Emperical Modeling of Growth Parameters in *Cellulosimicrobium cellulans* during Heavy Metal Tolerance

Nikhil Kumar Pipil, Sayan Chatterjee, Ram Singh Purty*

University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi 110078.

*Corresponding author: rspurty@ipu.ac.in

Abstract. In the present work we have studied the growth kinetics of *Cellulosimicrobium cellulans* under different metal stress. The strain used was Gram positive and non-pathogenic. Metal studied were Fe, Zn, Pb, Cu, Cd, and Ni. Growth curve of *Cellulosimicrobium cellulans* was studied under different concentrations of these metals. In the second part of our investigation we tried to establish growth equation which can define the growth curve of *Cellulosimicrobium cellulans*. The three parameters \( \mu_m, \lambda, X_m \) majorly contribute to growth equations. The changes in these parameters affect the location and duration of exponential or log phase \( t \). Thus, we have chosen these factors primarily to model our system. In each case we have observed little deviation from the predicted equation at the maximum error percentage below 10\% (~10) \( R^2\leq0.8 \). Next we have tried to model the cumulative effect of these metals simultaneously but our model has failed to reach an appreciable correlation with the experimental data set. In this case we may assume that more factors like mole fraction or partial molar volume of metal solution may have contributed to the growth profile.

1. Introduction

Heavy metals are the group of naturally occurring elements having high density greater than 5g/cm\(^3\) [1]. The toxicity of these elements depends on many factors which include type of chemical exposed, doses and duration of exposure on the individual. Amongst the various heavy metal, mercury (Hg), chromium (Cr), nickel (Ni), arsenic (As), lead (Pb) and cadmium (Cd), possess significant threat to human health [2]. These elements cause various physiological disorders and various life threatening diseases in humans i.e. cancer, cognitive impairment, chronic anemia, damage to kidney, nervous system, brain, skin, and bones even at very low concentration [3]. According to the US Environmental Protection Agency and the International Agency for Research on Cancer, heavy metals are grouped as human carcinogens [2]. There are many natural as well as human activities that lead to accumulation of heavy metals in the environment [4]. Human activities include mining, smelting, industrial production and use, domestic and agricultural use of metals and metal containing compounds [4]. Environmental sources are metal corrosion, atmospheric deposition, soil erosion of metal ions, leaching of heavy metals. Natural phenomena such as weathering and volcanic eruption are also a major source of heavy metals [5]. Waste from industries and fertilizers are the main source of heavy metals in water bodies. Nickel compounds are used in agriculture. It is also present in phosphate fertilizers. Nickel-cadmium batteries, smelters, absorber in nuclear reactors are some of the sources of Cd in environment [4].

Various methods have been developed for removal of heavy metals from the environment with the help of microorganisms or plants to re-establish the natural condition of contamination site (soil, water bodies). It has several benefits over other available options as it is cost effective, non-invasive, and provides a permanent solution. Phytoremediation make use of plants to remediate and revegetate heavy metal contaminated environment [6]. Phytoremediation is generally characterized into...
phytostabilization, phytoevaporation and phytoextraction based on different uptake mechanism. Other than plants, large numbers of microbes are employed to detoxify or remediating the metal polluted soil or water. Not only microbes but their enzymes are also used for remediating polluted areas. Metals which are in soluble form or diluted can be easily bio-accumulated. Technologies using microbes for remediating heavy metal pollution can provide an alternative to conventional methods and they seems to be future of heavy metal remediation. These types of technology are cheap and require less time [7]. In nature there are many bacteria which are heavy metal tolerant and they have developed mechanisms to tolerate the metal stress either by heavy metal efflux, complexation and reduction of metal ion or use them as terminal electron acceptor in anaerobic respiration [5]. Some of the naturally occurring heavy metal tolerant bacteria are *Enterobacter agglomerans*, *Bacillus* sp, *Enterobacteriaceae* strain and *Bacillus cereus*.

In our previous study, we have isolated multiple heavy metal tolerant bacteria from River Yamuna, New Delhi [8]. The isolated bacterial strain was characterized using 16S RNA gene sequencing and BLASTN analysis showed the strain to be *Cellulosimicrobium cellulans*. The strain used was Gram positive and non-pathogenic. In the present investigation, we have studied the growth kinetics of *C. cellulans* under different heavy metal stress. Growth curve of *C. cellulans* was studied under different concentration of heavy metals which include Fe, Zn, Pb, Cu, Cd, and Ni. In the second part of our investigation we tried to establish a growth equation which can define growth curve of *C. cellulans*.

2. Method and materials

2.1 Revival of Cellulosimicrobium cellulans

Glycerol stock of bacterial strain i.e, *Cellulosimicrobium cellulans* was obtained and incubated in LB media supplemented with 1mM concentration of ferric sulfate (Fe$_2$(SO$_4$)$_3$.7H$_2$O). The culture was then incubated overnight at 37°C. Overnight culture was then streaked plated in fresh LB agar plates supplemented with 1mM concentration of ferric sulfate (Fe$_2$(SO$_4$)$_3$.7H2O) and incubated overnight at 37°C. The colonies obtained after streaked plating was used for later experiments.

2.2 Chemicals and reagents

Stock solution of 1M were prepared in sterile deionised water separately for the heavy metals salts, cadmium chloride (CdCl$_2$.H$_2$O), nickel sulfate (NiSO$_4$.6H$_2$O), ferric sulfate (Fe$_2$(SO$_4$)$_3$.7H2O), cupric sulfate (CuSO$_4$.5H$_2$O), lead acetate (Pb(C$_2$H$_3$O$_2$)$_2$.3H$_2$O) and zinc sulfate (ZnSO$_4$.7H$_2$O).

2.3 Primary culture

For primary culture, LB media was prepared and autoclaved. A loop full of bacteria was taken from streaked plate and inoculated in 20 ml of LB media. The culture was then incubated overnight at 37°C and 180rpm.

2.4 Determination of growth curve

To study the effect of different heavy metals and their different concentrations on the bacterial growth, growth curve analysis was performed. Different concentrations of heavy metals were prepared using stock solution of 1M. In experimental set 1ml of primary culture was taken and inoculated in LB media with heavy metal stress. Control was maintained without heavy metal stress. Experimental sets and control were incubated at 37°C and 180 rpm and absorbance was measured after interval of every 3hr at 600 nm.

3. Results and discussions

In the present investigation growth curve of *C. cellulans* in response of different heavy was studied and compared with untreated control. The isolated bacterial strain was exposed to different concentration of single heavy metal and it growth was recorded at regular interval till 36 h. Different concentrations of heavy metals were tried ranging from 1–7 mM ferric sulfate, 1–8 mM lead acetate, 1-6 mM zinc sulfate, 1-4 mM cupric sulfate, 0.1and 0.5 mM for nickel sulfate and 0.1-2 mM cadmium chloride. Other than these growth curves of *C. cellulans* were also studied under 1 mM concentration of different heavy metals.
3.1 Growth curve of *Cellulosimicrobium cellulans* in response to heavy metal stress

In Fe stress condition, growth pattern in 1 mM stress is similar to that of control. Minimum growth was observed in 7 mM stress. Therefore, it can be concluded that stress of 7 mM ferric sulfate was lethal for *C. cellulans* (Fig. 1A). In Pb stress, growth pattern was inhibited in 6 mM. No further growth was observed on increasing the metal concentration (Fig. 1B). In case of Zn stress, 5 mM of zinc sulfate was found to be lethal as no growth was observed beyond that concentration (Fig. 1C). In case of Cu, 4 mM of cupric sulfate was lethal (Fig. 1D). In case of Ni, 1 mM nickel sulfate stress was found to be enough to inhibit the growth of the bacteria. In 2 mM and 3 mM Ni stress growth was not observed (Fig. 1E). In case of Cd, 2 mM cadmium chloride was found to be lethal as growth was inhibited.

**Figure 1A-F.** Growth curve of *Cellulosimicrobium cellulans* in presence of different concentration of each heavy metal.
3.2 *Cellulosimicrobium cellulans* under 1mM concentration of different heavy metals

*Cellulosimicrobium cellulans* reached its maximum growth at 18 hr. Reduction in growth was observed in presence of various metal stresses. The growth pattern in lead and iron stress was similar to that of control. The slowest growth was detected in cadmium and nickel stress (Fig. 2).

![Growth curve of *Cellulosimicrobium cellulans* under 1 mM concentration of different heavy metals.](image)

The objective of the present investigation was to study the effect of heavy metal stress on growth kinetics of *Cellulosimicrobium cellulans* as well to determine maximum biomass in presence of heavy metal stress and subsequent generation of growth equation. It has been observed that the variation in $\mu_m$ may not explain the difference in the growth profile of control and experimental set. Thus, we may assume that growth profile may depend on other factors such as X, $X_m$, $\lambda$, t [9]. These factors tend to define the metabolic system affected by the heavy metals [10]. The three parameters $\mu_m$, $\lambda$, $X_m$ majorly contribute to growth equation. The changes in these parameters affect the location and duration of exponential or log phase t [9]. Thus, we have chosen these factors primarily to model our system. For iron, till the range of 8mM of concentration our empirical equation holds good at 5% level of significant and $R^2 < 0.8$ similarly we had try to fit the equation with the dosage of Zn till the range of 6mM, Pb till the range of 8mM, Cd till the range of 3mM, Cu till the range of 4mM, Ni till the range of 3mM. In each case we have observed little deviation from the predicted equation at the maximum error percentage below 10% ($\alpha = 10$) $R^2<0.8$. Next we have tried to model the cumulative effect of these metals simultaneously but our model has failed to reach an appreciable correlation with the experimental data set. In this case we may assume that more factors like mole fraction or partial molar volume of metal solution may have contributed to the growth profile. Thus until we include more parameters which guide the growth profile in a real habitat where the presence of most of the heavy metals can be observed together. There are other growth parameters like $V_{max}$, $\tau$ etc. which when included may also help us to have a guiding equation for the growth profile.
4. Conclusions

The results of the experiments aimed at strengthening the mathematical proposal of dose dependent growth kinetics showed consistently that the response was majorly a direct function of $X$, $X_m$, $\lambda$, and obviously $t$. The results also gave empirical mathematical solutions to the kinetic profile when *Cellulosimicrobium cellulans* was incubated in an environment of various heavy metals and their different concentration. Our empirical model helps to study the effect of various heavy metals on the major growth parameters. The minimalistic mathematical proposal using a dose dependent growth profile minimizes the effect of experimental error by providing a guide equation in general. The proposal can be used to study the effect of the heavy metals Fe, Cu, Zn, Co, Pb, and Ni both individually as well as their cumulative effects on gram positive bacteria *Cellulosimicrobium cellulans*. However, our mathematical model fails to gauge the simultaneous effect of all these metals put together.

5. Acknowledgments

This investigation has been carried out under the Faculty Research Grant Scheme awarded to RSP from GGS Indraprastha University, New Delhi, India. Therefore, we wish to acknowledge Guru Gobind Singh Indraprastha University, New Delhi for infrastructural and financial support.

References

[1] Duffus J.H., 2002, *Pure Appl. Chem.*, 74, 793
[2] Rehman K., Fatima F., Waheed I., Akash M.S.H., 2017, *J Cellular Biochem.*, 119, 157
[3] Jaishankar M., Tseten T., Anbalagan N., Mathew B.B., Beeregowda K.N., 2014, *Interdisciplinary toxicology*, 7, 60
[4] He Z.L., Yang X.E., Stoffella P.J., 2005, *J Trace Elem. Med. Biol* 19:125–140
[5] Prabhakaran P., Ashraf M.A., Aqma W.S., 2016, *RSC Advances* 6, 109862
[6] DalCorso G., Fasani E., Manara A., Visioli G., Furini A., 2019, *Int. J Mol. Sci.* 20, E3412
[7] Rajkumar M., Sandhya S., Prasad M.N.V., Freitas H., 2012, *Biotecnol. Adv.* 30, 1562
[8] Bhati T., Gupta R., Yadav N., Singh R., Fuloria A., Waziri A., Chatterjee S., Purty R.S., 2019, *Microbiol. Biotechnol. Lett.* 47, 269
[9] Rial D., Vázquez J.A., Murado M.A., 2011, *Appl Microbiol Biotechnol.* 90, 1095
[10] Bruins M.R., Sanjay K., Frederick W.O., 2000, *Ecotox Environ Safe* 45, 198–207