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Impact of Microbially Treated Dye Wastewater on Growth of Pea Plant

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Abstract

Textile industry is known to discharge a significant amount of azo dyes in effluents which pollutes water and soil environment, and consequently may affect plant growth. Such wastewater must be treated prior to its discharge into wastewater stream. In this research work, azo dye contaminated water was treated with dye-degrading bacteria strains (Psychrobacter alimentarius KS23 and Staphylococcus equorum KS26) and its impact was studied on plant growth under controlled conditions. Pea (Pisum sativum) plant was used as a test crop. An attached growth sequencing batch reactor with the amendment of three different cosubstrates was used for bacterial treatment of 200mg/L concentration of Reactive Black-5 azo dye and pre-isolated bacterial strains capable of degrading azo dyes was used as an inoculum. Upto 94% color removal was observed with KS23 and KS26 in the presence of mineral salts including yeast extract or yeast extract only. Treated and untreated dye solution (control) was applied for about two weeks. Results revealed positive impact of treated azo dye with different cosubstrates and pre-isolated strains on shoot and root length and biomass in comparison to control. This study suggests that textile industry wastewater can be used for irrigation purposes after the amendment of mixed liquid suspended solids (sewage sludge) and co-substrates to achieve better biomass, but health risks associated with consuming such wastewater irrigated plants are still unknown.

Introduction

Environmental pollution is a matter of great concern and has been globally accepted as a serious problem because of adverse effects on human health, plants and animals. Plants being producers are vital unit of ecosystem and highly responsive to biotic and abiotic environmental factors which lead to stress condition. This stress can be expressed as stunted growth or germination inhibition (Garg and Kaushik, 2005).

Existing water and land resources are under immense pressure due to alarmingly increasing population rate: primary reason behind production of large volumes of sewage. In perspective of ongoing climate change and water shortages in near future, use of this raw sewage for irrigating crops is a considerable option. Particularly where effluent treatment facilities are lacking, sewage is used for irrigation purposes (Murtaza et al., 2010).

Textile industry is one of the major sources of industrial effluents affecting the environment. Untreated textile industry effluent contains high concentrations of consumed metal dyes, phenol and aromatic amines (Verma et al., 1969). Metal based colored dyes and foaming chemicals used retard the biological activity due to reduction in the availability of light and cause metal toxicity to the aquatic and terrestrial habitats (Tariq et al., 1996).

The colored effluents released from textile processing and dye-manufacturing industries have a huge amount of unreacted dyes as upto 15% of the dyestuff does not stick permanently to the fibers during dyeing processes and is therefore released into the environment (Tan et al., 2000).

This wastewater is discharged directly into water bodies without treatment leading to surface and ground water pollution and soil pollution. The contaminants such as Ni, Cr, Pb, Co, Fe, Ca, Na and K get added in water and soil, ultimately may be up taken by plants, as concentration of Cr and Pb has been reported to be 5.96 and 4.46 mg/ kg of soil irrigated with textile effluent (Manzur et al., 2006). Besides aesthetically displeasing, the discharge of colored effluents in water bodies lowers the photosynthesis rate as it hinders penetration of light in water. Untreated textile industrial water causes accumulation of carcinogenic compounds in plants, which ultimately become a part of food chain. Deflocculation of soil particles, salinization, alkalization, rise in N, P, K levels, pH (8.1-9.1) and outbreak of gastroschisis are outcomes of textile industrial wastewater (Root and Emch, 2010). Thus textile wastewater is more potent than any other industrial wastes and needs to be treated prior to its release (Nawaz et al., 2006). Existing effluent treatment techniques cannot remove recalcitrant azodyes from effluents fully because of color retreat, resistance to degradation and steadiness (Oliveira et al. 2010). Sequencing batch reactor containing suspended and attach film biocarriers is the most efficient and easy treatment option (Guo et al., 2009). Bacterial decolorization under certain conditions has gained popularity and trust as a method of treatment, is considered reasonable and ecofriendly, and can be pragmatic to a wide array of azo dyes (Saratale, 2011). Treated saline textile effluent has been used for irrigating olives. It was observed that leaf Cu, Na, N, Mn, Pb, Fe and Pb contents increased with increasing salinity of the treated wastewater. This increase was accompanied with a decrease in K and Mg contents. While roots indicated that the contents of Pb, Na, Mn, P, Cl and P increased while K decreased with the increase in salinity of treated wastewater (Al-absi, 2009). Bhatti and Singh, 2003 reported that textile effluent is rich in Na which reduces Mg and micronutrient concentration in seedlings, ultimately affecting root and leaf growth. Eucalyptus camaldulensis gave least growth when irrigated with textile effluent. Growth improved by the addition of municipal effluent to textile effluent.

This experiment will propose an idea that amendment of sewage sludge along with co-substrate (carbon source) into textile wastewaters can be a beneficial approach for agricultural irrigation, keeping in mind our limited fresh water resources. It also highlights the efficiency of different carbon sources added to enhance the biodegradation process and tests compatibility of pre-isolated strains with MLSS.

This experiment will reveal impact of treated wastewater on pea plant growth in terms of biomass but impact of consuming vegetables irrigated by such treated dye contaminated water on human health is yet to unleash.

Materials and methods

2.1 Analytical Techniques

Standard procedures described by APHA (2005) were followed for wastewater analysis. pH, DO and EC were analyzed by electrometric method, COD was obtained by close reflux method. Other techniques followed were moisture oven dry basis, nitrate nitrogen spectrophotometric method, organic method titration method, shoot length scale measuring method, root/shoot ratio dividing method, dry biomass dry weight basis and fresh biomass fresh weight basis.

2.2 Bacterial Treatment System for Wastewater

Attach growth sequencing batch reactor (mixed liquor suspended solids between 5000-6000mg/L under aerobic-anaerobic cyclic conditions) was set for treatment of 200mg/L reactive black dye contaminated wastewater. Previously isolated bacterial strains, namely Psychrobacter alimentrius KS23 and Staphylococcus equorum KS26, capable of decolorizing reactive azo dyes efficiently in liquid mineral salt medium (Khalid et al., 2012) were used to enhance the decolorization rate. The bacterial culture was prepared in conical flasks containing mineral salt medium and incubated at 30 °C. Dye decolorization was achieved with 1000 mg/L of MLSS, 20% (v/v) microbial culture and three co-substrates coded as C1 containing mineral salts including yeast extract (Khalid et al., 2008), C2 having yeast extract only and C3 containing glucose in synthetic wastewater composition (Khan et al., 2010) under anaerobic static conditions. Three replications were performed for every experimental unit and 24 h hydraulic retention time (HRTs) was set. All the reactor operations were followed as Saba et al., (2011). Sponge cubes of 1 cm3 were added in half of SG- MBR (suspended growth-membrane bioreactors), transforming it into attached system (Guo et al. 2010). Blocks provided attachment site to microbes thus enhancing the microbial growth. Treatment was evaluated in terms of percentage color and COD removal.

2.3 Impact of Treated Wastewater on Germination and Plant Growth

Both microbially treated and untreated dye polluted water was used for evaluating its effect on growth of the Pea (Climax) seedlings procured from local seed supplier. Before performing the experiment, the percent germination of these cultivars was checked using distilled water and was found to be between 90% and 100%. Only healthy seeds were chosen, sterilized (Khalid et al. 2004) and allowed to germinate at 25± 2 °C on Petri dishes containing a double layer Whatman No. 1 filter paper irrigated with 5 ml of respective treated dye contaminated water and untreated dye which was taken as control. The emergence of the radicle was taken as a criterion of germination. Percent germination was recorded.

Sand was selected as a growth medium instead of soil because sand has large pore spaces and it support proliferation of roots. When plants are not able to get nutrients from sand (which normally happens in case of soil) then they solely rely on the treatment applied for the nutrients. Thus sand provided an ideal medium to check impact of textile effluent on plant growth. Each treatment was replicated three times. Data regarding root and shoot growth i.e. root and shoot length was recorded after two weeks of incubation using scale. Number of leaves was counted for pea and number of spikes was counted for wheat by visual count method. Harvesting was done carefully so that no roots get broken. Fresh weights of shoot and root were taken using weighing balance. Dry weights were taken after drying the plant at 65 °C in oven for 24hr.

2.4 Statistical Analysis

All the data was subjected to analysis of variance and means were compared using Least Significance Difference test with the help of SPSS. 95 percent C.I. was set. Standard deviation was calculated to know the variability in the data.

Results and Discussion

3.1 Color Removal of Azo Dye

. Microbes use dye as a source of carbon and nitrogen, however addition of co-substrate enhanced the decolorization of Reactive black dye. 94% color removal with strain KS23 was attained in case of yeast extract as co-substrate followed by 91% when mineral salts including yeast extract was used as co-substrate and least color removal of 78% was observed with synthetic wastewater. Upto 93% color removal was observed with KS26 culture in presence of mineral salts including yeast extract followed by 85% with only yeast extract as co-substrate. Mix consortium on the other hand showed maximum of 92% color removal with only yeast extract as co-substrate, 87% and 81% color removal with mineral salts and synthetic wastewater (glucose as co-substrate) respectively.

3.2 Effect of Treated Wastewater on Germination

Dye contaminated wastewater treated with KS23 gave 100 percent germination with mineral salts and yeast extract as co-substrate while in presence of synthetic wastewater, none of seeds could germinate, whereas, application of dye treated with KS26 resulted in 75 and 25 percent germination with mineral salt medium and yeast extract as compared to control (75 percent germination). Dye treated with mix consortium showed 75 and 50 percent germination with yeast extract and synthetic wastewater while 25% with mineral salts including yeast extract. Thus, for pea, yeast extract seemed to be most effective carbon source and nutrient to support germination. This might be due to reason that yeast extract is better carbon source for bacteria which enhances biodegradation of toxic metabolites and ultimately this treated dye leads to better growth.

3.3 Effect of Treated Wastewater on Growth

.Wastewater treated in the presence of KS23 and mineral salt medium as co-substrate resulted in 3folds higher shoot length as compared to control. While 2 fold increase was observed in case of wastewater treated with KS26 and mineral salt medium. In the presence of C2, KS26 showed 2 fold greater shoot length while KS23 and consortium gave at least 90% higher shoot length as compared to control. Application of treated dye containing C3 as co-substrate showed 3 folds higher shoot length compared to control. Use of KS23 treated dye in the presence of C1 and C2 resulted in 2.5fold higher root length as compared to control. Synthetic wastewater proved to be most effective co-substrate to enhance the root length upto 4.5fold with KS26 and consortium. Highest root/shoot ratio was observed in case of dye treated with consortium in presence of C1 and C3.

In case of dye treated with consortium, 1.5-2 fold higher fresh shoot weight was observed in the presence of C1 and C2, while KS26 treated dye showed 2fold higher fresh shoot weight with C3 as co-substrate as compared to control. KS23 treated dye in the presence of C1 and C2 showed highest fresh root weight of 6-2 folds greater than control. Highest dry shoot and root weights were recorded for KS23 treated dye in the presence of co-substrate C3. Detailed data regarding all the studied growth parameters is given in Table 2 and Table 3.

Dyes have concentrate-dependent effect on the growth of plants (Liu et al. 2007). Probably, high concentration of dye from untreated wastewater may accumulate in roots which affect nutrient uptake and other metabolic activities resulting in reduction in plant growth.

Increased shoot and root length with treated dye may be due to presence of nitrogen (a vital nutrient) in dye solutions (Saravanamoorthy and Kumari, 2005). Increase in plant biomass of pea with the application of wastewater containing yeast as co-substrate can be attributed to activated synthesis of plant growth promoting hormones which trigger the activity of specific enzymes, which are capable of assimilating starch in plants that lead to increase in biomass (Arshad et al., 2008). Plants irrigated with treated water were healthier and dark green in color whereas leaves of plants irrigated with untreated dye wastewater showed high accumulation of the dye in leaves of pea plant leading to bluish color of plant. No paleness, inward leaf curling and dye color were observed in leaves of plants grown with treated water.

Addition of co-substrate resulted in better shoot length, root length; fresh and dry shoot and root weight as compared to untreated dye. Since extreme water scarcity is a major problem faced by majority of farmers in Pakistan, use of industrial effluent and sewage for irrigation is gaining popularity. By the amendments of co-substrate, sewage sludge and pre-isolated strains in textile industrial effluent, better results in terms of biomass can be achieved. Yeast extract is inexpensive as compared to mineral salts. Thus at the end of experiment, it can be concluded that yeast extract can be a better option to be used as a carbon source for attaining better biodegradation and subsequent use of industrial effluent for irrigating crops. Health aspects regarding consuming such crops are still unknown.

References

[1] APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington, DC.

[2] Al-Absi, K. M., F. M. Al-Nasir and A. Y. Mahadeen. 2009. Mineral content of three olive cultivars irrigated with treated industrial wastewater. Agri. Manag., 96: 616 – 626.

[3] Arshad, M., Shaharona B. and Mahmood. T., 2008. Inoculation with Pseudomonas spp. containing ACC deaminase partially eliminates the effects of drought stress on growth, yield and ripening of pea (Pisum sativum L.). Pedosphere, 18, 611-620

[4] Bhati, M. and G. Singh. 2003. Growth and mineral accumulation in Eucalyptus camaldulensis seedlings irrigated with mixed industrial effluents. Biores. Tech., 88: 221–228.

[5] Garg, V. K. and P. Kaushik. 2005. Influence of textile mill waste water irrigation on growth of Sorghum cultivars. Appl. Eco. Env. Res., 6(2): 1-12.

[6] Guo, W., H. H. Ngo, C. G. Palmer, W. Xing, A. Y. -J. Hu and A. Listowski. 2009. Roles of sponge sizes and membrane types in a single stage sponge-submerged membrane bioreactor for improving nutrient removal from wastewater for reuse. Desalination, 249: 672–676.

[7] Guo, W., H. H. Ngo, F. Dharmawan and C. G. Palmer. 2010. Roles of polyurethane foam in aerobic moving and fixed bed bioreactors. Bioresour. Technol., 101: 1435–1439

[8] Khalid, A., F. Kausar, M. Arshad, T. Mahmood and I. Ahmad. 2012. Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment. Appl. Micrbio. Biotech., DOI 10.1007/s00253-012-3877-7.

[9] Khalid, A., M. Arshad and Z. A. Zahir. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol., 96: 473–480.

[10] Khalid, A., M. Arshad and D. E. Crowley, 2008. Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains. Applied Microbiology and Biotechnology 78 (2), 361–369.

[11] Khan, S. J., S. Ilyas, S. Javed, C. Visvanathan and V. Jegatheesan. 2010. Performance of suspended and attached growth MBR in treating high strength synthetic wastewater. Biores. Tech., 102: 5331-5336.

[12] Liu, R., Q. Zhou, L. Zhang and H. Gao, 2007. Toxic effects of wastewater from various phases of monosodium glutamate production on seed germination and root elongation of crops. Frontiers of Environmental Science and Engineering in China. 1, 114–119.

[13] Manzur, S., M. H. Shah, N. Shaheen and A. Khalique. 2006. Multivariate analysis of trace metals in textile effluents, in relation to soil. J. Haz. Mat., 37: 105-113.

[14] Murtaza, G., A. G. Owen, M. A. Aziz, M. H. Zia and Saifullah. 2010. Disposal and Use of Sewage on Agricultural Lands in Pakistan: A Review. Pedosphere, 20(1): 23–34.

[15] Nawaz, S., S. M. Ali and A. Yasmin. 2006. Effects of industrial effluents on seed germination and early growth of Cicer arientum. J. Biol. Sci., 6(1): 49-54.

[16] Oliveira, G. A. R., E. R. A. Ferraz, F. M. D. Chequer, M. D. Grando, G. P. F. Angeli, J. C. Marcarini, M. E. Osugi, T. M. Lizier and M. V. B. Zanoni.2010. Chlorination treatment of aqueous sample reduces but doesnot eliminate mutagenic effect of azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. Mut. Res., 703: 200-208.

[17] Root, E. D. and M. E. Emch. 2010. Tracing drinking water to its source, an ecological study of relationship between textile mills and gastroschisis in North Carolina. Heal. Plac., 70: 794-802.

[18] Saba, B., T. Mahmood, B. Zaman and I. Hashmi. 2011. Reuse of treated wastewater using sequencing batch bioreactor for the improvement of wheat growth. J. Water Reuse and Desalinaton. Vol 1 no 3, 99 179-184.

[19] Saratale, R. G., G. D. Saratale, J. S. Chang and S. P. Govindwar. 2011. Bacterial decolorization and degradation, A review. J. Taiwan Instit. Chem. Eng., 42: 138-157.

[20] Sarawanamoorthy, M. D. and B. D. R. Kumari. 2005. Effect of cotton yarn dye effluent on physiological and biochemical contents of peanut (Arachis hypogaea L. cv. TMV-10) and green gram (Phaseolus radiatus L.cv. K1). Biochemical and Cellular Archives 5(1), 113-117

[21] Tan, N. C. G. and J. A. Field. 2000. Biodegradation of sulfonated aromatic compounds. In: P. Lens andL. W. Hulshoff-Pol. (Eds.). Environmental Technologies to Treat Sulfur Pollution. Principles and Engineering. IWA Publishing, London: pp 377–392.

[22] Tariq, J., M. Ashraf, M. Jaffar and M. Afzal. 1996. Pollution status of Indus River Pakistan through heavy metal and micronutrient content of fish, sediment and water. Water Res., 30: 1337-1344.

[23] Verma, S. R. and G. R. Shukla. 1969. Pollution in a perennial stream, 'Khala' the sugar factory effluent near lakes. Env. Health., 11: 145-162.

Tables

Table1. Color removal % of Reactive black 5 dye contaminated wastewater using microbial cultures

Treatments*	Color removal (%)		
	C1	C2	C3
KS23	91	94	78
KS26	93	85	79
Consortium	87	92	81

*Dye-contaminated wastewater with the amendment of either mineral salts containing yeast extract as a co-substrate (C1), yeast extract only (C2) or synthetic wastewater containing glucose as a co-substrate (C3).

Table 2: Impact of treated dye-contaminated wastewater on shoot and root length and root/shoot ratio of pea plant

Treatments*	Shoot length(cm)			Root length (cm)			Root/shoot ratio		
	C1	C2	C3	C1	C2	C3	C1	C2	C3
Control	2.7c**	3.2c	2.2c	3c	2.7c	2c	1.1b	0.84c	0.90c
KS23	9.6a	6.1b	4.3b	10.7a	9.1a	2.6c	1.11b	1.49a	0.60d
KS26	6b	9.3a	5.35ab	6.4b	9.05a	8.9b	1.06b	0.96b	1.6b
Consortium	3.8c	5.3b	6.4a	6.7b	5.5b	11.2a	1.76a	1.0b	1.75a

*Dye-contaminated wastewater with the amendment of either mineral salts containing yeast extract as a co-substrate (C1), yeast extract only (C2) or synthetic wastewater containing glucose as a co-substrate (C3).

**Mean values having similar letter(s) in a parameter do not differ significantly at $P<0.05$, according to LSD test.

Table 3: Impact of treated dye-contaminated wastewater on fresh shoot weight, fresh root weight, dry shoot weight and dry root weight of pea plant

Treatments	Fresh shoot weight (g/plant)			Fresh root weight (g/plant)			Dry shoot weight (g/plant)			Dry root weight (g/plant)		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
Control	0.22d	0.20b	0.12c	0.04d	0.06c	0.02c	0.03c	0.04c	0.03c	0.009c	0.01c	0.007d
KS23	0.47b	0.64a	0.25b	0.31a	0.16a	0.13ab	0.06b	0.09b	0.11a	0.09a	0.12a	0.17a
KS26	0.28c	0.10c	0.43a	0.21b	0.10b	0.10b	0.04c	0.09b	0.09b	0.07b	0.06b	0.01c
Consortium	0.56a	0.60a	0.33ab	0.15c	0.08c	0.15a	0.09a	0.10a	0.08b	0.06b	0.05b	0.06b

*Dye-contaminated wastewater with the amendment of either mineral salts containing yeast extract as a co-substrate (C1), yeast extract only (C2) or synthetic wastewater containing glucose as a co-substrate (C3)..

**Mean values having similar letter(s) in a parameter do not differ significantly at <0.05, according to LSD test.