Bio-Detoxification Treatment of Waste Water Containing Cadmium

Chandan Kumar Das and Harekrushna Sutar, *Member, IACSIT, IAENG*

***Abstract*—Cadmium is a highly toxic metal. The exposure to cadmium is primarily through food and water. Various conventional methods like flocculation, precipitation, ion exchange and membrane filtration are available for removal of cadmium from water at low concentrations and claimed to be expensive and inefficient. Here we have used pseudomonas fluorescens bacteria for removal of cadmium from waste water by adsorption. Analysis is done to study the growth rate of biomass with varying substrate composition with and without cadmium. Initial concentration of cadmium in feed water is varied with different residence time within the reactor and % removal of cadmium is observed. This proposed method is found to be efficient and economical for cadmium removal. Significant removal was observed. Removal was fast, metabolism independent surface process. Operations are carried out at aerobic conditions. High percentage of removal of cadmium by adsorption on bacteria is achieved, which rises up to 85%.**

***Index Terms*—Cadmium, pseudomonas fluorescens, adsorption, bioreactor.**

I. Introduction

The quality of life on earth is dependent on the overall quality of environment. The resources of the world are carelessly used by human being. The problems associated with contaminated water are now assumed to be increasing prominence in many countries. The problem covers worldwide and the estimated number of contamination sites for water is significant [1].

The urban sewage is a mixture of domestic and industrial waste water. The composition is very complex [2], [3].One among the point of gratification in the sewage effluent is the content of heavy metal like cadmium [4], [5].High concentration of cadmium in aquatic bodies pose a serious threat to the environment and public health because of their toxicity, accumulation in the food chain and persistence in nature[6].

Oral exposure to cadmium causes renal damage[7], osteoporosis[8],[9], prostate[10] and renal cancer[11]. Chronic exposure to even low levels of cadmium could also lead to adverse renal [12] and negative bone effects [8], [9]. The concentration level of cadmium in drinking water should not be more than 3μg/L [13].

The removal of toxic cadmium from water is an important issue for municipal authorities. The conventional methods for the removal of cadmium from water results in incomplete removal and high reagent or energy requirement, and generate toxic agents which are difficult to separate and other waste products that require careful disposal [14].

Steady progress in the elucidation of various microbial uptake mechanisms for cadmium results in the identification of specific microorganisms that are very promising for the treatment of cadmium contained water [15].Therefore interest in the exploitation of biotechnological methods for the removal of metal like cadmium using microorganisms is on the rise.

The literature reveals that biosorption by bacteria is an attractive and feasible option for treatment of waste water [16], [17].The main objective of this present study is to examine the percentage removal of cadmium from waste water by pseudomonas fluorescens at laboratory scale.

Manuscript received December 5, 2011; revised January 27, 2012. This work is supported in part by Jadavpur University, Kolkata, India.

Chandan Kumar Das and Harekrushna Sutar are with Indian Institute of Technology, Kanpur, 208016, India (e-mail: cdchandandas9@gmail.com; h.k.sutar@gmail.com).

II. Materials and Equipments

A. Chemical

(a) NaCl – E.Merck(India) Ltd. India.
(b) Sucrose – E.Merck(India) Ltd. India
(c) Yeast extracts powder – E.Merck(India) Ltd. India
(d) Beef extracts powder – E.Merck(India) Ltd. India
(e) Peptone – E.Merck(India) Ltd. India
(f) Alcohol (99.9%) – Bengal Chemical Ltd., India.
(g) HCl ,GR– E.Merck(India) Ltd. India.

B. Equipments

Equipments used in the present study and their specifications are given bellow;

(a) B.OD. Incubator cum shaker –S.C Dey and Co., Kolkata, India
(b) Hot air oven –Bhattacharya and Co., Kolkata ,India
(c) Cold centrifuge(C-24) –Remi Industries Ltd, Mumbai, India
(d) Laminar flow Hood–Bhattacharya and Co,Kolkata, India
(e) Autoclave –G.B.Enterprise, Kolkata, India.
(f) U.V. Shower – Bhattacharya and Co, Kolkata, India.
(g) Digital weighing machine –Sartorius
(h) Tulu Pump – G.B Enterprise , Kolkata ,India
(i) Compressor – G.B.Enterprise, Kolkata, India.
(j) Bioreactor (Height 1 m and diameter 5 cm)
(k) Refrigerator –S.C Dey and Co., Kolkata, India.

C. Analytical Instruments

(a) Double *beam* UV-VIS Spectrophotometer–Chemico.
(b) Atomic Absorption Spectrophotometer– Perkin Elmer.

D. Source of Isolation of Bacteria

(a) Wastes from Nickel-Cadmium removal battery industry.
(b) Wastes from cadmium pigmented plastics, ceramics, glasses, paints and enamels industries.
(c) Wastes from cadmium stabilized polyvinylchloride products.
(d) Wastes from cadmium coated ferrous and non-ferrous products.
(e) Wastes cadmium alloys.
(f) Wastes from cadmium electronic compounds.

These wastes are collected from an above mentioned product manufacturing industries in and around Kolkata. A clean dry screw capped tube was taken for collection of sample and was filled with it.

III. Experimental Method

In the present study the experimental procedure is typically consists of two phases.

1st phase (Batch process): In first part of the process the growth of bacteria (pseudomonas fluorescens) has been studied with varying cadmium concentration along with substrate (nutrient) concentration. The objective is to analyze the gain in bacterial mass with substrate and cadmium. The process indicates a negligible resistance of the increase of cadmium ion concentration on bacteria growth.

2nd phase: In this part the grown microorganism from the first phase is immobilized on suitable packing material (rice husk) to be used in the packed bed reactor. Continuous runs are conducted using the reactor and the behavior is observed with variable volumetric flow rate of feed water and initial concentration of cadmium ion in water. Finally cadmium concentration is determined in outlet water.

IV. Batch Process

Mother Inoculations: Under aseptic conditions, six 20ml suspended cultures inoculations were done using the medium and a pinch of fresh bacteria mass is added. The whole mass is kept in incubator shaker at 32℃ and 120rpm for 3 days.

1st inoculation: six 20 ml suspended cultures inoculation is done using the same media and 2 ml of mother inoculums. Then incubated at 32℃ and 120 rpm for 3 days.

2nd inoculation: Under similar conditions six 20 ml suspended cultures inoculation is done using the same media and 2 ml of 1st inoculums .Then the mass is incubated at 32℃ and 120 rpm for 3 days.

Finally batch study is done using the 2nd inoculums. A 48 hours batch is done and the incubation periods interval is 4 hours. First of all the conical flasks are plugged with non-adsorbent cotton and sterilized in autoclave. After that inoculation is done in laminar flow hood using 2 ml of 2nd inoculums and 20 ml of media. After inoculation is over all the conical flasks are kept in incubator shaker at 32℃ and 120 rpm. Then conical flasks are taken out from incubator shaker with their incubations periods and dry biomass is measured.

Biomass determination: Determination of cellular dry weight is done using direct method, and applicable only for cells grown in solid free medium. Samples of culture broth are centrifuged at 10000 rpm for 20 minutes. Filtered and washed with buffer solution. The washed cell mass is then dried at 80℃ for 24 hours. Then dry cell weight is measured.

V. Experimental Set-Up

The experimental set-up used for the experimentation is sketched in Fig. 1. Which consistes of 1) reservoir for cadmium containing water, 2) tulu pump for mainatining the flow to the reactor, 3) flow controlling valve, 4) rotameter for monitoring the flow rate, 5) bio reactor of 1 meter length and 5 cm diameter packed with rice husk, 6) compressor to provide air to the reactor to maintain aerobic condition for reaction, 7) sample collecting valves for outlet water analysis.

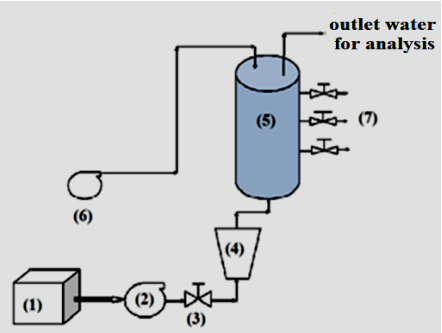

Fig. 1. Schematic diagram of experimental set-up

VI. Experimental Procedure

First microorganism is immobolized on suitable packing material .The packing material used for the experimentation is rice husk.Then the bioreactor is filled up with these packing materials immobolized with microorganism. Cadmium water is stored in the reservoir.Water enters by inlet nozzle at the bottom of the reactor via a tulu pump. Water moves in upward direction through the packed bed .Flow rate is controlled using the control valve and simultaneously monitored by rotameter.Air is supplied at the top of the reactor by a compressor .Outlet water is collected from outlet nozzle in a sample collecting tube.Continuous runs are conducted varying concentration of cadmium as well as flow rate of water .The change in volumetric flow rate changes the residence time in the reactor automatically. Different samples of different residence time for different concentrations are collected .Three residence time is followed as 4 hr, 8 hr and 16 hr respectively.As the water is passed through immobilized packing material cadmium is absorbed by bacteria cell surface.Cadmium is analysed by atomic absoption spectrometer (AAS) to know the percentage removal of cadmium.

VII. Results and Discussion

A. Inoculation without Cadmium

This phase consists of inoculation without the presence of cadmium.The composition of nutrient taken is tabulated in Table I. Here sucrose composition has been varied and taken

as 10 , 15 , 20 gm/L, respectively. The total amount of nutient broth is 20 ml.Inoculation has been done and the biomass growned with different inoculation time has been plotted in Fig. 2.

TABLE I: SUBSTRATE COMPOSITION WITHOUT CADMIUM.

Constituents of Nutrient	Amount (gm/l)	Total amount (ml)
Nacl	30	20
Yeast	10	
Sucrose	10, 15, 20	

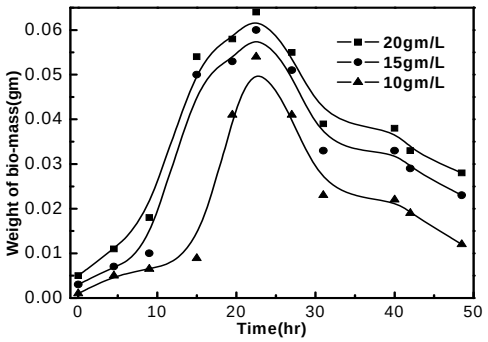

Fig. 2. Biomass growth kinetics for inoculation without cadmium, sucrose content: 10, 15 and 20 gm/l

It is analyzed that the growth rate of biomass increases with nutrient composition. The biomass weight produced increases with respect to increase in sucrose composition. The growth rate steadily increases with time up to a peak point and then falls down slowly as shown in Fig. 2.

B. Inoculation with Varying Cadmium Concentration

TABLE II: SUBSTRATE COMPOSITION WITH CADMIUM

Constituents of nutrient	Amount (gm/l)	Cadmium ion concentration (ppb)
Nacl	30	5 ,10,15
Yeast	10	
sucrose	10, 15 , 20	

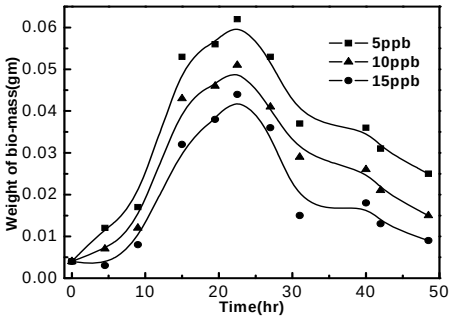

Fig. 3. Biomass growth characteristics curve for nutrient with cadmium, sucrose content: 10gm/L.

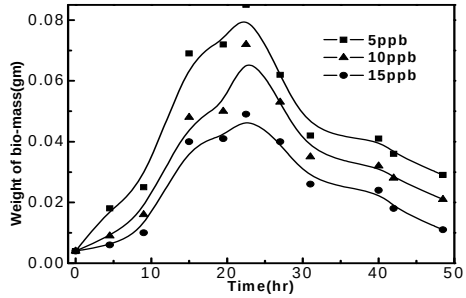

Fig. 4. Biomass growth characteristics curve for nutrient with cadmium, sucrose content: 15gm/l.

This phase plays an important role in experimental study. The objective is to analyze the effect of cadmium on biomass growth rate. Different cadmium concentrations are taken keeping the nutrient constituents constant and studied to measure the resistive power of bacteria growth towards cadmium. Cadmium concentration is varied as 5, 10, 15 ppb respectively. The growth kinetics curve is almost similar in behavior for the earlier case (i.e., without cadmium). It is observed that the presence of cadmium has negligible effect on growth rate. Although it is found that growth rate decreases from lower to higher cadmium composition.

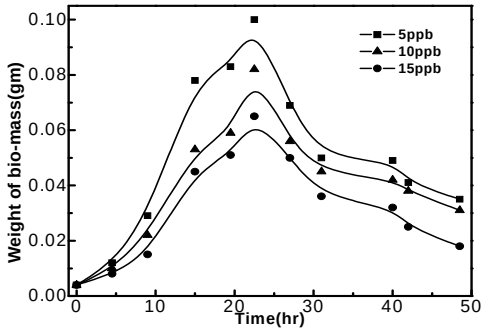

Fig. 5. Biomass growth kinetics curve for nutrient with cadmium, sucrose content: 20gm/l.

C. Cadmium Estimation

Bioreactor is operated continuously with aerobic condition. From the continuous runs of bioreactor, different samples are collected at different residence time. Samples are tested in AAS. The percentage removal of cadmium is shown in Table III.

TABLE III: CHARACTERISTIC DATA FOR CADMIUM REMOVAL FROM FEED WATER

Cadmium concentration of feed water (ppb)	Residence time (h)	% of removal of cadmium
10	4	73
	8	76
	16	81
15	4	73
	8	77
	16	83
20	4	74
	8	79
	16	85

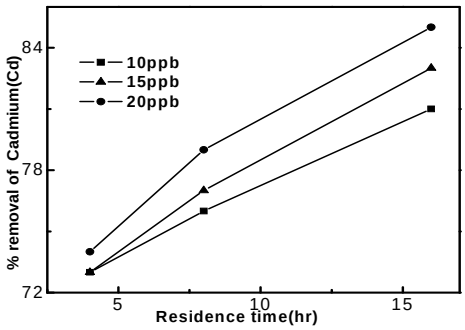

Fig. 6. % of cadmium removal with time.

Further analysis has been done to study the variation of cadmium and substrate concentration with respect to reactor height. For a fixed flow rate, the water samples are collected from the collecting nozzles at different height of the bioreactor (see Fig. 1).Analyzed in AAS.

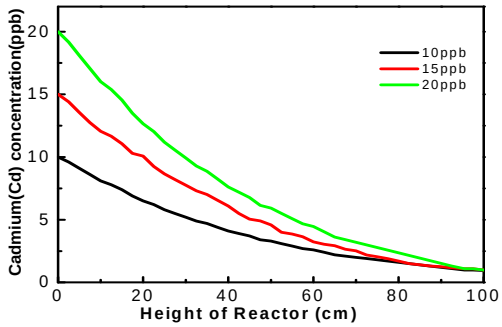

Fig. 7. Characteristic curve for variation of Cadmium with reactor height.

The result shows that the cadmium concentration decreases along the reactor length. On the other hand it is also found that more the cadmium content in feed water higher will be the % of removal along the reactor length. Simultaneous study also is carried for substrate concentration. It is observed that, in the similar fashion, the substrate concentration also shows a decreasing characteristic along the height of bioreactor.

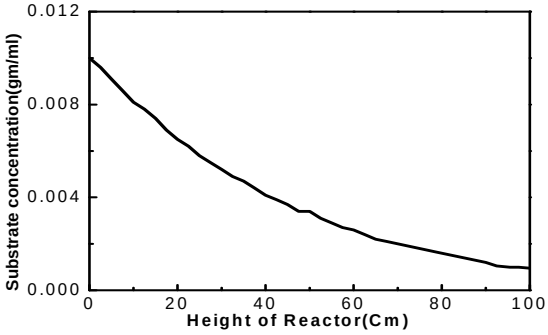

Fig. 8. Curve showing variation of substrate with reactor height.

VIII. Conclusion

Bio-detoxification treatment provides a technique for cleaning up pollutant by enhancing biodegradation process. These techniques are cost effective and dedicating sites which are set aside for long term research purpose. These opportunities offer potential for significant advances. The process offers an efficient way to treat contaminated ground water. Its advantages generally outweigh the disadvantages. The above batch study shows that cadmium is adsorbed by the bacteria cell surface. As a result cadmium is removed from water. During the continuous runs from bioreactor it is clear that as concentration of cadmium is more in water, the more will be the % removal of cadmium. Though it is a small scale laboratory process, it can act fruitfully in large scale industrial process, since it is easy to handle and used packing materials are readily available at low cost effect.

Acknowledgement

Both the authors are grateful to chemical Engineering department, Faculty of Engineering and Technology, Jadavpur University, Kolkata, India, for the kind technical support to conduct the experiments and for data analysis.

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Chandan Kumar Das was born on 25 January, 1983 at west Bengal state, India .Mr. Das has done B.E. & M.E in chemical engineering from Jadavpur University, Kolkata, India. Mr. das is presently pursuing Ph.D. in Chemical engineering at Indian Institute of Technolgy, Kanpur, U.P, India. He is involved to study the free energy calculation of solids and nano-crystals. Mr. Das has taken summer training at Dankuni coal complex (coal India Limited), Dankuni, West Bengal, India.

Harekrushna Sutar was born on 2 July 1982 at Dasarathpur, Jajpur district Orissa, INDIA. He has done B.E. in chemical Engineering from Utkal University, Bhubaneswar, India. M.Ch.E. from Jadavpur University, Kolkata, India. Mr. Sutar has worked in National Institute of Technology, Rourkela, India, as Technical Assistant for one year. He has also worked in Bhaba Atomic Research centre, Mumbai, India as a scientific officer for 2 year. Mr. Sutar has taken training at Haldia refinery, Haldia (Indian oil corporation limited), West Bengal, India & Rourkela steel plant , Rourkela(Steel Authority of India Limited) ,Orissa , India. Mr. Sutar has worked on effect of distributor design on drying kinetics of granular material using a fluidized bed drier. He is member of IACSIT, Singapore & IAENG (with membership number 118128).