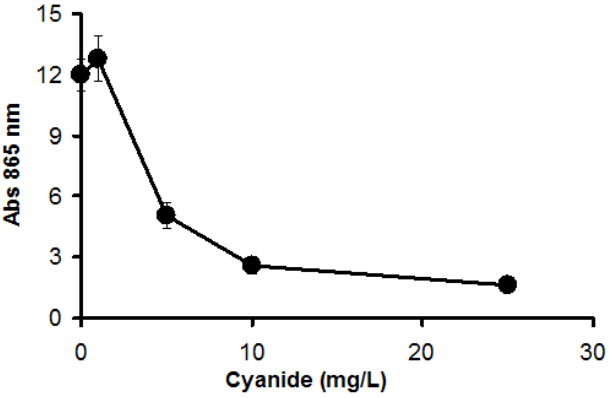

Fig. 7. The effect of cyanide on molybdenum reduction to molybdenum blue by *Serratia* sp. strain HMY1. Data presented as mean ± SD of triplicate determinations.

3.17. Effect of molybdenum reduction in the presence of cyanide

Although *Serratia* sp. strain HMY1 was able to grow and degrade cyanide, the molybdenum-reducing activity of this bacterium is severely inhibited by cyanide (Fig. 7). Previously, the inhibition by 10 mM (260 mg/L) cyanide in the molybdenum-reducing bacterium *Enterobacter cloacae* strain 48 was proposed to provide evidence that the molybdenum-reducing activity might involve the enzymes of the electron transport protein [45]. However, this conclusion was later questioned as the high cyanide concentration used has been demonstrated to lead to an increase in pH that indirectly effects molybdenum-reducing activity [59]. The result obtained in this work can be interpreted to indicate that the enzyme(s) of the electron transport chain from this bacterium might be probably involved in the reduction of molybdenum as the inhibiting concentration of cyanide was seen at a very low cyanide concentration; about 0.2 mM, which does not usually cause a dramatic increase in pH [59].

4. Conclusion

Strain HMY1 is the first bacterium exhibiting both molybdenum-reducing and cyanide degrading characteristics. Optimization of the molybdenum reduction through OFAT and RSM was successful with the latter further enhanced the molybdenum reduction property of this bacterium. Despite being able to grow on cyanide, this toxic compound cannot be used as an electron donor or nitrogen source to support molybdenum reduction, and this open future research in isolating molybdenum-reducing bacterium with such ability as this could alleviate the toxic effect of cyanide on molybdenum reduction observed in this work. The fact that molybdenum reduction is inhibited by cyanide is an interesting observation on the possible role of the electron transport chain as the potential site of molybdenum reduction. One potential application of this bacterium is in the remediation of gold mining effluents that are often contaminated with cyanide and other metal ions.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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References

[1] N. Kusnin, M.A. Syed, S.A. Ahmad, Toxicity, pollution and biodegradation of acrylamide – a mini review, J. Biochem. Microbiol. Biotechnol., 3 (2015) 6–12.
[2] M.I.E. Halmi, Rapid ecotoxicological tests using bioassay systems - A review, J. Biochem. Microbiol. Biotechnol., 4 (2016) 29–37.
[3] K.K. Ibrahim, M.A. Syed, M.Y. Shukor, S.A. Ahmad, Effect of heavy metals on cyanide biodegradation by resting cells of *Serratia marcescens* strain AQ07, J. Environ. Microbiol. Toxicol., 2 (2014) 58–60.
[4] M. Rosca, R.M. Hlihor, P. Cozma, E.D. Comaniţa, I.M. Simion, M. Gavrilescu, Potential of biosorption and bioaccumulation processes for heavy metals removal in bioreactors, 5th IEEE Int. Conf. E-Health Bioeng. Conf. EHB, (2015) 31–34.
[5] L.A. Sarubbo, R.B. Rocha, J.M. Luna, R.D. Rufino, V.A. Santos, I.M. Banat, Some aspects of heavy metals contamination remediation and role of biosurfactants, Chem. Ecol., 7540 (2015) 1–17.
[6] A.V. Singh, R. Singh, Synthesis, characterization, and application of tamarind nitrilotriacetic acid resin in removal of heavy metal ions from industrial effluent, Environ. Prog. Sustain. Energy, 32 (2013) 103–108.
[7] H. Yakasai, M. Rahman, N. Yasid, S. Ahmad, M. Halmi, M.Y. Shukor, Elevated molybdenum concentrations in soils contaminated with spent oil lubricant, J. Environ. Microbiol. Toxicol., 5(2) (2017) 1–3.
[8] S. Wu, C. Hu, Q. Tan, Z. Nie, X. Sun, Effects of molybdenum on water utilization, antioxidative defense system and osmotic-adjustment ability in winter wheat (*Triticum aestivum*) under drought stress, Plant Physiol. Biochem., 83 (2014) 365–374.
[9] R. Pandey, S.P. Singh, Effects of molybdenum on fertility of male rats, BioMetals, 15 (2002) 65–72.
[10] C. Neunhäuserer, M. Berreck, H. Insam, Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation, Water. Air. Soil Pollut., 128 (2001) 85–96.
[11] Q.H. Pei, S. Shahir, R.A.S. Santhana, Z.A. Zakaria, W.A. Ahmad, Chromium(VI) resistance and removal by *Acinetobacter haemolyticus*, World J. Microbiol. Biotechnol., 25 (2009) 1085–1093.
[12] B. Tiong, Z.M. Bahari, S.L. Irwan, J. Jaafar, Z. Ibrahim, S. Shahir, Cyanide degradation by *Pseudomonas pseudoalcaligenes* strain W2 isolated from mining effluent, Sains Malays., 44 (2015) 233–238.
[13] A. Husaini, H.A. Roslan, K.S.Y. Hii, C.H. Ang, Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites, World J. Microbiol. Biotechnol., 24 (2008) 2789–2797.
[14] M.I.E. Halmi, S.W. Zuhainis, M.T. Yusof, N.A. Shaharuddin, W. Helmi, Y. Shukor, M.A. Syed, S.A. Ahmad, Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate- degrading *Klebsiella oxytoca* strain dry14, BioMed Res. Int., 2013 (2013) 1–8.
[15] J.R. Lloyd, Microbial reduction of metals and radionuclides, FEMS Microbiol. Rev., 27 (2003) 411–425.
[16] A.R. Othman, N.A. Bakar, M.I.E. Halmi, W.L.W. Johari, S.A. Ahmad, H. Jirangon, M.A. Syed, M.Y. Shukor, Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi, BioMed Res. Int., 2013 (2013) 1–9.
[17] M.Y. Shukor, M.F. Rahman, N.A. Shamaan, M.S. Syed, Reduction of molybdate to molybdenum blue by *Enterobacter* sp. strain Dr.Y13, J. Basic Microbiol., 49 (2009) 43–54.
[18] A. Capaldi, B. Proskauer, BeiträgezurKenntniss der Säurebildungbei Typhus-bacillen und Bacterium coli - Eine differential-diagnostischeStudie, Z. FürHyg. Infect., 23 (1896) 452–474.
[19] A.M. Campbell, A. Del Campillo-Campbell, D.B. Villaret, Molybdate reduction by *Escherichia coli* K-12 and its chl mutants, Proc. Natl. Acad. Sci. USA., 82 (1985) 227–231.
[20] M.E. Khayat, M.F. Abd Rahman, M.S. Shukor, S.A. Ahmad, N.A. Shamaan, M.Y. Shukor, Characterization of a molybdenum-reducing Bacillus sp. strain khayat with the ability to grow on SDS and diesel, RendicontiLincei, 27 (2016) 547–556.
[21] R. Mansur, N. Gusmanizar, F.A. Dahalan, N.A. Masdor, S.A. Ahmad, M.S. Shukor, M.A.H Roslan, M.Y. Shukor, Isolation and characterization of a molybdenum-reducing and amide-degrading Burkholderiacepacia strain neni-11 in soils from west Sumatera, Indonesia, IIOAB, 7 (2016) 28–40.
[22] M.S. Shukor, A. Khan, N. Masdor, M.I.E. Halmi, S.R.S. Abdullah, M.Y. Shukor, Isolation of a novel molybdenum-reducing and azo dye decolorizing *Enterobacter* sp. strain Aft-3 from Pakistan, Chiang Mai Univ. J. Nat. Sci., 15 (2016) 95–114.
[23] M.K. Sabullah, M.F. Rahman, S.A. Ahmad, M.R. Sulaiman, M.S. Shukor, N.A. Shamaan, M.Y. Shukor, Isolation and characterization of a molybdenum-reducing and glyphosate-degrading Klebsiella oxytoca strain saw-5 in soils from sarawak, Agrivita, 38 (2016) 1–13.
[24] Y. Ibrahim, M. Abdel-Mongy, M.S. Shukor, S. Hussein, A.P.K. Ling, M.Y. Shukor, Characterization of a molybdenum-reducing bacterium with the ability to degrade phenol, isolated in soils from Egypt, Biotechnol. J. Biotechnol. Comput. Biol. Bionanotechnol., 96 (2015) 234–245.
[25] N. Masdor, M.S. Shukor, A. Khan, M.I.E. Halmi, S.R.S. Abdullah, N.A. Shamaan, M.Y. Shukor, Isolation and characterization of a molybdenum-reducing and SDS- degrading *Klebsiella oxytoca* strain Aft-7 and its bioremediation application in the environment, Biodiversitas, 16 (2015) 238–246.
[26] A.R. Othman, I.M. Abu Zeid, M.F. Rahman, F. Ariffin, M.Y. Shukor, Isolation and characterization of a molybdenum-reducing and orange G-decolorizing *Enterobacter* sp. strain Zeid-6 in soils from Sudan, Bioremediation Sci. Technol. Res., 3 (2015) 13–19.
[27] M.A. AbdEl-Mongy, M.S. Shukor, S. Hussein, A.P.K. Ling, N.A. Shamaan, M.Y. Shukor, Isolation and characterization of a molybdenum-reducing, phenol- and catechol-degrading *Pseudomonas putida* strain Amr-12 in soils from Egypt, Sci. Study

Res. Chem. Chem. Eng. Biotechnol. Food Ind., 16 (2015) 353–369.
[28] K.I. Karamba, M.Y. Shukor, M.A. Syed, A. Zulkharnain, N. Adeela, Isolation, screening and characterisation of cyanide-degrading *Serratia marcescens* strain AQ07, J. Chem. Pharm. Sci., 8 (2015) 401–406.
[29] K.I. Karamba, S.A. Ahmad, A. Zulkharnain, M.A. Syed, K.A. Khalil, N.A. Shamaan, F.A. Dahalan, M.Y. Shukor, Optimisation of biodegradation conditions for cyanide removal by *Serratia marcescens* strain AQ07 using one-factor-at-a-time technique and response surface methodology, RendicontiLincei, 27 (2016) 533–545.
[30] M.Y. Shukor, S.H.M. Habib, M.F.A. Rahman, H. Jirangon, M.P.A. Abdullah, N.A. Shamaan, M.A. Syed, Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain Dr. Y6, Appl. Biochem. Biotechnol., 149 (2008) 33–43.
[31] R. Devereux, S.S. Wilkinson, Amplification of ribosomal RNA sequences, Mol. Microb. Ecol. Man., 3 (2004) 509–522.
[32] J.D. Thompson, D.G. Higgins, T.J. Gibson, Clustal W: improving the sensitivity of progressive multiple sequence aligment through sequence weighting, position specific gap penalties and weight matrix choice, Nucleic Acids Res., 22 (1994) 4673–4680.
[33] N. Saitou, M. Nei, The neighbour-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol., 4 (1987) 406–425.
[34] T.H. Jukes, C.R. Cantor, Evolution of protein molecules, Mamm. Protein Metab., (1969) 21–123.
[35] J. Felsenstein, Confidence Limits on Phylogenies: An Approach Using the Bootstrap, Vol. 39, 1985.
[36] T. Margush, F.R. McMorris, Consensus n-trees, Bull. Math. Biol., 43 (1981) 239–244.
[37] R.D.M. Page, On consensus, confidence, and "total evidence," Cladistics, 12 (1996) 83–92.
[38] S. Nagashima, Spectrophotometric determination of cyanide with γ-picoline and barbituric acid, Anal. Chim. Acta, 91 (1977) 303–306.
[39] A. Campillo-campbell, D.B. Villaret, Correction: Molybdate reduction by *Escherichia Coli* K-12 and its chl mutants, Proc. Natl. Acad. Sci., 82 (1985) 3532–3532.
[40] M. Abo-Shakeer, L.K.A., Ahmad, S.A, Shukor, M.Y. Syed, Isolation and characterization of a molybdenum-reducing *Bacillus pumilus* strain lbna, J. Environ. Microbiol. Toxicol., 1 (2013) 9–14.
[41] S.A. Ahmad, M.Y. Shukor, N.A. Shamaan, W.P. Mac Cormack, M.A. Syed, Molybdate reduction to molybdenum blue by an antarctic bacterium, BioMed Res. Int., (2013) 1–10.
[42] M.F.A. Rahman, M.Y. Shukor, Z. Suhaili, S. Mustafa, N.A. Shamaan, M.A. Syed, Reduction of Mo(VI) by the bacterium *Serratia* sp. strain DRY5, J. Environ. Biol., 30 (2009) 65–72.
[43] M.Y. Shukor, M.F. Rahman, Z. Suhaili, N.A. Shamaan, M.A. Syed, Hexavalent molybdenum reduction to Mo-blue by *Acinetobacter calcoaceticus*, Folia Microbiol. (Praha), 55 (2010) 137–143.
[44] M.Y. Shukor, S.A. Ahmad, M.M.M. Nadzir, M.P. Abdullah, N.A. Shamaan, M.A. Syed, Molybdate reduction by *Pseudomonas* sp. strain DRY2, J. Appl. Microbiol., 108 (2010) 2050–2058.
[45] B. Ghani, M. Takai, N.Z. Hisham, N. Kishimoto, A.K.M. Ismail, T. Tano, T. Sugio, Isolation and characterization of a Mo^{6+}-reducing bacterium, Appl. Environ. Microbiol., 59 (1993) 1176–1180.
[46] M. Shukor, M.H. Hamdan, M.A. Othaman, N.A. Shamaan, M.A. Syed, Mo (VI) reduction to molybdenum blue by *Serratia marcescens* strain Dr. Y9, Pol. J. Microbiol., 58 (2009) 141–147.
[47] M.Y. Shukor, M.F. Rahman, Z. Suhaili, N.A. Shamaan, M.A. Syed, Bacterial reduction of hexavalent molybdenum to molybdenum blue, World J. Microbiol. Biotechnol., 25 (2009) 1225–1234.
[48] H.K. Lim, M.A. Syed, M.Y. Shukor, Reduction of molybdate to molybdenum blue by *Klebsiella* sp. strain hkeem, J. Basic Microbiol., 52 (2012) 296–305.
[49] A.B. Ariff, M. Rosfarizan, B. Ghani, T. Sugio, M.I.A. Karim, Molybdenum reductase in *Enterobacter cloacae*, World J. Microbiol. Biotechnol., 13 (1997) 643–647.
[50] R. Mansur, N. Gusmanizar, M.A.H. Roslan, S.A. Ahmad, M.Y. Shukor, Isolation and characterisation of a molybdenum-reducing and metanil yellow dye-decolourising *Bacillus* sp. strain Neni-10 in soils from West Sumatera, Indonesia, Trop. Life Sci. Res., 28 (2017) 69–90.
[51] M.Y. Shukor, M.A. Syed, Microbiological reduction of hexavalent molybdenum to molybdenum blue, in Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, A. Mendez-Vilas, ed., Formatex Research Center, Badajoz, Spain, 2010.
[52] S. Ibrahim, M.Y. Shukor, K.A. Khalil, M.I.E. Halmi, M.A. Syed, S.A. Ahmad, Application of response surface methodology for optimising caffeine-degrading parameters by *Leifsonia* sp. strain SIU, J. Environ. Biol., 36 (2015) 1215–1221.
[53] M.I.E. bin Halmi, S.R.S. Abdullah, H. Wasoh, W.L.W. Johari, M.S. bin M. Ali, N.A. Shaharuddin, M.Y. Shukor, Optimization and maximization of hexavalent molybdenum reduction to Mo-blue by *Serratia* sp. strain MIE2 using response surface methodology, RendicontiLincei, 27 (2016) 697–709.
[54] W.A. Ahmad, Z.A. Zakaria, Z. Zakaria, S. Surif, Hexavalent chromium reduction at different growth phases of *Acinetobacter haemolyticus*, Environ. Eng. Sci., 26 (2009) 1275–1278.
[55] J.L. Glenn, F.L. Crane, Studies on metalloflavoproteins. V. The action of silicomolybdate in the reduction of cytochrome c by aldehyde oxidase, Biochim. Biophys. Acta, 22 (1956) 111–115.
[56] M.Y.A. Shukor, C.H. Lee, I. Omar, M.I.A. Karim, M.A. Syed, N.A. Shamaan, Isolation and characterization of a molybdenum-reducing enzyme in *Enterobacter cloacae* strain 48, Pertanika J. Sci. Technol., 11 (2003) 261–272.
[57] Y.B. Patil, K.M. Paknikar, Development of a process for biodetoxification of metal cyanides from waste waters, Process Biochem., 35 (2000) 1139–1151.
[58] M. NallapanManiyam, F. Sjahrir, A.L. Ibrahim, A.E.G. Cass, Biodegradation of cyanide by *Rhodococcus* UKMP-5M, Biologia, 68 (2013) 177–185.
[59] M.Y. Shukor, Revisiting the role of the electron transport chain in molybdate reduction by *Enterobacter cloacae* strain 48, Indian J. Biotechnol., 13 (2014) 404–407.

Supplementary

Table S1
Plackett-Burman factorial model and analysis of variance (ANOVA) for molybdate reduction ANOVA for selected factorial model
Response 1: A865

Source		Sum of Squares		df	Mean Square	F-value	p-value	
Model			44.13	7	6.30	12.91	0.0132	Significant
A-Molybdate			16.03	1	16.03	32.82	0.0046	
B-Phosphate			4.31	1	4.31	8.82	0.0412	
C-pH			0.6864	1	0.6864	1.41	0.3015	
D-Temperature			0.0091	1	0.0091	0.0186	0.8982	
E-Sucrose			0.3040	1	0.3040	0.6224	0.4743	
F-Ammonium sulfate			0.3502	1	0.3502	0.7170	0.4448	
G-Time			22.44	1	22.44	45.94	0.0025	
Residual			1.95	4	0.4885			
Cor Total			46.08	11				
Std. Dev.	0.6989	R^2	0.9576					
Mean	10.00	Adjusted R^2	0.8834					
C.V. %	6.99	Predicted R^2	0.6884					
		Adeq Precision	11.4052					

Table S2
ANOVA for the fitted quadratic polynomial model to optimize molybdate reduction in strain HMY1

Source		Sum of Squares		df	Mean Square	F-value	p-value	
Model			288.78	9	32.09	28.23	< 0.0001	Significant
A-Molybdate Concentration			13.10	1	13.10	11.53	0.0068	
B-Phosphate Concentration			0.1985	1	0.1985	0.1746	0.6848	
C-Time			2.13	1	2.13	1.88	0.2005	
AB			1.22	1	1.22	1.07	0.3249	
AC			15.38	1	15.38	13.53	0.0043	
BC			21.11	1	21.11	18.58	0.0015	
A^2			31.96	1	31.96	28.12	0.0003	
B^2			134.81	1	134.81	118.62	< 0.0001	
C^2			118.59	1	118.59	104.35	< 0.0001	
Residual			11.36	10	1.14			
Lack of Fit			9.03	5	1.81	3.87	0.0818	Not significant
Pure Error			2.33	5	0.4664			
Cor Total			300.15	19				
Std. Dev.	1.06	R^2	0.9623					
Mean	8.35	Adjusted R^2	0.9285					
C.V. %	12.73	Predicted R^2	0.7675					
		Adeq Precision	15.6200					