" Biotechnology Solution for Water Treatment/ Industrial Waste Water "

Case study of textile industry wastewater treatment using a plant microbe integrated system

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The problem…

- Every single kilograms of the finished textile consumes about 100 L of water
- Wastewaters - colour, total dissolved solids, toxic metals, residual chlorine, BOD/COD, etc.
  - major constraint towards the reuse of the wastewater.
- Typical treatment: primary, secondary (biological treatment) and tertiary treatment (advanced treatment-including reverse osmosis, ultra-filtration and/or nano-filtration)

The problem…

- Cost - Rs. 34.08 to 73.22 per 1000 L
- Hazardous membrane rejects – need specialized disposal
- Many textile units are run as small-scale units - do not comply with regulations mainly because of cost
- Need for alternatives that are cost effective as well as reduce the rejects that require specialized disposal.

Key questions

- Will a plant-microbe system be able to treat the textile industry wastewater completely?
- How the proposed treatment cycles be managed?
- How does a plant microbe system compliment in the proposed treatment?
- What is the adsorption efficiency of vetiver roots for the pollutants, particularly textile dyes?

Objectives…

1. ***To determine the boundaries of process parameters of the proposed treatment methodology***

2. ***To design and optimize a lab scale textile industry wastewater treatment system to decolourize azo dyes and remove BOD, COD and other contaminants using vetiver and microbial consortia***

3. ***To evaluate the synergy between the bacteria and roots of vetiver in treating textile industry wastewater***

4. ***To assess the feasibility of the above system for large scale wastewater treatment***

Methodology - Trials

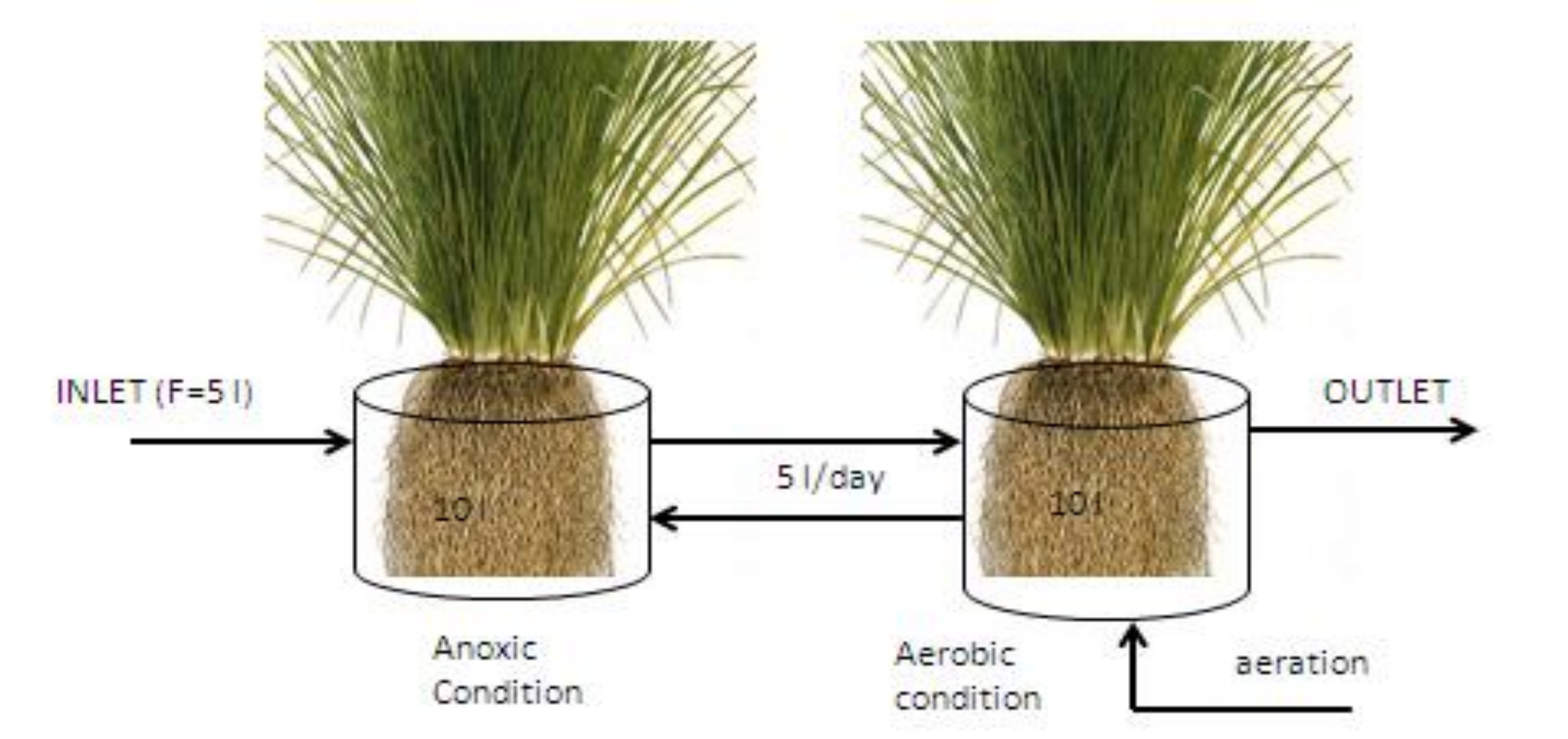
- Greenhouse studies with synthetic wastewater and single azo dye – methyl red
- Treatment of textile dye effluent
- Retention time optimization during the treatment of textile dye effluent

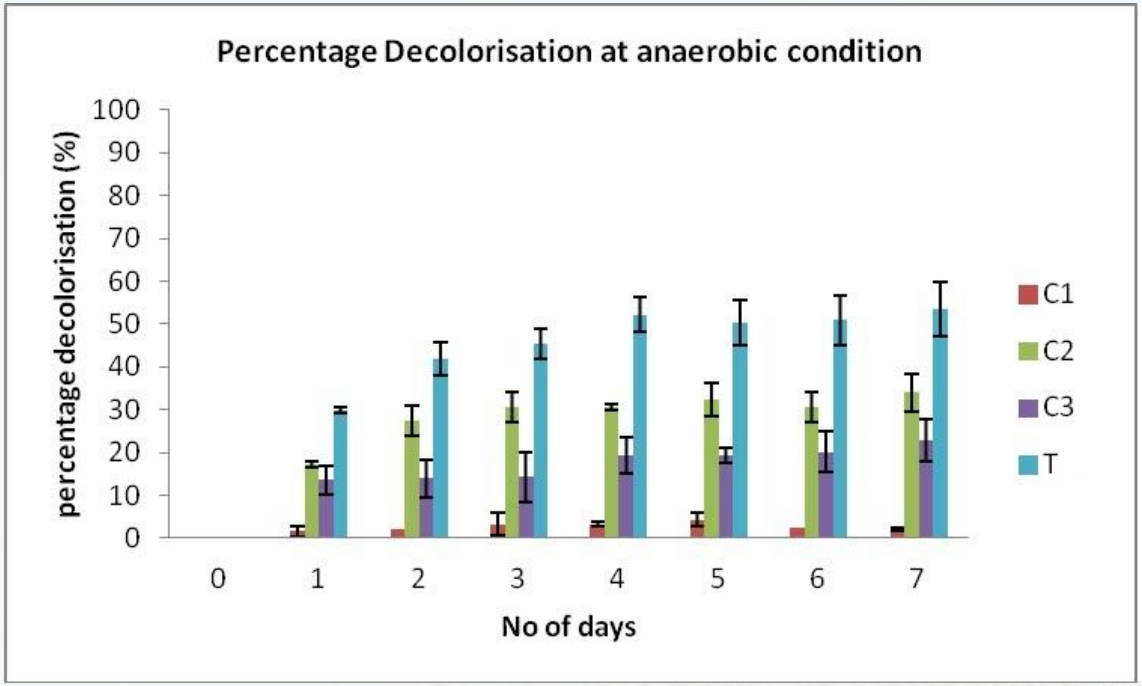
Greenhouse studies with synthetic wastewater and single azo dye – methyl red

Treatment	Description
C_1	Methyl red alone
C_2	Methyl red treated with microbial consortium
C_3	Methyl red treated with vetiver in Hoagland solution (0.25X)
T	Methyl red treated with both vetiver-microbe system in 0.25X Hoagland solution

Greenhouse studies with synthetic wastewater and single azo dye – methyl red

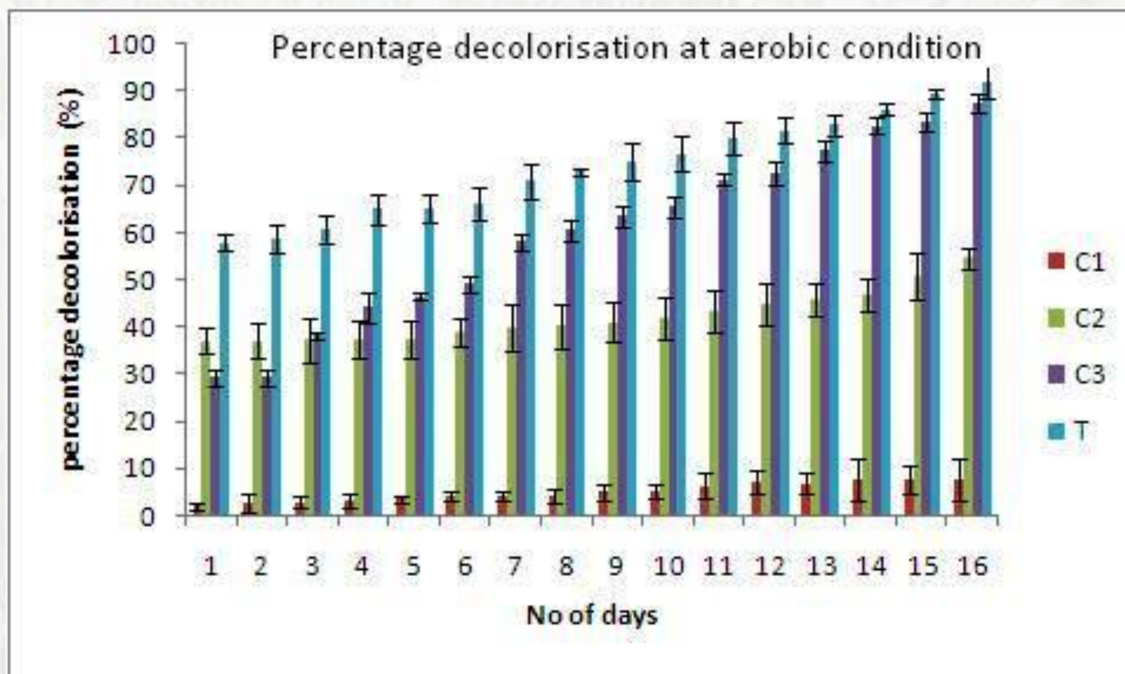
- Treatment volume: 10 L



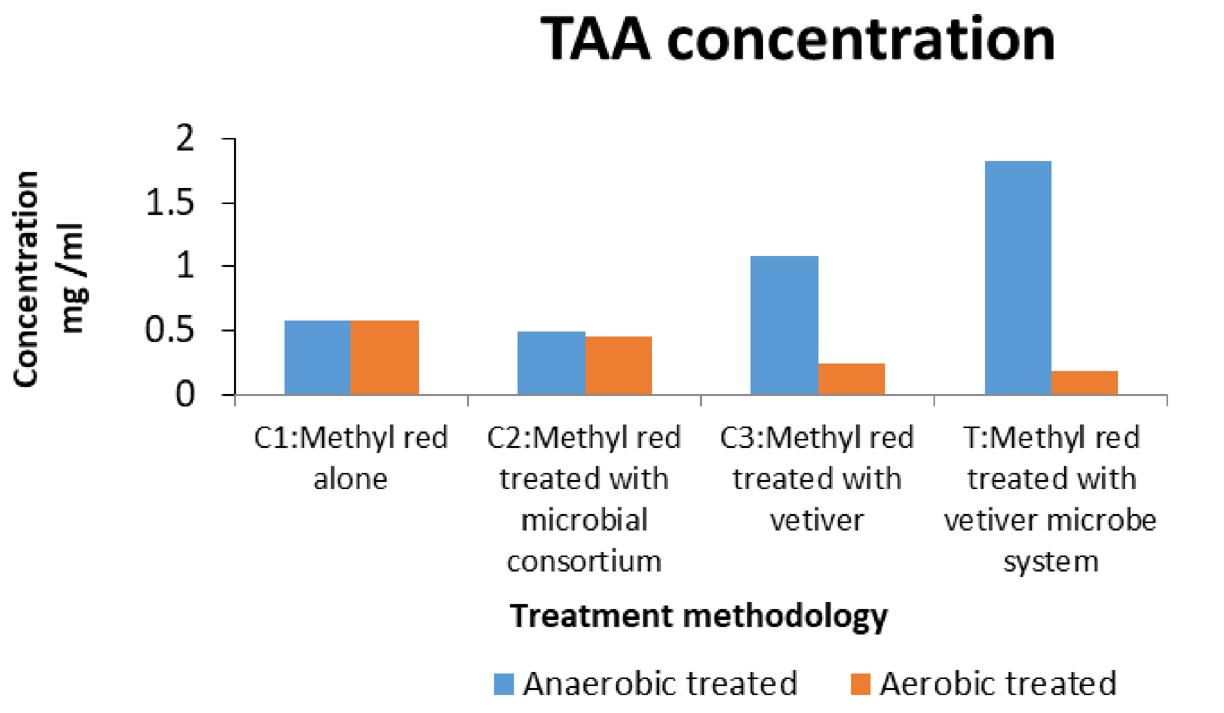


C1: Methyl red alone;
C2: Methyl red treated with microbial consortium;
C3: Methyl red treated with vetiver in Hoagland solution (0.25X);
T: Methyl red treated with both vetiver-microbe system in 0.25X Hoagland solution

7 days of anaerobic treatment followed by aerated treatment for 16 days till the decolourization rate stabilized



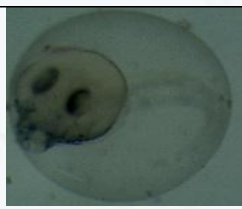
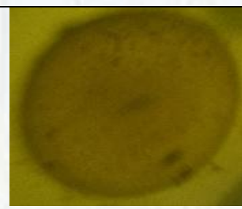
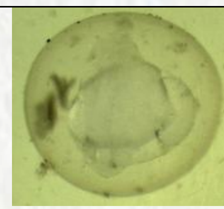
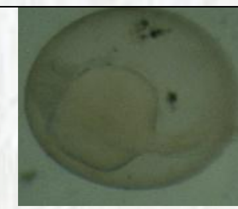
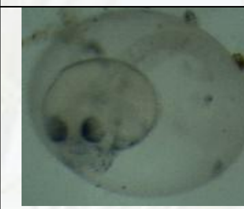
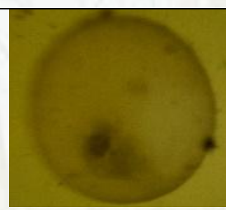
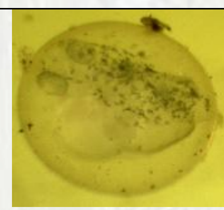
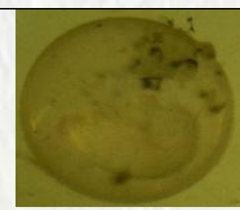
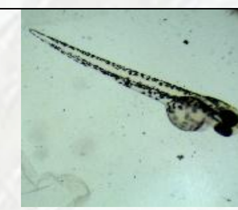
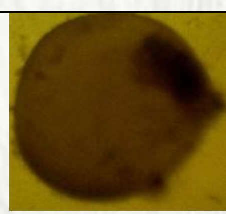
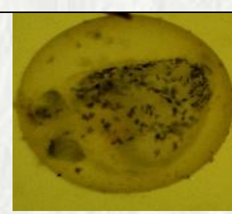
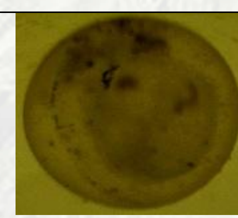
Roots before and after treatment



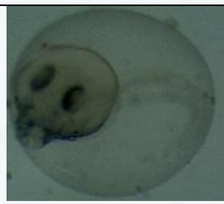
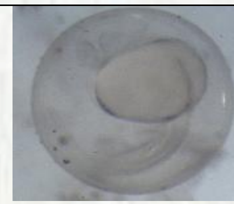
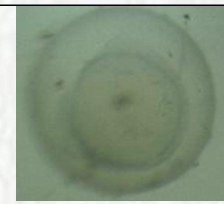
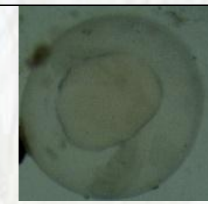
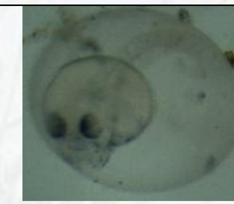
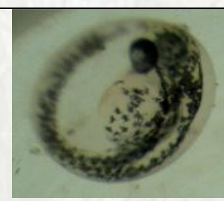
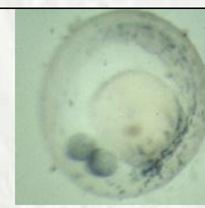
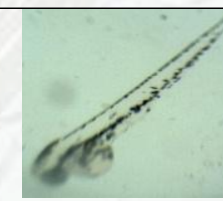
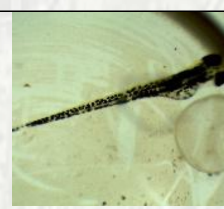
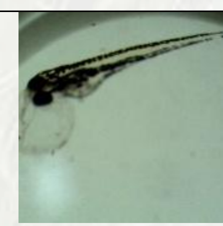
Formation of Aromatic amines during anaerobic treatment and their removal during aerobic conditions

S.No	m/z of metabolite	Retention time (min)	Name of metabolite
1	291.28	25.37	Methyl red
2	137.98	17.73	2-aminobenzoic acid (Anaerobic)
3	136	19.67	N, N'-dimethyl-p-phenylenediamine (Anaerobic)
4	123	16.23	Nitrobenzene (Aerobic)
5	122	15.75	Benzoic acid (Aerobic)

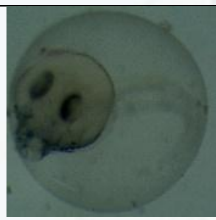
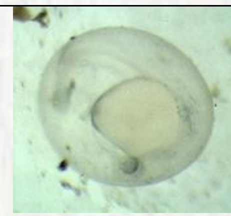
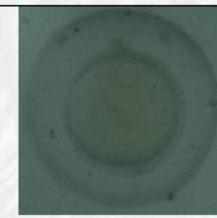
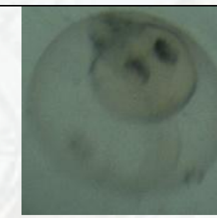
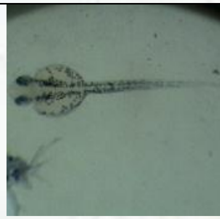
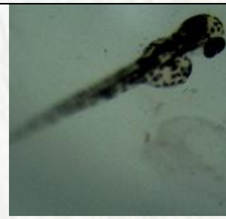
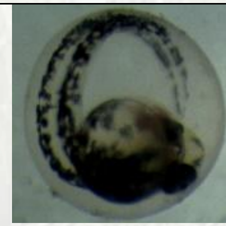
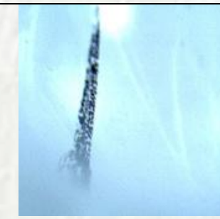
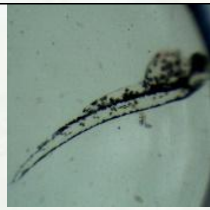
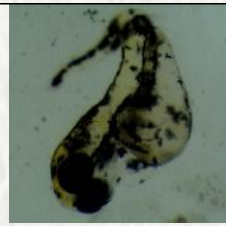
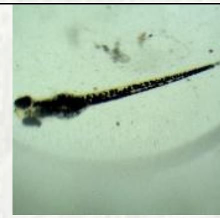
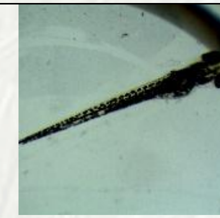
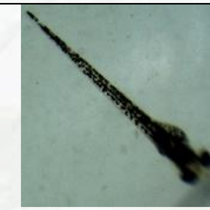
Zebrafish embryo development in treated water – 1X

	Fish water (Positive control)	Methyl red (40 mg/L) (negative control)	C1 WW with methyl red alone (1X concentration)	C2 WW with methyred and microbial consortia (1X concentration)	C3 WW with methyl red and vetiver (1X concentration)	T WW with methyl red + Vetiver + microbial consortia (1X concentration)
24 hpf						
48 hpf						
72 hpf						

Zebrafish embryo development in treated water – 0.1X

	Fish water (Positive control)	Methyl red Concentration (negative control)	C1 WW with methyl red alone (0.1 X) concentration	C2 WW with methyl red and microbial consortia (0.1 X) concentration	C3 WW with methyl red and vetiver (0.1 X) concentration	T WW with methyl red and Vetiver + microbial consortia (0.1 X) concentration
24 hpf						
48 hpf						
72 hpf						

Zebrafish embryo development in treated water – 0.01X

	Fish water (Positive control)	Methyl red (40 mg/L) (negative control)	C1 WW with methyl red alone (0.01 X) concentration	C2 WW with methyl red and microbial consortia (0.01 X) concentration	C3 WW with methyl red and vetiver (0.01 X) concentration	T WW with methyl red and Vetiver + microbial consortia (0.01 X) concentration
24 hpf						
48 hpf						
72 hpf						

Lethality and Malformations in developing embryos

- Lethality endpoints – coagulation of the embryo

- Teratogenicity endpoints – malformation of the head, tail, heart, scoliosis, and growth retardation

Survival percentage of embryos at time intervals of 24, 48 and 72 hour of post fertilization						
hpf	PC (Fish water)	NC (Methyl red)	Survival percentage			
			C1	C2	C3	T
24	100	0	16.67	33.3	33.33	50
48	100	0	16.67	33.33	33.33	50
72	100	0	16.67	33.33	33.33	50
Malformation percentage of embryos at time intervals of 24, 48 and 72 hour of post fertilization						
24	0	100	66.67	55.56	33.33	0
48	0	100	71.4	50	50	9.5
72	0	100	77.77	50	27.28	3.7

C1: Methyl red alone; C2: Methyl red treated with microbial consortium; C3: Methyl red treated with vetiver in Hoagland solution (0.25X); T: Methyl red treated with both vetiver-microbe system in 0.25X Hoagland solution

Total Aromatic Amines Vs Toxicity

	TAA	24 hpf			48 hpf			72 hpf		
	mg/mL	LC_{50}	EC_{50}	TI	LC_{50}	EC_{50}	TI	LC_{50}	EC_{50}	TI
C1	0.256	0.0274	0.0136	**2.022**	0.0274	0.0256	**1.067**	0.0274	0.0136	**2.022**
C2	0.102	0.004	0.004	**1**	0.0043	0.0043	**1**	0.0043	0.0039	**1.096**
C3	0.016	0.0013	0.0013	**1**	0.0013	0.0031	0.405	0.0013	0.0013	**1**
T	0.008	0.0028	0	0	0.0028	0.0029	**0.96**	0.0028	0.0033	**0.904**
MR	1.532	0.527	0.164	**3.2**	0.527	0.167	**3.15**	0.527	0.186	2.8

C1: Methyl red alone; C2: Methyl red treated with microbial consortium; C3: Methyl red treated with vetiver in Hoagland solution (0.25X); T: Methyl red treated with both vetiver-microbe system in 0.25X Hoagland solution

Wastewater collection tanks in the Industry

Wastewater Sample characteristics

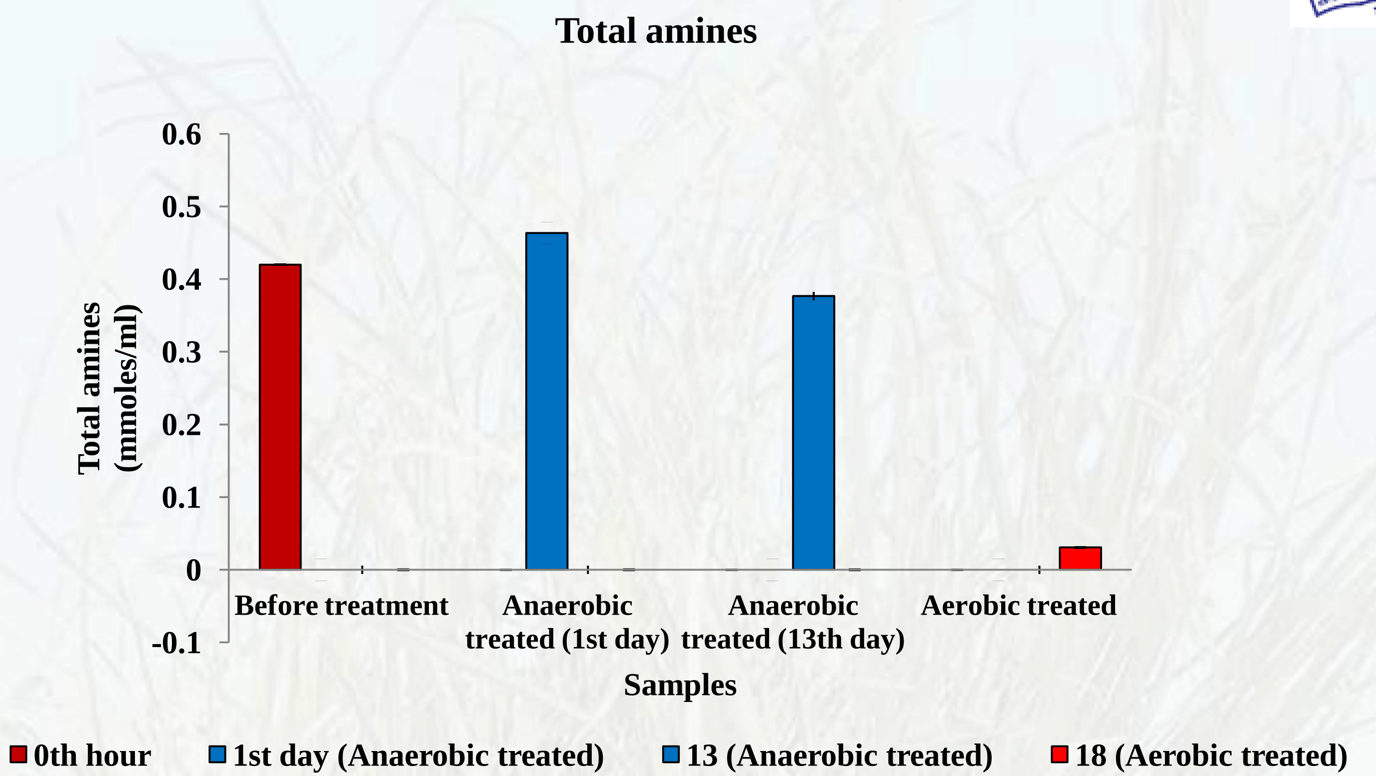
Sample from individual Process	Temp ˚C	pH	EC (µS)	TDS (mg/L)	TSS (mg/L)	BOD = DO_0 - DO_5 (mg/L)	COD (mg/L)
Acetic bath	45	3.32	3440	**2190**	4460	0.34	**6000**
Soap Wash	70	4.85	448	**285.33**	2564.67	2.11	**5600**
Acetic killer	45	4.67	1321	**858.33**	4891.67	3.72	**4400**
Soap out	90	3.82	1383.67	**900.67**	1899.33	3.24	**2000**
Dyes out	60	10.89	35330	**22830**	11720	2.37	**8400**
Peroxide out	90	11.39	2770	**1790**	7510	-	**4000**
Final wash	30	5.38	611.67	**242**	758	1.06	**3600**

Pilot scale set-up at the industry


AGNI

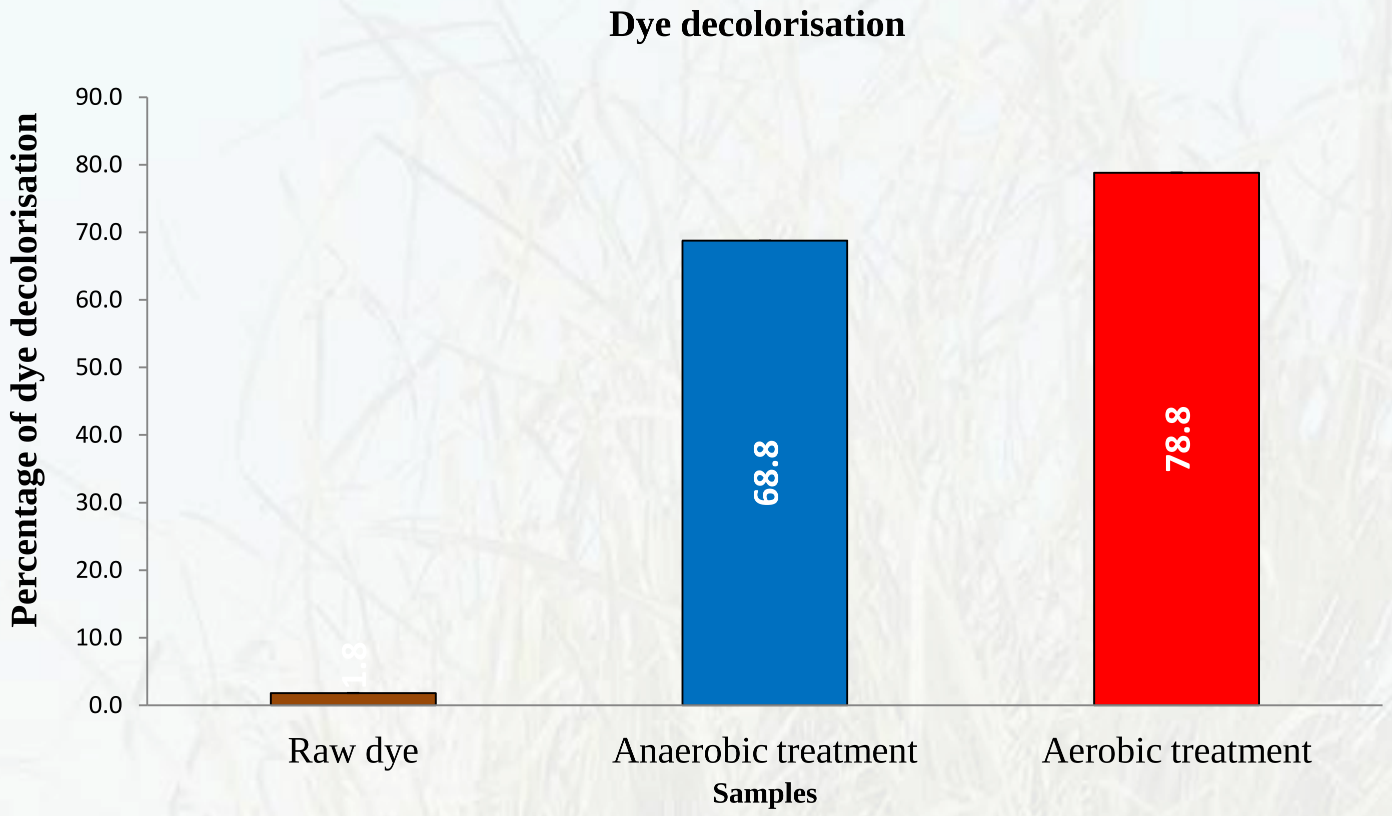
Vetiver used in the treatment

Total Amines – Sequential treatment of raw effluent from the industry



Decolorization and formation of aromatic amines at the end of 13 days of anaerobic treatment which were removed by 6 days of aerobic treatment

HRT Optimization



Dye decolourized maximum in anaerobic treatment by 4 hours followed by further decolourization in aerobic treatment

GC-MS data of textile dye effluent

Sample	m/z	RT(min)	Dye and its metabolites	Type of compounds present
Wastewater	168	17.12	Benzenamine,4-methoxy-2-nitro	Aromatic amines
	198	18.83	Azoxybenzene	Azo compound
	212	21.33	Diazene, (4-methoxyphenyl)phenyl-,(Z)-	Azo compound
	286	20.52	Diazene, bis(4-ethoxyphenyl)-, 1-oxide	Azo compound
	223	22.02	Monocrotophos	Toxic compound
Anaerobic treatment	135	17.12	Benzenamine, 2,4,5-trimethyl	Aromatic amines
	107	18.83	2-methyl-1-aminobenzene	Aromatic amines
	168	19.05	Benzenamine,4-methoxy-2-nitro	Aromatic amines
	121	20.53	Benzenamine,2,4-dimethyl-	Aromatic amines
	226	21.38	Benzenamine, 4,4',methylenebis[2-methyl	Aromatic amines
Aerobic treatment	228	17.17	Methyl tridecanoate	Aliphatic
	236	18.85	7,11-hexadecadienal	Aliphatic
	238	19.87	E-9,Hexadecenal	Aliphatic
	254	20.67	9-hexadecanoic acid	Aliphatic
	262	22.15	1,3,12-Nonadecatriene	Aliphatic

Survival percentage of embryos at time intervals of 24, 48 and 72th hour of post fertilization

Samples	Survival percentage		
	24 hpf	48 hpf	72 hpf
Positive control	91.67	91.67	91.67
Negative control	75	75	50
Anaerobic treated	91.67	88.89	69.44
Aerobic treated	100	97.22	83.33

Malformation percentage of embryos at time intervals of 24, 48 and 72th hour of post fertilization

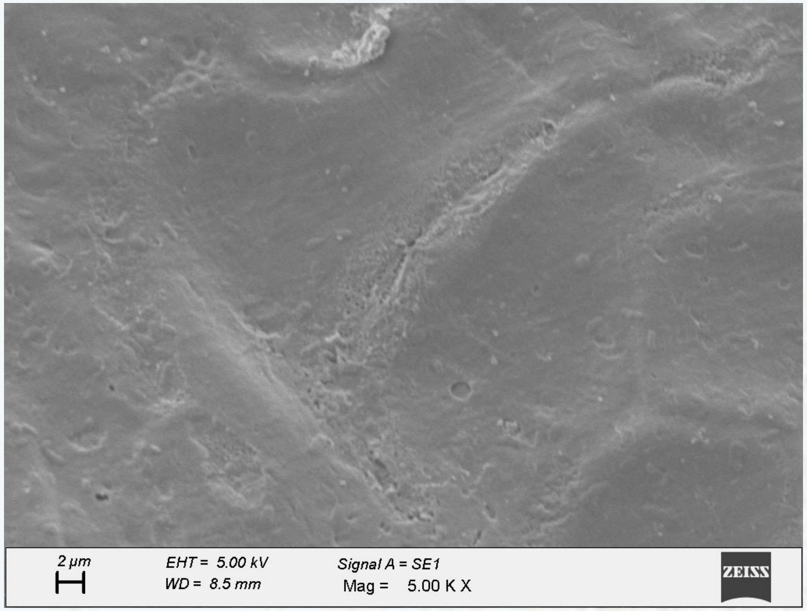
Samples	% Malformation in surviving embryos		
	24 hpf	48 hpf	72 hpf
Positive control	0	0	0
Negative control	66.67	66.67	50
Anaerobic treated	30.71	37.88	43.98
Aerobic treated	16.67	19.70	13.33

Teratogenecity index

Samples	72 hpf		
	LC_{50}	EC_{50}	TI
Raw dye	2.531	2.531	**1**
Anaerobic treated samples	2.939	0.5635	**5.215**
Aerobic treated samples	0.0059	4.889	0.00121
Fish water	0.0909	11	0.00826

SEM analysis of plant roots

a



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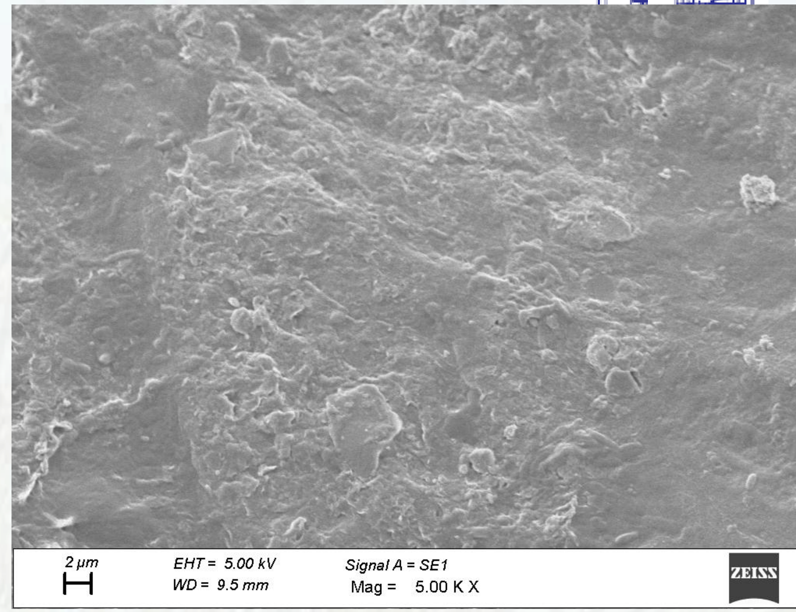

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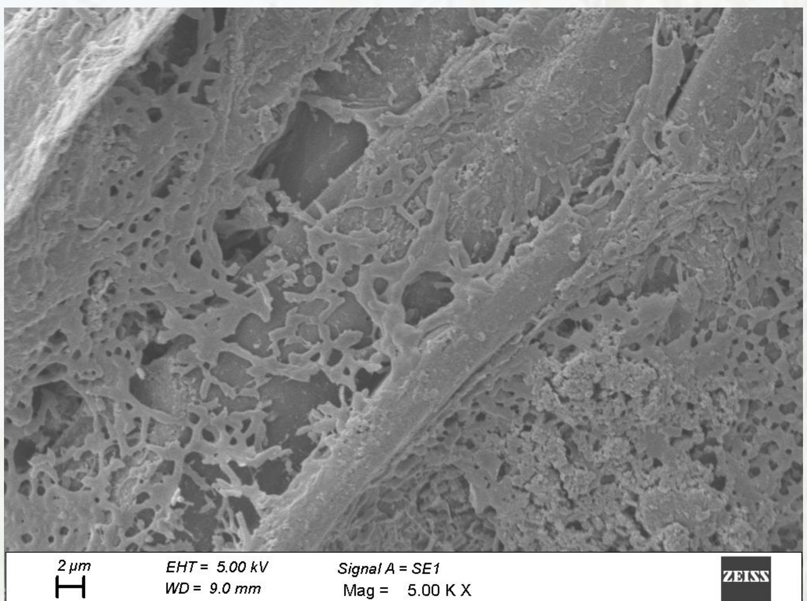

(a) bare vetiver root surface (b) morphological changes when treated with methyl red dye alone c) bacterial attachment onto root surface when treated under synergistic plant-microbe treatment system

Conclusion

- Decolourization – mainly during the anaerobic treatment
- Presence of tolerant plants – help in reducing the concentration of toxic compounds in water due to adsorption on to the surface of the roots

Conclusion

- Microaerophilic conditions in the root zones along with the biofilm formed on them involved in aerobic degradation of aromatic amines formed

- Sequential treatment was effective in reducing the toxicity of the treated water.

Limitations

- Total Dissolved Solids are not brought down below the CPCB standards (2100 mg/L)
- Time duration – 4 h for decolourization and more than 4 days for complete degradation

Translation and industry ready treatment

- The main constraint – time and TDS
- Reactor design optimization
- Integration with other processes like photocatalysis – we have undertaken this as part of the Newton – Bhabha Funded project
  - We are currently addressing constraints in this

Defluoridation

- Developed a composite capable of reducing the fluoride levels in groundwater
  - Patent applied
  - Technology transferred to industry

PSGTECH - CEAS

- PSG Tech **Centre for Environmental Analyses & Solutions**

- An initiative to address problems faced by the industry especially in terms of
  - Waste and wastewater analyses
  - testing of treated effluent quality and compliance with the norms
  - Troubleshooting and process parameter optimization

Acknowledgements

- DBT for support and funding for textile industry wastewater project

- DST for support for the defluoridation project

- RAE-Newton Bhabha Fund for the photocatalysis project

- PSG College of Technology for infrastructure and administrative support.

THANK YOU!