

Original Research

Effects of Hydraulic Retention Time (HRT) on the Performance of a Pilot-Scale Trickling Filter System Treating Low-Strength Domestic Wastewater

Abdul Rehman¹, Nasir Ayub¹, Iffat Naz^{1,2}, Irum Perveen¹, Safia Ahmed^{1*}

¹Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

²Department of Biology, Qassim University, Kingdom of Saudi Arabia

Received: 19 July 2018

Accepted: 16 October 2018

Abstract

Attached growth wastewater treatment systems are considered to be effective in developing countries due to their low energy, operational and maintenance costs. Thus the present study mainly focused on the treatment of domestic wastewater by a pilot-scale trickling filter system (TFs) installed in the natural environment at a residential area of Quaid-i-Azam University, Islamabad, Pakistan, and it was operated under three different hydraulic retention times (HRT), i.e., 24, 48 and 72 hrs. Furthermore, pilot-scale TFs showed significant efficiency regarding the removal of physico-chemical and microbiological parameters under different HRTs, i.e., 70.9 and 23.5% reduction in COD and EC contents, respectively, at HRT of 48 hrs., while significant removal of TDS (34%), SO₄ (37%), PO₄ (81.8%) and TN (66.6%) were noticed during operation of the system after 72 hrs of HRT. Likewise, a maximum of 86.4%, 82.5% and 83% decrease in total bacterial count were observed at HRT of 24, 48 and 72 hrs, respectively. Finally, it was suggested that pilot-scale TFs have great potential to be transferred to field scale in the areas disconnected from a centralized treatment system for handling sewage of small communities in underdeveloped and developing countries.

Keywords: stone media pilot scale TFs, domestic wastewater, hydraulic retention time (HRT), pathogen removal, chemical oxygen demand (COD)

Introduction

Water is exceptionally vital for the life and sustainability of all living organisms, but today, due to

adverse climatic conditions, it is considered a threatened resource worldwide [1]. Humans can stay alive without food for a number of weeks but without water, one cannot survive for more than a week [2]. It is a significant component of life being used for agricultural, industrial, domestic, recreational and other routine activities. According to Alkhamisi and Ahmed [3], about 70% of water is consumed in agricultural activities, 20% in

*e-mail: safiamrl@yahoo.com

industrial and 10% in residential, recreational and other daily events throughout the world. Due to misuse and mismanagement of such a precious resource, around 900 million people throughout the world have no access to drinking water reservoirs [4]. In developing countries like Pakistan, water availability levels declined from 1299 (1996-97) to 1100 m³ per capita in 2006, which was predicted to be 700 m³ per capita by the year 2025. Furthermore, extreme food deficiency (70 million tons) is anticipated due to a predicted severe shortage of water reservoirs (~32%) by 2025 [5].

In both rural and urban areas of developing countries, the quality of drinking water is severely affected due to microbial contamination. However, different factors are involved in the deterioration of drinking water reservoirs, including leaked pipes and their associated contamination, recurrent water supply and superficial water tables due to endless anthropogenic activities [6-7]. In addition, the USEPA [8] reported that the torrential rains, pesticides, unprocessed wastewaters, littoral water contamination and oil leakage are exceedingly toxic for drinking water reservoirs in Pakistan. Heavy metals also lead to health associated problems if its value exceeds permissible limits [9]. Global water scarcity, its competition and the negative influences on human life and the associated environment call for the development of appropriate strategies in wastewater treatment and water management sections [3, 8]. On the other hand, water accessibility, appropriateness and sustainability cannot be achieved abruptly via a single step. It needs integrated actions through government policy of priority.

Different treatment technologies are used today to treat wastewater, including physical treatment systems (filtration, precipitation), chemical treatment systems (flocculation, adsorption, coagulation) and biological treatment systems (attached growth and suspended growth systems). In comparison with physical and chemical methods, biological treatment systems are gaining attention due to its cost-effectiveness, easy operation and eco-friendly nature [10]. Biological treatment processes can be classified into attached and suspended growth. In attached growth systems, filter media are packed in the reactors to provide surface for the attachment of microorganisms to form slimy biofilm, for enhancing microbial absorptions and reduce contaminant rates [11-12]. Trickling filters (TFs), moving bed biological contactors, rotating biological contactors and membrane bioreactors are examples of attached growth systems. Moreover, such technologies offer several advantages such as easy handling, lesser hydraulic retention time (HRT), resistance to environmental variants, dynamic biomass and enhanced capacity to mineralize toxic substances [13-15].

Several studies have been conducted to evaluate the performance of bioreactors, including the influence of operating characteristics and packing media [16] in treating domestic or low-strength wastewater. Different researchers have examined the effect

of temperature and HRTs on the performance of attached growth systems while treating domestic sewage [14, 17-18]. When the temperature is high, the conversion rates of organic matters in TFs are also high, while at low temperature, more organic matter usually remains un-degraded as a result of slow hydrolysis of volatile solids at a given HRT [19-20]. Therefore, a longer HRT would be required in TFs in low-temperature conditions [21]. The present research study was designed to evaluate the effect of different HRTs, i.e., 24, 48 and 72 hrs on the performance of stone media pilot scale TFs constructed in an open environment under natural conditions at residential area of Quaid-i-Azam University (QAU) Islamabad Pakistan, treating domestic wastewater. Generally, HRT has significant effects on the efficiency of wastewater treatment systems, but several other factors such as seasonal variations, temperature, precipitation, humidity, organic loading and flow rates are obvious considerations. The temperature of the environment was routinely checked during biological operation and it was in the range of 16-38°C. This study will also help to determine and fix the most appropriate HRT for the treatment of wastewater through pilot scale TFs in future studies.

Experimental Section

Experimental Setup Scheme

The stone media pilot scale TF was installed in an open environment in a residential area of QAU Islamabad, Pakistan, as reported by Rasool et al. [22]. The system includes a primary sedimentation tank (PST) (diameter, 1.53 m and height, 1.37 m) to carry about 2300 gallons (8.7 m³) of influent, followed by the main body of TFs made of concrete (diameter 1.53 m and height 1.68 m, with total volume of 3.06 m³) to support the stones (average diameter of 4 inches or 0.1 m) used as filter media for bacterial growth. A recirculation tank (RCT) having the same dimensions as that of PST was installed next to the main body. A rotating arm distributor (length, 1.28 m) with numerous small pores was installed at the top of the main body of TFs to distribute wastewater uniformly over the surface of a filter bed. Electric pumps were connected to the wastewater distribution system through the polyvinylchloride (PVC) pipe system. To collect effluent and sludge, an underdrain system (diameter, 1.53 m and height, 0.46 m) was present at the bottom of the TF component and the total gap between the TF component and underdrain system was about 0.46 m in order to facilitate the oxygenation process. The stone bed has a voidage space of 35%. The schematic illustration of overall treatment units of pilot scale TFs is shown in Fig. 1.

This pilot-scale facility had the capacity to treat approximately 0.8 m³ (211.34 gallons) of wastewater

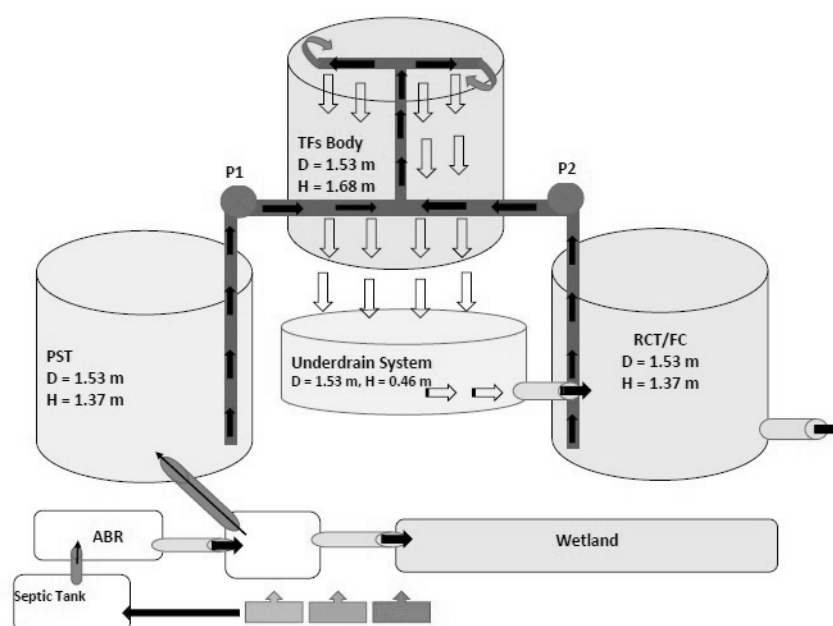


Fig. 1. Schematic illustration of overall treatment units of pilot-scale TFs.

depending upon the nature of flow rates maintained by both pumps. The study period covered the months from January 2016 to July 2016. For the development of active biofilter media, a mixture of wastewater and activated sludge (1:9) was pumped into the TFs for about 2 weeks before the start-up of the system for wastewater treatment. Environmental temperature was continuously monitored during the research study from January to July 2016, and it was in the range of 16-38°C.

Experimental Setup Operation

The operation of pilot scale TFs was the same as reported by Rasool et al. [22], but in the current study, experiments were conducted in three different phases in order to determine the effect of HRTs (24, 48 and 72 hrs) on the efficiency of pilot-scale TFs with respect to wastewater treatment. As pilot scale TFs contained two pumps (P1 installed on PST and P2 installed on RCT), initially influent was distributed for about 4.5 hrs from PST to the top of filter bed through P1 at a hydraulic flow rate of 0.64 m³/day, and after this P1 automatically switched off and pump P2 was turned on, which maintained the frequency of water distribution from RCT over the top of stone filter bed at a hydraulic flow rate of 0.04 m³/day. But here in the 1st phase of biological operation at HRT of 24 hrs, P2 continuously pumped and recirculated water from RCT for about 19.5 hrs while in the 2nd and 3rd phases of biological operation at HRT of 48 and 72 hrs, P2 recirculated water for about 43.5 and 67.5 hrs, respectively. After the completion of 24, 48 and 72 hr cycles, the effluent was allowed to flow into the sand bed compartment by gravitational force and then to be discharged into the

adjacent fields or stream. After this, P2 was switched off automatically and it turned P1 on, which pumped a new sample of influent from the primary sedimentation tank to the reactor, and next cycle begins. The digitally controlled system had the capacity to evaluate its running time, so in case of any load shedding, each pump finalized its running time.

Sampling of Wastewater

Standard methods were followed for sampling during study [23]. Influent and effluent samples of 24, 48 and 72 hrs HRT were collected in triplicate in 1000 mL separate clean plastic bottles and then shifted to the laboratory in an ice box in order to be preserved at 4°C in a refrigerator before determining changes in the concentration of different physicochemical and microbiological parameters.

Microbiological Analysis of Influent and Effluent Samples

Serial dilution and plate count method was used to determine colony forming units (CFU/mL) of aerobic bacteria according to standard methods [23]. Influent and effluent samples of 24, 48 and 72 hr HRT were collected, and dilutions (10⁻¹, 10⁻³, 10⁻⁵ and 10⁻⁷) were plated on nutrient agar plates. After inoculation, agar plates were kept in an incubator at 37°C for 48 hrs and then a number of bacterial colonies were counted by colony counter. CFU/mL was then determined using the formula:

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Inoculum size}}$$

Physicochemical Analysis of Influent and Effluent Samples

Physicochemical analysis of influent and effluent of 24, 48 and 72 hrs HRT were carried out by determining different parameters, i.e., pH was determined using digital pH meters, while electrical conductivity (EC) was measured by a PCS multi-test meter. Chemical oxygen demand (COD) was determined by COD kits (Merck Chemicals Inc.) using a spectroquant Pharo 100 instrument according to manufacturer instructions [23]. Standard protocols 1540-C, 4500-P and 0375 barium chrometry were used to determine total dissolved solid (TDS), phosphate (PO_4) and sulphate (SO_4) concentrations in influent and effluent samples, while total nitrogen (TN) was determined by kit method using Merck kits [23].

Results and Discussion

Pathogen Removal by Pilot Scale TFs under Different HRTs

The use of raw wastewater for irrigation purposes is an approach to utilize domestic wastewater with considerable benefits such as being rich in nutrients that plants require for their growth and, moreover, it has the capability of increasing soil fertility instead of using fertilizers, which makes irrigation cost-effective [24]. Besides, sewage water also contains toxic compounds, heavy metals and highly pathogenic microorganisms that have extremely adverse effects on terrestrial and aquatic ecology, the environment and public health [25-26]. A set of standard guidelines had been proposed by WHO [27] for the harmless use of treated water in agricultural sectors in order to secure agrarians and consumers of said crops. Therefore, it is essential to develop a suitable wastewater treatment technology in order to reduce the number of life-threatening and extremely pathogenic organisms before using treated

effluent in agricultural lands. In the present study, it was observed that pilot-scale TFs showed significant efficiency under different HRTs for removing pathogenic organisms from wastewater ($p = 0.0021$). About 37-86.4%, 41.6-82.5% and 42.4-83% reduction in total bacterial count were observed at HRTs of 24, 48 and 72 hrs respectively as shown in Fig. 2. The pattern of reduction in microbial count varied in different samples taken during the study period. The highest reduction (86.4%) was observed in S1 at 24 hrs HRT. While at 48 hrs and 72 hrs HRTs, the percentage reduction was lower in samples of low-temperature months (i.e., S1-S3), which was greater than 70% in samples of high-temperature months (i.e., S4, S5 and S6; Fig. 2). This pattern showed that environmental temperature also played a role in the pathogen reduction in the trickling filter [19-21]. The decrease in the number of pathogenic organisms within effluent samples might be due to the reduction of carbonaceous components in wastewater with treatment, or due to the confinement of microorganisms in the biofilm by adsorption [28]. Later on, it was associated with detachment and deactivation or natural die-off processes [29]. Furthermore, a similar percentage reduction (80-87%) in microbial count at HRT of 48 hrs were reported by Khan et al. [17] during their studies using a laboratory-scale stone media trickling filter system integrated with a sand column.

Carbonaceous and Nitrogenous Pollutant Removal by Pilot-Scale TFs under Different HRTs

Hydraulic retention time (HRT) is the time duration by soluble compounds to remain in a bioreactor for a specified period of time. It is directly related to the flow of wastewater toward a bioreactor [30]. Furthermore, adequate flow or recirculation rate and retention time offers sufficient contact between microbial biofilm and wastewater, resulting in an enhanced organic as well as nitrogenous pollutant removal efficiency of the treatment facility [30-31]. The effect of different HRTs on the performance of pilot-scale TFs are shown

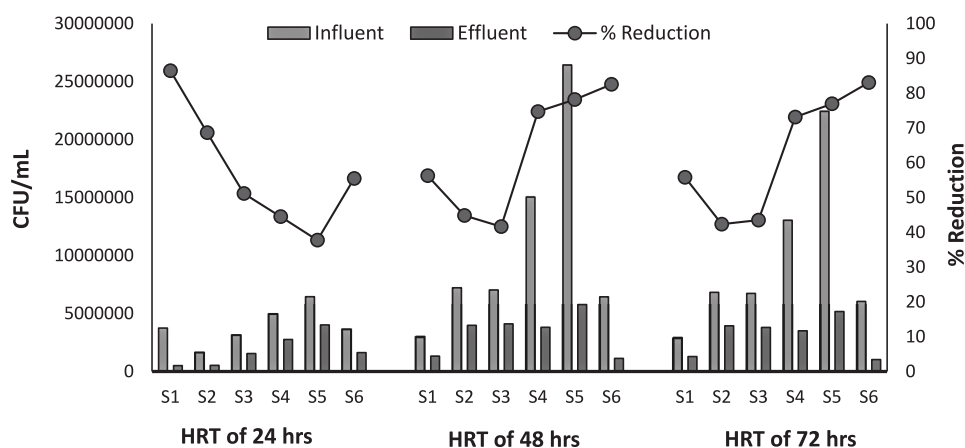


Fig. 2. Efficiency of pilot-scale TFs in terms of pathogen removal under different HRTs.

in Table 1. It was observed that increased HRT (from 24 hrs to 72 hrs) positively affected the efficiency of the system with respect to wastewater treatment. In the case of COD content reduction, the efficiency of pilot-scale TFs under an HRT of 24 hrs were in the range of 59.7-84.6% while at HRT of 48 and 72 hrs they were in the range of 62.2-85.3% and 23.5-80.8% respectively ($p = 0.003$), as shown in Fig. 3. This agreed with the findings of Ladu et al. [32], who studied the effects of three different HRTs on the efficiency of anaerobic filters while treating domestic wastewater, and found that the efficiency of the reactor increased by increasing HRT (from one to three days). Furthermore, they observed a 32% reduction in COD values at HRT of one day, 40% at HRT of two days and 44% at HRT of three days. The maximum removal percentage of COD at

higher HRT might be due to continuous recirculation of wastewater as a result microorganisms present in slime layer actively oxidizing organic compounds present in wastewater [14]. Moreover, Laing [33] reported an 84% reduction in COD values at HRT of 24 hrs while using sequential anaerobic batch reactors to treat synthetic winery wastewater. Leyva-Díaz et al. [34] reported an 85% reduction in COD contents at HRT of 18 hrs during their studies while using a hybrid moving bed membrane bioreactor.

In the current study, it was observed that the TDS removal efficiency of pilot-scale TFs increased with increases of HRT. About a 22% maximum reduction in TDS level was observed at HRT of 24 hrs while 38.8 and 57.8% reductions were found in TDS levels at HRT of 48 and 72 hrs, respectively ($p = 0.008$), as shown in Fig. 3.

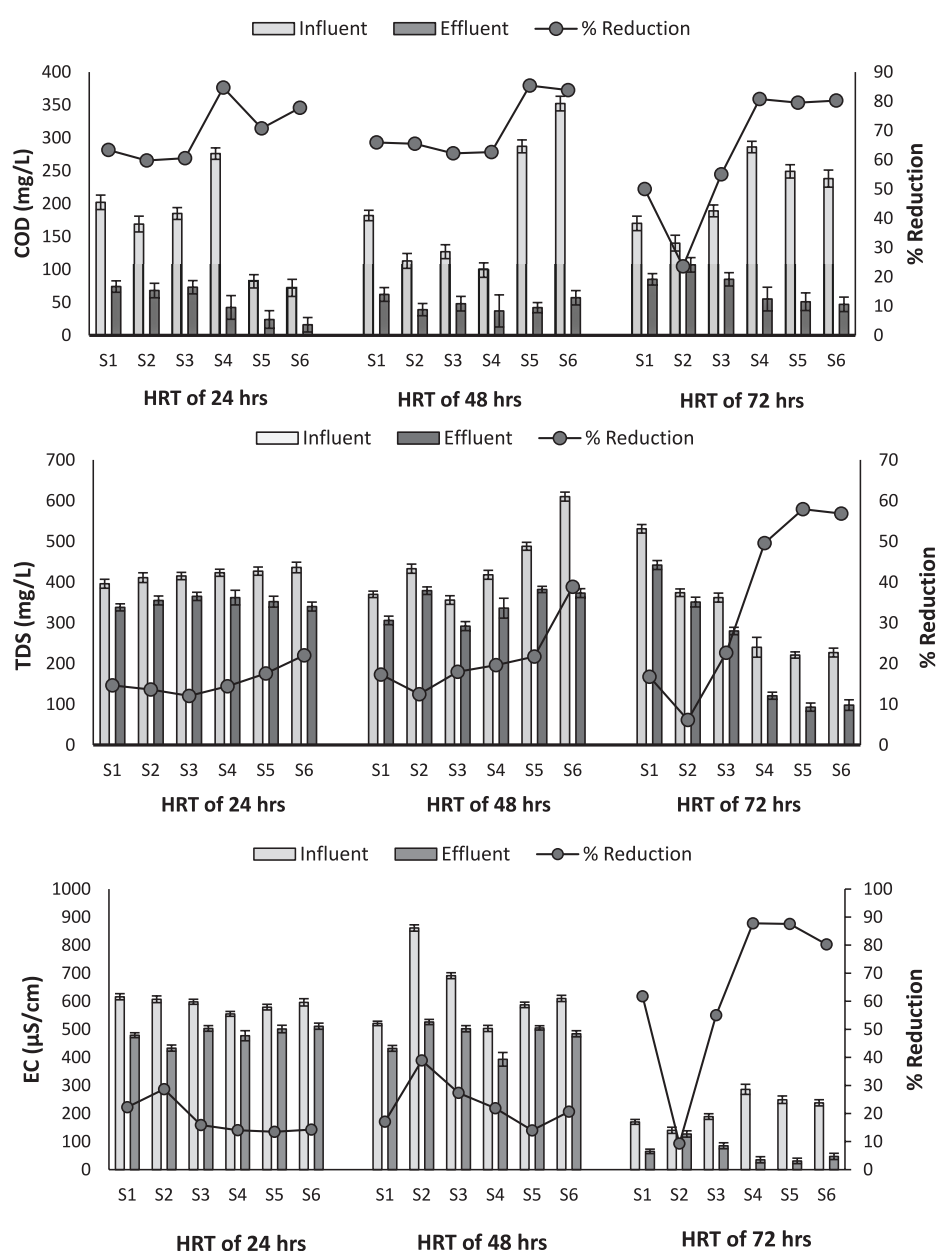


Fig. 3. Efficiency of pilot-scale TBF system in the average reduction of COD, TDS and EC values under different HRTs (bar indicates SD).

The reduction in TDS contents with increasing HRT was also due to continuous recirculation of wastewater for an extended period of time; as a result, microorganisms within biofilm got sufficient time to degrade dissolved organic components [18, 35]. Furthermore, Khan et al. [17] reported 23% and 66% reduction in the TDS values at HRT of 24 hrs and 48 hrs, respectively, during their study using stone media TFs integrated with sand column filter. According to WHO [27], the approved range of EC in wastewater is 400-1500 $\mu\text{S}/\text{cm}$, and furthermore it has a direct relationship with TDS, COD and fluorides present in water samples. Moreover, it was found that EC values of wastewater decreased up to 13.4-28.6% at HRT of 24 hrs while 14-39% and 5.87-23.04% reduction were observed at HRT of 48 and 72 hrs, respectively ($p = 0.03$), as shown in Fig. 3. A basic reason for the reduction in EC content might be that ammonium, nitrates and nitrites present in wastewater were converted to molecular nitrogen [22]. As a result, the concentration of free ions was reduced in order to conduct electrical current. Khan et al. [17] also reported a significant reduction in EC values by increasing HRT from 24-48 hrs while using a laboratory-scale fixed biofilm reactor.

Phosphate (PO_4) in domestic wastewater came in the form of polyphosphates, causing eutrophication in water bodies leading to adverse effects on aquatic life by minimizing light penetration (hypoxia) and

dissolved oxygen (DO) concentrations [14, 17]. Moreover, detergents, soaps, shampoos, oil and grease are being considered the most prominent source for PO_4 contamination in domestic wastewater [36]. In the present study, it was observed that an average reduction of 44.12%, 40.77% and 82% were achieved in PO_4 concentrations at HRT of 24, 48 and 72 hrs, respectively (for an overview, see Table 2). However, the overall efficiency of pilot-scale TFs in the reduction of PO_4 concentration is shown in Fig. 4, where individually up to 88.8%, 79.7% and 96.3% reduction were observed at HRTs of 24, 48 and 72 hrs, respectively, which was statistically highly significant ($p = 0.007$). These results showed consistency and compatibility with previous research work, where about 80-97% reduction in PO_4 concentrations were observed using “submerged membrane reactor” and “combined upflow anaerobic fixed bed in combination with suspended aerobic reactor having membrane unit” [36]. Khan et al. [17] showed an average reduction of 23% and 38% at HRT of 24 and 48 hrs, respectively, in PO_4 level using laboratory-scale stone media TFs to treat domestic wastewater and concluded that the removal of PO_4 was associated with metabolic activities of the microbial community flourishing on filter media. Furthermore, Zeng et al. [37] reported that nitrification and denitrification processes had promising effects on PO_4 elimination from wastewater during biological treatment.

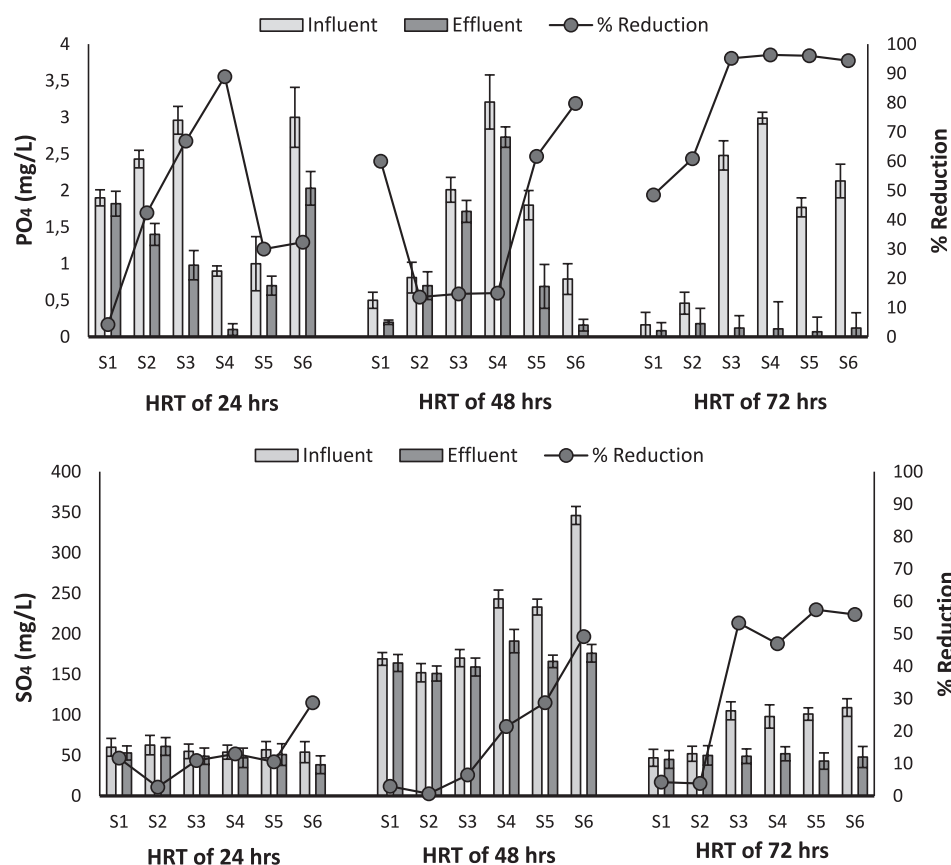


Fig. 4. Efficiency of pilot-scale TBF system in the average reduction of PO_4 and SO_4 content under different HRTs (bar indicates SD).

In the present study, the efficiency of the pilot-scale TFs to remove sulphates (SO_4) from wastewater increased with increases of HRT from 24-72 hrs. It was observed that during the 1st phase of biological operation, an average reduction of 12.9% whereas 18.2% and 36.96% reduction in SO_4 concentration were observed during the 2nd and 3rd phases of biological operations with respect to HRTs as shown in Table 2. However, the overall efficiency of pilot scale TFs in the reduction of SO_4 concentration is shown in Fig. 4, where significant ($p = 0.006$) reductions were observed in SO_4 content. Higher removal efficiency of SO_4 would be related to continuous recirculation and extended treatment time and, furthermore, higher HRT provides a favorable environment for sulphate-oxidizing bacteria because SO_4 become oxidized in the presence of oxygen [34]. Ehlers and Turner [38] reported that sulphate-oxidizing bacteria oxidize the sulphur to sulphates, which were then reduced to sulphide by sulphate-reducing bacteria. Sulphate-oxidizing bacteria were widely detected in domestic sewage and played a significant role in carbon, sulphur and nitrogen cycles. Laing et al. [33] reported that anoxic conditions developed within the biofilm reactor with the passage of time, and as a result sulphur-reducing bacteria actively participate in the reduction of sulphur compounds.

The pH value variations in the influent and effluent samples were monitored over time and it was

observed in the range of 6.3-7.5, 6.8-8.5 and 7.1-8.2 during the 1st, 2nd and 3rd phases of biological operation ($p = 0.01$) under different HRTs (i.e., 24, 48 and 72 hrs, respectively; Fig. 5). According to WHO [27] and the USEPA [39], the rate of ammonium oxidation decreased significantly in acidic pH range. Consequently, for optimum performance of treatment systems, it is best to maintain pH in the range of 6.8-8.0. The outcomes of a study in this regard revealed the same range indicating pilot scale TFs as one of the best options for domestic wastewater treatment. Likewise, Khan et al. [17] reported the equivalent pH range and its alteration with redox as well as nitrification and denitrification reactions and, furthermore, described that the pH values diminution after treatment through pilot-scale TFs might be due to the denitrification phenomenon converting nitrates to molecular nitrogen.

Untreated domestic wastewater contains a large amount of nitrogen either in organic or inorganic forms such as ammonia, nitrates and nitrites [40]. Therefore, total nitrogen (TN) refers to the total amount of organic and inorganic fractions of nitrogen present in wastewater, while the term Kjeldahl nitrogen refers to the sum of organic and inorganic fractions of nitrogen from ammonium (NH_4^+) [22]. The principle sources of TN in domestic wastewater are urea and proteins. Although it is an important component required for the growth of microorganisms and plants, the excess

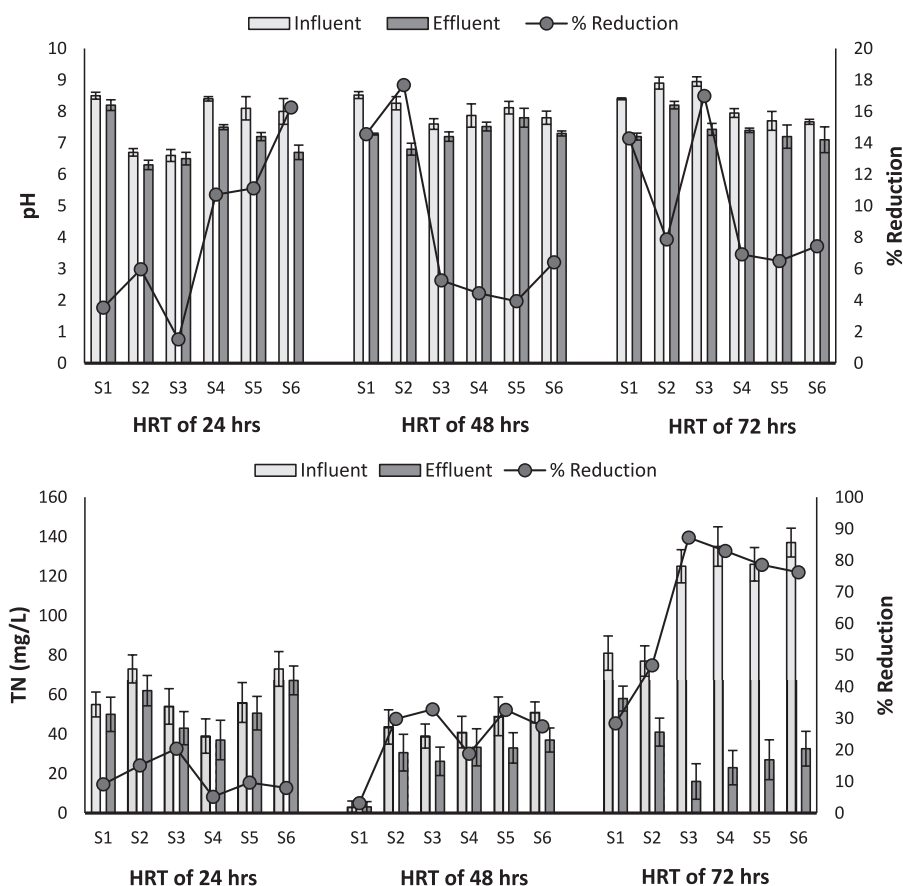


Fig. 5. Efficiency of pilot-scale TBF system in the average reduction of pH and TN content under different HRTs (bar indicates SD).

Table 1. Effects of different hydraulic retention times (HRT) on the performance of pilot-scale TFs with respect to average reduction of different physico-chemical parameters.

Parameters	S1			S2			S3			S4			S5			S6			
	I	E	% Efficiency	I	E	% Efficiency	I	E	% Efficiency	I	E	% Efficiency	I	E	% Efficiency	I	E	% Efficiency	
HRT-24 hrs	COD (mg/L)	202	74	63.4	169	68	59.8	185	73	60.5	276	42	84.6	82	24	70.7	72	16	77.7
	TDS (mg/L)	396	338	14.64	411	355	13.63	415	365	12.05	423	362	14.42	427	352	17.56	436	340	22.02
	EC (µS/cm)	616	479	22.24	607	433	28.66	598	503	15.88	555	477	14.05	579	501	13.5	596	511	14.26
	pH	8.5	8.2	3.5	6.7	6.3	5.97	6.6	6.5	1.52	8.4	7.5	10.7	8.1	7.2	11.11	8.0	6.7	16.25
	PO ₄ (mg/L)	1.9	1.82	4.21	2.43	1.4	42.38	2.96	0.98	66.89	0.9	0.1	88.8	1.0	0.7	30.0	3.0	2.03	32.33
	SO ₄ (mg/L)	60.0	53.0	11.67	62.7	61.0	2.71	55.0	49.0	11.0	54.0	47.0	12.96	57.0	51.0	10.53	54.0	38.48	28.74
HRT-48 hrs	TN (mg/L)	55.0	50.0	9.09	73.0	62.0	15.06	54.0	43.0	20.37	39.0	37.0	5.13	56.0	50.6	9.64	73.0	67.2	7.95
	COD (mg/L)	182	62	66	113	39	65.5	127	48	62.2	99	37	62.6	287	42	85.3	252	57	83.8
	TDS (mg/L)	370	306	17.3	433	379	12.5	356	292	18.0	418	336	19.6	488	382	21.7	610	373	38.85
	EC (µS/cm)	521	432	17.08	861	526	40.0	691	502	27.35	503	393	21.86	587	505	14.0	610	484	20.66
	pH	8.52	7.28	14.6	8.26	6.8	17.67	7.6	7.2	5.26	7.87	7.52	4.44	8.12	7.8	3.94	7.8	7.3	6.41
	PO ₄ (mg/L)	0.5	0.2	60.0	0.81	0.7	13.6	2.01	1.72	14.67	3.21	2.73	14.95	1.8	0.69	61.66	0.79	0.16	79.75
HRT-72 hrs	SO ₄ (mg/L)	169.0	164.0	2.96	152.0	151.0	0.66	170.0	159.0	6.47	243.0	191.0	21.4	233.0	166.0	28.75	346.0	176.0	49.13
	TN (mg/L)	3.2	3.1	3.13	43.6	30.6	29.82	39.0	26.2	32.82	41.0	33.3	18.78	49.0	33.0	32.65	51.0	37.0	27.45
	COD (mg/L)	170	85	50	140	107	23.57	189	85	55.02	286	55	80.76	249	51	80.25	238	47	80.25
	TDS (mg/L)	531	442	16.76	374	351	6.14	362	280	22.65	240	121	49.58	221	93	57.91	227	98	56.82
	EC (µS/cm)	744	620	16.66	528	497	5.87	512	394	23.04	338	310	8.28	353	303	14.16	339	300	11.50
	pH	8.4	7.2	14.28	8.9	8.2	7.86	8.95	7.43	16.98	7.95	7.4	6.91	7.7	7.2	6.49	7.67	7.1	7.43
	PO ₄ (mg/L)	0.165	0.085	48.5	0.46	0.18	60.86	2.48	0.12	95.16	2.99	0.11	96.32	1.77	0.07	96.05	2.13	0.12	94.36
	SO ₄ (mg/L)	47	45	4.25	52	50	3.84	105	49	53.33	98	52	46.93	101	43	57.42	109	48	55.96
	TN (mg/L)	81	58	28.39	77	41	46.75	125	16	87.2	135	23	82.96	126	27	78.57	137	32.6	76.20

Note: I = Influent sample, E = Effluent sample, S1, S2, S3, S4, S5, S6 = Codes of samples collected at different times during the study period.

Table 2. Overall efficiency of pilot-scale TFs in the average percentage reduction of different physico-chemical parameters under different HRTs.

HRTs	Avg. percentage (%) reduction in different parameters						
	COD (mg/L)	TDS (mg/L)	EC (μ S/cm)	pH	PO ₄ (mg/L)	SO ₄ (mg/L)	TN (mg/L)
24 hrs	69.45	15.72	18.09	8.17	44.1	12.93	11.20
48 hrs	70.9	21.32	23.49	8.72	40.77	18.22	24.10
72 hrs	61.64	34.98	13.25	9.99	81.87	36.96	66.68

amount of nitrogen in the effluent of a wastewater treatment system facilitates hypertrophication and algal growth and hence depletes the oxygen supply [41]. In the present study, it was observed that during the 1st phase of biological operation, an average reduction of 11.2% whereas 24.1% and 66.68% reduction in TN concentrations were observed during the 2nd and 3rd phases of biological operations, respectively (for an overview, see Table 2). Furthermore, in the present study the highest percentage reduction, i.e., 20.3, 32.8 and 87.2% in TN concentrations, were observed at HRTs of 24, 48 and 72 hrs, respectively, which was statistically highly significant ($p = 0.003$), as shown in Fig. 5. Different researchers have reported that the efficiency of a reactor for removing TN from wastewater largely depends on the ratio of COD to TN concentrations (COD/TN) along with reactor configuration [32, 42]. Ladu et al. [32] reported more than 75% TN removal efficiency in a sequencing batch reactor at a COD/TN ratio of greater than 3.9, while Wang et al. [42] reported a 37-41% reduction in TN concentration at a COD/TN ratio of 7.2. In the present study, 5.1-20.3% reduction in TN concentrations at COD/TN ratio of 0.9 were observed at HRT of 24 hrs and during the 2nd phase of operation (HRT of 48 hrs), 3.1-32.8% reduction in the concentrations of TN at COD/TN ratio of 1.75 while at HRT of 72 hrs, 28.4-87.2% reduction in the concentrations of TN at COD/TN ratio of 2.07 were observed. The subsequent increased in COD/TN ratio with HRT confirmed that the rate of nitrification increased, therefore in order to get optimum function of pilot scale TFs, maximum HRT of 72 hrs would be preferred.

Conclusions

We concluded from the present study that pilot-scale TFs showed significant efficacy regarding the removal of different physicochemical and microbiological parameters under HRTs of 24, 48 and 72 hrs. About 70.9 and 23.5% reduction in COD and EC content were observed at HRT of 48 hrs, respectively, whereas at HRT of 72 hrs the performance efficiency of pilot-scale TFs increased significantly in order to reduce different physicochemical parameters, i.e., TDS (34%), SO₄ (37%), PO₄ (81.8%) and TN (66.6%). Furthermore,

it was concluded that the efficiency of stone media pilot-scale TFs in terms of pathogen removal (CFU/mL) increased significantly with continuous recirculation of wastewater for an extended period of time. Moreover, stone as natural filter media showed proficiency at pilot-scale operations, thus stone media pilot scale TFs could be a promising and favorable technology for the treatment of wastewater – especially in water-stressed countries.

Acknowledgments

This research work was part of a project titled “Small-Scale Sewage Treatment and Wastewater Reuse System for Pakistan” and funded by the Higher Education Commission of Pakistan under the Pakistan-U.S. Science and Technology cooperation program Phase 4 projects [grant No. 4-428].

Conflict of Interest

The authors declare no conflict of interest.

References

1. PINTO U., MAHESHWARI B.L., GREWAL H.S. Effects of greywater irrigation on plant growth, water use and soil properties. *Resour Conserv Recycl.* **54** (7), 429, **2010**.
2. SALMA S., SHAH M.A., REHMAN S. Rainfall trends in different climate zones of Pakistan. *Pak J Meteorol.* **9** (17), **2012**.
3. ALKHAMISI S.A., AHMED M. Opportunities and challenges of using treated wastewater in agriculture. In *Environmental Cost and Face of Agriculture in the Gulf Cooperation Council Countries*. 109-123, **2014**.
4. WHO. Progress on sanitation and drinking water: 2015 update and MDG assessment. World Health Organization, **2015**.
5. QURESHI A.S. Water management in the Indus basin in Pakistan: challenges and opportunities. *Mt Res Dev.* **31** (3), 252, **2011**.
6. RAZA M., HUSSAIN F., LEE J.Y., SHAKOOR M.B., KWON K.D. Groundwater status in Pakistan: A review of contamination, health risks, and potential needs. *Crit. Rev. Environ Sci Technol.* **47** (18), 1713, **2017**.
7. PEREIRA A.S., CEREJEIRA M.J., DAAM M.A. Comparing ecotoxicological standards of plant protection

- products potentially toxic to groundwater life with their measured and modelled concentrations. *Ecotoxicol Environ Saf Abbreviation*. **102**, 152, **2014**.
8. USEPA. Guidelines for Water Reuse. United State Environmental Protection Agency, Washington, DC, **2012**.
 9. AWAN U.K., ANWAR A., AHMAD W., HAFEEZ M. A methodology to estimate equity of canal water and groundwater use at different spatial and temporal scales: a geo-informatics approach. *Environ Earth Sci*. **75** (5), 409, **2016**.
 10. ALI I., HADI F., BANO A. Microbial assisted phytoextraction of metals and growth of soybean (*Glycine max L. merrill*) on industrial waste water contaminated soil. *Pak J Botany*. **44** (5), 1593, **2012**.
 11. FINK R., ODER M., RANGUS D., RASPOR P., BOHINC K. Microbial adhesion capacity. Influence of shear and temperature stress. *Int J Environ Health. Res*. **25** (6), 656, **2015**.
 12. LOUPASAKI E., DIAMADOPOULOS E. Attached growth systems for wastewater treatment in small and rural communities: a review. *J Che Technol Biotechnol*. **88** (2), 190, **2013**.
 13. MAHMOUD M., TAWFIK A., EL-GOHARY F. Simultaneous organic and nutrient removal in a naturally ventilated biotower treating presettled municipal wastewater. *J Environ Eng*. **136** (3), 301, **2010**.
 14. NAZ I., ULLAH W., SEHAR S., REHMAN A., KHAN Z.U., ALI N., AHMED S. Performance evaluation of stone-media pro-type pilot-scale trickling biofilter system for municipal wastewater treatment. *Desalin Water Treat*. **57** (34), 15792, **2016**.
 15. XIANG Y., SHAO Z., KANG W., ZOU B., CHAI H. Effect of biofilm density on nitrous oxide emissions and treatment efficiency on sequencing batch biofilm reactor. *Wat Air Soil Pollut*. **227** (9), 304, **2016**.
 16. LADU J.L., LÜ X.W. Effects of hydraulic retention time, temperature, and effluent recycling on efficiency of anaerobic filter in treating rural domestic wastewater. *Water Sci Eng*. **7** (2), 168, **2014**.
 17. KHAN Z.U., NAZ I., REHMAN A., RAFIQ M., ALI N., AHMED S. Performance efficiency of an integrated stone media fixed biofilm reactor and sand filter for sewage treatment. *Desalin Water Treat*. **54** (10), 2638, **2015**.
 18. REHMAN A., NAZ I., KHAN Z.U., RAFIQ M., ALI N., AHMAD S. Sequential application of plastic media-trickling filter and sand filter for domestic wastewater treatment at low temperature condition. *Br Biotechnol J*. **2** (4), 179, **2012**.
 19. LUOSTARINEN S., SANDERS W., KUJAWA-ROELEVELD K., ZEEMAN G. Effect of temperature on anaerobic treatment of black water in UASB-septic tank systems. *Bioresour Technol*. **98** (5), 980, **2007**.
 20. SEGHEZZO L. Anaerobic Treatment of Domestic Wastewater in Subtropical Regions. Dissertation, Wageningen University, **2004**.
 21. DAIJA L., SELBERG A., RIKMANN E., ZEKKER I., TENNO T., TENNO T. The influence of lower temperature, influent fluctuations and long retention time on the performance of an upflow mode laboratory-scale septic tank. *Desalin Water Treat*. **57** (40), 18679, **2016**.
 22. RASOOL T., REHMAN A., NAZ I., ULLAH R., AHMED S. Efficiency of a locally designed pilot-scale trickling biofilter (TBF) system in natural environment for the treatment of domestic wastewater. *Environ Technol*. **39** (10), 1295, **2018**.
 23. APHA. Standard methods for the examination of water and wastewater. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington DC, USA, **2005**.
 24. HAMID H., ESKICIOGLU C. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. *Water Res*. **46** (18), 5813, **2012**.
 25. PERUJO N., ROMANÍ A.M., SANCHEZ-VILA X. Bilayer infiltration system combines benefits from both coarse and fine sands promoting nutrient accumulation in sediments and increasing removal rates. *Environ Sci Technol*. **52** (10), 5734, **2018**.
 26. PURNELL S., EBDON J., BUCK A., TUPPER M., TAYLOR H. Removal of phages and viral pathogens in a full-scale MBR: implications for wastewater reuse and potable water. *Water Res*. **100**, 20, **2016**.
 27. WHO. Guidelines for the safe use of wastewater, excreta and greywater (Vol. 1). World Health Organization, **2006**.
 28. LEONARD A. Management of wastewater sludge's: a hot topic at the European level. *J Residu Sci Technol*. **8** (2), **2011**.
 29. LUCAS F.S., THERIAL C., GONÇALVES A., SERVAIS P., ROCHER V., MOUCHEL J.M. Variation of raw wastewater microbiological quality in dry and wet weather conditions. *Environ Sci Pollut Res*. **21** (8), 5318, **2014**.
 30. RAJAKUMAR R., MEENAMBAL T., BANU J.R., YEOM I.T. Treatment of poultry slaughterhouse wastewater in up flow anaerobic filter under low up flow velocity. *Int J Environ Sci Technol*. **8** (1), 149, **2011**.
 31. LUIZ A., HANDELSMAN T., BARTON G., COSTER H., KAVANAGH J. Membrane treatment options for wastewater from cellulosic ethanol bio refineries. *Desalin Water Treat*. **53** (6), 1547, **2015**.
 32. LADU J.L.C., LU X.W., OSMAN A.M. Integrated Processes of Anoxic/Oxic Bioreactor and Artificial Wetland for Rural Domestic Wastewater Treatment. *Adv Mat Res*. (955), 2526, **2014**.
 33. LAING M. Investigating the performance of a novel Anaerobic Sequencing Batch Reactor (AnSBR) and optimization of operational parameters to treat synthetic winery wastewater (Doctoral dissertation, Stellenbosch: Stellenbosch University), **2016**.
 34. LEYVA-DÍAZ J.C., MUÑO M.M., GONZÁLEZ-LÓPEZ J., POYATOS J.M. Anaerobic/anoxic/oxic configuration in hybrid moving bed biofilm reactor-membrane bioreactor for nutrient removal from municipal wastewater. *Ecol Eng*. **91**, 449, **2016**.
 35. FERNANDES P.M., PEDERSEN L.F., PEDERSEN P.B. Influence of fixed and moving bed biofilters on micro particle dynamics in a recirculating aquaculture system. *Aquacult Eng*. **78**, 32, **2017**.
 36. SHI J., PODOLA B., MELKONIAN M. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *J Appl Phycol*. **19** (5), 417, **2007**.
 37. ZENG R.J., YUAN Z., KELLER J. Enrichment of denitrifying glycogen-accumulating organisms in anaerobic/anoxic activated sludge system. *Biotechnol Bioeng*. **81** (4), 397, **2003**.
 38. EHLERS G.C., TURNER S. Biofilms in wastewater treatment systems. *Microbial Biofilms-Current Research and Applications*, 99, **2012**.
 39. USEPA. Bureau of water supply and wastewater management: department of environmental protection

- agency, wastewater treatment plant operator training. United State Environmental Protection Agency, **2007**.
40. GANGAL M., KALDATE A., GRAVELEAU L., DESMOTTES C. Evaluation of Low Energy Requirements in Deammonification Systems. Proceedings of the Water Environment Federation. **2**, 1, **2015**.
41. SAKUMA T., JINSIRIWANIT S., HATTORI T., DESHUSSES M.A. Removal of ammonia from contaminated air in a biotrickling filter-denitrifying bioreactor combination system. Water Res. **42** (17), 4507, **2008**.
42. WANG H., ZHI W., DENG N., JI G. Review on the Fate and Mechanism of Nitrogen Pollutant Removal from Wastewater Using a Biological Filter. Pol J Environ Stud. **26** (5), **2017**.

