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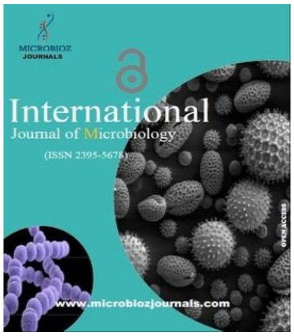

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Bioremediation of heavy metal in paper mill effluent using *Pseudomonas spp*

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Abstract

Heavy metals (Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Cu2) are the most toxic and common among the heavy metal pollutants of industrial effluents. In the present study, biosorption of heavy metal by some microorganisms from paper mill effluent was investigated. The efficient bacteria isolated from paper mill effluent were identified as *Pseudomonas spp*. The effect of pH and temperature on the biosorption capacity was investigated. The optimal pHs and temperatures were pH 7-9 and 25 - 35ºC in paper mill effluent for *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. The Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu2 content of the effluent was around 100 mg/L before remediation, after that the metal removal percentage was 86 and 74. The best activity was observed by *Pseudomonas aeruginosa* followed by *Pseudomonas fluorescens.*

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Introduction

Wastewater discharge by the industries is one of the major causes of environmental pollution particularly in the developing countries. These industrial effluents when constantly are contaminating our environment pose serious threat to human life (Buvaneswari et al., 2013). The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The released organic compounds and heavy metals are one of the key factors that exert negative influences on man and environment causing toxicity to plants and other forms of biotics and abiotics that are continually exposed to potentially toxic heavy metals (Paranthaman and Karthikeyan, 2013).

In India, around 905.8 million m3 of water is consumed and around 695.7 million m3 of wastewater is discharged annually by this sector. India’s current average fresh specific water consumption of about 150 m3/tonnes of product is far above the global best specific water consumption of 28.66 m3/tonnes and this large gap is primarily attributed to the use of obsolete technology / equipments and poor water management practices. Wastewaters from industries often contain more than one type of phenolic pollutant. Those with more complex structures are often more toxic than the simple phenol and yet little is known about the efficiency of treating wastewater containing a mixture of pollutants (Khan Nadeem et al., 2012). In Tamil Nadu, wastewater with varying levels of pollution load is generated at nearly all stages of paper production. An average of 30,000-40,000 litres of effluent is generated per ton of mills processed (Vaidhegi, 2013).

Heavy metal contamination due to natural and anthropogenic sources is a global issue. Rapid industrialization plays an important role in polluting the environment and causes severe degradation in pedosphere, hydrosphere and atmosphere. Effluents from industries contain appreciable amount of metallic cations like zinc, copper, iron, manganese, lead, nickel and cadmium (Vijendra et al., 1993). Long term irrigation with such effluents increases organic carbon content and heavy metal accumulation in soil and chances of their entrance in food chain and this ultimately causes significant bioaccumulation (Hakeem and Bhatnagar, 2008). To curtail metal pollution problems, many processes have been developed for the treatment and disposal of metals containing wastes like ion exchange, reverse osmosis in flotation and evaporation. The major shortcomings of the conventional treatments are low efficiency at low concentration of heavy metals, expensive handling and safe disposal of toxic sludge (Sub et al., 2008).

The different methods are used for the removal of heavy metals as important contaminants in water and wastewater. In chemical methods, to effectively decrease of heavy metals to acceptable levels require a large excess of chemicals, which increase the costs because of generating the voluminous sludge (Spearot and Peck, 1984).

On the other hand, a number of methods exist for the removal of heavy metals from liquid waste when they are present in high concentrations. These include methods such as precipitation, evaporation, electroplating, ion exchange and membrane processes (Matheickal and Yu, 1999). As an alternative to this method, recently, an eco-friendly method of treating contaminants by means of microorganisms has been focused. Microorganisms act includes biosorption, intracellular accumulation and enzyme-catalyzed transformation (redox reactions) (Lloyd, 2002). On the basis of energetic requirements, biosorption seems to be the most common mechanism (Haferburg and Konthe, 2007). Further more, this is the only option where dead cells can be applied as bioremediation agent. Nevertheless, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals via different mechanisms (Malik, 2004).Therefore, this work aimed to reduce or remove of heavy metal in paper mill effluent using bioremediation by some microorganisms.

Materials and methods

Collection of the paper mill industry effluent

One liter of effluent sample was collected in the plastic containers from the outlet of a Rajaganapathy Paper Mill Pvt. Ltd in Vadamangalam at Pondicherry. Its various physico-chemical characteristics were analyzed using standard methods (APHA, 2005). The effluents were stored at 4°C during storage period to avoid changes in its characteristics.

Isolates and identification of Fe^{2+}, Zn^{2+},Pb^{2+},Mn^{2+} and Cu^{2+}resistant bacteria

In order to check the tolerance level of the two isolated strains (Pseudomonas spp.) towards Fe, Zn, Pb, Mn and Cu nutrient medium was prepared, autoclaved and was amended with FeSo4 (Fe^{2+}), ZnSo4 (Zn^{2+}), Pb(NO3)2 (Pb^{2+}) MnSo4 (Mn^{2+}) and CuSo4 (Cu^{2+}),100 mg/L concentrations respectively. The isolated bacterial cultures were directly streaked on heavy metals incorporated Nutrient medium and incubated at 37˚C for 24 h. After the incubation period the plates were observed for growth on the media. The results were tabulated. Based on the maximum metals tolerance ability, of the best three strains were selected for further studies.

Growth of microorganisms and biosorption

Pseudomonas spp was incubated at 37ºC and at 150 rpm for 24 hrs in Nutrient broth. At the end of incubation, biomass was separated from medium by centrifuging at 5000 rpm and it was kept in the oven at 50ºC to remove the free water as much as possible.

Then it was suspended in deionized water separately in order to use it in the biosorption. 100 mL solutions containing 100 mg/L Fe, Zn, Pb, Mn and Cu was prepared from stock solution containing 1g/L FeSo4 (Fe^{2+}), ZnSo4 (Zn^{2+}), Pb(NO3)2 (Pb^{2+}) MnSo4 (Mn^{2+}) and CuSo4 (Cu^{2+}). Then 4.0 g microorganism (mg/L) was added to the medium (20 mg/L) and adsorptions of metals were investigated for different pH values adjusted by using HCl and NaOH at 37ºC. The solution containing the biomass was agitated in a shaker of 150 rpm during the adsoption. Samples taken at predetermined intervals were centrifuged and supernatants were analyzed. The analyses of Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+} ion was carried out by atomic adsorption spectrophotometer (Perkin- Elmer) at 0.01 ppm sensitivity level after dilution of the samples. By taking the determined optimum conditions into consideration, the capacity of microorganism to remove the mentioned metal from the tannery effluent was searched with the same method.

Statistical analysis

The data recorded in triplicate for the parameters were subjected to ANOVA using SPSS-statistical program at 5 % significance level.

Results and discussion

Some physicochemical characteristics of paper mill effluent were ascertained, from where Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+} tolerant bacteria were isolated. The colour of the effluent sample is brown, temperature is 39oC and pH is 8.4. When subjected to ICP (Intra-couple plasma), total Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+} was estimated to be about 100 mg/L. Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+} resistant isolates were identified as Pseudomonas fluorescens and Pseudomonas aeruginosa according to Bergey's manual Systematic Bacteriology. The effect of pH on heavy metals (Fe^{2+}, Zn^{2+} , Pb^{2+} ,Mn^{2+} ,Cu^{2+}) degrading by the bacterial strains (*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*) were investigated and the results were presented in the table - 1. The maximum degrading of heavy metal at pH 8 by the microbial *Pseudomonas aeruginosa* (93.7% for Fe^{2+}, 93.1% for Zn^{2+}, 92.6% for Pb^{2+}, 91.8% for Mn^{2+} and 92.2% for Cu^{2+}) respectively followed by *Pseudomonas fluorescens* in the same pH (89.6% for Fe^{2+}, 88.8% for Zn^{2+}, 88.1% for Pb2+, 87.1% for Mn^{2+} and 87.7% for Cu^{2+}). The effect of temperature on heavy metals (Fe^{2+}, Zn^{2+} , Pb^{2+} ,Mn^{2+} ,Cu^{2+}) degrading by the bacterial strains (*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*) were investigated and the results were presented in the table - 2. The maximum degrading of heavy metals at temperature of 30°C by the *Pseudomonas aeruginosa* strain (79.7% for Fe^{2+}, 79.4% for Zn^{2+}, 78.8% for Pb^{2+}, 77.7% for Mn^{2+} and 78.1% for Cu^{2+}) followed by *Pseudomonas fluorescens* (77.6% for Fe2+, 76.9% for Zn^{2+}, 76.4% for Pb^{2+}, 75.1% for Mn^{2+} and 75.8% for Cu^{2+}).

The pH ranging from 7 to 9 were studied for optimization of heavy metals degradation with the two efficient isolates were (*Pseudomonas fluorescens*) and (*Pseudomonas aeruginosa*) were initially supplied with 100 mg/ L of Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu^{2+} with inoculums volume of 2 ml, at optimum pH 8, the highest reduction was occured by the strain *Pseudomonas aeruginosa* for Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu^{2+} was 93.7, 93.1, 92.6, 92.2 and 91.8 for *Pseudomonas fluorescens* reduction percentage was 89.6, 88.8, 8.1, 87.7 and 87.1 Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu^{2+} respectively, where as in PMB 6 the removal of percentage was 83.6, 83.2, 83.1, 82.8 and 82.4 for Fe 2+, Zn2+, Pb2+,, Mn^{2+},and Cu^{2+} respectively. The similar results were reported by Shail Singh et al., (2007). In his report that the mixed culture of two bacterial strains Bacillus sp, Serratia marcescens of pulp paper effulent was found slightly alkaline, as the pH was recorded 8.0 that decreased upto 7.0. heavy metals such as Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were decreased upto zero level and effulent was also characterized by decrease concentration of ions such as No3 – (16.8), K+ (18) and Na + (59 mg/l) at 168h incubation period respectively.

The metal degradation the capability by the bacterial strain is greatly influenced by incubation temperature. The effects of temperature on heavy metals (25, 28 and 30ºC) removal by the three efficient organisms were assessed. The isolates showed higher removal percentage at 30°C rather than other temperatures. Among the three strains, at optimum temperature of 30°C, the highest reduction was occurred by the strain *Pseudomonas aeruginosa* for Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu^{2+} was 79.7, 79.4, 78.8, 78.1 and 77.7 *Pseudomonas fluorescens* the degradation percentage was 77.6, 76.9, 76.4, 75.8 and 75.1 for Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu^{2+} respectively. Similar results were reported by Sankar et al. (1998) who also reported maximum degradation at 30°C temperature.

S.No.	Name of the isolates	pH	Heavy metals biosorption (%)				
			Fe^{2+}	Zn^{2+}	Pb^{2+}	Mn^{2+}	Cu^{2+}
1.	***Pseudomonas fluorescens***	7	69.9	69.1	68.8	68.4	68.1
2.	***Pseudomonas aeruginosa***		73.5	72.7	71.2	70.1	69.8
1.	***Pseudomonas fluorescens***	8	89.6	88.8	88.1	87.1	87.7
`2.	***Pseudomonas aeruginosa***		93.7	93.1	92.6	91.8	92.2
1.	***Pseudomonas fluorescens***	9	73.7	73.6	73.2	72.8	72.4
2.	***Pseudomonas aeruginosa***		79.7	79.1	78.8	78.4	78.1
	SEd		1.96	2.06	2.16	2.11	2.11
	CD (P= 0.05)		4.12	4.34	4.55	4.44	4.44

Table 1: Effect of pH on biosorption of Fe $^{2+}$, Zn^{2+}, Pb^{2+},Mn^{2+},and Cu^{2+} mg/L by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa.*

S.No.	Name of the isolates	Temperature	Heavy metals biosorption (%)				
			Fe^{2+}	Zn^{2+}	Pb^{2+}	Mn^{2+}	Cu^{2+}
1.	***Pseudomonas fluorescens***	25^0C	49.1	48.4	48.2	47.4	46.9
2.	***Pseudomonas aeruginosa***		50.7	49.4	48.7	48.4	47.7
1.	***Pseudomonas fluorescens***	30^0C	71.7	70.9	70.4	69.9	69.4
`2.	***Pseudomonas aeruginosa***		74.8	74.2	74.1	73.3	73.5
1.	***Pseudomonas fluorescens***	35^0C	77.6	76.9	76.4	75.1	75.8
2.	***Pseudomonas aeruginosa***		79.7	79.4	78.8	77.7	78.1
	SEd		1.00	1.15	1.10	1.05	0.70
	CD (P= 0.05)		2.11	2.43	2.32	2.22	1.48

Table 2: Effect of Temperature on biosorption of Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+},and Cu2 mg/L by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*.

Conclusion

The present study establishes the role and efficiencies of *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* in the adsorption of Fe $^{2+}$, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Cu2 in paper mill effluents. The technology when upgraded will be a boon to effluent in tackling the pollution problem of paper mill waste water. The process would not only be economical but also eco-friendly and sustainable. However, further research is needed to establish the process with specific attention.

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