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Biosand Filter as a Point-of-Use Water Treatment Technology: Influence of Turbidity on Microorganism Removal Efficiency

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Abstract: The number of people living without access to clean water can be reduced by the implementation of point-of-use (POU) water treatment. Among POU treatment systems, the domestic biosand filter (BSF) stands out as a viable technology. However, the performance of the BSF varies with the inflow water quality characteristics, especially turbidity. In some locations, people have no choice but to treat raw water that has turbidity above recommended levels for the technology. This study aimed to measure the efficiency with which the BSF removes microorganisms from well water and from fecal-contaminated water with turbidity levels of 3, 25, and 50 NTU. Turbidity was controlled by the addition of kaolin to water. Turbidity removal varied from 88% to 99%. Reductions in total coliform (TC) and *Escherichia coli* ranged from 0.54–2.01 and 1.2–2.2 log removal values (LRV), respectively. The BSF that received water with a higher level of turbidity showed the greatest reduction in the concentration of microorganisms. Additional testing with water contaminated with four bacterial pure cultures showed reductions between 2.7 and 3.6 LRV. A higher reduction in microorganisms was achieved after 30–35 days in operation. Despite the filter's high efficiency, the filtrates still had some microorganisms, and a disinfection POU treatment could be added to increase water safety.

Keywords: biosand filter; point-of-use treatment; drinking water; microbiological contamination; turbidity; developing world

1. Introduction

Despite numerous investments, about 2.1 billion people in the world still consume drinking water from sources contaminated with feces [1], and about 2.3 billion people still lack basic health infrastructure. Furthermore, some 159 million people, mainly from rural areas and principally in developing countries, still drink water collected directly from surface sources and shallow, unsafe wells [1,2]. There are high rates of gastrointestinal and parasitic diseases, as well as malnutrition associated with the consumption of water contaminated by microbial pathogens [3,4].

Waterborne diseases remain the leading cause of mortality worldwide, registering more than 2.2 million deaths per year [5], with diarrhea being responsible for 1.5 million deaths [6]. Most cases occur in developing countries and affect children under five years of age [3]. Point-of-use (POU) water treatment technologies have been recommended as an effective solution for the provision of safe water in places where families do not have access to conventional systems for the treatment and supply of drinking water [7].

The biosand domestic filter (BSF) is one water treatment method that is applied at the point of use and has been classified as one of the main and most popular water treatment options, due to its effectiveness, simplicity of operation, ease of construction, and potential for using local materials. Between the years 1991 and 2015, more than 500,000 units were distributed worldwide [8,9].

The BSF is similar to a conventional slow sand filter (SSF), except that its operation is intermittent. It can be installed in plastic or concrete containers and has an elevated outlet tube located above the sand layer for discharge. This arrangement keeps water above the top sand layer, allowing saturation throughout the filter depth. A biofilm develops around sand particles on the filter top layer, where oxygen and nutrients are available [8,9]. This biofilm, also known by the German word schmutzdecke, is composed of a mat of slimy material, mostly organic, through which influent water must pass. The biofilm matrix includes bacteria, fungi, protozoa, algae, and aquatic insect larvae. The elimination of pathogens present in raw water is attributed to the biological action of this biofilm [10]. Further suspended solids removal from water is achieved by mechanical trapping [11,12]. Schmutzdecke matures in about 30 days, a period in which the filter does not operate optimally [13,14].

Several studies have shown that the BSF can reduce the occurrence of diarrhea by 47–54% [15,16], and it is capable of removing more than 95% of the turbidity, with 1 log removal value (LRV) for viruses, 2 LRV for bacteria, and more than 2 LRV for protozoa [17]. Turbidity in raw water is one of the factors that can limit the ability of the BSF to produce safe drinking water, creating conditions unfavorable for development of biofilm and filling pore spaces.

In Brazil, drinking water regulations require a turbidity level below 1 NTU for slow sand filtration (SSF). Di Bernardo et al. [18] suggest the use of the SSF for treating water with a turbidity level between 5 and 20 NTU. For values exceeding 50 NTU, the authors recommend full water cycle treatment. However, households do not always have the option to find raw water with less than 20 NTU, and they need to treat water at the point-of-use.

Considering the potential of the BSF, this study aimed to investigate the use of the BSF to treat fecal-contaminated waters with varying degrees of turbidity, and natural well water.

2. Materials and Methods

2.1. Filter and Media Preparation

The four filters used in the experiments were designed according to the Center for Affordable Water and Sanitation Technology (CAWST) [13]. The filters' containers were made of acrylics, a material that was available in the laboratory. The containers had 10 mm thickness, 300 mm inside diameter and 990 mm height. The containers were covered with low density polyethylene plastic to prevent algal growth on the walls. A diffuser plate was installed in each of the BSF by means of a trapezoidal polyethylene bucket with top and bottom diameters of 300 mm and 200 mm, respectively. The bucket was placed with its upper part at the top of the container. The bottom of the bucket was perforated with 2 mm holes, to ensure that feed water was evenly spread onto the filter top surface (Figure 1).

As support layers, the filters were filled with 8 cm of gravel of size 6–12 mm, 7 cm of granular gravel of size 1–6 mm, and 6 cm of coarse sand of size 1 mm. Above this support base, it was placed 46 cm of fine sand with an effective size (D10) of 0.11 mm and a uniform coefficient of 0.16 mm. All fillings were sieved, washed, and dried, before being placed into the filters. Granulometric tests of coarse and fine sands were made according to the methodology described in NBR 11799 [19]. Standing water was kept 4.8 cm above filter surface. The outlet tube with 19 mm diameter was elevated at the same standing water level above the filter sand layer. This elevation enabled a layer of water to be maintained above the top of the sand, regardless of whether the filter was operating or not. When in operation, water coming from the perforated bucket maintained pressure to force the flow through the pores of the filter. The flow rate slowed down as the operation approached the resting period. When the water finally stopped flowing, the standing water layer had the same height as that of the end of the outlet tube. The average surface application rate was approximately 1.44 $m^3/m^2/day$.

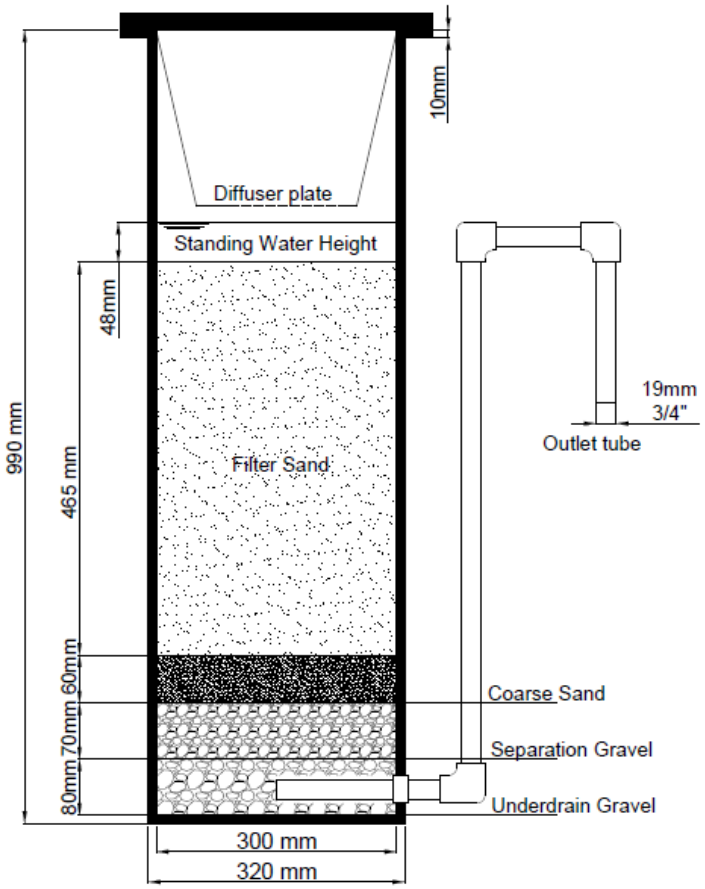


Figure 1. Cross section of the acrylic household scale biosand domestic filter (BSF) used in the experiments.

2.2. Feed Water

Filter influent consisted of water from a well (BSF1) and dechlorinated tap water that had been contaminated with filtered secondary effluent from a local wastewater treatment plant (BSF 2, 3, and 4). Dechlorination was achieved using sodium thiosulfate ($Na_2S_2O_3$). The ratio of dechlorinated tap water to effluent was 9:1. Kaolin solutions in concentrations of 5, 50 and 90 mg/L were added to BSF 2, 3, and 4 to achieve turbidity of 3, 25, and 50 NTU, respectively. The air and water temperature could not be controlled. While the average room temperature ranged between 12 and 25 °C during the experimental work, water temperature varied between 17 °C and 18 °C.

During a three-month period of operation, filters were dosed daily with 12 L of influent water. In the first 45 days, filters operated continuously for 24 h followed by a resting period of 24 h. From days 46 to 90, the BSFs were fed for 24 h with a pause period of 48 h. Images of the grains of sand were obtained by scanning electron microscopy (SEM) on days 1, 45, and 90 of BSF operation. BSF4 continued to operate for an additional 30 days after closing BSF 1, 2, and 3.

From days 120 to 125, BSF4 received 20 liters of water with 50 NTU turbidity, free of chlorine and bacteria. In days 124 and 125, samples of filtered water were collected and analyzed. No colony forming units (CFU) were detected. After confirmation that BSF4 effluent was free of CFU, 20 L of dechlorinated tap water with a turbidity of 50 NTU was contaminated by adding *Escherichia coli* ATCC® 25922 (*E. coli*), *Salmonella* Typhimurium DT177 (*S.* Typhimurium), *Enterococcus Faecalis* ATCC® 29212 (*E. faecalis)*, and *Pseudomonas aeruginosa* ATCC® 27853 (*P. aeruginosa)* to a final concentration of approximately 2.5×10^7 CFU/mL. Each bacterium was first cultivated in Tryptic Soy agar for 24 h at 37 °C. Two milliliters of brain heart infusion (BHI) culture medium was inoculated with three colonies of each bacteria and incubated at 37 °C for 18 h. Each bacteria species was cultivated separately.

BSF4 operated continuously while receiving the contaminated influent for five consecutive days with a resting period of 24 h. Samples from the filtrate were collected on days three, four and five, kept in 50 mL Falcon tubes and refrigerated. They were analyzed within a maximum of two hours after collection. They were submitted to five serial dilutions, as described in the literature [20]. The drop plate seeding technique was implemented to inoculate the selected agar plates, BEM (eosin methylene/blue agar—Levine), XLD (xylose deoxyclolate agar), BEA (Bili Scullin agar), cetrimide, and TSA (Tryptone Soy Agar) for *E. coli*, *S.* Typhimurium, *E. faecalis*, *P. aeruginosa* and TC, respectively. The plates were incubated at 35 °C and checked for CFU after 18 h. The non-growing plates remained in the incubator for additional 18 h. The number of colonies counted was multiplied by the respective dilution factor to calculate the concentration in CFU/mL. Three replicates were performed for each sample.

Figure 2 presents the experimental timeline and influent water preparation, including the concentrations of kaolin that were added to achieve the target turbidity.

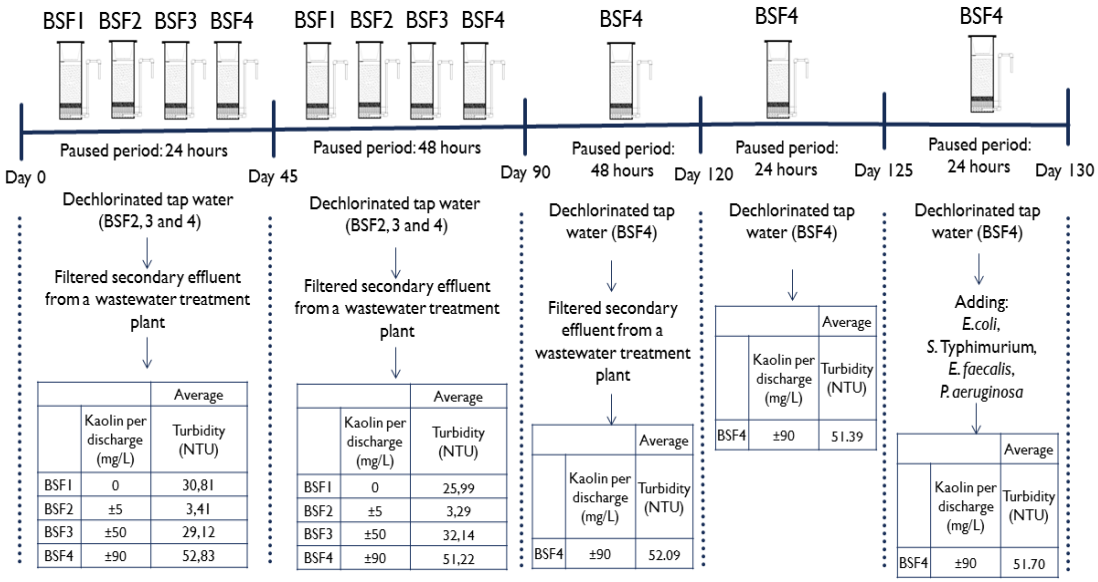


Figure 2. Experiment timeline and influent water preparation.

2.3. Statistical Analysis

The arithmetic mean and standard deviation were used for all data except microbiological data, for which the geometric mean was used. The percent reduction was calculated using source and filtered water (Influent-Effluent/Influent × 100) from each BSF for all water quality parameters. Logarithm removal values (LRV) and percent reductions were calculated for bacterial removal.

The Student's t-test (two-tailed) was used to evaluate the significance of the differences between the mean of most probable number (MPN) values detected before and after water filtration. A probability level of less than 0.05 (p-value < 0.05) was considered to be significant.

To analyze the influence of turbidity on the removal of total coliform (TC) and *E. coli*, an adjustment of mixed models was used. Separate means of responses were analyzed both for BSF and time (Time 1 before filtration and Time 2 after filtration), in addition to the interaction means between filter and time. Since the variable TC did not reach the assumption of normality, the log-normal distribution was used. Assumptions of normality are called mixed generalized linear models [21].

2.4. Monitoring

Table 1 presents the parameters that were analyzed, together with the methods and instruments used. The analyses were performed according to the methodologies of the Standard Methods for Examination of Water and Wastewater [22].

Table 1. Equipment and materials used for the water quality analysis.

Parameter/Analysis	Methodology	Model	Unit
Turbidity	2130 B—Nephelometric	HACH 2100—Turbidimeter	NTU
pH	4500-H+ B—Electrometric	DIGIMED, DM-22	-
Abs UV 254 nm	5910 B—UV Absorption	UV 1600	-
Alkalinity	2320 B—Titrimetric		mg $CaCO_3$/L
Total Coliforms	Colilert®	Colilert®	MPN/100 mL
E. Coli	Colilert®	Colilert®	MPN/100 mL
Nitrate	Ion chromatography	Metrohm™ 882	mg/L NO_3^-
SEM	-	MEC JSM 6060	-
E. coli *	Drop plate	BEM agar	CFU/mL
S. Typhimurium *	Drop plate	XLD agar	CFU/mL
E. Faecalis *	Drop plate	Bili Esculin agar	CFU/mL
P. aeruginosa *	Drop plate	Cetrimide agar	CFU/mL

* Only for BSF4.

3. Results and Discussion

Table 2 presents the results for water quality variables monitored in the influent and effluent of the filters.

Table 2. Summary of influent and effluent water quality variables measured in the BSF experiment.

	BSF1		BSF2		BSF3		BSF4	
	Influent	**Effluent**	**Influent**	**Effluent**	**Influent**	**Effluent**	**Influent**	**Effluent**
Temperature (°C)								
Mean	17.7	17.8	17.4	17.7	17.7	17.5	17.8	17.9
Std deviation	3.1	3.2	3.0	3.1	3.1	3.0	3.0	3.0
n	50	50	50	48	47	48	47	50
Turbidity (NTU)								
Mean	29.1	0.9	3.4	0.4	30.2	0.4	52.3	0.5
Std. deviation	7.3	0.4	0.9	0.2	6.1	0.2	10.5	0.2
n	54	54	53	53	53	52	53	52
pH								
Mean	7.3	7.4	6.7	6.2	6.5	6.1	6.2	5.9
Std. deviation	0.5	0.4	0.5	0.5	1.4	0.9	1.8	1.5
n	53	52	53	51	53	51	53	51
Alkalinity (mg $CaCO_3$/L)								
Mean	103	99	13	6	13	6	12	6.7
Std. deviation	16	14	5	4	5	4	5	4
n	50	49	49	47	48	47	48	47
UV_{254} Absorbance								
Mean	0.169	0.047	0.119	0.045	0.151	0.049	0.172	0.046
Std. deviation	0.048	0.037	0.043	0.018	0.032	0.017	0.049	0.017
n	41	42	42	42	39	40	40	40
Nitrate								
Mean	2.33	1.65	2.29	1.07	2.29	1.32	2.28	1.25
Std. Deviation	2.12	0.06	0.33	0.68	0.25	0.59	0.26	0.53
n	7	3	10	5	11	5	11	8
TC (MPN/100 mL)								
Mean	105	30	1706	99	1589	123	1779	17
Std. deviation	5	14	17	9.3	17	7	19	21
n	10	10	10	10	10	10	10	10
E. Coli (MPN/100 mL)								
Mean	15	1	447	22	471	4.3	506	3
Std. deviation	3	15	30	4	32	10	35	9
n	10	10	10	10	10	10	10	10

3.1. Turbidity Removal

Figure 3 shows turbidity values measured in the influent and effluent of each filter along the experiment. Most filtrates had turbidity lower than 1.0 NTU. Following day 30, the turbidity in filtrates remained approximately constant, except for some lower values measured after day 80. The ripening of the filters may have occurred after day 30, but this was not confirmed by SEM images. The removal rates measured in this study were comparable with values described in the literature [12,23,24].

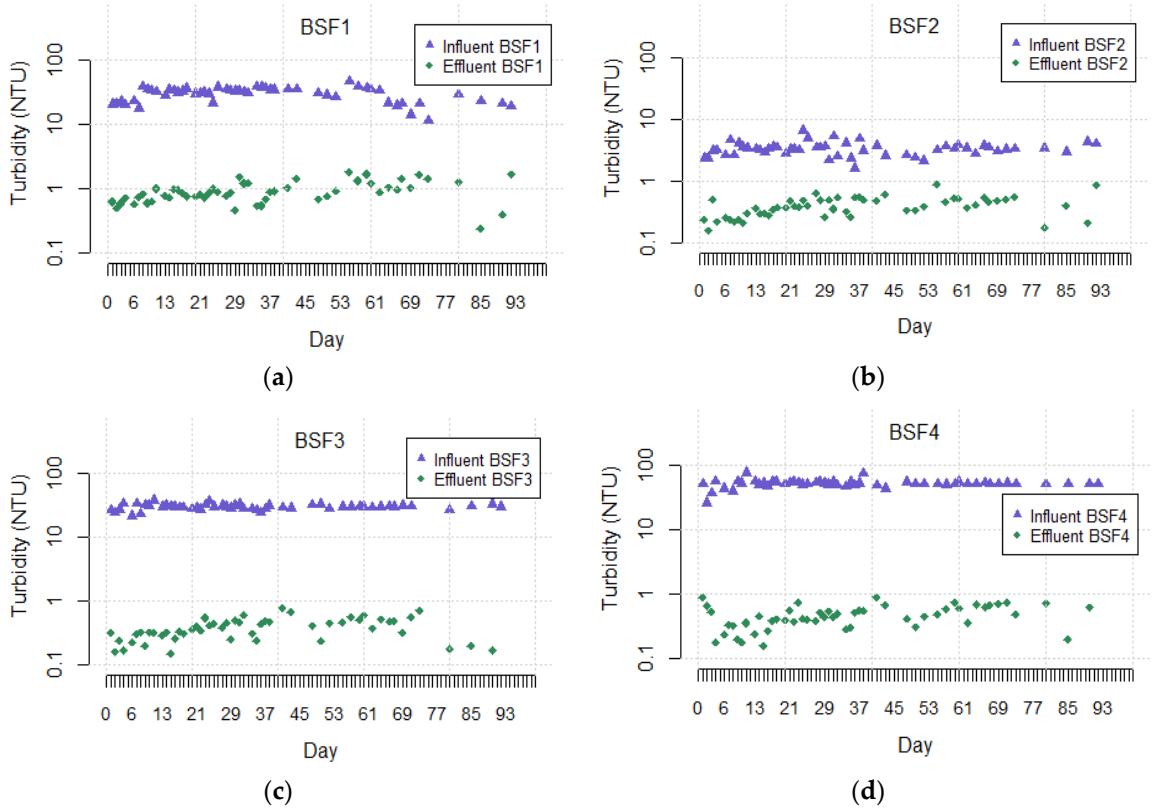


Figure 3. Influent and effluent turbidity over the course of the experiment: (**a**) BSF1, (**b**) BSF2, (**c**) BSF3 and (**d**) BSF4.

3.2. pH

Figure 4 shows the influent and effluent pH values for BSFs throughout the experiment. Filtrates from BSF2–4 had consistently lower pH values than the influent, showing that some acid-forming reaction was occurring within the filter medium. Reductions in pH values were also observed by Kennedy et al. [12].

3.3. Alkalinity

Figure 5 shows the alkalinity concentrations in the filters' influent and effluent. BSF1 had an average influent water alkalinity of 103 mg/L $CaCO_3$, and did not show a significant decrease throughout the entire operation. BSF2, BSF3 and BSF4 showed a reduction in alkalinity concentrations of approximately 50%. This suggests that an acid-forming reaction occurred within the filter media.

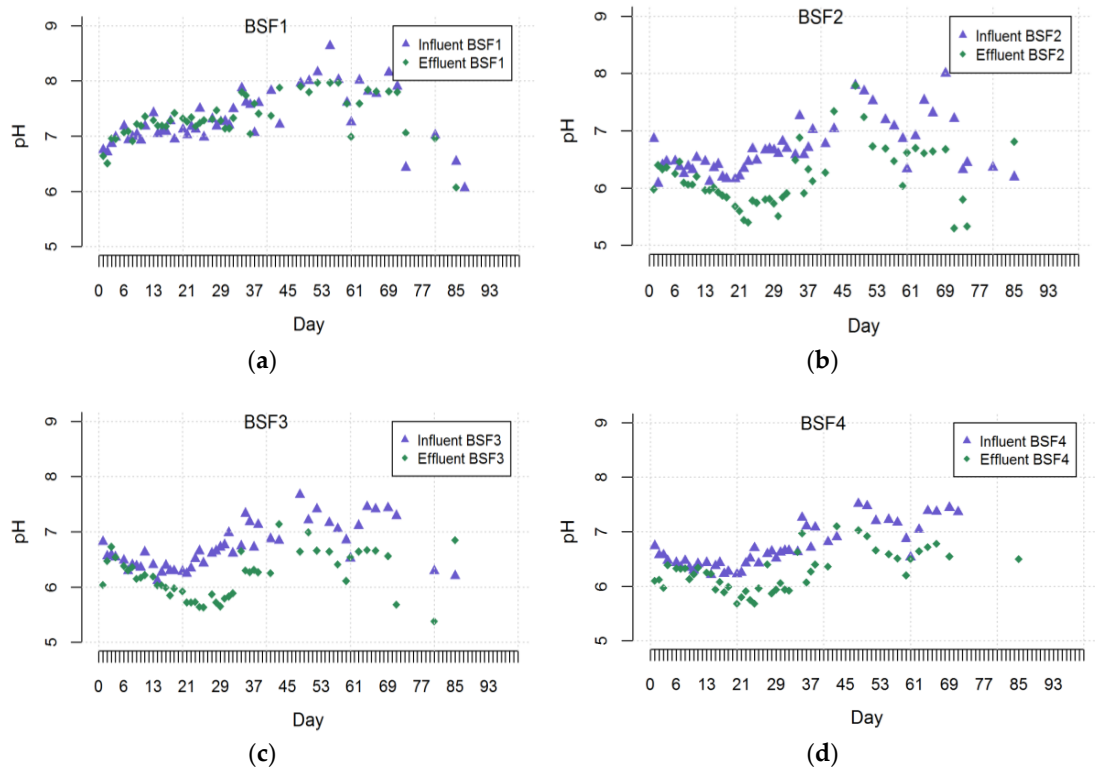


Figure 4. Influent vs. effluent pH over the course of the experiment: (**a**) BSF1, (**b**) BSF2, (**c**) BSF3, and (**d**) BSF4.

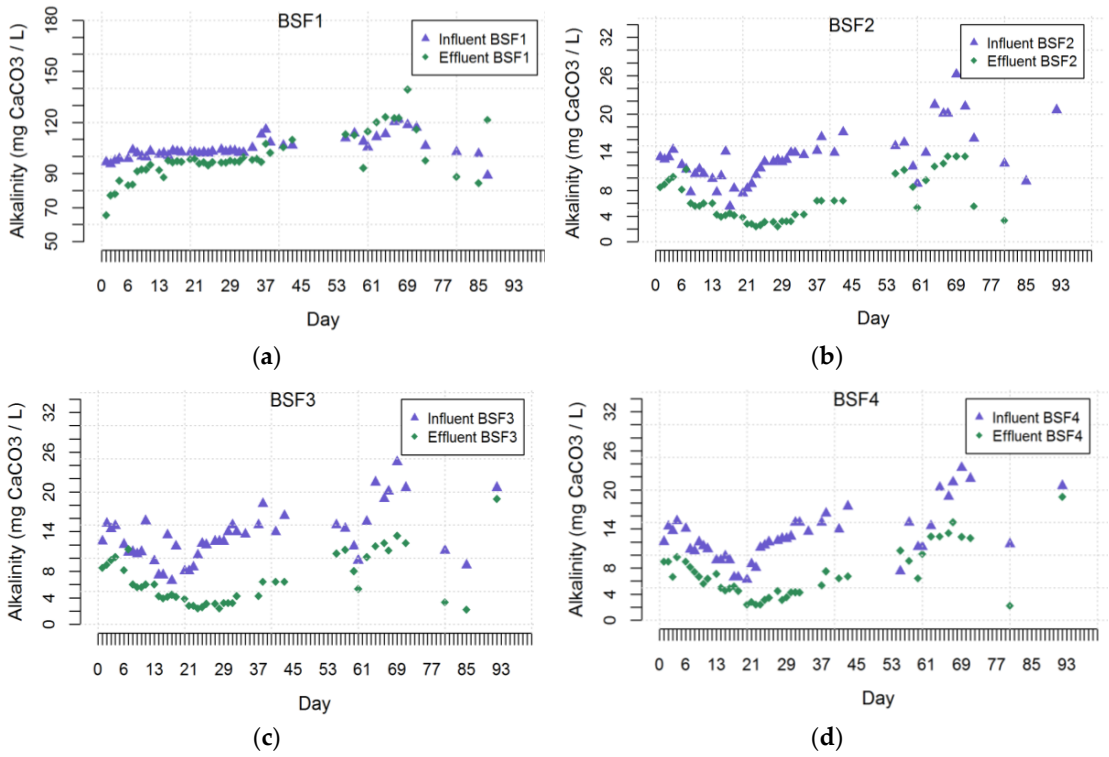


Figure 5. Comparison of alkalinity in influent vs. effluent over the course of the experiment: (**a**) BSF1, (**b**) BSF2, (**c**) BSF3, and (**d**) BSF4.

3.4. UV_{254} Absorbance

Figure 6 presents data on ultraviolet light absorption at a wavelength (UV_{254}) of 254 nm. Organic compounds with an aromatic structure or conjugated double carbon bonds are absorbed by UV_{254} [25]. Filtered absorbance values were reduced by 72%, 62%, 68% and 73%, with respect to influent absorbance for BSF1 to BSF4, respectively. The reductions showed that filters were retaining dissolved organic matter. Lynn et al. [23] measured a reduction in absorbance of 35% in BSF—lower than the observed in this study.

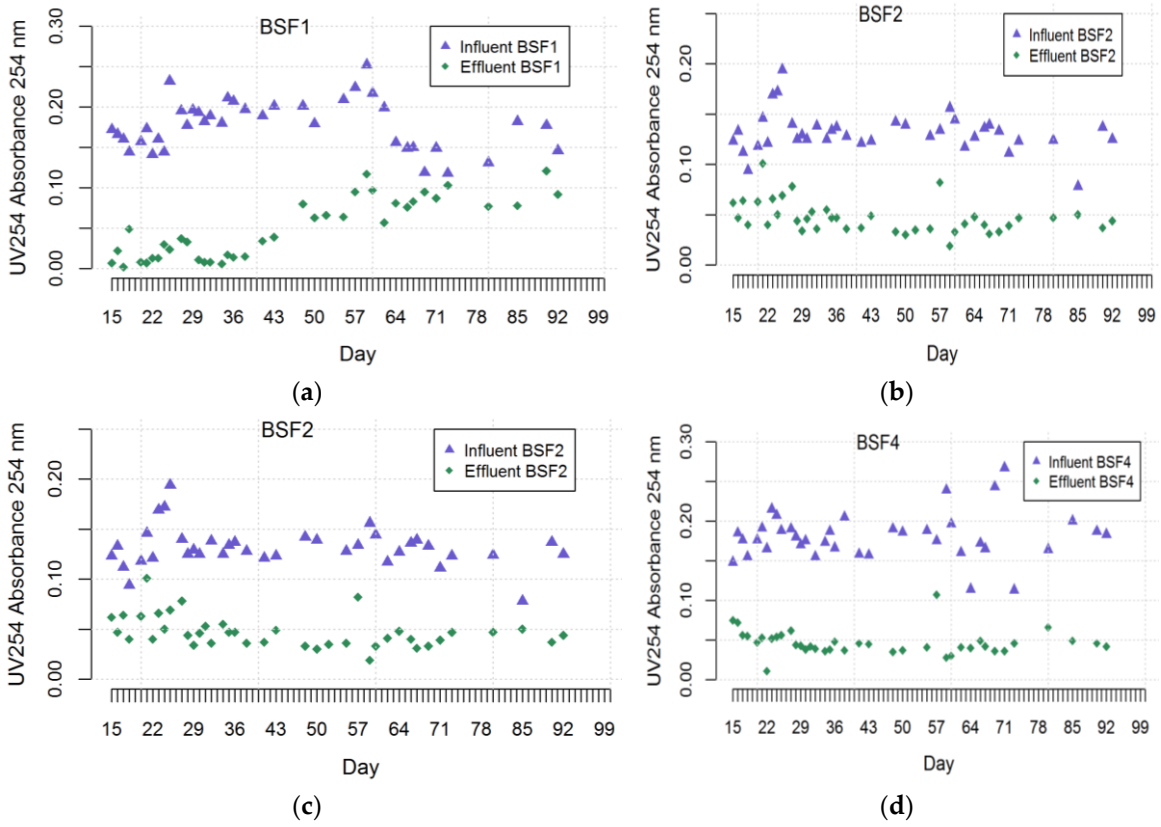


Figure 6. Influent vs. effluent absorbance at 254 nm over the course of the experiment: (**a**) BSF1, (**b**) BSF2, (**c**) BSF3 and (**d**) BSF4.

3.5. Nitrate

Nitrate concentrations in filtrates from BSF were consistently lower than those in influent, with reductions varying from 30–53%. The reductions in nitrate suggested that denitrification occurred in the lower parts of the filters, where an oxygen deficit could be present. This observation is compatible with the pH reduction and alkalinity consumption that occurred during filtration. In previous studies, the simultaneous occurrence of nitrification/denitrification in BSF has been suggested due to the availability of oxygen at the top layer, and its deficiency at lower depths [26,27].

3.6. Total Coliform Reduction

Figure 7 shows log removal values for total coliforms (TC) for the four filters during the experiment. Reduction efficiencies increased in each filter over the course of the experiment. The average reduction for BSF1 was 0.54 log (71.6%), while the other BSF showed greater average reductions, 1.23 log (94.2%), 1.11 log (92.3%) and 2.01 log (99.0%) for BSF2, BSF3, and BSF4, respectively.

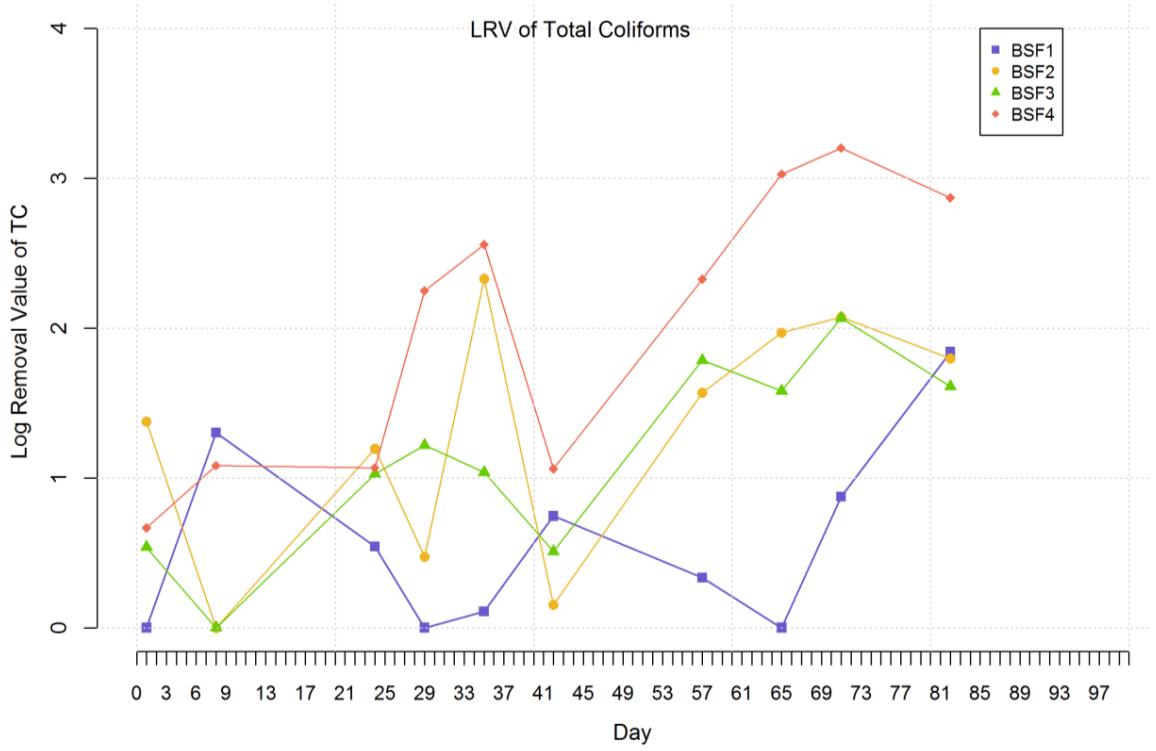


Figure 7. Log removal values of total coliforms throughout the experiment.

From day 0 to day 45, the TC reduction efficiencies for BSF2, BSF3 and BSF4 were 64.2%, 69.2% and 92.0%, respectively. From day 45 onwards, the operation cycle of the filters changed from a 24 h–24 h to a 24 h–48 h resting period. In Figure 7, it is possible to see the improvement in TC removal—98.4%, 98.1% and 99.8% for filters BSF2, BSF3 and BSF4, respectively. The higher efficiency in TC reduction implies that the BSF worked better with the change of the pause period from 24 h to 48 h. The improvements could also be related to the filter ripening.

In day 43, there were TC LRV drops in BSF 2, 3 and 4. In the same day, there was a rise in LRV for BSF1. There was no specific reason that could justify the decreases. Coliforms analyses have some variability, but it was unlikely to be the explanation.

In days 9 to 39, and 43 to 65, there were drops in TC LRV in BSF1. This filter, fed with well water, had influent with lower TC concentrations than BSF2, 3 and 4, which were contaminated with treated wastewater effluent. In general, rises and declines in TC LRV in BSF2, 3 and 4 occurred together and independently from BSF1.

BSF4, which had the highest level of turbidity (50 NTU), performed better than the other BSF in terms of coliform reduction. Its efficiency was comparable to the typical interval of 93–99% documented by other researchers [8,28–31].

To analyze the influence of turbidity on the removal of microorganisms, and to be able to differentiate the results according to BSF, an adjusted generalized mixed model was used with statistical analysis system (SAS) statistical software. The total coliforms variable did not reach the assumption of normality, and a log-normal distribution was used to generate the mixed generalized linear model. Figure 8 shows the results in terms of the variation in the mean value of the TC concentration of the four BSF at Time 1 (before filtration) and Time 2 (after filtration). It can be seen in this graph that the variation in TC concentrations during the two periods of BSF1 did not change significantly. However, the mean values of the responses of the other BSFs showed differences, meaning that there is statistical evidence to state that at least one filter average, time and filter interaction with time differed from the others.

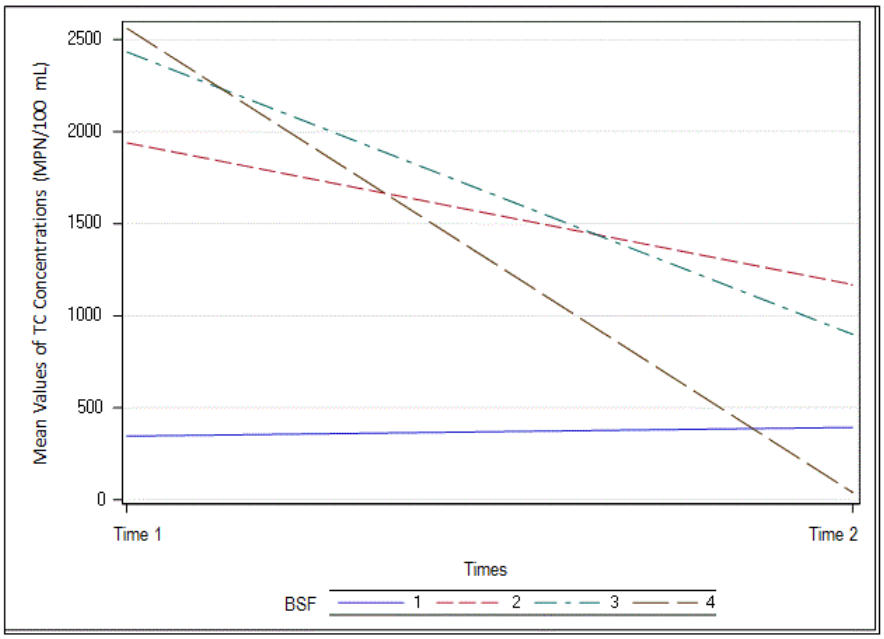


Figure 8. Analysis of variance for repeated measures by mixed models of total coliforms (TC).

As seen in Table 3, there is statistical evidence to state that at least one filter interaction with time (BSF*Time) differed from the others due to the fact that p is less than 0.05.

Table 3. Test of fixed effects of total coliforms.

Effect	Number Degrees of Freedom	F Value	$p > F$
BSF	3	4.13	0.0130
Time	1	95.99	<0001
BSF*Time	3	6.33	0.0015

Since some of the average values for the response variable (TC) of the BSF interactions with time were different from the others, a post-hoc test with the Tukey Kramer adjustment was performed, to determine where these differences were.

As shown in Table 4, for Time 1 (before filtration), BSF1 was significantly different from all other BSF, since all p values were less than 0.05. This was to be expected, since the water that was treated by BSF1 had a different origin than the others, namely, a well. At Time 1, there were no significant differences in the means of BSF2, BSF3 and BSF4, which were the filters that received influent water with similar concentrations of microorganisms: 1706, 1589 and 1779 MPN/100 mL, respectively.

For Time 2 (filtered water), the means of the response variables between BSF1 and other BSFs were not significant, because p values were always greater than 0.05. The interaction between BSF2 and BSF3, which had TC concentrations of 99 and 123 MPN/100 mL, respectively, was not considered significant ($p > 0.05$). However, the mean concentration of BSF4, 17 NMP/100 mL, was significantly different from that of BSF3.

Table 4. Simple effect comparisons of filter ime least squares means by time adjustment for multiple comparisons: Tukey.

Simple Effect Level	BSF	BSF	Standard Error	$p > \|t\|$
Time 1	1	2	0.4693	<0.0001
Time 1	1	3	0.4693	<0.0001
Time 1	1	4	0.4693	<0.0001
Time 1	2	3	0.4693	0.8801
Time 1	2	4	0.4693	0.9302
Time 1	3	4	0.4693	0.8116
Time 2	1	2	1.0199	0.2446
Time 2	1	3	1.0199	0.1727
Time 2	1	4	1.0376	0.4351
Time 2	2	3	1.0199	0.8361
Time 2	2	4	1.0376	0.0588
Time 2	3	4	1.0376	0.0378

3.7. *Escherichia coli*

Figure 9 presents log removal values for *E. coli* in the four filters over the course of the experiment. It shows that reduction efficiencies increased with time. The average reduction for the BSF1 was 1.2 log (93.5%), while the other BSFs showed greater reductions—1.3 log (95.0%), 2.0 log (99.1%) and 2.2 log (99.4%) for BSF2, BSF3 and BSF4, respectively. The *E. coli* concentration in BSF1 filtrate was the lowest among the filters, 1 MPN/100 mL, probably because it had a low influent concentration, 15 MNP/100 mL.

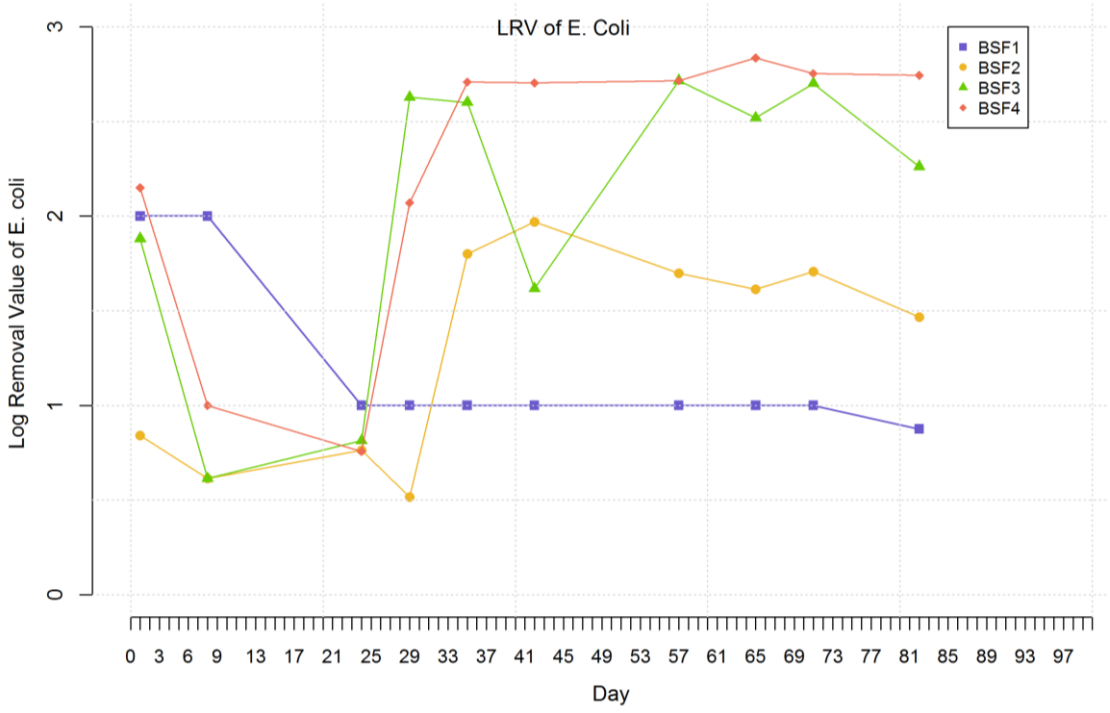


Figure 9. Log removal value of *E. coli* throughout the experiment.

From day 0 to 35, the average *E. coli* reduction efficiencies for BSF2, BSF3 and BSF4 were 78.4%, 89.7% and 92.7%, respectively. After day 35, the mean reductions for the same BSFs increased to 97.9%, 99.4% and 99.8%, respectively. These results showed that the BSFs worked better from day 35 onwards. According to the CAWST [13], the time required for the biofilm to mature is 30 days.

It is worth noting that BSF 3 and 4, which had higher influent turbidity, were more efficient than BSFs with lower turbidities. BSF4, in particularly, with a turbidity of 50 NTU, performed better than

all other BSFs. The *E. coli* reductions observed in this study compare well with the typical reduction interval of 93–99% documented by other researchers [8,29–31].

To analyze the influence of turbidity on the removal of *E. coli* and to be able to differentiate the results with respect to BSF, an adjustment of the generalized mixed models was used. The *E. coli* variable reached the assumption of normality, so it was decided to use the gamma distribution to generate the mixed generalized linear model.

For *E. coli*, the results were slightly different from the results found for total coliforms. Figure 10 shows the variations in the average values of the *E. coli* concentrations of the four BSFs at Time 1 (before filtration) and Time 2 (after filtration). The variation in the concentration of *E. coli* during the two periods of BSF1 did not change significantly. As mentioned before, the well water had low concentration of *E. coli*, with a geometric mean of 15 MPN/100 mL.

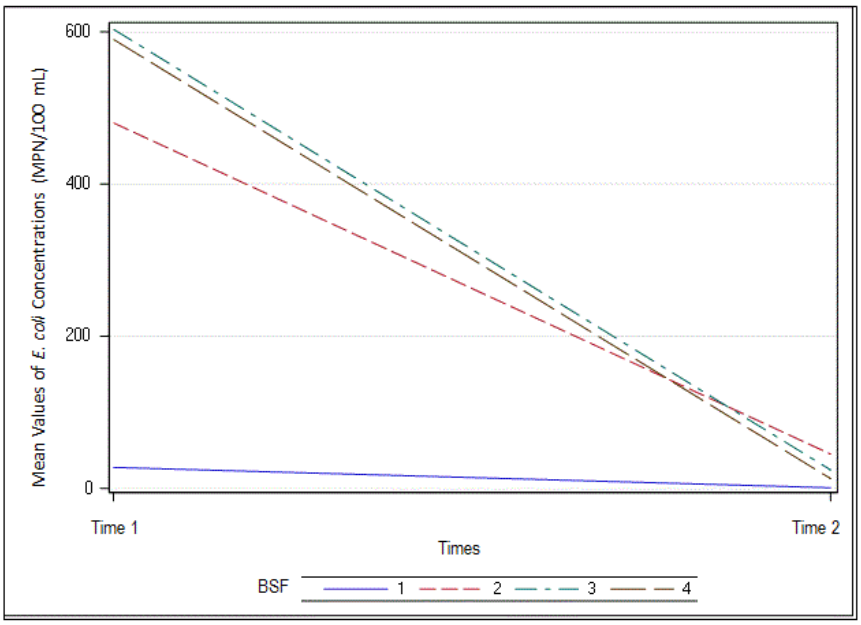


Figure 10. Analysis of variance for repeated measures by mixed models of *E. coli*.

Table 5 shows that there was no significant difference for the interaction of BSF and Time (BSF*Time) for the response variable *E. coli* concentration. There were significant differences between Time 1 and Time 2, which was expected since the influent water quality (Time 1) greatly differed from those of the BSF filtrates (Time 2). It is possible that there was no significant difference in the interaction of BSF and Time, because all BSFs had similar removal efficiencies. This might be due to the low *E. coli* concentration in all filtrates: 1, 22, 4, and 3 MPN/100 for BSF1, BSF2, BSF3 and BSF4, respectively.

Table 5. Test of fixed effects of *E. coli*.

Effect	Number Degrees Freedom	F Value	Pr > F
BSF	3	29.07	<0.0001
Time	1	132.41	<0.0001
BSF*Time	3	1.19	0.3270

Since none of the mean values for the response variable (*E. coli*) of the BSF interaction with time were different from the others, the post-hoc test with the Tukey Kramer adjustment for the variable *E. coli* was not performed.

3.8. Reduction in Salmonella Typhimurium DT177, Enterococcus faecalis ATCC® 29212, Pseudomonas aeruginosa ATCC® 27853 and Escherichia coli ATCC® 25922

Figure 11 shows the concentrations of bacteria in raw and filtered water in BSF4. There were significant reductions for all bacteria. The CFU of *P. aeruginosa* was not detected in the filtered water in all repetitions of the tests. The logarithmic removal units for *E. coli*, *S.* Typhimurium, *E. faecalis*, and TC were 2.7, 3.6, 2.8 and 3.0, respectively. The log removals for these bacteria were similar to those observed for *E. coli* in BSF4 after day 35 (Figure 9).

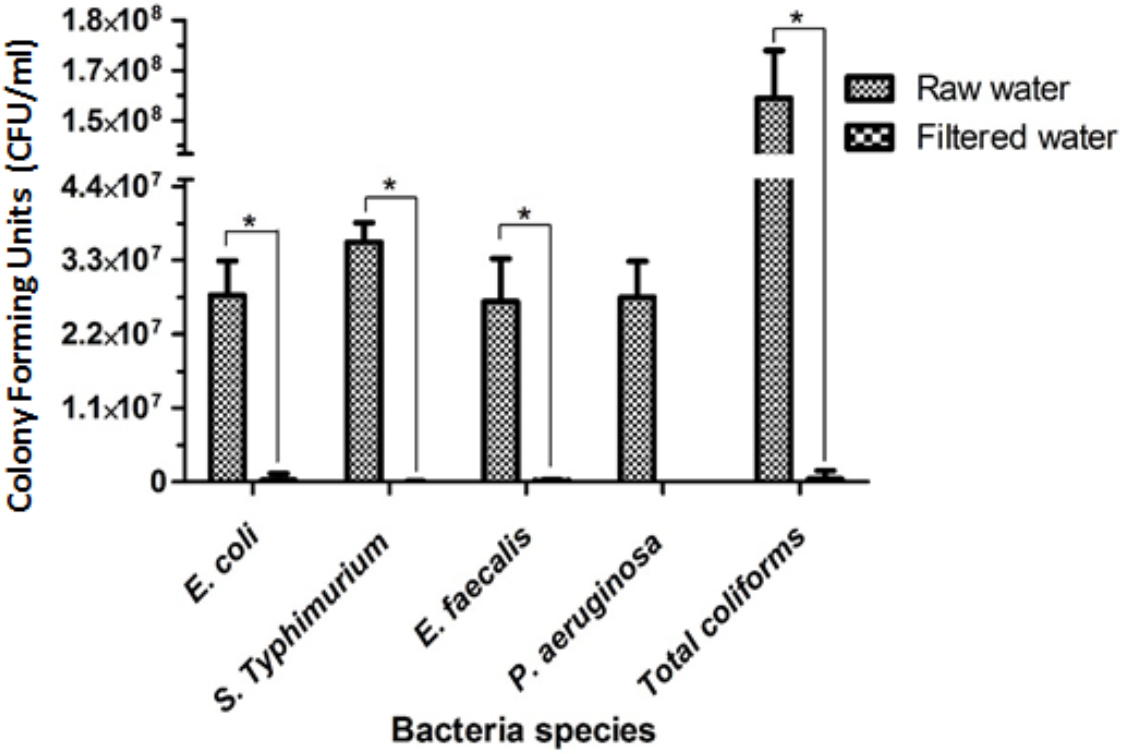
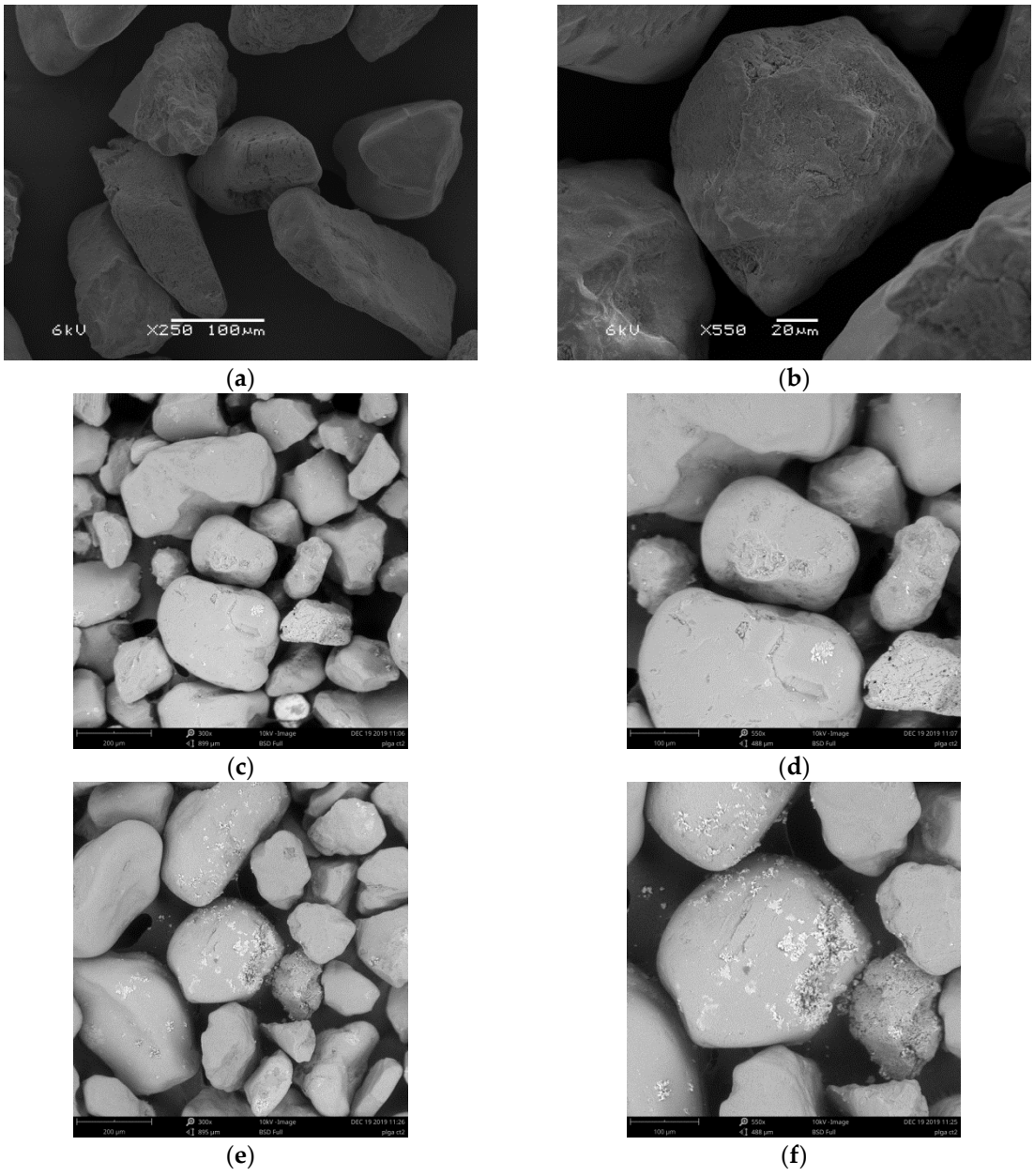


Figure 11. Concentrations of *E. coli*, *S.* Typhimurium, *E. faecalis*, *P. aeruginosa*, and TC of raw and filtered water.

3.9. Scanning Electron Microscopy

The top layers of sand from the BSF4 were visualized by SEM to monitor the growth of biofilms on the surfaces of the grains. At day 0, the grains were clean, without any material deposited (Figure 12a,b), after 45 days of operation the surface layer of sand was still clean, but a very small deposit could be noticed. On day 90, some grains showed formation of a small layer on their surfaces, which could be the initial development of biofilm. The SEM images showed little biofilm formation on the grain surfaces, which is not in accordance with the literature on either slow sand filters or biosand filters [10,13].



(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure 12. SEM images of the BSF4 top layer of sand in different magnifications. (**a**) Grains on day 0, 250×; (**b**) grains on day 0, 550×; (**c**) grains on day 45 of operation, 300×; (**d**) grains on day 45 of operation, 550×; (**e**) grains on day 90 of operation, 300×; (**f**) grains on day 90 of operation, 550×.

4. Conclusions

Four biosand filters were tested to evaluate the influence of turbidity on their ability to remove microorganisms present in feces. One filter received well water while the other three received water contaminated with treated wastewater with varying turbidity. Filters operated for 90 days and were capable of providing filtrates with turbidity that were almost always less than 1 NTU.

There were decreases in pH and alkalinity in filters contaminated with treated wastewater. Additionally, the nitrate concentrations were reduced, suggesting that denitrification might have occurred in the filter depth. The UV_{254} nm absorbance, which is related to the presence of organic matter, showed average removal efficiencies between 62 and 73%.

BSF had a reduction in total coliforms ranging from 0.54 to 2.01 LRV. BSF4, which received water with 50 NTU, showed the highest removal efficiency throughout the experiment, reaching up to 3 LRV

at its end. It was found statistical evidence to state that the efficiency of BSF4 was significantly greater than those from BSF1 (well water), BSF2 (3 NTU) and BSF3 (25 NTU). Reductions in *E. coli* ranged from 1.2 to 2.2 LRV. BSF4 also showed a higher removal efficiency throughout the experiment.

The experiment in which the BSF4 influent, which had higher turbidity, was contaminated with the bacterial species *S.* Typhimurium, *E. faecalis*, *E. coli*, and *P. aeruginosa* had filtrates with 2.7–3.6 LRV.

During most of the experimental period, the filter that received influent water with higher turbidity, induced by kaolin addition, consistently showed filtrates with lower concentrations of microorganisms. It is possible that kaolin particles provided a surface to which organisms adhered, with subsequent particle retention during filtration. The presence of aluminum atoms in the kaolin chemical structure may have a secondary role as coagulant, thus helping to remove microorganisms.

The biosand filter showed its capability to remove fecal microorganisms under different turbidity conditions. This is a reliable point-of-use water treatment. However, in some cases, filtrates still had some microorganisms. As an additional step for increasing microbiological safety, a complementary point-of-use disinfection step can be used.

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