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EFFECTS OF ZINC (Zn) AND CHROMIUM (Cr) ON THE PHENOL-DEGRADING BACTERIA GROWTH KINETICS

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Abstract

Heavy metals occur naturally within the earth crust; however, anthropogenic activities can artificially introduce these elements into the environment. Despite being the foremost isolated continent, Antarctica is not free from human contamination. Heavy metals are well-known to be the powerful inhibitors of xenobiotics biodegradation activities. A microbial growth model was presented for bacteria cell growth in the biodegradation of phenol containing heavy metals such as zinc (Zn) and chromium (Cr) ions. The Gompertz model was used to estimate three main growth parameters namely lag phase (λ), maximum growth rate (μ_{maz}), and maximum cell number at the stationary phase (N_{max}). Bacterial growth for both heavy metals was shown to be properly fit towards the curve with a high value of R^2 and low square root of the variance of residuals (RSME) value. The effect of heavy metals at 1.0 ppm showed that Cr has a considerable effect on bacteria consortium, inhibiting the degradation of phenol, while Zn has no effect, removing 100% of phenol. The predicted biokinetic from this model suggests the suitability of the bacteria consortium to be used in phenol removal.

INTRODUCTION

Antarctica, the foremost farther region on Earth, is mostly recognised as the last largely pristine and closed ecosystem. It has a crucial impact on the global atmosphere system and the study of past, present and future environments [1]. Despite its geographic isolation, growing human activities in Antarctica have caused different environmental emergencies in recent decades. Bargagli [2] and Burton-Johnson et al. [3] stated that only less than 2% of Antarctica is ice-free; yet, most human and terrestrial biological activities are packed in these regions. Tin et al. [4] reviewed that the most commonly found contaminants in the Antarctic ecosystem are those associated with fuel spills, possibly toxic elements (arsenic, cadmium, copper, lead, mercury, and zinc) and polychlorinated biphenyls (PCBs). Available data indicate that heavy metals, pesticides and other persistent organic pollutants (POPs) might reach Antarctica via Long-range Atmospheric Transport (LRAT) from other continents within the Southern Hemisphere and even beyond [2].

In East Antarctica, metals are the common contaminants of concern as a result of they had been proven to impact the native flora and fauna. Elevated concentrations of copper, cadmium, nickel and lead occur in marine sediments and tissues of benthic fauna in nearshore environments adjacent to Australia's Casey Station in East Antarctica [5]. Besides, changes within the community composition of local benthic diatoms are directly related to metal contamination (tin, lead copper, and iron) in sediments adjacent to Casey Station [6], whereas Majer et al. [7] noted the bioaccumulation of arsenic, cadmium and lead in invertebrates and macroalgae on the Antarctic Peninsula.

The existence of heavy metals, which may have been discharged into the environment by natural resources or

anthropogenic activities, can influence the exhibition of coupled biogeochemical structures [8]. Therefore, the interactions among heavy metals and local organisms are essential to be researched to understand the behaviour of organisms and biochemical ecosystems. According to Madoni et al. [9], Leduc et al. [10] and Gikas et al. [11], the addition of trace amounts of heavy metals to the environment of microbial cells often stimulates microbial growth due to utilization of small amount of heavy metals by microorganisms in various biochemical pathways. Yet, higher concentrations have brought about an extreme decrease of microbial activity, which was reflected by the reduction of the evident growth rate and increment in the lag phase. Since the microorganisms can adapt with time to relatively higher concentrations of heavy metals, the effects of cell growth do not depend only on the types of the microorganism and heavy metals. Situations wherein simultaneous contamination with the aid of heavy metals and organic compounds are present and have been detected in manufacturing areas [12].

Chromium is considered harmful to the environment and it is encountered in the oxidation states of tri-valent (III) and hexa-valent (VI). The key sources of Cr are the chemical and electroplating industry and the leather-based tanneries. Each oxidation states have different biological and toxicological properties. Tri-valent Cr accumulates within the cell membrane, while hexa-valent Cr is transported into the cells where it is diminished to tri-valent Cr and react with intracellular substance [13-15].

Zinc is an important component for plants, microorganisms, animals and humans. However, elevated concentrations of Zn are also repressing or toxic to cellular activities and growth, with the specific inhibitory concentration being dependent on the activity or cell being investigated. Zinc has been shown to inhibit respiration and growth of fungi, germination of fungal spores and bacterial conjugation, as well as block the adsorption of M13 coliphage to its host bacterium [16]. Concentrations of Zn rise unnaturally due to anthropogenic activities. Most Zn is added in the course of industrial activities, comprising mining, coal, as well as waste combustion and metal processing [17].

In this work, a simple experimental set up was used to determine the physiological and biochemical capabilities of phenol degradation by Antarctic bacteria consortium sensitivity and proof against heavy metals in the presence of two toxiferous known as Cr and Zn.

METHODS AND MATERIALS

Microorganism and media

Bacteria consortium were obtained from the soil of Bernardo O'Higgins Station, Antarctic. Phenol media (pH 7) included the following constituents in 1 L of distilled water: KH_2PO_4 (0.4), K_2HPO_4 (0.2), NaCl (2.0), $MgSO_4$ (0.1), $(NH_4)SO_4$ (1.0), $MnSO_4.H_2O$ (0.01), $Fe_2(SO_4).H_2O$ (0.01) and $NaMoO_4.H_2O$ (0.01). 1 ppm of heavy metals was added into each 100 mL flask. Two heavy metals were tested in this study, which were Cr and Zn. All tests were carried out in triplicates. The control has no heavy metal incorporation. The flasks were incubated in an orbital shaker at 150 rpm for 120 h at 10°C.

Biodegradation experiment

About 1 mL of the sample from each flask was transferred into a 1.5 mL Eppendorf tube. The sample was centrifuged using a tabletop centrifuge at 130 rpm for 10 min. The supernatant was carefully discarded into a test tube. Ammonium solution was dropped into each test tube using a dropper to obtain a pH of 10 and mixed. 10 µL of aminoantipyrene solution was pipetted to each test tube, followed by 10 µL of potassium ferricyanide ($C_6N_6FeK_3$) solution. The test tubes were incubated in a dark room for 15 min. After incubation, the samples were diluted to a factor of ten. 100 µL of the sample was transferred to 900 µL distilled water into a cuvette. The absorbance of the diluted samples was read at 510 nm using a UV-Visible Spectrophotometer.

Kinetics models

The Gompertz model [18] was carried out to estimate the main growth parameters namely, lag phase duration (λ), maximum growth rate (μ_{max}) and maximum cell number at the stationary phase (N_{max}).

RESULTS AND DISCUSSION

Soil as well as the water polluted by heavy metals lead to the aggregation of toxic particles in living beings through the food chain, which gave a negative impact on both physiological activities on flora and human health [19, 20]. Thavamani et al. [21] and Wong et al. [22] indicated that phenol-rich effluents generated by the industrial factories conjointly discharge heavy metals, inflicting in growth inhibition of most phenol-degrading microorganisms used for waste product disposal. Therefore, a lot of attention ought to be paid to the phenol removal performance of microorganisms within the media containing the presence of heavy metals.

Two different heavy metals, which were Cr and Zn, were tested against Antarctic bacteria consortium to determine their effects on phenol degradation (**Figure 1**). Based on the results shown, the consortium was able to stand the toxicity of Zn at 1 ppm concentration and degraded 100% of 0.5 g/L of phenol aside from for Cr contained medium. Zinc was able to degrade phenol and showed no inhibitory effect on the consortium because it is an essential micronutrient for bacteria, even though it has significant toxicity at high concentrations [23- 25].

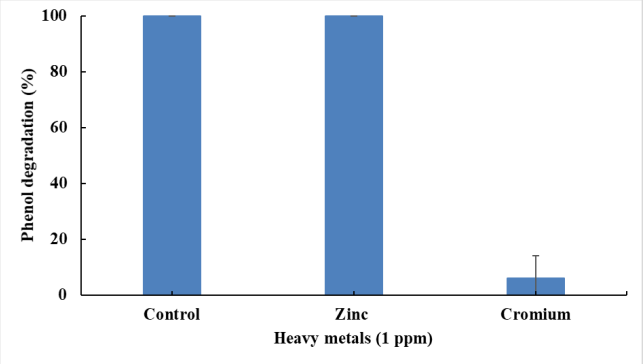

Figure 1. Effect of various heavy metals on biodegradation of 0.5 g/L phenol. Data represent mean ± SEM, $N = 3$.

The consortium degraded 6% of phenol within the Cr contained medium that illustrated a difference of about 94% compared to Zn that contained medium and the control with no heavy metal medium, but, able to grow in the presence of 1 ppm of Cr. Even though Cr can cause DNA damage, the bacteria consortium can resist them at a concentration of 1 ppm but unable to induce the phenol degradation. According to Ramírez-Díaz et al. [26], Viti and Giovannetti [27] and Zhitkovich [28], once Cr is inside the cells, it will undergo a reduction caused by various enzymes and non-enzymes activities, resulting in the production of several active intermediates that could directly cause alterations of DNA and toxic effects. Fatimah and Rao [29] declared that Cr is more damaging towards Gram-negative bacteria than Gram-positive bacteria because of the compositional variations within the cell membrane of the bacteria since Gram-negative bacteria incorporated a negatively charged lipopolysaccharide, it shows high binding affinity towards positively charged Cr (III) ions [30].

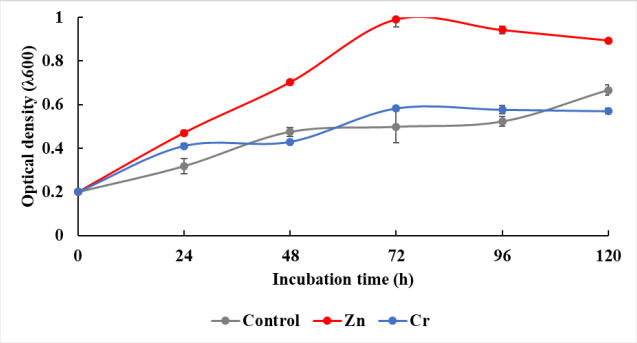

Figure 2. Growth curves (measured as optical density at 600 nm) for Zn contained medium and Cr contained media obtained by Antarctic bacteria consortium. Data represent mean ± SEM

Growth curves for Antarctic bacteria consortium obtained at different heavy metals (Zn and Cr) are illustrated in **Figure 2**, and growth parameters (λ, μ_{max}, and N_{max}) were calculated by applying the Gompertz model as shown in **Table 1**. These three parameters can concisely the population growth kinetics under specific environmental conditions and are therefore useful for evaluating consortium performance given their technological use. In general, low λ values indicate that a strain can rapidly begin multiplying, thus hindering the development of indigenous microbiota.

Table 1. Growth parameters of Antarctic bacteria consortium estimated by the Gompertz model

Parameters	Control	Zinc	Chromium
λ (h)	0.025	0.039	0.035
μ_{max} (h^{-1})	40.17	25.59	28.50
N_{max} (OD_{600})	0.660	0.974	0.591

High μmax values indicate that the bacteria are colonising the substrate rapidly and efficiently, while high Nmax values indicate that a high number of bacteria are present at the end of the growth process [31]. The Gompertz growth curve model for Antarctic bacteria consortium with the presence of Zn visually gave a better fit of experimental data (**Figure 3**) as the coefficient of determination (R^2) was 0.9541. The square root of the variance of residuals (RSME) value of this model was 0.0668, which indicates a better fit as it has a low value of RSME [32]. Meanwhile, the growth curve model for Antarctic bacteria consortium with the presence of Cr to degrade phenol was shaped properly as in **Figure 4** for Gompertz model where the RSME value was 0.0378. The graph was determined significantly as the value of R^2 was 0.9365.

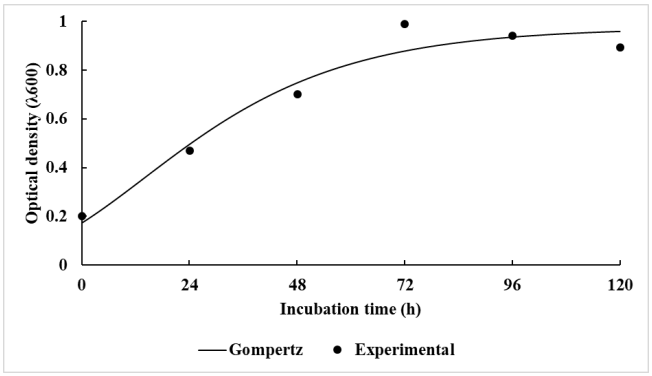

Figure 3. Gompertz growth curve on effect of Zn on Antarctic bacteria consortium.

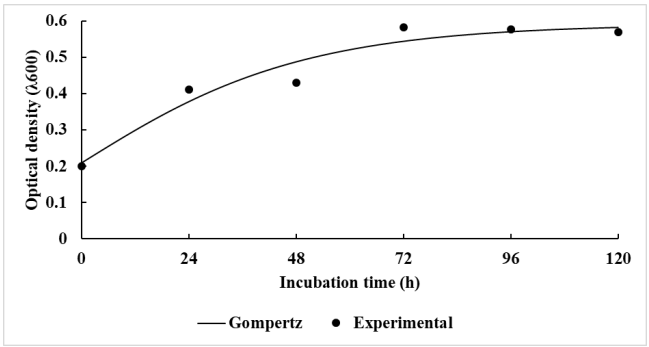

Figure 4. Gompertz growth curve on effect of Cr on Antarctic bacteria consortium.

Overall, the consortium showed less adaptability at Cr with respect to Zn (**Figure 2**). Clearly, Zn resulted in better growth rates, indicating that 1 ppm of Zn does not give any effect to the bacteria consortium growth. In Zn incorporated medium, the consortium showed a significantly shorter lag phase and reached higher N_{max} compared to that of with Cr incorporated medium. Moreover, the shapes of the consortium growth curve of Zn contained medium highlighted marked differences with the consortium growth curve of Cr contained medium. All the values for the lag phase (λ), maximum growth rate (μ_{maz}) and maximum cell number at the stationary phase (N_{max}) fell within the 95% confidence interval of the distribution for both growth curves on the effects of Zn and Cr.

CONCLUSION

In conclusion, we report for the first time the growth kinetics of the consortium for Zn contained medium and compare the kinetics with Cr contained medium under the same conditions. These data can help to better define and compare the effects of these two heavy metals toward the Antarctic bacteria consortium. Further studies will be needed on a greater number of concentrations of heavy metals to evaluate whether a higher concentration of heavy metals can affect the bacteria consortium performance in degrading phenol pollutants.

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REFERENCES

1. Chu, W., Dang, N., Kok, Y., Yap, K.I., Phang, S. and Convey, P. (2019). Heavy metals pollution in Antarctica and its potential impacts on algae. *Polar Science,* 20(1): 75-83.
2. Bargagli, R. (2008). Environmental contamination in Antarctic ecosystems. *Science of The Total Environment*, 400(1-3): 212-226.
3. Burton-Johnson, A., Black, M., Fretwell, P.T. and Kaluza-Gilbert, J. (2016). An automated methodology for differentiating rock from snow, clouds and sea in Antarctica from Landsat 8 imagery: a new rock outcrop map and area estimation for the entire Antarctic continent. *Cryosphere*, 10: 1665-1677.
4. Tin, T., Fleming, Z.L., Hughes, K.A., Ainley, D.G., Convey, P., Pfeiffer, S., Scott, J. and Snape, I. (2009). Impacts of local human activities on the Antarctic environment. *Antarctic Science,* 21(1): 3-33.
5. Duquesne, S. and Riddle, M. (2002). Biological monitoring of heavy-metal contamination in coastal waters off Casey Station, Windmill Islands, East Antarctica. *Polar Biology,* 25(3): 206-215.
6. Cunningham, L., Raymond, B., Snape, I. and Riddle, M.J. (2005). Benthic diatom communities as indicators of anthropogenic metal contamination at Casey Station, Antarctica. *Journal of Paleolimnology,* 33(4): 499-513.
7. Majer, A.P., Petti, M.A.V., Corbisier, T.N., Ribeiro, A.P., Theophilo, C.Y.S., Ferreira, P.A.D.L. and Figueira, R.C.L. (2014). Bioaccumulation of potentially toxic trace elements in benthic organisms of Admiralty Bay (King George Island, Antarctica). *Marine Pollution Bulletin,* 79(1-2): 321-325.
8. Sengòr, S.S., Barua, S., Gikas, P., Ginn, T.R., Peyton, B., Sani, R.K. and Spycher, N.F. (2009). Influence of heavy metals on microbial growth kinetics including lag time: Mathematical modelling and experimental verification. *Environmental Toxicology and Chemisry*, 28(10): 2020-2029.
9. Madoni, P., Davoli, D., Gorbi, G. and Vescovi, L. (1996). Toxic effect of heavy metals on the activated sludge protozoan community. *Water Research,* 30(1): 135-141.
10. Leduc, L.G., Ferroni, G.D. and Trevors, J.T. (1997). Resistance to heavy metals in different strain of *Thiobacillus ferrooxidans*. *World Journal of Microbiology and Biotechnology,* 13(4): 453-455.
11. Gikas, P., Sengör, S.S., Ginn, T., Moberly, J. and Peyton, B. (2009). The effects of heavy metals and temperature on microbial growth and lag. *Global NEST Journal,* 11(3): 325-332.
12. Al-Saleh, E.S. and Obuekwe, C. (2005). Inhibition of hydrocarbon bioremediation by lead in crude oil-contaminated soil. *International Biodeterioration and Biodegradation,* 56(1): 1-7.
13. Martell, A.E. (1981). Chemistry of carcinogenic metals. *Environmental Health Perspectives,* 40: 207-226.
14. Shen, H. and Wang, Y.T. (1994). Modeling hexavalent chromium reduction in *Escherichia coli* 33456. *Biotechnology and Bioengineering,* 43(4): 293-300.
15. Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Papanikolaou, E.C., Tsakon, A. and Lekkas, T.D. (2003). Effects of chromium (VI) addition on the activated sludge process. *Water Research,* 37(9): 2140-2148.
16. Babich, H. and Stotzky, G. (1978). Toxicity of zinc to fungi, bacteria, and coliphages: influence of chloride ions. *Applied and Environmental Microbiology,* 36(6): 906-914.
17. Wuana, R.A. and Okieimen, F.E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Network Ecology,* 2011: 1-20.
18. Zwietering, M.H., Jongenburger, I, Rombouts, F.M. and Riet, K.V. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology,* 56(2): 1875-1881.
19. Suciu, I., Cosma, C., Todică, M., Bolboacă, S.D. and Jäntschi, L. (2008). Analysis of soil heavy metal pollution and pattern in central Transylvania. *International Journal of Molecular Sciences,* 9(4): 434-453.
20. Fernández, P.M., Martorell, M.M., Blaser, M.G., Ruberto, L.A.M., de Figueroa, L.I.C. and Cormack, W.P.M. (2017). Phenol degradation and heavy metal tolerance of Antarctic yeasts. *Extremophiles,* 21(3): 445-457.
21. Thavamani, P., Megharaj, M. and Naidu, R. (2012). Bioremediation of high molecular weight polyaromatic hydrocarbons co-contaminated with metals in liquid and soil slurries by metal tolerant PAHs degrading bacterial consortium. *Biodegradation,* 23(6): 823-835.
22. Wong, K.K., Quilty, B., Hamzah, A. and Surif, S. (2015). Phenol biodegradation and metal removal by a mixed bacterial consortium. *Bioremediation Journal,* 19: 104-112.
23. Bruins, M.R., Sanjay, K. and Oehme, F.W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety,* 45(3): 198-207.
24. Blencowe, D.K. and Morby, A.P. (2003). Zn (II) metabolism in prokaryotes. *FEM Microbiology Reviews,* 27: 291-311.
25. Corbin, B.D., Seeley, E.H., Raab, A., Feldmann, J., Miller, M.R., Torres, J., Anderson, K.L., Dattilo, B.M., Dunman, P.M., Gerads, R., Caprioli, R.M., Nacken, W., Chazin, W.J. and Skaar, E.P. (2008). Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science,* 319: 962-965.
26. Ramírez-Díaz, M.I., Díaz-Pérez, C., Vargas, E., Riveros-Rosas. H., Campos-García, J. and Cervantes, C. (2008). Mechanisms of bacterial resistance to chromium compounds. *Biometals,* 21(3): 321-332.
27. Viti, C. and Giovannetti, L. (2007). Bioremediation of soils polluted with hexavalent chromium using bacteria: a challenge. *Environmental Bioremediation Technologies,* 57-76.
28. Zhitkovich, A. (2011). Chromium in drinking water: sources, metabolism, and cancer risks. *Chemical Research in Toxicology,* 24: 1617-1629.
29. Fathima, A. and Rao, J.R. (2018). Is Cr(III) toxic to bacteria: toxicity studies using *Bacillus subtilis* and *Escherichia coli* as model organism. *Achives of Microbiology,* 200: 453-462.
30. Khan, A.L., Ullah, I., Hussain, J., Kang S.M., Al-Harrasi, A., Al-Rawahi, A. and Lee, I.J. (2016). Regulations of essential amino acids and proteomics of bacterial endophytes *Sphingomonas* sp. Lk11 during Cadmium uptake. *Environmental Toxicology,* 31(7): 887-896.
31. Tarrah, A., Noal, V., Treu, L., Giaretta, S., Duarte, V.D.S., Corich, V. and Giacomini, A. (2018). Comparison of growth kinetics at different temperatures of *streptococcus macedonicus* and *Streptococcus thermophilus* strains of dairy origin. *Journal of Dairy Science,* 101(9): 7812-7816.
32. Heo, C., Kim, J. H., Kim, H. W., Lee, J., Hong, W., Kim, C. and Paik, H. (2010). The development of predictive growth models for total viable cells and *Escherichia coli* on chicken breast as a function of temperature. *Korean Journal Food Science of Animal Resources*, 30(1): 49-54.