Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2017.21701

99 (2017) 185–195
December

Assessment of an integrated tire-derived rubber media-fixed biofilm reactor (TDR-FBR) and sand column filter (SCF) for wastewater treatment at low temperature

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Received 21 April 2017; Accepted 19 November 2017

ABSTRACT

Due to the shortage of fresh water and extensive discharge of wastewater into aquatic environment, the present research work aimed to evaluate the wastewater treatment efficiency of an integrated tire-derived rubber media-fixed biofilm reactor (TDR-FBR) and sand column filter (SCF) at low temperature (5°C–15°C). Wastewater was continuously re-circulated in TDR-FBR for 48 h at a flow rate of 80 ± 2 mL/min for 120 d. A substantial reduction in the average concentration of different pollution indicators, such as biochemical oxygen demand (BOD_5, 92.7%), chemical oxygen demand (COD; 93.8%), total dissolved solids (TDS, 77.2%), phosphates (PO_4^{3-}, 42.2%), sulfates (SO_4^{2-}, 74.9%), nitrates (NO_3^-, 39.7%), nitrites (NO_2^-, 31.7%) and fecal coliform (80.9%) was achieved during 48 h of operational period through TDR-FBR. Subsequently, TDR-FBR effluent was further passed through SCF system and more than 95% of BOD_5 and COD removal was observed. While, 83.6%, 46.6%, 77.8%, 55.9%, 65.8% and 99.4% reductions were noticed in TDS, PO_4^{3-}, SO_4^{2-}, NO_3^-, NO_2^- and pathogenic indicators, respectively. However, increase in the concentrations of dissolved oxygen (DO) from 1.5 to 6.9 mg/L was noticed after the sequential treatment in TDR-FBR and SCF system. Moreover, cytotoxicity analysis of treated effluent on brine shrimp (*Artemia salina*) showed that TDR media has no hazardous effects on aquatic ecosystem. It can be concluded that this hybrid TDR-FBR and SCF system has potential for large-scale applications for small communities in the developing countries.

Keywords: Wastewater treatment; Tire-derived rubber media; Fixed biofilm reactor; Sand filter; Fecal coliform; Cytotoxicity

1. Introduction

A quarter of the world's population or a third of the population in developing countries will experience severe water shortages within the first quarter of the 21st century [1]. Extensive discharge of wastewater due to increase in urbanization, unmanageable water consumption and poor supervision of wastewater treatment tends not only to cause severe health as well as environmental issues but also to affect the quality and quantity of water resources throughout the world [2]. In the developing countries such as Pakistan, the quality of water is continuously deteriorating due to the uninterrupted and unintended input of waste from different sources, that is, industrial, agricultural and household,

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thereby affecting flora and fauna and its divergence, increase in water-borne infections and decrease in agricultural production [3]. Currently, in Pakistan more than 10% of municipal wastewater is treated up to primary level in treatment plants existing in Islamabad and Karachi. Due to the lack of sanitation and appropriate wastewater management, Pakistan has been included among those water stressed countries of the world which are likely to face severe fresh water shortage by 2020 [4]. Therefore, there is a growing demand for the development, installation and operation of cost-effective and efficient wastewater treatment technologies in different cities of Pakistan.

Different types of physical, chemical and biological wastewater treatment technologies and strategies have been designed for the management of water pollution [5]. Comparatively, biological wastewater treatment systems are considered to be more promising in terms of low operational cost and less hazardous nature to aquatic and terrestrial ecology. Depending on the composition of wastewaters, different biological technologies have been developed for their treatment. These include activated sludge, aerobic lagoons, extended aerobic systems, fixed biofilm reactor (FBR), rotating biological contractor and wetland [6]. Trickling biofilters are aerobic fixed biofilm reactors widely used in the wastewater treatment. FBR technology is usually ideal as compared with other biological treatment technologies as it requires low space, low cost of their construction, less maintenance requirements, has the capability to show resistance to toxins and shock loads and in return provides good treatment effects, mean of ecological restorations and simultaneous treatment of different kinds of pollutant [7,8]. In such systems, less washout of the biomass occurs, leading to high contact times and more stable operation compared with reactors with suspended bacteria. Moreover, the efficiency of FBR to treat wastewater is in direct relationship with biomass concentration, which play a vital role in the removal of carbonaceous compounds present in wastewater by mean of anaerobic digestion, aerobic oxidation, absorption and adsorption processes and hence produces an effluent of good quality [9].

As mentioned earlier that filter media is the heart of FBR because it provides a surface for the attachment of biomass [10]. Luo and Lindsey [11] reported that filter media having great definite surface area provide advance intensities of treatment proficiency. Moreover, porosity of filter media widely affects flow rates and mass transfer coefficient, which ultimately affect the performance of FBR [12]. Both natural and synthetic media are nowadays widely used in FBR for the development of biomass, but among synthetic filter media, rubber has been gaining attention due to its stable physical and chemical behaviour in response to wastewater clarification [13]. Moreover, experiments with TDR media have shown successful applications in the management of wastewater [13,14], because TDR is providing high surface area for the development of pollutants degrading microbial biofilms. The use of TDR in an FBR is a good option because of the financial benefit of reusing waste tire materials and its high potential for the higher growth of healthy biofilm [15].

Besides having a lot of advantages, FBR systems also possess several drawbacks such as further treatment of the water will be required for the removal of carbonaceous compounds and pathogens in order to meet discharge standards [6], particularly at low temperatures. For this purpose, sand column filters (SCF) can be used in combination with TDR-FBR systems. This type of system can form simple and robust technology that can be easily applied for final refining of pretreated wastewater to produce very high quality water, free of pathogens and other contaminants before discharge to the terrestrial or aquatic ecosystem [16]. Thus, the present research work is focused on the development of a simple, reliable, efficient and cost-effective remediation technology for the treatment of municipal wastewater in Quaid-i-Azam University, Islamabad, (Pakistan) as a representative situation for many developing countries. Presently, the municipal wastewater treatment performance of locally designed and constructed small-scale TDR-FBR followed by an SCF was investigated at low temperature range of 5°C–15°C for the removal of pollution indicators (BOD_5, COD, ammonia and pathogens). The inventiveness is to scale up this representation system for the treatment of wastewater in the university campus.

2. Materials and methods

2.1. Experimental setup and operation

2.1.1. Selection and evaluation of filter media

Discarded rubber tire (bus radial tire; Michelin, France) was cut into small cubical pieces, with each having a surface area ($6\ L^2$) of 21.66 cm^2. These TDR cubes were used as biofilter media. Energy dispersive X-ray spectroscope (EDS) was used for identification and quantification of elements with atomic mass greater than 12 g/mole in TDR medium (EDS, Jeole JSM-5910). For elemental analysis, a sample of TDR medium was placed in a carbon-based disk and was exposed to a beam (stream) of electrons. X-rays emitted from the TDR material after the bombardment of electrons were used for estimation of elements. Scanning electron microscopy (SEM) was carried out for surface characterization of TDR material under 1,000 magnifications (Jeole JSM-5910) [17].

2.1.2. Development and microbiological characterization of biofilm on TDR media

For the development of biofilm, tire-derived rubber (TDR) cubes were soaked in activated sludge for 2 weeks to reduce the start-up phase of FBR. After the development of slime layer on the stones, they were transferred to the FBR for wastewater treatment as biofilter media. Simultaneously, the biofilm was subjected to bacteriological analysis by pure culturing techniques [18].

2.1.3. Designing and operation of an integrated TDR fixed biofilm reactor and sand column filter

A low rate laboratory scale TDR-FBR was designed and constructed for the treatment of wastewater as described in our previous research [6,19]. It was consisted of a collection tank (35 L), polyvinyl chloride (PVC) pipe (height = 36 inches, outer diameter = 14 inches, inner diameter = 12.4 inches). Steel cage was used to hold filter media (height = 24 inches, diameter = 11 inches) along with hooks (height = 4 inches) used to suspend them in PVC pipe. Underdrain system also assisted as an intermediate clarifier (total height = 8 inches) with an outlet at a height of 3 inches from the bottom was used. A shower rose (diameter = 8 inches) acting as a wastewater distribution system was supported on the top of PVC pipe with a stand (height = 5 inches) and a net distance between the bottom of shower rose and top of filter bed surface was 9 inches. Rubber cubes were used as filter media for the development of biofilm in the reactor. Plastic container (having 25 L water holding capacity), water pump (voltage power 220 V) and a plastic pipe system (length = 125 inches, inner diameter = 2 cm) were used to facilitate the flow of wastewater though the bed of TDR-FBR. Down flow passive aeration through a space between outer court and inner core (steel cage) was utilized to ensure aerobic conditions. With the help of electric pump, 20 L of wastewater was pumped to TDR-FBR from primary clarifier. After passing through the TDR bed it gets collected in the recirculation/intermediate tank. Then, second electric pump was used to recirculate wastewater through the bed of TDR-FBR with hydraulic flow rate of 80 mL/min (retention time = 18 min). The wastewater treatment efficiency of the reactor was estimated after 12, 24, 36 and 48 h of operation. Then, 48 h TDR-FBR treated effluent was passed through SCF for further treatment and polishing. A laboratory scale SCF was designed and constructed, which consisted of a plastic column (height = 39 inches, inner diameter = 3 inches) filled with sand (size = 2 mm, used as filter media). A peristaltic pump (Vera varistaltic pump plus) was used for pumping TDR-FBR treated water into the SCF. The flow speed of the pump was adjusted that provide flow rate of 40 ± 2 mL/min with retention time across the filter bed of 15 min. The whole experiment was conducted for a period of 4 months (November to February) during the winter season (5°C–15°C) and the temperature was continuously monitored during operation. Schematic illustration of the whole process is shown in Fig. 1.

2.2. Wastewater treatment efficiency of the TDR-FBR and SCF

To determine the wastewater treatment efficiency of TDR-FBR and SCF, standard methods were followed for the characterization of influent and effluent to TDR-FBR and SCF during the study [20]. For microbiological characterization of wastewater, samples were collected in sterilized plastic

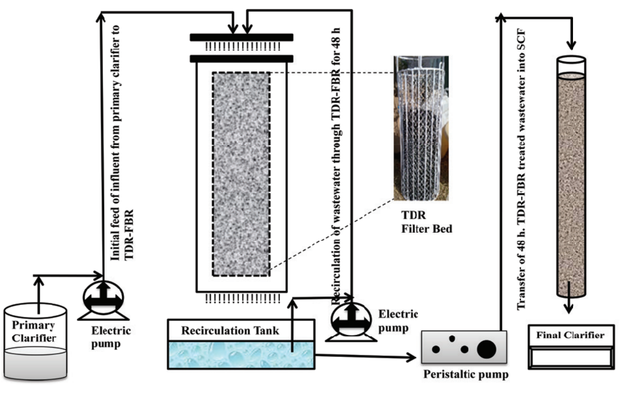

Fig. 1. Schematic illustration of the developed an integrated TDR-FBR and SCF wastewater treatment process.

bottles (250 mL). For physico-chemical analysis, about 1 L wastewater was collected in separate clean plastic bottles. These samples were immediately transferred to the laboratory for analysis of parameters such as dissolved oxygen (DO) and pH. However, the other physico-chemical parameters of water samples were then subsequently determined within 6 h of sampling.

2.2.1. Microbiological analysis of wastewater

Microbiological analysis of wastewater was carried out by a most probable number (MPN) technique [20]. For the investigation and enumeration of fecal coliform such as *Escherichia, Salmonella, Shigella, Klebsiella, Enterobacter* and *Citrobacter* sp., samples of both influent and effluent were incubated at 35°C–37°C for 24–48 h in MacConkey's broth using multiple tube technique having inverted Durham tubes. Positive tubes were sub-cultured on MacConkey's agar, nutrient agar, and mannitol salt agar plates and incubated at 35°C–37°C for 24–48 h. Negative plates were re-streaked before discarding tubes. Positive isolates were confirmed by general microscopy technique.

2.2.2. Physico-chemical analysis of wastewater

Physico-chemical analysis of wastewater was carried out by determining different parameters, that is, pH (D-25 Horiba), electrical conductivity (WTWcind330i) and DO (MM-60R, TOA-DKK) by their respective digital meters. Standard Titration method 2320 B was used to determine alkalinity in water [20]. BOD_5 was estimated by a 5-day BOD test (5210-B standard methods) while COD was determined by kit method (Merck, Germany). Standard methods 1540-C and 2540-D were used to estimate TDS and total suspended solids (TSS) in water samples, respectively. Standard method 4500-P, 0375 Barium chrometery, 4500 NO_3-N and 4500 NO_2-N were used to determine phosphates (PO_4^{3-}), sulfates (SO_4^{2-}), nitrates (NO_3^-) and nitrites (NO_2^-), respectively, in water samples [20].

2.2.3. Cytotoxicity assay

The cytotoxic effect of the treated wastewater by TDR media was detected by brine shrimp assay. For this assay, artificial seawater was prepared by dissolving 34 g commercial sea salt (Harvest Co., HK) in 1 L distilled water with continuous stirring. It was aerated for 2 h by vigorous shaking on magnetic stirrer and then poured into a rectangular dish (22 × 32 cm). Brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany) were hatched in a shallow rectangular dish filled with prepared seawater. A plastic divider of 2 mm containing several holes was clamped in the dish to make two unequal compartments. The eggs (25 mg approximately) were sprinkled in the larger compartment, which was kept in dark by covering with aluminum foil, while the smaller compartment was illuminated. Hatching began within 24 h, after which, phototropic nauplii (brine shrimp larvae) were collected by Pasteur pipette from the lightened side. Dram vials were used for this bioassay. Ten shrimps were transferred to each vial using a Pasteur pipette and volume was raised up to 5 mL with tap water, real wastewater and TDR-FBR treated effluents. The assay was performed in triplicate. The nauplii can be counted microscopically in the stem of a Pasteur pipette against a light background. The vials were maintained under illumination at room temperature 25°C–28°C. Survivors were counted with the aid of 3× magnifying glass after 24 h.

3. Results and discussions

3.1. Mineral detection and quantification in the filter media

The treatment efficiency of attached growth bioreactor depends on physiological activity of biomass found on the surface of carrier media. Many investigators have reported the properties of carrier materials that influence early microbial attachment and its growth, thereby affecting the performance of the reactors [19,21,22]. In the present research study, SEM of TDR media reveals rough, porous and uneven surface topography (Fig. 2(a)), which has increased surface area for colonization of microbes, aided by exogenous polysaccharide layer/glycocalyx which affixes more easily to the surface and provides anchoring points to protect the microbes from shear forces of fluid dynamics. Moreover, the EDS analysis of TDR reveals a cluster of peaks corresponding to constituent elements and their concentrations (Fig. 2(b)). Importance of the technique lies in its value in revealing the elemental composition of support media to determine the compatibility of microbes with it. In the EDS spectrum, the highest peak corresponded to carbon and was followed by the second core peak of sulfur. According to Naz et al. [13], TDR filter media has the potential due to presence of these non-toxic elements, to sustain development of a biofilm for wastewater treatment. While, the presence of less significant peaks in TDR particles used as the biofilter, indicated smaller concentrations of silica, mineral oils, sulfur, zinc oxide, stearic acid, sulfonamide, thiazoles, antioxidants and softeners, as investigated by Park et al. [23]. Moreover, by using X-ray photoelectron spectroscopic analysis, it was made possible to identify exact concentration of the elements, such as carbon (83.5%), oxygen (9.5%), silicon (3.7%), nitrogen (2.96%), sulfur (0.30%) and zinc (0.13%) both in small and large areas of the tested TDR material [13].

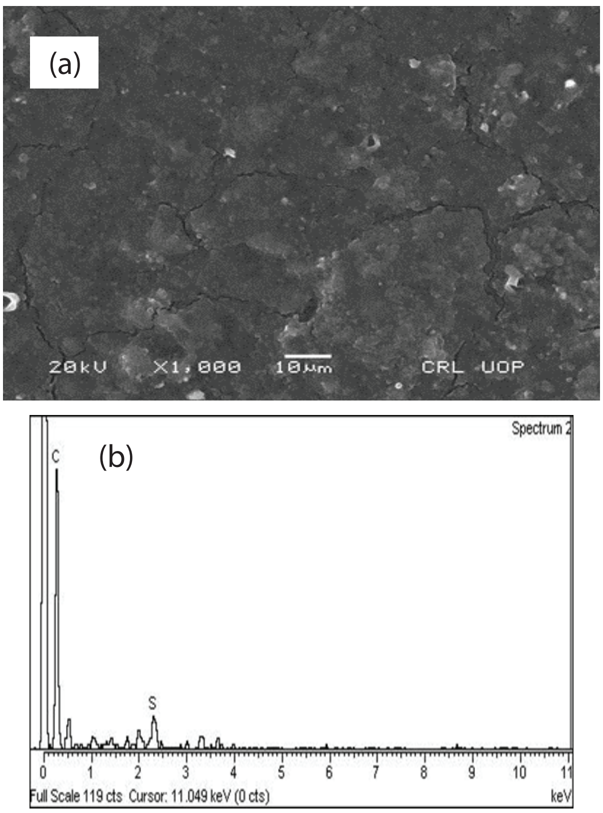

Fig. 2. (a) Scanning electron microscopic (SEM) analysis of tire-derived rubber media indicating rough surface, (b) EDS spectrum of TDR media indicating the cluster of elements.

3.2. Start-up phase of the TDR-FBR system

Biofilm and associated microbial community structure attached to specific filtration medium is an essential element of the FBR for achieving desired treatment efficiencies [24]. Previously, various reports mentioned that about 3–60 d were required to develop biofilm in FBR in order to initiate the wastewater treatment [25,26]. Furthermore, the start-up period of fixed-film wastewater treatment reactors was reduced by inoculating activated sludge from wastewater treatment plants with suspended growth processor attached biofilm reactors [27] or using purchased microorganisms specifically cultured for facilitating wastewater treatment in fixed biofilm treatment technologies [27,28]. Similar strategy was followed in the present study to develop biofilm on TDR filter media, used in the FBR system for the treatment of municipal wastewater. However, the start-up phase was reduced to 36 h at a temperature regime of 5°C–15°C for achieving effective treatment of wastewater as compared with previously reported bioreactors [25,29]. Leverenz et al. [25] observed that the reactors reached stable operation regarding removal of COD and suspended solids in 3 d. Various investigators have mentioned that the properties of the carrier materials significantly influenced the early microbial attachment and growth which thereby affected the performance of the biofilm reactors [15,19,30]. This shortening of start-up time and related effective treatment efficiency might be due to innovative carrier media (TDR) used for biofilm development in the FBR. The TDR media is able to carry physiologically active biofilms from wastewater in a very short duration of 14 d that are able to reduce pollutants in wastewater at different temperature ranges such as 10°C, 20°C and 30°C [19].

3.3. Characterization of biofilm on tire-derived rubber media

In the present study, bacteriological analysis of biofilm developed on TDR media was performed by pure culturing techniques and 11 different bacterial species were isolated from biofilm. Among these, three were gram positive (*Staphylococcus*, *Bacillus* and *Corynebacterium* sp.) and eight were gram negative (*Shigella*, *Salmonella*, *Pseudomonas*, *Micrococcus*, *Klebsiella*, *Enterobacter*, *Escherichia* and *Proteus* sp.) bacterial species, as shown in Table 1. These were mostly pathogenic in nature; however, Van Rijn et al. [30] verified denitrifying phosphate elimination by *Pseudomonas* sp. While, *E. coli* and *Pseudomonas* sp. were also identified to carry out

Table 1
Characterization of bacterial strains isolated from biofilm developed on TDR media after incubation with activated sludge

Strain code	Microscopic characterization				Biochemical characterization													
	Color	Shape	Arrangement	Gram's reaction	Fermentation			H_2S production	NO_3 reduction	Indole production	MR reaction	VP reaction	Citrate use	Urease activity	Catalase activity	TSI reaction	Microbial strains
					Lactose	Dextrose	Sucrose										
1	Pink	Rod	Scattered	–	AG	AG	A±	–	+	+	+	–	–	–	+	A/NC	*Escherichia coli*
2	Purple	Rod	Chain	+	–	A+	A+	–	+	–	–	–	–	–	+	–	*Bacillus subtilis*
3	Pink	Short rods	Scattered	–	–	AG±	A±	+	+	–	+	–	+	–	+	K/A,H_2S	*Salmonella typhimurium*
4	Pink	Rod	Scattered	–	–	A+	–	–	+	–	+	–	+	–	+	K/NC	*Pseudomonas fluorescens*
5	Pink	Rod	Scattered	–	AG	AG	AG	–	+	–	–	+	+	–	+	K/A	*Enterobacter aerogenes*
6	Purple	Cocci	Bunches	+	A	A	A	–	+	–	+	±	–	–	+	A/A	*Staphylococcus aureus*
7	Purple	Rod	Single/short chain	+	–	A	A	–	+	–	–	±	–	–	+	A/NC	*Bacillus cereus*
8	Purple	Cocci	Short chain	+	A	A	A	–	–	–	+	–	–	–	–	–	*Streptococcus lactis*
9	Pink	Cocco-bacillus	Scattered	–	–	–	–	–	–	–	–	–	±	–	+	–	*Alcaligenes faecalis*
10	Purple	Cocci	Diplococcic/tetrads	+	–	–	–	–	±	–	–	–	–	+	+	K/NC	*Micrococcus luteus*
11	Pink	Rod	Scattered	–	–	AG	AG±	+	+	+	+	–	±	+	+	–	*Proteus vulgaris*
12	Pink	Short rods	Scattered	–	–	A	A±	–	+	±	+	–	–	–	+	K/A,H_2S	*Shigella dysenteriae*
13	Pink	Rod	Scattered	–	AG	AG	AG	–	+	–	±	±	+	+	+	–	*Klebsiella pneumonia*
14	Purple	Rod	Phalliside	+	–	A±	A±	–	+	–	–	–	–	–	+	–	*Corynebacterium xerosis*
15	Pink	Rod	Scattered	–	–	–	–	–	+	–	–	–	+	–	+	–	*Pseudomonas aeruginosa*

Note: AG = Acid and gas; + = positive; – = negative; ± = variable reaction; A = acid production; K = alkaline reaction; NC = No change; H_2S = Sulfur reduction; K/A = red/yellow; K/NC = red/no color change; K/A, H_2S = red/yellow with bubble and black precipitate; A/NC = acid/no color change; A/A = yellow/yellow.

nitrification [31]. Besides, *Bacillus subtilis*, *Pseudomonas* and *Klebsiella* sp. were reported to be used as biosensors for BOD measurement in water [32]. Whereas the heterotrophic nitrification and aerobic denitrification capabilities of *Bacillus* sp. were reported under the aerobic condition by Zhao et al. [32]. Moreover, *Pseudomonas fluorescens* was reported to be involved in reducing excess sludge production in biological wastewater processes [33,34].

3.4. Wastewater treatment efficiency of an integrated TDR-FBR and SCF system

3.4.1. Microbiological characterization of influent and effluents

The quality of wastewater is associated with the presence or absence of fecal coliform, considered as indicator bacteria [35], as it indicates the presence of organic contamination. According to WHO guidelines [36], the quantity of fecal coliform (*Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter* and *Citrobacter* sp.) should not exceed 1,000/100 mL in water typically used for agriculture purposes. In the present research study, it was observed that the bacterial residual count (MPN/100 mL) in untreated wastewater was 1,100; however, it declined to 210 with a reduction rate of 80.9% after 48 h of treatment in TDR-FBR. While, further treatment of wastewater in SCF showed that the bacterial count decreased to 120 MPN/100 mL with a reduction rate of 89.1% (Table 2). Leverenz et al. [25] reported similar percentage removal of fecal coliform (80%–85%) by sand filtration. However, TDR-FBR in combination with SCF has generated higher removal efficiency of fecal coliform from wastewater than previously reported aerobic processes [37]. The decrease in number of fecal coliform was might be due to the removal of organic pollutants in biofilms reactors or due to retention in the biofilm by adsorption. It might be associated with detachment and deactivation or death of the microorganisms, [38] and further adsorption in the sand filters [39]. Moreover, Sundaresan and Philip [40] reported that the removal efficiency of pathogenic indicators has also been indirectly linked with parameters such as DO, pH, temperature and removal rates of suspended solids during biological treatment of wastewater.

3.4.2. Physico-chemical characterization of influent and effluents

Different physico-chemical parameters were used in the present research study to characterize the quality of wastewater samples before and after treatment and results were shown in Table 3. Among various physico-chemical parameters, BOD_5 is mostly used to determine organic pollution load and its biodegradation rates in wastewater [41]. In the present study, its level (382.68 mg/L) was high and significantly reduced to 27.8 mg/L with a reduction rate of 92.7% after 48 h of treatment in the TDR-FBR system. Sand filtration further reduced BOD_5 value to 9.4 mg/L and thus an overall removal rate of 97.5% was achieved by an integrated TDR-FBR and

Table 2
Microbiological characterization of influent and effluents using an integrated TDR-FBR and SCF system

Temperature	Wastewater samples	Fecal coliform			% Reduction
		MPN/100 mL	95% Confidence limits		
			Lower	Upper	
5°C–15°C	Untreated water	1,100	150	>4,800	
	TDR-FBR 48 h treated effluent	210	35	470	80.91
	SCF filtered effluent	120	30	380	89.09

Table 3
Efficiency of an integrated TDR-FBR and SCF system with respect to wastewater treatment along with physico-chemical characterization of influent and effluents

Parameters (mg/L)	Influent	Effluent characterization after treatment								Overall treatment efficiency (%) of the integrated system
		TDR-FBR (h)					SCF			
		12	24	36	48	Treatment efficiency (%)	Influent	Effluent	Treatment efficiency (%)	
DO	1.5	2.89	4.05	5.16	6.18	75.7	6.18	6.95	12	78.4
TDS	904	584	262	220	206	77.2	206	148	28.2	83.6
TSS	396	324	268	218	176	55.6	176	119	32.4	69.9
BOD_5	382.7	265.7	142	90.48	27.88	92.7	27.88	9.48	66.1	97.5
COD	376	265	141.4	89.8	23.1	93.8	23.1	8.8	61.9	97.7
PO_4^{-3}	2.04	1.79	1.52	1.34	1.18	42.2	1.18	1.09	7.63	46.6
SO_4^{-2}	82	65.3	55.8	37.8	20.6	74.9	20.6	18.2	11.65	77.8
NO_3^-	0.068	0.093	0.156	0.089	0.041	39.7	0.041	0.03	26.83	55.9
NO_2^-	0.041	0.067	0.144	0.103	0.028	31.7	0.028	0.014	50	65.8

SCF system (Fig. 3). The decrease in BOD_5 is typically related to the high biodegradation rate of organic contaminants by the microbial communities under aerobic condition [42,43]. COD is another important parameter also studied in this research and it was found that the initial concentrations COD in influent was high, that is, 376.3 mg/L. However, its load decreased to 23.1 and 8.8 mg/L with removal rates of 93.9% and 97.7% after 48 h of treatment in TDR-FBR and SCF systems, respectively (Fig. 3). The TDR-FBR proved to be significantly efficient ($P < 0.01$) even at a low temperature regime in acclimatizing microbial community in the form of biofilm on TDR media capable of treating wastewater. It was also reported by Sá and Boaventura [43] that COD/BOD_5 removal was positively correlated with an increase in DO levels. Before treatment operation, the concentration of DO in untreated wastewater was 1.5 mg/L but after sequential treatment in TDR-FBR and SCF system, its value considerably increased to 6.9 mg/L as shown in Fig. 3. In the present research study, the high DO levels in the effluent was due to sufficient aeration, provided by natural draft even without forced ventilation in the TDR-FBR system, recirculation of wastewater through filter bed and configuration of metabolically active biofilms on filter media. The physiologically competent microbes on the filter media are directly correlated with increased DO levels by decreasing carbonaceous pollutants (COD and BOD_5) in the effluent. Our results are in alignment with the research study of Naz et al. [44]. After treatment in TDR-FBR and SCF, the effluent has no adverse effect on terrestrial and aquatic life in the corresponding environment due to sufficient dissolved oxygen concentrations. Moreover, Nacheva et al. [42] also reported that the higher DO value up to 7.1 mg/L of effluent means that this water could carry on the oxygen requirements of the aquatic organisms.

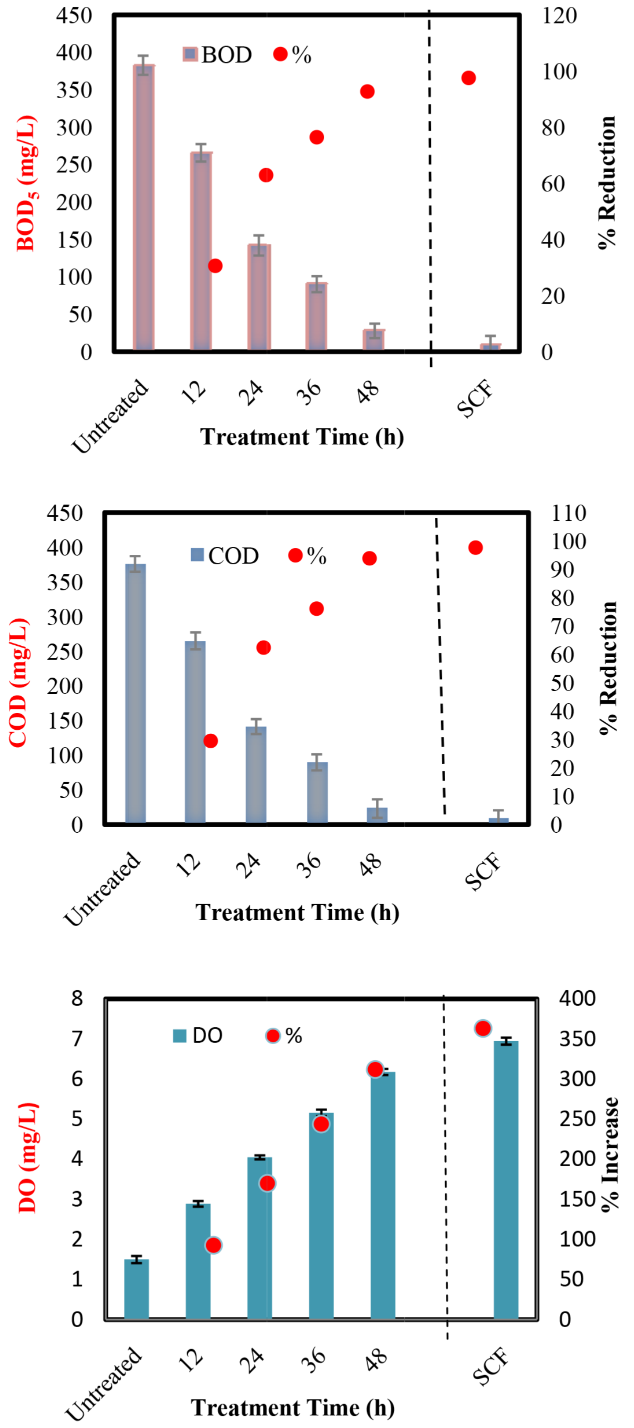

Fig. 3. Variation in the concentrations of carbonaceous pollutants (BOD_5 and COD) and dissolved oxygen (DO) in the wastewater during treatment in an integrated TDR-FBR and SCF system at 5°C–15°C.

The recommended value of TDS in water is less than 1,000 mg/L [37]; however, there is no standard guiding principle by WHO for TSS, but according to EPA [46] the recommended value of TSS lies in the range of 25–80 mg/L. The considerable percentage reduction was observed in TSS and TDS values during treatment. Highest percentage, that is, 55.5% and 70% reduction of TSS was carried out by TDR-FBR and SCF, respectively, while in case of TDS, TDR-FBR and SCF showed highest percentage reduction, that is, up to 77.2% and 83.6%, respectively, at a temperature range of 5°C–15°C (Fig. 4). Removal of the TDS might have been associated with constant recirculation of wastewater through the filter media leading to absorption, adsorption, oxidation and the digestion of carbonaceous compounds by the microorganisms present in biofilm [40,46].

Two different forms of nitrogen, that is, NO_2^- (0.041 ± 0.6 mg/L) and NO_3^- (0.068 ± 0.06 mg/L) were estimated in the wastewater samples before treatment. Fluctuations in their concentrations were noticed during treatment in the TDR-FBR over a period of 48 h. Initially, the concentrations of NO_2^- and NO_3^- dramatically increased to 0.144 and 0.156 mg/L, respectively, in TDR-FBR after 24 h of recirculation and then ultimately showed reduction after 48 h of treatment in the TDR-FBR to 0.028 and 0.041 mg/L, respectively (Fig. 5). Generally, decline in concentration of nitrite and raise in nitrate under aerobic conditions is attributed to the nitrite oxidizing activity of nitrifying bacteria (*Nitrosomonas* and *Nitrobactor* sp.). However, presently, the existence of *E. coli* and *Pseudomonas* sp. in the developing biofilm on TDR media in FBR, might be responsible for this nitrogen removal from wastewater. The fluctuations in concentration of nitrite and nitrate in the sludge by the existence of both kinds of bacteria were also reported by other researchers [48,49,31]. Moreover, decrease in COD and

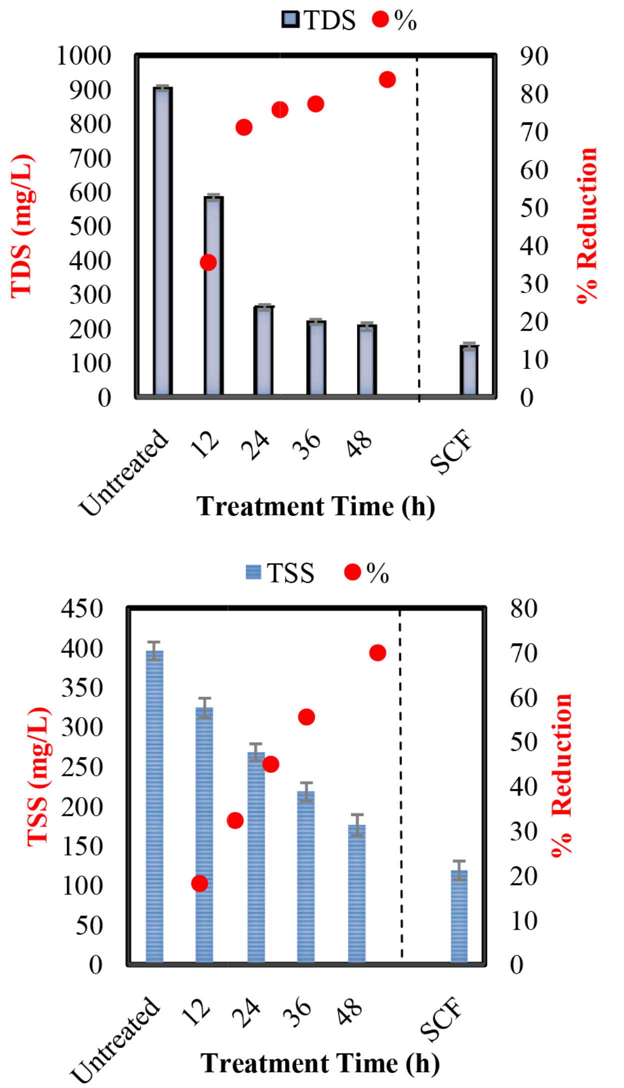

Fig. 4. Variation in the concentration of TSS and TDS during treatment at 5°C–15°C in an integrated TDR-FBR and SCF system.

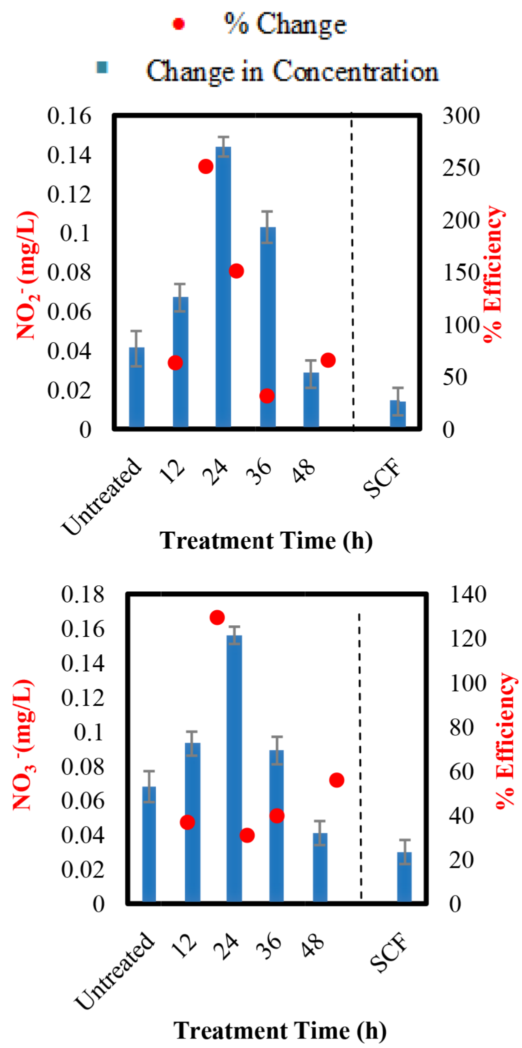

Fig. 5. Variation in the concentration of nitrites (NO_2^-) and nitrates (NO_3^-) in the wastewater during treatment in an integrated TDR-FBR and SCF system at 5°C–15°C.

BOD_5 values and increase in DO during treatment positively influence the growth of nitrifiers and thus triggered the process of nitrification. Helness and Odegaard [50] investigated simultaneous nitrification and phosphate uptake by aerobic biofilms. Further decrease in concentrations of NO_2^- and NO_3^- were noticed after sand filtration. Thus, sequential application of TDR-FBR and SCF has considerably removed about 65.85% NO_2^- and 44.12% NO_3^- from wastewater.

Presence of phosphates in the form of polyphosphates and organically bound forms relates to the input of detergents, chemicals and human excreta in the wastewater stream. It readily transformed into orthophosphates and causes eutrophication in the receiving water bodies. According to USEPA, the concentrations of PO_4^{3-} should not exceed more than 0.05 mg/L [51]. In the present study, the initial concentration of PO_4^{3-} in wastewater was 2.04 mg/L and it significantly decreased to 1.09 mg/L after sequential application of TDR-FBR and SCF system (Fig. 6). The removal of PO_4^{3-} has been associated with intake of microbial community flourishing on filter media for various cellular activities and biomass production. Garcia-Armisen et al. [52] demonstrated efficient removal of COD and phosphorus by a pilot-scale system comprising a vertically moving biofilm reactor followed by a stratified sand filter. In the present study, PO_4^{3-} removal can be ascribed to both denitrification and COD removal. Biological phosphorus removal population comprises at least two groups: one group capable of utilizing either oxygen or nitrate as an electron acceptor (denitrifying polyphosphate accumulating organisms (PAOs)), and the other group capable of utilizing only oxygen (aerobic PAOs). Simultaneous phosphorus and nitrogen removal processes require an aerobic phase of the nitrifying reaction to provide nitrate as an electron acceptor [53].

Sulfates (SO_4^{2-}) in different concentrations have always been an integral part of wastewater. In the present investigation, its initial concentration was 82 mg/L. However, a significant removal of 74.8% was achieved after 48 h treatment of wastewater in the TDR-FBR for 48 h. Further removal of SO_4^{2-} was observed in sand column filter and its final concentration reached to 18.2 mg/L (78% reduction) as shown in Fig. 6.

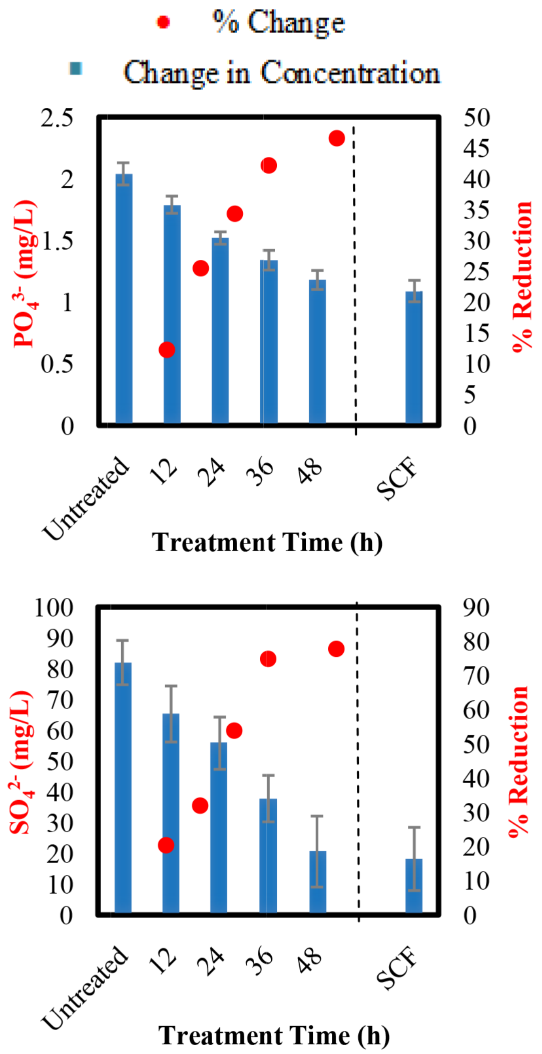

Fig. 6. Variation in the concentration of phosphates (PO_4^{3-}) and sulfates (SO_4^{2-}) in the wastewater during treatment in an integrated TDR-FBR and SCF system at 5°C–15°C.

TDR-FBR in combination with sand filtration showed higher treatment efficiency than Rehman et al. [54], who reported 65.8% sulfates removal from wastewater in a sequencing batch reactor using plastic as filter media at a temperature range of 5°C–15°C. Higher removal efficiency SO_4^{2-} might be related to continuous recirculation and higher treatment time of wastewater in the reactor. This provides a favorable environment for reducing the activity of bacteria because SO_4^{2-} becomes oxidized in the presence of oxygen. Normally the sulfate oxidizing bacteria (SOB) oxidize the sulfur to sulfates and then sulfate reducing bacteria reduce these sulfates into sulfide (H_2S) which then evaporates into the atmosphere. SOB are widely detected in domestic wastewater and play an important role in carbon, sulfur and nitrogen cycles [55].

3.5. Cytotoxicity assay of treated wastewater

In the present research, the effect of treated wastewater samples by using TDR as filter media in FBR system was

Table 4
Cytotoxicity assay of TDR-FBR treated water samples

Samples	Cytotoxicity test using brine shrimp			
	No. of brine shrimp	Dead	Alive	% of Larvae alive
Tap water	10	0	10	100
Untreated wastewater	10	9	1	10
TDR-FBR treated effluent	10	1	9	90

detected on brine shrimp. It was observed that all the larvae remain alive with tap water (100%) while 90% died and only 10% remained alive with untreated wastewater. It was due to the fact that tap water was free of toxic pollutants, whereas wastewater contains numerous carbonaceous, nitrogenous contaminants and low dissolved oxygen concentrations. All these factors exerted a negative effect on the shrimp. On the other hand, it was observed that TDR-FBR treated effluent has resulted in only 10% death of shrimp (90% remain alive), as shown in Table 4. It was obvious from these results that during treatment in FBR, wastewater becomes free of all types of contaminants to some extent. The differences in the effects of treated effluents on shrimp reflected to the biofilms activity, developed on TDR media of FBR system. It was also concluded from these results, that wastewater can be treated by using TDR media in FBR, it will produce effluent with less hazardous effects on the terrestrial and aquatic ecosystems.

4. Conclusions

The present research work proved that an integrated approach of wastewater treatment using TDR-FBR and SCF is quite effective even at a low temperature regime (5°C–15°C). Typically, the effectiveness of the reactors reveals through elimination of pollution indicators such as BOD_5/COD, TSS/TDS and microbial count. The reduction was also observed in nutrients such as SO_4^{2-}, PO_4^{3-}, NO_2^- and NO_3^- during treatment, giving indication of the presence of sulfate removing, phosphate accumulating, nitrifying and denitrifying microorganisms in the biofilm on TDR media. The overall performance of the reactor suggests a cost-effective, robust and environment friendly wastewater treatment solution for small communities in the developing countries. Thus, the present research work proved that this laboratory scale TDR-FBR and SCF has the potential to become the foundation for pilot scale wastewater treatment plant.

Acknowledgment

Authors sincerely acknowledge the Higher Education Commission of Pakistan (HEC, Pakistan) for providing financial assistance for this research work.

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