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Reduction of pathogenic microorganisms in an Imhoff tank–constructed wetland system

Mohammad Darvishmotevalli[a], Maryam Moradnia[b,a,*], Alireza Asgari[c], Mohammad Noorisepehr[d,e], Hamed Mohammadi[f]

[a]*Department of Environmental Health Engineering, Public Health School, Isfahan University of Medical Sciences, Isfahan, Iran, Tel. +989124256723; email: mohamad.darvish68@gmail.com*
[b]*Department of Environmental Health Engineering, School of Health, Kurdistan University of Medical Sciences, Sanandaj, Iran, Tel. +989100927010; Fax: +98(28) 33368778; email: moradnia@hlth.mui.ac.ir*
[c]*Department of Waste Management, Center for Research and Development, Farashetab Kian Aria (FSKAria) Co., Tehran, Iran, Tel. +989011925643; email: modiriatpasmand@gmail.com*
[d]*Department of Environmental Health Engineering, Public Health School, Alborz University of Medical Sciences, Alborz, Iran*
[e]*Research Center for Health, Safety and Environment (HSE), Alborz University of Medical Sciences, Alborz, Iran*
[f]*Torbat Jam Faculty of Medical Sciences, Torbat jam, Iran, email: hamedmohamdi36@gmail.com*

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ABSTRACT

Microbial quality is one of the most important quality characteristics associated with wastewater reuse in irrigation. This study was conducted in order to evaluate the removal of microbial parameters including Total coliform, fecal coliform, parasite eggs and protozoan cysts removal by hybrid Imhoff tank and constructed wetland (CW) systems. This experimental study was carried out during 6 months. A total number of 144 samples were collected from influent and effluent of hybrid Imhoff tank and CW systems. Parasite eggs and protozoan cysts count were performed based on the Bailenger method. Moreover, total coliform and fecal coliform counts were carried out using the most probable number (MPN) method. It appeared that the highest removal efficiencies were, respectively, achieved to be 99.999% (5 logs) and 99.999% (4 logs) for total coliform and fecal coliform and higher than 99% for intestinal nematode parasites eggs and protozoan cysts when the Imhoff tank and CWs systems were used as series. The *p*-value was statistically significant (*p*-value < 0.05) for all pathogenic microorganisms removed by the hybrid system of Imhoff tank–SSFCW. It is concluded that the hybrid system of the Imhoff tank–CW is effective in removal of cysts and parasite eggs. The use of a disinfection unit will be necessary for achieving the output standards for total coliform and fecal coliform.

Keywords: Intestinal microorganisms; Constructed wetland; Wastewater

1. Introduction

Generating wastewater with the least negative environmental impacts is one of the main goals of the wastewater treatment process. Microbial contaminant is considered as an important index related to wastewater effluent quality when it is supposed to be used for reclaimed wastewater applications such as irrigation of raw crops and vegetables, public lawns and parks [1–4]. However, there are various methods for wastewater treatment; natural treatment systems are considered as an environmentally friendly technology which can treat wastewater with high efficiency, minimum cost, low sludge production and simple operation and design [2]. Constructed wetlands (CWs) are among the applicable systems in small and medium communities (i.e., those with 2,000 households or fewer) which remarkably reduce various contaminants, especially microbial parameters based on natural processes involving wetland vegetation and soils

* Corresponding author.

[5,6]. All types of CWs are attached with growth bioreactors where wetland plants (including roots, stems, and leaves) and media material are used as the surface for microbial attachment. For effective and safe target levels of pathogen reduction, a one-stage system is usually not sufficient. One of the best methods for improving water quality with low concentrations of indicator microorganisms is the combined wastewater treatment system. CW systems are combined with other treatment systems to achieve higher efficiency, which are called "hybrid constructed wetlands" [7,8].

According to the results of some studies in recent years, the efficiencies up to 90% of microbial parameters such as *E. coli*, helminth eggs [2,9] and protozoan pathogens including *Cryptosporidium* and *Giardia* [10] were observed by subsurface CWs and biological anaerobic systems.

In Iran, a few studies have been carried out on the removal of parasite eggs and protozoan cysts by wastewater treatment plants. As there is no similar investigation on natural wastewater treatment systems in full scale in the small community of Iran, this study aimed to evaluate the efficiency of the combined systems of the Imhoff tank–SSFCW for sanitary wastewater treatment with the focus on the reduction of pathogenic microorganisms including total coliform, fecal coliform, intestinal nematode parasites and cysts.

2. Materials and methods

2.1. Specifications of Imhoff tanks and subsurface CW

This is an experimental study conducted in a 6-month period. A small community (a training camp in Qazvin) was selected as one of the sanitary sewers in Qazvin province. Based on the design fundamental, a prefabricated Imhoff tank unit (each unit had two separate sections including sedimentation and sludge digestion) and SSF system by a horizontal flow were constructed and operated as series, and also another system was applied as control. These systems were used to determine some biological parameters including total coliform, fecal coliforms, intestinal nematode parasites and cysts. A total number of 48 Imhoff tanks with 96 m^3 total volume were utilized as series. The dimensions of the CW were 1 m × 4 m × 20 m (D × W × L) where the plants such as the vetiver grass (*Chrysopogon zizanioides*) and Qazvin native straw (*Carex vulpinoidea*) were planted as the herbals of the wetland system. The total surface areas for the SSFCW and control systems (without plants) were 160 m^2 (80 m^2 for each system). The calculated retention residence time was 3 d for the Imhoff tank and 1.5–3 d for SSFCW.

2.2. Sampling and analysis of data

Wastewater samples were collected with 1 L volume from the Imhoff tank input (raw wastewater influent), the input to SSFCW and control system, and the output of the SSFCW and control system, twice per month (sampling points are determined in Fig. 1). A total number of 144 wastewater samples were gathered and transferred to a laboratory in less than 48 h at 4°C. The counts of total coliform and fecal coliform were measured according to American Public Health Association (APHA) et al. [11] using the MPN method. Intestinal nematode parasite and cyst counts were carried out based on the Bailenger method [12]. In this method, first, the samples were left for 4 h at room temperature to settle and then about 90% of the supernatant liquid was drained using a siphon and the remaining sediment at the bottom of tube was centrifuged at 1,000 × g for 15 min. Total sediment in the centrifuge tubes was returned to a single tube and centrifuged at 1,000 × g for 15 min. In the next step, as much as an equal volume of the sediment formed in the second step of centrifuge along with acetoacetic acid buffer (pH = 4.5) and acetyl acetate (twice the volume of the sediment) were added to the centrifuge tube. Afterward, the sample was homogenized by an agitator and centrifuged in 1,000 × g for 15 min. The final sediment was mixed and suspended in five volumes of zinc sulfate ($ZnSO_4$) 33%. The volume of the final product was recorded; subsequently, the final product was transferred to three McMaster slides each with 0.3 mL volume using the Pasteur pipette and observed using an SEM with magnification 10× and 40×.

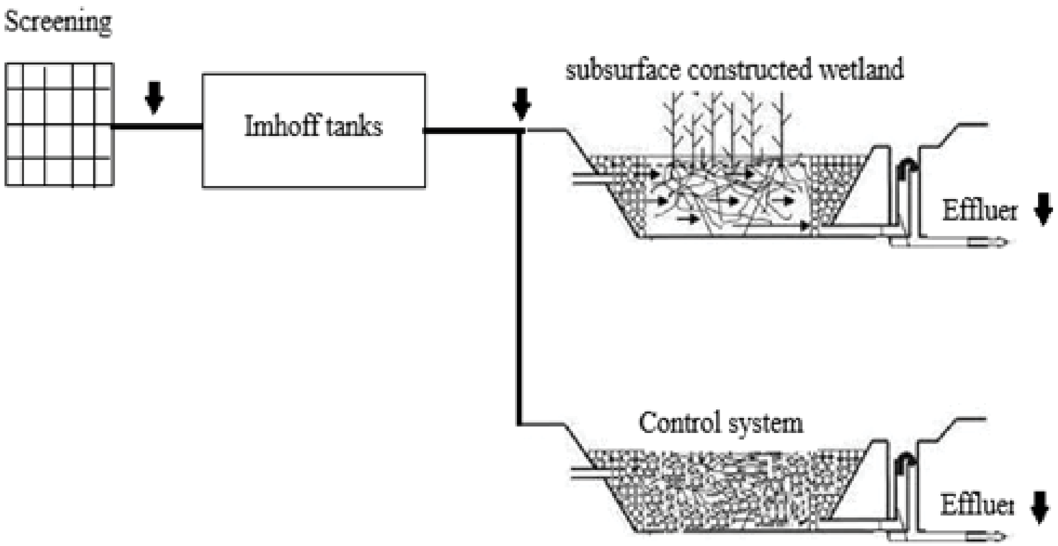

Fig. 1. Schematic of Imhoff tank–SSFCW and the sampling points.

The number of cysts and parasite eggs in 1 L of the sample was evaluated using the formula $N = AX/PV$ (N = number of parasite eggs or cysts in 1 L of the sample, A = average number of counted parasite eggs or cysts, X = the volume of final product [mL], P = the volume of McMaster slide [0.3 mL] and V = the volume of initial sample [L]).

Experimental errors were reduced with repeating the tests. Analysis of the samples was performed using paired-sample T-test via the SPSS16 software with a significance level of 0.05. The examined parameters were compared with the Iranian standards for use in irrigation and EPA [13].

The study systems (Imhoff tank and CW) are shown in Figs. 1 and 2.

3. Results

The mean microbial parameters of the raw wastewater, input and output of the control system and SSFCW as well as Iranian and EPA guidelines are provided in Table 1.

The variation of total coliform and fecal coliform counts during the study period by Imhoff tank, SSFCW and combined Imhoff tank–SSFCW systems are indicated in Figs. 3 and 4.

4. Discussion

Coliforms, intestinal nematode parasites and cysts are the common biological indicator in wastewater treatment. The World Health Organization has emphasized the risk of intestinal infections caused by intestinal microbes both for workers and for use of this type of wastewater for irrigation [14]. The obtained results (Table 1) showed that the greatest number of parasite eggs was *Ascaris lumbricoides* eggs in the raw wastewater samples. This issue can be due to the high resistance of *Ascaris* eggs than compared with parasites against unfavorable environment [12].

The efficiency of the Imhoff tank system to reduce total coliform, fecal coliform, *Ascaris lumbricoides*, *Enterobius vermicularis*, *Fasciola hepatica*, *Giardia* cyst and amoeba cyst was, respectively, obtained to be 92.39%, 88.83%, 75.83%, 73.54%, 73.83%, 81.44% and 82.18%. The ability of the Imhoff tank system to considerably reduce microbial parameters can be attributed to the sufficient retention time due to sedimentation and digestion mechanisms. Furthermore, the large number of the Imhoff tank units designed as series was effective in increasing the removal rate of microbial parameters. However, the reduction rate was not complete and the quality of the Imhoff tank effluent was not found to be within the permissible Iranian standards for irrigation and EPA. Therefore, it can be stated that the Imhoff tank system is considered as a temporary option for wastewater treatment and it is usually used as a pre-treatment [15]. The mean counts of total coliform, fecal coliform and nematode eggs (except *Enterobius vermicularis* and *Fasciola hepatica*) in the Imhoff tank effluent were not significant in comparison with the SSFCW, the Imhoff tank–SSFCW, and recommended standards (p-value > 0.05).

Further reduction of pathogenic microorganisms was obtained by treating the Imhoff tank effluent through the

(a) (b)

Fig. 2. (a) Subsurface constructed wetland and control system and (b) Imhoff tank.

Table 1
Average microbial parameters (±SD) of raw and treated wastewater samples

Parameter	Unit	Raw wastewater	Imhoff tank effluent	Control effluent	SSFCW effluent	Iranian standards for irrigation	EPA for irrigation
Total coliform	MPN/100 mL	9.3E8 ± 6.7E8	6.1E7 ± 2.7E7	6.68E5 ± 2E4	1.3E3 ± 374.8	1,000	200
Fecal coliform	MPN/100 mL	5.1E6 ± 9.3E5	6E5 ± 4.3E4	5E3 ± 2.3E2	4.01E2 ± 28.5	400	–
Ascaris lumbricoides	Number/L	10.11 ± 4.18	1.27 ± 1.03	1.3 ± 0.9	0	≥1	1
Enterobius vermicularis	Number/L	2.3 ± 1.75	0.54 ± 0.27	0	0	≥1	1
Fasciola hepatica	Number/L	1.1 ± 0.18	0.27 ± 0.08	0	0	≥1	1
Giardia cyst	Number/L	2.3 ± 1.12	1.05 ± 0.68	0	0	≥1	1
Amoeba cyst	Number/L	4.12 ± 2.15	2.4 ± 1.18	1.02 ± 0.18	0	≥1	1

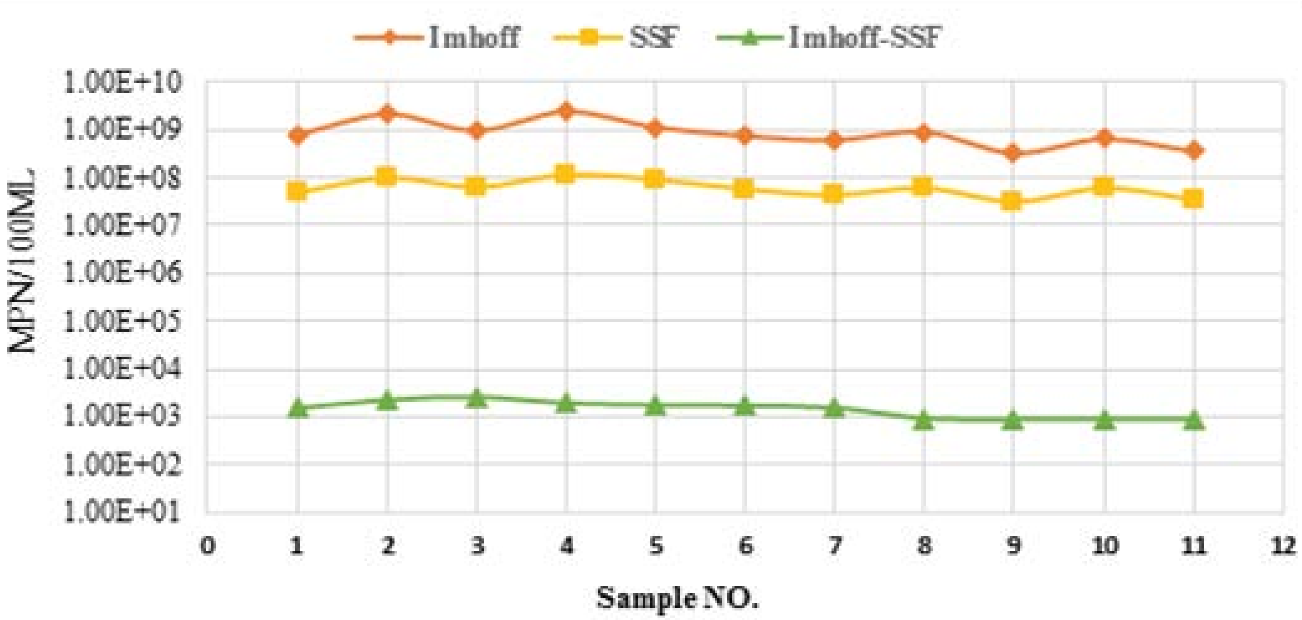

Fig. 3. Variation of total coliform counts during the study period by Imhoff tank, SSFCW and Imhoff tank–SSFCW systems.

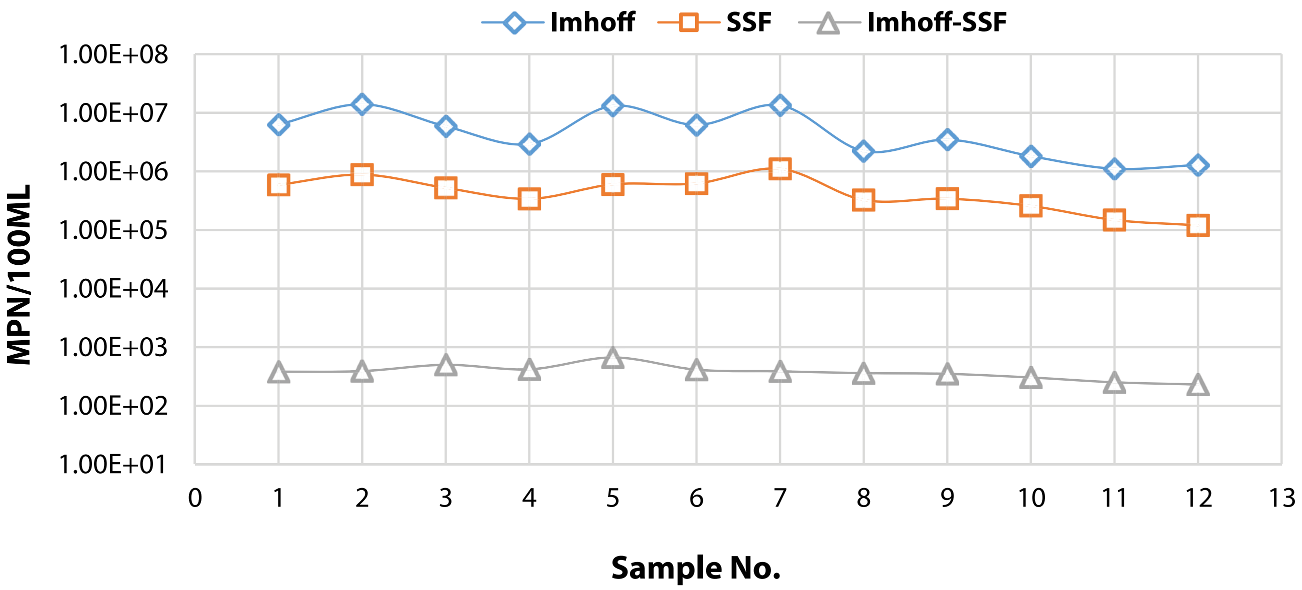

Fig. 4. Variation of fecal coliform counts during the study period by Imhoff tank, SSFCW and Imhoff tank–SSFCW.

SSFCW system. The main removal mechanism of microorganisms in wetlands include filtration, trapping in suspended solids, precipitation, sedimentation, and deactivation due to unfavorable environmental conditions [9,15]. The removal rate was, respectively, obtained by the SSFCW system to be 99.99% (4 log) and 99.93% (3 log) for total coliforms and fecal coliforms and above 99% for pathogenic nematodes and cysts. According to the results presented in Table 1, the count of total coliform and fecal coliform in the wetland effluent was not found within the acceptable limits regarding the Iranian guidelines and EPA for irrigation. Meanwhile, *p*-value was not significant for total coliform (p-value > 0.05). The SSFCW system was significantly effective in reducing fecal coliform, pathogenic nematodes and cysts (p-value < 0.05).

The mean number of *Ascaris Lumbricoides* eggs and amoeba cyst in the control system effluent was achieved to be 1.3 ± 0.9 number/L and 1.02 ± 0.18 number/L, respectively, which were both higher than the recommended standards for wastewater reuse in agricultural and irrigation (≤1 egg count per liter). Meanwhile, the mentioned microorganisms were removed completely. It can be concluded that wetland plants have significant performance in reduction of parasite eggs and protozoan cysts [16]. The *p*-value of the control system in reduction of all pathogenic nematodes and cysts was significant (p-value < 0.05). By comparing the performance of control system with Imhoff tank can be concluded that the media in control system has significant differences in pathogenic microorganism's reduction. The combined system of the Imhoff tank–SSFCW indicated higher removal rates compared with the single SSFCW and Imhoff tank systems with the corresponding efficiency of 99.999% (5 log), 99.99% (4 log) for total coliform and fecal coliform, respectively, and above 99% for parasite eggs and protozoan cysts. The overall performance of the system was found to be higher than that of the single Imhoff tank and SSFCW systems, which may be attributed to the combining of the treatment stage to two steps instead of one step and also to filtration and adsorption by plant roots which create suitable situations for the elimination of parasite eggs and protozoan cysts in CWs. The number of parasite eggs and protozoan cysts was within the permissible limits for irrigation according to the Iranian environmental standards and EPA. The p-value was statistically significant (p-value < 0.05) for all pathogenic microorganisms removed by the combined system of Imhoff tank–SSFCW. However, the level of total coliform and fecal coliform was not within the standards for irrigation. Therefore, it is necessary to apply a disinfection unit for achieving output standards for total coliforms and fecal coliforms after the SSFCW system. Studies employing combined systems such as hybrid systems wetland-aerated lagoons, horizontal subsurface flow–vertical subsurface flow (HSSF–VSSF) and anaerobic-SSF have demonstrated the effective removal of pollutants from wastewater [17,18]. Abdel-Shafy and El-Khateeb [19] studied the integration of the septic tank (anaerobic) and SSFCW for sanitary wastewater treatment. In the mentioned study, the total area of the CW was 200 m^2. The removal percentage was obtained by the septic tank as 89.89% for fecal coliform. However, the removal rates were obtained by the wetland system to be 99.99% (4 log) for this parameter. Moreover, the efficiency of the hybrid system was achieved to be 99.999% (5 log). The overall performance of our study was found to be slightly lower than that achieved by Abdel-Shafy and El-Khateeb [19]. The discrepancy can be due to the larger total surface area used in the mentioned study. Furthermore, investigation using experimental, pilot and full-scale CWs has revealed that fecal coliform inactivation typically ranged between 1.2 and 2.2 log units [20]. The mentioned study is in parallel with the study conducted by Morató et al. [21] who reported 1.2–2.2 log units of total coliforms and 1.4–2.3 log units of fecal coliforms via HSSF CWs.

Zurita and White [6] reported 99.99% reduction of *E. coli* by hybrid CWs (HF-VF) and the results confirmed that two-stage CWs can be utilized to reduce dangerous pathogenic microorganisms and produce a high quality effluent for reclaimed water uses.

Similarly, Sharafi et al. [22] reported the high performance of the CW system for removing parasite eggs (100%) and protozoan cysts (99.7%). They announced that removal efficiency of cysts and parasitic eggs by the CW was better than that of conventional activated system. Furthermore, studies conducted by Reinoso et al. [23] showed high performance for CW (i.e., 97%) in *Giardia* cysts removal. Molleda et al. [9] also indicated the removal efficiency of 100% for this parameter by the CW. The results of this study were found be in a good agreement with these studies. As shown in Figs. 3 and 4, the number of total coliform and fecal coliform in the Imhoff tank effluent was more than that in the wetland and hybrid system and the variation of these parameters had a constant trend in the hybrid system. That may be due to relatively stable inflow fluctuations and the larger total surface area when using the Imhoff tank and CW systems as series.

5. Conclusions

Based on to the obtained results, the Imhoff tank is a good option which can be considered as a pretreatment system of sanitary wastewater followed by the CW systems. Furthermore, by comparison of the control system and CW, it can be concluded that plants have a significant role in removal of pathogens and other detrimental water quality parameters. It is interesting to note that significant reduction of coliforms, parasite eggs and protozoan cysts was obtained when arranging two-stage combined wastewater treatment systems comprised of the Imhoff tank and CW (Imhoff tank–SSFCW).

For achieving output standards for total and fecal coliform, it will be necessary to use a disinfection unit before discharging to the environment. It can also be used for irrigation by considering the necessary standards if a disinfection unit is implemented. In the examined system, there is no chance for reproduction and proliferation of harmful insects on a layer of wetland. Thus, it is safe for social health system and small communities.

Moreover, in developing countries which deal with water shortage and also high-cost of wastewater treatment plant construction, applying such low-cost and eco-friendly wastewater treatment systems while maximizing reuse makes economic sense and enhances long-term sustainability of the coupled human-agricultural system.

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