Sludge Treatment Wetland (STW) as a Post-Treatment for Toilet-Linked Biogas Plant

A pilot-scale case study in Gujarat, India

Grover Hector Mamani Casilla

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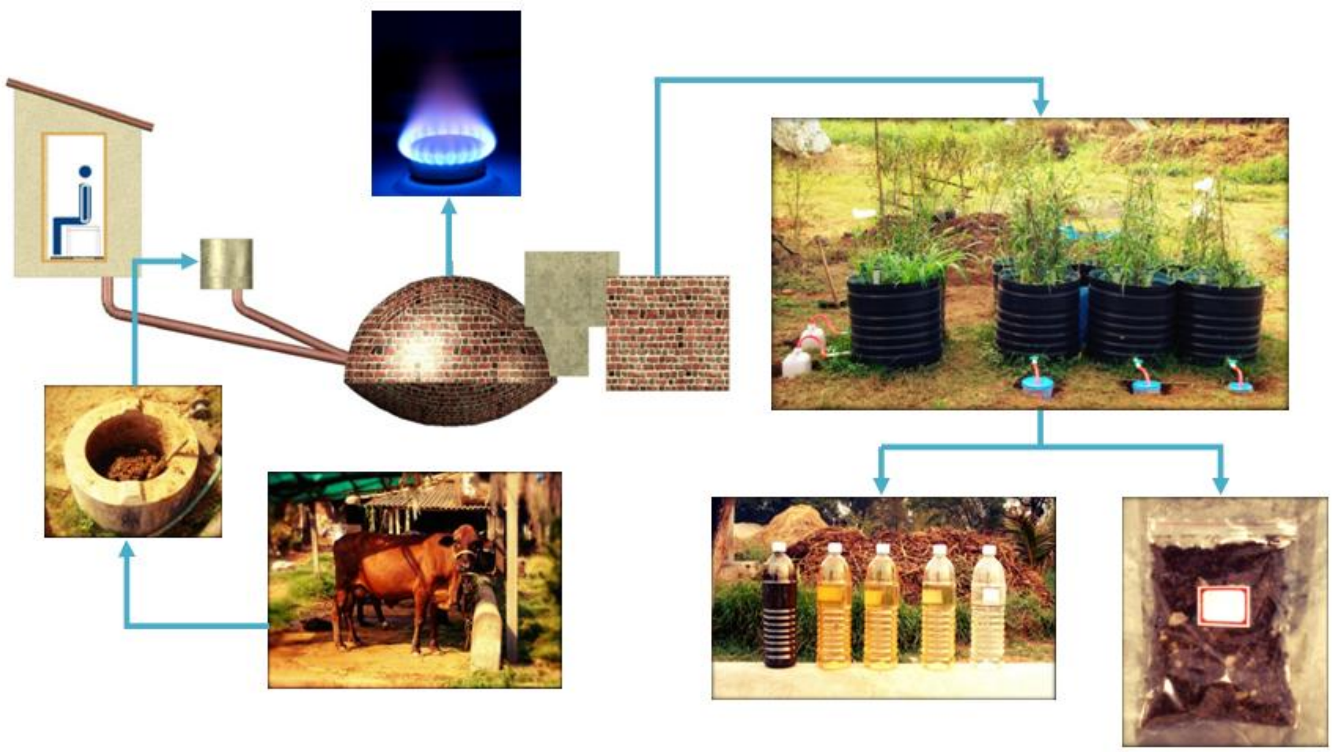

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Master of Science Thesis

by

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Abstract

Since 1900's, anaerobic biogas digester had been applied successfully in Asian countries like India, Nepal and China, treating cow manure, pig excreta, organic waste or a combination of them, providing not only biogas, which is used as source of energy, but also slurry used as soil conditioner.

During last 20 years in India, different government and private institutions promoted the use of toilet linked biogas plants (TLBPs) especially in rural area. TLBP is a modification of the household-size anaerobic biogas digester which includes toilet effluent connection through a pipeline. This system not only generates biogas and slurry, but also offers a new option for wastewater disposal and treatment. However, little is known about the slurry's properties, and previous research studies recommended a post-treatment of TLBPs effluent, prior its use as soil amendment. One of the options are sludge treatment wetlands (STWs).

This research characterized the TLBPs effluent by determining its physico-chemical properties and microbiological quality; and also, evaluated the performance of four pilot-scale STWs operated at different sludge loading rates (40.5, 81.0, 121.5 and 162.0 kgTSS/m^2-year). After sludge treatment two main products are generated: the biosolid accumulated at the STWs top layer and the water leachate collected at the bottom layer. Both products were evaluated from a reuse in agriculture point of view. Additionally the potential biomass production was compared for two plant species, *Phragmites karka* and *Napier Bajra* hybrid grass.

The four sludge treatment wetland configurations showed similar efficiencies in terms of nutrients concentration, mineralization and hygienisation, but regarding to sludge dryness, significant differences were observed at different loading rates. In fact, a loading rate of 108 kgTSS/m^2-year is recommended to obtain a product with appropriate moisture content. On the other hand, the water leachate collected at the bottom of STWs has a quality enough to comply with the standard limits for reuse in agriculture; it can be reused for irrigation.

The TLBP is a very good on-site sanitation example, applicable especially in rural area of India. If a STW is implemented as slurry's post-treatment, not only the slurry's quality is improved, but also treated water if produced, has a potential reuse for irrigation. Additionally, the plants used in STW (*Phragmites karka* and *Napier Bajra* hybrid grass) could be a good source of foliage for cows and buffalos; however more specific tests have to be conducted in order to evaluate the plants' pollutant accumulation.

Acknowledgements

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"I am able to do all things through Him who strengthens me" (Philippians 4:13)

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Table of Contents

List of Figures

List of Tables

Abbreviations

AFPRO	Action for Food Production
ADB	Asian Development Bank
APHA	American Public Health Association
BOD	Biochemical Oxygen Demand
BPL	Below Poverty Line
CFU	Colony-forming unit
COD	Chemical Oxygen Demand
DRI	Dynamic Respiration Index
EC	Electrical Conductivity
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FINISH	Financial Institutional Sanitation and Health
HRT	Hydraulic Retention Time
IS	Indian Standard
JMP	Joint Monitoring Program
K	Potassium
MPN	Most Probable Number
N	Nitrogen
NBA	Nirmal Bharat Abhiyan
P	Phosphorus
PVC	Polyvinyl chloride
SAR	Sodium-Adsorption Ratio
SLR	Sludge Loading Rate
STW	Sludge Treatment Wetland
T	Temperature
TAN	Total Ammonia Nitrogen
TLBP	Toilet Linked Biogas Plant
TSS	Total Suspended Solids
UNICEF	United Nation Children's Fund
UV	Ultraviolet

VSS	Volatile Suspended Solids
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

Nowadays it is well known that 2.5 billion people, which represents one third of the world's population, lacked access to an improved sanitation facility. According to the most recent Joint Monitoring Program Report by UNICEF and the World Health Organization, in India - the second largest population in the world - only 35% and 92% use improved sanitation facilities and drinking water sources respectively (WHO, 2013). Due to the lack of toilet facilities, fifty percent of India's population practice open defecation and three-fourths of India's surface water resources are polluted, being the main source of pollution (80%) sewage alone (ADB, 2009).

The impacts of the lack of sanitation on human health are very significant. Unsafe disposal of human excreta facilitates the transmission of oral-faecal diseases, including diarrhoea and a range of intestinal worm infections such as hookworm and roundworm (Soussan, 2006), causing an increase on: the diseases' incidence, higher mortality and morbidity rates, and health hazards. For this reason, the central government of India and different state governments are implementing numerous programmes to improve sanitation as well as to generate alternative energy sources in rural areas.

Most of the urban areas in India use centralized wet sanitation technologies, which involves some form of flush toilet connected to a sewer. Conversely, on-site sanitation technologies such as: traditional pit latrines, ventilated improved pits, urine diversion toilets and other, are implemented mostly in rural areas[1]. One of the most successful sanitation systems applied during the last 20 years in India's rural area is the toilet linked biogas plant (TLBP). This system consists of two components, the toilet (conventional cistern-flush or pour-flush toilets) and the household size anaerobic digester, which are connected through a PVC pipe.

Household size anaerobic digesters have been applied with the main purpose to generate biogas using cow dung mixed with water, and its initial design did not consider toilet connection. But, nowadays it was implemented successfully, not only to treat human excreta and cow dung, but also to generate biogas. This technology provides a sustainable source of energy in rural areas, and additionally the digested sludge (also called slurry) has a potential use for agricultural purposes because of its high nutrient value. However, previous studies reported that this treatment system has limitations regarding to pathogen removal (Michael, 2008, WASTE, 2013).

[1] 68.34% of India's population lives in rural area

Epidemiological studies of pathogens in sludge reported that bacteria, viruses, helminth eggs and protozoan cysts, present in the slurry, pose risks to human and animal health (de Lemos Chernicharo, 2005) and consequently the final product requires a post-treatment before its final disposal or reuse in agriculture.

Regarding to sludge stabilization, there are three main processes: i) biological stabilization, that could be anaerobic or aerobic digestion, ii) chemical stabilization, which is based on addition of chemicals, and iii) thermal stabilization, based on addition of heat. According to these processes, different kinds of technologies have been developed for sludge stabilization, for instance: sludge drying beds, centrifuges, filter press, belt press, thermal drying, constructed wetlands, composting and others. In regard to constructed wetlands, although its application is more popular in wastewater treatment, recent studies reported the successful application for sludge treatment (mainly sludge form WWTPs).

Sludge treatment wetlands (STW), also known as planted drying beds, are natural treatment systems where organic matter stabilization and pathogen reduction takes place, due to: solar radiation in the top layer (where sludge is accumulated), filtration through the sand and gravel beds, plants oxygen transfer, and microbial activity in the roots of the plants. This system has many advantages like: no energy requirement, local availability, easy to operate, on-site application and low investment cost, and the final product is suitable for land application, either directly or after additional composting to reach unrestricted product quality Uggetti et al. (2009).

On this research two main activities have been conducted; first, the TLBP digested sludge was characterized by monitoring its physical properties, organic matter, nutrients, and pathogens concentrations; and second, eight pilot-scale experimental units (sludge treatment wetlands) operated at four different loading rates were set-up, in order to evaluate not only the quality of biosolid accumulated at the top layer of each STW, but also the water leachate collected at the STW bottom layer.

1.1. BACKGROUND

In order to improve the sanitation conditions in India, the Ministry of Drinking Water and Sanitation is administering the flagship programme known as Nirmal Bharat Abhiyan (NBA). Under this scheme, the Ministry is providing financial support for the construction of individual household toilets targeted at people living Below Poverty Line (BPL), marginal farmers, and selected castes and tribes. In the state of Gujarat, the FINISH Society (a sanitation focussed project) is working to improve the health of the population in rural areas, through implementation of sanitation facilities.

Currently, FINISH Society is implementing the toilet linked biogas plants (TLBP) in Valsad and Navsari (districts of Gujarat) in two phases; the first phase comprises: 600 new TLBPs and 2000 toilet connections to existing biogas units, and the second phase: 1400 new TLBPs.

1.2. PROBLEM DEFINITION

The anaerobic digestion process has several advantages such as: low production of solids, low energy consumption, low land requirement, production of methane, generation of digested bio-solids and versatility to apply in small and large scale, yet conversely some of its main disadvantages are the unsatisfactory removal of nitrogen, phosphorus and pathogens.

The residual sludge (slurry), product of anaerobic digestion, has good characteristics to be used as soil conditioner and fertilizer (de Lemos Chernicharo, 2005). However, preliminary results of an ongoing project in India by WASTE (2013) found high concentration of pathogen indicators (*E. Coli* = 210 most probable number (MPN)/g in a wet sample). As a consequence, for safe reuse of the slurry, post-treatment is required to enhance the microbiology quality of this residue.

According with de Lemos Chernicharo (2005), the most important processes to reduce pathogens present in slurry are: composting, auto-thermal thermophilic aerobic digestion, alkaline stabilisation, pasteurisation and thermal drying, nevertheless recent studies identified sludge treatment wetlands as a potential alternative to conventional sludge treatments.

This research aims to evaluate the feasibility of vertical constructed wetlands as a sludge treatment at household size level, applied as a post-treatment for anaerobic digested sludge.

1.2.1. Research questions

1. What is the typical physical, chemical and microbiological quality of slurry produced at household size by anaerobic digesters?
    a. What are the limitations for reusing of the slurry for agricultural purposes (regarding human health)?
2. What is the pathogen removal efficiency of sludge treatment wetlands?
    a. What is the best applied sludge loading rate to operate a household size sludge treatment wetland?

1.3. RESEARCH OBJECTIVES

The main objective of this research is to investigate the feasibility of the application of a constructed wetland for sludge treatment, as a post-treatment of anaerobic digested sludge, to obtain a final product sufficiently safe from a human health perspective at household size level in Gujarat, India.

In order to achieve this objective, the following sub-objectives must be met.

1. Determine the microbiological characteristics of digested sludge produced by anaerobic digestion.
2. Implement a household-scale sludge treatment wetland.
    a. To establish the pathogen removal efficiency of sludge treatment wetland under different operating conditions (sludge loading rate).
    b. To evaluate the microbiological quality and quantity of dried sludge under local climate conditions.

CHAPTER 2

LITERATURE REVIEW

This research aims to assess the application of sludge treatment wetland as post-treatment of anaerobic digested sludge. To do this, information from different field is required. The following literature review describes important findings from previous research studies related with this topic and it is divided into three parts: anaerobic digestion, pathogens and sludge treatment wetlands.

2.1. ANAEROBIC DIGESTION

The microbiological processes which transform organic compounds into carbon dioxide and methane are called anaerobic digestion (Bitton, 2005).

Anaerobic digestion is carried out by specialized microorganisms, normally in two stages. In the first stage, complex organic compounds such as proteins, lipids and carbohydrates are converted into simple organic materials (volatile fatty acids, carbon dioxide and hydrogen gases) by hydrolysis and fermentation. In the second stage, performed by microorganisms named methanogens, the organic acids and hydrogen are converted into methane and carbon dioxide.

According to de Lemos Chernicharo (2005), it is estimated that millions of anaerobic digesters have been constructed around the world, treating not only solid wastes (including agricultural wastes, animal excrements and sludge from sewage treatment), but also effluents from industries (food, agricultural and beverage), because of the system's favourable characteristics. For this reason, anaerobic digesters have an enormous potential to be used as a treatment system especially for low-concentration wastewater.

The main advantages and disadvantages of anaerobic digestion process are summarized in *Table 2.1*.

In general the anaerobic treatment systems can be classified in two groups (de Lemos Chernicharo, 2007): conventional and high-rate systems. The first group, which includes sludge digesters, septic tanks and anaerobic ponds, has three main characteristics which are: i) absence of a solid retention mechanism in the system, ii) long hydraulic detention times and iii) low volumetric loads.

The second group is a consequence of the recent increase in research into anaerobic treatment. The main characteristics of high-rate systems are: i) they operate with short hydraulic detention time, ii) they have long solid retention time and iii) they incorporate biomass retention mechanisms. This group can be subdivided into two sub-groups:

a) Attached growth reactors: fixed bed reactors, rotating bed reactors and expanded bed reactors.

b) Dispersed growth systems: reactors with internal recirculation, two-stage reactors, baffled reactors, up-flow sludge blanket reactors and expanded granular bed reactors.

Table 2.1 *Advantages and disadvantages of the anaerobic process*

Advantages	Disadvantages
• Low production of solids, about 3 to 5 times lower than aerobic processes. • Low energy consumption, usually associated with an influent pumping station, leading to very low operational costs • Low land requirements • Low construction costs • Production of methane • Possibility of preservation of the biomass, with no reactor feeding, for several months • Tolerance to high organic loads • Application in small and large scale • Low nutrient consumption	• Anaerobic microorganisms are susceptible to inhibition by a large number of compounds • Process start-up can be slow in the absence of adapted seed sludge • Some form of post-treatment is usually necessary • The biochemistry and microbiology of anaerobic digestion are complex, and still require further studies • Possible generation of bad odours • Possible generation of effluents with unpleasant aspect • Unsatisfactory removal of nitrogen, phosphorus and *pathogens*

Source: Adapted from Chernicharo and Campos (1995); Von Sperling (1996); Lettinga et al. (1996)

Although most of the existing anaerobic treatment systems are applied on a large scale, it is also possible to apply it at household level. For example, the household size anaerobic biogas reactors - which initially were developed to produce biogas from cattle dung digestion - currently, are also used as a domestic wastewater treatment.

2.1.1. Anaerobic Biogas Reactor (Toilet Linked Biogas Plant TLBP)

An anaerobic biogas reactor is a compartment constructed as a fixed dome or floating dome reactor, where it is possible to degrade black water, sludge, and/or biodegradable waste. During the decomposition process, two products are generated: i) the biogas produced through fermentation that is collected at the top of the reactor and ii) the slurry which is rich in organics and nutrients, but it is not completely sanitized and still carries a risk of infection, so consequently it needs a treatment before its final disposal or reuse (Tilley et al., 2008).

Usually, the toilets are connected directly to the anaerobic biogas reactor and additionally there is an access point for organic materials. *See Figure 2-1.*

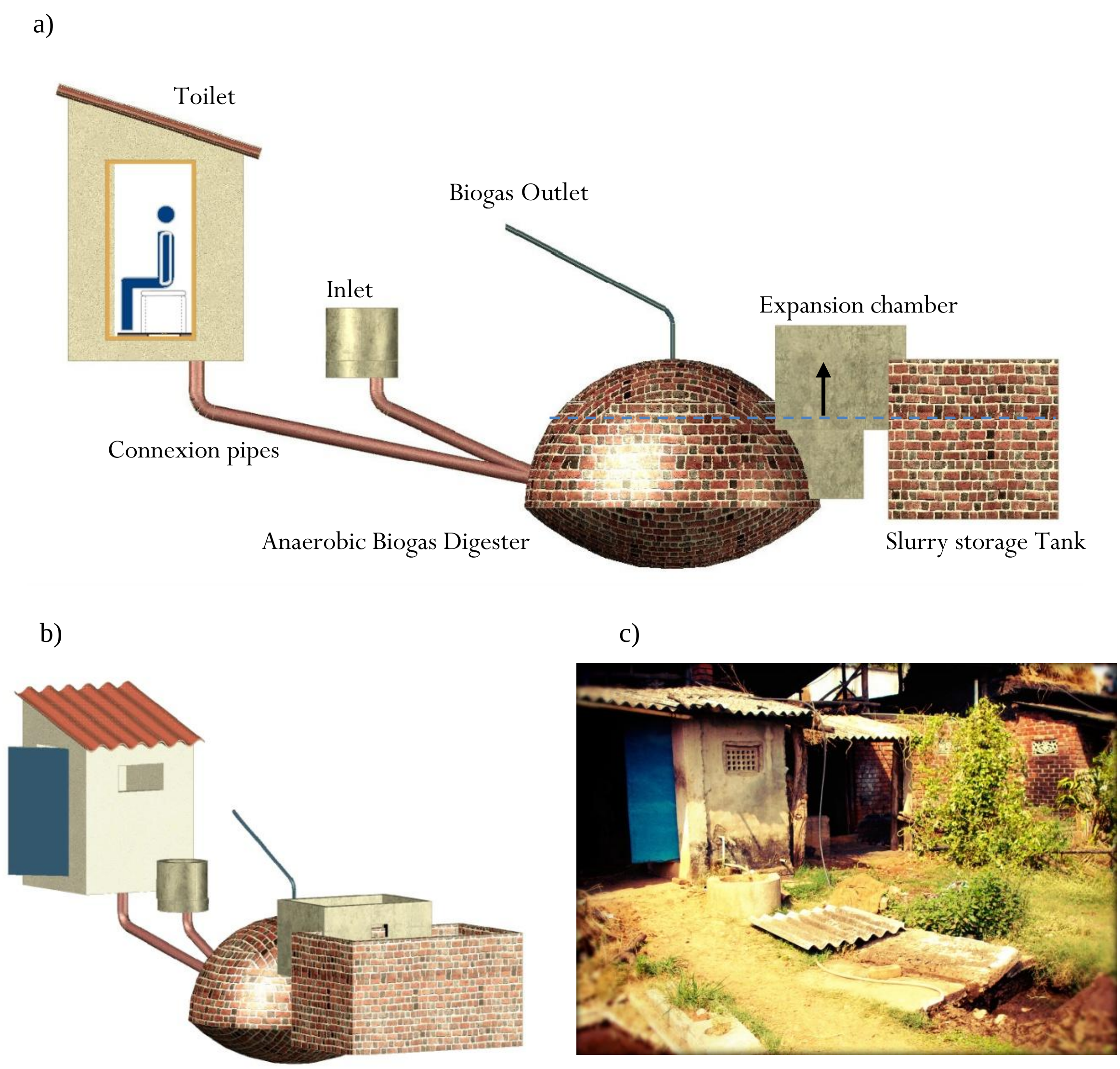


Figure 2-1 a) TLBP profile view. b) TLBP 3D view. c) TLBP selected

According to the FAO (1996), since 1936 Asian countries like China, India, Taiwan and Nepal developed different types of anaerobic biogas reactors where the main purpose is the same, to produce biogas energy. The difference between them is mainly the shape, size and construction materials. Some of the most used designs in Asia are described below.

Floating Drum Digester

Developed in 1956 by Jashu Bhai J Patel, the floating drum digester (popularly known as *Gobar Gas plant*) was implemented in India by the Khadi and Village Industries Commission after its approval in 1962. In this design, the digester chamber is made of brick masonry in cement mortar and in order to collect the biogas a mild steel drum is placed on top of the digester.

Fixed Dome Digester

This model, also known as drum less digester, was designed and built in China since 1936. The design includes an underground brick masonry chamber with a dome on the top for gas storage. Unlike the floating drum digester design; this model eliminates the use of mild steel gas which was costlier and susceptible to corrosion. Additionally the life of fixed dome digester is longer (from 20 to 50 years).

Deenbandhu Model

Whit the purpose of improving the Chinese fixed dome digester, the Action for Food Production (AFPRO) developed the *Deenbandhu* model on 1984, reducing the construction cost on 30%. It was also proved that this model is 45% cheaper than the first floating drum digester. *Deenbandhu* plants are made entirely of brick masonry work with a spherical shaped gas holder at the top and a concave bottom.

Bag Digester

In order to avoid the problems experienced with brick and metal digesters, bad digester was developed in 1960s in Taiwan. It consists of a long cylinder made of PVC. Because of pressure inside the digester is increased, welding facilities are required to implement successfully this model.

Plug Flow Digester

'The plug flow digester is similar to the bag digester. It consists of a trench (trench length has to be considerably greater than the width and depth) lined with, concrete or an impermeable membrane. The reactor is covered with either a flexible cover gas holder anchored to the ground, concrete or galvanized iron top. The first documented use of this type of design was in South Africa in 1957' (FAO, 1996).

Anaerobic Filter

This type of digester was developed in the 1950's to use relatively dilute and soluble waste water with low level of suspended solids. It is one of the earliest and simplest types of design developed to reduce the reactor volume. It consists of a column filled with a packing medium. A great variety of non-biodegradable materials have been used as packing media for anaerobic filter reactors such as stones, plastic, coral, mussel shells, reeds, and bamboo rings. The methane forming bacteria form a film on the large surface of the packing medium and are not earned out of the digester with the effluent. For this reason, these reactors are also known as "fixed film" or "retained film" digesters.

As it was mentioned on *Table 2.1*, the anaerobic digestion process has more advantages than disadvantages; however, one of the main limitations is the quality of the digested sludge, the so-called digester slurry, which usually does not comply with the standards established by environmental agencies. As a consequence, a post treatment is required to complete the removal of organic matter and nutrients but mainly pathogenic organisms such as viruses, bacteria, protozoan and helminths.

2.2. PATHOGENS

'The term pathogenic is applied to those organisms that either produce or are involved in the production of a disease' (Gray, 2004). The vast majority of water-related illnesses in developing countries are infectious and according to Bradley (1974) the action of pathogens are classified in four categories: i) Waterborne diseases, ii) Water-washed diseases, iii) Water-based diseases and iv) Water-related diseases.

This classification has been extended by Mara and Feachem (1999) who proposed a unitary environmental classification of water and excreta-related diseases in seven categories: i) Faecal-oral waterborne and water-washed diseases, ii) non-faecal-oral water-washed diseases, iii) Geohelminthiases, iv) Taeinases, v) Water-based diseases, vi) Insect-vector diseases and vii) Rodent-vector diseases.

From the point of view of environmental engineering, this classification is more useful than one based on biological type (virus, bacterium, protozoon, or helminth) because it groups the disease into categories of common environmental transmission routes. Consequently an environmental intervention designed to reduce transmission of pathogens in a particular category is likely to be effective against all pathogens in that category, irrespective of their biological type.

2.2.1. Pathogens in human excreta

Healthy people's excreta contain large numbers of non-pathogenic bacteria species, which corresponds to a common intestinal microbiota, while gastrointestinal pathogenic microorganisms are not naturally part of normal intestinal microbiota (Feachem et al., 1983). The presence of pathogenic microorganisms in faeces is an indication of infection among the population contributing to the faeces analysis. However, occasionally some of the commensal bacteria (referred as normal intestinal microbiota) may give rise to disease.

Lens et al. (2001) pointed out that the concentration of pathogenic microorganisms which cause illness depends on an individual immune system. *Table 2.2* shows a list of the main pathogenic bacteria and their infective dose.

2.2.2. Transmission

A large amount of intestinal pathogenic microorganisms are transmitted to a new host by ingestion (water, food, dirt on fingers and lips), through the lungs or the eye. Esrey et al. (1998) proposed a disease transmission route chart which explains the different routes from faeces to face as is shown in Figure 2-2.

Table 2.2 *Number of pathogenic bacteria required per healthy person for illness to occur*

Bacteria	Percentage ill			
	1-5	**26-50**	**51-75**	**76-100**
E. coli	10^6	10^8	10^8-10^{10}	10^{10}
Salmonella typhi	10^5	10^5-10^8	n.d.	10^8-10^9
S. meleagridis	10^6	10^7	10^7-10^8	n.d.
S. derby	n.d.	10^7	n.d.	n.d.
S. pullorum	n.d.	10^9	n.d.	10^9-10^{10}
Eneteroccus faecalis	10^9	10^{10}	n.d.	n.d.

Source: Kowal and Pahren (1982)

Note: n.d. = not determined

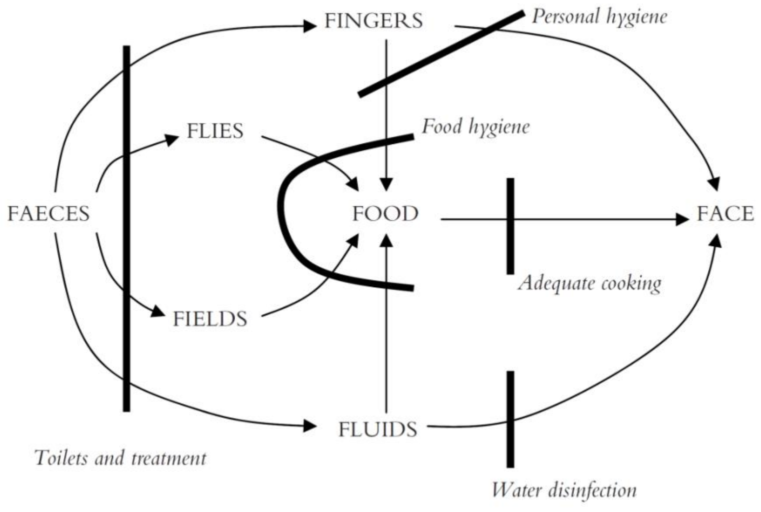


Figure 2-2 The F-diagram, showing the faecal disease transmission routes to a new host and the possible sanitation barriers

Source: Adapted from Esrey et al. (1998)

2.2.3. Methods to achieve die-off

There are a variety of methods to treat and sanitize faeces; most of them are considered unsafe due to the potential presence of high concentrations of pathogens. For each method, several factors affect the pathogen removal but there is a synergistic correlation between time and temperature, as shown in Figure 2-3, where the higher the temperature the less time is needed for elimination; and the longer the pathogens are left the lower the temperature that is needed (Lens et al., 2001).

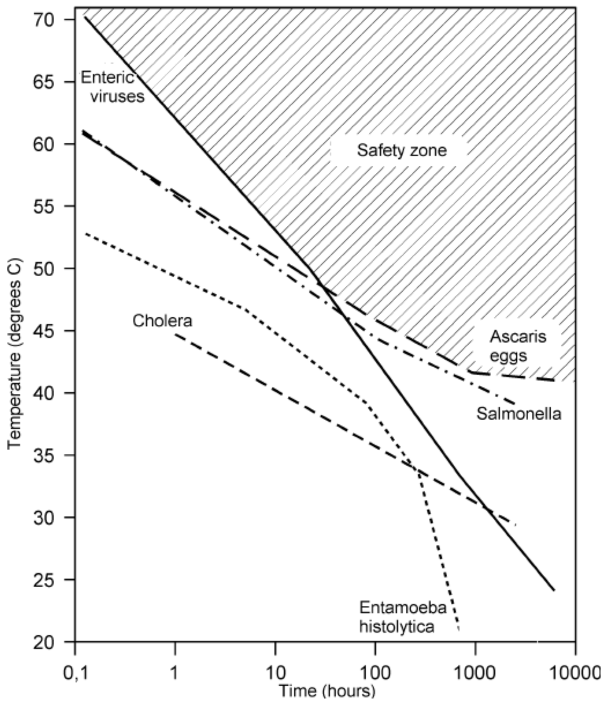


Figure 2-3 The influence of temperature and time on elimination of some common pathogens. The lines represent the necessary combination of time and temperature for total loss of each pathogen's capacity of infection. Thus, the hatched zone represents the combinations of time and temperature that are estimated to be lethal for all pathogens
Source: Lens et al. (2001)

Niwagaba (2009), examined different technologies for the treatment of source-separated human faeces and urine in developing countries under four criteria: simple, cheap, environmentally friendly and resource efficient; the methods for pathogen reduction in human excreta include storage, composting, incineration and chemical treatment.

Storage; the extent to which pathogens decrease in numbers during storage depends on factors such as pH, moisture, temperature, nutrient availability, oxygen availability, ammonia concentration and UV exposure (WHO, 2006). In areas where ambient temperatures reach up to 20 °C, a total storage time of 1.5 to 2 years will eliminate most bacterial pathogens, provided the faecal material is kept dry, and will substantially reduce viruses, protozoa and parasites. In areas with higher ambient temperatures (up to 35°C), a total storage period of one year will achieve the same result, as pathogen die-off is faster at higher temperatures (Schönning and Stenström, 2004).

Composting is the microbiological degradation of organic material to a humus-like stable product under aerobic, moist and self-heating conditions. When a well-conditioned substrate is composted, aerobic degradation of its organics occurs. The process is exothermic, i.e. heat is generated, resulting in increased temperature. The heat produced either remains in the compost mass or escapes by conduction, convection and radiation, or is lost with the outgoing gas. To keep the material undergoing composting hot enough for sanitation, sufficient amounts of the heat generated should remain in the compost matrix. This requires, at least on the small and medium scale, that the compost is well insulated. Niwagaba (2009) suggested that sanitation is attained for composts maintaining > 50°C for 2 weeks during which the compost is turned at least 4 times.

Incineration of faeces offers a treatment method that not only destroys pathogens, but is also a compact and rapid process (Niwagaba, 2009).

Incineration increases the temperature to high levels such that short exposure should be enough to inactivate any pathogens present. Literature on the burning of different types and sources of straw contains reports of 90-100% losses of N, S and C and 24% losses of P, 35% of K and 75% of S (Heard John, 2007). According to Jönsson and Vinnerås (2004), ash from the incineration of faeces contains large proportions of P and K, which, like plant ash, can fertilise the soil for agricultural purposes.

Chemical treatment; chemicals that can be used to treat faeces for pathogen reduction include acids, bases and oxidising agents. Some chemicals for disinfection contain substances of agronomic value, for example: $Ca(OH)_2$, NH_3, KOH and PO_4^{3-}. The use of these disinfecting chemicals is preferable for substrates that are to be recycled as fertilisers, as the nutrient content of the disinfectant increases the fertiliser value of the product (Vinneras et al., 2009).

The treatment by urea functions via enzymatic degradation, which produces uncharged ammonia, and thus ammonia is the disinfecting agent in urea treatment systems. When urea degrades the pH increases and when pH>9 is achieved, the majority of the ammonium/ammonia is uncharged ammonia and thus the disinfection of bacterial cells increases even more as a result of ammonia toxicity (Pecson et al., 2007).

In addition to the four methods of pathogen reduction mentioned above, there are also natural processes to remove pathogenic organisms in wastewater, for example de Lemos Chernicharo (2005) mentioned that maturation ponds and land infiltration are two of the main natural processes which are mainly used to remove pathogenic microorganisms. In natural processes, according to the type of pathogenic microorganism, the factors which contribute to their removal may be grouped in two. The first group of factors, which include bacteria and viruses, are: temperature, solar radiation, pH, food shortage, predator organisms and toxic compounds; and in the second group, that considers protozoan cysts and helminth eggs, the main factor is sedimentation.

2.2.4. Indicators of faecal contamination

Because direct detection of pathogenic microorganisms requires costly and time-consuming procedures, indicators for faecal contamination have been developed, which according to Bitton (2005) should have the following characteristics:

- Be one of the intestinal microflora of warm-blooded animals.
- Be present when pathogens are present, and absent in uncontaminated samples.
- Be present in greater numbers than the pathogen.
- Be at least equally as resistant as the pathogen to environmental conditions and to disinfection in water and wastewater treatment plants.
- Not multiply in the environment.
- Be detectable by means of easy, rapid, and inexpensive methods.
- The indicator organism should be non-pathogenic.

In a review of indicator microorganisms presented by Bitton (2005), there are eight groups of indicators that are shown in Figure 2-4.

Each indicator has its own methodology; some are more time-consuming and labour-intensive and may require more costly laboratory equipment than others. In order to monitor the hygiene quality after anaerobic processes, de Lemos Chernicharo (2007) recommends to measure faecal coliforms (*Escherichia coli*) and *helminth eggs*.

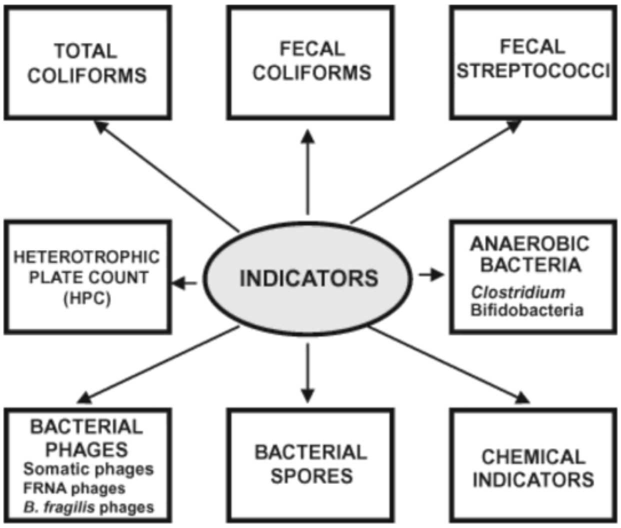


Figure 2-4 Microbial and chemical indicators
Source: Bitton (2005)

In most published research related to wastewater treatment, *Escherichia coli* have been used as an indicator of pathogenic contamination, because of two factors: firstly it can be distinguished promptly from the rest of the faecal group and secondly various strains cause different kinds of human health problems (Kadlec and Wallace, 2009).

2.3. SLUDGE TREATMENT WETLANDS (STW)

A constructed wetland is a complex system of saturated medium, designed and built by man, with submerged and emergent vegetation and aquatic animal life that simulates natural wetlands for human use and benefit (Peña Varón et al., 2011).

According to Vymazal and Kröpfelová (2008), constructed wetlands can be classified using three main criteria: i) the first is hydraulic criteria, which takes into account surface and sub-surface flow systems, ii) the second is the type of planting which includes submerged, emergent and floating plants and iii) the third is the flow path including horizontal and vertical.

Constructed wetlands have been used to treat different types of wastewater including municipal wastewater, industrial wastewater and landfill leachate. In the same way, constructed wetlands have been used also to treat sludge produced in wastewater treatment plants, and these systems are called sludge treatment wetlands (STW).

In wastewater treatment, the sludge generated after different processes such as: anaerobic digesters, conventional activated sludge systems, aerobic digesters, septic tanks, extended aeration systems and Imhoff tanks, have been treated in wetlands with beds constructed in rectangular concrete basins or constituted by soil basins (Uggetti et al., 2010).

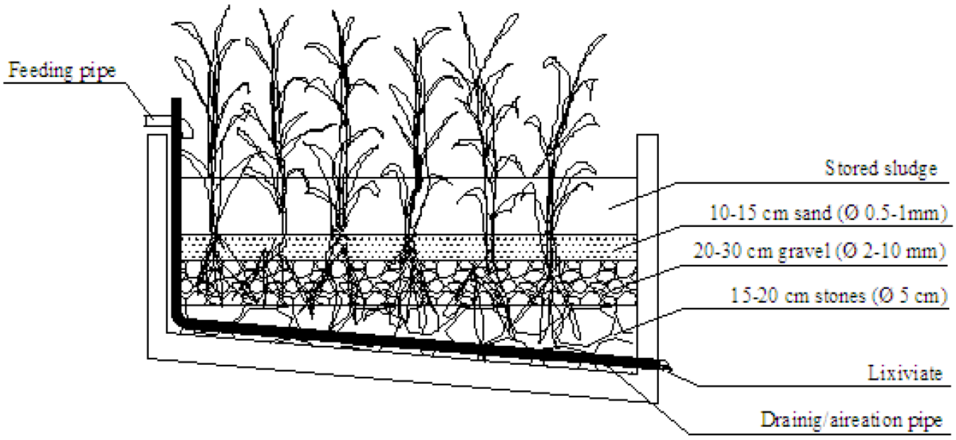


Figure 2-5 Schematic diagram of a sludge treatment wetland
Source: Uggetti et al. (2010)

The process of dewatering in sludge treatment wetlands is undertaken as a batch process with feeding and resting periods that 'may last a few days or weeks, depending on the treatment capacity, weather conditions, age of the system, dry matter content and thickness of the sludge' (Uggetti et al., 2010). The sludge's stabilization is basically achieved by filtration through different layers of granular medium and storage of filtered sludge in the top layer (*see Figure 2-5*). Additionally, the oxygen transfer by the plants to the stored sludge generates aerobic conditions improving sludge mineralization (Nielsen, 2005).

According to Uggetti et al. (2009), the final product is suitable for land application, either directly or after additional composting, although to reach a quality of an unrestricted product for application in agricultural crop field, additional hygienisation must be required.

2.3.1. Configuration and design

In a review of sludge treatment wetlands done by Uggetti et al. (2010), it was established that 'the main design factor is the sludge loading rate', although there are no standard values of design factors and configuration.

There is a variety of research on full-scale sludge treatment wetlands, with different recommendations regarding the sludge loading rate, as can be seen in Table 2.1. However, 60 kg dry matter/m^2*year is recommended in Europe. Additionally, Burgoon et al. (1997) recommends the application of a lower loading rate during the start-up period, to improve plant growth and vegetation development.

Also, parameters such as: resting period, shape and depth of the beds are not standardized.

2.3.2. Granular medium

The granular medium basically has two components: i) the filter composed by numerous layers of granular media and ii) the draining pipe.

The main purpose of the filter is to percolate water. The filter generally has three layers which are: stone, gravel and sand which commonly have heights of 15-20 cm, 20-30 cm and 10-15 cm respectively. Sand and gravel are located at the top of the filter and they help not only to retain solids and therefore preventing clogging processes, but also to provide a rooting medium for plants. In addition, stones are located at the bottom to protect the draining pipes.

Table 2.3 *Number of beds, total surface area, and sludge loading rates for different full-scale sludge treatment wetland studies.*

Number of beds	Surface of each bed (m^2)	Sludge loading rate (kg TS/m^2*year)	Reference
25	1,000	65	Burgoon et al. (1997)
8	500	60	Nielsen (2005)
10	1,050	60	Nielsen (2007)
8	468	22-44	Troesch et al. (2009)
3	66	55	Uggetti et al. (2009)
6	54	51	Uggetti et al. (2009)
7	25	125	Uggetti et al. (2009)

Source: Adapted from Uggetti et al. (2010)

Draining pipes are placed at the filter's base. They are opened to promote air circulation through the pipes and granular medium (Figure 2-6).

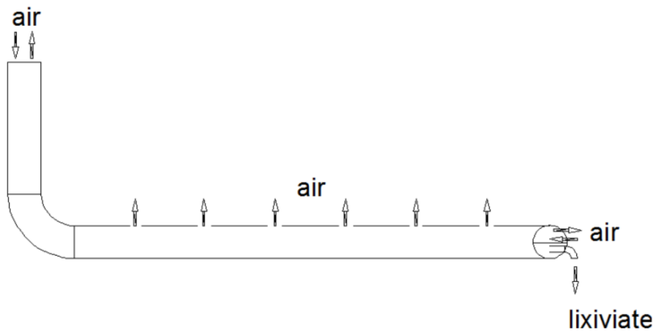


Figure 2-6 Detail of the draining and aeration pipe
Source: Uggetti (2011)

2.3.3. Plants

Plants are a key element of STWs, since they help both: sludge dewatering and its mineralization. Edwards et al. (2001) had investigated the impact of plants comparing planted and unplanted beds. In this study planted beds showed a higher TS concentration (20-21%) than unplanted (18%) and higher sludge height reduction (84-86% in planted beds and 81% in unplanted).

According to De Maeseneer (1997) plant species used in treatment wetlands have to be: able to grow in watery, muddy, anaerobic conditions and able to tolerate oscillations in water level, high salinity and variations between high and low pH. However, it is important to provide appropriate conditions for vegetation growth by applying the right sludge loading rate (SLR) during the start-up phase. Plantation density may vary between 4 rhizomes/m^2 (Edwards et al., 2001) and 15 rhizomes/m^2 (Magri, 2010).

The most widely used species in treatment wetlands for wastewater is the common reed (*Phragmites australis*) (Puigagut et al., 2007). Hardej and Ozimek (2002) evaluated the effect of sewage sludge on growth and morphometric parameters of *Phragmites australis* and demonstrated the high adaptation capacity of the common reed to the sewage sludge environment, observing that the shoot density was over two times greater than that commonly found in natural systems. Cattail (*Typha* sp.) has also been extensively used in wastewater treatment wetlands, in particular due to its high initial growth rate (De Maeseneer, 1997).

2.3.4. Treatment efficiency

Previous research in STW systems, evaluated its efficiency in terms of: i) sludge dewatering, ii) sludge stabilization and iii) microbial faecal indicators.

Sludge dewatering

Since one of the main objectives of the sludge treatment wetlands is dewatering, it has been seen that total solid concentration varies from 0.3 to 4% in influent sludge, and from 15 to 32% in the top layer of wetlands, as it is shown in *Table 2.4*.

Table 2.4 *Total and volatile solids concentration observed in different STWs*

System's location	Source of the sludge	TS (%)		VS (%TS)		Reference
		Influent	Wetlands	Influent	Wetland	
Fort Campbell, USA	Anaerobic digestion	3	32*	-	46*	Kim and Smith (1997)
Pilot plant in Rugeley, Staffordshire, UK	Biological Aerated filter and raw slurry solids	4	20	74	52	Edwards et al. (2001)
Apens, Spain	Activated sludge, extended aeration	0.7 - 1.5	22 - 25*	52 - 67	39 - 42*	Uggetti et al. (2009)
Sant Boi de Llucanes, Spain	Activated sludge, extended aeration	3	20 - 28*	52 - 42	36 - 40*	Uggetti et al. (2009)
Seva, Spain	Activated sludge, contact-stabilization	0.3 - 2	15 - 20*	58 - 59	46 - 50*	Uggetti et al. (2009)

Source: Adapted from Uggetti et al. (2010)
* Average from different depths

2.3.5. Sludge stabilization

Stabilization is generally determined through volatile solids of the sludge. As shown in *Table 2.4*, during sludge treatment, a reduction of volatile solids of 25-30% can be achieved, reaching a final volatile solid concentration between 40% and 50%.

Microbial faecal indicators

According to Obarska-Pempkowiak et al. (2003), applying sludge treatment wetlands for Imhoff tank sludge post-treatment, is possible to reduce *E. Coli* and inactivate *Salmonella* after 8 months. Also, Nielsen (2007) achieved concentrations below: 2 MPN/100g for *Salmonella*, 10 CFU/g for *Enterococci* and 200 MPN/100g for *E. Coli* for anaerobic digested sludge.

Finally, Uggetti et al. (2010) proposed that advanced treated sludge should not contain *Salmonella* in 50g (wet weight) and the treatment should achieve *E. Coli* concentration to less than $5*10^2$ CFU/g (dry-weight basis).

CHAPTER 3

METHODOLOGY

The present study has two main components; the first one is the toilet linked biogas plant (TLBP) effluent characterization, to determine the feasibility of its application as soil conditioner; and the second one is the implementation of pilot-scale sludge treatment wetlands operated at four different loading rates, to evaluate the efficiency for sludge dryness, stabilization, nutrients concentration and pathogen removal.

In order to characterize the anaerobic digested sludge (also called slurry), weekly samples were grabbed during a period of 2 months (from end of December 2013 to February 2014), assessing their physical properties (pH, temperature, electrical conductivity and total suspended solids), organic matter (volatile suspended solids and chemical oxygen demand), nutrients (nitrate, nitrite, ammonia, phosphate and potassium) and pathogen indicators (*Salmonella* and *E. Coli*).

To evaluate sludge treatment wetlands performance, eight pilot-scale experimental units were built where four different loading rates and two plants species were evaluated. After a short period of acclimatization (two weeks), seven campaign samples were conducted, testing two different elements: i) dried sludge and ii) water leachate.

The dried sludge accumulated at the top of each STW, was monitored in order to evaluate its quality for land application. The parameters tested in laboratory during the seven sampling campaigns were: pH, electrical conductivity, total suspended solids, volatile suspended solids, chemical oxygen demand, ammonia NH_3, nitrite NO_2^-, nitrate NO_3^-, phosphate P_2O_5 and *E.Coli*. On the other hand, water leachate (STW's effluent) was tested in laboratory to evaluate its feasibility to re-use for irrigation purposes. Unlike the parameters tested on dried sludge, for water leachate an additional parameter was determined in laboratory: Sodium-adsorption ratio (SAR).

The parameters for slurry's characterization as well as for dried sludge and water leachate analysis, were tested in a private laboratory (POLLUCON PVT.LTD.) located in Surat, approximately 50 km away from the project location.

Figure 3-1 illustrates the general framework of the research activities.

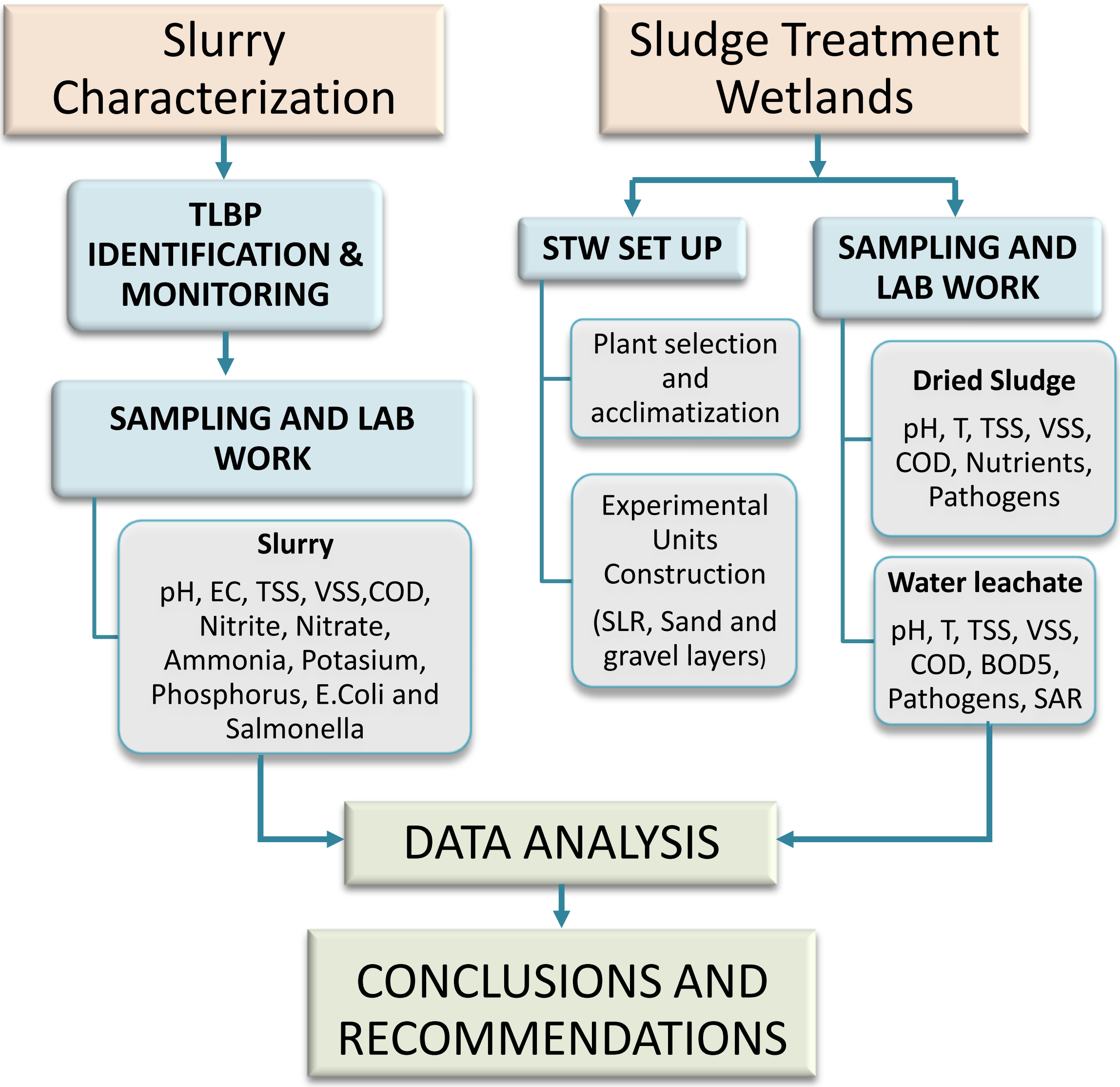


Figure 3-1 Research framework

3.1. EXPERIMENTAL SET-UP

3.1.1. Project location

The experiments took place outdoors at Pathri Village located in Valsad district of Gujarat, India (20°50'27"N, 72°59'56"E). *See Figure 3-2.* The project area air temperature during winter (from November to February) varies between 12 to 28°C, and during summer (from March to May) between 30 to 38°C. The region is also characterized for heavy rains among June and September, due to monsoon winds with an average annual rainfall of 845 mm.

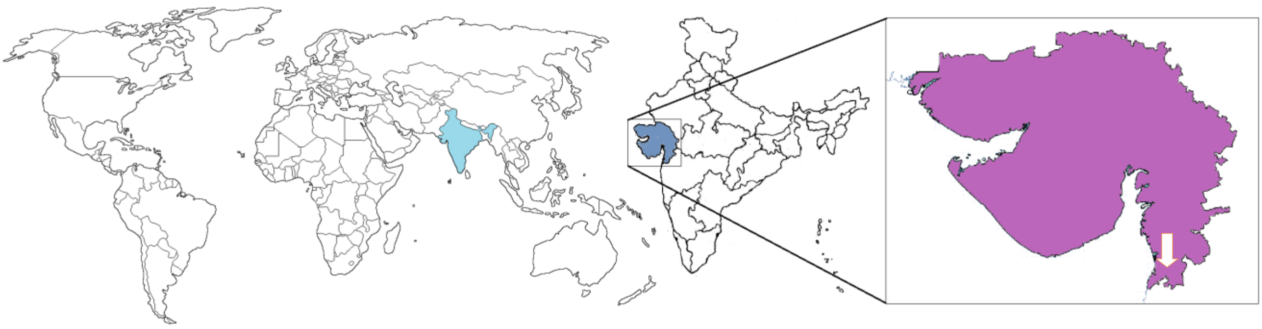


Figure 3-2 Project location

3.1.2. Pilot-scale experimental units

Eight sludge treatment wetland units (S1 to S8) at pilot-scale were constructed and operated during three months. Each unit was a plastic cylindrical tank with 0.70 m height and 0.75 m diameter. All units were constructed and planted in early December 2013, and started operation one month later. Table 3.1, summarizes the units characteristics and Figure 3-3 presents a scheme and a picture of the evaluated experimental units.

All units contained from bottom to top four layers: in the bottom 15-cm thick gravel was used (1.5 inches); in the middle a 10-cm thick of medium gravel (1 inch) and a 10-cm thick of fine gravel (0.5 inches) were employed; and in the top layer 10-cm thick coarse sand was used. Two perforated aeration PVC tubes (50mm in diameter) were installed to enhance the aeration, and at the bottom of each experimental unit a tap connected to a plastic container was fixed to collect the water leachate.

The majority of pilot-scale STWs were planted with common reed (S1 to S7 *Phragmites karka*) and one (S8) with a *Napier Bajra Hybrid grass* (NB-21). *See Figure 3-4*. Additionally, the experimental units were operated under four loading rates, as it is described on *Table 3.1*.

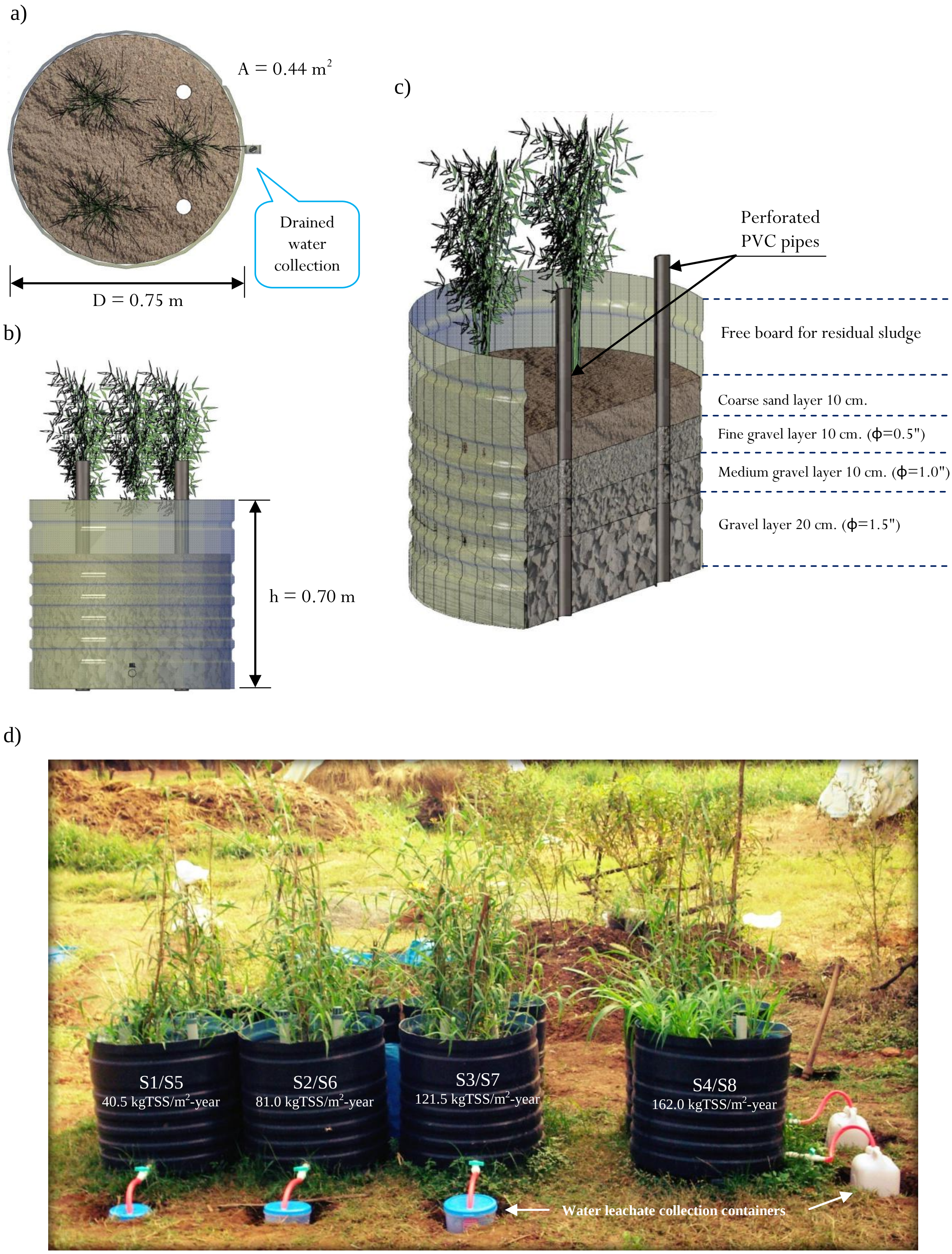


Figure 3-3 Experimental unit description (a) Top view, (b) Front view, (c) 3D section view and (d) Picture of eight pilot-scale sludge treatment wetlands after two months of operation.

Table 3.1 *Treatments and operational conditions*

Unit	Plant species	Nominal SLR (kgTSS/m2-year)	Real SLR (kgTSS/m2-year)
S1	*Phragmites karka*	30	40.5
S2	*Phragmites karka*	60	81.0
S3	*Phragmites karka*	120	121.5
S4	*Phragmites karka*	180	162.0
S5	*Phragmites karka*	30	40.5
S6	*Phragmites karka*	60	81.0
S7	*Phragmites karka*	120	121.5
S8	*Napier Bajra hybrid grass (NB-21)**	180	162.0

* Hybrid between *Pennisetum americanum L*. and *Pennisetum purpureum Schum*.

a) b)

Figure 3-4 Two different plants used in the STWs: a) *Napier Bajra* hybrid grass (NB-21) and b) *Phragmites karka*

Start-up phase.

Since plants are a key element in sludge treatment wetlands, previous research recommended a commissioning phase (acclimatization of the reeds), from 3 months to more than 1 year. Also, during this phase the use of a low loading rate (30 kgTSS/m^2-year) is advised (Troesch et al., 2009, Uggetti, 2011). In the present study, *Phragmites karka* and *Napier Bajra* hybrid grass were selected. These plants were locally available and they were already acclimatized to local weather conditions, although they were not used to grow in a granular media like in constructed wetlands treating slurry.

During a period of two weeks, the plants were kept in plastic bags irrigated with groundwater to promote root growth and establish the microorganism's communities in the rhizosphere (*See Figure 3-5*). The following two weeks they were planted in the experimental units and feed with a low loading rate (approximately 30 kgTSS/m^2-year). Additionally, before to start the operation under different sludge loading rates, the stems were cut at 30 cm above top layer, in order to compare the growth and biomass production between different configurations.

On the other hand, according to recommendations of previous studies the plantation density may vary between 4 and 15 rhizomes/m^2 (Edwards et al., 2001, Magri, 2010). On this study a density of: 6.8 rhizomes/m^2 was applied (3 plants in a STW of 0.44 m^2 of area). See the top view on Figure 3-3.

a)

b)

c)

Figure 3-5 Plants during nursery and acclimatization phase: a) front view, b) top view and c) experimental units

3.2. EXPERIMENTAL PROCEDURE

3.2.1. Anaerobic digested sludge (slurry)

The slurry was collected in the outlet of a household-size toilet linked biogas plant (TLBP). *See Figure 3-6*. The TLBP selected is operating since 1995 and it was built using the Deenbandhu model, which was put forth in 1984 by the Action for Food Production (AFPRO). Deenbandhu plants in India are made entirely of brick masonry work with a spherical shaped gas holder at the top and a concave bottom, it was designed to produce daily two cubic meters of biogas (Singh, 1989). Technical information and a typical design of Deenbandhu plant is shown in *Appendix A*.

The total suspended solid concentration of slurry varies day to day depending of: biogas consumption and digester feeding with dung and water mixture. After a period of two weeks feeding daily 60L (average) of dung and water mixture (30 L of water x 30 kg of cow dung) the slurry's TSS concentration was 14.71%. Since the slurry TSS concentration was significantly high compared with sewage sludge (TSS: 1 to 5%), it was diluted five times mixing with underground water.

Figure 3-6 Slurry at the outlet of toilet linked biogas plant

3.2.2. Feeding and resting period

The feeding was carried out manually using a graduated plastic container, which flooded uniformly the entire bed surface. Diluted slurry was introduced to the units in loading cycles: a feeding period of seven days in daily equal portions, followed by a resting period of one week. During the last four weeks the units S4 and S8 (loading rate equal to: 160 kgTSS/m^2-year), were fed with slurry without dilution.

3.3. SAMPLING CAMPAIGN AND ANALYTICAL METHODS

Seven sampling campaigns were carried out from January to February 2014. Each campaign comprised two different types of samples: i) Sludge (slurry and dried sludge), and ii) water leachate.

3.3.1. Sludge sampling and characterization

Samples of both types of sludge: slurry (influent) and dried sludge accumulated in the top layer of experimental units were analysed to study the system's performance. Additionally, the sludge accumulation depth inside the sludge treatment wetlands was measured.

The dried sludge samples were collected at the end of each resting period. To obtain representative composite samples, the top layer was divided in four sections and dried sludge samples were collected from each section being subsequently mixed. Samples were analysed using conventional methods according the procedures established by the American Public Health Association (APHA, 2012) and Indian standard methods (IS, 2003), on a private laboratory (POLLUCON PVT.LTD.), situated approximately 50 km away from experimental units' location.

Based on previous studies recommendations for sludge characterization and constructed wetlands performance evaluation (Uggetti et al., 2009, Obarska-Pempkowiak et al., 2003) the following parameters were analysed: pH, electrical conductivity (EC), total and volatile suspended Solids (TSS and VSS), chemical oxygen demand (COD), nitrite, nitrate, ammonia, total phosphorous (TP), and faecal bacteria indicators (*Salmonella* spp. and *E. Coli*). In *Table 3.2* it is indicated the specific method used for each parameter.

Table 3.2 *Test Methods used to test parameters for sludge analysis*

Parameter	Unit	Test Method
pH		Portable pH meter Multi 340i
T	°C	Mercury-in-glass thermometer
Total Suspended Solid	%	IS 3025 (P-17)84Re.02
Volatile Suspended Solid	%	APHA 2540 G
COD	%	APHA (22nd Edition) 5220-D (open reflux)
BOD (5 days at 20°C)	mg kg^{-1}	IS 3025 (P-44)
Ammonia as NH_3	%	IS 3025 (P-34)
Nitrate as NO_2	mg kg^{-1}	IS 3025 (P-34) 88 NEDA Method
Nitrite as NO_3	mg kg^{-1}	IS 3025 (P-34) 88 Chromotropic Acid
Potassium	mg kg^{-1}	Flame Photometer
Phosphorus as P_2O_5	%	Flame Photometer
E. Coli	CFU /g	IS 1622:1981 Edi. 2.4 (2003-05)
Salmonella	/g	IS 5887 (P-III)

Note: IS refers to Indian standards methods

The samples for *E. Coli* test were kept in sterilized containers of 100mL, and for other parameters 1L plastic bottles and plastic bags were used for slurry and dried sludge correspondingly. *See Figure 3-7.*

a) b) c)

Figure 3-7 a) 100 mL sterilized container, b) plastic bags for dried sludge sampling and c) 1L plastic bottles for slurry and water leachate samples

3.3.2. Water leachate sampling and characterization

Weekly samples were grabbed at the end of feeding period for each experimental unit. Two types of containers were used to collect the samples: a 100 ml sterilized container for pathogens test and 1 L plastic bottles for the other parameters. *See Figure 3-7.*

The parameters analysed at laboratory were: pH, electrical conductivity, total and volatile Suspended solids, chemical oxygen demand, biochemical oxygen demand (BOD_5 as easily biodegradable organic matter), ammonia, sodium, calcium, magnesium, and faecal bacteria indicators (*E. Coli*). In base of sodium, calcium and magnesium concentrations, the sodium-adsorption ratio was calculated using the equation 3.1.

$$SAR = \frac{Na^{+}}{\sqrt{\frac{1}{2}(Ca^{+2} + Mg^{+2})}} \tag{3.1}$$

Where: Na^{+} = Sodium (mg L^{-1}) Ca^{+2} = Calcium (mg L^{-1})
Mg^{+2} = Magnesium (mg L^{-1})

Additionally, in order to determine the water leachate volume generated during the feeding and resting period, the volume was measured daily with a graduated cylinder. Also, an artificial system to simulate precipitation was installed in S4 and S8 experimental units (SLR: 162 kgTSS/m^2-year), in order to estimate the evapotranspiration through system's water balance.

The system used to simulate precipitation consisted on a perforated plastic pipe installed at top level of STW container (approximately 20 cm above accumulated sludge level), which was connected to a plastic tank. A precipitation of 681 mm/month (equivalent to the highest monthly precipitation on August 2010: 677.8 mm) was graduated and applied during a period of two weeks. *See Figure 3-8*.

a) b)

Figure 3-8 a) Artificial precipitation system b) Perforated pipe

3.3.3. Plants biomass determination

In order to measure the plant biomass produced at different experimental units, fresh and dry weights were measured. After three months of sludge treatment wetland implementation, the plants were harvested cutting the stems 5 centimetres above the soil level, and their fresh weight were determined immediately with a digital scale (Ace 50 kg capacity by 0.001 kg resolution). The dry weight was determined at laboratory, after 24 hours of drying period in an oven at 70°C.

3.3.4. Statistical analysis

To evaluate and compare the achieved efficiencies at four different sludge loading rates on pollutant removal, mean and standard deviations were calculated for each configuration; subsequently, they were correlated through the curve that best fits the data trend. In addition, to identify outliers, data which can deviate markedly from other observations in the samples, Grubbs' test was applied.

Grubbs' test is used to detect a single outlier in a univariate data set that follows an approximately normal distribution. The Grubbs' test statistic is defined as:

$$G = \frac{max|Y_1 - \bar{Y}|}{\delta} \qquad (3.2)$$

Where: $\bar{Y}$ and δ are the sample mean and standard deviation, respectively

CHAPTER 4

RESULTS AND DISCUSSION

4.1. TLBP's EFFLUENT CHARACTERIZATION

In order to characterize the TLBP's effluent (slurry), physical properties, organic matter, nutrients and pathogen indicators were analysed in seven sampling campaigns during the winter season (December 2013 to February 2014). So as to identify outliers, data which can deviate markedly from other observations in the samples, Grubbs' test was applied. The results are presented and discussed below.

4.1.1. Physical properties

Four physical parameters were monitored: temperature (T), pH, electrical conductivity (EC) and total suspended solids (TSS). The mean, standard deviation, minimum, maximum and number of data for each parameter are summarized on *Table 4.1*.

Table 4.1 *Physical properties results*

Parameter	Mean	Standard deviation	Minimum	Maximum	Number of data
pH		-	6.72	7.32	55
T (°C)	23.89	2.05	20	28	55
EC (dS m^{-1})	1.09	0.60	0.538	1.91	4
TSS (%)	11.77	2.92	8.44	14	7

Temperature and pH

Temperature and pH were measured on daily basis at the field, using a portable pH-meter (WTW Multi 340i) and a mercury thermometer. The values of temperature fluctuated between 20 and 28°C (*See Figure 4-1*) and pH between 6.80 and 7.32 (*See Figure 4-2*).

In anaerobic digestion, temperature is one of the most important physical factors that affect microbial growth, since microorganisms are not able to control their internal temperature, the external ambient temperature has a direct impact on the inside cell's temperature (de Lemos Chernicharo, 2007). There are three temperature ranges associated with microbial growth: psychrophilic (4 to 15°C), mesophilic (20 to

40°C) and thermophilic (45 to 70°C, and above) (Batstone et al., 2002). The slurry's temperature data are within the mesophilic range.

Furthermore, considering that the ideal temperature range for mesophilic microbial formation of methane is 30 to 35°C and the slurry's temperature ranged between 20 and 28°C, it may be inferred that the production of methane in the digester is not the optimum. However, the optimum methane production depends not only of temperature, but also of other parameters like: pH, sulphates presence and COD/SO_4^{2-} ratio. In addition, it is probably that temperature inside the reactor would be higher than slurry's; and during the summer season an increase in the temperature is expected.

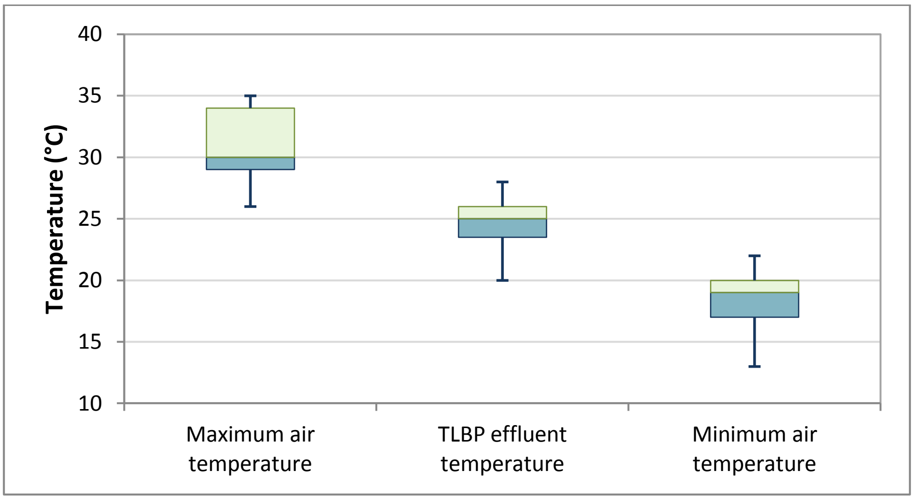


Figure 4-1 TLBP effluent temperature, maximum and minimum air temperatures

The pH is another key parameter to control the operation of anaerobic processes. According to de Lemos Chernicharo (2007) the pH range for an optimum growth of methane-producing microorganisms is between 6.6 and 7.4, as it could be seen in Figure 4-2 the pH measurements are between the recommended range, meaning that the TLBP probably operated under good condition.

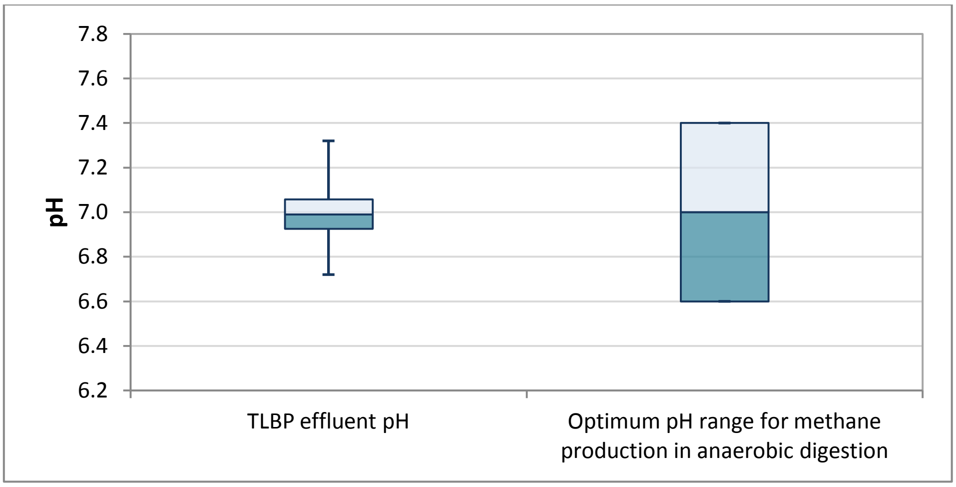


Figure 4-2 TLBP effluent pH and its recommended range for optimum growth of methane-producing microorganisms

Total Suspended Solids and Electrical Conductivity

Slurry's TSS and EC were determined in laboratory using the methods described on *Table 3.2*. The values of TSS (%) and EC (dS m^{-1}) were: 11.77 ± 2.92 and 1.09 ± 0.60 (mean ± standard deviation) respectively.

The slurry's TSS concentrations measured in the present study are lower than typical TSS of anaerobically digested sludge: TSS = 17.5% reported by Fang and Wong (1999) and higher than primary and secondary sludge[2] reported by Wang et al. (2008), who established the following ranges: 3 to 7% and 0.5 to 2% for primary and secondary sludge respectively. *See Figure 4-3.*

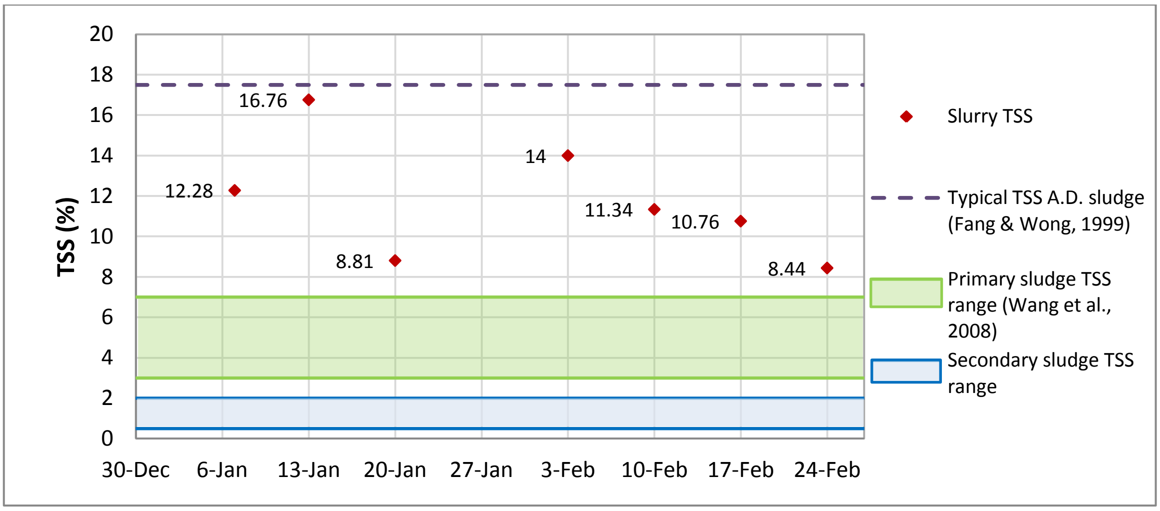


Figure 4-3 TLBP effluent's TSS, primary and secondary TSS ranges and typical anaerobically digested sludge TSS

Electrical conductivity (EC) is very important from an agricultural point of view since it can be a limiting factor of plant growth and seed germination. Santamaría-Romero and Ferrera (2001) indicated that electrical conductivity higher than 8 dS m^{-1} had a negative effect on soil microbial populations and in organic matter biotransformation. Additionally, Banegas et al. (2007) indicates that EC of composted anaerobic sludge ranges between 2.02 to 3.2 dS m^{-1}. In the present study the slurry's mean electrical conductivity is lower than composted anaerobic sludge and lower than the limit indicated by Santamaría-Romero and Ferrera (2001), consequently the EC is not a limiting factor for slurry application in agriculture.

In summary, the slurry's physical properties let us to infer that the household-size anaerobic digester is working under good operational conditions and it could be used in agriculture.

4.1.2. Organic matter

With the purpose to characterize the organic matter content two parameters were monitored: volatile suspended solids (VSS) and chemical oxygen demand (COD). The results for VSS and COD (both of them in g L^{-1}) are: 80.89 ± 20.29 and 93.08 ± 17.67 (mean ± standard deviation) respectively.

[2] Primary and secondary sludge are defined in a conventional WWTP as the product of the primary and secondary settling tank (Wang et al., 2008)

Volatile Suspended Solids

The VSS concentrations determined in seven sampling campaigns are very close to the typical VSS concentration for anaerobically digested sewage sludge reported by Fang and Wong (1999), VSS equal to 87.5 g L^{-1}. *See Figure 4-4.* However, the percentage of VSS in relation to TSS in the slurry (68.67%TSS) is significantly high compared with anaerobically digested sludge (50%TSS) or biosolids from sludge treatment wetland (45%TSS) (Uggetti et al., 2012). Due to the high organic matter concentration in the slurry, a post-treatment is required in order to stabilize it and make a final product suitable for re-use in agricultural crop fields.

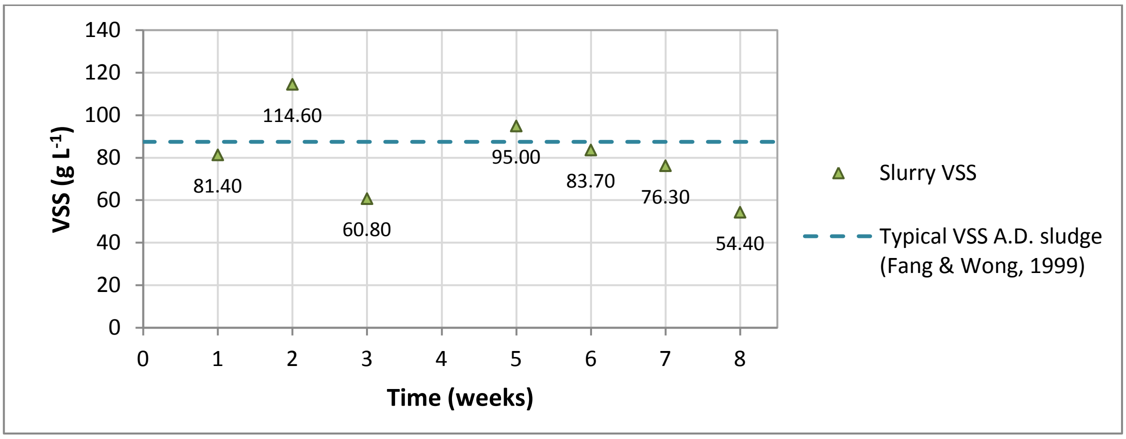


Figure 4-4 TLBP effluent's VSS and typical anaerobically digested sludge's VSS

Chemical Oxygen Demand

Regarding to COD data, a single outlier was identified and excluded from the analysis. *See Figure 4-5.*

The mean COD concentration of the slurry is very high (93.08 g L^{-1}). Henze et al. (2008) established two COD concentration limits for septic sludge, which are: 6.0 and 90.0 g L^{-1} for low and high respectively. Taking into account this range, the organic matter content in the slurry is slightly superior to the high COD concentration. Once again, COD like VSS are showing a high organic matter concentration in the slurry; indicating that a post-treatment is required for its stabilization.

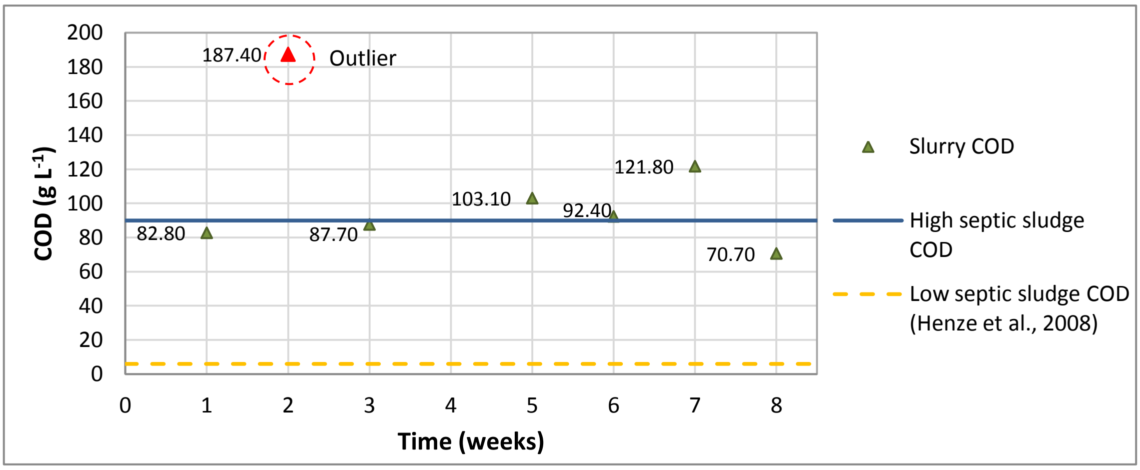


Figure 4-5 TLBP effluent's COD, high and low septic sludge COD concentrations

4.1.3. Nutrients

To characterize the slurry's nutrients content, bioavailable forms of nitrogen, (nitrate [NO_3^-], nitrite [NO_2^-], and ammonia [NH_4]), soluble reactive phosphate (fraction of TP that is available to organisms to growth) also called phosphate (PO_4) or orthophosphate (ortho-P), and potassium (K) were monitored.

Nitrogen

Under anaerobic conditions mainly ammonia is formed as a reduction product (due to microbially mediated biochemical breakdown of protein and non-protein nitrogenous compounds), being it the main source of nitrogen used by microorganisms (Hobson and Wheatley, 1994). Nitrate and nitrite are expected not to be available, since nitrogen is reduced to nitrogen gas and released to the atmosphere.

The mean values (in mg kg^{-1}) for nitrate, nitrite and ammonia are: 128.3, 0.47, and 968.33 respectively. In the case of ammonia, a single outlier was identified and excluded from the analysis. *See Figure 4-6.* As it was expected, very low concentrations of nitrate and nitrite, and high ammonia concentration were found.

With respect to ammonia and nitrate concentration, Sheng et al. (2013) reported that concentrations of total ammonia nitrogen (TAN) less than 1,540 mg L^{-1} does not have adverse effect on methane yield, whilst TAN concentrations higher than 3,780 mg L^{-1} cause serious inhibition of methanogenesis. Additionally, Sheng et al (2013) identified that nitrate concentrations from 100 to 750 mg L^{-1} enhanced the methane production concluding that concentrations of: 1,540 mg L^{-1} and 750 mg L^{-1} for ammonia and nitrate respectively, improve the methane production. In the present study, the concentrations of both: ammonia and nitrate are less than the recommended limits, which let us to infer that the operation of the TLBP in terms of nitrogen has good operational conditions.

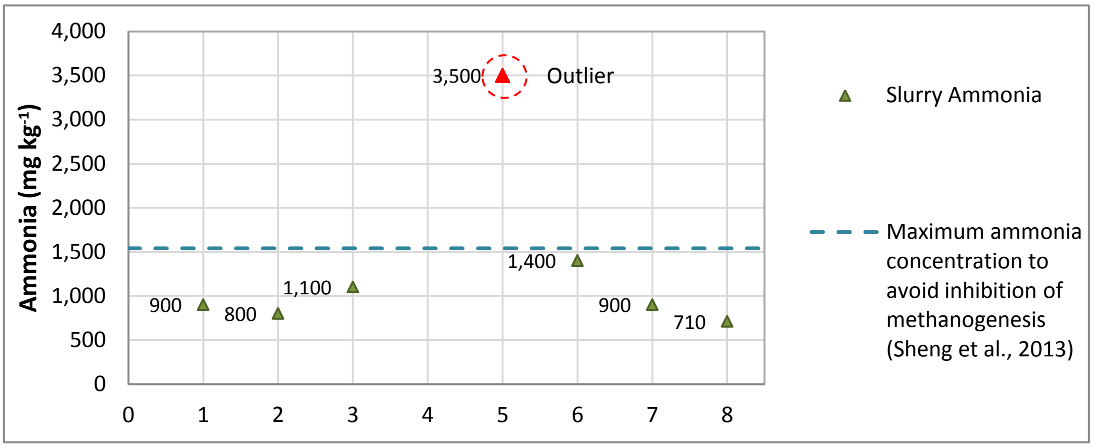


Figure 4-6 TLBP effluent ammonia and maximum ammonia concentration to avoid inhibition of methanogenesis

On the other hand, in anaerobic digestion of sewage sludge under mesophilic conditions, a direct relation between ammonia concentration and hydraulic retention time (HRT) was reported by Hindin and Dunstan (1960), who established that the concentration of ammonia increased as the HRT increased, from 500 mg L^{-1} to 1,200 mg L^{-1} at HRT of 5 and 90 days, respectively. According to the design of the monitored TLBP (*Deenbandhu* anaerobic digester), it has a HRT of approximately 40 days. However, based on the slurry ammonia concentration and the relation established by Hindin and Dunstan (1960), the HRT of the system might be more than 60 days. Consequently, it can be inferred that the system is not working under its maximum capacity increasing the HRT, which represent an advantage for pathogen removal due to high retention time.

Phosphorus and Potassium

The mean ± standard deviation values of phosphate and potassium are: 1,203.33 ± 766.88 mg kg^{-1} and 1,005.00 ± 107.39 mg kg^{-1} respectively. In anaerobic digestion processes, Kugelman and McCarty (1965) identified that concentrations of potassium less than 400 mg L^{-1} cause an enhancement in performance in both, the thermophilic and mesophilic ranges; while concentrations above 2,500 mg L^{-1} cause inhibition for methanogenesis bacteria. In the present study potassium concentration is high but it is less than the concentration which causes inhibition.

On the other hand, both the slurry's phosphate and potassium concentrations are very high compared with other materials such as: TLBP's sludge in Ethiopia, septic sludge and anaerobically digested sludge (*See Table 4.2*); it might be due to the high cow dung phosphorus and potassium concentrations. According to the Penn State Agronomy Guide, cow manure has concentrations of: phosphate equal to 1,500 mg kg^{-1} and potassium equal to 3,500 mg kg^{-1} (AGRICULTURE, 2013).

4.1.4. Pathogen indicators

Helminth eggs, *Salmonella* and *E. Coli* concentrations were determined in laboratory. The first two were absent in slurry and *E. Coli* concentration was: $2.62*10^5 \pm 7.94*10^4$ CFU/g dry-weight basis (mean ± standard deviation). *See Figure 4-7*.

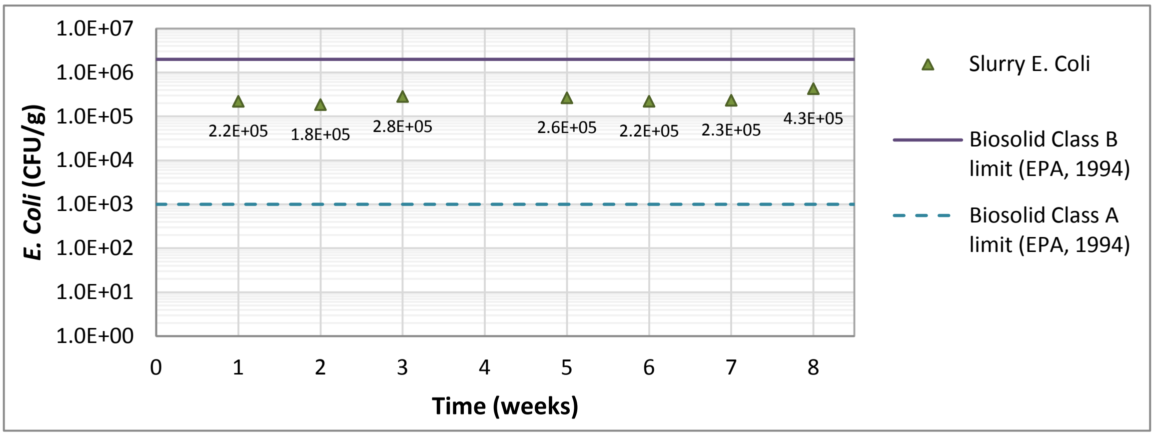


Figure 4-7 TLBP effluent *E. Coli* CFU/g (dry-weight basis) & biosolid class A and B limits (EPA, 1994)

Michael (2008), who characterized a school TLBP, reported a digested sludge's *E. Coli* concentration of 3E+06, and according to Henze et al. (2008), low and high pathogen concentration for septic sludge are: 1E+06 and 1E+08 CFU/g respectively. The slurry's *E. Coli* concentrations are considerably lower than concentrations reported before; as it was analyzed previously, it might be due to the high HRT.

According to the regulation for biosolids re-use in agriculture established by EPA (1994), the limits for class A and B are: 1E+03 CFU/g and 2E+06 CFU/g (dry-weight basis) respectively. The slurry *E. Coli* concentration might be classified as Class B, however, if it is required to achieve higher quality (Class A) a post-treatment is required.

Table 4.2 summarizes the main characteristics of influent and compares it with properties of different types of sludge.

Table 4.2 *Physico-chemical properties of different sludge*

Parameter	TLBP Slurry			TLBP Ethiopia Kokebe Tsebah School			Septic Sludge		Anaerobically digested sludge
							High	Low	
pH	7.00	±	0.11	8.07	±	0.24	8.50	7.00	7.45
T (°C)	23.89	±	2.05	-		-	-	-	-
EC (dS m^{-1})	1.09	±	0.60	-	±	-	-	-	1.86
TSS (%)	11.77	±	2.92	-	±	-	10.00	0.70	17.5
VSS (%)	8.09	±	2.03	-	±	-	6.00	0.40	8.75
COD (%)	9.31	±	1.77	0.12	±	0.05	9.00	0.60	-
Nitrate (mg kg^{-1})	128.30	±	110.19	38.70	±	2.33	-	-	-
Nitrite (mg kg^{-1})	0.47	±	0.38	-	±	-	-	-	-
Ammonia (mg kg^{-1})	968.33	±	248.22	-	±	-	-	-	-
Potassium (mg kg^{-1})	1,005.00	±	107.39	498.30	±	4.41	-	-	-
Phosphorus as P_2O_5 (mg kg^{-1})	1,203.33	±	768.33	82.00	±	3.78	300	40	113.33
E. Coli (CFU/g)	2.94.E+4	±	5.29E+3	3E+06	±	1E+06	1E+08	1E+06	-
Salmonella	Absent			-			-	-	-
Reference	This study			Michael (2008)			Henze et al. (2008)		Fang and Wong (1999)

4.2. BIOSOLIDS FROM SLUDGE TREATMENT WETLANDS

On previous studies in constructed wetlands, an acclimatization phase (also called commissioning phase) from 3 months to more than 1 year was considered before to start the testing period (Edwards et al., 2001, Magri, 2010, Uggetti, 2011, Troesch et al., 2009). The results below correspond to this phase, since the experimental units was operated under real sludge loading rates (See *Table 4.3*) after two weeks of field-harvested *Phragmites karka* transplantation.

4.2.1. Sludge loading rate SLR

As it was mentioned in the point 3.2.2 the experimental units were fed daily at different sludge loading rates. Since the loading rates are based on slurry's total solids concentrations and it changed every day, a daily TSS tests were required in order to calculate the volume to feed on each unit.

Due to restrictions on access to the lab, daily TSS tests were not possible to perform. However, in order to estimate the slurry TSS concentrations, daily settled sludge volumes (SSV) were determined based on the sludge volume index method. Analysing the slurry's TSS concentrations, measured on TLBP's effluent characterization, and their respective settled sludge volumes, a linear correlation was calculated and used as calibration curve to estimate the loading rates applied on each experimental unit (*See Table 4.3*). The correlation between TSS (%) and settled sludge volume (mL/L) is shown in Figure 4-8.

Table 4.3 *Nominal and estimated SLR (kgTSS/m^2-year)*

Unit	SLR (kgTSS/m^2-year)	
	Nominal	Estimated
S1/S5	30	40.49
S2/S6	60	80.99
S3/S7	120	121.48
S4/S8	180	161.97

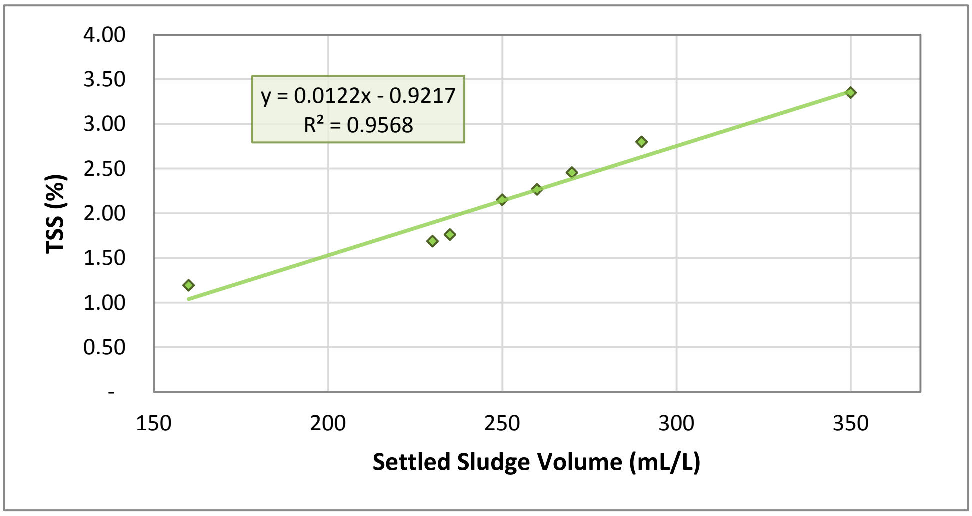


Figure 4-8 Correlation TLBP effluent TSS (%) vs. Settled Sludge Volume (mL/L)

4.2.2. Sludge dryness

As it was explained in the point 3.2.1, the slurry collected at the outlet of TLBP was diluted five times and it was spread manually on the STW's, the water content of diluted slurry (STW influent) ranged between 96.65% and 98.31%. The slurry moisture was significantly reduced to 19.5 - 49% at different STW's configurations. *See Figure 4-9.*

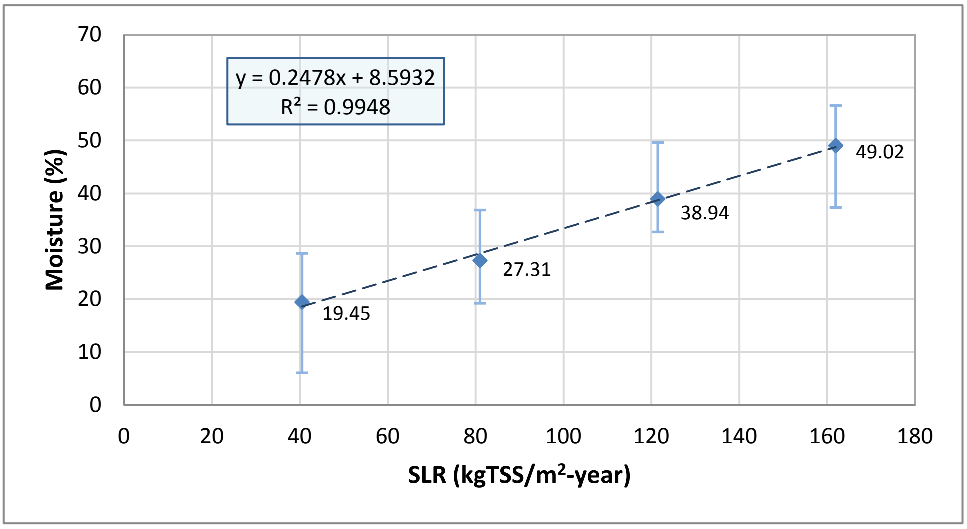


Figure 4-9 STW dried sludge moisture content under different loading rates

As it is shown in Figure 4-9, there is a linear correlation of dried sludge moisture content at different SLRs. This interrelation represents the system's dewatering capacity under different operating conditions, which might be function of parameters like: sludge accumulation at the top layer, plants height and evapotranspiration (discussed in the point 4.3)

The dryness of the final product (TSS about 51-80.5%) is higher than the observed in other research studies. For instance, Melidis et al. (2010), who studied the STW's dewatering capacity in the north of Greece, reported a TSS of 50% (SLR equal to 100 kgTSS/m^2-year) and Uggetti et al. (2012) reported a TSS around 18-25% in Spain (SLR equal to 125 kgTSS/m^2-year).

The difference on dewatering efficiency between this and previous studies might be due to the weather conditions. Stefanakis and Tsihrintzis (2011) found that the main water loss mechanism in STWs is the evapotranspiration which is affected by meteorological parameters such as temperature, precipitation, depth and solar radiation. In this sense, since the present study was conducted during winter season, higher dewatering efficiency is expected during summer.

From dried sludge re-use in agriculture point of view, the moisture content in dried sludge determines its bulk and therefore may affect transportation cost. Moisture content can also affect product handling, if it is too dry can be dusty and irritating to work with, whereas if it is excessively wet can be heavy and difficult to apply uniformly. Darlington (2005) recommended a moisture content between 35% and 60% to apply compost as soil amendment. In this sense, experimental units operated under 121.5 and 162.0 kgTSS/m^2-year (S3/S7 and S4/S8) achieved moisture contents between the recommended range (38.9 and 49%). *See Figure 4-9.*

4.2.3. Organic matter and stability

The dried sludge (accumulated at the top of experimental units) organic matter content was measured in terms of volatile suspended solids as percentage of total suspended solids VSS (%TSS) in seven sampling campaigns. The results for the first set of samples were identified as outliers and excluded for the analysis.

The influent mean ± standard deviation is: 68.77 ± 1.32 %TSS and it fluctuated between 68.07 and 70.52 %TSS. In all STWs there was a reduction of VSS (*Figure 4-10*).

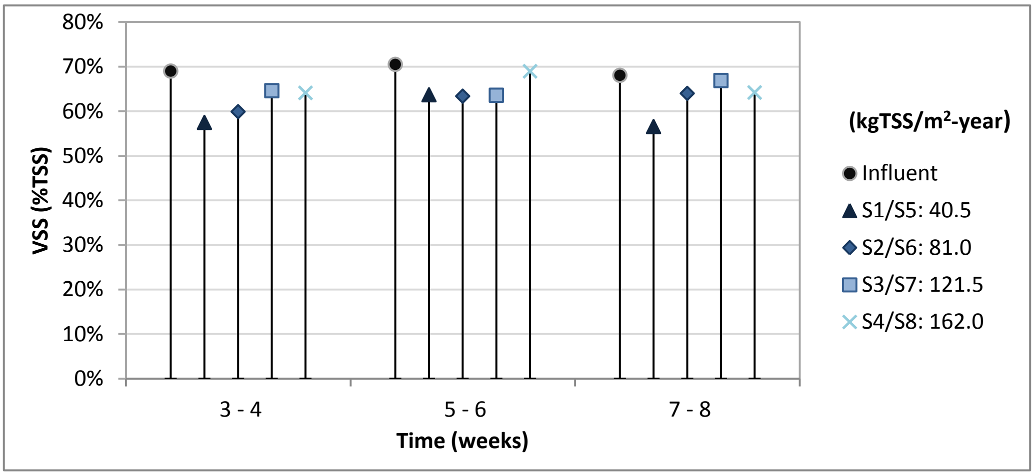


Figure 4-10 VSS concentration at the influent and its reduction at different loading rates

Considering the mean VSS (%TSS) reduction at different experimental unit's configurations, there is a reduction of: 9.51%, 6.33%, 3.74% and 3.01% for STWs working at: 40.5, 91, 121.5 and 162 (kgTSS/m^2-year) respectively. A second order correlation (that fits best the data set) describes the stabilization capacity at different SLRs, where higher VSS reduction is achieved at lower loading rate and vice versa, indicating a potential effect of sludge loading rate on wetlands performance. *See Figure 4-11*.

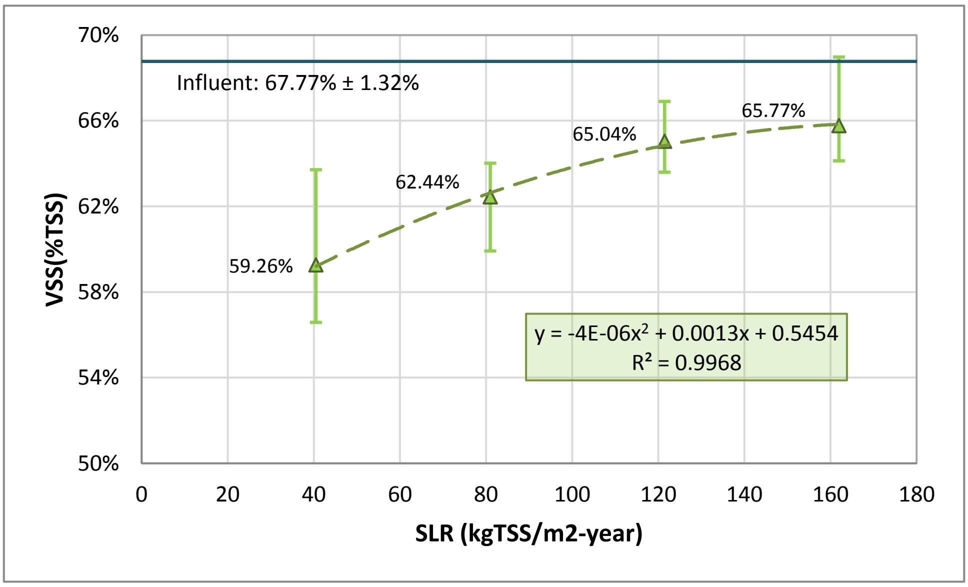


Figure 4-11 Sludge VSS (%TSS) reduction at different STWs

Plants have an important role in STW, since them contribute for sludge mineralization through oxygen transport from the atmosphere to rhizosphere. The plant's rhizosphere creates aerobic microsites in the dried sludge layer, generating the conditions for aerobic degradation processes and other oxygen-dependent reactions such us nitrification, which potentially will take place (Vymazal, 2005).

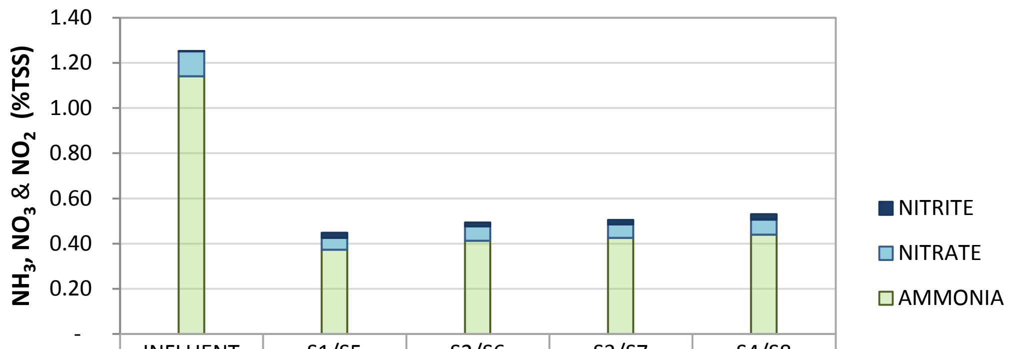
In a STW (which treats sewage sludge in Spain) working at 40 kgTSS/m^2-year with a resting period of 3 months, two different VSS reduction rates were reported, 10 and 2 %TSS, for summer and winter season respectively (Uggetti et al., 2012), concluding that the organic matter mineralization is function of temperature, since high temperature enhances microorganisms' activity. In the present study, the VSS reduction achieved at similar SLR (40.5 kgTSS/m^2-year), was 9.51% (from 68.77 to 59.26 %TSS). This reduction is very similar to the one achieved in Spain, despite the resting period was only one week, but the climate conditions are different.

On the other hand, the compost organic matter content is commonly high; for instance, Ruggieri et al. (2008) reported 71% VS/TS for compost of sewage sludge and Bertran et al. (2004) 62% VS/TS for compost of sewage sludge mixed with vegetable wastes. The higher organic matter content in compost is due to humic-like substances produced during the composting process.

4.2.4. Nutrients

Nitrogen

Due to crops highest response to nitrogen, it represents the highest economic value in biosolids. Nitrogen is present in both forms: inorganic that represents between 10 and 30% of total nitrogen (nitrite, nitrate, ammonia) and organic (proteins, amino acids, amino sugars, starches, associated with polymers and others) (Andreoli et al., 2007). In the present study, inorganic forms of nitrogen, which are readily available to plants, were monitored. The concentrations in the influent and STWs are shown in Figure 4-12.



	INFLUENT	S1/S5	S2/S6	S3/S7	S4/S8
NITRITE	0.0005	0.0222	0.0178	0.0200	0.0245
NITRATE	0.11	0.05	0.06	0.06	0.07
AMMONIA	1.14	0.37	0.41	0.43	0.44

Figure 4-12 Influent and sludge treatment wetlands inorganic nitrogen composition

As it is shown in Figure 4-12, the ammonia concentration is reduced from 1.14 NH_3%TSS (influent ammonia concentration) to 0.37, 0.41, 0.43 and 0.44 NH_3%TSS at S1/S5, S2/S6, S3/S7 and S4/S8 respectively. Nitrate is also reduced from 0.11 NO_3%TSS (influent nitrate concentration) to 0.05, 0.06, 0.06 and 0.07 NO_3%TSS in the four STWs. However, nitrite concentration increases in the experimental units.

The variation of ammonia, nitrite and nitrate concentration might be due to three main factors: i) sludge mineralization, ii) nitrification process and iii) plant uptake.

Phosphorus

The main sources of phosphorus in sewage sludge are microorganism cells and phosphate-containing detergents and soaps (Andreoli et al., 2007). On this study, phosphate from detergents and soaps might be in very few concentrations, since the influent of TLBP does not include grey water.

As in the case of nitrogen, the TP available fraction for organisms to growth (P_2O_5) was measured. The influent phosphate concentration (1.89 ± 1.29 P_2O_5%TSS) was reduced in two STW configurations and increased on the other two.

In the STWs that were operated under 40.5 and 81 kgTSS/m^2-year (S1/S5 and S2/S6) there was a slightly reduction to 1.7 and 1.84 P_2O_5 (%TSS), respectively. However, in the STWs which were operated under 121.5 and 162 kgTSS/m^2-year (S3/S7 and S4/S8) there was a significant increase of phosphate concentration of: 4.85 and 3.16 P_2O_5 (%TSS) respectively. (*See Figure 4-13*)

The phosphate concentration variation at different STWs indicates that the plants' phosphorus requirements are covered with the influent phosphorus concentration. Nonetheless, when the system operates at high SLR (higher than 81 kgTSS/m^2-year), the system stores additional concentration of phosphorus in the biosolid accumulated at the top layer. Sakadevan and Bavor (1998) suggested that the major P-removal mechanisms in constructed wetlands is via substratum, in the present study it makes sense since the sludge accumulated at the top becomes part of the bed, and at high depth the phosphorus concentration is also high. On the other hand, probably adsorption and desorption processes take place on the different gravel layers, however the gravel' adsorption and desorption are very low (Li et al. (2013a) reported gravel's adsorption and desorption of 0.032 and 0.002 %TP respectively).

In addition, the results for the last two STWs (S3/S7 and S4/S8) show a better phosphate concentration compared with composted sludge reported by Bertran et al. (2004), who found a phosphate concentration of 2.33 P_2O_5(%TSS).

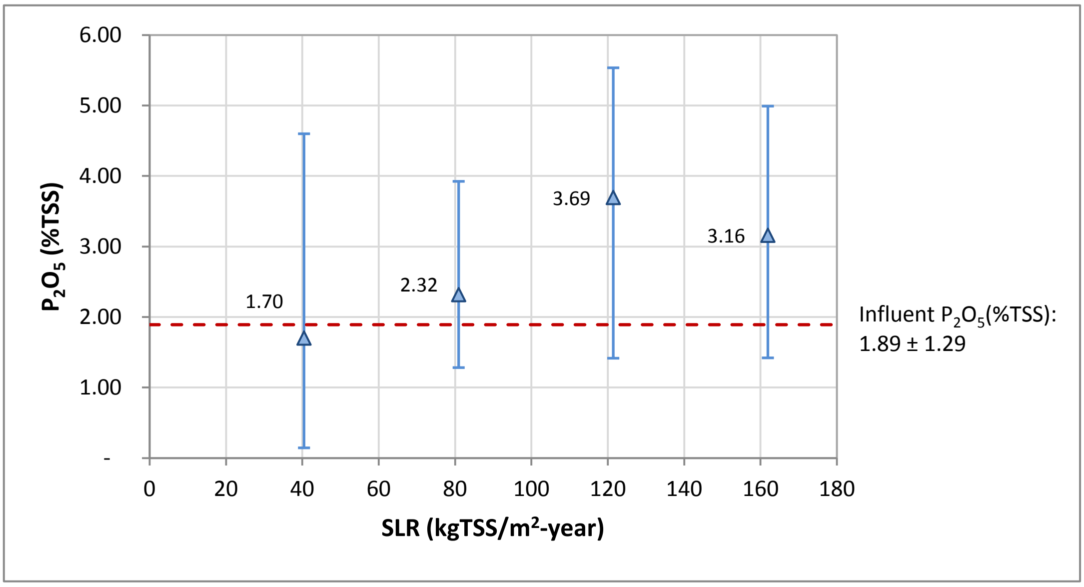


Figure 4-13 Influent and STWs phosphate concentrations

Potassium

The Potassium concentration in the influent (0.83 ± 0.24 %K/TSS) was reduced significantly to: 0.26, 0.27, 0.30 and 0.39 %K/TSS at different SLRs: 40.5, 81, 121.5 and 162 kgTSS/m^2-year respectively. (*See Figure 4-14*)

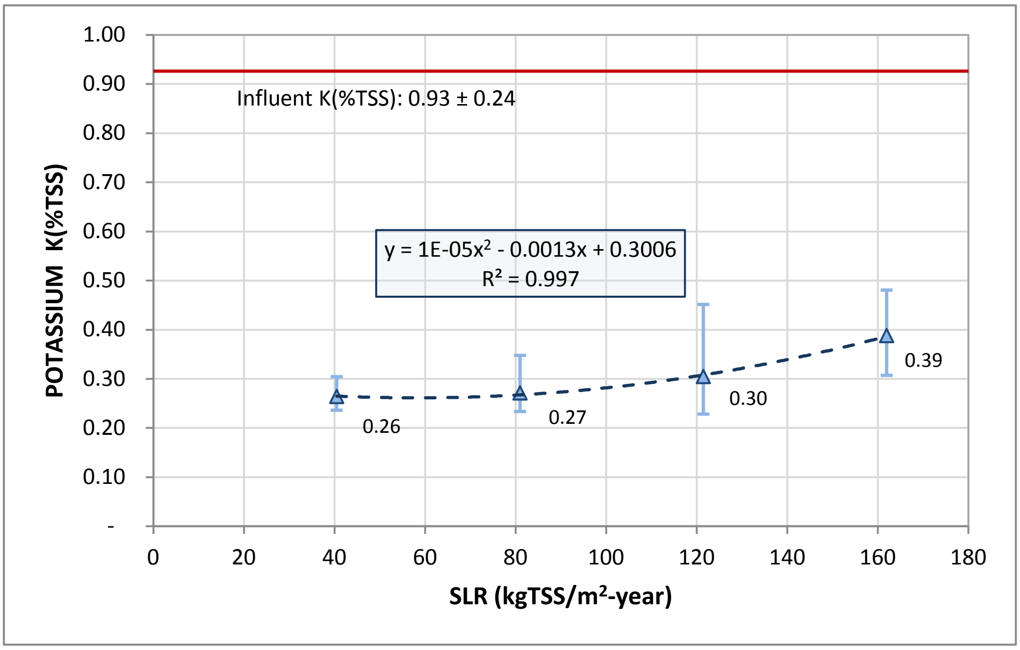


Figure 4-14 Influent and STWs potassium concentrations

As it can be seen in Figure 4-14, a second-order correlation is well adjusted between potassium concentration and SLR, since the potassium concentration increases at high sludge loading rates.

In comparison with a STW operated under 125 kgTSS/m^2-year, where a reduction of potassium concentration of 66.6% was found (from 0.27 to 0.18 %K/TSS) (Uggetti et al., 2012), in the present study the percentage of reduction at different STWs were lower (28.48, 29.27, 32.87 and 41.87% at 40.5, 81, 121.5 and 162 kgTSS/m^2-year respectively). The difference might be due to variation on potassium concentration in the influent.

On the other hand, Bertran et al. (2004) found a potassium concentration of 0.65 %K/TSS in composted sewage sludge, which is higher than the concentrations found in the present study. However, the required agricultural doses are frequently dependent on the fertilizer and soil characteristics (Pomares and Canet, 2001; Andreoli et al., 2007).

4.2.5. Faecal bacteria indicators

In order to determine the pathogen concentration in the dried sludge, two faecal bacteria indicators were analysed: *Salmonella and E. Coli*; *Salmonella* was not detected, while *E. Coli* was present in small quantities from 2.06E+03 to 5.68+03 CFU/gTSS at different sludge loading rates. *See Figure 4-15*.

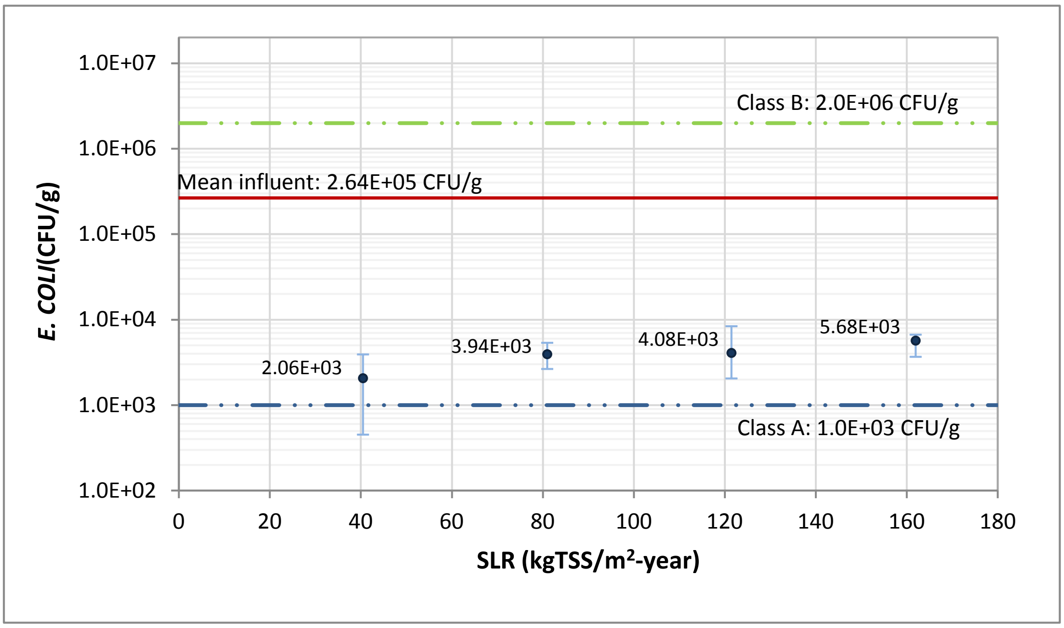


Figure 4-15 *E. Coli* concentration at different STWs and EPA limits for class A and B

The U.S. Environmental Protection Agency (EPA) developed a regulation[3] for use or disposal of sewage sludge biosolids, which in terms of pathogen requirements establishes two limits: less than two million and less than one thousand colony-forming units (CFU) per gram of biosolids (dry-weight basis) for class B and A, respectively. The biosolids that are under the limit for class A, does not have restrictions for use, and it could be used whether in bulk or sold, or given away in bags or other containers; and biosolids under class B limit, could be only applied in bulk, however, tracking of pollutant loadings to the land is not required (EPA, 1994). In the present study, the mean reductions achieved in the four STWs are higher than class A and lower than class B limits. *See Figure 4-15.*

Notwithstanding the mean pathogen's concentration exceed the limits for class A in the four STWs, the pathogen reduction capacity of each system increases along the time. As it can be seen in Figure 4-16, the biosolids of STWs which were operated under 40.5, 81.0 and 121.5 kgTSS/m^2-year, have a clear reduction of pathogen concentration during the first two months of system's implementation; the tendency for the STW which was operated at higher SLR (162.0 kgTSS/m^2-year) is not the same.

In the case of S1/S5 (operated at 40.5 kgTSS/m^2-year), after one month of its implementation, the pathogen concentration is less than 1,000 CFU/g (dry-weight basis), consequently it can be classified as Class A. The same tendency it is observed on the other STWs, however a system monitoring and its evaluation for a longer period are required.

[3] *The Standards for the Use or Disposal of Sewage Sludge* (Title 40 of the Code of Federal Regulations [CFR], Part 503) 2007

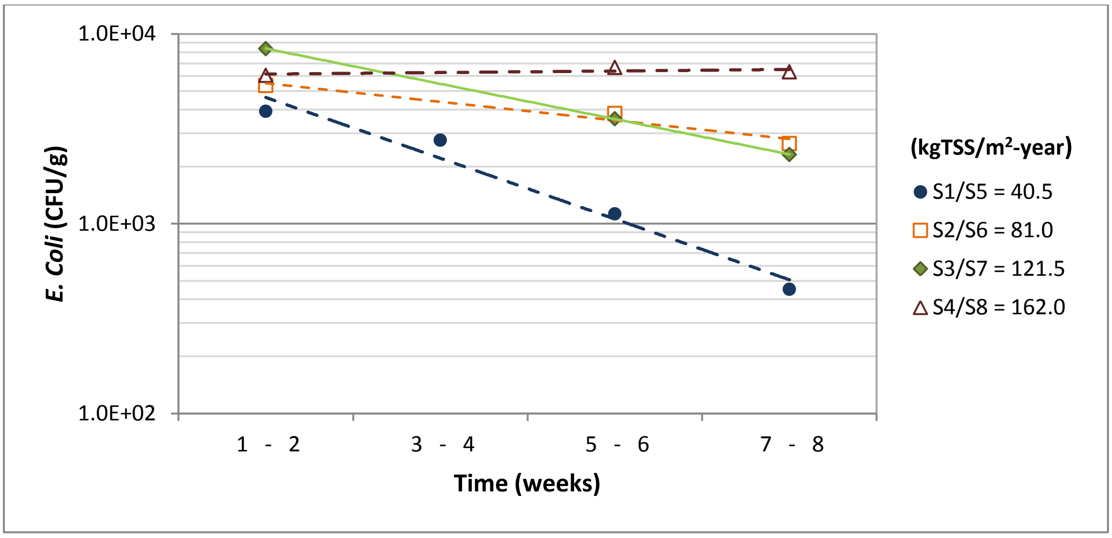


Figure 4-16 Pathogen reduction during the first two months of system implementation

4.2.6. Sludge accumulation

The sludge accumulation at the top layer, was measured by a ruler fixed on the wall of each STW, the total accumulation in the period of two months are shown on *Table 4.4*.

Table 4.4 *Sludge layer height*

STW kgTSS/m2-year	Sludge height (cm)				
	Week 0	Week 1 - 2	Week 3 -4	Week 5 - 6	Week 7 - 8
S1/S5 = 40.5	0	0.4	0.85	1.5	1.95
S2/S6 = 81.0	0	0.75	1.5	2.25	3.1
S3/S7 = 121.5	0	1.25	2.35	3.1	3.95
S4/S8 = 162.0	0	1.55	2.7	3.6	4.85

After two months of operation, the biosolids' accumulation in the top layer of different STWs were: 1.55, 2.70, 3.60 and 4.85 cm at 40.5, 81.0, 121.5 and 162 kgTSS/m^2-year, respectively.

Furthermore, it seems that the sludge accumulation rates in the four STWs (10.10, 17.60, 23.46 and 31.61 cm/year), are directly related with the SLRs, which can be related through a linear correlation. *See Figure 4-17*.

The sludge accumulation rates are in accordance with other studies conducted with sludge treatment wetlands. For instance, Nielsen (2007) reported a sludge layer height increase of approximately 10 cm/year, in a constructed wetland operated under 60 kgTSS/m2-year; and Uggetti et al. (2009) reported an accumulation of 25 to 30 cm/year in a constructed wetland operated at 125 kgTSS/m2-year. Additionally, Uggetti et al. (2009) observed that sludge height accumulation is opposite to the temperature and solar radiation trend, which influence the evapotranspiration rate, consequently a decrease in the accumulation depth is expected during summer season.

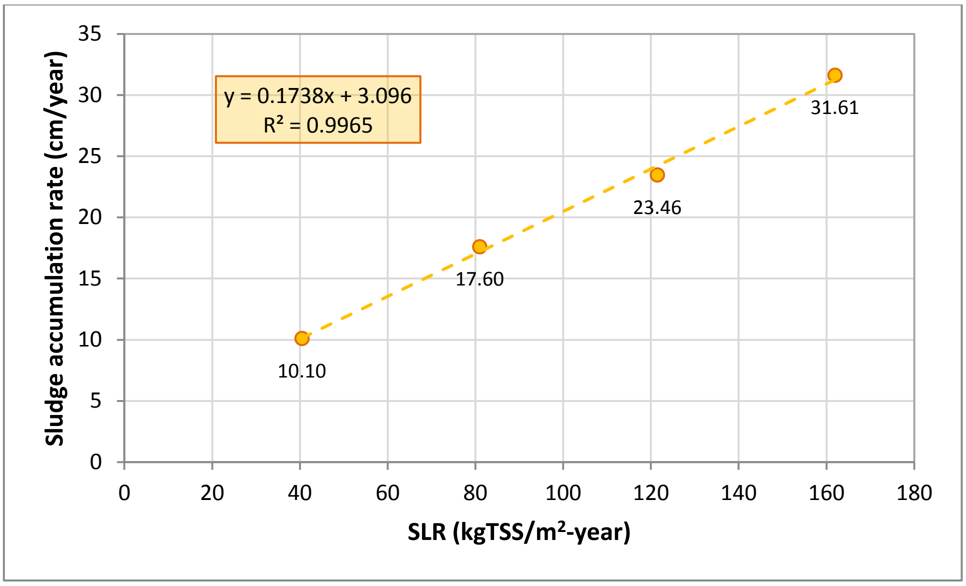


Figure 4-17 Sludge accumulation rates (cm/year)

On the other hand, the trends of sludge accumulation at distinct loading rates are clearly differentiated through linear correlations, where the high slope corresponds to the high sludge loading rate. *See Figure 4-18.*

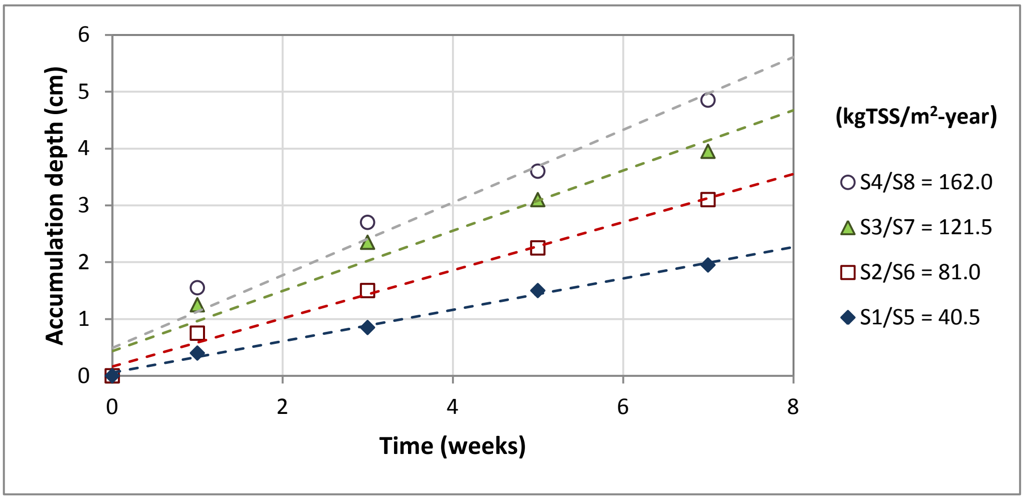


Figure 4-18 Accumulation trends at different SLRs

Finally, it is very important to consider the sludge accumulation rate as one of the main parameters to determine the dimensions of constructed wetlands. Usually, in sewage sludge treatment, heights around 1.5 to 2 meters are considered for dried sludge storage (for approximately 10 years of operation); however, in the case of the present study, such high depths are not required, since the biosolid accumulated in the top layer might be used at least once per year as organic fertilizer.

4.3. LEACHATE QUANTITY AND QUALITY

The dewatering process in sludge treatment wetlands is carried out mainly as the combination of two mechanisms: evapotranspiration (which combines the effect of evaporation and transpiration of plants) and water percolation through the granular medium and water. Stefanakis and Tsihrintzis (2011) found that between 58 and 84% of total water losses in sludge drying reed beds (SDRBs) are due to evapotranspiration, and the remaining percentage of water are either: stored in the sludge layer (1% to 4%) and/or drained (13% to 41%).

In the present study, two phases were identified (regarding to water leachate generation). The first phase covers the first five weeks of operation, when water leachate was collected and tested to determine its physical and chemical parameters and pathogen indicators. And the second phase after fifth week, when the amount of water collected was reduced significantly (volume of water leachate less than 200 mL/week in all experimental units) and only pathogen indicators were analyzed. *See Figure 4-19*.

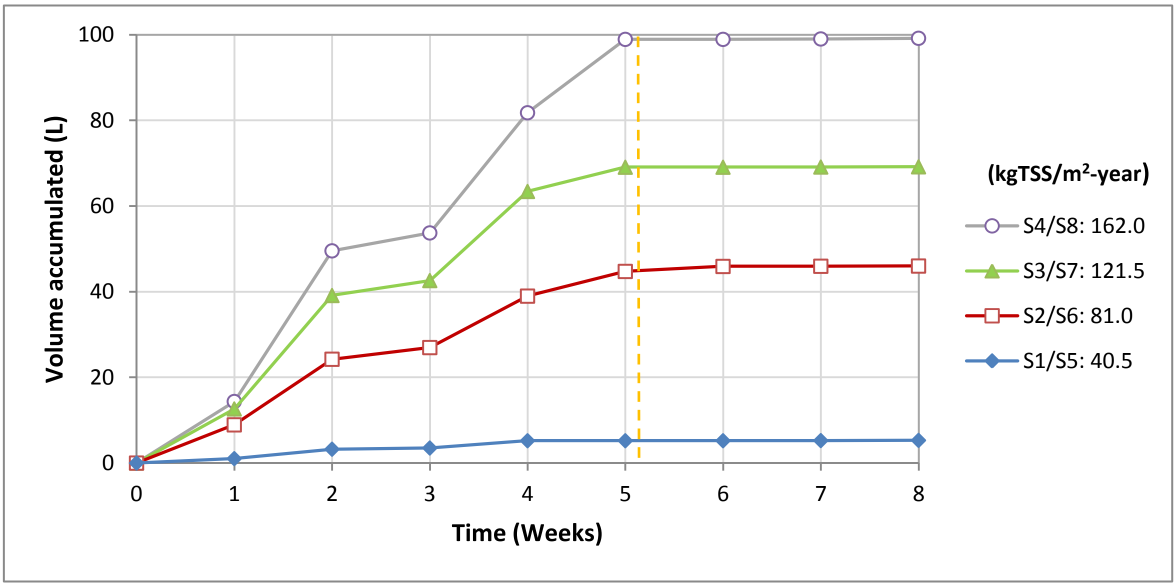


Figure 4-19 Water leachate accumulated volume at different loading rates

4.3.1. Water leachate quantity

In order to determine the volume of water leachate generated during the feeding and resting period, the volume collected in a plastic tank at the bottom of the each constructed wetland was measured daily.

The volumes of water leachate after two months of STWs' implementation were: 5.30, 46.01, 69.18 and 99.19 L to STWs operated at 40.5, 81, 121.5 and 162 kgTSS/m^2-year, respectively. As it was mentioned before, after five weeks the water leachate drained at the bottom of each experimental unit decreased significantly.

As the experimental units were evaluated during the acclimatization phase, the decrease of water leachate might be due to two factors. First, the evapotranspiration which is influenced by weather conditions and directly related the plants type and growth; and second, the accumulation of sludge at the top layer that may change the hydraulic conductivity.

Evapotranspiration (ET)

Evapotranspiration includes the atmospheric losses from a wetland that occur as a result of direct evaporation from water and soil, and the moisture that passes through vascular plants to the atmosphere (Kadlec and Wallace, 2009). As evapotranspiration is a complex process it is difficult to measure directly, however several methods have been developed to estimate wetland ET loss, ranging from simple empirical equations to more complex modelling approaches which usually require the input of multiple meteorological data from the site under investigation (Headley et al., 2012).

In the present study a water balance for a period of two weeks (feeding and resting period) have been determined, in the experimental units S4 and S8 where artificial precipitation was applied. The water balance can be represented by equation 4.1.

$$In + P = Out + ET \tag{4.1}$$

Where:

In = Influent
P = precipitation
Out = effluent
ET = evapotranspiration

The evapotranspiration was 11.90 mm/day. This result is higher than ET in a sludge drying reed bed (in Greece) equal to 7.8 mm/day, reported by Stefanakis and Tsihrintzis (2011), and lower than ET in a sub-surface constructed wetland (in Morocco) equal to 57 mm/d (El Hamouri et al., 2007). The difference might be mainly due to variations in the meteorological parameters in different locations, especially solar radiation and photoperiod time.

Since during the research period there was no precipitation, the only source of water in the STWs was the influent (diluted slurry). Based on the influent average TSS concentration, the amount of water entering the system in mm/day was estimated at different sludge loading rates as it is shown in Figure 4-20. Through this relation, it can be inferred that loading rates lower than 108 kgTSS/m^2-year could lead to plant water starvation. This situation is also confirmed by the plants' health. In fact, during the last two weeks of the research period, the *Phragmites* leaves of the experimental unit S1 (operated at 40.5 kgTSS/m^2-year) changed to a yellowish colour indicating water stress. *See Figure 4-21.*

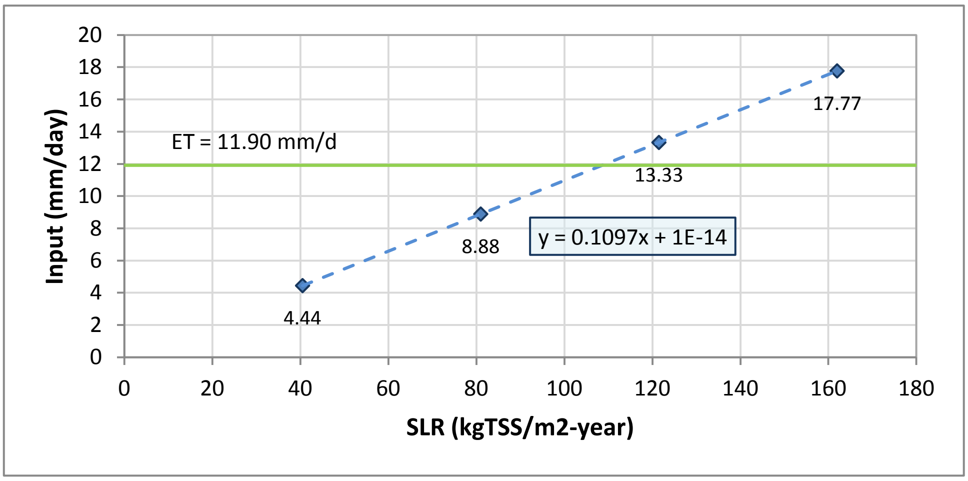


Figure 4-20 Volume of water fed daily at different SLRs

Figure 4-21 Leaves' yellowish colour at S1 (SLR: 40.5 kgTSS/m^2-year)

To finalize, the evapotranspiration has a key role in the STW's performance; consequently it is required to monitor the system during other seasons of the year, also to check the influence of precipitation on it.

4.3.2. Water leachate quality

As it was discussed before after five weeks of operation, the amount of water collected at the bottom of each experimental unit, was decreased significantly; therefore, the results of physico-chemical parameters corresponds to the first phase; however, pathogen indicator (*E. Coli*) was measured along the research period.

The means results are summarized in *Table 4.5*.

Table 4.5 *Water leachate quality*

Parameter	STW (kgTSS/m^2-year)			
	S1/S5: 40.5	S2/S6: 81.0	S3/S7: 121.5	S4/S8: 162.0
pH	8.54	7.88	8.28	8.13
EC (dS/m)	1.607	0.422	0.419	0.002
TSS (mg L^{-1})	48.00	15.50	17.50	16.63
VSS (mg L^{-1})	40.00	13.00	15.00	8.67
COD (mg L^{-1})	53.18	160.34	142.61	227.00
BOD_5 (mg L^{-1})	12.00	33.55	28.22	37.23
SAR	7.33	21.64	25.08	23.76
E. Coli (CFU/100mL)	151.25	39.11	106.49	120.56

According to the water-quality standards for India and water reuse guidelines elaborated by U.S. Environmental Protection Agency (USEPA, 2004), water for irrigation is classified as Class E, which has the following limits regarding to its quality: pH between 6.0 and 8.5, electrical conductivity (EC) less than

2.250 dS/m, Sodium-adsorption ratio less than 26, total suspended solids (TSS) less than 30 mg L^{-1}, BOD_5 less than 30 mg L^{-1} and *E. Coli* less than 200 CFU/100ml.

In general high efficiency removal was achieved in the monitored parameters, consequently the water leachate quality in under the limits of Class E; therefore it might be used for agricultural irrigation.

Additionally, once again a clear tendency to increase the removal capacity was observed in the experimental units along the time. For instance, in Figure 4-22 shows that the lowest *E. Coli* concentration in water leachate was during the last two weeks of operation.

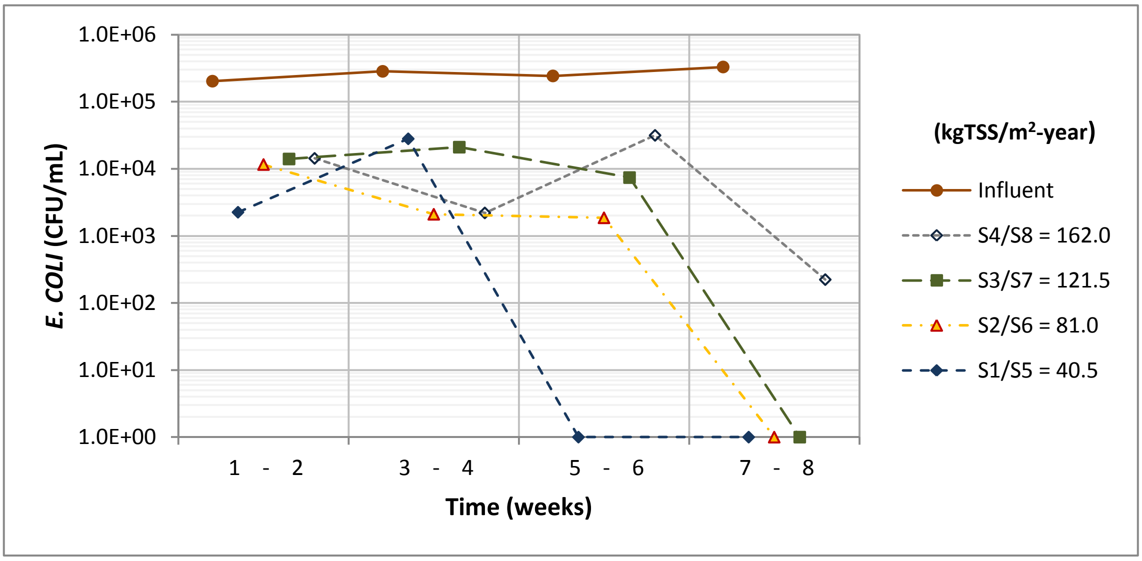


Figure 4-22 *E. Coli* reduction on water leachate at different loading rates during the research period of time

4.4. PLANTS

4.4.1. Plants fresh and dry weight

Fresh and dry weight was measured in the field and at laboratory, respectively. The results are shown in *Table 4.6.*

Table 4.6 *Fresh and dry above-dried sludge plant's weight*

Experimental Unit	Weight (g)		SLR (kgTSS/m2-year)	Plant specie
	Fresh	Dry		
S1	245.00	133.26	60	*Phragmites Karka*
S2	495.00	127.41	120	*Phragmites Karka*
S3	835.00	172.76	200	*Phragmites Karka*
S4	375.00	153.56	250	*Phragmites Karka*
S5	295.00	67.85	60	*Phragmites Karka*
S6	575.00	119.89	120	*Phragmites Karka*
S7	675.00	130.41	200	*Phragmites Karka*
S8	735.00	117.23	250	*Napier Bajra* hybrid grass (NB-21)
P-K_i	65.00	17.38	n.a.	*Phragmites Karka*
NB-21_i	55.00	36.22	n.a.	*Napier Bajra* hybrid grass (NB-21)
P-K_f	110.00	10.27	n.a.	*Phragmites Karka*

Note: P-K_i and NB-21_i correspond to *Phragmites Karka* and *Napier Bajra* hybrid grass initial weights, respectively. And P-K_f corresponds to Phragmites Karka weight which was fed only with underground water.

To compare the effect of different loading rates on plants' growth, the relative growth rate have been determined, and is analyzed in the point 4.4.2.

4.4.2. Relative growth rate (RGR)

In order to quantify the speed of plant growth and observe the influence of different loading rates on it, relative growth rates (RGR) have been calculated for each experimental unit (See *Table 4.7*). The relative growth rate is measured as the mass increase per aboveground biomass per day (g g^{-1} d^{-1}). It is considered to be the most widely used way of estimating plant growth, although it also has been criticised as calculations typically involve the destructive harvest of plants (Hoffmann and Poorter, 2002).

The relative growth rates calculations were based on the following equation:

$$RGR = \frac{\ln W_2 - \ln W_1}{t} \tag{4.2}$$

Where:

W_1 = initial dry weight
W_2 = final dry weight
t = treatment time in days.

Table 4.7 *Plants' growth of eight experimental units operated under four different loading rates*

Experimental Unit	Above-dried sludge biomass (g DW m^{-2})	RGR (d^{-1})	SLR ($kgTSS/m^2$-year)	Plant specie
S1	908.56	0.037	60	*Phragmites Karka*
S2	868.73	0.036	120	*Phragmites Karka*
S3	1,177.92	0.042	200	*Phragmites Karka*
S4	1,047.02	0.040	250	*Phragmites Karka*
S5	462.61	0.025	60	*Phragmites Karka*
S6	817.41	0.035	120	*Phragmites Karka*
S7	889.16	0.037	200	*Phragmites Karka*
S8	799.31	0.021	250	*Napier Bajra* hybrid grass (NB-21)

As it can be shown in Figure 4-23, there is an optimum plant biomass production in the experimental unit which was operated at 120 kgTSS/m^2-year. Also a tendency to increase the biomass production at high loading rates is observed. However, in the STW S4/S8 operated at 162 kgTSS/m^2-year there is tendency to decrease the biomass production, it might be due to two factors: first, these experimental units started their operation two weeks later than others; and second, two different plant species have been used on these units. In addition, the high relative growth rate variance on these units shows the different biomass production on different plant species.

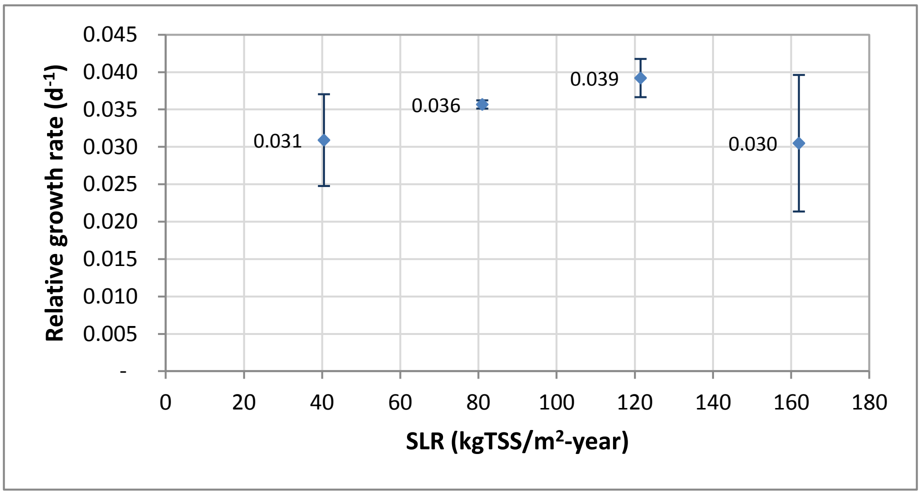


Figure 4-23 Plants' relative growth rate at different loading rates

On the other hand, if the relative growth rates of experimental units S4 and S8 are compared, a significant difference in biomass production is observed, since the S4's RGR (planted with *Phragmites karka*) is almost twice S8's RGR (planted with *Napier Bajra* hybrid grass). On this sense, the use of *Phragmites karka* is recommended rather than *Napier Bajra* hybrid grass.

Finally, the biomass production (measured in terms of relative growth rate) at different loading rates calculated in the present study, are comparable with biomass production of other plant species like: *Pennisetum Purpureum* (0.045) and *Pontedaria cordata* (0.031), used in constructed wetlands to treat undiluted wastewater (Li et al., 2013b).

CHAPTER 5

CONCLUSIONS

This study characterised the properties of a toilet linked biogas plant (TLBP) effluent, comparing it with other similar products like septic sludge, anaerobically digested sludge and composted sludge; and also, evaluated the performances of eight pilot-scale sludge treatment wetlands operated at four different loading rates, applied as post-treatment for TLBP's digested sludge, by analysing the properties of dried sludge accumulated on the top layer and assessing the quality of drained water collected at the bottom of experimental units. Focus was put on: the quality of biosolids for agricultural reuse as soil conditioner; and the quality of drained water for water reuse. From this work, the following conclusions can be drawn.

1. The TLBP effluent (also called slurry) has a high nutrient content which gives an economic value to this product, but the organic matter and pathogen concentrations indicate that the TLBP effluent requires a post-treatment prior to use in agriculture.

2. The slurry's pH (6.8-7.32), temperature (20-28 °C), ammonia (968.33 mg kg^{-1}) and nitrate (128.3 mg kg^{-1}) concentrations, indicate that the household-size anaerobic digester is working under good operational conditions from the methane production point of view. The range of pH for the optimum methane production is between 6.6 and 7.4, also ammonia and nitrate concentrations less than 1,540 mg L^{-1} and 750 mg L^{-1} are recommended for an optimum methane yield (Sheng et al., 2013).

3. Very high moisture reduction was achieved on the dried sludge accumulated at the top layer of all experimental units, (TSS from 50.98% to 80.56%) compared with conventional dewatering methods like centrifuges (14-18% TSS on conventional waste activated sludge (Andreoli et al., 2007)). Furthermore, considering the recommended range of moisture from agricultural reuse point of view (between 35% and 60%), loading rates higher than 121.5 kgTSS/m^2-year (moisture content equal to 38.94%) are recommended.

4. On the whole, the four sludge treatment wetland configurations showed similar efficiencies in terms of mineralisation and hygienisation. According to the U.S. Environmental Protection Agency (EPA) regulation for use or disposal of sewage sludge biosolids, the dried sludge could be classified as class B.

5. Due to the high STW's evapotranspiration (ET is 11.9 mm d^{-1}) a minimum loading rate of 108 kgTSS/m^2-year is recommended to avoid plants' starvation under project's local climate conditions.

6. The water leachate collected at the bottom of experimental units, has very good quality. According to Indian standards and USEPA (2004) regulations for water reuse, it could be classified as Class E. Consequently, reuse for irrigation is recommended.

7. There are no significant differences between growth biomass rates (0.031, 0.036, 0.039 and 0.030 at different loading rates). However, between *Phragmites karka* and *Napier Bajra* hybrid grass, there is a difference of almost 50% (Phragmites GRG is twice than NB), consequently the use of *Phragmites karka* is recommended

CHAPTER 6

RECOMMENDATIONS

1. Further research should be carried out to study the STWs performance in a longer period of time. Since constructed wetlands performance are directly influenced by climate conditions (winter, summer), and an improvement of pathogen removal efficiency was observed at longer time, it would be interesting to have a system's evaluation at least during one year.

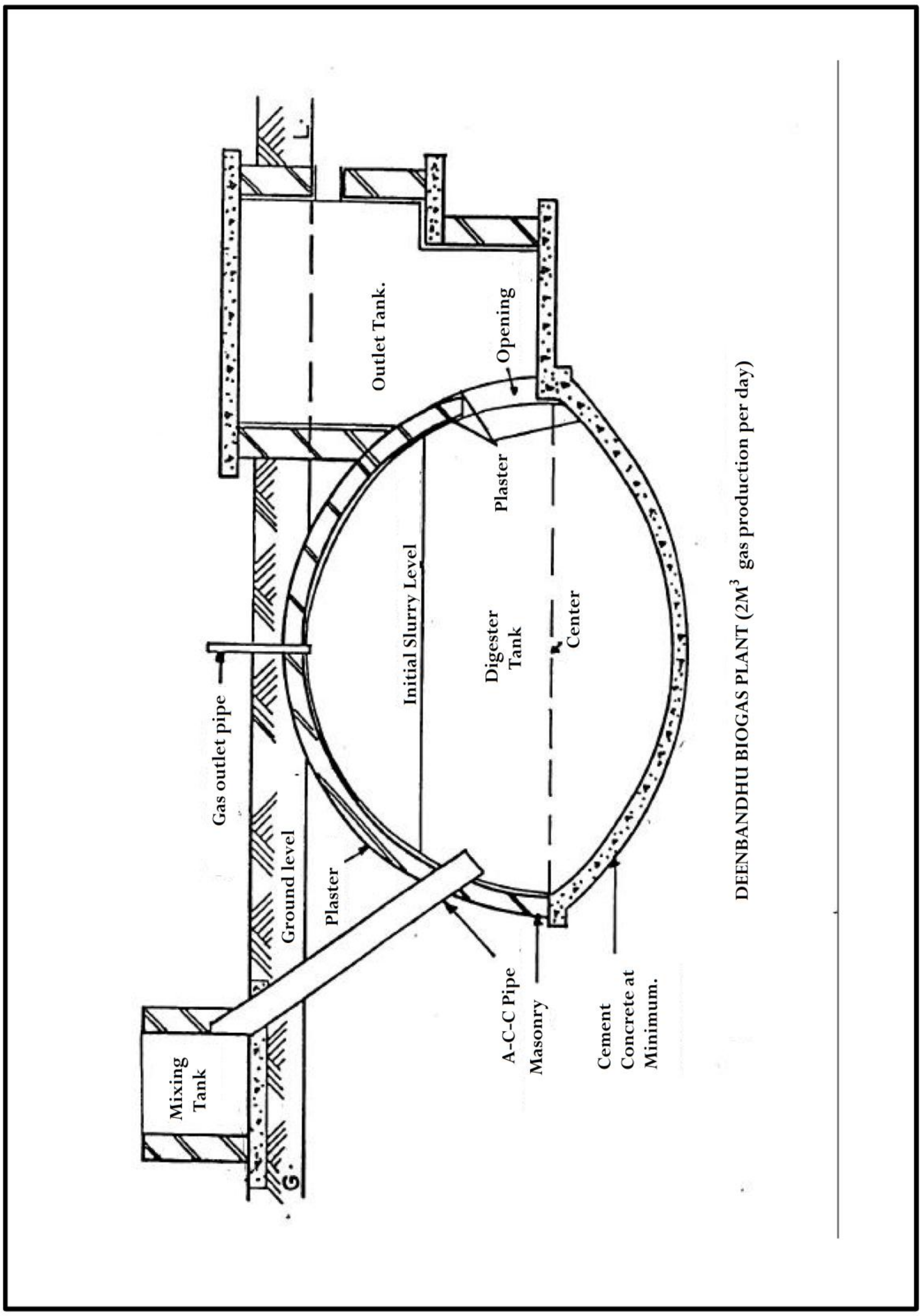
2. Further investigations are required to evaluate the pollutants concentration on plants, because they represent a potential source of foliage to feed cows and buffalos.

3. Constructed wetlands are recognized for their potential to treat different types of wastewater, one of them is greywater. Since the slurry concentration was high and the STW plants' water requirements are also high, due to India climate conditions, it would be interesting to evaluate the system's performance treating slurry diluted with greywater.

4. A study can be carried out to evaluate the influence of bed materials and its configuration. In the present study four layers of gravel (different diameters) and sand was used, however it is expected that only two layers would be enough. Additionally, it would be interesting to evaluate the use of recyclable materials in construction or other locally available and cheap material, instead of gravel.

Appendices

Appendix A Typical design of Deenbandhu plant



Source: Singh (1989)

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