Available online at www.elixirpublishers.com (Elixir International Journal)

Pollution

Elixir Pollution 47 (2012) 8921-8923

ISSN: 2229-712X

Bioremediation of Copper present in waste water using isolated Micro-organism *Stenotrophomonas sp.* PD2 from Soil of Dhapa, Kolkata, India

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ARTICLE INFO

Article history:

Received: 21 April 2012;

Received in revised form:

25 May 2012;

Accepted: 14 June 2012;

Keywords

Copper (II) resistant bacteria,

Biosorption,

Copper,

Dhapa soil.

ABSTRACT

Copper is one of the essential trace nutrients in all high plants and animals. But it can be toxic in high concentration. It has been found that marine life is damaging due to high copper concentration in water. This study investigated that degradation of copper by a group of bacteria isolated from copper polluted areas, Dhapa, Kolkata. Copper resistant bacteria i.e. *Stenotrophomonas sp.* PD2 was isolated from soil of Dhapa had shown maximum copper resistance upto 200 mg L^{-1} Cu(II). The influence of different parameters responsible for the bioremediation of copper by the copper-resistant bacteria, such as initial copper concentration and initial pH, contact time of the solution was studied. From experimental results it was observed that almost 95% copper can be reduced from wastewater using the isolated micro-organism.

Introduction

Speedy industrialization continuously insists environmental contamination and pollution cause by heavy metals in the industrial effluents [1,2]. Copper is present in various industrial effluents [3,4].

Though Copper is one of the essential trace nutrients. But it can be toxic if present in higher concentration. It has been found that marine life is damaging due to high copper concentration in water [5,6] and high copper uptakes may cause liver, kidney, brain damage and even death. Wilson's disease, Schizophrenia also occurs for high copper uptakes [7].

Copper removal from wastewaters has a valid importance and its toxicity to human body is at levels of 100–500 mg day^{-1} [8-9].

There are various formal methods of copper (II) removal from wastewaters. But these methods require high capital and operating costs, high energy requirement and also suffer from incomplete removal in some cases [10].

There is a need of new cost-effective process to remove heavy metals from waste water to reduce the environmental pollution causes by the heavy metal. Bioremediation is an effective process to reduce environmental pollution due to heavy metals. Biosorption is a cost-effective microbial process to remove heavy metal from the waste water [1, 11-25]. There are two types of biosorption- a) Active biosorption, b) passive biosorption. Live bacteria used in active biosorption whereas in passive biosorption dead bacterial biomass used to remove heavy metal from aqueous solution. In active biosorption bacteria uptakes the heavy metal metabolically whereas in passive biosorption heavy metals gets adsorb on cell surface of the bacterial cell.

The objective of the present study was to investigate the capacity of active biosorption of copper by using the copper resistant bacteria isolated from soil of polluted wasteland (Dhapa). The batch study involves changing one independent parameter (pH, heavy metal concentration) while maintaining others at a fixed level.

Materials and methods:-

Bacteria Isolation and cultivation:-

At first, soil was collected from polluted wasteland (Dhapa) at Kolkata. At first soil was diluted to sterile normal saline water and serial dilution was followed upto 10^{-7} times. The nutrient agar plates containing various copper concentration was inoculated by the soil serial dilution's solution of 10^{-7}. These plates were incubated at 30 degree centigrade for 24 hours. Maximum copper resistance of the bacteria was obtained at 200 mg L^{-1} Cu(II) , using $CuSO_4,5H_2O$ in nutrient agar (Himedia) plate. Cu(II) concentration was varied from 10 mg L^{-1} upto 200 mg L^{-1} in the nutrient agar plates..

Biochemical tests and staining of the isolated bacteria:- The isolated bacteria was gram-negative, coccus shaped bacteria.

Bacterial characterization:-

On the basis of nucleotide homology and phylogenetic analysis the isolated micro-organism was *Stenotrophomonas* sp. The bacterial nucleotide sequence has been submitted to the GenBank under Accession Number: PD2 JQ809229.

Chemicals and media:-

A stock solution of copper was prepared using Pentahydrate copper sulfate ($CuSO_4,5H_2O$) dissolving in distilled water. 1000 mg L^{-1} Cu(II) stock solution was prepared by diluting 3.93 gm of $CuSO_4,5H_2O$ (obtained from Merck) in 1 liter distilled water. Using this stock solution different copper concentration media was prepared. Nutrient broth was used as the media(obtained from Himedia).

Biosorption Experiments:-

Nutrient broth consists of different concentration of Cu solution and different pH was prepared for the experiment. For pH adjustment 0.1(N) HCl and 0.1(N) NaOH solutions were used. After that media was autoclaved in 250 ml conical flasks containing 100 ml media. The media was inoculated with the

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isolated bacteria. Conical flasks were kept in rotary shaker incubator at 30°C, 120 rpm. After different time intervals sample was collected and centrifuged at 6000 rpm for 10 minutes. Supernatant fractions was analyzed for the remaining copper ions in the biosorption media were determined spectrophotometrically (Techcomp UV 2300 Spectrophotometer) at 460 nm by using sodium diethyl dithiocarbamate (obtained from Loba) as the complexing agent [26]. Reduction of copper ion present in solution was analyzed using the following equation-

$$\%Degradation = \frac{(C_0 - C_1)}{C_0} x100 \quad \text{---------- (1)}$$

Where, C_0=initial copper concentration, mg/L
C_1=final copper concentration, mg/L

Results and Discussions:-

The effect of initial copper concentration on Biosorption:-

Bioremediation of copper was affected by several factors such as initial pH, initial copper ion concentration, and temperature. The percentage of reduction was a function of the initial metal concentration. The increase in initial copper concentration resulted in increase in the capacity of metal biosorption from 10 to 100 mg/L. With increasing copper concentration, copper reduction increased and meanwhile the reduction decreased [Fig 1].

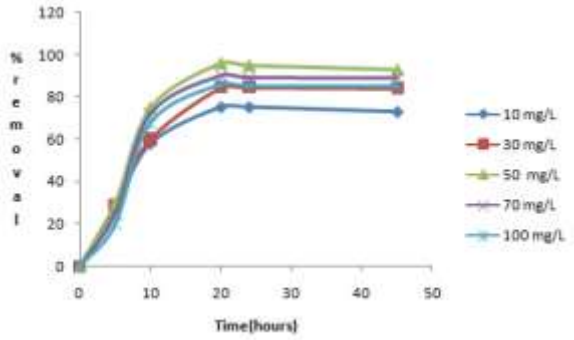

Fig 1: copper reduction at different initial copper concentration at different time intervals.

Effect of pH:-

Bacterial cell surface was negatively charged whereas Cu(ll) was positively charged, and as a result copper may attached with the bacterial cell surface [27]. The batch study with varying pH was performed in 100 ml (50 mg L^{-1}) copper concentrated nutrient broth solution. Maximum removal was obtained at high pH, i.e. 6. But copper salt's solution started precipitating above pH 6 as observed in the laboratory. For this problem in this experiment high pH range was kept at 6 [Figure 2]. It was observed that as the pH increased, the % removal of copper increased.

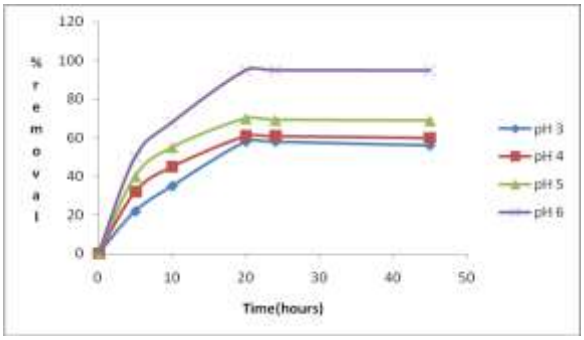

Fig 2: Copper reduction at different pH and at different time intervals for 50 mg/l copper present in wastewater

Effect of contact time on Biosorption:-

Bacterial population in the culture may increase with increasing time, so bacterial metabolic uptakes of copper may increased with time. Upto 20 hours time the copper biosorption increased, after that the removal of copper starts decreased. It seems that the after 20 hours bacteria may be in death phase due to lack of food supply.

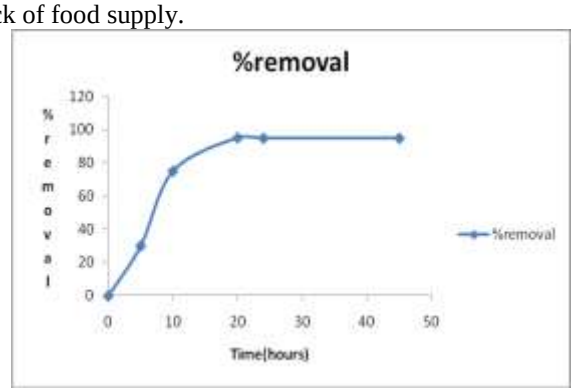

Fig 3: % Reduction of copper at different time intervals.

Conclusions:-

The active biosorption characteristics of Cu(II) ions were studied using isolated *Stenotrophomonas sp.* PD2. From experimental results it was observed that almost 95% of copper present in solution can be degraded by this species. So, this result indicated that the isolated novel bacteria may be used for effective degradation of Cu(II) ions present in wastewater.

References:-

[1] Cetinkaya Do¨nmez. G, Aksu Z , Ozturk A, Kutsal T (1999) A comparative study on heavy metal biosorption characteristics of some algae. Process Biochemistry 34 : 885–892.

[2] Tekin S_ahan a, Hasan Ceylan b, Nurettin S_ahiner c, Nahit Aktas_ a,* Optimization of removal conditions of copper ions from aqueous solutions by Trametes versicolor. Bioresource Technology 101 (2010) 4520–4526

[3] Y.S. Ho, J.F. Porter, G. McKay, Equilibrium isotherm studies for the sorption of divalent metal ions onto peat: copper, nickel and lead single component systems,Water, Air Soil Pollut. 141 (2002) 1–33.

[4] A. Özer, D. Özer, A. Özer, The adsorption of copper(II) ions on to dehydrated wheat bran (DWB): determination of the equilibrium and thermodynamic parameters, Process Biochem., 39(2004) 2183–2191.

[5] P. Saha, S.Datta, S. K. Sanyal, Assessment of soil admixture membrane used as liner for waste landfill. Indian Science Cruiser, 22(5) (2008) 40–52.

[6] P. Saha, S. Datta, S. K. Sanyal, Hazardous waste pollution prevention using clay with admixture. Clean—Soil, Air, Water, 36(2) (2008) 230–238.

[7]P. Saha, S. Datta and S. K. Sanyal, Application of Natural Clayey Soil as Adsorbent for the Removal of Copper from Wastewater. Journal of Environmental Engineering © ASCE, (2010)1409-1417.

[8] Chong, K.H., Volesky, B., 1995. Description of 2-metal biosorption equilibria by Langmuir-type models. Biotechnology and Bioengineering 47, 451–460.

[9] Norton, L., Baskaran, K., McKenzie, S.T., 2004. Biosorption of zinc from aqueous solutions using biosolids. Advanced Environmental Resource 8, 629–635.

[10] S. Karthikeyan, R. Balasubramanian, C.S.P. Iyer, Evaluation of the marine algae Ulva fasciata and Sargassum sp. for the biosorption of Cu(II) from aqueous solutions, Bioresour. Technol. 98 (2007) 452–455.

[11] Y.S. Ho, J.F. Porter, G. McKay, Equilibrium isotherm studies for the sorptionof divalent metal ions onto peat: copper, nickel and lead single component systems,Water, Air Soil Pollut. 141 (2002) 1–33.
[12] J. Wang, C. Chen, Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review, Biotechnol. Adv. 24 (2006) 427–451.
[13] A.I. Zouboulis, K.A. Matis, M. Loukidou, F. ˇSebesta, Metal biosorption by PANimmobilized fungal biomass in simulated wastewaters, Colloids Surf. A 212 (2003) 185–195.
[14] K. Parvathi, R. Nagendran, R. Nareshkumar, Lead biosorption onto waste beer yeast by-product, a means to decontaminate effluent generated by battery manufacturing industry, Electronic J. Biotechnol. 10 (2007) 92–105.
[15] E.L. Cochrane, S. Lu, S.W. Gibb, I. Villaescusa, A comparison of low-cost biosorbents
and commercial sorbents for the removal of copper from aqueous media, J. Hazard. Mater. B137 (2006) 198–206.
[16] J.C. Igwe, A.A. Abia, A bioseparation process for removing heavy metals from waste water using biosorbents, Afr. J. Biotechnol. 5 (2006) 1167–1179.
[17] M. Mapolelo, N. Torto, Trace enrichment of metal ions in aquatic environmentsby *Saccharomyces cerevisiae*, Talanta 64 (2004) 39–47.
[18] P. Vasudevan, V. Padmavathy, S.C. Dhingra, Kinetics of biosorption of cadmium on Baker's yeast, Bioresour. Technol. 89 (2003) 281–287.
[19] V. Padmavathy, P. Vasudevan, S.C. Dhingra, Thermal and spectroscopic studies on sorption of nickel(II) ion on protonated baker's yeast, Chemosphere 52 (2003) 1807–1817.
[20] V. Padmavathy, P. Vasudevan, S.C. Dhingra, Biosorption of nickel ions on baker's yeast, Process Biochem. 38 (2003) 1389–1395.
[21] L.N.L. Vianna, M.C. Andrade, J.R. Nicoli, Screening of waste biomass from *Saccharomyces cerevisiae*, *Aspergillus oryzae* and *Bacillus lentus* fermentations for removal of Cu, Zn and Cd by biosorption, World J. Microbiol. Biotechnol. 16 (2000) 437–440.
[22] B. Volesky, Advances in biosorption of metals: selection of biomass types, FEMS Microbiol. Rev. 14 (1994) 291–302.
[23] R. Ashkenazy, S. Yannai, R. Rahman, E. Rabinovitz, L. Gottlieb, Fixation of spent *Saccharomyces cerevisiae* biomass for lead sorption, Appl. Microbiol. Biotechnol. 52 (1999) 608–611.
[24] M. Mapolelo, N. Torto, B. Prior, Evaluation of yeast strains as possible agents for trace enrichment of metal ions in aquatic environments, Talanta 65 (2005)930–937.
[25] Y. Goksungur, S. Uren, U. Guvenc , , Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass, Bioresour. Technol. 96 (2005) 103–109.
[26] Snell FD, Snell, CT. Colorimeric Method of Analysis. 3rd edition. Vol. 2. Canada: D. Van Nostrand Company, 1959
[27] S. Chowdhury and P. Das Saha, Scale-up of a dye adsorption process using chemically treated rice husk: optimization using response surface methodology, Desalination and Water Treatment, 37(2012) : 1–6.