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Bioremediation using artificial constructed wetland in combination with efficient microbial cultures

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Abstract

The paper highlights the use of constructed wetland in combination with effective microbial cultures (Bacterial and fungal) for the removal of BOD, COD and faecal coliform from untreated sewage water of STP2 of Karnal city (India). The constructed wetland consists of iron drums of 220-litre capacity with 60 litres of average loading rate having 85-cm length and filled with 35-cm coarse sand, 10-cm pebbles of 2-5 mm diameter, 35 cm gravel from top to bottom, was installed at CSSRI, Karnal in the state of Haryana. Drums in triplicate were planted with *Phragmite* and inoculated with bacterial cultures SWB1 (*Alcaligenes cupidus*, MTCC 6850), SWB19 (*Enterobacter intermedius*, MTCC 6849) and fungal culture SWF1 (*Aspergillus flavus*, MTCC 6589) added at an interval of one month having 1 litre of culture broth and control without inoculation. Other treatments comprised of: no plantation, *Eichhornia* and *Typha* both uninoculated. Constructed wetland was found to be influenced by season, retention time and inoculated microorganisms.Inoculated *Phragmites* showed better BOD, COD and faecal coliform reduction as compared to uninoculated *Phragmites* because of higher biomass buildup. The system being easy to operate and low cost, can provide an economical viable solution for wastewater management.

Keywords:*BOD, COD, faecal coliform, contructed wetland, Phragmites*, *Eichhornia*, *Typha, Alcaligenes cupidus, Enterobacter intermedius, Aspergillus flavus*

Introduction

Bioremediation is the use of microorganisms or plants to detoxify an environment, mostly by transforming or degrading, pollutants. Four basic techniques may be used: (1) stimulation of the activity of indigenous microorganisms by the addition of nutrients, regulation of redox conditions, optimizing pH conditions; (2) inoculation of the sites with microorganisms of specific biotransforming abilities; (3) application of immobilized enzymes; and (4) use of plants (phytoremediation) to remove, contain, or transform pollutants. *In situ* bioremediation involves the use of organisms to remove pollutants at the site of contamination. Often, these organisms are indigenous to the area and may even be adapted for growth on the chemical contaminants in that particular environment. An alternative to the enhancement of bioremediation by indigenous microorganisms is the use of an inoculum of an appropriate pure or mixed culture of degrading microorganisms to effect removal of the undesired compound (s) (Gibson and Sayler, 1992). Constructed wetland aim to control systematically and optimize the ability of a wetland system to remove or transform wastewater pollutants and in many cases to also create an aesthetic environment for the development of wildlife and social objectives. In recent years interest has increased in wastewater treatment through constructed wetlands because of their low cost and energy requirement (Gersberg *et al.*,1986).Several investigators have reported that wetlands may act as efficient water purificiation system and nutrient sink . Wetlands remove aquatic pollutants through bacterial transformation and physio-chemical processes like adsorption, precipitation and sedimentation .

Constructed wetlands are of low cost, simple to operate and are more suitable for treatment of domestic waste water. However, insufficient information is available on the design and operation of wetlands in the country. Therefore laboratory investigation was carried out to optimize conditions for bioremediation

using microbes in combination with aquatic plants best found under lab conditions using constructed wetlands.

Materials and Methods

Modified form (Wood, 1995) of wetland was constructed using iron drums of 220 lit capacity having 85-cm length and filled with 35-cm coarse sand, 10-cm pebbles of 2-5 mm diameter, 35 cm gravel from top to bottom. Drums in triplicate were planted with *Phragmite* and inoculated with SWB1, SWB19, consortium of SWB1 and SWB19, SWF1 and control without inoculation. Other treatments comprised of: no plantation, *Eichhornia* and *Typha* both uninoculated. Where SWB1&SWB19 were bacterial cultures *Alcaligenes cupidus* (MTCC 6850) and *Enterobacter intermedius* (MTCC 6849) while SWF1 was fungal culture *Aspergillus flavus* (MTTC 6589). It was down flow system with 60 litres of average loading rate.It was loaded regularly from inlet of STP2 sewage water of Karnal city having average BOD (161mg/l) and COD (340 mg/l). Efficient bacterial and fungus cultures were added at an interval of one month. Inoculum level was maintained at 1 litre culture broth per 60 litre of loaded sewage. Sampling was done in two different seasonal temperatures.

(1) Sampling was done in slightly moderate seasonal temperature (January-March, 2004) employing retention time of 72 hr. (January-February), 48 hr. (February-March) and 24 hr. (March) respectively for percent BOD and COD reduction.

(2) Sampling was made at high seasonal temperature (May-June, 2004) for retention time of 24 hr., 48 hr. and 72 hr. respectively for percent BOD and COD reduction. Faecal coliform count was also observed after retention time of 72 hr. Influent and effluent samples were collected regularly and BOD, COD and faecal coliform were analysed as per Standard Methods for the Examination of Water and Wastewater (1985).

Results and Discussion

Sub samples for total outlet were collected after retention time of 24 hr during March, May and June (Fig. 1a and 1b). The mean percent BOD, COD for different treatments having moderate seasonal temperature showed maximum percent BOD reduction by *Typha* (68.4%) followed by SWB19 inoculated *Phragmite sp*. (64.9%). Whereas maximum mean COD reduction was observed in *Phragmite sp*. (61.4%) inoculated with consortium and SWF1 inoculated. For samples at high seasonal temperature maximum percent BOD reduction was observed in *Typha* (69.6%) followed by SWB19 inoculated *Phragmite sp*. (68.0%) similarly percent COD reduction was maximum in *Typha sp*., 67.5%.Sub samples for total outlet were collected after retention time of 48 hr during February-March and May-June respectively (Fig. 2a and 2b). During moderate seasonal temperature the mean percent BOD reduction was observed maximum for SWB1 inoculated *Phragmite sp*. (82.8%) followed by SWB19 (82.7%) and maximum percent COD reduction was observed in consortium inoculated *Phragmite sp*. (76.9%) followed by SWB1 and SWB19 inoculated *Phragmite sp*. (75.5%). For samples at high seasonal temperature (extreme summer) maximum mean percent BOD reduction was observed in consortium inoculation (85.5%) followed by *Typha sp*. (85.0%) and COD percent reduction was maximum in SWB1 inoculation, 79.5%.Sub samples for total outlet of constructed wetland were collected after retention time of 72 hr during January-February and May-June respectively (Fig. 3a and 3b). The mean percent BOD reduction was maximum in consortium inoculation (46.3%) followed by SWF1 inoculated *Phragmite sp*. (45.5%) and COD reduction observed maximum in SWB19

inoculation (50.2%) during moderate seasonal temperature. While during high seasonal temperature maximum percent BOD reduction observed in *Typha sp*. (88.6%) followed by SWB19 inoculation (86.5%) and also COD reduction maximum in *Typha sp*. (83.6%) followed by consortium inoculated Phragmite sp. 83.8%.Thus it is concluded that mean percent reduction (BOD and COD) increased with increase in seasonal temperature with same retention time. However percent BOD and COD reduction increased with increase in retention time when subjected to same seasonal temperature. Percent BOD and COD reduction increased from 24 hr. retention time to 72 hr. retention time for outlet samples at high seasonal temperature (May-June, 2004). Long retention time and an extensive surface area in contact with the flowing water provides for effective removal of particulate and organic matter as reported by Wood (1995). Reduction in BOD and COD with three days retention during January-February was less as compared to two days retention in February-March. Similarly, for one day retention time in end of March, values of reduction were slightly less than two days but more than three days retention time in month of January-February .This indicates that bioremediation is a temperature dependent process. Maximum reduction obtained in summer can be easily ascribed to ideal temperature available for oxidation process (Trivedy and Nakate, 2002). Wetlands performance is affected by rainfall, temperature (Juwarkar *et al*. 1995). Heritage *et al*. (1995) suggested improvement in BOD reduction over the spring and summer. It might be due to temperature increase and increased plant growth over the period. Season is a significant factor in the removal of BOD (Kuehn and Moore , 1995). Faecal coliform reduction by constructed wetland after retention time of 72 hr. during high seasonal temperature (May-June, 2004) was tried (Table 1). The mean inflow of faecal coliform were $95x10^5$ per 100 ml which reduced maximum in SWF1 inoculated Phragmites ($71x10^2$/100 ml) followed by SWB19 inoculated *Phragmite* ($12x10^3$/100 ml). Unvegetated control observed reduction having mean outflow value of $48x10^4$/100 ml. This reduction in bacterial load may be because of (1) the bacteria are sedimented or trapped in the root hairs of wetland plants (2) wetland plants may have the capacity to secrete a chemical substance which could have bactericidal or bacteriostatic effects. Mandi (1994) reported faecal coliform reduction of 98.6% and 78% during summer and winter respectively by *Eichhornia crassipes*. Bavor *et al*. (1988) cited an average value of 1 million counts per 100 ml in the effluent from 9 trickling filter in NSW. Chick and Mitchell (1995) reported marked reduction in faecal coliform count from mean inflow value of 12 million counts per 100 ml to a mean for all VFWs of less than 150,000.

Inoculated *Phragmites* showed better BOD and COD and Faecal coliform reduction as compared to uninoculated *Phragmite* because of higher biomass of inoculated *Phragmite* compared to uninoculated *Phragmite* (Table 2). Ramesh *et al*. (1990) reported substitution of mango rhizosphere soil with dominant bacterial isolate resulted in maximal improvement of height and biomass of ber seedling. Fungal treatment alone or in combination with bacteria was less effective. Further studies will be required to optimize these conditions for effective treatment of wastewater under field conditions.

Conclusion

The results showed that constructed wetland with an average loading rate of 60 litre sewage in combination with effective bacterial cultures SWB1 (*Alcaligenes cupidus*, MTCC 6850), SWB19 (*Enterobacter intermedius*, MTCC 6849) and fungal culture SWF1 (*Aspergillus flavus*, MTCC 6589) added at an interval of one month having 1 litre of culture broth in *Phragmites* showed increased BOD,COD reduction with retention time of 24hr,48hr and 72hr and faecal coliform reduction with 72hr retention time (May-June 2004) as compared to uninoculated *Phragmites* because of increased biomass of culture supplemented Phragmites. Other treatments comprised of: no plantation, *Eichhornia* and *Typha* both uninoculated.

Mean percent reduction (BOD and COD) increased with increase in seasonal temperature with same retention time. However percent BOD and COD reduction increased with increase in retention time when subjected to same seasonal temperature. BOD and COD reduction with three days retention during January-February 2004 were less as compared to two days retention in February-March,2004. Similarly, for one day retention time in end of March,2004 values of reduction were slightly less than two days but more than three days retention time in month of January-February 2004. Faecal coliform reduction by constructed wetland after retention time of 72 hr. during high seasonal temperature (May-June, 2004) was tried. The mean inflow of faecal coliform were $95x10^5$ per 100 ml which reduced maximum in SWF1 inoculated *Phragmites* ($71x10^2$/100 ml) followed by SWB19 inoculated *Phragmite* ($12x10^3$/100 ml). Unvegetated control observed reduction having mean outflow value of $48x10^4$/100 ml. The constructed wetland seems to be cost- effective alternative to conventional treatment processes which involves huge cost and its efficacy further gets improved by addition of efficient microbial cultures. Wetland performance is affected by temperature, retention time etc. Since it is not site specific, the system can be implemented near the wastewater source.

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Table 1. Faecal coliform count in miniature wetland inlet and outlet, having retention time of 72 h (May-June 2004).

Plant type	Inoculation @1.0l/60l	Faecal coliform (MPN/100ml) range	Faecal coliform (MPN/100ml) (Mean)*	
			Inlet	Outlet
		$5x10^3$-$8x10^7$	$95x10^5$	
Phragmite	SWB1	$6x10^3$-$7x10^4$	---do-----	$22x10^3$
---do-----	SWB19	$4x10^3$-$5x10^4$	---do-----	$12x10^3$
---do-----	Consortium	$3x10^3$-$4x10^4$	---do-----	$15x10^3$
---do-----	SWF1	$2x10^3$-$5x10^4$	---do-----	$71x10^2$
---do-----	Nil	$2x10^3$-$5x10^4$	---do-----	$29x10^3$
Typha	Nil	$6x10^2$-$11x10^5$	---do-----	$28x10^3$
Eichhornia	Nil	$8x10^4$-$8x10^5$	---do-----	$23x10^4$
Unvegetated (UV)Control		$14x10^4$-$13x10^5$	---do-----	$48x10^4$

Table 2: Harvested fresh Biomass of miniature wetland.

Plant type	Inoculation @1.0l/60l	Range of biomass (kg)	Average* biomass (kg)
Phragmite	SWB1	3.5-4.0	3.6
---do-----	SWB19	2.75-4.5	3.6
---do-----	Consortium	3.5-4.5	4.0
---do-----	Cow dung	3.5-4.0	3.6
---do-----	SWF1	3.5-4.0	3.91
---do-----	Nil	3.0-3.0	3.0
Typha	Nil	4.0-6.0	5.0
Eichhornia	Nil	1.25-1.75	1.5

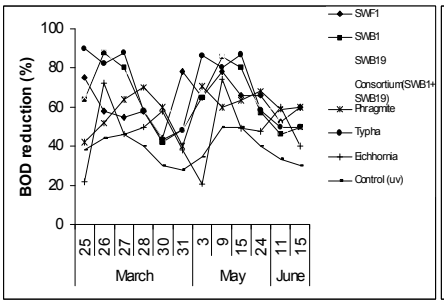

Fig 1a: Percent BOD* reduction in different treatments of wetland having retention time (24hr)
*** Values are mean of three replicates**

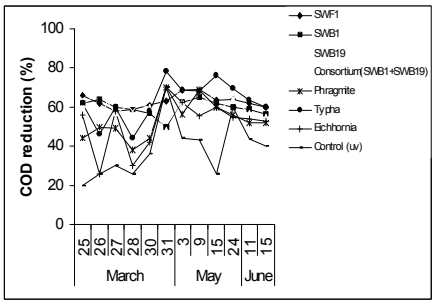

Fig 1b: Percent COD* reduction in different treatments of wetland having retention time (24hr)
*** Values are mean of three replicates**

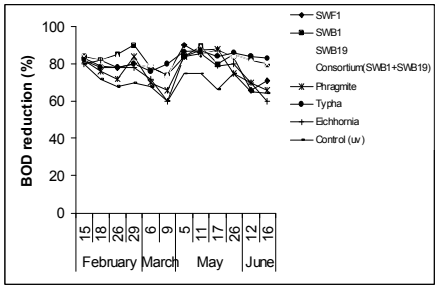

Fig 2a: Percent BOD* reduction in different treatments of wetland having retention time (48hr)
Values are mean of three replicates

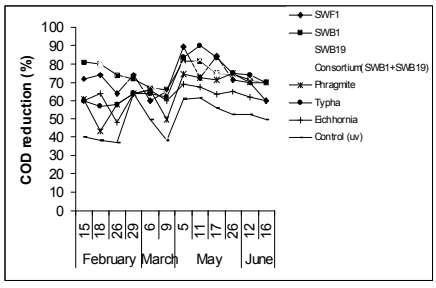

Fig 2b: Percent COD* reduction in different treatments of wetland having retention time (48hr)
*** Values are mean of three replicates**

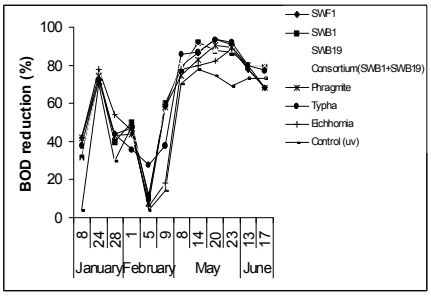

Fig 3a: Percent BOD* reduction in different treatments of wetland having retention time (72hr)
*** Values are mean of three replicates**

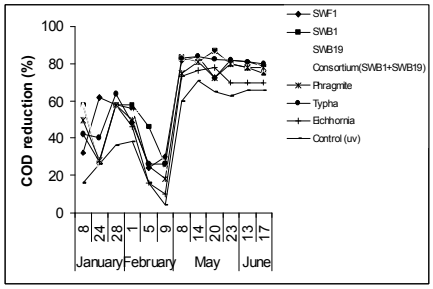

Fig 3b: Percent COD* reduction in different treatments of wetland having retention time (72hr)
*** Values are mean of three replicates**