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*Short Communication*

# Bioremoval of Molybdenum from Aqueous Solution

**Mohd Izuan Effendi Halmi[1], Helmi Wasoh[2], Surani Sukor[1], Siti Aqlima Ahmad[1], Mohd Termizi Yusof[3] and Mohd Yunus Shukor[1*]**

[1]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Malaysia*
[2]*Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia*
[3]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Malaysia*
*For correspondence: yunus.upm@gmail.com; mohdyunus@upm.edu.my

## Abstract

Molybdenum is very toxic to ruminants with level as low as 2 parts per million can cause severe scouring. Its contamination of waters and soils in agricultural areas needs novel removal technology. In this work we demonstrated a novel method of molybdenum removal from aqueous solution using the dialysis tubing method coupled with molybdenum-reducing activity of *Serratia* sp. strain Dry5. The enzymatic reduction of molybdenum is molybdenum blue, a colloid that does not pass through dialysis tubing. The calculated maximal rate of molybdenum blue production ($V_{MoblueMax}$) was 0.264±0.034 mM (Mo-blue h)$^{-1}$ and the concentration of molybdate resulting in the half-maximal rate of reduction ($K_{Mo}$) was 21.78±3.89 mM molybdate and the specific maximal rate of Mo-blue production was approximately 80 mM (Mo-blue.hr.mg cells)$^{-1}$ indicating an efficient system with high tolerance towards molybdenum. © 2014 Friends Science Publishers

**Keywords:** Agriculture; Molybdenum; Molybdenum blue; Dialysis tubing; *Serratia* sp.

## Introduction

Molybdenum is one of the heavy metals of which its pollution is reported globally (Davis, 1991). For example, the sea of the Tokyo Bay in Japan is contaminated with molybdenum reaching up to several hundreds of ppm (Davis, 1991). Molybdenum is relatively low in toxicity to human but it is known to be very toxic to ruminant animals. The scouring in cows was reported after grazing in areas contaminated with molybdenum at up to 5 ppm (Sas, 1989). In Tirol, Austria, large pasture areas have been found to be contaminated with molybdenum with level as high as 200 ppm. It is here that the first case of bioremediation of molybdenum was attempted using a combination of plants and microbes (Neunhäuserer *et al*., 2001). In Malaysia, several cases of heavy metals pollution including molybdenum has been reported due to accidental leakage of effluents from broken pipings of a copper mining that is closed now. Heavy metals pollution from this source has been reported in the surrounding paddy field areas and jeopardizing the agricultural sector in this area (Kosaka and Wakita, 1978; Yong, 2000). The use of bacteria in metal removal has been extensively studied. One of the mechanisms of metal removal is via enzymatic reduction of metal into a less toxic precipitable form. Molybdenum reduction to molybdenum blue (Mo-blue) is a striking example with the reduced product exhibiting an intense blue precipitable mass (Campbell *et al*., 1985). Molybdate reduction by microbes to Mo-blue has been reported since the last one hundred years (Capaldi and Proskauer, 1896; Campbell *et al*., 1985; Sugio *et al*., 1988; Ghani *et al*., 1993; Shukor *et al*., 2000; Rahman *et al*., 2009; Shukor *et al*., 2008, 2010a, 2010b). Komori *et al*. (1990) were the first to report on the bioremoval of chromate using dialysis tubing. The dialysis tubing method is an attractive bioremoval system as other immobilized systems tend to get clog or become impossible to remove the entangled mass of matrix, cells and reduced heavy metals precipitates. We report on the potential use of this method in molybdenum removal.

## Materials and Methods

### Growth and Maintenance of *Serratia* sp. Strain DRY5

*Serratia* sp. strain DRY5 was originally isolated from an abandoned metal recycling ground near the King Edward VII (2$^{nd}$) Primary School in the city of Taiping, State of Perak, Malaysia. The bacterium exhibits strong Mo-reducing capacity (Rahman *et al*., 2009). The growth and maintenance of *Serratia* sp. strain DRY5 was maintained on a solid agar of low phosphate (2.9 m*M* phosphate) media (pH 7.0) containing (%w/v) sucrose (1.0), $MgSO_4.7H_2O$ (0.05), $(NH_4)_2SO_4$ (0.3), yeast extract (0.05), NaCl (0.5), $Na_2HPO_4$ (0.073) and $Na_2MoO_4.2H_2O$ (0.726) (Rahman *et al*., 2009). The carbon source was autoclaved separately. For growth in liquid media 100 mM phosphate was used

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and this is called high phosphate media (HPM). For large-scale growth, *Serratia* sp. strain DRY5 was grown in 5 L of HPM at 30°C for 48 h on an orbital shaker at 100 rpm (Kubota). The production of molybdenum blue from the media was measured at 865 nm using the specific extinction coefficient of 16.7 $mM^{-1}cm^{-1}$ (Shukor *et al*., 2000).

### Dialysis Tubing Experiment

Cells were centrifuged for 10 min at 15,000 g. The pellet was resuspended in low phosphate solution at pH 7.0 containing (%w/v) $MgSO_4.7H_2O$ (0.05), $(NH_4)_2SO_4$ (0.3), $Na_2HPO_4$ (0.05), NaCl (0.5) and yeast extract (0.05) (Shukor *et al*., 2002) to an absorbance of 1.0 at 600 nm. About 10 mL of this suspension was transferred into a dialysis tubing that had been previously boiled for 10 min. The tubing was then immersed in 100 mL of sterile LPM media at pH 7.0 containing various concentrations of sodium molybdate and incubated statically at 30°C. For the control, 10 mL of the cell suspension was placed in a polypropylene tube and boiled for 10 min and cooled down and placed inside the dialysis tubing. Aliquots (1 mL) of the media were periodically taken and then centrifuged for 15 min at 15,000 g. The supernatant was read at 865 nm. Experiments were carried out in triplicate.

### Data and Statistical Analysis

Data were analyzed using the statistical software Graphpad Prism available from www.graphpad.com. Values are reported as mean ± SE. a Student's t-test or a one-way analysis of variance with post hoc analysis by Tukey's test was used for comparison between groups and $P < 0.05$ was considered statistically significant.

## Results

### Mo-blue Production Rate

Fig. 1 shows the increase in the production of Mo-blue inside of the dialysis tubing at different concentrations of sodium molybdate. The increase in Mo-blue production was approximately linear at all molybdate concentrations. Boiled cells showed no Mo-blue production (Data not shown). When the initial rate of increase of Mo-blue product was plotted against molybdate concentration, a saturation kinetics profile similar to the Michaelis-Menten enzyme kinetics (Eqn. 1) was obtained (Fig**.** 2). The calculated maximal rate of Mo-blue production ($V_{MoblueMax}$) was 0.264±0.034 mM $(Mo\text{-}blue.hr)^{-1}$ and the concentration of molybdate resulting in the half-maximal rate of reduction ($K_{Mo}$) was 21.78±3.89 mM molybdate. The high cell density in the dialysis tube probably allows for maximal reduction in molybdate concentrations that should be inhibitory to free cells as seen in Fig. 2. As the initial cell density was about 0.0033 mg dry cells $mL^{-1}$ at an OD 600 nm of 1.0, the specific maximal rate of Mo-blue production was approximately 80 mmole $(Mo\text{-}blue.hr)^{-1}$ per mg cells.

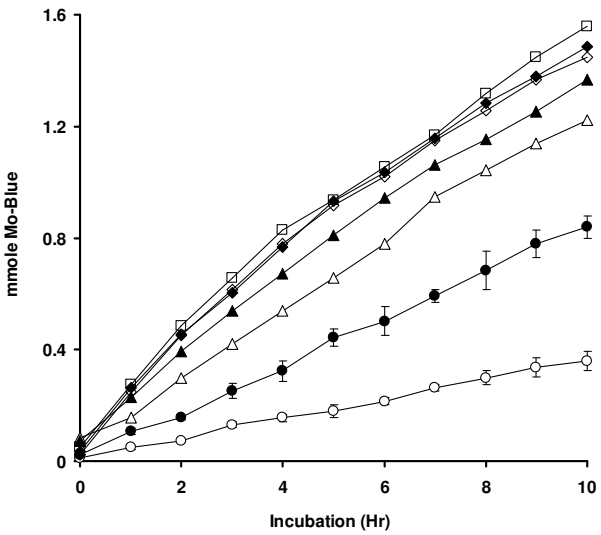


**Fig. 1:** Mo-blue produced in dialysis tubing by *Serratia* sp. strain DRY5 at 10 (○), 20 (●), 30 (△), 40 (▲), 50 (◇), 60 (◆), and 70 (□) m*M* sodium molybdate.

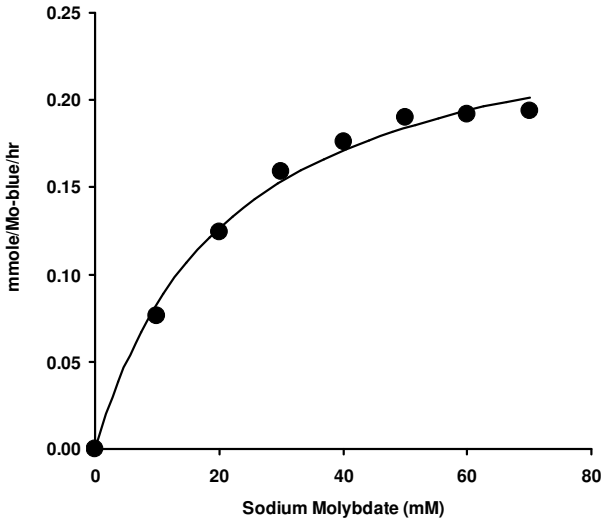


**Fig. 2:** The rate of increase of molybdenum blue at various substrate (molybdate) concentrations

## Discussion

Mo-blue is a sensitive test for the presence of chemical-reducing agents (Killefer and Linz, 1952). This means that many reducing agents, organic or inorganic are capable of reducing molybdate (and molybdophosphate) to Mo-blue. Hence, it would be difficult to know whether the reduction is either enzymatic or due to bioreductants produced by the cells. Both processes could also contribute simultaneously to the overall Mo-reducing activity. We have shown previously that the dialysis tubing method could be used as a distinguishing technique for this purpose.

We exploited the colloidal property of the molybdenum blue product in the removal process for molybdenum from aqueous environment.

This high rate or Mo-blue production is not surprising since *E. cloacae* strain 48 could tolerate and reduce sodium molybdate at concentration as high as 200 mM (Ghani *et al*., 1993). Theoretically, the maximal rate of reduction of molybdate should be achieved using saturated molybdate concentrations at approximately 5 times the $K_{Mo}$ value (~100 mM). For practical purposes, the highest concentrations of molybdate ever reported in soil or water bodies as a pollutant is approximately 2000 ppm or 20.8 mM (Runnells, 1976). Metal removal using similar strategy of membranous bacterial biofilm supported on PVC have been attempted for the removal of the toxic chromate with 72.6% removal rate achieved with an influent concentration of 200 ug $L^{-1}$ Cr (Zhang *et al*., 2011).

$$v_{MoBlue} = v_{MoBlueMax} \frac{Mo}{K_{Mo} + Mo} \quad (1)$$

In conclusion, the dialysis tubing method could be exploited as a tool for bioremediation especially for molybdenum in waste water effluents or pretreatment system. Heavy metals could affect production of agricultural products and their removal or remediation is highly sought (Angin and Yaganoglu, 2012; Abdollahi *et al*., 2011). Hence, this novel technology in the case of molybdenum could be pursued further in dealing with actual water bodies or soils contaminated with molybdenum. The removal rate reflects an efficient removal system and would benefit molybdenum-polluted agricultural areas and water bodies that are used as drinking water for ruminants and for irrigation.

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