Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.903521

54 (2015) 2638–2647
May

Taylor & Francis
Taylor & Francis Group

Performance efficiency of an integrated stone media fixed biofilm reactor and sand filter for sewage treatment

Zia Ullah Khan[a,b], Iffat Naz[a], Abdul Rehman[a], Muhammad Rafiq[a], Naeem Ali[a], Safia Ahmed[a,*]

[a]*Microbiology Research Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan, Tel. +92 051 9064 30; email: safiamrl@yahoo.com*
[b]*Department of Food Science and Nutrition, College of Biosystem Engineering and Food Science, Zhejiang University, Hangzhou 310058, P.R. China*

Received 3 August 2013; Accepted 1 March 2014

ABSTRACT

In this study, the efficiency of stone media fixed biofilm reactor (FBR) and sand column filter (SCF) was checked for domestic sewage treatment of university area. Sewage was continuously recirculated through FBR during different time intervals of 12, 24, 36, and 48 h followed by a SCF. There was reduction in odor, alkalinity, pH, turbidity, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total dissolved solids, total suspended solids, electrical conductivity, phosphates (PO_4), sulfates SO_4^{2-}, nitrate NO_3^-, nitrite NO_2^-, and bacterial count, while dissolved oxygen concentration significantly increased after FBR and SFC treatments. Results revealed that the efficiency of FBR was improved by increasing the treatment time. The removal of BOD_5, COD, and turbidity (89.67, 89.62, 99.84%), respectively, was achieved in FBR treatment. While 97.12, 97.15, and 100% reduction in BOD_5, COD, and turbidity was attained in SCF treatment. Moreover, over 80% removal of coli forms and *Enterococcus faecalis* was maintained after FBR and SCF treatments. Our results suggested that combined application of FBR and SCF may serve as a promising approach for the treatment of sewage and has potential to be scaled up for large-scale application.

Keywords: Fixed-biofilm reactor; Sand-column filter; Sewage treatment; BOD_5; COD; Coliforms

1. Introduction

Sewage treatment is a phenomenon of removing contaminants from domestic wastewater for generating treated waste stream and sludge to be discharged in the environment or able to reuse for agricultural activity [1]. In Pakistan, flow of untreated sewage into fresh water bodies or land (2,000 million gallons of sewage/d) has badly affected the quantity and quality of fresh water resources [2]. It was estimated that 26% of the total domestic vegetable production in Pakistan is cultivated with sewage, which is directly consumed by people [3]. Approximately, 35–40% of deaths were also reported due to water borne diseases [4]. Presently, proper management of wastewater is required just like cost-effective indigenous remediation technologies to save the fresh water reservoirs.

*Corresponding author.

Biological processes are considered very effective for wastewater treatment due to their low-cost, easy operational and environmental friendly compared to physical and chemical treatments [5]. The wetland, activated sludge, anaerobic digestion, and fixed biofilm bioreactor (FBR) are biological operational systems for wastewater treatment, whereas the activated sludge process and microbial suspension are used for the removal of nitrogen and phosphorus [6]. Among different biological treatment technologies, FBR is quite effective in developing areas of the world due to their low operational energy, small size, maintenance, simplicity, resistance to toxins, and shock loads. Moreover, due to its simple and reliable processing, it has the ability to produce effluents of high quality [7].

Generally, a bioreactor may refer to any engineered system that can maintain a biologically active environment for long time. In such system, biochemical process (aerobic and anaerobic) is carried out by microbes to degrade the pollutants [8,9]. In suspended growth systems, such as activated sludge and fluidized beds, contaminated water (used water) is circulated in an aeration basin and microbial population aerobically degrades organic matter and produces CO_2, H_2O, and new cells. The cells form a sludge, which is settled out in a clarifier, is either recycled to the aeration basin, or disposed and in this way to help clean wastewater [10]. In bioreactor, biofilm developed on an inert support matrix aerobically degraded contaminants, and showed high efficiency for degradation of pollutants in wastewater compared to reactors [11].

A simple and well-established attached growth bioreactor is used for municipal wastewater treatment [12]. It mainly consists of three parts i.e. distribution system for monitoring hydraulic load rates, filter media for the development of biofilm, and under drain system for collecting treated wastewater and solids (sludge) from filter. Filter media is the main component of FBR which provides surface area of the biofilm [13]. Aerobic and anaerobic microbes contained in the system remove the pollutants by degradation. The aerobic condition is maintained by supplying air to the filter media bed [14]. Slow sand filters are used as secondary treatment process for the removal of pathogens and solids from the wastewater. It is a simple and robust water treatment technology especially used to supply safe drinking water but now it has been adopted as a final polishing step in wastewater treatment [15].

Previously, it has been reported that two streams, (1) Quaid-i-Azam University (QAU), Islamabad, Pakistan and (2) Nur-pur-Shahan carry sewage into Rawal Lake [16], caused severe pollution due to higher load of microbes [17] as well as heavy metals [18]. Rawal Lake water reservoir of Islamabad also benefited Rawalpindi which is the third largest city of Pakistan with up to two million population [19]. In order to protect Rawal Lake water reservoir from polluted discharge, in this study we focused to treat the QAU, Islamabad by locally designed bench-scale stone media FBR and sand column filter (SCF) at mesophilic temperature range i.e. 25–35˚C. Moreover, earlier studies showed that much work has done on FBR and sand filter, but this study is different from the previous work, which investigated the integrated FBR and sand filter system for the wastewater treatment. This combined treatment provides an efficient tool for the wastewater treatment, and can be a pilot-scale facility, to improve the public and environmental health by removing the pollutants and pathogens from wastewater. This treated sewage can be used for agriculture purposes without any hesitation in university campus.

2. Materials and methods

2.1. Experimental setup and operation

Low rate FBR was designed and used for the treatment of QAU sewage. Stones/pebbles of fresh water stream without pores on surface area were collected, having 1.5 inches diameter (surface area = 47.72 cm^2) and used as a filter media for microbial biofilm development. Water pump was used to recirculate 20 L of wastewater in FBR (hydraulic flow rate = 80 mL/min and retention time = 18 min). The flow rate was controlled by electric dimmer connected to the water pump. Passive aeration was provided between outer and inner core of the reactor. The SCF was made up of plastic column (height = 39 inches and inner diameter = 3 inches) filled with sand (0.2 mm). A peristaltic pump was used for pumping 48 h FBR treated sewage from intermediate tank into the SCF with flow rate (43 mL/min), while the retention time across the filter bed was 15 min.

2.2. Development and characterization of biofilm on stones

Stones were incubated in tub for two weeks in activated sludge collected from wastewater treatment plant, Islamabad in order to develop active biofilm for sewage treatment. Total surface area of stone media (approximately 750 stones) available for the attachment of microbes was approximately 35,790 cm^2. After 14 d of incubation, biofilm was observed on stones, which is subjected to bacteriological analysis by pore plate technique, microscopy, cultural characteristics, and biochemical tests [20].

2.3. Sampling of sewage

Sewage samples were collected in sterilized glass bottles (250 mL) and subjected to microbial analysis within 24 h. For physico-chemical analysis, about 1 L sewage water sample was collected in separate clean plastic bottles. Samples were immediately transferred to the laboratory for dissolved oxygen (DO) and pH analysis. Samples were preserved at 4˚C for further analysis. However, other physico-chemical parameters were measured within 6 h.

2.4. Treatment of sewage

Sedimentation was carried out by keeping sewage water for 2–3 h in tank, and the suspended solids and large particulate matters present were removed manually. Then 20 L of wastewater was recirculated through FBR (hydraulic flow rate = 80 mL/min, retention time = 18 min). The flow rate was controlled by electric dimmer connected with pump and subsamples collected after 12, 24, 36, and 48 h. After 48 h treatment through FBR, water was passed through SCF by peristaltic pump and flow rate was adjusted 43 mL/min, while the retention time across was 15 min.

This experiment was performed at lab scale from March to June (2011), where the temperature was continuously monitored during whole the treatment process, ranged from 25 to 35˚C. Schematic diagram of the whole treatment is presented in Fig. 1.

2.5. Physico-chemical analysis

Physico-chemical analysis of wastewater was carried out by determining different parameters, i.e. pH measured by a digital pH meter (D-25 Horiba) and turbidity with nephelometric turbidity units (NTU) by using water analyzer 2000 N (Nippon Denshoku). The electrical conductivity (EC) was determined by a conductivity meter (WTWcind330i in μS/cm), DO by a digital DO meter. The biochemical oxygen demand (BOD_5) was measured by the 5-day BOD test, i.e. 5210B standard method and chemical oxygen demand (COD) by kit (high range 14541 and low range 14560 CSB/COD kits) (Merck Co.). Total dissolved solids (TDS) and total suspended solids (TSS) were measured by standard methods 1540C and 2540D, respectively, while the orthophosphate (PO_4) contents in water were measured by 4500-P (standard method). The sulfate (SO_4) content in water was measure by environmental protection agency (EPA) 0375 Barium chrometery, EPA 4500 NO_3-N used for the determination of nitrate (NO_3) and EPA 4500 NO_2-N for nitrite (NO_2) (APHA, 2005). All parameters were measured in triplicates, the treated sewage samples were compared by T-test with zero time value and $p < 0.05$ was considered as the minimum value for statistical significance by using Microsoft Excel Program (2007).

The sewage treatment efficiency (considering concentrations of each physico-chemical parameter) after

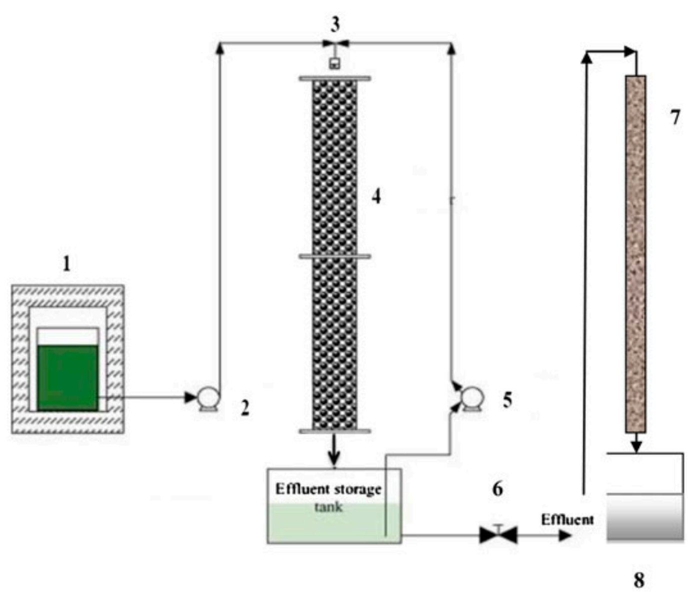

Fig. 1. Schematic diagram of treatment process: (1) sedimentation tank for collection of untreated wastewater, (2) feed pump, (3) shower rose as a wastewater distribution system, (4) FBR filled with stone media, (5) feed pump for recirculation of sewage through the bed of FBR, (6) peristaltic pump for pumping 48 h FBR treated sewage into SCF, (7) SCF, and (8) final clarifier.

12, 24, 36, and 48 h in the FBR and sand filtration was determined by using formula.

$$\text{Treatment efficiency}\,(\%) = 100 \times \left[(\text{concentration of pollutant})_i - (\text{concentration of pollutant})_e\right]/(\text{concentration of pollutant})_i$$

where the subscripts "i" and "e" denoted influent and effluent, respectively.

2.6. Microbiological analysis

Microbiological investigation of sewage was carried out by most probable number technique (MPN/100 mL index of pathogenic indicators i.e. fecal coliforms and *Enterococcus faecalis*) and average bacterial count (CFU/mL) according to Bergey's Manual of Determinative Bacteriology [20]. For determining the MPN index of fecal coliforms (*Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter* and *Citrobacter* sp.), the sample of influent (untreated) and effluent (treated) sewage (48 h treated in FBR) was incubated in MacConkey's broth (MacB) using multiple tube technique with inverted Durham tubes. Positive tubes were sub-cultured on MacConkey's agar (MacA), nutrient agar (NA), and Mannitol salt agar (MSA) plates. Finally, positive isolates were confirmed by microscopic analysis (Gram's staining) and MPN index was calculated according to standard MPN table. For investigation and enumeration of *E. faecalis*, the untreated and treated sewage samples were incubated in azide dextrose broth at 45˚C using same multiple tube technique. Then positive tubes were subcultured on MacA and NA plates. Finally, positive growth of coagulase-negative, Gram-positive cocci on bile esculin agar and at 45˚C in Brain Heart infusion broth verifies its fecal nature and MPN index was determined according to standard MPN.

The CFU/mL of bacterial colonies in the influent and effluent samples was determined by conventional serial dilution method. These wastewater samples, i.e. 1 mL, were serially diluted up to 10^{-5}. From dilutions of 10^{-1}, 10^{-3}, and 10^{-5}, 0.1 mL of sample was pipette out, spreaded on NA and incubated at 37˚C for 24 h. The colonies appeared on plates were counted with colony counter and the CFU of each colony was then calculated by using formula; CFU/mL = number of colonies x dilution factor/inoculum size.

3. Results and discussion

The physical, chemical, and bacteriological parameters exhibited considerable variations at different treatment times in stone media FBR and SCF. All the measurements were carried out in temperature range (25–35˚C). The average results of the physico-chemical and microbiological parameters for untreated and treated wastewater samples were discussed as follows.

3.1. Microbiological characterization of biofilm

In this study, the bacteriological characterization of biofilm was performed by pure culturing technique and was directly streaked on NA plates, incubated at 37˚C for 24 h. For the isolation of pure cultures, different colonies appeared on NA plates were further sub-cultured on selective media (eosin methylene blue (EMB), MaCA, *Salmonella Shigella* agar (SSA), Pseudomonas cetrimide agar (PCA), MSA, blood agar (BA), etc.) and incubated at 37˚C for 24 h. On the basis of microscopy (Gram staining and shapes), cultural characteristics (size, form, pigmentation, margins, elevation, and opacity) on different media and biochemical tests (Triple sugar iron test, Indole/H2S motility test, Citrate utilization test, catalase test, urease test, methyl red Voges-Proskauer test (MR-VP), and Oxidase test), different bacterial strains identified in the biofilm were *E. coli*, *B. subtilus*, *S. typhimurium*, *P. fluorescens*, *P. aeruginosa*, *E. aerogenes*, *S. aureus*, *B. cereus*, *S. lactis*, *A. faecalis*, *M. luteus*, *P. vulgaris*, *K. pneumonia*, *C. xerosis*, *Actinomycetes*, *Nitrosomonas*, *Nitrobacter*, and *Thiobacillus* sp. Andersson et al. [21]. The 13 bacterial strains studied in wastewater have the capability to make a biofilm on solid matrix. These microbial communities are mostly involved in degradation of contaminants in sewage, because they rapidly oxidize soluble organic and nitrogenous compounds in wastewater [22,23].

3.2. Sequential treatment of sewage by FBR and SCF

3.2.1. Odor

Odor is one of the most important parameter for the prediction of water quality. The untreated domestic wastewater sample had dark gray color and unpleasant smell. There are many causes of odor including excessive organic substances in heavily polluted water with high level of nutrients. The degradation of organic substances by bacteria produces a wide variety of unpleasant odors. In this study, odor of the water was improved significantly after treatment, due to the removal of contaminants from wastewater [24]. During wastewater recirculation in FBR, more retention time between microbes and wastewater increased the rate of contaminant removal, which has been designated to the increased DO concentration, and facilitated the oxidation of pollutants [25].

Table 1
Characteristics of sewage before treatment and its comparisons with WHO (1993) and USEPA (1986) Guidelines; treatment efficiency (%) of combined FBR and SCF system for sewage treatment

				Treatment efficiency (%)				
				FBR (h)				SCF
Parameters tested	WHO standard	USEPA standard	Mean values of raw sewage	12	24	36	48	
pH	6.5–8.5	6.5–9.5	7.82	1.15	1.41	1.79	1.92	2.81
TDS (mg/L)	1,000	500–1,000	942 ± 2.22	5.73	8.81	16.88	24.63	27.70
EC (μS/cm)	400–1,215	NGV	979.2	8.40	11.87	19.30	26.68	29.43
TSS (mg/L)	NGV	25–80	795 ± 3.00	15.50	29.43	42.76	66.0	100
Turbidity (NTU)	5	10	1125.78	62.50	76.22	97.80	99.84	100
BOD_5 (mg/L)	NGV	5–8	223.87 ± 1.00	48.03	57	72.38	89.67	97.12
COD (mg/L)	10	8–10	327.66 ± 1.52	47.80	56.56	72.23	89.62	97.15
Alkalinity (mg/L)	NGV	NGV	382 ± 3.00	8.12	21.73	34.55	47.12	55.50
Phosphate (mg/L)	NGV	0.05	2.02 ± 0.005	7.92	23.76	31.68	38.12	41.58
Sulfate (mg/L)	250	NGV	0.635 ± 0.001	12.44	39.48	55.60	69.60	79.69
Nitrite (mg/L)	3	0.5	0.053± 0.001	137.7	323.3	175.4	18.86	62.26
Nitrate (mg/L)	50	0.5	0.134 ± 0.001	134.7	291.3	250.0	58.7	71.74

Note: NGV, not given value.

3.2.2. Alkalinity

Alkalinity is the hardness of water, due to HCO_3^- and OH^- [26]. In this study, alkalinity of raw sewage (386 mg/L) gradually reduced with different treatment retention times, i.e. 12, 24, 36, and 48 h in FBR and SCF, as shown in Fig. 2(a). The alkalinity reduced 47.12% after 48 h of FBR treatment (Table 1) to raw sewage water. Reduction in alkalinity is very important because the process of nitrification requires slight alkaline condition for the conversion of ammonia to nitrates and at higher alkaline condition, the microbe loses their ability to convert ammonia into nitrates [27]. Moreover, reduction of alkalinity is due to the denitrification and microbial degradation of bicarbonate in wastewater [26], as in this investigation maximum reduction in alkalinity was obtained after 48 h in FBR and SCF treatments, indicating that more retention time of wastewater with biofilm, and accelerated ammonia and carbonate decomposition.

3.2.3. pH

pH always used to express the intensity of the acidic and alkaline nature of solutions, and any variation to the recommended range (6.5–8.5) may affect living things [28]. Our results revealed that the pH of sewage was reduced from 7.82 to 7.60–7.67 after FBR and SCF treatments, as shown in Fig. 2(a). It has been designated to denitrification in FBR, which probably resulted in a decrease in pH as previously documented by Sakuma et al. [29].

3.2.4. Turbidity

Colloidal and extremely fine dispersions of pollutants cause turbidity in water. Removal of turbidity is very important in wastewater treatment, as it is understood that the pathogenic micro-organisms flourished in turbid water and cause contaminations which results in epidemics [30]. The untreated sample had turbidity of 1125.78 NTU and the permissible limit of turbidity is 10 NTU by USEPA and < 5 NTU as prescribed by WHO [28]. Our results elaborated that FBR treatment of sewage significantly reduced the turbidities, 1125.78, 422.1, 267.6, 24.3, and 1.8 NTU, with different treatment times (12, 24, 36, and 48 h), respectively. Furthermore, SCF treatment completely removed turbidity from sewage water (Fig. 2(b)). This study indicated that recirculation of wastewater in FBR mostly removed organic and inorganic contaminants from wastewater [25]. The presence of large amount of colloidal particles in sewage increases its turbidity in one hand and on the other hand, it may reduce the effect of disinfectant by protecting the pathogenic organisms [31].

3.2.5. Dissolved oxygen concentration

The DO reflects the physical and biological processes prevailing in water. The DO indicates the degree of pollution in water bodies [30]. In this study, untreated sewage water contained 1.63 mg/L DO value, indicated high pollution load. However, it was gradually increased with increasing retention time in

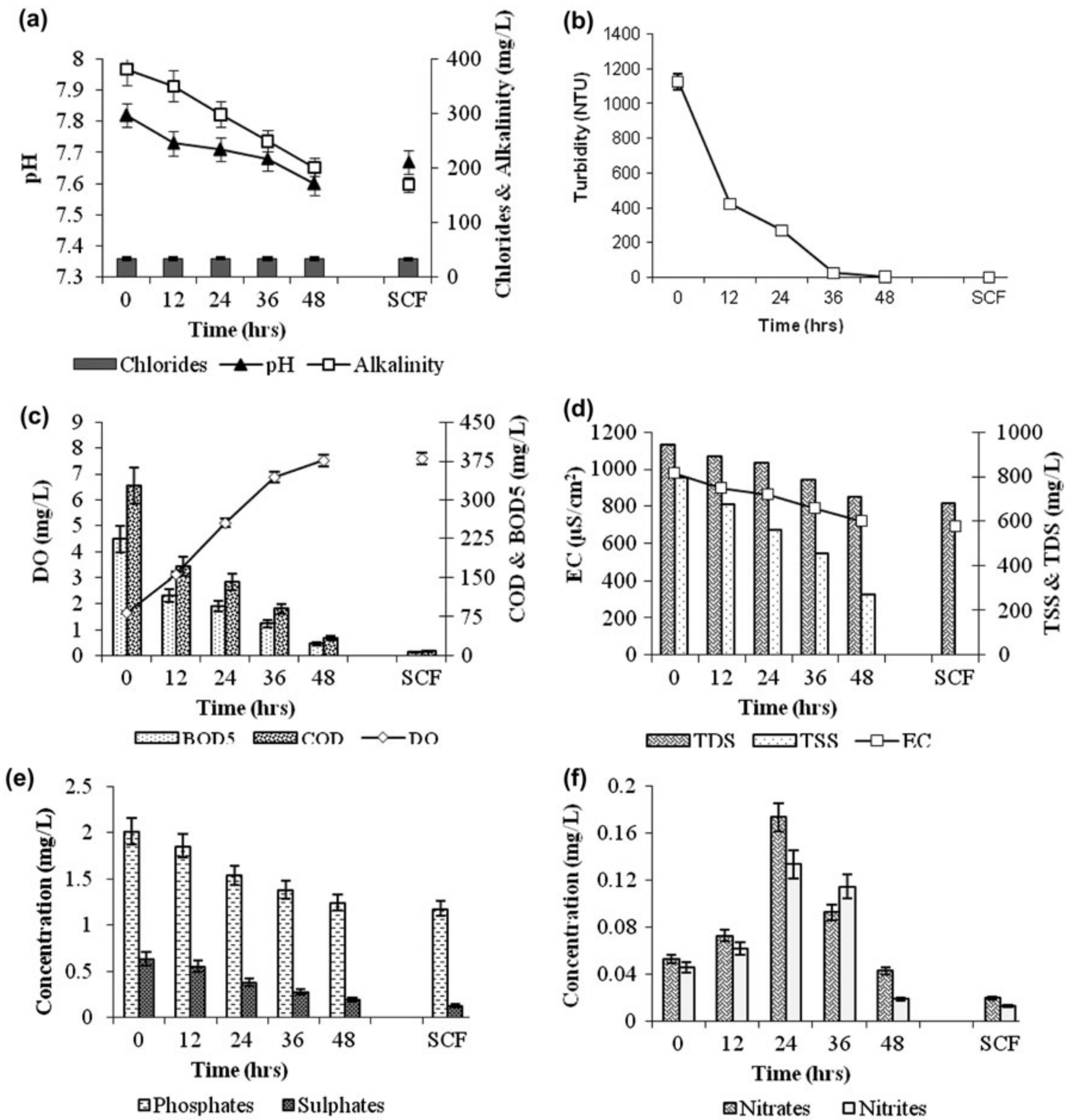

Fig. 2. Changes in the levels of different parameters of sewage tested before and during treatment in the FBR and SCF at 25–35˚C, (a) pH, alkalinity, and chlorides, (b) turbidity, (c) DO, BOD_5, and COD, (d) EC, TSS, and TDS, (e) phosphates and Sulfates, and (f) nitrites and nitrates.

FBR. The DO values significantly increased to 3.13, 5.11, 6.88, and 7.52 mg/L with retention time in FBR treatments, respectively, and reached to 7.59 mg/L after SCF treatment, as shown in Fig. 2(c). The increase in DO might be due to the decrease in COD, BOD_5, TSS, and TDS levels because it is already understood that these parameters have indirect relation with DO, any decrease in them increased the DO value [32]. From this study, it is clear that FBR with SCF have the ability to increase DO. The higher DO values 7.1 ± 0.8 mg/L of wastewater indicates that this water could support the oxygen requirements of the aquatic organisms [33].

3.2.6. Biochemical oxygen demand

BOD_5 is the amount of oxygen required for microbes to degrade organic and inorganic waste in the sewage. The initial value of BOD_5 (223.87 mg/L) was higher while the DO (1.63 mg/L) contained lower concentration due to the huge amount of contaminants. FBR and SCF treatments of sewage water significantly reduced the BOD_5 up to 89.67% and consequently DO values (7.52 mg/L) increased with retention time (48 h), indicating that sufficient amount of oxygen was available for microbes in FBR (stone filter media) to degrade organic contaminants in wastewater, as shown in Fig. 2(c). Thus, the heterotrophic bacteria of biofilm rapidly oxidized the organic matters and converted them into biomass and gas. Similar results of BOD_5 reduction were found in earlier study [34]. The reduction of BOD_5 due to the microbial activity in FBR treatment was also reported by Shipin et al. [35]. Furthermore, the FBR treated water when retreated with SCF, BOD_5 significantly reduced (97.12%) due to wastes adsorption. The overall sequential treatment showed highly significant results for 48 h treatment in FBR ($p < 0.001$) and SCF ($p < 0.001$).

3.2.7. Chemical oxygen demand

COD is an indicator of organic contaminants in water and usually expressed in milligrams per liter (mg/L) [30]. In this work, COD of sewage water (327.66 mg/L) gradually reduced with the increasing treatment time in FBR, 171, 142.33, 91, 34, and 9.33 mg/L after 12, 24, 36, and 48 h, and SCF, respectively (Fig. 2(c)). After 48 h treatment of sewage water, COD value was within range (9.33 mg/L) as mentioned by USEPA (8–10 mg/L). The value of COD decreased up to 89.62% in FBR treatment (Table 1) was a significant ($p < 0.001$) reduction. These results indicated that micro-organisms of biofilm are responsible in the degradation of carbon-containing compounds. Furthermore, results revealed that retention time of sewage water in FBR and SCF plays a significant role in COD reduction, due to microbial degradation of contaminants. Similar results of COD reduction were found in FBR for domestic wastewater by early investigators Sa and Boaventura [36]. The sequential application of FBR and SCF proved to be very effective as to treat wastewater within international limits and can be discharged without any health hazards into the natural streams. The process of nitrification is also stimulated by reduction of COD. Numerous bench-scale studies have shown that nitrification is increasingly suppressed with increased organic carbon loading [37]. As carbon decreased, the concentration of DO increased (Fig. 2(c)), and the possibility of oxygen diffusion to the autotrophic nitrifiers increases, which consequently triggered nitrification (Fig. 2(f)). The decrease in COD values may also due to DO and COD_5, any increase in DO and decrease in BOD_5 affect the COD values, reduction in alkalinity also provides good media for microbial activity and has positive effects in COD reduction.

3.2.8. Total dissolved solids and total suspended solids

In this investigation, TDS and TSS loads before treatment were 942 and 795 mg/L, respectively (Fig. 2(d)). However, 66% reduction was observed after 48 h treatment in FBR, while SCF complicity (100%) reduced the TSS ($p < 0.001$), as shown in Fig. 2(d), and also discussed by Szogi et al. [38]. On the other hand, TDS showed 24.63% reduction after 48 h of treatment in FBR and 27.7% after SCF, as shown in Table 1 (Fig. 2(d)).

3.2.9. Electrical conductivity

The EC before treatment was (979.2 $\mu S/cm^2$) higher than WHO limits (400–1,200 $\mu S/cm^2$). The FBR and SCF treatments decreased 26.86 and 29.43% after 48 h treatments, respectively, as shown in Fig. 2(d) and Table 1. The decrease in EC was designated to nitrate (NO_3^-) conversion into diatomic molecular nitrogen (N_2), which escaped to the atmosphere. There is a logical relationship between the various parameters which characterize the wastewater, such as COD, color, turbidity, conductivity, and suspended solid, which are interlinked to each other [39]. The decline of other parameters also resulted in the reduction of EC. The EC was also found to be associated with the amount of TSS and fluoride; any decrease in their values decreased the EC [31].

3.2.10. Phosphates (PO_4)

Polyphosphates from detergents, animal, and human excreta into sewage, are the main source of phosphates, causes eutrophication in lakes. It is removed from wastewater by the intracellular microbial accumulation for cellular activities and biomass production [38]. No prescribed values are available for orthophosphate in sewage by WHO. But according to US/EPA, it should not exceed 0.05 mg/L if streams discharge into lakes. This study showed around 2.02 mg/L of phosphate in raw sewage (Fig. 2(e)). However, 38.12% removal efficiency was obtained in FBR after 48 h treatment time and 41.58% in SCF (Table 1). It indicates that polyphosphate accumulating bacteria might be present in the biofilm. Jin et al. [40] also reported 80% phosphate removal efficiency of FBR. But in other investigation, combination of anaerobic fixed bed reactor and suspended aerobic activated sludge reactor showed 97% efficiency [41]. Maximum Phosphates and its binary compounds were removed easily at neutral pH, but its higher and lower values are not considered to be suitable in batch system [42]. Zeng et al. [43] Reported that nitrification and denitrifcation process also have positive effects on phosphate removal, our results are also consistent with the reported study.

3.2.11. Sulfates (SO_4^{2-})

Sulfates are present in all types of contaminated wastewater including natural run-off, domestic sewage, and industrial effluent. Salts containing sulfates are more soluble in water and impact hardness [44]. In the present study, the average percentage reduction in sulfate was 12.44, 39.84, 55.60, and 69.60% ($p < 0.001$) after 12, 24, 36, and 48 h, respectively, of treatment in FBR and 79.69% ($p < 0.001$) in SCF (Table 1). It might be due to the increase in DO concentration in treated sewage during treatment, which is required for oxidation of reduced sulfur compounds [44].

3.2.12. Nitrate (NO_3^-) and nitrite (NO_2^-)

Urea and proteinaceous substances in sewage contribute ammonia (NH3), nitrites (NO_2^-), and nitrates (NO_3^-) concentrations. The permissible range of NO_2^- and NO_3^- in drinking water is 50 and 3 mg/L, respectively [28]. In present study, the level of NO_2^- and NO_3^- reduced to 0.013 and 0.02 mg/L compared to its initial values 0.053 and 0.134 mg/L, respectively. Initially, the amount of NO_2^- increased to 323.3% in 24 h followed by reduction (18.86%) after 48 h and then showed further reduction (62.26%) after SCF treatments. Similar trends were also presented by NO_3^- (291.3%) after 24 h of treatment and then reduced to 71.74% after SCF (Fig. 2(f)). The first increase of NO_2^- and NO_3^- in 24 h treated water designated to the breakdown of ammonia to NO_3^- and NO_2^- by nitrifying bacteria and then reduction in their values was due the conversion of nitrite and nitrate to free nitrogen molecule by denitrifying bacteria. The increase of DO and decrease of COD are supportive for nitrifying bacteria to use dissolved carbon dioxide for new biomass production compared to heterotrophs. Nitrification and denitrification play significant role in the removal of nitrogen in wastewater treatment [27]. Theoretically, anaerobic condition within FBR plays an important role in nitrogen removal by gentrification. The pH also play an important role in denitrification at pH (7.0–8.0) [45] and high nitrification and denitrication process also help in phosphate removal from wastewater [43]. In this investigation, decrease in NO_3^- due to their conversion to nitrogen molecule (N2) and decrease in EC due to conversion of ionic form of nitrogen i.e. NO_3^- into the diatomic molecular nitrogen (N_2), as shown in the Fig. 2.

3.3. Microbiological analysis of sewage before and after treatment

The strength of bacterial population was determined in terms of CFU/mL. The average number of bacteria in untreated, 48 h, and SCF treated sewage samples were 5.33×10^{10}, 2.88×10^6, and 1.32×10^3, respectively. There was gradual reduction in its count, which might be due to retention of pathogenic micro-organisms (present in waste influent) on filter media by adsorption and later removed or deactivated by predation or natural die-off process [21]. MPN test for fecal coliforms (*Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *and E. coli*) showed that untreated sewage limit was between 150 and 4,800 probably more than 1,100 while that of 48 h FBR treated were in the range of 120–870/100 mL. On the other hand, MPN results for *E. faecalis* in untreated sewage were in the range 140–4,800 probably more than 1,100. Whereas, 48 h treated sewages were in the range of 55–450 per 100 mL (Table 2). The microbiological analysis of untreated and treated sewage by MPN index and CFU not only determined the percentage reduction of pathogenic bacteria but also

Table 2
MPN index of total fecal coliforms and *E. faecalis* of raw, FBR, and SCF-treated sewage samples at 25–35˚C

Pathogenic indicators	Sewage samples	MPN/100 mL	95% confidence limit	
			Lower	Upper
Fecal coliforms	Untreated wastewater	>1,100	150	>4,800
	48 h treated	210	120	870
	Sand filter	140	60	190
E. faecalis	Untreated	>1,100	150	>4,800
	48 h treated	220	55	450
	Sand filter	130	60	190

showed different types of bacterial strains present in wastewater. It might be due to peak metabolic activity of microbes at the given temperature. Moreover, sewage also contained human excreta and large amount of nutrients which support large microbial populations. Approximately, 80–87% reductions in fecal coliforms and 80–88% reduction in *E. faecalis* were observed after combined application of FBR and SCF. Almost similar reduction in the pathogenic indicators after treatment by fixed film bioreactor was reported by various investigators [46]. The sand bed filter proved to be efficient in reduction of fecal coliforms and approximately, 80–85% reduction in fecal coliforms was reported by Hill et al. [15].

4. Conclusions

This study concluded that FBR and SCF treatment system proved to be efficient for sewage water treatment at decentralized scale. A significant association was found between the percentage removal of contaminants and pathogens with a different treatment time and highest percentage removal was found after 48 h retention time. The quality of treated water was found to be considerably improved in terms of BOD_5, COD, TSS, and pathogen indicators i.e. fecal coliforms and *E. faecalis* after sand filtration. A significant reduction was observed in SO_4, PO_4, NO_2, and NO_3 concentrations during treatment in FBR and SCF, indicating the presence of sulfate-reducing, phosphate-accumulating, nitrifying, and denitrifying microbes in the biofilm on stone media. This study suggests that maximum retention time of sewage water with bioflim of trackling filter reduces the microbial load and chemical impurities from sewage water and it can be scaled up for larger application.

Acknowledgments

The authors extend their gratitude to Pak-EPA, H-9 Islamabad for providing facility to perform physicochemical analysis of water samples. Special gratitude to Higher Education Commission (HEC) of Pakistan and US/AID for providing financial support to carry this research study.

References

[1] K.M. Evans, T.G. Ellis, Fundamentals of the Static Granular Bed Reactor, PhD’s Thesis, Iowa State University, Ames, IA, 2004.

[2] WWF, Pakistan’s Waters at Risk, Water and Related Health Issues in Pakistan and Key Recommendations, 2007. Available 21 September 2012 from: http://www.wwfpak.org/freshwater/pdf/water-report

[3] J.H.J. Ensink, T.W. Mahmood Hoek, L. Raschid-Sally, F.P. Amerasinghe, A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one, Water Policy 6 (2004) 197–206.

[4] A. Mashiatullah, M.Z. Chaudhary, M.S. Khan, T. Javed, R.M. Qureshi, Coliform bacterial pollution in Rawal Lake, Islamabad and its feeding streams/river, The Nucleus 47 (2010) 35–40.

[5] I.M.I. Shalaby, A.D. Altalhy, H.A. Mosallam, Preliminary field study of a model plant for sewage water treatment using gravel bed hydroponics method, World Appl. Sci. J. 4 (2008) 238–243.

[6] K.V. Gernaey, M.C.M. Loosdrecht, M. Henze, M. Lind, S.B. Jorgensen, Activated sludge wastewater treatment plant modelling and simulation: State of the art, Environ. Modell. Softw. 19 (2003) 763–783.

[7] G.A. Lewandowski, L.J. DeFilippi, Biological Treatment of Hazardous Wastes, John Wiley, ISBN no. 978-0-471-04861-9, 1997.

[8] A.C. Yeh, C. Lu, M.R. Lin, Performance of an anaerobic rotating biological contactor: Effects of flow rate and influent organic strength, Water Res. 31 (1997) 1251–1260.

[9] J.C. Akunna, C. Jefferies, Performance of family-size sequencing batch reactor and rotating biological contactor units treating sewage at various operating conditions, Water Sci. Technol. 41 (2000) 97–104.

[10] P. van der Steen, A. Brenner, J. van Buuren, G. Oron, Post-treatment of UASB reactor effluent in an integrated duckweed and stabilization pond system, Water Res. 33 (1999) 615–620.

[11] C. Novotny, K. Svobodova, O. Benada, O. Kofronova, A. Heissenberger, W. Fuchs, Potential of combined fungal and bacterial treatment for color removal in textile wastewater, Bioresour. Technol. 102 (2010) 879–888.

[12] M. Eddy, Wastewater Engineering. Treatment, Disposal, Reuse, 3rd ed., McGraw-Hill Int. Ed., Singapore, 1991.

[13] M. Rodgers, Organic carbon removal using a new biofilm reactor, Water Res. 33 (1999) 1495–1499.

[14] S. Pal, U. Sarkar, D. Dasgupta, Dynamic simulation of secondary treatment processes using trickling filters in a sewage treatment works in Howrah, West Bengal, India, Desalination 253 (2009) 135–140.

[15] V.R. Hill, A. Kantardjieff, M.D. Sobsey, P.W. Westerman, Reduction of enteric microbes in flushed swine wastewater treated by a biological aerated filter and UV irradiation, Water Environ. Res. 74 (2002) 91–99.

[16] M. Ahmad, M.I.A. Khan, Nisar, M.Y. Kaleem, Study of pollution in Rawal Lake, J. Chem. Soc. Pak. 21 (1999) 47–49.

[17] T. Ahmed, R. Kanwal, S.S. Tahir, N. Rauf, Bacteriological analysis of water collected from different dams of Rawalpindi/Islamabad region in Pakistan, Pak. J. Biol. Sci. 7 (2004) 662–666.

[18] R.N. Malik, M. Nadeem, Spatial and temporal characterization of trace elements and nutrients in the Rawal Lake reservoir, Pakistan using multivariate analysis techniques, Environ. Geochem. Health 33(6) (2011) 525–541.

[19] N. Aftab, 5 M gallons of sewage flows into rawal dam every day, 2010 Available 23 August 2011 from: http://www.thenews.com.pk
[20] G.J. Holt, P.H. Sneath, N.R. Krieg, Bergey's Manual of Determinative Bacteriology, 9th ed., Lippincott Williams and Wilkins, Baltimore, MD, 1994.
[21] S. Andersson, G. Kuttuva Rajarao, C.J. Land, G. Dalhammar, Biofilm formation and interactions of bacterial strains found in wastewater treatment systems, FEMS Microbiol. Lett. 283 (2008) 83–90.
[22] R.B. John, W.S. Robert, Aerobic Treatment of Wastewater and Aerobic Treatment Units. National Decentralized Water Resources Capacity Development Project, University of Arkansas, Fayetteville, AR, 2004.
[23] M. Leonard, Biotransformation of Sewage in a Trickling Filters, Science and Technology's Enviro Link Scheme, Foundation for Research, Institute of Environmental Science and Research ("ESR"), 2009.
[24] Government of Pakistan, Pakistan Environmental Protection Agency (Ministry of Environment), National Standards for Drinking water Quality (NSDWG), 2008.
[25] USEPA, Wastewater Technology Fact Sheet Trickling Filter Nitrification, Office of Water, 832-F-00-015, United States Environmental Protection Agency, Washington, DC, 2000.
[26] P.K. Gupta, Methods in Environmental Analysis Water, Soil and Air, 1st ed., UpdeshPurohit for Agrobios, India Jodhpur Agro House, 2004, pp. 47–48.
[27] R. Almstrand, P. Lydmark, F. Sörensson, M. Hermansson, Nitrification potential and population dynamics of nitrifying bacterial biofilms in response to controlled shifts of ammonium concentrations in wastewater trickling filters, Bioresour. Technol. 102 (2011) 7685–7691.
[28] World Health Organization (WHO), Guidelines for Drinking Water Quality, vol. 1, World Health Organization, Geneva, 2004.
[29] T. Sakuma, S. Jinsiriwanit, T. Hattori, M.A. Deshusses, Removal of ammonia from contaminated air in a biotrickling filter—Denitrifying bioreactor combination system, Water Res. 42 (2008) 4507–4513.
[30] S. Sehar, I. Naz, I. Ali, S. Ahmed, Monitoring of physico-chemical and microbiological analysis of underground water samples of district Kallar Syedan, Rawalpindi-Pakistan, Res. J. Chem. Sci. 1(8) (2011) 24–30.
[31] M. Pritchard, T. Mkandawire, J.G. O'neill, Biological, chemical and physical drinking water quality from shallow wells in Malawi: Case study of Blantyre, Chiradzulu and Mulanje, Phys. Chem. Earth 32 (2007) 1167–1177.
[32] H. Gullicks, H. Hasan, D. Das, C. Moretti, Y.T. Hung, Biofilm fixed film systems, Water 3 (2011) 843–868.
[33] M.A. Belmont, E. Cantellano, S. Thompson, M. Williamson, A. Sánchez, C.D. Metcalfe, Treatment of domestic wastewater in a pilot-scale natural treatment system in central Mexico, Ecol. Eng. 23 (2004) 299.
[34] K. Soontarapa, N. Srinapawong, Combined membrane-trickling filter wastewater treatment system, J. Sci. Res. Chula. Univ. 26 (2001) 59–70.
[35] O.V. Shipin, P.D. Rose, P.G.J. Meiring, Microbial processes underlying the PETRO concept (trickling filter variant), Water Res. 33 (1999) 1645–1651.
[36] C.S.A. Sá, R.A.R. Boaventura, Biodegradation of phenol by *Pseudomonas putida* DSM 548 in a trickling bed reactor, Biochem. Eng. J. 9 (2001) 211–219.
[37] P. Pearce, S. Williams, A nitrification model for mineral-media trickling filters, Water Environ. J. 13(2) (2007) 1747–1793.
[38] A.A. Szogi, F.J. Humenik, J.M. Rice, P.G. Hunt, Swine wastewater treatment by media filtration, J. Environ. Sci. Health 32 (1997) 831–843.
[39] A. Chavez, B. Jimenez, C. Maya, Particle size distribution as a useful tool for microbial detection, Water Sci. Technol. 50 (2004) 179–186.
[40] Y.L. Jin, W.N. Lee, C.H. Lee, I.S. Chang, X. Huang, T. Swaminathan, Effect of DO concentration on biofilm structure and membrane filterability in submerged membrane bioreactor, Water Res. 40 (2006) 2829–2836.
[41] B. Kocadagistan, E. Kocadagistan, N. Topcu, N. Demircioğlu, Wastewater treatment with combined upflow anaerobic fixed-bed and suspended aerobic reactor equipped with a membrane unit, Process Biochem. 40 (2005) 177–182.
[42] A. Converti, M. Rovatti, M.D. Borghi, Biological removal of phosphorus from wastewaters by alternating aerobic and anaerobic conditions, Water Res. 29 (1995) 263–269.
[43] R. Zeng, R. Lemaire, Z. Yuan, J. Keller, A novel wastewater treatment process: Simultaneous nitrification, denitrification and phosphorus removal, Water Sci. Technol. 50 (2004) 163–170.
[44] P. Lens, A. Massone, A. Rozzi, W. Verstraete, Effect of sulfate concentration and scraping on aerobic fixed biofilm reactors, Water Res. 29 (1995) 857–870.
[45] M. Sanchez, A. Mosquera-Corral, R. Mendez, J.M. Lema, Effect of ammonia on the performance of denitrifying reactors, Proceedings of the European Conference on New Advances in Biological Nitrogen and Phosphorus Removal for Municipal or Industrial Wastewaters, Narbonne, France, 12–14 October, 1998, pp. 119–126.
[46] J. Darby, G. Tchobanoglous, M.A. Nor, D. Maciolek, Shallow intermittent sand filtration: Performance evaluation, The Small Flows J. 2 (1996) 3–14.