



Sequential Application of Plastic Media-Trickling Filter and Sand Filter for Domestic Wastewater Treatment at Low Temperature Condition

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AR, IN and ZUK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MR managed the microbiological analyses of the study. Authors NA and SA supervised this research study and manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Present research was aimed towards designing and construction of efficient plastic media-trickling filter (TF) for the treatment of domestic wastewater. The hydraulic flow rate through the TF was maintained at 80 ± 2 ml/min at a temperature range of 5-15°C by selecting treatment time of 12, 24, 36 and 48 hrs. Parameters like COD, BOD₅, TSS, turbidity, NO₃, NO₂, SO₄, PO₄ and pathogenic indicator microbes were monitored after treatment of 12, 24, 36 and 48 hrs. The efficiency of the TF was improved with increase of time from 12 to 48 hrs. Maximum efficiency of TF was observed after 48 hrs treatment viz. 93.45, 93, 86.25, 57.8, 63.15, 25, 32.43, 99.95 and 86.3% reduction from the zero time value for BOD₅, COD, TSS, PO₄, SO₄, NO₃, NO₂, turbidity and fecal coliforms respectively. Finally 48 hrs treated sample was passed through sand filter (SF) for further final polishing and approximately, 95.72, 95, 100, 73.5, 65.8, 58.3, 37.83, 100 and 91.5% reduction in BOD₅, COD, TSS, PO₄, SO₄, NO₃, NO₂, turbidity and fecal coliforms was observed. This study showed that plastic media-trickling filter along with sand filter is a

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promising technology for wastewater treatment and can be scaled up for small communities in the developing countries.

Keywords: Trickling filter; domestic wastewater; BOD₅; COD; plastic media.

1. INTRODUCTION

Wastewater is liquid end product of municipal, agricultural and industrial activity [1]. It is comprised of 99.3% water, small amount of organic, in-organic compounds and numerous pathogenic/non-pathogenic microbes that provide harmful threats to the associated human community. In developing countries like Pakistan, rapid population growth, industrialization and urbanization from the last twenty years have placed stress on water availability. The high pollution level of rivers and groundwater due to discharge of untreated wastewater has led to different environmental consequences such as reduction of biodiversity, increase in water related diseases and decrease in agricultural productivity [2]. The problem of water shortage is expected to become more acute in the future, so effective measurements should be taken to overcome this calamity but economic considerations are so much important in selecting a suitable treatment technology [3].

Wastewater treatment is based on physical, biological and thermal specific processes (chemical oxidations, ion exchange, desorption) specifically when compounds are present in suspension, biodegradable and non-biodegradable forms respectively [4]. However, biological processes for the treatment of wastewater are best option as compared to physical and chemical treatment processes due to their low operational cost, easy handling and cause almost no harmful effects to the corresponding environment [5]. Biological treatment technologies are further classified into extensive and intensive processes. Extensive processes such as constructing wetlands are used for small communities while, intensive processes such as trickling beds and activated sludge are used for large industrial plants or large cities.

Trickling filter is an attached growth bioreactor in which wastewater trickles through the bed of matrix media, considered as heart of the system. As water passes through the media having microbial biomass in the form of biofilm, concentration of nutrients in wastewater decreases. Various types of bacteria, fungi, protozoa etc are present in biofilm which degrade most of the organic compounds present in wastewater and the efficiency of TF increases by maintaining a high biomass concentration in the biofilm [6]. Moreover, TF reliably produced a high quality of secondary wastewater effluent with very high level of nitrification because it provides longer retention time for wastewater within column. It is relatively expensive as compared to other treatment options and having low space, energy and maintenance requirements.

The present research is focused on designing and construction of lab scale plastic media-trickling filter (TF) and sand filter (SF) for effective treatment of domestic wastewater from residential colony, Quaid-i-Azam University, Islamabad. That water contaminating two streams which enters Rawal Lake [7]. This lake provides drinking water to third largest city of Pakistan, Rawalpindi [8]. Recently it is reported that this lake has been contaminated with high levels of metals, nutrients and fecal coliform through direct discharge of wastewater [9]. Presence of fecal coliform shows that the source water may be contaminated by pathogens

or disease producing bacteria or viruses, which can also exist in fecal material. Pak-EPA has advised QAU for stoppage of such wastewater [10].

2. MATERIALS AND METHODS

2.1 Experimental Set-up and Operation

2.1.1 Plastic media-trickling filter (TF)

The outer court of TF was constructed from PVC pipe (height = 36cm, outer diameter = 14cm, inner diameter = 12.4cm). A steel cage was fabricated (height = 24 inches and diameter = 11 inches) to hold filter media i.e., plastic balls (surface area = 1.766 inch²) as shown in Fig. 1 (A, B). Underdrain system (total height = 8 inches) with an outlet at a height of 3 inches was positioned at bottom below steel cage to collect treated wastewater. A shower rose (diameter = 8 inches) was used as wastewater distribution system supported on the top of stone media bed. A net distance between the bottom of shower rose and top of filter bed surface was 9 inches. A plastic container (bath room tub of 25-L capacity) was used to hold treated wastewater and water pump (power 220 volts used for recirculation of water). A plastic pipe (length = 125 inches, inner diameter = 2 cm) connected with pump was employed to facilitate flow of water. Down flow aeration through a space between outer court and inner core (steel cage) was utilized to ensure aerobic conditions. With the help of water pump, 20 liter of wastewater was passed through bed of TF (hydraulic flow rate = 80±2 ml/min, retention time = 18 min). The flow of water was controlled by electric dimmer connected to the water pump. It was run under different treatment times (12, 24, 36 and 48 hrs) at 5-15°C (Continuously operated from Nov, 2011 to Feb, 2012 in Islamabad, PK). After 48 hrs HRT, treated wastewater was then passed through SF.



Fig. 1. (A) Plastic balls used as filter media (B) Trickling filter steel cage filled with plastic balls as a media

2.1.2 Sand filter (SF)

It consisted of plastic column (height = 39", inner diameter = 3") filled with sand (size = 0.2 mm in diameter) to depth of 30". A peristaltic pump was used for pumping TF 48 hrs treated wastewater into the SF. Flow speed of pump was adjusted to 40±2 ml/min. The retention time across the filter bed was 15 min.

2.1.3 Development of biofilm on plastic media

For the development of biofilm, 340 plastic balls were incubated in activated sludge for two weeks as shown in Fig. 1(A). After the development of slime layer on plastic balls, they were subjected to bacteriological analysis by pure culturing techniques (Microscopy, cultural characteristics and biochemical tests).

2.1.4. Treatment of wastewater

After collection, the wastewater was allowed to pass through TF and then through SF. Preliminary, wastewater used for treatment was characterized and was given a retention time of 2-3 hrs in collection tanking in order to sediment the suspended solids and large particulate matters. It was then recirculate through TF by giving a treatment time of 12, 24, 36 and 48 hrs. After 48 hrs of treatment in TF, this treated water was passed through SF for further treatment. A schematic diagram of overall treatment process is shown in Fig.2. Temperature was continuously monitored during the study by using thermometer and found to be in the range of 5-15°C.

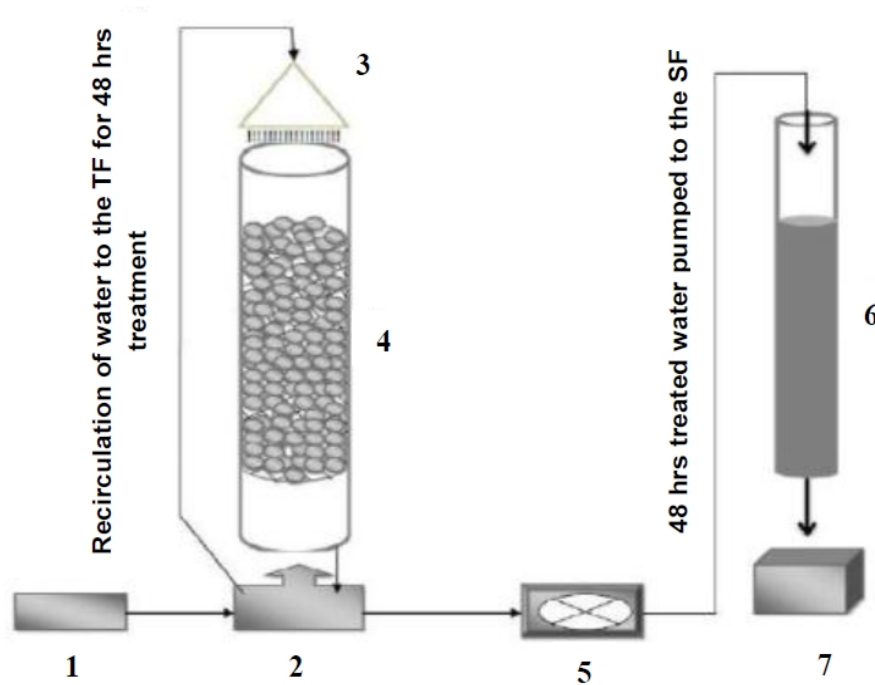


Fig. 2. Schematic diagram of overall treatment process

Legends:

1 = Sedimentation Tank for collection of untreated wastewater; 2 = Plastic container with water pump for recirculation of wastewater; 3 = Shower rose for distribution of wastewater; 4 = TF filled with plastic media (balls); 5 = Peristaltic pump for pumping TF treated wastewater into sand filter; 6 = Sand column Filter; 7 = Treated wastewater collection tank.

2.2 Physico-Chemical Analysis

2.2.1 pH

The pH of samples was determined in the laboratory by using “Digital pH meter (D-25 Horiba)”. Before measurement, the instrument was calibrated using distilled water.

2.2.2 Turbidity

Turbidity was measured by using “Water analyzer 2000N, Nippon Denshoku”. Before measurement, turbidity meter was calibrated with standard turbidity suspension of 10, 100 and 1000 (Nephelometric turbidity unit) NTU.

2.2.3 Electrical conductivity (EC)

The EC of water samples was determined with the help of “Conductivity meter model WTWcind330i” in micro Siemens per centimeters.

2.2.4 Dissolved oxygen (DO)

The dissolved oxygen (DO) was measured by using “Digital DO meter model MM-60R, TOA-DKK” in mg per liter. The electrode of the DO meter was washed with distilled water each time before analysis of new samples.

2.2.5 Biological oxygen demand (BOD₅)

The BOD₅ of the water samples was determined by using 5-Day BOD test i.e. 5210 B. Standard Method [11]. It was measured by using the following formula;

$$\text{BOD}_5 \text{ (mg/l)} = (\text{DO1} - \text{DO2}) / \text{volume of dilution sample} \times 1000$$

Where,

DO1 = DO of diluted sample immediately taken after preparation

DO2 = DO of the diluted sample after 5 days incubation at 20°C

2.2.6 Chemical oxygen demand (COD)

The chemical oxygen demand of samples was determined by kit method; high range 14541 and low range 14560 CSB/COD kits (Merck, Germany).

2.2.7 Total dissolved solids (TDS)

Standard method 1540 C was used to determine total dissolved solids in water [11]. The TDS were determined by using the formula;

$$\text{Total dissolved solids (mg/l)} = (\text{A-B}) \times 1000 / \text{sample (ml)}$$

Where,

A = weight of dried residue + dish (mg)

B = weight of empty dish (mg)

2.2.8 Total suspended solids (TSS)

Standard method 2540 D was used to determine total suspended solids in water [11]. The value of TSS was calculated by using the formula;

$$\text{TSS} = (A-B) \times 1000 / \text{ml of sample}$$

Where,

A = weight of filter + residue in mg

B = weight of filter paper in mg

2.2.9 Chlorides

Titration method using silver nitrate was used to determine Chloride concentrations in water. Following formula was used to calculate its levels,

$$\text{Chloride (mg/l)} = \frac{D \times \text{Normality of AgNO}_3 \times \text{equivalent weight of chlorine} \times 1000}{\text{Volume of sample used}}$$

Where,

D = Volume of AgNO₃ used in the sample – volume of AgNO₃ used in blank

Normality of AgNO₃ = 0.0141N

Equivalent weight of chlorine = 35.5g

2.2.10 Alkalinity

Standard Titration method, 2320 B was used to determine alkalinity in water [11].

$$\text{Total alkalinity} = B \times N \times 50 \times 1000 / \text{volume of the sample}$$

Where,

B = volume of titrant used

N = normality of H₂SO₄

50 = equivalent weight of CaCO₃

2.2.11 Orthophosphates and sulphates

Standard method 4500-P and EPA method 0375 Barium chrometry was used to determine orthophosphate and sulphates respectively in water samples [11].

2.2.12 Nitrate-nitrogen and nitrite-nitrogen

EPA method 4500 NO₃-N and 4500 NO₂-N was used to determine nitrates and nitrites in water samples (Standard Methods, 2005).

2.3 Microbiological Analysis

Microbiological analysis of wastewater was carried out by determining MPN/100ml index of pathogenic indicators i.e. fecal coliforms and average bacterial count (CFU/ml) according to Bergey's Manual of Determinative Bacteriology [12].

2.4 Statistical Analysis

Statistical analysis of each treatment was carried out by using Microsoft excel program. In order to find treatment efficiency, the water samples were compared by t-test and $P < .05$ was considered the minimum value for statistical significance.

3. RESULTS AND DISCUSSION

3.1 Bacteriological Analysis of Biofilm

Plastic media is highly efficient as compared to other media in wastewater clarification, allowing good microbial growth due to high specific surface area and low molecular weight [13]. Bacteriological analysis of biofilm was performed by pure culture techniques and different bacterial species such as *Salmonella sp.*, *Pseudomonas sp.*, *Enterobacter sp.*, *Klebsiella sp.*, *Shigella sp.*, *Proteus vulgaris*, *Alcaligenes faecalis*, *Staphylococcus sp.*, *Streptococcus sp.*, *Micrococcus sp.*, *Corynebacterium sp.* and *Bacillus sp.*, were identified in biofilm. Microscopic and biochemical characterization of these isolated bacterial strains was shown in Table 1. Andersson et al. [14] also studied the capability of 13 bacterial strains found in wastewater to form biofilm on solid matrix.

Table 1. Microscopic and biochemical characterization of isolated bacterial strains

Strains	Morphology/ Gram staining	Fermentation											Identified Organisms	
		Lactose	Dextrose	Sucrose	H ₂ S test	NO ₃ test	Indole test	MR test	VP test	Citrate test	Urease test	Catalase test		TSI test
1	Rod/Negative	AG	AG	AG	-	+	-	-	+	+	-	+	K/A	<i>Enterobacter sp.</i>
2	Rod/Negative	AG	AG	AG	-	+	-	±	±	+	+	+	-	<i>Klebsiella sp.</i>
3	Cocci/Positive	-	-	-	-	±	-	-	-	-	+	+	K/NC	<i>Micrococcus sp.</i>
4	Rod/Positive	-	A±	A±	-	+	-	-	-	-	-	+	-	<i>Corynebacterium sp.</i>
5	Rod/Negative	AG	AG	A±	-	+	+	+	-	-	-	+	A/NC	<i>Escherichia coli</i>
6	Short rod/Negative	-	A	A±	-	+	±	+	-	-	-	+	K/A, H ₂ S	<i>Shigella sp.</i>
7	Short rod/Negative	-	AG±	A±	+	+	-	+	-	+	-	+	K/A, H ₂ S	<i>Salmonella sp.</i>
8	Rod/Positive	-	A	A	-	+	-	-	±	-	-	+	A/NC	<i>Bacillus sp.</i>
9	Cocci/Positive	A	A	A	-	+	-	+	±	-	-	+	A/A	<i>Staphylococcus sp.</i>
10	Rod/Negative	-	-	-	-	+	-	-	-	+	-	+	-	<i>Pseudomonas sp.</i>
11	Rod/Negative	-	AG	AG	+	+	+	+	-	±	+	+	-	<i>Proteus vulgaris</i>
12	Cocci/Positive	A	A	A	-	-	-	+	-	-	-	-	-	<i>Streptococcus sp.</i>
13	Cocco- Bacillus/Negative	-	-	-	-	-	-	-	-	±	-	+	-	<i>Alcaligenes faecalis</i>

Key: AG = Acid and gas; + = Positive; - = Negative; ± = Variable reaction; A = Acid production; K = alkaline reaction; NC = No change; H₂S = Sulfur reduction; K/A = Red/yellow; K/NC = Red/no color change; K/A, H₂S = Red/yellow with bubble and black precipitate; A/NC = Acid/no color change; A/A = Yellow/yellow.

3.2 Microbiological Characterization of Wastewater before and after Treatment

For the quantitative and qualitative analysis of pathogenic indicators in wastewater before and after treatment, the spread plate and MPN techniques were used. The strength of bacterial population was determined in terms of CFU/ml. The average number of bacterial in untreated, 48 hrs treated and SF treated wastewater samples were 8.03×10^6 , 4.06×10^4 and

4.31×10^3 respectively. Approximately, 85-90% reduction in fecal coliforms was observed after treatment.

The MPN index of untreated wastewater samples were >1100 per 100 ml while for 48 hrs and SF treated wastewater was 150 and 93 per 100 ml respectively. The presumptive and confirmatory tests were positive for MPN positive tubes (Table 2). This large microbial load in untreated colony wastewater was due to the presence of human excreta and heavy nutrient loads. After treatment pathogenic microorganisms present in waste influent, retained in the filter media by adsorption and are later removed or deactivated by predation or natural die off process. Sand bed filter proved to be more efficient in reduction of fecal coliforms as compared to trickling filter and approximately, 80-85% reduction in fecal coliforms was reported by Harold et al. [15] during wastewater treatment using sand filter.

Table 2. Average number of bacteria (CFU/ml) and MPN index of fecal coliforms in wastewater (95% confidence limits) before and after treatment by using plastic media-TF and sand filter (SF) at a low temperature range (5-15°C)

Wastewater samples	CFU/ml	MPN/100 ml (Fecal coliforms)	95% confidence limits	
			Lower	Upper
Untreated sample	8.03×10^6	>1100	150	>4800
48 hrs treated sample	40.6×10^4	150	30	440
SF treated sample	4.31×10^3	93	15	380

3.3 Treatment of Residential Colony Wastewater

3.3.1 Analysis of wastewater before treatment

The quality of domestic wastewater was examined in triplicates and was remained almost same with a few small variations in different parameters. Apparently, it was grey in color with mordant smell. Different parameters such as pH (7.86), NO_2 (0.037 mg/L), NO_3 (0.06 mg/L), orthophosphates (0.083 mg/L), SO_4 (0.038 mg/L), TDS (692 mg/L), TSS (800 mg/L) and EC (699 $\mu\text{S}/\text{cm}$) of the residential colony wastewater were within the standard limits of WHO. However, DO (1.86 mg/L), BOD_5 (100.41 mg/L), COD (147.66 mg/L) and turbidity (1013.9 NTU) considerably showed deviation from the prescribed limits indicating the high level of contamination. Average loading and performance values are shown in Table 3.

3.3.2 Analysis of water after treatment

Among different physico-chemical parameters, pH is of prime importance to characterize the quality of wastewater. Although pH has no direct effect on aquatic as well as terrestrial life but it has been expected that pH has negative impact on the microbial consortia involved in wastewater clarification because pH affects the solubility of many toxic and nutritive chemicals therefore, affect the availability of these substances to microorganisms. According to WHO [16] 6.5-8.5 is the prescribed range of pH for drinking water. The pH of untreated colony wastewater was slightly high due to human excreta. In the study, a slight decrease was observed in pH during treatment but remained within prescribed range. A possible reason for this might be that denitrification process occurred within TF due to which a decreased in pH was observed [17].

Table 3. Wastewater treatment efficiency of plastic media-TF and sand filter (SF) at a low temperature range (5-15°C)

Parameters analyzed	WHO standards (2004)	Untreated water sample	Treatment efficiency of plastic media-TF					Treatment efficiency of sand filter (SF)		
			Treatment time (hrs)				%treatment	Influent	Effluent	%treatment
			12	24	36	48				
DO (mg/L)	6-8	1.86	3.23	3.72	6.08	7.97	328.5	7.97	8.04	332.3
pH	6.5-8.5	7.86	7.82	7.80	7.78	7.62	3.05	7.62	7.49	4.7
TDS (mg/L)	< 1000	692	674	639	623	601	13.15	601	588	15.03
EC (µS/cm)	400-1215	699	680	647	631	610	12.73	610	596	15.03
TSS (mg/L)	NGV	800	600	400	200	110	86.25	110	0	100
Turbidity (NTU)	< 5	1013	9.32	4.7	0.84	0.43	99.95	0.43	0	100
BOD₅ (mg/L)	NGV	100.4	35.8	15.1	10.65	6.57	93.45	6.57	4.3	95.72
COD (mg/L)	NGV	147.66	52.6	22.3	15.66	9.66	93	9.66	6.33	95
PO₄ (mg/L)	NGV	0.083	0.07	0.05	0.045	0.035	57.83	0.035	0.022	73.5
SO₄ (mg/L)	250	0.038	0.03	0.02	0.02	0.014	63.15	0.014	0.013	65.8
NO₃ (mg/L)	50	0.06	0.07	0.14	0.109	0.045	25	0.045	0.025	58.33
NO₂ (mg/L)	3	0.037	0.06	0.15	0.121	0.025	32.43	0.025	0.023	37.83

The untreated wastewater showed very low value of DO i.e. 1.86 mg/L and according to [16] the prescribed limit of DO for drinking water is 6-8 mg/L. In the present study a statistically highly significant improvement in the quality of wastewater was observed in terms of DO after treatment ($P = .0003$; 332.3%). DO values rose to 3.23, 3.72, 6.08 and 7.97 mg/L after 12, 24, 36 and 48 hrs of treatment in TF respectively and further increased to 8.04 mg/L after sand filtration. This might be due to biodegradation of compounds present in wastewater that previously used dissolved oxygen for various oxidation-reduction reactions. After treatment the higher DO values 7.1 mg/L of wastewater means that this water could support the oxygen requirements of the aquatic organisms [18].

A slight decrease was observed in the mean values of TDS and EC during treatment i.e. up to 13.1 and 12.73% after 48 hrs of treatment in TF ($P = .04$) respectively and up to 15.03% ($P = .03$) for both TDS and EC after sand filtration. The level of total suspended solids (TSS) showed significant improvement with increasing HRT ($P = .0001$) where 86.25% reduction in TSS was observed after 48 hrs of treatment and 100% after sand filtration. According to [16], the prescribed value of EC, TDS and TSS in drinking water is 400-1215 $\mu\text{S}/\text{cm}$, <1000 mg/L and 25-80 mg/L respectively. EC is directly related to the suspended solids, dissolved solids and COD i.e. larger the number of suspended and dissolved solids in wastewater, higher will be the EC and vice versa [19]. In this study, it was found that EC value of untreated wastewater decreased gradually during treatment due to decrease in TDS and TSS levels. It might be related to the conversion of NO_3 into diatomic molecular nitrogen (N_2), which also decreases EC levels of domestic wastewater. The EC is also found to be associated with the amount of chlorides as reported by [20] but the values of chlorides were very low in the water under study.

Turbidity is another important physical parameter that may cause the growth of pathogens leading to the outbreaks of water borne diseases throughout the world [21]. The average value of turbidity of untreated wastewater was very high i.e. 1013 NTU and it was due to the presence of particulate and organic matters which make the water turbid. During the treatment in the TF, turbidity of wastewater was completely removed with an efficiency of 100%. This might be due to the degradation of compounds in the wastewater by microorganisms during treatment [19,20,22].

Biochemical oxygen demand (BOD_5) is a measure of the amount of oxygen that bacteria will consume while decomposing organic matters present in wastewater over the period of five days. No specific criterion is described by WHO for BOD_5 range but according to [23] standards; the BOD_5 of normal drinking water should be in the range of 5-8 mg/L. Initially the average concentration of BOD_5 in untreated wastewater was high i.e. 100.4 mg/L. It was due to the presence of organic compounds that used large amount of oxygen for their oxidation. If effluent with high BOD_5 levels is discharged into a stream or river, it will accelerate bacterial growth in the river and consume the oxygen levels in the river to the extent that are lethal for aquatic life. But it was observed during study that the BOD_5 value of wastewater decreased up to 95.72% after treatment ($P = .00009$) and can be safely discharge. This decrease in BOD_5 value was due to high biodegradation of organic contaminants of wastewater during constant recirculation in TF. Similarly 86-97.8% of BOD_5 reduction in TF for domestic wastewater was reported by Soontarapa and Srinapawong [24].

Chemical oxygen demand (COD) does not differentiate between biologically available and inert organic matters, and it is a measure of the total quantity of oxygen required to oxidize all organic materials into carbon dioxide and water. The untreated wastewater showed very high level of COD i.e. 147.66 mg/L. This highest COD of untreated wastewater was due to

the presence of large amount of organic compounds. According to [16], no specific criterion is available for COD range but [23], described limits of 8-10 mg/L drinking water. In the present study, it was observed that COD values of residential colony wastewater decreased up to 95% after treatment ($P = .00009$). A basic reason for this removal of COD might be the degradation of organic compounds by the microorganisms attached to the matrix in TF. Similar results of COD removal were reported by Sakuma et al. [17] in their study.

Municipal sewage, industrial discharge along with agricultural runoff is the major source of orthophosphate (PO_4) in wastewater. Microbes are unable to receive phosphorus as it binds readily to particles. Soluble phosphorus which is available for uptake is called orthophosphate and the increase in orthophosphate level in wastewater causes eutrophication, leading to a potential increase in biomass [25]. According to WHO [16] no standard values are available for phosphate removal. But the [23] water quality criteria state that phosphate should not exceeds 0.05 mg/L if streams discharge into lakes or reservoirs, 0.025 mg/L within a lake or reservoir, and 0.1 mg/L in streams of flowing waters not discharging into lakes or reservoirs to control algal growth [23]. The amount of orthophosphate was not very high in colony sewage i.e. 0.083 mg/L. When orthophosphate removal was checked after different time interval during treatments a reduction of 57.83% ($P = .002$) was observed after 48 hrs of treatment in TF and further decreased to 73.5% after sand filtration ($P=.0008$). This showed that polyphosphorus accumulating bacteria might be present in the biofilm that used soluble phosphorus as a substrate and thus reduced the level of orthophosphate in wastewater after treatment. Szogi et al. [25] observed similar results of orthophosphate removal during their study.

The sulfates (SO_4) are present in all types of contaminated wastewater including, natural runoff, domestic sewage and industrial effluent. In this study untreated wastewater contains small concentration of (0.038 mg/L) of SO_4 . It was within the permissible limit of WHO. After treatment, a significant improvement was observed in the level of SO_4 ($P = .0009$; 65.87%). This might be due to the presence of sulfate reducing or oxidizing bacteria in biofilm. [26] also showed similar results in terms of SO_4 removal during wastewater treatment.

Untreated wastewater contained 0.037 and 0.06 mg/L of nitrite (NO_2) and nitrate (NO_3) nitrogen respectively. It was observed during study that initially the concentration of NO_2 and NO_3 increased up to 0.156 and 0.14 mg/L respectively till 24 hrs, which suggest the process of nitrification. Then there was drastic decrease up to 0.023 and 0.025 mg/L respectively after treatment which showed denitrification process i.e. conversion of NO_3 into the molecular nitrogen (N_2) and due to which a decreased was observed in EC of colony wastewater. Nitrification process plays an important role in wastewater treatment [27]. The first step of nitrification process is the oxidation of ammonia into NO_2 , performed by two different microbes i.e. Ammonia oxidizing bacteria and Ammonia oxidizing archea while the second step of nitrification is the oxidation of NO_2 into NO_3 by *Nitrobacteria* [28]. Sandip et al. [29] showed 40-90% NO_3 and 55-92% NO_2 reduction in their study.

4. CONCLUSION

In the present study, simultaneous application of plastic media-trickling filter and sand filter proved to be efficient for the treatment of domestic wastewater at low temperature regimes. A significant association was found between the percentage removals of contaminants at different HRT in the TF. While, highest percentage removal was found after 48 hrs of treatment in TF. The quality of treated wastewater was found to be improved in terms of BOD_5 , COD, TSS and pathogen indicators i.e. fecal coliforms and *Enterococcus faecalis*

after sand filtration. A substantial reduction was also observed in SO_4 , PO_4 , NO_3 and NO_2 concentrations during study which indicates the presence of sulfate reducing/oxidizing, phosphate accumulating, nitrifying and denitrifying bacteria in the biofilm.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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