Effect of different metals on the chromate reductase activity of *Magnetospirillum gryphiswaldense* MSR-1

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Abstract Bioreduction of hexavalent chromium Cr(VI) from industrial wastewater has been extensively studied because of the process's low cost and high effectiveness. While several bacteria were found to be capable of reducing Cr(VI) to the less toxic Cr(III), reduction in the presence of other pollutants is usually not studied. This study explores the effect of other metals on the chromium reduction capabilities of the magnetotactic bacteria *Magnetospirillum gryphiswaldense* (MSR-1). The bioreduction ability was measured using the activity of the bacteria's chromate reductase, an enzyme group that enables them to perform reduction from Cr(VI) directly to Cr(III). The results showed that the presence of other heavy metals significantly limited the activity of chromate reductase. Mercury was identified to be the strongest inhibitor, reducing the chromium reduction efficiency from 28.27% to 13.67%, followed by copper, zinc, and then nickel. These findings can be used to identify which heavy metals should be treated first to achieve the best chromium removal from wastewater.

Keywords: magnetotactic bacteria, heavy metal, chromate reductase, enzyme crude extract

1. Introduction

Hexavalent chromium Cr(VI) in the environment poses great risks due to its toxicity, carcinogenicity, and mutagenicity (Ye et al., 2023). Reduction of Cr(VI) to the less hazardous Cr(III) is an essential method to manage chromium contamination in wastewater.

Utilizing bioremediation to address this has gained attention due to its cost-effectiveness, high efficiency, and environmental compatibility (Ye et al., 2023). Use of magnetotactic bacteria (MTB) gives an advantage of being separable from the effluent due to its magnetosomes (MS). These are membrane-bound crystals that allow MTB to employ magnetotaxis, which is the movement directed by local magnetic field (Gareev et al., 2021). Results from Wu et al. (2023) have already proven that *Magnetospirillum gryphiswaldense*, an MTB strain, is capable of removing to 40 mg/L Cr(VI).

Chromate-bearing wastewaters are usually mixed with other heavy metals that can be toxic to different organisms such as mercury, nickel, copper, and zinc (Ayele and Godeto, 2021). These coexisting ions affect the chromium reduction abilities and mechanisms of bacteria (Chen and Tian, 2021). Understanding the interactions of such contaminants with the bacterial enzymes is crucial to the control of chromium pollution using microbial methods. Thus, the present work explores the effect of the presence of different heavy metals on the chromate reductase activity of MSR-1.

2. Materials and methods

2.1. *Bacterial strains and medium preparation*

The bacteria *Magnetospirillum gryphiswaldense* MSR-1^T (DSMZ 6361) was obtained from Leibniz-DSMZ (Deutsche Sammlung van Mikrooganismen und Zellkulturen GmbH, Germany). The pre-cultures were grown in flask standard medium (FSM) as prepared by Heyen and Schuler (2003). Control is grown in sterilized ionic washing buffer (IWB) added with potassium lactate and soybean peptone. All other samples use this formulation as their base. IWB is composed of 0.1 g KH_2PO_4, 0.34 g $NaNO_3$, and 1 mL EDTA chelated trace mixture (Widdel and Bak, 1992) solution per liter. Potassium lactate serves as the carbon source and soybean peptone was selected because of MSR-1's preference to it. The added amounts of these two were based on FSM.

2.2. *Sample preparation and measurements*

MSR-1 was shaken cultured in microaerobic (1%O_2:99%N_2) serum bottles sealed with butyl-rubber stopper containing 5 mL FSM for 48 hours at culture conditions of 30ºC and shaking at 120 rpm (Wu et al., 2023). This was then combined with 35 mL FSM in 100 mL-serum bottles and shaken-cultured under the same conditions for 16 more hours. The resulting optical density was measured using UV-Vis spectrophotometer at 565 nm

(OD_{565}). The cells were washed twice with 40 mL IWB and centrifuged at 4°C and 8500 rpm for 10 minutes.

The enzyme crude extracts were prepared by resuspending the washed cells to IWB and sonicating with cycle of alternating 30s ultrasonic wave and 30s rest for a total of 10 minutes. Using the calculated OD, samples were extracted and resuspended to appropriate amounts of IWB to make 100 mL of samples with supposed OD_{565} of 0.5. The sulfate salts of the other metal ions (Cu(II), Zn(II), Ni(II), and Hg(II)) were added at 1 mM concentrations. Cr(VI) as potassium dichromate ($K_2Cr_2O_7$) was supplemented to a final concentration of 10 mg/L. The 1,5-diphenylcarbazide (DPC) method was used to calculate the Cr(VI) concentration of the samples and absorbance values were measured at 540 nm (Su et al., 2021).

3. Results and Discussions

Cr(VI) reduction efficiencies of MSR-1 in the presence of different heavy metals are shown in Figure 1.

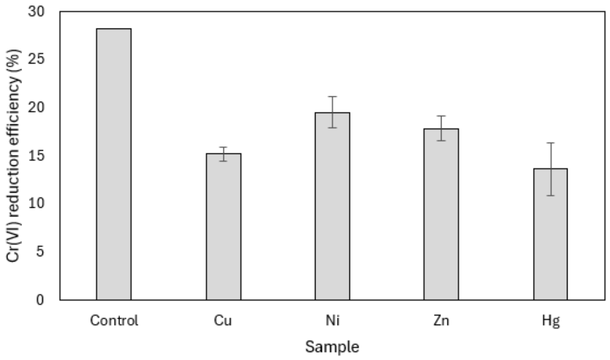

Figure 1. Cr(VI) reduction efficiencies of MSR-1 enzyme crude extract exposed to different heavy metals.

The addition of the metal sulfates led to chromium reduction inhibition of MSR-1's chromate reductase on varying degrees. Metal ions, in general, affect microbial chromate reduction either by destroying cells or by inhibition of the enzyme responsible for reduction. The latter usually occurs when metals form complexes that lead to inactivation of the reductase enzymes (Soni et al., 2012).

The presence of mercury resulted in the biggest decrease in efficiency to 13.67% versus 28.27% efficiency of the control. Mercurial salts also decreased chromate reductase activities in other bacteria such as *P. putida*, *Bacillus* sp., and *E. coli* ATCC 33456. These results may be due to mercury binding on the enzyme systems and causing denaturation to the reductase protein (Xu et al., 2012).

On the other hand, nickel had the least influence on the chromate at 19.53% reduction efficiency. Meanwhile, the addition of zinc resulted in a 17.87% reduction. Zinc had varying effects on different bacterial species' chromium reduction, but its inhibition was mostly credited to the possibility of it forming mercaptide bonds with sulfhydryl groups of the enzyme molecule (Li et al., 2016).

Various studies showed that copper in low concentrations stimulated the chromate reduction in several bacteria citing its ability to act as an electron redox center and aid in shuttle of electrons between enzyme subunits (Xu et al., 2012). However, excessive concentration may have led to inhibitory effects (Chen and Tian, 2021). In this study, the addition of copper still resulted in the inhibition of chromate reductase of MSR-1. The trend of the effects of the metal ions based on the reduction efficiencies is Ni(II) > Zn(II) > Cu(II) > Hg(II).

References

Ayele, A. and Godeto, Y.G. (2021). Bioremediation of Chromium by Microorganisms and Its Mechanisms Related to Functional Groups. Journal of chemistry.

Branco, R., Chung, A. P., Johnston, T., Gurel, V., Morais, P., and Zhitkovich, A. (2008). The Chromate-Inducible chrBACF Operon from the Transposable Element TnOtChr Confers Resistance to Chromium(VI) and Superoxide. Journal of Bacteriology, 190(21), 6996–7003. d

Chen, J., and Tian, Y. (2021). Hexavalent chromium reducing bacteria: mechanism of reduction and characteristics. Environmental Science and Pollution Research, 28(17), 20981–20997. doi: 10.1007/s11356-021-13325-7

Gareev, K. G., Grouzdev, D. S., Kharitonskii, P. V., Kosterov, A., Koziaeva, V. V., Sergienko, E. S., & Shevtsov, M. A. (2021). Magnetotactic Bacteria and Magnetosomes: Basic Properties and Applications. Magnetochemistry, 7(6), 86. doi: 10.3390/magnetochemistry7060086

Heyen, U. and Schuler, D. (2003) Growth and magnetosome formation by microaerophilic *Magnetospirillum* strains in an oxygen-controlled fermentor. Appl. Microbio. Biotechnol. 61, 536-544. doi: 10.1007/s00253-002-1219-x

Li, N., Pan, Y., Zhang, N., Wang, X., and Zhou, W. (2016). The bio-reduction of chromate with periplasmic reductase using a novel isolated strain Pseudoalteromonas sp. CF10-13. RSC Advances, 6(108), 106600–106607.

Soni, S. K., Singh, R., Awasthi, A., Singh, M., and Kalra, A. (2012). In vitro Cr(VI) reduction by cell-free extracts of chromate-reducing bacteria isolated from tannery effluent irrigated soil. Environmental Science and Pollution Research, 20(3), 1661–1674.

Su, Y.-Q., Yuan, S., Guo, Y.-C., Tan, Y.-Y., Mao, H.-T., Cao, Y., and Chen, Y.-E. (2021). Highly efficient and sustainable removal of Cr (VI) in aqueous solutions by photosynthetic bacteria supplemented with phosphor salts. Chemosphere, 283, 131031.

Widdel, F., and Bak, F. (1992). Gram-Negative Mesophilic Sulfate-Reducing Bacteria. The Prokaryotes, 3352–3378.

Wu, S., Hsiao, W.-C., Zhao, Y-C., and Wu, L.-F. (2023). Hexavalent chromate bioreduction by a magnetotactic bacterium *Magnetospirillum gryphiswaldense* MSR-1 and the effect of magnetosome synthesis. Chemosphere 330, 138739. doi: 10.1016/j.chemosphere.2023.138739

Xu, L., Luo, M., Jiang, C., Wei, X., Kong, P., Liang, X., Zhao, J., Yang, L., & Liu, H. (2012). In vitro reduction of hexavalent chromium by cytoplasmic fractions of Pannonibacter phragmitetus LSSE-09 under aerobic and anaerobic conditions. Applied biochemistry and biotechnology, 166(4), 933–941. doi: 10.1007/s12010-011-9481-y

Ye, Y., Hao, R., Shan, B., Zhang, J., Li, J., and Lu, A. (2023). Reduction and Removal Mechanism of Cr(VI) by a Novel *Penicillium Rubens* LR6. Geomicrobiology Journal. doi: 10.1080/01490451.2023.2293736