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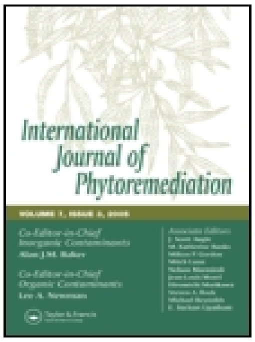

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METHANE EMISSION POTENTIAL AND INCREASED EFFICIENCY OF A PHYTOREMEDIATION SYSTEM BIOAUGMENTED WITH *BACILLUS FIRMUS* XJSL 1–10

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Horizontal subsurface flow constructed wetland mesocosms (HSSCW) designed to treat municipal waste water were bioaugmented with* Bacillus firmus *XJSL 1–10. The efficiencies of the three HSSCW mesocosms (non-vegetated HSSCW,* Schoenoplectus validus *HSSCW and* Bambusa vulgaris *HSSCW) were assessed. Bioaugmentation not only enhanced the efficiency of the phytoremediation system but also reduced methane emission from an average of 51.3 mg/m^2/d to 21.6 mg/m^2/d in* Schoenoplectus validus *HSSCW and from an average of 1708 mg/m^2/d to 1473 mg/m^2/d in* Bambusa vulgaris *HSSCW. Each of the three types of bioaugmented HSSCWs showed higher purification efficiency with respect to the removal of BOD and NH_4-N than the non-bioaugmented HSSCWs. The performance enhancement was most significant in bioaugmented* Schoenoplectus validus *HSSCW mesocosm with 48.8 and 44.8% lower BOD, and NH_4-N, respectively than the non-bioaugmented HSSCW.

KEY WORDS: purification, municipal waste water, constructed wetland, mesocosms

INTRODUCTION

Constructed wetlands are cost effective and eco-friendly systems harboring a number of essential microorganisms in various parts of the planted system. Microbes play a vital role as they help in removal of organic matter, eutrophicants and are also involved in many transformations responsible for the generation of various greenhouse gases in a wetland. Hence, creation of wetlands may have undesirable environmental consequences, as anaerobic conditions and low redox potential in wetlands favor GHG production and emission (Thiere *et al.* 2011). As one of the main greenhouse gases, methane emission from natural wetlands is recognized to contribute 20–39% of global warming (Laanbroek 2010). It has a global warming potential of 25 relative to CO_2 on a 100-year time range (Sha *et al.* 2011). In the presence of anoxia and metabolic activity of the methanogenic microbes the accumulated organic carbon or CO_2 emitted from heterotrophic consumption can be reduced to methane (Conrad 1996). Methane emissions have been studied in constructed wetland designed for waste water treatment (Teiter and Mander 2005) and reports on methane

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emission from constructed wetlands have been variable. In a study by Maltais-Landry *et al.* (2009) GHG emissions were found to be 2 to 10 fold more in constructed wetlands than in natural wetlands. Carbon and oxygen availability is spatially and temporally heterogenous in constructed wetlands resulting in high and variable fluxes of methane (−377 to 38,000 mg C – CH4/m^2/d) (Sovick *et al.* 2006; Ström *et al.* 2007). The emissions of methane from a constructed wetland is also a function of the type of plants used as different plant species have widely divergent (deleted number) microbes in their rhizosphere. Different wetland plants have varying capacity to transport methane and to oxidize it. It is known that different varieties of wetland rice plants emit different amounts of methane (Singh *et al.* 1997).

Bioaugmentation is the application of indigenous or allochthonous wild type or genetically modified organisms to polluted hazardous waste sites or bioreactors in order to accelerate the removal of undesired compounds (Limbergen *et al.* 1998). Fantroussi and Agathos (2005) defined bioaugmentation as the technique for improvement of the capacity of a contaminated matrix to remove pollution by the introduction of specific competent strains or consortia of microorganisms. There is limited information on bioaugmentation studies in constructed wetland for waste water treatment. Rustige and Nolde (2007) had performed laboratory experiments with specially adapted biomass that has been added to columns imitating constructed wetlands. In another study by Paredes *et al.* (2007) a subsurface flow constructed wetland was inoculated with anammox biomass and the rate of nitrogen removal was increased by 4–5-fold. Zou *et al.* (2008) reported that bioaugmentation caused an enhanced nitrogen removal in lab scale subsurface waste water infiltration system.

In this study, an attempt was made to increase the efficiency of a phytorememdiation system consisting of horizontal subsurface flow constructed wetland (HSSCW) mesocosms designed to treat municipal waste water by bioaugmenting them with a bacterial species identified as *Bacillus firmus* XJSL 1–10. As wetlands are also known to be a source of methane, the bioaugumented HSSCWs (non-vegetated, with wetland plant species and with a non-wetland plants species) were also assessed for their methane emission potential. A non-wetland plant species was used in the study considering the advantage it offers due to the lack of methane transmission potential owing to the absence of aerenchymatous tissue in its shoots.

MATERIALS AND METHODS

Constructed Wetland Design

The experimental wetland system was a horizontal sub-surface flow constructed wetland (HSSCW) mesocosm located at National Environmental Engineering Research Institute (NEERI), at Mumbai, India. There were three types of HSSCWs (non-vegetated HSSCW, *Schoenoplectus validus* HSSCW and *Bambusa vulgaris* HSSCW). Each type of HSSCW, made up of acrylic sheets had dimensions of 80 cm × 30 cm × 30 cm and baffles were used thus providing continuous flow and increasing the rate of sedimentation. The bottom layer of the unit comprised of gravels of 5.4–6.2 cm followed by graded gravels with reducing size. The top layer was filled with gravels of 1.3–1.6 cm diameter. The use of graded gravel bed helped in either avoiding or reducing the problem of clogging over a period of time. A plant density of 16 plants /m^2 in each planted wetland was maintained and the study was conducted in triplicates.

Scheme of Operation

Municipal wastewater collected from a nearby treatment plant was used as the influent. The system was operated at hydraulic retention time (HRT) of 24 h in each unit, with a flow rate of 12 ml/min to analyze the extent of BOD, NO_3-N, NH_4-N removal and methane emission. An HRT of 24 h was found to be optimum in the said design from the preliminary experiments.

Bacillus firmus XJSL 1-10 was isolated from the effluent of an established *Schoenoplectus validus* HSSCW as best results in bioaugmentation have been achieved with microbial strains that have been isolated from the same polluted environment since they are adapted to it (Nurk *et al.* 2009). Amongst the various strains isolated *Bacillus firmus* XJSL 1-10 was found to be the most efficient in utilizing organic matter from the waste water. Bacterial culture grown in Nutrient Broth Media (NBM) and incubated for 2 h (log phase) was used for bioaugmentation. To the bioaugmented system, 10 ml of bacterial inoculum was added per liter of sewage while in the non-bioaugmented system. 10 ml of NBM devoid of the bacterial isolate was added per liter of sewage.

Sampling and Analysis

A comparative analysis between the influent and the effluent after 24 hours was carried out for Biochemical Oxygen Demand (BOD), ammoniacal nitrogen, and nitrate nitrogen according to the Standard Methods for Examination of Water and Wastewater (APHA-AWWA-WEF, 1995).

Estimation of Methane Flux

Methane flux was estimated by the "closed chamber technique" (Pathak *et al.* 2003). The closed chamber technique envisaged the use of chambers of 80 cm $\times$ 30 cm $\times$ 100 cm size (according to experiment) made of 6 mm acrylic sheets for gas sampling. The chambers were inserted into the groove or channel of the HSSCW and the channel was filled with water to make the system air-tight. One 3-way stopcock (Eastern Medikit Ltd. India) was fitted at the top of chamber to collect gas samples. The chambers were thoroughly flushed several times with a 50 ml syringe to homogenize the inside air. Gas samples at 0 and 1 h were collected from the chamber. Gas samples were drawn with a syringe with the help of hypodermic needle. After drawing sample, chambers were made air tight with three way stop cock. Head space volume inside the box were recorded to calculate flux of methane. The gas samples were analyzed by Gas Chromatograph (GC 2014 Shimadzu) equipped with Porapak Q column and FID. Injector temperature at 150°C, column temperature at 40°C and detector temperature at 100°C was maintained.

Statistical Analysis

ANOVA and Pearsons correlation was determined using SPSS 13.

RESULTS AND DISCUSSION

Methane emissions from *Schoenoplectus validus* HSSCW was found to be less than the emissions from *Bambusa vulgaris* HSSCW and non-vegetated HSSCW (Table 1). One

Table 1 Response to bioaugmentation in three types of CW mesocosms in terms of BOD, NH_4-N and NO_3-N in the effluent and methane emissions from the mesocosms

		S. validus Effluent		*B. vulgaris* Effluent		Non-vegetated Effluent	
Influent	mg/l	SN	SB	BN	BB	NN	NB
BOD	180	21.3 ± 1.47*	10.95 ± 0.65	36 ± 2	31.5 ± 1.58	27.1 ± 1.52	24.8 ± 1.35
NH_4-N	13.1	2.9 ± 0.11	1.6 ± 0.04	3.43 ± .08	2.35 ± 0.05	3.7 ± 0.11	2.55 ± 0.05
NO_3-N	1.8	0.76 ± 0.11	0.52 ± 0.15	0.97 ± 0.14	0.57 ± 0.08	1.12 ± 0.17	0.54 ± 0.58
CH_4 emission mg/m^2/d		51.3 ± 2.85	21.6 ± 1.2	1708 ± 94	1473 ± 82	897 ± 47	226 ± 12

SN: *Schoenoplectus validus* non-augmented, SB: *Schoenoplectus validus* bioaugmented.
BN: *Bambusa vulgaris* non-augmented, BB: *Bambusa vulgaris* bioaugmented.
NN: Non-vegetated non-augmented, NB: Non-vegetated bioaugmented.
*SD: Standard Deviation.

Table 2 ANOVA table showing significant difference in BOD, Ammoniacal Nitrogen, and methane emission between three types of mesocosms: *Schoenoplectus* (S), *Bambusa* (Bam), and non-vegetated (NV)

		BOD			Ammoniacal Nitrogen			Nitrate Nitrogen			Methane		
	df	SS	F	Sig	SS	F	Sig	SS	F	Sig	SS	F	Sig
NB (S,Bam,NV)	2	343	570	0.0001	1.17	52	0.0001	0.182	4.42	0.06	4120648	552	0.0001
B (S,Bam, NV)	2	668	218	0.0001	1.42	254	0.0001	0.218	0.868	0.467	3703759	807	0.0001

NB: Non-bioaugmented.
B: Bioaugmented.

way ANOVA was done and the methane emission values were found to be significantly different (at 1% level of significance) between *Schoenoplectus validus, Bambusa vulgaris,* and non-vegetated HSSCW (in both bioaugmented and non-bioaugmented mesocosms) (Table 2).

This is attributed to the presence of aerenchymatous tissue in the shoots of the *Schoenoplectus validus* which supplies atmospheric oxygen to the roots and the rhizosphere. The supply of oxygen favors the establishment of oxygen rich zones. Consequently, the emission of methane was less due either inhibition of methanogenesis or /and promotion of methane oxidation. It is known that dissolved oxygen acts as an electron acceptor to constrain methane production in the oxidation of organic carbon in the sediment (Stadmark and Leonardson 2005). The supply of oxygen via the shoots of *Schoenoplectus validus* was such that the net methane emission (methane production- methane oxidation) was less than from the non-vegetated and *Bambusa vulgaris* HSSCWs. Due to the absence of aerenchymatous tissue in *Bambusa vulgaris* there was more anaerobicity in *Bambusa vulgaris* HSSCW which led to more emission of methane than from *Schoenoplectus validus* HSSCW. Higher methane emission from *Bambusa vulgaris* HSSCW than from non-vegetated HSSCW can be explained by the presence of root exudates which comprises of amino acids, simple sugars and nucleic acid derivatives and have a stimulatory effect on the growth of the microbes (Kraffczyk *et al.* 1984; Lynch and Whipps 1990). In addition, exudates may act as primers for the degradation of existing organic matter (Bottner *et al.* 1988; Dormaar 1990). The CO_2 released further gets reduced to methane by methanogens (Conrad 1996). Also gases such as ethylene and CO_2 are a part of low molecular weight root exudates (Grayston and Jones 1996). On bioaugmenting the wetlands there was reduction in the methane emission

Table 3 ANOVA table showing significantly lower mean values of methane emission (mg/m^2/d) in bioaugmented mesocosms than the non-bioaugmented system in *Schoenoplectus* (S), *Bambusa* (Bam), and non-vegetated (NV)

Mean		SD	SE	SM	df	F	Sig
S (NB)	57.3	2.85	1.64	1327	1	277.6	0.0001
S (B)	21.6	1.2	0.69				
Bam NB)	1708	94.5	54.5	83072	1	10.61	0.031
Bam(B)	1473	82	47.34				
NV (NB)	897	47.5	27.42	674690	1	539.2	0.0001
NV(B)	226	12.5	7.21				

SD: Standard Deviation, SE: Standard Error, SM: Square of Means.

Table 4 ANOVA table showing significantly lower values of BOD, Ammoniacal Nitrogen and Methane in bioaugmented mesocoms (*Schoenoplectus* (S), *Bambusa* (Bam), and non- vegetated (NV)) than the corresponding non-bioaugmented mesocosms

	BOD				Ammoniacal Nitrogen				Nitrate Nitrogen				Methane			
	dF	SS	F	Sig	dF	SS	F	Sig	dF	SS	F	Sig	dF	SS	F	Sig
S (NB,B)	1	160.1	123	0.0001	1	2.38	316.1	0.0001	1	0.089	4.9	0.09	1	1327.5	277.6	0.0001
Bam NB,B)	1	119.7	34.9	0.004	1	1.89	365.1	0.0001	1	0.236	17.5	0.01	1	83072	106.1	0.0001
NV (NB,B)	1	68.6	32.9	0.005	1	2.16	259.2	0.0001	1	0.084	0.44	0.57	1	6746	539.2	0.0001

NB: Non-bioaugmented, B: bioaugmented, S: *Schoenopleetus*, Bam: *Bambusa,* NV: Non-vegetated, df: Degree of freedom, SS: Sum of Squares.

in all the wetlands (Table 1). On way ANOVA was done and the methane emission from bioaugmented mesocosms were significantly less (at 1% level of significance) than the non-bioaugmented mesocosms (Table 3).

Our results have shown methane emission as 21.6 mg/m^2/d from bioaugmented *Schoenoplectus validus* HSSCW and 51.3 mg/m^2/d from non-bioaugmented *Schoenoplectus validus* HSSCW with an influent BOD of 180 mg/l. This was found to be lower than methane emission (96.5 mg/m^2/d) from another horizontal flow constructed wetland with a lower influent BOD of 62.8 mg/l as reported by Sovik *et al.* (2006). Less methane emission is attributed to the plant species used and the bioaugmentation approach.

As stated earlier the selected bacterial species identified as *Bacillus firmus* XJSL 1–10 was most efficient in removing organic matter from waste water amongst all the isolated strains. Therefore, the added aerobic bacterial species led to efficient aerobic degradation of the organic matter present in the waste water. Considering all the bioaugmented mesocosms (*Schoenoplectus validus, Bambusa vulgaris* and non-vegetated HSSCW) the Pearsons correlation between BOD removal and methane emission was −0.607 which was significant at 0.05 level (1-tailed). Even in non-bioaugmented mesocosms the Pearsons correlation between BOD removal and methane emission was negatively correlated which was significant at 0.05 level (1-tailed). A negative correlation indicates that BOD removal was also aerobic in all the mesocosms. BOD removal was enhanced in bioaugmented mesocosms as indicated by significantly different BOD values observed in bioaugmented and non-bioaugmented mesocosms (Table 4).

In bioagumented *Schoenoplectus validus* HSSCW there was significant enhancement in the removal of various constituents present in municipal waste water. There was 48.8 and 44.8% lower BOD and NH_4-N values, respectively observed in bioaugmented *Schoenoplectus validus* mesocosm than in non-bioaugmented *Schoenoplectus validus* mesocosm (Table 1). Also, when the same bacterial species was used for bioaugmenting non-wetland plant species *(Bambusa vulgaris)* mesocosm an enhancement in the removal of the waste water constituents was observed. There was 12.5 and 31.4% lower BOD and NH_4-N, respectively in bioaugmented *Bambusa vulgaris* mesocosm than in non-bioaugmented *Bambusa vulgaris* mesocosm (Table 1). In bioaugmented non-vegetated mesocosm there was 8.4 and 31% lower BOD and NH_4-N, respectively than in non-bioaugmented mesocosm (Table 1). As per the ANOVA, BOD and NH_4-N values were significantly lower (at 1% level of significance) in bioaugmented mesocosms as compared to the non-bioaugmented mesocosms (Table 4). Nitrifiers are chemoautotrophs which derive carbon from carbon dioxide and energy from the oxidation of NH_4-N. Hence, the increase in the removal of NH_4-N by nitrifiers is attributed to the abundance of carbon dioxide which is released by the metabolic activity of the added bacterial species or through the uptake of ammonical nitrogen by the added bacterial species.

CONCLUSION

The added bacterial species *Bacillus firmus* XJSL 1-10 was effective in increasing the municipal waste water treatment efficiency in terms of BOD and NH_4-N removal in the designed horizontal subsurface flow constructed wetland system with a wetland plant species, *Schoenoplectus validus*. Even in the non-wetland plant (*Bambusa vulgaris*) mesocosm the response was significant. Besides, the performance enhancement the bioaugmentation approach in the phytoremediation system undertaken effectively reduced methane emission from all the three types of HSSCW mesocosms.

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