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Decentralized Biological Compact Unit for the Removal of Parasitic Helminth Ova During Sewage Treatment

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Abstract

A decentralized cost effective biological compact unit (BCU) wastewater treatment system was operated to investigate its capability in removing parasitic helminth ova. A total of twenty four batches of four wastewater stages (raw sewage, anaerobic effluent, aerobic effluent and BCU effluent) were collected. The samples were concentrated and purified then examined by light microscopy to detect and identify helminths ova. Other parameters for examining the wastewater quality as COD, BOD, TSS and FC were measured. The highest prevalence of helminth ova was recorded in raw wastewater (75%), followed by 37.5%, 25% and 4.2% in anaerobic, aerobic and BCU effluents, respectively. *Ascaris* ova were the most prevalent nematode genus in raw and treated wastewater. The overall removal of helminth ova reached 50% and 66.7% by anaerobic and aerobic treatment steps, respectively. The BCU had a strong significant effect in reducing the count of the parasitic helminth ova (P=0.002), but it did not eliminate them completely. The results also indicated that the treated wastewater quality produced in terms of COD, BOD, TSS, FC and plastic nematode ova after 12 h reached 78 mg/L, 38 mg/L, 19 mg/L, 560 MPN index /100ml and one ova/L, respectively. These values proved to be satisfactory and complying with the Egyptian law for restricted crops irrigation. In conclusion, the BCU was easy to operate. Nevertheless, its effluent quality in terms of parasitic with treated wastewater helminth ova was found to be comparable with typical centralized wastewater treatment units.

***Keywords**:* Reduction; Parasitic helminthes; Biological compact unit; Sewage; Reuse

1. Introduction

In 1989, World Health Organization (WHO, 1989) paid attention to diarrheal diseases caused mainly by the reuse of wastewater and sludge, contaminated by helminthes, for agriculture and set guidelines for safe reuse. Once again by the end of 2006, WHO set up a limit for the presence of helminth egg in wastewater and sludge reused in agriculture or aquaculture (WHO, 2006). Based on the highest risks of wastewater related disease caused by helminthes and the available epidemiological data, the introduced limits intestinal nematode eggs were applied. Considerably, of intestinal nematode eggs have low infective dose and long stability in the environment. In terms of their control in both wastewater and sludge, a little attention has been given to helminth ova (Hindiyeh, 2004). Water resources available for human consumption and irrigation are significantly decreased over the foregoing decennium. This fact a worldwide phenomena, has major implications on the developing countries owing to the high rate of population growth, the fragile economy and almost the arid climate. It becomes inherent, therefore, to recycle wastewater after receiving adequate treatment to compensate for the serious water resource shortage hitting all developing countries (WHO, 2002). Installation, maintenance and running costs of conventional centralized wastewater treatment plants and distribution networks prohibit their use in rural regions in developing nations (Massoud *et al*., 2009; Chirisa *et al*., 2016). Most developing countries suffer from lack of adequate sanitation in their rural and suburbs of urban areas, a real health hazard challenge that must be mitigated. The health hazards linked with the use of untreated sewage affect the health of human and animals (WHO, 2002).

Discharge of untreated or partially treated wastewater into water bodies causes contamination by pathogenic organisms similar to those in the original human excreta such as viruses, bacteria, protozoa and helminthes. These organisms survive in wastewater for days, weeks, and possibly months in the soil and on crops that come in contact with wastewater (WHO, 2002). Therefore, improvements in wastewater treatment technologies are continuously needed to reach optimal designs having low capital investment and maintenance costs, simple operation requirements and high capability for removal of pathogenic organisms including parasitic helminthes (Ichinari *et al*., 2008; Mohammed and Elbably, 2016).

Decentralized wastewater treatment plants are generally small in size and do not need a long distance piping network which makes them the right option for villages or small towns (Paraskevas *et al*., 2002; Sharma and Kazmi, 2016). Subject treatment plants are designed for small communities producing rather low amount of raw sewage (2000–10,000 persons) and are considered a viable solution (Easa and Abou-rayan, 2010). All cost elements of these treatment units are dramatically less than centralized treatment units, which present them as the alternative solution for rural and remote areas in developing countries (Nasr *et al*., 2009; Djuma *et al.,* 2016).

Over the last decennium, most arid and semi-arid countries used wastewater for crop irrigation due to the paucity of alternative water sources and necessity to increase the production of the food. In many countries of the world, treated or even raw wastewater and sludge are being used for agricultural activities. Worldwide, there are 5 million people infected with helminth parasites (WWAP, 2003; Amoah *et al*., 2018), mainly in developing countries. Infections with helminths are particularly common in regions where poor hygienic conditions are predominant and poverty, reaching occurrence percentages of up to 90% (Bratton and Nesse, 1993). Contamination of crops with helminthes can take place through direct fecal contamination with both human and animal excreta, or through the use of contaminated sludge or wastewater for agriculture (Moodley *et al*., 2008). Ascariasis is considered one of the most common parasitic diseases endemic in Latin-America, the Far East, Australia and Africa. The mortality rate of ascariasis was low. Children under 15 years were the most infected ones and about 1.5 million of these children suffered from body growth problems (de Silva *et al.*,1997; Irwin *et al.*, 2017).

In general, helminth parasites are transmitted through ingestion of contaminated food or water (in most helminthes) and contact with infective larvae polluting water and wastewater (in hook worm infection) (Feachem *et al.*, 1983).

The ecology of parasitic helminth ova was poorly understood in the environmental engineering field due to difference in biology and structures between helminth ova other infective stages of protozoa, bacteria and viruses. Helminth ova contribute one of the main health risk-associated pathogens according to the WHO guidelines dealing with aquaculture and agriculture water reuse (Fuhrimann *et al.*, 2016). In environmental study in Egypt, *Ascaris* ova were found in 28% of raw wastewater, and after treatment with gravel bed hydroponic (GBH) constructed wetlands the effluent samples were free from *Ascaris* ova (Stott *et al.*, 1997). In spite of this, limited data were available for efficiency of economically biological compact unit for removal of parasitic helminth ova from domestic wastewater to produce final effluents capable of complying criteria for use in agriculture purposes.

2. Material and methods

2.1 Treatment units and wastewater characteristics

A compact treatment unit of 110L capacity was designed and manufactured from PVC material. The treatment unit consists of three stages. In the 1st stage (vol. 60L), sewage is precipitated and anaerobic treatment occurs. In the 2nd stage (vol. 40L), aerobic biological treatment takes place where packing materials are stacked. The packing is composed of equal length plastic tubes (3 cm) having similar sizes. The tubes are engraved on both surfaces to create crests at equal pitch in order to maximize the contact surfaces where bacteria build up. In the 3rd stage (vol. 10L), the sewage is settled and the excess of settled sludge returns to the contact aeration stage (Figure 1). The compact unit is located at National Research Centre (NRC) pilot area, Dokki, Giza governorate, Egypt. The system is fed continuously with domestic sewage via a connection from the public sewerage system. Physical–chemical and bacteriological analyses for the influent and effluent from each stage were carried out according to the standard method for examination of water and wastewater (APHA, 2012).

2.2 Parasitological analysis

Twenty four batches of four wastewater samples (raw sewage, anaerobic effluent, aerobic effluent and BCU effluent) were collected. The samples were processed briefly according to South Africa national standard method as follow; each sample (5 liters volume) was filtered through the 150 μm filter onto the 20μm, consquently. Then the matter held back by the 150 μm filter was discarded, whilst the solids collected on the 20 μm filter are kept and rinsed off into a plastic beaker. The two stainless steel sieves having pore size content of the beaker was poured into as many test tubes, and then centrifuged at 1389 g for 3 min. The deposits were combined into a 50 ml test

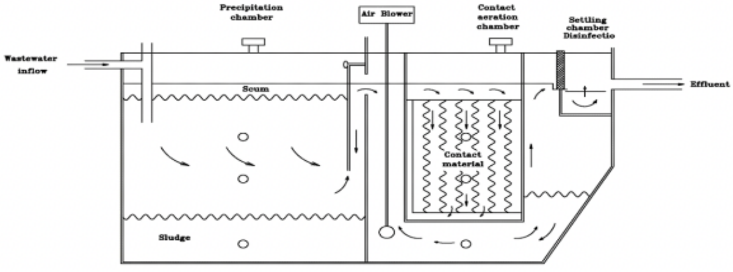

Figure 1. Schematic diagram of compact biological unit system (BCU).

tube. The sediments in the 50 ml test tube were floated using zinc sulphate (ZnSO4) solution (specific gravity 1.3). After adding ZnSO4 solution, the mixture was centrifuged at 617 g for 3 min. The obtained supernatant containing the helminthes ova was added to distilled water and concentrated by centrifugation at 964 g for 3 min. At the final step the deposit was transferred to one or more microscope slides, covered with a cover slip, and examined under the microscope to differentiate and enumerate parasitic helminth ova using the 10x objective lens and the 40x objective to confirm any uncertainties (Moodley *et al*., 2008).

2.3 Statistical analysis

The obtained data were analyzed using one-way analysis of variance (ANOVA), and paired t-test using Minitab statistical program (Minitab Inc., Pennsylvania, USA). P values less than 0.05 were considered significant (Wild, 2005).

3. Results

3.1 BCU Treatment System

The BCU utilizes both anaerobic and aerobic treatment technologies for wastewater treatment. A spool pipe was connected between the nearby sewage intake header and the BCU to afford for continuous feed of the unit at 12hr HRT, a flow rate of 0.22 m3/day, hydraulic loading rate 2 m3/m3/day and organic loading rate of 0.94 kgCOD/m3/day. The BCU was able to remove 80% of chemical oxygen demand (COD), 90% of total suspended solids (TSS), 85% of biological oxygen demand (BOD), 48.5% of Total Kjeldahl nitrogen (TKN) and 52% of total phosphorous (TP) (Table 1). The results indicated that the treated wastewater quality produced in terms of COD, BOD, TSS , FC and nematode ova after 12 hr reached 78mg/L, 38mg/L, 19mg/L, 560 MPN index/100ml and one ova/L.

3.2 Removal of parasitic helminth ova

The highest prevalence of the helminth ova was recorded in raw wastewater (75%), followed by 37.5% in anaerobic effluent and 25% in aerobic effluent. *Ascaris* ova were the most prevalent parasite in raw and treated wastewater, followed by those of *Hymenolepis*, *Enterobius* and *Trichuris*. Statistical analysis showed the prevalence of *Ascaris* ova had a strong significance (P=0.000) in wastewater samples in comparison with other helminths ova. The prevalence of *Ascaris* ova in raw wastewater was 37.5%, while it reached 20.8%, 12.5% and 4.2% in anaerobic, aerobic and BCU effluents, respectively. *Trichuris* ova occurred in raw and anaerobic effluents by the same percentage (4.2%). On the other hand it disappeared in the aerobic effluent samples. The prevalence of *Hymenolepis* ova reached 12.5% in each of raw wastewater, anaerobic and aerobic effluents. *Enterobius* ova were present only in raw wastewater in percentage 20.8%, while there was no ovum related to *Enterobius* in anaerobic, aerobic effluents and BCU effluents. Anaerobic treatment step had no significant effect on the removal of pathogenic helminth ova (P = 0.186). By conventional statistical criteria, the removal of helminth ova by both anaerobic and aerobic treatment steps was considered to be not significant (P=0.134)

4.Discussion

The BCU was able to remove 85% of biological oxygen demand (BOD). Results of the present investigation were in accordance with that obtained by Ichinari *et al.* (2008) who used anaerobic followed by aerobic biofilm for treatment of domestic wastewater (BOD 88%) and in line with those obtained by Zhidong *et al.* (2009) and Imura *et al.* (1995). The present results were lower than those mentioned by Abou-Elela *et al.* (2015) who obtained 93% BOD removal when treating domestic wastewater using up flow anaerobic sludge blanket (UASB) followed by aerobic treatment. Furthermore, a drop of Three logs in fecal coliform counts had been achieved in the final effluent at 28ºC (Pant and Mittal, 2007). The majority of FC removal was found to have happened in the aerobic and settling compartments resulting to an average count of 9.4×102/100 ml for TC and 5.6×102/100ml for FC in the final effluent at 28°C(Nasr *et al*., 2016). Temperature and BOD concentration of wastewater that contained pathogens were observed to have major influence on the reduction rates. The main portion of TC and FC removal in the BCU occurred in the aerobic compartment enhanced by the large contact surface of the packing materials (Metcalf and Eddy, 1991). These results are similar to that obtained by Ueda and Horan (2000) and Stevik *et al*. (2004) who found that the adsorption to the biofilm was the main cause of removal of different types of pathogenic bacteria, protozoa and helminthes in porous media, membrane bioreactor and biological sand filters.

The results of the current study declared that parasitic helminth ova related to two categories of pathogens according to the environmental classification of excreted pathogens (Hindiyeh, 2004). *Enterobius* and *Hymenolepis* follow Category I and *Ascaris* and *Trichuris* follow category II. Category I infections are brought about by pathogens infective immediately upon excretion, have a low infectious dose and cannot replicate outside the host. This category includes excreted viruses, protozoa and the helminths (such as *Hymenolepis* and *Enterobius*). Transmission of these pathogens takes place primarily via direct transport from person to person in the immediate municipal environment, especially when low grades and crowding of personal hygiene dominate, although the survival time of excreted viruses and protozoa may be long enough for them to pose a health risk in schemes for the use of excreta and wastewater. The diseases in category II are brought about by the soil-transmitted intestinal nematodes, which do not require an intermediate host. Their ova need a latent period of development maturation in the environment before they ready to be infective. On the other hand, these parasites are only weakly affected by host immunity and their infectious dose is only one organism. The most important of these are the human roundworm *Ascaris lumbricoides*, the hookworms (*Ancylostoma duodenale* and *Necator americanus*), and the human whipworm (*Trichuris trichiura*). These soil-transmitted

Table 1. Characteristic of the BCU compartments effluent at HRT 12h (Nasr *et al*., 2016).

Parameter	Measuring unit	Raw wastewater	Treatment steps						Egyptian law for re-strictedd irrigation
			Anaerobic effluent	Removal%	Aerobic effluent	Removal %	BCU effluent	Removal %	
pH		7.6	7.4	-	7.8	-	7.8	-	
Temp	°C	28	28	-	28.4	-	28	-	-
TSS	mg /L	237	64	71	36	44	19	90	-
COD	mg O_2/L	512	247	52	145	40	78	80	40
BOD	mg O_2/L	261	131	54	72	42	38	85	80
T.K.N	mg N /L	56	44.6	18	34	20	27	48.5	40
AMN	mg N /L	25	28	-13	16	35	12	52	-
TP	mg P /L	4.8	2.92	21	1.8	42	1.42	65	-
NO3	mg N /L	0.24	0.05	-	2.1	-	1.8	-	-
TC	MPN index/100ml	6.2×10^6	5.6×10^5	88.7	4×10^4	92.2	9.4×10^2	97.5	-
FC	MPN index/100ml	5.75×10^5	4.5×10^4	89.6	3.5×10^3	92.8	5.6×10^2	97.6	1000
Nematode ova*	Ova/L	12	7	41.7	3.6	48.6	1	91.7	1

*: indicates results of the present

intestinal nematodes are readily conveyed

Table 2. Prevalence of helminth ova in raw and stepwise treated wastewater

Helminth ova	Wastewater samples for											
	Raw sewage			Anaerobic effluents			Aerobic effluents			BCU effluents		
	Ex.	+ve	%	Ex.	+ve	%	Ex.	+ve	%	Ex.	+ve	%
Ascaris		9	37.5		5	20.8		3	12.5		1	4.2
Trichuris		1	4.2		1	4.2		0	0		0	0
Hymenolepis	24	3	12.5	24	3	12.5	24	3	12.5	24	0	0
Enterobius		5	20.8		0	0		0	0		0	0
Total		**18**	**75**		**9**	**37.5**		**6**	**25**		**1**	**4.2**

Ex.: Examined samples, +ve: Positive samples, %: Percent of positivity

Table 3. Removal of the detected helminth ova through different treatment steps

Helminth ova	Positive raw wastewater samples	Anaerobic effluents			Aerobic effluents			BCU effluent		
		+ve	-ve	%	+ve	-ve	%	+ve	-ve	%
Ascaris	9	5	4	44. 4	3	2	40	1	2	66.7
Trichuris	1	1	0	0	0	1	100	0	-	-
Hymenolepis	3	3	0	0	3	0	0	0	3	100
Enterobius	5	0	5	100	0	0	0	0	-	-
Total	**18**	**9**	**9**	**50**	**6**	**3**	**33.3**	**1**	**5**	**83.3**

Table 4. Range and mean count of helminths ova per liter in positive wastewater samples.

Helminth Ova	Raw sewage		Anaerobic treatment		Aerobic treatment		BCU final effluent	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Ascaris	1-7	4. 3	2-4	3	2-3	2. 3	1	1
Trichuris	3	3	2	2	0	0	0	0
Hymenolepis	2-4	3. 3	1-3	2	1-2	1. 3	0	0
Enterobius	1-5	2.6	0	0	0	0	0	0
Total		**13.2**		**7**		**3.6**	**1**	**1**

through the use of raw or insufficiently treated wastewater in agricultural purposes (Hindiyeh, 2004). Results of the present study showed that *Ascaris* ova were the most prevalent in raw and treated wastewater, followed by ova of *Hymenolepis*, *Enterobius* and *Trichuris*. In a Syrian study concerning parasitic infestation and the use of untreated sewage for irrigation of vegetable crops, it was found that the domestic sewage of Aleppo district contained 3340 *Ascaris* eggs/liter, which represents an *Ascaris* prevalence rate of 42% of the total Aleppo population excreting an average of 800,000 eggs/day/person (Bradley and Hadidy, 1981). The correlation between the irrigation of vegetables with sewage and the number of parasites in Aleppo is that irrigation completes the cycle by returning the parasites back to the community. Identification of helminth ova in the influent of Tehran, Iran revealed the presence of hookworms, *Ascaris lumbricoides*, *Enterobius* vermicularis, *Taenia spp.*, *Trichostrongylus spp.*, *Dicrocoelium dendriticum* and *Hymenolepis* nana, while in Isfahan district only *Trichostriogylus H. nana* and *A. lumbricoides*, were isolated (Mahvi and Kia, 2006). The results of the literature confirm that helminth ova content in wastewater is very different due to the difference in health conditions of people living in developing and developed countries, and therefore the used treatment methodology for wastewater should be different.

The present study showed that the *Ascaris* ova removal reached 44.4% and 33.3% after anaerobic and aerobic treatment steps, respectively. The removal of *Enterobius* was achieved completely after the first treatment step (anaerobic). Anaerobic treatment step had no removal effect on *Trichuris* ova, while the aerobic treatment step removed it completely. *Hymenolepis* ova were resistant to the removal by both anaerobic and aerobic treatment steps. Other investigation declared that the up flow anaerobic sludge blanket with 5.5 h retention time, with waste water containing between 64 and 320 helminth ova (HO)/l produced an effluent with 1.3–45 helminth ova/l with a mean value of 16 HO/l and a mean removal efficiency of 96%. The researchers recommended coupling the UASB system with stabilization ponds in order to completely remove the fluctuations observed in the effluent (Von Sperling *et al*., 2002).

The results obtained from the BCU showed 94.4% removal of helminth ova. these results are compatible with those obtained by Keawvichit *et al*. (2001) in the treatment of domestic wastewater of Chiang Mai municipality by centralized conventional wastewater treatment processes using aerated lagoon system that releases more than 25,000 m3/ d of treated water. They recorded the disappearance of parasitic stages in treated wastewater after investigating 12 samples in 6 months. Also, our results were similar to those obtained by Sharafi *et al*. (2012) who achieved 100% removal of protozoan cyst for both of Kermanshah activated sludge wastewater treatment plant and Gilan-e-Gharb stabilization ponds. Similar results were reported by Jimenez (2007) who recorded 90–99% removal of parasitic helminths ova and protozoan cysts through processes of conventional wastewater treatment plants. Therefore, the reuse of treated wastewater in agriculture was considered efficient, especially in tropical countries or in drought zones.

5. Conclusion

The use of designed BCU system with the established criteria for domestic wastewater treatment produced effluents having physico-chemical, bacteriologic and parasitological criteria complied with the Egyptian code for wastewater treatment and compatible with WHO guidelines for wastewater treatment and reuse. This BCU system is very easy to operate and can be applied successfully in rural areas and small communities.

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