Research in Biotechnology, 6(5): 16-23, 2015 ISSN: 2229-791X
www.researchinbiotechnology.com

Regular Article

Bioremediation of wastewater using various sorbents and vegetable enzymes

Yadav Anu*

Department of Biotechnology, K. C. College, 124, Dinshaw Wachha Road, Churchgate, Mumbai, Maharashtra 400020
*Corresponding author E-mail: **anudabur_18@yahoo.com**

The world community is facing a problem related to the management of wastewater very severely due to the extensive industrialization, increasing population density and a highly urbanized society. The quality of wastewater effluents is responsible for the degradation of receiving water bodies, such as lakes, rivers, streams. The two main processes for the removal of impurities from wastewater influents are chemical and biological. Because of the many drawbacks of chemical wastewater treatment, biological treatment is advocated in the last few decades. In this paper, an attempt has been made to use various alternative, inexpensive sorbents like sugarcane bagasse, spent tea leaves, fly ash and peroxidase enzyme isolated from the waste of vegetables to help in the bioremediation of wastewater samples. Among the various sorbents, fly ash was found to be the most effective and helped in the removal of organic matter as well as the inorganic ions. The combination of fly ash and charcoal helped drastically in lowering the chemical oxygen demand (COD) in the wastewater samples. Immobilized peroxidase enzyme was ideal for minimizing biological oxygen demand (BOD) and for the removal of phenols from wastewater.

Keywords: Wastewater, sorbents, immobilized peroxidase enzyme, BOD, COD.

Water is one of the most essential components of all our lives. Water is not only the universal solvent but it also acts as one of the most important components of all our life processes. We know that the sources of drinking water are very limited. Nowadays even the amount of water available is becoming too less for the ever growing population due to a variety of reasons. The only solution to all these problems is the recycling of the wastewater to yield much precious water, which is otherwise lost.

Most of the industries discharge wastewater and their effluents containing toxic materials into rivers without adequate treatment. Environmental pollution particularly from heavy metals and minerals in the waste water is the most serious problem in India. Heavy metals are major pollutants in marine, ground, industrial and even treated wastewater. Most of the point sources of heavy metal pollutants are industrial wastewater from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, lumber and wood products. The heavy metals are transported by runoff water and contaminate water sources downstream from the industrial site. To avoid health hazards it is essential to remove these toxic heavy metals from waste water before its disposal. Most of the heavy metals discharged into the wastewater are found toxic and carcinogenic and cause a serious threat to the human health (Srivastava *et al*., 2006). The release of large quantities of hazardous materials into the natural environment has resulted in a number of environmental problems and due to their non-biodegradability and persistence, gets accumulated in the environmental elements such as food chain, and thus may pose a significant threat to the human health (Lakherwal, 2014).

Given the inadequacy of biological methods for effective bioremediation of wastewater, adsorption has come to stay as one of the popular physical/chemical method successfully employed. Adsorption process is advantageous because of its sludge free clean operation and complete removal of ions, even from the diluted solutions. The emphasis has of late, however shifted towards low-cost adsorbents which serve as viable alternatives to the more expensive activated carbon. The most widely used adsorbent is activated carbon. The adsorption capacity of activated carbon is on the higher side. But it is very expensive and also regeneration results in the loss of adsorbent which is undesirable.

In recent years, many studies have been carried out to find out inexpensive alternatives such as coir pith, banana pit, rice husk, clay, groundnut shell, maize cob, peat, orange peel, coconut husk, wheat straw dust, etc. (Ashoka and Inamdar, 2010).

Various inexpensive adsorbents used in this study for decontamination of water are fly ash, charcoal, sugarcane bagasse and spent tea leaves. Charcoal is one of the best adsorbents for many organic chemicals because of its hydrophobicity, high specific surface (800- 1200 m^2/g), and microporous structure. This adsorbent is widely used for cleaning up drinking water and contaminated wastewater (Vasilyeva *et al*., 2006). Nowadays many researchers are using fly ash as an adsorbent for the removal of various pollutants. It is revealed from the studies that the treatment of domestic wastewater can be done by fly ash generated from thermal power plant to reduce the organic load. It is physically and economically viable approach (Singh *et al*., 2013). The use of treated sugarcane bagasse has also been studied as an adsorbent. Instead of just disposing the bagasse as a waste, the efficient utilization can be made for bioremediation of wastewaters (Ashoka and Inamdar, 2010). In recent years, tea waste is also gaining grounds due to its potential to overcome heavy metal pollutants. Insoluble cell walls of tea leaves are largely made up of cellulose and hemicelluloses, lignin, condensed tannins and structural proteins. In other words, one- third of the total dry matter in tea leaves has good potential as metal scavengers from solutions and wastewaters since the above constituents contain functional groups. The responsible groups in lignin, tannin or other phenolic compounds are mainly carboxylate, aromatic carboxylate, phenolic hydroxyl and oxyl groups (Wasewar, 2010).

Enzymes as biocatalysts have been used in many biological reactions but they mostly suffer from certain disadvantages. Enzymatic removal of phenolic compounds have been investigated by many researchers and it has been shown that peroxidases are able to react with aqueous phenolic compounds and form non-soluble materials that could be easily removed from the aqueous phase, however; these processes suffer from enzyme inactivation. Therefore attentions came into immobilization of peroxidases for the purpose of wastewater treatment (Alemzadeh *et al*., 2009).

In the present research work, various cheap adsorbents were used as an alternative to the commercially available expensive adsorbents for the treatment of wastewaters. Also, immobilized vegetable enzymes extracted from vegetable peels are used in the study for the removal of phenol and to reduce the BOD of wastewater.

Materials and Methods

1. The wastewater samples used in the present study were collected from Mithi River (Santacruz), Steel industry- Tavadiya, Vasai and Paper coating industry- Vasai.

2. **Chemical sorbents** used for treatment procedure were Fly ash and Charcoal.

3. **Biological sorbents** used for present work were spent tea leaves and Sugarcane bagasse.

4. **Peroxidase enzyme** extracted from radish and potato peels were also used. A 2% enzyme solution was prepared using phosphate buffer; the mixture was homogenized and then centrifuged to remove unwanted debris. The filtered supernatant was used as the crude extract. The extracted enzyme was immobilized in 6% sodium alginate beads for further treatment.

5. Various parameters were considered for the characterization of wastewater samples, which are listed below.

- **Physical parameters**- Color, pH, Optical density, Total solids, Total dissolved solids, Total suspended solids.

- **Chemical parameters**- BOD, COD, Phenol and metal ions.

a. Total Solids, Total Dissolved Solids and Total Suspended Solids

Total solids: Take an evaporating dish and weigh, put 250 ml unfiltered sample in it and evaporate on water bath. After evaporation dry it in oven for 1 hour, cool and weigh again. TS is calculated using the following formula-

$$TS = \frac{(a-b) \times 1000 \times 1000}{V}$$

Where, a= final weight of the dish in gm; b= initial weight of the dish in gm; V= volume of sample evaporated in ml.

Total dissolved solids: Take an evaporating dish and weigh. Filter 250 ml sample through Whatman paper no. 1 so that the filtrate should not have any turbidity. Evaporate the clear filtrate in the evaporating dish on a water bath. After evaporation dry it in oven for 1 hour, cool and weigh again. TDS is calculated using the following formula-

$$TDS = \frac{(a-b) \times 1000 \times 1000}{V}$$

Where, a= final weight of the dish in gm; b= initial weight of the dish in gm; V= volume of sample evaporated in ml.

Total suspended solids: TSS is calculated as the difference between Total solids and Total dissolved solids.

b. **Biological oxygen demand**: Dissolved oxygen content was determined in the wastewater samples using the Winkler's method. After an initial measurement of the dissolved oxygen, the bottle was sealed and stored in dark incubator at room temperature for five days. The dissolved oxygen is measure again after 5 days and the difference between the two is estimated as biological oxygen demand.

c. **Chemical oxygen demand**: COD was determined using the redox titration method. In this method, 10ml of 0.25N $K_2Cr_2O_7$ was taken and mixed with 20 ml diluted wastewater sample. A pinch $HgSO_4$ and $AgSO_4$ was added along with 30ml conc. H_2SO_4. The whole mixture was refluxed for 2 hours in a water bath. The mixture was removed from the water bath and cooled, and then distilled water was added to make up the volume to 140ml. 2-3 drops of Ferroin indicator was added. 10 ml solution from the above mixture was taken and titrated against 0.1N ferrous ammonium sulphate. End point was bluish-green to red wine.

d. **Test for the presence of Phenol**: Add alcoholic $FeCl_3$ to the wastewater sample and check for color development. The formation of violet color indicates the presence of phenol.

Results and Discussions

Physical parameters

1. Color of the wastewater samples

The three samples used in the present study were collected from three different sites. They varied drastically in color. The colors of the three samples are tabulated in table 1.

Table 1: Color of the three wastewater samples used

Sr. No.	Wastewater samples	Color
1.	Mithi river	Pale yellow
2.	Steel industry wastewater	Green
3.	Paper coating industry wastewater	White

2. pH of the wastewater samples

One of the first parameter to be studied of any wastewater sample should be its pH. The pH of a sample determines its acidity or alkalinity. The wastewater before disposal in to the environment should be brought to near neutral pH. Here in this study, we used peroxidase enzyme extracted from radish and potato peels to treat the wastewaters and the pH is tabulated in table 2. The vegetable enzyme used was effective on all the three wastewater samples. It brought the pH of the samples to near neutral except for in case of Steel industry sample. Immobilized enzyme was more effective as compared to the free enzyme in the wastewater treatment.

3. Optical density of wastewater samples

All the wastewater samples discarded in the environment has some or the other suspended waste in it along with some dissolved waste. This suspended waste is responsible for the turbidity of wastewater. This turbidity is measured as the optical density which is mentioned in table 3. Here, various adsorbents are used to treat the wastewater to get rid of the turbidity. Among the various sorbents, fly ash and charcoal together is the best effective treatment for Mithi river sample, spent tea leaves and sugarcane bagasse are not at all effective. In case of Steel industry samples, the combination of fly ash + charcoal and spent tea leaves are equally effective and sugarcane bagasse is the least effective. Fly ash + charcoal, spent tea leaves and sugarcane bagasse are equally effective on Paper coating industry wastewater.

Table 2: pH of wastewater before and after treatment with peroxidase enzyme

Sr.No.	Wastewater samples	pH before treatment	pH after treatment with	
			Immobilized enzyme	Free enzyme
1.	Mithi river	6.7	7.5	6.9
2.	Steel industry	1.95	5.5	3.5
3.	Paper coating industry	11.28	8.5	10

Table 3: OD of wastewater samples after treatment with the three adsorbents

Sr. No.	Wastewater samples	OD before treatment	OD after treatment with		
			Fly ash & Charcoal	Spent tea leaves	Sugarcane bagasse
1.	Mithi river	0.04	0.01	0.04	0.04
2.	Steel industry	0.49	0.09	0.09	0.14
3.	Paper coating industry	0.33	0.15	0.1	0.1

4. Total solids, Total dissolved solids & Total suspended solids of wastewater samples

The term solids is generally used when referring to any material suspended or dissolved in water or wastewater that can be physically isolated either through filtration or through evaporation. Total solids are nothing but summation of total dissolved solids and total suspended solids. Water with total solids generally is of inferior palatability and may induce an unfavorable physiological reaction. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

In the present study estimation of total solids (Table 4) was carried out and was found to be highest in the Paper coating industry wastewater and lowest in Mithi river sample. The wastewater samples were treated with immobilized peroxidase enzyme and total solids was calculated again after the treatment. Immobilized peroxidase enzyme was most effective on the solids of Paper coating industry sample, followed by Steel industry sample. It gave a difference of 10.88 gm/L, 8.66 gm/L and 2.22 gm/L in the total solids, total dissolved solids and total suspended solids, respectively, for the Paper coating industry wastewater.

Table 4: TS, TDS and TSS in the wastewater samples after treatment with immobilized peroxidase enzyme

Sr. No.	Wastewater samples	Total solids treatment (gm/L)		Total dissolved solids (gm/L)		Total suspended solids (gm/L)	
		Before	After	Before	After	Before	After
1.	Mithi river	0.37	0.31	0.24	0.19	0.13	0.12
2.	Steel industry	91.1	84.12	65.7	60	25.4	24.12
3.	Paper coating industry	141.8	130.92	18.16	9.5	123.64	121.42

Table 5: BOD levels of wastewaters after treatment with vegetable extracted peroxidase enzymes

Sr. No.	Wastewater samples	BOD before treatment (mg/L)	BOD after treatment with vegetable enzymes (mg/L)			
			Radish free enzyme	Radish immobilized enzyme	Potato free enzyme	Potato immobilized enzyme
1.	Mithi river	40.55	20.27	13.5	30.33	20.3
2.	Steel industry	994.35	212.87	142.1	354.79	213
3.	Paper coating industry	40.5	20.24	20.2	20.26	20.3

Chemical parameters

5. Biological oxygen demand (BOD)

Biological Oxygen Demand (BOD) is the amount of oxygen consumed by the organic and inorganic compounds which were oxidized by biological-oxidation effect in a certain condition. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose the waste. In that case, the demand for oxygen will be high so the BOD level will be high.

In the present study, the wastewater samples were treated with peroxidase enzymes extracted from radish and potato peels, both in the free form and immobilized form. The highest BOD levels were found in the Steel industry sample (994.35 mg/L) and other two samples had equal levels of BOD levels. After treatment with peroxidase enzyme, the radish extracted peroxidase immobilized enzyme reduced the biological oxygen demand to a great effect as compared to the other treatments for the Mithi river sample. In case of the Steel industry sample also radish extracted peroxidase immobilized enzyme gave the best result (142.10 mg/L). But in case of Paper coating industry sample, all the treatments gave somewhat equal results. From the data tabulated in table no. 5, it is clear that the treatment of wastewater with peroxidase enzyme in the immobilized form is the best way to reduce the levels for biological oxygen demand.

6. Chemical oxygen demand (COD)

Chemical Oxygen Demand (COD) is the amount of oxygen consumed by the organic compounds and in-organic matter which were oxidized in water. COD is related to BOD; however BOD only measures the amount of oxygen consumed by microbial oxidation and is most relevant to waters rich in organic waste. COD and BOD do not measure the same type of oxygen consumption. Fly ash waste can be utilized as adsorbent regarding dyes containing wastewater treatment to remove COD, color, suspended solids and turbidity (Shah *et al*., 2013). Hence, after treatment of the wastewaters with fly ash it was seen that the COD levels were drastically lowered as given in table 6. Fly ash acted as the best adsorbent for steel industry sample, thus reducing the waste load in the sample. Fly ash treatment reduced the COD demand by 40 mg/L, 96 mg/L and 56 mg/L for Mithi river sample, Steel industry sample and Paper coating industry sample, respectively.

7. Presence of Phenol

In this study, presence of phenol was found in all the three wastewater samples. The wastewater was then treated with immobilized peroxidase enzyme and after treatment the phenol was found to be absent in the samples. A major drawback of enzymatic wastewater treatment process is that it requires a large amount of enzyme to achieve high removal efficiency due to enzyme inactivation. But to increase the potential use of enzymes in wastewater treatment we applied the immobilization step. Enzyme immobilization is usually targeted at improving operational stability, while preventing contamination of the solution being treated. In addition, immobilized enzymes can be easily separated from the solution for reuse or for use in continuous reactors (Chiong *et al*., 2014).

Table 6: COD of wastewaters after treatment with fly ash

Sr. No.	Wastewater samples	COD before and after treatment with Fly ash (mg/L)	
		Before treatment	After treatment
1.	Mithi river	52	12
2.	Steel industry	132	36
3.	Paper coating industry	84	28

Enzymatic treatment is seen as a potential alternative to conventional methods for the removal of phenolic content. Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products. They also can change the characteristics of a given waste to render it more amenable to treatment or aid in converting waste material to value-added products (Karam and Nicell, 1997). The enzymatic method is suitable for treating high concentration phenol solution because the process can be completed without dilution within a short time. This is advantageous in instances where space cannot be secured for wastewater treatment plants based on biological oxidation

(Nakamoto and Machida, 1992). Some other potential advantages of enzymatic treatment as compared to conventional treatment include operation over a wide range of pH, temperature and salinity, and the ease and simplicity of controlling the process. Enzymes function with high specificity, are very efficient in removing targeted compounds, and are easier to handle and store (Wilberg *et al*., 2002). Amongst the various types of enzymes, peroxidases have been identified as a suitable candidate for the treatment of phenolic contaminants.

8. Presence of various ions in the wastewater samples

Many of the ions which are harmless or even beneficial at relatively low concentrations may become toxic to plants and animals at high concentration, either through direct interference with metabolic processes or through indirect effects on other nutrients. Toxicity normally results in impaired growth, changes in the morphology and even its death. The degree of damage depends on the concentration of the toxic ion, environment and other factors. Batch adsorption experiments conducted using fly ash for the removal of zinc and cadmium showed that the maximum adsorption of Zn^{2+} and Cd^{2+} was found to occur at pH 7.0–7.5 (Bayat, 2002). Zeolites derived from Indian fly ash were used for the removal of Cu^{2+}, Co^{2+}, and Ni^{2+} from aqueous solutions by conducting batch studies (Mishra and Tiwari, 2006). It was observed that the metal uptake increased with the increasing concentration, pH and temperature.

In the Mithi river sample, Magnesium and Aluminium ions were detected, in the Steel industry sample Iron and Nickel ions were detected and in the Paper coating industry sample only Cadmium ions were detected (Table 7). The three wastewater samples were treated with 1:1 proportion of fly ash and charcoal. After treatment with fly ash and charcoal the Mithi river sample and the Paper coating industry sample were free from the ions, but the Steel industry sample still contained Fe^{3+} ions. Thus, fly ash and charcoal acts as good adsorbing agents.

Table 7: Presence of ions in the wastewater samples after treatment with Fly ash & Charcoal

Sr. No.	Wastewater samples	Before treatment	After treatment
1.	Mithi river	Mg^{2+}, Al^{3+}	-
2.	Steel industry	Fe^{3+}, Ni^{2+}	Fe^{3+}
3.	Paper coating industry	Cd^{2+}	-

Conclusion

A wide range of inexpensive adsorbents have been investigated in the present study. Large quantities of fly ash and sugarcane bagasse are produced in India which creates serious disposal problems. This paper has provided a view of the use of sugarcane bagasse, spent tea leaves and fly ash as adsorbents for the treatment of wastewater samples. Fly ash and charcoal have been found to be very effective for the removal of heavy metals from wastewaters and also to reduce the COD and optical density of samples. Spent tea leaves were found to be the least effective of all the adsorbents. Apart from the adsorbents, peroxidase enzymes isolated from vegetable peels and immobilized have also been used. Treatment with peroxidase enzymes lead to the lowering of total solids, BOD and phenol concentrations in the wastewater samples. Further research is required to utilize and optimize fly ash, sugarcane bagasse and peroxidase enzyme in improved ways for getting maximum bioremediation of wastewater.

References

Alemzadeh I, Nejati S and Vossoughi M. 2009. Removal of Phenols from Wastewater with Encapsulated Horseradish Peroxidase in Calcium Alginate. Engineering Letters. 17(4): 13-16.

Ashoka H.S. and Inamdar S.S. 2010. Adsorption removal of methyl red from aqueous solutions with treated sugarcane bagasse and activated carbon- a comparative study. Global Journal of Environmental Research. 4(3):175-182.

Bayat B. 2002. Combined removal of Zinc (II) and Cadmium (II) from aqueous solutions by adsorption onto high calcium turkish fly ash. Journal of water, air and soil pollution. 136:69-92.

Chiong T, Lau S.Y, Khor E.H. and Danquah M.K. 2014. Enzymatic approach to phenol removal from wastewater using peroxidases. OA Biotechnology. 10(1):3-9.

Karam J. and Nicell J.A. 1997. Potential Applications of Enzymes in Waste Treatment. Journal of Chemical Technology & Biotechnology. 69(2):141-153.

Lakherwal D. 2014. Adsorption of Heavy Metals: A Review. International Journal of Environmental Research and Development. 4(1):41-48.

Mishra T and Tiwari S.K. 2006. Studies on sorption properties of zeolite derived from Indian fly ash. Journal of Hazardous Materials. B137:299–303.

Nakamoto S and Machida N. 1992. Phenol removal from aqueous solutions by peroxidase-catalyzed reaction using additives. Water Research. 26(1):49-54.

Shah A.K, Ali Z.M, Memon A.R, Laghari A.J, Mughal M.A, Shah S.F.A. and Saleem H. 2013. Exploitation of Low Cost Coal Fly Ash Adsorbent with Coagulants for the Treatment of Industrial Complex Nature Dyes Wastewater. International Journal of Scientific & Engineering Research, 4(9):109-119.

Singh R.K, Gupta D.K, Kumar S and Kumar S. 2013. Utilization of fly ash as biologically activated carbon on microbial consortium for the removal of pollutants from waste water. International Journal of Advanced Biotechnology and Research. 4(1):20-28.

Srivastava V.C, Swamy M.M, Mall I.D, Prasad B. and Mishra I.M. 2006. Adsorptive removal of phenol by bagasse, fly ash and activated carbon: Equilibrium, kinetics and thermodynamics. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 272:89-104.

Vasilyeva G.K, Strijakova E.R. and Shea P.J. 2006. Use of activated carbon for soil bioremediation. Soil and Water Pollution Monitoring, Protection and Remediation. 3(23):309-322.

Wasewar K.L. 2010. Adsorption of metals onto tea factory waste: A review. International Journal of Research and Reviews in Applied Sciences. 3(3):303-322.

Wilberg K, Assenhaimer C. and Rubio J. 2002. Removal of aqueous phenol catalysed by a low purity soybean peroxidase. Journal of Chemical Technology & Biotechnology. 77(7):851-857.