International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-2539, Volume 5, Issue 2; April-June, 2018, pp. 58-63

http://www.krishisanskriti.org/Publication.html

Kinetic Study of Removal of Toxic Metals by a Mixed Bacterial Culture Isolated from East Calcutta Wetlands

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Abstract—*Nowadays, one of the most prominent contributors in environmental pollution, specifically in case of soil and ground water pollution is industrial effluent which is laden with heavy metals like lead, cadmium, mercury etc. Heavy metals in hazardous waste cause serious health issues. Removal of heavy metals by chemical processes results in residual toxicity. Thus, bioremediation is much more beneficial as an economic and environment friendly method. The present study deals with lead and cadmium. Mixed bacterial cultures were obtained from the soil collected from East Calcutta Wetlands. The cultures were acclimatized in presence of lead and cadmium separately. The pure cultures capable of growing in presence of lead or cadmium were isolated from the mixed bacterial culture. After isolation, the strains were identified to be Stenotrophomonasmaltophilia (tolerating lead) and Bacillus subtilis (tolerating cadmium) by 16s rRNA sequence data analysis method. The μ_{max} (maximum specific growth rate) value, without lead inhibition, was found to be 0.321 hr^{-1} and with lead inhibition, was found to be 0.182 hr^{-1}. Growth kinetic study was done for cadmium tolerating culture as well. For the culture tolerating cadmium, μ_{max} value, without cadmium inhibition, was found to be 0.241 hr^{-1} and with cadmium inhibition, was found to be 0.187 hr^{-1}. Metal removal kinetic study of the corresponding pure cultures were done in presence of respective metals. Atomic absorption spectroscopy (AAS) was used to determine the concentration of the metals. The removal kinetics of lead by Stenotrophomonasmaltophilia was investigated. With respect to lead concentration, the order was found to be 1.63 and the rate constant was 0.01967 $mg^{-0.63}l^{0.63}h^{-1}$. By Similar experiment, the removal rate of cadmium by Bacillus subtilis with respect to cadmium concentration was determined and the order of the reaction was 1.43 and the rate constant was found to be 0.01076 $mg^{-0.43}l^{0.43}h^{-1}$.*

Keywords: *Bioremediation, East Calcutta Wetlands, Lead, Cadmium.*

1. INTRODUCTION.

Metal pollution plays a key role in environmental pollution caused by toxic heavy metal deposition in the ground or water. Toxic heavy metals are relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental contexts. There are several heavy metals like Cadmium, Lead, Arsenic, Mercury, Antimony, Thallium, Silver etc. found in the ground water due to human activities as well as found naturally in the earth. They can enter plant, animal, and human tissues via inhalation, diet, and manual handling and can bind to and interfere with the functioning of vital cellular functions [1-4].

The present study deals with removal of lead and cadmium. Cadmium (Cd), a widely dispersed metal in environment as cadmium sulfide, is refined during zinc production and occurs in association with zinc. Lead is widely dispersed in air (as smelters), water, soil, food, dust and affects human. Cd is an extremely toxic industrial and environmental pollutant classified as a human carcinogen. Acute exposure to cadmium fumes may cause flu-like symptoms including chills, fever, and muscle ache sometimes referred to as "the cadmium blues." More severe exposures can cause tracheo-bronchitis, pneumonitis, and pulmonary edema. Symptoms of inflammation may start hours after the exposure and include cough, dryness and irritation of the nose and throat, headache, dizziness, weakness, fever, chills, and chest pain [5-6]. Lead (Pb) poisoning is a type of metal poisoning caused by introduction of lead in the body. Exposure to lead can occur by contaminated air, water, dust, food, or consumer products. In acute poisoning, typical neurological signs are pain, muscle weakness, numbness and tingling, and, rarely, symptoms associated with inflammation of the brain abdominalpain, nausea, vomiting, diarrhea, and constipation are other acute symptoms [7-9].

Lead and Cadmium can be removed by chemical methods which results in byproduct or residual toxicity in most cases. There are studies where extremophils had been used for their removal but extremophilic conditions are difficult to maintain and are costly. In the present work removal kinetics study of bioremediation of lead and cadmium bymesophilic bacterial culture have been studied. The bacterial culture was isolated

from East Calcutta Wetlands (a Ramsar site receiving both domestic and industrial wastes). Removal kinetics give an idea of the rate of removal of Pb and Cd which is the basis for the economics of any removal treatment.

2. MATERIALS AND METHODS.

2.1 Chemicals and reagent.

Nutrient broth media with metal salt and without metal presence used in experimental study, was prepared from analytical grade chemicals procured from Merck, India. Lead was added in the media in the form of lead acetate and cadmium as cadmium chloride.

2.2 Bacterial strain selection

Soil sample collected from East Calcutta Wetlands was suspended in 0.8% saline water and kept in incubator shaker, the supernatant was collected and used as an inoculum for nutrient broth, kept in the incubator to form the mother culture. This mother culture was inoculated in the lead and cadmium containing media, kept in the incubator for bacterial growth. Serial dilutions and plating were done to isolate single colonies (cadmium and lead tolerating strains)based on size, shape, color and transparency. These single colonies were grown overnight in the nutrient broth for identification and for a comparative growth and removal kinetic study.

2.3. Bacterial Identification.

The isolated single bacterial strains were identified by 16srRNAanalysis.

2.4 Growth and removal kinetic study and determination of the kinetic parameters

For growth kinetic study, nutrient broth media was prepared with and without metal salts (lead acetate, cadmium chloride).10%(v/v) of the pure cultures were inoculated in the media without metal salts, kept for 24h in the incubator shaker (37°C , 100rpm). Aliquots were collected at every 1-hour interval and absorbance was measured at 600nm. For growth kinetic study with inhibition, nutrient broth media was prepared containing 5ppm lead acetate and cadmium chloride separately. 10% (v/v) of cadmium and lead tolerating cultures were added to respective media, kept in the incubator overnight, aliquots were collected at every 1hr interval and absorbance was measured at 600nm. For removal kinetic study, nutrient broth media with 10ppm lead acetate and 30ppm cadmium chloride were prepared separately. 10% (v/v) respective pure cultures were added to the respective media and incubated overnight.15ml aliquots of the media were collected every hour the aliquots were centrifuged at6000 rpm for 5 mins. The pellet (cells) was discarded and the supernatant was used for atomic absorption spectroscopy. Decrease in the metal salts (lead and cadmium salts) concentration with respect to time were plotted. Kinetic parameters of removal coefficient with respect to cell concentration and with respect to metal concentration along with the orders of the reaction were calculated for both the pure cultures.

3. RESULT AND DISCUSSIONS.

3.1 Bacterial identification.

The two isolated strains constituting the mixed bacterial culture identified by 16rRNA analysis were found to be most similar with Stenotrophomonasmaltophilia (Genbankentry: FJ772015) for removal of lead and Bacillus sp. (Genbank entry:AF500205) for cadmium.

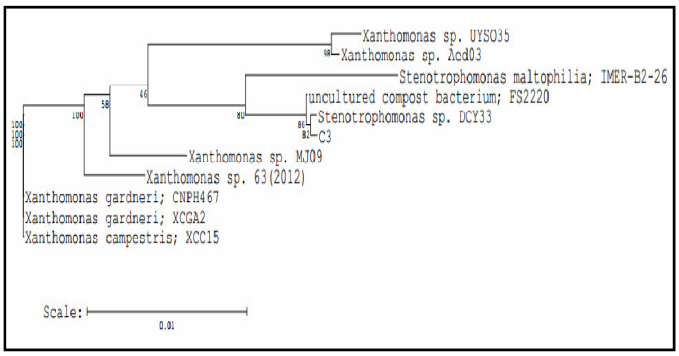

Fig. 1: Phylogenetic tree of lead tolerating culture

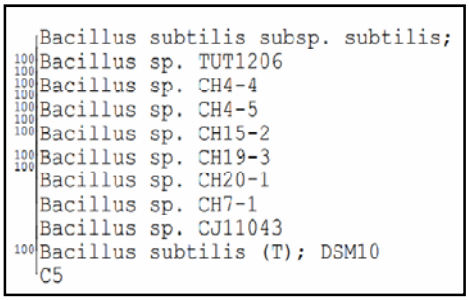

Fig. 2: Phylogenetic tree for cadmium tolerating culture

3.2. Removal kinetic study and determination of kinetic parameters.

3.2.1. Kinetic study of removal of lead (Pb) from media.

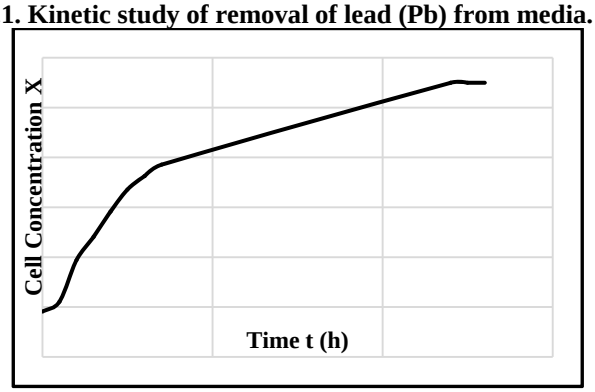

Fig. 3: Growth Curve of Lead tolerating Culture (without metal presence)

Fig. 3 shows the growth curve of Lead (Pb) tolerating culture while it was grown without the presence of any metal in the media. After 25hrs(approx) the cell concentration became constant.

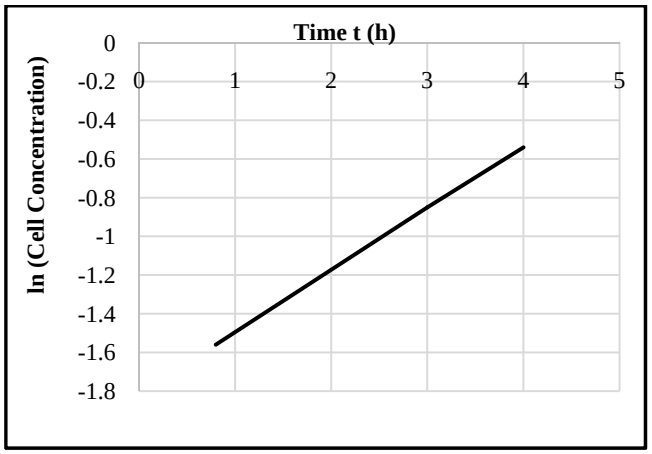

Fig. 4: Determination of maximum specific growth rate in absence of any heavy metal

Fig.4 shows a plot of ln X vs t to find the μ_{max}(maximum specific growth rate) value for the lead (Pb) tolerating culture when grown without inhibition.The μ_{max} value was found to be 0.321 h^{-1}.

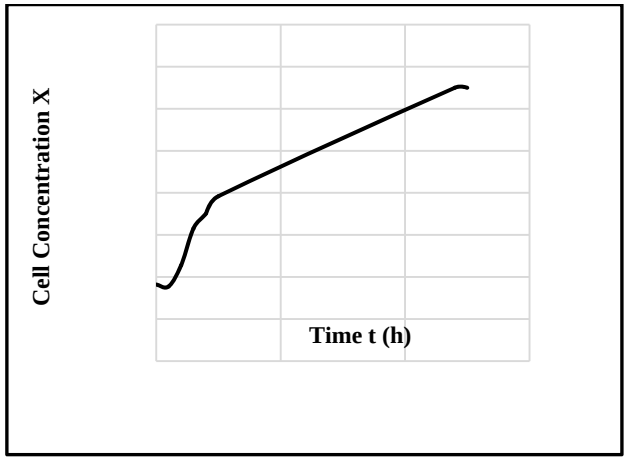

Fig. 5: Growth Curve of Lead tolerating Culture (In presence of metal in media)

Fig. 5 shows the growth curve of Lead (Pb) tolerating culture while it was grown in the presence of 5ppm lead (Pb) in the nutrient media and the stationary phase was observed after 25h approximately.

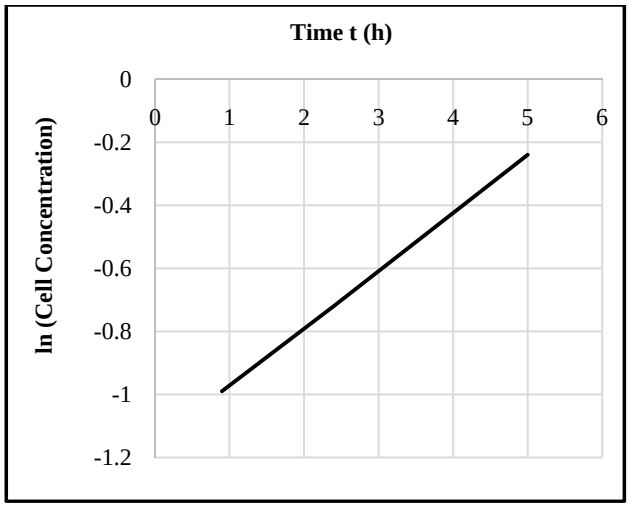

Fig. 6: Determination of μ_{max} from slope for Lead (Pb) tolerating culture

Fig.6 is a graph has been plotted for ln X vs t to find the μ_{max} value for the lead (Pb) tolerating culture when grown in the presence of lead (Pb).The μ_{max}value was found to be 0.182 h^{-1}.

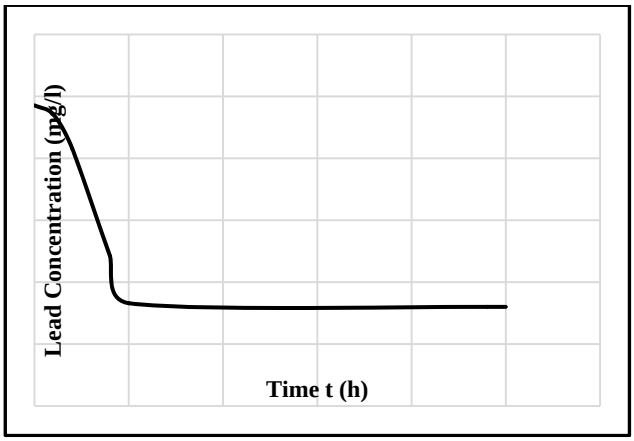

Fig. 7: Change in Lead (Pb) concentration (mg/l) in Lead removing culture with respect to time(h)

Fig. 7 shows the decrease in lead (Pb) concentration with respect to time from the data obtained by atomic absorption spectroscopy (AAS). It can be seen that for a certain concentration of lead (here, 10ppm), there is a threshold concentration of metal (~3.2 ppm) beyond which the metal cannot be removed. The threshold concentration of metalis reached at about 5hours of growth.

The probable reasons for this could be that AAS has a lower limit of 3 ppm beyond which it cannot measure. So, even if lead (Pb) has been removed, it will not be detected. Moreover, concentration of lead (Pb) might be too low to result in effective adsorption on the cell surface.

Here, the experiment was done at a higher concentration of lead (Pb) than generally found in the environmental pollutants to factor in the increasing level of metal pollutants in the near future.

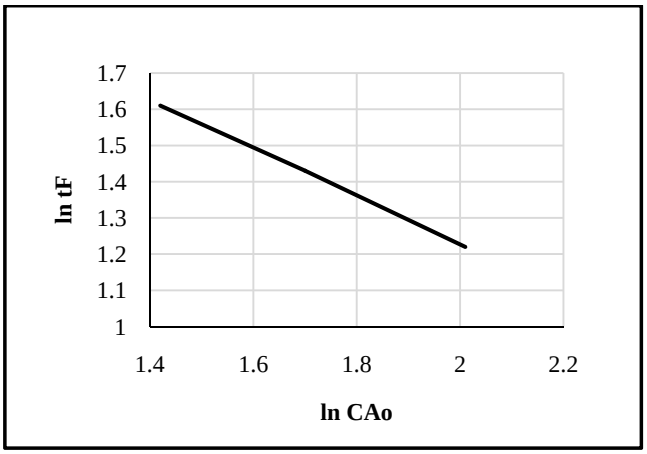

Fig. 8: Determination Kinetic parameters of Lead (Pb) removalwith respect to Lead concentration

From the graph, the kinetic equation comes to be as follows.

$$-\frac{d[CAo]}{dt} = 0.01967 C_{Ao}^{1.63}$$

From Fig. 8, we can determine the kinetic parameters of lead (Pb) removal with respect to lead concentration. From the graph, the order of the reaction is 1.63 and the **rate constant** is **0.01967 $mg^{-0.63}l^{0.063}h^{-1}$**.

Though it was found that the culture could tolerate lead but the bacterial growth rate had decreased to 0.182 h^{-1}. It shows lead has an inhibitory effect on the growth.

3.2.2 Kinetic study of removal of Cadmium (Cd) from media.

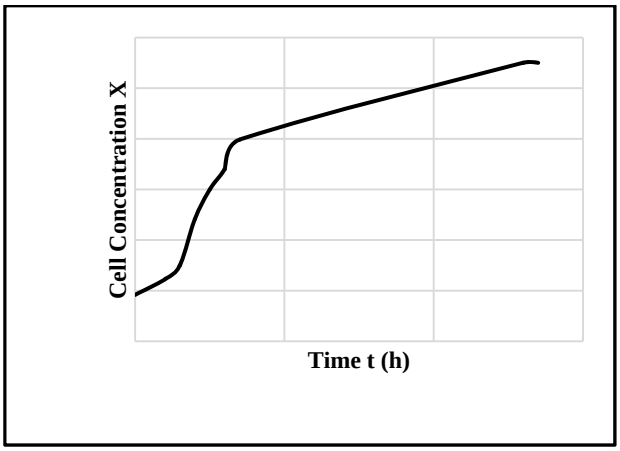

Fig. 9: Growth Curve of Cadmium (Cd) tolerating Culture in absence of any heavy metal

Fig. 9 shows the sigmoidal growth curve with lag,log and advent stationary phase obtained after 27h approximately.

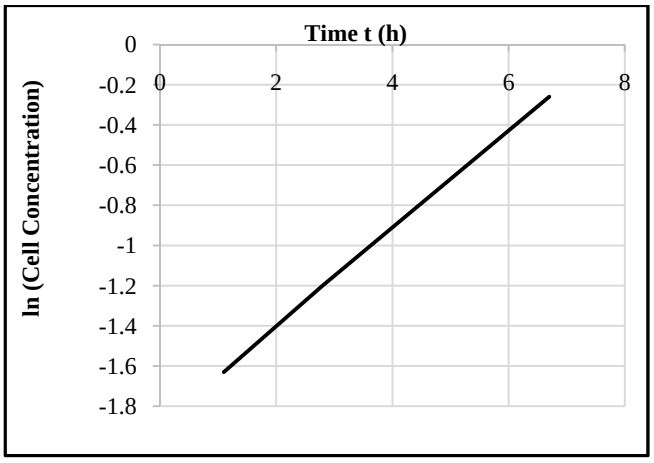

Fig. 10: Determination of maximum specific growth rate from slope for Cadmium (cd) tolerating culture in absence of any heavy metal.

A graph (Fig. 10) has been plotted for ln X vs t to find the μ_{max} (maximum specific growth rate) value for the cadmium (Cd) tolerating culture when grown without inhibition.The μ_{max} value was found to be 0.241 h^{-1}.

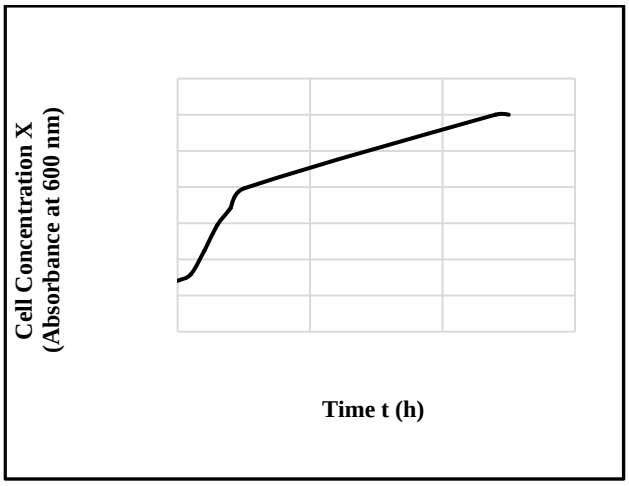

Fig. 11: Growth Curve of Cadmium (Cd) tolerarting Culture in presence of heavy metal

Fig. 11 shows the sigmoidal growth curve with lag,log and advent stationary phase obtained after 27h approximately.

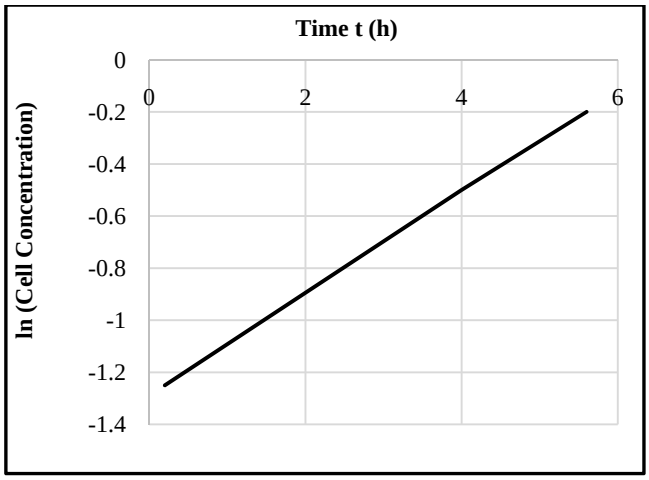

Fig. 12: Determination of μ_{max} from slope cadmium (cd) tolerating culture in presence of heavy metal.

A graph (Fig. 12) has been plotted for ln X vs t to find the μ_{max} value for the cadmium (Cd) tolerating culture when grown without inhibition.The μ_{max}value was found to be 0.187 h^{-1}.

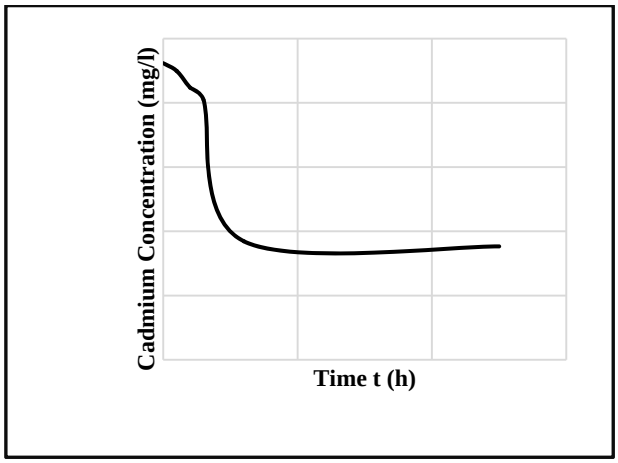

Fig. 13: Change in Cadmium (Cd) concentration (mg/l) in Cadmium removing culture with respect to time (h)

Fig. 13 shows the decrease in cadmium (Cd) concentration with respect to time from the data obtained by atomic absorption spectroscopy (AAS). It can be seen that for a certain concentration of cadmium (here, 30 ppm), there is a threshold concentration of metal (~17 ppm) beyond which the metal cannot be removed. The threshold concentration of metal is reached at about 6 hours of growth.

The probable reason for this could be that the concentration of cadmium (Cd) is too low for surface attachment of cadmium to the cells. Probably, that is why, they are not removed.

Here, the experiment was done at a higher concentration of cadmium (Cd) than generally found in the environmental pollutants to factor in the increasing level of metal pollutants in the near future.

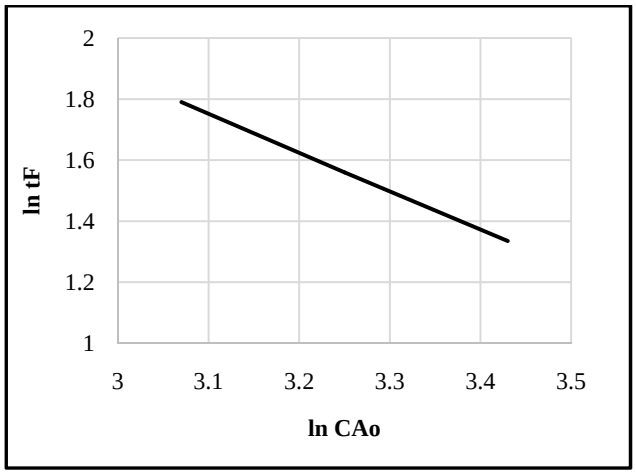

Fig. 14: Determination of kinetic parameters of cadmium(Cd) with respect to cadmium Concentration.

From the graph, the kinetic equation comes to be as follows.

$$-\frac{d[CAo]}{dt} = 0.01076 C_{Ao}^{1.43}$$

From Fig. 14, we can determine the kinetic parameters of cadmium (Cd) removal with respect to cadmium concentration. From the graph, the order of the reaction is found to be 1.43 and the **rate constant** is calculated as **0.01076 $mg^{-0.43}l^{0.43}h^{-1}$**. Though it was found that the culture could tolerate cadmium but the bacterial growth rate had decreased to 0.187 h^{-1}. It shows cadmium has an inhibitory effect on the growth.

4. CONCLUSION

We found that mixed bacterial culture could successfully tolerate lead and cadmium.The pure culture that could tolerate Pb was *Stenotrophomonasmaltophilia* (Genbankentry: FJ772015). The order of the reaction (lead (Pb) removal with respect to lead concentration) is 1.63 and the **rate constant** is **0.01967 $mg^{-0.63}l^{0.063}h^{-1}$**. For cadmium the pure culture was *Bacillus subtilise* (Genbankentry: AF500205) and the order of the reaction of cadmium removal is 1.43 and the **rate constant** is calculated as **0.01076 $mg^{-0.43}l^{0.43}h^{-1}$**.

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