

Bio-filtration of helminth eggs and coliforms from municipal sewage for agricultural reuse in Peru

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Thesis

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Abstract

Where fresh water resources are scarce, treated wastewater becomes an attractive alternative for agricultural irrigation. However, the presence of large amounts of pathogens, even in treated wastewater, constraints its productive use, which is aggravated when sanitation and public health are poor. Among pathogenic indicators, helminth eggs are one of the most persistent microorganisms in treated effluents that may survive for several months in the irrigated fields. Application of upflow anaerobic sludge blanket (UASB) reactors could contribute to decrease the pathogenic content in wastewater due to physical and biological interactions with the anaerobic sludge bed, such as filtration and entrapment. In this thesis, the potential of the anaerobic sludge bed to particularly remove helminth eggs, was investigated in four phases. In the first phase, a temperature of 4° C was fixed in the UASB reactors in order to reduce the biological activity of the sludge. Hence, the anaerobic sludge filtration capacity at different upflow velocities was studied. This phase of the research was performed in two experiments. The first one using latex beads, simulating helminth eggs, and the second one using real helminth eggs, predominating in Peruvian wastewater. First experimental results show that increasing the upflow velocity led to a decrease in the removal efficiency of latex beads. At the lowest upflow velocity of 0.3 m·h⁻¹, 100% removal of latex beads was reached. At an upflow velocity higher than 1 m·h⁻¹, the removal efficiency dropped under 90 %. The degree of stabilisation of the sludge nor the sludge bed volume did not have a significant effect. Second experiment's results show that with upflow velocities below 1.5 m·h⁻¹ real helminth eggs removal is greater than 70 %. Simultaneously tested, total and faecal coliforms removal was less than 83 %. The most common helminth eggs species found in the studied wastewater were *Ascaris lumbricoides*, *Trichuris spp.* and *Strongyloides spp.* The second phase was performed using two lab-scale UASB reactors at average ambient temperatures between 16.7 °C and 28.5 °C in the city of Lima (Peru). *Ascaris suum* eggs originating from infected pigs were selected as model organisms, considering their similarity, in terms of size and morphology, with *Ascaris lumbricoides*, a human pathogen. The sludge filtration capacity was determined, applying upflow velocities between 0.09 and 0.68 m·h⁻¹. Average helminth eggs removals varied between 26 and 93 %, depending on upflow velocity and sludge bed height. 93 % removal was achieved when applying an upflow velocity of 0.09 m·h⁻¹ and a sludge bed height reaching 19-25 % of the total reactor height. The third phase was conducted to test the effect of lower operational temperatures in the UASB reactor on the pathogen removal from domestic wastewater. Thus, a lab scale UASB reactor in the city of Puno (Peru), treating wastewater with temperatures varying between 11.3 and 14.3 °C for a period of 22 weeks after the start-up of the reactor, was used. Upflow velocities varied between 0.12 and 0.41 m·h⁻¹. Results confirmed outcomes of the first phase of this research concerning helminth eggs removal, and consequently show that the sludge bed filtration capacity varied between 89 and 95 %. Faecal coliform removal varied between 0.9 and 2.1 log₁₀ and *E. coli* removal between 0.8 and 1.6 log₁₀. In

general, removal efficiencies regarding helminth eggs and faecal coliforms, are not sufficient to comply with reuse standards. Finally, the capacity of Down Flow Hanging Sponge (DHS) reactors for removing faecal coliforms from domestic UASB reactor effluent for agricultural reuse in developing countries was investigated. Applied reactors were the cube type DHS (G1) without recirculation, the cube type DHS (G1) with recirculation and the curtain type DHS (G2). Results reveal an average faecal coliform removal of 4.74, 3.42 and 1.25 \log_{10} respectively. These results comply with categories A, B and C of WHO (1989) standards, correspondingly. Therefore, treatment trains consisting of UASB-DHS reactors can possibly be applied when agricultural reuse is contemplated.

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This thesis is dedicated
to my beloved parents, sisters
and brother

CHAPTER 1

General Introduction

1.1 Introduction

Adequate sanitation, together with hygiene and safe water, are fundamental for good health and social and economic development (Mara et al., 2010). Since 1990 sanitation coverage has increased only by 20% in developing regions. It is reported in literature (Yi et al., 2011; Uwidia and Ejeomo, 2014) that municipal and industrial wastewaters, produced in developing countries, are in many cases just discharged into water bodies without any treatment. Likewise, in 2012, 1 billion people (15 % of the world's population) still continue practising open defecation (WHO, 2014). Globally, more than 50% of the rivers, oceans, and lakes are polluted with untreated wastewater (Mara, 2003; Baum et al., 2013). The non-treated discharge, also contaminates water supplies and food, causing illness, particularly among the poorest population. Clean natural fresh water sources are also becoming increasingly scarce (Corcoran, 2010). Particularly, in Latin America approximately 86% of the total municipal wastewater flow is discharged untreated. It brings into play significant environmental and human health impacts and deterioration of vital water sources for human, agricultural and industrial activities (Mara, 2003; Qadir et al., 2010). Main reasons for the lack of wastewater treatment are financial issues and ignorance of low-cost wastewater treatment processes and economic benefits of treated water reuse (Mara, 2003).

Wastewater can be viewed as both a harmful effluent and a renewable resource. While non-treated municipal wastewater can cause pollution and illness, properly treated wastewater can become a source of water and nutrients, for example agriculture and landscape irrigation or aquaculture. Main applications of treated wastewater are in agriculture and aquaculture (WHO, 2006). Wastewater management systems should include appropriate treatment technologies in combination with methodologies and techniques for applying safe use of the treated wastewater and included nutrients (Corcoran, 2010).

Using reclaimed water also diminishes the demands on conventional water sources, offering the possibility to use them for drinking water purposes while protecting the environment (Quinlan et al., 2008; Yi et al., 2011). Where water is used several times, society saves costs, and where wastewater is used for productive purposes, like (ferti-) irrigation, society gains additional value from the crops produced and from the improvements in livelihoods (Qadir et al., 2010).

For the use of treated wastewater in agriculture, several factors need to be considered during the selection and consequent design of technologies. These factors include the presence of pathogens, nutrients, heavy metals and chemical contaminants as well as salinity and the impact on soil structure. (Norton-Brandão et al., 2013). Though any required water quality can be attained by adding existing advanced treatment steps (Tchobanoglous et al., 2003; Lahnsteiner and Lempert, 2007), financial constraints

often limit the application of these technologies. Therefore, treated municipal wastewaters might, amongst other components, still contain human pathogens (Mara, 2003; Jiménez and Asano, 2008)

In industrialised countries, enforced restricted irrigation practices prevent that raw eaten vegetables and salad crops are irrigated with raw or insufficiently treated wastewater. In developing countries, of which many have adopted the same strict regulations, public health authorities “officially” do not approve the use of wastewater for irrigation of vegetables and salad crops eaten raw. However, when water is scarce, such crops are widely irrigated illegally with raw or poorly treated wastewater. Especially in regions with high water scarcity, the use of domestic wastewater or excreta and grey water is an important component of integrated water and nutrient resource management (IWRM)(WHO, 2006). This usually occurs in the vicinity of major cities, particularly in semi-arid regions. It was estimated that in 2011 approximately 50 countries throughout the world irrigated 50 million hectares of crops using raw wastewater. It resulted in contamination of 12% of the world's crop production and as a consequence affected the public health (Shuval, 2011).

1.2 Pathogens present in wastewater

Pathogenic organisms from human faeces contained in wastewater are very diverse and can be classified in four groups, viz. bacteria, protozoa, viruses and helminths. This diversity and number of pathogens in wastewater depend upon the general health of the contributing population (Feachem et al., 1983; Sidhu and Toze, 2009). Once in the wastewater, waterborne pathogens are transmitted by the ingestion of contaminated water with faeces. They also can be transmitted by ingestion of contaminated food, dermal contact, or by inhalation (Tchobanoglous et al., 2003; Santo Domingo et al., 2007; Jiménez et al., 2010). The ecological and survival characteristics of pathogenic organisms depends on environmental conditions. Thus, no single indicator organism can predict the presence of all enteric pathogens in contaminated waters. (Savichtcheva and Okabe, 2006; Santo Domingo et al., 2007).

Bacteria are microscopic organisms ranging from approximately 0.2 to 10 μm in length. Municipal wastewater can contain a wide variety and concentration range of pathogenic bacteria. One of the most common pathogens in domestic wastewater is the genus *Salmonella*. Other bacteria isolated from wastewater include *Vibrio* (i.e. *Vibrio cholerae*), *Mycobacterium*, *Clostridium*, *Leptospira* and *Yersinia* species. Faecal and total coliforms bacteria are commonly used as indicators of potential water-borne bacterial pathogens (Gronewold and Wolpert, 2008; Bohra et al., 2012). Coliforms are usually detected in higher concentrations than pathogenic bacteria and generally respond similar to environmental conditions and treatment systems as many pathogenic bacteria (Rompré et al., 2002). However, bacteriological determination, relying only on coliforms bacteria, unfortunately does not predict the presence of all

pathogenic organisms (Watanabe et al., 1997; Asano, 1998; Tchobanoglous et al., 2003). Coliforms bacteria concentration is measured using the most probable number (MPN) method or the colony forming units (CFU) method. Therefore it is expressed as MPN. 100 mL⁻¹ or CFU. 100mL⁻¹ respectively (APHA et al., 1998; Gronewold and Wolpert, 2008).

Protozoa is a collective term for unicellular eukaryotes, lacking cell walls. Protozoans are often classified along with algae and other simple unicellular eukaryotes in the kingdom Protista. *Giardia lamblia* and *Cryptosporidium parvum* are examples of common protozoa detected in contaminated water. They prevail in water as cysts with approximately 15 µm or oocysts growing from 3 to 6 µm. These cysts are insensitive to disinfectants at the concentration commonly used in water treatment plants, i.e. between 0.05 and 1 mg. L⁻¹ Cl₂ to reduce bacterial contamination (Caccio et al., 2003; Tchobanoglous et al., 2003).

Viruses are extremely small parasitic microbes which vary between 20 and 200 nm. They can be reproduced only by invading a host cell whose reproductive processes they redirect to produce more viruses (Mara, 2003). The most important human enteric viruses are enteroviruses like *poliovirus*, hepatitis A, echo, and coxsackie (Asano, 1998; Payment et al., 2001; Tchobanoglous et al., 2003).

Helminths are pluricellular worms. Helminths worms come in different types and sizes (from around 1 mm to several metres in length) with various life cycles and optimal living environments. Their life cycles are very complex and very different from other pathogens present in domestic wastewaters. Their eggs are microscopic and range in size from 10 µm to more than 100 µm (Tchobanoglous et al., 2003).

When a person ingests the eggs, they stick to the duodenum, where the larvae leaves the egg, crossing the wall into the blood stream. The different types of helminths eggs that can be found in municipal wastewater are shown in Table 1.1 and the most important helminth egg characteristics are presented in Table 1.2. The eggs contained in wastewater are not infective itself. However, infective larvae can be developed in terms of days at temperatures less than 45°C and moisture higher than 5 % as usually found in soils, crops and human body (Feachem et al., 1983; Koné et al., 2007). The most important hosts which can transport helminth eggs to inhabitants are described in Table 1.3.

Amongst the waterborne diseases, which are caused by pathogens present in raw wastewater, helminth eggs have been identified as those posing a major health risk for humans. Intestinal helminthiasis is an important public health problem in developing countries (Cabirol and Noyola, 2002). Helminth eggs have the ability to survive in adverse environmental conditions in which most pathogenic bacteria cannot survive. Helminth eggs are infective at minimal infective dose (Peasey et al., 2000; Bitton,

2005). In addition, humans did not develop self-immunity (Westcot, 1997). Helminths are usually present in high concentrations in wastewater and excreta, especially in developing countries where hygiene and proper water treatment is lacking (Méndez Vega and Marchán Peña, 2008).

Table 1.1 Taxonomic classification of main helminths found in wastewater

Kingdom	<i>Animalia</i>												
Subkingdom	<i>Eumetazoa</i>												
Infrakingdom	<i>Bilateria</i>												
Phylum	<i>Platyhelminthes</i>					<i>Nematoda</i>							
Class	<i>Tremátoda</i>				<i>Cestoidea</i>	<i>Phasmidea o Secernentea</i>			<i>Adenophorea</i>				
Subclass	<i>Digenea</i>				<i>Eucestoda</i>								
Superorder	<i>Anepitheliocystidia</i>		<i>Epitheliocystidia</i>										
Order	<i>Echinostomida</i>	<i>Strigeatida</i>	<i>Plagiorchiida</i>	<i>Opisthorchiida</i>	<i>Cyclophyllidea</i>		<i>Ascaridida</i>		<i>Rhabditida</i>		<i>Trichurida o Enoplida</i>		
Suborder	<i>Echinostomata</i>	<i>Strigeata</i>	<i>Plagiorchiata</i>	<i>Opisthorchiata</i>			<i>Ascaridina</i>		<i>Rhabditina</i>	<i>Strongylina</i>	<i>Enoplina</i>		
Superfamily	<i>Echinostomatoidea</i>	<i>Schistosomatoidea</i>	<i>Allocreadioidea</i>	<i>Opisthorchioidea</i>			<i>Ascaridoidea</i>	<i>Oxyuroidea</i>	<i>Rhabditoidea</i>	<i>Strongyloidea</i>	<i>Trichuroidea</i>		
Family	<i>Fasciolidae</i>	<i>Schistosomatidae</i>	<i>Troglorematidae</i>	<i>Opisthorchiidae</i>	<i>Taeniidae</i>	<i>Dipylidiidae</i>	<i>Ascarididae</i>	<i>Oxyuridae</i>	<i>Strongyloidae</i>	<i>Ancyllostomatidae</i>	<i>Trichuridae</i>		
Genus	<i>Fasciola</i>	<i>Schistosoma</i>	<i>Paragonimus</i>	<i>Clonorchis</i>	<i>Taenia</i>	<i>Dipylidium</i>	<i>Ascaris</i>	<i>Enterobius</i>	<i>Strongyloides</i>	<i>Ancylostoma</i>	<i>Trichuris</i>		
Specie	<i>F. hepática</i>	<i>S. mansoni</i>	<i>Paragonimus spp</i>	<i>C. sinensis</i>	<i>T. solium</i>	<i>T. saginata</i>	<i>D. caninum</i>	<i>A. suum</i>	<i>A. lumbricoides</i>	<i>E. vermicularis</i>	<i>S. stercoralis</i>	<i>A. duodenale</i>	<i>T. trichitura</i>

Source: After Olsen (1974), Soulsby (1968), Neves (2000), Beltrán *et al.* (2005), Llop *et al.* (2001), Botero and Restrepo (2003)

Table 1.2 Main characteristics of helminths present in wastewater

Specie	EP	Egg Size (microns)		EC	EEW	LGT	T	pH	Host	Human pathology
		Length	wide							
<i>F. hepática</i>	8000 - 25000 daily	130 - 150	63 - 90	NE	Has esclerotine (proliferol and proteins)	2 months	39 °C	basic	human (D); snail (I)	Fascioliasis
<i>S. mansoni</i>	300 daily	114 - 180	45 - 70	E	Transparent covering with a lateral spine	34 days	36.5	neutral	human (D); snail (I)	Esquistosomiasis o bilharzia
<i>Paragonimus spp</i>	2500 daily	68 - 118	39 - 67	NE	Narrow layer, asimetric and lightly compressed	22 – 24 weeks	36.5	basic	human (D), snail (1 st I), crustaceans (2 nd I)	Paragonimiasis
<i>C. sinensis</i>	2880 daily	27 - 35	11-20	E	Oval shape with Operculum convex	28 days	36.5	basic	human (D), snail (1 st I), fish (2 nd I)	Clonorchiasis
<i>T. solium</i>	30000 for each PS	4-8	6-7	E or mature PS	Hardcover and easy to stick to the host	50 days	36.5	basic	human (D), pig (I)	Cisticercosis
<i>T. saginata</i>	80000 for each PS	4-8	6-7	E or mature PS	Striated wall and oncosphere	50 days	36.5	basic	human (D), cow (I)	Teniasis
<i>D. caninum</i>	25 – 30 for each PS	35	60	E o mature PS	Containing lipids, mitochondria, glycogen	3 – 4 weeks	36.5	basic	human (D), flea (I)	Dipilidiasis.
<i>A. suum</i>	20000 daily	45 - 75	35 - 50	E	Lipoproteins and a layer of chitinous	12 – 15 months	36.5	neutral	human	Ascariasis
<i>A. lumbricoides</i>	200000 a 240000	40 -80	85-95	E	Desiccation-resistant layer	2 months	36.5	basic	human	Ascariasis
<i>E. vermicularis</i>	11000 - 16000	50 - 60	20 - 30	E and N.E	Transparents with a thick layer	8 weeks	36.5	basic	human	Enterobiasis
<i>S. stercoralis</i>	11000	40 - 60	32 - 40	E	thick layer	3 days for direct route, 10 days indirect route	36.5	basic	human	Strongyloidiasis
<i>A. duodenale</i>	10000 - 30000	40	32	E	Resistent layer	3 – 4 weeks	36.5	basic	human	Anquilostomiasis
<i>T. trichiura</i>	2000 – 10000	30	50	N.E and E	Long, tough	2 – 3 weeks	36.5	basic	human	Tricuriasis

Notes: EP: egg production; EC: egg characteristic produced in the parasite life cycle; EEW: Structure of the egg wall; LGT: Time of activation and development stage of the parasite (usually a larvae) in the final host; T: Temperature for larvae growing; NE: not embryonated; E: embryonated; PS: proglotide segments; I: intermediary host; D: definitive host.

Source: After Olsen (1974), Soulsby (1968), Neves (2000), Beltrán *et al.* (2005), Llop *et al.*(2001), Jimenez (2007); Botero and Restrepo (2003)

Table 1.3 Main intermediate hosts for different species of helminth eggs

Specie	Main intermediate host
<i>F. hepática</i>	Snail
	<i>Stagniola bulimoides</i> <i>Fossaria modicella</i>
<i>S. mansoni</i>	snail
	<i>Stagniola palustris</i>
	<i>S. exilis</i>
	<i>S. reflexa</i>
	<i>S. emarginata</i>
	<i>Lymnaea stagnalis</i>
	<i>Physa parkeri</i> <i>Physa gyrina</i>
<i>Paragonimus spp</i>	snail (first host):
	<i>Pomatiopsis lapidaria</i>
	<i>P. cincinnatiensis</i>
	<i>Oncomelania nosophora</i>
	crustaceans (second host):
	<i>Cambarus propinquis</i>
	<i>C. robustus</i>
	<i>C. virilis</i>
	<i>C. diogenes</i>
	<i>C. rusticus</i>
<i>C. sinensis</i>	snail :
	<i>Ammicola limosa</i>
	fish:
	<i>Catostomus comersoni</i>
<i>T. solium</i>	<i>Sus scrofa doméstica</i>
<i>T. saginata</i>	<i>Bos taurus</i>
<i>D. caninum</i>	flea:
	<i>Trichodectes canis</i>
	<i>Ctenocephalides canis</i> <i>C. catis</i>

Source: After Olsen (1974), Soulsby (1968), Neves (2000), Beltrán *et al.* (2005), Llop *et al.* (2001), Botero and Restrepo (2003)

Regarding the content of helminth eggs in raw domestic wastewater and sludge in different countries, a summary of the existing literature (Mahvi and Kia, 2006; García Palacio, 2010; Navarro and Jiménez, 2011; Gil et al., 2013; Verbyla et al., 2013) is presented in Table 1.4. It can be distinguished that the knowledge of helminth egg content in raw domestic wastewater is scarce. The helminth eggs content shows a wide variation between 1 and 3006 eggs. L⁻¹. Moreover, the information about helminth eggs content in the excess sludge is more limited and presented a variability between 67 and 735 eggs. g TSS⁻¹.

Table 1.4 Helminth eggs content in wastewater and excess sludge in different countries.

Country or region	Domestic wastewater	Sludge	References
	eggs. L ⁻¹	eggs. g TSS ⁻¹	
Developing countries in general	70-3000	70-735	a
Bolivia	306-3006	N.D.	b
Brazil	166-202	75	a
Colombia	16-43 (Neves)	N.D.	c
Egypt	N.D.	Mean: 67 maximum: 735	a
Ghana	N.D.	76	a
Iran	2-21(Teheran)	N.D.	d
Jordan	300	N.D.	a
Mexico	6-98(cities)	N.D.	a
	Up to 330(rural areas)	N.D.	a
Venezuela	270 (Aragua)	N.D.	e
Morocco	840	N.D.	a
Ukraine	60	N.D.	a
France	9	5-7	a
Germany	N.D.	<1	a
Great Britain	N.D.	<6	a
United States	1-8	2-13	a

Notes:

- N.D. means no data available
- References a: Navarro and Jiménez (2011); b: Verbyla (2013); c: García Palacio (2010), d: Mahvi (2006), e: Gil *et al.* (2013)

Furthermore helminth eggs are spread through the environment in areas where access to sanitation (i.e. safe storage, collection, treatment and safe disposal/reuse of faeces and urine) is insufficient (Koné et al., 2007). There are several ways of transmission of helminth eggs to humans. A scheme of the main transmission ways, elaborated based on Table 1.1, 1.2 and 1.3 is shown in Figure 1.1:

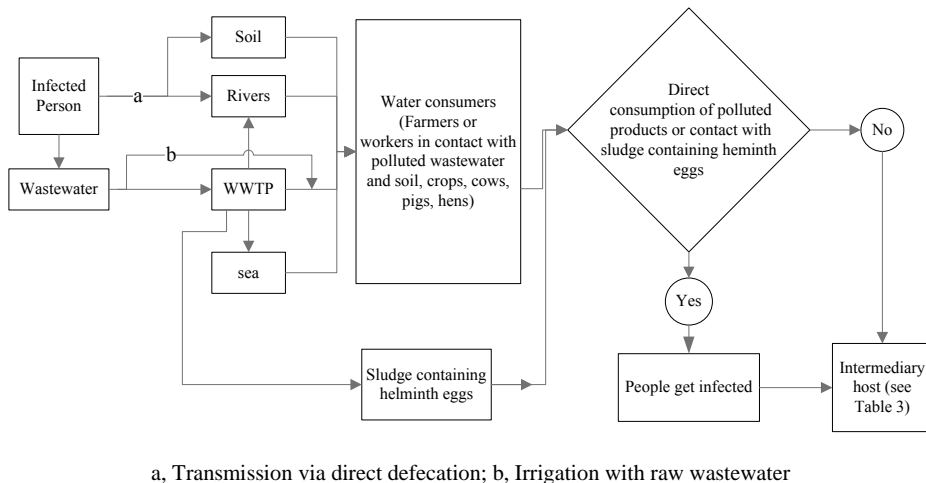


Figure 1.1 Schematic representation of transmission pathways for helminth eggs infections via direct defecation and wastewater.

Source: After Ensink (2007), Olsen (1974), Soulsby (1968), Neves (2000), Beltrán *et al.* (2005), Llop *et al.* (2001), Feachem (1983, Botero and Restrepo (, 2003 #280).

Among enteric pathogens, helminth eggs are very resistant and can survive in water, soil and crops between 10 and 12 months upon excretion in tropical climates (Koné et al., 2007). Helminth eggs are transmitted to humans by direct ingestion of polluted products or via an intermediary host (Figure 1.1). Breaking the transmission cycle of helminths is crucial to prevent parasitic infection (Ruff, 1999; Bergquist and Lustigman, 2010). It can be done by hygiene routines during irrigation (WHO, 2006) or removing them in the wastewater treatment plant (Scott et al., 2004; von Sperling et al., 2005; Jiménez, 2006; Ensink et al., 2007).

1.3 Wastewater treatment technologies applied for wastewater reclamation in developing countries

This section discusses the possibilities to treat domestic wastewater to such an extent that safe reuse for different types of crops and irrigation methods is feasible.

1.3.1 Pathogen removal and/or inactivation

The removal of pathogens in wastewater treatment plants (WWTP) can be performed by physical separation and inactivation. The first mentioned process includes one or more phase separation processes where pathogens are retained as sludge independently of their viability. During inactivation, pathogens die and consequently becomes non-viable, consequently the biological potential of them to successfully duplicate is destroyed (Sobsey, 1989; de Victorica and Galván, 2003; Beutel and Larson, 2014).

Examples of physical pathogen separation techniques are sedimentation and filtration (Feachem et al., 1983; Jimenez et al., 2001; von Sperling et al., 2005; Qu et al., 2013; Beutel and Larson, 2014). Also biological treatment techniques like activated sludge or UASB include a sludge separation step where adsorbed pathogens are separated from the liquid phase.

Due to environmental conditions, like temperature and pH, the ideal environment for human pathogenic organisms is the human intestinal track (Botero and Restrepo, 2003; Jimenez, 2007). Outside the human body, i.e. in the sewer system, wastewater treatment plant or the receiving water body, the pathogenic organisms will not grow and tend to decay (von Sperling et al., 2005). Main control factors for inactivation of bacteria and viruses are temperature, solar radiation, pH, food shortage, predators and toxic compounds (von Sperling et al., 2005; Qu et al., 2013). Helminth eggs, as indicated earlier, are an exception because they can survive for many months. Inactivation of protozoan cysts and helminth eggs mainly occurs at temperatures above 45°C (Koné et al., 2007) and at pH values higher than 12 or lower than 7 (Jimenez et al., 2001; Jimenez, 2007). Koné (2007) reported an *Ascaris* inactivation of 90 to 100% during composting of faecal sludge at temperatures between 45 and 68 °C and an exposure time varying between 30 and 80 days. Cabirol *et al.* (2002) reported that exposure to a temperature of 60°C for 30 minutes was sufficient to inactivate *Ascaris* eggs. Furthermore, Feachem *et al.*, (1983) reported that inactivation of helminth eggs occurs at a moisture content lower than 5%.

1.3.2 Treatment technologies

The so-called land based or extensive technologies were in the past widely implemented in developing countries for wastewater treatment. Extensive technologies are effective in removal and inactivation of helminth eggs (von Sperling

et al., 2005) but require large land areas and therefore cannot be applied in densely populated or urbanised regions (Brissaud, 2010). Existing land-based technologies are often over-loaded as a result of growing population, while possibilities for extension are limited by increased land prices. For example, in Lima (Peru), urban areas strongly expanded and the costs of land increased 1.87 times from 2002 to 2010 (Webb and Baca, 2009).

When selecting a treatment technology for application in developing countries, investment, operation and maintenance costs and simplicity are most important criteria (von Sperling, 1996; von Sperling and Chernicharo, 2002; Massoud et al., 2009).

Conventional extensive sewers combined with activated sludge systems, are in general too complicated, energy intensive and expensive to provide a sustainable solution in developing countries (Massoud et al., 2009). Growing population and urbanisation urgently asks for development of on-site, compact, low cost WWTP that enable reuse of treated water in (peri) urban agriculture (von Sperling, 1996; Mara, 2003; WHO, 2006; Mara et al., 2010).

Anaerobic treatment of sewage is regarded as a pre-treatment process removing organic matter and converting its biodegradable fraction into methane. The Upflow Anaerobic Sludge Blanket (UASB) reactor has been applied in several warm (sub) tropical countries. BOD removal efficiencies of 50 to 78% were reported for 25 UASB reactors, operated at HRTs of 5-11 hours in India, Colombia and Brazil, (Mungray et al., 2010). Soluble nutrients, like ammonia and phosphate, are released with the liquid effluent (von Sperling et al., 2005; Chernicharo, 2006) and can be used for fertilisation. Pathogens are, however, insufficiently removed (Jimenez, 2007). Though, von Sperling et al. (2002) reported that helminth eggs could be removed by filtration through the sludge bed of the UASB. Produced excess sludge in well-designed UASB reactors is 'stable' and can be used as a soil conditioner in agriculture after disinfection (i.e. drying, co-composting). The UASB reactor is considered a promising technology for domestic wastewater treatment in developing countries, since it can be designed at very short HRT compared to extensive technologies (Chernicharo and Machado, 1998; von Sperling et al., 2005; Khan et al., 2011). It is characterised by energy production instead of energy use, low investment, operating and maintenance costs, small foot print and flexible scale (van Lier and Lettinga, 1999; von Sperling et al., 2005; Chernicharo, 2006).

The UASB reactor has been selected as a combined primary and partial secondary treatment of domestic sewage within the current research. UASB effluents are generally post-treated for irrigation purposes, considering water reclamation and reuse regulations (von Sperling and Chernicharo, 2002).

The down flow hanging sponge reactor, trickling filter, subsurface flow constructed wetland, rotating biological contactor and polishing pond (Machdar et al., 1997; Tandukar et al., 2005; von Sperling et al., 2005; Fleifle et al., 2013) are attractive post-treatment techniques that need limited or no energy for mechanical aeration (Table 1.5).

1.3.3 “UASB treatment chains” for pathogen removal

Von Sperling et al. (2005) and Chernicharo (2006) reported different “treatment chains”, including a UASB reactor, for domestic wastewater treatment (Table 1.5). It can be observed that some of these "UASB treatment chains" reach pathogen contents that comply with WHO (1989) guidelines (see Table 1.5). For example a UASB combined with overland flow, constructed wetland or polishing ponds can achieve a helminth egg content less than 1 egg. L⁻¹ and a faecal coliform removal higher than 2.5 log₁₀. Depending on the reuse conditions (WHO, 1989), the remaining faecal coliforms must be removed in a polishing step to achieve WHO standards.

Table 1.5 Pathogen effluent content, faecal coliforms content in the effluent, land requirement, construction costs, O&M costs, consumed power for aeration, FC-LA ratio and O&M-FC ratio for chains treatment regarding domestic wastewater treatment

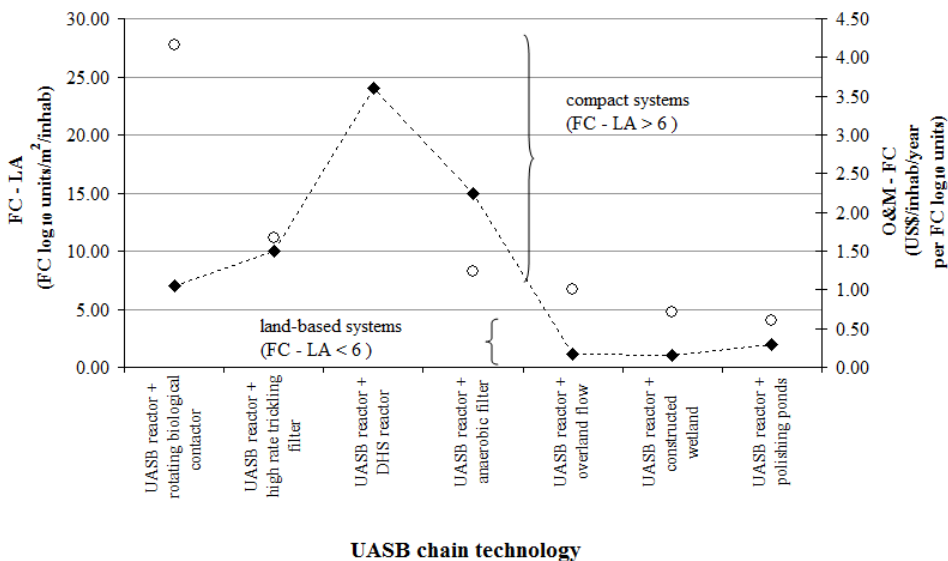
Treatment chains	Pathogen Effluent content		Faecal coliforms removal efficiency	Land requirement	Construction Costs	O&M Costs	Consumed Power for aeration	FC-LA	O&M-FC
	Faecal Coliforms MPN/100 mL	Helminth eggs eggs/L	average log ₁₀ units	average m ² /inhab	average US\$/inhab	average US\$/inhab	average kWh/inhab/year	log ₁₀ units per m ² /inhab (8)=(3) / (4)	US\$/inhab/year per FC log ₁₀ units (9)=(6) / (3)
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)=(3) / (4)	(9)=(6) / (3)
UASB reactor + activated sludge	10 ⁶ -10 ⁷	>1	1.5	0.14	37.50	3.75	17.00	10.71	2.50
UASB reactor + submerged aerated biofilter	10 ⁶ -10 ⁷	>1	1.5	0.10	32.50	3.75	17.00	15.00	2.50
UASB reactor + complete mix aerated lagoon + sedimentation pond	10 ⁶ -10 ⁷	>1	1.5	0.20	25.00	2.75	6.00	7.50	1.83
UASB reactor + dissolved air flotation	10 ⁶ -10 ⁷	>1	1.5	0.10	27.50	2.75	1.25	15.00	1.83
UASB reactor + rotating biological contactor	10 ⁶ -10 ⁷	>1	1.5	0.22	71.00	6.25	0.00	6.98	4.17
UASB reactor + high rate trickling filter	10 ⁶ -10 ⁷	>1	1.5	0.15	35.00	2.50	0.00	10.00	1.67
UASB reactor + DHS reactor	10 ⁴ -10 ⁷	>1	2.4	<0.10	N. D.	N. D.	0.00	>24.00	N. D.
UASB reactor + anaerobic filter	10 ⁶ -10 ⁷	>1	1.5	0.10	25.00	1.85	0.00	15.00	1.23
UASB reactor + overland flow	10 ⁴ -10 ⁶	<1	2.5	2.25	27.50	2.50	0.00	1.11	1.00
UASB reactor + constructed wetland	10 ² -10 ⁵	<1	3.5	3.32	41.00	2.50	0.00	1.06	0.71
UASB reactor + polishing ponds	10 ² -10 ⁴	<1	4	2.00	22.50	2.40	0.00	2.00	0.60

* Adapted from Chernicharo (2006), Von Sperling (2005), Tafwik et al. (2006a), Tandukar et al. (2005), Tandukar et al. (2007), Onodera et al. (2014) and Tawfik *et al.* (2015)

Notes:

- O&M: Operation and maintenance costs
- FC-LA: Ratio between faecal coliform removal efficiency and Land requirement per inhabitant. The calculation of these values were performed dividing the average faecal coliform removal efficiency (3) by the average land requirement (4).
- O&M-FC: Ratio between operation and maintenance costs per inhabitant and faecal coliform removal efficiency. The calculation of these values was performed dividing the average operation and maintenance costs (6) by the average of the faecal removal efficiency (3).
- N.D.: Not data found.

The “UASB technology chain” selection for municipal wastewater treatment for reuse will depend on local conditions, required effluent standards and economic resources (Campos and Von Sperling, 1996) next to social and political aspects. Additional selection criteria could be the FC-LA value, the relation between faecal coliform removal and land requirement per inhabitant and the O&M-FC value, the relation between operating and maintenance costs per inhabitant and faecal coliform removal, as developed by Von Sperling et al. (2005) and Chernicharo (2006) (Figure 1.2). They characterised systems with a FC-LA value < 6 as land-based systems and those with a FC-LA value >6 as compact systems. The O&M – FC value is higher for compact than land based systems (Figure 1.2). Unlike the lower O&M – FC value for land-based systems, compact systems will generally be more cost-effective due to the high FC-LA value and high land prices in urbanised areas. Particularly for the chain UASB reactor and DHS reactor, the FC-LA value is greater than 21. Then, the latter value represents a promising option when available area is limited.



◆ FC - LA : ratio between faecal coliforms removal efficiency and land requirement per inhabitant

○ O&M - FC : ratio between operation and maintenance costs per inhabitant and faecal coliforms removal efficiency

Notes:

- The figure was elaborated after Chernicharo (2006), Von Sperling (2005), Tafwik *et al.* (2006a), Tandukar *et al.* (2005), Tandukar *et al.* (2007), Onodera *et al.* (2014) and Tawfik *et al.* (2015)
- Particularly for the DHS reactor Tawfik *et al.* (2015) worked with an upflow anaerobic hybrid (AH) reactor instead of a UASB reactor

Figure 1.2 FC-LA and O&M-FC value for different “UASB technology chains”

1.3.4 Down flow hanging sponge reactors

According to the literature (Tandukar et al., 2005; Tawfik et al., 2006b; Tandukar et al., 2007), some types of DHS reactors (so called generations: G3, G4, G5 and G6) remove faecal coliforms between 79.0 and 99.7 % at an HRT varying between 2 and 2.7 h. DHS reactors cube type (G1) and curtain type (G2) have not been studied for their capacity to remove faecal coliforms. The G1 and G2 type have however shown their simplicity in terms of construction (Agrawal et al., 1997; Machdar et al., 1997; Machdar et al., 2000). Since the investment, operating and maintenance costs, and simplicity are the most important criteria when selecting a technology in developing countries (von Sperling, 1996; von Sperling and Chernicharo, 2002), DHS reactors type G1 and G2 were selected for the current research to polish UASB reactors effluents.

1.4 Scope of this thesis

The literature review shows that UASB reactors can play a role in the removal of helminth eggs and faecal coliform removal, but little information is available on the effect of different environmental and process conditions on the removal process, especially for helminth eggs. After an analysis of the different "UASB treatments chains" for developing countries, it can be concluded that compact systems could offer advantages compared to land-based systems. The main advantage is the low land requirement for sufficient pathogen removal, as characterised by the high FC-LA value.

Therefore, the aim of this thesis was to study the pathogen removal in compact systems considering an anaerobic step as pre-treatment.

Chapter 2 studies the potential of anaerobic sludge, which behaves as a filter bed in a UASB reactor, for the physical removal of helminth eggs at 4°C (when the bioactivity is negligible). Chapter 3 describes in detail the helminth egg removal capacity of UASB reactors at different upflow velocities under subtropical conditions. Depending on the upflow velocity, helminth eggs can be scavenged by the sludge bed. Chapter 4 focuses on the helminth egg content of domestic wastewater in Peruvian highlands and the filtration capacity of an anaerobic sludge bed in a UASB reactor for helminth eggs and faecal coliform removal at low temperatures as prevailing in mountainous areas in Peru. Chapter 5 presents the results of the down flow hanging sponge (DHS) reactor as a post-treatment of UASB reactors' effluent with special emphasis on faecal coliform removal. Depending on the type of DHS reactor used, different categories of water reuse can be applied. Chapter 6 discusses the results of this thesis and the importance of pathogen removal from domestic wastewater in order to enable reuse of water for irrigation and discusses the achieved results and presents the main conclusions of the whole thesis.

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CHAPTER 2

Filtration of an anaerobic sludge bed
for the removal of helminth eggs

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Filtration capacity of an anaerobic sludge bed for the removal of helminth eggs

Abstract

This research was conducted to elucidate the anaerobic sludge filtration capacity for helminth eggs at different operational conditions in order to estimate the removal of helminth eggs in upflow anaerobic sludge blanket (UASB) reactors. During the trials a low operational temperature of 4°C was applied to minimise the bioactivity in the sludge bed. The study was performed in two stages: the first one using latex beads simulating helminth eggs and the second one using helminth eggs. The filtration capacity of two types of sludge was evaluated: digested primary sludge and flocculent UASB reactor sludge. Filtration tests were conducted under different upflow velocities. A control test without sludge was used to distinguish between settling and sludge filtration. The experiments included measurements of the total and faecal coliforms removal and identification of the most common helminth eggs species.

The results of the experiments using latex beads confirmed that hydraulic properties during the settling experiments are different than those during sludge bed filtration, due to the fluid properties. The sludge filtration capacity regarding latex beads and helminth eggs removal is reciprocally correlated to the upflow velocity. Then lower removal of latex beads and helminth eggs is achieved at higher upflow velocities. Results show helminth eggs removal between 79 -100 % when using anaerobic sludge with upflow velocities in the range of 0.39-1.6 m·h⁻¹ at 4°C. Average total and faecal coliforms removal was respectively less than 80 and 76 %. The most common helminth eggs found in the studied wastewater was *Ascaris lumbricoides*.

Keywords

Helminth eggs; municipal wastewater; pathogens; UASB reactor; sludge bed filtration capacity

2.1 Introduction

The presence of pathogens in partly treated wastewater poses a considerable health risk to the farmers and general public, when this water is directly or indirectly used for agricultural irrigation and household appliances (WHO, 2006). As described in Chapter 1, pathogenic organisms are very diverse and include bacteria, viruses, protozoa and helminth eggs. Microorganisms and helminth eggs are adhered to the solids present in wastewaters (Jiménez, 2006; Jimenez, 2007). Processes to remove solids are i) plain sedimentation and sedimentation using gravitational forces including centrifugal methods, ii) flotation including dispersed or dissolved air flotation methods, and iii) filtration, which includes deep bed and membrane filtration (Gregory, 2004). Sedimentation and filtration processes are for part considered primary treatment and as such essential in a domestic wastewater treatment plant (Campos and Von Sperling, 1996; von Sperling, 1996; Tchobanoglous et al., 2003; von Sperling et al., 2005). In addition, post filtration processes can be applied using e.g. sand filtration or membrane filtration (Jimenez et al., 2001; Chernicharo, 2006).

Since the late eighties of the past century, upflow anaerobic sludge blanket (UASB) reactors have been successfully applied for treatment of municipal wastewater at tropical and semi-tropical conditions (Seghezzo et al., 1998; van Lier et al., 2010; Souza et al., 2011; Heffernan et al., 2012; Chernicharo et al., 2015), but only few studies are available that research the removal of helminth eggs during anaerobic treatment (Cabirol and Noyola, 2002). Von Sperling *et al.* (2002) reported that UASB reactors, operating at 5.5 hours HRT and treating domestic wastewater, produced an effluent with 1.3-45 egg·L⁻¹, while the influent contained 64-320 egg·L⁻¹. Paulino et al. (2001) observed a variable removal efficiency of 60 to 93% in an anaerobic fluidized bed. Although these values seem already a significant amount, it is insufficient for agricultural reuse according to the WHO guidelines for safe use of reclaimed wastewater for agricultural irrigation (WHO, 2006). Von Sperling *et al.* (2002) recommend a secondary treatment after the UASB reactor to efficiently remove pathogens from municipal sewage, according to the defined standards.

Particle removal in UASB reactors is dependent on the applied hydraulic regime as well as on the prevailing sludge bed properties (Mahmoud et al., 2003). The latter can be differentiated in physical and biological characteristics of the sludge bed (Mahmoud et al., 2003; Mahmoud et al., 2006). Physical sludge characteristics relate to e.g. density and viscosity (Seysiecq et al., 2003; Mori et al., 2006). Biological characteristics relate among others to biomass activity, microbial composition, presence of extracellular polymers and so on. The prevailing operational conditions such as temperature, applied organic loading rate, hydraulic retention time, and upflow velocity will impact both the biological and physical sludge characteristics (Mahmoud et al., 2003; Mahmoud et al., 2006).

The settling velocity of suspended solids (particles) in the wastewater depends on their diameters, density, shape, roughness and the prevailing hydraulic conditions such as Reynolds number and the water viscosity (Loch, 2001). Based on Stokes' law, Ayres and Mara (1996) determined an approximation of the settling velocity (at 20° C) of the three most common helminth eggs: viz. *Ascaris lumbricoides* at 20 mm·min⁻¹, *Trichuris trichiura* at 16 mm·min⁻¹ and *Hookworms*: at 6 mm·min⁻¹. Sengupta *et al.* (2011) reported that Stokes' law sometimes overestimates the settling velocity of helminth eggs. In the same research the settling velocity of *Ascaris*, *Trichuris* and *Oesophagostomum* eggs from pigs, in wastewater was assessed as 9.558, 5.196 and 6.372 mm·min⁻¹, respectively. The relatively low removal rate of *Trichuris* and *Oesophagostomum* could probably be related to their smaller size and therefore, their associated lower settling velocity (Cheng, 1997; Loch, 2001) compared to *Ascaris* eggs. However, more research is needed to find an exact explanation for the difference in the observed settling velocities (Sengupta *et al.*, 2011).

In order to measure the sludge filtration capacity for solids removal, Mahmoud (2006) developed "the sludge filterability technique". This technique can also be used for identifying the mechanisms that are involved in the removal of solids in an upflow sludge bed system. Using this technique, the main objective of this research was to study the sludge filtration capacity of anaerobic sludge in a lab scale UASB reactor using latex beads to simulate helminth eggs, followed by a research that used helminth eggs containing raw wastewater. Secondary objectives included the identification of the common species of helminth eggs, and the assessment of the sludge filtration capacity for faecal and total coliforms in a flocculent anaerobic sludge. Two types of sludge were tested, viz. primary digested sludge from a sludge digester in the Netherlands and flocculent sludge from a full scale UASB reactor in Peru.

2.2 Materials and methods

2.2.1 Influent

Experiment 1 was performed using domestic settled wastewater from the influent of the wastewater treatment plant (WWTP) of Bennekom village (The Netherlands) as influent. Experiment 2 was performed using the domestic wastewater from the WWTP located at San Juan de Miraflores district (Lima, Peru). The main characteristics of the wastewater are presented in Table 2.6.

Table 2.6 Main characteristics of the domestic wastewater of Bennekom (The Netherlands) and San Juan de Miraflores district (Peru)

Parameter	Units	Bennekom		San Juan de Miraflores	
		n ¹	influent	n	influent
Biochemical Oxygen Demand	mg·L ⁻¹	N.M.	N.M.	40	521 ± 110
Chemical Oxygen Demand	mg·L ⁻¹	7	400 ± 56	30	1408 ± 399
Total Suspended Solids	mg·L ⁻¹	7	291 ± 18	56	490 ± 490
Volatile Suspended Solids	mg·L ⁻¹	7	195 ± 12	56	389 ± 85
Total Solids	mg·L ⁻¹	4	709 ± 21	56	550 ± 93
Volatile total Solids	mg·L ⁻¹	4	281 ± 16	56	399 ± 80
Faecal Coliforms	MPN·100mL ⁻¹	N.M.	N.M.	56	4.1 x 10 ⁸ ± 1.2 x 10 ⁹
Total Coliforms	MPN·100mL ⁻¹	N.M.	N.M.	56	6.8 x 10 ⁸ ± 1.2 x 10 ⁹
Helminth eggs	egg·L ⁻¹	4	<1 ± 0	17	15 ± 5

Notes

¹ n: number of analysed samples² N.M.: not measured.

Data from Bennekom was measured within the research from December 1st to February 27, 2009. Data from San Juan de Miraflores was measured from January 1st, 2009 and October 30th, 2013.

2.2.2 Reactors

For Experiment 1, four identical acrylic lab scale 2.5 L UASB reactors with a height of 0.40 m and a diameter of 0.09 m were used. During Experiment 1, different tests were conducted applying different amounts of sludge, i.e., 350 mL, 700 mL and 1000 mL. These volumes correspond to 0.06, 0.11 and 0.15 m in the sludge bed height.

For Experiment 2, two identical 1.60 L lab scale UASB reactors made of Pyrex were used. Reactor height was 1.25 m and diameter 0.04 m. Both reactors were inoculated with 140 mL of sludge, which corresponds to 0.110 m in the sludge bed height.

2.2.3 Inoculum

Inoculum for Experiment 1 consisted of digested sludge (DS) from a primary sludge digester, operated at 30 days HRT and 35°C of the WWTP of Ede (The Netherlands). Twenty litres of the latter sludge was placed in a 30°C room for an additional 20 days of digestion, and is further referred to as extended digested sludge (EDS).

Inoculum for Experiment 2 consisted of anaerobic flocculent sludge (Mahmood et al.), taken from the pilot-scale 536 m³ UASB reactor located at CITRAR. The inoculum was

taken at 1.5 m height from the bottom of the reactor (total height of the reactor was 6.0 m).

Main characteristic of each inoculum is indicated in Table 2.7.

Table 2.7 Main characteristic of the inoculum applied in experiment 1 and 2

Experiment	Sludge quality ¹	Total solids (TS) g·L ⁻¹	Volatile solids (VS) g·L ⁻¹	Density at 4°C g·L ⁻¹	Stability (g·CH ₄)·(gVS) ⁻¹
1	DS	52 ± 1.4	33 ± 1.1	1120± 3.0	0.025± 0.0000
1	EDS	49 ± 1.2	30 ± 0.8	N.M.	0.015± 0.0012
2	FS	163±25	106±24	1096± 1.5	N.M. ²

Notes:

¹ DS: Digested sludge; EDS: Extended digested sludge; FS: Flocculent sludge

² N.M. means not measured.

2.2.4 The latex beads

For Experiment 1, latex beads, Coulter® CC Size standard L90: $\phi = 90 \mu\text{m}$, with a density of $1.05 \text{ mg}\cdot\text{L}^{-1}$ (Miami, USA) have been used to simulate helminth eggs, as their shape, size and density are very similar to the actual helminth eggs (Jimenez, 2007; Quinzanos et al., 2008; Sengupta et al., 2011). According to the supplier, latex beads are discrete spherical particles, uniform in material composition and contain smooth surfaces. Thus considering the fact that latex beads do not interfere with each other, their settling velocity under creeping flow conditions (Reynolds number less than 1) in a Newtonian fluid can be described using the Stokes law.

$$V = \left[\frac{g \cdot (\rho_p - \rho_w) \cdot d^2}{18 \cdot \eta} \right] \quad \text{eq. 2.1}$$

Where V is the particle settling (or terminal) velocity ($\text{m}\cdot\text{s}^{-1}$); g is the acceleration of gravity ($\text{m}\cdot\text{s}^{-2}$); d is the particle diameter (m); ρ_p is the density of the particle ($\text{kg}\cdot\text{m}^{-3}$); ρ_w is the density of the fluid which is water ($\text{kg}\cdot\text{m}^{-3}$) and η is the dynamic viscosity of the medium ($\text{kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$).

Based on Stokes' law, it is theoretically calculated from eq. 2.1 that the latex beads with a diameter of $90 \mu\text{m}$ have a settling velocity of $13.7 \text{ mm}\cdot\text{min}^{-1}$ ($0.8 \text{ m}\cdot\text{h}^{-1}$) at 20°C , which is close to the settling velocity of the helminths eggs (Ayres and Mara, 1996). Correcting for temperature, at 4°C , these latex beads with a diameter of $90 \mu\text{m}$ have a settling velocity of $8.4 \text{ mm}\cdot\text{min}^{-1}$ ($0.5 \text{ m}\cdot\text{h}^{-1}$).

2.2.5 Latex beads counting

In the beginning of Experiment 1, the method suggested by WHO (Ayres and Mara, 1996) was planned to be applied for the latex beads counting, as this method is used to count helminth eggs (von Sperling et al., 2002; von Sperling et al., 2003; Mahvi and Kia, 2006; Sanz et al., 2009; Zacarias Sylvestre et al., 2014). Unfortunately, this method turned out to be not applicable to synthetic media because the used chemicals (ethyl acetate and zinc sulphate) react with the latex beads. Therefore, based on the WHO method (Ayres and Mara, 1996) and the settling properties of the latex beads, a multi-step methodology was developed. This method consisted on the collection of a 1 L sample for settling during 2 hours in a 1 L graduated cylinder with a height of 40 cm, to concentrate the settled particles. Subsequently, removal of 90% of the supernatant volume (900 mL), followed by an additional settling of the remaining sample (100 mL) for 2 hours in an graduated cylinder of 100 mL. After that, 90% of the supernatant volume (90 mL) was removed. Finally, a well mixed aliquot is taken from the remaining 10 mL and placed in a two chamber counting cell to proceed to count the latex beads.

The two chambers counting cell (Mc Master worm eggs 2 cell, Hawksley, Lancing, UK). for micro/macroscope, especially designed for helminth eggs, has two chambers with grids (Figure 2.3). Under each grid a sample with a volume of 0.15 mL can be placed. It is then possible to count the amount of eggs per grid, and therefore per volume, in order to know the helminth egg concentration (or the latex beads concentration) of a sample.

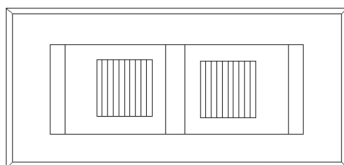


Figure 2.3 The Mc Master worm counting eggs 2 chamber cell

To obtain a reliable mean latex bead or helminth egg concentration (in number per sample volume), each sample was analysed four times (2 times with the two chambers counting cell). The counting observed was the concentration of particles in a volume of 0.15 mL of a 10 mL sample resulting from a multi-step methodology (see below). It is then possible to obtain the number of particles that is present in the 10 mL, which is equal to the number of latex beads in the original sample of 1L. It is assumed that all particles that were in the original sample of one litre were concentrated in the 10 mL sub-sample (equation 2.2).

$$C = 10 * \frac{X}{0.15} \quad \text{eq. 2.2}$$

where: C is the particle concentration expressed in number of particles per litre, 10 is the volume of the final concentrated sample in mL, X is the number of particles counted in one grid (or the mean of four samples) and 0.15 is the volume under the grid in mL.

2.2.6 Macroscope

A macroscope (Nikon SM7800, Japan) was used to count the latex beads connected with the Mc Master counting cell. Macroscope was chosen instead of a microscope because of the possibilities it offers in terms of colour filters, magnification, direction of the mirror and addition of extra lights. Those different configurations made the latex beads easier to be recognized and differentiated from other particles.

2.2.7 Physicochemical and bacteriological analysis

Total Chemical Oxygen Demand (COD) was measured according to Standard Methods (APHA et al., 1998) using Dr. Lange test kits. Total suspended solids (TSS) and volatile suspended solids (VSS) were performed according to Standard Methods (APHA et al., 1998). Density of sludge was measured using a Gay-Lussac-Pycnometer of 24.822 ml (LMS, Germany). Viscosity of the sludge was measured using a viscometer FANN model 32, USA, using a shear rate of 600 rpm. For helminth eggs analysis, the flotation method described by Ayres and Mara (1996) was used. Total coliforms were determined according to the standard total coliform fermentation technique (APHA et al., 1998). Faecal coliforms were analysed according to the Faecal Coliform Procedure (Eaton et al., 2005). Stability of unfed digested and well digested sludge was performed with serum bottles of 1L. The tests were conducted in duplicate. Each type of sludge were placed in the bottles and incubated in shakers (120 rpm) in the dark at 30°C. For each series, 200 mL of sludge was added to each serum bottle. After adding the sludge, the serum bottles were flushed with nitrogen gas. The tests lasted for 15 days when the biogas production rate had ceased and the biogas was measured using Oxitop pressure measuring heads. Biogas composition (in terms of CH₄ and CO₂) was analysed in a Fisons Instrument GC 8340 gas chromatogram equipped with a 30 m × 0.53 mm × 25 µm Molsieve column (Alltech 13940), and a 2 × 25 m × 0.53 mm × 10 µm PoraBond Q column (Varian 7354). The columns were connected in parallel. Helium was the carrier gas and its flow rate was 42.5 mL·min⁻¹. The temperatures of the oven, the injection port, the thermal conductivity detector and the filament were 40, 110, 100 and 140°C, respectively.

2.2.8 Experimental set-up

For Experiment 1, every week fresh domestic wastewater was delivered by a tractor and pumped into a cooling storage tank (former milk tank, MEKO, Assen, The Netherlands) with a total volume of approximately 3500 L. The tank's content was continuously stirred and kept at 4°C (average room temperature: 18.5°C). Before each new fill-up, the tank was cleaned to avoid any mixing between 'old' and 'fresh' wastewater. Then, the

unsettled influent was pumped from the cooling storage tank into a 100 L tank, where it was settled for approximately a day (simulating primary clarification). The supernatant (70 L) was pumped into a second tank to obtain wastewater free from larger particles. The wastewater was mixed with the latex beads using a stirrer (Heidolph, Germany). For Experiment 2, 50 L fresh wastewater was daily delivered and kept in a stirred tank at 4°C (reactor temperature less than 5°C). All reactors in both experiments were fed by the same influent tank.

Before each experiment (Experiment 1 and Experiment 2), a sludge washing phase was applied according to the method described by Mahmoud et al. (2006). Introduction of a washing phase minimizes the impact of the previous conditions on the new test conditions in the sludge. During the washing phase, effluent COD and VSS values were monitored until stabilized values were attained. Tap water was the washing medium. The sludge washing phase was applied for both the digested sludge and the extended digested sludge as the first step during each experiment. Trial results showed that it was necessary to ‘wash’ the sludge for at least an elapsed time period of fourfold the HRT. The washing phase from each experiment was then conducted in total for an elapsed time period of six fold the HRT value. After the sludge washing phase, the latex beads could be introduced in the influent tank.

The general set up of both experiments with latex beads and wastewater containing helminth eggs is shown in the Figure 2.4.

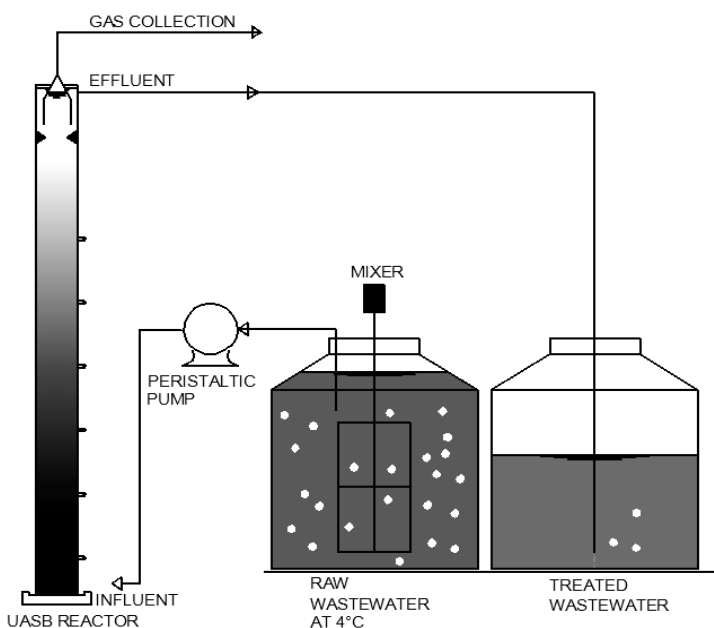


Figure 2.4 Set up of the experiments 1 and 2. Latex beads mixed with wastewater were used for experiment 1 and wastewater containing helminth eggs for experiment 2.

2.2.9 Sludge bed filtration of latex beads (Experiment 1)

Four types of test series were conducted to study the sludge filtration capacity to remove latex beads simulating helminth eggs: (a) impact of upflow velocity, (b) impact of degree of sludge stabilisation (c) impact of sludge bed volume and (d) control tests. All tests except (d) control tests, included a washing phase procedure at the beginning. The digested sludge was used in the upflow velocity test and the sludge bed volume tests. The extended digested sludge was only used in the experiment testing the impact of degree on sludge stabilisation.

a. The impact of the upflow velocity

Four upflow velocities, viz. 0.3, 0.5, 1, 1.5 $\text{m}\cdot\text{h}^{-1}$, were tested. Experiments were performed in triplicate. The four reactors were fed with exactly the same influent by means of four peristaltic pumps (2 Masterflex, UK, and 2 Watson Marlow, USA). The number of latex beads per litre in the influent varied from 3141 to 3159 (Table 2.8).

The test was carried out considering a washing phase during a time equivalent to fourfold the HRT value. After that, latex beads were added to the influent tank. Subsequently, the four reactors were started gradually at different times to finish in the same time. Then, it was followed by effluent sampling after a total time equivalent of six times the HRT value from the moment the reactor was started. Finally, in order to determine the variation of the influent in terms of latex beads, COD, and solids concentration, samples of the influent were taken at three different times: when the test started, after the washing phase and at the end of the test;

Table 2.8 Set up of reactors to assess the impact of the upflow velocity and sludge bed digestibility on the helminth eggs filtration capacity, COD and VSS concentration.

Description	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5	Reactor 6
Upflow velocity ¹ ($\text{m}\cdot\text{h}^{-1}$)	0.3	0.5	1.0	1.5	1	0.5
Average number of latex beads in the influent	3159	3159	3159	3159	3141	3141
Sludge quality ²	DS	DS	DS	DS	EDS	EDS

Notes:

¹ The corresponding hydraulic retention time (HRT) for upflow velocities of 0.3, 0.5, 1.0 and 1.5 $\text{m}\cdot\text{h}^{-1}$ were 1.33, 0.84, 0.42 and 0.28 respectively. A volume of 700 mL of sludge was used in all reactors

² DS: Digested sludge; EDS: Extended digested sludge

b. The impact of degree on sludge stabilisation

In order to assess the impact of degree of sludge stabilisation on the sludge filtration capacity to remove helminth eggs, two upflow velocities were tested, i.e. 0.5 and 1.0 $\text{m}\cdot\text{h}^{-1}$, using digested sludge and extended digested sludge in two reactors of 700 mL. Reactors were fed with exactly the same influent by means of two peristaltic pumps (2 Masterflex, UK).

The test procedure was the same as that for to the impact of the upflow velocity tests. The experiments were performed in triplicate. The effluent was collected separately from each reactor in order to be able to take separate samples for assessing the latex beads concentration. The results of latex bead removal were then compared to those obtained in the impact of the upflow velocity test for the corresponding upflow velocity (see Reactor 2 and 3 in Table 2.8).

c. Impact of sludge bed volume

In order to study the influence of the sludge bed height and upflow velocity on the helminth eggs filtration capacity, two different volumes of sludge, i.e. 350 mL and 1000 mL, were applied at two different upflow velocities, i.e. 0.5 and 1 $\text{m}\cdot\text{h}^{-1}$, as indicated in Table 2.9. Assays were done in triplicate. The four reactors were fed with exactly the same influent by means of four independent peristaltic pumps (2 Masterflex, UK, and 2 Watson Marlow, USA) and latex beads were introduced at once.

Table 2.9 Set up of reactors for testing the impact of the sludge bed volume

Description	Reactor 1	Reactor 2	Reactor 3	Reactor 4
Upflow velocity ¹ ($\text{m}\cdot\text{h}^{-1}$)	1	0.5	1.0	0.5
Volume of sludge (mL)	350	350	1000	1000

¹ The corresponding hydraulic retention time (HRT) for upflow velocities of 0.5 and 1 $\text{m}\cdot\text{h}^{-1}$ were 0.84 and 0.42 h respectively.

The test was carried out in the same way as explained for the upflow velocity test, except that the four reactors where started in groups of two, according to their HRT: first reactors with HRT of 0.84 h (reactor 2 and 4) and later reactors with HRT of 0.42 h (reactors 1 and 3). Reactors started at two different times, so that they reached an elapsed time equivalent to four times the HRT value at the same time.

d. Control reactors

In order to study the removal of the latex beads only by sedimentation, control tests were performed. Two upflow velocities were tested, i.e. 0.3 and 1.0 m·h⁻¹ in control UASB reactors, in absence of sludge. Tests were performed in duplicate. The two reactors were fed with exactly the same influent by means of two peristaltic pumps (2 Masterflex, UK). The results of latex bead removal by sedimentation were then compared to those obtained in the upflow velocity test in the presence of a sludge bed for the same two upflow velocities (see test - a, reactors 1 and 3 in Table 2.8).

The test was carried out in three phases. Firstly, during the blank phase, feeding of the UASB reactor with settled wastewater during a time equivalent to one time the HRT value. Secondly, adding latex beads to the influent tank. Finally effluent samples were taken after an elapsed time equivalent to three times the HRT value.

The two reactors were started at two different times, so that they reached an elapsed time equivalent to one time the HRT value, both at the same time. The two reactors were fed by the same influent tank. Then latex beads were introduced simultaneously in the feed tank.

In order to study the removal of the latex beads by plain settling, 2 samples were taken from the influent and the effluent. The influent concentration of latex beads was measured by taking one influent sample of one litre. The sampling was performed immediately after the two reactors reached an elapsed time of one time the HRT value. The effluent concentration of latex beads was measured by taking an effluent sample of one litre from each reactor when an elapsed time of three times the HRT value was reached.

2.2.10 Sludge bed filtration of helminth eggs (Experiment 2)

This experiment was performed to assess the sludge bed filtration capacity to remove helminth eggs using inoculum from a domestic wastewater UASB reactor. Five upflow velocities were applied (0.39, 1.58, 2.83, 3.16 and 4.12 m·h⁻¹) based on the laboratory facilities. The indicated upflow velocities of 0.39 and 1.58 m·h⁻¹ are in the range recommended by von Sperling *et al.*(2005). The remaining values were selected considering the influence of high peak flows which would lead to low values of HRTs. Experiments were done in triplicate. Raw cooled (4°C) wastewater containing helminth eggs was placed in a vessel with permanent mixing, using a stirrer, then it was pumped to the UASB lab scale reactors by using 2 peristaltic pumps (Masterflex, USA).

2.3 Results

2.3.1 Sludge bed filtration of latex beads (Experiment 1)

a. Impact of the upflow velocity

The results of the upflow velocity test on helminth egg removal, described in Table 2.8, are shown in Figure 2.5. Each bar, made of three points, corresponds to one of the four reactors operated at upflow velocities of 0.3, 0.5, 1.0, and 1.5 $\text{m}\cdot\text{h}^{-1}$, respectively. Results show decreased removal efficiency at increased upflow velocity.

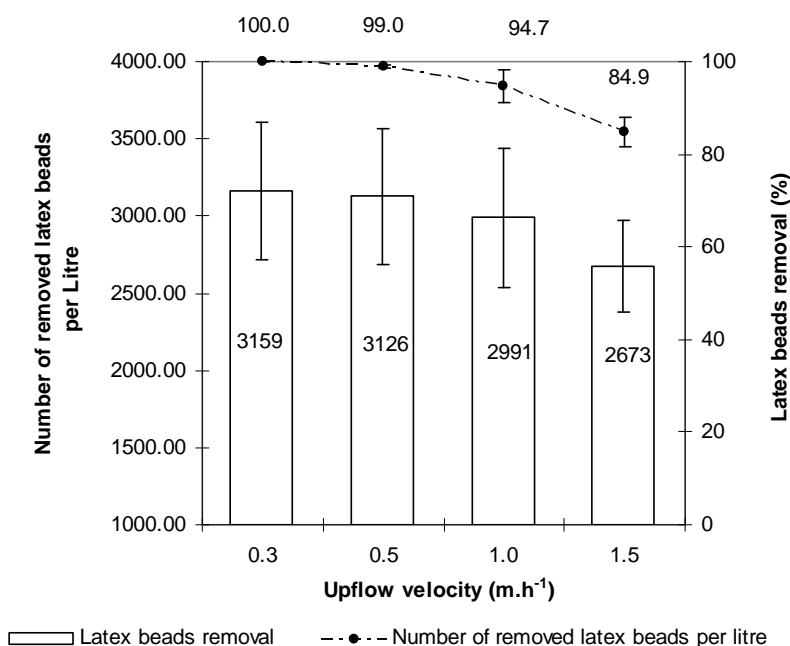


Figure 2.5 Latex beads removal efficiency and the average number of removed latex beads per litre as a function of the upflow velocity. Results show the average of three repetitions and standard deviation.

b. Impact of degree on sludge stabilisation

Regarding the impact of degree on sludge stabilisation on latex beads removal, no significant effect was observed (see Table 2.10).

Table 2.10 Effect of the degree of sludge stabilization on latex beads removal efficiency. Each result shows the average of three samples and standard deviation.

Sludge quality ¹	Upflow velocity (m·h ⁻¹)	Latex beads removal efficiency (%)
DS	0.5	99.0 ± 0.4
EDS	0.5	99.1 ± 0.5
DS	1.0	94.7 ± 3.5
EDS	1.0	97.2 ± 0.9

Notes:

¹ DS: Digested sludge; EDS: Extended digested sludge; FS: Flocculent sludge

c. Impact of sludge bed volume

The main objective of this test was to measure the effect of the sludge bed volume size on latex beads removal and COD removal. Figure 2.6 shows the results for latex beads removal in relation to the different upflow velocities and sludge bed volumes. Figure 2.7 shows the results of the effect of the sludge bed volume size on total COD removal.

It can be observed that increasing the sludge bed volume did not have a significant effect on the latex beads removal. Also COD removal efficiency was not significantly altered for different sludge bed heights, neither at an upflow velocity of 0.5 nor at 1 m·h⁻¹.

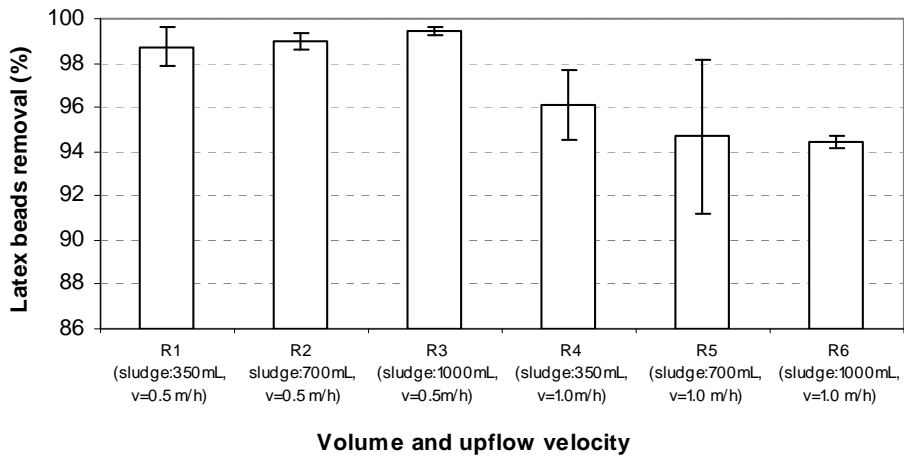


Figure 2.6 The effect of different sludge bed volumes at two upflow velocities on latex beads removal (%) in lab scale UASB reactors. Each result shows the average of three samples and standard deviation.

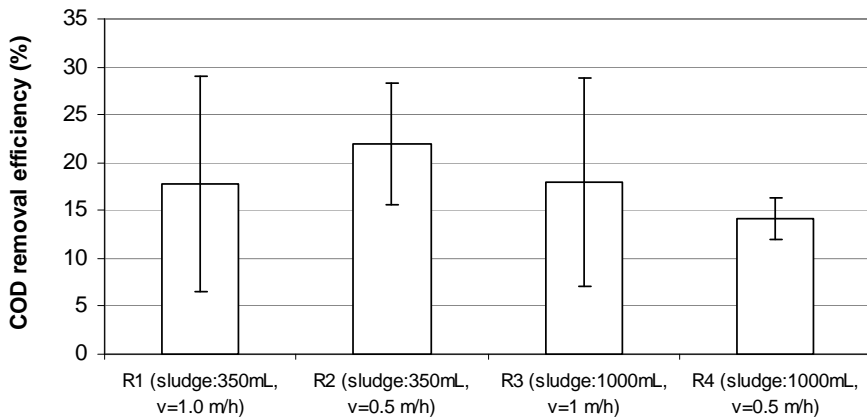


Figure 2.7 Total COD removal for different sludge bed heights at two different upflow velocities. Results show the average of three samples and standard deviation.

d. Control reactors

At an upflow velocity of $0.3 \text{ m}\cdot\text{h}^{-1}$, no significant differences were observed in latex beads removal efficiency between the control reactor in plain settling (average of $99.1 \pm 1.3 \%$) and the reactor with a sludge bed (average of $100.0 \pm 0.0 \%$) (Figure 2.8).

At an upflow velocity of $1 \text{ m}\cdot\text{h}^{-1}$, the difference in latex bead removal efficiency between the sludge bed reactor ($94.7 \pm 3.5 \%$) and the control reactor ($82.1 \pm 2.8 \%$) amounted to about $12.5 \pm 4.5 \%$.

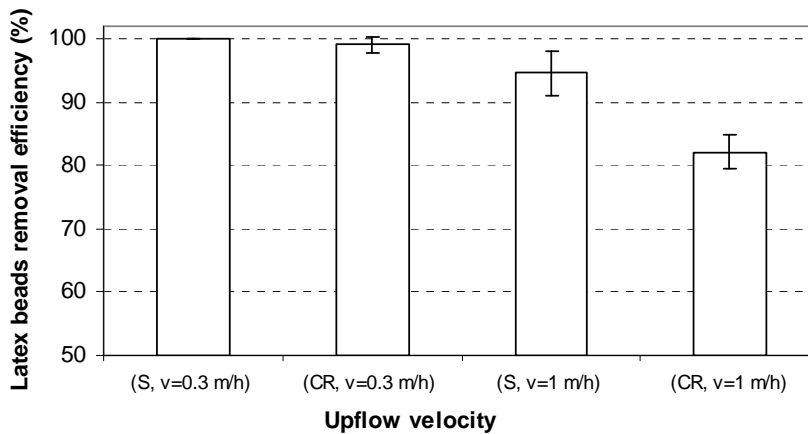


Figure 2.8 Effect of the presence of the sludge bed on latex beads removal (%). S: presence of a sludge bed, CR: control reactors. Each result shows the average of three samples and standard deviation

2.3.2 Sludge bed filtration of helminth eggs (Experiment 2)

The characteristics of the studied wastewater and the results for the different applied upflow velocities in terms of helminth eggs, COD, faecal coliforms and Total Coliforms concentrations in Experiment 2 are presented in Table 2.11.

Table 2.11 Results of the experiment conducted with helminth eggs

Upflow velocity (*)		m·h ⁻¹	0.39	1.58	2.83	3.16	4.12
Helminth eggs	I	egg·L ⁻¹	2.33 ± 0.58	4.67 ± 0.58	4.33 ± 1.15	4.67 ± 0.58	4.67 ± 0.58
	E	egg·L ⁻¹	0 ± 0	1 ± 0	1.67 ± 0.58	3.67 ± 1.15	3.67 ± 0.58
	R	(%)	100 ± 0	78.57 ± 2.89	61.54 ± 3.85	21.43 ± 20.21	21.43 ± 2.89
	Ie		T, A	T,A	T,A,S	A	T,A,S
COD	I	mg·L ⁻¹	748 ± 10	748 ± 12.5	866.33 ± 0.58	1016 ± 6	866 ± 34
	E	mg·L ⁻¹	400 ± 2	510 ± 103.94	704.33 ± 97.99	641.33 ± 81.85	585 ± 94.92
	R	(%)	46.52 ± 0.88	31.82 ± 14.11	18.7 ± 13.33	36.88 ± 11.63	32.45 ± 9.26
Faecal Coliforms	I	MPN·100mL ⁻¹	9.2E+07 ± 0E+07	9.2E+07 ± 0E+07 7.93E+07 ±	7.93E+07 ± 2.19E+07	5.4E+07 ± 3.8E+07	1.8E+07 ± 3.12E+07
	E	MPN·100mL ⁻¹	2.23E+07 ± 1.1E+07	2.19E+07	5.4E+07 ± 3.29E+07	5.4E+07 ± 3.8E+07	1.8E+07 ± 3.12E+07
	R	(%)	75.72 ± 11.92	13.77 ± 42.99	31.93 ± 31.55	0 ± 23.85	0 ± 22.29
Total Coliforms	I	MPN·100mL ⁻¹	92E+07 ± 0E+07	92E+07 ± 0E+07	63.13E+07 ± 50E+07	66.67E+07 ± 43.88E+07	8.93E+07 ± 6.12E+07
	E		68.84 ± 23.85	55.07 ± 41.3	80.25 ± 47.69	76 ± 47.69	3.73 ± 47.69
	R	(%)	68.84 ± 23.85	55.07 ± 41.3	80.25 ± 47.69	76 ± 47.69	3.73 ± 47.69

(*): The area of the UASB reactor was $1.075 \times 10^{-3} \text{ m}^2$

I: Influent; E: Effluent; R: Removal efficiency; Ie: Identified specie; T: *Trichuris spp.*; A: *Ascaris lumbricoides*; S: *Strongyloides spp.*

For each upflow velocity 3 samples were analysed

The density of the used sludge at 4°C was measured to be 1096 g·L⁻¹. Analysis of UASB reactor sludge showed an inverse relationship between both sludge density and viscosity versus temperature (Figure 2.9).

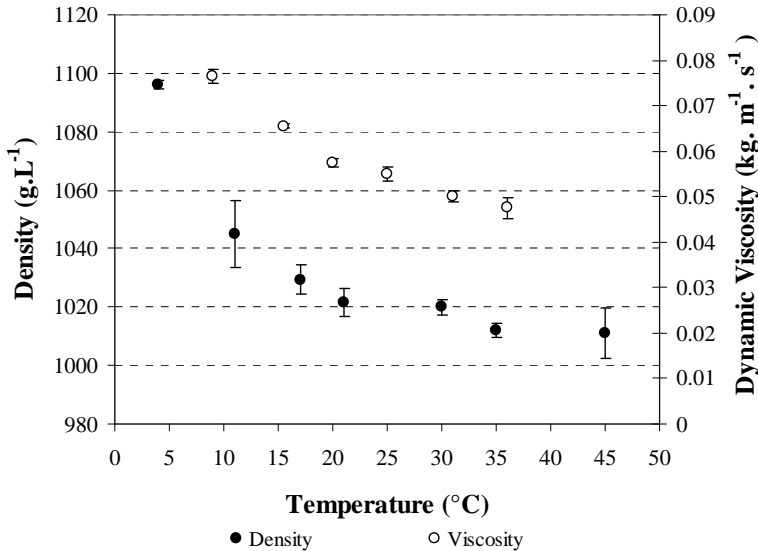


Figure 2.9 Density (●) and dynamic viscosity (○) of the anaerobic flocculent sludge as a function of the temperature. Tested temperatures for viscosity were 9, 15.5, 20, 25, 31 and 36°C and for density 4, 11, 17, 21, 30, 35 and 45°C. Each result shows the average of three samples.

2.4 Discussion

2.4.1 Sludge bed filtration of latex beads

An approximation of the theoretical latex beads settling, assuming discrete settling through the sludge bed, can be calculated using eq. 2.1. Under the given conditions, i.e. $d=90\ \mu\text{m}$, $\rho_p = 1.05\ \text{kg}\cdot\text{m}^{-3}$ and sludge density (ρ_s) = $1.12\ \text{kg}\cdot\text{m}^{-3}$, latex beads have a theoretical settling velocity of $-0.014\ \text{m}\cdot\text{h}^{-1}$ which is very close to zero. Dynamic sludge viscosity at 4°C was estimated to be $\eta = 0.078\ \text{kg}\cdot\text{m}^{-3}$, assuming a linear extrapolation based on the reported results in Figure 2.9. The negative value of the settling velocity may suggest an upward movement of the beads through the sludge bed instead of settling. Likely in terms of densities, latex beads may settle in water on top of the sludge bed under creeping flow conditions. However, the discrete settling theories are possibly, not applicable under the described experimental condition, as suggested by Seyssiecq *et al.*, (2003), who reviewed the rheological characteristics of activated sludge and sewage sludge. In fact, the used types of sludge behave as non-Newtonian flows, which might imply that application of eq. 2.1 is not valid (Seyssiecq *et al.*, (2003).

The surface charge and viscosity of the sludge and the extracellular polymeric substances (Johansen et al., 2013) content present in the fluid (Seyssiecq et al., 2003; Mori et al., 2006; Pevere et al., 2006) may affect the sludge filtration capacity of latex beads. The indicated characteristics might be reflected in resistance against movement of particles (i.e. latex beads) inside the sludge. The latter hypothesis needs to be tested. Additionally, sludge properties like porosity, pore pressure and particle size distribution, can influence the filtration capacity for latex beads (Lee and Wang, 2000; Redman et al., 2001).

a. Impact of the upflow velocity

As shown in Figure 2.5, increasing the upflow velocity led to a decrease in the removal efficiency of latex beads. At the lowest upflow velocity of $0.3 \text{ m}\cdot\text{h}^{-1}$, in all three replicates, 100% removal of latex beads was reached; latex beads were not found in any of the effluent samples. At an upflow velocity higher than $1 \text{ m}\cdot\text{h}^{-1}$ the removal efficiency dropped below 90% for the three runs.

The very low upflow velocity of $0.3 \text{ m}\cdot\text{h}^{-1}$, apparently did not affect the sludge filtration capacity. As soon as the upflow velocity increased, the resistance against movement of particles through the sludge bed apparently decreased as well as the associated viscosity (Pevere et al., 2006). Consequently, the sludge filtration capacity decreased.

b. Impact of sludge bed volume and stabilisation.

No significant effect of increasing the sludge bed volume or degree of stabilisation on latex bead removal was observed. Sludge properties, governing the resistance towards particle movement, are apparently not impacted by the applied upflow velocities between $0.5\text{-}1 \text{ m}\cdot\text{h}^{-1}$ and the sludge bed volumes between 350 -700 mL in the UASB reactor. The presence of extracellular polymeric substances (Johansen et al., 2013) might be relevant in eggs' filtration and depends on the applied sludge retention time (Mahmoud et al., 2006). A different degree of sludge stabilisation relates to different sludge retention times. Unfortunately, EPS concentrations were not analysed within this study.

The observed low values for total COD removal, could be related to only the physical filtration capacity, since at the imposed operational temperature of 4°C , the biological activity was minimised, meanwhile some sludge washed out.

c. Settling of latex beads versus sludge bed filtration

At an upflow velocity of $0.3 \text{ m}\cdot\text{h}^{-1}$, 100% efficiency was observed for the sludge bed filtration test. Although the control reactor (plain settling) showed a slightly lower removal of latex beads, the observed differences are not significant (Figure 2.8). The

slightly lower latex beads removal efficiency in plain settling and sludge filtration might be attributed to a possible high dynamic viscosity of the sludge compared to the water ($\eta = 0.001569 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ at 4°C). The dynamic viscosity of sludge, which was not measured, is expected to be high since, the viscosity reported in the current research for the flocculent sludge was high ($\eta = 0.0765 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ at 9°C). An increased dynamic viscosity, might contribute to the observed filtration capacity of the sludge layer for latex bead removal. However, the latter hypothesis needs to be tested.

According to Stokes' law the used latex beads have a theoretical settling velocity of $0.5 \text{ m}\cdot\text{h}^{-1}$ at 4°C (see the control reactor test in §2.2.4), resulting in a net discrete settling velocity of $0.2 \text{ m}\cdot\text{h}^{-1}$ in plain water. Therefore, full removal of the latex beads in control reactors was expected and results confirm theory. However, the fact that some latex beads washed out in the control reactor means that either the particle size distribution of the latex beads is non-uniform, or flow conditions in the test columns are non-laminar. Additionally, a current is generated in the opposite movement direction of the particles in a system where simultaneously, particles are settling in a fluid (Salinas-Salas, 2012). Such upward current could also contribute to reduced latex beads settling.

At an upflow velocity of $1 \text{ m}\cdot\text{h}^{-1}$, an increased difference in removal efficiency was expected because the applied upward velocity of $1 \text{ m}\cdot\text{h}^{-1}$ exceeds the theoretical settling velocity of the latex beads of $0.5 \text{ m}\cdot\text{h}^{-1}$ by a factor 2. The observed high retention of the latex beads at the applied high upflow velocities might again be attributed to a non-uniform particle size distribution of the beads in the medium or to non-laminar flow conditions in the used test column. Non-laminar and turbulent flow patterns may cause downward flows and even dead zones in specific parts of the test column. Also, as a result of prevailing wall shear stress, decreased upflow velocities may occur from the centre to the reactor wall (Figure 2.10), similar to pipe flow patterns discussed by Streeter *et al.* (1988) and Smits (2003). Non-laminar flows may also be caused by non-homogeneous influent distribution. Upward flows deviating from presumed laminar conditions could allow settling of latex beads since applied upflow velocity would be smaller than $1 \text{ m}\cdot\text{h}^{-1}$ in some parts of the reactor. The laminar flow regime is difficult to achieve in water systems due to the low water viscosity (Avila *et al.*, 2011). In fact, when liquid viscosity is low, turbulence may occur in the form of localized puffs (Streeter *et al.*, 1988). Additionally, when other conditions prevail, *viz.* variations in particle size, presence of non-Newtonian fluids, turbulence fluctuations due to biogas production and so on, discrete settling explanations cannot be used anymore (Mori *et al.*, 2006; Pevere *et al.*, 2006).

Also in full scale UASB reactors, a perfectly homogeneous influent flow distribution cannot be expected (Van Haandel and Lettinga, 1994; Lettinga, 1995; Seghezzi, 2004), particularly when realizing that the number of influent feed pipes will be as low as possible to reduce the construction costs.

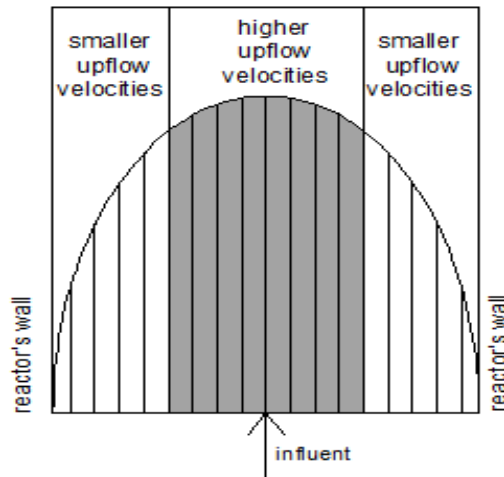


Figure 2.10 Possible influent distribution in the used lab scale UASB reactor
Source : Adapted from Streeter *et al.*(1988) and Smits (2003).

2.4.2 Sludge bed filtration of helminth eggs

For the studied domestic wastewater at 4°C, results show that at an upflow velocity of 0.39 and 1.58 m·h⁻¹ a mean removal of 100 and 79% of helminth eggs is expected, respectively. At higher upflow velocities, reaching 4.1 m·h⁻¹, which in practice is impossible to apply for flocculent sludge beds, the mean helminth egg removal dropped to 21 %. The sludge filtration capacity is strongly affected by a high upflow velocity.

Results reveal that *Ascaris lumbricoides* was the most common helminth egg present in the studied wastewater which is in line with the literature (Ayres and Mara, 1996; O'Lorcain and Holland, 2000; Brownell and Nelson, 2006). Consequently, the predominant availability of *Ascaris lumbricoides* in wastewater in comparison to other species like *Trichuris trichiura* and *Hookworms*, allowed us to use *Ascaris lumbricoides* as the helminth egg indicator in further research. The removal efficiency of faecal and total coliforms was below 80% under all test conditions. Insufficient adsorption of the pathogenic organisms to the sludge occurred and the applied HRT was too short for a significant die-off at the prevailing temperature (Raangeby et al., 1996; Uemura et al., 2002).

Dynamic viscosity results show a mean value of $0.0575 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ at a shear rate of 600 rpm (10 s^{-1}) at 20°C . These results are higher than the results reported by Pevere *et al.* (2006), who showed a viscosity of anaerobic granular sludge increasing from 0.0033 to $0.0058 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ for a shear rate decreasing from 1000 - 200 s^{-1} . Analysis of UASB reactor sludge showed an inverse relationship between both density and dynamic viscosity versus temperature

Sludge dynamic viscosity at 4°C is higher than $0.0765 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ (viscosity measured at 9°C) which is distinctly higher than the water dynamic viscosity at 4°C , i.e. $\eta = 0.001569 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$. The increased viscosity and density at low temperature, increases the hydraulic shear stress on the sludge particles, resulting in a decreased hydraulic turbulence in the reactor (Mahmoud *et al.*, 2003; Pevere *et al.*, 2006). Density and shape of particular helminth eggs in combination with the prevailing sludge characteristics, may have an impact on the UASB reactor's filtration capacity for this type of eggs.

2.4.3 Use of latex beads as a model for helminth eggs

For both latex beads and helminth eggs 100% removal is achieved at 4°C and the used low upflow velocity of respectively 0.3 and $0.39 \text{ m}\cdot\text{h}^{-1}$. Similar removal efficiencies were also achieved at upflow velocity of 1.5 and $1.59 \text{ m}\cdot\text{h}^{-1}$ for respectively latex beads and helminth eggs. Latter results do indicate that latex beads are a good alternative for studying sludge filtration of helminth eggs. Considering the high infectiousness of helminth eggs, which complicates any experimental set-up in terms of health risks (Feenstra *et al.*, 2000; Fatta-Kassinos *et al.*, 2011); the use of latex beads is recommended.

2.4.4 UASB field operational conditions

Results demonstrated that operating UASB reactors at a reduced upflow velocity while aiming at developing a dense sludge bed with a high viscosity can significantly improve worm eggs removal in domestic wastewater treatment plants. However, under field operational conditions, viz. upflow velocities between $0.5\text{-}1.0 \text{ m}\cdot\text{h}^{-1}$ and temperatures between $20\text{-}30^\circ\text{C}$, the volumetric biogas production ($\text{m}^3\cdot\text{m}^{-3}\cdot\text{d}^{-1}$) will be higher than at low temperatures (Lettinga *et al.*, 2001; von Sperling *et al.*, 2005; Chernicharo *et al.*, 2015). This biogas production will likely impact the sludge bed filtration capacity. In fact, biogas production, at least locally, increases the Reynolds number. The higher degree of turbulence compared to the current research, will likely induce inertial lift of particles. Therefore the sludge filtration capacity is expected to decrease at higher temperatures.

2.5 Conclusions

Sludge filtration capacity is reciprocally correlated to upflow velocity.

For both latex beads and helminth eggs 100% removal is achieved at 4°C and low upflow velocity of respectively 0.3 and 0.39 m·h⁻¹. A decreased removal is achieved at increased upflow velocities.

Under conditions of plain settling, 100% latex beads removal is achieved at low upflow velocity of 0.3 m·h⁻¹ due to a theoretical settling velocity of 0.5 m·h⁻¹, at 4°C, of the latex beads.

Hydraulic fluid properties are different for sludge filtration in comparison to plain settling, resulting in different removal mechanisms.

Use of latex beads is a good alternative for studying sludge filtration of helminth eggs.

2.6 Acknowledgments

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CHAPTER 3

Helminth egg removal capacity of
UASB Reactors under subtropical
conditions

Yaya Beas, R.E., Ayala-Limaylla, C., Kujawa-Roeleveld, K., van Lier, J. B. and Zeeman, G.

Helminth egg removal capacity of UASB reactors under subtropical conditions

Abstract

This research was conducted to study the anaerobic sludge filtration capacity regarding helminth egg removal in upflow anaerobic sludge blanket (UASB) reactors. Two 25 L lab-scale UASB reactors were operated at an ambient temperature which varied between 17.1 °C and 28.6 °C. *Ascaris suum* egg was selected as the model egg considering its similarity in terms of size and morphology to *Ascaris lumbricoides*, a human pathogen. *Ascaris suum* eggs were obtained from female parasites of infected pigs. The anaerobic sludge filtration capacity was performed applying upflow velocities between 0.09 and 0.68 m·h⁻¹. Three sludge bed heights in the range of 0.30–0.40 m, 0.50–0.60 m and 0.60–0.70 m were applied. These sludge bed heights corresponded to 19%–25%, 31%–38% and 38%–44% of the total reactor height, respectively. Under the mentioned conditions, the average helminth egg removal efficiency was reciprocally correlated to the imposed upflow velocity. The studied lab-scale reactors reported an average helminth egg removal between 34%–100%, 30%–91% and 34%–56%, when the sludge bed in the UASB reactor was 19%–25%, 31%–38% and 38%–44% of the total reactor height, respectively. The decreased filtration capacity at increasing sludge bed heights might be likely related to biogas production and channeling formation. The average helminth egg removal efficiency in the control experiments performed without any sludge bed, by plain sedimentation, varied between 44% and 66%.

Keywords

Helminth eggs; *Ascaris suum*; pathogens; UASB reactor; sludge bed filtration capacity.

This chapter is based on

Yaya-Beas, R.E., Ayala-Limaylla, C., Kujawa-Roeleveld, K., van Lier, J. B. and Zeeman, G. (2015). "Helminth Egg Removal Capacity of UASB Reactors under Subtropical Conditions." Water 7(5): 2402-2421"

3.1 Introduction

When treated wastewater is intended to be used for agricultural purposes, the presence of pathogens may limit its application potential (Jimenez, 2007; Navarro and Jiménez, 2011). Due to their shell resistance, helminth eggs are the most persistent pathogens to inactivation (Jimenez, 2007; Maya et al., 2012). Particularly in developing countries, high concentrations of helminth eggs are present in domestic wastewater, which cause parasitic diseases like ascariasis, taeniasis and trichuriasis (Cooper et al., 2000; Blumenthal et al., 2001; Cruz Toribio, 2010). The prevailing symptoms caused by these diseases include diarrhea, effects on mental development, and anemia (de Bonilla, 1990; Santiso, 1997; WHO, 2006). Within the group of pathogenic organisms, helminth eggs are infective agents which range in size from 10 μm to more than 100 μm (Tchobanoglous et al., 2003; Jimenez, 2007; Qadir et al., 2010).

Most literature regarding removal of helminth eggs is related to inactivation of helminth eggs contained in excess sludge (Borrely et al., 1998; Gantzer et al., 2001; Keller et al., 2004; de Souza et al., 2011; Maya et al., 2012) and physical removal from wastewater (Mara, 2003; von Sperling et al., 2005; Jimenez, 2007). Technologies to inactivate helminth eggs in sludge are aimed at destroying the structure of the egg (mainly damages in its lipid layer) which prevents further development and survival of the eggs (Jimenez et al., 2001; Koné et al., 2007; Maya et al., 2012). The best technologies for inactivation of helminth eggs present in sludge are thermal treatment at 108 °C (Gantzer et al., 2001), irradiation at 3500 Gy (Borrely et al., 1998; de Souza et al., 2011), pasteurization at 70 °C (Cabaret et al., 2002; Keller et al., 2004) or chemical treatment using sulfuric, hydrochloric, propionic, acetic or peracetic acid (Jimenez et al., 2001). For example processes like alkaline pre- and post-stabilization, by adding lime or other alkaline compound to the sludge, and thermophilic anaerobic digestion have shown high residual concentrations of worm eggs, *i.e.*, more than 1 $\text{egg}\cdot\text{g}^{-1}$ TS, and 0.99–1.1 $\text{egg}\cdot\text{g}^{-1}$ TS in the sludge, respectively (Jimenez, 2007; Maya et al., 2012). These values are higher than the restrictive limit in developing countries, where the use of treated waste and wastewater in (irrigated) agriculture is commonly applied. (WHO, 1989; von Sperling et al., 2005; WHO, 2006; Jimenez, 2007). Maya *et al.* (2012) reported that four genera of helminth eggs, *i.e.*, *Ascaris lumbricoides*, *Ascaris suum*, *Toxocara canis* and *Trichuris trichiura*, are sensitive to environmental conditions in the larval state in the sludge. Furthermore, a proper combination of pH, dryness and contact time with temperatures above 60 °C can be applied to inactivate the eggs efficiently (Brownell and Nelson, 2006; Maya et al., 2012). Unfortunately, external energy and chemical-dependent technologies are in general not feasible for developing countries because they are complex, not sustainable and expensive in terms of investment, operating and maintenance costs (von Sperling, 1996; Mara, 2003; von Sperling et al., 2005; Maya et al., 2012).

Within the group of technologies applied to physical helminth egg removal (not inactivation) from wastewater, land-based post-treatment technologies such as sand

filtration, wetlands and polishing ponds are reported to achieve helminth egg removal of 90–99%, 100% and 100%, respectively (von Sperling et al., 2002; Chernicharo, 2006; Jimenez, 2007). In addition, Jimenez (2001) reported that grit removal followed by a coagulation flocculation process in what is known as advanced primary treatment (APT), combined with an upflow sand filtration, reduced the amount of helminth eggs from 1.2 to 0.2 egg·g⁻¹. Additionally, a study using APT followed by a sand filter combined with a synthetic medium reduced the amount of helminth eggs in average from 26 to 1.2 egg·g⁻¹. Furthermore, APT followed by a multimedia filter and inclined parallel plates reduced the concentration from 27.0 to 1.2 egg·g⁻¹ (Jimenez et al., 2001). Limited research is executed on physical helminth egg removal in UASB reactors (von Sperling et al., 2002; Jimenez, 2007; Jimenez, 2007). Filtration and sedimentation has been considered the main mechanism of helminth egg removal in UASB reactors (von Sperling et al., 2002; Mara, 2003; Jimenez, 2007). During filtration and sedimentation, helminth eggs are respectively accumulated in the sludge bed and on the bottom of the reactor (von Sperling et al., 2003; Jimenez, 2007).

The removal of helminth eggs in UASB reactors has been reported to amount to 60–90% (Jimenez, 2007). UASB reactor technology is relatively cheap and compact and could contribute to domestic wastewater treatment in a sustainable way to improve environmental protection, resource recovery and public health protection (Uemura and Harada, 2000; van Lier et al., 2001; von Sperling et al., 2005; Chernicharo, 2006; van Lier et al., 2010; Jorsaraei et al., 2013). However, the effect of different operational conditions of UASB reactors on helminth egg removal has not been evaluated thus far. Helminth egg removal through sedimentation and filtration would give an added value to UASB reactors. Mahmoud *et al.* (2006) described the sludge bed filtration of UASB reactors as a mechanism for solids removal in domestic wastewater. Similar processes might affect the removal of helminth eggs in UASB reactors.

Pig helminths like *Ascaris suum*, *Trichuris suis* and *Oesophagostomum spp.* are often used in research as model organisms for human intestinal parasites, because they are very similar in morphology and size to the corresponding human parasite eggs and are relative easy to obtain in high numbers from infected pigs (Boes and Helwich, 2000). Maya *et al.* (2012) reported that no significant differences were found between *Ascaris lumbricoides* and *Ascaris suum* regarding the inactivation conditions. In addition, *Ascaris* eggs were found to be the most resistant helminth egg genus to inactivation, combining unfavorable pH, dryness and temperature conditions, in comparison with *Taenia sp.* and *Toxocara sp.*, *Trichuris sp.* and *Hymenolepis* (Maya et al., 2012). In previous work (Yaya-Beas et al., 2010; Yaya-Beas et al., Unpublished results), it has been shown that in the municipal wastewater in Peru, *Ascaris lumbricoides* was the predominant specie. Therefore, this research was conducted using *Ascaris suum* as helminth eggs as surrogate for the human parasite.

Mature *Ascaris sp.* eggs have an ovoid shape with average sizes of 40–70 µm (O’Lorcain and Holland, 2000; Jimenez, 2007). This helminth egg is very resistant to

inactivation under different environmental conditions (Jimenez, 2007; Maya et al., 2012). This resistance is related to their four-layered shell composed of a lipid layer with a total thickness of about 4.5 μm , a mechanically rigid chitinous layer, a vitelline membrane and an external coat (O'Lorcain and Holland, 2000; Quilès et al., 2006; Jimenez, 2007). The shell is sensitive to lipid solvents and shows reduced surfaces and ridges. This mammillated layer is bile-stained to a golden brown color, and its high hydration makes it limp in the natural environment (Quilès et al., 2006; Maya et al., 2012). Microorganisms present in anaerobic sludge may play a role in degrading nematode eggs, though limited research results are available. For example, it has been reported that *Duddingtonia flagrans* and *Angiostrongylus Cantonensis* (nematofagous fungi) feed on free-living nematodes at the larval stage at 27 °C (da Cruz et al., 2011; Federica et al., 2012; Arias et al., 2013). These fungi could survive in the digestive tract of different animal species and kill parasite larvae as they develop in the feces. Evidence exists that they are able to degrade the eggshell enzymatically and infect the helminth eggs (Larsen, 2000; Manzanilla-López et al., 2013).

Sludge bed density, extracellular polymeric substances (Seysiecq et al., 2003; Mori et al., 2006; Pevere et al., 2006; Johansen et al.), stability (Seghezzo, 2004) and methanogenic conversion capacity (Seghezzo, 2004; De Graaff et al., 2010) are some of the parameters that may impact the sludge bed filtration capacity for helminth eggs. Depending on the applied solids retention time (SRT) and the concentration of helminth eggs in the influent, long-term filtration may lead to saturation of the sludge bed, possibly lowering the filtration capacity. According to reviewed literature (Chernicharo et al., 2001; Jimenez et al., 2001; Mendez et al., 2002; Jiménez, 2005; von Sperling et al., 2005; Jimenez, 2007; Jiménez et al., 2010; Jiménez et al., 2010), no studies have been done thus far to characterize the sludge bed capacity for helminth egg filtration. Therefore, the main aim of this research was to study the sludge bed filtration capacity of UASB reactors with respect to the physical retention of helminth eggs under different upflow velocities at the prevailing subtropical temperatures. Filtration capacity is defined in this research as the physical process to retain helminth eggs using anaerobic sludge as a filtration medium.

3.2 Materials and methods

3.2.1 Influent

The research was carried using raw wastewater from two urban villages called El Angel and El Milagro located in Lima (Peru). This wastewater was fed into a pilot plant located at the Research Center for Wastewater Treatment and Hazardous Wastes (CITRAR) at the campus of the National University of Engineering (Lima, Peru). The main characteristics of the wastewater are shown in Table 3.1.

Table 3.1 Influent wastewater characteristics from two urban villages called El Angel and El Milagro located in Lima (Peru), used for this research

Parameter	Units	Average	<i>n</i>
Chemical Oxygen Demand	mg·L ⁻¹	723.2 ± 320.3	90
Suspended Solids	mg·L ⁻¹	126.5 ± 28.5	36
Oils and Grease	mg·L ⁻¹	30.8 ± 14.1	36
Total Phosphorous—P	mg·L ⁻¹	6.6 ± 2	35
Total Kjeldahl nitrogen—TKN	mg·L ⁻¹	16.2 ± 6.5	36
Dissolved Oxygen	mg·L ⁻¹	6.8 ± 0.4	36
Temperature	°C	22.8 ± 4.1	233
pH	-	7.1 ± 0.3	233
Fecal Coliforms	MPN/100mL	9.67 × 10 ⁸ ± 1.89 × 10 ⁸	36
Helminth eggs	egg·L ⁻¹	2.4 ± 1.4	90

Note: Where *n* is a number of grab analyzed samples.

The wastewater was pumped daily into a 200 L tank. The tank was filled with fresh wastewater every morning for all cases except when the upflow velocity of 0.68 m·h⁻¹ was tested. For the latter situation, it was filled again in the afternoon when the remaining volume of the wastewater was 20 L. After filling the tank, the wastewater was mixed using a mechanical stirrer (18 RPM) with a stock solution containing *Ascaris suum*. The helminth egg concentration in the tank varied between 20–50 egg·L⁻¹. The tank was kept at ambient temperatures and its content was used to continuously feed the UASB reactors. The pH and temperature of the wastewater was measured daily at 9:00, 12:00 and 16:00. The setup of the experiments is shown in Figure 4.1.

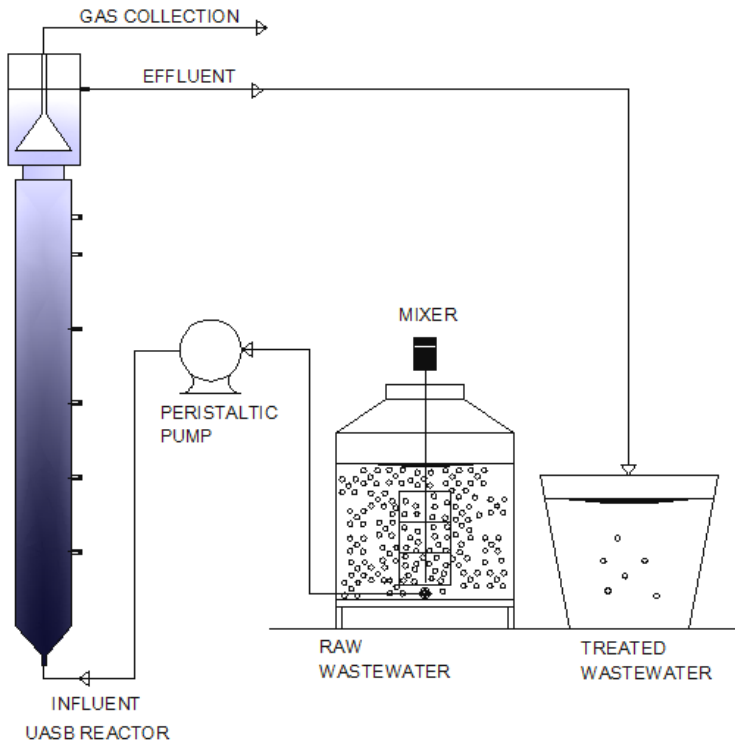


Figure 3.1 Set up of the filtration experiments in UASB (upflow anaerobic sludge blanket) reactors using wastewater inoculated with *Ascaris suum* eggs.

3.2.2 Upflow Anaerobic Sludge Blanket (UASB) Reactors

Two 25 L identical acrylic cylindrical lab-scale UASB reactors with a total height of 1.60 m and a diameter of 0.15 m were used separately in parallel. They were located at CITRAR. The experiments were performed from January 2010 to August 2013.

3.2.3 Inoculum

The inoculum was anaerobic flocculent sludge sampled from the 536 m³ pilot-scale UASB reactor located at CITRAR. The inoculum was taken at a height of 1.5 m from the bottom of this reactor (total height of the reactor was 6.0 m). The total solids and volatile solids concentration of inoculum was 163 ± 37 and 106 ± 44 g·L⁻¹ respectively.

3.2.4 Helminth Eggs

The experiment was conducted using *Ascaris suum* as helminth egg surrogate for the human parasite. The *Ascaris suum* helminth eggs were collected from female parasites of infected pigs (*Sus crofa domesticus*). In order to collect helminth eggs, dissections of the female parasite were performed according to Diawara *et al.* (2009) by means of a longitudinal incision to obtain the reproductive system (womb and ovary). The womb and ovary were placed in 50 mL of physiological whey solution where they were opened to extract the helminth eggs. The optimal morphology and viability of the eggs of *Ascaris suum* were verified by microscopic observation according to Johnson *et al.* (1998) and by using the staining procedure applied by de Victorica and Galván (2003), respectively. Helminth eggs were added to the 200 L wastewater tank, which was fed to the UASB reactors.

3.2.5 Helminth Egg Counting

A multi-step methodology using local materials was developed from the modified Bailenger method (Ayres and Mara, 1996) and (Bailenger, 1979). This method was chosen due to its simplicity and the low cost of materials, in addition to the fact that it allows recovery of a wide range of helminths from the sample. The detailed methodology consists of collection of a 1 L sample, followed by settling for 24 h in a 1 L clear borosilicate glass bottle with graduations to concentrate the helminth eggs and to remove 90% of the supernatant (900 mL) by using a siphon. Then, 60 mL of the sediment are transferred to six centrifuge tubes of 10 mL each. Afterwards, the tubes are centrifuged at 1000 g for 15 min, and 70% of the supernatant (7 mL) is removed without shaking the tubes to avoid mixing the pellet with the supernatant. The remaining 40 mL of sediment is distributed over the same centrifuge tubes until the tubes are filled with 10 mL. Next, the bottle is rinsed two or more times with 10 mL of distilled water until it is completely clean. The corresponding rinse water is spread over the same centrifuge tubes or in new tubes. Distilled water is used to complete the remaining volume to fill 10 mL of water in each centrifuge tube. Again, the centrifugation step is repeated. Subsequently, 2 mL of saturated sodium chloride solution with a specific gravity of 1.18 is added as flotation solution and, the tubes are shaken vigorously laterally. Afterwards, it is controlled whether all solids are located in the liquid phase. After 10 minutes, two phases are distinguished in the tubes. Finally, the top phase (1.5 mL) formed in the tubes is transferred to glass slides to be observed under the microscope (objectives lens 4× and 10×) and to count the eggs.

3.2.6 Physicochemical and Bacteriological Analysis

Total chemical oxygen demand (COD), suspended solids, volatile solids, oil/grease, pH, temperature, biochemical oxygen demand (BOD₅) and fecal coliform analysis were

determined following standard methods (APHA et al., 1998). Gravimetric and extractive-gravimetric methods carried out with hexane as a solvent were executed for solids and oil/grease determination, respectively. COD analysis was executed using high range Hach's COD digestion vials as well as a digester reactor DR 200, and program 17 from colorimeter DR 890. Dissolved oxygen, total nitrogen Kjeldahl and total phosphorous were measured according to Method HACH 10360, 8038 and 8048, correspondingly (HACH, 2008). Microscopic views were performed with an optical microscope ZEISS Primo Star Serial number 3122001719.

3.2.7 UASB Operational Conditions

In order to study the influence of different upflow velocities and sludge bed heights in the UASB reactors, four experiments were carried out as indicated in Table 3.2. Each experiment was performed in duplicate (two reactors).

In order to facilitate the statistical interpretation of the results, it is assumed that at an upflow velocity near to zero, all helminth eggs are removed in the UASB reactor in experiment 1, 2 and 3. This assumption is in line with the results described in previous research (Yaya-Beas et al., 2010; Yaya-Beas et al., Unpublished results).

Table 3.2 Setup of experiments in lab-scale UASB reactors to test the sludge filtration capacity to remove helminth eggs.

Experiment	SB Height Variation	SBp	Upflow Velocities
	(m)	(%)	($\text{m}\cdot\text{h}^{-1}$)
1	0.30 to 0.40	19 to 25	0.09, 0.17, 0.23, 0.34 and 0.68
2	0.50 to 0.60	31 to 38	0.09, 0.11, 0.17, 0.23, 0.34 and 0.68
3	0.60 to 0.70	38 to 44	0.09, 0.14, 0.17, 0.23, 0.34, 0.45 and 0.68
4 (blank experiment)	0	0	0.09, 0.11, 0.14, 0.17, 0.23, 0.34, and 0.68

Notes: Where SB means sludge bed and SBp is the sludge bed expressed as a percentage of the total reactor height. The upflow velocities of 0.09, 0.11, 0.14, 0.17, 0.23, 0.34, 0.45 and 0.68 $\text{m}\cdot\text{h}^{-1}$ correspond to an hydraulic retention time (HRT) of 15, 12, 10, 8, 6, 4, 3 and 2 h, respectively. Each experiment was repeated three times.

The startup of the UASB reactors was performed at an upflow velocity of 0.34 $\text{m}\cdot\text{h}^{-1}$ and hydraulic retention time (HRT) of 4 h. Each upflow velocity for every experiment was applied during seven days and samples were taken on the last day. The samples were taken after an elapsed time equivalent to one HRT, after introducing a known wastewater corresponding in the influent tank. The effluent of UASB reactors was collected separately from each reactor in order to be able to take separate samples. For

every upflow velocity, six samples were analyzed for COD and helminth egg content and temperature in the influent and effluent. A total of six samples were performed per upflow velocity, which were collected respectively from three measurements in each UASB reactor.

Experiment 1 was performed 85 days after the start of the UASB reactor. Before starting experiment 2, reactors were operated for approximately 30 days and continuously fed with domestic wastewater containing an average helminth egg concentration of $2.4 \text{ egg}\cdot\text{L}^{-1}$ and an HRT of 4 h. The two reactors were fed with exactly the same influent using two peristaltic pumps (2 Masterflex, Oldham, UK). Some samples from the effluent in experiment 2 were taken for each upflow velocity in order to do microscopic observations. Experiment 3 started immediately after finishing experiment 2. Experiment 4 (control experiment) was performed without sludge in the acrylic UASB reactor 7 days after all experiments were finished. All experiments were performed at ambient temperatures. Sludge was removed in each UASB reactor in order to maintain the established sludge bed height variation according to Table 2.

3.3 Results and discussion

A summary of the results of experiments 1, 2, 3 and 4 is listed in Table 3.1. The sludge filtration capacity at ambient temperatures and sludge bed heights in the ranges of 0.30–0.40 m and 0.50–0.60 m, showed a negative linear function between the average helminth egg removal efficiency and upflow velocity with a coefficient of determination of 0.94 and 0.91, respectively. When the sludge bed height increased to 0.60–0.70 m, the negative linear correlation is still present but the coefficient of determination (R^2) decreased to 0.57.

Table 3.3 Results of helminth egg removal and chemical oxygen demand (COD) removal efficiencies at applied upflow velocities and wastewater temperatures. Each upflow velocity was applied three times in each UASB reactor. Then a total of six samples per upflow velocity was analyzed.

Experiment	Upflow Velocity (m·h ⁻¹)	Temperature (°C)	Helminth Egg removal (%)	COD (%)
Experiment				
1	0.09	24.6 ± 2.4	93 ± 5	71.9 ± 7.1
	0.17	28.6 ± 2	77 ± 4	66.4 ± 8.2
	0.23	25.6 ± 3.5	61 ± 7	63.1 ± 8.6
	0.34	23 ± 3.3	52 ± 9	60.3 ± 6.4
	0.68	26.5 ± 2	26 ± 7	45.4 ± 6.3
Experiment				
2	0.09	22 ± 6	91 ± 3	71.6 ± 10.3
	0.11	22.5 ± 5.3	75 ± 10	71.6 ± 2.2
	0.17	24.2 ± 1.5	71 ± 11	66.2 ± 12.7
	0.23	23.2 ± 3.1	61 ± 10	65 ± 7.4
	0.34	26.1 ± 0.5	51 ± 7	63.7 ± 15.1
	0.68	25.5 ± 3	30 ± 15	63 ± 19.1
Experiment				
3	0.09	23.3 ± 0.9	55 ± 1	80.3 ± 2.4
	0.14	21.4 ± 2.9	53 ± 5	80.2 ± 15.5
	0.17	27.1 ± 0.5	56 ± 7	80.2 ± 8.1
	0.23	23.3 ± 5.7	56 ± 8	79.3 ± 0.8
	0.34	22.1 ± 4.2	55 ± 11	69.5 ± 14.8
	0.45	28.5 ± 2	46 ± 8	60.5 ± 0.4
	0.68	26.2 ± 2.3	34 ± 8	45.3 ± 3.4
Experiment				
4	0.09	16.9 ± 1	66 ± 3	77.6 ± 2.4
	0.11	16.9 ± 0.5	48 ± 3	44.8 ± 9.8
	0.14	17.3 ± 1	57 ± 3	84 ± 1.9
	0.17	17.3 ± 2	44 ± 3	50.7 ± 5.7
	0.23	16.9 ± 0.8	53 ± 3	64.9 ± 5
	0.34	18.1 ± 1	52 ± 10	71.1 ± 3.7
	0.68	17.7 ± 1	54 ± 8	55.2 ± 7.3

Experiment 1: Upflow velocity between 0.09 and 0.68 m·h⁻¹ and sludge bed height between 0.30 and 0.40 m (19 to 25% of the total reactor height)

The efficiencies of helminth egg removal as a function of the upflow velocity, applying a sludge bed height between 0.30 and 0.40 m in two reactors, are shown in Figure 3.2, both operated at five upflow velocities of 0.09, 0.17, 0.23, 0.34 and 0.68 m·h⁻¹. Results show a decreasing trend for helminth egg removal efficiency at an increasing upflow velocity. A negative linear relationship was observed between upflow velocity and helminth egg removal with a high coefficient of determination ($R^2 = 0.92$). The current results of the experiment applying a low sludge bed height of 19–25% show that an increment of the upflow velocity leads to a decrease of the sludge filtration capacity. The latter statement could be explained because as soon as the wastewater upflow velocity increases, the associated sludge viscosity probably decreases (Pevero et al., 2006). Analogous to the removal of helminth eggs, the COD removal efficiency is decreasing at an increasing upflow velocity (Figure 3.3). A negative linear relationship was observed between upflow velocity and COD removal with a high coefficient of determination ($R^2 = 0.99$). Average ambient temperature varied between 23 and 28.6 °C in both UASB reactors.

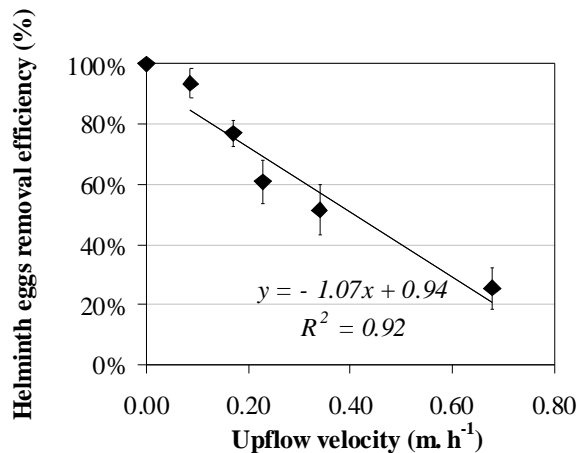


Figure 3.2 Helminth egg removal efficiencies (♦) versus upflow velocity at a sludge bed height between 0.30 and 0.40 m.

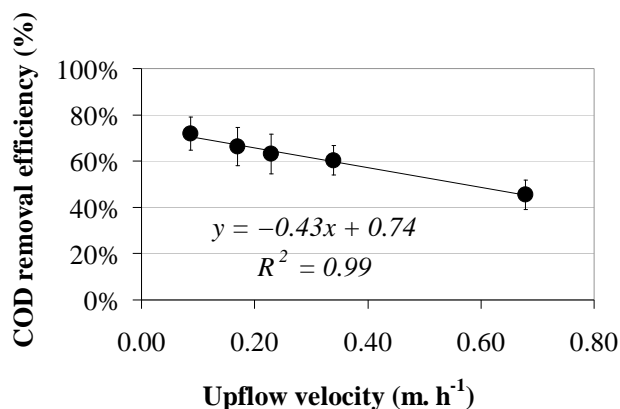


Figure 3.3 Total COD removal efficiencies in two UASB reactors, characterized by a sludge bed height between 0.30 and 0.40 m.

Experiment 2: Upflow velocity between 0.09 and 0.68 m·h⁻¹ and sludge bed height between 0.50 and 0.60 m (31 to 38% of the total reactor height)

The efficiencies of helminth egg removal as a function of the upflow velocity, applying a sludge bed height between 0.50 and 0.60 m in two reactors, are shown in Figure 3.4, both operated six different upflow velocities: 0.09, 0.11, 0.17, 0.23, 0.34 and 0.68 m·h⁻¹. Results show a decreasing trend for helminth egg removal efficiency at an increasing upflow velocity (Figure 3.4). A negative linear function was observed between upflow velocity and helminth egg removal with a high coefficient of determination ($R^2 = 0.91$). The observed results applying a sludge bed height of 31–38% were similar to those at a sludge bed height of 19–25%.

In contrast, the COD removal efficiency did not show a clear trend at an increasing upflow velocity (Figure 3.5) when applying a sludge bed height of 0.50–0.60 m. Average ambient temperature varied from 22.0 to 26.1 °C.

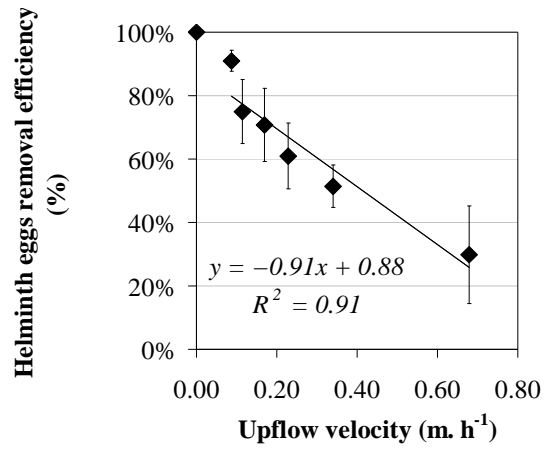


Figure 3.4 Helminth egg removal efficiencies (◆) versus upflow velocity using a sludge bed height between 0.50 and 0.60 m.

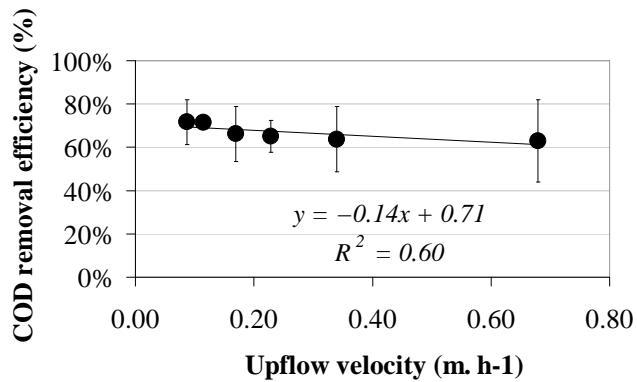


Figure 3.5 Total COD removal efficiencies *versus* upflow velocity using a sludge bed height between 0.50 and 0.60 m.

Experiment 3: Upflow velocity between 0.09 and 0.68 m·h⁻¹ and sludge bed height between 0.60 and 0.70 m (38 to 44% of the total reactor height)

Results of the helminth egg removal as a function of the upflow velocity at a sludge bed height between 0.60 and 0.70 m, in two UASB reactors operated at 0.09, 0.14, 0.17, 0.23, 0.34, 0.45 and 0.68 m·h⁻¹, are shown in **Figure 3.6**. Though a slightly decreasing trend is shown with increasing upflow velocity, the coefficient of determination is low ($R^2 = 0.83$). Moreover, standard deviations are large.

Results on COD removal efficiency show a decreasing trend at an increasing upflow velocity (Figure 3.7). A negative linear correlation was observed between upflow velocity and COD removal with a high coefficient of determination ($R^2 = 0.97$). Average ambient temperature varied from 21.4 to 28.5 °C.

Although counterintuitive, the decreasing trend in helminth egg removal efficiency at an increasing sludge bed height in the studied lab-scale reactor might be explained by an increase in turbulence, created by the biogas production and formation of channels through the sludge bed (Lettinga et al., 1984; Abdelgadir et al., 2014) during all studied velocities. The possible saturation with helminth eggs during previous experiments could also have influenced the stability of the system with respect to helminth egg removal. Since none of the eggs are spherical (O'Lorcain and Holland, 2000; Quilès et al., 2006; Jimenez, 2007; Jimenez, 2007), it is likely that they settle with different, but unknown orientations (Sengupta et al., 2011).

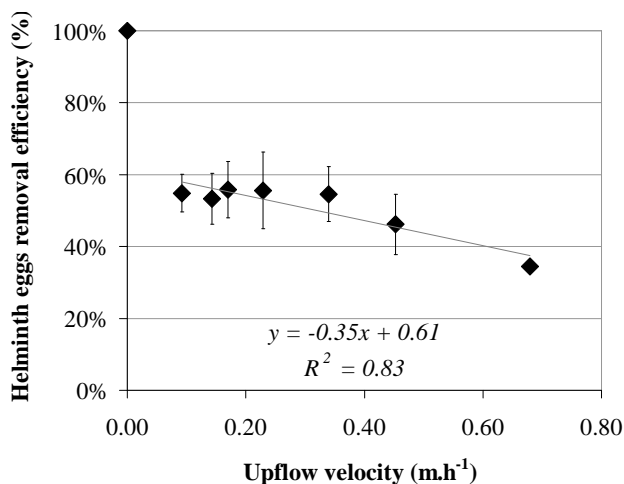


Figure 3.6 Helminth egg removal efficiencies (♦) versus upflow velocity using a sludge bed height between 0.60 and 0.70 m.

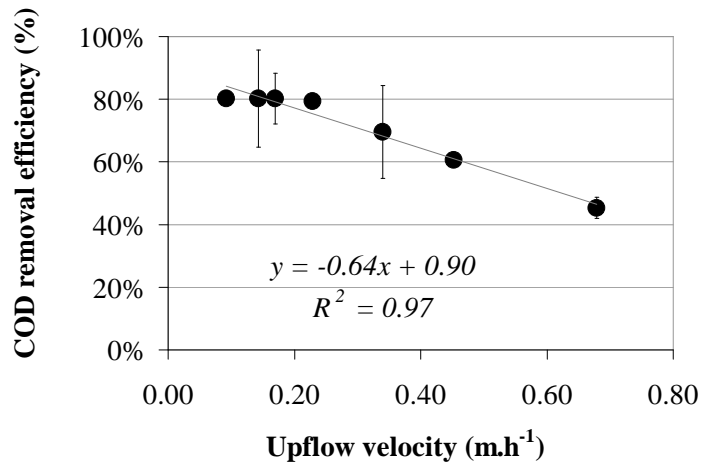


Figure 3.7 Total COD removal efficiencies versus upflow velocity using a sludge bed height between 0.60 and 0.70 m. Results show the average of two reactors and standard deviation and three measurements per reactor.

Experiment 4: Blank experiment using upflow velocity between 0.09 and 0.68 m·h⁻¹ and no sludge bed

The effect of the upflow velocity on the settling of helminth eggs is demonstrated by the results of the control experiment, applying a UASB reactor without sludge. Results for seven different upflow velocities, 0.09, 0.11, 0.14, 0.17, 0.23, 0.34, and 0.68 m·h⁻¹, are shown in Figures 3.8 and 3.9. Each point is the average of three samples in two reactors.

Figure 3.8 indicates that the best efficiency for helminth egg removal was obtained at 0.09 m·h⁻¹, when the removal efficiency reached 66 ± 3%. For upflow velocities higher than 0.09 m·h⁻¹, the helminth egg efficiency removal was lower but always exceeded 44 ± 3%. It should be noted that standard deviations were large, so no significant differences were observed for helminth egg removal at an increasing upflow velocity. Figure 3.9 shows the trend for TSS and VSS removal. The best TSS and VSS removal efficiency (about 80%) was obtained at the lowest upflow velocities. For the higher upflow velocities the TSS and VSS removal efficiencies dropped to 40–50% and 30–40% for TSS and VSS, respectively.

The control experiment was executed in winter at a relatively low temperature and average ambient temperature varied from 16.9 to 18.1 °C. Latter temperatures were colder compared to previous experiments since experiment 4 was carried out coincidentally during winter.

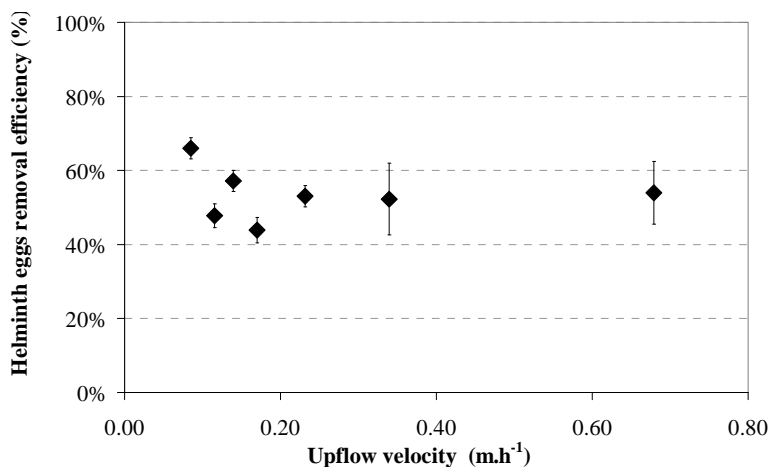


Figure 3.8 Helminth egg removal efficiencies (◆) versus upflow velocity without sludge in the control experiment. Results shows the average of three samples and standard deviation; no sludge bed.

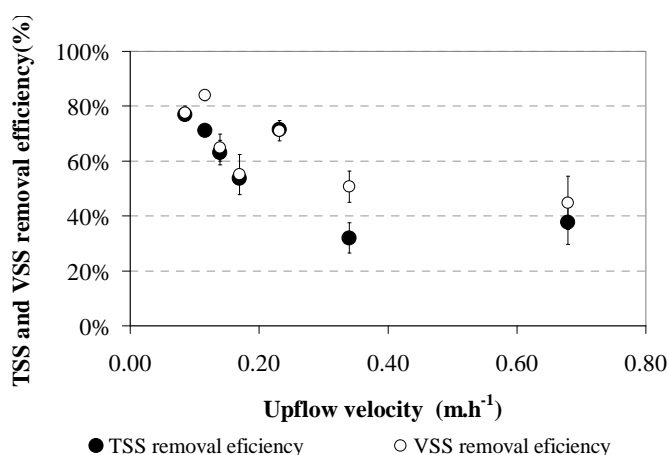


Figure 3.9 TSS (●) and VSS (○) removal efficiencies in the control experiment—No sludge bed. Results show the average of three samples and standard deviation.

Results of experiments 1, 2, 3 and 4 show that the sludge bed in a UASB reactor is an inappropriate and unreliable filter medium for helminth eggs. Therefore, for achieving a complete helminth egg removal, a UASB reactor must be followed by an adequate post-treatment unit like land-based settling units or a post-filtration step (Chernicharo et al., 2001; von Sperling et al., 2005; Chernicharo, 2006). For the control experiment (without sludge), average helminth egg removal efficiency varied between 44 and 66% at upflow velocities between $0.09 \text{ m}\cdot\text{h}^{-1}$ and $0.68 \text{ m}\cdot\text{h}^{-1}$. Unexpectedly, these values exceed the removal efficiencies of the reactors filled with high volumes of sludge,

particularly at the high upflow velocities. Previous research (Yaya-Beas et al., 2010; Yaya-Beas et al., Unpublished results) showed that viscosity of the flocculent anaerobic sludge is approximately more than 50 times higher than the viscosity of the liquid water, thereby theoretically leading to a better retention of helminth eggs.

The explanation in the control experiments for why the levels of helminth egg removal were so high is not very clear. Likely, the better retention might be associated to the absence of biogas production and thus turbulence. Therefore, in the absence of turbulence, the wastewater flow is more homogeneous (Bolle et al., 1986; Elmitwalli et al., 1999; Mahmoud, 2002; Lew et al., 2004), and helminth egg settling follow a discrete settling pattern. Another remarkable observation is that even at the lowest upflow velocity ($0.09 \text{ m}\cdot\text{h}^{-1}$) helminth eggs do not settle completely nor are retained completely by the sludge bed. The wash out of helminth eggs under these conditions may indicate that either the egg density is much less than expected, or the flow distribution is far from laminar (Bolle et al., 1986; Ojha and Singh, 2002; Seghezzo, 2004). A higher degree of channeling, which is expected at higher volumes of sludge (Lettinga et al., 1984; Jeison and Chamy, 1999; Seghezzo, 2004), will aggravate the extremes in the flow distribution patterns. The latter will certainly lead to poorer filtration performances, as was also observed in the conducted experiments.

COD removal efficiencies showed a similar trend to the helminth egg removal efficiency. An increased removal of COD with decreasing upflow velocity was shown by Mahmoud (Mahmoud, 2002; Mahmoud et al., 2003). Though completely different in nature, helminth eggs are also particles that could be expected to behave similarly.

Microscopic observations in the effluent

Microscopic observations were performed only for experiment 2. The presence of helminth eggs was detected in the sludge samples. In addition, several damages have been microscopically observed in the morphology of helminth eggs in the effluent (Figure 3.10a,c,f) with respect to the influent (Figure 3.10b–e,g,h) of the UASB reactor. The observed damages in the internal morphological structure of the eggs might be related to a possible loss in egg viability. These damages could be possibly caused by the retention of the eggs in the sludge bed for the applied HRT prior to their washout. The indicated hypotheses need to be confirmed in further research. Figure 3.10c, d presents respectively some microscopic views of helminth eggs in the influent and effluent of the UASB at an applied upflow velocity of $0.09 \text{ m}\cdot\text{h}^{-1}$. There are some changes in the internal morphology like probable larval development but without progression to the next stage (Figure 3.10c–e). Figure 3.10g,h shows some observed damages in the structure of helminth eggs.

The percentage of damaged helminth eggs present in the effluent of the UASB reactor that operated under different conditions was not determined, but visually they only were present in the effluent and not in the influent.

Following microscopic observations of the sludge sampled at different upflow velocities in experiment 2, it is shown that damages to helminth eggs occurred in all applied upflow velocities. It is shown in Figure 3.10h that some helminth eggs formed clusters of eggs. The mechanisms behind this phenomenon are not known.

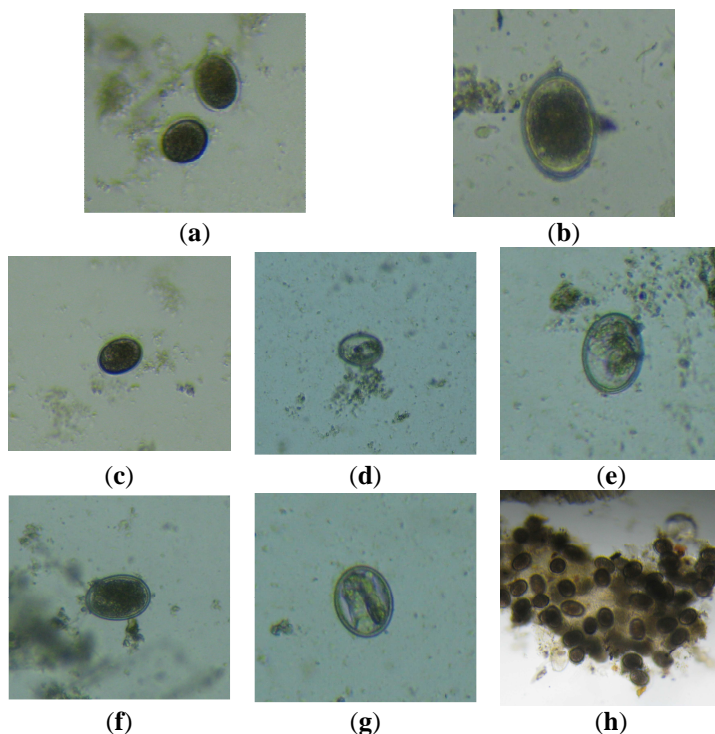


Figure 3.10 Helminth eggs in the influent (a) and in the effluent (b) for an applied upflow velocity of $0.09 \text{ m}\cdot\text{h}^{-1}$. Helminth eggs in the influent (c) and in the effluent (d) and (e) for an upflow velocity of $0.34 \text{ m}\cdot\text{h}^{-1}$. Helminth eggs from the effluent show an internal morphology likely affected by the experimental conditions. Helminth eggs in the influent (f) and in the effluent (g) and (h) of lab-scale UASB reactor for an upflow velocity of $0.68 \text{ m}\cdot\text{h}^{-1}$. Helminth eggs from the effluent (g) show an apparently deteriorated semi-crystalline internal morphology (possible larval development but interrupted by the conditions of the experiment) and (h) group of attached helminth eggs.

The observed damages on *Ascaris suum* might be attributed to the prevailing physicochemical conditions in the direct vicinity of the eggs or to other microorganisms, which could be present in the anaerobic sludge like nematofagous fungi (da Cruz et al., 2011; Federica et al., 2012; Arias et al., 2013). The relatively low

helminth egg removal in the experiment with the highest sludge bed might also be related to a high percentage of damaged helminth eggs as a result of a long retention of helminth eggs in the sludge bed. Damaged helminth eggs might have a decreased density and thus a lowered settleability. The latter hypothesis could also explain why the removal of helminth eggs in the blank experiment (without sludge bed) is relatively high compared to the sludge bed reactors. This hypothesis needs to be verified in future research.

The results showed that helminth egg removal will not be sufficient for UASB systems operated with conventionally collected domestic wastewater where relatively high upflow velocities need to be applied as a result of the low COD concentration (Dong et al., 2013; Ozgun et al., 2013). New trends in domestic wastewater collection and transport like uncoupling rainwater (Rulkens, 2006) and source separation (Kujawa-Roeleveld and Zeeman, 2006; Udert and Lienert, 2013; Zeeman and Kujawa-Roeleveld, 2013) increase wastewater concentration and therefore reduce applied upflow velocities. The observed increased helminth egg removal at reduced upflow velocity might imply that application of UASB reactors, with similar loading rates in source separated domestic wastewater, leads to improved helminth egg removal.

3.4 Conclusions

This study demonstrated that with an increased sludge bed height there is a reduction in the sludge filtration capacity for helminth egg removal. If treated wastewater is used for irrigation purposes, the UASB reactor must be followed by an adequate post-treatment unit. The sludge filtration capacity at ambient temperatures and sludge bed height in the range of 0.30–0.40 m and 0.50–0.60 m, which agrees with 19–25% and 31–38% of the total height reactor, respectively, showed a negative linear function between the average helminth egg removal efficiency and upflow velocity. This study reported an average helminth egg removal between 34–100%, 30–91% and 34–56% when the sludge bed height was 19–25%, 31–38% and 38–44%, respectively, of the total height in the UASB reactor at upflow velocities varying between 0.09 and 0.68 $\text{m}\cdot\text{h}^{-1}$. Several damages were observed during microscope observations in the morphology of helminth eggs present in the sludge and the effluent of UASB reactors at upflow velocities between 0.09 and 0.68 $\text{m}\cdot\text{h}^{-1}$.

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CHAPTER 4

Presence of helminth eggs in
domestic wastewater and its removal
at low temperature UASB reactors
in Peruvian highlands

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Presence of helminth eggs in domestic wastewater and its removal at low temperature UASB reactors in Peruvian highlands

Abstract

This work studied the anaerobic sludge filtration capacity for pathogen reduction in a 29 L and 1.65 m height lab-scale UASB reactor treating domestic wastewater at low temperatures in the city of Puno (Peru). The anaerobic sludge filtration capacity was performed applying upflow velocities of 0.12, 0.14, 0.16, 0.20, 0.27 and 0.41 m·h⁻¹. Results show that the helminth egg removal varied between 89 and 95% and the most common specie was *Ascaris lumbricoides*. Faecal coliform and *E. coli* removal varied in the range of 0.9–2.1 and 0.8–1.6 log₁₀ respectively. Likely related to the low operational temperatures, the total COD removal varied between 37 and 62%. The best performance in terms of removal of helminths eggs, total COD and turbidity was obtained at the lowest upflow velocity of 0.12 m·h⁻¹. In order to meet WHO standards for water reuse a post-treatment unit will be required to polish the effluent.

Keywords

Helminth eggs; municipal wastewater; pathogens; UASB reactor; sludge bed filtration capacity

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4.1 Introduction

Treated domestic wastewater is an attractive alternative water source for the production of irrigation water (WHO, 2006; McCarty et al., 2011; Mohd, 2013). Pathogen content might hinder the safe use of domestic wastewater for irrigation purposes. Pathogenic organisms from human faeces contained in wastewater are very diverse and can be classified in four groups, viz. bacteria, protozoa, viruses and helminths (Tchobanoglous et al., 2003; von Sperling et al., 2005; Santo Domingo et al., 2007; Jiménez et al., 2010). Within the group of pathogens present in domestic wastewater, helminth eggs have been identified as the most resistant pathogens because of their insufficient removal during wastewater treatment (WHO, 1989; Jiménez, 2006; WHO, 2006). Regarding reuse of treated domestic wastewater the World Health Organisation (WHO) recommend less than 1 egg/L of helminth eggs for restricted and unrestricted irrigation (WHO, 1989; WHO, 2006). A relatively low Helminth egg content, varying between 2 and 5 egg/L, is reported in urban domestic wastewater in Peru (Yaya-Beas et al., 2010). Much higher concentrations between 16 and 43 egg/L were reported by Garcia Palacio (2010) in Colombia. In Brazil, Navarro and Jimenez (2011) reported a helminth egg concentration between 166 and 202 egg/L. These levels of helminth eggs represent a risk of parasitism transmission. Parasitic infections are endemic and very common among Andean countries like Peru, Colombia, Ecuador and Venezuela (Cooper et al., 2000; García Palacio, 2010; González et al., 2011; Gil et al., 2013).

Sanitation conditions in highlands from low income regions in Latin American developing countries are in general poor (Escobar Ramirez and Barg, 1990; Reynolds, 2001; WHO, 2012). Some examples are evidenced in Puno (Peru) (Maco et al., 2001; Marcos et al., 2003; Rossi Luna, 2010), Nariño (Colombia) (Sánchez-Triana et al., 2006; Botero-Garcés et al., 2009; Gomez-Duarte et al., 2013), Quito (Ecuador) (Weber et al., 1994; Da Ros, 1995; Fernández and Buitrón Cisneros, 2011) and La Paz (Bolivia) (Benavides and Mendoza, 2003; Escobari, 2003). For example, a coproparasitological study conducted in Puno on subjects, whose ages were between 4 and 98 years showed that the overall prevalence of parasitism in the study population was 91.20% (Maco et al., 2001). Poverty and lack of health protection programs, and insufficient education, especially in Andean locations, favour parasitism transmission in comparison to other parts of Peru (Maco et al., 2001; Marcos et al., 2003). Regarding Peru, approximately 9 million people, 32% of the national population (INEI, 2007), live in the highlands. The treatment of domestic wastewater in the Andean highlands is additionally challenged by the relatively low ambient temperatures and the very low availability of large areas of flat land, which makes the application of land-based systems very cumbersome. A typical example is the city Puno located at 3810 m.a.s.l in the Peruvian highlands which contaminates its inner bay in the ancient Titicaca lake with insufficiently treated domestic discharges (Northcote et al., 1991). The prevailing low ambient temperatures in Puno impose difficulties for any biological wastewater treatment system as biochemical reaction rates decrease distinctly. WWTP based on only aerobic

technologies in low income locations usually generates financial constraints associated to high capital and operational costs (von Sperling, 1996; von Sperling et al., 2005; Kassab et al., 2010). Anaerobic wastewater treatment processes can, however, increase overall energy recovery efficiency and carbon emission savings (Lettinga, 2008; Verbyla et al., 2013).

The application of Upflow Anaerobic Sludge Blanket (UASB) reactors for domestic wastewater treatment is so far restricted to tropical and semi-tropical conditions (Seghezzeo et al., 1998; van Lier et al., 2010; Souza et al., 2011; Heffernan et al., 2012; Chernicharo et al., 2015). For temperatures below 15 °C, application of UASB reactors is possible when long hydraulic retention times (HRT) and long sludge retention times (SRT) are applied. For these temperatures, an SRT longer than 100 days is necessary to provide sufficient methanogenic activity (Zeeman and Lettinga, 1999). Such long SRT will concomitantly result in a long HRT, and consequently, the upflow velocity would be relatively low. Density and shape of particular helminth eggs in combination with anaerobic sludge predominant characteristics at low temperatures, could impact positively on the sludge filtration capacity for helminth eggs (Yaya-Beas et al., Unpublished results). Thus, a low upflow velocity may on one hand cause short circuiting in the sludge bed, and therefore affect negatively the helminth egg removal. On the other hand, depending on the density and shape of helminth eggs and the influence of the quality of the sludge, helminth eggs might be retained in the sludge bed.

Most investigations applying UASB technology at temperatures below 15 °C mainly focussed on COD removal and not on pathogens removal. Lew *et al.* (2004) reported a COD removal of 48%, and 70% respectively at 10 and 14 °C, when applying a UASB reactor for the treatment of domestic wastewater (HRT varied between 3 and 24 hours). Grin (1983) reported that UASB reactors treating raw wastewater can only achieve a total COD removal between 30 and 50% at an HRT of 8 hours at 11–12 °C. Luostarinen *et al.* (2007) showed that UASB septic tanks can remove 65% of the total COD in black water at temperatures between 5 to 13 °C at an HRT of 4.3 days. Elmitwalli *et al.* (2007) described a 31% of total COD removal in a UASB reactor treating grey water at an HRT of 20 hours and temperatures between 14 and 21.8 °C. These low removal efficiencies will pose the necessity to apply a post-treatment to comply with the discharge or reuse requirements. Previous research (Yaya-Beas et al., 2015; Yaya-Beas et al., Unpublished results) reported that 100% removal of helminth eggs is achieved at 4 °C and low upflow velocities between 0.3 and 0.39 m·h⁻¹. Yaya-Beas *et al.* (2015) elucidated that helminth egg removal varied between 93% and 26% respectively at average wastewater temperature between 16.7 and 28.6 °C for upflow velocities between 0.09 and 0.68 m·h⁻¹. Chernicharo (2006) reported that helminth egg removal varied between 60–90%.

Filtration processes for solids removal in domestic wastewater using the anaerobic sludge bed from UASB reactors were researched by Mahmoud *et al.* (2006). Similarly,

these processes might affect the removal of pathogen indicators like helminth eggs, faecal coliforms and *Escherichia coli* in UASB reactors. Thus, the anaerobic sludge filtration capacity regarding pathogens at temperatures lower than 15 °C would give an added value to UASB reactors.

The main objectives of this research were to identify the common species of helminth eggs in wastewater in the Andean city of Puno (Peru) and to study the anaerobic sludge bed filtration capacity for pathogens removal under low temperatures. The sludge bed filtration capacity was assessed in a lab-scale UASB reactor under different upflow velocities and temperatures, varying between 11.3 and 14.3 °C. The selected pathogen indicators were helminth eggs, faecal coliforms and *Escherichia coli* (*E.coli*).

4.2 Materials and methods

4.2.1 Influent

The research was performed in the city of Puno situated in the Peruvian Andes at an altitude of 3810 m.a.s.l. Puno is characterised by an average ambient temperature of 8 °C and an annual precipitation of 750 mm (Olarde Calsina and Olarte Daza, 2013). A volume of 500 L of fresh domestic wastewater containing helminth eggs was daily delivered from the WWTP 'El Espinar' located in the same city and stored in a stirred tank (15 rpm) at ambient temperatures. Subsequently, it was pumped to the UASB lab-scale reactor using a peristaltic pump (Masterflex, USA). The main characteristics of the wastewater determined in this research are presented in Table 4.1.

Table 4.1 Main characteristics of the domestic wastewater of El Espinar (Puno-Peru)

Parameter	Units	n ¹	Influent
pH		33	7.8 ± 0.2
Temperature	°C	26	12.5 ± 2.0
Turbidity	NTU	33	370 ± 130
Total COD	mg·L ⁻¹	19	621± 146
Soluble COD	mg·L ⁻¹	18	226± 79
Biochemical Oxygen Demand (BOD ₅)	mg·L ⁻¹	18	243 ± 59
Total Solids (TS)	mg·L ⁻¹	16	1532 ± 282
Volatile Solids (VS)	mg·L ⁻¹	16	467 ± 162
Helminth eggs	mg·L ⁻¹	17	194 ± 79
Total coliform	CFU·100mL ⁻¹	29	5.3E+10 ± 5.5E+10
Faecal coliform	CFU·100mL ⁻¹	29	2.5E+10 ± 3.17E+10
<i>E. coli</i>	CFU·100mL ⁻¹	29	3.4E+10 ± 4.0 E+10

¹n: number of analysed grab samples during the research period

4.2.2 The UASB reactor

A 29 L acrylic lab-scale UASB reactor was used. The reactor height was 1.65 m and diameter 0.15 m. The research was performed from 26th December 2012 to 7th July 2013.. The scheme of the reactor set up is shown in Figure 4.1.

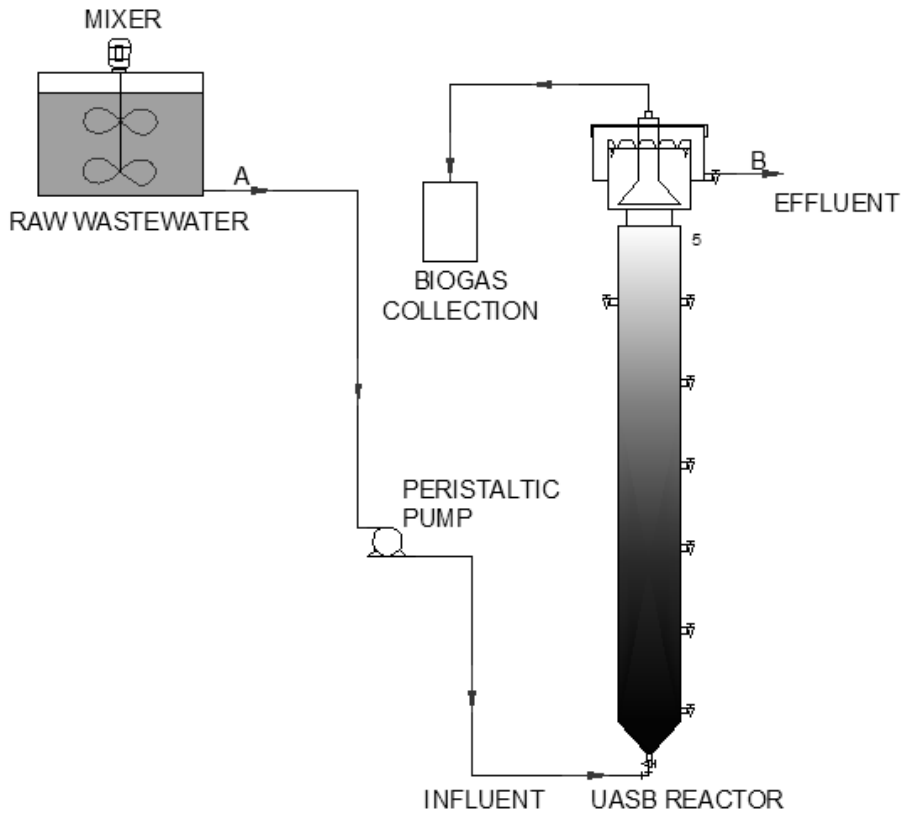


Figure 4.1 Schematic diagram for the set up of the filtration experiments in the UASB reactor at low temperatures. A and B indicate the

4.2.3 Inoculum

The inoculum consisted of 6L of anaerobic flocculent sludge sampled from a 536 m³ pilot-scale UASB reactor located at CITRAR (Yaya-Beas et al., 2015). The inoculum was sampled at a height of 1.5 m from the bottom of that reactor (total height and average upflow velocity of the reactor were 6.0 m and 1 m·h⁻¹ correspondingly). The total solids and volatile solids concentration of the inoculum were 163 ± 37 and 106 ± 44 g·L⁻¹, respectively.

4.2.4 Physicochemical and bacteriological analysis

Suspended solids, total solids, volatile solids, oil and grease analysis were performed according to Standard Methods (APHA et al., 1998). The pH, temperature and faecal coliforms determinations were performed according to Standard Methods (Eaton et al., 2005). Soluble COD was determined in a filtered sample through a membrane filter type Millipore and pore size of 0.45 μm . Chemical oxygen demand (COD) was measured according to Hach method 8000 (HACH, 2008) following the reactor digestion method using COD digestion vials high range (20–1500 $\text{mg}\cdot\text{L}^{-1}$) and low range (0–150 $\text{mg}\cdot\text{L}^{-1}$), digester reactor DRB 200 and a DR 890 Hach Colorimeter. Dissolved oxygen measurement was performed according to Hach method 10360 (HACH, 2008). Total coliforms and *E. coli* were performed according to Membrane Filtration Method using m-ColiBlue24 Broth PurRite Ampoules (HACH, 2008). Faecal coliforms were performed according to Membrane Filtration Method using m-FC with Rosolic Acid Broth ampoules (HACH, 2008). The produced colonies by total coliforms, *E. coli* and faecal coliforms were counted and reported in colony forming units per 100 ml ($\text{CFU}\cdot 100\text{mL}^{-1}$) of wastewater sample, having a level of detection of 1 $\text{CFU}\cdot 100\text{mL}^{-1}$. Helminth egg counting was performed according to the same methodology described by Yaya-Beas *et al.* (Yaya-Beas et al., 2015). Helminth eggs were identified using a combination of the keys given by USF (2005) and by Thienpont (1979). Microscopic observations were performed with an optical microscope ZEISS Primo Star. Biogas was continuously collected in 5 litres gas bag. Methane in the collected biogas was determined by gas displacement in Mariotte flasks, using a 16 % NaOH solution to remove the CO_2 at ambient temperatures which varied between 8.4 and 11.5 $^{\circ}\text{C}$.

4.2.5 UASB operational conditions

After the inoculation, the UASB reactor started up with the domestic wastewater from Puno as the influent at an upflow velocity of 0.41 $\text{m}\cdot\text{h}^{-1}$. The research on the effect of various upflow velocities on the filtration capacity of the anaerobic sludge bed was initiated after 41 days, when a stable turbidity removal was reached of 83 ± 5 %.

a. Set up of experiments

The research was performed during 152 days (22 weeks). Six upflow velocities, i.e. 0.12, 0.14, 0.16, 0.20, 0.27 and 0.41 $\text{m}\cdot\text{h}^{-1}$ were applied. The number of weeks applied for each upflow velocity and number of analysis are given in Table 4.2. Grab samples were collected for each upflow velocity and measurements of all replicates were identical.

Table 4.2 Set up of upflow velocity tests and number of analysis and measurements performed for each week of the research in the lab-scale UASB reactor. A total number of 3 analyses/day were performed for turbidity and pH while 5 analyses/day for temperature.

*W	Upflow Velocity ($\text{m}\cdot\text{h}^{-1}$)	Hydraulic retention time (h)	Total coliforms	Faecal coliforms	<i>E. coli</i>	Helminth eggs	Total COD
1	0.12	14.2	1	1	1	0	1
2	0.12	14.2	0	0	0	0	0
3	0.27	6.0	1	1	1	1	1
4	0.27	6.0	0	0	0	0	0
5	0.27	6.0	1	1	1	0	1
6	0.20	8.1	1	1	1	1	1
7	0.20	8.1	1	1	1	0	0
8	0.20	8.1	1	1	1	1	1
9	0.20	8.1	2	2	2	1	1
10-11	0.16	10.1	2	2	2	1	1
12-13	0.14	12.1	2	2	2	1	1
14-15	0.12	14.2	1	1	1	1	1
16	0.20	8.1	1	1	1	1	1
17	0.20	8.1	2	2	2	1	1
18-19	0.27	6.0	2	2	2	1	1
20	0.41	4.0	2	2	2	1	1
21	0.41	4.0	1	1	1	1	1
22	0.12	14.2	1	1	1	1	1
Total			29	29	29	17	19

*W = week number.

The samples were taken after an elapsed time equivalent of one HRT, after introducing a new batch of wastewater in the influent tank. Samples from influent and effluent were collected in glass bottles and analytical determinations were performed immediately.

Depending on the type of analysis, intervals during sample collection varied over the whole sampling period, ranging from 0.5 hours to 3 hours. In order to guarantee a steady state, sampling regarding, helminth eggs and total COD were performed in the seventh day after applying a specific upflow velocity. Regarding total and faecal coliforms and *E. coli*, samples were taken on the third day, after applying a specific upflow velocity.

Influent and effluent temperature were measured daily five times (9:00, 11:00, 13:00; 15:00 and 17:00 hours), ambient temperature was measured hourly. Turbidity and pH of influent and effluent were measured three times a day (9:00, 12:00 and 17:00 hours). Methane production for all upflow velocities except for $0.16 \text{ m}\cdot\text{h}^{-1}$ was measured once a day. Due to technical failure no data for biogas production is available for an upflow velocity of $0.16 \text{ m}\cdot\text{h}^{-1}$.

4.2.6 Calculations

The HRT at a predetermined SRT is calculated using the formula given by Zeeman & Lettinga (1999):

$$\text{HRT} = \left[\frac{24 * C * SS * R * (1 - H)}{X} \right] * \text{SRT} \quad (\text{eq. 4.1})$$

where C is the COD concentration in the influent ($\text{COD}_{\text{total}}$, in $\text{kgCOD} \cdot \text{m}^{-3}$), SS is the fraction of suspended solids in the influent ($\text{COD}_{\text{ss}}/\text{COD}_{\text{total}}$), X is the sludge concentration in the reactor (in $\text{kgCOD} \cdot \text{m}^{-3}$), R is the fraction of COD_{ss} removed and H is the level of hydrolysis of the removed solids. Values of C and SS are determined from influent conditions given in Table 4.1.

4.3 Results and discussion

Total helminth egg removal varied between 89 ± 11 and $95 \pm 3\%$ for different upflow velocities (see Table 4.3). The average wastewater temperature varied between 11 and 14 °C during the research period. The wastewater during the research period was characterised as a medium strength domestic wastewater in terms of COD. Concerning pathogens' content in terms of helminth eggs, faecal coliforms and *E. coli* the domestic wastewater of Puno is considered very concentrated compared to results reported in literature (Jimenez et al., 2001; Elmitwalli et al., 2002; Tawfik et al., 2006b). The observed high helminth egg content might be related to the previous reported very poor sanitation in Puno (Northcote et al., 1991; Maco et al., 2001; Marcos et al., 2003).

A summary of obtained results of the experiments for treating domestic wastewater in a UASB reactor at different upflow velocities and low temperature, is presented in Table 4.3. The Table 4.3 presents influent end effluent characteristics and removal efficiencies for helminth eggs, total coliforms, faecal coliforms, *E. coli*, total COD, BOD5 and turbidity.

Table 4.3 Helminth eggs, total coliforms, faecal coliforms, *E. coli*, total COD, BOD and turbidity in influent and effluent when treating domestic wastewater from Puno (Peru) in a UASB at low temperature and different upflow velocities.

Upflow velocity	m·h ⁻¹	0.12	0.14	0.16	0.20	0.27	0.41
Flow	L·d ⁻¹	49.0	57.6	69.1	86.4	115.2	172.8
Ambient temperature	°C	10 ± 0.6	10.7 ± 1	10.4 ± 0.2	11.5 ± 0.5	7.5 ± 1	8.4 ± 1.1
Wastewater temperature	°C	12.3 ± 1.6	12 ± 1.8	12.3 ± 2.1	14.3 ± 2	11.3 ± 0.9	12.3 ± 2.5
Total Coliforms							
n		3	4	4	8	6	4
Influent	CFU·100mL ⁻¹	5.6E+10 ± 6E+10	1.6E+10 ± 0.8E+10	9.4E+10 ± 7.8E+10	5.7E+10 ± 5E+10	6.9E+10 ± 5.8E+10	2.7E+10 ± 2.8E+10
Effluent	CFU·100mL ⁻¹	0.8E+09 ± 0.7E+09	1.1E+09 ± 1.2E+09	2.4E+09 ± 2.2E+09	3.2E+09 ± 2.3E+09	7E+09 ± 7.1E+09	3.5E+09 ± 1.7E+09
Efficiency	%	91 ± 13	95 ± 5	95 ± 5	91 ± 8	89 ± 8	66 ± 41
Faecal Coliforms							
n		3	4	4	8	6	4
Influent	CFU·100mL ⁻¹	4E+10 ± 6.1E+10	6E+09 ± 4.6E+09	4.9E+10 ± 4.6E+10	3.1E+10 ± 2.4E+10	1.7E+10 ± 2.3E+10	0.9E+10 ± 1.1E+10
Effluent	CFU·100mL ⁻¹	2.8E+08 ± 1.9E+08	5.5E+07 ± 4.8E+07	5.1E+08 ± 2.8E+08	6.7E+08 ± 4.9E+08	6.6E+08 ± 2.3E+08	5.9E+08 ± 2.9E+08
Efficiency	%	76 ± 37	99 ± 0	96 ± 7	88 ± 28	82 ± 27	85 ± 10
<i>E. coli</i>							
n		3	4	4	8	6	4
Influent	CFU·100mL ⁻¹	4E+10 ± 4.3E+10	1E+10 ± 0.4E+10	6.4E+10 ± 5.5E+10	3E+10 ± 4E+10	4.2E+10 ± 4.8E+10	1.7E+10 ± 1.8E+10
Effluent	CFU·100mL ⁻¹	5.5E+08 ± 4.7E+08	9.3E+08 ± 6.6E+08	1.4E+09 ± 1.2E+09	1.3E+09 ± 0.9E+09	4.8E+09 ± 6.4E+09	2.3E+09 ± 1.7E+09
Efficiency	%	91 ± 12	89 ± 9	95 ± 5	83 ± 19	85 ± 13	81 ± 9

continuation of Table 4.3.

Helminth eggs							
n		2	2	2	5	3	2
Influent	egg·L ⁻¹	113 ± 37	256 ± 16	222 ± 18	166 ± 92	244 ± 105	181 ± 48
Effluent	egg·L ⁻¹	5 ± 1	18 ± 6	15 ± 4	21 ± 26	35 ± 44	9 ± 0
Efficiency	%	95 ± 3	93 ± 2	94 ± 1	89 ± 8	89 ± 11	95 ± 1
Total COD							
n		3	2	2	5	4	2
Influent	mg·L ⁻¹	554 ± 84	710 ± 98	606 ± 49	610 ± 149	597 ± 246	726 ± 111
Effluent	mg·L ⁻¹	211 ± 70	354 ± 105	294 ± 17	309 ± 52	287 ± 121	457 ± 34
Efficiency	%	62 ± 8	51 ± 8	51 ± 7	48 ± 6	52 ± 6	37 ± 5
BOD ₅							
n		3	2	2	5	4	2
Influent	mg·L ⁻¹	211 ± 59	255 ± 4	213 ± 18	242 ± 76	259 ± 77	276 ± 43
Effluent	mg·L ⁻¹	86 ± 25	114 ± 6	112 ± 1	113 ± 49	141 ± 62	231 ± 27
Efficiency	%	55 ± 26	55 ± 3	47 ± 5	53 ± 17	47 ± 13	16 ± 3
Turbidity							
n		105	42	42	126	105	42
Influent	NTU	379 ± 31	345 ± 152	309 ± 73	356 ± 171	406 ± 185	408 ± 48
Effluent	NTU	52 ± 26	56 ± 21	78 ± 20	53 ± 23	66 ± 28	123 ± 25
Efficiency	%	87 ± 6	77 ± 24	74 ± 9	84 ± 5	81 ± 10	69 ± 9

Notes:

- The upflow velocities of 0.12, 0.14, 0.16, 0.20, 0.27 and 0.41 m·h⁻¹ correspond to an HRT of 14.2, 12.1, 10.1, 8.1 6.0 and 4.0 hours respectively

4.3.1 Helminth eggs content in the wastewater

Results for the whole research period showed a very high average total helminth egg concentration in the wastewater, reaching $194 \pm 79 \text{ egg}\cdot\text{L}^{-1}$ (Figure 4.2). These results agree with previous research in Peru, Colombia and Brazil (García Palacio, 2010; Navarro and Jiménez, 2011). The presence of helminth eggs in the studied wastewater, evidence the high health risks for getting intestinal parasitism in the current exposed population to direct contact with the wastewater.

The helminth egg content for each applied upflow velocity is shown in Figure 4.3. Results show the predominance of four species of helminth eggs in the sewage influent: *Ascaris lumbricoides*, *Toxocara spp.*, *Hymenolepis nana* and *Enterobius vermicularis*. *Ascaris lumbricoides* was the most common helminth egg present in the influent studied wastewater with an average concentration of $142 \pm 106 \text{ egg}\cdot\text{L}^{-1}$ during the whole research. This helminth egg specie was also the most predominant in domestic wastewater from Lima (Yaya-Beas et al., Unpublished results) *Ascaris lumbricoides* frequently has been reported as the most common helminth egg in domestic wastewater in Mexico, Morocco and Colombia (Cifuentes et al., 1999; Habbari et al., 2000; Blumenthal et al., 2001; Bethony et al., 2006; García Palacio, 2010)

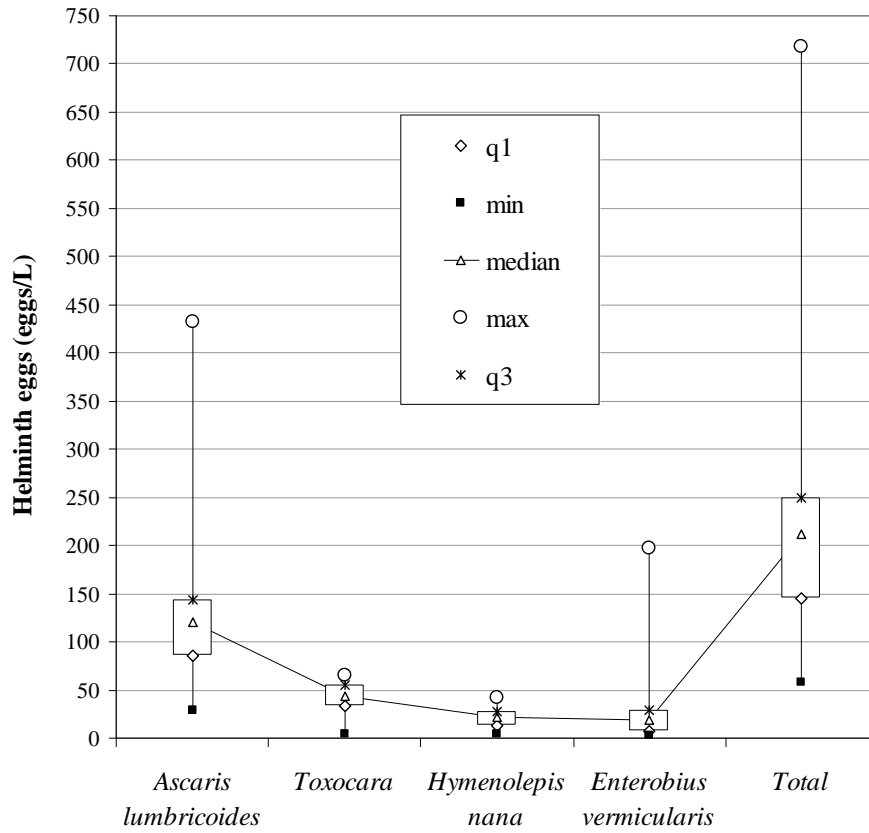


Figure 4.2 Box plot of helminth egg content in the influent during the research.

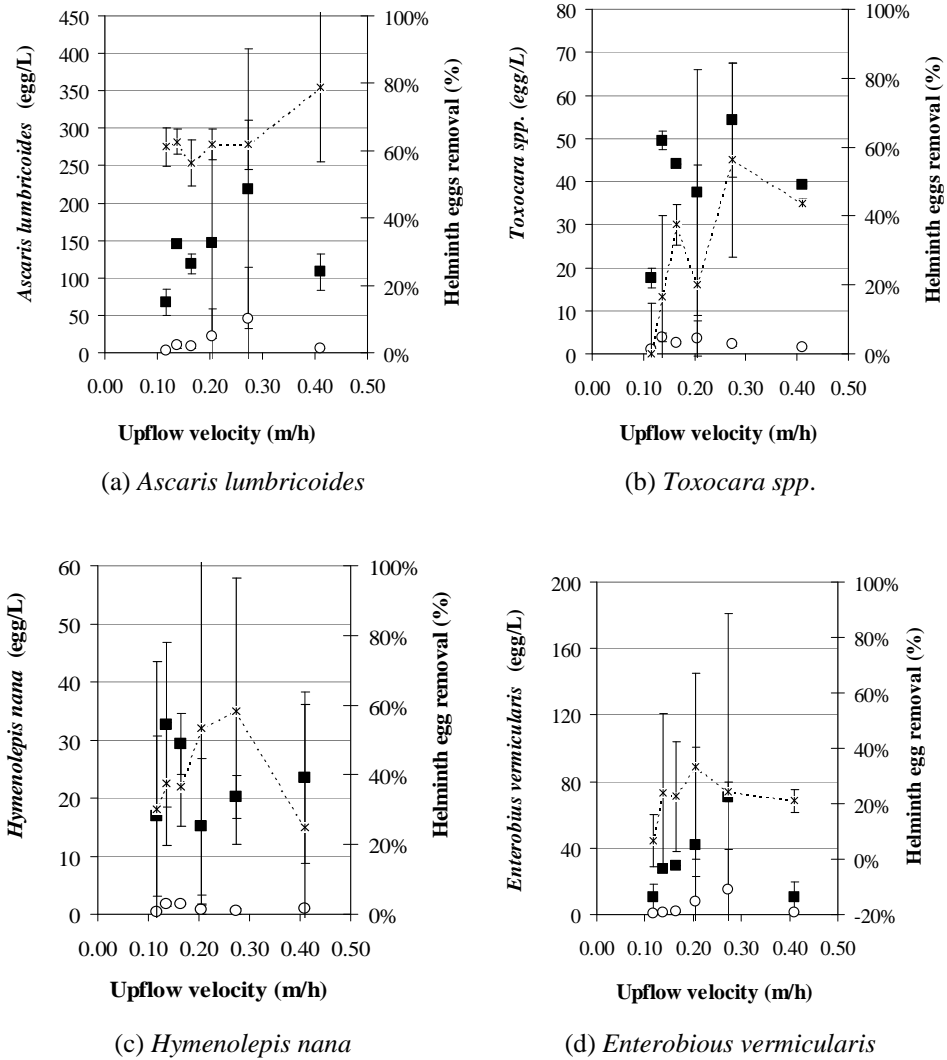


Figure 4.3 Helminth eggs content in the influent (■) and effluent (○) and helminth eggs removal efficiencies (*) of a lab scale UASB reactor at low temperatures and different upflow velocities: a) *Ascaris lumbricoides*, b) *Toxocara spp.*, c) *Hymenolepis nana*,; and d) *Enterobius vermicularis*.

4.3.2 Filtration capacity of anaerobic sludge for pathogens

The current research showed an average helminth egg removal efficiency between 89 and 95%. However, the average helminth egg concentration in the effluent varies between 5 and 35 egg/L. Although, the observed reduction can be considered high, the final effluent values are also very high, if compared to the low infective dose of 1 egg per person (WHO, 2006). Likewise, the survival time of helminth eggs at ambient conditions reported in the literature is very high (WHO, 1989; WHO, 2006). The longest survival times for *Ascaris lumbricoides*, *Toxocara*, *Hymenolepis nana* and *Enterobius vermicularis* are 3 years (Strauss, 1996), 2 years (Dunsmore et al., 1984; Gillespie et al., 1991; Mizgajska, 2001), 7 months (O'Donnell et al., 1984) and 70 hours (Grice and Procriv, 1993) respectively. For the indicated survival time, the corresponding temperature ranges are [10, 30 °C], [-40, 37 °C], [-4, 25 °C] and [-10, 40 °C] (Dunsmore et al., 1984; O'Donnell et al., 1984; Gillespie et al., 1991; Grice and Procriv, 1993; Strauss, 1996; Mizgajska, 2001).

The observed helminth egg removal under low temperature conditions was distinctly higher than those reported in previous research conducted under subtropical conditions in Peru (Yaya-Beas et al., 2015) and Brazil (Chernicharo, 2006). Possibly, the observed high helminth egg removal is related to the applied low upflow velocities compared to the full scale reactors in Brazil (Chernicharo, 2006), as well as the lower biogas production at low temperatures (Lettinga et al., 2001), which limits the degree of turbulence in the reactor. No significant difference in helminth egg removal efficiency was found between the applied upflow velocities and during their fluctuations. Additionally, no relation between helminth egg removal and turbidity removal was found. It could be associated to the fact that turbidity measured all suspended solids present in the wastewater and helminth eggs are the smallest fraction of these suspended solids (Mahmoud et al., 2003; von Sperling et al., 2005; Mahmoud et al., 2006).

Likely, due to large standard deviations related to non-laminar flow conditions, it was not possible to establish a statistical correlation between the removal of each specie of helminth egg and the upflow velocity. However, it can be observed that average removal efficiency of *Ascaris lumbricoides* was higher than the other species. The latter efficiency removal could be related to the higher size of *Ascaris lumbricoides*, which is 40–80 × 25–50 µm, thus having a higher settling velocity or entrapment potential compared to other species (Ayres and Mara, 1996). The size for *Enterobius vermicularis* is 50–65 length x 20–30 width µm while the diameter of *hymenolepis nana* and *Toxocara spp.* is respectively 30–60 and 85–85 µm (USF, 2005; Jimenez, 2007).

The collected methane varied between 16 ± 1 and 34 ± 3 NL/kg COD removed, which was very low compared to the 90 NL/kg COD removed reported at 15 °C by Mahmoud (2002). The estimated amount of dissolved methane in the effluent as percentage of the amount of total produced methane varied between 18–54%, using the methane solubility

in water of 33–36 mL/L at the measured effluent temperatures, a CH₄ partial pressure of 80% and the applied effluent flow, belonging to the respective HRTs.

Average of faecal coliforms and *E. coli* content in the raw wastewater are 2.5 E+10 and 3.4E+10 CFU·100mL⁻¹ respectively. These values are between 1 and 4 log₁₀ higher compared to other researches (Jimenez et al., 2001; Elmitwalli et al., 2002; Jiménez Cisneros et al., 2002; Tawfik et al., 2006b). Similar values has been reported in Mexican and Latin American's wastewaters and were associated to the existing poor sanitation conditions and low water use (Saéñz-Forero, 1999; Hernández-Acosta et al., 2014).

Unlike the strong variation in the influent content (see Table 4.3), removal efficiencies of faecal coliforms and *E. coli* were very similar. The average faecal coliforms removal varied between 76 and 99% (equivalent to 0.94 and 2.09 Log₁₀) (Figure 4.4). Chernicharo (2006) and Seghezzi (2004) reported similar results in previous research, working with UASB reactors under tropical and subtropical conditions. Average *E. coli* removal varied between 81 and 95% (equivalent to 0.77 and 1.64 Log₁₀). There seems to be a slight reciprocal correlation between the faecal coliforms and *E. coli* removal and applied upflow velocities, however R² is only 0.6.

Low temperatures has been regarded as favourable for bacterial survival, possibly as a result of low decay rates and reduced predation by protozoa and bacterial predators (Cools et al., 2001; Zhang et al., 2012). As the effluent still contains helminth eggs and the lowest concentration of pathogenic indicator is still very high, i.e. 5.5E+07 ± 4.8E+07 CFU·100mL⁻¹ for faecal coliforms (see Table 4.3), a post treatment unit will be required to meet the WHO guidelines (WHO, 1989; WHO, 2006) in the case of water reuse for irrigation.

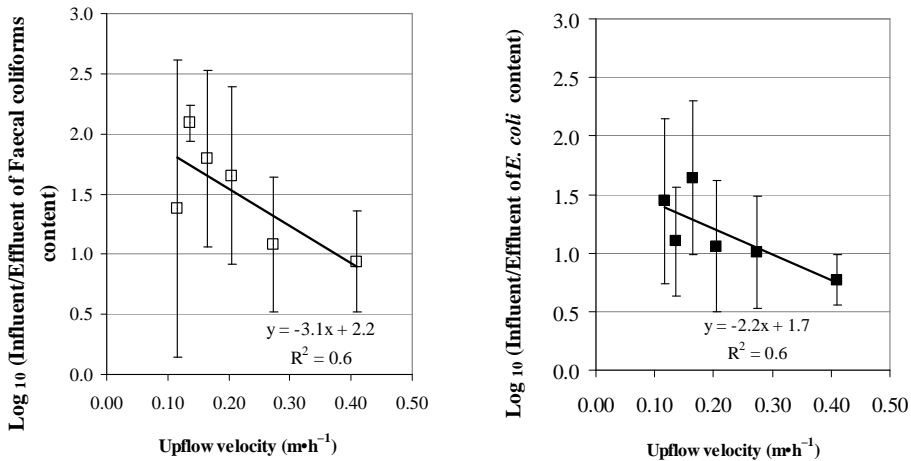


Figure 4.4 Log₁₀ of Faecal coliforms (□) and *E. coli* (■) removal in a UASB reactor at low temperatures at different applied upflow velocities.

4.3.3 Total coliform, COD and turbidity removal

Total coliform removal was always insignificant. It varied between $66 \pm 41\%$ and $95 \pm 5\%$ (equivalent to $0.67 \pm 0.46 \log_{10}$ and $1.57 \pm 0.57 \log_{10}$).

Total COD removal varied between 37 ± 5 and $62 \pm 8\%$. These results (Figure 4.5) are similar to the observed COD removal of 30–50% at 11–12 °C reported by Grin (1983). Elmitwalli (2007) observed a similar COD removal of 31% at 14–21 °C. Even though there is an apparent negative reciprocal trend between total COD removal and applied upflow velocities, due to the large standard deviations, the correlation is statistically not significant (R^2 of 0.35).

Turbidity removal varied between 69 ± 9 and $87 \pm 6\%$. Similar to COD, a negative slight relationship between the average turbidity removal and applied upflow velocities (Figure 4.5) is observed. Also, a low correlation coefficient R^2 of 0.72 was observed. The seemingly slight negative trend might be explained by an imposed increased hydraulic shear in the sludge bed at the time when the influent flow of the UASB reactor increased (Mahmoud et al., 2003). An increased upflow velocity will result in lifting settled particles from the sludge bed when settling velocities of smaller particles are exceeded. Consequently, captured solids are detached and the solids removal efficiency deteriorates.

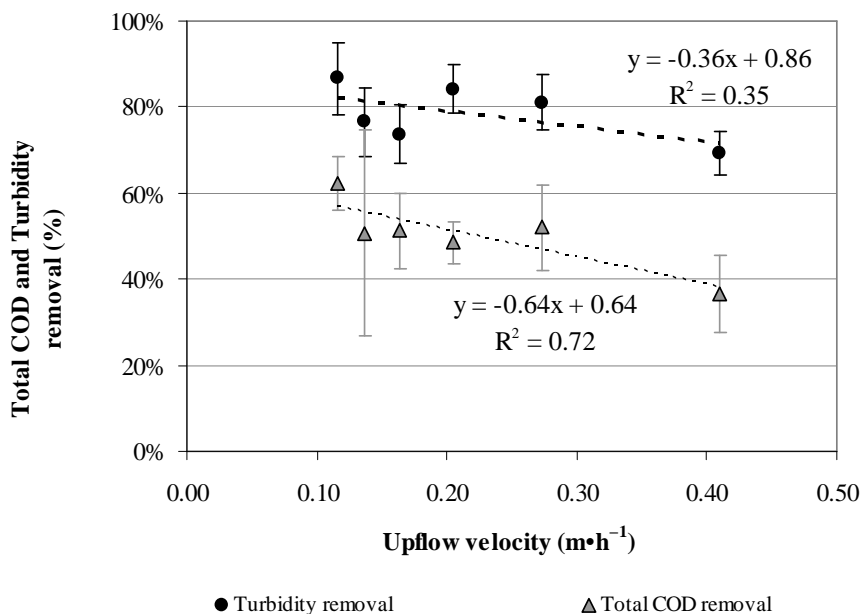


Figure 4.5 Total COD and turbidity removal in a UASB reactor at low temperatures at different applied upflow velocities.

4.3.4 The sludge filtration capacity for full scale conditions

The highest total helminth egg removal and highest COD removal was obtained at an upflow velocity of $0.12 \text{ m}\cdot\text{h}^{-1}$ (see Table 4.3). In order to allow hydrolysis and methanogenesis, the SRT in an anaerobic system should at least become 150 days for temperatures less than $15 \text{ }^\circ\text{C}$ (Zeeman, 1991; Zeeman and Lettinga, 1999; Lettinga et al., 2001). A theoretical HRT of 26.7 hours for an SRT of 150 days in a full scale reactor can be determined through eq.4.1. At such HRT, an upflow velocity of $0.23 \text{ m}\cdot\text{h}^{-1}$ is obtained for a total reactor height of 6 m as generally applied for full scale conditions. The latter calculation was performed considering one phase UASB reactor, X and H were 15 kgVSS/m^3 (Zeeman and Lettinga, 1999) and 53% (de Graaff, 2010) respectively. R was 84% which is the average turbidity removal at an upflow velocity of $0.20 \text{ m}\cdot\text{h}^{-1}$ (see Table 4.3). According to Table 4.3, an average helminth egg removal efficiency of $89 \pm 11\%$ can be expected at an upflow velocity of $0.20\text{--}0.27 \text{ m}\cdot\text{h}^{-1}$. Therefore, for the above mentioned full scale conditions, with an upflow velocity of $0.23 \text{ m}\cdot\text{h}^{-1}$, *ca.* 89% of helminth egg removal can be expected. Remaining helminth eggs have to be removed in a post-treatment step when agricultural irrigation is foreseen (WHO, 1989; WHO, 2006). For developing countries such post-treatment step could include high rate trickling filters, rotating biological contactor, anaerobic filters,

constructed wetlands, polishing ponds or overland flow systems (Seghezzo, 2004; von Sperling et al., 2005).

4.4 Conclusions

The average total helminth egg content in the influent wastewater varied between 166 and 256 egg·L⁻¹ where *Ascaris lumbricoides* was the prevailing helminth egg specie.

The sludge bed filtration capacity of a UASB operated at low temperatures varies between 89 ± 11 and 95 ± 1 % for helminth eggs. No significant difference is shown between upflow velocities of 0.12–0.41 m·h⁻¹.

The UASB effluent with a helminth egg content varying between 5 and 35 egg·L⁻¹ does not meet the WHO standards for reuse.

The total and faecal coliforms, and *E. coli* reduction is insignificant in a UASB operated at low temperatures and upflow velocities of 0.12–0.41 m·h⁻¹.

4.5 Acknowledgments

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CHAPTER 5

A Downflow Hanging Sponge (DHS) reactor for faecal coliform removal from an Upflow Anaerobic Sludge Blanket (UASB) effluent

Yaya Beas, R.E., Kujawa-Roeleveld, K., van Lier, J. B. and Zeeman, G.

A Downflow Hanging Sponge (DHS) reactor for faecal coliform removal from an Upflow Anaerobic Sludge Blanket (UASB) effluent

Abstract

This research was conducted to study the faecal coliforms removal capacity of Downflow Hanging Sponge (DHS) reactors as a post-treatment for an Upflow Anaerobic Sludge Blanket (UASB) reactor. Three long-term continuous lab-scale DHS reactors i.e. a reactor with cube type sponges without recirculation, a similar one with recirculation and a reactor with curtain type sponges. The porosities of the applied medium were 91, 87 and 47% respectively. The organic loading rates were 0.86, 0.53 and 0.24 kgCOD·m⁻³·d⁻¹ correspondingly at hydraulic loading rates of 1.92, 2.97 and 1.32 m³·m⁻²·d⁻¹, respectively. The corresponding averages for faecal coliform removal were 99.997, 99.919 and 92.121 % respectively. The WHO (1989) standards, in terms of faecal coliform content for unrestricted irrigation (Category A), was achieved with the effluent of the cube type DHS (G1) without recirculation. Restricted irrigation, category B and C is assigned to the effluent of the cube type with recirculation and the curtain type, respectively. Particularly for organic compounds, the effluent of evaluated DHS reactors complies with USEPA standards for irrigation of so called non-food crops like pasture for milking animals, fodder, fibre, and seed crops.

Keywords

reactor, domestic wastewater, faecal coliforms, UASB, BOD, COD

This chapter is based on

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5.1 Introduction

Proper concepts and technologies for attaining a sustainable, robust and socio-economically affordable protection of the environment need to be applied for wastewater treatment (Chernicharo et al., 2015). Use of treated wastewater, particularly in agriculture, is driven by the interest in increasing water availability and recycling nutrients in soils with poor fertility (van Lier and Huibers, 2010). Insufficiently treated wastewater for agricultural water reuse, may create human and environmental health risks especially when water reuse is becoming a more practised activity as a result of water scarcity (van Lier and Huibers, 2010). Health constraints become critical in developing countries, where helminth infections are endemic (WHO, 2006). Thus, the monitoring of waterborne pathogens indicators are crucial when treated wastewater is used for agricultural irrigation. Waterborne coliforms, which are generally detected in higher concentrations than pathogenic bacteria, are used as a critical indicator for the potential presence of entero-pathogens in water (von Sperling et al., 2005; Uemura and Harada, 2010).

The regulatory limits for the use of reclaimed wastewater for irrigation are best illustrated by the guidelines of the World Health Organization (WHO) and the United States Environmental Protection Agency (WHO, 1989; USEPA, 2004; WHO, 2006).

The WHO guideline 2006 does not provide limits for viral pathogens, bacterial, protozoan and organic matter (WHO, 2006), but contains sanitary measures for public health based on risk assessment. The WHO guideline 1989 considers the control of helminth eggs and faecal coliform content (WHO, 1989). It distinguishes three categories of water reuse viz., unrestricted (A), restricted (B) and restricted localised irrigation (C) (see Table 5.2). The USEPA standard, differentiates three types of agricultural reuse viz.: 1) Non commercially processed food crops (non-CPFC), 2) CPFC and 3) non-food crops (USEPA, 2004).

Standards for the two presented guidelines are given in Table 5.1 and Table 5.2. The WHO guideline 1989 has been applied in Ecuador, Argentina, Brazil and Peru (Jiménez and Asano, 2008). Moreover, most developing countries prefer to use the WHO guideline 1989 and USEPA because of their financial constraints to perform requested analysis and assessments in the new WHO (2006) guideline (Angelakis et al., 1999; Jiménez and Asano, 2008).

Table 5.1 WHO microbiological quality guidelines (1989) for water use in agriculture.

Category	Reuse conditions ^a	Exposed group	Helminth eggs indicator ^b (egg·L ⁻¹)	Faecal coliforms indicator ^c (in number per 100 ml)
A	Unrestricted irrigation: crops to be eaten uncooked, sport fields, public parks ^d	Workers, consumers, public	≤ 1	≤1000
B	Restricted irrigation. Cereal, industrial fodder crops, pasture or trees ^e	Workers		Not standard recommended
C	Restricted irrigation: Localised irrigation of crops in category B, if exposure of workers and the public does not occur.	None	Not applicable	

^a In specific cases, local, epidemiological, sociocultural and environmental factors should be taken into account and guidelines modified accordingly

^b Arithmetic mean for *Ascaris* and *Trichuris* species and hookworms

^c Geometric mean during the irrigation period

^d A more stringent guideline limit (≤ 200 faecal coliforms/100 mL) is appropriated for public lawns, with which the public may come into direct contact

^e In the case of fruits trees, irrigation should cease two weeks before fruits is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

Table 5.2 USEPA Standard (2004) for water use in agriculture.

Type	Agricultural reuse	Reclaimed water quality	
		Physicochemical indicators	Faecal coliform indicator (number per 100 ml) ^b
1	Not CPFC ^a : surface or spray irrigation of any food crop including crops eaten raw	pH = 6-9 BOD ₅ ≤ 10 mg·L ⁻¹ Turbidity ≤ 2 NTU Cl ₂ residual ≥ 1 mg·L ⁻¹	No detectable
2	CPFC: surface irrigation of orchards and vineyards	pH = 6-9 BOD ₅ ≤ 30 mg·L ⁻¹ TSS ≤ 30 mg·L ⁻¹ Cl ₂ residual ≥ 1 mg·L ⁻¹	≤ 200
3	Non-food crops: pasture for milking animals, fodder, fibre and seed crops		

^a Commercially processed food crops (CPFC) are those that, prior to sale to the public or others, have undergone chemical or physical processing sufficient to destroy pathogens.

^b Either the membrane filter or fermentation-tube technique may be used

Within wastewater treatment, anaerobic treatment offers advantages over other conventional processes, such as the activated sludge process for biochemical oxygen demand (BOD₅) removal. These advantages include lower energy consumption, lower excess sludge production and simple operation and maintenance (van Lier et al., 2010; Chernicharo et al., 2015). Among the anaerobic reactors, the UASB reactor has been found most suitable for domestic wastewater treatment because of its simplicity in construction and compactness. In addition, it neither requires mechanical mixing and effluent recirculation (von Sperling et al., 2005). UASB reactors alone are, however, not able to meet the wastewater reuse standards particularly when treated effluents are used for agricultural purposes (Chong et al., 2012; Chernicharo et al., 2015). The limitation of UASB reactors regarding the agricultural use of treated wastewater is expressed mainly by an insufficient or negligible faecal coliform removal (van Lier et al., 2010; Chernicharo et al., 2015). Furthermore, the helminth egg concentration usually exceed 1 egg·L⁻¹ in the effluent (von Sperling et al., 2005). Consequently, the effluent does not comply with the WHO and USEPA guidelines for the use of treated wastewater in irrigated agriculture with exposure to workers and public.

In order to polish the UASB reactors effluent, several low-cost aerobic technologies based on suspended or attached growth systems without power consumption for aeration, are proposed in literature (Agrawal et al., 1997; Machdar et al., 1997; Tandukar et al., 2005; von Sperling et al., 2005). The proposed technologies are the downflow hanging sponge (DHS) reactor, conventional trickling filter, subsurface flow constructed wetlands, rotating biological contactors, and polishing ponds.

The combined UASB-DHS reactor can remove faecal coliforms between 79.0 % and 99.98 % (Tandukar et al., 2005; Tawfik et al., 2006a; Tandukar et al., 2007; Uemura and Harada, 2010; Onodera et al., 2014). DHS reactors are characterised by little material and energy requirement, whereas systems are very compact, having an HRT less than 3 hours (Agrawal et al., 1997; Mahmoud et al., 2011). Another advantage is that the DHS reactor only requires little maintenance, since clogging of filter media does not occur. The latter is attributed to the prevailing hydraulic shear stress that dislodges parts of the attached material when growth reaches a saturated level (von Sperling et al., 2005). Subsequently, the excess sludge should be removed by sedimentation.

Within the DHS reactor, the influent percolates down through the sponge medium. During passage, the water gets almost saturated with oxygen without the need of mechanical aeration. Originally the DHS reactor was constructed by using cube shaped polyurethane foam sponges that hang freely in the air (Machdar et al., 2000). Due to its high porosity, polyurethane sponges could retain significantly more biomass in a DHS reactor compared to the biomass hold-up in a traditional trickling filter system. The retained biomass in the DHS consists of a wide range of microbial organisms, whose composition depends on wastewater characteristics and environmental conditions

(Mahmoud et al., 2011). The active immobilized biomass consumes organic compounds and nutrients from the wastewater for their metabolism utilising the dissolved oxygen (Mahmoud et al., 2011). The most important features of DHS reactors are the natural aeration by convective flows only, the short hydraulic retention time (HRT), and the very long sludge retention time (SRT) (Tawfik et al., 2010). The mechanisms for faecal coliforms and pathogenic bacteria reduction in the DHS reactors are different than those for organics removal; adsorption to biomass and/or predation by higher organisms might play a role (Tawfik et al., 2006a).

The DHS reactor was developed in six different configurations, named generations (G1-G6), to test the practical applicability. The generations differ in orientation and distribution of the sponges inside the DHS reactor and, therefore, in practical applicability and dead zone volume.

Results of previous research (Tandukar et al., 2005; Tawfik et al., 2006b; Tandukar et al., 2007) demonstrated that DHS reactors type G3, G4, G5 and G6 remove between 79.0 and 99.7 % faecal coliforms at an HRT between 2 and 2.7 h. The capacity to remove faecal coliforms of DHS reactor type G1 and G2 was not studied. The G1 and G2 type has however shown their simplicity in terms of construction (Agrawal et al., 1997; Machdar et al., 1997; Machdar et al., 2000). Basically, investment, operation and maintenance costs and simplicity are the most important criteria when selecting a technology in developing countries (von Sperling et al., 2005). Therefore, the aim of the present research was to define the capacity of DHS reactors (G1 and G2) for removing faecal coliforms from domestic UASB reactor effluent for agricultural reuse in developing countries.

5.2 Materials and methods

5.2.1 Influent wastewater

The research was carried out using wastewater from two urban villages called El Angel and El Milagro located in Lima (Peru). This wastewater was fed into a pilot plant located at the Research Centre for Wastewater Treatment and Hazardous Wastes (CITRAR) at the campus of the National University of Engineering (Lima, Peru). The effluent from a 536 m³ pilot-scale UASB reactor located at CITRAR was used as influent wastewater for the constructed DHS reactors.. The main characteristics of the wastewater fed into the DHS reactors are shown in Table 5.3:

Table 5.3 Effluent wastewater characteristics of the 536 m³ UASB reactor located at CITRAR

Parameter	Units	Average	n ¹
Total coliforms	MPN·100mL ⁻¹	2.6 E+08 ± 2.9 E+08	10
Faecal coliforms	CFU·100mL ⁻¹	3.4 E+07 ± 9.8 E+07	45
Dissolved oxygen (DO)	mg·L ⁻¹	0.7 ± 0.7	160
Biochemical oxygen demand (BOD ₅)	mg·L ⁻¹	102 ± 44.2	42
Total chemical oxygen demand (Total COD)	mg·L ⁻¹	227.1 ± 103.1	67
Soluble chemical oxygen demand (soluble COD)	mg·L ⁻¹	128.9 ± 52.9	40
Turbidity	NTU	133.1 ± 82.2	638
Temperature	°C	20.9 ± 5.1	640
pH		7.4 ± 1.2	636

¹n: number of grab analysed samples

The wastewater was daily pumped into three 200 L independently stirred tanks (18 rpm) and from there, transported by gravity to each DHS reactor.

5.2.2 DHS Reactors

Two types of DHS reactors were applied, viz. the cube type without (G1) and with recirculation (G1) and the curtain type (G2). Different types of polyurethane sponges were used as biomass carrier media and the densities of the sponges were 18, 12 and 20 Kg·m⁻³, according to manufacturer specifications, respectively for each reactor. The sponge porosities were determined according to the water saturation method performed by Chen *et al.* (2004) with the difference that a volume of sponge (Vol) was immersed in a known volume of distilled water (V) under vacuum for 5 hours. The saturated sponge was removed, and then the remaining volume was measured (V-Vv). The porosity (n) was calculated by dividing the volume of pores (Vv) by the corresponding volume of the sponge (Vol). The measurement was repeated five times. The sponges were cut in small pieces as will be described for each type of reactor.

Three experiments were executed. For experiment 1, two identical cube type (G1) DHS systems were constructed and operated in parallel; each one was composed by two modules in series (01 and 02) as shown in Figure 5.1. Each module was made of acrylic with a total height of 0.29 m and a diameter of 0.09m. Each module contained five columns of cube type sponges. Each column comprised six sponge cubes (Figure 5.1).

For experiment 2, one cube type (G1) DHS reactor, composed of two modules in series (01 and 02) was built as is indicated in Figure 5.1. Each module was made of glass with a total height of 0.55 m and a diameter of 0.115 m. Each module contained 12 columns of cube type sponges and each column comprised 12 sponge cubes. The side of each

cube, and distance between each sponge cube were respectively, 0.030 and 0.005 m for experiment 1, while for experiment 2 these measurements were correspondingly 0.025 and 0.005 m. In order to allow natural aeration, the distances between module 01 and 02 were 0.10 and 0.15 m respectively, for experiment 1 and 2. DHS reactors were operated from 6th July 2009 to 30th June 2010 for experiment 1, and from 1st July 2011 to 2nd March 2012 for experiment 2.

For experiment 3 one curtain type (G2) DHS reactor, composed of 2 modules in series (01 and 02) was built as indicated in Figure 5.1. Each module consisted of 1 acrylic vessel containing 10 sponge rectangular parallelepipeds (sponge-columns) whose sides were 0.50, 0.050 and 0.038 m for height, length and width, respectively. Each vessel had a total length of 0.59 m and a height of 0.74 m. The width of the vessel was 0.09 m. The horizontal distance between each sponge strip inside each vessel was 0.002 m. A funnel was placed at the end of each sponge, to allow proper conduction of the effluent from module 01 to module 02. In order to allow natural aeration, the vertical separation between module 01 and 02 was 0.010 m. No recirculation was applied in this experiment. The DHS reactor in experiment 3 was operated from 2nd April 2011 to 30th October 2011. In order to retain possibly produced sludge, in all experiments, a settler was included after the DHS reactor. The settler was cleaned every week. The settler's volume was 0.5, 2.6 and 3.6 L for experiments 1, 2 and 3 respectively.

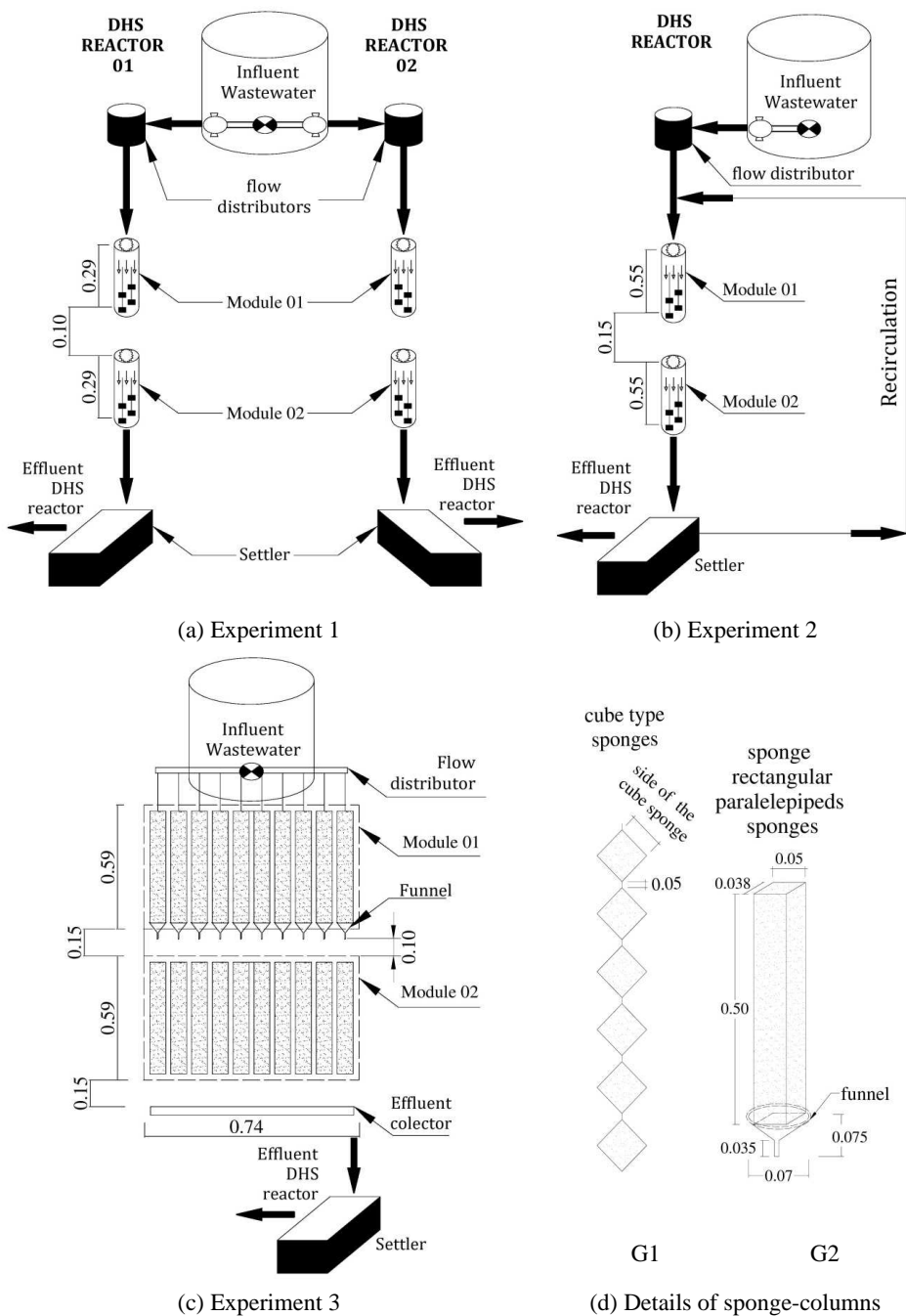


Figure 5.1 Set up of experiments in the lab-scale DHS reactors as a post-treatment for a UASB reactor effluent with special emphasis on faecal coliform removal: experiment 1 using two cube type DHS (G1) reactors (a), experiment 2 using one cube type DHS reactor (G1) with recirculation (b) experiment 3 using one lab scale curtain type DHS (G2) reactor.

5.2.3 Operational conditions

Feeding of DHS reactors was obtained by using equal distribution of influent wastewater over the sponges via the influent inlet (Figure 5.1). Flow distributors were calibrated three times a day in all experiments in order to guarantee the established flow indicated in Table 5.5. The flow distributors were replaced by new and clean ones every two weeks. No inoculation was applied in any of the reactors.

The influent flows were $12.2 \text{ L}\cdot\text{d}^{-1}$ for experiment 1, and $30.9 \text{ L}\cdot\text{d}^{-1}$ for experiment 2. Only for experiment 2, a recirculation was introduced from the settler back to the first module. Recirculation of settled wastewater was applied in order to guarantee i) an homogeneous hydraulic load ii) an increase of dissolved oxygen in the influent through the contact of the effluent, and iii) probably less dead zones than reactors without recirculation (von Sperling et al., 2005). The recirculation (R) flow of $30.9 \text{ L}\cdot\text{d}^{-1}$ was equal to the influent flow (Figure 5.1). The recirculation flow was calibrated three times a day. For experiment 3, the flow was $86.4 \text{ L}\cdot\text{d}^{-1}$ and ten pipes were installed in order to equally divide the wastewater over the whole sponge area.

The end of the start-up period was considered to be achieved when a stable turbidity content was reached, which was 30 ± 24 , 17 ± 8 and 19 ± 2 NTU for experiment 1, 2 and 3, respectively, in the last 10 weeks. In order to research the performance of the three DHS reactors, grab samples of 1L were taken from each experiment after 90, 70 and 57 days of the start-up of the reactors, respectively. The sampling frequency was determined based on the laboratory facilities and is indicated in Table 5.4.

Table 5.4 Frequency of sampling and performed analysis for the three experiments. The pH and temperature was daily analysed.

Parameter	Experiment 1	Experiment 2	Experiment 3
DO	twice a week		daily
Turbidity	daily		once a week
BOD ₅	each three weeks	each four weeks	
COD total		each two weeks	
COD soluble			
Faecal coliforms			

Experiment 1, 2 and 3 were performed during 269, 175 and 154 days respectively after the end of the start-up period. The experimental duration was influenced by the availability of laboratory facilities. Faecal coliform content was selected as main microbiological quality indicator. BOD₅ and COD were selected as physicochemical quality indicators for organic compounds.

The effluent quality in terms of faecal coliform content of the evaluated reactors was then compared with the WHO (1989) standards. Regarding BOD₅ content, turbidity and pH, the effluent quality was compared to USEPA standards since the WHO guideline 1989 does not include these parameters. A summary of the main operational conditions for each DHS experiment is given in Table 5.5.

Table 5.5 Main operating characteristics of the DHS reactors.

Experiment¹	Units	1	2	3
<u>Reactors</u>				
DHS type		cubes	cubes with recirculation	curtain
Generation		G1	G1	G2
Flow	L·d ⁻¹	12.2	30.9	86.4
R ²		0	1	0
Surface area ³	m ²	0.0064	0.0104	0.0657
DHS Volume ⁴	m ³	0.0037	0.0114	0.0775
HRT ⁵	h	2.90	1.52	2.49
HLR ⁶	m ³ ·m ⁻² ·d ⁻¹	1.92	2.97	1.32
OLR ⁷	kgCOD·m ⁻³ ·d ⁻¹	0.86	0.53	0.24
<u>Settler</u>				
HRT	h	1	2	1
Volume	m ³	0.0005	0.0026	0.0036
<u>Data of the medium</u>				
Total volume of the medium	m ³	0.0016	0.0023	0.0191
OLRm ⁸	kgCOD·m ⁻³ ·d ⁻¹	1.96	2.69	0.97

Notes:

¹ Experiment: number of the Experiment.

² R: recirculation factor expressed as the relation Q_r/Q_i. Where Q_r and Q_i are the recirculation and influent flow respectively.

³ Surface area of the reactor: it corresponds to the surface area of module 01 and is also equal to the cross sectional area of the acrylic modules.

⁴ DHS Volume: DHS reactor volume which corresponds to the volume of module 01 plus module 02 excluding the separation between modules.

⁵ HRT: hydraulic retention time of the reactor which implies the HRT of module 01 plus module 2. Both modules have the same HRT.

⁶ HLR: hydraulic surface loading rate of the reactor, based on the flow rate over the surface area of the reactor.

⁷ OLR: organic loading rate, based on the average COD mass flow over DHS volume.

⁸ OLRm: Medium organic loading rate, based on the flow rate, average COD and total volume of the medium.

5.2.4 Physicochemical and bacteriological analysis

Total and faecal coliforms, total chemical oxygen demand (COD), biochemical oxygen demand (BOD₅), dissolved oxygen (DO), analysis were determined following Standard Methods (Eaton et al., 2005). Faecal coliforms were measured by the membrane filtration technique using m-FC agar base as the medium. The agar was prepared in accordance with manufacturer specifications (Criterion, Hardy Diagnostics, Santa Maria, CA). The mixture of agar and appropriately diluted wastewater sample on the petri dishes was uniformly spread to avoid trapping air bubbles. The cultured petri dishes were inverted and incubated at 44.5 °C for 24 h. The produced colonies by faecal coliforms were then counted and reported in colony forming units per 100 ml (CFU·100 mL⁻¹) of wastewater sample, having a level of detection of 1 CFU·100mL⁻¹. Total COD was determined from unfiltered samples. Soluble COD was measured after filtering the sample through a 0.45 µm membrane filter, type Millipore. COD analysis was executed using high range Hach COD digestion vials high range (20-1500 mg·L⁻¹) and low range (0-150 mg·L⁻¹) as well as a digester reactor DR 200, and programme 17 from colorimeter DR 890. Nephelometric and electrometric method were applied for turbidity and DO determination. BOD₅ was determined as a difference of DO content at the beginning of the experiment and after 5 days incubation at 20°C (bottle method). The pH and temperature were measured with a Hach HQ411d laboratory meter.

5.3 Results and discussion

The water saturation method showed that the porosities of sponges were $91 \pm 0.5\%$, $87 \pm 0.25\%$ and $47 \pm 0.45\%$ respectively for experiment 1, 2 and 3. Effluent faecal coliform content was hundred times smaller when operating the cube type DHS reactor without recirculation ($2.1\text{E}+02 \pm 4.1\text{E}+02$ vs $3.4\text{E}+04 \pm 5.1\text{E}+04$). However, the mean BOD₅ content was reduced from 19 to 6 mg·L⁻¹ by using recirculation for the cube type DHS reactor. Also soluble COD decreased from 62 to 47 mg·L⁻¹. A total COD, soluble COD and BOD₅ removal of respectively $67.2 \pm 3.1\%$, $53.5 \pm 1.1\%$ and $80.9 \pm 2.0\%$ was achieved for experiment 1. Somewhat, higher removal efficiencies were accomplished in experiment 2, viz. $74.6\% \pm 8.2\%$, $71.1\% \pm 10.6\%$ and $93.6 \pm 3.4\%$ for total COD, soluble COD and BOD₅, correspondingly. The removal of total COD, soluble COD and BOD₅ for experiment 3 was respectively $68.8 \pm 8.2\%$, $84.9 \pm 5.3\%$ and $84.9 \pm 5.3\%$. A summary of the results for all experiments, after the start-up period, is presented in Table 5.6.

Table 5.6 Experimental results obtained using DHS reactors**A. Experiment 1: cube type DHS reactor without recirculation**

Parameter	units	n ¹	Influent	Effluent ²	Efficiency
Temperature	°C	266	20.9 ± 2.3	20.5 ± 3.1	
pH		266	7.4 ± 0.3	7.7 ± 0.4	
DO	mg·L ⁻¹	134	0.8 ± 0.7	5.6 ± 1	
Turbidity	NTU	266	145.3 ± 50.1	47.1 ± 35.3	67.2 ± 1 %
BOD ₅	mg·L ⁻¹	12	104.4 ± 13.7	19.5 ± 6.5	80.9 ± 2 %
COD total	mg·L ⁻¹	26	260.8 ± 77.7	85.9 ± 62.6	67.2 ± 3.1 %
COD soluble	mg·L ⁻¹	26	133.3 ± 31.5	62 ± 38.1	53.5 ± 1.1 %
Faecal coliforms	CFU·100mL ⁻¹	10	6.1E+06 ± 3.4E+06	2.1E+02 ± 4.1E+02	99.997 ± 0.000 %

B. Experiment 2: cube type DHS reactor with recirculation

Parameter	units	n	Influent	Effluent 1 ³	Effluent 2 ⁴	Efficiency
Temperature	°C	130	23.3 ± 4.3	23.4 ± 4.3	23.2 ± 4.2	
pH		126	7.6 ± 0.3	7.7 ± 0.3	7.5 ± 0.5	
DO	mg·L ⁻¹	26	0.4 ± 0.3	5.9 ± 1.1	6.1 ± 0.9	
Turbidity	NTU	9	144.1 ± 63.7	26.1 ± 17.9	10.3 ± 4.7	92 ± 4.3 %
BOD ₅	mg·L ⁻¹	8	107.4 ± 39.1	20.4 ± 5.2	6.2 ± 2.8	93.6 ± 3.4 %
Total COD	mg·L ⁻¹	19	196.2 ± 51.3	68.8 ± 33.8	47.3 ± 15.2	74.6 ± 8.2 %
Soluble COD	mg·L ⁻¹	12	113.2 ± 25.8	43 ± 7.9	31.4 ± 9.3	71.1 ± 10.6 %
Faecal coliforms	CFU·100mL ⁻¹	11	1.1E+08 ± 2E+08	5E+06 ± 3.1E+06	3.4E+04 ± 5.1E+04	99.919 ± 0.117 %

C. Experiment 3: curtain type DHS reactor

Parameter	units	n	Influent	Effluent 1 ³	Effluent 2 ⁴	Efficiency
Temperature	°C	154	18.8 ± 1.4	18.9 ± 1.2	18.9 ± 1.1	
pH		154	7.3 ± 1.1	7.5 ± 0.4	6.6 ± 0.5	
DO	mg·L ⁻¹	154	2.3 ± 0.4	4.2 ± 0.7	4.9 ± 0.7	
Turbidity	NTU	154	104.1 ± 13.8	30.8 ± 12.3	18.9 ± 4.8	81.8 ± 4.4 %
BOD ₅	mg·L ⁻¹	22	98.8 ± 15.5	23.4 ± 5.3	14.9 ± 5.8	84.9 ± 5.3 %
Total COD	mg·L ⁻¹	22	214.1 ± 44.2	N. M. ⁵	77.6 ± 18.6	62.8 ± 9.9 %
Soluble COD	mg·L ⁻¹	22	136.2 ± 33.7	N. M.	59.3 ± 14.1	55 ± 10.9 %
Faecal coliforms	CFU·100mL ⁻¹	22	7.2E+06 ± 5.6E+06	1.7E+06 ± 1.7E+06	5.9E+05 ± 7.5E+05	92.121 ± 6.210 %

Notes:

¹n means number of grab samples.

²Results show the average values for the effluent of the two DHS reactors.

³Results show the average values for the effluent after the module 01.

⁴Results corresponds to the effluent of the DHS reactor.

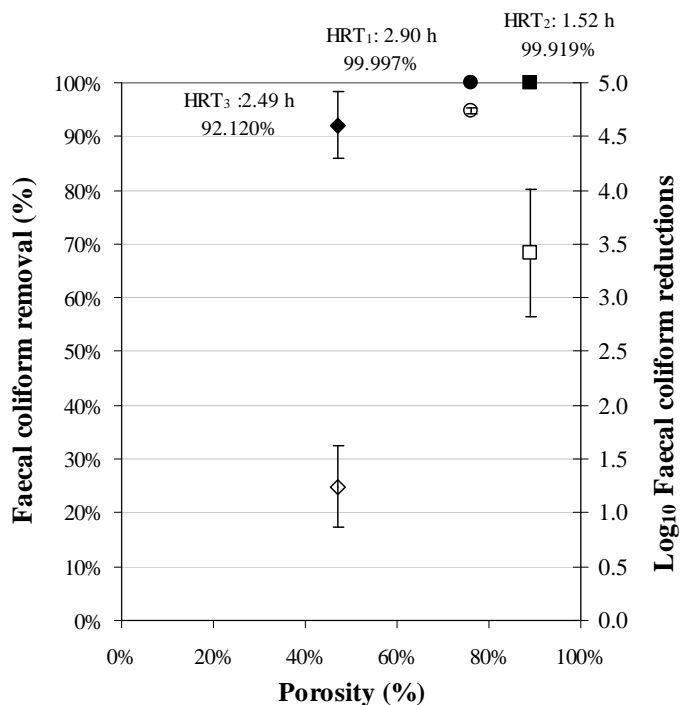
⁵N.M. means not measured

5.3.1 The DHS reactor capacity for removing faecal coliforms

The main emphasis of this research was to study the removal efficiency of three types of DHS systems for faecal coliforms. The pathogenic indicator, faecal coliforms, showed a significant reduction in all experiments and the best results were obtained for the cube type DHS reactor. The highest faecal coliforms reduction of 99.997 ± 0.000 % was obtained in experiment 1. A lower removal efficiency of 99.919 ± 0.117 % was achieved in experiment 2. Experiment 3, showed the lowest faecal coliforms reduction, viz. 92.121 ± 6.210 %.

Despite the relatively long HRT of 2.49 hours, the curtain type DHS reactor evidenced the lowest average faecal coliform removal ($1.25 \log_{10}$) as compared to that in the cube type configuration with ($3.42 \log_{10}$) and without recirculation ($4.74 \log_{10}$). This significantly lower removal efficiency could be associated to a much lower porosity and possibly occurrence of dead zones and short circuiting compared to experiment 1 and 2. The latter might be attributed to the experimental set-up and must be further investigated. Results indicate that it is necessary to analyse the flow distribution in the studied DHS reactors. The porosity of the medium characterises the available adsorption sites of the carrier material as previously reported by (Tawfik et al., 2010; Tawfik et al., 2011). A low porosity implies low biomass adsorption. It also implies low substrate conversion rates because of the non-optimised contact between wastewater pollutants and the low amount of biomass. Consequently, a low porosity will lead to a low biomass yield and low substrate conversion capacity. The sponge porosity may affect the type of biomass, the permissible hydraulic loading rate and the degree of clogging of the surface area of the carrier material. Clogging of the sponge surface area could result in dead zones during the filtration process since no biomass will grow in the sponge interior areas (Tawfik et al., 2011).

A significant difference in faecal coliform removal is shown between the cube type DHS reactors without recirculation among experiments 2 and 3. The best performance in experiment 1 could be ascribed to the longer HRT in the cube type DHS reactor compared to the other two experiments (Figure 5.2).



Faecal coliform removal efficiency in %	● Experiment	■ Experiment	◆ Experiment
Log ₁₀ faecal coliform reductions	○ 1	□ 2	◇ 3

Figure 5.2 Faecal coliforms removal expressed in terms of percentage and log₁₀ reductions versus porosity. Log₁₀ reductions were determined by dividing faecal coliform content in the influent between faecal coliform in the effluent.

5.3.2 The compliance with water reuse guidelines

Following the WHO guideline 1989, the effluent with a faecal coliform content of $2.1\text{E}+02 \text{ CFU}\cdot 100\text{mL}^{-1}$, produced in experiment 1 can be used for unrestricted irrigation (category A). Restricted irrigation, category B and C is assigned to the effluent of experiment 2 with a faecal coliform content of $3.4\text{E}+04 \pm 5.1\text{E}+04 \text{ CFU}\cdot 100\text{mL}^{-1}$ and the effluent of experiment 3 with a faecal coliform content of $5.9\text{E}+05 \pm 7.5\text{E}+05 \text{ CFU}\cdot 100\text{mL}^{-1}$, respectively.

Average BOD₅ content in the effluent of the evaluated DHS reactors, varied between 6.2 and 19.5 $\text{mg}\cdot\text{L}^{-1}$, which is lower than the USEPA standard of 30 $\text{mg}\cdot\text{L}^{-1}$ for treated effluents applied for CPFC and non-food crops (types 2 and 3). The average pH variation between 6.6 and 7.7, complies with USEPA standards for each type of agricultural use. Average turbidity variation between 10.3 and 47.1 NTU in all evaluated DHS reactors, exceeds significantly the limit of 2 NTU for irrigation of food

crops (type 1). Therefore, in terms of BOD₅, turbidity and pH, the effluents meet the USEPA standard for agricultural reuse, type 2 and 3.

5.3.3 The performance of DHS reactors with respect to organic matter removal

Generally, the DHS is a good polishing step in terms of total COD, soluble COD and BOD₅ removal. No significant differences were found regarding total and soluble COD removal between the three experiments. The average BOD₅ of 80.9 to 93.6 % and total COD removal efficiency of 67.2 to 74.6 %, observed in the DHS G1 reactors were in close proximity to results of Agrawal *et al.* (1997) and Machdar *et al.* (1997). The latter were 97 % and 78 % for the BOD₅ and total COD removal efficiency, respectively. The total COD removal efficiency of 62.8 %, obtained in DHS G2 reactor was similar to the 59 % reported by Machdar *et al.* (2000).

The turbidity, a measure for the suspended solids content, was reduced by 67.2 ± 1 %, 92.0 ± 4.3 % and 81.8 ± 4.4 % in experiment 1, 2 and 3, respectively. Results illustrate a significant increase in average DO in the effluent of the DHS reactors, viz. respectively 0.8 - 5.6, 0.4 - 6.1 and 2.3 - 4.9 mg·L⁻¹ for experiments 1, 2 and 3 (see Table 5.6). The latter can be attributed to convective flow natural aeration.

The lowest BOD₅ and turbidity removal was obtained in experiment 1. BOD₅ removal in experiment 2 was 12.7% higher compared to the value obtained in experiment 1. BOD₅ removal in experiment 2 was slightly higher than experiment 3. The highest BOD₅ removal efficiency was observed in experiment 2, applying could be attributed to the recirculation of the settled wastewater. Recirculation enhances the contact between organic matter and microorganisms present in the biofilm (von Sperling *et al.*, 2005). Additionally, turbidity removal in experiment 2 was higher than that in experiment 3, with a significant difference of 10% (see Table 5.6). No correlation was found between BOD₅ and faecal coliform removal.

The pH in the effluent of experiment 2 is slightly lower compared to the pH of the influent. This reduction could be associated to some degree of nitrification as observed in the lowest part of trickling filters when the BOD₅ concentration is near 15 mg·L⁻¹ (Agrawal *et al.*, 1997; von Sperling *et al.*, 2005).

During the experimental trials, the operation and maintenance activities of the lab-scale DHS reactors were relatively simple and consisted of cleaning of pipelines to maintain a constant flow and to prevent clogging. Sponges remained in good condition (no visual damage observed) during the research period.

5.4 Conclusions

Cube type (G1) DHS reactors showed the best capacity for faecal coliform removal. The cube type system without recirculation complies with WHO (1989) standards for unrestricted irrigation (Category A). Restricted irrigation, category B and C is assigned to the effluent of the cube type DHS reactor with recirculation and the curtain type DHS reactor, respectively. Regarding organic compounds, the effluent of the evaluated DHS reactors complies with USEPA standards in terms of BOD₅, pH and turbidity for irrigation of only non-food crops, like pasture for milking animals, fodder, fibre, and seed crops. Results did not show a correlation between BOD₅ removal and faecal coliform removal.

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CHAPTER 6

General discussion

6.1 Introduction

Freshwater availability is a complex issue that affects economy, society and ecology. Water shortages rapidly increase with increasing urban population and might be further impacted by climate change. Due to fresh water scarcity, treated urban wastewater is considered as an alternative water source for agricultural, industrial and municipal uses in urban and peri-urban areas of both developing and industrialised countries (Jiménez and Asano, 2008; McDonald et al., 2011; Mohd, 2013; Mosteo et al., 2013; Norton-Brandão et al., 2013). Irrigation with treated wastewater has several advantages. For instance, if not removed during treatment, treated wastewater contains nutrients which can be beneficially used in agriculture instead of artificial fertilisers (Van Lier and Huibers, 2004; van Lier and Huibers, 2010; Mohd, 2013). However, care should be taken to not over-fertilise crops since irrigation water demand is generally determined by crop water requirements and not by nutrient demands (Boom et al., 2008). Another constraint of treated water reuse is the presence of contaminants that can affect the quality of soils, crops, and human health, such as pathogens, salts, metals, organic compounds and pharmaceutical and personal care residues (Van Lier and Huibers, 2004; Fatta-Kassinos et al., 2011; Abargues et al., 2012). From the previous list, the main concern for the agricultural use of treated wastewater is the presence of pathogens like bacteria, helminth eggs, protozoa and enteric viruses (see Chapter 1).

Particularly in developing countries, wastewater treatment systems are often non-existent or abandoned altogether due to high energy requirements, lack of skilled operators and investment, and/or too high operation and maintenance costs (Verbyla et al., 2013). Due to fresh water scarcity the effluent of wastewater treatment plants (WWTP) is used by farmers to irrigate all kind of crops (Jiménez and Asano, 2008; Méndez Vega and Marchán Peña, 2008). Thus, untreated or partially treated wastewater that is used for agricultural irrigation becomes a source of pathogens transmission of increasing importance (Balcázar, 2007). Therefore, farmers and consumers are more and more at risk of getting infected due to the exposure to pathogen-rich irrigation waters and/or polluted crops (Feachem et al., 1983; Ayres and Mara, 1996; Márquez-Hernández et al., 2010).

In industrialized countries, the commonly applied wastewater technologies use high amounts of energy, sometimes even with the addition of chemicals for advanced nutrients removal and/or effluent disinfection (Tchobanoglous et al., 2003; Lettinga, 2008; Verbyla et al., 2013). A treatment technology based on mainly aerobic treatment in low income countries usually generates financial constraints (von Sperling et al., 2005). These constraints are associated with the high capital and operational costs (Campos and Von Sperling, 1996; Kassab et al., 2010). Anaerobic wastewater treatment processes can, however, increase overall energy recovery and fossil carbon emission savings (Lettinga, 2008; Verbyla et al., 2013). Therefore, combined anaerobic–aerobic systems could be more effective in terms of capital and operational costs than full aerobic systems (Chan et al., 2009; Kassab et al., 2010). Anaerobic treatment of

domestic wastewater is a viable and cost-effective alternative under subtropical conditions (Seghezzi, 2004; Chernicharo, 2006). The main advantages are the relatively low construction and operational cost, operational simplicity and low sludge production. Additional advantages comprise energy production as biogas and the applicability in small and large scales (Lettinga et al., 1984; Seghezzi et al., 1998; Lettinga et al., 1999). Within anaerobic technologies, the upflow anaerobic sludge blanket (UASB) reactor is mostly applied and considered a more sustainable alternative for domestic wastewater treatment in developing countries for cities and small communities (Khan et al., 2011). Though, UASB reactors alone are not able to meet WHO reuse standards, particularly when treated wastewater is used for agricultural purposes (Khan et al., 2013).

Several aerobic post-treatment processes have been proposed to enhance the quality in the UASB reactors effluents. Typical selections encompass the activated sludge process, rotating biological contactor (RBC), trickling filter, down-flow hanging sponge (DHS) reactor, sequencing batch reactor (SBR), dissolved air flotation, constructed wetland, anaerobic and aerobic filter (Machdar et al., 2000; Chernicharo, 2006; Tawfik et al., 2006a; Khan et al., 2011). During the selection of appropriate technologies in developing countries, economic limitations should be considered (Chernicharo, 2006). Particularly, due to their compactness and the application of convective airflows without energy use, DHS reactors have several advantages over the other mentioned aerobic processes. These advantages are its low investment cost and energy requirement, as well as its limited maintenance. (Machdar et al., 2000; Uemura et al., 2002; Machdar and Faisal, 2011; Fleifle et al., 2013; Fleifle et al., 2013). Although, not thoroughly studied, the DHS reactor is considered based on literature as a promising technology for pathogen removal. Consequently, the DHS reactor has been selected in the present research to polish UASB reactor effluents (see Chapter 5).

The largest part of this research (Chapter 2, Chapter 3, Chapter 4 and Chapter 5) was conducted in Lima (Peru). Lima is characterized by a semi-arid climate and a water supply network coverage of 91 %. Additionally, Lima is currently struggling with water scarcity (Fritzmam et al., 2007; Wirth, 2010; Ioris, 2015). The remaining population (9%) is located mainly in the hilly parts of the city without access to the drinking water network, whereas the wastewater is discharged into water sources or infiltrated in the soil located in the vicinity (Liwa, 2008; Ioris, 2015). Even though 91 % of the population is connected to the public sewer network, only about 51 % of the wastewaters receive some treatment (Vergara León, 2013). The raw or partially treated wastewater is illegally discharged in the rivers, directly discharged into the Pacific Ocean, or used for crops (Schoppmann, 1996; Peasey et al., 2000; Liwa, 2008). Only 5 % of the treated wastewater is used for irrigation (Liwa, 2008). However, there is an increasing demand for irrigation water especially along the Peruvian desert coast (Bartone, 1985; León and Moscoso, 1995; Schoppmann, 1996; Nava, 2001). About 33 WWTP apply water reuse in irrigated agriculture, covering approximately 3010 Ha (Bartone, 1985). For example in the city of Tacna, effluents from stabilization ponds

have been used since 1975 to irrigate 200 Ha of potatoes, maize, alfalfa and olives (Nava, 2001). Unfortunately, public health risks exist when WWTP are not correctly working, due to the lack of operation and maintenance or higher organic and pathogenic loads in the influent, compared to the design loads (León and Moscoso, 1995; Nava, 2001). The health risks are expressed as the probability of getting infected by direct contact between humans (or animals) and pathogens present in wastewater (Nava, 2001; WHO, 2006).

Within the group of pathogens, helminth eggs has been identified as a crucial indicator because of their low infective dose of 1 egg per person. Additionally, helminth eggs are present in wastewater in most developing countries (Jimenez, 2007; Jimenez and Asano, 2008; Navarro and Jiménez, 2011). The situation in highlands from low income regions is aggravated when poor sanitation conditions prevail due to the increased pathogen content (see Chapter 4). Then, in order to prevent occurrence of intestinal parasitosis, the removal of helminth eggs is an important criteria when selecting a technology for domestic wastewater treatment. Consequently, the current research gives more insight into the filtration capacity of sludge from UASB reactors with particular emphasis on helminth egg removal.

6.2 The importance of removing helminth eggs in wastewater treatment plants

6.2.1 The helminth egg content in wastewater and sludge

Helminthiasis remains a major cause of diseases in countries with poor sanitary facilities (Santamaría and Toranzos, 2003; Larson et al., 2010). Probably because countries can not afford the measurement costs, the helminth egg concentration in raw wastewater and wastewater sludge has not been reported in detail, (Mahvi and Kia, 2006; García Palacio, 2010; Navarro and Jiménez, 2011; Gil et al., 2013; Verbyla et al., 2013). The helminth egg concentration in domestic wastewater varied widely between 1 and 3006 eggs·L⁻¹ in Bolivia, Brazil, Colombia, Iran, Jordan, Mexico, Venezuela, Morocco, Ukraine, France and United States (Mahvi and Kia, 2006; García Palacio, 2010; Navarro and Jiménez, 2011; Gil et al., 2013; Verbyla et al., 2013). Moreover, the information of helminth eggs in excess sludge is more scarce than in wastewater and varied between 1 and 735 eggs per g TSS in Brazil, Egypt, Ghana, France , Germany, Great Britain and United States (Navarro and Jiménez, 2011). The results of the current research showed that the average helminth egg concentration in raw domestic wastewater varies between 4 ± 1 and 194 ± 79 egg·L⁻¹ in Lima and Puno (Peru), respectively. Based on the infective dose of 1 helminth egg per person (WHO, 2006), it could be expected that the probability of getting infected with parasitosis is much higher in Puno than in Lima. Latter difference could be attributed to the prevailing different sanitation conditions (see Chapter 2, 3 and 4). Thus, the capacity to remove helminth eggs until a level of less than 1 egg·L⁻¹ is an important criterion when selecting a

technology for domestic wastewater treatment when humans come in direct contact with treated wastewater.

6.2.2 Predominance of helminth eggs

The results of the present research, show that *Ascaris lumbricoides*, *Trichuris sp.* and *Strongyloides sp.* are the most common helminth eggs present in domestic wastewater in subtropical Lima (see Chapter 2). Additionally *Ascaris lumbricoides*, *Toxocara sp.*, *Hymenolepis nana* and *Enterobius vermicularis* were the most common helminth eggs present in the low temperature domestic wastewater in Puno (Peru), situated at an altitude of 3800 m.a.s.l. The content of the corresponding helminth eggs were 142 ± 106 , 41 ± 19 , 21 ± 11 and 36 ± 55 egg·L⁻¹ respectively. (see Chapter 4). *Ascaris lumbricoides* and *Trichuris trichiura* are considered the worldwide most prevailing helminth eggs (Cifuentes et al., 1999; Habbari et al., 2000; Blumenthal et al., 2001; Santamaría and Toranzos, 2003; Bethony et al., 2006; García Palacio, 2010). In the present research *Ascaris lumbricoides* was always present in the largest numbers in Peruvian wastewater.

6.2.3 Removal of pathogens from domestic wastewater

The reduction of pathogens like helminth eggs, bacteria and viruses as recommended in the WHO guidelines (WHO, 2006), can be achieved by applying so called land-based, extensive wastewater treatment processes, like stabilization ponds and constructed wetlands (Jiménez et al., 2010). Such land-based treatment processes usually require a large surface area, which varies between 1 and 5 m² per population equivalent (von Sperling et al., 2005; Moelants et al., 2008). Unfortunately, this area demand is a major drawback since large areas of flat land are not available in hilly areas, whereas land prices, especially in the urban and per-urban areas are generally too high. (Moelants et al., 2008). Thus, the current research is focused on so-called compact technologies, evaluating the potentials of particularly UASB reactors for removing (filterable) pathogens .

UASB reactors are reported to remove 60-90% of the helminth eggs in domestic wastewater (Jimenez, 2007). Sedimentation and filtration have been considered the main mechanisms of helminth egg removal in UASB reactors (von Sperling et al., 2002; Jimenez, 2007). Chapter 1 presents an overview of pathogen removal efficiencies, applying different treatment trains that include a UASB reactor for treating domestic wastewater. As existing information on the UASB sludge filtration capacity is mainly related to solids (Mahmoud et al., 2003) and not to pathogen removal, the current research is focused on the filtration capacity for helminth egg removal. The research considered the influence of different environmental and operational conditions like temperature, upflow velocities, and wastewater characteristics on the filtration process (see Chapters 2, 3 and 4).

UASB reactors are known to have a limited removal capacity for pathogenic bacteria (Khan et al., 2013), indicating that an additional treatment step is required for meeting the reuse restrictions. Selection of the most proper post treatment system depends on local conditions. In the present research, the DHS reactor was selected as the post treatment step for the UASB reactor (see Chapter 5), basically for its high faecal coliform removal capacity at a relatively low HRT. According to literature (Uemura et al., 2002; Chong et al., 2012), it can remove up to 2.57 log₁₀ of faecal coliforms at short HRTs (between 0.5 to 1.3 h), at temperatures between 7 and 30°C. For the current study, DHS reactors removed between 1 and 4 log₁₀ of faecal coliforms, also applying a relatively short HRT (less than 3 h) at temperatures between 19 and 23°C (see Chapter 5). By using the proposed sequence of UASB-DHS reactors for treating the sewage prior to agricultural reuse, the human health risks will be distinctly reduced compared to untreated reuse or treatment with solely a UASB reactor. The analysis of health risks is a very complex research that depends on the environmental, economic, social, and epidemiological characteristic of each location (WHO, 2006; Drechsel et al., 2008; Mara and Kramer, 2008). The health risks associated with the use of effluents from UASB and DHS reactors are further discussed in item 6.5 *Health risks associated to water reuse in agriculture* in view of the WHO guidelines (2006).

6.3 The anaerobic sludge filtration capacity for helminth eggs

6.3.1 UASB sludge filtration capacity and the effect of upflow velocity

In this research the filtration capacity of anaerobic sludge to remove helminth eggs under different conditions was studied. Following the literature (Dietrich, 1982; Cheng, 1997), the removal of a mixture of differently sized particles is very complex, because they would settle at different velocities. Using a UASB sludge bed as a filter for the removal of particles, the complexity increases due to prevailing reactor conditions, such as biogas production, applied liquid upflow velocity and fluctuations in temperature.

The results presented in Chapter 2 describe the influence of upflow velocity on helminth egg removal in UASB reactors, excluding factors like temperature and related gas production. This research was divided in two parts. In the first part latex beads were used with a uniform size (standard L90: $\phi = 90 \mu\text{m}$) and density (1.05 mg/L) (Coulter® CC, Miami, USA). The latex beads were used to simulate helminth eggs, as their shape, size and density are similar to the helminth egg characteristics (Quinzanos et al., 2008). The main objective was to determine the filtration capacity of digested sludge (from a primary sludge digester) for retaining helminth eggs from domestic wastewater. A temperature of 4 °C was selected to limit biodegradation minimise gas production. As a result, the sludge bed performance was mostly influenced by the different, applied upflow velocities. Supplementary experiments without sludge bed showed that settling played a major role in the removal, rather than filtration. Results show a decreased latex

beads removal efficiency at increased upflow velocity. Additionally, the degree of sludge digestibility did not show a significant effect on the sludge filtration capacity.

During the second part of the research, the filtration capacity of the UASB sludge was evaluated using five upflow velocities, namely 0.39, 1.58, 2.83, 3.16 and 4.12 m·h⁻¹. For both latex beads and helminth eggs, 100% removal is achieved at 4°C using an upflow velocity of 0.3 and 0.39 m·h⁻¹, respectively. Therefore, a 100% filtration capacity of flocculent sludge in UASB reactors is expected for wastewater temperatures nearly to 4°C when operating the UASB at an upflow velocity of about 0.3 m·h⁻¹. Finally, the use of latex beads in university laboratories to study more filtration tests of sludge in UASB reactors is recommended to prevent the risks of parasitic infection

6.3.2 UASB sludge filtration capacity for helminth eggs at low temperature domestic wastewater treatment

Chapter 4 presents the filtration results under field conditions, performed in the city of Puno located at an altitude of 3800 m.a.s.l. Experiments were conducted at the prevailing low average wastewater temperatures between 11.3 and 14.3 °C. The average helminth eggs influent content was 194 ± 79 egg·L⁻¹. Irrespective of the applied upflow velocity between 0.12 and 0.41 m·h⁻¹, the helminth egg filtration capacities were very similar between 89 and 95%. Although the applied upflow velocity seems to be at the low side, proper treatment of domestic sewage at this low temperature can only be expected using an upflow velocity of 0.23 m·h⁻¹, which is calculated based on Zeeman & Lettinga (1999). The lower helminth egg removal compared to the filtration capacity achieved at 4°C, at similar upflow velocities (Chapter 2), might be due to the observed, though low, gas production. Results show that the average helminth egg content in the effluent was 19 ± 23 egg·L⁻¹. Then, with the prevailing high influent helminth eggs content, the observed filtration capacities are not sufficient for applying unrestricted irrigation according to the WHO guidelines (WHO, 1989). Regarding faecal coliforms and *E. coli* removal, results showed an insignificant removal of less than 1.7 Log₁₀ for all applied conditions. Therefore, disinfection is needed as a post treatment step to remove remaining helminth eggs, faecal coliforms and *E. coli*, if treated wastewater is used for irrigation purposes and has to reach WHO guidelines.

6.3.3 UASB sludge filtration capacity for helminth eggs under subtropical conditions

This part of the research is presented in Chapter 3. The prevailing, average ambient temperature was 22.8 °C in the city of Lima (Peru). UASB sludge filtration experiments were performed using a stock solution, containing *Ascaris suum*, a model organism for human helminth eggs. The helminth egg concentration in the influent tank varied between 20-50 egg·L⁻¹.

The study demonstrates that, when applying temperatures between 17.1 and 28.6 °C and, if the sludge bed height increases, then filtration capacity of anaerobic sludge inside UASB reactors for helminth eggs is reduced.

When the system was operated at a sludge bed height of 19–38% of the total reactor height, a reciprocal correlation between the average helminth egg removal efficiency and upflow velocity (between 0.09 and 0.68 m·h⁻¹) was observed. The reported average helminth egg removal varied between 30 and 100 %. The average helminth egg removal efficiency in the control experiment without a sludge bed, representing plain sedimentation, varied between 44% and 66%. The decreasing trend in helminth egg removal efficiency at an increasing sludge bed height was explained by a possible increment in turbulence, created by the biogas production and channel formation in the sludge bed (Lettinga et al., 1984; Abdelgadir et al., 2014). Importantly an additional settling step after the UASB is therefore suggested, for practical purposes, to improve the helminth egg removal to below restrictive standards. More research is needed to understand the exact influence of biogas production in the flow of wastewater inside the UASB reactor and in the filtration capacity of the UASB sludge.

Microscopic observations showed the deteriorated semi-crystalline morphological structure of *Ascaris suum* eggs present in effluents and sludge. These damages might be attributed to the contact of microorganisms present in the UASB sludge bed and helminth eggs at the experimental conditions. Unfortunately, damaged *Ascaris suum* eggs in effluent and sludge were not quantified or tested on viability. The question whether effluents of UASBs, containing *Ascaris* eggs, are still infectious, therefore still needs to be answered.

6.4 Post treatment of UASB reactors

The main aim of this phase of the research (see Chapter 5) was to study the capacity of a DHS reactor for removing faecal coliforms from the effluent of a UASB reactor treating domestic sewage. Then based on the WHO standards and the quality of the produced wastewater different agricultural reuse possibilities could be assigned. The DHS reactor was selected among several types of high rate trickling filters as a promising technology for post treatment of UASB effluents. Among its main features reported in the literature is the capacity to effectively retain colloidal material (Tandukar et al., 2005; Tawfik et al., 2006a; Tandukar et al., 2007; Uemura and Harada, 2010; Onodera et al., 2014). In general, they showed a faecal coliform reduction between 79.0 % and 99.98 %.

Regarding faecal coliform removal, the results of this study have been compared to the WHO guidelines of 1989, which contains the maximum permissible limits in absolute values that most developing countries employ (Angelakis et al., 1999; Jiménez and Asano, 2008; González González and Chiroles Rubalcaba, 2011). The WHO guidelines

of 2006 for unrestricted irrigation, provides recommended restrictions for faecal coliforms based on a quantitative microbial risk assessment (QMRA) approach, i.e. no absolute values are given. The recommended restrictions are accompanied with the introduction of proper sanitary measures and recommendations to calculate the referred limits based on a risk assessment for a specific location. The latter assessment was not available in this study. Further, analysis regarding health risks is described in section 6.5 *Health risks associated to water reuse in agriculture*.

Results evidenced that a DHS reactor can remove faecal coliforms between 92.121 and 99.997 % (equivalent to 1.25 to 4.74 \log_{10}) at a relatively short HRT (between 1.25 and 2.28 h). The highest faecal coliform reduction of 99.997 ± 0.000 % was obtained in cube type DHS (G1) reactors without recirculation. A lower removal efficiency of 99.919 ± 0.117 % was observed in the cube type DHS (G1) reactors with recirculation. The curtain type DHS (G2) reactor, showed the lowest faecal coliforms reduction, viz. 92.121 ± 6.210 %.

The rather efficient removal of faecal coliforms from the UASB effluent by the investigated DHS reactors, proves a high DHS efficiency when compared to extensive technologies, like constructed wetlands and stabilization ponds (De Sousa et al., 2001; Cavalcanti, 2003; von Sperling et al., 2005) as a polishing alternative for the UASB reactors. The best results of faecal coliform removal were obtained for the cube type DHS reactors, which probably can be attributed to the more even flow distribution and observed media porosity in this configuration. The observed higher porosity might lead to the occurrence of less dead zones, more adsorption areas compared to the curtain type reactor.

Based on the average faecal coliform content in the effluent of the three evaluated DHS reactors, different reuse possibilities can be assigned to the treated wastewater following the WHO guidelines (WHO, 1989; WHO, 2006). The effluent with an average faecal coliform content of $2.1E+02$ CFU·100mL⁻¹, produced in the cube type DHS reactor without recirculation can be used for unrestricted irrigation (Category A). Unrestricted irrigation includes crops likely to be eaten uncooked, sport fields and public parks. The effluent of the cube type DHS reactor with recirculation, with an average faecal coliforms content of $3.4E+04$ CFU·100mL⁻¹ can be used for restricted irrigation, of Category B. Restricted irrigation Category B comprises cereal crops, fodder crops, pasture and trees. The effluent of the curtain type DHS reactor without recirculation, with an average faecal coliform content of $5.9E+05$ CFU·100mL⁻¹ is assigned to restricted irrigation, Category C, which includes crops not exposed to workers and public.

6.5 Health risks associated to water reuse in agriculture

The new WHO guideline of 2006 does not provide limits for viral pathogens and bacteria. In order to apply this WHO guideline it is recommended to apply a health risk study in all situations of water reuse (WHO, 2006). Risk is the likelihood of identified hazards causing harm in exposed populations in a specified time frame including the severity of the consequences (CAMRA, 2015).

Based on exposure scenarios of vegetable consumption and the epidemiological context, a tolerable Disability Adjusted Life Years loss per person per year (DALY loss pppy) of $\leq 10^{-6}$ DALY pppy is recommended for irrigation using treated wastewater in agriculture (WHO, 2006; Drechsel et al., 2009). This value corresponds to a tolerable risk of fatal cancer of 10^{-5} per person from consuming drinking water containing a carcinogen. Then this infected person has a 1: 100 000 lifetime chance of developing fatal cancer. One DALY loss means one year of illness or one year lost due to premature death (WHO, 2006). DALYs are an important tool for comparing health outcomes because they account health effects and delayed and chronic effects, including morbidity and mortality (Bartram et al., 2001). Thus, when risk is described in DALYs, different health outcomes can be compared and risk-management decisions prioritized (WHO, 2006; Drechsel et al., 2009).

According to the WHO guidelines of 2006, food crops, irrigated with treated wastewater, especially those eaten uncooked, are expected to be as safe as drinking water in order to prevent infection of people due to direct contact with irrigated crops. Thus, the tolerable disease burden of $\leq 10^{-6}$ DALY pppy should be applied (WHO, 2006). In order to achieve the indicated tolerable DALY loss pppy, the removal of pathogenic organisms is the main objective of domestic wastewater treatment for developing countries as it expresses the risk factor for public health (Mahmoud et al., 2011). Helminth eggs are of particular interest because a person only requires a minimum infective dose of 1 egg to be infected (Jimenez, 2007). Additionally, the survival time of helminth eggs at ambient conditions is long and varies from months to more than 3 years (Shuval, 1990; de Victorica and Galván, 2003; Khan et al., 2008). Therefore, a content of helminth eggs less than $1 \text{ egg} \cdot \text{L}^{-1}$ is requested in most situations except for high stem crops applying localised drip irrigation, when no crops are picked up from the soil like fruit trees. Gravity-fed irrigation and pressurised irrigation are main types of irrigation system distinguished in the WHO guidelines. They differ in the way water is applied, in their uniformity and application efficiency, in the cost of the system components, and in the water quality which they transport (Eisenberg et al., 2014). Localised irrigation employs drippers and micro-spray heads. The water is applied directly at one point, or under the soil surface, which is closest to the plant (Oron et al., 1999; Eisenberg et al., 2014). Particularly, for localised drip irrigation no recommendation regarding helminth eggs content is given because there is not direct contact between the treated wastewater and crops (WHO, 2006).

6.5.1 Risks associated with the use of treated wastewater

Based on the tolerable DALY loss pppy, the DALY loss per case (of the disease) and the disease-infection ratio (dir), the tolerable disease pppy [P_D] and the annual risk of infection [$P_{I(A)}$], so called tolerable infection risk can be determined according to the following equations (WHO, 2006):

$$P_D = \frac{\text{Tolerable DALY loss pppy}}{\text{DALY loss per case}} \quad (\text{eq. 6.1})$$

$$P_{I(A)} = \frac{\text{Tolerable disease risk pppy}}{\text{dir}} \quad (\text{eq. 6.2})$$

Where the disease-infection ratio (dir) $\in [0,1]$

Additionally, in order to determine $P_{I(A)}$, the probability of infection in an individual or in a community from a single dose of pathogen $P_{I(d)}$, must be determined using the quality of health's report, the so called "health outcome" for a specific location. The health outcome can be obtained by epidemiological studies or quantitative microbial risk assessment (QMRA) when no database containing information of a specific disease is available (Drechsel et al., 2009; Navarro and Jiménez, 2011). For the QMRA the first step is to establish the best distribution model fitting observed infection rates as a function of pathogen exposure doses (Navarro and Jiménez, 2011).

For the dose-response relationships, the beta-Poisson dose-response model (see eq. 6.3) was used for the risk calculation of getting infected by 'ingesting' helminth eggs, virus and bacteria (Haas et al., 1999; Drechsel et al., 2009; Navarro and Jiménez, 2011). The beta-Poisson dose-response model considers that the pathogen-host survival probability vary according to a beta probability distribution). This model was selected by Navarro *et al.* (2009) and Haas *et al.* (1999) as it best describing the dose-response relationships for the *Ascaris lumbricoides*, rotavirus and *Salmonella* (non-typhe):

$$P_{I(d)} = 1 - \left[1 + \left(\frac{d}{N_{50}} \right) \times \left(2^{\frac{1}{\alpha}} - 1 \right) \right]^{-\alpha} \quad (\text{eq. 6.3})$$

$$P_{I(A)} = 1 - \left[1 - P_{I(d)} \right]^n \quad (\text{eq. 6.4})$$

In eq. 6.3 $P_{I(d)}$ is the risk, expressed as probability of becoming infected by ingesting 'd' number of organisms (dose) from a single exposure. N_{50} is the median infection dose, representing the number of organisms that will infect 50 per cent of the exposed population; and α is the dimensionless infectivity constant (Drechsel et al., 2009). This dose (d) is the number of pathogens ingested with the crop (i.e. lettuce, carrots or onion) and is assumed to be a volume of treated wastewater that remains on the crop after irrigation, for example, 11mL to remain on 100g of lettuce (Shuval et al., 1997). In eq. 6.4 $P_{I(A)}$ is the annual risk of infection (or tolerable infection risk) in an individual from "n" multiple exposures per year to a pathogen dose. Particularly for helminth eggs, the beta-Poisson model ($\alpha = 0.104$ $\beta = 1.096$) was used in previous research (Navarro et al., 2009; Navarro and Jiménez, 2011) to estimate risk of *Ascaris lumbricoides* infection for a child who consumes raw crops once per week.

The WHO guidelines of 1989 suggests the number of pathogens allowed per 100 mL of treated wastewater (WHO, 1989). Thus, eq. 6.1, 6.2, 6.3 and 6.4 allows the calculation of the pathogen dose that can be ingested by an exposed individual to the wastewater (containing the pathogens) without exceeding the $P_{I(A)}$ (WHO, 2006). It considers that pathogens in raw wastewater can be reduced by applying treatment technologies and sanitary measurements. Treatment technologies encompass the application of physical, biological and chemical processes through wastewater engineering in order to improve the quality of the water. Sanitary measurements combine the application of activities, which can be used by inhabitants to protect their health and to reduce the level of exposure of a particular dose of contaminants in crops (WHO, 2006). The exposure assessment is very complex and involves a combination of addressing the methods used to measure the microbes and their content in the water, air or soil and the duration of the exposure (CAMRA, 2015).

Results of this research demonstrates that within anaerobic treatment, UASB reactors can provide an average helminth egg removal in the range of 26 - 93% and 89 - 95 % at subtropical and low temperature conditions. Therefore, the expected risk $P_{I(d)}$ in an individual from a single dose of pathogens using wastewater from the influent and effluent of the UASB reactor for irrigation of crops was evaluated using the beta-Poisson dose-response model (eq. 6.3). The α and β values ($\alpha = 0.104$ $\beta = 1.096$) were taken from another parameterisation on previous research (Navarro et al., 2009; Navarro and Jiménez, 2011). Additionally, the expected risk $P_{I(d)}$ was determined assuming an *Ascaris lumbricoides* content of $100 \text{ egg} \cdot \text{L}^{-1}$ in the influent and a wastewater volume between 1 and 10 mL (dose) that remain on the crops. The number of ingested helminth eggs is directly proportional to the applied doses. Results of $P_{I(d)}$ are shown in Figure 6.1. After $P_{I(d)}$ calculation, the annual risk of infection $P_{I(A)}$ was determined using eq. 6.4 and results are shown in Figure 6.2.

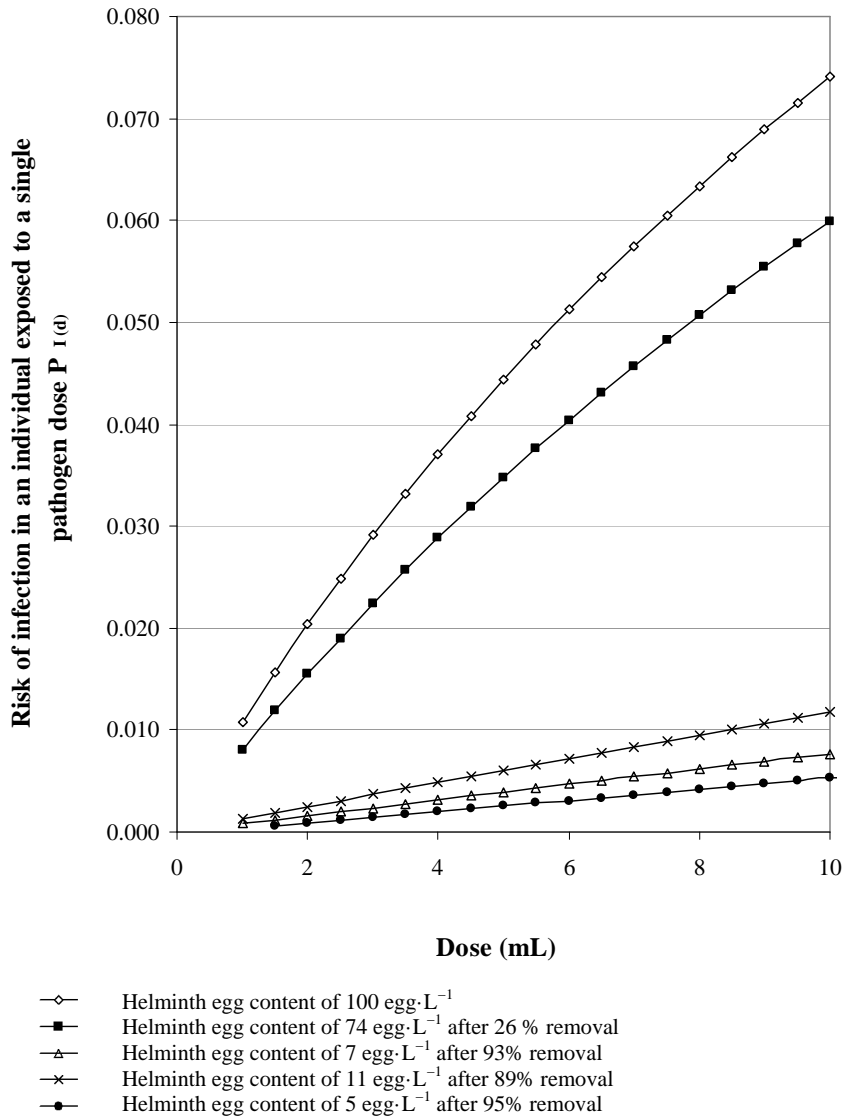


Figure 6.1 Risk of infection from a single dose of pathogens using wastewater containing helminth eggs for irrigation of crops following the beta-Poisson dose-response model.

An *Ascaris lumbricoides* content of 100 egg·L⁻¹ in the influent, different helminth egg removal of 26, 89, 93 and 95 %, and doses of wastewater volume between 1 and 10 mL that remain on the crops per exposure were assumed. The exposure group consumes raw crops once per week.

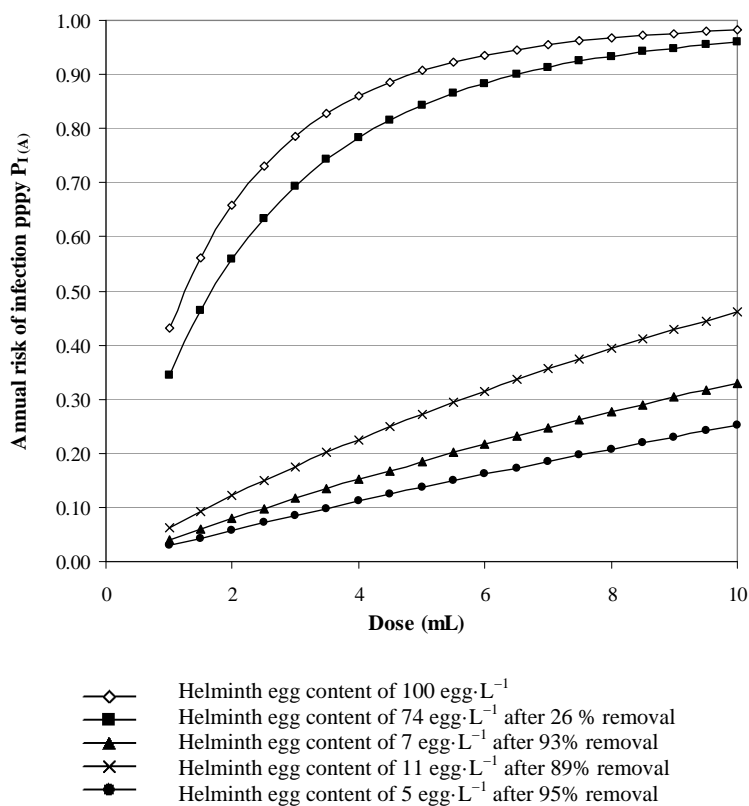


Figure 6.2 Annual risk of infection of an individual per year to helminth egg doses. An *Ascaris lumbricoides* content of 100 egg·L⁻¹ in the raw influent, different rates for helminth egg removal (26, 89, 93 and 95 %) in treated wastewater and doses of wastewater volume between 1 and 10 mL that remain on the crops per exposure were assumed. The current results considers that the exposure group consumes raw crops once per week.

Particularly, the results of $P_{I(d)}$ and $P_{I(A)}$ for 10mL of wastewater remaining in the ingested crops which were previously contaminated with helminth eggs are shown in Table 4.1. According to Table 4.1, on the one hand, it can be expected that 981900 of 1000 000 inhabitants would get infected if they ingest a 1 - 10 mL dose of raw wastewater which remains on the crops. On the other hand, if a UASB reactor is used in the Peruvian highlands, the amount of infected people can be reduced to 5500 inhabitants if they ingest a 1 - 10 mL dose of a effluent wastewater from a UASB reactors which remains on the crops. It means that annual risks of infection can be reduced by 74%. Therefore, the application of UASB technology as (pre)treatment will significantly reduce the annual risks, $P_{I(A)}$, of becoming infected. The reduction of $P_{I(A)}$ is expressed in the range of 19 - 90 % and 84 - 93%, respectively, when helminth egg removal varies in the range of 26 - 93% and 89 - 95 %.

Table 6.1 Different levels of risks and removal of risks associated to helminth eggs in the effluent of UASB reactors and raw wastewater for 10mL of wastewater remaining in the ingested crops.

Helminth eggs content in wastewater (egg·L ⁻¹)	Helminth eggs removal (%)	P _{I(d)}	P _{I(A)}	P _{I(d)} removal (%)	P _{I(A)} removal (%)	Operational characteristics
100	0%	0.0741	0.9819	0%	0%	a
74	26%	0.0599	0.9601	19%	2%	b
7	93%	0.0077	0.3304	90%	66%	b
11	89%	0.0118	0.4607	84%	53%	c
5	95%	0.0055	0.2513	93%	74%	c

Notes

a: correspond to null removal of helminth eggs present in raw wastewater

b: correspond to the removal of helminth eggs in UASB reactors at subtropical conditions

c: correspond to the removal of helminth eggs at low temperature UASB reactors in Peruvian highlands

Regarding faecal coliforms, it must be realised that they are not necessarily pathogenic but the number of faecal coliforms gives a satisfactory indication whether the water has been contaminated by faeces (von Sperling et al., 2002; von Sperling et al., 2005). Therefore, the presence of faecal coliforms in a water source indicate a risk of getting infected by possible pathogens present in the water. The faecal coliform group includes the genus *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter*. *Escherichia coli* is a major enteric pathogen particularly in developing countries (Guentzel, 1996). It resides as a commensal gram negative bacterium in the intestinal tract and is excreted in faeces. Enterohemorrhagic *Escherichia coli* (EHEC; particularly serotype O157:H7) is a highly pathogenic variant and has been the cause of many diseases from faecally polluted food (Strachan et al., 2005).

For the dose-response relationship of faecal coliforms, no specific model was found in the revised literature (Rose and Gerba, 1991; Shuval et al., 1997; Tellez et al., 1997; Haas et al., 1999; Bartram et al., 2001; Howard et al., 2002; Westrell et al., 2004; Beltran and Jiménez, 2008; Drechsel et al., 2009; Devleesschauwer et al., 2014). For this reason it is assumed that *Escherichia coli* (EHEC) is present in the wastewater in the same number as the faecal coliforms content.

The dose-response model (see eq. 6.3) which best describes the pathogen-host survival risks Enterohemorrhagic *Escherichia coli* (EHEC) is the exponential dose-response model shown in eq. 6.5 (CAMRA, 2015):

$$P_{(d)} = 1 - e^{-r \times d} \quad (\text{eq. 6.5})$$

In eq. 6.5 "r" is a model parameter. Then, the exponential dose-response model ($r = 0.000218$) was used to estimate risk EHEC infection (Cornick and Helgerson, 2004; Strachan et al., 2005; CAMRA, 2015).

One of the limitations of UASB reactors regarding the direct agricultural use of treated wastewater is expressed mainly by an insufficient or negligible faecal coliform removal capacity (see Chapter 2 and 4) compared to the WHO guidelines of 1989. Therefore, the capacity of a DHS system for removing faecal coliforms from a domestic UASB reactor's effluent to produce wastewater quality suitable for agricultural reuse was studied in Chapter 5. Results of this research demonstrated an average faecal coliform removal for cube type DHS reactors without and with recirculation of 4.74 and 3.42 \log_{10} respectively. The curtain type DHS reactor showed the lowest performance for faecal coliforms removal (average removal of 1.25 \log_{10}).

Assuming an EHEC content of $1 \text{ E}+08 \text{ CFU} \cdot 100\text{mL}^{-1}$ in the influent of a DHS reactors and 3 scenarios of 4.74, 3.4 and 1.25 \log_{10} of EHEC removal, the expected risks of infection $P_{I(d)}$ and $P_{I(A)}$ are calculated and presented in Figure 6.3 and Figure 6.4 respectively. The three scenarios correspond to the effluents of the cube type DHS reactors without recirculation and with recirculation, and the curtain type DHS reactor respectively. It should be noticed that EHEC content of $1 \text{ E}+08 \text{ CFU} \cdot 100\text{mL}^{-1}$ in the influent wastewater is rather exaggerated considering the fact that EHEC is part of the larger group *Escherichia coli*, many of which cause little or no disease (Strachan et al., 2005; CAMRA, 2015). Therefore, the calculated risks of infection will be high.

For the $P_{I(d)}$ the exponential dose-response model was used (Figure 6.3). During the current calculation a wastewater volume between 1 and 10 mL that remains on the crops (dose) was assumed, however, to confirm it, further research is needed for a specific location. The number of ingested colony forming units (CFU) is directly proportional to the applied doses.

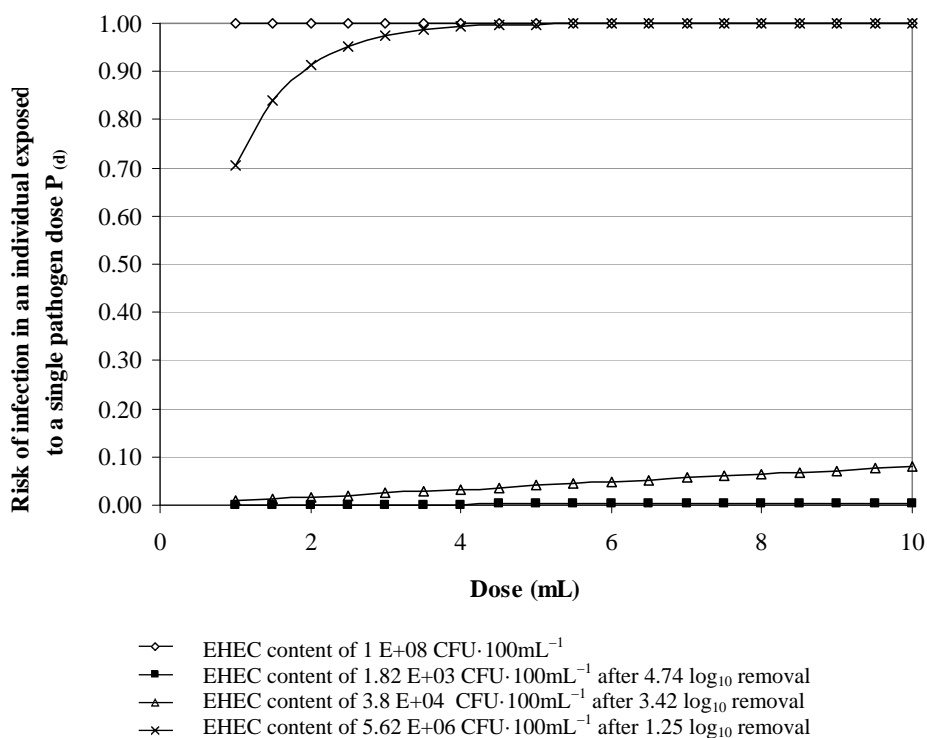


Figure 6.3 Risk of infection from a single dose of pathogens using wastewater containing Enterohemorrhagic *Escherichia coli* (EHEC) for irrigation of crops following the exponential dose-response model.

An EHEC content of 1 E+08 CFU·100mL⁻¹ in the influent, different rates for EHEC removal (4.74, 3.42 and 1.25 log₁₀) in treated wastewater and doses of wastewater volume between 1 and 10 mL that remain on the crops per exposure were assumed. The current results considers that the exposure group consumes raw crops once per week.

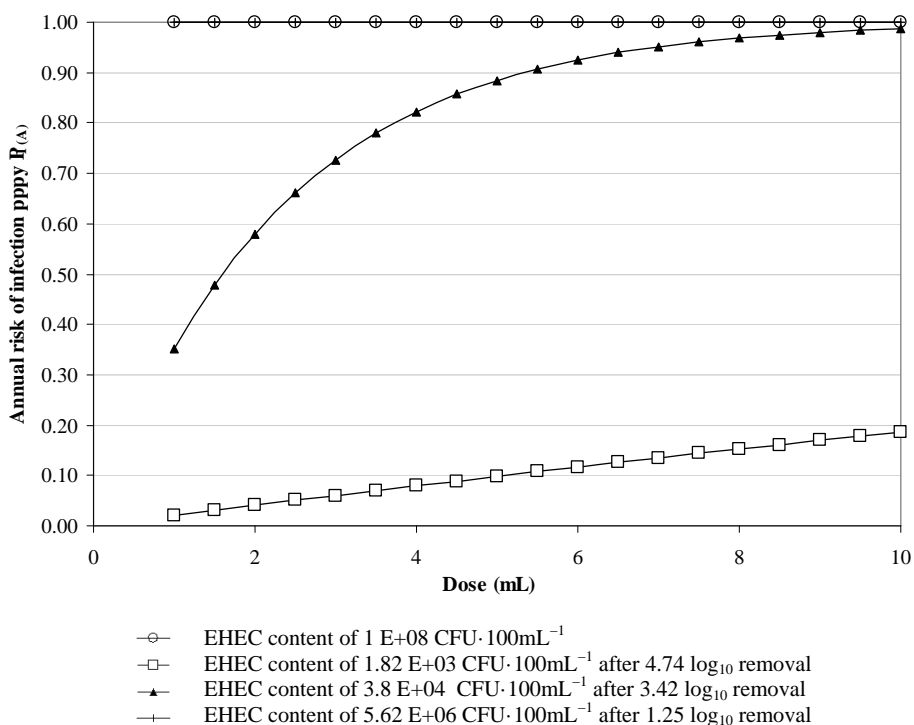


Figure 6.4 Annual risk of infection of an individual per year to Enterohemorrhagic *Escherichia coli* (EHEC) dose.

An EHEC content of $1 \text{ E}+08 \text{ CFU} \cdot 100\text{mL}^{-1}$ in the influent, different EHEC removal of 4.74, 3.42 and 1.25 \log_{10} , and, doses of wastewater between 1 and 10 mL that remain on the crops per exposure were assumed. The exposure group consumes raw crops once per week.

Particularly, the results of $P_{I(d)}$ and $P_{I(A)}$ for 10mL of wastewater remaining in the ingested crops and contaminated with EHEC are shown in Table 6.2. According to Table 6.2, on the one hand, it can be expected that all people from a group of 1000 000 inhabitants would get infected with EHEC if they ingest a 1 - 10 mL dose of raw wastewater which remains on the crops. On the other hand, if a cube type DHS reactor without recirculation is used to polish the effluent of UASB reactor, the amount of infected people can be reduced to 186900 inhabitants. For the latter calculation it was assumed that they ingest a 1 - 10 mL dose of a effluent wastewater from cube type DHS reactor which remains on the crops. It means that annual risks of infection can be reduced by approximately 81%. Then, it can be contemplated from Table 6.2 that the annual risks of becoming infected $P_{I(A)}$ can be highly reduced using a cube type DHS reactor without recirculation compared to the other studied DHS reactors. The risks of infection from a single dose of EHEC can be reduced 253 times with respect to the influent (from 1 to 0.004) after using a UASB+DHS reactor in cube type DHS reactor

without recirculation. For the DHS reactor with recirculation the risks can be reduced 13 times with respect to the anaerobically treated influent (from 1 to 0.0795). Finally for certain type DHS reactor, no significant reduction of risks may be expected (Table 6.2).

Table 6.2 Different levels of risks and removal of risks associated to EHEC in the effluent of UASB reactors and without UASB reactors for 10mL of wastewater remaining in the ingested crops.

EHEC in the effluent (CFU·100mL ⁻¹)	Log ₁₀ EHEC reductions	P _{I(d)}	P _{I(A)}	P _{I(d)} removal (%)	P _{I(A)} removal (%)	DHS type
100 000 000	0.00	1.0000	1.0000	0.0%	0.0%	a
1820	4.74	0.0040	0.1869	99.6%	81.3%	b
38019	3.42	0.0795	0.9867	92.0%	1.3%	c
5623413	1.25	1.0000	1.0000	0.0%	0.0%	d

Notes

a: No DHS reactor was used

b: cube type DHS reactors without recirculation

c: cube type DHS reactors with recirculation

d: curtain type DHS reactor

6.5.2 Approaches to achieve targets of WHO guidelines

The WHO guideline (2006) distinguish between different achieved pathogen reduction in terms of log₁₀ units for restricted and unrestricted irrigation. Irrigated crops included in these two groups were clearly described by Andreadakis *et al.*, (2006). Crops for restricted irrigation comprise forests and areas where access to the public is not expected, fodder, industrial crops, pastures, trees (whose fruits do not come into contact with the ground during collection), seed crops, crops that produce products which are processed before consumption. Unrestricted irrigation includes all other crops such as vegetables, vineyards and crops, with products that are consumed raw and produced in greenhouses.

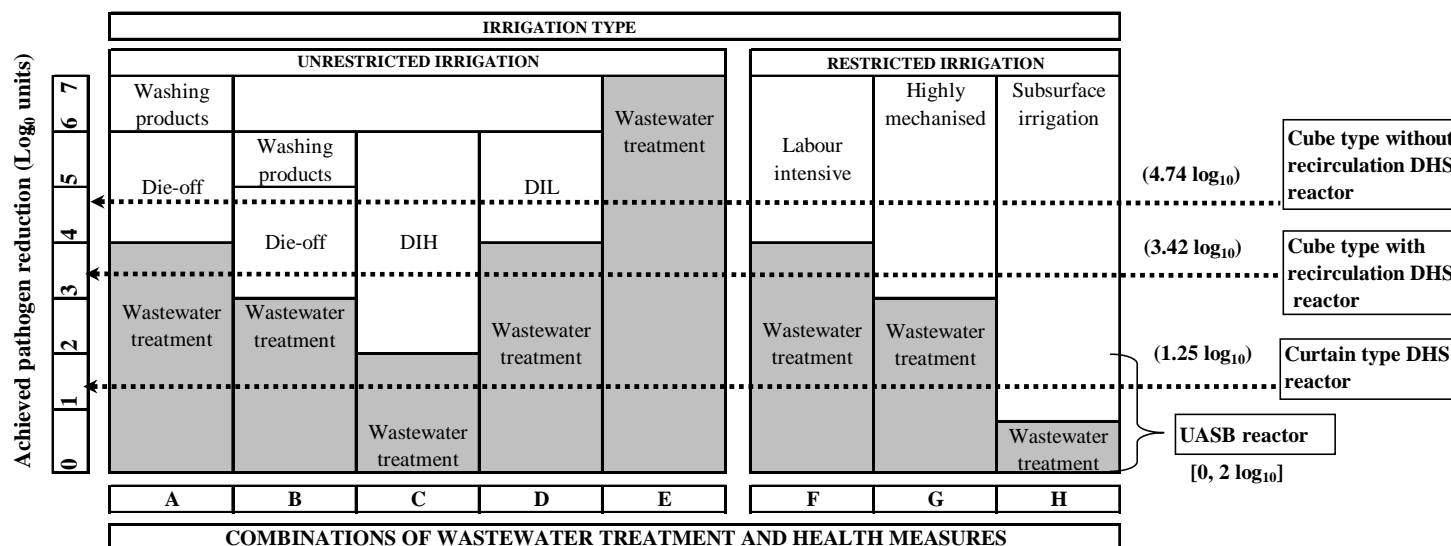
The WHO guideline (2006) recommends 6-7 and 3-4 log₁₀ units of pathogen reduction for unrestricted and, restricted irrigation in order to achieve a tolerable annual risk of infection of $\leq 10^{-6}$ DALY pppy. These recommendations need to be adjusted to a particular location after a health risk assessment. The indicated targets for pathogen reduction can be achieved by a combination of wastewater treatment and sanitary measures. Sanitary measures includes natural die-off of pathogens under field conditions, washing products before eating, combination of the filtering properties of the soil and type of irrigation (Oron *et al.*, 1999; WHO, 2006). Helminth eggs must be removed in all cases except for drip irrigation for high growing crops (DIH) like fruit trees, pecans trees (WHO, 2006).

During the current research, faecal coliforms were removed by the UASB reactor between 1 and 2 log₁₀ units (see Chapter 2 and 4). Therefore, according to the WHO guidelines of 2006, the effluent of UASB reactors can be used for restricted irrigation via subsurface irrigation (Type G) or for unrestricted irrigation of high growing crops using drip irrigation only when no crops are picked up from the soil (Type C). However, it can be noticed that each case should be analysed for a particular situation since probably drip irrigation is not feasible because of high maintenance costs in consideration of their easily clogging by suspended matter.

The results of Chapter 5 revealed that faecal coliform removal in a DHS reactor varied between 1 and 4 log₁₀ at relatively short HRT (between 1.3 and 2.3 h). Following the WHO guidelines of 2006 in terms of only faecal coliforms, the effluent of different water reuse options can be stated (Figure 6.5). The effluent of the cube type DHS reactor without recirculation with 4.74 log₁₀ average reduction of faecal coliforms can be used for all types of irrigation except type D. Second, the effluent of the cube type DHS reactor with recirculation and 3.42 log₁₀ average reduction of faecal coliforms can be used for both, unrestricted irrigation (types B and C) and restricted irrigation (types G and H). Finally, the effluent of the curtain type DHS reactor without recirculation with 1.25 log₁₀ average reduction is only suitable for unrestricted irrigation through a subsurface irrigation system which allow 7 log₁₀ reduction required for root crops.

The application of an appropriate post-treatment technology after UASB reactors and DHS reactors could further enhance the level of pathogen reduction. Particularly for UASB reactors, in order to get $\leq 10^{-6}$ DALY pppy, the remaining helminth eggs should be removed by post treatment technologies such as polishing ponds, constructed wetlands, overland flow, coagulation and sand filtration (Jimenez et al., 2001; Tchobanoglous et al., 2003; von Sperling et al., 2005). In order to reduce faecal coliforms, some disinfection technologies, depending of the water use, were suggested by previous researchers (Cheremisinoff, 2001; Zhou and Smith, 2002; Von Sperling, 2005; Bracho et al., 2006). These disinfection technologies include maturation ponds, slow sand filters, advanced oxidation processes (AOPs), UV irradiation, chemical disinfectants such as bromine, chlorine and iodine. Regarding chemical disinfection methods, care should be taken for the formation of disinfection by-products, such a organochlorides, which are carcinogenic and persistent and accumulate in the field.

Regarding other constituents content, the guidelines developed by WHO does not set any value regarding BOD, COD, metals or nutrients content for water reuse. It should be realised that organic matter as such is not harmful and nutrients are even beneficial for the farmers (Van Lier and Huibers, 2004; Van Lier and Huibers, 2004; van Lier and Huibers, 2010). Additionally, the WHO guidelines suggest to take into account good agricultural practices to minimize the environmental impacts like salinisation of soil and contamination of water resources.



- A: Treatment is followed by pathogen die-off between the last irrigation and consumption. Root crops that can be eaten uncooked
- B: Treatment is followed by pathogen die-off between the last irrigation and consumption and washing products. Non-root salad crops and vegetables can be eaten uncooked
- C: Treatment is followed by drip irrigation for high growing crops (DIH)
- D: Treatment is followed by drip irrigation for low growing crops (DIL)
- E: Crops can be irrigated immediately after treatment
- F: Treatment is followed by labour intensive restricted irrigation
- G: Treatment is followed by highly mechanised restricted irrigation
- H: Treatment is followed by sub-surface irrigation

Source: Adapted from WHO guideline (2006)

Figure 6.5 Options for water reuse after the studied UASB and DHS reactors according to WHO guidelines of 2006 in order to achieve the health based target of $\leq 10^{-6}$ DALY per person per year. All combinations require a helminth eggs content less than $1 \text{ egg} \cdot \text{L}^{-1}$, except when treatment is followed for drip irrigation for high growing crops (DIH).

6.6 Prospects for water reuse and excess sludge

Application of an appropriate wastewater treatment technology is the key component for increasing the coverage of wastewater treatment in developing countries. It often requires proven technology, achievement of targeted parameters, low investment costs, low operation and maintenance costs, compared with fully mechanical or aerated technologies that are often applied in industrialised countries (Libhaber and Orozco-Jaramillo, 2012). Based on the current research it can be concluded that the UASB reactor can be an appropriate technology in developing countries. However, it is necessary to add a post treatment unit to polish the wastewater until achieving the established target regarding pathogen removal, especially to minimize the associated health risks when the treated water is considered for agricultural reuse.

Special care should be taken into account for the excess sludge coming from the wastewater treatment process, since it contains high amounts of pathogens (Navarro et al., 2009). Then this sludge must be stabilised, dewatered (reaching minimally 25 % DS) and disinfected (Ødegaard et al., 2002). The disinfection step must include inactivation of viable helminth eggs. Different alternatives has been described in the literature (Ødegaard et al., 2002; Jimenez, 2007; Koné et al., 2007; Fidjeland, 2010; Fidjeland et al., 2013; Magri et al., 2015) to disinfect the sludge. These technologies include alkaline post stabilization, acid treatment, anaerobic digestion, thermal drying of anaerobically digested sludge, ammonia sanitisation, composting, dehydration and electron beam irradiation.

6.7 Recommendations for future research

- The presence of helminth eggs in the treated wastewater poses risks to people if the treated wastewater is used for agricultural irrigation (WHO, 2006). During the present research, it was demonstrated that from the technological point of view the filtration capacity of sludge in UASB reactors (denominated in this research “sludge filtration capacity”) can contribute to reduce the helminth egg content from the influent. In wastewater streams characterised by high helminth egg content, anaerobic sludge filtration would provide advantages for environmental protection specially by reducing health risks. However, during the sludge filtration process in the UASB reactor containing a flocculent anaerobic sludge bed, it is necessary to study the hydraulic influence of density, viscosity, upflow velocity, the biogas production and temperature. The resulting sludge filtration capacity might be insufficient to attain the restrictive residual helminth egg concentration.
- During the research on the sludge filtration capacity under subtropical conditions several unexpected damages were observed in the morphology of helminth eggs in the anaerobic environment. Therefore, it is recommended to study the disinfecting

microbial capacity of anaerobic sludge from UASB reactors over helminth eggs viability. Results can provide useful information to optimise pathogen removal.

- Helminth eggs removed during the anaerobic filtration process are accumulated in the excess sludge (Navarro et al., 2009). Therefore, more investigation using low cost technologies must be performed in full scale plants in order to inactivate helminth eggs from the indicated excess sludge, especially in developing countries.
- This thesis has shown that DHS reactors were able to remove faecal coliforms in the range of 1.25 to 4.74 \log_{10} using HRT values of less than 2.5 hours. These results makes this technology apparently feasible to apply when land area is limited. Further research is needed on an appropriate scale in order to study the influence of media porosity, type of flow, short circuiting and biofilm formation on and inside the medium during the treatment process on faecal coliform removal. The identification of microorganism and mechanisms involved in faecal coliform removal should be addressed. The removal of specific pathogens may also be studied in order to determine the extent of wastewater treatment, different types of treated wastewater reuse and risks to which humans are exposed to. Further research is also needed to study the viability of helminth eggs through the developed biofilm in a DHS reactor.
- In order to address the water reuse approach using WHO guidelines from 2006, the removal capacity of DHS reactors with respect to the most known pathogens in developing countries like *Salmonella spp.*, *Shigella spp.*, *Escherichia coli* (for example EHEC serotype O157:H7) and helminth eggs should be investigated.
- Further research needs to be performed in developing countries to determine the DALY loss per case, the median infection dose (N_{50}) and the effect on pathogen reduction by combining wastewater treatment technologies and health measures proposed by the WHO (2006). The obtained information will allow to calculate the risk of becoming infected by ingesting a dose from a single exposure [$P_{I(d)}$] and the annual risk of infection [$P_{I(A)}$], and therefore apply WHO guidelines 2006 in developing countries.

6.8 References

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General summary

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The use of treated wastewater in agricultural irrigation becomes an attractive alternative, especially when water resources are scarce. However, since domestic wastewater includes discharges from toilet, kitchen and shower, it contains human pathogens. The presence of pathogens in wastewater increases human health and environmental risks. Anaerobic wastewater treatment will only limitedly reduce the pathogenic content and thus may need an additional post treatment step to fulfil reuse criteria. So far, the exact pathogen removal capacity of anaerobic reactors remains unclear and so does risk reduction by implementing anaerobic treatment with complementary post treatment. Since at present, raw or partially treated sewage is commonly used in irrigated agriculture, a detailed insight in the pathogen removal capacity of compact, cost-effective treatment systems is of crucial importance. This research describes the effect of Upflow Anaerobic Sludge Blanket (UASB) reactors in combination with specific post-treatment steps during the wastewater treatment. During the research, particular emphasis was placed on the use of UASB reactors due to its compactness and low operation and maintenance costs compared to aerobic technologies, such as activated sludge, that are commonly used in industrialised countries.

Chapter 1 describes the main pathogens, prevailing in wastewater. It also shows the main benefits of the use of reclaimed domestic wastewater. This chapter includes an overview of existing wastewater treatment technologies applied for wastewater reclamation in developing countries. Various “treatment trains” are presented consisting of combinations of a UASB reactor with different post treatment techniques. These "treatment trains" were categorized in systems that require a significant amount of land area (land-based dimensioned design) and those that do not (volumetric based design). Latter systems are much more compact.

Chapter 2 presents the research on determining the filtration capacity of anaerobic sludge for helminth eggs at different operational conditions in UASB reactors. During the experiments an operational temperature of 4 °C was applied to minimise the bioactivity in the sludge bed. Filtration tests were conducted under different upflow velocities. Before filtration tests, a sludge washing phase was applied to minimise the impact of the preceding experimental conditions. The study was performed in two phases: the first one, using latex beads simulating helminth eggs, and the second one, using real helminth eggs. During the first phase of the research, the anaerobic sludge filtration capacity was evaluated using digested sludge from a primary sludge digester operated at a hydraulic retention time (HRT) of 30 days at 35 °C. The digester is part of the wastewater treatment plant (WWTP) located in Ede, The Netherlands. Four types of test series were conducted to study the sludge filtration capacity to remove latex beads: impact of upflow velocity, impact of degree of sludge stabilisation, impact of sludge bed volume, and control tests. During the experiments, four upflow velocities, namely 0.3, 0.5, 1, and 1.5 m·h⁻¹, were tested. For the second phase, a control test without

sludge was used to study the removal of the latex beads solely by sedimentation. For the second phase, a flocculent UASB sludge was used. The latter sludge was taken from the 536 m³ pilot-scale UASB reactor located at the Research Center for Wastewater Treatment and Hazardous Wastes (CITRAR) at the campus of the National University of Engineering (Lima, Peru). Microbiological analysis included total and faecal coliforms and the identification of the most common helminth eggs species.

Results from the first phase showed a decreased removal efficiency of latex beads at increased upflow velocities. With regards to the impact of degree of sludge stabilisation, no significant effect was observed. Increasing the sludge bed volume did not have a significant effect on the latex beads removal. At an upflow velocity of 0.3 m·h⁻¹, no significant differences were observed in the latex beads removal efficiency between the control reactor and the reactor with a sludge bed. Results of the second phase showed that the most common helminth eggs found in the studied wastewater were *Ascaris lumbricoides*, *Trichuris spp.* and *Strongyloides spp.* It was demonstrated that at 4°C and low upflow velocities of 0.30 and 0.39 m·h⁻¹, respectively, 100% removal for both latex beads and helminth eggs is achieved. Lower removal percentages were found at higher upflow velocities. Additionally, 100% latex beads removal was obtained at plain settling at a theoretical settling velocity of 0.5 m·h⁻¹ at 4°C. Total and faecal coliform removal was less than 80% at all studied upflow velocities.

Chapter 3 describes the experiments on assessing the anaerobic sludge filtration capacity regarding helminth eggs under subtropical conditions. Two lab-scale UASB reactors at average ambient temperatures between 17°C and 29°C were used. *Ascaris suum* helminth eggs were selected as model eggs, considering their similarity in terms of size and morphology to *Ascaris lumbricoides*, a human pathogen. *Ascaris suum* eggs were obtained from female parasites of infected pigs. The helminth egg concentration in the influent tank varied between 20–50 eggs·L⁻¹. The sludge filtration capacity tests were performed applying upflow velocities between 0.09 and 0.68 m·h⁻¹. The sludge filtration capacity test was performed at ambient temperatures. The sludge bed thickness was in the range of 0.30–0.40 and 0.50–0.60 m. This range is coincided with 19–25% and 31–38% of the total UASB reactor height. The tests showed a reciprocal correlation between the average helminth egg removal efficiency and upflow velocity. The average helminth egg removal was between 34–100%, 30–91% and 34–56%, when the sludge bed height in the reactor was 19–25%, 31–38% and 38–44%, respectively, at upflow velocities varying between 0.09 and 0.68 m·h⁻¹.

Chapter 4 presents the research on assessing the anaerobic sludge filtration capacity of UASB reactors at low ambient temperatures. The research was performed using a lab-scale UASB reactor of 29 L and domestic wastewater with temperatures varying between 11 and 14°C for a period of 22 weeks after the start-up of the reactor. The scenario of the research was the city of Puno situated in the Peruvian Andes at an altitude of 3810 m.a.s.l. The effect of several upflow velocities, viz. 0.12, 0.14, 0.16,

0.20, 0.27 and 0.41 $\text{m}\cdot\text{h}^{-1}$, on the reduction of pathogens was tested. The pathogens indicators were helminth eggs, faecal coliforms and *E. coli*. The average helminth eggs influent concentration was $194 \pm 79 \text{ eggs}\cdot\text{L}^{-1}$. *Ascaris lumbricoides* was the most common helminth egg found in the influent (average of $142 \text{ eggs}\cdot\text{L}^{-1}$) and effluent (average of $19 \text{ eggs}\cdot\text{L}^{-1}$). Results show that the sludge filtration capacity varied between 89 and 95% for helminth egg removal. The observed high helminth egg removal could be related to the lower biogas production at low temperatures that probably limited the degree of turbulence in the reactor. Faecal coliform removal varied between 0.9 and 2.1 \log_{10} and *E. coli* removal varied between 0.8 and 1.6 \log_{10} . The total Chemical Oxygen Demand (COD) removal was low and varied between 37 and 62%. The best performance in terms of removal of helminth eggs, total COD and turbidity was obtained at the lowest upflow velocity of $0.12 \text{ m}\cdot\text{h}^{-1}$. The results confirmed that post-treatment is required to further remove pathogens to achieve the World Health Organization (WHO) guidelines.

Chapter 5 describes experiments to determine the capacity for faecal coliform removal by Down Flow Hanging Sponge (DHS) reactors as a post-treatment alternative for the effluent of UASB reactors by conducting three long-term continuous lab-scale experiments. Three different DHS reactors were evaluated. These reactors were the cube type DHS (G1) without and with recirculation and the curtain type DHS (G2). The porosity of the applied medium was 91, 87 and 47% while the respective HRT was 2.9, 1.5 and 2.5 h. The organic loading rate was 0.86, 0.53 and 0.24 $\text{kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ while their corresponding hydraulic loading rate was 1.92, 2.97 and 1.32 $\text{m}^3\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ correspondingly. Cube type (G1) DHS reactors showed the best capacity for faecal coliform removal. According to the WHO guidelines (1989), the effluent with an average faecal coliforms content of $2.1\text{E}+02 \text{ CFU}\cdot 100 \text{ mL}^{-1}$, produced in the cube type reactors without recirculation, can be used for unrestricted irrigation (Category A). Restricted irrigation of category B is assigned to the effluent of cube type reactor with recirculation and an average faecal coliforms content in the effluent of $3.4\text{E}+04 \text{ CFU}\cdot 100\text{mL}^{-1}$. Restricted irrigation of category C is ascribed to the effluent of curtain type reactor with an average effluent coliform content of $5.9\text{E}+05 \text{ CFU}\cdot 100\text{mL}^{-1}$. The average Biochemical Oxygen Demand (BOD_5) reduction varied between 80.9 and 93.6 with a BOD_5 content in the effluent of 19.5 and 6.2 $\text{mg}\cdot\text{L}^{-1}$ in cube type without and with recirculation. For the curtain type reactor, the average BOD_5 reduction was 84.9% which correspondent to a BOD_5 content of 14.9 $\text{mg}\cdot\text{L}^{-1}$ in the effluent. With regards to the effluent BOD_5 concentrations, all researched DHS reactors complied with the United States Environmental Protection Agency (USEPA) standards for restricted irrigation of non-food crops, like pasture for milking animals, fodder, fibre, and seed crops.

Chapter 6 includes the results and discussion and reflects on the presented work in the whole thesis. The results show that especially the helminth egg removal by anaerobic sludge filtration is a promising alternative for pre-treatment of wastewater especially for locations with space limitations where the application of large land-based treatment

systems is simply not possible. Firstly, special attention is given to the residual public health risks after the application of UASB reactors for water reclamation in subtropical conditions and low temperatures. Secondly, the residual health risk after the application of DHS reactors for polishing the UASB reactor effluents, under subtropical conditions, was analyzed. The health risks analysis was carried out following the recommendations of the 2006 WHO guidelines. The corresponding annual risks of infection (named $P_{I(A)}$) in an individual, due to the ingestion of an average number of organisms in a specified dose was determined. It was assumed that a dosage of wastewater volume between 1 and 10 mL remained on the crops per exposure. A reduction of the annual risks of infection can be expected when applying any of the researched treatment systems. The assessed reduction is in the range of 19-90% at a helminth egg removal in UASB reactors between 26 and 93%, at subtropical conditions, i.e. average wastewater temperature between 17 °C and 29 °C. The observed reduction of annual risks is in the range of 84-93% when helminth egg removal in UASB reactors varies in the range of 89-95% at low temperature conditions, i.e. average wastewater temperatures between 11 and 14 °C.

For DHS reactors, the annual risks analysis was performed using *Enterohemorrhagic Escherichia coli* (EHEC) as a pathogen indicator. Results show that annual risks are lowest using the effluent of a cube type DHS reactor compared to the effluent of curtain type reactors. During this theoretical health risk analysis it was concluded that when using a cube type DHS reactor without recirculation, the risks of infection for water reuse can be reduced 253 times compared to untreated wastewater reuse. Similarly, for the DHS reactor with recirculation, the risks of infection can be reduced 13 times compared to untreated reuse. However, for the curtain type DHS reactor no significant reduction of health risks is expected.

This research clearly shows the application potentials of the compact wastewater treatment system consisting of a UASB reactor followed by a cube type DHS reactor for the reclamation of domestic sewage for agriculture irrigation. Results show a distinct reduction in human health risks compared to the use of untreated sewage, but also compared to the use of solely a UASB reactor.

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effective treatment systems is of crucial importance. This research describes the effect of Upflow Anaerobic Sludge Blanket (UASB) reactors in combination with specific post-treatment steps during the wastewater treatment. During the research, particular emphasis was placed on the use of UASB reactors due to its compactness and low operation and maintenance costs compared to aerobic technologies, such as activated sludge, that are commonly used in industrialised countries.

Chapter 1 describes the main pathogens, prevailing in wastewater. It also shows the main benefits of the use of reclaimed domestic wastewater. This chapter includes an overview of existing wastewater treatment technologies applied for wastewater reclamation in developing countries. Various “treatment trains” are presented consisting of combinations of a UASB reactor with different post treatment techniques. These "treatment trains" were categorized in systems that require a significant amount of land area (land-based dimensioned design) and those that do not (volumetric based design). Latter systems are much more compact.

Chapter 2 presents the research on determining the filtration capacity of anaerobic sludge for helminth eggs at different operational conditions in UASB reactors. During the experiments an operational temperature of 4 °C was applied to minimise the bioactivity in the sludge bed. Filtration tests were conducted under different upflow velocities. Before filtration tests, a sludge washing phase was applied to minimise the impact of the preceding experimental conditions. The study was performed in two phases: the first one, using latex beads simulating helminth eggs, and the second one, using real helminth eggs. During the first phase of the research, the anaerobic sludge filtration capacity was evaluated using digested sludge from a primary sludge digester operated at a hydraulic retention time (HRT) of 30 days at 35 °C. The digester is part of the wastewater treatment plant (WWTP) located in Ede, The Netherlands. Four types of test series were conducted to study the sludge filtration capacity to remove latex beads: impact of upflow velocity, impact of degree of sludge stabilisation, impact of sludge bed volume, and control tests. During the experiments, four upflow velocities, namely 0.3, 0.5, 1, and 1.5 m·h⁻¹, were tested. For the second phase, a control test without sludge was used to study the removal of the latex beads solely by sedimentation. For the second phase, a flocculent UASB sludge was used. The latter sludge was taken from the 536 m³ pilot-scale UASB reactor located at the Research Center for Wastewater Treatment and Hazardous Wastes (CITRAR) at the campus of the National University of Engineering (Lima, Peru). Microbiological analysis included total and faecal coliforms and the identification of the most common helminth eggs species.

Results from the first phase showed a decreased removal efficiency of latex beads at increased upflow velocities. With regards to the impact of degree of sludge stabilisation, no significant effect was observed. Increasing the sludge bed volume did not have a significant effect on the latex beads removal. At an upflow velocity of 0.3 m·h⁻¹, no significant differences were observed in the latex beads removal efficiency between the

control reactor and the reactor with a sludge bed. Results of the second phase showed that the most common helminth eggs found in the studied wastewater were *Ascaris lumbricoides*, *Trichuris spp.* and *Strongyloides spp.* It was demonstrated that at 4°C and low upflow velocities of 0.30 and 0.39 m·h⁻¹, respectively, 100% removal for both latex beads and helminth eggs is achieved. Lower removal percentages were found at higher upflow velocities. Additionally, 100% latex beads removal was obtained at plain settling at a theoretical settling velocity of 0.5 m·h⁻¹ at 4°C. Total and faecal coliform removal was less than 80% at all studied upflow velocities.

Chapter 3 describes the experiments on assessing the anaerobic sludge filtration capacity regarding helminth eggs under subtropical conditions. Two lab-scale UASB reactors at average ambient temperatures between 17°C and 29°C were used. *Ascaris suum* helminth eggs were selected as model eggs, considering their similarity in terms of size and morphology to *Ascaris lumbricoides*, a human pathogen. *Ascaris suum* eggs were obtained from female parasites of infected pigs. The helminth egg concentration in the influent tank varied between 20–50 eggs·L⁻¹. The sludge filtration capacity tests were performed applying upflow velocities between 0.09 and 0.68 m·h⁻¹. The sludge filtration capacity test was performed at ambient temperatures. The sludge bed thickness was in the range of 0.30–0.40 and 0.50–0.60 m. This range is coincided with 19–25% and 31–38% of the total UASB reactor height. The tests showed a reciprocal correlation between the average helminth egg removal efficiency and upflow velocity. The average helminth egg removal was between 34–100%, 30–91% and 34–56%, when the sludge bed height in the reactor was 19–25%, 31–38% and 38–44%, respectively, at upflow velocities varying between 0.09 and 0.68 m·h⁻¹.

Chapter 4 presents the research on assessing the anaerobic sludge filtration capacity of UASB reactors at low ambient temperatures. The research was performed using a lab-scale UASB reactor of 29 L and domestic wastewater with temperatures varying between 11 and 14°C for a period of 22 weeks after the start-up of the reactor. The scenario of the research was the city of Puno situated in the Peruvian Andes at an altitude of 3810 m.a.s.l. The effect of several upflow velocities, viz. 0.12, 0.14, 0.16, 0.20, 0.27 and 0.41 m·h⁻¹, on the reduction of pathogens was tested. The pathogens indicators were helminth eggs, faecal coliforms and *E. coli*. The average helminth eggs influent concentration was 194 ± 79 eggs·L⁻¹. *Ascaris lumbricoides* was the most common helminth egg found in the influent (average of 142 eggs·L⁻¹) and effluent (average of 19 eggs·L⁻¹). Results show that the sludge filtration capacity varied between 89 and 95% for helminth egg removal. The observed high helminth egg removal could be related to the lower biogas production at low temperatures that probably limited the degree of turbulence in the reactor. Faecal coliform removal varied between 0.9 and 2.1 log₁₀ and *E. coli* removal varied between 0.8 and 1.6 log₁₀. The total Chemical Oxygen Demand (COD) removal was low and varied between 37 and 62%. The best performance in terms of removal of helminth eggs, total COD and turbidity was obtained at the lowest upflow velocity of 0.12 m·h⁻¹. The results confirmed that post-

treatment is required to further remove pathogens to achieve the World Health Organization (WHO) guidelines.

Chapter 5 describes experiments to determine the capacity for faecal coliform removal by Down Flow Hanging Sponge (DHS) reactors as a post-treatment alternative for the effluent of UASB reactors by conducting three long-term continuous lab-scale experiments. Three different DHS reactors were evaluated. These reactors were the cube type DHS (G1) without and with recirculation and the curtain type DHS (G2). The porosity of the applied medium was 91, 87 and 47% while the respective HRT was 2.9, 1.5 and 2.5 h. The organic loading rate was 0.86, 0.53 and 0.24 kg COD·m⁻³·d⁻¹ while their corresponding hydraulic loading rate was 1.92, 2.97 and 1.32 m³·m⁻²·d⁻¹ correspondingly. Cube type (G1) DHS reactors showed the best capacity for faecal coliform removal. According to the WHO guidelines (1989), the effluent with an average faecal coliforms content of 2.1E+02 CFU·100 mL⁻¹, produced in the cube type reactors without recirculation, can be used for unrestricted irrigation (Category A). Restricted irrigation of category B is assigned to the effluent of cube type reactor with recirculation and an average faecal coliforms content in the effluent of 3.4E+04 CFU·100mL⁻¹. Restricted irrigation of category C is ascribed to the effluent of curtain type reactor with an average effluent coliform content of 5.9E+05 CFU·100mL⁻¹. The average Biochemical Oxygen Demand (BOD₅) reduction varied between 80.9 and 93.6 with a BOD₅ content in the effluent of 19.5 and 6.2 mg·L⁻¹ in cube type without and with recirculation. For the curtain type reactor, the average BOD₅ reduction was 84.9% which correspondent to a BOD₅ content of 14.9 mg·L⁻¹ in the effluent. With regards to the effluent BOD₅ concentrations, all researched DHS reactors complied with the United States Environmental Protection Agency (USEPA) standards for restricted irrigation of non-food crops, like pasture for milking animals, fodder, fibre, and seed crops.

Chapter 6 includes the results and discussion and reflects on the presented work in the whole thesis. The results show that especially the helminth egg removal by anaerobic sludge filtration is a promising alternative for pre-treatment of wastewater especially for locations with space limitations where the application of large land-based treatment systems is simply not possible. Firstly, special attention is given to the residual public health risks after the application of UASB reactors for water reclamation in subtropical conditions and low temperatures. Secondly, the residual health risk after the application of DHS reactors for polishing the UASB reactor effluents, under subtropical conditions, was analyzed. The health risks analysis was carried out following the recommendations of the 2006 WHO guidelines. The corresponding annual risks of infection (named P_{I(A)}) in an individual, due to the ingestion of an average number of organisms in a specified dose was determined. It was assumed that a dosage of wastewater volume between 1 and 10 mL remained on the crops per exposure. A reduction of the annual risks of infection can be expected when applying any of the researched treatment systems. The assessed reduction is in the range of 19-90% at a helminth egg removal in UASB reactors between 26 and 93%, at subtropical conditions,

i.e. average wastewater temperature between 17 °C and 29 °C. The observed reduction of annual risks is in the range of 84-93% when helminth egg removal in UASB reactors varies in the range of 89-95% at low temperature conditions, i.e. average wastewater temperatures between 11 and 14 °C.

For DHS reactors, the annual risks analysis was performed using *Enterohemorrhagic Escherichia coli* (EHEC) as a pathogen indicator. Results show that annual risks are lowest using the effluent of a cube type DHS reactor compared to the effluent of curtain type reactors. During this theoretical health risk analysis it was concluded that when using a cube type DHS reactor without recirculation, the risks of infection for water reuse can be reduced 253 times compared to untreated wastewater reuse. Similarly, for the DHS reactor with recirculation, the risks of infection can be reduced 13 times compared to untreated reuse. However, for the curtain type DHS reactor no significant reduction of health risks is expected.

This research clearly shows the application potentials of the compact wastewater treatment system consisting of a UASB reactor followed by a cube type DHS reactor for the reclamation of domestic sewage for agriculture irrigation. Results show a distinct reduction in human health risks compared to the use of untreated sewage, but also compared to the use of solely a UASB reactor.

Algemene samenvatting

Algemene samenvatting

Het gebruik van gezuiverd huishoudelijk afvalwater in de landbouw wordt als een aantrekkelijk alternatief gezien, vooral wanneer water schaars is. Huishoudelijk afvalwater bevat echter toilet-, keuken- en douchewater en veel ziekteverwekkers (pathogene organismen). De aanwezigheid van ziekteverwekkers in gezuiverd afvalwater kan bij waterhergebruik leiden tot grote risico's voor de volksgezondheid. Anaerobe afvalwaterzuivering kan het aantal pathogenen in het afvalwater weliswaar verlagen, maar voor een vergaande verwijdering van pathogenen is een na-geschakelde zuivering nodig. Hergebruik van vergaand gezuiverd stedelijk afvalwater in de geïrrigeerde landbouw zal de risico's voor milieu en volksgezondheid in grote mate reduceren in vergelijking met de veelvoorkomende toepassing van ongezuiverd rioolwater. Dit onderzoek beschrijft de mogelijkheden van Upflow Anaerobic Sludge Blanket (UASB) reactoren in combinatie met specifieke nabehandelingsstappen ten behoeve van de verwijdering van pathogene organismen. Tijdens het onderzoek werd met name gekeken naar UASB-reactoren vanwege hun compactheid en lage exploitatie- en onderhoudskosten in vergelijking met aerobe technologieën, zoals actief slib.

Hoofdstuk 1 beschrijft de belangrijkste ziekteverwekkers die voorkomen in afvalwater. Het beschrijft tevens de belangrijkste voordelen van het gebruik van gezuiverd huishoudelijk afvalwater. Ook bevat het hoofdstuk een overzicht van technologieën die gebruikt worden voor de zuivering van afvalwater in ontwikkelingslanden. De diverse behandelingsketens, bestaande uit een combinatie van een UASB-reactor met verschillende nazuiveringstechnieken, worden in dit hoofdstuk nader toegelicht. De zuiveringscombinaties zijn ingedeeld in systemen die op basis van landoppervlak zijn gedimensioneerd (land-based) en systemen die volumetrisch zijn gedimensioneerd (compact).

Hoofdstuk 2 beschrijft onderzoek naar de filtratie capaciteit van anaeroob slib voor wormeitjes onder verschillende operationele omstandigheden in UASB-reactoren. Tijdens de experimenten werd een temperatuur van 4°C toegepast, om de biologische activiteit van het slib-bed te minimaliseren. Filtratie proeven werden uitgevoerd bij verschillende opwaartse snelheden. Voor de proeven werd een slib spoelfase toegepast om het effect van de voorafgaande experimentele omstandigheden te minimaliseren. Het onderzoek werd uitgevoerd in twee fasen. In de eerste fase werden latex korrels gebruikt als surrogaat voor wormeitjes in afvalwater. De tweede fase bevatte afvalwater met echte wormeitjes. Gedurende de eerste fase van het onderzoek werd de filtratiecapaciteit van slib onderzocht, waarbij gebruikt werd gemaakt van uitgegist primair slib afkomstig van een 35°C slibgistingstank die bedreven werd met een verblijftijd van 30 dagen op de rioolwaterzuiveringsinstallatie (rwzi) in Ede, Nederland. Er zijn 4 testen uitgevoerd om de slibfiltratiecapaciteit van latex korrels te bestuderen. De toegepaste testvariabelen waren opwaartse snelheid, mate van slibstabilisatie, en de invloed van het slib-bed volume. Er zijn 4 opwaartse snelheden getest, namelijk 0,3, 0,5, 1, en 1,5 m·h⁻¹. Voor

de tweede fase, werd een controle test zonder slib gebruikt om de verwijdering van latex korrels door alleen bezinking te bestuderen. Voor de tweede fase werd vlokkig slib uit een UASB gebruikt. Dit slib was afkomstig van een 536 m³ UASB testreactor van CITRAR in de stad Lima (Peru). Met behulp van microbiologische analyses werden de totale en fecale colibacteriën en de meest voorkomende worm ei soorten geïdentificeerd.

Resultaten uit de eerste fase lieten een verminderd verwijderingsrendement zien van latex korrels bij een verhoogde opwaartse snelheid. De mate van slibstabilisatie had geen significant effect. Het verhogen van het slib-bedvolume had geen significant effect op de verwijdering van de latex korrels. Bij een opwaartse snelheid van 0.3 m·h⁻¹, werden geen significante verschillen gevonden in verwijderingsrendement van latex korrels tussen de referentiereactor met alleen water en de reactor met slibbed. Uit de resultaten van de testen uit de tweede fase blijkt dat de meest voorkomende eitjes in het onderzochte afvalwater, *Ascaris lumbricoides*, *Trichuris spp.* en *Strongyloides spp* waren. Bij 4 °C en lage opwaartse snelheden van respectievelijk 0.30 en 0.39 m·h⁻¹, werd 100% verwijdering bereikt van zowel latex korrels als wormeitjes. Hogere opwaartse snelheden resulteerden in een verminderde verwijdering van korrels en eitjes. Daarnaast werd 100% van de latex korrels verwijderd met behulp van eenvoudige bezinking bij een theoretische bezinksnelheid van 0.5 m·h⁻¹ bij 4 °C. Bij alle toegepaste opwaartse snelheden was de verwijdering van zowel totaal coliformen als fecale coliformen minder dan 80%.

Hoofdstuk 3 beschrijft de resultaten van onderzoek naar de filtratiecapaciteit van anaeroob slib voor wormeitjes onder subtropische omstandigheden. Hiertoe, werden twee labschaal UASB-reactoren met gemiddelde temperaturen tussen de 17°C en 29°C gebruikt. *Ascaris suum* wormeitjes werden geselecteerd als model eitjes, aangezien ze qua grootte en morfologie op humane pathogene wormeitjes lijken. *Ascaris suum* eitjes werden verkregen uit vrouwelijke parasieten van besmette varkens. De concentratie van wormeitjes in de influent tank varieerde tussen 20 en 50 eitjes·L⁻¹. De slibfiltratiecapaciteitstesten werden uitgevoerd bij omgevingstemperatuur en opwaartse snelheden tussen 0.09 en 0.68 m·h⁻¹. De hoogte van het slibbed was tussen 0.30 en 0.40 m en tussen 0.50 en 0.60 m. Deze slibbedhoogte komt overeen met 19–25% en 31–38% van de totale UASB-reactor hoogte. De testen toonde een omgekeerde correlatie tussen de opwaartse snelheid en de verwijdering van wormeitjes. De gemiddelde verwijdering van wormeitjes lag tussen 34–100%, 30–91%, en 34–56% bij een slibbed hoogte van respectievelijk 19–25%, 31–38%, en 38–44%. De opwaartse snelheden varieerden tussen 0.09 en 0.68 m·h⁻¹.

Hoofdstuk 4 presenteert het onderzoek naar de anaerobe slibfiltratiecapaciteit van UASB-reactoren bij lage omgevingstemperaturen, dat werd uitgevoerd in de plaats Puno (Peru) op een hoogte van 3810 m boven zeeniveau. De filtratie experimenten zijn uitgevoerd in een labschaal UASB-reactor van 29 L met huishoudelijk afvalwater als influent bij temperaturen tussen 11 en 14 °C. Het verwijderingsrendement van

wormeitjes, fecale coliformen en *E. coli*. is vastgesteld bij opwaartse snelheden van 0.12, 0.14, 0.16, 0.20, 0.27, en 0.41 m·h⁻¹. De gemiddelde concentratie van wormeitjes in het influent was 194 ± 79 eitjes·L⁻¹. *Ascaris lumbricoides* was het meest voorkomende wormeitje in het influent (gemiddeld 142 eitjes·L⁻¹) en effluent (gemiddeld 19 eitjes·L⁻¹). Uit de resultaten blijkt dat de slibfiltratiecapaciteit voor wormeitjes varieert tussen 89 en 95%. De waargenomen hoge verwijdering van de wormeitjes kan worden toegeschreven aan de lage opstroomsnelheid en aan de lage biogasproductie bij lage temperaturen, hetgeen de turbulentie in de reactor beperkt. Verwijdering van fecale coliformen varieerde tussen 0.9 en 2.1 log₁₀ en voor *E. coli* tussen 0.8 en 1.6 log₁₀. De totale verwijdering van chemisch zuurstof verbruik (CZV) was laag en varieerde tussen de 37 en 62%. De beste prestatie met betrekking tot verwijdering van wormeitjes, totale COD, en troebelheid werd verkregen bij de laagste opwaartse snelheid van 0.12 m·h⁻¹. Om te voldoen aan de pathogenen richtlijnen van de World Health Organization (WHO), is nabehandeling noodzakelijk.

Hoofdstuk 5 beschrijft de verwijdering van fecale coliformen met behulp van Down Flow Hanging Sponge (DHS) reactoren als nabehandeling van het UASB-reactor effluent. Hiervoor werden drie, lange termijn, continue labschaal experimenten uitgevoerd. De DHS reactoren waren van het ‘kubus’ type, DHS (G1) met en zonder recirculatie en ‘gordijn’ type DHS (G2). De porositeit van de toegepaste media was 91, 87 en 47%, terwijl de respectievelijke hydraulische verblijftijd (HRT) 2.9, 1.5 en 2.5 uur was. De organische belasting bedroeg 0.86, 0.53 en 0.24 kg CZV·m⁻³·d⁻¹, terwijl de bijbehorende hydraulische belasting 1.92, 2.97 en 1.32 m³·m⁻²·d⁻¹ bedroeg. De ‘kubus’ type (G1) DHS reactoren hadden het hoogste rendement voor de verwijdering van fecale colibacteriën. Volgens de in 1989 vastgestelde WHO richtlijnen kan het effluent geproduceerd in een ‘kubus’ type reactor zonder recirculatie, met een gehalte aan fecale coliformen van 2.1E + 02 CFU·100 mL⁻¹, voor niet-restrictieve irrigatie worden gebruikt (categorie A). Restrictieve irrigatie, categorie B, is toegestaan voor effluent van de ‘kubus’ type reactor met recirculatie met effluent fecale coliform concentraties van gemiddeld 3.4E+04 CFU·100 mL⁻¹. Restrictieve irrigatie, categorie C, is toegestaan voor effluent van een ‘gordijn’ type reactor met een gemiddelde concentratie van 5.9E+05 CFU·100 mL⁻¹. De reductie in biochemisch zuurstof verbruik (BZV₅) varieerde tussen 80.9 en 93.6 % met een BZV₅ gehalte in het effluent van 19.5 en 6.2 mg·L⁻¹ in de ‘kubus’ type reactoren zonder en met recirculatie, en 14.9 mg·L⁻¹ voor de ‘gordijn’ type reactor. Dus, wat betreft BZV verwijdering kan worden geconcludeerd dat het effluent van de onderzochte DHS reactoren voldoet aan de United States Environmental Protection Agency (USEPA) normen voor irrigatie van gewassen die niet als voedsel dienen voor mensen. Voorbeelden van deze toepassingen zijn weilanden voor melkvee, veevoer, vezels, en zaadgewassen.

Hoofdstuk 6 beschrijft de resultaten en discussie en reflecteert op het gepresenteerde werk in het proefschrift. De resultaten laten zien dat de verwijdering van wormeitjes door anaerobe slibbed filtratie een veelbelovend alternatief is voor behandeling van

afvalwater wanneer er onvoldoende ruimte beschikbaar is om op oppervlakte basis gedimensioneerde systemen te installeren. In het hoofdstuk wordt nader ingegaan op de gezondheidsrisico's van de toepassing van UASB-reactoren in subtropische klimaten en bij lage temperaturen. Daarnaast zijn de gezondheidsrisico's geanalyseerd van DHS reactoren als nazuivering van UASB reactoren onder sub-tropische omstandigheden. De gezondheidsrisico-analyse werd uitgevoerd volgens de 2006 WHO-richtlijnen. Het overeenkomstige jaarlijkse risico op infectie (genoemd $P_{I(A)}$) als gevolg van de inname van een gemiddeld aantal pathogene organismen in een dosis werd bepaald per individu. Er werd aangenomen dat per blootstelling 1 tot 10 mL (behandeld) afvalwater met de gewassen werd ingenomen. Bij toepassing van elk van de onderzochte zuiveringssystemen wordt een vermindering van de jaarlijkse infectierisico's verwacht. De berekende vermindering van het jaarlijkse risico bedraagt 19–90%, bij een verwijdering van wormeitjes in de UASB-reactoren tussen 26 en 93%, voor subtropische gebieden (gemiddelde afvalwater temperatuur tussen 17 °C en 29 °C). Bij lage temperaturen (gemiddelde afvalwater temperatuur tussen 11 en 14 °C) varieert de verwijdering van wormeitjes in de UASB-reactoren van 89–95%, en ligt de waargenomen vermindering van het jaarlijkse risico tussen de 84 en 93%.

De analyse van het jaarlijkse risico bij toepassing van DHS reactoren werd uitgevoerd met *Enterohemorrhagic Escherichia coli* (EHEC) als een ziekteverwekker indicator. De resultaten laten zien dat het jaarlijkse risico lager is bij het gebruik van het effluent van de 'kubus' type DHS reactor in vergelijking met de 'gordijn' type reactor. Uit deze theoretische gezondheidsrisico-analyse kan worden geconcludeerd dat de risico's van besmetting bij gebruik van effluent van de 'kubus' type DHS-reactor, zonder recirculatie, 253 maal lager zijn dan bij gebruik van ongezuiverd water. Met een 'kubus' type DHS-reactor met recirculatie kan het infectiegevaar 13 keer worden verminderd in vergelijking met gebruik van ongezuiverd water. Er kan geen significante vermindering van gezondheidsrisico's worden verwacht bij het gebruik van een 'gordijn' type DHS-reactor. Dus, in vergelijking met hergebruik van ongezuiverd afvalwater in de landbouw of behandeling met uitsluitend een UASB-reactor, zullen de gezondheidsrisico's duidelijk worden verminderd indien de zuivering bestaat uit een UASB reactor gevolgd door een 'kubus' type DHS reactor.

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