

Biotreatment of Textile Effluent for Irrigation Purpose Using Indigenous Bacterial Consortium (BMP1/SDSC/01): A Step Towards Reducing Fresh Water Scarcity

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Abstract A bioreactor was designed to attain efficient, cost-effective and environmentally reliable bioremediation system for the biotreatment of textile effluents to produce irrigation water. The consortium BMP1/SDSC-01 (having six indigenous bacterial isolates: *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus* sp., *Micrococcus* sp. and *Pseudomonas* sp.) was used in the bioreactor for the treatment of effluents. Bacterial obliteration from the biotreated textile effluents was done by using acetic acid, which proved efficient and result showed that bacterial growth was not possible in the presence of acetic acid. The treated and untreated textile effluents were applied on two common crops of Pakistan *Zea mays* L. CV C1415 (Maize) and *Sorghum vulgare* Pers. CV SSG5000 (Sorghum) to monitor efficacy of bioremediated textile effluents. During experiment plant height, number of leaves, photosynthesis and transpiration rates, and biomass were measured. The results clearly indicated that in untreated effluents maize plant height, biomass, photosynthesis and transpiration was 93.26, 45.38, 9.11 and 0.41, respectively. Under irrigation of treated effluents, plant height, biomass, photosynthesis and transpiration were 124, 44.07, 16.95 and 0.84 respectively. Similar trend was observed in sorghum, its plant height increased to 115.8 instead of 85 (untreated). Simultaneously, number of leaves, biomass, photosynthesis and transpiration were 8.62, 44.45, 13.29 and 0.51, respectively. The results are proving that bioreactor successfully reduced the toxicity level of textile effluents and can be used for irrigation purposes. This study will help to produce irrigation water from textile effluents at large scale in Pakistan by applying bioremediation.

Keywords Bioreactor, Textile Effluents, Acetic Acid, Bacterial Consortium, Phytotoxicity

1. Introduction

Freshwater has become a serious global issue because of its overexploitation in the world which is the major restraint to the future food security and sustainability. Presently water crises are common feature of the modern world. About 34 % of the world population is suffering with moderate to high water stress, this may increase to 50 % till 2030. Water becomes a limited resource at regional and local scales as consumption is exceeded to its renewability or is polluted [10,9,13]. The total world colorant production is estimated 8x10⁶ kg per year, of which 10 to 15% released into effluent during different processes. The textile effluent has been one of the main sources of severe pollution worldwide. The release of dye effluents into the environment is undesirable, not only because of their visual effect, but also because many dyes are made from known carcinogens, and their breakdown products are toxic and mutagenic to life. As a feasible alternative, biological processes have received increasing interest due to their low cost and eco-friendly nature. It also has low energy requirements, easy process control, operation over a wide range of conditions, and a minimal environmental impact[30].

The toxicity of textile effluent is the major constrain to its biological treatment. It is necessary to know, how the action of pollutants occur on the life stages of organism in an ecosystem. All over the world the researchers have highlighted the significance to appraise effluent eco-toxicity[29]. The literature showed that the effect of detoxified textile dyes and effluent is evaluated on common crops only at germination stage [22,11,26]. To get comprehensive consequences it is necessary to check the effects of detoxified textile effluent on the whole growth period of the crops. For this purpose we isolated the indigenous bacteria and develop a consortium capable of detoxifying the textile effluent, then the detoxified effluent check on two common cash crops for whole the growth

period. And finally concluded that biotreatment of textile effluent by the consortium BMP1/SDSC/01 made it suitable for irrigation purpose.

2. Materials and Methods

2.1. Sample Collection and Bacterial Consortium

Textile effluents were collected in screw capped sterilized plastic cans from Hudiara drain near Nishat Mills Limited 5Km Off - 22Km Ferozepur Road Lahore, Pakistan [6]. Consortium BMP1/SDSC-01 (having six indigenous bacterial isolates: *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus* sp., *Micrococcus* sp. and *Pseudomonas* sp.) selected in previous studies[24, 25]) was used as inoculum for the bioremediation of textile effluent.

2.2. Bioreactor Design

A bioreactor was primarily designed to attain efficient, cost-effective and environmentally reliable bioremediation system. This bioreactor was a glass jar (20L) having tight screw cap at top and a tap at bottom to collect bio-remediated water and was used for the bioremediation of textile effluent carried out at lab scale. The textile effluent (16L) was taken into glass jar; the Consortium BMP1/SDSC-01 in nutrient broth was added at rate of 100ml/L of effluent. The pH (7.5 to 8.5) and temperature (32°C to 37°C) was maintained throughout the experiment [16].

2.3. Bacterial obliteration by induced environmental stress

Bacterial obliteration was done by inducing acetic acid stress. The acetic acid was added at the rate of 0.05ml/L to the bio-remediated textile effluent and pH was measured. The biotreated effluent having acetic acid was used as inoculum to check the colony forming ability(CFA) of the consortium on nutrient agar. The bacterial growth was observed after 24 hours of inoculum to assess bacterial elimination [20].

2.4. Physicochemical Characterization of Textile Effluents

Characterization of the textile effluent for various parameters such as temperature, color, pH, EC, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS) and total suspended solids (TSS) was carried out before and after the bioremediation by bacterial consortium BMP1/SDSC-01 in the bioreactor [17].

2.5. Experiment Design to Check Efficacy of Bio-remediated Textile Effluent

Experiment was conducted in pots at Botanic Garden, GC

University Lahore on *Zea mays* L. CV C1415 (Maize) and *Sorghum vulgare* Pers.CV SSG5000(Sorghum) to check efficacy of bio-remediated textile effluent. The textile effluent and bio-remediated textile effluent was added in the pots having experimental plants at regular time intervals. A control set of pots was irrigated with tap water. During experiment plant height, number of leaves, photosynthesis and transpiration rate, and biomass were measured[15].

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using software package Co-stat version 3.03 [7].

3. Results

3.1. Monitoring of Bioreactor and Bacterial Obliteration

In order to control the bacterial growth acetic acid was used, which proved efficient. Throughout the experimentation acetic acid was tested and result showed that bacterial growth is not possible in presence of acetic acid. The survival ability of the consortium was also tested throughout 7 days. All the bacterial isolates in consortium survived, which represent their potential in bioremediation. Contrary to this these bacterial isolates did not survived in presence of acetic acid (Table 1).

Table 1. Colony Formation Ability (CFA) of indigenous bacterial isolates

Bacterial isolates	CFA of bacterial isolates after 24h incubation (Days)						
	1	2	3	4	5	6	7
<i>Bacillus mycoides</i>	+	+	+	+	+	+	+
<i>Bacillus cereus</i>	+	+	+	+	+	+	+
<i>Bacillus subtilis</i>	+	+	+	+	+	+	+
<i>Bacillus</i> sp.	+	+	+	+	+	+	+
<i>Pseudomonas</i> sp.	+	+	+	+	+	+	+
<i>Micrococcus</i> sp.	+	+	+	+	+	+	+
CFA of bacterial isolates after 24h incubation after the addition of acetic acid							
<i>Bacillus mycoides</i>	-	-	-	-	-	-	-
<i>Bacillus cereus</i>	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-
<i>Bacillus</i> sp.	-	-	-	-	-	-	-
<i>Pseudomonas</i> sp.	-	-	-	-	-	-	-
<i>Micrococcus</i> sp.	-	-	-	-	-	-	-

+Bacterial colony appeared; - Bacterial colony not appeared

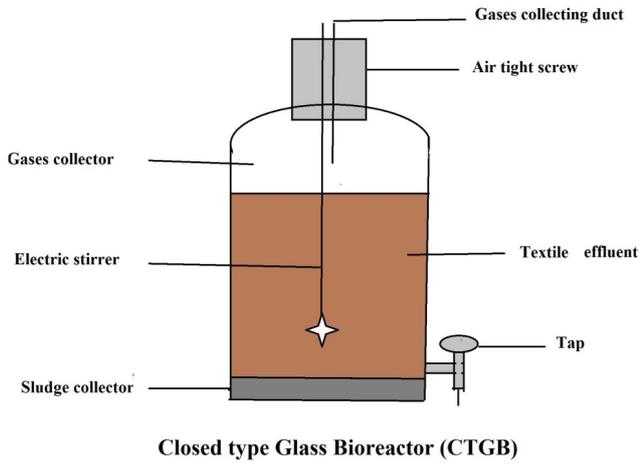
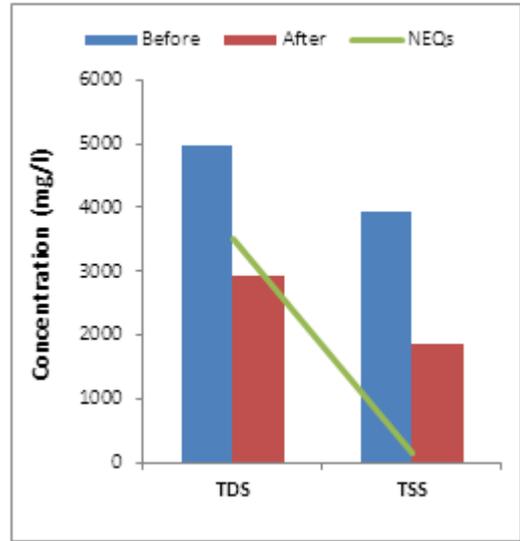
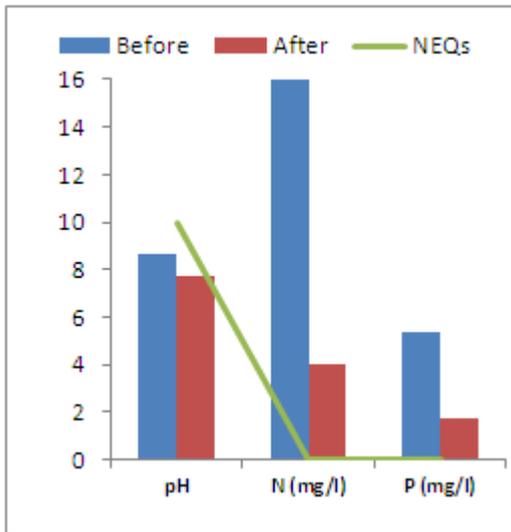


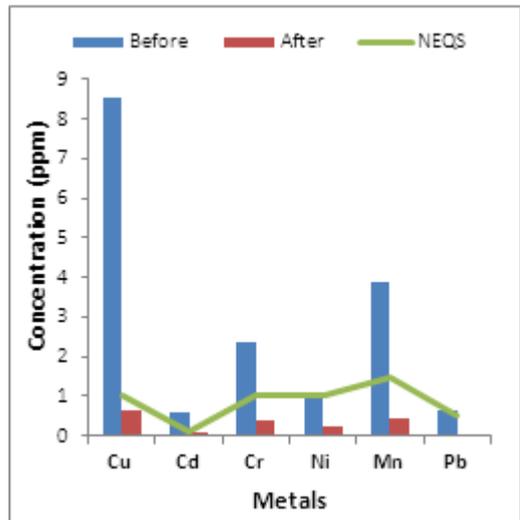
Figure 1. Lab scale bioreactor for the biotreatment of textile effluents by bacterial consortium BMP1/SDSC/01



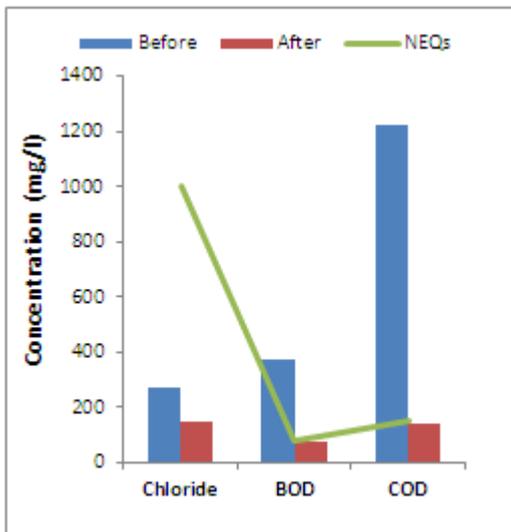
c



a



d



b

Figure 2 a-d. Characteristics of textile effluent before and after Bioremediation and comparison with NEQs

3.2. Treatment Efficiency of Bioreactor

The designed bioreactor successfully treated 16L textile effluent in 7 days (Figure 1).The effluents were brownish black in color, pungent smell, high temperature (35.6 °C) and alkaline pH (8.66).Electric conductivity of effluents was 5.78ms/cm, indicating the high amount of dissolve salts. TSS (3920 mg/l) and TDS (4960 mg/l) were quite high. Nitrogen, phosphorus, and chloride of the textile effluents were 16mg/l, 5.4mg/l and 273mg/l respectively. A high load of COD (1219mg/l) and BOD (370mg/l) was also observed. The analysis for heavy metals showed high amount as compared to National Environmental Quality Standards [19]. The average amount (ppm) of metal like Cu, Cd, Cr, Ni, Mn and Pb was 8.54, 0.59, 2.34, 1.12, 3.87 and 0.62, respectively (Figure 2).

After treatment effluent EC was brought to 3.18 (ms/cm), similarly pH was reduced up to 7.72. The values of nitrogen, phosphorous and chloride were 4, 1.74 and 149 (mg/l), respectively. The designed bioreactor tremendously reduced BOD (from 273 to 149mg/l) and COD (1219 to 143mg/l). Similarly, TDS and TSS were reduced to 2930 and 1859, respectively. This bioreactor also proved efficient for the removal of heavy metals. Heavy metals like, Cu, Cd, Cr, Ni, Mn and Pb were reduced to a limit of 0.65, 0.06, 0.38, 0.21, 0.45 and 0.04, respectively. Surprisingly, all the values of treated effluent were under NEQS. This proved the efficiency and usefulness of the current bioreactor in textile effluent treatment (Figure 2).

3.3. Phytotoxicity Test of Textile Effluent and Bio-remediated Textile Effluent on Maize and Sorghum

Phytotoxicity test of treated effluent was conducted on maize and sorghum. In order to conclude, plants were given untreated, treated effluent and tap water (as control). The results indicated that plant show better growth at treated effluent rather than untreated effluent. Both maize and sorghum show better plant height with treated effluent. In untreated effluents maize biomass, photosynthesis and transpiration was 45.38, 9.11 and 0.41, respectively. Under irrigation of treated effluents, biomass, photosynthesis and transpiration were 44.07, 16.95 and 0.84 respectively. These values depict that untreated effluent is more toxic as compared to the treated effluent. Similar trend was observed in sorghum, its plant height increased to 115.8 instead of 85 (untreated). Simultaneously, number of leaves, biomass, photosynthesis and transpiration were 8.62, 44.45, 13.29 and 0.51, respectively (Table 2). These all parameters were high in treated effluent than untreated effluent. These results are proving that the present study is successful in reducing the toxicity level of textile effluent. And the treated effluent can be safely used for irrigation purposes. This will solve two main problems of Pakistan, firstly it will reduce the water pollution and secondly it will facilitate in overcoming irrigation water shortage.

4. Discussion

Different parameters of the textile effluents were studied both before and after the treatment in the bioreactor. A significant reduction of 52.98% in EC of the effluent was observed, this was may be due to utilization of different salts by the bacterial consortium [17]. In the treated effluents 11.85% reduction in pH was observed which was might be due to bio-remediating action of bacterial consortium on different pollutants in the effluent [17,1]. In the treated effluent concentration of Nitrogen and Phosphorous was 79.2% and 68.78% less than the untreated effluents, respectively. The change in the concentration of nitrogen and phosphorous was due to the utilization of the compounds as nitrogen and phosphorous sources by the bacterial isolates present in the consortium [27,21]. BOD and COD value of treated effluent was 50% and 61%, respectively less than the untreated effluents. After the treatment values of the both parameters became within the NEQS limits. This reduction in BOD and COD was due to degradation of textile dyes and other pollutants present in the untreated effluent [31]. High value of chloride was measured in untreated effluents while 46.42% reduction was observed in treated effluents. Reduction in chloride concentration in textile effluents by bacterial treatment was also observed by [4]. It was noted that values of TSS and TDS in the untreated effluent were higher than the permissible limits (NEQS) while after treatment 45% and 53%, reduction was observed, respectively. This decrease in both parameters was mainly due to utilization of compounds by the bacteria present in the consortium [4].

The consortium BMP1/SDSC-01 was also able to reduce the Cu, Cd, Cr, Ni, Mn and Pb up to permissible limits (Figure 2). Metal containing textile dyes used in dyeing process are responsible for high metal concentration in textile effluent, which causes a lot of problems associated with human and ecosystem health [8]. A number of mechanisms exist for heavy metal removal from aquatic environment by bacteria, fungi, algae, macrophytes and higher plants. Biosorption, active cell transport and enzymatic binding are the processes mainly used by bacteria to remove heavy metals from the effluents [3,5]. The consortium efficiency might be depended on different metals binding sites present on the bacterial cells. The consortium BMP1/SDSC-01 represented a marked increase in bioremediation of Cu, Cd, Cr, Ni, Mn and Pb.

Table 2. Phytotoxicity test of textile effluents and Bio-remediated textile Effluents on maize and sorghum

Parameters	Maize				Sorghum			
	Control	Effluents	Bio-remediated Effluents	LSD	Control	Effluents	Bio-remediated Effluents	LSD
Plant Height	131.4±0.67a	93.26±1.78c	124.4±2.00b	3.07	127.3±0.94a	85.12±1.58c	115.3±0.89b	6.23
Number of Leaves per Plant	11.49±0.72a	7.62±0.17b	9.41±0.81ab	3.42	8.90±0.13a	8.12±0.16b	8.62±0.16ab	0.76
Biomass (Dry weight)	59.40±2.28a	45.38±0.34b	44.07±1.45b	8.05	52.86±0.77a	43.66±0.62b	44.45±1.2b	2.60
Photosynthesis	19.52±0.82a	9.11±0.15b	16.95±0.35c	4.01	15.25±0.38a	8.73±0.12b	13.29±0.51c	3.87
Transpiration	0.96±0.01a	0.41±0.01b	0.84±0.02c	0.12	0.56±0.02a	0.32±0.01b	0.51±0.03c	0.09

Mean followed by different letters in the same row differ significantly at p=0.05 according to the Duncan's Multiple Range Test, ± standard deviation, LSD: least significance difference

The untreated textile effluents imposed high load COD and complex coloration due to the presence of textile dyes, which ultimately diminish the quality of water bodies used for irrigation. This practice reduces the soil fertility and crop yield [14]. The biodegradation of effluents by isolated bacteria may lead to generate a variety of products [23]. Therefore it is virtually important to study the toxicity impact of these degradation products during the life stages of crops so that to overcome yield reduction. [22, 11, 26] studied the effect of detoxified textile dyes and effluent on common crops only at germination stages. Table 2 represents the Phytotoxicity impact of textile effluents and biotreated effluents by consortium BMP1/SDSC-01 on plant height, number of leaves, photosynthesis and transpiration rate and biomass of *Zea mays* L. CV C1415 (Maize) and *Sorghum vulgare* Pers. CV SSG5000 (Sorghum), common crops of Punjab, Pakistan. The result clearly indicated that biotreated textile effluents are less toxic as compared to untreated effluents which are in agreement with results reported [28]. The results advocate that using consortium BMP1/SDSC-01 (having six indigenous bacterial isolates: *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus* sp., *Micrococcus* sp. and *Pseudomonas* sp.) to treat textile effluents in glass bioreactor is safe and feasible in practical applications. The study revealed that reuse potential of effluent is suitable for irrigation as current source are depleting day by day, but it eventually required regular monitoring [2]. Water scarcity has a great impact on human life as it becomes one of the most pressing problems. The global challenges to meet future demand are constrained by sustainable freshwater availability [12]. Therefore new and novel methods are required for efficient and eco-friendly treatment of existing polluted resources of water [18]. Promising results obtained from designed bioreactor upscale the reuse of textile effluents after biotreatment. On the basis of results it is concluded that bioreactor successfully reduced the toxicity level of textile effluents and can be used for irrigation purposes. It will help to produce irrigation water from textile effluents at large scale in Pakistan by applying bioremediation.

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