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Removal of Zn^{2+} and Pb^{2+} using new isolates of *Bacillus spp.* PPS03 and *Bacillus subtilis* PPS04 from Paper mill effluents using indigenously designed Bench-top Bioreactor

Pushpendra Pal Singh* and A. K. Chopra

Department of Zoology and Environmental Science, Gurukula Kangri University, Haridwar-249404 (UK), INDIA
*Corresponding author. E-mail: pps09gangwar@gmail.com

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Abstract: Biosorption processes have the potential to decrease environmental hazards through their factors such as initial metal ion concentration, temperature, pH and biomass concentration in the solution. In the present study biosorption process was performed using the strains of *Bacillus spp.* PPS 03 (KF710041) and *Bacillus subtilis* PPS 04 (KF710042) isolated from sediment core of Paper mill effluent (PME) for the removal of Zn^{2+} and Pb^{2+} in an indigenously designed Bench-top Bioreactor. The temperature, initial pH, biomass and incubation period of PME for Zn^{2+} and Pb^{2+} reduction was standardized. The strains exhibited significant reduction in Zn^{2+} and Pb^{2+} of PME to the extent of 73.29% and 85.64% with PPS 03 and 78.15% and 87.57% respectively with PPS 04 after 120 hrs of aerobic treatment. The reduction in the metals occurred from first day of the treatment, but the maximum reduction in these metals was observed after 120 hrs. at pH (7.0±0.2), temperature (35±1.0℃) and biomass (5% v/ v) of the bacterial strains. The removal of metals with strain PPS 04 was more in comparison to the strain PPS 03. The Freundlich isotherms on the data showed that it was linearly fitted for Zn^{2+} and Pb^{2+}. The values of correlation coefficient (R^2) of Freundlich isotherms were greater than 0.812 for Pb^{2+} and Zn^{2+}. The kinetic study for the rate of removal of Pb^{2+} and Zn^{2+} by both species was found to best fit a Pseudo first order reaction. The rate constant was found to be inversely proportional to the concentration of parameters. Thus, the microbial strains were found efficient for the biosorption/removal of Pb^{2+} and Zn^{2+}.

Keywords: Aerobic treatment, Biosorption, Freundlich isotherms, Pb^{2+} and Zn^{2+}, Pseudo first order, PME

INTRODUCTION

Heavy metals are difficult to remediate and adversely affect the ecosystems and human health. The high costs of traditional remedial approaches have driven interests in applications of bioremediation technology (Wu *et al.*, 2009). Pulp paper industries are the sixth largest effluent generating industries of the world (Ugurlu *et al.*, 2007). These effluents have been found to contain more than 200-300 different organic compounds and approximately 700 organic and inorganic compounds (Tambekar *et al.*, 2008). Organic and inorganic contents of the effluent provide ample opportunity to flourishing a variety of pathogenic microorganism (Chandra *et al.*, 2006). Release of heavy metals without proper treatment poses a significant threat to public health because of its persistence, biomagnifications, and accumulation in food chain. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency, and eco-friendly nature (Rajendran *et al.*, 2003; Wasi *et al.*, 2011a,b). The microorganisms have the capacity to remove, immobilize, or detoxify metals and radionuclides through various mechanisms (Ji and Silver, 1995).

Bioremediation is the process of breaking down or transforming hazardous materials into simple nontoxic substances by biological treatments (Sivasubramanian, 2006). The basis for biosorption is the metal binding abilities of various biological materials. Algae, bacteria, fungi and yeasts have been shown to be potential metal biosorbents (Parungao *et al.*, 2007). The capacity of any biosorbent is mainly influenced by biomass characteristic, physiochemical properties of the target metals and the micro environment of contact solution including pH, temperature and interaction with other ions (Murthy *et al.*, 2012). The 16S rRNA gene is the most widely used sequence in bacterial identification (Weisberg *et al.*, 1991; Drancourt *et al.*, 2000). Sequence comparison of 16S rRNA has been used as a powerful tool for establishing phylogenetic and evolutionary relationships among organisms (Amer, 2003).

Sequestration is also a detoxification mechanism for Pb^{2+}. Several bacterial species used for intra and extracellular binding of Pb^{2+} to avoid toxicity. Earlier studies done by Levinson *et al.*, 1996; Levinson and Mahler, 1998 using *Staphylococcus aureus*; Mire *et al.*, 2004 using *Citrobacter freundii*, *Vibrio harveyi* and Roane, 1999 using *Bacillus megaterium* and

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Pseudomonas marginalis lower the concentration of free Lead ions by precipitating Lead as a phosphate salt. However, the molecular mechanisms responsible for the formation of lead precipitates in these strains are not known. Many bacteria have a cell wall or envelope that is capable of passively adsorbing high levels of dissolved metals, usually via a charge-mediated attraction (Mohamed, 2001; Chatterjee *et al.*, 2014). Thus, Zn^{2+} and Pb^{2+} resistance microorganisms can be used to remediate heavy metal containing effluent, by a process known as biosorption which is nothing but a pollution treatment technology that uses biological systems to catalyze the destruction or transformation of various chemicals to less harmful forms. Therefore, the aim of the present study was to isolate and identify heavy metal resistant bacteria and to evaluate their efficiency to remove Zn^{2+} and Pb^{2+} from Paper mill effluent (PME) using indigenously designed Bench top aerobic bioreactor under laboratory conditions.

MATERIALS AND METHODS

Sample collection: The PME was collected from the discharge point of the effluents of Star Paper Mill Ltd. (29.964°N 77.546°E) situated at about 5 km from the Saharanpur city (U.P.) which produces paper as its main product from agro based residues. The collected samples were filled in a dry sterilized 35 liter polypropylene container. The samples were preserved at 4°C in the refrigerator to retard biological activity prior to use, till its processing for isolation of heavy metals resistant bacteria.

Isolation and identification of bacterial strains

Enumeration of cultured microbial populations: Bacterial populations were estimated following the method described by Aneja (1996). For that, 10 g of soil sample were transferred to 100 ml sterile DW (w/v) and mixed thoroughly by shaking the flask for 5 minutes on a rotary shaker. Similarly 10 ml effluent sample was added in 90 ml DW (v/v) and mixed thoroughly by shaking the flask for 5 minutes on a rotary shaker. Serial dilutions of the suspension were made upto 10^{-1} to 10^{-7} using sterile DW. Nutrient agar media was prepared and poured into sterile petriplates. A sample of 0.1 ml from the appropriate dilutions was spread over cooled agar medium in petriplates. The Nutrient agar media plates were incubated at 37±1°C for 24-48h. All the results were noted in triplicates.

Microorganisms and culture condition: The different bacterial strains were isolated and (PPS_b 01 to PPS_b 12) cultured from the soil/water of the nearby area of the sampling site. The stock cultures/ subcultures of these strains were maintained. The bacterial strains were enriched in nutrient broth 24-48 hours. Further sub-culturing of bacterial strains were performed from the stock culture for maintaining the strains and incubated at pH 7.0 and temperature 37°C for 24 to 48 hours.

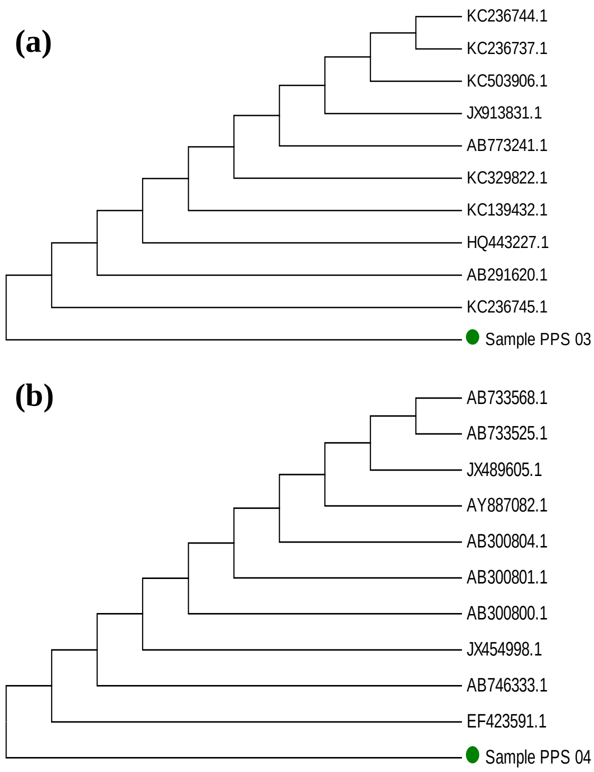

Fig.1. *Phylogenetic tree of heavy metal removal using bacterial strains, PPS 03 (a) and PPS 04 (b) isolated from paper mill industry. Bootstrap consensus tree (1,000 copies) was drawn by multiple sequence alignment with neighbour-joining method using software Mega, version 4.0.*

Screening of potential strains of heavy metal tolerant bacteria: Different bacterial isolates from soil were grown on the Nutrient agar media. Screening of bacterial isolates capable for heavy metal removal was carried out using the Standard soil embadation technique (Mali *et al*., 2000). For that, a loopful growth from grown culture were streaked on nutrient agar plate containing stress amount of PME and incubated at 37±1°C for 24-48 hrs. Heavy metal degrading ability of bacteria isolates was confirmed by the presence of clear zone around the colonies. The selected bacterial cultures were maintained on NAM plates or slants at 4°C. The bacterial inoculum prepared was taken at the rate 5 percent (v/v) of the effluent used for treatment. The effluent was supplemented with carbon source (glucose), nitrogen source (peptone) and pH adjusted to 7.0±0.2. The effluent (150 ml) in a sterilized Erlenmeyer flasks inoculated with individual bacterial isolates was then inoculated at 27°C in a rotary shaker for 24-48 hrs. The heavy metals such as Pb^{2+} and Zn^{2+} were observed and measured at an interval of 0 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs and 168 hrs. On the basis of comparative analysis of percentage reduction of different parameters studied by the individual isolates, the first two most potential bacterial strains PPS 03 and PPS 04 were screened out and selected for further analysis. This procedure was repeated thrice to find the

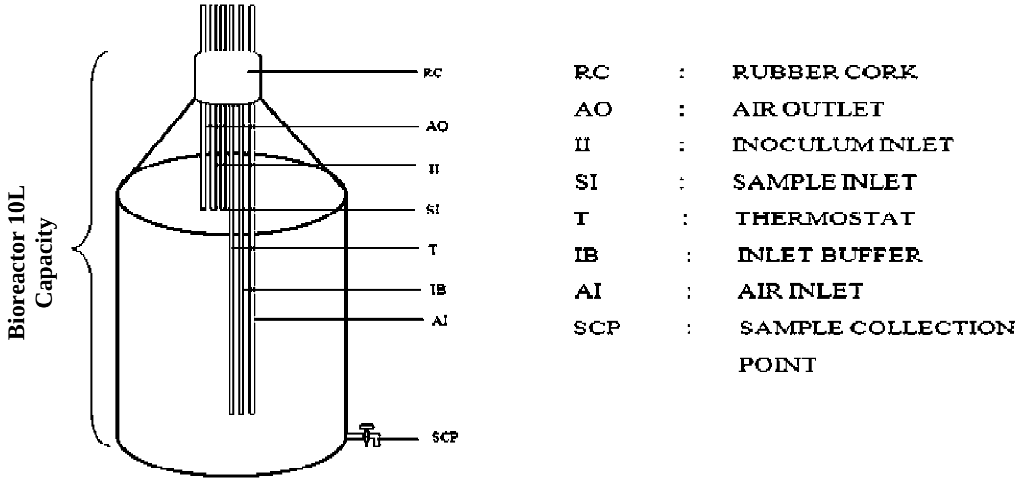

Fig. 2. *Bioreactor design used for biosorption process.*

best potential strain for degradation of pollution parameter under study.

Molecular identification of bacterial isolates by 16S rDNA sequence analysis: Sequence data amplified genomic DNA of selected bacterial strain was analyzed and compared with the existing data base of gene bank, National Centre for Biotechnology Information. Bootstrap consensus sequence was drawn by multiples sequence alignment with neighbour-joining method using software mega version 4.0 with different species of bacteria. It was identified as *Bacillus sp.* PPS03 (accession no. KF710041) and *Bacillus subtitles* PPS04 (accession no. KF710042) having 98% identity of the 16S rDNA sequence. Phylogenetic tree clearly indicates that both the isolates belong to the genera *Bacillus sp.* (Figs. 1a,b).

Experimental setup

Bioreactor design for the treatment of the effluent: The biosorption of PME was carried out in a specially designed Bench top bioreactor (Fig.1) that consisted of glass (Borosil) aspirator bottle (10 litre) as the reaction vessel, fitted with rubber cork (air tight with silicon grease) having six ports connected with sterilized hollow tubes, which had provision for acid/alkali tubes for adjustment of pH. The tubings were attached to an aerator to bubble sterilized air in the effluent, air outlet, sample inlet for injecting sample in the reactor, inoculum inlet for culture transfer and thermostat for maintaining the temperature. The lower side opening of the vessel was used as sample collection system. The reaction vessel was filled with 7.0 liter effluent, inoculated with selected bacterial strains and mouth of the glass vessel was properly sealed with rubber cork, wrapped with parafilm. Initially, the pH, temperature, biomass and values in the reactor vessel were maintained during the experiment.

Heavy metals analysis: The heavy metal concentration of Zn^{+2} and Pb^{+2} in the effluents was determined by digesting 200 ml samples with a mixture of concentrated HNO_3 and $HClO_4$ acid (10 ml + 2 ml). The digested samples were filtered through Whatman filter No. 42 and finally volumes were made 10 ml with 0.1 N HNO_3 and analyzed for heavy metals using Atomic Absorption Spectrophotometer (AAS) (Model ECIL-4129) following standard methods (APHA, 2005).

Table 1. Physico-chemical and heavy metal characteristics of Star paper mill effluent.

S.N.	Parameters	Initial concentrations	BIS for irrigation water
1.	Tempera-ture (°C)	34.70±8.28	-
2.	Colour (PCU)	3268.67±26.69	25.0
3.	TSS (mg L^{-1})	792.0±1637	200.0
3.	TDS (mg L^{-1})	2146±205.72	1900
4.	Turbidity (NTU)	671.67±10.41	10.0
5.	pH	7.90±0.56	5.5-9.0
6.	DO (mg L^{-1})	Nil	-
7.	Zn^{2+} (mg L^{-1})	7.69±0.39	15.0
8	Pb^{2+} (mg L^{-1})	2.26±0.19	1.0

Mean ± of three values; BIS- Bureau of Indian standard.

Percent biosorption and efficiency estimation: Experiments were carried out over a wide range of experimental conditions and % metal removal of PME with use of different bacterial strains, i.e. *R* (%) was calculated using the following equations 1 and 2.

$$R\,(\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \qquad (1)$$

Where, C_0 and C_e represent initial and final heavy metal concentration respectively.

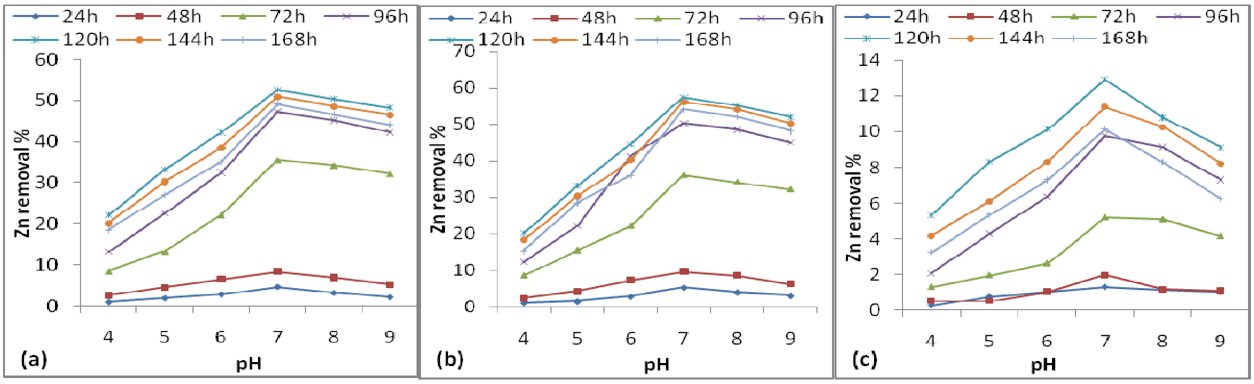

Fig. 3. *% removal of Zn^{2+} using (a) PPS 03, (b) PPS 04 strains and (c) control with different pH at constant operating conditions of temperature 30°C, biomass 3.0 v/v and time 24-168 hrs.*

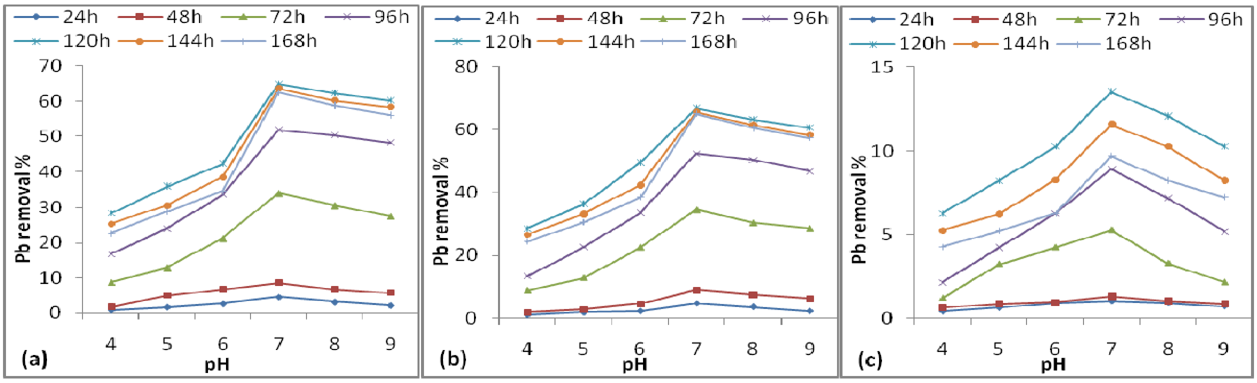

Fig. 4. *% removal of Pb^{2+} using (a) PPS 03, (b) PPS 04 strains and (c) control with different pH at constant operating conditions of temperature 30°C, biomass 3.0 v/v and time 24-168 hrs.*

The biosorption efficiency/ equilibrium was estimated as

$$q_e = \frac{(C_0 - C_e)}{M} \times V \quad (2)$$

Where,

q_e - Amount of adsorbed metal ion onto the biomass at equilibrium (mg/L);

M - Amount of biomass in the suspension (L); and

V - Volume of the suspension.

Freundlich isotherm model: The linearized form of the Freundlich isotherm model was used in the present work. The model was based upon the assumption that the surface of the biosorbent is heterogeneous and the metal ions bind on the surface of biosorbent and one over another in a multilayer fashion. The linearized form of Freundlich isotherm model has been represented in the following equation 3 (Freundlich, 1907).

$$In\ q_e = In\ K_F + b_F\ In\ C_e \quad (3)$$

Where, K_F - Freundlich adsorption constant related to adsorption capacity (mg/g); and b_F-Freundlich adsorption constant related to adsorption intensity (affinity between the sorbent and sorbate). A plot of In q_e versus In C_e gives a straight line with slope K_F and intercept b_F.

Biosorption rate kinetics: In this study, experimental batch biosorption kinetic data was modeled using Pseudo first order kinetic, to analyze the adsorption rates of heavy metal by isolated bacterial biomass. The pseudo-first-order kinetic equation was employed to analyze biosorption data obtained from various experiments using different adsorbents and biosorbents (Liu and Liu, 2008). The linear form of Pseudo first order kinetic equations (4) is given as-

Pseudo first order model

$$\log (q_e - q_t) = \log (q_e) - \frac{k_1 t}{2.303} \quad (4)$$

Where, q_t and q_e are the amount of metal adsorption per unit weight of biosorbent (mg/l) at time t (min) and at equilibrium respectively, k_1 is the adsorption rate constant. The pseudo first order rate constant k_1, estimated by plotting log (q_e- q_t) against time (t).

Statistical analysis: The data for all heavy metals were collected and were analyzed for calculating mean, standard deviation of each parameter with the help of Microsoft Excel 2007.

RESULTS AND DISCUSSION

Concentration of Zn^{+2} and Pb^{+2} in Paper Mill Effluent (PME): The present study observed that the concentration of Zn^{+2} (7.75 mg/l) and Pb^{+2} (2.04 mg/l) in Paper mill wastewater were far beyond the limits of Bureau of Indian irrigation standards (BIS, 1991) for

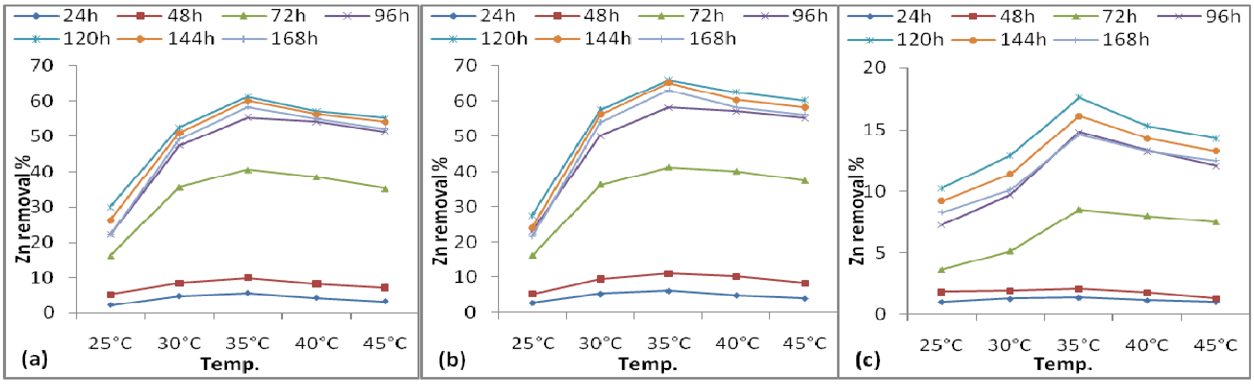

Fig. 5. *% removal of Zn^{2+} using (a) PPS 03, (b) PPS 04 strains and (c) control with different temperatures at constant operating conditions of pH 7.0, biomass 3.0 v/v and time 24-168 hrs.*

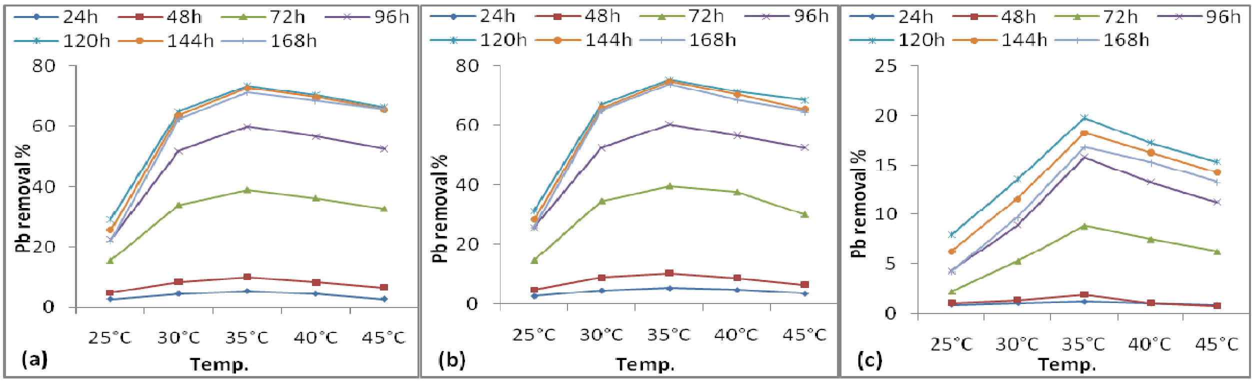

Fig. 6. *% removal of Pb^{2+} using (a) PPS 03, (b) PPS 04 strains and (c) control with different temperatures at constant operating conditions of pH 7.0, biomass 3.0 v/v and time 24-168 hrs.*

its disposal on land and water bodies (Table 1).

Removal of Zn^{+2} and Pb^{+2} by isolated bacterial strains

Effect of pH: pH plays an important role in degradation of contaminants. The biosorption of the cell remains sensitive to pH (Simie *et al.*, 1998). The cell surface metal binding sites and availability of metal in solution are affected by pH. Bacterial species have the ability to adsorb maximum heavy metal contaminants at pH range (5 to 9). Thus, pH range is widely accepted as being optimal for uptake of heavy metal contaminants (Rani *et al.*, 2010; Al-Daghistani, 2012 and Olaniran *et al.*, 2013).

The removal % of Zn^{2+} and Pb^{2+} after 168 hrs of aerobic treatment with different pH (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) being temperature 30±1.0°C and biomass 3.0% v/v constants in comparison to control (Effluent without inoculum) with PPS 03 and PPS04 is shown in Figs. 3-4. The maximum removal of Zn^{2+} (52.61%) and Pb^{2+} (64.96%) of PME was recorded with PPS 03 and Zn^{2+} (57.48%) and Pb^{2+} (66.90%) of PME was recorded with PPS 04 as compared to control. The removal of the metals with PPS 03 and PPS 04 was found to be slow at an early stage of aerobic treatment which increased to its maximum at 120 hrs with pH 7.0±0.2 and after that it decreased from 144 hrs onwards with an increase in pH upto 9.0 ±0.2. Among PPS 03 and PPS 04, the maximum removal of Zn^{2+} and Pb^{2+} of PME was better obtained with PPS 04 at maximum pH of 7.0±0.2 being temperature 30±1.0°C and biomass 3.0% v/v as constants.

Similarly, reduction of Cu (54% to 67%) at optimum pH (2), temperature (37°C) in 24 hrs using mixed culture strains of *Acidithiobcillus caldus*, *Leptospirillum* spp., *Ferroplasma* spp. and *Sulphobacillus* spp. was recorded by Mamba *et al.* (2009). Rani *et al.* (2010) reported that biosorption of immobilized cells had potential for reduction of different heavy metals from electroplating industrial effluent samples using different strains of bacteria such as *Bacillus* sp. for Cu (69.34%), *Pseudomonas* sp. for Cd (90.41%) and *Micrococcus* sp. for Pb (84.27%) than the dead bacterial cells at pH (5-9), biomass (1-5%), temperature (37°C) and 24 hrs incubation time. Kumar *et al.* (2010) reported Cu and Pb removal from Tannery effluent by three bacterial species. *Staphylococcus* sp. was used at pH (6.5) and temperature (37°C) under optimum conditions, with highest uptake for Pb accumulation ranging from 0.186-0.183 mg/g and for Cu from 0.72-1.536 mg/g. In case of *Streptomyces* sp., Cu reduction ranged from 0.386-6.42 mg/g and Pb reduction ranged from 0.063-0.286 mg/g under optimum conditions of pH 6.5 and temperature 37°C and with the use of *Flavobacterium* sp. at pH 7.0 and temperature 30°C, maximum reduction of Cu ranged from 0.358-1.194 mg/g and Cd reduction from 0.050-0.161 mg/g.

Effect of temperature: Temperature plays a major

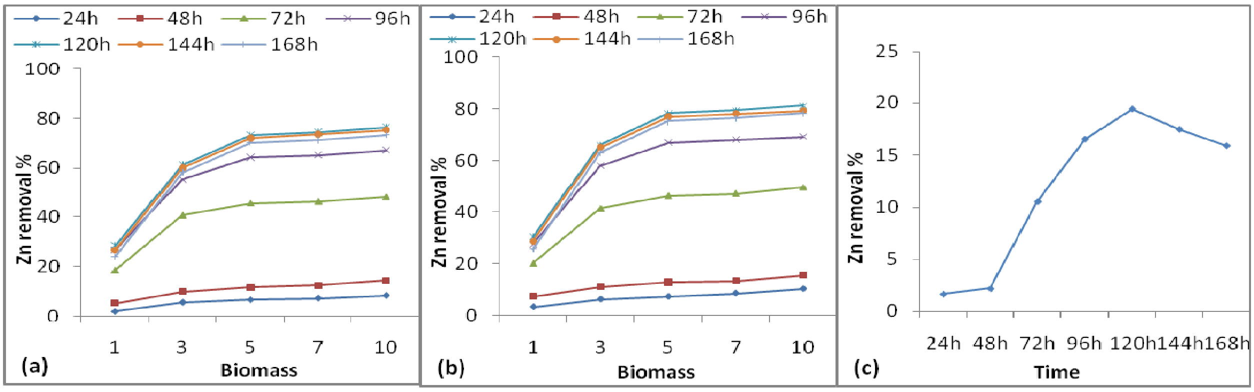

Fig. 7. *% removal of Zn^{2+} using (a) PPS 03, (b) PPS 04 strains with different biomass (v/v) and (c) control at constant operating conditions of pH 7.0, temperature 35°C and time 24-168 hrs.*

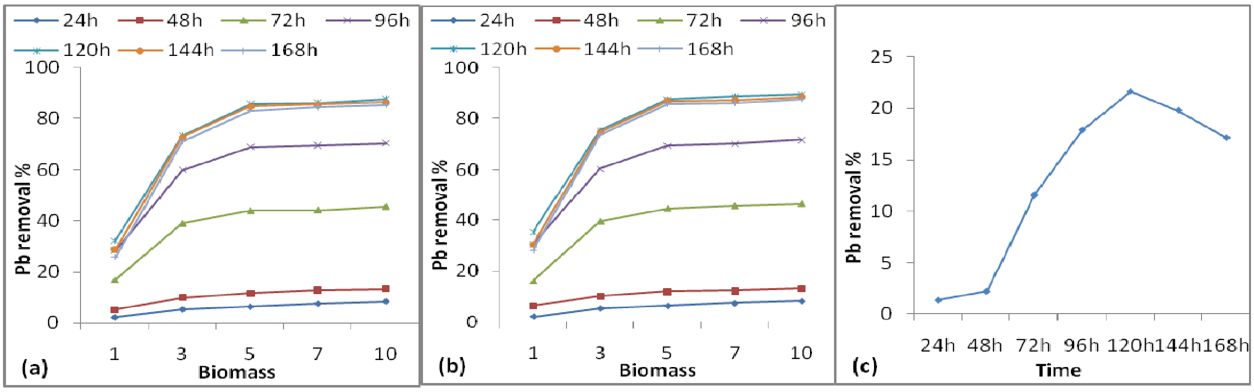

Fig. 8. *% removal of Pb2+ using (a) PPS 03, (b) PPS 04 strains with different biomass (v/v) and (c) control at constant operating conditions of pH 7.0, temperature 35°C and time 24-168 hrs.*

role in the adsorption of heavy metals. Although, the magnitude of the heat effect for the biosorption process is one of the most important criteria for the efficient removal of heavy metals from the wastewater. Temperature changes affect a number of factors which are important in heavy metal ion biosorption (Kumar *et al.*, 2009; Marandi *et al.*, 2010 and Kamsonlian *et al.*, 2011).

In the present study, the removal of Zn^{2+} and Pb^{2+} with PPS 03 and PPS 04 after 168 hrs of aerobic treatment with different temperatures (25, 30, 35, 40 and 45°C) being pH 7.0±0.2 and biomass 3.0% v/v as constants in comparison to control is shown in Figs. 5-6. The maximum removal efficiency of Zn^{2+} (61.14%) and Pb^{2+} (73.50%) of PME was recorded with PPS 03 and Zn^{2+} (66.01%) and Pb^{2+} (75.44%) of PME was recorded with PPS 04 as compared to control. At the early stage of aerobic treatment, the removal efficiency of heavy metals was observed to be slow which increased to its maximum at 120 hrs with temperature 35±1.0°C and then decreased from 144 hrs onwards with an increase in temperature upto 45±1.0°C. Among the bacterial strains PPS 03 and PPS 04, the maximum removal of Zn^{2+} and Pb^{2+} of PME was found better with a PPS 04 at maximum temperature of 35±1.0°C being pH 7.0±0.2 and biomass 3.0% v/v as constants.

Selvi *et al*. (2012) have also reported higher reduction of Pb (90%), Cu (85%), Zn (80%), Cr (70%) and Hg (80%) from Tannery effluent using isolated strains of *Pseudomonas* sps. Kumar *et al.* (2010) reported lower reduction of heavy metals with acclimated bacterial strains that were used to remediate the waste by biosorption process in a Batch culture. *Pseudomonas* sp. and *Bacillus* sp. reduced Cu (68% / 56%) and Ni (65% / 48%).

Effect of biomass: Earlier studies done by Vijayaraghavan and Yun, (2008); and Suryan and Ahluwalia (2012) on removal of heavy metals revealed that biomass is the key factor, which affects the biosorption process for removal of heavy metals concentrations. Increase in biomass concentration leads to interference between the binding sites. The heavy metals and salts present in the effluent behave differently with change in biomass concentration. The influence of biomass concentration on the percentage sorption of physico-chemical parameters to achieve the maximum biosorption capacity of the biosorbent for different parameters depends on optimization of biomass which may be due to the unavailability of binding sites to the metal and also due to the blockage of binding sites with excess biomass.

During present study, the removal of Zn^{2+} and Pb^{2+} heavy metals with PPS 03 and PPS 04 after 168 hrs of aerobic treatment with different biomass (1.0, 3.0, 5.0, 7.0 and 10.0% v/v) being temperature 35±1.0°C and

Table 2. Freundlich adsorption isotherm and Pseudo first order rate kinetic for Zn^{+2} and Pb^{+2} after treatment with biomass of PPS 03 and PPS 04 bacterial strains.

Parameters	**Bacteria culture**	**Freundlich adsorption isotherm parameters**			**Pseudo first order Kinetics**		
		k_F (ml L^{-1})	**b_F**	**R^2**	**K_1**	**q_e(ml L^{-1})**	**R^2**
Zn^{2+}	PPS 03	4.649E+09	1.754	0.901	0.06448	269.61	0.891
	PPS 04	2.164E+07	1.473	0.881	0.05988	266.93	0.888
Pb^{2+}	PPS 03	1.288E+01	1.184	0.826	0.04836	75.41	0.884
	PPS 04	1.256E+01	1.074	0.812	0.04836	74.96	0.888

pH 7.0±0.2 as constants in comparison to control is shown in Figs. 7-8. The maximum removal of Zn^{2+} (73.29%) and Pb^{2+} (85.64%) of PME was recorded with PPS 03 and in case of PPS 04, the removal for Zn^{2+} Pb^{2+} was recorded to be 78.15% and 87.57% respectively. The removal of heavy metals was recorded to be slow at an early stage of aerobic treatment which increased to its maximum at 120 hrs with biomass 5.0% v/v and after that it decreased from 144 hrs onwards (Figs. 7-8). Among PPS 03 and PPS 04, the maximum removal efficiency of Zn^{2+} and Pb^{2+} of PME was obtained better with a PPS 04 at maximum biomass of 5.0% v/v being temperature 35±1.0°C and pH 7.0±0.2 as constants.

Subhashini *et al.* (2011) reported the maximum removal of Cu (73%) using *Shizosaccharomyces pombe* at an initial concentration of 100 ppm with (1% v/v) of inoculum concentration at a temperature of 30° C from aqueous solutions. Yadav and Chandra (2013) reported a reduction of Cu (91.47%), Zn (93.41%), Fe (78.38%), Ni (66.66%) and Mn (78.91%) from Biomethanated distillery spent wash with the specific ratio (4:3:1:1) of *Proteus mirabilis* (FJ581028), *Bacillus* sp. (FJ581030), *Raoultella planticola* (GU329705) and *Enterobacter sakazakii* (FJ581031) within 192 hrs in the presence of glucose (1%) and peptone (0.1%). Suriya *et al.* (2013) reported that isolated strain of *Enterobacter cloacae* AB6 reduced Pb (II) 65.68% at 200 mg l^{-1} and Cu (II) 74.46 % at 300 mg l^{-1} of initial metal ion concentrations under optimized conditions. Biomass concentration of these strains varied from 0.5 to 3.0 mg/ml and it was found that a concentration of 1.5mg/ml strain was sufficient for maximum biosorption of around 74.87% for Cu (II).

Javaid *et al.* (2010) has reported lower reduction of Cu (II) (72.01%), Ni(II) (53.16%) and Zn(II) (7.08%) using *Schizophyllum commune fries*, a wood rotting fungus under pre-optimized conditions of biomass (2% malt extract broth) at 150 rpm and 25°C for 1 hrs isolated from Electroplating industry effluent. Amera *et al.* (2012) reported that biomass of *Bacillus* spp. and *Pseudomonas* spp. isolated from wastewater of second campus of Mosul University with adding 1% glucose showed maximum removal of Zn (72.00%) with *Bacillus* spp. and *Pseudomonas* spp., Ni (32.00%) with *Bacillus* spp. and (36.00%) with *Pseudomonas* spp., while Pb (8.00%) with both *Bacillus* spp. and *Pseudomonas* spp. in 24 hrs incubation period. Thippeswamy *et al.* (2012) also reported lower reduction of Pb (45.00%), Ni (43.00%), Zn (38.00%) and Cu (37.00%) using the yeast *Saccharomyces* sp. isolated from PME using 4% ethanol as carbon source and 0.01% glucose in 72 hrs of treatment.

Adsorption isotherm and rate kinetics with strains PPS 03 and PPS 04 biomass: The Freundlich adsorption isotherm and Pseudo first order rate kinetic for removal of heavy metals viz. Zn^{2+} (7.69±0.39 mgL^{-1}) and Pb^{2+} (2.26±0.19 mgL^{-1}) using PPS 03 and PPS 04 are shown in Table 1. The data obtained was linearly fitted for all parameters. The values of correlation coefficient (R^2) of Freundlich isotherms were greater than 0.812 for the Zn^{2+} and Pb^{2+}. The maximum k_F values were observed for Zn^{2+} (4.649E+09) and Pb^{2+} (1.288E+01) with the use of the biomass of PPS 03 followed by the biomass of PPS 04. The maximum b_F was obtained for Zn^{2+} (1.754) and Pb^{2+} (1.184) using the biomass of PPS 03 followed by the biomass of PPS 04 (Figs. 9-10 and Table 2).

Ambrósio *et al.* (2012) reported that Freundlich isotherm yielded a better linear fit (R^2>0.99) than the Langmuir model (R^2>0.95) suggesting that the adsorption surface presents heterogeneity. The mycelial surface of *Cunninghamella elegans* was observed to be more selective towards the red dye followed by reactive black and orange II. At equilibrium, the mycelium adsorbed 23.5, 4.18 and 6.94 mg/g of red, black and orange dyes respectively. It can be seen that the adsorption by *C. elegans* depended upon their genus and the adsorbed organic compound. Accordingly, the inactive mycelium of *C. elegans* was considered a good adsorbent. Oves *et al.* (2013) reported that *Bacillus thuringiensis* strain OSM29 showed an obvious removing potential for Cu, Pb and Ni. Furthermore for the data of heavy metals, the Langmuir and Freundlich adsorption constants evaluated from the isotherms with correlation coefficients (R^2>0.98) showed a better absorption process.

Rate kinetics: Pseudo first order rate kinetic parameters and correlation coefficients (R^2) for Zn^{2+} and Pb^{2+} by PPS 03 and PPS 04 are given in table 2. The first order equations were plotted for ln (q_e-q_t) against (t). The values of ln (q_e-q_t) calculated from the experimental data for Zn^{2+} and Pb^{2+} using PPS 03 and PPS 04 and the k_1

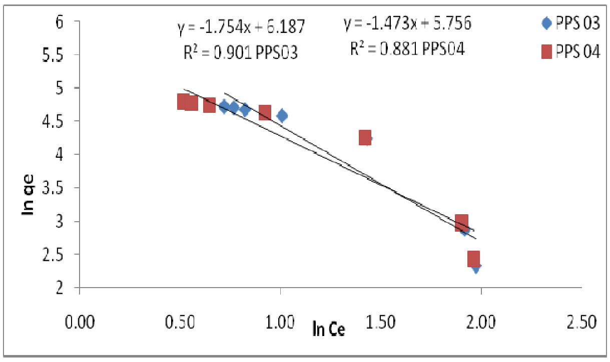

Fig. 9. *Freundlich adsorption isotherm for Zn^{2+} by the biomass of PPS 03 and PPS 04 isolated bacterial strains at optimum conditions of pH 7.0±0.2, biomass 5 ml L^{-1}, temp. 35±1.0°C and contact time 168 hrs.*

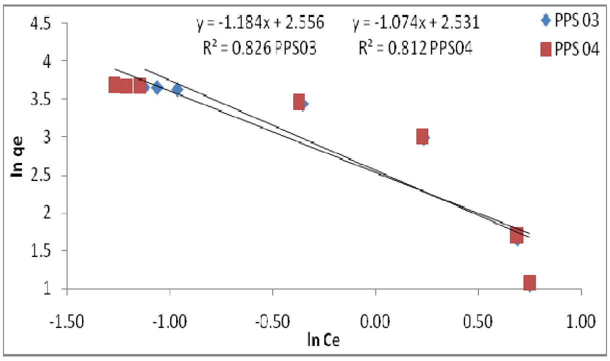

Fig. 10. *Freundlich adsorption isotherm for Pb^{2+} by the biomass of PPS 03 and PPS 04 isolated bacterial strains at optimum conditions of pH 7.0±0.2, biomass 5 ml L^{-1}, temp. 35±1.0°C and contact time 168 hrs.*

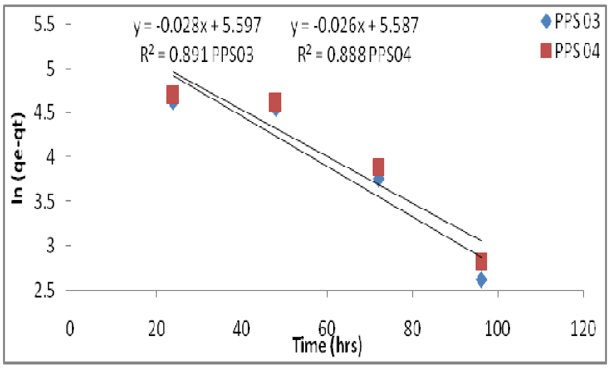

Fig. 11. *Pseudo first order rate kinetics for Zn^{2+} by the biomass of PPS 03 and PPS 04 isolated bacterial strains at optimum conditions of pH 7.0±0.2, biomass 5 ml L^{-1}, temp. 35±1.0°C and contact time 168 hrs.*

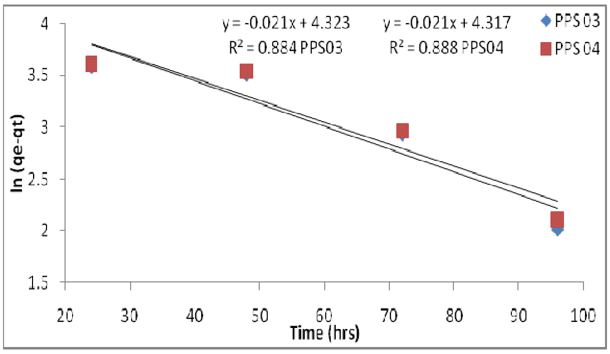

Fig. 12. *Pseudo first order rate kinetics for Pb^{2+} by the biomass of PPS 03 and PPS 04 isolated bacterial strains at optimum conditions of pH 7.0±0.2, biomass 5 ml L^{-1}, temp. 35±1.0°C and contact time 168 hrs.*

and q_e (estimated) values calculated from the slope of these plots are given in figs. 11-12. The values of correlation coefficient (R^2) of Pseudo first order were >0.884, which indicated that kinetic model was well suited. The maximum rate constants were obtained for Zn^{2+} (0.064484) and Pb^{2+} (0.048363) using the biomass of PPS 03 followed by the biomass of PPS 04. The maximum estimated equilibrium concentrations (q_e) were observed for Zn^{2+} (269.61) and Pb^{2+} (75.41) with the use of the biomass of PPS 03 followed by the biomass of PPS 04. (Figs. 11-12 and Table 2).

In the earlier studies (Anjaneya *et al*., 2009; Nandi *et al*., 2009; Chopra and Singh, 2012) on applicability of rate kinetics on biosorption of effluent parameters revealed that kinetics studies are important to evaluate adsorption dynamics.

Sethuraman and Balasubramanian (2010) revealed that kinetic models were examined with Pseudo first order and pseudo second order kinetics. The results revealed that the Cr(VI) is considerably adsorbed on bacterial biomass and it could be an economical method for the removal of Cr(VI) from aqueous solution. In this investigation it was noticed that the Pseudo second order kinetics match satisfactorily with the experimental data.

El-Hassouni *et al.* (2013) reported that experimental data for biosorption characteristics of Cu(II) ions from aqueous solution using the red alga (*Gigartina acicularis*) biomass were investigated as a function of pH, biomass dosage, contact time and metal concentrations at room temperature (20-23°C) were tested in terms of biosorption kinetics using Pseudo first order and Pseudo second order kinetic models. The results showed that the biosorption processes of both metal ions followed well Pseudo second order kinetic.

Conclusion

The present indigenously prepared Bench-top Bioreactor showed better removal of Zn^{2+} and Pb^{2+} from PME using isolated *Bacillus spp.* PPS03 (KF710041) and *Bacillus subtilis* PPS04 (KF710042) strains. The strain PPS 03 exhibited significant reduction in Zn^{2+} and Pb^{2+} of the PME to the extent of 73.29% and 85.64% respectively and with PPS 04 to the extent of 78.15%, 87.57% respectively after 120 hrs of aerobic treatment. The strain of PPS 04 was more effective in removal of Zn^{2+} and Pb^{2+} from the PME at optimum conditions of pH (7.0), temperature (35°C) and biomass (5% v/v) for the time duration of 120 hrs. The synergic effect of these three factors like pH, temperature and biomass was the important one for the removal of Zn^{2+} and Pb^{2+}. The Adsorption isotherm model, Freundlich adsorption isotherm study showed maximum correlation coefficient (R^2) of Freundlich adsorptions model was mostly greater than 0.80 and close to 1. The Freundlich model showed linearity for Zn^{2+} and Pb^{2+} with isolated bacterial strains PPS 03 and PPS 04. Freundlich isotherm model fitted the results quite well which are in agreement with the heterogeneity of sorbent surface. The rate kinetics evaluation study showed maximum correlation of coefficient (R^2 >0.884), which signifies good fit of Pseudo first order kinetic model for the removal of Zn^{2+} and Pb^{2+} with bacterial strains PPS 03 and PPS 04.

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