Assessing the Reuse Potential of Wastewater for Irrigation: The Removal of Helminth Eggs from a UASB Reactor and Stabilization Ponds in Bolivia

by

Matthew Eric Verbyla

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Master of Science in Environmental Engineering
Department of Civil and Environmental Engineering
College of Engineering
University of South Florida

Major Professor: James R. Mihelcic, Ph.D.
Sarina J. Ergas, Ph.D.
Ricardo Izurieta, M.D., Dr.PH
Stewart M. Oakley, Ph.D.

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DEDICATION

This thesis is dedicated to my family—to my parents, Roger and Roberta, who taught me the value of hard work and have provided me with support on so many levels throughout my life; to my sisters Katherine and Allison; and to my lovely wife, Wendy. You have all provided me with encouragement and support which has helped me to succeed academically. I am truly fortunate to have such amazing people in my life. I would also like to dedicate this thesis in memory of my friend, Lic. Luis Gamboa, who valued academic integrity and made learning enjoyable.

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ABSTRACT

Extreme hunger, malnutrition, and the lack of access to sanitation are among the most pressing development challenges, but the world is not on track to meet the targets that have been established by the Millennium Development Goals. The integration of wastewater treatment and food production systems allows for the recovery of resources from wastewater, and can provide an important solution to meet the sanitation needs of growing urban populations and provide periurban farmers with a consistent supply of water and nutrients. Stabilization ponds have been long considered to be an appropriate technology for wastewater reuse systems in developing countries, but advanced anaerobic treatment technologies, such as upflow anaerobic sludge blanket (UASB) reactors, are also becoming common. The objective of this study is to evaluate the reuse potential of wastewater for irrigation from two community-managed treatment systems in Bolivia: one consisting of three stabilization ponds in series (three-pond system) and the other consisting of a UASB reactor and two stabilization ponds in series (UASB-pond system).

Specifically, the removal of helminth eggs and thermotolerant coliform bacteria is measured in both systems and evaluated with respect to the World Health Organization (WHO) guidelines for the safe use of wastewater in agriculture, which are based on health targets. Results indicate that both systems provide good removal of conventional water quality parameters but poor removal of nutrients, discharging effluents with 37 to 54 mg/L of total nitrogen and 5.7 to 9.4 mg/L of total phosphorus. The three-pond system provided >92% removal of helminth eggs and 3.4-log removal of thermotolerant coliforms, and no geohelminth eggs were detected in the system effluents. However, *Ascaris* eggs were detected in the effluents of the UASB-pond system and the overall removal of thermotolerant coliforms was only 2.3 log units. Viability estimates based on the use of a vital stain indicate that eggs detected in pond effluents are less likely to be viable than eggs detected in the raw wastewater, in the sludge, or in the

effluent of the UASB reactor. Sludge samples from the facultative pond in the three-pond system had higher concentrations of helminth eggs than sludge samples from the UASB reactor.

Based on these results, the effluents from the three-pond system can be reused for irrigating any crop with the exception of root crops and low-growing crops that can be consumed raw (i.e. onions and strawberries). Effluents from the three-pond system may be used to irrigate salad crops or high-growing crops that are consumed raw, but additional public health interventions must be implemented throughout the food production process to meet WHO recommendations for protecting the health of farmers and consumers. The effluents from the UASB-pond system should not be reused unless improvements to the system increase its pathogen removal efficiency. The results from this study indicate that a system consisting of stabilization ponds in series may produce a higher quality effluent that is more suitable for wastewater irrigation than a system with a UASB reactor.

RESUMEN EJECUTIVO

El hambre, la malnutrición, y la falta de acceso al saneamiento básico son algunos de los desafíos más importantes actualmente para el desarrollo internacional. Sin embargo, el mundo no está en camino para lograr los retos establecidos por los Objetivos de Desarrollo del Milenio. La integración de los sistemas de tratamiento de aguas residuales y los sistemas de producción agrícola permite la recuperación de recursos de las aguas residuales. Estos sistemas integrados pueden proveer el saneamiento básico a la población urbana, y una fuente consistente de agua y nutrientes a los agricultores periurbanos. Generalmente se ha considerado que las lagunas de estabilización son una tecnología apropiada para el reuso de aguas residuales en los países en desarrollo, no obstante algunas tecnologías anaeróbicas avanzadas tal como los reactores anaeróbicos de flujo ascendente (RAFA, o UASB por sus siglas en inglés), últimamente son más comunes que antes. El objetivo del presente estudio es evaluar dos sistemas de tratamiento de aguas residuales en Bolivia, para determinar si se puede reusar los efluentes para el riego de cultivos. Un sistema tiene tres lagunas de estabilización en serie y el otro sistema tiene un reactor UASB seguido por dos lagunas de estabilización en serie.

Específicamente, se ha medido la remoción de los huevos de helmintos y los coliformes termotolerantes en los dos sistemas. Los resultados son evaluados según las recomendaciones establecidas por la Organización Mundial de la Salud (OMS) para el uso seguro de las aguas residuales en la agricultura, las cuales son basadas en objetivos de salud. Los resultados indican que ambos sistemas tienen buena remoción de los parámetros convencionales de calidad de agua, pero mala remoción de los nutrientes. Los sistemas actualmente descargan efluentes con concentraciones de 37 a 54 mg/L de nitrógeno total y de 5.7 a 9.4 mg/L de fósforo total. El sistema de tres lagunas remueve >92% de los huevos de helmintos y 3.4-log de los coliformes termotolerantes, y no se ha detectado ningún huevo de geohelmintos (helmintos que son

transmitidos por el suelo) en el efluente del sistema. Pero, los huevos de *Ascaris* fueron detectados en los efluentes del sistema con el reactor UASB y este sistema solo tenía una remoción total de 2.3-log para los coliformes termotolerantes. Los cálculos aproximados de viabilidad, basados en la tinción con azul tripán, indica que es menos probable que los huevos detectados en los efluentes de las lagunas fueran viables que los huevos detectados en el agua cruda, en los lodos o en el efluente del reactor UASB. Las concentraciones de huevos de helmintos en los lodos de la laguna facultativa del sistema de tres lagunas eran más altas que las concentraciones en los lodos del reactor UASB.

Según los resultados de esta investigación, los efluentes del sistema de tres lagunas en serie pueden ser reusados para la irrigación de los cultivos, con la excepción de los cultivos de raíces comestibles y los cultivos con una fruta que crezca cerca de la tierra, y que se puede consumir crudos (cebollas, fresas, etc.). Por ejemplo, los efluentes del sistema de tres lagunas pueden ser usados para regar cultivos de ensalada o cultivos con una fruta que no crezca tan cerca de la tierra (lechuga, tomate, etc.). Sin embargo, la presencia de los huevos de *Taenia* que se ha detectado en varias muestras puede presentar un riesgo a la salud, y se ha hecho varias recomendaciones para proteger la salud de las personas. Por ejemplo, para satisfacer los requisitos de la OMS, será necesario implementar varias intervenciones de salud adicionales en el proceso de producción y cosecha de cultivos para dar una mayor protección a los agricultores y a los consumidores. A diferencia del sistema con tres lagunas, el agua el sistema del reactor UASB no debe ser reusada para la agricultura a menos que se hagan mejoras al sistema para aumentar la remoción de los patógenos. Los resultados de este estudio indican que en el contexto de estas dos comunidades, el sistema de lagunas de estabilización en serie produce un efluente de mejor calidad para el reuso que el sistema del reactor UASB.

1.0 INTRODUCTION

1.1 Present Development Challenges

At the turn of the 21st century, leaders from 189 countries gathered at United Nations general assembly and made commitments to eradicate extreme poverty, malnutrition, and disease, adopting eight Millennium Development Goals (MDGs) with targets to be met by the year 2015 (United Nations 2000). However, according to a recent report, the world's progress on Goal #1, eradicating extreme hunger, was hindered by the global food crisis of 2008 (United Nations 2011) (Figure 1). The cause of this crisis has been partially attributed to unstable fertilizer prices (Cordell et al. 2009), increasing populations and affluence, and low crop yields due to land constraints, water scarcity, and extreme weather (Food and Agriculture Organization 2008; Christiaensen 2009). Finding sustainable long-term solutions to these problems is a challenge, especially since the global food demand is expected to double by 2050 (Tilman et al. 2002).

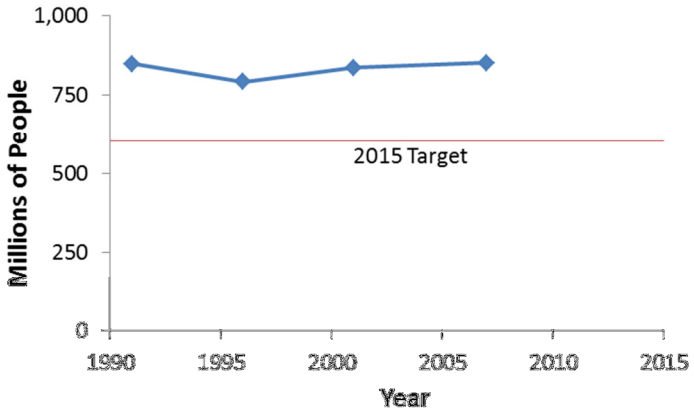


Figure 1: Trend for the number of people in developing regions who are undernourished Source of data: United Nations (2012)

Biogenetics has been viewed as one possible solution to feed the world's growing population. Since the 1970s, advances in genome technologies have contributed to the development of new varieties of wheat, rice, and corn that are capable of providing higher yields

than conventional varieties on the same amount of land. Many of these new genetically-modified crop varieties, which have been adopted globally, require more water to produce the same amount of food, thus creating a water crisis (Pearce 2006). The global use of groundwater for irrigation has been growing (Siebert et al. 2010), especially given recent increases in access to low-cost pumps and energy supply networks in developing countries (Scott and Sharma 2009). However, the water in many aquifers is not being recharged at the same rate at which it is being withdrawn. This is a problem since one-fifth of the global irrigation demand in 2000 was attributed to non-sustainable groundwater withdrawals (Wada et al. 2012). This unsustainable extraction of freshwater for irrigation has led to the desiccation of ecosystems that have provided people with renewable food sources and economic livelihoods for thousands of years (Pearce 2006). In addition, changes in climate and land use threaten to place further stress on limited freshwater resources (Zimmerman et al. 2008), which can impact agriculture (Turral et al. 2011).

Sanitation has been another challenge. Developing countries are not on track to meet the target defined by MDG #7, which is to halve the proportion of people without access to improved sanitation by 2015 (United Nations 2012). Meeting this goal has been challenging in regions such as Southern Asia and Sub-Saharan Africa, where population has increased at very high rates. In fact, while the fraction of people without access to sanitation in these two regions has decreased since 1990, the total number of people without access has actually increased (Figure 2).

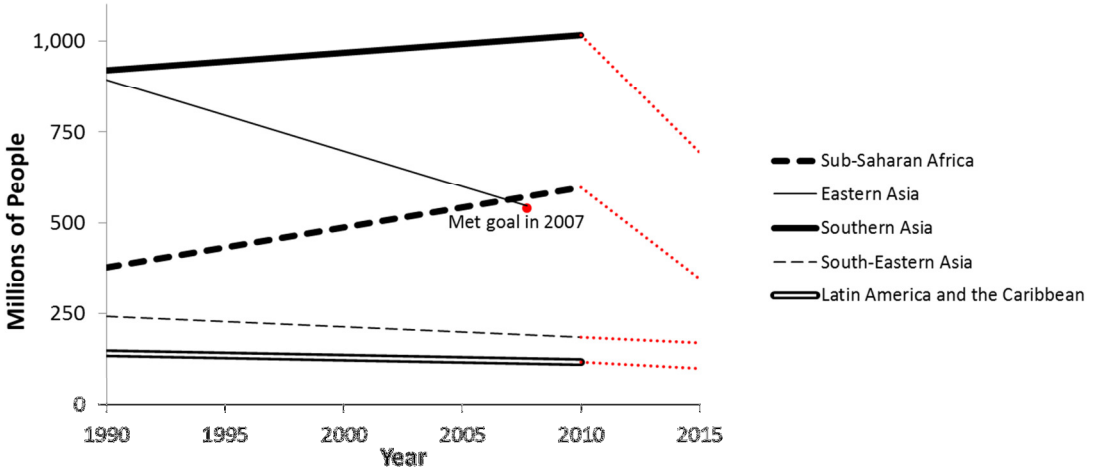


Figure 2: Regional trends for people without access to improved sanitation (Dotted lines represent progress required in order to meet MDG target by 2015, assuming populations continue to grow at rates similar to the last 20 years) Source of data: WHO-UNICEF (2012)

Although 72% of the 2.5 billion people in the world without access to improved sanitation live in rural areas (WHO-UNICEF 2012), the need for sanitation investments for urban populations in developing countries should not be overlooked. Since the 1950s, the number of people living in urban areas has been increasing at an exponential rate (United Nations 2007), which has led to the creation of mega-cities with sprawling urban slums. As urban areas become more congested, decentralized sanitation becomes less practical, and sewer systems are installed. In Latin America and the Caribbean, where 80% of the population lives in urban areas (United Nations 2007), three out of every four people that gained access to improved sanitation between 1990 and 2000, gained it by means of a connection to a sewer system (Figure 3) (WHO-UNICEF 2000). A total of 184 million people in Asia and Africa also connected to sewer systems for the first time between 1990 and 2000. The Millennium Development Goal monitoring program counts a connection to a sewer system as *improved sanitation* whether or not the sewage receives treatment before being discharged back into the environment (WHO-UNICEF 2012). Sewer systems without wastewater treatment do not necessarily ensure the hygienic separation of pathogens from human contact. This is especially alarming, considering that approximately 90% of the wastewater in developing countries is discharged without any treatment (Raschid-Sally and Jayakody 2008; Corcoran et al. 2010).

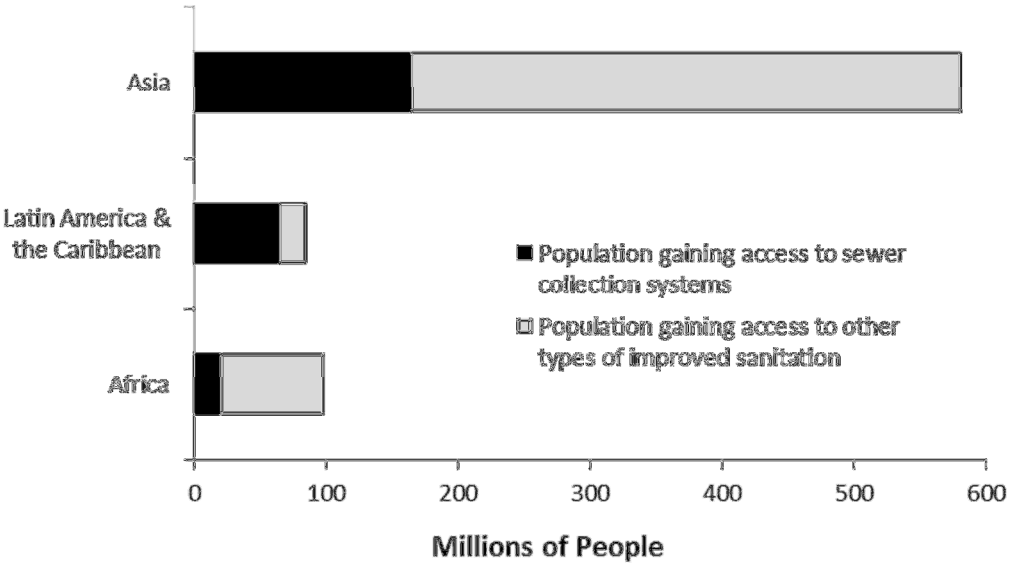


Figure 3: New sewer users in Asia, Africa, and Latin America between 1990 and 2000 Source of data: WHO-UNICEF (2000)

The use of sewer systems will likely continue to be an important part of the solution for urban populations without access to improved sanitation. Cultural preferences in some areas favor the use of flush toilets over dry sanitation technologies such as composting latrines (Santos et al. 2011). This is especially true for Muslim and Hindu communities, where water is traditionally used for anal cleansing (Nawab et al. 2006; Avvannavar and Mani 2008). Muslims and Hindus account for more than half of the people living in India, China, Indonesia, Nigeria, Pakistan, and Bangladesh—six countries that have two-thirds of the total population currently lacking access to improved sanitation (Figures 4 and 5).

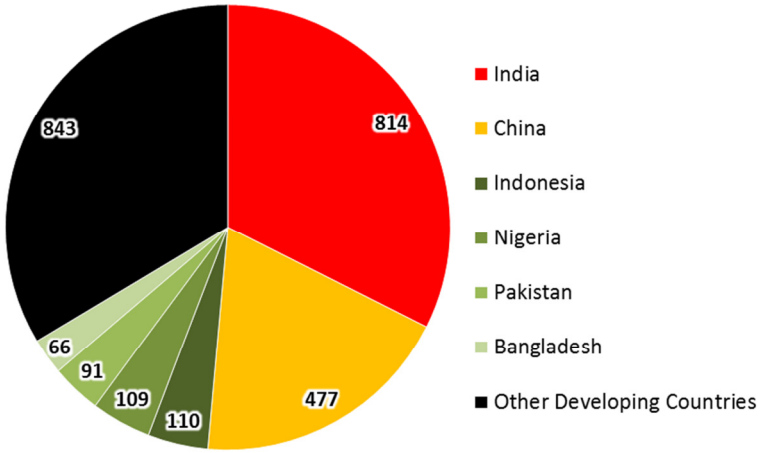
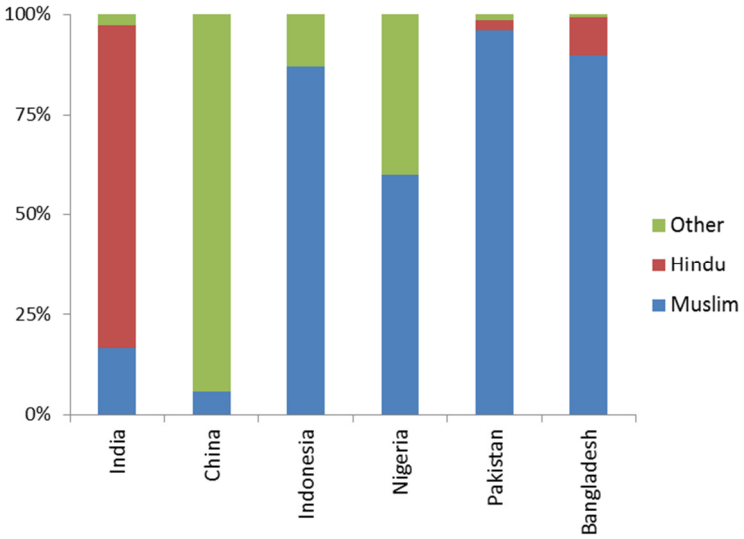


Figure 4: Population without access to improved sanitation (millions of people)
Source of data: WHO-UNICEF (2012)



Millions of People

Country	Muslim	Hindu	Other
India	205	986	34
China	80	0	1,261
Indonesia	209	0	31
Nigeria	95	0	63
Pakistan	167	4	3
Bangladesh	133	14	1
Total	**889**	**1,005**	**1,393**
Percentage	**27%**	**31%**	**42%**

Figure 5: Muslim and Hindu populations in six developing countries
Source of data: Central Intelligence Agency (2011); Muslim Population in the World (2011)

As sewer system coverage in developing countries increases, the quality of surface waters will likely decrease, and the number of regions with extreme water stress conditions will likely increase (Fry et al. 2008). Water quality is of particular importance to eliminate extreme poverty and hunger especially in developing countries where vulnerable populations rely on environmental income that depends on water quality, such as fishing (Fry et al. 2008).

1.2 Resource Recovery from Wastewater as Part of the Solution

The traditional objectives of wastewater treatment include the removal of contaminants with highly-mechanized systems at a central treatment plant, and the discharge of treated effluents to waterbodies (Figure 6a). While this has allowed many developed countries to eliminate the health burden from diarrheal diseases, the discharge of wastewater via point sources contributes to the eutrophication of waterbodies, as nutrient removal is inefficient in conventional systems without large material and energy inputs. The conventional approach to wastewater treatment and the use of highly-mechanized systems have been largely unsuccessful in developing countries, despite investments made by the international development sector (Salguero et al. 2004; Oakley 2005). However, recent sustainable development initiatives have led to the proposal of a new paradigm for wastewater treatment, which is focused not only on the removal of contaminants, but also on the recovery and reuse of water, material, and energy resources (Guest et al. 2009) (Figure 6b). The recovery of these resources is achieved by integrating wastewater treatment and food production systems, which can provide an important solution for the global development challenges described above. For example, wastewater can be reused for irrigation, making freshwater available for water supply systems and ecosystem restoration.

Agriculture is the largest consumer of water globally, and can account for up to 90% of the freshwater extracted in some developing countries (Food and Agriculture Organization 2003). In addition to providing farmers with a constant, year-round supply of water, wastewater also contains nitrogen, phosphorus, potassium, and micro-nutrients in forms that are readily available to plants, making it a resource that is appreciated and even preferred by some farmers (Qadir et al. 2007; Raschid-Sally and Jayakody 2008; Corcoran et al. 2010).

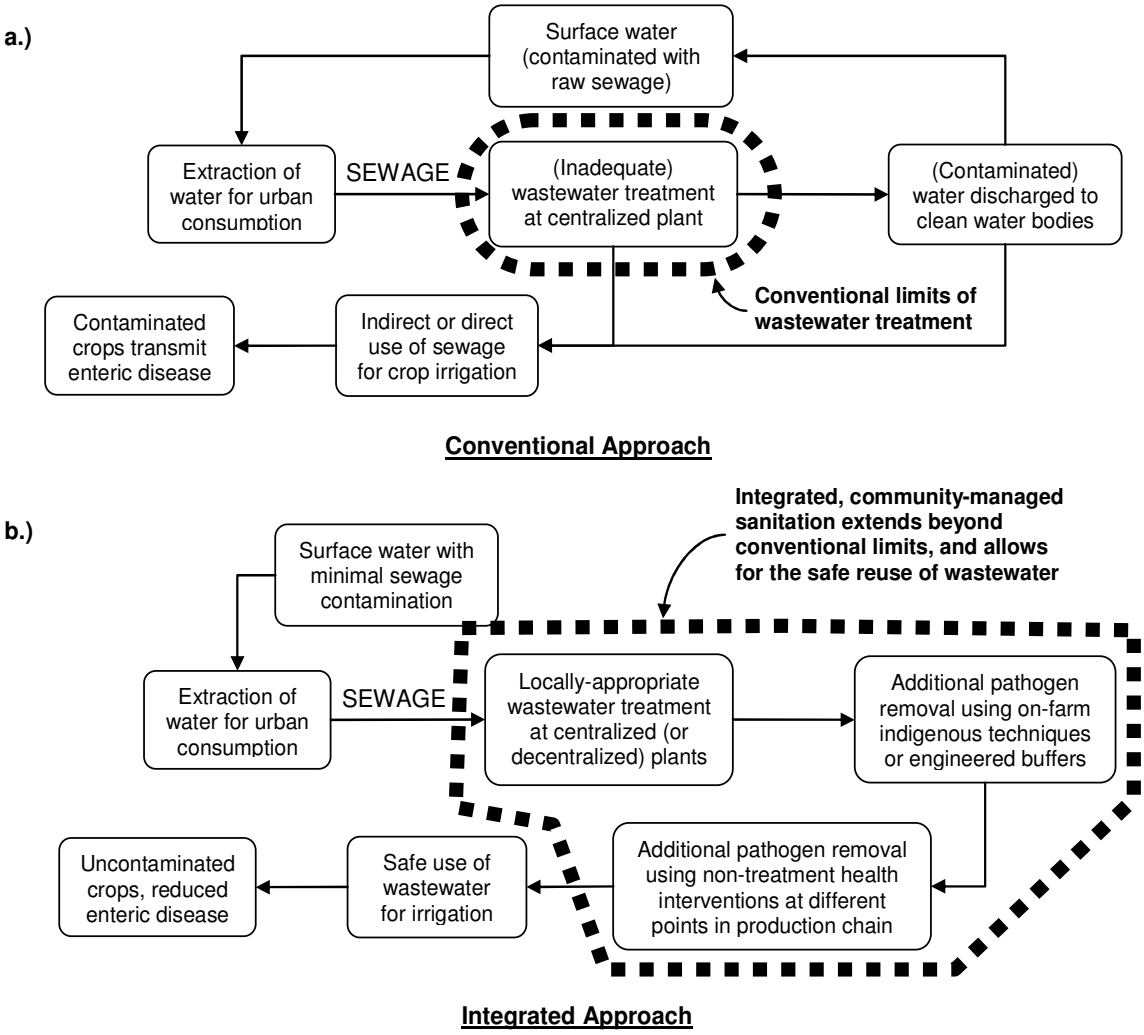


Figure 6: The conventional approach (a) to wastewater treatment, and the integrated approach (b) for wastewater treatment and food production systems

From a life cycle perspective, the reuse of wastewater for irrigation conserves embodied energy by offsetting the need for synthetic fertilizers (Buonocore et al. 2012). The production and distribution of fertilizers that use Haber-Bosch nitrogen and mined phosphate rock generates large quantities of waste products and greenhouse gases, and requires substantial energy and material inputs. This has also made the cost of these fertilizers highly dependent on the price of fossil fuels (Rosset 2008). On the other hand, the use of reclaimed wastewater and human waste in agriculture returns nutrients to their surrounding environment and reduces the eutrophication of surface waterbodies (Muga and Mihelcic 2008). Reclaiming nutrients from wastewater and human waste is a particularly important part of the solution to the looming phosphorus crisis—the

phosphorus in human urine and feces alone accounts for one-fifth of the global demand (Mihelcic et al. 2011).

The integration of sanitation and food production systems is not a new concept. Human excreta was used in China to increase soil fertility as early as 475 B.C. (Shiming 2002). The presence of flow regulation devices found in sewer canals of ancient Athens indicates that wastewater may have been sold to farmers (Semple 1928). Today, reclaimed water is used throughout the world, especially when groundwater and surface water sources are not sufficient. The problem is that this practice is often unsanctioned in developing countries (Williams 2003). The irrigation of food crops with untreated wastewater has been documented in middle-income and developing countries throughout the world, including Ghana (Drechsel et al. 2006), Pakistan (van der Hoek et al. 2002), Mexico (Scott et al. 2000; Environmental Protection Agency 2004; Corcoran et al. 2010), Peru (Moscoso and Alfaro 2007; Moscoso et al. 2008), and Bolivia (Moscoso and Coronado 2002; Huibers et al. 2004). Irrigation with untreated wastewater is largely underreported, but global estimates of the total area irrigated range from 3 million hectares (Drechsel et al. 2006) to as many as 20 million hectares (Raschid-Sally and Jayakody 2008).

1.3 Appropriate Technologies to Mitigate Health Risks

The use of untreated wastewater in agriculture can severely impact human health, as water is central to the transmission of fecal-oral diseases. Epidemiological studies have linked the use of untreated or partially-treated wastewater in agriculture with increased incidences of ascariasis (Cifuentes 1998; Amahmid and Bouhoum 2005), shigellosis (Porter et al. 1984), giardiasis (Srikanth and Naik 2004), cholera, and typhoid fever (Shuval 1993). Children are the most vulnerable to these gastrointestinal diseases (Qadir et al. 2007) and chronic diarrhea is the leading cause of child malnutrition (World Health Organization 2009a). The World Health Organization (WHO) has published guidelines that provide low-income countries with a risk-based framework for creating policies that permit the reuse of wastewater in agriculture, while still achieving reasonable health targets (World Health Organization 2006a). To meet these targets,

WHO recommends the use of appropriate wastewater treatment, coupled with non-treatment health protection measures such as produce washing, irrigation scheduling, and crop restrictions.

Waste stabilization ponds (also known as oxidation ponds or lagoons) have been described as the most appropriate technology for integrated wastewater treatment and irrigation systems in developing countries with sufficient topographically-suitable land (Feachem et al. 1983; Shuval et al. 1986; Peña Varon et al. 2000; Mara and Horan 2003; Egocheaga and Moscoso 2004a, b; Mara 2004; Moscoso et al. 2008; Oakley 2005a). Although there is little information about the number of stabilization pond systems in developing countries, they are very common throughout the world, especially in tropical climates (Mara 2004). When analyzed using social, environmental, and economic indicators, stabilization ponds and the land application of wastewater are more sustainable than conventional mechanized wastewater treatment systems such as activated sludge, especially for systems treating less than 5,000 MGD (Muga and Mihelcic 2008). Despite the ubiquitous use of stabilization ponds in developing countries, improved anaerobic technologies such as upflow anaerobic sludge blanket (UASB) reactors have recently become popular for the treatment of municipal wastewater in developing countries with tropical climates. These reactors have small footprints, which may make them suitable for land-constrained urban communities, but they are also hydraulically less robust than stabilization ponds and require more frequent attention for operation and maintenance. Furthermore, some maintenance and troubleshooting activities can be expensive for low-income communities, and may require the need for external support from skilled professional labor, such as engineering or laboratory services (Peña Varon et al. 2000). On the other hand, biogas recovered from these reactors could be an energy resource for communities.

1.4 Research Goal and Hypotheses

Based on the challenges and opportunities described above, the overall goal of this study is to evaluate the potential for safely reclaiming the effluents from two community-managed domestic wastewater treatment systems in Bolivia to use for irrigation. Specifically, the two systems are compared with respect to their ability to remove helminth eggs, bacterial pathogen indicators, conventional water quality parameters, and nutrients. Pathogen removal is assessed

with respect to the WHO Guidelines (World Health Organization 2006a). The first system (from here on referred to as the three-pond system) consists of three stabilization ponds in series. The second system (from here on referred to as the UASB-pond system) consists of an upflow anaerobic sludge blanket (UASB) reactor followed by two stabilization ponds in series.

This study addresses the following hypotheses:

1. Both sanitation systems can remove helminth eggs and produce effluents that meet the 2006 World Health Organization Guidelines for reuse in agriculture.
2. The three-pond system can remove helminth eggs and bacterial pathogen indicators more efficiently than the UASB-pond system.
3. The three-pond system can remove nitrogen and phosphorus more efficiently than the UASB-pond system.

The results of this study will provide insight into the reuse potential of wastewater treated in sanitation systems that incorporate stabilization ponds or UASB reactors, not only for communities in this region of Bolivia, but throughout other regions of the world as well. Assessing wastewater reuse in this particular region of Bolivia is particularly important however, since a recent study of the watersheds linked water stress to changes in climate (Fry et al. 2012).

2.0 BACKGROUND

2.1 Stabilization Ponds and UASB Reactors

The treatment of wastewater in stabilization ponds results from a variety of processes, including: the sedimentation of suspended solids; the photochemical oxidation or biodegradation of organic material (measured in terms of oxygen demand); the removal or inactivation of pathogens; the incorporation of soluble elements or compounds into microbial biomass; and the removal of nutrients (i.e., nitrogen, phosphorus) via sedimentation, volatilization, or incorporation into biomass and conversion by bacteria and algae. Because the oxygen needed to biodegrade organic material is provided by algae through photosynthesis rather than mechanical aeration, reaction rates are slower than in mechanized systems, requiring ponds to have larger volumes and longer hydraulic retention times. Typical removal rates for nutrients and conventional parameters are shown in Table 1.

Table 1: Typical removal rates of conventional parameters in stabilization ponds

Parameter	Anaerobic Ponds	Facultative Ponds	Maturation Ponds
BOD_5	40-70%[1]	65-95%[2]	minimal
Suspended Solids	40-80%	40-95%	minimal[3]
Total Nitrogen	30-80%		
Total Phosphorus	30-50%		

Source: Environmental Protection Agency (1983); Peña Varon (2002); Mara (2004); Oakley (2005b); Mihelcic et al. (2009); Oliveira and von Sperling (2011)

[1] For loading rates of 100 – 350 g/m^3/day, removal varies with temperature (Mara 2004)

[2] Removal rates depend on factors such as surface loading rate, temperature, insolation, etc.

[3] The majority of suspended solids leaving a maturation pond typically consist of algae biomass

Non-aerated stabilization ponds are classified as either anaerobic, facultative, or maturation ponds, based on their depths, treatment objectives, and dissolved oxygen content. Typical pond systems either consist of facultative ponds followed by maturation ponds, or

anaerobic ponds followed by facultative ponds and maturation ponds. Anaerobic ponds are the deepest (2.0 – 5.0 meters), and are sized based on the volumetric loading rate of biochemical oxygen demand (BOD). Facultative ponds are shallower (1.5 – 2.5 meters), and are typically designed based on a BOD surface loading rate which allows for the development of an algae population in the top part of the water column, where sunlight can penetrate. Facultative ponds tend to maintain anaerobic conditions at the bottom and aerobic conditions at the top of the water column. Maturation ponds are the shallowest (1.0 – 1.5 meters) and are designed for additional nutrient and pathogen removal. Stabilization pond systems are used globally in middle income and developing countries, but are also common in small towns and rural areas of developed countries (Table 2). Advanced pond systems incorporate mechanical aeration and mixing to increase treatment efficiency. However, this requires the need for additional maintenance and energy inputs, making their application less suitable for developing countries (Mara 2004).

Table 2: Global use of stabilization pond systems for wastewater treatment

Country	Approximate number of pond systems
France	2,500
United States	2,000
Germany	1,100
Portugal	300
New Zealand	100
Denmark	50

Source: Mara (2004); Okafor (2011)

Over time, solids accumulate at the bottom of stabilization ponds, especially near the entrance. The removal of sludge from stabilization ponds is required every two to five years for anaerobic ponds and every five to 15 years for facultative ponds (Oakley 2005b). Desludging constitutes the most expensive and technically-challenging task associated with the operation and maintenance of pond systems. Unfortunately, many pond systems in developing countries operate without operational or financial plans in place for desludging, and over time have accumulated sludge depths greater than one meter (Oakley et al. 2012). This not only increases

the cost and difficulty of desludging, it also seriously affects the hydraulics of ponds, thereby reducing their treatment efficiencies. Nevertheless, low-cost methods have been developed to more easily desiccate deep sludge, such as the use of local wetland plants (Oakley et al. 2012).

Anaerobic reactors, such as septic tanks and Imhoff tanks, have been used for hundreds of years to treat domestic wastewater, and are still commonly used in systems serving small communities and in developing countries. A variety of high-rate anaerobic systems, including upflow anaerobic sludge blanket (UASB) reactors, have been developed more recently and may offer new opportunities for urban wastewater treatment. UASB reactors distribute wastewater uniformly in the bottom of the reactor, where it flows upward, as shown in Figure 7. The diameter of the reactor increases as the wastewater flows upward, causing the velocity to decrease. This allows for the creation of a layer of suspended solids with similar settleability characteristics, known as a "sludge blanket." Over time, these suspended particles floc together and granulize, providing a surface for the development of biofilms with different bacterial populations grouped in layers, which is the key to the high efficiency of the UASB reactor (Chernicharo 2007). These reactors were developed in Holland in the 1970s to treat high-strength industrial wastewaters, but were later found suitable to treat domestic wastewater in warm climates and have since been implemented globally, especially in Brazil, Colombia, and India (Aiyuk et al. 2006).

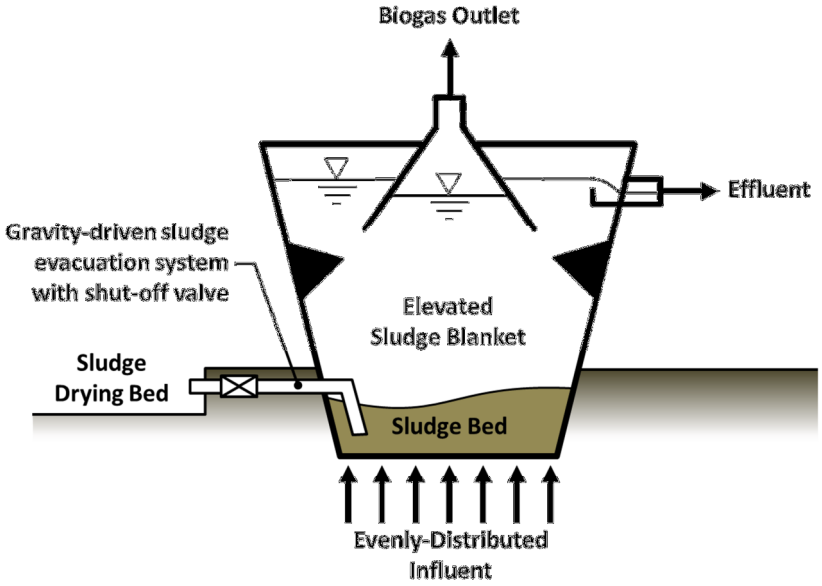


Figure 7: Schematic representation of a UASB reactor

Anaerobic conditions allow for the hydrolization of complex organic material into soluble sugars, amino acids and fatty acids. Fermentative and acetogenic bacteria convert these compounds into volatile fatty acids, hydrogen gas, and carbon dioxide. Different species of methanogenic bacteria can use the fatty acids and hydrogen gas to produce methane. In wastewater with high concentrations of sulfur compounds, hydrogen sulfide is also produced by sulfate-reducing bacteria. A bell-shaped phase separator catches the biogas, where it can be harvested and used as fuel. The heavier sludge that accumulates at the bottom of the reactor must be removed every few weeks, which is typically done by gravity with large-diameter pipes. However, in order to maintain treatment efficiency, it is important to retain a portion of high-activity biomass with microbial communities that are already acclimated to the conditions within the reactor. This high-activity biomass typically consists of the flocculated sludge granules with good settleability characteristics, described above (Chernicharo 2007).

There are several important limitations to the use of UASB reactor systems, such as the reactor's sensitivity to inhibiting substances (i.e. cationic salts, ammonia, sulfides and metals). The efficiency of UASB reactors is often inconsistent, which may reflect the presence of inhibiting substances, seasonal fluctuations in temperature, or inconsistent operation and maintenance practices (Chernicharo 2007). Because of their variable efficiency and technical limitations, UASB reactors require post-treatment in order to remove conventional parameters to levels that are comparable to other wastewater treatment technologies (Table 3). In developing countries, this post-treatment is often achieved with stabilization ponds, trickling filters, or constructed wetlands.

Table 3: Typical removal of conventional parameters in wastewater treatment systems

Parameter	UASB reactor only	UASB Reactor and Post Treatment[1]	Stabilization Ponds	Constructed Wetlands	Activated Sludge
BOD_5	60-80%[2]	75-95%	65-95%	65-95%	85-95%
Suspended Solids	60-80%	70-95%	50-95%	50-90%	85-95%
Total Nitrogen	minimal	30-65%	30-80%	20-80%	20-80%
Total Phosphorus	minimal	0-50%	30-50%	30-65%	35-55%

Source: Peña Varon et al. (2000, 2002); Keller et al. (2004); Mara (2004); Mihelcic et al. (2009); Bastos et al. (2010); Oliveira and von Sperling (2011)

[1] Post treatment can consist of polishing ponds, trickling filters, constructed wetlands, etc.

[2] Removal rates increase with greater hydraulic retention times and higher temperatures.

UASB reactors and anaerobic waste stabilization ponds essentially perform the same function. UASB reactors cannot be compared directly to facultative ponds, because facultative ponds also incorporate an aerobic zone, which offers different treatment capabilities. In developing countries, the capital investment, operation, and maintenance costs associated with UASB reactors are almost always higher than stabilization ponds, provided that topographically-suitable land is available (Peña Varon et al. 2000), which raises the question: what are the advantages of using a UASB reactor? The advantages and disadvantages of UASB reactors and stabilization ponds are summarized in Table 4. For example, UASB reactors require smaller volumes than stabilization ponds and therefore require less land space. UASB reactors also produce more sludge per kilogram of BOD removed than stabilization ponds, and the sludge in UASB reactors has to be removed more frequently than solids produced from waste stabilization ponds (Mara 2004). This is due to the fact that the sludge in stabilization ponds is stored for years, during which time the organic fraction degrades, reducing its overall volume. The sludge from stabilization ponds and UASB reactors can contain high concentrations of viable pathogens and must be managed appropriately to prevent the spread of excreta-related diseases.

Table 4: Comparison of stabilization ponds and UASB reactors

Stabilization Ponds	UASB Reactors
• More land space required	• Less land space required
• More robust (hydraulically, pH conditions)	• Less robust (hydraulically, pH conditions)
• Lower capital costs	• Higher capital costs
• Easier day-to-day operation and maintenance, less need for post-construction technical support	• More difficult day-to-day operation and maintenance, more need for post-construction technical support
• Sludge must be removed every 2-4 years (anaerobic ponds) or every 5-10 years (facultative ponds), typically requires heavy equipment or large manual effort	• Sludge must be removed every 1-2 weeks, typically done by gravity, total volume of sludge produced per kg BOD removed is larger than in pond systems
• Biogas produced (anaerobic ponds) is difficult to collect and reuse	• Biogas produced can easily be collected and reused
• Open-air system may create social perceptions of unpleasant odors and views. Alternatively, the creation of new habitats for animals or birds might be a positive community resource.	• An enclosed reactor may or may not be aesthetically pleasing to a community, depending on social or cultural preferences.
	• Requires post-treatment of some sort

Social factors may also play a role in the decision process, to choose the installation of a UASB reactor system or a stabilization pond system. Communities might be more apt to accept an enclosed reactor instead of an open pond, due to perceptions about unpleasant odors and unsightly views. On the other hand, stabilization ponds can provide habitats for animals and birds, which may become a positive natural resource for an urban community. Finally, the amount of post-construction technical support needed to maintain, troubleshoot, and operate a UASB reactor may make this technology less favorable than stabilization ponds. In community-managed water supply systems, post-construction support is known to positively influence the sustainability of the system (Schweitzer and Mihelcic 2012).

2.2 Transformation and Removal of Nutrients

The majority of the nutrients in raw wastewater originate from urine, fecal matter, and food scraps. Some soaps and detergents also contain nutrient compounds. Nitrogen is excreted in feces in the form of proteins, amino acids or ammonium. In urine, nitrogen is excreted as urea ($CO(NH_2)_2$). The majority of organic nitrogen in raw sewage is converted to ammonia-nitrogen under aerobic conditions in the sewer pipes. Ammonium (NH_4^+) dissociates at higher pH levels to form ammonia (NH_3), which can be stripped to the atmosphere at high pH levels. Phosphorus in wastewater is organic or inorganic, and it can be in either particulate (>0.45 µm) or dissolved (<0.45 µm) forms. Urine contains almost entirely inorganic, dissolved orthophosphate ions (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$), while human feces primarily contain organic and sediment-bound phosphorus. As the organic material in wastewater decomposes, some of the organically-bound phosphorus will be converted to orthophosphates, which is the form that is readily available to most plants and algae.

The ammonia-nitrogen entering a pond system can be converted to nitrate under aerobic conditions via nitrification. Nitrate is the principal form of nitrogen used by plants and algae, but ammonium is also available to some species. Nitrate is converted to nitrogen gas (N_2) by microorganisms under anoxic conditions via denitrification, if a source of soluble BOD is also readily available. There is a general lack of consensus in the literature about which mechanisms for the removal and transformation of nitrogen in stabilization pond systems are the most

important, and several studies have resulted in contradictory conclusions. Some have concluded that the most important mechanism is ammonia stripping, especially in ponds with high algal activity that causes pH values greater than 9.3 and oversaturated dissolved oxygen conditions that allow for the release of oxygen bubbles (Catunda and van Haandel 1996). Others claim that the most important mechanism is the incorporation into biomass and its subsequent removal via sedimentation, where a portion of the organic nitrogen from dead cells is ammonified and retained in the sediments (Camargo Valero 2008). Some studies have concluded that nitrogen removal via nitrification and denitrification is likely negligible in ponds, since low concentrations of nitrifying bacteria in wastewater tend to associate with organic matter and settle to the bottom of the pond, where there is a lack of dissolved oxygen needed for nitrification (Ferrara and Avci 1982; Pano and Middlebrooks 1982; Reed 1985). Other studies however, have documented significant removal via nitrification and denitrification, especially in pond systems with long hydraulic retention times (Hurse and Connor 1999; Lai and Lam 1997; Picot et al. 2004; Zimmo et al. 2003). Based on the aforementioned theoretical considerations and results from previous research, it is reasonable to assume that stabilization pond systems should cause an overall decrease in nitrogen and especially in ammonia-nitrogen.

In pond systems, phosphorus is primarily removed via mineralization, precipitation, and incorporation into pond sediments (Figure 8). Phosphorus associated with flocs of organic material settles out into the sediments. Inorganic phosphorus can also settle out of the water column by becoming incorporated into biomass or by forming precipitates with cations such as calcium; the latter would primarily occur at pH levels above 9 (Mara 2004). A portion of the phosphorus in pond sediments can also be re-released and resuspended into the water column. Houng and Gloyna (1984) found that the uptake of inorganic phosphorus by algae occurred to a greater extent in facultative and maturation ponds than in anaerobic ponds. Phosphorus in the sediments of anaerobic ponds was released at a rate that was two times higher than in facultative ponds and more than 25 times higher than in maturation ponds. Therefore, in a typical stabilization pond system, anaerobic and facultative ponds are a source of phosphorus, while maturation pond sediments retain mineralized phosphorus (Houng and Gloyna 1984). Another

study of a pond system in France concurred with this finding, reporting higher phosphorus concentrations in the sediments of the final maturation pond (mainly bound to iron hydroxides and calcium) than in the sediments from the first two ponds (Gómez et al. 2000). High levels of dissolved oxygen in maturation ponds protect the phosphorus in the sediments from being re-released to the water column.

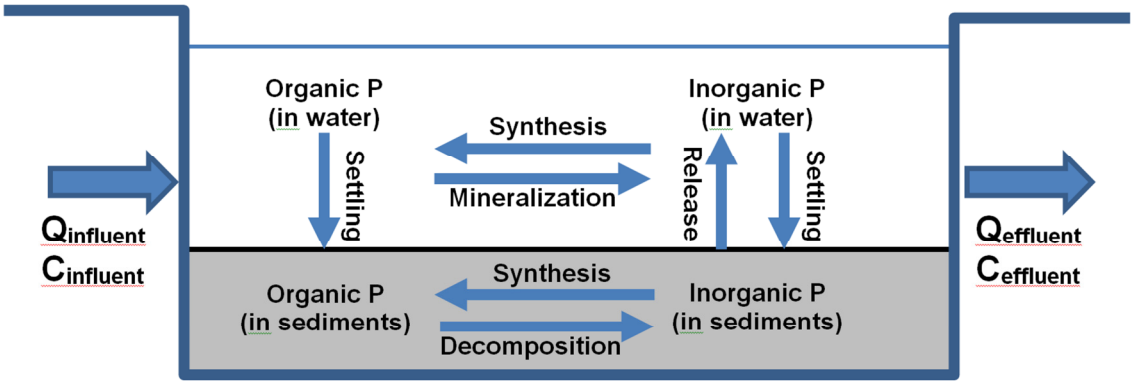


Figure 8: Phosphorus cycle in stabilization ponds (adapted from Houng and Gloyna 1984)

The removal of nutrients in UASB reactors can be assumed to be much less than in stabilization ponds, considering that they typically have much shorter hydraulic retention times. The anaerobic conditions in these reactors would prevent nitrifying bacteria from converting ammonia into nitrate. However, any nitrate present in the wastewater as it enters the reactor may be denitrified to form nitrogen gas. Significant phosphorus removal in UASB reactors treating domestic wastewater is even more unlikely. While biological phosphorus removal has been reported in pilot scale batch reactors treating phosphorus-rich (60-100 mg/L) industrial wastewater with high concentrations of volatile fatty acids (Comeau et al. 1996), it is unlikely to occur in a UASB reactor treating domestic sewage. Any measureable reduction of nutrients in a UASB reactor would likely be due to the incorporation of nutrients into biomass and subsequent removal via desludging.

2.3 Health Risks from Pathogens in Wastewater

The health risks of reusing wastewater for irrigation are based on the amount of viable pathogens in the wastewater, the characteristics of the pathogens, and the amount of exposure

that people have to these pathogens. The enteric pathogens of concern in municipal wastewater include viruses, bacteria, helminth eggs, and protozoan parasites. Some zoonotic pathogens may also enter wastewater from livestock operations, animal slaughterhouses, household pets, and pests. Health risks associated with vector-borne pathogens can also be an important factor for wastewater treatment systems in which the wastewater is open to the surrounding environment.

Mara and Feachem (1999) present an environmental classification system for the transmission of water-related diseases, which groups diseases into seven categories based on their environmental transmission route. For example, the first two categories include fecal-borne and non-fecal-borne diseases that are transmitted via water and whose transmission is best prevented by improving potable water quality, availability, and reliability. Other categories include water-based diseases (those that require intermediate aquatic hosts), and vector-based diseases. The transmission of these diseases is prevented through hygiene interventions, and by providing urban drainage and limiting the contamination of surface and recreational waters.

The third and fourth categories described by Mara and Feachem (1999) include two different types of helminthiases (parasitic intestinal worm infections). Eggs from the worm species in Category 3 develop into an infective stage in a soil environment, and are generally transmitted directly from one human host to another via ingestion or penetration of skin. Category 4 described by Mara and Feachem (1999) is *taeniases* (tapeworm). In Table 5, this category has been expanded to include neurocysticercosis, which is another disease that can be caused by *Taenia*. The many species of tapeworms each have slightly different life cycles, which all typically involve an intermediate herbivorous host and a definitive carnivorous host. The intermediate host consumes *Taenia* eggs and develops cysts in their muscle tissue. When the definitive host consumes raw or undercooked meat of the intermediate host with the *Taenia* cysts, they can develop intestinal tapeworm. Only definitive hosts excrete *Taenia* eggs in their feces. Humans are the definitive hosts for *Taenia saginata* and *Taenia solium*, for which cows and pigs are the intermediate hosts, respectively. Humans can develop tapeworm therefore, by consuming undercooked beef or pork. However, humans can also develop muscle cysts by ingesting *Taenia* eggs. These cysts are most dangerous if the eggs are from the *Taenia solium* species, because

they can travel to the brain, causing neurocysticercosis, a disease which is linked to epilepsy. Soil-transmitted helminthiasis, taeniasis and neurocysticercosis are best prevented by improving the treatment of wastewater and biosolids prior to reuse in agriculture.

Table 5: Helminthiases that can be transmitted via wastewater irrigation

Disease & Helminth Species			Transmission Route(s)	Global Importance
Category 3: Soil-transmitted (geo-) helminthiases	*Ascaris lumbricoides*	Roundworm (Ascariasis)	Human → Soil → Human (ingestion)	Geo-helminthiases are listed by the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) as one of seventeen Neglected Tropical Diseases; *Strongyloidiasis* is listed by WHO as a "neglected" condition. *Strongyloides stercoralis* eggs typically hatch inside the gastrointestinal tract prior to excretion, where they can reinfect the host (autoinfection). *S. stercoralis* is also an opportunistic parasite, capable of completing its life cycle in a soil environment outside of a host (Mahmoud 1996).
	Ancylostoma duodenale	Hookworm	Human → Soil → Human (skin penetration)	
	Necator americanus	Hookworm	Human → Soil → Human (skin penetration)	
	Strongyloides stercoralis[1]	Threadworm (Strongyloidiasis)	Human → Soil → Human (skin penetration) Human → Human (autoinfection)	
	Trichuris trichiura	Whipworm (Trichuriasis)	Human → Soil → Human (ingestion)	
Category 4: *Taenia*-related diseases	*Taenia saginata*	Beef tapeworm (Taeniasis)	Human → Cow → Human (ingestion of meat)	Taeniasis is a health concern especially if irrigating fodder crops. The ingestion of undercooked meat infected with *Taenia* cysticerci causes Taeniasis (tapeworm), but the direct ingestion of *Taenia solium* eggs can cause neuro-cysticercosis, the leading cause of acquired epilepsy worldwide.
	Taenia solium[2]	Pork tapeworm (Taeniasis)	Human → Pig → Human (ingestion of meat)	
		Neuro-cysticercosis	Human → Soil → Human (ingestion of eggs)	

Source: Adapted from Feachem et al. (1983); Mara and Feachem (1999)

Engineers traditionally use bacterial indicator organisms such as thermotolerant (fecal) coliforms to estimate the removal of pathogens in wastewater. However, different types of pathogens behave very differently in certain environments and have different infective doses. For example, helminth eggs are larger than bacteria, protozoa, and viruses, and therefore are removed more easily from wastewater via sedimentation. However, ensuring the removal of helminth eggs is of particular importance when wastewater is reclaimed for irrigation. The infective dose can be as low as one egg, and the eggs of some species, such as *Ascaris lumbricoides*, can survive for months or even years in soil, sediments, or biosolids (Feachem et al. 1983; Moe and Izurieta 2003; Nelson and Jiménez 2000). Other species of geohelminths, such as *Strongyloides stercoralis*, are also capable of behaving as opportunistic parasites—they can complete their life cycle as free-living organisms outside of a host before infecting a new host (Mahmoud 1996). Different species of helminths infect a new host in different ways. For the soil-transmitted helminths, some species infect their hosts via ingestion, while other species (i.e. hookworms) infect their hosts by penetrating the skin. Therefore, consumers of crops irrigated with wastewater can be at risk if viable helminth eggs are ingested, and farmers can be at risk if their skin comes into contact with contaminated soil. Helminths infect a total of 5 million people globally, the majority of which live in developing countries (Jimenez 2007). In some impoverished regions, incidence rates can reach 90%. Although the mortality rate associated with helminthiasis is low (Jiménez 2007b), the high morbidity rates in developing countries can have secondary health and developmental impacts. For example, helminth infections can impact the nutritional status of pregnant mothers (Mara et al. 2010). It has been estimated that globally, *Ascariasis* alone may put as many as 1.5 million children under the age of 15 at risk for permanent growth retardation (de Silva et al. 1997). Therefore, it is important to understand the removal of helminth eggs, especially from wastewater treatment systems where the effluent is to be evaluated for reuse in irrigation.

In 1973, the World Health Organization (WHO) published Technical report No. 517, entitled *Reuse of Effluents: Methods of Wastewater Treatment and Health Safeguards*, which addressed public health concerns associated with the reuse of wastewater. This report

recommended using chlorine to reduce coliforms to concentrations below 100 CFU per 100 ml, in order for wastewater to be reused for unrestricted irrigation with a “limited health risk” (World Health Organization 1973). These recommendations, which were developed without relevant epidemiological research, were not based upon actual health risks (Carr 2005), and were impractical for many farmers, especially those in developing countries. In 1989, WHO published updated guidelines (Mara and Cairncross 1989), which relaxed the recommended concentration of fecal coliform bacteria in wastewater for unrestricted irrigation from 100 to 1,000 coliforms per 100 ml, and acknowledged the threat of soil-transmitted helminth eggs, recommending a concentration of less than one egg per liter for both restricted and unrestricted irrigation. Unlike the recommendations from the 1973 report, the 1989 Guidelines were adopted globally and even modified by some countries to meet their own specific needs (Carr 2005).

In 2006, WHO published their most current set of recommendations, the *Guidelines for the Safe Use of Wastewater, Excreta and Greywater*, a document consisting of four volumes that address the use of these waste streams in agriculture and aquaculture, and provide guidance on policy, regulation, and institutional arrangements (World Health Organization 2006b). The 2006 Guidelines are based on new health evidence and contemporary risk management approaches, such as quantitative microbial risk assessment (QMRA). Instead of quantifying the health impact in terms of the total number of diseases, it is measured in disability adjusted life years (DALYs), which is a weighted impact that considers the context of the overall disease burden (Fattal et al. 2004). Volume 2 of the 2006 Guidelines addresses the reuse of wastewater in agriculture, recommending an integrated, systems approach that accounts not only for wastewater treatment, but also for non-treatment health interventions applied at different stages in the crop production process (Drechsel et al. 2010). A concentration of less than 1 helminth egg per liter of wastewater is still recommended in the 2006 Guidelines. Furthermore, when children under 15 years are present, wastewater should have less than 0.1 helminth eggs per liter, or additional health interventions should be implemented, such as the regular administration of antihelminthic drugs or regular deworming.

Instead of requiring a specific concentration of fecal coliform bacteria, the 2006 Guidelines require a target log removal of pathogens via wastewater treatment and other health protection measures, such that wastewater irrigation does not contribute more than a 10^{-6} DALY loss per person per year (World Health Organization 2006a). Figure 9 shows eight reuse scenarios (labeled A through H) that demonstrate how treatment, crop restrictions, and health protection measures achieve a combined 6 to 7 log-unit removal of pathogens.

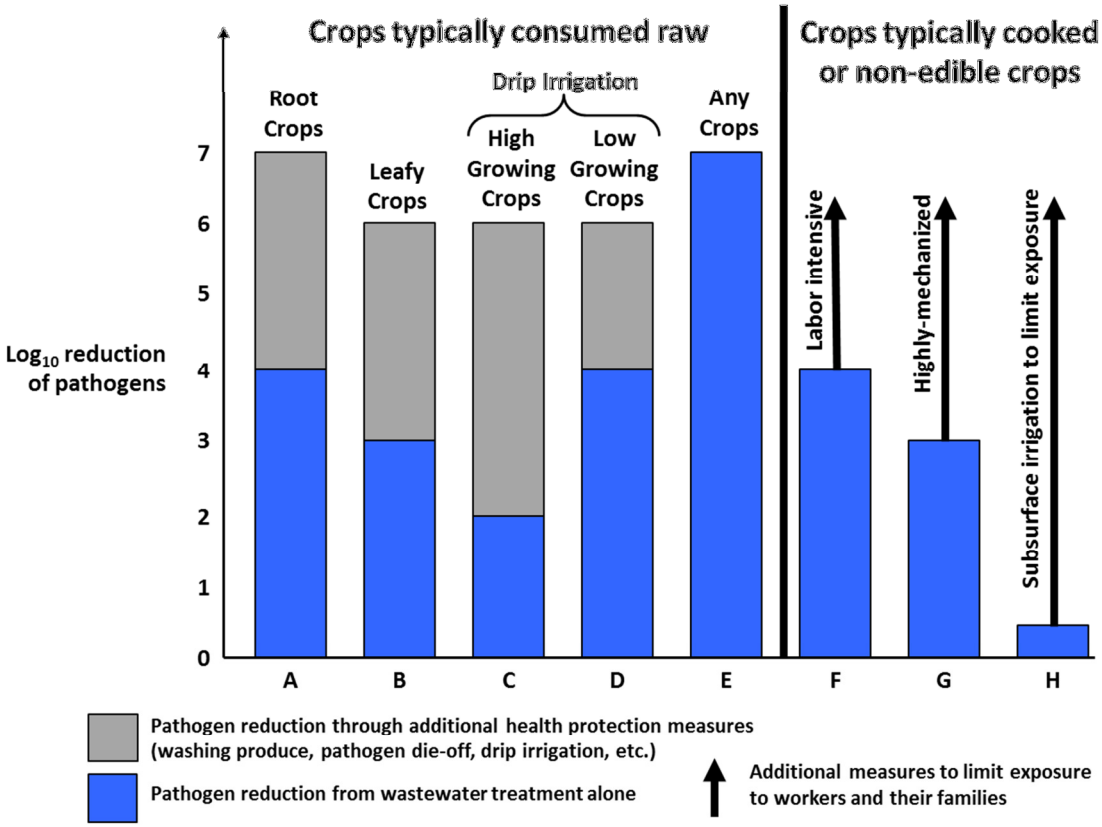


Figure 9: Wastewater irrigation scenarios presented in the 2006 WHO Guidelines (generated by author, after World Health Organization 2006a)

Figure 9 shows that root crops (such as onions) require a 7 log-unit reduction of pathogens, but leafy crops (such as lettuce) only require a 6 log-unit reduction of pathogens. This is based on epidemiological evidence (Shuval et al. 1997; Fattal et al. 2004) and risk estimates of infection from viral, bacterial, and protozoan pathogens, using QMRA with a β-Poisson dose-response model and 10,000-trial Monte Carlo simulations (World Health Organization 2006a). As shown in Scenario A of Figure 9, if a four log reduction is achieved from wastewater treatment, the remaining three log reduction can be achieved via additional health protection measures,

such as washing produce or pathogen die-off between the last irrigation and consumption. In Scenario C of Figure 9, a two-log reduction is achieved from wastewater treatment, and the other four-log reduction is achieved by using drip irrigation and restricting irrigation to high growing crops, such as tomatoes. These crops are less likely to become re-contaminated after a rain event than low-growing crops which come into contact with the soil, such as strawberries. The scenarios in Figure 9 are not the only possibilities (other scenarios could be developed based on local practices); however, they do provide an indication of the level of treatment required for wastewater to become a viable resource for agriculture.

2.4 Pathogen Removal in Stabilization Ponds and UASB Reactors

Stabilization ponds and UASB reactors can remove and inactivate human pathogens via a variety of mechanisms, which vary depending on the type of pathogen. The removal of bacterial pathogen indicators occurs via a variety of mechanisms, including both light-dependent and light-independent processes. Light-dependent processes include sunlight-mediated inactivation (Davies-Colley et al. 1999; Maïga et al. 2009), temperature increases caused by heating from sunlight, and high pH values caused by increased algal activity (Mara 2004). Light-independent processes include sedimentation of bacteria associated with flocs or other settleable particles, predation by protozoa and small micro-invertebrates such as water fleas, and die-off from starvation (Mara 2004). Thermotolerant coliform bacteria removal in pond systems can be modeled using pseudo-first-order kinetics in a completely mixed reactor, as shown in Equation 1 (Marais 1974); or in a reactor with dispersed flow, as shown in Equation 2 (Wehner and Wilhelm 1956; von Sperling 1999, 2002, 2003).

$$N_i = \frac{N_e}{1 + K_b t} \tag{1}$$

N_e = concentration of fecal coliforms (or *E. coli*) in the effluent
N_i = concentration of fecal coliforms (or *E. coli*) in the influent
T = water temperature
K_b = first-order rate constant for bacterial removal $K_b = 2.6 \cdot 1.19^{T-20}$

$$N_e = N_i \left(\frac{4a}{(1+a)^2} \right)^{\frac{1-a}{2(L/B)^{-1}}} \quad (2)$$

N_e = concentration of fecal coliforms (or *E. coli*) in the effluent

N_i = concentration of fecal coliforms (or *E. coli*) in the influent

$a = \sqrt{1+4k_{B(T)}\theta(L/B)^{-1}}$

$\theta = V/Q$ (i.e. the nominal hydraulic retention time)

$k_{B(T)}$ = rate constant, $= \left(0.92D^{-0.88}\theta^{-0.33}\right) \cdot 1.07^{T-20}$

T = water temperature

L, B, D = length, width, and depth of the pond

Helminth eggs are mainly removed via sedimentation, due to their large size and settling properties (Feachem et al. 1983; Shuval et al. 1986; Horan 2003). Theoretical settling velocities of particles can be calculated using Stokes' Law, based on the particle's size and shape, its relative density compared to the water, and the viscosity of the water. Table 6 provides theoretical settling velocities for several types of helminth eggs. However, one study found that theoretical settling velocities were as much as three times higher than measured settling velocities of *Ascaris* eggs in clean water (Sengupta et al. 2011). In other words, helminth eggs in pond systems may require more time to settle out than predicted by Stokes' law, regardless of the pond hydraulics. On the other hand, helminth eggs that are stuck to or that associate with larger flocs of organic material in the wastewater may settle out at faster rates than free-floating eggs.

Table 6: Theoretical settling velocities for helminth eggs

Species	Diameter Range (µm)	Density (g/cm^3)	Assumed Shape	Theoretical Settling Velocity (m/h)
Ascaris lumbricoides	55 – 70	1.11	Sphere	0.65 – 0.99
Hookworms	40 – 60	1.055	Sphere	0.39
Taenia saginata	30	1.1	Sphere	0.26
Trichuris trichiura	22 – 30 (dia.) 50 – 76 (length)	1.15	Cylinder	0.47 – 1.53

Source: Feachem et al. (1983); Sengupta et al. (2011)

The long hydraulic retention times and quiescent flow conditions typically encountered in stabilization ponds favor the settling of helminth eggs. For example, according to Ayres et al. (1992), pond systems with hydraulic retention times of at least 11-12 days should produce an effluent with less than one egg per liter, and pond systems with hydraulic retention times of at least 19 days should ensure the complete removal of helminth eggs. This has been generally accepted by the academic and international public health community as a standard guideline for pond systems. In fact, the WHO even goes so far as to suggest that the hydraulic retention time of stabilization ponds can be used as a surrogate to ensure compliance with the <1 egg per liter requirement (World Health Organization 2006b). The problem with this assumption is that in developing countries, stabilization ponds are often constructed with a variety of configurations and inefficient hydraulics. For example, a tracer study of one stabilization pond system revealed a mean hydraulic retention time that was only one-fifth of the nominal hydraulic retention time, resulting in high concentrations of helminth eggs in the effluent (Lloyd and Frederick 2000).

Figure 10 shows that data assembled from the literature for stabilization ponds reveals a weak correlation between the concentration of helminth eggs in pond effluents and nominal hydraulic retention times, especially those that are less than 10 days (Saqqar and Pescod 1992; Dixo et al. 1995; Ouazzani et al. 1995; Silva et al. 2000; Soares et al. 2000; Mara et al. 2001; Madera et al. 2002; von Sperling et al. 2002, 2003; Stott et al. 2003; Oakley 2004; Bastos et al. 2010). This lack of correlation is likely due to variations in the concentrations of helminth eggs in the system influents, as well as variations in the flow conditions and the configurations of pond influents and effluents. The WHO does not address whether or not a tracer study is required to determine the mean hydraulic retention time. The studies cited also utilize a variety of methods for the detection of helminth eggs, which have varying rates of recovery and minimum levels of detection. Therefore, some of the variability in the data may be due to the different methods used to enumerate and detect helminth eggs.

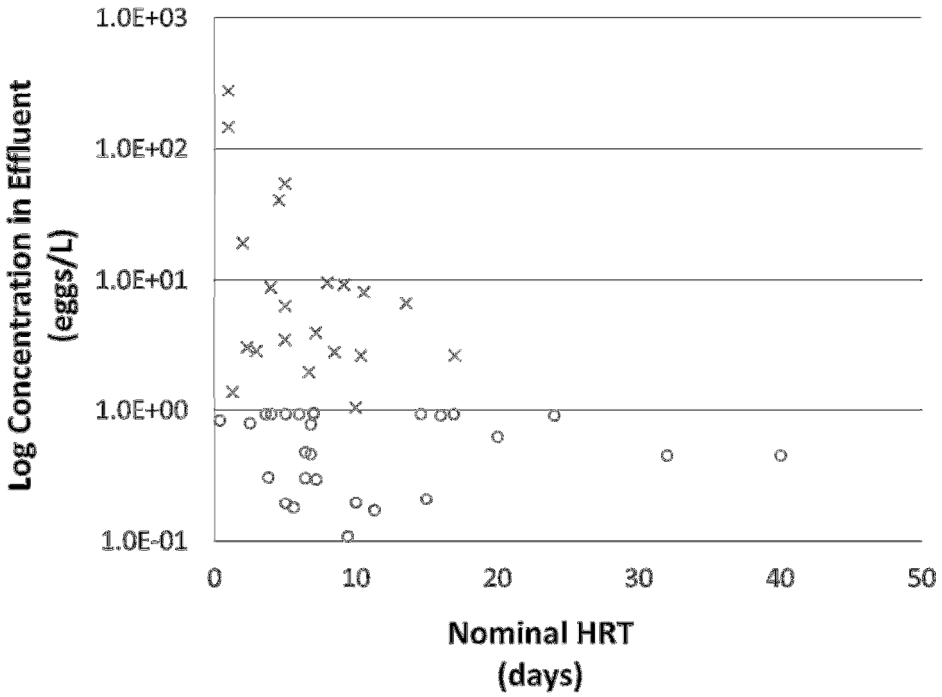


Figure 10: Nominal hydraulic retention times of stabilization ponds and corresponding concentrations of helminth eggs in pond effluents that (o) meet and (x) do not meet recommendations by the 2006 WHO Guidelines for reuse in irrigation

UASB reactors are advanced anaerobic treatment systems that were originally developed to treat industrial wastewater with high concentrations of COD, but have since been adapted for the treatment of domestic wastewaters, especially in developing countries with warm climates. These reactors are typically sized for the removal of organic material and suspended solids, not pathogens. Therefore, pathogen removal in UASB reactors is not frequently reported in the literature. The hydraulic retention time (HRT) for a typical UASB reactor, which is measured in hours, is typically much less than retention times of stabilization ponds. Because of this, pathogen removal in a UASB reactor with an HRT of several hours is theoretically going to be lower than pathogen removal in a stabilization pond with an HRT of several days. According to Chernicharo (2007), UASB reactors typically reduce the concentration of thermotolerant coliforms by one log unit or less. As shown in Table 7, the majority of studies identified in the literature have reported 60% to 90% removal of helminth eggs (Chernicharo et al. 2001; Dixo et al. 1995; Keller et al. 2004; Soares et al. 2000; von Sperling et al. 2002, 2003). However, no removal of helminth eggs was found for at least one full-scale system (von Sperling et al. 2005). Many factors influence the

removal of helminth eggs in a UASB reactor, including the velocity and turbulence of the flow through the reactor, pH conditions, and sludge characteristics. Furthermore, densely-packed sludge at the bottom layer of reactors may actually act as a filter for larger pathogens such as helminth eggs, which may become trapped in the interstitial spaces of the sludge bed (Chong et al. 2012). UASB reactors also produce less turbid effluent than anaerobic ponds, which allows for greater light penetration into any maturation ponds used for post-treatment. This can induce higher levels of algal activity, a higher pH, and greater removal of pathogens and nutrients (Catunda and van Haandel 1996).

Table 7: Removal of helminth eggs in UASB reactors treating domestic wastewater

Source	Scale	Helminth Egg Removal			
		Avg. Influent (eggs/L)	Avg. Effluent (eggs/L)	Nom. HRT (hours)	Observed Removal
Dixo et al. (1995)	Full	16,774 Range: (8720 – 34,000)	1740 Range: (400 – 4125)	7.0	90%
Chernicharo et al. (2001)	Full	47.3	14.0	NR	70%
		120.7	21.3	NR	82%
von Sperling et al. (2002) Soares et al. (2000)	Pilot	64.3 Range: (0 – 320.0)	16.2 Range: (1.3 – 45.0)	5.5	75%
von Sperling et al. (2003)	Pilot	254	37	5.0	85%
		76	16	5.0	79%
		75	10	7.5	87%
		26	4	7.5	85%
Keller et al. (2004)	Pilot	19.5 Range: (16.7 – 13.3)	5.0	4.9	74%
von Sperling et al. (2005)	Full	NR	NR	NR	71%
		NR	NR	NR	0%
		NR	NR	NR	88%
	Pilot	NR	NR	NR	86%
		NR	NR	NR	63%

NR = not reported

2.5 Knowledge Gap in Literature

Evaluating the reuse potential of wastewater in agriculture is an important part of the solution to meet the sanitation and hunger targets of the Millennium Development Goals. Helminth eggs in particular present an important health concern for wastewater reuse systems in developing countries. Stabilization ponds have been recommended as appropriate for the treatment of wastewater in developing countries. The results from the literature review summarized above demonstrate that stabilization pond systems can remove helminth eggs from wastewater to levels that are recommended by the WHO for reuse in agriculture. This is especially true when hydraulic retention times are longer than 20 days. However, there is weak correlation between hydraulic retention time and effluent concentration of helminth eggs, which may represent a lack of understanding about the influence of pond configuration and hydraulics. High-rate anaerobic technologies, such as the UASB reactor, represent newer technologies with growing popularity in developing countries. If combined with additional post-treatment, UASB reactors may offer some advantages and some disadvantages when compared to systems consisting only of stabilization ponds. The number of previous studies available in the literature that have reported the removal of helminth eggs and pathogens in UASB reactors is limited.

Based on the needs and limitations described above, the present study will fill the knowledge gap in the literature by measuring the removal of helminth eggs and pathogen indicators from a wastewater treatment system consisting of three stabilization ponds in series, and a system consisting of a UASB reactor followed by two stabilization ponds in series. The advantages of this study are that both systems are approximately the same age, are community-managed, and serve two small communities located in the same geographical region, with similar populations and health conditions.

3.0 MATERIALS AND METHODS

The study was conducted at the wastewater treatment plants of two small towns in the Alto Beni region of Bolivia (Figure 11). The first system, consisting of three stabilization ponds in series (three-pond system), serves a population of approximately 777 people, and is located at latitude 15º 39' 06" south, longitude 67º 10' 29" west, at an elevation of 460 meters above sea level. The second system, consisting of a UASB reactor followed by two stabilization ponds in series (UASB-pond system), serves a population of approximately 1310 people, and is located at latitude 15º 33' 36" south, longitude 67º 20' 19" west, at an elevation of 405 meters above sea level. These systems have been described in detail by others (Muga et al. 2009a, 2009b; Fuchs and Mihelcic 2011). It is important to note that, from 2010 until the completion of the study, community members had been unable to remove the sludge from the UASB reactor because of a clogged discharge pipe. By June 2012, the sludge had nearly reached the top of the reactor; however water still passed through at normal flow rates during the time of the sample collection.

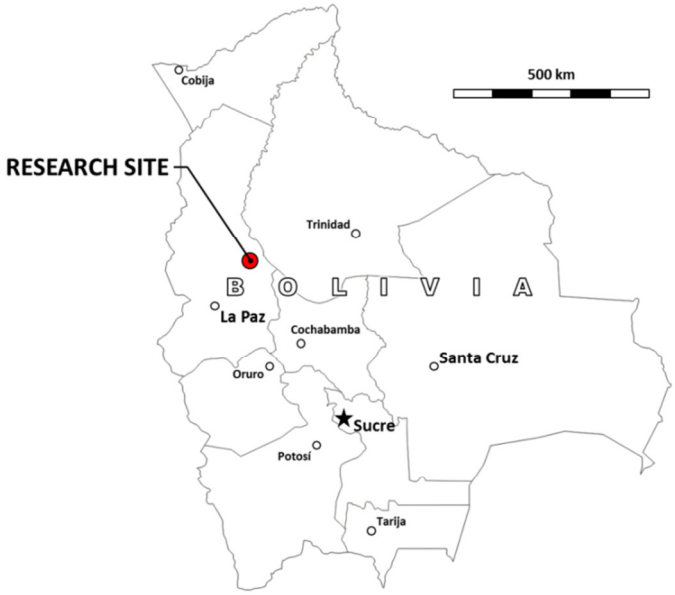
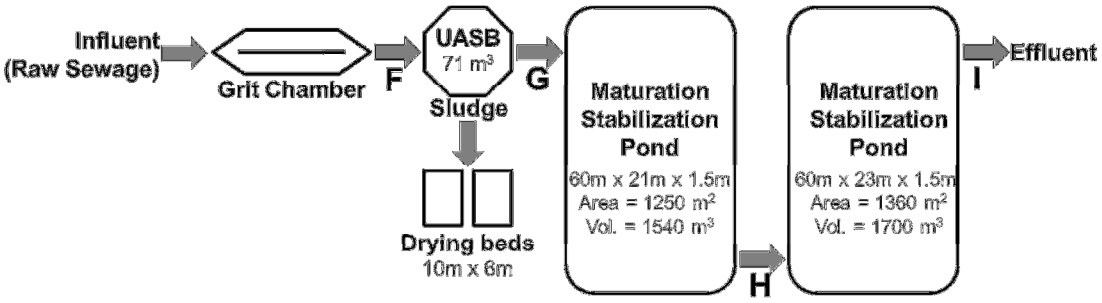


Figure 11: Location of research field site in Bolivia

3.1 Collection of Samples

Water samples were collected at four locations in both systems between 2007 and 2012. Sludge samples were collected near the influent of the facultative pond (in 2011 and 2012), and from the UASB reactor (in 2012). Water samples were analyzed for physical-chemical water quality parameters and thermotolerant coliforms once or twice per year (except in 2012). Water and sludge samples were analyzed for helminth eggs in 2011 and 2012. Figure 12 depicts a schematic of both systems, showing each of the eight sampling locations. In the three-pond system (Figure 12a), samples were collected at A) the influent of the facultative pond; B) the effluent of the facultative pond; C) the effluent of the first maturation pond; and D) the effluent of the second maturation pond. In the UASB-pond system (Figure 12b), samples were collected at F) the influent of the UASB reactor; G) the effluent of the UASB reactor; H) the effluent of the first maturation pond; and I) the effluent of the second maturation pond.

(a)



(b)

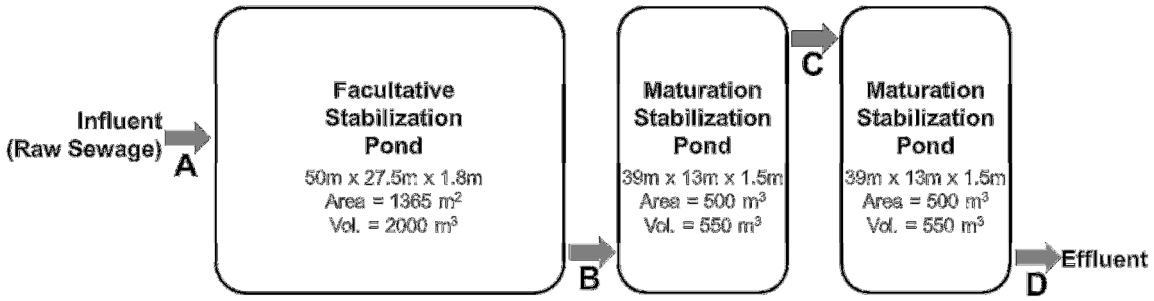


Figure 12: Schematic diagram of a.) sampling points A through D in the three-pond system; and b.) sampling points F through I in the UASB-pond system

Samples were either grab samples or 24-hour composite samples, depending on the purpose of the sample. Composite water samples were collected at hourly intervals. The volume collected each hour was calculated based on the flow rate measured during that hour, in order to obtain a more representative composite sample, as described in Figure 13. By using this procedure to collect composite samples instead of collecting the same volume for every hourly subsample, a more representative sample of the wastewater discharged throughout the day is provided. Grab samples were collected during the peak daily flow, typically between 7:00 a.m. and 10:00 a.m. All sampling events occurred during the month of June, which is in the middle of the dry season, when irrigation would most likely occur.

Total volume needed for 24-hour sample: *z* liters

Estimated daily flowrate: *y* liters per day

The following ratio ($r_{z/y}$) is calculated:

$$\frac{z \text{ liters}}{y \text{ liters}} = r_{z/y}$$

If composite samples are to be collected hourly, one composite sample will require 24 subsamples, with each subsample representing the wastewater discharged over the course of one hour. The volume needed for each subsample is equal to the ratio $r_{z/y}$ multiplied by the measured flow rate, multiplied by one hour.

If flow at hour *x* is q_x, the volume v_x collected for that hourly subsample is: $v_x = r_{z/y} \cdot q_x \cdot (1 \text{ hr})$

If the average daily flowrate equals the estimated daily flowrate, then the total volume *V* of the sample at the end of the 24 hours can be calculated as shown below, and should be equal to *x*:

$$V = \sum_{x=1-24} r_{z/y} \cdot q_x \cdot (1 \text{ hr}) = v_1 + v_2 + v_3 + ... + v_{23} + v_{24} \approx z$$

Figure 13: Composite sample collection procedure

Separate equipment was used at each collection point to avoid cross-contamination of samples. Grab samples for bacterial analysis were collected with sterile plastic bottles provided by the laboratory. All equipment used for composite samples was made of inert plastic, and was disinfected prior to use with the following procedure: first, equipment was wiped down with a 10% sodium hypochlorite solution; then, it was rinsed in a soapy water solution; finally, equipment was triple-rinsed with potable water and left to air-dry. Flow rates were measured using a stopwatch and 8,000-ml or 10,000-ml graduated plastic pitchers. Subsamples were collected in the plastic pitchers and the appropriate volumes were measured using a smaller plastic graduated bottle with 5-ml marks. Funnels were used to transfer subsamples into 10-liter sample jugs, which were

stored on ice in a Styrofoam cooler in the shade for the duration of the sampling period and during transportation to the laboratory. Samples collected from both systems were brought back to a central staging area for processing, and finally shipped to the appropriate laboratory for analysis or analyzed on-site with field equipment.

Composite samples were analyzed for the following parameters, as shown in Table 8: total suspended solids (TSS); five-day biochemical oxygen demand (BOD_5); chemical oxygen demand (COD); total nitrogen (TN); total Kjeldahl nitrogen (TKN); ammonia-nitrogen (ammonia-N); nitrate (NO_3); total phosphorus (TP); orthophosphate, and helminth eggs. Grab samples were analyzed for thermotolerant coliforms (TTC) and some grab samples were also analyzed for helminth eggs. Sludge samples were collected from the inside of the UASB reactor and from several points near the entrance to the facultative pond in the three-pond system, and were analyzed for the following parameters: total solids (TS) and total volatile solids (TVS).

Table 8: Collection dates and number of samples analyzed

Parameter	Collection Date						Type of Sample[1] (n=# samples)
	6/2007	6/2008	6/2009	6/2010	6/2011	6/2012	
TSS	x	x		x x	x		Composite (5)
BOD_5	x	x		x x	x		Composite (5)
COD	x	x		x x	x		Composite (5)
TN	x	x	x	x x	x		Composite (6)
TKN					x		Composite (1)
Ammonia-N			x		x		Composite (2)
NO_3					x		Composite (1)
TP	x	x			x x x x		Composite (6)
Orthophosphate					x		Composite (1)
TTC	x	x	x	x x	x		Grab (6)
Helminth Eggs					x	x x	Composite (3) Sludge[2] (3)
TS					x	x x	Sludge[2] (3)
TVS					x	x x	Sludge[2] (3)

[1] All samples refer to water samples unless otherwise noted

[2] Sludge samples were only collected at the three-pond system in 2011

3.2 Conventional Parameter Analysis

Composite samples were distributed into two-liter plastic bottles that were rinsed with water prior to sample collection, and shipped on ice overnight to the Instituto de Ingenería Sanitaria laboratory, located at the Universidad Mayor de San Andres (UMSA) (La Paz, Bolivia). The samples were further divided at the laboratory and analyzed for TSS, COD, and BOD_5. Portions of these samples were also analyzed for nutrients, as described below. Analyses were performed in accordance with Standard Methods for the Examination of Water and Wastewater (APHA et al. 2012).

3.3 Nutrient Analysis

A portion of the two-liter composite samples used for the analyses of conventional parameters (described above) were also used for the following nutrient analyses, performed at the Instituto de Ingenería Sanitaria laboratory: TN, TKN, ammonia-N, nitrate, TP, and orthophosphate. All nutrient analyses, unless otherwise noted, were performed in accordance with Standard Methods for the Examination of Water and Wastewater (APHA et al. 2012). Samples collected in 2011 were analyzed in the field with a spectrophotometer (Hach DR 2800) for TN, ammonia-N, nitrate, TP, and orthophosphate. Deionized water was used in all cases as a negative control. Total nitrogen was measured via the persulfate digestion method, as described in the Hach DR 2800 Spectrophotometer Procedures Manual (Hach Company 2007). Briefly, 0.5-ml aliquots of the samples were added to vials containing a solution of sodium hydroxide and potassium persulfate, shaken vigorously for 30 seconds, and heated to 100℃ for 30 minutes with a thermoreactor (Orbeco Hellige TR125, Sarasota, FL) to allow all forms of nitrogen in the sample to be converted to nitrate. After the vials were cooled to room temperature, a powder packet containing sodium metabisulfite was added, and the vials were shaken for 15 seconds. The vials were placed to rest for three minutes to allow the sodium metabisulfite to eliminate halogen oxide interferences. A second powder packet, containing a mixture of sodium metabisulfite, urea, chromotropic acid disodium salt, and white quartz sand, was added and the vials were shaken for 15 seconds, and placed to rest for two minutes. Finally, a portion of the solution from the vials was added to a solution of sulfuric acid to bring down the pH, and the vials were slowly inverted

ten times to mix. The vials were placed to rest for a ten minute reaction time, which allowed for the chromotropic acid to react with the nitrates in the sample, forming a yellow complex with an absorbance maximum at 410 nm. The spectrophotometer was zeroed at 410 nm using the vial containing the negative control, and the absorbance was read at 410 nm for each of the vials containing samples. Results were interpolated from a standard curve prepared in the field, using a solution containing a known concentration of total nitrogen.

Nitrate was measured using the chromotropic acid method, as described in the Hach DR 2800 Spectrophotometer Procedures Manual (Hach Company 2007). This procedure is very similar to the total nitrogen procedure, only omits the step where organic nitrogen is digested with the alkaline solution of potassium persulfate. Briefly, 1.0-ml aliquots of the samples were added to vials containing a solution of sodium metabisulfite and sulfuric acid, and slowly inverted ten times to mix. A second powder packet, containing a mixture of sodium metabisulfite, urea, chromotropic acid disodium salt, and white quartz sand, was added and the vials were slowly inverted 10 times to mix, and placed to rest for five minutes, which allowed for the chromotropic acid to react with the nitrates in the sample, forming a yellow complex with an absorbance maximum at 410 nm. The spectrophotometer was zeroed at 410 nm using the vial containing a negative control, and the absorbance was read at 410 nm for each of the vials containing samples. Results were interpolated from a standard curve prepared in the field, using a solution containing a known concentration of nitrates.

Ammonia nitrogen was measured with the salicylate method, as described in the Hach DR 2800 Spectrophotometer Procedures Manual (Hach Company 2007). Briefly, 0.1-ml aliquots of the samples were added to vials containing a diluent solution, to which powder packets containing sodium dichloroisocyanurate, lithium hydroxide, sodium salicylate, sodium citrate, sodium tartrate, and sodium nitroferricyanide were added. The vials were then shaken thoroughly and allowed to sit for 20 minutes to allow several reactions to take place. The ammonia compounds in the samples react with chlorine to form monochloramine, which reacts with the salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of the sodium nitroferricyanide to form a blue-colored compound, which mixes with the yellow-colored

excess reagent to make a green-colored solution that can be measured in the spectrophotometer at a wavelength of 655 nm. The negative blank should remain a yellowish color, since it should be free of ammonia compounds. The spectrophotometer was zeroed at 655 nm using the negative control, and the absorbance was read for each of the vials containing samples. Results were interpolated from a standard curve prepared in the field, using a solution containing a known concentration of ammonium.

Total phosphorus was measured using the acid hydrolysis method, as described in the Hach DR 2800 Spectrophotometer Procedures Manual (Hach Company 2007). Briefly, a 5.0-ml aliquot of the sample was added to test vials along with potassium persulfate, shaken to mix, and then heated to 150°C for 30 minutes with a thermoreactor (Orbeco Hellige TR125, Sarasota, FL) to allow all forms of phosphorus in the sample to be converted to reactive orthophosphates. After being cooled to room temperature, a 1.54N solution of sodium hydroxide was added to the vials, and they were placed in the spectrophotometer, which was zeroed at 880 nm. The vials were then removed from the spectrophotometer and a powder packet containing potassium pyrosulfate, sodium molybdate, and ascorbic acid was added to the solution. The vials were shaken for 30 seconds to mix, and then placed to rest for two minutes, to allow the orthophosphates in the solution to form complexes with the molybdate, which are then reduced by the ascorbic acid, producing an intense molybdenum blue color, which was then read by the spectrophotometer at 880 nm no more than 8 minutes after the powder packet was added to the solution. The absorbances read were interpolated from a standard curve prepared in the field, using a solution containing a known concentration of phosphorus.

Orthophosphates were measured using the same method as the total phosphorus analysis, only omitting the step where the inorganic phosphates are hydrolyzed to form reactive orthophosphates. Samples were placed in vials and the spectrophotometer was zeroed at 880 nm. Powder packets containing potassium pyrosulfate, sodium molybdate, and ascorbic acid were added to the samples, which were shaken and then placed to rest for two minutes to allow orthophosphates to form complexes with the molybdate and produce the molybdenum blue color, which was read by the spectrophotometer at 880 nm no more than 8 minutes after the powder

packet was added to the solution. The absorbances read were interpolated from a standard curve prepared in the field, using a solution containing a known concentration of orthophosphates.

3.4 Thermotolerant Coliform Analysis

Grab samples were shipped to the Instituto de Ingenería Sanitaria laboratory in La Paz and analyzed within 24 hours for thermotolerant coliforms, using either the membrane filtration method (for samples with turbidity <50 NTU) or the multiple tube fermentation technique (for samples with turbidity >50 NTU), as described in the Standard Methods for the Examination of Water and Wastewater (APHA et al. 2012). Briefly, for the membrane filtration method, the samples were diluted and filtered through a 0.45-µm membrane filter under partial vacuum. Approximately 60-ml of sterile dilution water was subsequently passed through each filter. The filters were then placed with sterile forceps onto culture dishes that had been previously prepared with sterile absorbent pads saturated with an mFC medium consisting of: 10.0 g tryptose; 5.0 g polypeptone; 3.0 g yeast extract; 5.0 g sodium chloride; 12.5 g lactose; 1.5 g bile salts; 15.0 g agar; and reagent-grade water. Culture dishes were then incubated for 24 hours at a temperature of 44.5 ±0.2℃. After the incubation period, dishes were removed from incubator and colonies were enumerated and concentrations were calculated based on the dilution of each sample. For the multiple tube fermentation technique, ten aliquots of each wastewater sample were added to multiple tubes at several dilutions and incubated for 24 hours at 44.5 ±0.2℃ with an EC medium consisting of: 20.0 g tryptose; 5.0 g lactose; 5.0 g sodium chloride; 1.5 g bile salts; 4.0 g dipotassium hydrogen phosphate; 1.5 g potassium dihydrogen phosphate; and reagent-grade water. Gas production and growth within 24 hours was considered a positive reaction and failure to produce gas (with little or no growth) a negative reaction. The number of positives for each dilution was interpreted using a Most Probable Number table (APHA et al. 2012).

3.5 Helminth Eggs Analysis

Composite samples collected in 2011 and 2012 were analyzed for helminth eggs at the Centro de Aguas y Saneamiento Ambiental (CASA) laboratory, at the Universidad Mayor de San Simon (UMSS) in Cochabamba, Bolivia. The volumes of water samples collected are shown in

Table 9. The volumes of samples collected from the system effluents were generally higher than the volumes of raw wastewater samples at the influents because the concentrations of helminth eggs in the influents were anticipated to be much higher and therefore require less volume to achieve a countable number of eggs.

Table 9: Volumes of samples collected for helminth egg analysis

Treatment System	Location of Sample[1]		Sample Date and Volume (liters)		
			6/13/2011	6/17/2012	6/20/2012
Three-Pond	A	Raw Wastewater (System Influent)	5.0	2.3	2.0
	B	Effluent of Facultative Pond	-	9.1	2.0
	D	Effluent of 2nd Maturation Pond	-	32.1	2.0
UASB-Pond	F	Raw Sewage (System Influent) [2]	2.5	8.0	2.0
	G	Effluent of UASB Reactor	-	7.9	2.0
	I	Effluent of 2nd Maturation Pond	-	22.5	2.0

[1] The locations of samples correspond with Figure 12

[2] This sample point was actually located after a grit removal chamber

All samples were collected using 10-liter jugs in the field and then brought back to a central processing area. Here, samples were either transferred to buckets for concentration via gravity settling (6/13/2011 and 6/17/2012) or transferred directly to pre-rinsed 2-liter water bottles to ship to the laboratory (6/20/2012). Any material sticking to the jugs was washed into the buckets or the 2-liter bottles with drinking water. The buckets used for settling were placed on a flat surface for a minimum of four hours, to allow the eggs to settle to the bottom. Any floating scum or bubbles were broken up by gently stirring the sample. After the settling period, the top 50 to 90 percent of the supernatant was decanted using a siphon (Figure 14). The remaining sediments were transferred into empty 2-liter potable water bottles with a funnel. Any materials left in the bottom of the settling buckets were washed into the 2-liter bottles using potable water. The samples were then transported within 48 hours in a Styrofoam cooler on ice to the Centro de Aguas y Saneamiento Ambiental laboratory for further processing and analysis.

At the Centro de Aguas y Saneamiento Ambiental laboratory, samples were analyzed in accordance with the Mexican Test Method for the Determination of Helminth Eggs in water

samples (Secretaria de Comercio y Fomento Industrial 1999), with a few notable exceptions, as described below. This method, which is a modified version of the EPA method 625/R-92/013 (Environmental Protection Agency 2003), allows for the isolation of helminth eggs from wastewater or sludge samples based on their relative density, which differs from the density of many other substances typically present in wastewater and sludge. The method uses coagulation, sedimentation, flotation, decantation, and two-phase separation, to concentrate the eggs in a volume of solution small enough so that a representative portion of it can be transferred to a counting slide and observed under a light microscope by a trained microbiologist, who measures, identifies, and enumerates helminth eggs.

Figure 14: Samples settling (left); supernatant decanted manually with siphon (right)

A magnesium sulfate ($MgSO_4$) solution with a specific gravity of 1.3 was prepared at the laboratory in advance using sterile, reagent-grade water (Figure 15). The Mexican guidelines recommend using a zinc sulfate solution, but this was substituted with magnesium sulfate because of the fact that Epsom salt is readily available at a low cost in Bolivia. An alcohol-acid solution was also prepared in advance, by mixing 650 ml of 0.1N H_2SO_4 and 350 ml of ethyl

alcohol. All wastewater samples were distributed into centrifuge tubes. Sludge samples were first washed through a 160 µm filter with 5 liters of sterilized water. The water and material that passed through the filter was then collected and left to settle in a bucket for a minimum of three hours, after which time the supernatant was siphoned off and the sediment was distributed into centrifuge tubes. The settling bucket was washed three times with sterile water to collect any eggs that may have been stuck to the sides of the bucket. Positive control samples were prepared using sterile reagent water and a known concentration of *Ascaris suum* eggs, which were harvested from the uterus of adult female worms extracted from the intestines of a pig.

All samples were centrifuged at 400*g* for 3-5 minutes, and the supernatant was siphoned off, leaving behind a concentrated pellet (Figure 16). The pellet was then re-suspended in 150 ml of the magnesium sulfate solution, homogenized, and centrifuged again at 400*g* for three to five minutes (Figures 15 and 16). The supernatant was then poured off into a 2,000-ml container, diluted with 1,000 ml of distilled water and left to settle for a minimum of three hours. At this point, the samples were noticeably cleaner than they were at the beginning (Figure 17).

Figure 15: Preparation of magnesium sulfate solution (left) and centrifuge tubes (right)

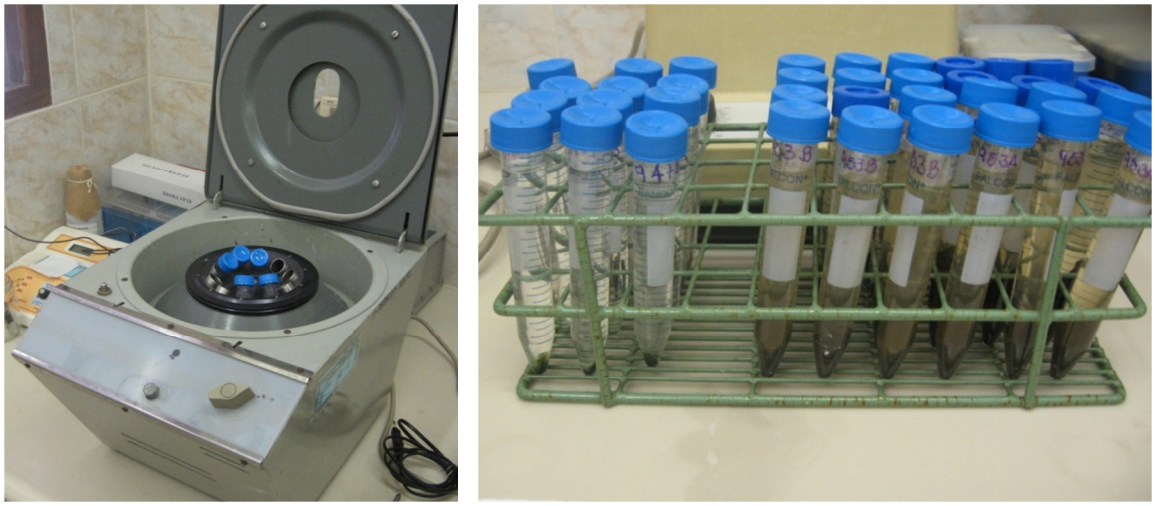


Figure 16: Centrifugation of samples (left); pellets in tubes after centrifugation (right)

Figure 17: Diluted samples settling after centrifugation with magnesium sulfate solution

After samples were left to settle, the top of the sample was carefully siphoned off, leaving approximately 150 ml at the bottom. These volumes were poured into a 200-ml centrifuge tube, along with 50 ml of sterile reagent water, which was used to wash the bottom and the sides of the containers in the event that any eggs remained stuck to the containers. The samples were then centrifuged at 480*g* for three minutes, the supernatants were decanted, and the pellets were resuspended in sterile reagent water in other centrifuge tubes, where they were centrifuged again at 480*g* for three minutes. The supernatants of the samples were decanted again, only this time, the pellets were resuspended in centrifuge tubes with 15 ml of the alcohol-acid solution. Ten ml of

ether was carefully added to the centrifuge tubes, which were shaken softly while removing the cap periodically between shaking, to allow gases to escape. The samples were then centrifuged one last time at 660*g* for three minutes, and the supernatants were decanted as much as possible without removing the pellet at the bottom. The final volumes of the pellets varied from sample to sample, but after homogenizing the pellets, a small volume was pipetted from each sample and transferred to a counting slide (Neubauer improved bright-line) (Figure 18). Counting slides were observed under a light microscope methodically to identify and count helminth eggs. A minimum of two repetitions were done, and the average was taken. Results were reported as eggs per liter and the concentrations of the samples were back-calculated, based on the original sample volumes.

Figure 18: Preparing the counting slides for helminth egg enumeration under microscope

There are several different ways to measure the viability of helminth eggs in the laboratory, including the use of incubation, microscopic observation and vital stains. In this study, some of the helminth eggs from each sample location were isolated from the counting slide and a 0.1-ml solution of 0.4% Trypan Blue stain (Fisher Scientific, #ICN1691049) was added. Trypan Blue selectively stains dead cells a dark blue color, while living cells remain unstained. It is important to note some of the eggs may have been inactivated or destroyed during the sample concentration process: the use of ether in the final step of the sample concentration process can be particularly harmful to helminth eggs (Nelson and Darby 2001). Trypan Blue may also over-predict the number of non-viable eggs, since the amount of stained eggs increases with respect to time (World Health Organization 2004). Results can be improved by examining samples within five minutes of adding the stain (World Health Organization 2004). In this study, eggs that were stained within the first five minutes were counted as non-viable, while non-stained eggs were assumed to be viable.

3.6 Statistical Analysis

When there were more than five data points, the mean and the 90% confidence interval was calculated for each sample location, assuming normal distribution of the data. All "non-detect" samples were replaced with a value equal to half of the limit of detection (Wendelberger and Campbell 1994). In order to compare the data between systems, Microsoft Excel was used to perform an unpaired, two-tailed *t*-test on each set of sample points. A homoscedastic *t*-test was used if the variance of the data points in each system was found to be equal, and a heteroscedastic *t*-test was used if the variance was found to be unequal, based on an F-test. An alpha value of 0.10 (instead of 0.05) was used to estimate significance, based on the assumption that samples analyzed from full-scale natural treatment systems (such as the ones studied) are likely to have results that vary much more than a controlled experiment in a laboratory setting.

4.0 RESULTS AND DISCUSSION

4.1 Organic and Hydraulic Loading

In order to make a meaningful comparison between the two systems studied, it is necessary to demonstrate that their organic loading rates and flow conditions do not drastically differ. An overloaded system will not remove pathogens or other constituents as well as a system operating at optimal loading rates. For the purposes of this study, to account for poor hydraulics and hydraulic short-circuiting, it is assumed that the mean hydraulic retention times of the ponds in these systems are approximately 50% of the theoretical retention times (the volume divided by the average flowrate). Tracer studies of stabilization ponds have produced varying results. Frederick and Lloyd (1996) found that the mean hydraulic retention time of one facultative pond was reduced by 75% of its theoretical value. Macdonald and Ernst (1986) found reductions of 59% and 25% for two maturation ponds, while Herrera and Castillo (2000) found a 55% reduction in a three-pond system. Torres et al. (1999) reported mean hydraulic retention times for three different facultative ponds in Spain that were only 13%, 10%, and 4% lower than their theoretical retention times. For this study, the overall hydraulic retention times for the three-pond system and the UASB-system are estimated to be 20 days and 28.7 days, respectively (Table 10). Although the UASB-pond system has almost twice as many users as the three-pond system, the per capita flow of the UASB-pond system is less than half of the per capita flow of the three-pond system (44.4 L/capita-day versus 98.5 L/capita-day). This is likely explained by the differences in water usage between the two communities. The UASB-pond system users have water meters and pay monthly fees based on usage, whereas the three-pond system users pay a flat fee regardless of how much water they use. This difference in water usage causes the UASB-pond system to receive higher-strength wastewater than the three-pond system (average BOD_5 is 235 mg/L versus 191 mg/L).

Table 10: Flow conditions and estimated hydraulic retention times (HRT) for each system

Treatment System	Avg. Flow Rate	Population Served	Per Capita Flow	Avg. BOD_5 Influent	Theoretical HRT (Estimated HRT)			
					UASB/FP	MP #1	MP #2	Total
	(m^3/day)	(people)	(L/p/day)	(mg/L)	(days)	(days)	(days)	(days)
Three-Pond	76.6	775	98.5	191	26.0 (13.0)	7.0 (3.5)	7.0 (3.5)	40.0 (20.0)
UASB-Pond	58.1	1300	44.4	235	1.2 (1.2)	26.0 (13.0)	29.0 (14.5)	56.2 (28.7)

UASB = upflow anaerobic sludge blanket reactor; FP = facultative pond; MP = maturation pond

The organic surface loading of a pond is calculated as the average flow entering the pond, multiplied by the average concentration of BOD_5 at the pond influent, divided by the area of the pond. This surface loading rate can be compared to the theoretical rate of oxygen production, which is based on the solar insolation and the estimated efficiency of algae in the pond. Monthly insolation data was obtained from the Surface Meteorology and Solar Energy website (Appendix A), which is maintained by NASA (2011). The theoretical maximum surface loading rate was calculated for the facultative pond (three-pond system) and the first maturation pond (UASB-pond system) since these two ponds receive the highest-strength wastewater. The calculation is based on Equations 3 and 4 (shown below), assuming that 24,000 kilo-Joules of sunlight is required to produce one kilogram of algae (Rittmann and McCarty 2001; Oakley 2005b). The conversion efficiency for algae in stabilization ponds is assumed to be 3.0% (Oakley 2005b).

$$106CO_2 + 65H_2O + 16NH_3 + H_3PO_4 \rightarrow C_{106}H_{181}O_{45}N_{16}P + 118O_2 \quad (3)$$

$C_{106}H_{181}O_{45}N_{16}P$ represents algae in the pond

$$SLR = \frac{I_s \cdot CE \cdot \left(1.55 \frac{kg\ O_2}{kg\ \text{algae}}\right)}{24{,}000 \frac{kJ}{kg\ \text{algae}}} \quad (4)$$

I_s = solar insolation ($\frac{kJ}{ha \cdot day}$) and CE = conversion efficiency (%)

In this region of Bolivia, the month with the lowest solar radiation is June (Appendix A). Therefore, the maximum theoretical surface loading rate is also the lowest in June. In this region, it is estimated that algae can produce approximately 270 kilograms per hectare per day during

the month of June. This value is then compared to the average mass of biodegradable organic material entering the pond to determine if the pond is organically overloaded. The surface loading rate is calculated by dividing the product of the influent BOD and the influent flow, by the area of the pond. The concentrations of ultimate BOD at the influents of the facultative pond (three-pond system) and the first maturation pond (UASB-pond system) are higher than the measured concentrations of BOD_5 (0.191 kg/m^3 and 0.121 kg/m^3, respectively) and lower than the measured concentrations of COD (0.467 kg/m^3 and 0.317 kg/m^3, respectively). If the ultimate BOD in untreated wastewater is assumed to be 150% of the BOD_5 (Mara 2004), then the organic surface loading rates for the facultative pond (three-pond system) and the first maturation pond (UASB-pond system) would be approximately 161 kg/ha·day and 85 kg/ha·day, respectively. These rates are lower than the theoretical maximum surface loading rate for the month of June, which is the month with the least amount of solar insolation in the year for this region of Bolivia (Tables 11 and 12). In fact, if the surface loading rates were calculated using the observed COD concentrations, they would still be lower than the maximum surface loading rate for June, which is a strong indication that the ponds are not organically overloaded, and that pond surfaces are likely saturated with oxygen during the day.

Table 11: Surface organic loading rates for the first pond in each system

Pond (System)	Pond Area (ha)	Influent BOD_u (kg/m^3)	Influent Flow (m^3/d)	SLR (kg BOD_u per ha·d)
Facultative Pond (Three-Pond)	0.1365	0.287	76.6	161
Maturation Pond 1 (UASB-Pond)	0.1250	0.182	58.1	85

BOD_u = Ultimate BOD; SLR = surface loading rate

Table 12: Monthly theoretical maximum surface loading rates based on solar insolation and algal oxygen production

Theoretical Monthly Maximum Surface Loading Rate (kg O_2 produced per hectare·day)											
Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
330	330	320	300	280	270	280	310	330	350	350	340

4.2 Removal of Conventional Parameters and Nutrients

Table 13 provides the observed removal of conventional water quality parameters (BOD_5, COD, and TSS), based on five years of sampling (2007-2011), as well as a range of expected removal based on reports in the literature. The mean values are calculated based on a Student's *t*-distribution. There is no significant difference between the overall removals of these parameters for the two systems studied. Furthermore, the observed removals are consistent with findings from similar systems reported in the literature.

Table 13: Mean concentrations and removal of conventional parameters

Parameter		Three-Pond		Literature[1]	UASB-Pond		Literature[1]
		Mean	df		Mean	df	
BOD_5 (mg/L)	Influent	191	4		235	4	
	Effluent	20	4		34	3	
	Removal %	**90%**		**65-95%**	**86%**		**75-95%**
COD (mg/L)	Influent	467	4		598	4	
	Effluent	140	4		140	3	
	Removal %	**70%**		**65-80%**	**77%**		**65-90%**
TSS (mg/L)	Influent	244	4		355	4	
	Effluent	36	4		36	3	
	Removal %	**85%**		**50-95%**	**90%**		**70-95%**

[1] **Source: Peña Varon et al. (2000, 2002); Keller et al. (2004); Mara (2004); Mihelcic et al. (2009); Bastos et al. (2010); Oliveira and von Sperling (2011)**

df = degrees of freedom

Percent removals for total nitrogen and total phosphorus in each system were computed based on averages from between five and seven data points per sample location, collected over the course of five years. The observed removals of total nitrogen in the three-pond system and the UASB-pond system were 20% and 23%, respectively. The observed removals of total phosphorus in the three-pond system and the UASB-pond system were 37% and 20%, respectively. According to the literature, pond systems typically remove between 30% and 80% total nitrogen and between 30% and 50% total phosphorus, while systems with UASB reactors and ponds typically remove between 30% and 65% of total nitrogen and between 0% and 50% total phosphorus (Peña Varon et al. 2000, 2002; Keller et al. 2004; Mara 2004; Mihelcic et al.

2009; Bastos et al. 2010; Oliveira and von Sperling 2011) When compared to removal rates reported in the literature, the rates measured for these two systems are lower for nitrogen and similar for phosphorus.

Figure 19 shows the mean concentrations of total nitrogen at different points in the system, with error bars representing the 90% confidence interval, based on Student's *t*-distribution. Figure 20 shows the breakdown of the different forms of nitrogen at the influent, midpoint, and effluent points of the two systems, based on data from 2011 (the only year that samples were analyzed for ammonia-nitrogen, nitrate-nitrogen, and total Kjeldahl nitrogen). Based on this very limited data, there appears to be little overall change in the breakdown of the different forms of nitrogen in both systems, but it is not possible to reach a significant conclusion. The high percentages of ammonia throughout the systems indicate that there is a lack of nitrification happening in both systems, and a high discharge of ammonia-nitrogen, which can be toxic to aquatic life in the river to which the system discharges.

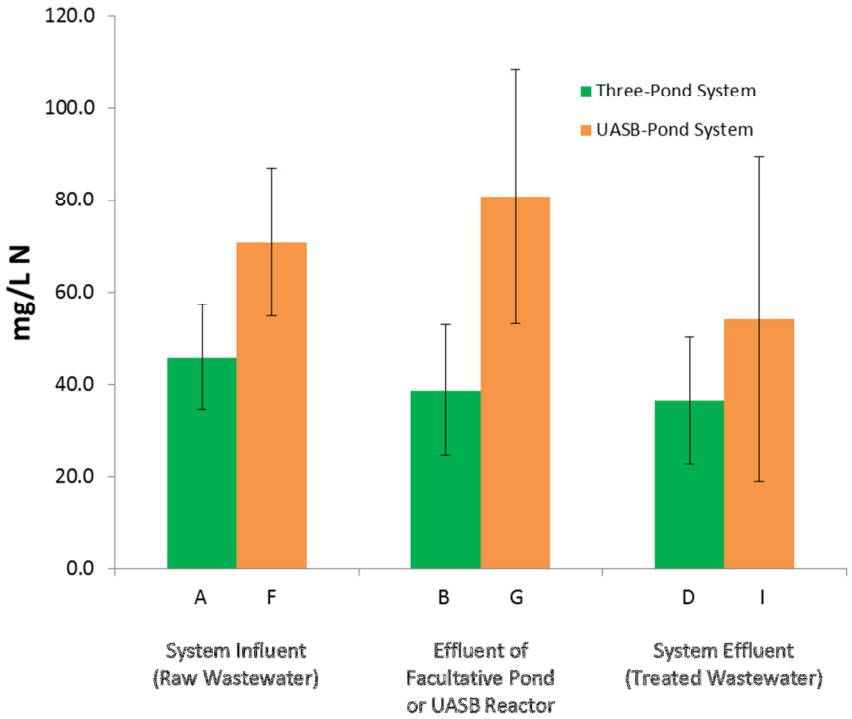


Figure 19: Concentrations of total nitrogen in the three-pond and UASB-pond systems

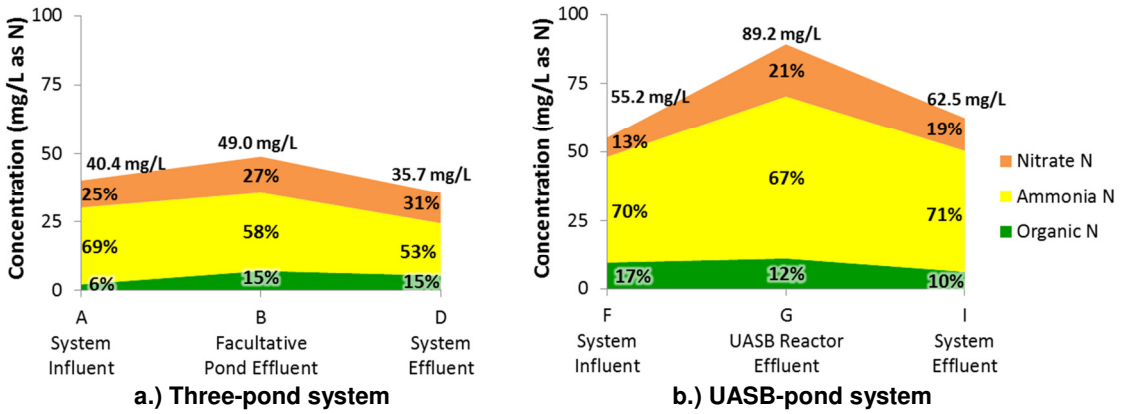


Figure 20: Breakdown of the different forms of nitrogen along different sampling points in a) the three-pond system and b) the UASB-pond system in 2011

There was a significant difference between the concentrations of total phosphorus at the influent (9.1 mg/L) and effluent (5.7 mg/L) of the three-pond system. The concentration of orthophosphate also steadily decreased in this system (from 6.6 to 4.6 mg/L as P). This finding makes sense since orthophosphate is the form of phosphorus that is readily available to algae, and the mechanism expected to contribute to the removal of dissolved phosphorus is uptake into biomass and subsequent settling. There was no significant difference between the concentrations of total phosphorus at the influent (11.8 mg/L) and effluent (9.4 mg/L) of the UASB-pond system, but the slight reduction in the average concentrations appears to have been achieved in the maturation ponds as opposed to the UASB reactor. Figure 21 shows the mean concentrations of total phosphorus in both systems, with error bars representing the 90% confidence intervals.

The treatment systems are functioning as expected in terms of the removal of physical-chemical parameters (COD, BOD_5, TSS), but the removal of nitrogen and phosphorus is much lower than expected, especially for the UASB-pond system. This is unfavorable considering that the effluents from both systems are discharged to streams that are less than a kilometer upstream from the Beni River, which is an important local resource for fishing and transportation. In 2007, the year the UASB-pond system was constructed, samples taken in the stream at a location downstream of the wastewater discharge point had 3.4 mg/L and <0.1 mg/L of total nitrogen and total phosphorus, respectively. In 2009, after the UASB-pond system had been up and running for two years, concentrations of total nitrogen and total phosphorus at this point were

13.3 mg/L and 12.4 mg/L, respectively. The wastewater treatment systems from this study are two of several that have been constructed in this region during the past decade (Fuchs and Mihelcic 2011). Fishing in the nearby stretch of the Beni River has reportedly decreased in recent years due to a decline in fish populations—a phenomenon that locals have attributed to the prior use of blast fishing techniques (Gobierno Municipal de Palos Blancos and ACDI/VOCA 2008).

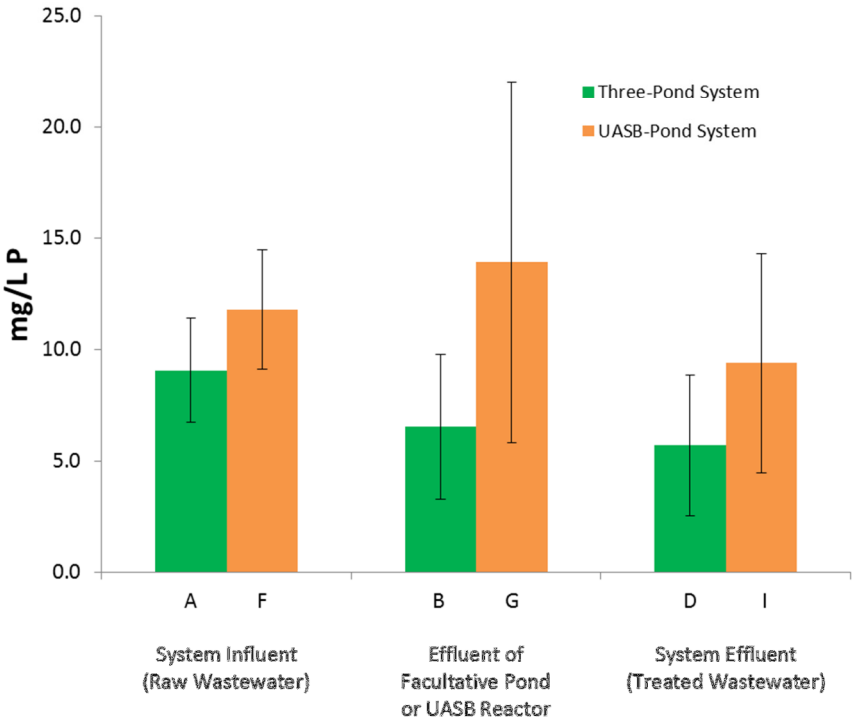


Figure 21: Concentrations of total phosphorus in the three-pond and UASB-pond systems

4.3 Removal of Helminth Eggs

Helminth eggs were detected in the raw wastewater at the influents of both systems, at concentrations that exceeded 1,000 eggs/L, which is slightly higher than concentrations detected in some other middle-income and developing countries. In Mexico, untreated wastewater in cities typically has less than 100 eggs/L, but can have up to 330 eggs/L in peri-urban and rural areas (Jiménez 2007a). Average concentrations of helminth eggs from the influents of ten different wastewater treatment systems in Honduras ranged from 2 to 744 eggs/L (Oakley 2004). In Brazilian cities, raw wastewater typically contains between 166 and 202 eggs/L, but one study measured average concentrations of almost 17,000 eggs/L in a low-income periurban

neighborhood (Dixo et al. 1995). Lloyd and Frederick (2000) reported concentrations between 10,000 and 20,000 eggs per liter in wastewater from a refugee camp in Bangladesh. However, helminth egg concentrations in developing countries will not usually exceed 1,000 eggs/L, according to Mara and Horan (2003).

The concentrations of helminth eggs detected in the raw wastewater in this study are consistent with local health reports. A local clinical study found helminth eggs in fecal samples from 71 out of approximately 150 children under the age of five from the town that utilizes the UASB-pond system (Ajata 2006). Fifty of these children were infected with *Ascaris lumbricoides*. In our study, approximately 450 *Ascaris* eggs per liter were detected in the raw wastewater. Given that an adult female *Ascaris* worm lays approximately 200,000 eggs per day (Jiménez 2007c), in a town that discharges 58.1 m^3 of wastewater per day, fifty children with one worm each would contribute almost 200 eggs/L. Children that are infected with more than one worm would excrete higher concentrations of eggs. Additionally, other community members not included in the study may have *Ascaris* infections. Therefore, the average concentration of 450 *Ascaris* eggs per liter in the raw wastewater samples appears to be a reasonable result that reflects the reported health conditions of the users of this system.

The most common helminth species detected in the raw wastewater samples from both communities were *Taenia spp.* (78.9%), followed by *Ascaris lumbricoides* (19.1%), *Trichuris trichiura* (1.7%), and Hookworm species (0.3%). In contrast, the species documented by the clinical study referenced above included *Ascaris lumbricoides* (50 of 71 cases), *Strongyloides stercoralis* (13 of 71 cases), and *Trichuris trichiura* (3 of 71 cases). *Taenia* eggs and Hookworm eggs were not detected in fecal samples from the clinical study (Table 14). Based on this information, and considering that *Taenia* species worms have several non-human definitive hosts including cats and dogs which can excrete eggs in their feces, it is plausible to assume that the *Taenia* eggs identified in the samples may not be human in origin. Furthermore, the recommendations for helminth eggs in the WHO Guidelines only refer to four species, collectively known as the geohelminths: *Ascaris lumbricoides*, *Trichuris trichiura*, and two Hookworm species: *Ancylostoma duodenale* and *Necator americanus* (Mara 2007). Nevertheless, *Taenia* eggs can

also present human health risks, especially if wastewater is used to irrigate pasture or fodder crops to feed cows or pigs, since the ingestion of *Taenia* cysts in undercooked pork or beef can cause human tapeworm (World Health Organization 2004), and the ingestion of *Taenia solium* eggs by humans can cause neurocysticercosis, which is the leading cause of acquired epilepsy in developing countries (García et al. 2003). Therefore, if wastewater with *Taenia solium* eggs is used to irrigate crops that may be consumed raw by humans, they may present a risk for neurocysticercosis. Unfortunately, it is almost impossible to distinguish between *Taenia* species using the standard helminth egg detection methods based on microscopic identification, as eggs from different *Taenia* species are almost identical in appearance and size.

Table 14: Species of helminth eggs detected in wastewater and stool samples from one of the two communities involved in the study

Species	UASB-Pond		Three-Pond
	Raw Wastewater (this study)	Stool Samples (Ajata 2006)	Raw Wastewater (this study)
Ascaris spp.	40.3%	70.4%	7.6%
Hookworm spp.	0%	NR [2]	0.8%
Strongyloides stercoralis[1]	n/a	18.3%	n/a
Taenia spp.	54.5%	NR [2]	91.7%
Trichuris spp.	5.2%	4.2%	0%
Other species[4]	n/a	7.0%	n/a

[1] This species of helminth is present in feces as a filariform larvae, and is therefore not detectable in water samples using the methods applied in this study

[2] NR = Not reported

[3] Number of samples, n = 71

[4] The names of other species were not provided

Figure 22 shows the average concentrations of helminth eggs at each of the points from both treatment systems, including only the geohelminths. Figure 23 shows the average concentrations of the geohelminths and *Taenia spp.* eggs. The whiskers represent minimum and maximum concentrations found in the samples (except for point D from Figure 22, where the upper whisker represents the limit of detection). If eggs of all species are considered, the three-pond system and the UASB-pond system achieved average overall removals of 93% and 32%, respectively. However, if only geohelminth eggs are considered, the three-pond system still

removed >92% of the geohelminth eggs overall, with no geohelminth eggs detected in the system effluents; and 81% of the geohelminth eggs were removed in the UASB-pond system. *Taenia* eggs were detected in the effluents of the three-pond system in one out of two samples (average of 116 eggs/L). However, both *Taenia* eggs and *Ascaris* eggs were detected in the effluents of the UASB-pond system in one out of two samples (1,227 *Taenia* eggs/L and 116 *Ascaris* eggs/L). The fact that eggs were detected at all in the effluents from either system would be unexpected considering that the theoretical HRT for the two systems are 40 and 56 days. However, given that hydraulic short-circuiting was observed (Lizima 2011), it is feasible that the mean HRT are much lower than the theoretical HRT. It is also important to note that heavy rains during one of the sample dates caused higher-than-usual flow rates into the systems, indicating stormwater infiltration, which may partially explain the large variability in the data for the UASB-pond system.

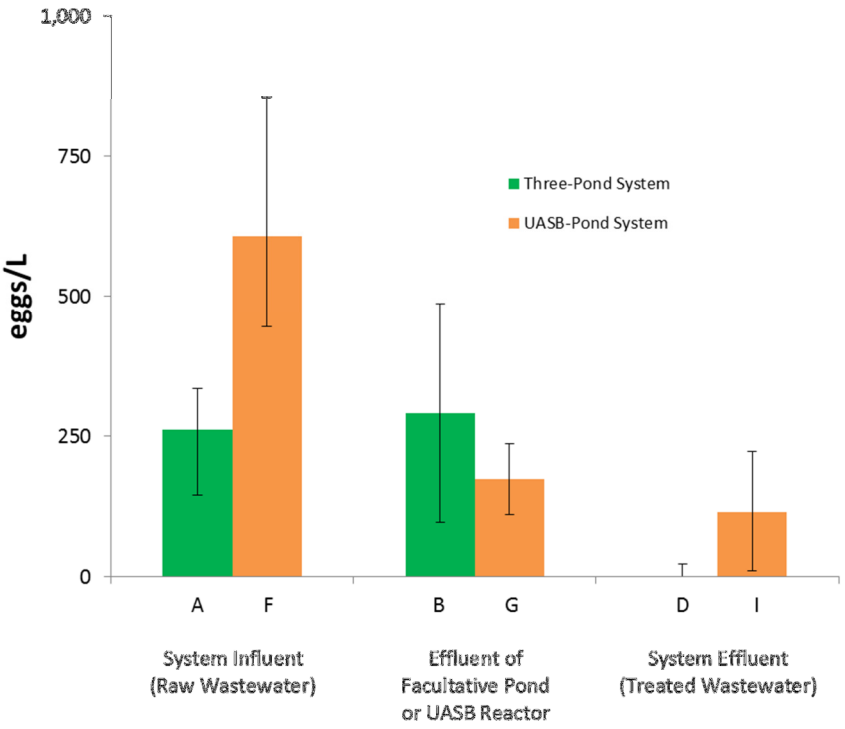


Figure 22: Concentrations of geohelminth eggs at different points in the three-pond and UASB-pond systems

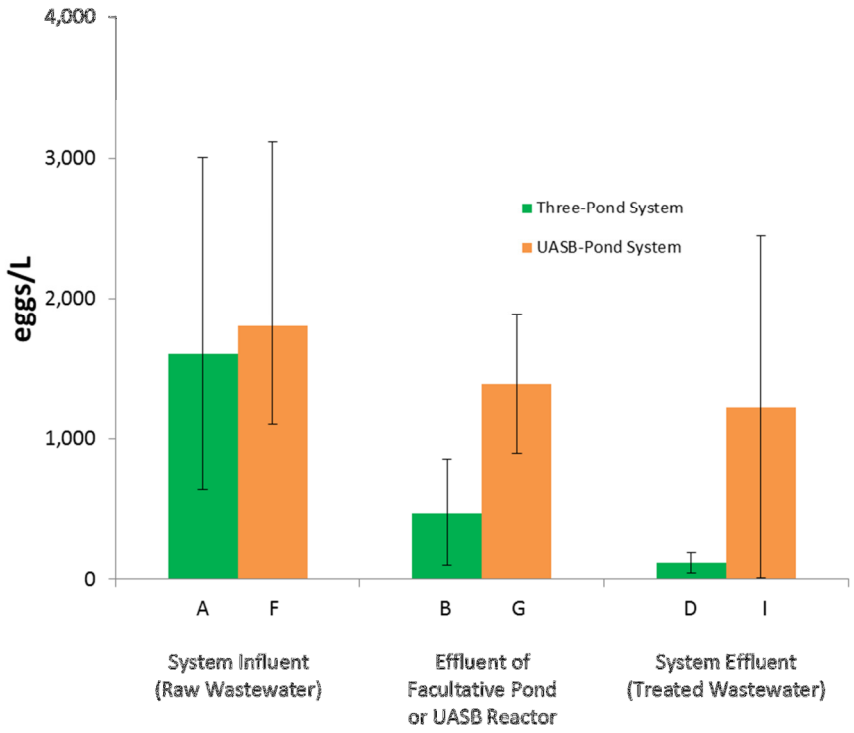


Figure 23: Concentrations of geohelminth and *Taenia* eggs at different points in the three-pond and UASB-pond systems

It is difficult to draw conclusions about the effectiveness of helminth egg removal from the facultative pond or the UASB reactor alone, due to the small number of samples, and the large variations observed in the samples. If eggs from all helminth species are considered, the average observed removal was 70% in the facultative pond and 23% in the UASB reactor. However, if only geohelminth eggs are considered, there was little to no removal in the facultative pond and an average observed removal of 71% in the UASB reactor. These results are poor compared to other findings reported in the literature, especially for the facultative pond, which has a theoretical hydraulic retention time of 26 days. This may indicate that the hydraulic performance of the pond is poor, and the mean hydraulic retention time may be much shorter than originally anticipated. Preliminary results from a dye study performed on the facultative pond in 2011 of the three-pond system confirm this, as peak concentrations of dye were measured in effluents of the facultative pond within the first 48 hours (Lizima 2012). The observed removal of helminth eggs in the UASB reactor agrees somewhat with values reported in the literature.

Helminth eggs in the environment only pose a risk to human health if they remain viable long enough to develop into an infective stage. Therefore, it is important to understand not only how many eggs are present in wastewater or biosolids, but also what percentage is still viable. In this study, the Trypan Blue stain penetrated the cell walls of five out of six eggs isolated from raw wastewater samples in the three-pond system, and seven out of eight eggs from raw wastewater samples in the UASB-pond system. Two-thirds of the eggs isolated from the UASB reactor effluents (n=9) and from the UASB reactor sludge (n=15) were also stained. In the facultative pond sludge, a little more than half of the eggs isolated were stained (n=24). All of the eggs isolated from water samples at the effluents of both systems were stained, as shown in Table 15. These results indicate that the percent of viable eggs in the raw wastewater entering both systems may be similar, and also that eggs detected in the effluents of the systems might be more likely to be non-viable than eggs detected in the raw wastewater. Non-viable eggs have a lower density than viable eggs, so any non-viable eggs entering the system may settle at a slower rate than viable eggs. In one study, a greater percentage of non-viable eggs were found in sludge samples located closer to the pond outlet than in samples collected closer to the pond inlet (Nelson et al. 2004). Also, eggs that have settled to the bottom of a pond may become inactivated over time, and then later they may become resuspended by turbulent flow, biogas bubbles, or the activity of fish or other animals such as turtles (Oakley 2005b). Turtles, dogs, and a variety of other animals were spotted in several of the ponds studied at different times during the day and the night (Figure 24).

Table 15: Fraction of eggs that were stained with Trypan Blue within five minutes

System and Sample Location	Raw Wastewater	UASB/FP Effluent	MP 1 Effluent	MP 2 Effluent	Sludge[2]
Three-Pond System	5 / 6	1 / 1	3 / 3	1 / 1	15 / 24
UASB-Pond System	7 / 8	6 / 9	3 / 3	10 / 10	10 / 15

[1] UASB = upflow anaerobic sludge blanket reactor;
FP = facultative pond; MP = maturation pond

[2] Sludge samples were taken at a point near the influent pipe of the facultative pond (three-pond system) and from a core of sludge built up in the UASB reactor (UASB-pond system)

Figure 24: Olingo or coati spotted in one of the maturation ponds

Helminth eggs were detected in all sludge samples from the facultative pond (three-pond system) near the entrance pipe (n=5); and in the UASB reactor (n=3). The average concentrations of geohelminth and *Taenia* eggs in the sludge from the facultative pond and the UASB reactor were 899 and 236 eggs/g total solids, respectively; the average concentrations of only geohelminth eggs in the sludge from the facultative pond and the UASB reactor were 183 and 121 eggs/g total solids, respectively (Figure 25, the whiskers represent the minimum and maximum values observed in any one sample). The most common species detected in the sludge were *Taenia* and *Ascaris*, but *Trichuris* and Hookworm eggs were also detected in some samples. The results of this study also indicate that the sludge from the facultative pond has a similar percentage of potentially-viable eggs as the sludge from the UASB reactor. Treatment is required before sludge with viable helminth eggs can be land-applied. Approximately 115 m^3 and 60 m^3 of sludge were measured in the facultative pond (from the three-pond system) and the UASB reactor, respectively. Neither of the two community water committees had a desludging plan for the systems at the time of the study. The build-up of sludge in stabilization ponds can contribute to poor hydraulics and potentially the resuspension of helminth eggs.

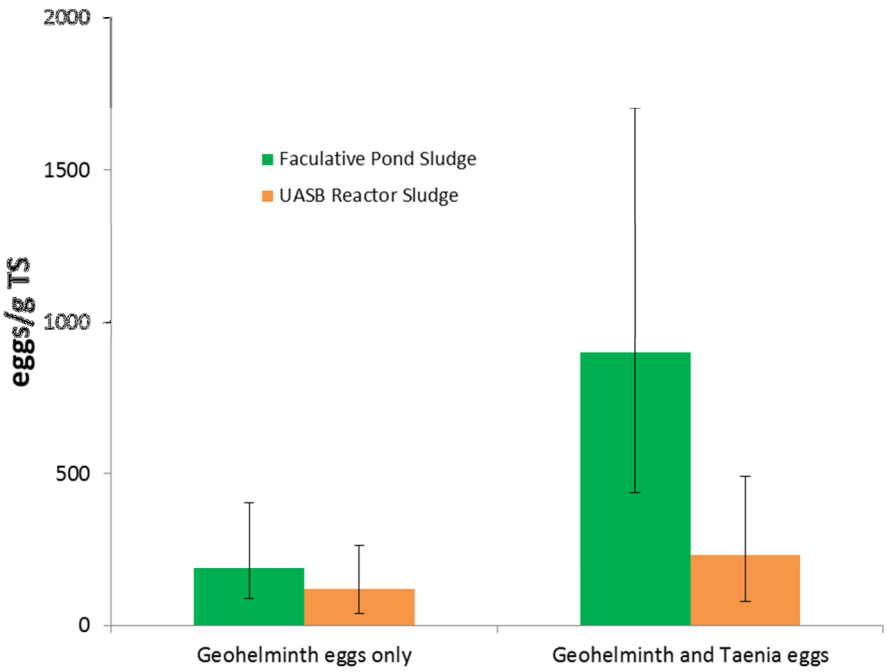


Figure 25: Helminth egg concentrations in sludge from facultative pond and UASB reactor

4.4 Removal of Thermotolerant Coliforms

Helminth eggs are not the only pathogenic organism in wastewater that can present a public health risk if wastewater is used for irrigation. Thermotolerant coliforms were used in this study to estimate the removal of viral, bacterial, and protozoan pathogens. The average concentrations of thermotolerant coliforms at different points in the two treatment systems are presented in Figure 26 (the whiskers represent the minimum and maximum concentrations detected in any one sample). The three-pond system removed an average of 3.4 log units, while the UASB-pond system removed an average of 2.3 log units.

Predicted removal rates for thermotolerant coliforms, based on models developed by Marais (1974) and von Sperling (1999; 2002; and 2003), are provided in Table 16. Based on a comparison of estimations made by these two models to observed removal data, the complete mix model by Marais (1974) appears to more accurately predict the thermotolerant coliform removal in these pond systems.

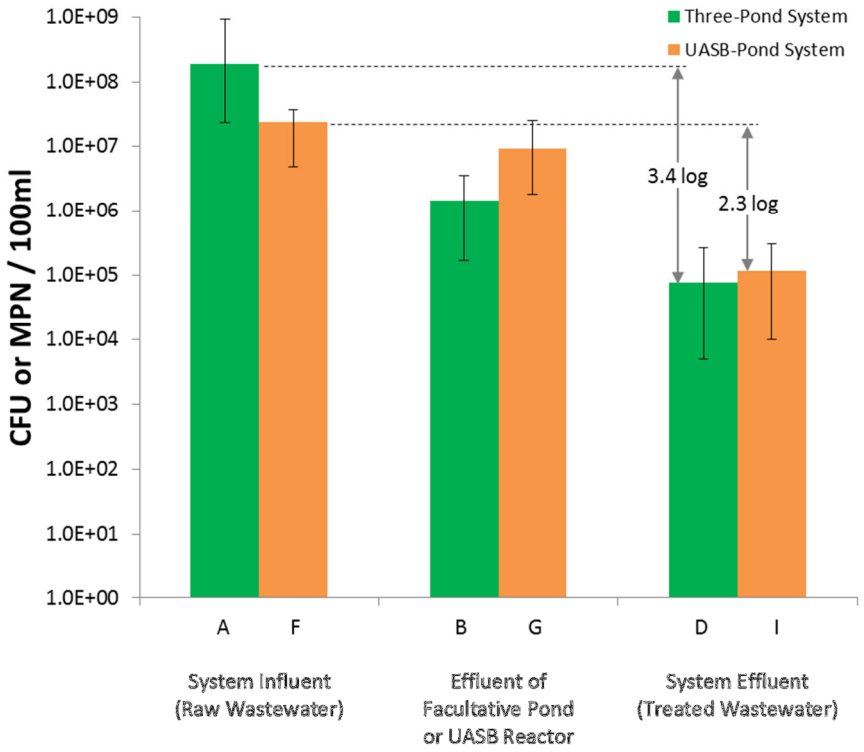


Figure 26: Concentrations of thermotolerant coliforms at different points in the three-pond and UASB-reactor systems

Table 16: Predicted versus observed log removal of thermotolerant coliform bacteria

System	Pond	Predicted Log Removal		Observed Log Removal
		Dispersed Flow[1]	Complete Mix[2]	
Three-pond	Facultative Pond	1.4	1.8	2.1
	Maturation Ponds	1.6	1.5	1.3
	Total System	**3.1**	**3.3**	**3.4**
UASB-pond	UASB Reactor	n/a	n/a	0.4
	Maturation Ponds	3.0	2.2	1.9
	Total System	**3.0**	**2.2**	**2.3**

[1] von Sperling (1999; 2002; and 2003);

[2] Marais (1974)

4.5 Assumptions and Limitations

In this study, helminth eggs were measured directly and thermotolerant coliforms were used as an indicator for other bacterial, viral, and protozoan pathogens. There are certain limitations associated with the method for quantifying concentrations of helminth eggs in wastewater and sludge samples, which are discussed in Section 3.5. Another limitation of this

study is the use of thermotolerant coliforms as an indicator for other pathogens. The concentration of thermotolerant coliforms in wastewater does not always correlate with the concentration of viral and protozoan pathogens (Savichtcheva et al. 2007; Rosario et al. 2009; Symonds et al. 2009). The risk assessments simulated in the 2006 WHO Guidelines use three index pathogens: *Campylobacter* (bacterial); *Cryptosporidium* (protozoan); and rotavirus (viral). Risk is estimated with quantitative microbial risk assessment (QMRA), and assuming that there are between 0.01 and 1 rotavirus, between 0.01 and 1 *Campylobacter*, and between 0.01 and 0.1 *Cryptosporidium* oocysts per every 10^5 *E. coli* in wastewater (World Health Organization 2006a). Also, the removal of thermotolerant coliforms does not necessarily correspond with equivalent removals of *E. coli* bacteria. If the concentrations of viral and protozoan pathogens in the systems from this study do not correspond with the assumptions made in the 2006 WHO Guidelines, then the risk associated with wastewater irrigation may differ from the WHO's recommended target of $<10^{-6}$ DALY per person per year.

5.0 CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to evaluate the reuse potential of wastewater for irrigation from two community-managed treatment systems in Bolivia: one consisting of three stabilization ponds in series (three-pond system) with an estimated overall hydraulic retention time (HRT) of 20 days; and the other consisting of a UASB reactor and two stabilization ponds in series (UASB-pond system) with an estimated overall HRT of 28 days. While farmers in this region do not currently irrigate with wastewater, the local population growth rate exceeds 3% (Fuchs et al. 2008), and water resources in this region may decrease by more than 28% in the near future due to anticipated changes in land use and climate (Fry et al. 2012). Thus, wastewater may soon become an important resource to these communities.

In this study, the performance of the two treatment systems was compared with respect to the removal of helminth eggs, bacterial pathogen indicators, conventional parameters and nutrients. The systems did not appear to be organically or hydraulically overloaded at the time of the study. Nevertheless, the UASB reactor was nearly filled with sludge that had been accumulating for more than a year at the time of sampling, and the stabilization ponds in both systems appeared to have hydraulic conditions that indicated short-circuiting and dead zones. While these conditions are not optimal for operation and performance, it is not unusual to find wastewater treatment systems in developing countries that are operating at less-than-optimal conditions.

5.1 Summary of Key Findings

Despite the fact that the systems were operating under less-than-optimal conditions, both systems removed conventional wastewater parameters to levels that are consistent with those reported in the literature. Removal of TSS and BOD_5 was between 85% and 90% and removal of

COD was 70% or higher for both systems. However, both systems had poor nutrient removal, discharging effluents with high concentrations of total nitrogen (37 – 54 mg/L) and phosphorus (5.7 – 9.4 mg/L). In particular, both systems discharged high concentrations of ammonia-nitrogen (19 – 44 mg/L), which can be toxic to aquatic life in the river to which the systems discharge. Alternatively, these nutrients can be seen as a potential resource for local organic farming.

The three-pond system removed helminth eggs (>92%) and thermotolerant coliforms (3.4 log units) better than the UASB-pond system (32% – 81% and 2.3 log units, respectively). Geohelminth eggs were not detected in the effluents of the three-pond system (limit of detection 22 eggs/L), but they were detected in the effluents of the UASB-pond system (~116 eggs/L). *Taenia* eggs were detected in the effluents of both the three-pond system (~116 eggs/L) and the UASB-pond system (~1,227 eggs/L). The fact that *Taenia* eggs were detected more frequently than other species in the effluents of both systems makes sense, since the theoretical settling velocity for *Taenia* eggs is lower than the settling velocities of geohelminth eggs. The membranes of eggs detected in the effluents of both systems were stained with Trypan Blue more frequently (18 of 18) than eggs detected in the influents of both systems (12 of 14) and in sludge samples (25 of 34), which may indicate that the eggs from the effluents were less likely to have been viable at the time of sample collection. A summary of the key findings is provided in Figure 27.

Because the limit of detection in this study was 22 eggs/L, it is not possible to conclude that the effluents of the three-pond system meet the WHO recommendation of <1 geohelminth egg per liter. However, recent microbial risk studies indicate that this recommendation may be too conservative for some regions (Mara and Sleigh 2010). For example, Ensink and van der Hoek (2009) recommend a maximum of 15 eggs per liter for the unrestricted use of wastewater for irrigation in Pakistan. The recommendations presented in the 2006 WHO Guidelines for pathogen reduction are based on the assumption that wastewater irrigation will not create an additional health burden exceeding 10^{-6} DALYs per person per year. However, 10^{-4} or 10^{-5} DALYs per person per year may be a more appropriate target for some regions (World Health Organization 2007), and the WHO encourages countries to design policies reflecting their own socioeconomic situations and health goals (World Health Organization 2006b). To provide some perspective, the

overall estimated environmental burden of disease in Bolivia is 0.06 DALYs per person per year, which represents 24% of the total health burden in the country (World Health Organization 2009b). Bolivia has not yet adopted national guidelines for the reuse of wastewater in irrigation.

THREE-POND SYSTEM

- Overall helminth egg removal: >92%
- No geohelminth eggs detected in effluents (minimum level of detection = 22 eggs/L)
- Overall removal of thermotolerant coliforms: 3.4 log units

Recommendations for reuse potential:

- Suitable for irrigation of any crops except for root crops and low-growing crops that may be consumed raw (i.e. onions, strawberries), provided additional health interventions are implemented.
- *Taenia* eggs detected in effluents may present risk for cows and pigs if pastures or fodder crops are irrigated. People that consume undercooked meat could be at risk for tapeworm. Education and meat inspection programs should be implemented if irrigating pastures or fodder crops.
- The ingestion of *Taenia solium* eggs is linked to neurocysticercosis. It is not possible to visually distinguish *T. solium* eggs from other *Taenia* species. Before using this wastewater to irrigate crops that are consumed raw, hospital records should be reviewed to see if the incidence of epilepsy in this region is higher than national averages, which may indicate that neurocysticercosis is a problem.

Health Interventions to Protect Consumers

- Wash produce in a weak detergent solution and rinse with clean water (2-log pathogen reduction).
- When irrigating crops that may be consumed raw, any combination of the following health interventions should be implemented to provide an additional 3- to 4-log reduction of pathogens:
  - Use drip irrigation techniques (2-log pathogen reduction)
  - Ensure that produce is peeled (2-log pathogen reduction)
  - Allow for die-off of pathogens on crop surfaces (~1-log pathogen reduction, varies with weather and exposure to sun)

Health Interventions to Protect Farmers and Families

- Effluents should be monitored every 3 – 6 months for helminth eggs (as recommended by the WHO).
- Unless crops are harvested with highly-mechanized systems, farmers should wait two weeks from the last day of wastewater irrigation before harvesting, and should get regular deworming treatment.
- If spray irrigation is used, there should be a minimum 50-meter buffer zone from residential areas.

UASB-POND SYSTEM

- Overall helminth egg removal: 32% – 81%
- *Ascaris* eggs detected in the system effluents (~116 eggs/L)
- Overall removal of thermotolerant coliforms: 2.3 log units

Not recommended for irrigation, unless system improvements increase treatment efficiency.

BOTH SYSTEMS

- Both systems had good removal of TSS, BOD_5, COD; but little removal of nitrogen and phosphorus, which can damage surface waters if discharged, but are a potential resource for organic agriculture.
- Ponds appear to have poor hydraulics; multiple inlets and outlets may improve treatment efficiency.
- Both systems have built-up sludge (~60 m^3 in the UASB reactor, ~115 m^3 in the facultative pond) with >100 geohelminth eggs/g TS, at least a third of which are potentially viable. The concentration of helminth eggs in the facultative pond sludge is higher than in the UASB reactor sludge.

Figure 27: Summary of key findings and recommendations

5.2 Recommendations for the Communities in this Study

Because geohelminth eggs were not detected in the final effluents of the three-pond system, it may be appropriate to safely use these effluents for irrigation in accordance with WHO guidelines, provided that additional health protection measures are simultaneously implemented. While the 2006 WHO Guidelines do not specifically address the public health significance of *Taenia* eggs, they can survive for up to six months in pastures (World Health Organization 2004) and can cause sickness in humans through a variety of mechanisms. For example, if wastewater is used to irrigate fodder crops, pigs or cows consuming these crops can develop cysts in their muscle tissue. Humans that consume undercooked meat with these cysts can then acquire tapeworm. Eggs from the *Taenia solium* species are of particular concern for human health. The ingestion of *T. solium* eggs is linked to neurocysticercosis, the leading cause of epilepsy in many developing countries (García et al. 2003). Since it is nearly impossible to visually distinguish between *T. solium* eggs and eggs from other *Taenia* species, advanced and more expensive laboratory methods would be required, such as PCR analysis. Alternatively, the incidences of epilepsy could be monitored at the local hospital and compared to national averages. If acquired epilepsy is currently a problem in this region, it might indicate that the eggs found in the wastewater were *T. solium* eggs. Assuming that the eggs do not belong to the *T. solium* species, the three-pond system effluents may be suitable for the irrigation of any crops, with the exception of root crops (i.e. onions) and low-growing crops (i.e. strawberries) that may be consumed raw. Additional health interventions, described below, should be implemented simultaneously to further protect the health of consumers and farmers.

Crop restrictions and additional health interventions implemented on farms protect the health of consumers. Although no geohelminth eggs were detected in the effluent of the three-pond system, the minimum level of detection was greater than 1 egg/L. Therefore, any produce irrigated with treated wastewater from the three-pond system that may be consumed raw, should be washed in a weak detergent solution and rinsed with clean water prior to consumption. This can provide an additional 2-log reduction of pathogens and helminth eggs (Mara 2007). Figure 28 depicts the eight reuse scenarios presented in the 2006 WHO Guidelines, with an overlay

showing the average reduction of thermotolerant coliforms observed in the systems from this study. As seen in Scenario B, the effluents from the three-pond system could be used to irrigate salad crops, provided that an additional 3-log reduction of pathogens is achieved from health interventions on the farm. Effluents from this system should not be used to irrigate root crops or low-growing crops (i.e. onions or strawberries), unless additional health protection measures can ensure a total pathogen reduction of 6 or 7 log units. Examples of health interventions that can be implemented on the farm include using drip irrigation techniques (provides an additional 2- to 4-log reduction of pathogens), peeling produce such as carrots (reduces pathogens by 2 log units), or allowing for the die-off of pathogens on crop surfaces (can provide from 0.5- to 2-log reduction, depending on time and exposure to sunlight).

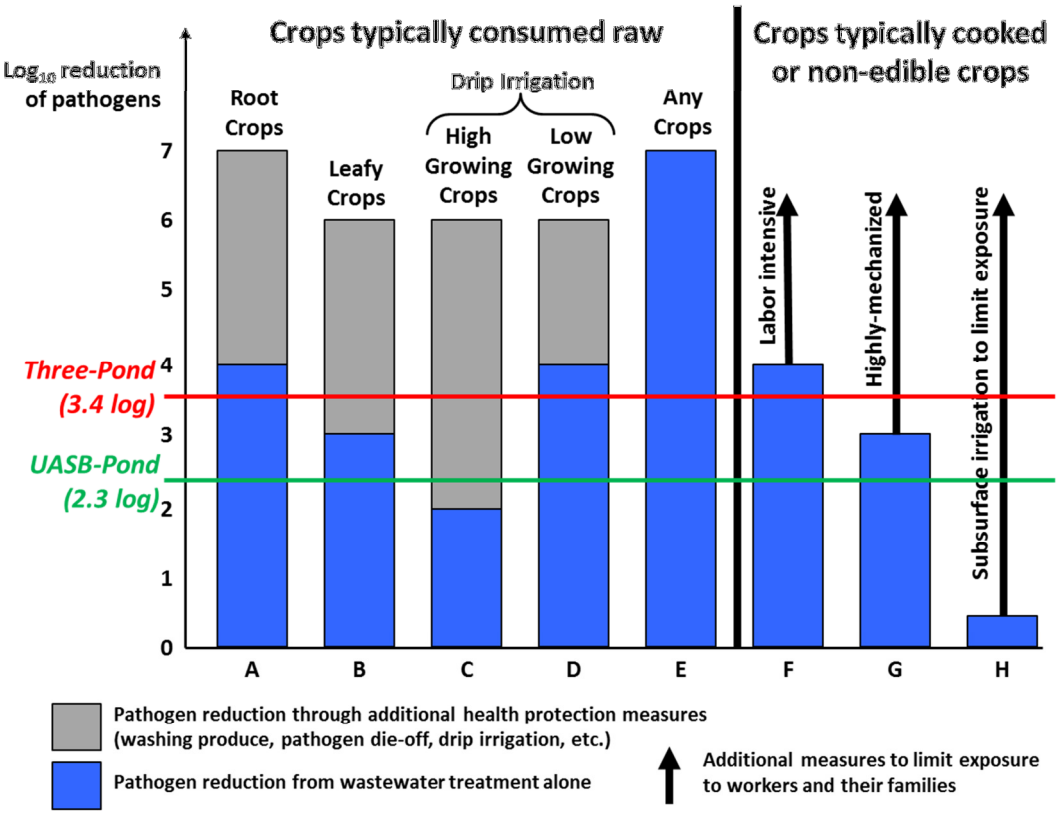


Figure 28: Removal of thermotolerant coliforms compared to WHO recommendations (generated by author, after World Health Organization 2006a)

Other types of health interventions are necessary to protect the health of farmers and their families. As shown in Scenario G from Figure 28, if the effluents of the three-pond system are used to irrigate non-edible crops or crops that are cooked prior to consumption, additional

health protection measures should be implemented to protect the health of farmers and their families. Mechanized harvesting processes help farmers minimize contact with wastewater used for irrigation, but in low-income developing countries, these types of systems are uncommon. Farmers using wastewater for irrigation should wait at least two weeks from the last irrigation before harvesting, and should always wear close-toed shoes and gloves while harvesting. The use of gloves may be particularly important for farmers in Bolivia that chew coca leaves while they work, so that they avoid touching their mouths with contaminated hands. Farmers irrigating with wastewater should also be offered regular deworming treatments. The local hospital in this region currently administers a deworming program free of charge every six months for children under five years old, which can be expanded to serve farmers as well. Finally, if spray irrigation methods are used, a 50-meter buffer should be maintained between fields and residential zones.

Geohelminth eggs were detected in the final effluents of the UASB-pond system. Therefore, the effluents from this system should not be used for irrigation, unless improvements to the system are made to increase its treatment efficiency. The WHO suggests that hydraulic retention times can be used as a surrogate to ensure the removal of helminth eggs. Previous studies have concluded that pond systems with overall HRTs of 12 days or more will produce an effluent with less than one egg per liter, and ponds with overall HRTs of 19 days or more will produce an effluent that is free of helminth eggs (Ayres et al. 1993; Mara 2004). However, as observed by the results of the systems in this study, this assumption is not always true, at least when considering the theoretical hydraulic retention time, which may overestimate the mean hydraulic retention time. The pond hydraulics in both systems studied should be improved, to increase treatment efficiency. For example, all ponds from the two systems studied had only one inlet and one outlet, which causes short-circuiting and allows for the formation of hydraulic dead zones in the corners of the pond, as shown in Figure 29a. An alternative configuration with multiple inlets and outlets (Figure 29b) would create conditions that more closely approximate plug flow. Unfortunately, a simple fix like this may end up being rather expensive for the two communities in this study, since ponds from both systems are lined with HDPE geomembranes. Any modification to this lining requires the geomembrane to be cut and welded back together.

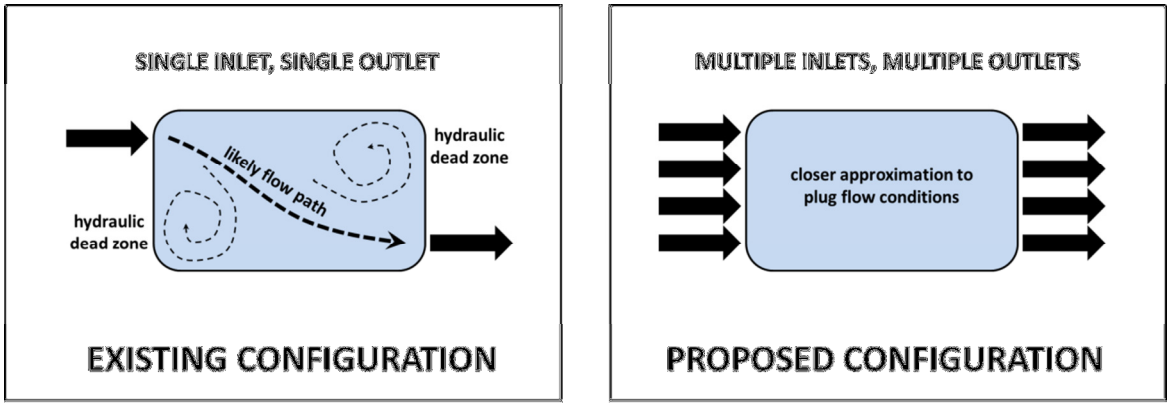


Figure 29: Schematic drawing of a) existing and b) proposed pond configurations

5.3 Recommendations for Laboratories in Developing Countries

There is not a single method for the detection of helminth eggs in wastewater samples that is internationally accepted, and studies from developing countries that are reported in the literature use a variety of methods. Many of these reports do not state their detection limits, often claiming that systems provide "100% removal" of helminth eggs or that the effluents contained concentrations of "0 eggs/L". In reality, the percent recovery and detection limits of different methods that are commonly used can vary greatly, as shown in Table 17. Technicians from laboratories in developing countries who are responsible for monitoring wastewater for helminth eggs need to be aware of limits of detection for the method they are utilizing. They should also understand the difference between "non-detect" results and what it means to measure a true concentration of zero (which is technically impossible for all practical purposes). Furthermore, the viability of the eggs should be taken into consideration, as non-viable eggs will not present a health risk. Many reports from the literature do not take viability into account. Different methods commonly used to detect helminth eggs in wastewater often call for the use of flotation solutions with different specific gravities. This may cause some methods to favor the recovery of certain species of helminth eggs over others, as the specific gravity of eggs can vary between and even within species. In this study, eggs from the following species of helminths are reported: *Ascaris* (roundworm), *Trichuris* (whipworm), *Ancylostoma duodenale* (hookworm), *Necator americanus* (hookworm), and *Taenia* (tapeworm).

Table 17: Detection limits for methods commonly used and adapted in developing countries to measure concentrations of helminth eggs in wastewater samples

Method	Nominal Detection Limit[1] (eggs/L)	Reported Percent Recovery (%)	Estimated Detection Limit[2] (eggs/L)	Precision[3]	
				5 eggs/L	40 eggs/L
Modified Bailenger (Ayres and Mara 1996)	2 – 5[5]	30 – 74%[7]	2.7 – 16.7	ND	ND
Leeds I (Ayres 1989)	1 – 1.5[6]	24%[8]	4.2 – 6.3	0.42[8]	1.99[8]
Leeds II (Ayres et al. 1989)	1	50 – 80%[9]	1.3 – 2.0	ND	ND
US EPA (Yanko 1987)	1	82%[8]	1.2	0.69[8]	2.72[8]
Membrane Filter (de Victorica and Galván 2003)	1	75%[8]	1.3	0.81[8]	4.15[8]
Centrifugation and Flotation (World Health Organization 1989)	1	33 – 70%[9]	1.4 – 3.0	ND	ND

Notes:

[1] The nominal detection limit is defined as the lowest number of eggs that can be detected in a one-liter water sample (some methods call for larger volume samples, which would allow for a lower limit of detection, but for comparative purposes, all values were adjusted for a one-liter sample). The nominal limit of detection shown here is based on the assumption that samples are concentrated down to a volume of 1 ml and that the portion of that concentrated volume that is observed under the microscope as recommended by each protocol. If the entire concentrated sample is transferred to the slide and observed under the microscope, the nominal detection limit is 1 egg/L for a one-liter sample.

[2] Estimated detection limit is calculated based on assumptions for the nominal detection limit and the average recovery rates; it is adjusted for all methods to consider one-liter samples.

[3] Precision is shown here by the standard deviation for multiple analyses of a sample with a known quantity of eggs. The standard deviation is provided for samples containing lower concentrations (5 eggs/L) and higher concentrations (40 eggs/L) of helminth eggs. Methods with lower standard deviations have higher precision, which is more preferable.

[4] ND = Not determined

[5] In this method, the eggs are floated to the top of the meniscus in 15 ml centrifuge tubes, and are collected for examination by “lifting” the meniscus from the tube using the coverslip from a glass slide, which is then observed under the microscope. According to the protocol, four slide coverslips should be used per tube, and Ayres et al. (1989) found that by doing these four repetitions, 71% or more of the eggs are observed (up to 29% are left in the meniscus).

[6] In this method, if the final concentrated volume is 1 ml, it is mixed with 5 equal volumes of flotation salt (i.e. total volume of 6 ml). Aliquots of that 6 ml concentrated sample are placed on McMaster slides with one or two chambers, each chamber holding 0.15 ml of solution. The protocol recommends observing two or three slides to increase accuracy (i.e. volume observed under microscope is from 0.6 ml to 0.9 ml, if dual-chamber slides are used).

[7] Sanz et al. (2009)

[8] Maya et al. (2006)

[9] World Health Organization (2004)

Some studies from the literature only report geohelminth eggs (*Ascaris*, *Trichuris*, and Hookworm), while others report *Taenia* species or other helminth species that are not specifically referenced by the WHO Guidelines, such as *Hymenolepis*, *Toxocara* and *Enterobius*. Some methods, such as the US EPA method, the Leeds II method, and the Membrane Filter method, have nominal limits of detection of one egg per liter (for a 1-liter sample). This is because the protocol requires that the entire concentrated portion of the sample is observed under a microscope. Other methods, such as the Modified Bailenger method, the Leeds I method, or the method used in this study, have nominal limits of detection that are higher than one egg per liter of water sampled, since only a portion of the concentrated sample is observed under a microscope. For these methods, the nominal limit of detection depends on the volume of the final concentrated pellet, the percentage of the final concentrated volume that is transferred to the counting slide, and the amount of time the laboratory microbiologist is able to spend at a microscope for each particular sample. For the method used in this study, in order to reduce the limit of detection, samples with larger volumes were collected. The actual limit of detection for any method however, is always higher than the nominal limit, due to the fact that no method recovers 100% of the eggs from the original sample during the concentration process. Rates of recovery reported in the literature for the methods that are most commonly used and adapted for studies in developing countries range from 24% to 82%. Some methods even report that the recovery rate is dependent on the concentration of total suspended solids, or the number of eggs in the sample. Malicki et al. (2001) demonstrated that the detection limit for all conventional methods can be cut in half if an internal standard is used. However, this requires laboratories to maintain stock solutions of helminth eggs stained with crystalline violet to spike samples.

The percent recovery for the method used in this study has not been determined, but based on the fact that the method used in this study uses flotation, sedimentation, and biphasic separation steps in a manner similar to the US EPA method and the modified Bailenger method, the percent recovery may be similar to those two methods. The detection limits in this study were higher because time and equipment limitations in the laboratory enabled only small aliquots of the final concentrated samples to be observed under the microscope.

5.4 Broader Implications

Developing countries are currently not on track to meet targets for sanitation and hunger eradication, set by the Millennium Development Goals. Prices of commercial fertilizers fluctuate with the cost of fossil fuels and affect global food production. Wastewater contains nutrients in forms that are readily available to plants, and the majority of sewer systems in developing countries do not provide wastewater treatment, dumping nutrients into rivers and streams. The two systems from this study utilize wastewater treatment technologies that are commonly used in developing countries and are often financed by international banks and bilateral development agencies. Based on the results of this study, the three-pond system appears to provide better treatment for wastewater reuse in irrigation than the UASB-pond system. Furthermore, appropriate technologies used for wastewater reclamation and reuse may offer a unique opportunity to finance sanitation in developing countries and make advances towards the Millennium Development Goal targets, especially for growing urban areas located near agricultural production systems.

The two systems from this study were constructed with a combination of local and international funds, and are owned, operated, and maintained by an elected board of community members. As shown in Table 18, the total capital cost of the UASB-pond system is almost twice as high as the cost of the three-pond system. Annual operation and maintenance costs for UASB reactors are also higher than operation and maintenance costs for stabilization ponds (Peña Varon et al. 2000). This is because the operation of UASB reactors requires more inputs from skilled human resources and laboratories. For example, a trained operator of a UASB reactor should periodically monitor the operational stability of the reactor (i.e. pH, alkalinity, concentration of volatile acids), to make sure that acid fermentation does not outcompete the methanogens (Chernicharo 2007), which could cause the reactor to go sour. Sludge also has to be evacuated every few weeks from a UASB reactor, while ponds only have to be dredged once every few years. The quality of the UASB reactor sludge should be monitored periodically to determine its stability, its specific methanogenic activity, and its settleability (Chernicharo 2007). Therefore, the

entity responsible for operating and maintaining a UASB reactor will likely spend more on payroll and laboratory fees than the community operating a system of stabilization ponds.

The UASB-pond system in this study was designed with more than twice the capacity than the three-pond system, and therefore was less expensive per beneficiary. However, the users of the UASB-pond system have a metered water supply system, and use less water per capita than users of the three-pond system. Thus, the average flowrate of the UASB-pond system is actually lower than that of the three-pond system, and the cost per cubic meter of treated wastewater is lower for the three-pond system than for the UASB-pond system (Table 18). Yet, the three-pond system produces a lower-cost potential resource while the UASB-pond system produces a higher-cost waste stream and potential environmental and public health liability. It is important to note that the cost and the appropriateness of implementing stabilization pond systems for the treatment and reuse of domestic wastewater will be contingent on topographical and geographical limitations as well as the cost of land, which differs from region to region.

Table 18: Implementation costs for each system

Parameter	Three-Pond	UASB-Pond
Total Implementation Cost (per beneficiary)	$180,875 ($431)	$321,959 ($310)
Average Flow Rate (m^3/yr)	28,000	21,200
% Hydraulic Design Capacity	66%	23%
Implementation Cost (per m^3 over 20 year period)	$0.32	$0.76
Cost of potable water per m^3 in some regions of Bolivia	$0.40	

Source of data: Fuchs et al. (2008)

6.0 RECOMENDACIONES PARA LAS COMUNIDADES

El presente estudio fue realizado en los años 2011 y 2012, con el objetivo de evaluar los sistemas de aguas residuales de las comunidades de San Antonio (Caranavi) y Sapecho (Palos Blancos), y para determinar el potencial para el reuso de los efluentes en la irrigación de cultivos. Específicamente, se midió la remoción de los huevos de helmintos y de los coliformes termotolerantes en los dos sistemas. El sistema de San Antonio tiene tres lagunas de estabilización en serie y el sistema de Sapecho tiene un reactor anaeróbico de flujo ascendente (RAFA, o UASB por sus siglas en inglés) seguido por dos lagunas de estabilización en serie. Los dos sistemas no estaban sobrecargados hidráulica u orgánicamente en el momento del estudio. Tenían una buena remoción de los parámetros convencionales de calidad de agua, pero muy poca remoción de los nutrientes nitrógeno y fósforo (Tablas S1 y S2).

Tabla S1: Remoción de parámetros convencionales físico-químicos de calidad de agua

Comunidad y Sistema	DBO_5 (mg/L)			DQO (mg/L)			SST (mg/L)		
	Aflu.	Eflu.	Rem.	Aflu.	Eflu.	Rem.	Aflu.	Eflu.	Rem.
San Antonio – Tres Lagunas	191	20	90%	467	140	70%	244	36	85%
Sapecho – UASB y Lagunas	235	34	86%	598	140	77%	355	36	90%

DBO_5 = demanda bioquímica de oxígeno; DQO = demanda química de oxígeno;
SST = sólidos suspendidos totales; Aflu. = Afluente; Eflu. = Efluente; Rem. = Remoción

Tabla S2: Remoción de nutrientes

Comunidad y Sistema	Nitrógeno (mg/L N)			Fósforo (mg/L P)		
	Aflu.	Eflu.	Rem.	Aflu.	Eflu.	Rem.
San Antonio – Tres Lagunas	45.9	36.6	20%	9.1	5.7	37%
Sapecho – UASB y Lagunas	70.9	54.2	23%	11.8	9.4	20%

Aflu. = Afluente; Eflu. = Efluente; Rem. = Remoción

La remoción de los coliformes termotolerantes era de 3.4-log (99.96%) y la remoción de los huevos de helmintos era mayor de 92% en el sistema de San Antonio. En los efluentes de este sistema, la concentración de coliformes termotolerantes era de ~10,000 unidades por 100

ml, y no se pudo detectar ningún huevo de helminto (de las especies que presentan más riesgo de parasitosis para las personas). Al contrario, en los efluentes del sistema de Sapecho, se ha detectado los huevos de la lombriz intestinal *Ascaris*. La remoción de los coliformes termotolerantes en este sistema solo era de 2.3-log (99.52%). La Tabla S2 es un resumen de los resultados y las recomendaciones de reuso.

Según lo que se ha encontrado en esta investigación, los efluentes del sistema de San Antonio parecen ser aptos para el reuso en la irrigación de cualquier cultivo con la excepción de los cultivos de raíces comestibles y los cultivos con una fruta que crezca cerca de la tierra, que se puede consumir crudos (cebollas, fresas, etc.). Por ejemplo, los efluentes del sistema de San Antonio pueden ser usados para regar cultivos de ensalada o cultivos con una fruta que no crezca tan cerca de la tierra (lechuga, tomate, etc.). Es importante que también se implementen intervenciones de salud adicionales para dar una mayor protección a los agricultores y a los consumidores. Por ejemplo, todos los productos que se pueden comer crudos y que se rieguen con aguas residuales deben de ser lavados con detergente y agua limpia.

Debido a la presencia de los huevos de *Ascaris* en los efluentes del sistema de Sapecho, no se deben usar los efluentes de este sistema para la agricultura a menos que se hagan algunas mejoras al sistema para aumentar la remoción de los patógenos. Por ejemplo, en vez de tener una sola entrada y una sola salida en las lagunas, se pueden construir varias entradas y salidas para evitar la creación de las zonas muertas en las esquinas de las lagunas (Figura S1). La Figura S2 presenta un resumen de los resultados y las recomendaciones del estudio.

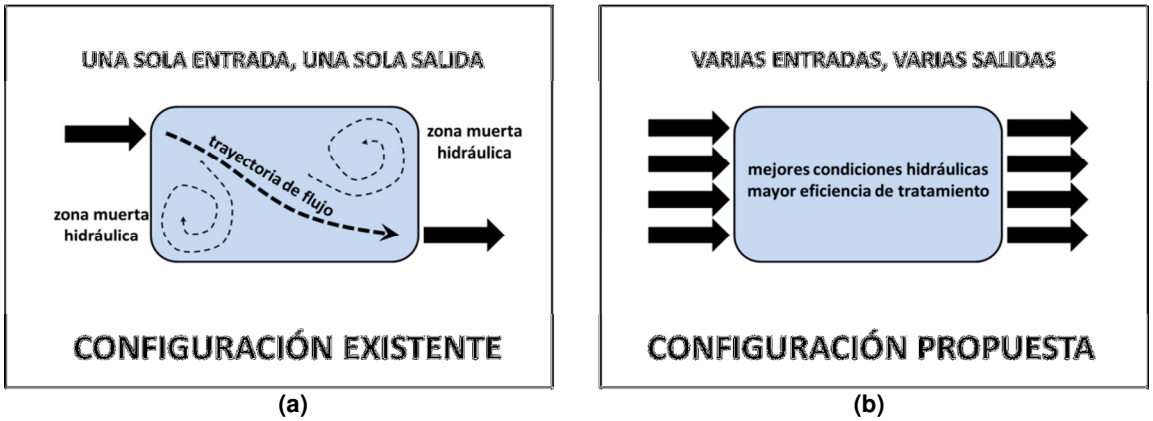


Figura S1: Representación esquemática de a) la configuración existente de las lagunas y de b) la configuración propuesta para las lagunas

SISTEMA DE SAN ANTONIO

- Remoción de huevos de helmintos: >92%
- Ningún huevo de geohelmintos (*Ascaris*, *Trichuris*, *Ancylostoma*, y *Necator*) fue detectado en los efluentes (límite de detección de 22 huevos/litro)
- Remoción de coliformes termotolerantes: 3.4 unidades logarítmicas (99.96%)

Recomendaciones para el reuso en irrigación:

- Los efluentes son aptos para usar en la irrigación de cualquier cultivo menos los que tienen raíces comestibles y los que tienen una fruta que crezca cerca de la tierra y que se come crudos (cebollas, fresas, etc.), con la condición que se implemente intervenciones de salud adicionales, las cuales son resumidas seguidamente con más detalle.
- Los huevos de *Taenia* que se ha detectado en los efluentes pueden presentar un riesgo para las vacas y los cerdos, si se usa para regar forraje. Las personas que consuman la carne cruda pueden contraer lombrices intestinales. Se debe implementar programas de inspección de carne para las personas que decidan regar forraje con las aguas residuales.
- El consumo de los huevos de *Taenia solium* puede causar el neurocisticercosis, una enfermedad que provoca la epilepsia (los huevos de otras especies de *Taenia* no presentan este riesgo). Es imposible distinguir los huevos de *Taenia solium* de los demás especies de *Taenia* en el microscopio, entonces antes de usar esta agua para el riego de las plantas, se debe consultar con el hospital y las clínicas locales para averiguar si la epilepsia (o el neurocisticercosis) es un problema existente en la zona.

Intervenciones de salud para proteger a los consumidores

- Lavar los cultivos con detergente y enjuagar con agua limpia
- Usar el riego de goteo cuando sea posible
- Pelar las frutas y vegetales cuando sea posible

Intervenciones de salud para proteger a los agricultores y sus familias

- Se debe monitorear los efluentes cada 3 a 6 meses para los huevos de helmintos (con una muestra de 5 litros que se puede mandar a la Lic. Iriarte del laboratorio de CASA-UMSS en Cochabamba).
- Si no usan equipo mecanizado, deben esperar al menos dos semanas para cosechar después de regar con aguas residuales; los agricultores deben tener acceso a medicinas desparasitantes.
- Si se usan métodos rociadores para regar, se debe mantener una distancia mínima de 50 metros entre el campo y las residencias.

SISTEMA DE SAPECHO

- Remoción de huevos de helmintos: 32% – 81%
- Huevos de *Ascaris* fueron detectados en los efluentes (~116 huevos/litro)
- Remoción de coliformes termotolerantes: 2.3 unidades logarítmicas (99.52%)

Los efluentes no son aptos para el riego a menos que se mejora el sistema y la remoción de patógenos.

AMBOS SISTEMAS

- Ambos sistemas tenían una buena remoción de sólidos suspendidos y demanda de oxígeno, pero tenían poca remoción de los nutrientes nitrógeno y fósforo, los cuales pueden dañar a los cuerpos receptores de agua, o alternativamente pueden ser un recurso para la agricultura orgánica.
- Parece que las lagunas en ambos sistemas tienen una baja eficiencia hidráulica. Se puede mejorar la eficiencia hidráulica y probablemente la eficiencia de la remoción de patógenos si se construyen varias entradas y salidas en cada laguna, en vez de una sola entrada y una sola salida.
- Los dos sistemas tienen lodos acumulados (~60 m^3 en el reactor UASB de Sapecho, ~115 m^3 en la laguna facultativa de San Antonio) con >100 huevos de helmintos por gramo. La concentración de huevos de helmintos es más alta en los lodos de la laguna de San Antonio que en los lodos del reactor UASB. Por lo menos, un tercio de los huevos son viables. Las dos comunidades deben tener planes y presupuestos para vaciar los lodos de las lagunas y del reactor. La acumulación de los lodos en estos sistemas es normal, pero puede reducir la eficiencia de los sistemas. Generalmente, se debe evacuar los lodos de las lagunas, cada 3 a 5 años y del reactor UASB, cada 2 a 4 semanas.

Figura S2: Resumen de los resultados y recomendaciones para las comunidades

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APPENDICES

Appendix A – Insolation Data for Research Field Site from NASA

NASA Surface meteorology and Solar Energy - Available Tables

Latitude -15.6 / Longitude -67.25 was chosen.

Geometry Information

Elevation: **1483** meters
taken from the
NASA GEOS-4
model elevation

Northern boundary
-15

Western boundary
-68

Center
Latitude -15.5
Longitude -67.5

Eastern boundary
-67

Southern boundary
-16

Parameters for Sizing and Pointing of Solar Panels and for Solar Thermal Applications:

Monthly Averaged Insolation Incident On A Horizontal Surface (kWh/m^2/day)

Lat -15.6 Lon -67.25	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual Average
22-year Average	4.68	4.76	4.57	4.36	4.07	3.92	3.95	4.41	4.69	5.07	5.05	4.93	4.53

Minimum And Maximum Difference From Monthly Averaged Insolation (%)

Lat -15.6 Lon -67.25	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Minimum	-14	-16	-16	-11	-14	-14	-16	-17	-14	-17	-8	-13
Maximum	22	17	16	7	13	13	13	13	17	17	21	25

Monthly Averaged Insolation Incident on a Horizontal Surface: Monthly amount of total solar radiation incident on a horizontal surface at ground level on earth, averaged over 22-year period (Jul 1983 - Jun 2005). Each monthly averaged value is evaluated as the numerical average of 3-hourly values for the given month. This is also referred to as global horizontal radiation.

- **Units:** kWh/(m^2·day) **note: 1 kWh/(m^2·day) = 3.6 x 10^7 kJ/ha·day)
- **Reference:** SSE Methodology for detailed discussion of the methodology for deriving the SSE horizontal surface insolation from satellite observations.
- **Minimum and Maximum Difference from Monthly Averaged Insolation:** The minimum and maximum values for a given month indicate the percent difference between the year that has the least (minimum) or most (maximum) monthly averaged insolation and the 22-year monthly averaged insolation. The values are expressed in percent.

Appendix B – Images of Helminth Eggs

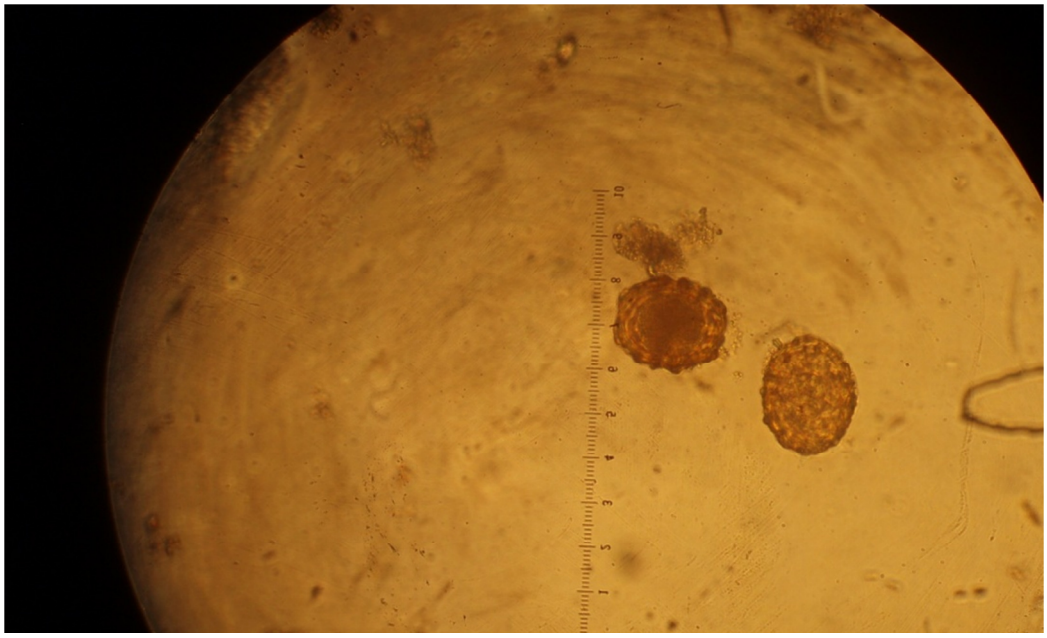

Figure B1: *Ascaris* species eggs from wastewater sample

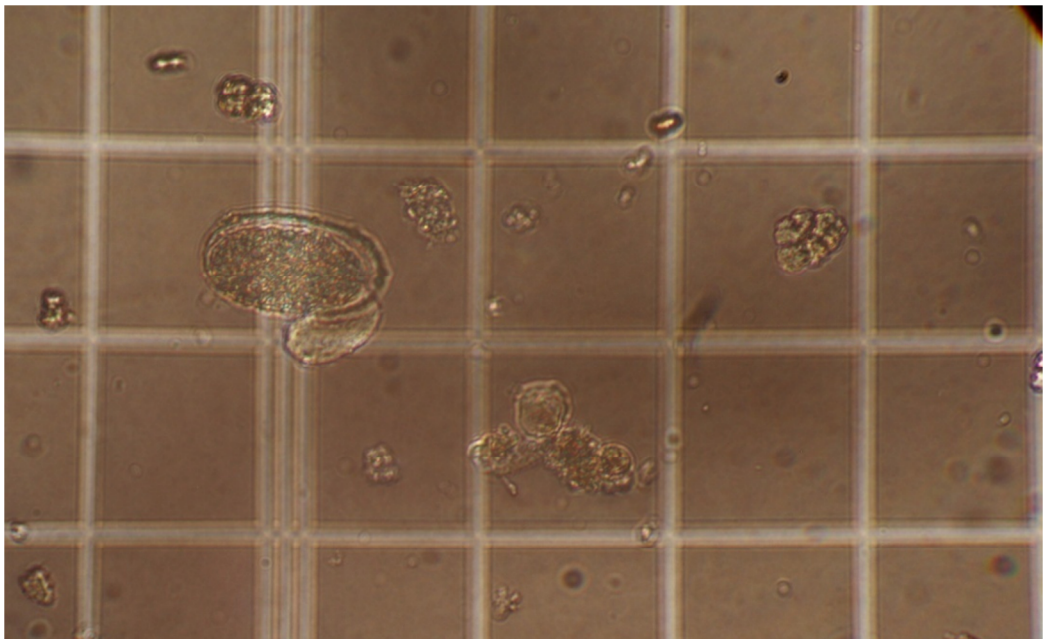

Figure B2: Hookworm species egg from wastewater sample

Appendix B (Continued)

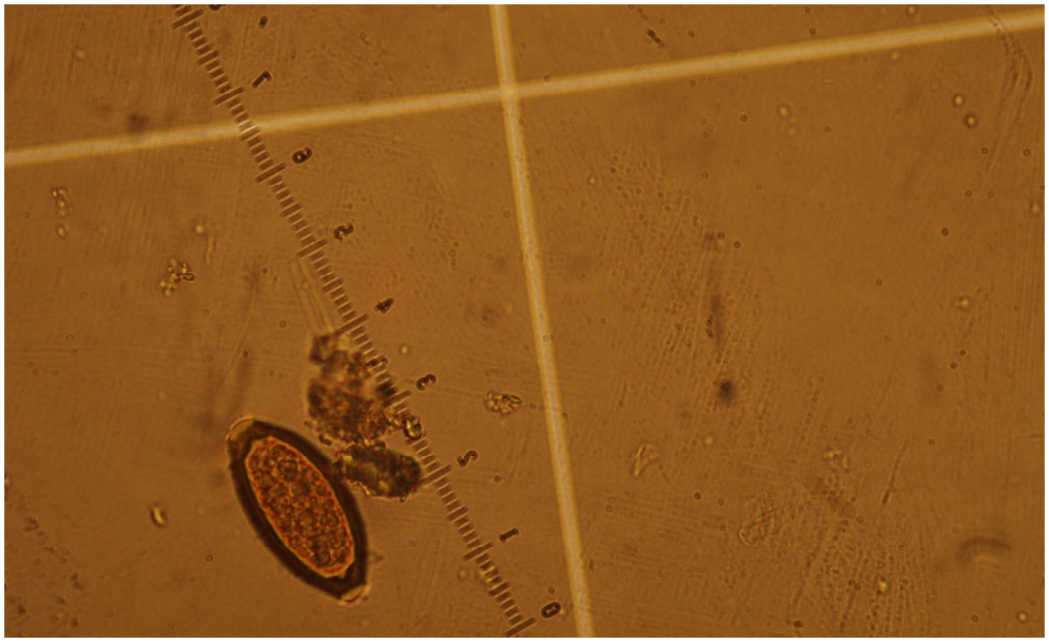

Figure B3: *Trichuris* species egg from wastewater sample

Appendix C – Laboratory Results

Table C1: Concentrations for physical-chemical parameters

Parameter		Sample Point		6/12/2007	6/24/2008	6/22/2009	6/22/2010	6/28/2010	6/13/2011
BOD_5 (mg/L)	THREE-POND	A	Influent	190.0	340.0	88*	115.0	156.0	168.0
		B	Facultative	58.0	38.0	108*	32.0	34.0	20.0
		C	Maturation 1	54.0	22.0	88*	16.0	14.0	
		D	Maturation 2	42.0	14.0	78*	8.0	16.0	20.0
	UASB-POND	F	Influent	190.0	240.0	3,890*	285.0	195.0	232.0
		G	UASB		28.0	68*	212.0	184.0	60.0
		H	Maturation 1			22*	135.0	74.0	
		I	Maturation 2		12.0	12*	56.0	55.0	12.0
COD (mg/L)	THREE-POND	A	Influent	444.0	700.0	218*	318.0	368.0	536.0
		B	Facultative	168.0	102.0	423*	166.0	192.0	138.0
		C	Maturation 1	166.0	76.0	702*	142.0	222.0	
		D	Maturation 2	134.0	68.0	446*	114.0	242.0	140.0
	UASB-POND	F	Influent	354.0	448.0	12,680*	746.0	736.0	598.0
		G	UASB		74.0	172*	550.0	426.0	219.0
		H	Maturation 1			160*	400.0	287.0	
		I	Maturation 2		68.0	108*	198.0	198.0	96.0
TSS (mg/L)	THREE-POND	A	Influent	215.0	453.0	1.2*	144.0	155.0	245.0
		B	Facultative	56.0	26.0	8.5*	80.0	65.0	37.0
		C	Maturation 1	59.0	15.0	15.2*	55.0	72.0	
		D	Maturation 2	33.0	18.0	10.4*	42.0	46.0	40.0
	UASB-POND	F	Influent	156.0	483.5	46,490*	385.0	495.0	230.0
		G	UASB		17.0	42*	175.0	76.0	65.0
		H	Maturation 1			210*	112.0	80.0	
		I	Maturation 2		15.0	16*	42.0	62.0	25.0

* Based on the fact that the results from 2009 for the physical-chemical analyses shown above were so much different from all the other data points, these values were subjectively decided to be outlying data, and were therefore not included in the calculations of percent removal, etc.

Table C2: Concentrations for thermotolerant coliforms

Parameter		Sample Point		6/12/2007	6/24/2008	6/22/2009	6/22/2010	6/28/2010	6/13/2011
Thermotolerant Coliforms (CFU or MPN/100ml)	THREE-POND	A	Influent	9.2E+08	6.4E+07	5.0E+07	2.3E+07	3.5E+07	4.9E+07
		B	Facultative	1.1E+06	1.4E+06	1.7E+05	3.5E+06	1.5E+06	8.0E+05
		D	Maturation 2	5.0E+03	6.1E+04	8.5E+03	2.6E+05	1.1E+05	7.0E+03
	UASB-POND	F	Influent		3.5E+07	4.8E+06	9.0E+06	3.7E+07	3.2E+07
		G	UASB		1.8E+06	6.0E+06	2.5E+07	2.1E+06	1.0E+07
		I	Maturation 2		8.0E+04	1.1E+05	3.0E+05	1.0E+04	7.0E+04

Appendix C (Continued)

Table C3: Concentrations for nutrients in the three-pond system

Parameter		Sample Point		6/12/2007	6/24/2008	6/22/2009	6/22/2010	6/28/2010	6/13/2011	6/26/2011	6/26/2011	6/26/2011
THREE POND	Total Kjeldahl Nitrogen (mg/L N)	Raw Wastewater (System Influent)	A						30.2			
		After Facultative Pond	B						35.7			
		After MP 2 (System Effluent)	D						24.5			
	Ammonia-Nitrogen (mg/L NH_3-N)	Raw Wastewater (System Influent)	A			30.8			28.0			
		After Facultative Pond	B			30.1			28.6			
		After MP 2 (System Effluent)	D			29.4			19.0			
	Nitrate (mg/L NO_3-N)	Raw Wastewater (System Influent)	A						10.1			
		After Facultative Pond	B						13.3			
		After MP 2 (System Effluent)	D						11.2			
	Total Nitrogen (mg/L N)	Raw Wastewater (System Influent)	A	53.2		35.0	43.4	57.4	40.4			
		After Facultative Pond	B	25.2	29.4	25.2	54.6	49.0	49.0			
		After MP 2 (System Effluent)	D	22.4	31.2	40.6	29.4	60.2	35.7			
	Total Phosphorus (mg/L P)	Raw Wastewater (System Influent)	A	8.4	11.8	7.9			7.9	5.0	11.1	11.5
		After Facultative Pond	B	4.0	6.1	7.2			8.9			
		After MP 2 (System Effluent)	D	3.2	5.2	9.7			7.6	1.6	6.9	
	Orthophosphate (mg/L PO_3)	Raw Wastewater (System Influent)	A						20.4			
		After Facultative Pond	B						19.7			
		After MP 2 (System Effluent)	D						14.0			

Appendix C (Continued)

Table C3: Concentrations for nutrients in the UASB-pond system

UASB POND	Total Kjeldahl Nitrogen (mg/L N)	Raw Wastewater (System Influent)	F						48.2			
		After UASB	G						70.3			
		After MP 2 (System Effluent)	I						50.4			
	Ammonia-Nitrogen (mg/L NH_3-N)	Raw Wastewater (System Influent)	F						38.6			
		After UASB	G			60.5			59.4			
		After MP 2 (System Effluent)	I			45.1			44.2			
	Nitrate (mg/L NO_3-N)	Raw Wastewater (System Influent)	F						7.0			
		After UASB	G						18.9			
		After MP 2 (System Effluent)	I						12.1			
	Total Nitrogen (mg/L N)	Raw Wastewater (System Influent)	F		85.8	67.2	82.6	63.7	55.2			
		After UASB	G		48.3	68.3	95.2	103.0	89.2			
		After MP 2 (System Effluent)	I		41.0	17.9	95.2	54.6	62.5			
	Total Phosphorus (mg/L P)	Raw Wastewater (System Influent)	F		10.0	11.1			14.7	9.9	15.4	9.8
		After UASB	G		10.2	16.1			15.5			
		After MP 2 (System Effluent)	I		9.1	5.1			14.0	12.9	6.0	
	Orthophosphate (mg/L PO_3)	Raw Wastewater (System Influent)	F						32.7			
		After UASB	G						43.0			
		After MP 2 (System Effluent)	I						33.8			

Appendix C (Continued)

Table C4: Calculations for helminth egg concentrations in water samples from three-pond system

			Microscopy Egg Counts (same volume observed for each repetition)					Initial Sample Volume	Final Sample Volume (concentrated)	Volume Observed (Microscopy)	Estimated Total Number of Eggs in Sample	Minimum Level of Detection	Concentration in Sample
			[1]					[2]	[3]	[4]	[5]	[6]	[7]
			(counted from microscope)					(measured)	(measured)	(measured)	=[1]*[3]/[4]	=[3]/[4]/[2]	=[5]/[2]
			R1	R2	R3	R4	Average	mL	mL	uL	eggs	eggs/L	eggs/L
A_{comp}	2011	*Ascaris sp.*	2	3	0	5	2.5	5,000	1.1	1.8	1,528	31	306
A_{comp}	2011	*Hookworm sp.*	0	0	1	0	0.25	5,000	1.1	1.8	153	31	31
A_{comp}	2011	*Taenia sp.*	0	2	4	4	2.5	5,000	1.1	1.8	1,528	31	306
A_{comp}	2011	*Trichuris sp.*	0	0	0	0	0	5,000	1.1	1.8	<153	31	<31
A_{comp}	2012a	*Ascaris sp.*	0	0	-	-	0	2,310	5.0	1.8	<1389	601	<601
A_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	2,310	5.0	1.8	<1389	601	<601
A_{comp}	2012a	*Taenia sp.*	3	2	-	-	2.5	2,310	5.0	1.8	6,944	601	3,006
A_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	2,310	5.0	1.8	<1389	601	<601
A_{comp}	2012b	*Ascaris sp.*	0	0	-	-	0	2,000	2.1	1.8	<583	292	<292
A_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	2.1	1.8	<583	292	<292
A_{comp}	2012b	*Taenia sp.*	0	4	-	-	2	2,000	2.1	1.8	2,333	292	1,167
A_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	2.1	1.8	<583	292	<292
B_{comp}	2012a	*Ascaris sp.*	4	0	-	-	2	9,130	4.0	1.8	4,444	122	487
B_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	9,130	4.0	1.8	<1111	122	<122
B_{comp}	2012a	*Taenia sp.*	1	2	-	-	1.5	9,130	4.0	1.8	3,333	122	365
B_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	9,130	4.0	1.8	<1111	122	<122
B_{comp}	2012b	*Ascaris sp.*	0	0	-	-	0	2,000	1.4	1.8	<389	194	<194
B_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	1.4	1.8	<389	194	<194
B_{comp}	2012b	*Taenia sp.*	0	0	-	-	0	2,000	1.4	1.8	<389	194	<194
B_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	1.4	1.8	<389	194	<194

Appendix C (Continued)

Table C4: (Continued)

			Microscopy Egg Counts (same volume observed for each repetition)					Initial Sample Volume	Final Sample Volume (concentrated)	Volume Observed (Microscopy)	Estimated Total Number of Eggs in Sample	Minimum Level of Detection	Concentration in Sample
			[1]					[2]	[3]	[4]	[5]	[6]	[7]
			(counted from microscope)					(measured)	(measured)	(measured)	=[1]*[3]/[4]	=[3]/[4]/[2]	=[5]/[2]
			R1	R2	R3	R4	Average	mL	mL	uL	eggs	eggs/L	eggs/L
C_{comp}	2012a	*Ascaris sp.*	4	0	-	-	2	16,760	2.0	1.8	2,222	33	133
C_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	16,760	2.0	1.8	<556	33	<33
C_{comp}	2012a	*Taenia sp.*	0	0	-	-	0	16,760	2.0	1.8	<556	33	<33
C_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	16,760	2.0	1.8	<556	33	<33
C_{comp}	2012b	*Ascaris sp.*	0	0	-	-	0	2,000	2.3	1.8	<639	319	<319
C_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	2.3	1.8	<639	319	<319
C_{comp}	2012b	*Taenia sp.*	0	0	-	-	0	2,000	2.3	1.8	<639	319	<319
C_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	2.3	1.8	<639	319	<319
D_{comp}	2012a	*Ascaris sp.*	0	0	-	-	0	32,100	2.6	1.8	<722	22	<22
D_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	32,100	2.6	1.8	<722	22	<22
D_{comp}	2012a	*Taenia sp.*	1	1	-	-	1	32,100	2.6	1.8	1,444	22	45
D_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	32,100	2.6	1.8	<722	22	<22
D_{comp}	2012b	*Ascaris sp.*	0	0	-	-	0	2,000	2.7	1.8	<750	375	<375
D_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	2.7	1.8	<750	375	<375
D_{comp}	2012b	*Taenia sp.*	0	0	-	-	0	2,000	2.7	1.8	<750	375	<375
D_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	2.7	1.8	<750	375	<375

Appendix C (Continued)

Table C5: Calculations for helminth egg concentrations in water samples from UASB-pond system

			Microscopy Egg Counts (same volume observed for each repetition)					Initial Sample Volume	Final Sample Volume (concentrated)	Volume Observed (Microscopy)	Estimated Total Number of Eggs in Sample	Minimum Level of Detection	Concentration in Sample
			[1]					[2]	[3]	[4]	[5]	[6]	[7]
			(counted from microscope)					(measured)	(measured)	(measured)	=[1]*[3]/[4]	=[3]/[4]/[2]	=[5]/[2]
			R1	R2	R3	R4	Average	mL	mL	uL	eggs	eggs/L	eggs/L
F_{comp}	2011	*Ascaris sp.*	3	0	0	4	1.75	2,500	2.2	1.8	2,139	122	856
F_{comp}	2011	*Hookworm sp.*	0	0	0	0	0	2,500	2.2	1.8	<306	122	<122
F_{comp}	2011	*Taenia sp.*	1	0	1	0	0.5	2,500	2.2	1.8	611	122	244
F_{comp}	2011	*Trichuris sp.*	0	0	0	0	0	2,500	2.2	1.8	<306	122	<122
F_{comp}	2012a	*Ascaris sp.*	1	1	-	-	1	8,000	5.0	1.8	2,778	174	347
F_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	8,000	5.0	1.8	<1389	174	<174
F_{comp}	2012a	*Taenia sp.*	1	3	-	-	2	8,000	5.0	1.8	5,556	174	694
F_{comp}	2012a	*Trichuris sp.*	1	0	-	-	0.5	8,000	5.0	1.8	1,389	174	174
F_{comp}	2012b	*Ascaris sp.*	1	1	-	-	1	2,000	1.6	1.8	889	222	444
F_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	1.6	1.8	<444	222	<222
F_{comp}	2012b	*Taenia sp.*	6	6	-	-	6	2,000	1.6	1.8	5,333	222	2,667
F_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	1.6	1.8	<444	222	<222
G_{comp}	2012a	*Ascaris sp.*	0	1	-	-	0.5	7,930	3.2	1.8	889	112	112
G_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	7,930	3.2	1.8	<889	112	<112
G_{comp}	2012a	*Taenia sp.*	3	4	-	-	3.5	7,930	3.2	1.8	6,222	112	785
G_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	7,930	3.2	1.8	<889	112	<112
G_{comp}	2012b	*Ascaris sp.*	1	0	-	-	0.5	2,000	1.7	1.8	472	236	236
G_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	1.7	1.8	<472	236	<236
G_{comp}	2012b	*Taenia sp.*	1	6	-	-	3.5	2,000	1.7	1.8	3,306	236	1,653
G_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	1.7	1.8	<472	236	<236

Appendix C (Continued)

Table C5: (Continued)

			Microscopy Egg Counts (same volume observed for each repetition)					Initial Sample Volume	Final Sample Volume (concentrated)	Volume Observed (Microscopy)	Estimated Total Number of Eggs in Sample	Minimum Level of Detection	Concentration in Sample
			[1]					[2]	[3]	[4]	[5]	[6]	[7]
			(counted from microscope)					(measured)	(measured)	(measured)	=[1]*[3]/[4]	=[3]/[4]/[2]	=[5]/[2]
			R1	R2	R3	R4	Average	mL	mL	uL	eggs	eggs/L	eggs/L
H_{comp}	2012a	*Ascaris sp.*	0	0	-	-	0	11,760	3.4	1.8	<944	80	<80
H_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	11,760	3.4	1.8	<944	80	<80
H_{comp}	2012a	*Taenia sp.*	0	0	-	-	0	11,760	3.4	1.8	<944	80	<80
H_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	11,760	3.4	1.8	<944	80	<80
H_{comp}	2012b	*Ascaris sp.*	1	5	-	-	3	2,000	1.2	1.8	2,000	167	1,000
H_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	1.2	1.8	<333	167	<167
H_{comp}	2012b	*Taenia sp.*	1	2	-	-	1.5	2,000	1.2	1.8	1,000	167	500
H_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	1.2	1.8	<333	167	<167
I_{comp}	2012a	*Ascaris sp.*	0	0	-	-	0	22,510	1.6	1.8	<444	20	<20
I_{comp}	2012a	*Hookworm sp.*	0	0	-	-	0	22,510	1.6	1.8	<444	20	<20
I_{comp}	2012a	*Taenia sp.*	0	0	-	-	0	22,510	1.6	1.8	<444	20	<20
I_{comp}	2012a	*Trichuris sp.*	0	0	-	-	0	22,510	1.6	1.8	<444	20	<20
I_{comp}	2012b	*Ascaris sp.*	0	1	-	-	0.5	2,000	1.6	1.8	444	222	222
I_{comp}	2012b	*Hookworm sp.*	0	0	-	-	0	2,000	1.6	1.8	<444	222	<222
I_{comp}	2012b	*Taenia sp.*	4	6	-	-	5	2,000	1.6	1.8	4,444	222	2,222
I_{comp}	2012b	*Trichuris sp.*	0	0	-	-	0	2,000	1.6	1.8	<444	222	<222

Appendix C (Continued)

Table C6: Calculations for helminth egg concentrations in sludge samples from facultative pond

			Microscopy Egg Counts (same volume observed for each repetition)					Initial Sample Volume	Final Sample Volume (concentrated)	Volume Observed (Microscopy)	Estimated Total Number of Eggs in Sample	Minimum Level of Detection	Concentration in Sample
			[1]					[2]	[3]	[4]	[5]	[6]	[7]
			(counted from microscope)					(measured)	(measured)	(measured)	=[1]*[3]/[4]	=[3]/[4]/[2]	=[5]/[2]
			R1	R2	R3	R4	Average	mL	mL	uL	eggs	eggs/L	eggs/L
A_1	2011	*Ascaris sp.*	2	5	-	-	5	1,000	0.6	0.2	15,500	1,550	15,500
A_1	2011	*Hookworm sp.*	0	0	-	-	0	1,000	0.6	0.2	<1550	1,550	<1550
A_1	2011	*Taenia sp.*	22	26	-	-	26	1,000	0.6	0.2	80,600	1,550	80,600
A_1	2011	*Trichuris sp.*	0	0	-	-	0	1,000	0.6	0.2	<1550	1,550	<1550
A_2	2011	*Ascaris sp.*	2	3	-	-	3	1,000	0.9	0.2	12,900	2,150	12,900
A_2	2011	*Hookworm sp.*	0	1	-	-	1	1,000	0.9	0.2	4,300	2,150	4,300
A_2	2011	*Taenia sp.*	61	40	-	-	50.5	1,000	0.9	0.2	217,150	2,150	217,150
A_2	2011	*Trichuris sp.*	0	0	-	-	0	1,000	0.9	0.2	<2150	2,150	<2150
A_3	2011	*Ascaris sp.*	1	8	-	-	8	1,000	0.8	0.2	32,000	2,000	32,000
A_3	2011	*Hookworm sp.*	0	2	-	-	2	1,000	0.8	0.2	8,000	2,000	8,000
A_3	2011	*Taenia sp.*	5	14	-	-	14	1,000	0.8	0.2	56,000	2,000	56,000
A_3	2011	*Trichuris sp.*	0	1	-	-	1	1,000	0.8	0.2	4,000	2,000	4,000
A_4	2012	*Ascaris sp.*	0	5	-	-	2.5	50	2.3	1.8	3,194	12,778	63,889
A_4	2012	*Hookworm sp.*	0	0	-	-	0	50	2.3	1.8	<639	12,778	<12778
A_4	2012	*Taenia sp.*	9	7	-	-	8	50	2.3	1.8	10,222	12,778	204,444
A_4	2012	*Trichuris sp.*	0	0	-	-	0	50	2.3	1.8	<639	12,778	<12778
A_5	2012	*Ascaris sp.*	1	0	-	-	0.5	50	2.5	1.8	694	13,889	13,889
A_5	2012	*Hookworm sp.*	0	0	-	-	0	50	2.5	1.8	<694	13,889	<13889
A_5	2012	*Taenia sp.*	2	2	-	-	2	50	2.5	1.8	2,778	13,889	55,556
A_5	2012	*Trichuris sp.*	0	0	-	-	0	50	2.5	1.8	<694	13,889	<13889

Appendix C (Continued)

Table C7: Solids analysis for facultative pond sludge

				Sample 1	Sample 2	Average
Facultative Pond	A	2011	*Total Solids (g/L)*	184.45	181.46	182.96
	A	2011	*Volatile Solids (g/L)*	n/a	n/a	n/a
	A	2011	*Fixed Solids (g/L)*	n/a	n/a	n/a
Facultative Pond	A	2012	*Total Solids (g/L)*	166.73	148.59	157.66
	A	2012	*Volatile Solids (g/L)*	132.87	119.81	126.34
	A	2012	*Fixed Solids (g/L)*	33.86	28.88	31.37

Table C8: Calculation of helminth egg concentrations per dry weight of facultative pond sludge

$$Conc_{DW} = \frac{Conc_L}{TS}$$

where $Conc_{DW}$ = dry weight concentration (eggs/g TS)

$Conc_L$ = liquid concentration (eggs/L)

TS = total solids (g/L)

				eggs/g TS	eggs/g FS
Facultative Pond	A1	2011	*Geohelminths only*	178	676
	A2	2011	*Geohelminths only*	141	536
	A3	2011	*Geohelminths only*	361	1371
	A4	2012	*Geohelminths only*	405	1539
	A5	2012	*Geohelminths only*	88	335
Facultative Pond	A1	2011	*Geohelminths and Taenia Eggs*	788	3,960
	A2	2011	*Geohelminths and Taenia Eggs*	1,921	9,655
	A3	2011	*Geohelminths and Taenia Eggs*	820	4,121
	A4	2012	*Geohelminths and Taenia Eggs*	1,702	8,554
	A5	2012	*Geohelminths and Taenia Eggs*	440	2,214

Appendix C (Continued)

Table C9: Calculations for helminth egg concentrations in sludge samples from UASB reactor

			Microscopy Egg Counts (same volume observed for each repetition)					Initial Sample Volume	Final Sample Volume (concentrated)	Volume Observed (Microscopy)	Estimated Total Number of Eggs in Sample	Minimum Level of Detection	Concentration in Sample
			[1]					[2]	[3]	[4]	[5]	[6]	[7]
			(counted from microscope)					(measured)	(measured)	(measured)	=[1]*[3]/[4]	=[3]/[4]/[2]	=[5]/[2]
			R1	R2	R3	R4	Average	mL	mL	uL	eggs	eggs/L	eggs/L
F_1	2012	*Ascaris sp.*	2	4	-	-	3	50	2.3	1.8	3,833	12,778	76,667
F_1	2012	*Hookworm sp.*	0	0	-	-	0	50	2.3	1.8	<639	12,778	<12778
F_1	2012	*Taenia sp.*	3	2	-	-	2.5	50	2.3	1.8	3,194	12,778	63,889
F_1	2012	*Trichuris sp.*	0	0	-	-	0	50	2.3	1.8	<639	12,778	<12778
F_2	2012	*Ascaris sp.*	0	0	-	-	0	50	4.0	1.8	<1111	22,222	<22222
F_2	2012	*Hookworm sp.*	0	0	-	-	0	50	4.0	1.8	<1111	22,222	<22222
F_2	2012	*Taenia sp.*	1	0	-	-	0.5	50	4.0	1.8	1,111	22,222	22,222
F_2	2012	*Trichuris sp.*	0	0	-	-	0	50	4.0	1.8	<1111	22,222	<22222
F_3	2012	*Ascaris sp.*	2	0	-	-	1	50	1.4	1.8	778	7,778	15,556
F_3	2012	*Hookworm sp.*	0	0	-	-	0	50	1.4	1.8	<389	7,778	<7778
F_3	2012	*Taenia sp.*	1	2	-	-	1.5	50	1.4	1.8	1,167	7,778	23,333
F_3	2012	*Trichuris sp.*	0	0	-	-	0	50	1.4	1.8	<389	7,778	<7778

Table C10: Solids analysis for UASB reactor sludge

				Sample 1	Sample 2	Average
UASB Reactor	F	2012	*Total Solids (g/L)*	290.42	280.05	285.24
	F	2012	*Volatile Solids (g/L)*	214.15	206.15	210.15
	F	2012	*Fixed Solids (g/L)*	76.28	73.90	75.09

Appendix C (Continued)

Table C11: Calculation of helminth egg concentrations per dry weight of UASB reactor sludge

$$Conc_{DW} = \frac{Conc_L}{TS}$$

where $Conc_{DW}$ = dry weight concentration (eggs/g TS)

$Conc_L$ = liquid concentration (eggs/L)

TS = total solids (g/L)

				eggs/g TS	eggs/g FS
UASB Reactor	F1	2012	***Geohelminths and Taenia Eggs***	493	2,477
	F2	2012	***Geohelminths and Taenia Eggs***	78	392
	F3	2012	***Geohelminths and Taenia Eggs***	136	685
UASB Reactor	F1	2012	***Geohelminths only***	269	1,021
	F2	2012	***Geohelminths only***	39	148
	F3	2012	***Geohelminths only***	55	207

Appendix D – Sludge Volume Measurements

The levels of sludge were measured in the facultative pond from the three-pond system in June 2011, by using a boat, a measuring tape, and a PVC pipe with white cloth tied to the end of it. The PVC pipe was dipped into the sludge at different locations in the pond, and the depth of the sludge at each location was estimated by measuring the length of the portion of the white cloth that was stained. The location of each measurement was measured from the banks of the pond. The measured volume of accumulated sludge in the facultative pond, which had never been evacuated since the system started operation in 2007, was approximately 115 m^3.

Table D1: Measured sludge depths in the facultative pond

Number	Sludge Depth (cm)	Vertical distance* (m)	Horizontal distance* (m)	Notes
1	46	5	8	
2	13	5	19	
3	132	5	13	
4	13	11	8	
5	104	11	11	May have gone past 30". Dark sludge to 30?
6	15	11	19	
7	124	11	12	
8	20	16	9	
9	30	13	13	In line with center of lagoon entrance
10	15	13	16	
11	23	12	9	
12	7	32	6	
13	61	4	15	
14	79	4	9	
15	51	7	9	
16	127	7	13	
17	127	7	15	Maybe deeper
18	13	7	17	
19	66	6	15	

* = distance from bottom right corner of facultative lagoon

Appendix D (Continued)

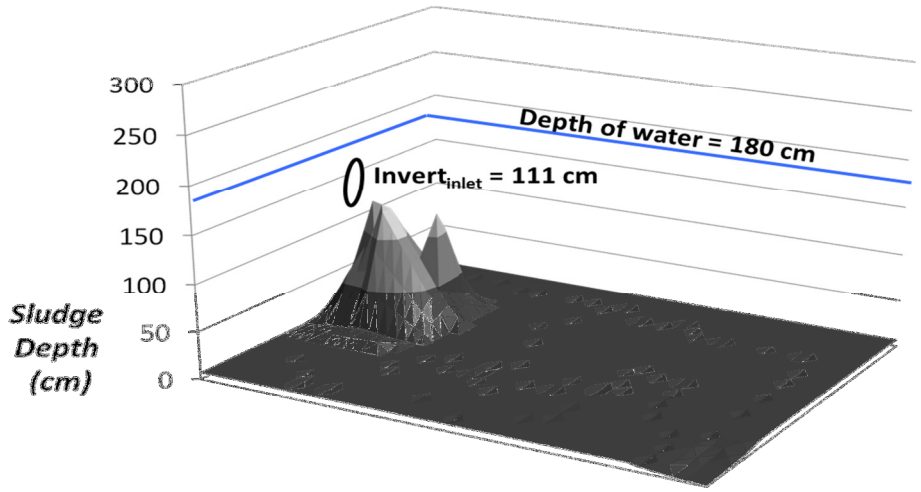

Figure D1: Plot of sludge build-up in facultative pond

The approximate volume of sludge in the UASB reactor was measured in June 2012 by dipping a PVC pipe into the reactor to determine the depth of the built-up sludge, and multiplying that depth by the average interior diameter of the reactor. The measured depth of sludge was approximately 85% of the height of the reactor, which has a volume of 71 m^3 meters. Therefore, the accumulated sludge in the reactor is estimated at 60 m^3, although is likely less due to the fact that the sludge bed was expanded when it was measured. The sludge had reportedly not been emptied in approximately three years at the time of measurement.

ABOUT THE AUTHOR

Originally from Connecticut, Matthew Verbyla graduated from Lafayette College in 2006 with a Bachelor of Science in Civil and Environmental Engineering and a minor in Spanish. He has over three years of engineering consulting experience, mostly with the preparation of site plans and permit applications for industrial, commercial and municipal clients; he currently provides engineering support for HRP Associates, Inc. in New Port Richey, Florida. He is currently an EIT, a LEED Green Associate, and a member of AWWA, WEF, and AIDIS.

Matthew also has several years of experience working on water and sanitation projects in developing countries in Latin America. He received a U.S. Student Fulbright Fellowship in 2007 to complete a nine-month study of the sustainability of community-managed rural water systems in Honduras, in the context of decentralization policies. Matthew subsequently worked in Honduras as the Project Director for the 503(c)(3) organization Global Community Development, where he helped coordinate designs for water and sanitation projects for low-income rural and peri-urban communities. In 2012, Matthew was awarded a National Science Foundation Graduate Research Fellowship, and will continue at USF to pursue a Ph.D. in Environmental Engineering, working with Dr. James Mihelcic. He is also a member of the USF Doctoral Student Leadership Institute, and participates on two task forces for USF World. Matthew currently lives in Tampa with his wife, Wendy Antunez, whom he met while working in Honduras. In their free time, Matthew and Wendy enjoy visiting friends and family, traveling, and cooking.