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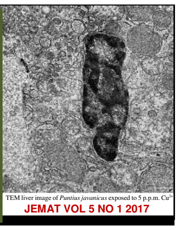

TEM liver image of *Puntius javanicus* exposed to 5 p.p.m. Cu^{2+}

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Modelling the Effect of Copper on the Mo-reduction Rate of the Antarctic Bacterium *Pseudomonas* sp. strain DRY1

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ABSTRACT

Molybdenum reduction by the Antarctic bacterium *Pseudomonas* sp. strain DRY1 is strongly inhibited by copper. Mo reduction by this bacterium at 10 mM sodium molybdate shows a sigmoidal pattern with lag periods ranging from 7 to 10 h at various concentrations of copper. As the concentration of copper was increased, the overall Mo reduction rate was inhibited with 1.2 mg/L causing the cessation of Mo reduction rate. The modified Gompertz model was utilized to obtain Mo reduction rates at different concentrations of copper. The Mo reduction rates obtained from the modified Gompertz model was then modelled according to the modified Han-Levenspiel, Wang, Liu, modified Andrews and the Amor models. Out of the five models, only Wang, modified Han-Levenspiel and the Liu models were able to fit the curve, whilst the modified Andrews and Amor models were unable to fit the curves. Both the Wang and modified Han-Levenspiel models show acceptable fitting while the Liu model shows poor fitting. Results of the statistical analysis showed that the modified Han-Levenspiel model was the best model based on the lowest values for RMSE and AICc, highest adjusted correlation coefficient (adR^2) and values of AF and BF closest to unity. The parameters obtained from the modified Han Levenspiel model, which were C_{crit}, μ_{max} and m which represent critical heavy metal ion concentration (mg/l), maximum reduction rate (nmole Mo blue/h) and empirical constant values were 0.225 (95%, confidence interval from 0.198 to 0.251), 1.200 (95%, confidence interval from 1.180 to 1.220) and 0.443 (95%, confidence interval from 0.261 to 0.626). The modified Han-Levenspiel accurately predicted the critical copper concentration that completely inhibited molybdenum reduction rate in this bacterium.

INTRODUCTION

The existence of toxic metal ions in polluted comprising wastewater displayed an inhibition influence on the bacterial growth and utilization of toxic substance. The presence of heavy metals can inhibit biodegradation and ultimately inhibit the bioremediation process. It is because of the fact that in contrast to a number of other inhibitors, heavy metal ions cannot be degraded and once accrued by microorganisms to a poisonous amount, this results in inhibition to the microorganism's growth rate. Therefore, modifications to the substrate inhibition model can be used to examine the inhibitory parameters caused by toxic ions. Numerous models such as the modified Han-Levenspiel [1], Wang [2], Liu [3], modified Andrews [4], Amor [5] have been utilised [6] to evaluate the result of heavy metal on the bacterial degradation of toxic substance. From these models inhibition related constants, which include C, C_{crit}, μ, μ_{max}, K_c, K_s, K_i and m which represent heavy metal ion concentration (g/l), critical heavy metal ion concentration (g/l), initial growth rate (g/l h), maximum growth rate (g/l h), inhibition constant (g/l), Monod constant (g/l), metal inhibition constant (g/l) and empirical constant values, respectively, can be found.

To date aside from these reports, there are almost no other reports on the effect of heavy metals on the growth rate of microorganisms as most reports on the effect of heavy metals on the primary models of the growth of microorganisms and not on secondary models. Furthermore, there is no report on the use of the above models in modelling the effect of metals on the rate of Mo reduction, a phenomenon that can be utilized for molybdenum bioremediation. As numerous Mo-reducing bacteria are affected strongly by coper [7–18], the aim of this study is to model the effect of this metal on the rate of reduction. A previously isolated Mo-reducing Antarctic bacterium was chosen for this study.

MATERIALS AND METHODS

Growth and maintenance of Mo-reducing bacterium

The Mo-reducing bacterium—*Pseudomonas* sp. strain DRY1 has been previously reported [19]. The growth and Mo reduction were carried out in a microplate format as before [20] utilizing the low phosphate molybdate medium (LPM) [19]. The microplates (Corning® microplate) were incubated and sealed at 15 °C and the absorbance after 72 h was read at 750 nm (BioRad reader, model 680, Richmond, CA).

Primary modelling on the rate of Mo reduction

The modified Gompertz model was utilized to obtain specific reduction rates and lag periods, all of which are important parameters that can be further used in modelling the effect of copper on the reduction rate [21–23,23–28]. The equation (**Eqn. 1**) is as follows;

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

The value obtained from this primary modelling exercise was then used to model the effect of metal as follows;

Models to study the Effect of metal on Mo reduction rate

The models (**Table 1**) utilized in this study are as follows;

Table 1. Various metal inhibition models.

Models	Equation	Authors
Modified Han-Levenspiel	$r = u_{max}\left(1 - \frac{C}{C_{crit}}\right)^m$	[1]
Wang	$r = \frac{u_{max}}{1 + \left(\frac{C}{K_C}\right)^m}$	[2]
Liu	$r = \frac{u_{max}K_C}{K_C + C}$	[3]
Modified Andrews	$r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[4]
Amor	$r = \frac{u_{max}C}{C + \left(\frac{C^2}{K_i}\right)}$	[5]

Fitting of the data

The nonlinear equations were fitted with a Marquardt algorithm using CurveExpert Professional software (Version 1.6). The algorithm searches the best method that minimizes the sum of the squares between predicted and measured values. The software calculates the starting values automatically via the steepest ascent method.

Statistical analysis

The use of numerous statistical methods such as the corrected AICc (Akaike Information Criterion), Root-Mean-Square-Error (RMSE), bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (R^2) is important as a criterion to select for the best model, and these statistical discriminatory functions will be used throughout this study [24].

RESULTS AND DISCUSSION

Molybdenum reduction by the bacterium exhibits a sigmoidal profile, which is a common theme seen in many Mo-reducing bacteria [29–31]. As the concentration of copper was increased a significant decrease in the maximum Mo reduction was observed together with the increase in lag periods (**Fig. 1**). A concentration of copper at 1.0 mg/L leads to a complete cessation of reduction. This was also observed in many Mo-reduction works [32–41].

To obtain Mo reduction rates at different concentrations of copper, the modified Gompertz model was utilized (**Fig. 2**), which shows close fitting to the model with adjusted correlation coefficients of between 0.96 and 0.99 indicating good fittings. The modified Gompertz model has been successfully utilized to model Mo reduction in various bacteria [30,41–43] and is much more accurate than the normal method of linearizing and otherwise sigmoidal model through log or natural log transformation. The model also shows that as the concentration of copper was increased, this led to a decrease in Mo reduction rates and an increase in the lag period as well.

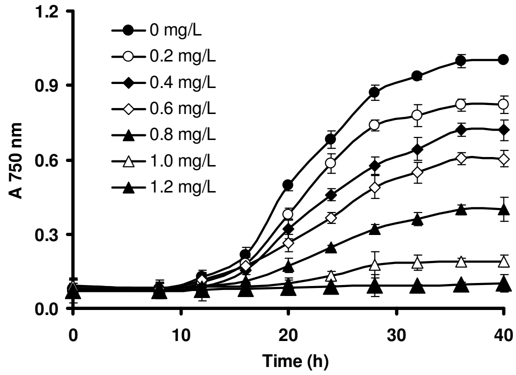

Fig. 1. Mo reduction rate by *Pseudomonas* sp. strain DRY1 at 10 mM sodium molybdate under various concentrations of copper (from 0.2 to 1.2 mg/L). The error bars represent the mean ± standard deviation of triplicates.

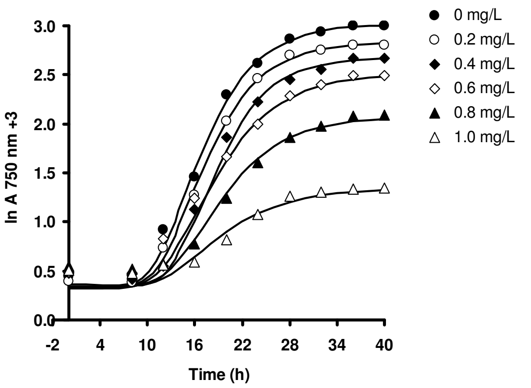

Fig. 2. Mo reduction rate (log transformed) of *Pseudomonas* sp. strain DRY1 at 10 mM sodium molybdate under various concentrations of copper (from 0.2 to 1.2 mg/L) as modelled using the modified Gompertz model.

The Mo reduction rates were then modelled using the available metal inhibition models at various copper concentrations. Wang, modified Han-Levenspiel and the Liu models were able to fit the curve, whilst the modified Andrews and Amor models were unable to fit the curves (**Figs. 3** to **5**). The Liu model shows poor fitting while both the Wang and modified Han-Levenspiel models show visually acceptable fitting. Results of the statistical analysis showed that the modified Han-Levenspiel model was the best model based on the lowest values for RMSE and AICc, highest adjusted correlation coefficient (*adR*^{2}) and values of AF and BF closest to unity (**Table 2**).

Table 2. Statistical analysis of the metal inhibition model.

Model	*p*	RMSE	*adR*^{2}	AF	BF	AICc
mod H-L	3	0.012	0.879	1.041	0.973	-47.20
Wang	3	0.014	0.837	1.063	0.979	-44.28
Liu	2	0.028	0.453	1.117	0.937	-43.78
Andrews	4	n.a.	n.a.	n.a.	n.a.	n.a.
Amor	3	n.a.	n.a.	n.a.	n.a.	n.a.

Note:
p no of parameter
mod H-L modified Han-Levenspiel
adR^2 adjusted correlation coefficient
RMSE Root mean square error
AF Accuracy factor
BF Bias factor
AICc corrected Akaike Information Criteria
n.a. not available

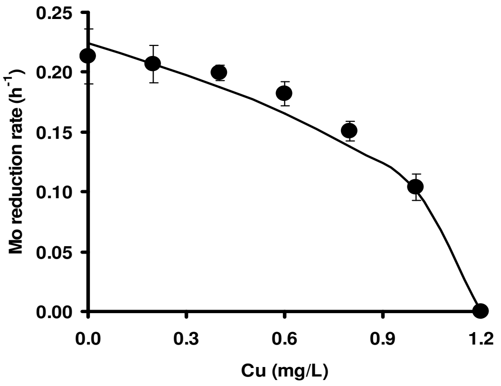

Fig. 3. Mo reduction rate by *Pseudomonas* sp. strain DRY1 at 10 mM sodium molybdate under various concentrations of copper (up to 1.2 mg/L) as modelled using the modified Han-Levenspiel model.

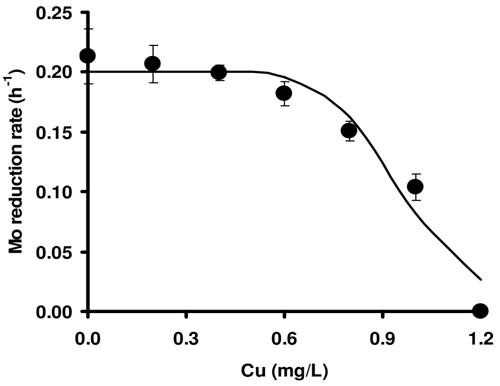

Fig. 4. Mo reduction rate by *Pseudomonas* sp. strain DRY1 at 10 mM sodium molybdate under various concentrations of copper (up to 1.2 mg/L) as modelled using the Wang model.

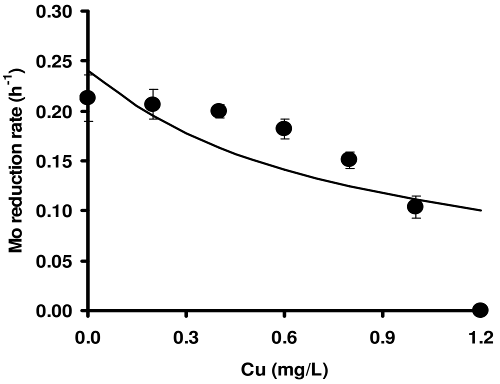

Fig. 5. Mo reduction rate by *Pseudomonas* sp. strain DRY1 at 10 mM sodium molybdate under various concentrations of copper (up to 1.2 mg/L) as modelled using the Liu model.

The parameters obtained from the modified Han Levenspiel model, which were C_{crit}, μ_{max} and m which represent critical heavy metal ion concentration (mg/l), maximum reduction rate (nmole Mo blue/h) and empirical constant values were 0.225 (95%, confidence interval from 0.198 to 0.251), 1.200 (95%, confidence interval from 1.180 to 1.220) and 0.443 (95%, confidence interval from 0.261 to 0.626). The modified Han-Levenspiel accurately predicted the critical copper concentration that completely inhibited molybdenum reduction rate in this bacterium. Previous researches have shown that the modified Han-Levenspiel model is the best model to fit the effect of inhibitor including salts and heavy metals on the growth or product formation rates [1,6].

In a related study, the growth rate of the bacterium *Enterobacter* sp. strain Neni-13 on SDS in the presence of various concentrations of mercury was modelled according to the various metal inhibition model similar to this study [42]. The best model was Wang. The Wang model can predict the critical mercury concentration that completely inhibited bacterial growth on SDS. The parameters obtained which are C_{crit}, μ_{max} and m, representing critical heavy metal ion concentration (mg/l), maximum growth rate (g/l h) and empirical constant values were 0.216, 1.05 and 0.389, respectively.

The usage of metal inhibition models is inadequately depicted in the literature regardless of the significance of such research considering the reality that heavy metals are ubiquitously found in contaminated waters together with organic pollutants. A number of research has investigated on the inhibitory effect of heavy metals of the growth rate of bacteria growing on toxic substances. In one such study, zinc and nickel inhibit the biodegradation rate of monoaromatic hydrocarbons by *Bacillus* sp. and *Pseudomonas* sp. and the Andrews model was successfully utilized to model the inhibition [5].

These heavy metals including copper inhibit the degradation pathway by binding to functional groups of proteins and enzymes, rendering these enzyme inactive [6]. In order to reduce metal ions toxic effect, bacteria use a battery of mechanisms including complexation with the binding of metal ions to cell surfaces, metallothionen, biomethylation, development of efflux pumps, and the removal of metal ions by precipitation [43]. These strategies are important for bacterial resistance against toxic metal ions in future selection of copper tolerant Mo-reducing bacteria.

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CONCLUSION

To conclude, the usage of metal inhibition models to model the result of metal ions to the rate of growth of bacteria or rate of transformation including metal reduction rate on toxic substances is extremely uncommon and generally overlooked regardless of the significance of these kinds of study. In this study, the effect of copper on the molybdenum reduction rate was modelled in accordance with several metal inhibition models, with the modified Han-Levenspiel model identified as the most effective model. The model permits the forecast of the crucial copper concentration which can totally inhibit the rate of molybdenum reduction. It is anticipated that in the existence of heavy metals, the reduction rate is going to be actually highly afflicted as the bacterium need to withstand the toxicity of both kind of toxicants simultaneously. The outcomes out of this study can be quite essential in field trial works where molybdenum reduction for bioremediation is desired in areas co-contaminated with copper.

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REFERENCES

1. Wang J, Wan W. Kinetic models for fermentative hydrogen production: a review. Int J Hydrog Energy. 2009;34(8):3313–3323.
2. Wang Y, Zhao Q-B, Mu Y, Yu H-Q, Harada H, Li Y-Y. Biohydrogen production with mixed anaerobic cultures in the presence of high-concentration acetate. Int J Hydrog Energy. 2008;33(4):1164–1171.
3. Liu X, Zhu Y, Yang S-T. Construction and characterization of ack deleted mutant of *Clostridium tyrobutyricum* for enhanced butyric acid and hydrogen production. Biotechnol Prog. 2006;22(5):1265–1275.
4. Andrews JF. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnol Bioeng. 1968 Nov 1;10(6):707–23.
5. Amor L, Kennes C, Veiga MC. Kinetics of inhibition in the biodegradation of monoaromatic hydrocarbons in presence of heavy metals. Bioresour Technol. 2001 Jun 1;78(2):181–5.
6. Gopinath KP, Kathiravan MN, Srinivasan R, Sankaranarayanan S. Evaluation and elimination of inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by *Pseudomonas* sp. mutant. Bioresour Technol. 2011;102(4):3687–3693.
7. Shukor MY, Shamaan NA, Syed MA, Lee CH, Karim MIA. Characterization and quantification of molybdenum blue production in *Enterobacter cloacae* strain 48 using 12-molybdophosphate as the reference compound. Asia-Pac J Mol Biol Biotechnol. 2000;8(2):167–72.
8. Shukor MY, Habib SHM, Rahman MFA, Jirangon H, Abdullah MPA, Shamaan NA, et al. Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain Dr. Y6. Appl Biochem Biotechnol. 2008;149(1):33–43.
9. 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.
10. Shukor MY, Bakar NA, Othman AR, Yunus I, Shamaan NA, Syed MA. Development of an inhibitive enzyme assay for copper. J Environ Biol. 2009;30(1):39–44.
11. 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.
12. Yunus SM, Hamim HM, Anas OM, Aripin SN, Arif SM. Mo(VI) reduction to molybdenum blue by *Serratia marcescens* strain Dr. Y 9. Pol J Microbiol. 2009;58(2):141–147.
13. 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.
14. Shukor MY, Rahman MF, Suhaili Z, Shamaan NA, Syed MA. Hexavalent molybdenum reduction to Mo-blue by *Acinetobacter calcoaceticus*. Folia Microbiol (Praha). 2010;55(2):137–43.
15. 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.
16. Ahmad SA, Halmi MIE, Shukor MY, Shamaan NA, Syed MA. Effect of metal ions on the molybdenum-reducing activity of *S. marcescens* strain DrY6. J Environ Microbiol Toxicol. 2013;1(1):5–8.
17. Halmi MIE, Zuhainis SW, Yusof MT, Shaharuddin NA, Helmi W, Shukor Y, et al. Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate- degrading *Klebsiella oxytoca* strain DRY14. BioMed Res Int. 2013;2013:Article number 384541.
18. Othman AR, Bakar NA, Halmi MIE, Johari WLW, Ahmad SA, Jirangon H, et al. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi. BioMed Res Int. 2013;2013:Article number 371058.
19. Ahmad SA, Shukor MY, Shamaan NA, Mac C, Syed MA. Molybdate reduction to molybdenum blue by an Antarctic bacterium. BioMed Res Int. 2013;2013:Article number 871941.
20. Shukor MS, Shukor MY. A microplate format for characterizing the growth of molybdenum-reducing bacteria. J Environ Microbiol Toxicol. 2015;2(2).
21. Christen P, Vega A, Casalot L, Simon G, Auria R. Kinetics of aerobic phenol biodegradation by the acidophilic and hyperthermophilic archaeon *Sulfolobus solfataricus* 98/2. Biochem Eng J. 2012;62:56–61.
22. Basak B, Bhunia B, Dutta S, Chakraborty S, Dey A. Kinetics of phenol biodegradation at high concentration by a metabolically versatile isolated yeast *Candida tropicalis* PHB5. Environ Sci Pollut Res. 2014;21(2):1444–1454.
23. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modeling of the growth kinetics of *Bacillus* sp. on tannery effluent containing chromate. J Environ Bioremediation Toxicol. 2014;2(1):6–10.
24. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Evaluation of several mathematical models for fitting the growth of the algae *Dunaliella tertiolecta*. Asian J Plant Biol. 2014;2(1):1–6.
25. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Modeling the growth kinetics of *Chlorella vulgaris* cultivated in microfluidic devices. Asian J Plant Biol. 2014;2(1):7–10.

26. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Modeling the growth curves of *Acinetobacter* sp. strain DRY12 grown on diesel. J Environ Bioremediation Toxicol. 2014;2(1):33–7.
27. Shukor M. Modelling the growth of Moraxella sp. B on monobromoacetic acid (MBA). Bull Environ Sci Manag. 2015;3(1):1–6.
28. Halmi MIE, Shukor MS, Masdor NA, Shamaan NA, Shukor MY. Evaluation of several mathematical models for fitting the growth of sludge microbes on PEG 600. J Environ Microbiol Toxicol. 2015 Oct 30;3(1):1–5.
29. Halmi MIE, Ahmad SA, Syed MA, Shamaan NA, Shukor MY. Mathematical modelling of the molybdenum reduction kinetics in *Bacillus pumilus* strain Lbna. Bull Environ Sci Manag. 2014;2(1):24–9.
30. Othman AR, Johari WLW, Dahalan FA, Shukor MY. Mathematical modeling of molybdenum blue production from *Serratia marcescens* strain DR. Y10. Bioremediation Sci Technol Res. 2015;3(2):1–6.
31. Yakasai MH, Ibrahim KK, Yasid NA, Halmi MIE, Rahman MFA, Shukor MY. Mathematical modelling of molybdenum reduction to mo-blue by a cyanide-degrading bacterium. Bioremediation Sci Technol Res. 2016;4(2):1–5.
32. Halmi MIE bin, Abdullah SRS, Wasoh H, Johari WLW, Ali MS bin M, Shaharuddin NA, et al. Optimization and maximization of hexavalent molybdenum reduction to Mo-blue by *Serratia* sp. strain MIE2 using response surface methodology. Rendiconti Lincei. 2016 Dec 1;27(4):697–709.
33. Mansur R, Gusmanizar N, Dahalan FA, Masdor NA, Ahmad SA, Shukor MS, et al. Isolation and characterization of a molybdenum-reducing and amide-degrading *Burkholderia cepacia* strain neni-11 in soils from west Sumatera, Indonesia. IIOAB. 2016;7(1):28–40.
34. Mansur R, Gusmanizar N, Roslan MAH, Ahmad SA, Shukor MY. 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. 2017 Jan;28(1):69–90.
35. Mohamad O, Yakasai HM, Karamba KI, Halmi MIE, Rahman MF, Shukor MY. Reduction of molybdenum by *Pseudomonas aeruginosa* strain KIK-11 Isolated from a metal-contaminated soil with sbility to grow on diesel and Sodium Dodecyl Sulphate. J Environ Microbiol Toxicol. 2017;5(2):19–26.
36. Sabullah MK, Rahman MF, Ahmad SA, Sulaiman MR, Shukor MS, Gansau AJ, et al. Isolation and characterization of a molybdenum-reducing and phenolic- and catechol-degrading *Enterobacter* sp. strain saw-2. BIOTROPIA - Southeast Asian J Trop Biol. 2017 May 22;24(1):47–58.
37. Abo-Shakeer LKA, Rahman MFA, Yakasai MH, Bakar NA, Othman AR, Syed MA, et al. Kinetic studies of the partially purified molybdenum-reducing enzyme from *Bacillus pumilus* strain lbna. Bioremediation Sci Technol Res. 2017 Jul 31;5(1):18–23.
38. Sabullah MK, Rahman MF, Ahmad SA, Sulaiman MR, Shukor MS, Shamaan NA, et al. Assessing resistance and bioremediation ability of *Enterobacter* sp. strain saw-1 on molybdenum in various heavy metals and pesticides. J Math Fundam Sci. 2017 Oct 3;49(2):193–210.
39. Yakasai MH, Rahman MFA, Rahim MBHA, Khayat ME, Shamaan NA, Shukor MY. Isolation and characterization of a metal-reducing *Pseudomonas* sp. strain 135 with amide-degrading capability. Bioremediation Sci Technol Res. 2017 Dec 31;5(2):32–8.
40. Yakasai MH, Ibrahim KK, Yasid NA, Halmi MIE, Rahman MFA, Shukor MY. Mathematical modelling of molybdenum reduction to mo-blue by a cyanide-degrading bacterium. Bioremediation Sci Technol Res. 2016 Dec 31;4(2):1–5.
41. Shukor MS, Shukor MY. Bioremoval of toxic molybdenum using dialysis tubing. Chem Eng Res Bull. 2015;18(1):6–11.
42. Shukor MY, Gusmanizar N, others. Modelling the effect of heavy metals on the growth rate of *Enterobacter* sp. strain neni-13 on SDS. J Environ Microbiol Toxicol. 2018;In press.
43. Porcheron G, Garénaux A, Proulx J, Sabri M, Dozois CM. Iron, copper, zinc, and manganese transport and regulation in pathogenic Enterobacteria: correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. Front Cell Infect Microbiol. 2013;3:90.