

Removal of Pathogenic Microorganisms in Pilot-scale Uasb-septic Tanks and Albireh Urban Wastewater Treatment Plant in Palestine

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Abstract: *Inadequate wastewater treatment facilities and a general lack of data on hygienic aspects of treated wastewater have hampered the successful implementation of effluent reuse schemes at the national level; in addition, illegal irrigation practices with partially treated effluent impose serious health hazards and environmental problems. The main objective of this research is to investigate the pathogens removal in Upflow Anaerobic Sludge Blanket (UASB)-septic tanks and Albireh wastewater treatment plant (AWWTP). The UASB-septic tanks, located at AWWTP, were fed continuously with raw municipal wastewater from the aerated grit chamber of AWWTP. The two pilot scale UASB-septic tanks (R1 and R2) were operated at two different hydraulic retention times (HRT) of 2 and 4 days for R1 and R2, respectively. At AWWTP, as an extended aeration system with aerobic sludge stabilization, the HRT was about one day. Both raw wastewater and treated effluent were tested for microbial pathogens, including indicator bacteria, protozoa and trophozoite using microscopic and specific culture media. The removal efficacies of R1 and R2 were 15.5% and 15% for fecal coliform and 6.9% and 11% for fecal streptococcus, respectively, while the removal efficacy of the oxidation ditch was 38% for fecal coliform and 16% for fecal streptococcus. Though Salmonellae was detected in 30% of analyzed influent samples, it was not detected in any sample of the effluent of both treatment systems. Also, the treated effluent of R1, R2 and oxidation ditch was parasite- (cysts or trophozoite) free. This information can assist municipalities and village councils in the implementation of rational and efficient treatment strategies for sustainable effluent reuse. Finally, the installation of post treatment stages, including filtration and disinfecting units, is recommended in order to comply with prescribed national guidelines for effluent reuse.*

Keywords: *Albireh oxidation ditch, effluent reuse, indicator bacteria, pathogens removal, UASB-septic tank*

Introduction

Wastewater management in Palestine is a high priority from the perspectives of both environmental protection and resource conservation. In many cases, traditional wastewater treatment technology, such as

aerobic activated sludge processes, are inappropriate for the physical and economic characteristic of the small communities (Al-Sa'ed, 2005). Moreover, the existing treatment systems are poorly operated and the effluent is no better than the influent in most of cases, where raw or partially treated wastewater is discharged into

Wadis. This water is then used for agricultural purposes in regions with a shortage of natural water resources, such as the Hebron, Jenin and Nablus districts in the West Bank.

Pathogens (indicator) removal – including fecal coliform, fecal streptococcus, *Salmonellae Shigella*, *Helminthes eggs, cysts (Amoeba, Giardia)* – using technology, such as UASB-septic tank as anaerobic system or oxidation ditch as aerobic technology, in Albireh wastewater treatment plant was not investigated under the ambient conditions in Palestine. As indicated in Table 1, wastewater contains three major types of human pathogens: bacteria, protozoa, and helminthes (Feachem *et al.*, 1983, Shuval *et al.*, 1986).

World Health Organization (2006) and EPA (2004) guidelines for irrigation specify a maximum concentration of 1 helminthes egg per liter and 10³ fecal coliform colonies per 100 ml. Several countries have adopted reuse rules based on WHO guidelines and for various applications of reclaimed effluent. Data regarding effluent reuse in restricted and unrestricted agricultural irrigation, as taken from some regional standards (WHO, Palestine, Jordan, and Israel), is

shown in Table 2.

Enhanced primary sewage treatment using large-scale, high rate anaerobic reactors, including Upflow Anaerobic Sludge Blanket (UASB), have been investigated in tropical climates, mainly in India and Latin America, over the last two decades (Hulshoff Pol *et al.*, 1997). They can achieve high COD removal rates and SS in relatively short HRTs, are capable of producing less sludge, require small area demand and have moderate construction costs. Nevertheless, single stage UASB reactors tested at pilot-scale under temperate climate conditions have showed unsatisfactory results with regard to nutrient and pathogens removal rates. Investigations have shown that cold winter periods impaired the hydrolysis rate and also caused the accumulation of SS and microbial pathogens in the reactor (Zeeman and Lettinga, 1999; Elmitwalli *et al.*, 2002). To overcome these disadvantages, recent research has indicated the benefits of combining the UASB-septic tanks with aerobic treatment stages, including suspended and attached growth processes (Sousa and Foresti, 1996; von Sperling *et al.*, 2001). However, all these studies have aimed at achieving effective organic and nutrient removal rates with no attempt to investigate the pathogens removal rates of

Type of pathogen	Unit	Species or kind
Indicator/pathogenic bacteria	CFU/100 ml	Fecal coliform Fecal streptococcus <i>Salmonella, Shigella</i>
Filamentous bacteria	CFU/100 ml	<i>Nocardia</i>
Protozoa (cysts)	Eggs/L	<i>Entamoeba histolytica, Entamoeba coli</i> <i>Giardia lamblia, Amoeba</i>
Helminthes	Eggs/L	<i>Ascaris lumbricoides</i> <i>Trichuris trichiura</i> and other ova
Hookworms	Eggs/L	<i>Strongyloides sterorlasis</i>
Trophozoite	Eggs/L	<i>Trichomonas</i> <i>Enterbeous vermicularis</i>

Table 1. Possible pathogens in the municipal wastewater of Albireh city.

Parameter	Jordan	Israel	Palestine	WHO
TSS (mg/l)	50-150	10	20-50	-
BOD ₅	30-300	10	20-30	-
NO ₃ -N	30-45	20-25	25-50	-
Fecal coliforms (CFU/100ml)	100-1,000	10-200	200-1,000	10 ³ -10 ⁴
Parasites (eggs number/L)	≤ 1	≤ 1	≤ 1	≤ 1

Table 2. Adopted effluent reuse guidelines for restricted and unrestricted agricultural irrigation in some countries based on WHO guidelines (WHO, 2006; PSI, 2003; Government of Jordan, 2002; Cikurel, 2003).

the integrated anaerobic-aerobic treatment processes. Using RBC as a post-treatment stage for UASB treated effluent, results published recently by Tawfik *et al.*, (2003) showed that RBC produced effluent with *E. coli* count of 1.2×10^5 CFU/100 ml, which could only be used for restricted agricultural irrigation, according to WHO standards (1989). Previous studies on environmental factors affecting the natural decay processes of microbial indicators in natural and mechanized post-treatment systems considered the operational parameters (temperature, pH, light intensity and radiation, and algal content) as key factors (Steen *et al.*, 2000).

A few local studies were conducted in order to investigate the feasibility of UASB septic tanks, particularly when followed by natural treatment systems, with a special focus on design and operational parameters (Al-Juaidy *et al.*, 2003; Fuqaha and Al-Sa`ed, 2006). However, there is no Palestinian comprehensive data on the pathogens content of treated effluent suitable for agricultural reuse with respect to Fecal coliform (FC), Fecal streptococcus, *Salmonellae*, *Shigella*, *Helminthes eggs (HE)*, *Amoeba* cysts, *Giardia* cysts, or pathogens in trophozoite form (Al-Sa`ed, 2007). Hence, the main goal of this research is to investigate the occurrence and efficacy of microbial pathogens removal in UASB-septic tanks and to compare it with the efficiency of Albireh oxidation ditch under variable HRT. In addition, the suitability of the treated effluent from the treatment systems under study was also evaluated for agricultural irrigation in light of WHO guidelines.

Materials and Methods

Materials and system description

Two pilot scale UASB-septic tanks, reactor (R1) HRT 2-days and reactor (R2) HRT 4-days, with 0.8 m^3 volume for each, were operated in parallel and fed with municipal wastewater from Al-Bireh city. Detailed design and material description can be found in the M.Sc. thesis of Al-Shayah (2005). The reactors were operated at ambient temperature between 30/3/2004 - 15/09/2004. The results for influent of wastewater treatment plant and the effluent from UASB-septic tank reactors R1, R2 and oxidation ditch were tested for

the removal efficiency of solids and pathogens (Fecal coliform, fecal streptococcus, *Salmonellae*, *Shigella*, *Helminthes* eggs and *Amoeba* and *Giardia* cysts).

Albireh oxidation ditch entails a two lain modified activated sludge process and the operational lain is serving, at present, about 32000 population equivalent (PE), which considerably exceeds the designed treatment capacity (25000 PE). Due to mismanagement and the current financial strategy of Albireh Municipality, which is aimed at reducing annual operational costs, the second existing oxidation ditch lain has never been put into operation. The organic materials and nutrients are microbial degraded at a low sludge loading rate ($\leq 0.05 \text{ kg BOD/kgTSS.d}$) with simultaneous nitrification and denitrification processes (surface aerated zone = 4900 m^3 and mechanically mixed anoxic zone = 2100 m^3). The excess biosolids (sludge) are aerobically stabilized at an average sludge age of 25 days, thickened by a gravity thickener, filter press dewatered and, finally, disposed off at Albireh sanitary landfill. The average hydraulic retention time (HRT) in the oxidation ditch is usually about one day (dry weather) and around 20 hours during wet weather conditions (Al-Sa`ed and Hithanwi, 2006).

Sampling and Analytical Techniques

Ayres and Mara (1996) recommend taking samples two times per week depending on the type of pathogens in question. During this study, 1-2 grab samples were taken per week at 0900 from a continuously running system. The sampling program entailed one influent and two effluent samples from the UASB-septic tanks at 2-days HRT (R1) and at 4-days HRT (R2), as well as one sample from the effluent of the oxidation ditch. Samples were placed in an icebox, cooling during the transportation period (30 minutes) before analysis. The total suspended solids (TSS), turbidity, the fecal coliforms (FC) and the fecal streptococcus were detected according to the Standard Methods (APHA, 1995).

Salmonella spp. was isolated by first placing the sample in dulcitol selenite enrichment broth and incubating at 37°C for 24 h. This was followed by

spread plating on Hektoen enteric agar and incubation at 37°C for 24 h as outlined by APHA (1995). Suspected Salmonella colonies were stabbed into Lysine Iron Agar (LIA) and incubated at 37°C for 24 h. Black colonies resulting from the LIA agar were further isolated after being streaked onto TSA and confirmed with an API 20E biochemical test strip specific for Salmonella.

To ensure the efficient collection of all eggs, Ayres and Mara (1996) recommend the application of at least double the theoretical settling time for any container depth. Also, using centrifugation leads to a good recovery of protozoa, eggs, and larvae. Hence, after setting for 1 hour, about 800 ml of the settled sediment sample was centrifuged for 5 min at 1500 RPM, and then one sediment drop was taken to the slide and tested using a ZEISS microscope with X10 and X40 magnification (UNRWA, 1999).

Results and discussion

Turbidity and total suspended solids (TSS)

Turbidity in water and wastewater is caused by suspended and colloidal matter, such as clay, silts, finely divided organic and inorganic matter, as well as other microorganisms. Turbidity is an expression for the optical property that causes light to be scattered and absorbed, rather than transmitted, with no change in direction through the sample (APHA, 1995). The correlation of turbidity with the weight or particle number concentration of suspended matter is difficult

because the size and shape of the particle affect the light-scattering properties of the suspension. Variations in turbidity values are shown in Figure 1.

Turbidity was reduced by only 70% to 73% between the inflow and outflow from the UASB-septic tanks (R1 and R2 respectively), with the largest removal (98 percent) occurring after the secondary settling tank of the oxidation ditch. Turbidity removal percentage is directly related to TSS removal. The average removal values are shown in Table 3. Turbidity is an important parameter for reuse and agricultural purposes, since further treatment processes depend on the turbidity results; in order for the disinfection process to take place, for example, turbidity must be less than 2 NTU according to WHO guidelines (WHO, 2006). Total Suspended Solids (TSS) can lead to the development of sludge deposits and anaerobic condition when poorly treated wastewater is discharged into the aquatic environment. In a reuse context, it has negative impacts on the maintenance of irrigation system, especially sprinkler and drip systems (Pettygrove *et al.*, 1985). The average TSS values for influent and effluent of R1, R2 and oxidation ditch are shown in Table 3, where the analysis, using SPSS $q < 0.05$ for R1, R2 and oxidation ditch for the removal of TSS, was found.

As shown in Table 3, a wide variation in TSS in the influent and effluent of the oxidation ditch appears in Standard deviation values, while the values for the UASB were stable. The average TSS values (mg/l) for influent and effluent of R1, R2, and oxidation ditch were 574 ± 131 , 121 ± 16 , 114 ± 18 and 25 ± 23 , respectively.

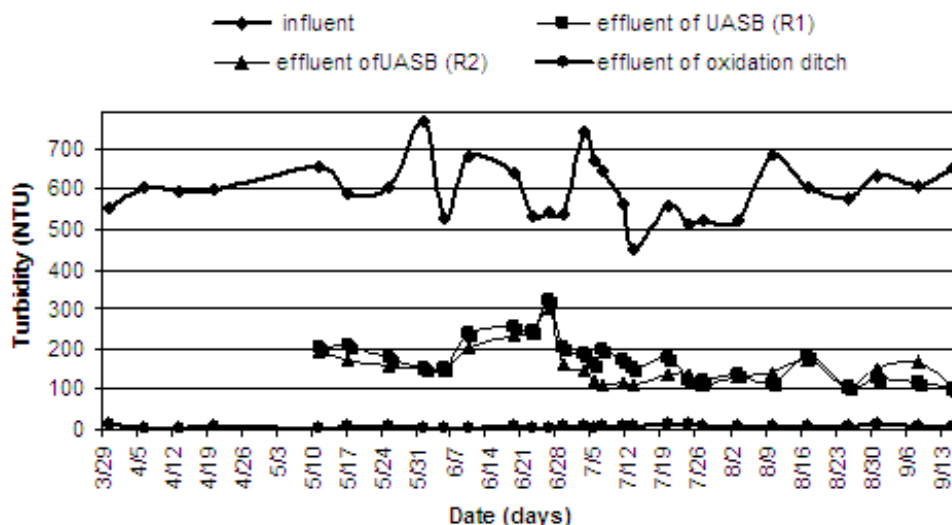


Figure 1. Turbidity values for influent, effluent of R1, R2 and oxidation ditch.

The influent TSS value was high compared with other TSS values reported by Kerstens (2001) in Jordan (420 mg/l), and Orhon *et al.* (1997) for rural areas in Egypt (310 mg/l). This might be due to the difference in population habits and in type of sewerage systems. In addition, low water consumption might make Albireh municipal wastewater of a high strength with reference to TSS parameter. The effluent TSS values of the two UASB-septic tank and oxidation ditch varied, but were still acceptable according to CEC (1991) which considers values > 150 mg/l as a desirable quality and also lies within the Jordanians' guidelines (30-300

mg/l). However, the obtained effluent values are not acceptable according to Israeli (30 mg/l) or Palestinian guidelines (150 mg/l) used for irrigation and for groundwater recharge or discharge, respectively.

The TSS removal efficiency was affected by HRT and upflow-velocity prevailing in both R1 and R2, as shown as shown in Figure 2. Having a secondary settling tank and a long solids retention time (above 20 days) in the aeration tank of the oxidation ditch might be the reason for the high TSS removal rates compared with UASB-septic tanks.

Turbidity removal (%)			
UASB-septic tank		AWWTP oxidation ditch	
R1 (2-days HRT)	R2 (4-days HRT)		
(n= 25) 70.48 ±9.87	(n= 25) 73.23 ±8.81	(n= 18) 98.31 ±0.52	
TSS removal (%)			
(n= 18) 79.66 ±5.01	(n= 19) 80.67 ±5.62	(n= 19) 95.43 ± 4.94	
Fecal bacteria removal (log₁₀)			
Influent:	UASB-septic tank effluent		AWWTP-Effluent (oxidation ditch)
<i>Fecal streptococcus</i>	R1 (2-days HRT)	R2 (4-days HRT)	
(n= 14) 6.29 ±0.64	(n= 16) 5.76 ±0.51	(n= 15) 5.47 ±0.78	(n= 13) 5.10 ±0.64
<i>Fecal coliforms</i>			
(n= 23) 7.33 ±0.62	(n= 18) 6.19 ±0.93	(n= 19) 6.10 ±0.64	(n= 19) 4.45 ±0.270

* Data are presented as average value (± Standard deviation; n= samples number)

Table 3. Turbidity and TSS removal (%) and pathogenic reduction (log₁₀) for R1, R2 and oxidation ditch*.

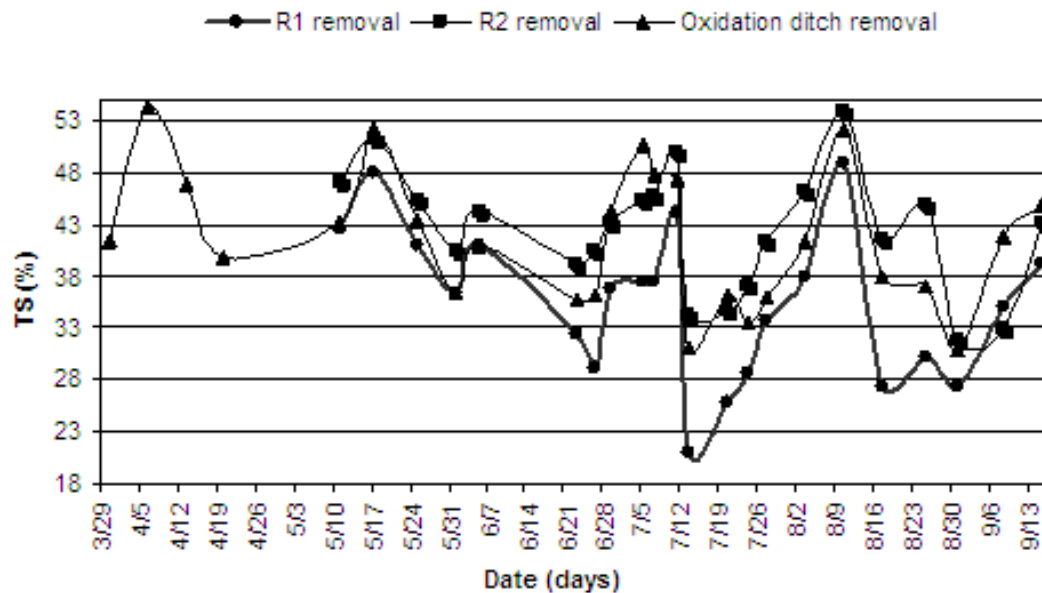


Figure 2. TSS removal from the pilot scale at Albireh, R1, R2 and oxidation ditch.

Microbial indicators and parasitic pathogens
Fecal streptococcus and fecal coliforms

The results obtained for fecal streptococcus analysis on influent and effluent of both UASB-septic tanks (R1 and R2) and the oxidation ditch are summarized as average logarithmic values in Table 3, shown above.

indicator for reuse and agricultural purposes, as fecal streptococcus causes infection on the mouth and throat area and is a common flora in the stomach and intestines. As illustrated in Figure 3a, the removal efficiency for FS varied, since the standard deviation is greater than values. This phenomenon has two possible interpretations: (1) the FS values of the effluent of R1, R2 and oxidation ditch are greater than the influent values or (2) streptococcus survives for a longer time where cell duplication and accumulation might occur.

Fecal streptococcus is considered as a pathogenic

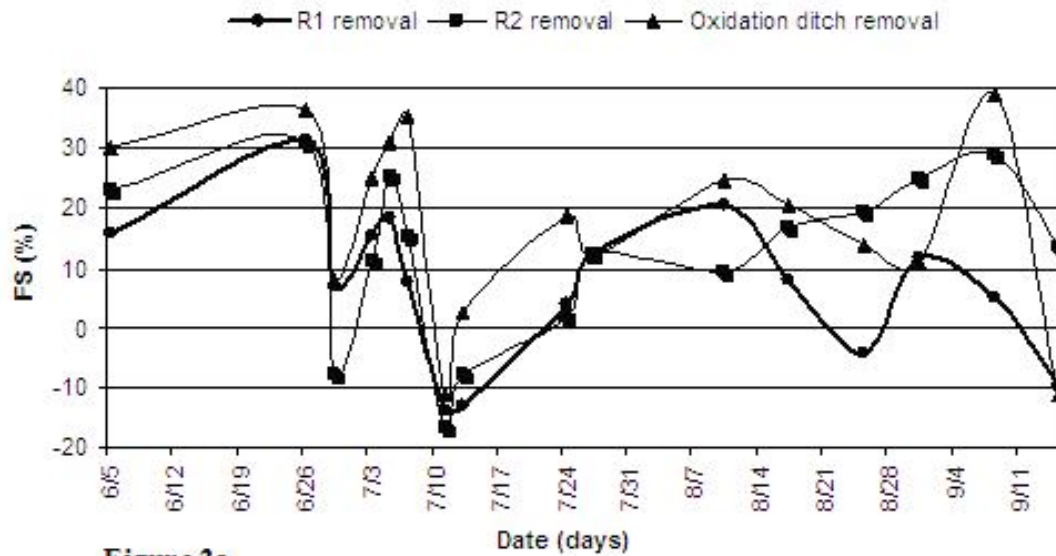


Figure 3a.

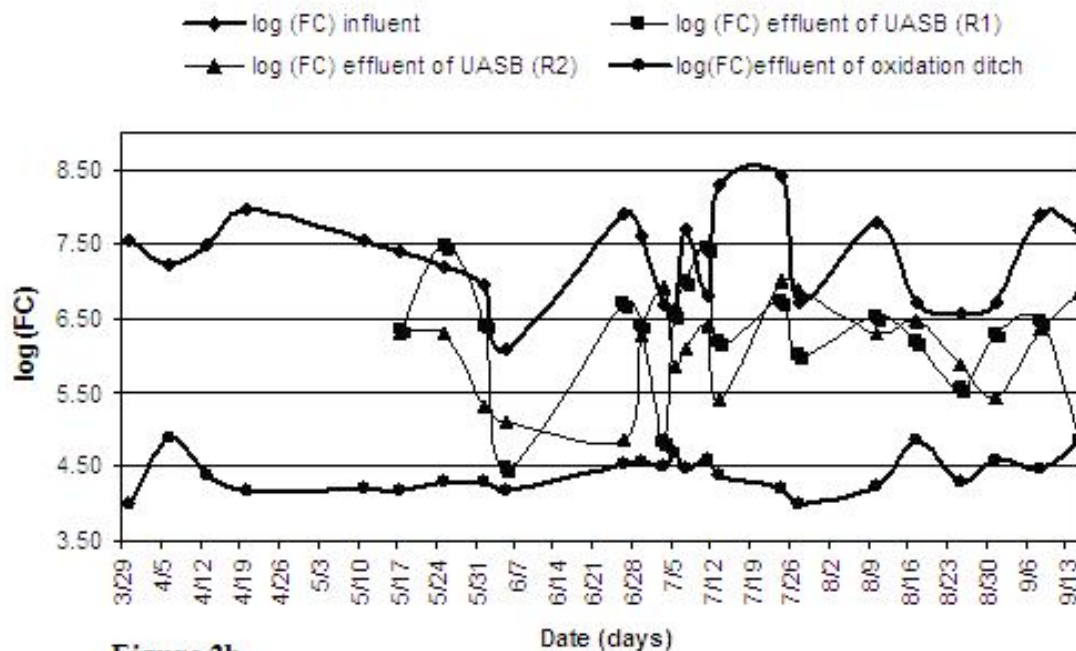


Figure 3b.

Figure 3 (a & b). Fecal streptococcus removals for R1, R2 and oxidation ditch.

There are variations in fecal coliform in the influent when treated with two processes using anaerobic (UASB)-septic tank and aerobic system using oxidation ditch. The average values for FC in logarithmic form are shown in Figure 3b. Fecal coliform results are plotted against time in logarithmic format, where the significant results, using SPSS, were $q > 0.05$ for R1 and R2, while Albireh oxidation ditch was $q < 0.05$. Similar findings were reported by Payment *et al.*, (2001) on fecal indicator bacteria occurrence and removal at the Montreal Urban Community wastewater treatment facility. He found that fecal coliforms were the most numerous of the indicator bacteria and their removal averaged 25%, while the *Fecal streptococci* removal was 29%.

The removal efficacy (%) for FC by the UASB-septic tanks was lower than the oxidation ditch, since the removal of FC depends upon the technology used. The behavior of *Fecal coliform* and *Fecal streptococcus* was the same in terms of concentration and removal values, but the difference between them was the survival time: FS survives the environmental condition over a long time compared with FC, which is more sensitive to the environmental conditions change. Moreover, the content of both microorganisms in the effluent exceeded the accumulated influent values in the reactors, and

hence affected the removal values (negative values), as shown in Figure 4.

Our results are in accordance with the findings of Ottoson (2004), who investigated the fecal coliforms removal in compact treatment (activated sludge) and in innovative systems (MBR and UASB). He found that the efficacy of the UASB reactor treating municipal Swedish wastewater was significantly lower ($1.8 \log_{10}$) than in the aerobic treatment lines ($2.1-3.2 \log_{10}$) with <24 h retention times. Based on the obtained data, it is obvious that both the anaerobically treated effluent (R1 and R2) and the undisinfected tertiary treated effluent of AWWTP are unsuitable for unrestricted agricultural irrigation. Recent results published by Tawfik *et al.*, (2003) reveal the importance of integrating disinfecting and filtration units after fixed film reactors in order to obtain an effluent that comply with WHO guidelines for restricted agricultural irrigation.

Salmonellae removal

The results obtained from *Salmonellae* analysis reveal its presence in only 30% of the total samples (13 samples) analyzed for the influent during the whole study period. However, the treated effluent of both pilot scale (R1 and R2) and Albireh oxidation ditch (AWWTP) were free of *Salmonellae*. The removal rate

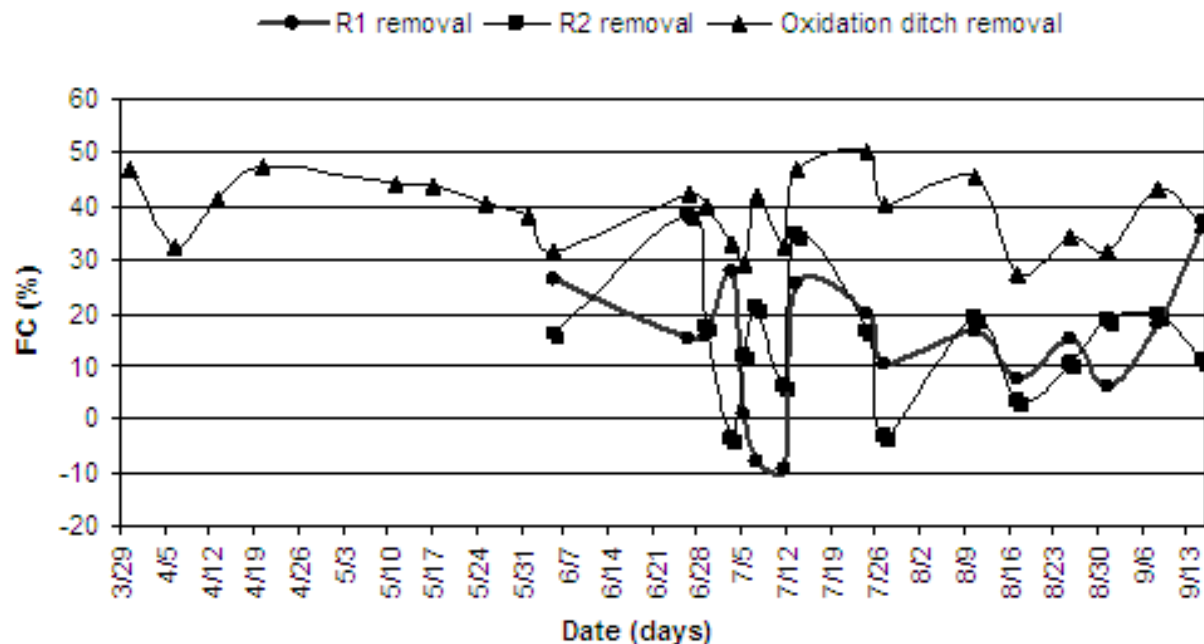


Figure 4. Fecal coliforms removal for R1, R2 and oxidation ditch.

of *Salmonellae* was less significant than that of fecal coliform or fecal streptococcus in the same reactor, as *Salmonellae* bacteria is less resistant than fecal coliform or fecal streptococcus. The complete removal of *Salmonellae* observed in both treatment systems may be attributed mainly to cell die-off, the degradation of organic matter, competition for limiting nutrients or trace elements, filtration, adsorption, aggregate formation, predators, and toxins emitted by other microorganisms. While investigating the fate of indicator pathogens, *Giardia* and *Cryptosporidium*, similar arguments were introduced by Thurston *et al.*, (1996) on factors that may have contributed to the large pathogens removal rates observed in integrated anaerobic-duckweed ponds systems.

Removal of parasitic pathogens in UASB-septic tanks and Albireh oxidation ditch

A summary of all potential pathogens found during the study period (190 days) are shown in Figure 5. However, the presence of these pathogens was not detected on a regular frequency; indeed, some were detected only 2 to 5 times. It is worth mentioning that not all of the pathogens detected in the influent were found in the effluent of the investigated systems.

Moreover, *Ascaris lumbricoides* were absent and not detected during the analysis. This is an indication that Ascariasis infection is not common among the inhabitants of Albireh city or in other communities connected to Albireh wastewater treatment plant.

The methodology applied in this study for the detection of parasites has limitations and did not detect all possible pathogenic parasites, such as *Cryptosporidium parvum*, a coccidian protozoan parasite. Because AWWTP lacks a physical (sedimentation and filtration units) or a chemical pre-treatment stage, *C. parvum* oocysts might be considered a potential contaminant of Albireh's reclaimed effluent. Using the focus detection method-most-probable-number cell culture technique, Gennaccaro *et al.*, (2003) reports that reclaimed effluent contained, on average, seven infectious oocysts per 100 liters *C. parvum* oocysts, where 40% of the final disinfected effluent samples contained infectious *C. parvum* oocysts. Therefore, if planned agricultural irrigation is intended in Ramallah-Albireh district, reclamation units, including filtration and disinfection, is required in order to produce reclaimed water that does not have a negative impact on public health or irrigated produce.

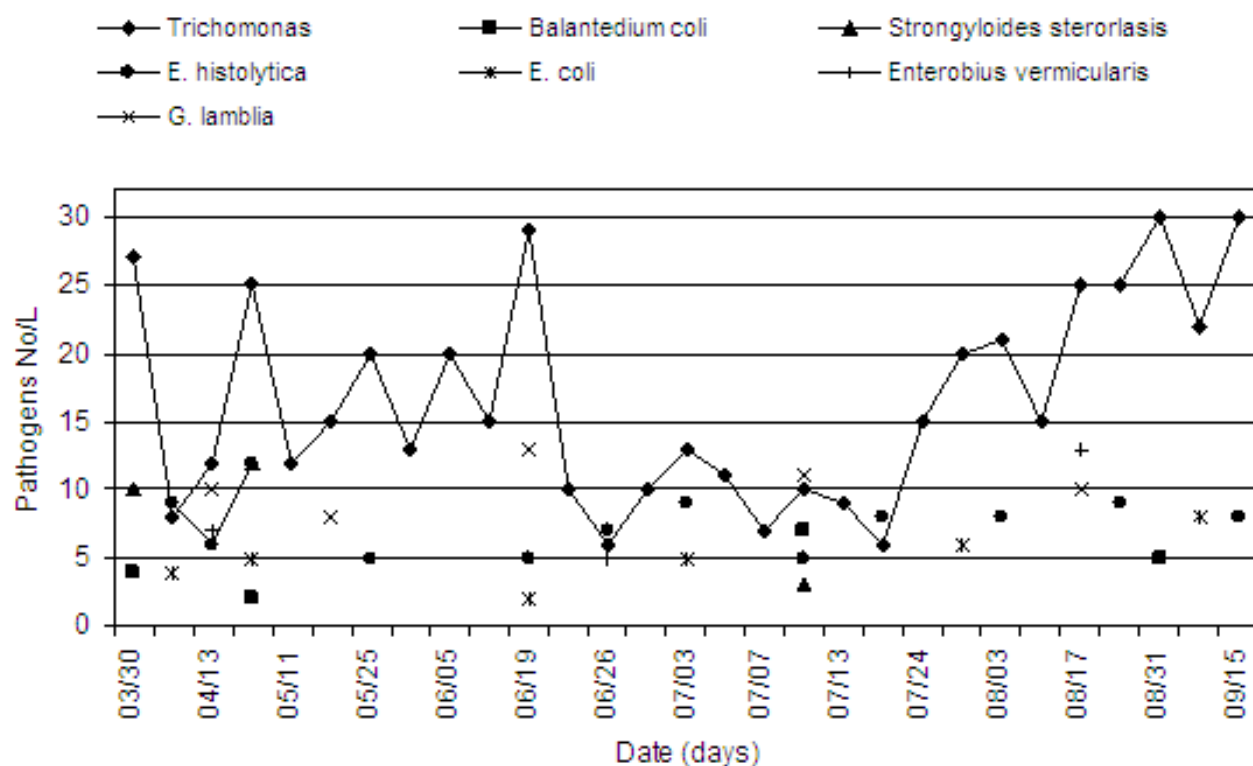


Figure 5. Pathogens in the influent of UASB pilot scale at Albireh oxidation ditch site.

Compared with influent content of parasites, Figure 6 shows that the accumulation of pathogenic *Trichomonas* has occurred in both UASB-septic tanks (R1 and R2 reactors). The pathogenic microbial accumulation observed in the UASB-septic tanks may be attributed primarily to the settling of the materials, degradation of organic matter, filtration, adsorption and aggregate formation. The short HRT of 2 to 4 days in the UASB-septic tanks also suggests that settling and subsequent re-suspension caused by the upflow velocity may have been major contributors to the increased numbers of some pathogenic microorganisms like *Trichomonas*.

Although AWWTP did not yield large reductions in indicator bacteria, complete reductions were observed for parasitic pathogens as compared to the outflow from the anaerobic UASB-septic tanks. As discussed above, long sludge age prevailing in the extended aeration and removal of daily excess sludge might have played a key role in this reduction tendency. The significant reductions in TSS content and turbidity justify the complete reduction of parasitic pathogens in the oxidation ditch. Pathogens' ability to infect depends on a large number of factors: both host and parasite are living creatures and therefore do not respond to environmental factors in a similar mode. The analysis for wastewater in term of pathogens must then be done on a regular basis by monitoring the endemic parasite and by controlling the disease associated with. It is

worth mentioning that it is difficult to determine the infectious dose, as there are several factors that must be taken into consideration.

Conclusions and Recommendations

Overall, the UASB-septic tanks did not perform well with regards to the removal of fecal indicator bacteria and parasitic pathogens, especially compared with AWWTP, which showed better removal efficacy for parasitic pathogens only. Hence, supplementary effluent reclamation units are needed to protect public health and the receiving aquatic environment. Based on the results obtained, the following conclusions were drawn:

- No helminthes eggs were detected in the influent of Albireh AWWTP, as the *Ascaris lumbricoides* was not found during the analysis period (April - September). However, in terms of parasitological analysis for the influent of AWWTP, the result revealed that the presence of *Entamoeba histolytica*, *Entamoeba coli*, *Giardia lamblia*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Enterobius vermicularis*, *Trichomonas*, and *Balantidium coli* indicating that the wastewater must be treated before being discharged into the Wadis or being reused for irrigation.
- The effluent from R1, R2 and oxidation ditch were free from the aforementioned pathogens except for *Trichomonas* from the effluent of R1 and R2 where enrichment for its growth was noticed. The fecal coliform (*FC*), fecal streptococcus (*FS*) and *Salmonellae* varied

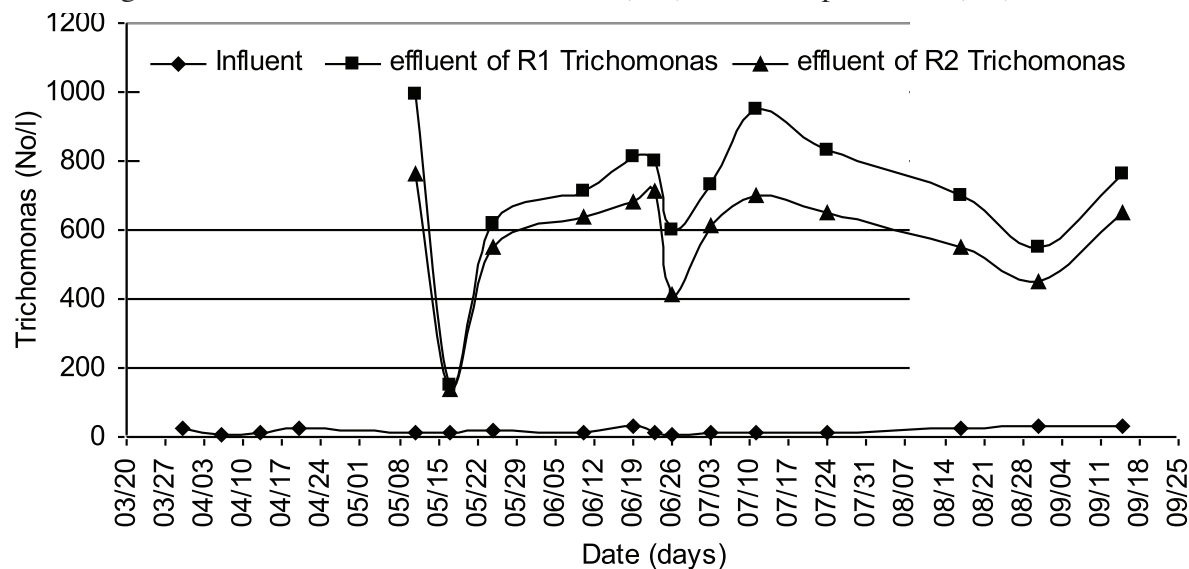


Figure 6. *Trichomonas* content in the UASB-septic tank effluents (R1 and R2 reactors).

influent of AWWTP as log (FC) and log (FS) and were 7.325, 6.293, respectively, where *Salmonellae* was confirmed three time during the analysis. Likewise, the removal efficiency for *Fecal coliform* in R1, R2 and oxidation ditch were 16%, 15%, and 39%, respectively. Moreover, the removal efficiency for fecal streptococcus using R1, R2 and oxidation ditch were 7%, 11% and 16%, respectively. Finally, the removal efficiency for *Salmonellae* was 100% since the effluent from R1, R2 and oxidation ditch are free from *Salmonellae*.

- The average value for turbidity in the influent of R1, R2 and oxidation ditch was 598, 174, 158 and 9 NTU, respectively. Moreover, the removal efficiency for R1, R2 and oxidation ditch was 71%, 73% and 98%. Moreover, ($q < 0.05$) which is significant for R1, R2 and oxidation ditch. High NTU values might impair UV-disinfecting units. Moreover, the value of less than 2 NTU is considered to be appropriate water for recycled water, as this permits effective disinfection.

Recommendations

The results obtained from this study can be used to determine the adequacy of the current municipal wastewater treatment technologies for the control of microbial indicators and wastewater-borne pathogens. The information also can assist Palestinian municipalities in the implementation of efficient treatment strategies to achieve sustainable agricultural effluent reuse. While the anaerobic UASB-septic tanks was less effective in reducing the microbial pathogens of interest, adequate post-treatment units need to be considered in order to improve the overall treatment capability. In addition, to minimize public health risks and improve the environmental quality of receiving water bodies, disinfecting units must be installed in Albireh oxidation ditch if effluent reuse is envisaged. As this study was conducted during summer period, further investigations on UASB-septic tank efficacy for the removal of pathogens during winter months is highly recommended. Further studies for on adequate post-treatment of the UASB effluent is crucial to reduce the turbidity and TSS that directly related to the pathogens. Health risk studies on the types of pathogens caused by direct use of domestic wastewater in some rural areas should be carried out especially

where hygienic services are poor and more infected people are expected. Therefore, further analysis on pathogens identification and characterization should be made as only *Salmonellae* were identified during this study. Finally, raising awareness and training operators and farmers on possible waterborne diseases are necessary protective measures if treated effluent is used for agricultural irrigation, irrespective of whether it is planned or unplanned reuse.

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