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Removal of Heavy Metals by Indigenous Microorganisms and Identification of Gene Responsible for Remediation

Research Article

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Abstract

The present study deals with the removal of heavy metals from solid waste disposal sites. The waste generated from the small scale industrial operations such as grinding, milling, cutting, rubbing, painting, washing are collected and dumped in open grounds. The waste containing heavy metals was found subjected to action of rain, sun and atmospheric conditions prevailing in the environment. The characterization of waste was done and indigenous microorganisms were isolated and developed as culture for remediation of selected heavy metals Fe, Cu, Cd. The microbial consortium was subjected to exposure of metals at increasing concentrations separately in a minimal salt medium using incubator shaker under controlled environmental conditions and potential microorganisms were identified for each of the selected heavy metals using 16Sr-RNA technique and bioinformatic tools. Bioremediation of heavy metals have been carried out at selected concentrations viz.25ppm, 50ppm and 100ppm indigenous potential microorganisms *Klebsiallasp.* MHF ENV III using shake flask incubation method. The potential organism *Klebsiallasp.* MHF ENV III have been found effective for bioremediation of heavy metals (Fe, Cu and Cd). *Klebsiallasp.* MHF ENV III being a potential microorganism for bioremediation of heavy metals has been further studied for identification of gene responsible for bioremediation.

Keywords: Bioinformatics; Bioremediation; Heavy Metals; Potential Microorganisms.

Introduction

Environmental Pollution has become a major global concern due to rapid growth of industrialization, globalization, urbanization, modern agricultural development. Environmental pollution sources include coal-fired plants, power plants, oil refinery, incinerator, PVC factory, nuclear plants, metal production factories, plastic factories and many industrial processes/operations which involve the use of chemicals and metals. The wastes generated from the various activities are being treated by physical, chemical and biological treatment to meet the standards prescribed under the Environmental Protection Act (EPA). In spite of the present treatment, the organic and inorganic- pollutants are found persisting in soil-water environment. The organic compounds can be degraded by natural bioremediation but metals do not biodegrade by microbial activities and found in our environment. Metals pose a very different pollution processes, earth' crust, soil erosion, mining, industrial discharge, urban runoff, sewage effluent, air pollution fallout, pest or disease control agents applied to plants and other toxic levels. Effects of toxic metals on living organisms have been considered as exclusive-environmental problem.

The solid waste generated from small scale industries containing metals pose an environmental problem than the waste generated by large scale chemical industries. In a waste disposal site of small scale industries, microorganisms develop multiple ways of using both essential and unwanted toxic metals. Microbial-metal interaction in waste disposal is of interest to environmentalist to use the adapted microorganisms as a source of biomass for bioremediation of heavy metals by developing laboratory techniques that could be applicable to the field for decontamination of environment. The study of heavy metals and microbe-metal interaction in the physical-chemical environment including the mechanism of microorganism sequester to immobilize the metals from solid waste disposal site will be of high significance to the environmentalist for developing a remediation technology.

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The present research studies deal with the waste dumping due to the processes/operations of the small scale industries. Waste contains metals which are being bioaccumulate by the micro-organisms present in dumping sites. Waste is characterized for physicochemical and microbial status. Microbial consortium was developed, and potential microorganism was identified by 16Sr-RNA technique for the remediation of metals Fe, Cu and Cd. Bioremediation of heavy metals was studied using microbial consortium and potential organism separately. The comparison has been made as to ensure effectiveness of microbial consortium or potential microorganism for bioremediation of heavy metals. Further, genomic study was carried out and gene identified in potential microorganism responsible for bioremediation of heavy metals. The study has significance for removal of heavy metals to clean up environment.

Materials and Methods

Microbial Biomass

Microorganisms used for bioremediation for bioremediation of heavy metals, were isolated from solid waste samples collected from various locations of metal contaminated waste disposal site at Mira Road and Bhayander. *Klebsiallasp.*MHF ENV III had been isolated from this heavy metal contaminated site and identified as potential microorganism [2].

Experimental Set-up for Bioremediation of Heavy Metals

Bioremediation of selected heavy metals was carried out in a shake flask bioreactor. Analytical grades of metal salts (cadmium nitrate, copper sulfate and ferrous sulfate) were used to prepare stock solutions (1000mg/L) Each stock solution was sterilized by autoclaving and stored for further use.

Laboratory Scale-Up Technique for adaptation of identified Potential Microorganism *Klebsiallasp.*MHF ENV III

1ml subcultured *Klebsialla pneumonia* MHF ENV III (nutrient broth) was inoculated into Erlenmeyer flasks (250ml) containing nutrient culture media with a metal concentration of 5mg/L. The inoculated flasks were kept in orbital shaker incubator at 160rpm, 30°C for a period of 7 days. After 7 days, 1ml of this culture media was added with a metal concentration of 25mg/L, the flasks were again kept on orbital shaker incubator at 160rpm, 30°C for a period of 7 days. The microbial culture was subcultured into nutrient culture media with the metal concentration of 50mg/L, 75mg/L and 100mg/L and was kept in orbital shaker incubator at 160rpm, 30°C for increasing a total period of 35 days. The culture was adapted from lower concentration to higher concentration upto 100mg/L. The identified microorganism was found to adapt to heavy metals after carrying out adaptation by scale up process technique was able to survive at higher concentrations of metal. The adapted microbial culture of *Klebsialla pneumonia* MHF ENV III was further used for bioremediation experiment of heavy metals

Bioremediation of Iron, Copper and Cadmium

Bioremediation of the selected heavy metals (Iron, Copper and Cadmium) by *Klebsialla pneumonia* MHF ENV III was carried out in a 250ml Erlenmeyer flask containing sterile Minimal Salt Medium (MSM). In a batch biodegradation experiment with 25, 50 and 100mg/L concentrations of the metals were taken into 100ml MSM. The experiment was conducted on a shaker incubator at 25°C and continuous shaking at 130rpm. Biodegradation was assessed by comparing the disappearance of the metals in the sample and controls over the period of microbial growth. The metal concentrations were monitored over a time to compare lag periods and biodegradation rates for different concentrations. The lag period was determined as the time during which the metal concentrations remained relatively constant. Microbial growth was observed in terms of CFU and O.D. The samples (5ml) were withdrawn hourly from 0 to 6 h and then every 24 h for 14 days. Samples were transferred to 10 ml vials and capped for AAS analysis. The physicochemical parameters such as pH, temperature, electrical conductivity, alkalinity, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand were observed and maintained daily and then after an interval of a one week for consecutive weeks.

Analytical Procedure and Sample Preparation

For degradation studies of heavy metals in liquid medium, samples were centrifuged (10min, 213 1000rpm, Plastocrafts, Rota 6R-V/Fm) to separate cell mass and the supernatant. Both supernatants were digested using acid mixture of $HClO_4$:HNO_3 (3:1). Acid digestion was carried out on hot plate at 70-100°C until yellow fumes of HNO_3 and white fumes $HCLO_4$ were observed. The digestion process was continued until a clear solution remained after volatilization of acids, and was stopped when the residue in the flask was clear and white. The digested sample was dissolved in distilled water, filtered to remove impurities [3] and made up to the desired volume. The samples were analyzed by GBC 932 B+ Atomic Absorption Spectrophotometer (Australia) using air acetylene flame to estimate cadmium, copper and iron contents in the microbial samples.

Genomics Studies: Identification of Copper resistant gene pcoA responsible for bioaccumulation of copper

Bacterial plasmids contain specific genes for resistances to toxic heavy metal ions including Ag+, AsO_2, AsO_1, Cd^{2+}, $CrO4_2$, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Sb^{3+} and Zn^{2+}. pco is a structural gene which is present on pcoA operon which encodes for PCOA protein. PCOA protein is a periplasmic protein that binds copper. The plasmid pco determinant is thought to moderate periplasmic binding and oxidation of excess copper carrying out transmembrane transport.

Genomic DNA Extraction

DNA was extracted from 25 ml overnight grown *Klebsialla sp.* MHF ENV III by using the phenol: chloroform DNA extraction method. The quantity and quality of the extracted DNA were checked spectrophotometrically (UV-VIS Shimadzu) by measuring the UV absorption spectrum of the DNA solution at 260nm and 280nm and the DNA was finally dissolved in sterile Tris-EDTA buffer. The electrophoresis unit was used to carry out the electrophoresis. The resolved gel was visualized on a gel documentation unit (Bio Rad, VersaDoc MP 5000 system).

Plasmid DNA Extraction

Plasmid DNA extraction also extracted to amplifying the target gene. DNA was finally dissolved in sterile Tris-EDTA buffer. The Bangalore GeNei electrophoresis unit used to carry out the electrophoresis. The resolved gel was visualized on a gel documentation unit (Bio RAd, VersaDoc MP 5000 system)

PCR primers used for amplifying the pocA gene fragments

Three primer pairs were designed for amplification of pcoA gene using PRIMER3 software. ….. DNA fragments coding for the structural gene pcoA were amplified by using eppendorf master cycler pro and 25µl reaction mixture. The comparison of Dream Taq PCR 2X master mix was as follows: Taq DNA polymerase: 0.05 units/ µl, MgCL2: 4mM, dATP: 0.4mM, dTTP: 0.4mM, dCTP: 0.4mM, dCTP: 0.4mM. The PCR reaction mixture was prepared by mixing 6 µl of master mix, 2 µl of 250 pmol each primer, 1 µl of 50 ng template DNA, and 6 µl nuclease free DDW. PCR cycling started with a 5 min at 95°C hot step after which cycling was paused, 0.5 µl Taq polymerase added and cycling resumed. Amplifications were run for 30 cycles in a thermal cycler PxE 0.2 following a denaturation step at 95°C for 30 s, an annealing step at 65°C for 45 s and elongation step at 72°C for 90 s, followed by a final extension, for 10 min at 72°C.ThepcoA gene was amplified using three sets of primers and the amplified product was resolved by a final extension for 10 min at 72°C. Nucleotide sequence of the pcoA gene was determined by DNA sequencing with the help of both forward and reverse PCR primers, and a DNA sequencer. The NCBI/GenBAnk database search was conducted with the BLAST program. The translation of the nucleotide sequence was done with the help of online molecular biology tools, ExPASy. The restriction endonuclease sites of the gene was determined with the help of online analysis tools. The promoter region was determined by Softberry-BRPROM program.

Result and Discussion

The present research study deals with bioremediation of heavy metals with special reference to Cd, Cu and Fe using identified microorganisms from solid waste metal contaminated waste disposal site. The potential organism identified for each metal were sequenced and identified by BLAST techniques [1]. Bioremediation of metals (Fe, Cu and Cd) have been studied using microbial consortium as well as potential organism i.e. *Klebsialla sp.* MHF ENV III.*Klebsialla sp.* was identified as potential microorganism and used for the bioremediation of iron, cadmium and copper at selected concentrations viz. 25ppm, 50ppm and 100ppm in minimal salt medium using shake flask method under controlled environmental conditions. Samples were assessed for bioremediation of these selected heavy metals at a interval of every hour for a day: thereafter for a period of 7 days at interval of each day and later assessed at 14th day and 21st day. The environmental parameters have also been studied under which these heavy metals were remediated.

Bioremediation of Iron by *Klebsialla* sp. MHF ENV III

Iron being nutrient was remediated by *Klebsialla sp.* even up to a concentration of 100ppm. Iron at a concentration of 25ppm was bioaccumulated 100% after a period of 21days, whereas at an initial concentration of 50ppm iron was bioaccumulated 100% after a period of 21days. Whereas in case of 100ppm percentage biosorption was slower this showed that 100ppm after a span of 21 days (Figure 2). The environmental parameters have also been studied under which iron, cadmium and copper was remediated using shake flask method under controlled environmental conditions in an incubation shaker. pH was monitored throughout the experiment which was found to be decreasing from 7 to 5.6, 5.7 and 5.8 after 1 day of the experiment, in case of 25ppm, 50ppm and 100ppm respectively. A research study done by Qaiser et.al. has reported that at pH values close to five, the adsorbent surfaces are negatively charged due to release of H+ ions, therefore these attract cations. At pH close to 5, the binding sites became negatively charged due to presence of hydroxyl, carboxylic and amino groups [4]. Nasseri et al. have observed maximum biosorption of metals at pH5. With further increase in pH, the percent removal of metal was decreased [5]. Electrical conductivity was found to vary from 16mS/cm-15.8 mS/cm, 15.3mS/cm-15.9 mS/cm, 15.3 mS/cm-15.9 mS/cm at a concentration of 25ppm, 50ppm and 100ppm of Fe respectively. Temperature studied at varying concentration of metal is a good indicator for solubility of metals, electricity conductively. Besides, lower pH as decreased from 35°C to 29°C in 25ppm containing iron but from 29.3°Cto 30°C respectively. In another study done by Green-ruiz et al., shows that an increase of temperature resulted in higher maximum biosorption of Cd, with the highest one at 35°C. BOD was found to increase from 5mg/L to 8mg/L at 25ppm, 3.9mg/L to 6mg/L at 50ppm and 2.5 mg/L to 4.3mg/L at 100ppm after a period of 21 days. Decrease in DO concentration during bioremediation of each concentration of iron was found to be inversely proportional to the BOD values. COD was found to decrease 63.7% in case of 25ppm, 62.5% in case of 50ppm and 60.8% case of 100ppm after a period of 21days of experiment (Figure 1).

Bioremediation of Copper by *Klebsialla sp.* MHF ENV III

Copper at a concentration of 25ppm was bioaccumulated 100% after a period of 21 days, whereas at an initial concentration of 50ppm copper was bioaccumulated 92.1% after a period of 21 days. Whereas in case of 100ppm percentage biosportion was slower which shows that 100ppm concentration of copper was toxic of *Klebsialla sp.* and biosportion was found to be 86.7% after a period of 21days (Figure 4). This shows that *Klebsialla sp.* This shows that *Klebsialla sp.* can be used to remediate copper to decontaminate the environment. Similar research done by Semra Ilhan et al., shows the *Staphyloccus saprophyticus* could adsorb 105ppm of copper upto 44.94ppm. Our findlings are also in agreement with [6] who reported Cu+2, biosorption potential of three isolates 15,16 17 of *Pseudomonas sp.* at concentrations 50ppm and 100ppm beyond which saturation was achieved. Isolate 15 showed maximum biosorption (73.8%) at 50ppm metal concentration while further increasing the concentration to 100ppm decreased the potential to 67.1%. Fahmy et al. have also reported potentially of *Enterobacter sp.* Cu 1 strain to resist 275mg Cu/l where *Stenotrophomonas sp.* [7]. pH was monitored throughout the experiment which was found to be decreasing from 7 to 7.2, 8.3 and 8 after 21 days of experiment in case of 25ppm, 50ppm and 100ppm respectively. The variation in pH is due to the interaction of microorganism with iron at a concentration of 25, 50 and 100ppm. Electrical conductivity represents dissolved solids which have also been studied as environmental parameter at varying concentra-

Figure 1. Variation In Environmental Parameters During Bioremediation of Iron.

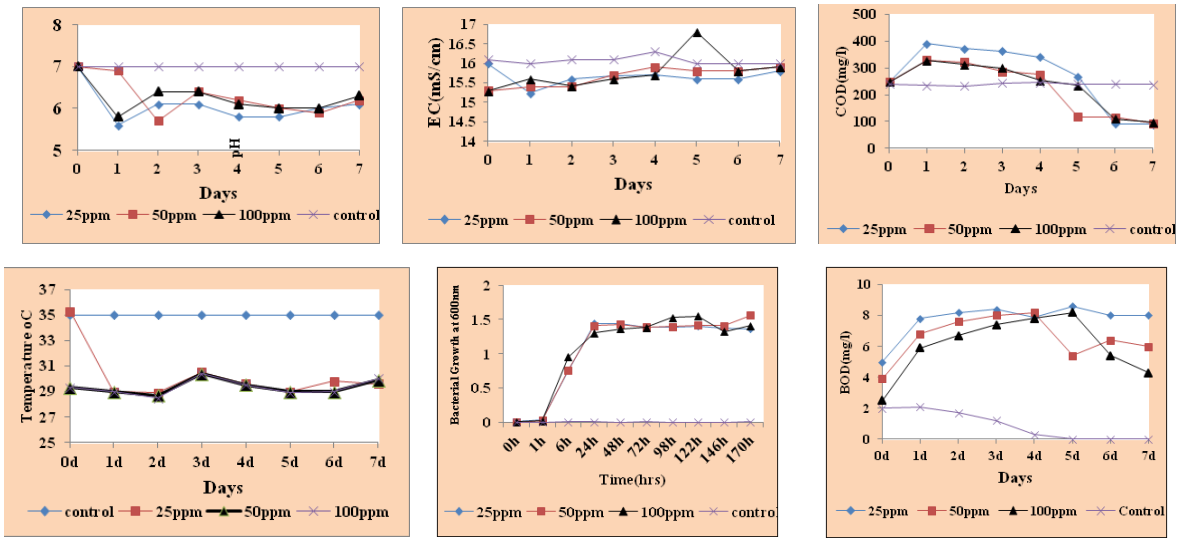
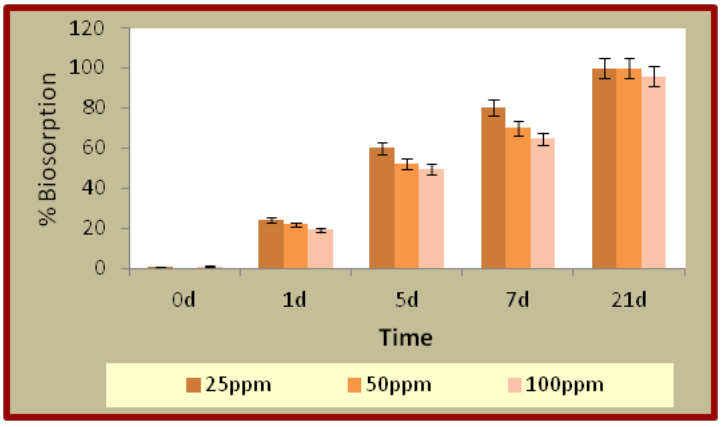


Figure 2. Biosorption Potential of *Klebsiella* sp. MHF ENV III For Bioremediation of Iron.



tions of copper over a period of 21 days. Electrical conductivity was found to vary from 16.6 mS/cm-16.9 mS/cm, 16.3 mS/cm-16mS/cm, 16.1 mS/cm-16 mS/cm at a concentration of 25 ppm, bioremediation experiment from 34°C to 32°C at 25 ppm, from 35°C to 30.7°C at 50ppm and from 35°C to 31°C at 100ppm [11]. have studied the effect of temperature of heavy metal adsorption. BOD was found to increase from 5mg/L to 5.6mg/L at 25ppm, 4.6mg/l to 6mg/L at 50ppm and 3mg/L to 6.9mg/L at 100ppm after a period of 21 days. In case of control, BOD was found to decrease after a period of 7 days upto non detectable amount which shows there was no growth of microorganisms. Majid Sa'idi has been reported that BOD5 is suppressed significantly by even concentrations (12mg/L) of copper or chromium [8]. Percentage COD reduction was found to be 95.5% in case of 25ppm concentration after a period of 21 days of experiment (Figure 3).

Bioremediation of Cadmium by *Klebsialla sp.* MHF ENV III

Cadmium at a concentration of 25ppm was bioaccumulated 92% after a period of 21 days, wheras at an initial concentration of 50ppm cadmium was bioaccumulated 89.2% after a period of 21 days. Whereas in case of 100ppm percentage biosorption was slower which shows that 100ppm concentration of cadmium was toxic to *Klebsiallasp.* and biosorption was found to be 67.3% after a period of 21 days. Bioremediation of cadmium was found to be effective by *Klebsialla sp.* at 25, 50 and 100ppm (Figure 5). *C.tropicalis* was also able to remove Cd+2 56% and 73% from the waste water after 6 and 12 days respectively [9]. pH was monitored throughout the experiment which w as found to be decreasing from 7 to 6.8, 6.4 and 6.7 after 21 days in case of 25ppm, 50ppm and 100ppm. Decrease in pH increases solubility of metal at lower concentration of cadmium [10]. have correlated cadmium biosorption with the variation in pH and iilustrated that the amount of Cd+2 metal ion adsorbed increases with its initial concentration as well with increase in the solution pH or basicity. Say et al., have also correlated biosorption of cadmium with pH values [12]. Temperature was found to be optimum for the growth and prolification of microorganism for bioremediation of cadmium. It was found to vary from 31.5°C to 29.3°C at 25ppm, 31.6°C to 28.6°C at 50ppm and 31.6°C to 28.6°C at 100ppm of cadmium concentration. Electrical conductivity was found to vary from 16.4 mS/cm-16.3 mS/cm, 15.9 mS/cm-15.6mS/cm, 16.1mS/cm-15.8mS/cm at a concentration of 25ppm, 50ppm and 100ppm of cadmium respectively. BOD was found to increase from 4mg/L to 3.9mg/L in 25ppm, from 3mg/L to 4mg/L in 50ppm and from 1.6mg/L to 2.6mg/L in 100ppm of cadmium concentration after a period of 21 days. The observed decrease in BOD is primarily due to the consumption of oxygen by the microbial biomass for their growth and proliferation. Microbial growth, measured in

Figure 3. Variation In Environmental Parameters During Bioremediation of Copper.

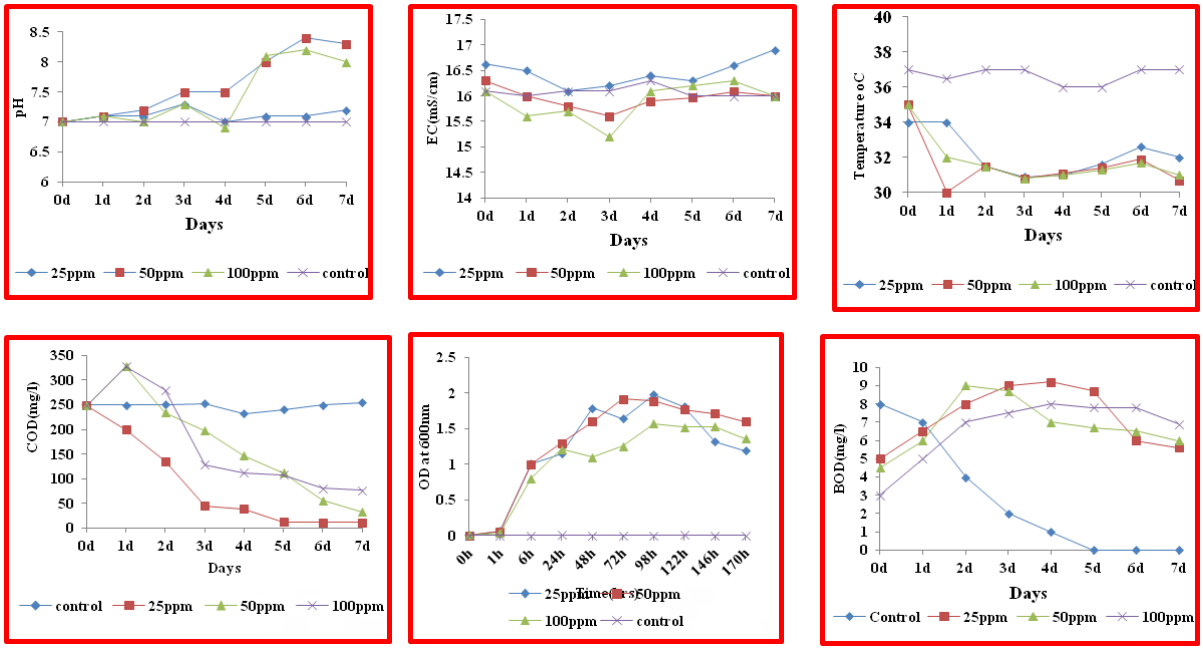


Figure 4. Biosorption Potential of *Klebsiella* sp. MHF ENV III For Bioremediation of Copper.

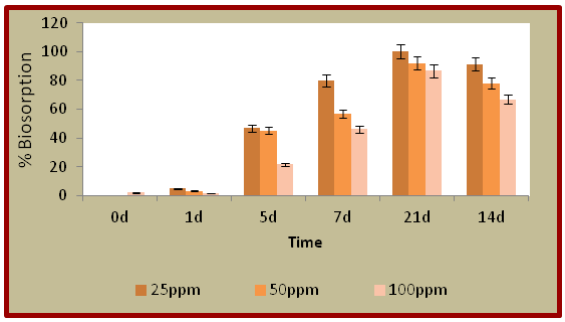
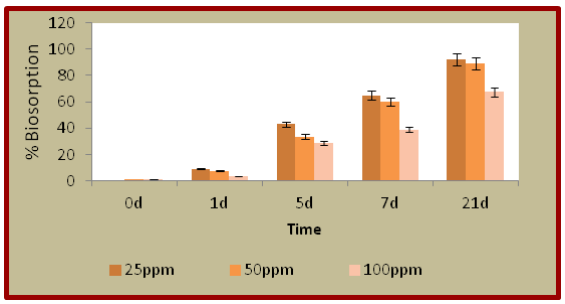


Figure 5. Biosorption Potential of *Klebsiella* sp. MHF ENV III For Bioremediation of Cadmium.



terms of absorbance (600nm) was found higher at a concentration of 25ppm as compared to 50ppm whereas in throughout the bioremediation experiment. It was observed that 50ppm and 100ppm of cadmium concentration were inhibitory for *Klebsiallasp* in comparison to 25ppm. Growth was very slow in 100ppm of cadmium concentration. Maximum growth was observed after 2nd day in case of 25ppm and after 3rd and 5th day at initial concentration of 50ppm and 100ppm respectively. After 7 days, microbial growth declined upto 1.091 at initial concentration of 25ppm, 1.089 at 50ppm and 0.373 at 100ppm of cadmium. Reduction in O.D. values shows the death of microorganism and lack of nutrients available after a certain period. COD decreased upto 99.1% in 25ppm, 75.8% in 50ppm and 75% in 100ppm concentration of cadmium containing medium after a period of 21 days of experiment (Table 1).

Comparative Study of Bioremediation of Selected Heavy Metals (Fe, Cu and Cd) with Microbial consortium isolated and *Klebsialla sp.* MHF ENV III

The bioremediation of heavy metals was studied at a selected concentration of 25, 50 and 100ppm over a period of 7 days, 14 days and 21 days using potential microorganism *Klebsialla sp.* MHF ENV III and microbial consortium. After a period of 21 days, complete biosorption of 25ppm of Fe was found by both potential microorganism and microbial consortium, similarly, 25 ppm of copper was found to be completely biosorped after a period of 21 days by microbial consortium as well as *Klebsialla sp.* Whereas in

Table 1. Variation In Environmental Parameters During Bioremediation of Iron, Copper and Cadmium.

Parameters		25ppm			50ppm			100ppm		
		Cu	Cd	Fe	Cu	Cd	Fe	Cu	Cd	Fe
pH	0day	7	7	7	7	7	7	7	7	7
	21day	7.2	6.8	6.1	8.3	6.4	6.2	8	6.7	6.3
Temperature (°C)	0day	34	31.5	35.3	35	31.6	29.3	35	31.6	29.3
	21day	32	29.3	29.6	30.7	28.6	29.9	31	28.6	30
Electrical conductivity	0day	16.6	16.35	16	16.3	15.9	15.3	16.1	16.14	15.3
	21day	16.9	16.3	15.8	16	15.6	15.9	16	15.8	15.9
COD (mg/l)	0day	64	78	248	73	89	248	93	137	248
	21day	11	27	90	33	60	93	76	62	97
BOD (mg/l)	0day	5	4	5	4.5	3	3.9	3	1.6	2.5
	21day	5.6	3.9	8	6	4	6	6.9	2.6	4.3
OD600	0day	0.004	0.05	0.004	0.003	0.05	0.002	0.005	0.053	0.006
	21day	1.19	1.091	1.365	1.6	1.089	1.56	1.35	0.373	1.411

Table 2. Comparison between Bioremediation of Heavy Metals By Potential Microorganism And Microbial consortium In Bioreactor for 25 ppm concentration.

Metals	0 Day		7th Day		14th Day		21st Day	
	Klebsiella pneumoniae	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium
Iron (%)	0.39	0	80	99.6	98	100	100	100
Copper (%)	0	1.	80	99.8	91.3	100	100	100
Candmium (%)	0	5.55	59.3	98	78	100	92	100

Table 3. Comparison between Bioremediation of Heavy Metals By Potential Microorganism and Microbial consortium In Bioreactor for 50 ppm concentration.

Metals	0 Day		7th Day		14th Day		21st Day	
	Klebsiella pnamioniae	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium
Iron (%)	0	7.8	70	100	88.9	100	100	101
Copper (%)	0	8.0	80	100	78	100	100	100
Candmium (%)	0.4	18.3	60.3	99.9	65	100	89.2	100

Table 4. Comparison between Bioremediation of Heavy Metals by Potential Microorganism and Microbial consortium In Bioreactor for 100 ppm concentration.

Metals	0 Day		7th Day		14th Day		21st Day	
	Klebsiella pnamioniae	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium	*Klebsiella pnamioniae*	Microbial consortium
Iron (%)	1.04	1.15	64.4	98	76	100	95.9	100
Copper (%)	2	0	46.2	99.4	67	99.9	86.7	99.9
Candmium (%)	1	9.09	38.7	99.6	53	99.9	67.3	100

case of cadmium, 100% biosorption was found at 25 ppm using microbial consortium while it was found inhibitory for *Klebsialla sp.* and biosorption found to be 92%. At 50ppm, Fe and Cu were found to be completely biosorped by both microbial consortium and potential microorganism while 82.2% biosorption in case of Cd and 100% in case of microbial consortium. At 100ppm concentration of Fe, 95.9% biosorption rate was found using microbial consortium *Klebsialla sp.* and 100% biosorption in case of microbial consortium; while 99.9% in case of Cu by microbial consortium and 86.7% at 100ppm concentration. 67.3% for Cd was found to be biosorped by potential microorganism and 100% by microbial consortium. Table 1 gives information regarding the behaviour of potential microorganism with different metals Fe, Cu and Cd at different metal concentrations. Iron was found to be effectively remediated by the potential microorganism [1]. It was shown that *Klebsialla sp.* was getting acclimatized to cadmium and

copper and then remediated both the metals (Cd and Cu). Thus, *Klebsialla sp.* MHF ENV III could be used as a potential microorganism for remediation of heavy metals-Fe, Cu and even toxic metal-Cd. Comparative data for bioremediation indicates that microbial consortium used for bioremediation of heavy metals (Cd, Cu and Fe) at selected concentrations of 25ppm (Table 2), 50ppm (Table 3)and 100ppm (Table 4)was effective using microbial consortium and potential organism in laboratory bioreactor in comparison to bioremediation of these metals carried at the same selected concentration by potential microorganism. This research could be beneficial for remediation of heavy metal present solid waste by prevailing the environmental conditions.

Genomics Studies

Isolation of pcoA gene responsible for heavy metal uptake with special reference to copper

Bacteria have genes specific for transport of needed nutrients and for resistances to the toxic ions of most heavy metal elements. These bacteria not only resist pollutants but show great promise for practical use in bioremediation due to the genetic basis of resistance mechanisms. Such heavy metal resistant microorganisms are very useful in biotechnology for the remediation of metal contaminated environments and can also be used in the construction of biomarkers for the detection of the presence of metals. However, a more basic understanding of adaptation can be achieved if the molecular mechanism were understood. They thus provide a useful means of investigating bacterial responses to environmental stress and the molecular mechanisms of adaptation. Therefore, the present research has been extended to understand the molecular basis of heavy metal accumulation into microbial cell; therefore, pcoA gene was isolated and identified from *Klebsialla sp.* MHF ENV III which has proved to be versatile microorganism for bioaccumulation of heavy metals. pcoA is a structural gene which is located on pco operon. PcoA substitutes for chromosomally-determined CueO and therefore is likely to have copper oxidase activity. The plasmid pco determinant is thought to moderate periplasmic binding and oxidation of excess copper cations carrying out transmembrane transport. Genomic DNA was purified using agarose gel electrophoresis and a fragment of a pcoA gene was amplified from *Klebsialla sp.* by PCR using the designed gene-specific primers. *Klebsialla sp.* MHF ENV III possesses copper resistant gene pcoA which encodes copper oxidase protein PcoA. Presence of a 165bp band indicates that microbial strain contain pcoA gene (Figure 6). The partial sequence of the copper resistance gene pcoA was amplified from the strain using gene specific primers with Cu-resistant genes from other bacteria. pcoA gene identified as responsible gene for resistance of copper can be cloned into bacterium with high plasmid numbers or bacterium with low generation time and bacterium which has shown multiple metal resistances (Figure 7 and 8).

Figure 6. Isolation of Genomic DNA from PCR Amplification of gene.

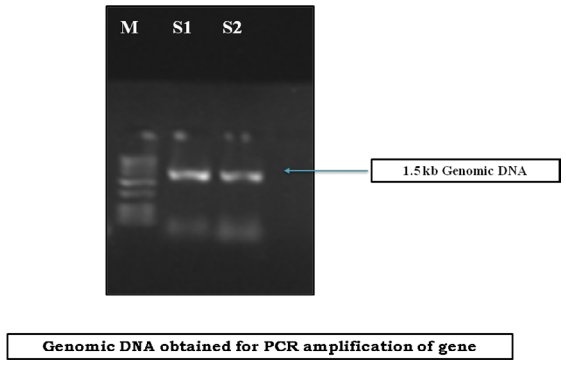


Figure 7. PCR amplification of a pcoA fragment- Lane 1: represents the MassRulerTM DNA ladder (Fermentas); Lane 2 represents the pcoA gene fragment.

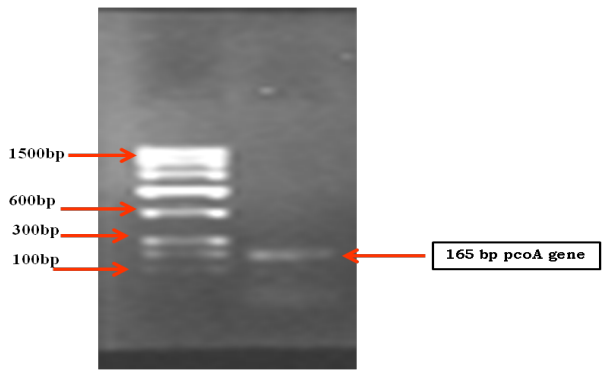


Figure 8. Gene Sequence: 165bp Long Product.

>ACGCCTGCGCATTACCCTGGTGAACGATACCATGATGACCCATCCGATTCATCTGCATGGCATGTGGAGCGATCTGGAAGATGAAAACGGCAACTTTATGGTGCGCAAACATACCATTGATATGCCGCCGGGCAGCAAACGCAGCTATCGCGTGACCGCGGATGC

Conclusion

The potential microorganism-*Klebsialla* sp. MHF ENV III has been found effective for bioremediation of heavy metals (Fe, Cu, Cd). *Klebsialla* sp. MHF ENV III being a potential microorganism for bioremediation of heavy metals has been further studied for identification of gene responsible for bioremediation. The laboratory technique developed for bioremediation of heavy metals would be beneficial to clean up the environment.

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