Low-cost physico-chemical disinfection of human excreta in emergency settings

Emanuele Sozzi

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School of Environment and Technology
University of Brighton
United Kingdom

DEDICATION

"But he said unto them: 'Except I shall see in his hands
the print of the nails, and put my finger into the print of the nails,
and thrust my hand into his side, I will not believe'"
John 20:25

This thesis is dedicated to my mother, my sister
and to all the true friends I have been blessed to have

"The hottest places in hell are reserved for those
who in time of moral crisis preserve their neutrality"
From John F. Kennedy's Presidential Library and Museum,
based upon a similar quotation from the third canto of Dante's Inferno

ABSTRACT

The operation of a health-care facility, such as a cholera or Ebola treatment centre in an emergency setting, results in the production of pathogen-laden wastewaters that may potentially lead to onward transmission of the disease. The research presented here outlines the results of field and laboratory studies devised to inform the design and operation of a novel full-scale treatment protocol to disinfect pathogen-laden hospital wastewaters *in situ*, thereby eliminating the need for potentially hazardous road haulage and disposal of human excreta or wastewater to poorly-managed waste facilities. The approach investigated has the potential to provide an effective barrier to disease transmission by means of a novel but simple sanitary intervention.

During Phase I of this research, a fieldwork study in Haiti focused on the design and operation, at short notice and within a disaster setting, of a new treatment technology that aimed to obviate the transport of untreated human excreta from emergency cholera treatment centres (CTC) to poorly-managed waste facilities. The results of this fieldwork period were validated and further optimised during Phase II: a detailed laboratory-based study in the UK that assessed the performance of the novel treatment technology in order to improve its efficacy.

The performance of two physico-chemical protocols was monitored, first in the field (Port-au-Prince, Haiti), by means of both bench-scale and full-scale batch treatment of real highly-contaminated faecal waste from a cholera treatment centre (Phase I), and subsequently during more detailed laboratory studies (Phase II) using a 'faecal-waste matrix' that was created by mixing various municipal wastewaters and sludges in a proportion that aimed to mimic the composition of wastewaters produced at health-care facilities in emergency settings.

The two investigated protocols achieved coagulation/flocculation and disinfection by exposure to high– or low–pH environments, using thermotolerant coliforms, intestinal enterococci, and somatic coliphages as indices of disinfection efficacy, and several physico-chemical parameters as indicators of treatment performance. In the high–pH treatment protocol, the addition of hydrated lime resulted in wastewater disinfection and coagulation/flocculation of suspended solids. In the low-pH treatment, disinfection (and partial colloidal destabilization followed by sedimentation) was achieved by the addition of hydrochloric acid, followed by pH neutralisation. A potential further step in this

second protocol was the coagulation/flocculation of suspended solids using aluminium sulphate.

During Phase II, removal rates achieved for the high pH treatment protocol, in terms of physico-chemical parameters, were: COD > 80%; suspended solids > 85%; turbidity > 85%. Removal rates in terms of microbiological parameters were: thermotolerant coliforms > 5 Log_{10}, intestinal enterococci >2 Log_{10} and somatic coliphage > 2 Log_{10}. Removal rates achieved for the low-pH treatment protocol in terms of physico-chemical and microbiological parameters were: COD > 80%; thermotolerant coliforms between 0.2 and 1.2 Log_{10}, with a mean removal of 0.75 Log_{10} and > 3 Log_{10} removal for intestinal enterococci. The removal of somatic coliphage was in excess of 4 Log_{10}.

The quantity and density of the sedimented sludge and several other physico-chemical parameters (such as total nitrogen, total phosphorous, ammonia and ammonium, etc.) for the analysis of the supernatant were also monitored.

This study represented the first known successful attempt to disinfect wastewater in a disease outbreak setting without resorting to the alternative, untested, approach of 'super-chlorination' which, it has been suggested, may not consistently achieve adequate disinfection. In addition, a basic costs analysis demonstrated significant savings in the use of reagent compared with super-chlorination. The approach to sanitation for cholera treatment centres and other disease outbreak settings presented here offers a timely response to a UN call for *in situ* disinfection of wastewaters generated in such emergencies. Further applications of the method to other emergency settings have been actively explored in discussion with the World Health Organization (WHO) in response to the ongoing Ebola outbreak in West Africa, and with the UK-based non-governmental organization (NGO) Oxfam.

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SYMBOLS AND ABBREVIATIONS

BOD_5	Biochemical oxygen demand over 5 days
CFU	Colony-forming units
COD	Chemical oxygen demand
CTC	Cholera treatment centre
DAF	Dissolved air flotation
EVD	Ebola virus disease
G_{ave}	Average velocity gradient
HAV	Hepatitis A virus
HEV	Hepatitis E virus
MDG	Millennium development goals
NGO	Non-governmental organisation
NTU	Nephelometric turbidity units
PFU	Plaques-forming units
RPM	Rotations per minute
SDG	Sustainable Development Goals
SS	Suspended solids
TSS	Total suspended solids
UN	United Nations
WHO	World Health Organization

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I would like to thank my supervisor, Professor Huw Taylor, for his support throughout my PhD, beginning with our first chat inside the MSF hospital in Port-au-Prince in 2011, when he encouraged me to start this journey. I still remember him saying: “No, I don’t agree with your concern that this would be ‘too simple for a PhD’. It doesn’t have to be complicated, it has to be smart’.

I am also very grateful to my other supervisor, Dr James Ebdon for his valuable input and constant support during this journey.

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My sincere gratitude is also for the fantastic people who have been the best desirable companions over these years: Francesco and Magda, Marjorie, Lorenzo, Camino, Mario, Marco, Professor Rozzi and Edgard. Finally, I would like to thank Dr. Mark Erickson for his beautiful verses

And Patroklus armed himself in gleaming silver.
He put about his chest the labcoat of his mentor,
White as snow on the mountain peaks,
With secure fastenings, and fitted it to himself.
And about his hands he cast the purple gloves of nitrile,
And then his goggles, clear and sturdy,
And he took the valiant sharp-beaked pipette that fitted his grasp, preparing for the membrane filtration to come.

With gloved hand Patroklus turns the tap to bring forth mighty vacuum,
But dismayed he was that no suction came forth.
And he spoke winged words saying: "The fucking vacuum pump is broken again!"

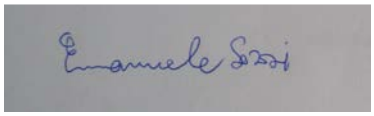
Hephaestus, high on Mount Olympus, was aggrieved at the insult he had received,
And also that no Achaean had made sacrifice to him that day.
Vowing vengeance mighty Mulciber in his magnificence flew to the silver tower.
And Patroklus, hard at work on the membrane filtration, piled high the membrane's sheaths and saw not the god at his shoulder
As Hephaestus's breath blew the leaves into his fire

As a burning forest's flames and smoke can be seen from afar, and the rushing roar of flames brings fear to the heart of the bravest warrior, so the bench took light
And as Hephaestus's wrath took hold Patroklus, fearless, smote the flames with his terrible armour.
And Odysseus and Nestor too smote the flames, and tamed the ash and smoke, praying to Hephaestus to still his wrath.
Panic and Strife departed and the Achaeans gave thanks for their health.

From 'Patroklus Fights the Fire', Dr Mark Erickson, 24th June 2015

AUTHOR'S DECLARATION

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed 

Dated 15th May 2016

1. CHAPTER ONE – INTRODUCTION

1.1 THE CONSEQUENCES OF DIARRHOEAL DISEASE

Enteric and diarrhoeal diseases have been estimated to cause the death of 1.33 million children aged under five each year [1]. These diseases, which include cholera, are the second most common cause of infantile mortality (i.e., death during the first five years of life from the end of the neonatal stage) worldwide [2]. Malnutrition, which is observed to be very highly correlated with enteric and diarrhoeal diseases, is thought to be an underlying contributing cause of more than three million deaths each year, accounting for approximately 45% of the deaths of children younger than five, and making surviving children more vulnerable to other diseases [3, 4].

The most recently available detailed studies into the causes of global child mortality are from 2014. Although mortality rates have significantly improved between 2000 and 2013 (the years to which the two major studies referred) and appear to have further improved since the last report was published, Liu et al. (2015) recently estimated that diarrhoeal deaths still correspond to 9.2% of the 6.3 million deaths of children under five occurring worldwide (0.58 million deaths in total) [1, 5, 6]. More than half of these early childhood deaths are due to conditions that could be prevented or treated by access to simple, affordable interventions and more than 70% of all diarrhoeal deaths occurring among children occur within only fifteen developing countries [6, 7].

From a broader global health perspective, it is important to note not only the importance of child mortality, but also the issue of easily avoidable deaths in general, as between 842,000 and 1.4 million diarrhoea deaths are estimated to be caused by inadequate drinking water, sanitation and hygiene. This corresponds to 0.9 to 1.5% of the total disease burden (calculated as 'Disability Adjusted Life Years' or DALYs) and 58% of diarrhoeal diseases [8-10].

Furthermore, it is important to appreciate what are the consequences of diarrhoeal diseases. Enteric and diarrhoeal diseases are not only a significant cause of death in emergency settings, but are also a major cause of malnutrition. Diarrhoea can be one of the main causes of a deficiency in vitamin A, iron, zinc, and iodine. Over the longer term, such nutritional problems can lead to multiple negative effects, including disabilities, impaired fitness, cognitive development and fluency, lower

school achievement and even malabsorption of drugs and other long-term health problems [11, 12]. In order to demonstrate the magnitude of the issue, it may be useful to highlight specific findings from detailed studies of the impacts of diarrhoea on the growth and development of a child:

- Moore et al. (2001) estimated that the average growth shortfall from diarrhoea and enteric parasites in the first two years of life is around 8.2 cm.
- Guerrant et al. (2002) reported that the fitness impairment associated with diarrhoea may equate to a subsequent decrease in work productivity of 17% for the person affected by the disease [13].
- According to Guerrant et al. (1999 and 2008) and Niehaus et al. (2002), the cognitive impairment resulting from the average diarrhoea burden equates, for the victim of this disease, to nearly 10 IQ points [12, 14, 15].

One of the underlying explanations of these observations is probably that malnutrition reduces immunological capacity to defend against infectious disease, which deplete and deprive the body of essential nutrients [11]. For instance, intestinal infections lead to malnutrition and malnutrition worsens intestinal infections [15]. Furthermore, a 2008 WHO bulletin suggested a “decline in appropriate diarrhoea case management among children less than five years old” and suggested one reason to be that the recent growth in attention and resources dedicated to the eradication of HIV, tuberculosis and malaria has not been matched for other important causes of childhood death, including diarrhoea [16].

1.2 THE BURDEN OF DISEASE ASSOCIATED WITH EXCRETA-BORNE PATHOGENS

Although mortality rates have in recent years further declined, the challenge remains to reduce the total global disease burden in low-income countries by improving access to clean drinking water and basic sanitation [17-19].

As a consequence of the issue of diarrhoeal disease, the United Nations (UN) Millennium Summit in 2000, set out to reduce by 50% the population without sustainable access to 'improved' drinking water and basic sanitation by 2015 (the 'baseline' year being 1990) as a target within the eight agreed Millennium Development Goals (MDG – target 7C) [17, 18]. The participants of this Summit also agreed the target of reducing child mortality by two thirds by 2015 ('baseline' year 1990) [20]. As many childhood deaths are directly or indirectly caused by diarrhoeal diseases leading to malnutrition, it was agreed that a common strategy to reach both targets was required.

1.2.1 THE MDG AND SDG DRINKING WATER TARGETS

The final Millennium Development Goals (MDG) report [17] indicated that, during the past 24 years, more than 2.6 billion people have gained access to 'improved' drinking water sources and that the proportion of the global population using such sources was likely to reach 91% by the end of 2015, up from 76% in 1990. This means that the MDG drinking water target was met five years ahead of the target date.

Figure 1.1 shows in detail how the target was achieved; it also demonstrates that the target was achieved on a global scale, but that considerable work still remains to be done in the countries of the Oceania, Sub-Saharan Africa, and Caucasus and Central Asia regions [17].

The new ('post-2015') Sustainable Development Goals (SDG) address these issues as part of Goal 6, aiming to 'ensure access to water and sanitation for all'. With regard to water availability, the new target aims to achieve universal and equitable access to safe and affordable drinking water for all (UN, 2015b).

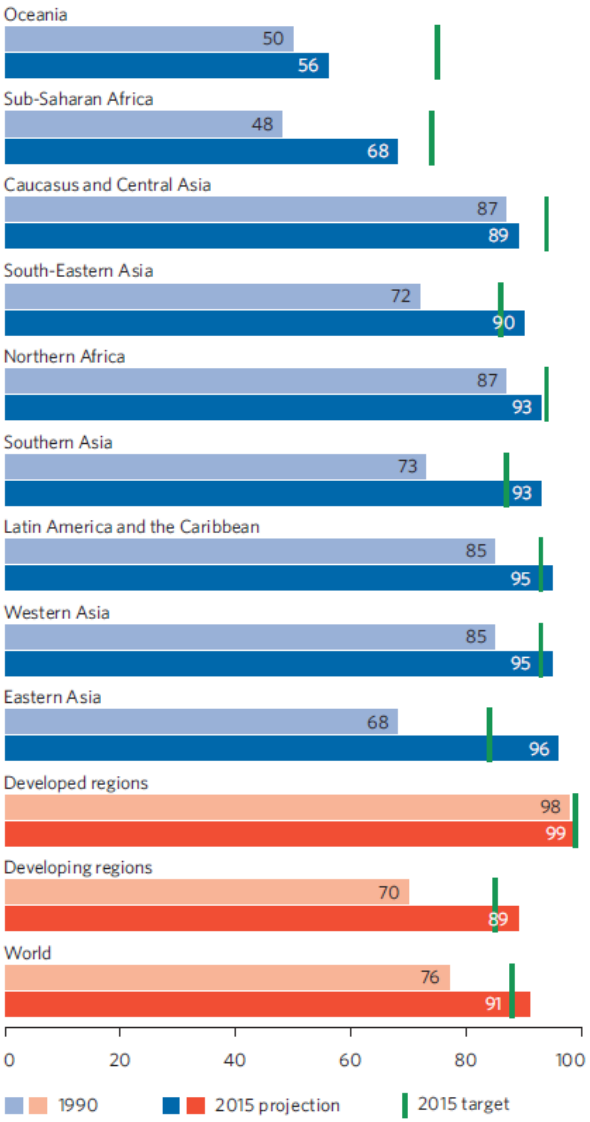


Figure 1.1: Proportion of the population using an improved water source: 1990 vs. 2015 [17]

1.2.2 THE MDG AND SDG SANITATION TARGETS

The seventh MDG (target 7c) not only aimed to improve access to drinking water, they also targeted the availability of basic sanitation as an essential means to tackle all the issues previously mentioned. Unfortunately, although gains in sanitation have been impressive, they were not sufficient to meet the target set [17].

Figure 1.2 details what has been achieved in terms of improved sanitation since 1990 and how Oceania, Southern Asia and Sub-Saharan Africa are experiencing the greatest challenges in meeting the target set for sanitation. In 1990, only 49% of the global population had access to improved sanitation and 24% of the global population was still resorting to open defecation [17]. Coverage should therefore have been extended to 75% to meet the target 7c, which was therefore missed [17, 18, 21].

From 1990 to 2013, approximately 2.1 billion people gained access to a latrine, flush toilet or other improved sanitation facility and the proportion of the global population resorting to open defecation declined from 24 to 13% (Figure 1.3) [17, 19]. Still, in 2015 one in three people (2.4 billion) still use unimproved sanitation facilities, including 946 million people who still practise open defecation, a practice that poses serious health and environmental risks to themselves and their communities [22]. The new SDG are addressing these gaps as part of Goal 6. With regard to sanitation development, the new target aims to achieve access to adequate and equitable sanitation and hygiene for all and to end open defecation by 2030, paying special attention to the needs of women and girls and those in vulnerable situations. The same target also aims to halve the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally [23].

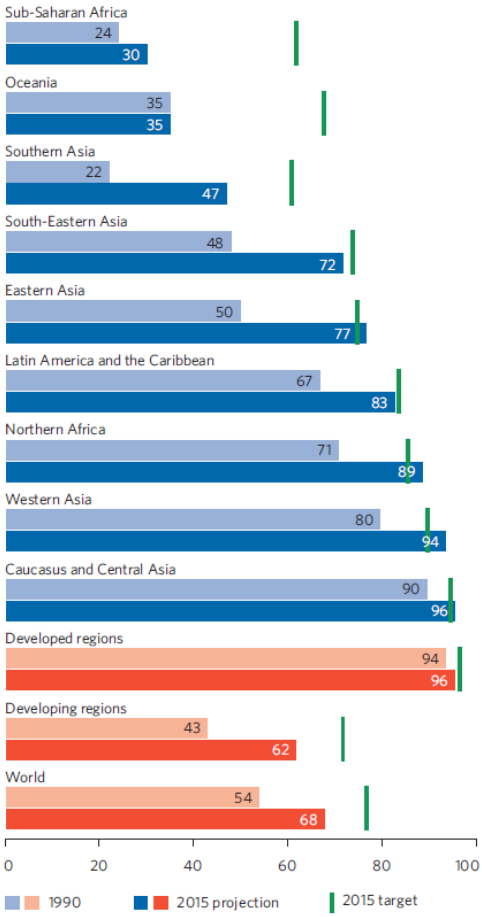


Figure 1.2: Proportion of the population using an improved sanitation facility: 1990 vs. 2015 [17]

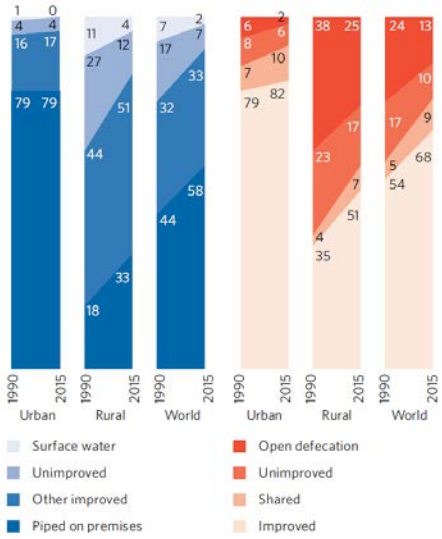


Figure 1.3: Proportion of population by water and sanitation practices: 1990 vs. 2015 [17]

1.2.3 CHOLERA AS A COMPONENT OF THE GLOBAL 'WATER AND SANITATION' PROBLEM

The WHO estimates that cholera alone is responsible for three to five million illnesses and between 100,000 and 120,000 deaths each year [24-27]. More conservative estimates identify 1.4 billion people at risk of cholera in countries in which the disease is endemic. Here, an estimated 2.8 million cholera cases are occurring annually. For countries in which the disease is not endemic, the same study estimates that 87,000 cases of cholera occur each year. The incidence is considered to be highest in children below five years of age, the consequence being that about 91,000 people are thought to die of cholera every year in countries in which the disease is endemic and 2,500 people die of the disease in countries in which the disease is not endemic (see Figure 1.4) [28]. The WHO estimates that the officially reported cases represent only 5 to 10% of the actual number occurring annually worldwide [29, 30]. Based on the hypothesis that 0.5% of total diarrhoea cases are caused by cholera, a 2002 literature review of diarrhoeal reports, which aimed to provide detailed estimations of the causes of diarrhoeal diseases worldwide, concluded that 11 million cholera cases occur globally every year among children under five years of age [28, 31]. Therefore, it can be argued that a global effort aiming to achieve the aforementioned SDG needs to include an intervention to tackle this disease as one of its priorities.

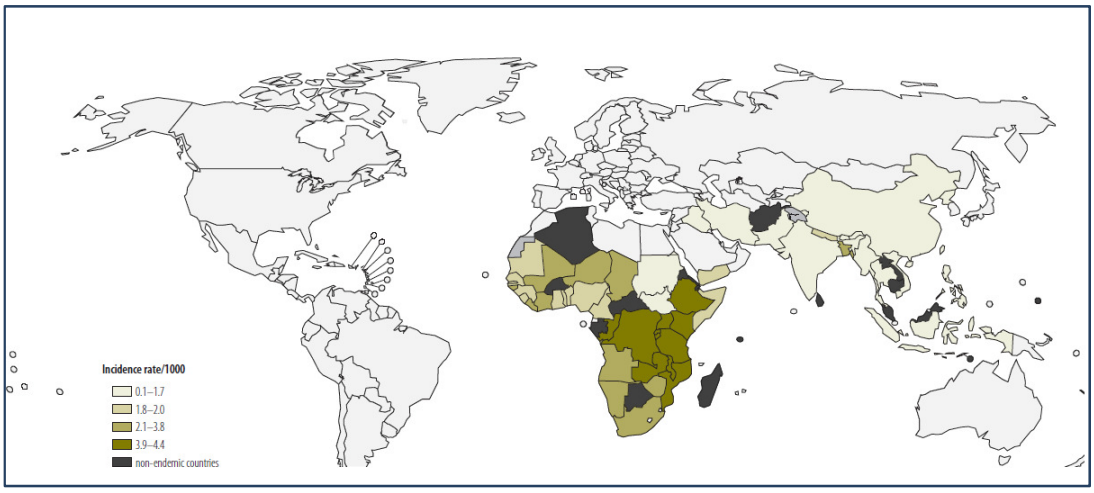


Figure 1.4: Geographical patterns of estimated cholera incidence for the entire population in endemic countries [28, 29]

1.2.4 THE OTHER EMERGENCY DISEASES PART OF THE 'WATER AND SANITATION' GLOBAL ISSUES

In general, cholera is only one of the specific infectious diseases that may potentially be transmitted by human excreta, an outbreak of which presents a challenge to existing WASH (Water, Sanitation and Hygiene) practices. Of these diseases, Ebola haemorrhagic fever and hepatitis A and E are the most relevant in terms of morbidity and mortality. A greater focus on practical *in situ* disinfection of human waste may offer an effective first step in the development of a longer-term sanitation ladder to support infection control. The research presented here focuses on an innovative *in situ* disinfection technique, which to date has mainly been applied in the context of a cholera outbreak, but which could potentially, and in the near future, provide a health protection intervention within the context of other outbreaks of neglected tropical diseases, including the two forms of water- and faecal-borne hepatitis and, most importantly, Ebola.

In other words, this research developed in response to a cholera outbreak, but at a later stage the potential scope expanded beyond cholera to include viral infections. Though the pathogens (hepatitis A-virus, hepatitis E-virus, Ebola virus and *Vibrio cholera*) present very different pathologies and transmission mechanisms, over the course of this work it became evident that this research might provide valuable knowledge relevant to these and other diseases that may cause, or result from, human disasters.

2. CHAPTER TWO – LITERATURE REVIEW

2.1 CHOLERA: THE AETIOLOGY OF THE DISEASE

Cholera is a severe, acute, dehydrating diarrhoeal disease of humans, which, in the absence of adequate rehydration, can lead to death in both children and adults within 12 hours [25, 26]. The disease results from infection by a pathogenic strain of the bacterium *Vibrio cholerae*, which is capable of producing a potent toxin [32]. *Vibrio cholerae* is a member of the Vibrionaceae family of curved, gram-negative rods [24]. It is a 'facultative pathogen' that has both environmental and human stages in its life cycle [26, 33]. *Vibrio cholerae* infection displays a clinical spectrum that ranges from asymptomatic infection to severe cholera, known as *cholera gravis* [25, 26].

After ingestion of *Vibrio cholerae*, the majority of the bacteria are killed by gastric acid, but the surviving organisms can colonise the small intestine and elaborate cholera toxin, the major virulence factor for pathogenic strains. Although this review does not aim to provide a comprehensive overview of how the bacterium causes the disease, it is sufficient to mention that cholera toxin is a protein consisting of one A subunit associated with five B subunits. Through a few complex biochemical processes, the A subunit causes chloride secretion through the apical chloride channel and decreased sodium chloride absorption. This results in a net movement of electrolytes and water into the lumen of the intestine, which causes secretory diarrhoea [34-36].

The infectious dose of *Vibrio cholerae* O1 has been estimated to be 10^5 to 10^8 units in experimental human infections, but could be as low as 10^3 in the presence of achlorhydria, a medical state in which the production of gastric acid in the stomach is low [24]. The incubation period ranges from 12 h to 5 days [37]. Approximately 75% of people infected with *Vibrio cholerae* do not develop any symptoms. Nevertheless, the bacteria remain present in their faeces for one to two weeks, which are released into the environment and can potentially infect surrounding communities [27].

Among the 25% of the population that develop symptoms, 80% present mild or moderate symptoms and 20% develop acute watery diarrhoea [25, 26] . The severe dehydration that follows can lead to death if untreated [25]. People with low immunity present the highest risk of death when infected [25-27]. The case

fatality rate for severe cholera without treatment can be as high as 50% [25], although today, if the cholera outbreak is well-managed, this can be kept below 1% [27]. Cholera is often described as the classic water- or faecal-borne disease because it is commonly associated with water; however, the bacterium can also be transmitted by contaminated food and directly from person to person [25].

Since the first recognised cholera pandemic, the pathogen has demonstrated its ability to spread rapidly, both regionally and internationally. According to the WHO, control of the disease requires a combination of interventions and barriers, ranging from water supply, sanitation and hygiene improvements to medical interventions, including the use of currently available vaccines [27, 38, 39].

2.2 SCIENTIFIC AND ENGINEERING RESPONSE TO EXCRETA-BORNE CHOLERA TRANSMISSION

This section presents an overview of the nature of the disease from a public health perspective with the goal of demonstrating how the international community has historically faced the challenge of cholera. It is beyond the scope of this chapter to provide a comprehensive overview of the history of cholera pandemics, so it is therefore useful only to summarise briefly how the seven cholera pandemics originated, their consequences and what the scientific community has progressively learned in facing them.

2.2.1 FIRST DOCUMENTED PANDEMICS

The first descriptions of a disease latterly identified as cholera are found in Sanskrit documents dating back to the 5th century BC, which provide “good reasons to assume that the disease has existed on the Indian subcontinent for centuries” [24]. The earliest Western record of cholera dates from the 16th century and refers to cases observed in India [40, 41]. The first recorded global cholera pandemic of ‘Asiatic cholera’ began in Bengal in 1816 and spread across India four years later, when the outbreak extended to other parts of southern Asia. The total deaths from this first epidemic remain unknown.

Cholera reached Europe for the first time in 1831 during the second cholera pandemic, originating in India in 1826, only three years after the first pandemic had ended there. Exactly as the first, it started with outbreaks along the Ganges river delta in India and moved along trade and military campaign routes to Central Asia, the

Middle East, Europe and eventually to North America. This epidemic later become called the 'Asiatic Cholera Pandemic' and receded only in 1851 [40, 42]. It is interesting to note that, if the scientific community was fundamentally powerless during the first pandemic, only a few years later, during the second, medical science developed a major tool still used today as a first remedy to rehydrate patients with severe cholera: the intravenous saline drip. This effective technique was developed from the work of the Scottish doctor Thomas Latta [43, 44].

2.2.2 FROM THE 'MIASMA' THEORY TO THE CLASSIFICATION AS 'WATER- AND FAECAL-BORNE DISEASE'

During the Florence outbreak of 1845–1846, also part of the same 'Asiatic Cholera Pandemic', the Italian anatomist Filippo Pacini identified for the first time the causative agent of the disease. His 1854 paper, '*Microscopical observations and pathological deductions on Asiatic cholera*', was ignored by the scientific community for 30 years until the work of the German physician Robert Koch became famous [45].

This delayed reaction was a result of the prevailing belief of scientists in the miasma theory of disease during Pacini's time. In several of his papers, Pacini even proposed various effective treatments for the new disease [46]. John Snow, who disproved the miasma theory of cholera transmission, and Robert Koch, who was credited with the discovery of the causative bacterium, were both unaware of Pacini's work. More than 100 years after Pacini's discovery in 1965, the 'International Committee on Systematics of Prokaryotes' adopted the formal name *Vibrio cholerae Pacini 1854* to honour the original work of the anatomist [47].

However, the most fundamental steps in the direction of medical and infrastructural intervention to defeat the disease were made during the subsequent pandemic: the third pandemic started in 1852 and severely affected Russia, with over one million deaths. The pandemic reached China, Japan, Europe and the US [41, 42]. It was during this pandemic, in 1853, that the physician John Snow contributed to resolving the 'Soho outbreak' of cholera in London, thanks to his identification of a neighbourhood public water pump, which was subsequently found to have been contaminated with the faeces of a baby with cholera. His obstinacy was finally successful in convincing the local officials to remove its handle, contributing to the end of the outbreak and indirectly demonstrating to the scientific community the benefits of environmental barriers to the transmission of the disease [48]. Snow's study demonstrated that contaminated water was the main agent for the spread of

cholera. Although he did not directly identify the causative agent, his discovery demonstrated for the first time that the disease did not originate from the miasma emanating from untreated sewage, but that it was instead waterborne.

During the same period, once cholera had struck the United States, and in particular Chicago, some US scientists came to the conclusion that poor sanitary infrastructure and poor health care were playing an important role in the spread of the disease. They began to realise that cholera prevalence was higher in some regions of the southern States, where the prevailing African-American population was under-served in terms of sanitary infrastructure and health care [49]. It would take many years and at least two more pandemics for this message to be understood and acted upon.

2.2.3 THE IMPORTANCE OF IMPROVEMENTS IN WATER AND SANITATION TO DISEASE CONTROL

Other important steps towards a structured intervention to fight the disease occurred during the fourth cholera pandemic, which spread mostly in Europe and Africa between 1863 and 1875, as the work of John Snow was for the first time used by other epidemiologists to save lives [50-53]. It was during the fifth pandemic, which struck Europe between 1881 and 1896, that Robert Koch managed to isolate the bacterium *Vibrio cholerae* and later proposed his postulates that explain how the bacterium causes the disease. His work helped to establish the germ theory of disease [54]. As previously mentioned, until this time the scientific community had believed that the disease was caused by direct exposure to 'miasma'. Koch's discovery was fundamental to establishing that the disease was more specifically contagious and was transmitted via the faecal-oral route, through the direct exposure to the faeces of an infected person, including those present in contaminated wastewater.

During the 1880s, European governments managed to improve their sanitation and water systems significantly, and this may be identified as one of the reasons why the following pandemic (i.e., the sixth – 1899 to 1923) was less devastating for the continent, especially when compared with regions less advanced from a health and sanitation perspective, such as the Russian and the Ottoman Empires, the Philippines and India [42].

2.2.4 THE CLASSIFICATION OF THE PATHOGEN

The seventh cholera pandemic officially began in 1961 in Indonesia. This pandemic was caused by a new biotype of *Vibrio cholerae* first isolated in 1905 in El Tor, Egypt [24]. The pandemic is therefore also called El Tor after the dominant strain causing it (even though it has to be pointed out that it was not the only strain present during the pandemic). The El Tor strain is distinguished from the classic strain, as explained in Table 2.1.

Vibrio cholerae is classified into more than 200 serogroups, based on the O antigen of the lipopolysaccharide; of these cholera toxin-producing (toxigenic) strains, the O1 and O139 serogroups cause the vast majority of disease outbreaks [24, 55]. The O1 serogroup is subdivided into two distinct biotypes, El Tor and classical, the second of which is associated with earlier pandemics. Two major serotypes exist, namely Ogawa and Inaba [25].

The El Tor biotype is the causative agent of the current seventh cholera pandemic; the classical biotype, associated with the previous pandemics, now appears to be extinct [56]. However, clinical evidence exists of the increasing severity of diseases linked to the emergence of atypical *Vibrio cholerae* organisms; these have incorporated genetic material from classical biotype strains into an El Tor biotype background [55, 56].

In 1992, *Vibrio cholerae* O139 was first recognised in south Asia as a cause of epidemic cholera [57]. According to Ramamurthy et al. (2013) “this organism is derived from *Vibrio cholerae* O1 El Tor by lateral transfer of a genomic island substituting the O139 for the O1 antigen” [58]. In the 1990s, the O139 serogroup caused devastating outbreaks, but the O1 El Tor strain remains the dominant strain on a global scale [55].

Table 2.1*: Classification of* Vibrio cholerae *according to serogroups, strains and serotypes [26]*

Serogoup													
O1						O2 ... O138				O139			
Strain													
Classical			El Tor			Analogous sub-structures: no epidemic cholera	Classical			El Tor			
Serotype													
Inaba	Ogawa	Hikojima*	Inaba	Ogawa	Hikojima*	Analogous sub-structures: no epidemic cholera	Inaba	Ogawa	Hikojima*	Inaba	Ogawa	Hikojima*	

* very rare

The seventh cholera pandemic is considered by some to have occurred between 1961 and 1975 but in reality it continues, with lower intensity, to the present day. During the past decade the spread of the disease has also been helped by modern transportation and mass migration, whereby devastating epidemics of cholera have occurred in Angola, Ethiopia, Zimbabwe, Pakistan, Somalia, Sudan, Vietnam, and Haiti [24]. According to these authors, among immunologically naïve populations, cholera affects all age groups, and epidemics can be associated with high case fatality rates. This was, for example, the case in Haiti, where cholera had been absent for approximately a century before 2010. Population density, poor sanitation and health infrastructure increase the case fatality rates in any epidemic setting [24].

However, global cholera mortality rates have dropped markedly, mainly as a result of modern medical responses (principally oral rehydration therapy or ORT [59]) and preventative measures, such as the provision of clean water and the adoption of effective hygiene practices and infrastructural improvements. The case fatality rate of 50%, typical of the first pandemics, dropped to approximately 10% by the end of the 1980s, and is now considered to be between 1 and 2% (e.g., the case fatality rate of the Haitian outbreak currently stands at 1.7%) [60]. The case fatality rate in a well-managed, cholera outbreak could in fact be less than 1% [36, 59].

2.3 THE 2010 HAITIAN CHOLERA OUTBREAK

With the goal of briefly outlining the context in which the fieldwork experiments took place, this section presents an overview of the Haitian outbreak, from both a microbiological and a public health perspective.

Ten months after the devastating earthquake of 12th January 2010, cholera appeared in Haiti for the first time in nearly a century. The first cases were confirmed on October 22nd, 2010 [61]. The outbreak escalated, and as of 30th September 2015, the resulting mortality had reached 9,031 and the cumulative morbidity had reached 750,752 – equivalent to more than 7% of the country's population [60, 62]. According to the WHO, the outbreak accounted for 57% and 53% of global cholera cases, and 58% and 37% of global cholera deaths reported in 2010 and 2011 respectively [61] Morbidity levels have probably been higher than these figures suggest because, as previously mentioned, only a small fraction of total cholera cases may be reported to the relevant authorities [63].

The source of the Haitian outbreak has been the subject of heated debate. Three hypotheses were proposed, based on the previous theories of Colwell [64]. The first

hypothesis holds that an environmental strain of *Vibrio cholerae* that normally inhabits the Gulf of Mexico travelled to Haiti naturally via ocean currents as a consequence of the earthquake and caused the present cholera epidemic [65]. The second hypothesis holds that a local, non-toxigenic *Vibrio cholerae* strain endemic to the Haitian environment naturally mutated into a virulent pathogenic strain [65]. The third one holds that the source of the outbreak was an infected human who carried a pathogenic strain of *Vibrio cholerae* into Haiti from a cholera endemic region outside the country [65]. The United Nations Stabilization Mission (MINUSTAH) in Haiti was created in April 2004 by the United Nations Security Council. Following the January 12th, 2010 earthquake, the United Nations passed additional resolutions that served to increase the number of international forces in the country in order to support recovery, reconstruction, and stability efforts [61]. A more specific hypothesis as to the source of the cholera outbreak, and one that is commonly believed in Haiti, advances that soldiers at the Mirebalais MINUSTAH camp were the direct source of the cholera outbreak. The Mirebalais MINUSTAH camp was located upstream of the area in which the first cholera cases were identified and a new contingent of soldiers had recently arrived at the time of the first cases. Witnesses reported sanitation practices at the camp that allowed the faeces of soldiers to enter the nearby river untreated.

According to Chin et al. (2011), there is now good molecular evidence to suggest a close relationship between the Haitian isolates of *Vibrio cholerae* and variant *Vibrio cholerae* El Tor O1 strains isolated in South Asia (specifically in Bangladesh) in 2002 and 2008, and a more distant relationship with isolates currently circulating in South America [66]. Hendriksen et al. (2011) used whole-genome sequence typing (WGST) technology to characterise 24 recent *Vibrio cholerae* isolates from Nepal and to evaluate the suggested epidemiological link with the Haitian outbreak. The study showed that all *Vibrio cholerae* isolates from Nepal "belonged to a single monophyletic group that also contained isolates from Bangladesh and Haiti", therefore providing support for the hypothesis that the isolates were brought to Haiti from South Asia [67]. The cholera epidemic began in the upstream region of the Artibonite River served by the Mirabalais Hospital. Inhabitants of the region have little or no consumption of fish or shellfish products, which are known to have been associated with outbreaks of cholera worldwide. A sudden cholera outbreak began on October 20th, 2010 in the Artibonite River Delta, indicating that cholera had spread throughout the Artibonite River Delta within two to three days of the first cases being recorded in the upstream region.

2.4 THE IMPORTANCE OF WASTEWATER TREATMENT IN EMERGENCY SETTINGS

Before giving a brief description of the characteristics of wastewaters produced in emergency settings, with particular reference to the specific case of cholera and a brief reference to hepatitis E and Ebola epidemics, it is worthwhile briefly considering the ecology of *Vibrio cholerae*. This will help to elucidate why it is considered important, in the case of infectious disease outbreaks, to disinfect contaminated human excreta before disposal to the environment.

Miller et al. (1985), West (1989) and Borroto (1997) reviewed the evidence that *Vibrio cholerae* is not, as had previously been assumed, simply a human bacterial pathogen that occasionally enters bodies of water, but is, rather, "a bacterium that has a distinct aquatic habitat, but which is capable of infecting humans" [68-70]. Further, Islam et al. (1993a, b; 1990) suggested that in a low–income country where cholera exists as a human disease, certain waters may act as a reservoir for *Vibrio cholerae* [71-73].

2.4.1 THE ROLE OF AQUATIC RESERVOIRS IN MAINTAINING CHOLERA ENDEMICS

The experimental evidence seems to support the hypothesis that aquatic reservoirs play a major part in maintaining all cholera endemics. This hypothesis is strengthened by the inadequacies of the alternative explanations of the mechanism responsible for maintaining endemic cholera. The most important of these is based on the 'continuous transmission theory', which suggests "the maintenance of *Vibrio cholerae* by low–level continuous transmission through people with asymptomatic infection or mild disease" [69].

In their review of the environmental reservoirs of *Vibrio cholerae* and their role in cholera transmission, Vezzulli et al. (2010) point out that the pathogen may not only survive in environmental reservoirs for a relatively long time, but also under certain circumstances may attach to the chitin-containing shells of crustaceans in coastal waters, and under certain climatic conditions may even multiply within this environmental niche [74]. According to Nelson et al. (2009), endemic cholera occurs in regions "with natural aquatic reservoirs of *V. cholerae*, where the bacteria can persist either in a free-living state or in association with phytoplankton, zooplankton or detritus" [55].

2.4.2 THE IMPORTANCE OF WASTEWATER TREATMENT UNDER CONDITIONS OF EPIDEMIC CHOLERA

If it is true that aquatic reservoirs play the major role in making the disease endemic, under epidemic conditions rapid transmission between humans certainly occurs by the faeco-oral route and therefore effective disinfection of drinking waters, and indeed wastewaters, remains an essential barrier against disease transmission. The underlying principle of water, sanitation and hygiene intervention in cholera epidemics is therefore one of using 'multiple barriers'. No single intervention is considered an infallible barrier to transmission but, through several interventions in the water cycle, the risk of transmission of the pathogen in infectious doses is substantially reduced [38, 39]. Figure 2.1 summarises the model proposed by Morris (2011) for the lifecycle and transmission of *Vibrio cholerae* [36].

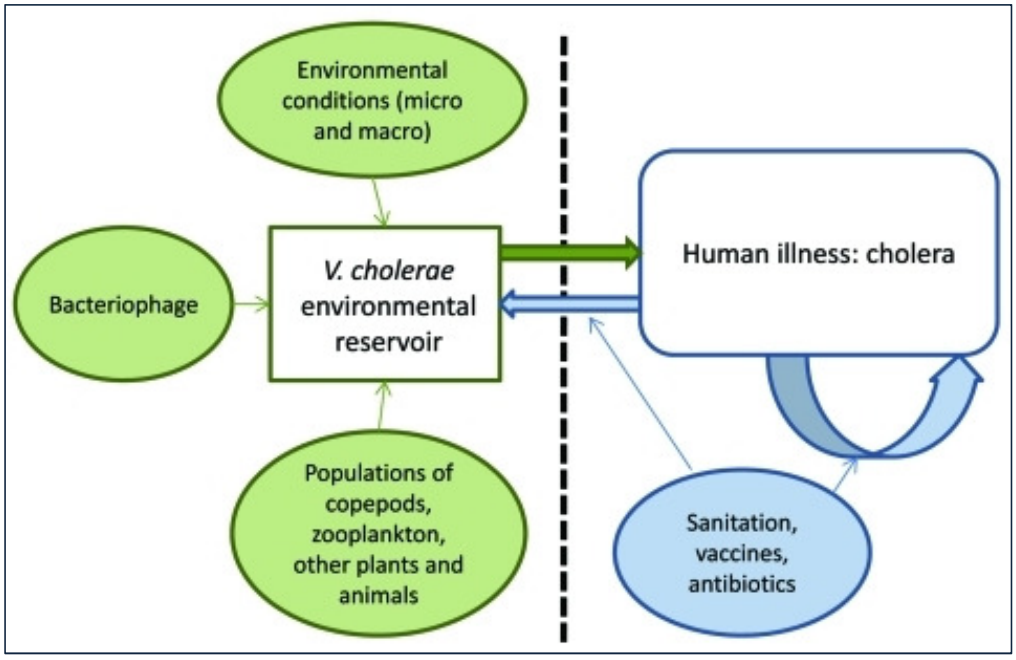


Figure 2.1*: Model of the lifecycle and transmission of* Vibrio cholerae *[36]*

According to Merrell et al. (2002) and Nelson et al. (2009) *V. cholerae* strains associated with epidemic disease are able to respond to changes in their immediate environment as they move from environmental reservoirs to humans and back. Human colonisation by the pathogen creates a hyper-infectious bacterial state that may contribute to the epidemic spread of cholera. The reason for this is that the competitive advantage of stool-derived bacteria persists after dissemination back into the environmental reservoir and, probably, back to

humans [55, 75]. It is important to bear in mind, however, that, once the microorganism is introduced into a human population, transmission occurs primarily by “fast” transmission from person to person (taking advantage of the hyper-infectious state), without returning to the aquatic environment. Therefore there is the need to treat contaminated wastewaters in order to block this route [55].

2.4.3 THE IMPORTANCE OF WASTEWATER TREATMENT UNDER CONDITIONS OF ENDEMIC CHOLERA

Returning to geographical areas in which the disease is endemic (i.e., the infection is maintained in the population without the need for external inputs) rather than epidemic (i.e., the infection is introduced in the population by an external input), the model proposed by Morris (2011) highlights the importance of drinking and wastewater treatment under these conditions because:

- The effective treatment of drinking water (and fishery products) before human consumption is the best way to avoid the transmission of the pathogen from the environmental reservoir to the human reservoir (from left to right – see green arrow in Figure 2.1)
- The effective treatment of contaminated wastewater is the best way to reduce, as much as possible, the transmission of the pathogen from the human to the environmental reservoir (from right to left – see blue arrow in Figure 2.1), in other words, to avoid the continuous ‘recharge’ of the aquatic reservoirs that play an essential role in making the disease difficult to eradicate.

2.4.4 SANITATION AS KEY PART OF A MULTIPLE APPROACH TO CHOLERA INTERVENTION

For the reasons previously outlined, once a cholera epidemic strikes, established cholera control strategies call for a combination of interventions, including improvements to the quality and quantity of drinking water supplies, promotion of effective hygiene practices and provision of consistently functional sanitation chains. Under certain circumstances, the administration of oral vaccines to ‘at risk’ communities may also be recommended [26, 39]. Treatment of infected individuals is largely based on oral (or in more serious cases, intravenous) rehydration [25]. For the most severe cases, a suitable antibiotic, such as

tetracycline, doxycycline or azithromycin, may be administered [76]. Only a multifaceted approach in which several anti-diarrhoea measures are implemented simultaneously “with mutually reinforcing and complementary impacts” can achieve the ambitious UN target of eradicating the disease [38, 39, 77].

2.4.5 THE RISK TO HUMAN HEALTH POSED BY HUMAN EXCRETA CONTAINING *VIBRIO CHOLERAE* IN AN EMERGENCY CONTEXT

The provision of a consistently functional sanitation chain is currently one of the most difficult goals to achieve, as part of a successful strategy to control cholera. In order to make clear the main objectives of the research described here, it is useful to elucidate briefly why hospital wastewater effluent, with a particular focus on the case of wastewater from cholera treatment centres (but also hepatitis A and E treatment centres and Ebola care units), should be disposed of in a manner that does not pose an unacceptable risk to human health.

Human excreta can potentially transmit many infectious diseases. The pathogens leaving the body of an infected person through the excreta can contaminate one or more healthy individuals, reaching them through one of many possible paths. Prüss et al. (2002) developed a simple and effective model to represent the transmission pathways of faecal–oral diseases (Figure 2.2).

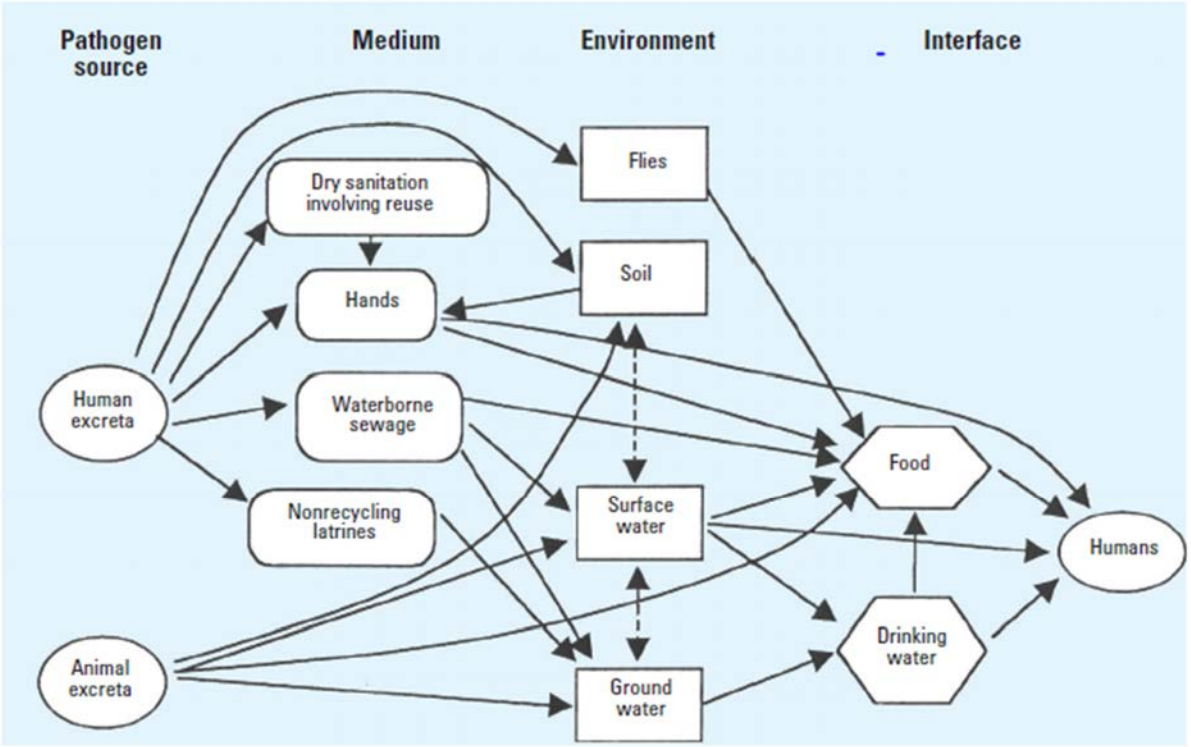


Figure 2.2: Transmission pathways of faecal–oral diseases [78]

The *in situ* treatment of pathogenically-contaminated wastewater within the challenging context of medical emergencies (the most relevant examples being cholera, Ebola, hepatitis A and E outbreaks) therefore needs to be capable of removing microbial pathogens significantly more effectively than do conventional treatment technologies [79]. The resulting technology also needs to be relatively low–cost, logistically simple, rapid to deploy and immediately effective. For the specific case of a cholera emergency, such systems have rarely been established, and no peer-reviewed literature that critically evaluates their operational performance is available. However, the concentration of *Vibrio cholerae* in Cholera Treatment Centre (CTC) wastewaters and the potential risk to public health that the pathogen represents may be estimated from previous studies.

2.4.6 CONCENTRATION OF *VIBRIO CHOLERAE* IN CONTAMINATED WASTEWATERS

During a two-year investigation of cholera carriers in the Philippines, Dizon et al. (1967) measured the numbers of *Vibrio cholerae* per gramme of faeces among human populations in areas of the country in which the disease was endemic or epidemic. The faeces of 'simple carriers' contained between 10^2 and 10^5 *Vibrio cholerae* per gramme of faeces, whereas the faeces of patients presenting symptoms of 'mild cholera' were shown to contain between 10^6 and 10^9 *Vibrio cholerae* per millilitre of stool (on their first day of illness) [80]. Figure 2.3 shows the appearance of the very dense 'rice-water' stool from cholera patients.

Figure 2.3: Typical rice-water stool from cholera patients [25]

Howard et al. (1975 and 1977) examined the wastewater from a hospital operated by the international NGO Oxfam in Bangladesh, which admitted between two and 40 confirmed cholera cases per day. The authors recorded levels of *Vibrio cholerae* between 5 x 10^5 and 5 x 10^7 colony-forming units per 100 ml of wastewater. It is worth noting that the level of *Vibrio cholerae* was demonstrated to exceed that of thermotolerant coliforms in this instance [81, 82]. A study performed during an outbreak in Peru reported similar figures [83]. Further, Nelson et al. (2009) reported the rice water stools of cholera patients to harbour between 10^{10} and 10^{12} vibrios per litre [55].

2.4.7 TRANSMISSION ROUTES OF EXCRETA-RELATED DISEASES

In 1983 Feachem et al. [77, 84] developed an environmental classification of excreta-related diseases that was later refined by Mara and Alabaster (1995) (see Figure 2.4) [85].

Category (1)	Environmental transmission features (2)	Example (3)
1. Nonbacterial feco-oral diseases	Nonlatent Low to medium persistence Unable to multiply High infectivity No intermediate host	*Viral:* Hepatitis A and E Rotavirus diarrhea *Protozoan:* Amoebiasis Cryptosporidiosis Giardiasis *Helminthic:* Enterobiasis Hymenolepiasis
2. Bacterial feco-oral diseases	Nonlatent Medium to high persistence Able to multiply Medium to low infectivity No intermediate host	Campylobacteriosis Cholera Pathogenic *Escherichia coli* infection Salmonellosis Shigellosis Typhoid Yersiniosis
3. Geohelminthiases	Latent Very persistent Unable to multiply No intermediate host Very high infectivity	Ascariasis Hookworm infection Strongyloidiasis Trichuriasis
4. Taeniases	Latent Persistent Able to multiply Very high infectivity Cow or pig intermediate host	Taeniasis
5. Water-based helminthiases	Latent Persistent Able to multiply High infectivity Intermediate aquatic host(s)	Schistosomiasis Clonorchiasis Fasciolopsiasis
6. Excreta-related insect-vector diseases		Infections in 1−3 transmitted mechanically by flies and cockroaches Bancroftian filariasis transmitted by *Culex quinquefasciatus*
7. Excreta-related rodent-vector diseases		Infections in 1−3 transmitted mechanically by rodents Leptospirosis

Figure 2.4: Environmental classification of excreta-related diseases

According to Cairncross, Feachem and Mara [86, 87] and Médecins Sans Frontières guidelines for infection control in healthcare settings [88], there are three fundamental transmission routes of excreta-related diseases associated with contaminated wastewater:

- Faecal-oral transmission;
- Disease transmission through water-based helminths eggs and helminths (worms) transmitted by the soil; and
- Excreta-related diseases transmitted by insect vectors.

The proper treatment of the wastewater produced by a healthcare structure in an emergency setting can significantly contribute (as part of the so-called ‘multiple barrier approach’) to preventing the spread of a disease in the context of an epidemic [38, 39]. The reason for this is that effective treatment can directly break the first two transmission routes mentioned above, and can indirectly play a role in breaking the third, as detailed below:

- The most important public health risk for the population surrounding a health structure is the transmission of pathogens through contamination of hands, water, food and objects by excreta. The pathogens involved may be viruses, bacteria, protozoa and certain helminths. The list of faecal-

orally transmitted diseases is long, and includes typhoid fever, cholera, amoebic dysentery, giardia, and hepatitis A (see Figure 2.4);

- In any emergency context, parasitic worms can be transmitted through wastewater if this is not properly treated;
- Médecins Sans Frontières (MSF) public health guidelines (1994) note that all the diseases mentioned in the previous categories can, in principle, be transmitted by insects, in particular mosquitoes breeding in polluted water [88].

It is therefore evident that treatment of contaminated wastewater should be part of a comprehensive strategy to tackle endemic infectious diseases in low–income countries, in particular in areas affected by epidemics and so in conclusion, the main objective of excreta treatment and disposal in emergency settings should be to reduce the transmission of diseases resulting from environmental contamination by faecal matter or the proliferation of vectors, mainly by means of containment. The methods of excreta disposal in the context of an emergency should remove microbial pathogens more effectively than conventional treatment technologies and be as simple and cheap as possible.

2.4.8 THE IMPORTANCE OF GOOD SANITATION PRACTICES TO CONTROL EBOLA AND HEPATITIS A AND E OUTBREAKS

The research presented here initially focused on a new technique to be specifically applied to the *in situ* sanitation of wastewater from cholera treatment centres. During the course of the project, especially following the Ebola outbreak in West Africa, it became apparent that further applications of the method under study to other emergency settings should be explored. These opportunities have been actively investigated in discussion with the WHO, and with the UK-based NGO, Oxfam. The following section provides a brief description of three disease outbreaks that represent potential applications of the approach.

As stated by the WHO, the provision of water and sanitation plays an essential role in protecting human health during all disease outbreaks, including outbreaks of Ebola Virus Disease (EVD). “Good and consistently applied water, sanitation and hygiene (WASH) practices, both in health-care settings and the community will further help to prevent human-to-human transmission of EVD and many other infectious diseases” [89].

Ebola viruses are the causative agents of a severe form of viral haemorrhagic fever, designated Ebola haemorrhagic fever (also Ebola virus disease or EVD). The infection is characterised by systemic inflammatory response and immune suppression, both causing impairment of the vascular, coagulation, and immune systems, leading to multi-organ failure and shock [90]. The characteristics of the virus suggest that it is unlikely to survive for extended periods outside of the body and the virus is fragile in the environment compared with the enteric viruses commonly causing diarrhoeal disease. There is no evidence for transmission of Ebola viruses via drinking-water contaminated by faeces or urine. However, although Ebola is not 'enteric', internal bleeding can potentially lead to significant quantities of blood in the faeces of Ebola patients. Although survival of the virus within the excreta is likely to be short, its exact survival characteristics have not been accurately defined yet [91, 92]. Therefore the excreta of Ebola patients need to be treated with extreme caution and *in situ* disinfection may represent a useful barrier to disease transmission, especially considering the very low infective dose [93]. A multi-barrier approach to the issue of controlling the transmission of this disease, within the rational risk management framework of 'sanitation safety planning' is advisable [94].

The hepatitis A virus (or HAV) is the causative agent of an acute infectious disease of the liver. The virus can cause debilitating symptoms but does not cause chronic liver disease and is normally not fatal. The infected person normally recovers fully from hepatitis A within a few weeks, but in a very few cases the virus is the cause of fulminant hepatitis (or acute liver failure), associated with high mortality. There is no specific treatment for the disease. Therapy is aimed at maintaining comfort and adequate nutritional balance, including replacement of fluids lost from vomiting and diarrhoea [95]

The hepatitis E virus (or HEV) is the causative agent of a form of viral liver inflammation. It mostly causes an acute and self-limiting infection and mortality rates are low, but it may evolve into chronic hepatitis in immune-compromised patients. The disease severely impairs a person's ability to work and occasionally develops into an acute, liver disease, which is fatal in about 2% of cases. hepatitis E is clinically comparable to hepatitis A, but in pregnant women the disease can become more severe [96, 97].

Both hepatitis A and hepatitis E viruses have a faecal-oral transmission route and are associated with a lack of safe water and poor sanitation. As with Ebola haemorrhagic fever, outbreaks of hepatitis A and E are a significant public health concern, examples including frequent outbreaks of hepatitis E in East and South Asia, occurring most

commonly during the rainy season when water sources become contaminated by faecal material [97]. As the hepatitis E treatment centres result in wastewaters that represent a potential disease transmission route, it is important to consider the *in situ* physicochemical treatment of wastewater to be an essential part of the response to outbreaks of this disease. More complex is the strategy for the control of hepatitis A, as in developing countries with very poor sanitary conditions and hygienic practices, most children have been infected with the hepatitis A virus before the age of 10 years. Therefore “epidemics are uncommon because older children and adults are generally immune, symptomatic disease rates in these areas are low and outbreaks are rare” [95]. As the outbreaks are not as ‘acute’ and dramatic as for Ebola and hepatitis E, there are generally no treatment centres exclusively dedicated to the control of this disease. Nevertheless the *in situ* disinfection of hospital wastewater may represent a useful barrier to the transmission of the disease, as part of a multi-barrier approach.

2.5 WASTEWATER TREATMENT IN EMERGENCY SETTINGS

In low–resource settings, outbreaks of disease are highly likely to challenge the often limited existing healthcare provision, hence the necessity for rapid intervention provided by external NGOs that specialise in the rapid construction and operation of health care facilities in emergency contexts. The successful operation of these facilities undoubtedly saves lives. However, their operation also results in large volumes of pathogen-laden human faecal waste. As previously mentioned, this waste may contain high concentrations of pathogens: *Vibrio cholerae* in the case of a CTC, Ebola virus in the case of a Ebola Care Unit or ECU, hepatitis E virus in the case of a hospital in which hepatitis E is treated and hepatitis A virus in the case of any hospital in a region in which hepatitis A is endemic. These pathogens have the potential to contaminate drinking water supplies, and thereby to further the transmission of the diseases in the area surrounding the health facility.

According to Harvey et al. (2002) [98], the most common low–cost technology choices available to dispose of hospital wastewater in emergency situations without resorting to chlorine-based disinfection are:

- Soakaway pits;
- Infiltration trenches; and
- Evaporation pans and evapo-transpiration beds.

It is worth pointing out that the first two of the abovementioned options are considered suitable to deal with a wastewater containing high levels of enteric pathogens only if the water table is at least one and half metres lower than the lowest point of the excavated pit or trench [99].

Where wastewater has high content of solids, oil or detergent, it may be necessary to separate/deactivate these components prior to soil infiltration [100]. The main categories of such treatment are [99, 100]:

- Removal of settleable and suspended solids (by passing the wastewater through strainers or filters or coagulant-aided sedimentation) in order to prevent soil pores from quickly becoming clogged and thereby preventing infiltration into the ground;
- Removal of grease or oil, generally achieved through grease traps;

- Settlement tanks, *de facto* combining the two treatment techniques described above;
- Septic tanks, which are a small-scale anaerobic biological treatment system.

2.6 STUDIES INTO PHYSICO/CHEMICAL WASTEWATER TREATMENT SYSTEMS RELEVANT TO THE CURRENT RESEARCH

Prior to the Phase II experimental design, a review of available studies into physico/chemical wastewater treatment systems relevant to the current research was performed. The so called 'super-chlorination' of faecal waste has not been yet fully evaluated by a comprehensive peer reviewed study. The studies relevant to this research are briefly summarized at the beginning of this section.

The few available papers are mainly related to two technologies for the physico/chemical treatment of wastewater: the treatment methods developed by Gambrill et al. [101, 102] and later optimised by Taylor et al. [103, 104] focussing on pH-based physico-chemical treatment as an alternative to waste stabilisation ponds and the work of Rattanapan et al. [105] on dissolved air flotation. The most relevant literature regarding both technologies is briefly discussed as follows. Later in this section, the concept of velocity gradient and some important aspects of the hydrated-lime induced coagulation-flocculation theory are also briefly summarised, both concepts being essential for a detailed understanding of the methodology chapter.

2.6.1 SUPER-CHLORINATION OF FAECAL WASTE

It would seem sensible that wastewaters from hospitals, in particular those in which cholera patients are treated, are disinfected through chlorine-based products in order to prevent the further spread of the disease in the area surrounding the treatment centre. However, it is widely-recognised that chlorination itself may potentially lead to additional public health and environmental hazards, such as the production of carcinogenic compounds and impeded biological oxidation processes in receiving waters. Furthermore, the contaminated wastewater arising from CTC is characterised by extremely high concentrations of readily-oxidisable matter. It would therefore be imprudent to assume that a wastewater disinfection process based on chlorination would

consistently disinfect the waste to an adequate degree [106], given that the ability of these *in situ* disinfection strategies to reduce target pathogens had not been formally assessed beforehand [107].

Furthermore, it has been suggested that certain strains of *V. cholerae* (the 'rugose' phenotype) may be more resistant to chlorine-based disinfection, as a result of exo-polysaccharide production. According to Yildiz and Schoolnik (1999), Ali et al. (2005) and Liang et al. (2007), the 'rugose' colonial variant of *V. cholerae* O1, biotype El Tor produces "a unique extracellular polysaccharide, designated EPSETr", which confers cell aggregation, promotes biofilm formation and in particular promotes resistance to chlorine [108-110]. Such strains may therefore pose a further elevated risk to human health, even following 'super-chlorination' [111-113].

Finally, even if 'super-chlorination' were able to reduce *Vibrio cholerae* numbers to levels that did not pose a significant risk to those living downstream of CTC operations, the production of combined chlorine residuals and the relatively high operational costs associated with this process are likely to make it both environmentally and financially unacceptable in the medium- to long-term (see cost analysis as part of Chapter 4 and within Appendix 1). Moreover, this approach to disinfection does not significantly remove suspended material.

2.6.2 PHYSICO-CHEMICAL TREATMENT OF MUNICIPAL WASTEWATER BASED ON CONTINUOUS FLOW

It has been suggested that it may be possible to treat municipal wastewater successfully using physico-chemical methods, as an alternative to the waste stabilisation ponds (WSP) that are widely used in low–income countries (including Haiti), especially in situations in which sufficient land for a WSP is unavailable [101, 102]. Further to this, Taylor et al. (1994) investigated the efficacy of this approach and highlighted the difficulties associated with operating a system that requires an automatic pH control feedback mechanism to control the operation of a continuously running treatment system. The authors demonstrated that a continuous flow wastewater treatment system, based on chemically-induced removal of excreted pathogens, with a mean hydraulic retention time of approximately nine hours, was capable of achieving an effluent quality equivalent to that of a waste stabilisation pond with a retention time of 20 to 40 days [103, 104].

2.6.3 ENHANCED EFFICACY OF DISSOLVED AIR FLOTATION (DAF) BY ACIDIFICATION FOLLOWED BY COAGULATION

Rattanapan et al. (2011) successfully tested a physical/chemical technology to remove COD from wastewater with extremely high levels of COD, suspended solids (SS) and Biochemical Oxygen Demand (BOD_5), representing influent characteristics similar to those encountered in the first field study described in this thesis (although the technique was not designed to treat hospital wastewater, but to treat biodiesel wastewater). The technique was based on acidification followed by coagulation/flocculation aided by various flocculation agents (a process similar, though not identical, to the one that will be further referred to as 'low–pH' treatment). The authors tested various treatment protocols and the results were compared between acidifying with hydrochloric acid (as in the experiments executed as part of the current study), and sulphuric acid. The performance of various coagulants was also compared, *vis-a-vis* an aluminium-based coagulant – polyaluminium chloride and ferric chloride [105]. Two fundamental differences between the technology described by Rattanapan et al. and that tested within this research study are highlighted below:

1. **Reactor type**: The study was based on a dissolved air flotation (DAF) technology, and the treatment for the current study is based on a batch reactor technology;
2. **Influent characteristics**: Rattapan's study analysed the treatment of an industrial wastewater that was rich in grease and oil, whereas the wastewater treated in this research study was derived from a hospital. Nevertheless, it is interesting to highlight that, even though derived from different sources, the two wastewaters presented very similar physico-chemical characteristics, both having very high values for COD (above 10,000 mg/l), suspended solids (above 1,000 mg/l), and turbidity.

A key conclusion of the above-mentioned study is that the search for a technology capable of treating wastewater with very high COD, SS, turbidity and BOD_5 levels, should consider physical/chemical treatment based on acidification performed with hydrochloric and sulphuric acid followed by aided coagulation/flocculation as one of the practicable options [105].

Lefebvre et al. (2011) analysed the role played by low–pH environments on effluent quality during the treatment of domestic wastewater, through a physical/chemical technology based on a microbial fuel cell system [114]. Suzuki et al. (1997) analysed

the role played by acidification during the anaerobic treatment of brewery wastewater [115]. Xing et al. (2010) analysed the role played by acidification pre-treatment in the production of bio-hydrogen from dairy manures through anaerobic fermentation [116]. All the studies mentioned above confirm that hydrochloric acid is a good option for the reduction of pH in a wastewater treatment system, as it is easy to find, cheap and relatively safe to use.

2.6.4 SEPTIC TANK FAECAL WASTE TREATMENT

As later explained in Chapter 4.2 'Shortlisted technologies for full-scale treatment', septic tank treatment would be an option for the disinfection of highly contaminated faecal waste, prior to its infiltration into the ground or its discharge into a waterbody. A brief review of the literature,focussing on this approach, demonstrated that this treatment option was unsuitable for this project for a number of reasons, and as such should not be pursued any further. The reasons for this were as follows:

- Several studies into the composition of wastewater derived from various infectious (including tropical) disease hospitals have previously reported the presence of high concentrations of antimicrobial compounds (and resistance genes) in untreated hospital wastewater [117], [118], [119], [120];
- These compounds are known to have the potential capacity to 'destabilise' the anaerobic removal processes that are essential for the effective and efficient removal, decomposition or mineralisation of wastewater;
- Therefore, it was considered that septic tank treatment, based on either aerobic or anaerobic biological oxidation, would have been difficult, if not impossible, to establish and would have been insufficiently robust to operate effectively and reliably within this setting.

2.6.5 THE CONCEPT OF VELOCITY GRADIENT

The concept of average velocity gradient (G_{ave}) is briefly summarised as follows, as G_{ave} is an important parameters for the 'replicability' of the laboratory scale tests on a full scale. The concept of velocity gradient was developed by Camp and Stein [121] and an easy to understand definition of average velocity gradient is provided by Bridgeman et al. [122]:

- The floc size is dependent on the turbulence energy dissipation rate. The absolute velocity gradient G (s^{-1}) encapsulates the turbulence energy dissipation rate
- G is defined as:

$$G = \sqrt{\frac{P/V}{\mu}} = \sqrt{\frac{\varepsilon}{\nu}}$$

Where:

P = power dissipated

V = tank volume

μ = dynamic viscosity of the water,

ε = energy dissipation rate per unit mass

v = kinematic viscosity of the water.

- As the flow characteristics and the energy dissipation vary within the mixing vessel from point to point, G is a function of time and position, and is therefore extremely difficult to calculate and represent. Therefore it is convenient to replace the absolute velocity gradient with an approximation of its value, i.e., its average value throughout the vessel. This can be defined as G_{ave}:

$$\mathrm{G_{ave}} = \sqrt{\frac{P_{\mathrm{ave}}}{V\mu}}$$

Where:

P_{ave} = average power consumption = $P_0 \rho N^3 D^5$

P_0 = impeller power number

q = fluid density

N = rotational speed of the impeller

D = impeller.

- G_{ave} is universally used to characterise mixing regimes in flocculators.

2.6.6 IMPORTANT ASPECTS OF THE HYDRATED-LIME INDUCED COAGULATION-FLOCCULATION THEORY

It is beyond the scope of this review to detail the theory of hydrated-lime induced coagulation-flocculation, as explained previously by other authors, particularly

Semerjian and Ayoub (2003) [123]. It is sufficient to point out briefly the following reactions [124, 125], that are essential to understand the mechanisms of chemical hydrated-lime induced coagulation-flocculation, in order to fully appreciate the methodology as set out in Chapter 3 and the results, particularly those presented in Chapters 6, 7 and 8.

At a pH range of 9.1 to 9.5, as explained by equation (1) and equation (2) (below), the calcium carbonate formed precipitates out of solution, entrapping suspended and colloidal particles through the 'sweep coagulation' mechanism.

$$Ca(OH)_2 + H_2CO_3 \leftrightarrow CaCO_3 \downarrow + 2H_2O \quad (1)$$

$$Ca(OH)_2 + Ca(HCO_3)_2 \leftrightarrow 2CaCO_3 \downarrow + 2H_2O \quad (2)$$

Following incremental lime addition, at a pH range of 10.5 to 11.0, precipitation of calcium phosphates becomes the major cause of 'sweep coagulation', as explained by equation (3) and equation (4) (below).

$$3Ca(OH)_2 + 2PO_4^{3-} \leftrightarrow Ca_3(PO_4)_2 \downarrow + 6OH^- \quad (3)$$

$$4Ca(OH)_2 + 3PO_4^{3-} + H_2O \leftrightarrow Ca_4H(PO_4)_3 \downarrow + 9OH \quad (4)$$

In terms of the precipitation of magnesium compounds, some precipitation occurs at pH 9.5, becoming significant above 10.5 and complete at a pH range of between 11.0 and 11.5.

$$Mg^{2+} + 2OH^- \leftrightarrow Mg(OH)_2 \downarrow \quad (5)$$

The precipitating floc "acts as a 'weighting agent' by increasing the density of the settleable particles, thereby enhancing their settlement" [123].

2.7 THE SELECTION OF A SUITABLE FAECAL INDICATOR ORGANISM

In order to explain why specific indicators organisms were chosen for analysis in this study, it is useful to outline briefly how an appropriate faecal indicator organism is defined as such, and what the main criteria are for its selection.

Towards the end of the nineteenth century, the idea of 'indicator organisms' started to be explored after Robert Koch and Theodor Escherich suggested focusing future medical research, not only on the identification of the microorganisms causing diseases, but also on non-pathogenic microorganisms present in the human intestine. Both microbiologists were implying that a

relationship between the concentration of such microorganisms present in the intestines and the concentration of the pathogen itself may be established [45, 126, 127] and used as a tool for indicating the potential presence of enteric pathogens in the environment.

In a review of extant studies into the monitoring of pathogens for drinking water quality control, Gray (2008) explained that pathogens are normally outnumbered by the normal bacterial flora in the human intestines, and that the isolation of pathogens often requires complex tests, making routine monitoring of individual pathogenic microorganisms impracticable [128]. There is therefore a clear need for a rapid and, if possible, single test that indicates the presence of faecal contamination in the environment and suggests a potential risk of the presence of human pathogens. In addition, the review points out that most cases of water contamination occur infrequently and therefore an examination method, based on frequent, simple tests is generally more effective than an examination method based on occasional, but more complicated and detailed, tests for specific pathogens [128].

2.7.1 THE VALIDITY OF THE INDICATOR PRINCIPLE

The above-mentioned considerations have led to the development of the use of 'indicator organisms', which can be used to determine the likelihood of water contamination by faeces. Analogous considerations to those that Gray (2008) reviews for the selection of an appropriate faecal indicator organism for drinking water are undertaken within the current study for wastewater treatment. The aim has invariably been to select one (or a few) indicator(s) that are easy to measure and at the same time 'robust', giving the user of the proposed wastewater treatment technology the possibility to estimate the level of faecal contamination in the treatment system influent and effluent both easily and effectively.

Based on the so-called ‘Ingram principle’ [129] and on the distinction between ‘faecal indicator’ and ‘surrogate for the assessment of a contamination risk’ [130], Gray (2008) (for water), Griffin et al. (2001) (for marine water) and Miescier and Cabelli (1982) (for wastewater) identify the main criteria for the selection of the most suitable indicator organism. [128, 131, 132]. In temperate regions, *Escherichia coli* is widely assumed to fulfil these requirements and is therefore widely used as a faecal indicator microorganism [133].

At this stage, it is useful to outline the main differences between the concepts of 'process indicator', 'faecal indicator', 'model organism', 'tracer' and 'surrogate' for the assessment of an environmental risk. According to Banks and Board (1983) and Sinclair et al. (2012), a process indicator is a parameter (and not necessarily an organism) used in food and water industries to demonstrate the efficacy of a process [134]. Ashbolt et al. (2001) define a faecal indicator, mentioned above, as an organism, such as *Escherichia coli*, that indicates the presence of faecal contamination [135]. According to Sinclair et al. (2012) and Mossel (1995), a model organism is one that behaves in the same manner as a pathogen in a given environment or set of conditions; coliphages can be for example used to model the behaviour of human enteric viruses [130, 136]. According to Sinclair et al. (2012) and Bales et al. (1989), a tracer is an organism used in transport studies, such as a "coliphage or spores to trace groundwater movement or spore transport in aerosol" [130, 137]. A surrogate for the assessment of an environmental risk is an organism, particle, or substance used to study the fate of a pathogen in a specific environment [130].

Today the indicator paradigm underlies many aspects of measures to control the transmission of waterborne disease and consequently forms the basis of legislation that governs public health interventions, such as the control and management of bathing waters, drinking water and wastewater reuse systems. The validity of this rationale has not yet been demonstrated for some of the pathogens responsible for outbreaks of emerging infectious diseases, including *Vibrio cholerae* and the Ebola virus, but has been corroborated by Oragui et al. (1987; 1993a; 1993b) and Miescier and Cabelli (1982) for other pathogenic organisms, namely enteroviruses, rotaviruses, salmonellae etc. [131, 138-140], .

The main limitations of the classical indicator paradigm have been pointed out by several authors:

- Griffin et al. (2001) point out that no microbial indicator has yet been identified that can be used effectively in all regions [132, 141];
- The technology developed in this study were intended to tackle public health problems more commonly encountered in tropical and sub-tropical climates. Gray, Anderson and Wolf's reviews of the extant literature suggest that, when the average ambient temperature is relatively high, as is the case in tropical climates, *E. coli* and intestinal enterococci may be capable of multiplying outside of the human body [128, 142, 143]. For instance, as a consequence of a study of soil faecal contamination, based

on the use of faecal coliform as an indicator, Griffin et al. (2001) reported that coliforms deposited in soils may survive in substrates, and thus their presence may not mean that faecal wastes are continuously entering the tested area. This would make *E. coli* less compatible with the criteria for the definition of an ideal indicator [132, 141];

- The limitations of the currently most widely-used indicators are already recognised, as summarised by Griffin et al. [141]. The most interesting limitation is that current media-based techniques may not detect 'viable but non-culturable organisms' and that current indicators are shed by non-human animals, as well as by humans. Therefore occurrence does not always indicate that human pathogens are present;
- Many authors, including Leclerc et al. (2001) and Harwood et al. (2005), have challenged the validity of the classical indicator organism paradigm, suggesting that coliform bacteria do not always adequately reflect the occurrence of pathogens [144, 145]. This is particularly true for the wastewater effluents of disinfection treatment because coliform bacteria are, in general, more susceptible to chemical disinfection than are enteric pathogens [142, 145]. As the current study is focused on chemical disinfection, these limitations need to be carefully considered;
- Harwood et al. [145] analysed correlations between several indicator-pathogen combinations, using six different reclaimed water facilities effluents. As indicator organisms, they analysed:
    i. Total coliform bacteria;
    ii. Faecal coliform bacteria;
    iii. Enterococci;
    iv. *Clostridium perfringens*; and
    v. F-specific coliphages.

  The pathogens they considered were:
    i. Enterovirus;
    ii. *Cryptosporidium* spp.; and
    iii. *Giardia* spp.

  All the possible combinations of indicators and pathogens listed above (i.e., Cartesian product) were analysed and the surprising conclusion was that no combinations of indicator vs. pathogen showed a strong correlation. This point must be considered carefully, for the simple reason that, in the context of an emergency (e.g., a cholera epidemic), any effluent of a wastewater treatment plant is potentially at risk of being used by the

local population immediately after its discharge. In other words, a simple wastewater treatment plant, even if not designed to operate as a wastewater reclamation facility, may indirectly be used as such, therefore indicating the relevance of the Harwood et al. (2005) case study to this research [145]. On the other hand, bearing in mind the limitations of the indicator paradigm, it is important to note that, if *E. coli* is reduced by four or even five log values, then it may be reasonable to suggest that *V. cholerae* will also be significantly reduced.

- Leclerc et al. (2001) suggest that, for the same reasons, the shortcomings of other indicators, including *Enterococcus* spp. should also be considered, although no specific studies have yet been reported [144, 146].

2.7.2 LIMITATIONS OF THE INDICATOR PARADIGM IN THE SPECIFIC CASE OF CHOLERA

Curtis (1996), in a review of studies that considered the validity of the indicator organism paradigm, refers to comparisons of the behaviour of *E. coli* and *Vibrio cholerae* in estuarine waters made by Peterson et al. (1984): these studies pointed out some of the limitations of the studies that correlated *E. coli* and levels and those of *Vibrio cholerae* [147]. A comparison of the behaviour of *E. coli* and *Vibrio cholerae* in microcosms of estuarine water by Hood and Ness (1982) and Guthrie and Scovill (1984) came to similar conclusions [148, 149]. These limitations need to be considered in the search for the most appropriate available indicator of *Vibrio cholerae* in water and wastewater engineering contexts .

Early studies by Woese et al. (1985) and the further development of this area through the work of MacDonell et al. (1986) compared the ribosomal RNA (rRNA) of *E. coli* and *Vibrio cholerae*, the results suggesting that the two bacteria have a common evolutionary ancestor but, more importantly for this research, that substantial divergence has occurred at a later evolutionary stage. The most interesting conclusion of their research is that *E. coli* is no more related to *Vibrio cholerae* than it is to any other bacterium of the *Vibrionaceae* group [150, 151]. Based on the conclusion of Woese and MacDonell, as part of a review of the fate of *Vibrio cholerae* in wastewater treatment systems, Curtis (1996) suggests that, on the basis of existing microbiological knowledge of the behaviour of *Vibrio cholerae*, there is, in principle, no reason to assume – without having verified this

through laboratory studies – that *E. coli* is the best available surrogate for *V. cholerae*, and hence the best indicator among all those potentially available for the purposes of this research [79].

Finally, it should be noted that it is also important to remember that most wastewater disinfection efficacy testing makes no claims that the indicator organism reduction is correlated to the reduction of a specific pathogen. A 'faecal indicator organism' should not be confused with a 'surrogate'. Nevertheless, a significant reduction of the indicator presence allows the author to suggest that the level of enteric bacteria is being significantly reduced, therefore significantly reducing any hazard to human health associated with subsequent soil infiltration of the effluent.

2.8 RATIONALE FOR THE STUDY

As previously mentioned, the options available today for the treatment of wastewater in an emergency context may be suitable to deal with a bacteriologically-contaminated wastewater only if the water table is at least one and half metres lower than the lowest point of the excavated pit or trench [99]. This has historically been the case for most of the emergency settings in which the treatment of wastewater contaminated with enteric pathogens has been necessary. Therefore, the traditional approach of humanitarian organisations dealing with contaminated excreta has been to infiltrate all contaminated wastewater using *in situ* infiltration pits, latrines or an equivalent form of treatment. This is the reason why the manuals for water and sanitation field workers, which provide instructions on how to treat contaminated excreta, only mention these approaches. Unfortunately, as detailed in section 2.5, page 43, this approach is not always applicable, as soakaway pits and infiltration trenches are only suitable for dealing with a wastewater that contains high levels of enteric pathogens if the water table is significantly lower than the lowest point of the excavated pit or trench. Evaporation pans and evapo-transpiration beds, on the other hand, do not require the same water table depth, but are definitely not suitable for dealing with such a highly contaminated wastewater: given the quantities and high concentration of enteric microorganisms (and potentially pathogens) in this type of wastewater, both technologies would require the faecal waste to be dried for weeks (if not months) in an open area, inside or in proximity to a hospital, potentially putting the health of people living and working in the area at significant

risk. Therefore a new technology is needed to fill this engineering and health protection gap [99, 152]. The situation in Port-au-Prince, Haiti, perhaps offers the best example of the limitations of previous approaches because here the water table (or ground water level) is, in many areas of the city, higher than that required to operate a sanitary intervention in a safe way according to the classical approach (only 30-40 cm below ground level – see Figure 2.5).

Therefore, there is a need to develop a new method for the treatment of the highly contaminated wastewater deriving from an emergency health facility or, in the specific case of a cholera epidemic, from a CTC. Such a method must be designed so that it can, in principle, be applied to the treatment of any wastewater contaminated with pathogenic enteric microorganisms in any location in the world in which a high water table makes it impossible to rely on one of the standard techniques mentioned in the previous sections.

Figure 2.5: High groundwater level at the MSF hospital site in Port-au-Prince: the traditional approach for wastewater disposal was therefore not applicable

2.9 THE HAITIAN CASE STUDY: THE HUMAN HEALTH HAZARD OF WASTE DISCHARGE WITHOUT PRIOR DISINFECTION

The initial stages of this research project took place within a post-disaster setting. The need to develop an effective *in situ* treatment and disinfection process rapidly led to an operational system but the experience also raised a number of fundamental questions about the best way resolve a problem that is likely to be common to many other disaster settings. Therefore, the disaster response led the author, in consultation with his supervisors, to develop the research questions that

underlie this PhD study and it is therefore worth summarising the broader context of this first stage of the research here.

By October 2010, the rapid spread of the Haitian cholera outbreak had resulted in a pressing need for CTC facilities throughout the country and a novel, low–cost and consistently-effective way to treat and disinfect the wastewaters from MSF CTC operations was therefore urgently required. In Port-au-Prince, a partly-commissioned MSF maternity hospital ('Delmas 33') was converted by the organisation into a CTC within a matter of days. By the time its operational life ceased in early March 2011, more than 3,000 cholera in-patients had been treated at the facility. It was then converted back into a maternity hospital, and a new MSF CTC was established on nearby tennis courts. In total, at these two CTCs, MSF water and sanitation engineers were required to treat and dispose safely of over 620,000 litres of wastewater, which were potentially infected with high levels of *Vibrio cholerae*. Such wastes therefore needed to be treated and disposed of with extreme caution. Within the Haitian context, rapid intervention to provide effective disinfection of this wastewater was essential, in order both to control disease transmission and to respond to the prevailing concerns of the local populace with regard to the management of cholera wastes by international organizations [153].

Because of the non-availability of an accepted technique for the low–cost treatment of contaminated faecal waste in the context mentioned, the response of many of the international organizations operating in the wake of the Haiti cholera outbreak from the outset was to instigate road haulage (by tanker) of all faecal waste originating from cholera patients (chlorinated or otherwise) to a centralised waste pit at the Truitier landfill site on the outskirts of Port-au-Prince. This facility was situated close to the impoverished and densely populated community of Cité Soleil on the western outskirts of the capital, a few hundred metres from the coast and on the aquifer of the Cul-de-Sac plain, traditionally a source of raw drinking water for the city of Port-au-Prince [154]. This suggests that the practice of disposal of cholera wastes to the environment represents a potential hazard to human health in this context.

The main reasons why it would be imprudent to assume that a wastewater disinfection process based on 'super-chlorination' would consistently disinfect the waste to an adequate degree have been explained in section 2.6.1. Moreover, road haulage (by tanker) of significant quantities of contaminated wastewater to a centralised waste pit

can reasonably be considered to be hazardous to human health, particularly within the complex, often chaotic, urban context of many post-emergency scenarios.

Although the work undertaken in Haiti successfully provided a novel and effective treatment system in an emergency setting, the experience raised important questions regarding the treatment mechanisms and process optimisation that clearly required detailed and well-managed laboratory studies to answer.

2.10 AIM OF THE STUDY

In the work described here, the author aimed to develop an innovative evidence framework that would contribute to the design and operation of a novel treatment system to disinfect highly contaminated hospital faecal waste in the context of an emergency, with a particular, but not exclusive, focus on the context of a cholera epidemic. Treating the contaminated faecal waste effectively *in situ* eliminates the need for road haulage and disposal to poorly-managed waste facilities. The ultimate aim was therefore the protection of the health of the inhabitants of the area surrounding the health facility from the potential risk of disease associated with contaminated wastewaters. This implied the achievement of following objectives:

- To design a treatment technology capable of treating highly contaminated wastewaters, achieving a degree of microbial inactivation that will enable it to be infiltrated into soil or discharged to surface waters without presenting an elevated risk of human disease transition in neighbouring communities;
- To quantify by means of carefully designed laboratory studies the degree of microbial inactivation and suspended solids removal efficacy that can be achieved within a low–cost treatment process;
- To evaluate whether the level of faecal indicator bacteria in CTC wastewater effluents can be reduced to levels that meet international effluent consent standards

This framework aimed to ensure that future emergency wastewater treatment technologies remain low–cost, robust and easy to set up within a few days. Further, it is essential that this innovative technology should be subjected to a robust critical risk evaluation of each stage of the treatment, in order to maximise human health protection at the time of the emergency and to enable the organisation applying it to gain the fullest possible benefit from the resulting evidence-base.

It is important to point out that during the Phase 1 of this study (field work) a significant amount of effort was put by the author into the treatment of the sedimentation sludge prior to the discharge into the environment. The sludge was dried and transformed into a so called ‘cake’ [125] and partially burned into a furnace at 600^{o}C in order to make it safe to be discharged into an infiltration pit. The sludge treatment is not the main focus of this research and therefore its results are not detailed within this thesis. During Phase 2 of the current study it was decided how to prioritize the research, rather focussing the laboratory research to the characteristics of the supernatant. Further study is needed to more accurately assess the characteristics of the sedimentation sludge and possibly optimize the discussed protocols to improve its characteristics prior to the discharge into the environment.

3. CHAPTER THREE – MATERIALS AND METHODS

3.1 FIELD STUDIES: THE HAITI TREATMENT SYSTEM

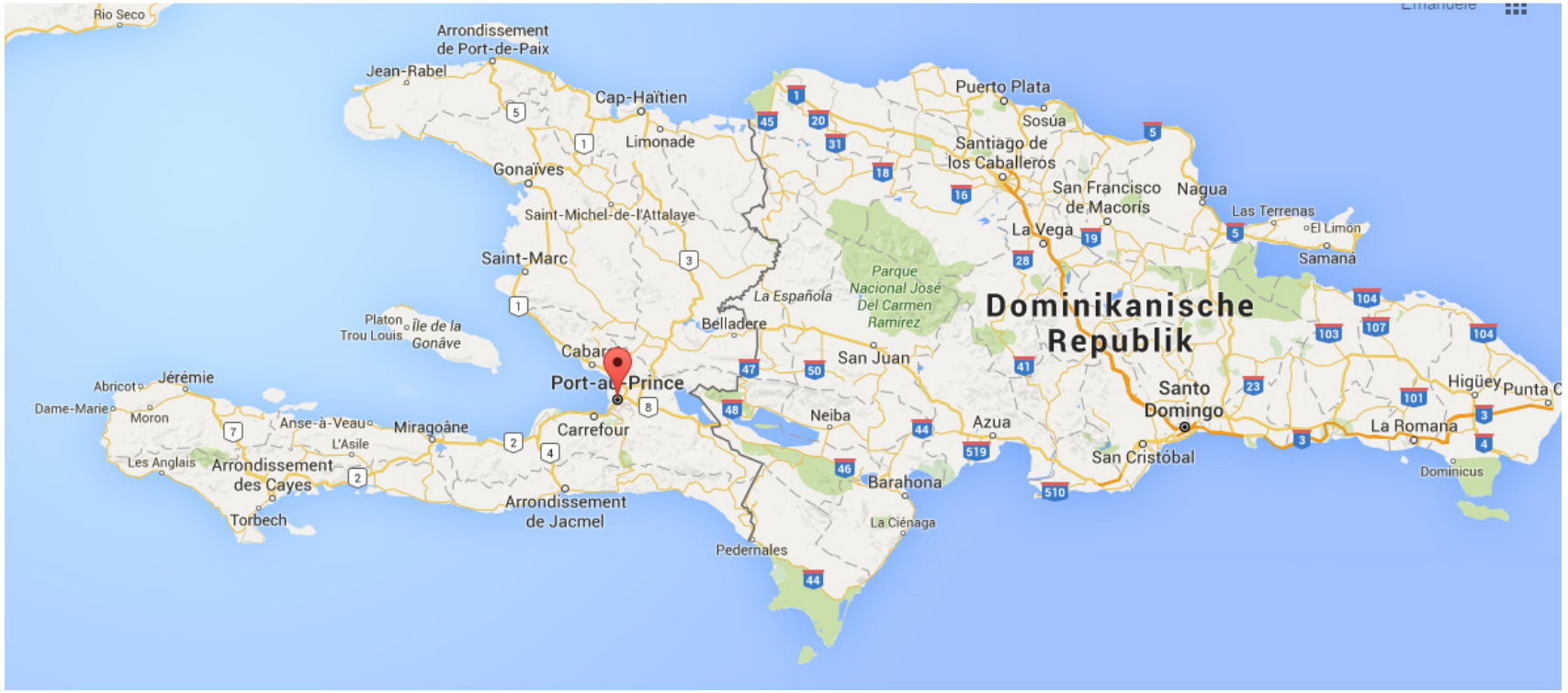
The following section describes the studies undertaken during 'Phase I' of the research project in Port-au-Prince, Haiti, between January and October 2011. During the first phase of the research, the author was actively involved in the research pilot project, having the role of 'Water, Sanitation and Hygiene' field-expert, and working on the design and management of the pilot project described below in response to the Haitian cholera outbreak. The author was supported by a multi-disciplinary team of experts, who remotely provided advice and logistical support from the UK (University of Brighton) and from Amsterdam (MSF OCA Head Quartier). The author's supervisor took a leading support role within the same team, was constantly in contact with the author during the entire duration of the field work and was also present in the field for two weeks to supervise the design of the pilot treatment plant, supporting the authors efforts to ensure that the intervention was evidence-based and appropriate to the emergency context at hand.

The next sections briefly describe how the author formulated and elaborated the final treatment protocol, in consultation with his advisor. At the beginning of the field work presented in this section, the author needed to recruit six staff members to help him to perform the most physically-challenging components of the work. The staff members were recruited according to MSF policies and from within the population living in proximity to the two field locations.

3.1.1 SITE DESCRIPTION

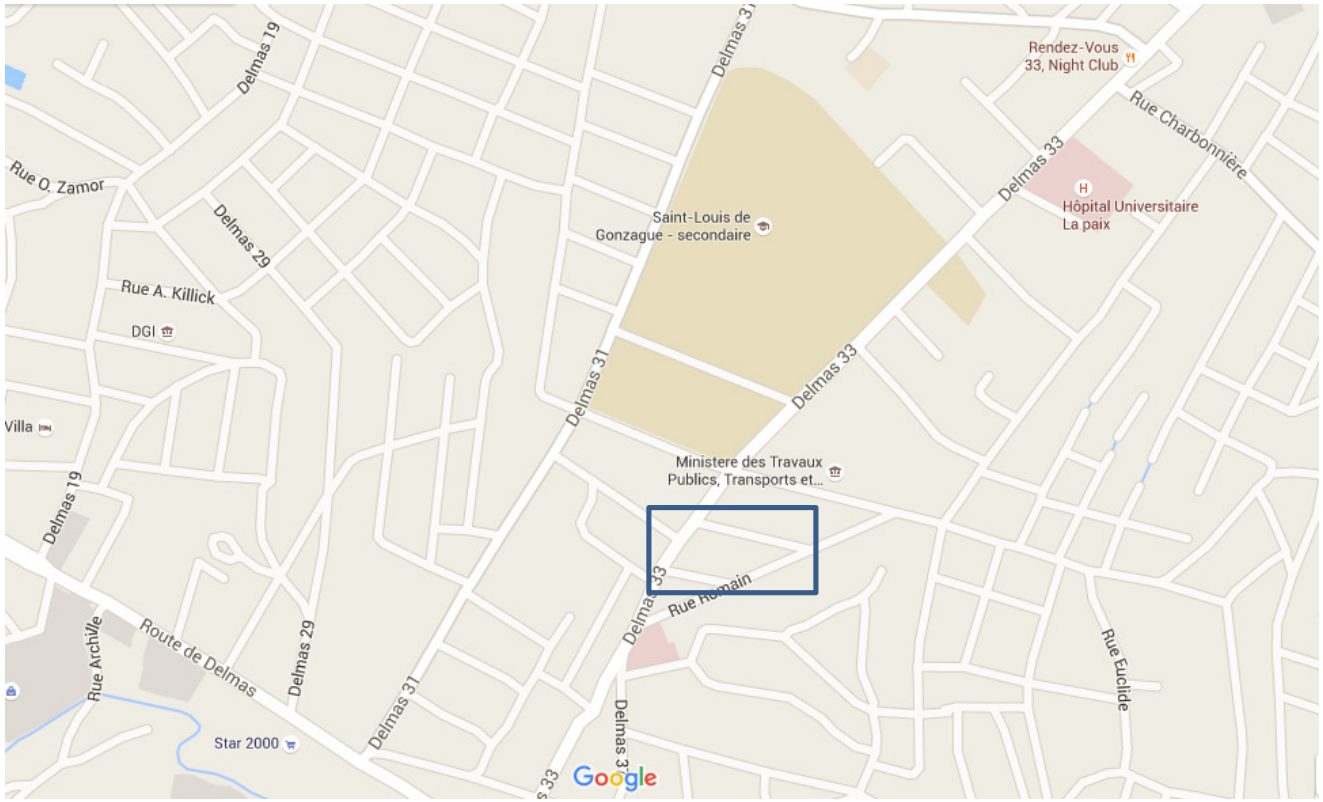
As mentioned in Chapter 2, batch treatment systems based on high– ('Protocol A') and low–pH ('Protocol B') physico-chemical treatment were designed, operated, and monitored within two CTC operations in Port-au-Prince, over a period of more than six months.

The first site at which the fieldwork experiments took place was a partly-commissioned MSF maternity hospital converted by the organisation into a CTC. The hospital was located in the 'Delmas 33' city district. Figures 3.1, 3.2 and 3.3 show the approximate location of the site (located at latitude: +18.552 and longitude: -72.303).



Figures 3.1: The location of Port-au-Prince, Haiti, in which the fieldwork took place (source: Googlemap®,4th March 2016)

3.2: Location of Delmas 33 District in Port-au-Prince (see rectangle - source: Googlemap®,4th March 2016)



3.3: Location of the first site at the partly-commissioned MSF maternity hospital at latitude: +18.552 and longitude: -72.303 (see rectangle - source: Googlemap®,4th March 2016)

Initial laboratory pilot-scale studies of high– and low–pH treatment, using simple five-litre beakers, were followed by full-scale batch treatment of the wastewater using both protocols, initially at the 'Delmas 33' CTC. At a later stage, and following closure of this facility, a new, full-scale wastewater treatment facility was established at the nearby 'Delmas-Tennis Court' CTC. Figure 3.4 shows the approximate location of the site (located at latitude: +18.558 and longitude: -72.300).

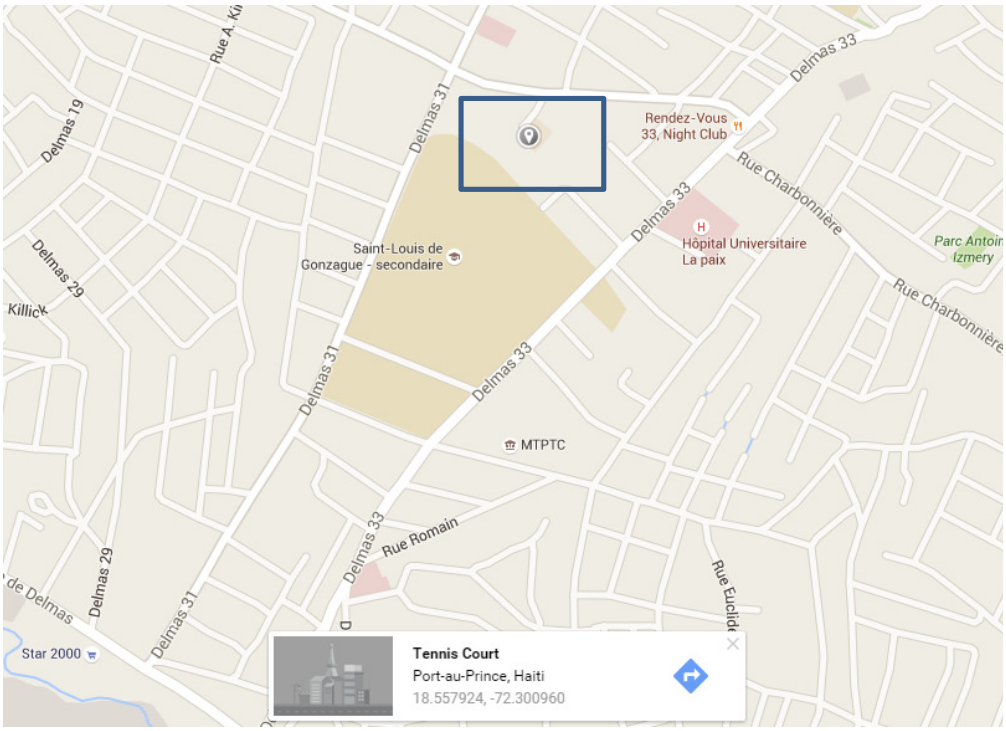


Figure 3.4: Location of the 'Delmas-Tennis Court' CTC site at latitude: +18.558 and longitude: -72.300 (see rectangle - source: Googlemap®,4th October 2015)

The results reported in Chapter 4 refer exclusively to the analysis of batches that were treated when adequate monitoring equipment had become available in the field. Protocol A was used for the treatment of two batches of wastewater and Protocol B was used for the large-scale treatment of six batches of CTC wastewater, the batch volumes being in all cases between 10 and 15 m^3.

A detailed risk assessment was undertaken for each stage of the project. This included details of operator hygiene requirements and the appropriate use of personal protective equipment to minimise operator contact with potentially corrosive chemicals [153].

3.1.2 FULL-SCALE OPERATION OF THE TWO PROTOCOLS

Laboratory jar-testing of the high–pH treatment process (Protocol A) using hydrated lime ($Ca(OH)_2$) and, at a later stage, the low–pH treatment process (Protocol B) using aluminium sulphate, was followed by full-scale batch treatment. Here, wastewater and coagulants (added at concentrations suggested by the jar-tests) were combined within regimes that mimicked, as closely as possible, initial 'rapid-mixing' followed by slow-mixing, and finally settlement for a minimum period of 14 hours, all within a 30 m^3 circular open tank.

At this stage it is important to point out that for this type of project the classical working sequence for the experimental work is as follows:

1. Bench scale experiments
2. Pilot scale experiments
3. Full scale experiments

As previously mentioned in Chapter 2 and in particular in section 2.9 and 2.10, an important aspect of this study was the fact that it was partly limited by the constraints of an emergency setting. The urgency to find a rapid solution for the disinfection of a significant amount of highly contaminated faecal waste at the very beginning of the project forced the author of the current study to reduce the initial bench scale (or pilot scale) test to a limited amount of experiments, immediately followed by the full scale treatment cycles. These had the 'double' aim to quickly treat the contaminated faecal waste and test the performance of the system at the same time. Only at a later stage during Phase 2 of the research it was possible to assess in detail, through laboratory based bench scale experiments, the performance of both protocols and optimize it. Therefore, the circumstances

forced the author to follow an unusual working sequence for the experimental work. Although, chronologically, the pilot study took place first, the treatment procedures and diagrams, with particular focus on the full scale processes, are presented here first. Subsequently the laboratory procedure followed during the pilot-scale study is explained.

Sections 3.3.1 and 3.3.2 illustrate how the full-scale operation of high– and then low–pH treatment was performed in the field. Sections 3.3.3 and 3.3.4 then illustrate the laboratory procedures used in the pilot-scale (or bench-scale) field study of high–and then low–pH treatment.

3.1.3 FULL-SCALE OPERATION OF HIGH–pH TREATMENT

Figure 3.5 outlines the full-scale treatment procedures adopted for the high–pH treatment. The 30 m^3 treatment tank (reactor) was filled to a maximum level of approximately two-thirds of the total capacity of the tank. The wastewater was then mixed by re-circulation, using a petrol-fuelled centrifugal pump, so as to obtain a homogenous mix. The established set of bacteriological and physico-chemical parameters measured during the pilot-studies was determined for the wastewater influent from grab samples of approximately 30 to 50 ml. In addition, the COD (mgO_2/L) of the reactor influent was measured.

The lime slurry was prepared by adding hydrated (slaked) lime to chlorinated drinking water (as this was the only available water at the full-scale treatment location), at a concentration of approximately 20 g/L, in a 200 litre drum, placed on a platform above the reactor tank, directly above the influent pipe (see Figure 3.6). Lime slurry was continuously added to the wastewater, with the inflow hose running parallel to the tank wall by means of an 'elbow-joint', in an attempt to achieve 'rapid-' or 'flash-mixing', until the pH level of the circulating wastewater was measured to be greater than, or equal to, 11.4. This stirring phase is defined as the coagulation phase (also commonly called 'flash-mixing' or 'rapid-mixing' phase). Subsequent stirring was defined as the 'flocculation phase' (which is also commonly referred to as a 'slow-mixing' phase) [125, 155]. 'Rapid-' or 'flash-mixing' is defined as the phase of the coagulation-flocculation process that immediately follows the addition of the coagulant and is intended to achieve effective coagulation. During this phase, high–energy, turbulent, rapid-mixing enables the system to disperse the coagulant effectively and promote particle collisions. Only a fraction of a second is required in theory to achieve complete particle collisions in an ideal system, if perfect mixing conditions are achieved. In practice, this phase takes up to one to three minutes in most batch or continuous physico-chemical treatment processes [155].

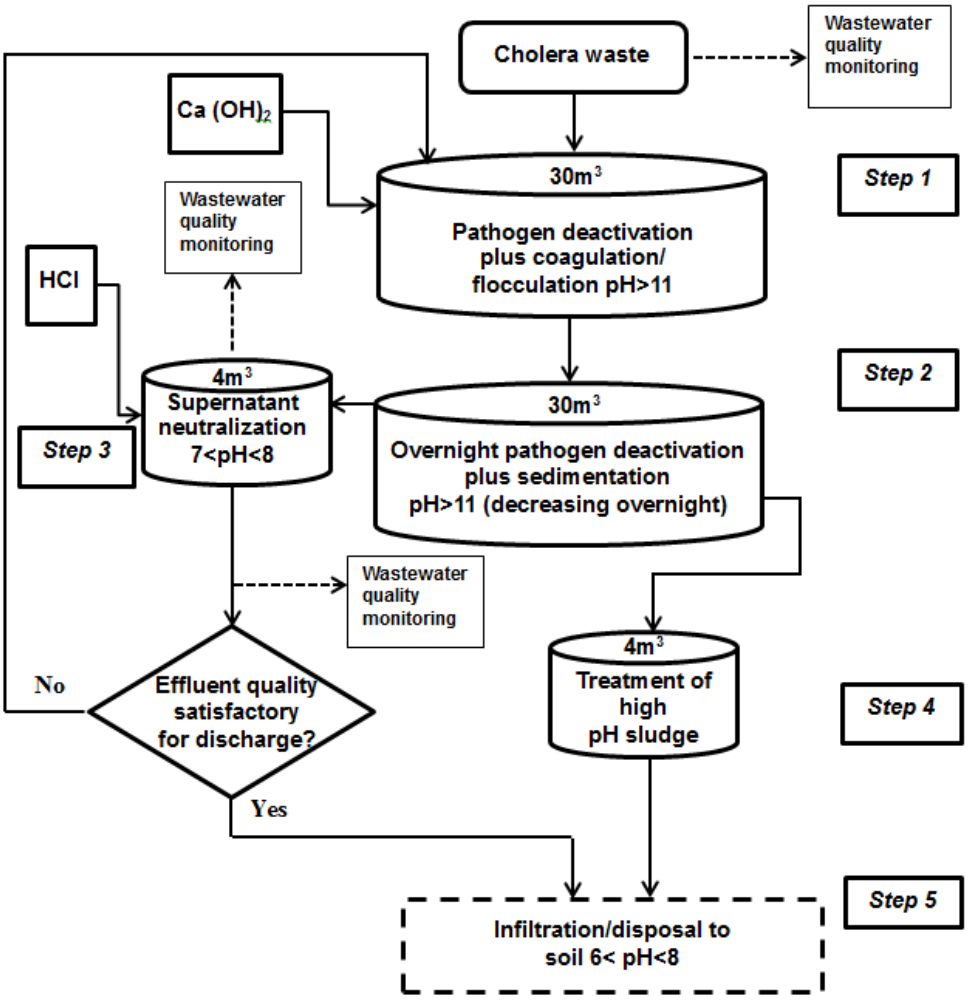


Figure 3.5: Schematic overview of the high–pH treatment protocol.

Figure 3.6: Lime slurry preparation: mixed lime slurry was added continuously to the contents of the sedimentation tank in order to approximate a rapid-mixing regime. The inflow hose was positioned to run parallel to the tank wall by means of an 'elbow-joint'

Once the target pH level had been reached, the pump was operated continuously at a relatively low revolution rate for approximately 15 minutes, in order to achieve 'slow-mixing' [155], and consequently to aid flocculation of the reactor contents. 'Slow-mixing' is defined as the phase that aims to increase the particle size from micro-flocs to visible suspended particles (flocculation). When the floc-particles collide, they bond to produce larger, visible flocs (through 'bridging', 'binding', and 'strengthening') increasing the weight and settling rate. Once they have reached their optimum size and strength, the 'matrix' is ideally ready for sedimentation. Contact times for

flocculation range typically from 15 to 30 minutes. To prevent flocs from tearing apart or shearing, the mixing velocity and energy should ideally be reduced as the floc-size increases.

The pump was then switched off and the wastewater left to settle for at least 14 hours. A small grab sample of the resulting ('partially treated') supernatant was removed for analysis using the same set of parameters used to test the untreated wastewater influent (the sample was adjusted to a pH level of between 7 and 8 by the addition of HCl in the field laboratory before analysis). After measuring the depth of sludge in the tank, the supernatant was carefully pumped into a nearby 3.8 m^3 tank, taking care not to re-suspend the sludge. The contents of this tank were then adjusted to a pH level of between 7 and 8, by the addition of HCl. A final sample of supernatant was removed for analysis as before.

Providing that the effluent had reached a quality considered to be 'satisfactory' (defined as having achieved a turbidity level of less than 50 NTU, a pH level of between 6 and 8, and containing fewer than 1,000 thermotolerant coliform colony-forming units (CFU) per 100 ml), this 'final effluent' was then carefully infiltrated into *in situ* soil trenches. If the effluent quality failed to meet these quality criteria, the entire treatment procedure was repeated before the final effluent was allowed to be infiltrated to the soil.

3.1.4 FULL-SCALE OPERATION OF LOW–pH TREATMENT

Figure 3.7 outlines the full-scale treatment procedures adopted for the low–pH treatment. The process of tank filling was identical to that followed for high–pH treatment and grab samples of the influent were analysed for the same parameters prior to treatment.

HCl was then added to the tank contents until the pH level of the circulating wastewater was recorded to be equal to, or lower than, 3.9. Once the target pH level had been reached, the contents were recirculated slowly using a petrol-fuelled centrifugal pump for five minutes to ensure that the pH level within the reactor was as homogenous as possible. The pump was then switched off, and the tank contents were left to stand for a minimum period of no less than 14 hours.

When the depth of sludge in the tank had been measured, the supernatant was carefully pumped (taking care not to re-suspend the limited quantity of sludge that had been produced at this stage) into a nearby smaller tank (3.8 m^3). The contents of this

tank were adjusted to a pH level of between 6 and 7, by the addition of lime slurry (prepared as previously explained for the high–pH treatment protocol), before a grab sample was removed for analysis using the same set of parameters, as before. The wastewater at this stage was considered to be ‘partially treated’.

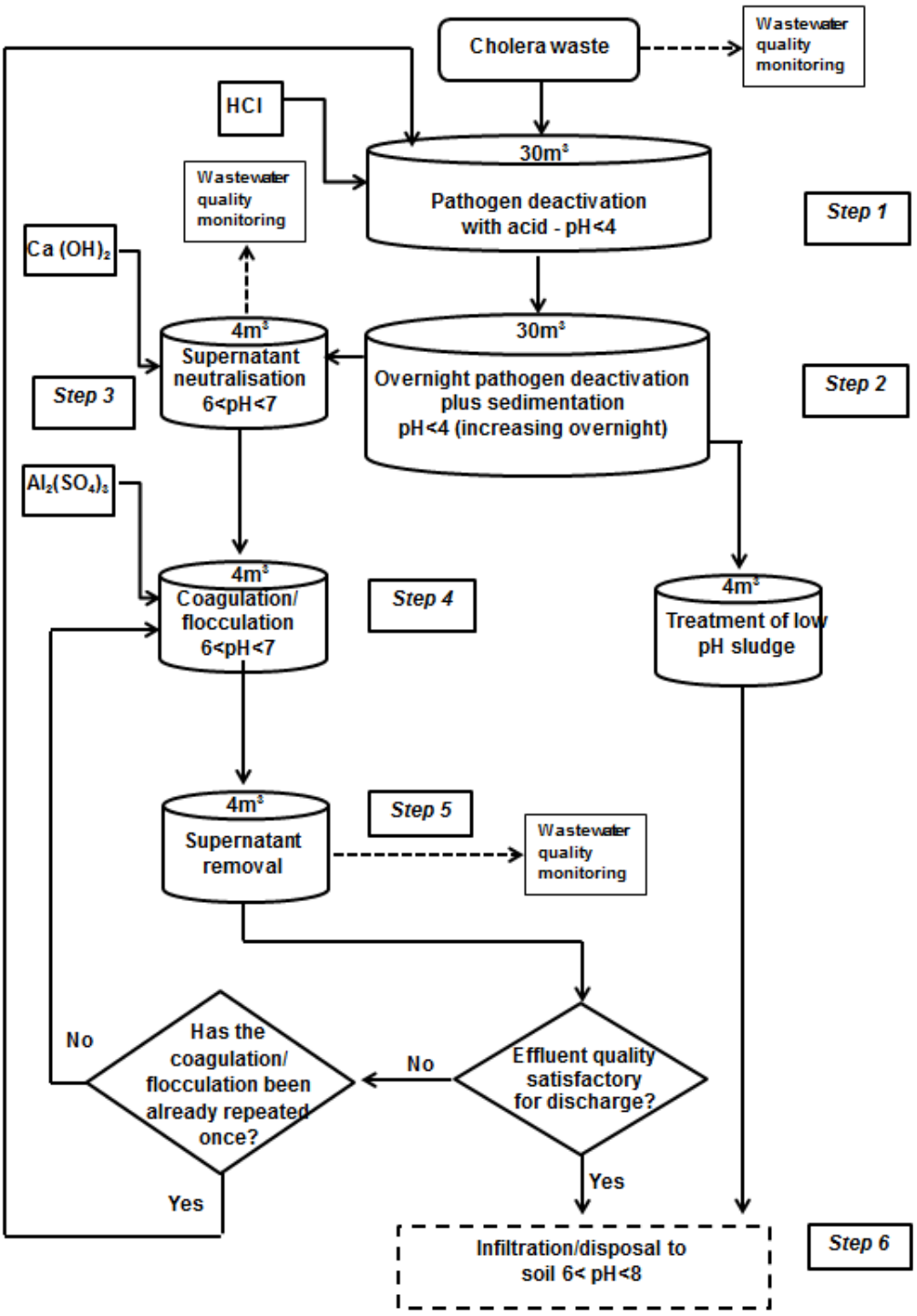


Figure 3.7: Schematic overview of the low–pH treatment protocol.

It is perhaps worth noting that, while the addition of HCl, as described above, did not in itself result in coagulation/flocculation, it was considered useful to take advantage of unaided overnight sedimentation before the supernatant was removed for subsequent coagulation/flocculation the next day. The remaining, relatively small quantity of 'low–pH (disinfected) sludge' removed from the bottom of the treatment tank in Protocol B was blended with the much larger volume of 'high–pH sludge' produced by Protocol A, effectively producing a pH-neutral blend.

A concentrated solution of aluminium sulphate was prepared by dissolving approximately 300 g of the hydrated salt in 1 litre of chlorinated drinking water. Four transparent beakers, each containing 1 litre of wastewater, were used as simplified jar-tests, with the aim of determining the quantity of coagulant needed to achieve adequate sedimentation. This was found to be approximately 100 mg/l. The aluminium sulphate solution was added to each 3.8 m^3 tank, with manual 'rapid-mixing' achieved using a short stirring rod for approximately five minutes ('flash-mixing'), followed by a manual slow–mixing phase of about 15 minutes, using a longer stirring rod to improve the formation of flocs (slow–mixing phase shown in Figure 3.8).

Figure 3.8: Slow mixing phase for the low–pH treatment.

The wastewater was then left to settle for approximately one hour and a grab sample of approximately 30 ml of supernatant ('final treated effluent') was tested for the standard parameters, as before. Provided that the effluent had achieved the 'satisfactory' quality previously specified under Protocol A, the supernatant was carefully removed (taking care not to re-suspend the limited quantity of sludge that had been produced at this stage) and then infiltrated to the soil. Again, if the quality criteria for satisfactory final effluent had not been met, the coagulation/flocculation procedure, using aluminium sulphate, was repeated. If the effluent quality level had still not met the specified quality standards at this stage, the entire treatment process, including low–pH disinfection and coagulation/flocculation, would have been repeated, but in practice this was never required.

The remaining, relatively small quantity of neutral sludge removed from the bottom of the smaller tank during this treatment phase was blended with the much larger volume of sludge produced by high–pH treatment and the small volume of sludge produced during the first phase of low–pH treatment.

3.1.5 PILOT-SCALE STUDY OF HIGH–pH TREATMENT

Simple jar-test studies, using five-litre beakers, were initially used to investigate the efficacy of high–pH treatment with regard to the removal of thermotolerant coliforms and suspended solids (or turbidity). At the inception of each jar-test, a small sample of untreated wastewater (approximately 30 ml) was taken and the following parameters were tested for: turbidity – recorded as nephelometric turbidity units (NTUs); presumptive thermotolerant coliforms – recorded as colony-forming units (CFUs) per 100 ml; pH level; and quantities of chemical reagents used – recorded as grammes or milligrammes per litre. The first step of each jar-test experiment involved the step-wise addition of hydrated lime slurry ($Ca(OH)_2$) to the wastewater, until the pH of the mix reached a level between 11.4 and 12.2. This was immediately followed by three minutes of 'rapid-mixing', followed by 15 minutes of slow-mixing. Both steps were achieved manually in the absence of a mechanical jar-test rig.

The contents of the beaker were then left to settle overnight. The supernatant was subsequently removed and its pH level adjusted to approximately 7 (within a 6 to 8 pH range) by the addition of concentrated hydrochloric acid (HCl). At the end of each 'jar-test' process, a small sample (approximately 30 ml) of supernatant was removed and tested for the same set of wastewater quality parameters as mentioned previously.

3.1.6 PILOT-SCALE STUDY OF LOW–pH TREATMENT

Simplified 'jar-test' studies were similarly performed in order to investigate the efficacy of low–pH treatment. At the inception of each jar-test, a small sample of untreated wastewater (approximately 30 ml) was tested for the same set of parameters as in the high–pH treatment.

The first step of the jar-test experiment for Protocol B involved the addition of hydrochloric acid (HCl), at a quantity that was sufficient to decrease the wastewater pH to a level between 3.7 and 3.9, so as to achieve disinfection of the wastewater. This was immediately followed by 'rapid-mixing' for one minute. Following overnight sedimentation, the wastewater was adjusted to a pH level of approximately 7, by the addition of the hydrated lime slurry that was also used for Protocol A. At this point, another small sample (approximately 30 ml) of supernatant was removed for analysis as before.

Aluminium sulphate (75 to 150 mg/L - either as $Al_2(SO_4)_3 * 16H_2O$ or as $Al2(SO_4)_3 * 18H_2O$) was next added to the beaker as a coagulating agent in order to aid suspended solids removal, and consequently, to achieve a further reduction in bacterial levels. The addition of aluminium sulphate was immediately followed by three minutes of 'rapid-mixing', followed by 15 minutes of slow–mixing.

Following the slow–mixing phase, the wastewater was allowed to settle in the five-litre beaker reactor for one hour. Once again, a small sample of supernatant (approximately 30 ml) was removed for analysis, as before.

3.2 MONITORING OF FIELD TEST TREATMENT PERFORMANCE

The following set of physico-chemical and bacteriological analyses were performed on all high–pH and low–pH treatment samples (both during the pilot-scale studies and full-scale plant operation). The main aim of all analyses was to determine the degree of reduction in turbidity (NTU) or total suspended solid (TSS), and thermotolerant coliforms (CFU per 100 mL). Measurements of COD concentration were only achieved during full-scale operation of high–pH treatment. All analyses were undertaken on grab samples, typically 30-50 ml of the wastewater, taken either from the five-litre beakers (pilot-scale trials) or from the full-scale treatment tanks. The type, quality and amount (as mass of solid or volume of liquid chemical) of all reagents used were recorded in order to determine the performance of both studied protocols, in terms of cost of treatment per unit of raw liquid waste volume.

3.2.1 PHYSICO-CHEMICAL PARAMETERS

Initially, turbidity levels were recorded (as NTU) following a simplified 'turbidity tube' method [156]. This method was later replaced by a nephelometric method, using a Hach portable turbidimeter (model 2100P), which operated within a wavelength range of 400 to 600 nm [157]. All turbidity data reported in this thesis were recorded nephelometrically.

Measurement of total suspended solids (as mg/L) was achieved by filtration of the sample through a glass-fibre filter, according to standard methods [158]. As an oven was not available in the field, filters were dried at ambient temperature (normally greater than 30 °C) until constant weight was achieved (normally within 48 hours). During the follow–up laboratory phase of this study, a bench-scale test was performed in order to compare the efficacy of filter drying at ambient temperature with filter drying at 105Cº in an incubator. This test was undertaken to demonstrate that the non-standard protocol used in Haiti achieved an acceptable result.

pH levels were measured several times during both protocols in order to minimise the quantity of reagents used to achieve adequate disinfection (and in the case of Protocol A, to ensure effective coagulation and flocculation). The pH level was also frequently measured during the later neutralisation phases (both protocols) in order to achieve a final pH level of between 6 and 8. A Palintest Micro 500 pH meter was used for all measurements, according to standard methods [159]. pH buffers (7.0 and 4.0) were used for pH meter calibration and pH probes were stored in a saturated KCl solution. In addition, because of the potential for damage to the probe at high–and low–pH levels, simple pH litmus paper strips were frequently used to verify the pH values obtained.

COD (as mgO_2/L) was measured according to a standard *closed reflux, colorimetric method* [160]. A simplified spectrophotometric field kit (Palintest), operating at a wavelength of 600 nm, was used. The samples were digested at 150 ºC for two hours in a strong solution of sulphuric acid, in the presence of chromium and silver salts. The tubes were then cooled and the colour was measured using the Palintest photometer. Four test kits were used, with a maximum detection level of either 2,000 mg/l or 20,000 mg/l, for analysis of the influent, and either 150 mg/l or 400 mg/l, for analysis of partially, or fully-treated wastewaters.

Despite the practical field constraints encountered in Haiti, the quantities of all chemical reagents were considered to have been recorded as accurately as possible during all operations.

3.2.2 BACTERIOLOGICAL PARAMETERS

Presumptive counts of thermotolerant coliforms were recorded as CFU per 100 ml, using a DelAgua water-testing kit. The kit procedure uses the 'thermotolerant (faecal) coliform membrane filter procedure', which is based on standard methods [161]. The procedure involves membrane filtration through a sterile nitrose-cellulose membrane filter (0.45 µm), sterilised by the production of formaldehyde, which is formed by burning methanol. Acidic and alkaline samples were washed through the filter with an excess of distilled water for one minute, to ensure that the pH level of the membrane prior to incubation approximated neutrality. Following filtration, the filters were incubated at 44 °C ±1 °C for 18 to 24 hours, on sterile absorbent pads, soaked in membrane lauryl sulphate broth (Oxoid). Samples were diluted according to their predicted bacterial counts, using de-ionised water. Following incubation, all yellow colonies of greater than 2 mm diameter were enumerated and recorded as CFU of presumptive thermotolerant coliforms per 100 ml of the original sample.

3.3 LABORATORY TRIALS IN THE UK

The following section describes the studies undertaken during 'Phase II' of the research project in the laboratories of the University of Brighton, UK.

3.3.1 DESIGN AND OPERATION OF LABORATORY TREATMENT TRIALS IN THE UK

During the laboratory-scale trials undertaken in the UK from 2012 to 2015, various concentrations of hydrated lime (or hydrochloric acid) solution were used to increase (or decrease) the pH of 2 L of a 'faecal waste matrix' mix. The rationale for the use of a defined 'waste matrix' for these experiments is briefly elucidated here and further discussed in Chapters 5 and 9.

Following initial laboratory trials, and as a consequence of the results, the decision was taken to prepare the 'faecal waste matrix' by the addition of 80% (vol/vol) municipal wastewater to 20% (vol/vol) highly concentrated sludge.

The wastewater was collected before each test from the influent prior to preliminary screening, grit chamber and primary sedimentation at Southern Water's Hailsham wastewater treatment plant in East Sussex, UK. Sludge was regularly collected from two stabilisation tanks located at the same wastewater treatment facility. Both tanks

are used for the storage and partial stabilisation of untreated sedimentation sludge transported to the Hailsham facility from wastewater treatment plants in the local vicinity that are not equipped with facilities for the stabilisation of primary and secondary sedimentation sludge. The Hailsham wastewater treatment site is located in Hailsham at latitude: +50.87426 and longitude: +0.27238.

The sludge was added to the municipal wastewater with the goal of increasing the concentration of enteric microorganisms, suspended solids and COD in the experimental treatment matrix in order to generate a 'faecal waste mix' that was as similar as possible to that produced by a health facility in an emergency setting.

During the initial test cycles, which were designed to optimise the laboratory test procedure and to define the test methodology, the matrix used for the experiments was prepared immediately after wastewater and sludge collection, refrigerated at 4 ºC (+/- 2 ºC) and used within approximately two weeks. The quality of the faecal waste mix was monitored during this time period in order to estimate any reduction in levels of enteric microorganisms, COD and any change to other physico-chemical parameters resulting from biologically-mediated degradation processes that were slowly occurring in the matrix with time. Further details of the rationale for the chosen composition of the faecal waste matrix are given in Chapter 9.

For all the batch treatments for which results are presented in this dissertation, wastewater and sludge were mixed and processed within 24 to 72 hours of collection in order to maximise the concentration of indicator organisms, pathogens, suspended solids and COD at the beginning of each jar-test.

3.3.2 EXPERIMENTAL SET-UP FOR THE HIGH–pH TREATMENT PROTOCOL

Initially, various concentrations of hydrated lime solution were used to increase the pH level of 2 L of selected 'faecal waste matrix' mix. The laboratory experimental procedures that were then followed for the validation and optimisation of the high–pH treatment protocol are summarised in Figure 3.9.

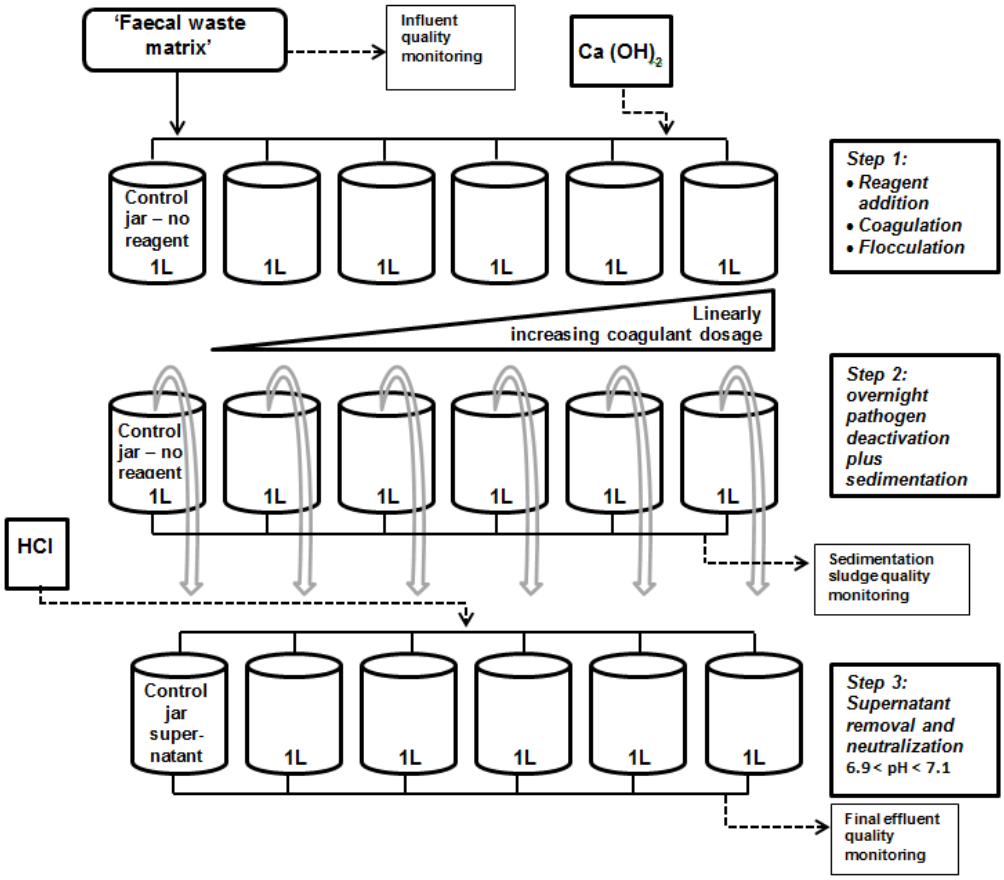
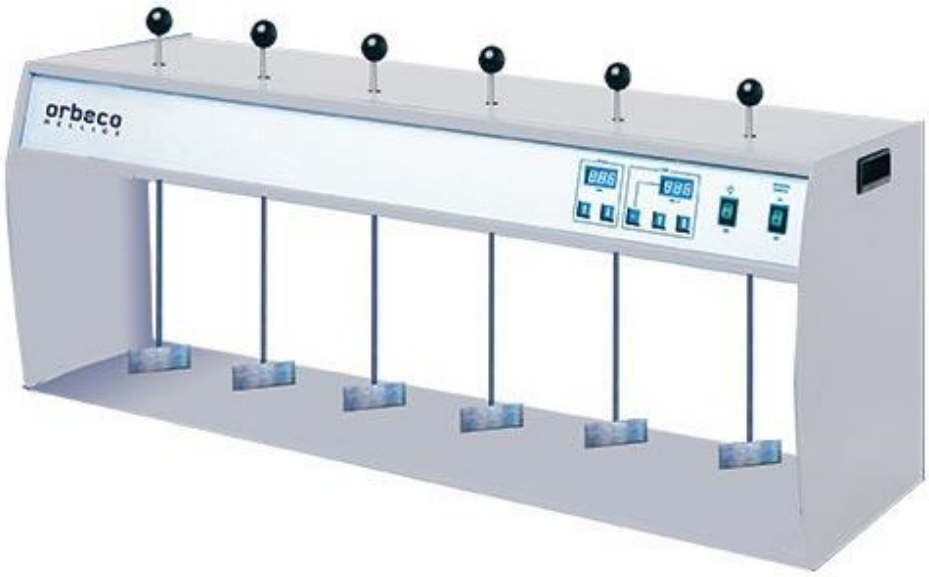


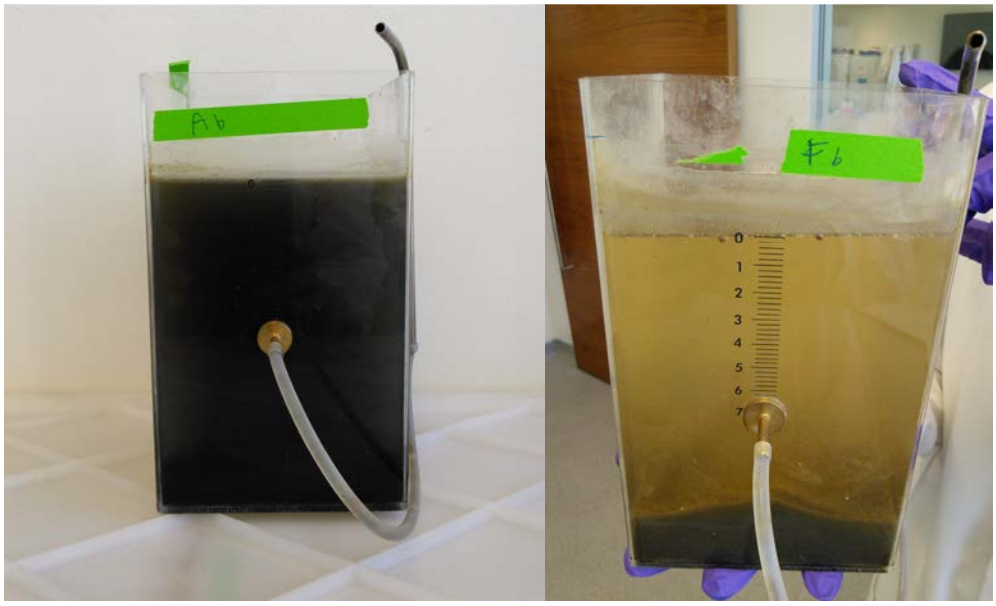
Figure 3.9: Schematic overview of the high–pH laboratory experimental procedure

The experiments were conducted using an Orbeco Hellige standard jar-test unit (Figure 3.10) that comprises six paddle rotors (24.5 mm x 63.5 mm), equipped with six square beakers, each of 2000 mL volume.



(Figure 3.10*: Orbeco Hellige standard jar-test unit (source: Orbeco Hellige website – 20th August 2015)*

Figures 3.11 and 3.12 show the 2000 mL square beakers



(source: *Orbeco Hellige website – 20th August 2015)*

Figures 3.11*: Square beakers at the end of the treatment*

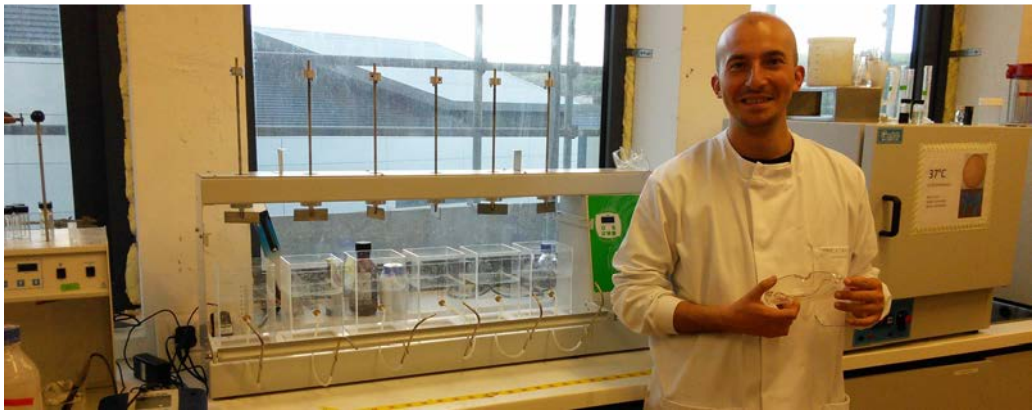


Figure 3.12: Square beakers aligned before treatment commencement

The standard jar-test unit and the square beakers used were part of a kit that allows the user to monitor the average velocity gradient [as s^{-1}] during each mixing phase. The definition of velocity gradient and its significance is illustrated in section 2.6.3. The correlation between G_{ave} [as s^{-1}] and paddle rotation frequency, expressed as 'rotations per minute' (RPM) [as min^{-1}], was defined by the manufacturer of the selected machine. The correlation is linear and is represented in Figure 3.13.

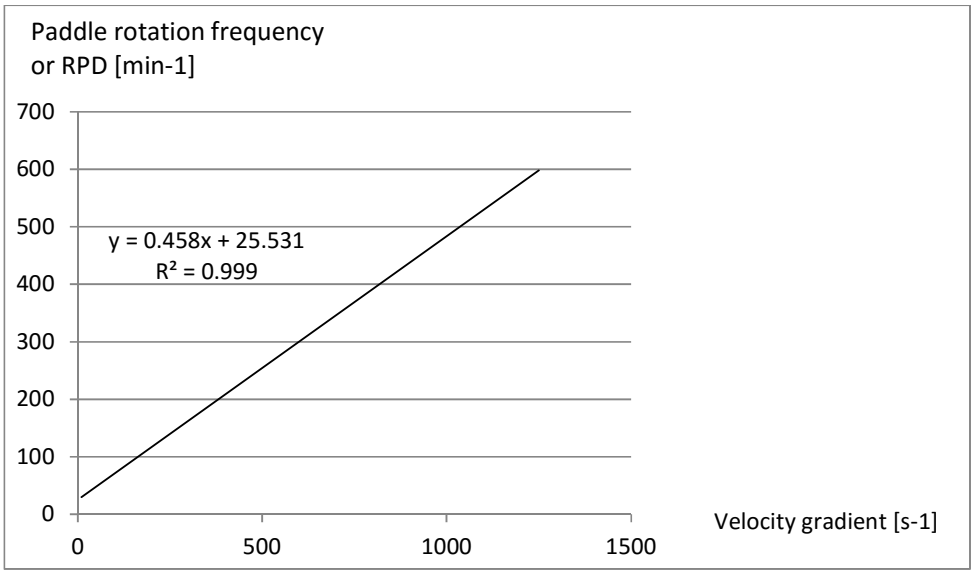


Figure 3.13: Linear correlation between velocity gradient [as s^{-1}] on the axis of abscissae and paddle rotation frequency or RPM [as min^{-1}] on the axis of ordinates (source table printed on Orbeco Hellige standard jar-test unit)

The quantity of hydrated lime added to each jar varied slightly over the course of this study. The reason for this is further discussed in Chapters 5 and 9. A pH target was determined during the design phase of the study. Subsequently, a preliminary test was performed before each cycle, to take into account the quality of the new faecal waste mix with regard to: its magnesium and alkalinity content, the amount of solid

and organic matter and, perhaps most importantly, its buffer capacity. The ultimate goal of the preliminary test was to determine with a degree of accuracy the quanitity of hydrated lime to be added to each new faecal waste mix in order to reach the target pH.

The preliminary test was performed the same day as each jar-test, normally two (maximum four) hours before and consisted of the incremental addition of lime to a 100 ml sample of faecal waste mix, followed by rapid manual mixing, recording of the pH level and fresh lime addition. The incremental addition of coagulant was, on average, repeated ten to 12 times until a pH level greater than 12 was achieved. The aim was to plot a graph that correlated the amount of added hydrated lime with the resulting pH level. An example of such a graph is presented in section 5.3.

The approximate quantity of hydrated lime solution added to each jar, the resulting concentration of reagent and the target pH level are presented in Table 3.1.

Table 3.1: Quantity of hydrated lime solution added to each jar for each target pH level

Jar no.	**Amount of 10% $Ca(OH)_2$ solution added [mL] – approximate values**	**Conc. $Ca(OH)_2$ inside the jar immediately after coagulant addition [g/L] – approximate values**	**Target pH at flocculation end**
1	0.0	0.00	Initial pH (average 6.7)
2	17.2	0.86	8.6
3	28.2	1.41	9.5
4	39.2	1.96	10.4
5	50.2	2.51	11.3
6	61.2	3.06	12.2

Each sample was stirred at 250 RPM for an average of 45 seconds (with an uncertainty range of 35 to 50 seconds), followed by a second stage of stirring at 50 RPM for 20 minutes (with an uncertainty range of 19:50 to 20:10 minutes). The velocity gradients of each phase are detailed in Table 3.2.

Table 3.2: Velocity gradient as a function of the paddle rotation frequency during high pH physico-chemical treatment

Phase	**Rotation speed or RPM [as min^{-1}]**	**Velocity gradient [as s^{-1}]**
Flash mixing (coagulation)	250	430
Slow mixing (flocculation)	50	34

Following completion of the flocculation phase, the treated matrix was left to settle for 24 hours (with an uncertainty range of 20 to 26 hours) and the supernatant was then carefully removed to be neutralised and the microbiological and physico-chemical characteristics of supernatant and sludge determined.

During the course of two jar-test experiments, a sample of the treated influent matrix was collected immediately after completion of the coagulation-flocculation phase (flash-mixing) and before the onset of the sedimentation. Collection was undertaken within 120 seconds of completion of the rapid mixing (with an uncertainty range of 80 to 160 seconds). The sample was analysed for its microbiological quality and a limited set of physico-chemical analyses were also performed.

3.3.3 EXPERIMENTAL SET-UP FOR THE LOW–pH TREATMENT PROTOCOL

The experimental procedure followed for the validation and optimisation of the low–pH treatment protocol is presented in Figure 3.14. First, hydrochloric acid solution was used to reduce the pH level of 2 L of influent matrix. As with the protocol for the high–pH treatment process, wastewater and sludge were mixed and processed within 24 to 72 hours of collection in order to maximise the concentration of indicator organisms, pathogens, suspended solids and COD at the beginning of each jar-test, and the experiments were conducted using the same jar-test equipment and the same matrix, as mentioned previously.

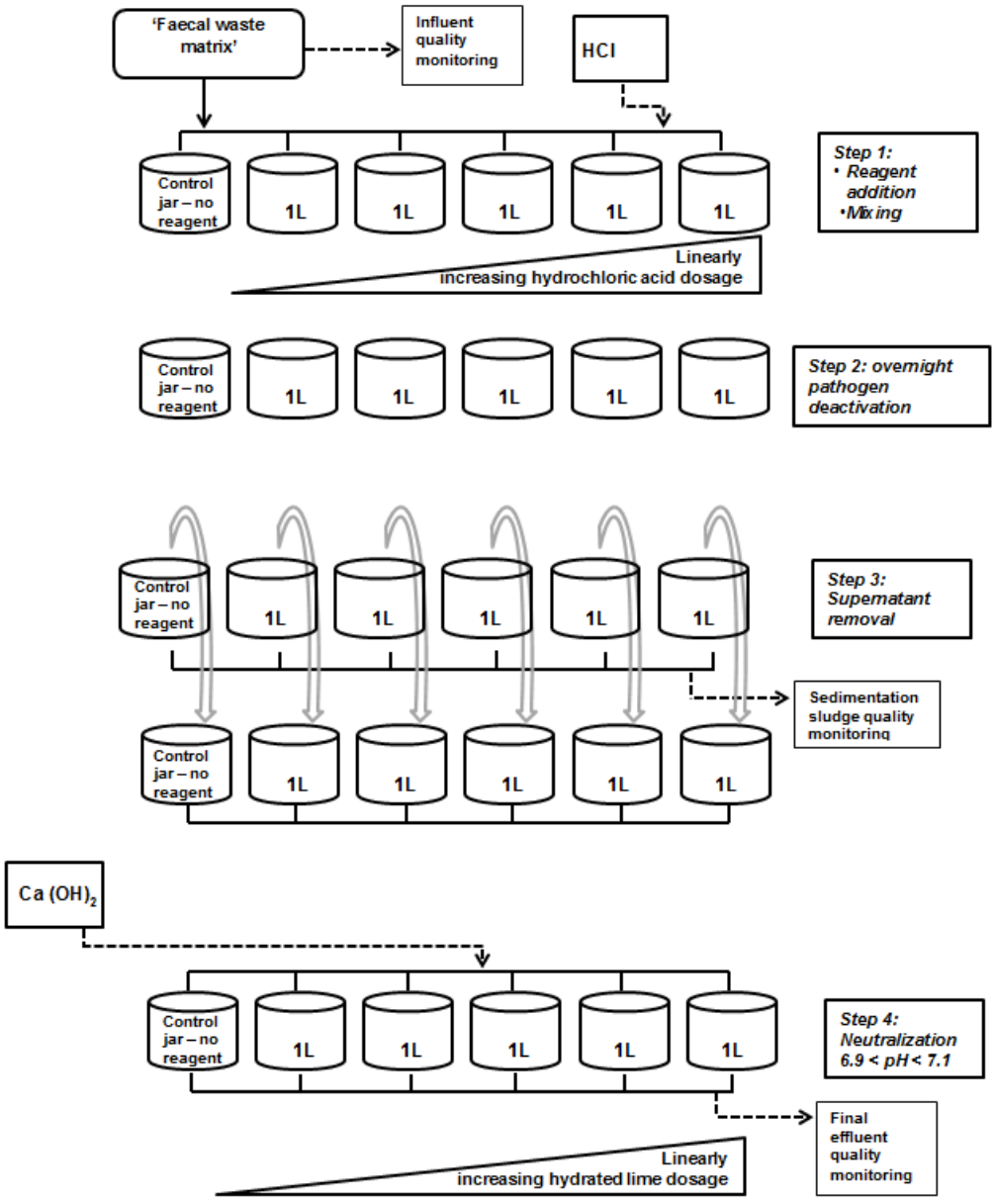


Figure 3.14: Schematic overview of the low–pH laboratory experimental procedure

The amount of hydrochloric acid added to each jar varied slightly over the course of this study. The reason for this is further discussed in Chapters 5 and 9. A pH target was first determined during the design phase of the study. Subsequently a preliminary test was performed before each cycle, with the aim of taking into account the quality of the new faecal waste mix as specified above. The ultimate goal of the preliminary test was to determine with a degree of accuracy the amount of hydrochloric acid to be added to each new faecal waste matrix in order to reach the target pH level.

The preliminary test consisted of the incremental addition of HCl to a 100 ml sample of faecal waste matrix, followed by rapid manual mixing, recording of the pH level and the addition of acid. The incremental addition was, on average, repeated eight to ten times until a pH level lower than 3 was reached. The aim was to plot a graph that correlated the quantity of added hydrochloric acid with the resulting pH level. An example of such a graph is presented in section 5.4, Figure 5.1.

The average quantity of 4 molar hydrochloric acid added to each jar, the resulting concentration of acid and the target pH level are presented in Table 3.3. The rationale for the choice of the specified dosages and target pH levels is discussed later.

Table 3.3: 4 molar hydrochloric acid added to each jar [mL/L] and target pH

Jar no.	**4 mol HCl added to the jar [mL/L faecal waste matrix]**	**Target pH at end of acidification**
1	0.0	Initial pH (average 6.6)
2	0.83	5.8
3	1.6	5.0
4	2.45	4.2
5	3.2	3.4
6	3.9	2.6 – 2.8

Each sample was stirred at 80 rpm during the acidification phase to ensure complete mixing of the acid into the solution and to ensure that the pH level was uniform at every point of the beaker. The acidification phase was followed by stirring at 50 rpm for 20 minutes (with an uncertainty range of 19 to 21 minutes).

The velocity gradients of each phase are provided in Table 3.4.

Table 3.4: Velocity gradient as a function of the paddle rotation frequency during low–pH physico-chemical treatment

Phase	**Rotation speed or RPM [as min^{-1}]**	**Velocity gradient [as s^{-1}]**
Acidification	80	90
Slow mix to homogenize the matrix	50	34

As stated for the high–pH treatment, the estimation of average velocity gradients (G_{ave}) for bench and full-scale treatment provided essential information when scaling up the low–pH physico-chemical treatment. Basic guidelines on how to estimate its value for full-scale reactors are provided in section 3.3.2.

The supernatant was removed after a median settlement time of 24 hours (within a range of 22 to 26 hours) for neutralisation. Following neutralisation, the microbiological and physico-chemical characteristics of supernatant and sludge were determined.

During the course of one jar-test experiment, a sample of the influent matrix treated through a pH range of 3.6 to 3.8 was collected immediately after completion of the acidification phase (at the end of the mix at 50 rpm) and before sedimentation. The sample was taken within 120 seconds of completion of the slow mixing (within a range of 80 to 160 seconds). The sample was analysed for its microbiological quality and a limited set of physico-chemical analyses was also performed.

The results from some preliminary tests, which aimed to inform and optimise the full laboratory procedure, led to the decision not to include a further and unnecessary aluminium-based coagulation-flocculation stage into the laboratory-based experimental protocol.

3.4 PERFORMANCE MONITORING OF THE LABORATORY-SCALE TREATMENT

3.4.1 PHYSICO-CHEMICAL PARAMETERS

Measurement of turbidity levels (as NTU) was performed according to the standard nephelometric method using a Hach portable turbidimeter (model 2100P), which operated within a wavelength range of 400 to 600 nm [157].

Measurement of total suspended solids (or TSSs - as mg/L) was achieved by filtration of the sample through a 47 mm diameter Whatman glass-fibre filter of GF/C grade and 1.2 µm porosity, followed by desiccation at 103 – 105 ºC for at least one hour and until constant weight was achieved, according to standard methods [158].

As a result of the limited availability of laboratory equipment during the first phase of the project (fieldwork analyses), it was not possible to dry filters for suspended solids analysis at 103 – 105 ºC as recommended by the standard methods. Neither was it possible to dry the filters in a microwave, applying one of the protocols adapted from the standard methods as proposed by some studies investigating the physico-chemical analyses of food samples [162, 163]. Filters were instead dried at ambient temperature (in general greater than 30 °C) until constant weight was achieved. In order to quantify the error possibly introduced by this adaptation of the standard method, a simple test was performed during 'Phase II' of the project. A few filters from samples presenting different concentrations of total suspended solids (samples from the raw faecal waste matrix before treatment and samples from jars representing different levels of treatment) were first dried at ambient temperature, in the EPHReG laboratories. Their weight was recorded and the same filters were then dried at 103 – 105 ºC for at least one hour and until constant weight was achieved, as recommended by the standard methods, with the aim of monitoring the variation of filter weight obtained through this second step.

Where the determination of fixed and volatile suspended solids (or FSS and VSS - as mg/L) was required, this was based on the weight of the filter residue after completion of the TSS measurement. The filter was further ignited at 550 ºC for at least 15 minutes and until constant weight was achieved, according to standard methods [164].

Measurement of settleable solids (or SS - as mL/L) was based on a gravimetric test performed using an Imhoff cone, according to standard methods.

Figure 3.15 shows an example of the methodology followed for settleable solids measurement for six effluent samples at the end of the gravimetric test.

Figure 3.15: Final result of gravimetric test for the measurement of settleable solids

pH levels were measured using a Palintest Micro 500 pH meter, according to standard methods [159]. pH buffers (10.01, 7.01 and 4.01) were used for pH meter calibration and pH probes were stored in a saturated KCl solution.

COD (as mgO_2/L) was measured according to a standard closed-reflux colorimetric method [160]. The samples were digested at 150 ºC for two hours in a strong solution of sulphuric acid, potassium dichromate and mercury sulphate. Following digestion, the tubes were cooled and the change in colour was determined using the Hach photometer model DR 3900 operating at 600 nm wavelength. The concentrations of sulphuric acid, potassium dichromate and mercury sulphate, added to prepare the vials, were selected to determine COD concentration within the range 100 to 900 mgO_2/L. The test was not performed using commercially available reagents or pre-made vials, but using reagents prepared within the laboratory to achieve the high number of test repetitions required at a reasonable cost. A brief description of materials used and methods applied is provided in Chapter 5.

The total nitrogen concentration (as mgTN/L) was measured using a spectrophotometric laboratory kit (Hach-Lange – LCK 338), which is based on the same principle as that followed for the standard persulphate method for the measurement of the concentration of inorganically- and organically-bonded nitrogen [165]. Following digestion, the tubes were cooled and the change in colour was determined using the Hach photometer model DR 3900. A test kit that supported the analysis of samples containing between 20 and 100 mg/L of total nitrogen was used.

The total phosphorous concentration (as mg PO_4-P/L or mg PO_4/L) was measured using a spectrophotometric laboratory kit (Hach-Lange – LCK 350), which is based on the same principle as that followed by the standard ascorbic acid method for the measurement of the concentration of the dissolved orthophosphate [166]. Following digestion, the tubes were cooled and the change in colour was determined using the Hach photometer model DR 3900. A test kit that allowed the analysis of samples containing 2 to 20 PO_4-P/L or 6 to 60 mg PO_4/L was used.

The concentration of ammonium (as mg NH_4-N/L or mg NH_4/L) was measured using a spectrophotometric laboratory kit (Hach-Lange – LCK 303). The Hach-Lange procedure is based on a principle similar to that followed for the standard automated phenate method for the measurement of the concentration of dissolved ammonia [167]. The reaction is promoted by simple agitation and does not require digestion. The change in colour is determined using the Hach photometer model DR 3900. A

test kit that allowed the analysis of samples containing 2 to 47 NH_4-N/L or 2.5 to 60 mg NH_4/L was used.

3.4.2 BACTERIOLOGICAL PARAMETERS

Presumptive counts of thermotolerant coliforms were recorded as colony-forming units (CFU) per 100 ml, following the 'thermotolerant (faecal) coliform membrane filter procedure' using standard methods [161]. The procedure requires membrane filtration through a sterile nitrose-cellulose membrane filter (0.45 µm). The samples were always neutralised before analysis. Following filtration, the filters were incubated at 44 °C ±1 °C for 18 to 24 hours, soaked on sterile Petri dishes filled with Merck® m-FC agar with rosolic acid, as recommended by standard methods [161]. Samples were diluted according to their predicted bacterial counts using quarter strength Ringer's solution. Following incubation, all yellow colonies greater than 2 mm in diameter were enumerated and recorded as CFU of presumptive thermotolerant coliforms per 100 ml of the original sample.

Presumptive counts of intestinal enterococci (closely related to the term 'faecal streptococci') were recorded as CFU per 100 ml, following the intestinal enterococcus membrane filter procedure from the standard method [168]. Membrane filtration was undertaken as described for thermotolerant coliforms. Following filtration, the filters were incubated at 37 °C ±1 °C for 44 to 48 hours, soaked on sterile Petri dishes filled with Merck® membrane filter enterococcus selective agar according to Slanetz and Bartley's method [169] and [170] [168]. Samples were diluted according to their predicted bacterial counts using quarter strength Ringer's solution. Following incubation, all pink to red colonies greater than 2 mm in diameter, were enumerated and recorded as CFU of presumptive intestinal enterococci per 100 ml of the original sample.

Somatic coliphage were detected and enumerated by incubating the sample with an appropriate host strain and recorded as the number of plaque-forming units of somatic coliphages per 100 mL (pfu/100mL), following the EN ISO European Standard Method [171] [172]. The host strain used was an *E. coli* strain CN (ATCC 700078), also known as WG-5 [173]. The procedure was based on the mixing of a 1 ml sample (pure or diluted) with a small volume of semi-solid nutrient medium and a host strain culture. The mix was then plated on a solid nutrient medium. Following incubation at 37 °C ±1 °C for 17 to 19 hours the number of relatively large – up to 7 mm – plaque-forming units per ml sample was counted. All samples were neutralised before

analysis. Samples were diluted according to their predicted bacterial counts using quarter strength Ringer's solution.

During the course of a single experiment for the analysis of the high–pH treatment and a single experiment for the analysis of the low–pH treatment performance, F-specific RNA bacteriophage were detected and enumerated by incubating the sample with an appropriate host strain and recorded as the number of plaque-forming units of F-RNA bacteriophage per 100 mL (pfu/mL), following the EN ISO European Standard Method [174]. The host strain used was a *Salmonella typhimurium* strain (NCTC 12484), known as WG-49 [175]. The procedure is similar to the one previously explained for the detection of somatic coliphage and is based on the mixing of 1 ml sample (pure or diluted) with a small volume of semi-solid nutrient medium and a (different) host strain culture. The mix is then plated on a solid nutrient medium. Following incubation at 37 °C ±1 °C for 17 to 19 hours the number of 1.5 mm diameter (or smaller) plaque forming units per ml sample was counted. All samples were neutralised and diluted as previously described.

3.4.3 VISUAL ASSESSMENT OF TREATMENT PERFORMANCE

During the final part of the laboratory study, an attempt was made to replace turbidity – as a surrogate for other, presumably more reliable, but also time- and resource-consuming indicators of treatment efficacy – with a visual assessment of effluent clarity. The aim of this small study was to assess whether visual assessment of treatment performance might be an acceptable surrogate for laboratory analyses, such as turbidity testing, in the resource-limited environment of emergency settings.

The assessment was performed by volunteers who had little or no understanding of the technology being tested, in order to minimise the risk of biased assessments. The objective was to try to establish a correlation between a numerical value assigned by volunteers to determine the clarity of different types of effluent after overnight sedimentation (different quantities of reagent, i.e., different levels of treatment) and levels of an indicator of treatment performance that may be considered to be a surrogate of certain pathogens.

The first test was a so-called 'stand-alone' (alternatively referred to as a 'single comparison' test or quasi 'double blind') visual assessment, whereas the second test was a so-called 'all in' (also referred to as a 'grouped comparison' test, or quasi 'single blind') visual assessment. The difference between these was that the first test was slightly more complex, performed according to a procedure that allowed the volunteer

to only see one jar of treated effluent at a time. The aim was to avoid (or to minimise) any bias introduced by a comparison, involuntarily made by the participant, between an assessed jar and that of other jars of the same set in proximity to it.

During the second type of test, the volunteers were allowed to assign, subjectively, a numerical value to each jar to assess the clarity of a sample collected at the end of a six-jar-test experiment (i.e. effluent), with the jars aligned next to each other, inside the jar-test rig, at the end of the sedimentation phase.

The stand-alone (or 'double blind') test was performed as follows:

1. A standard jar-test (high– or low–pH treatment) was performed as explained above and the overnight sedimentation was completed. The volunteers were invited to the laboratory in the morning, at the end of the overnight sedimentation process;

2. Before allowing the volunteers to have access to the jar-test rig, all jars were covered with a black plastic sheet;

3. Each volunteer was instructed as to how to perform the visual assessment. The volunteers were asked to assess the level of clarity of different effluent samples, giving a whole value numerical response between zero (for 'completely transparent' samples) to ten (for 'completely opaque' samples). Semi-unitary numerical values (such as 6.5, 7.0 etc.) were also used, if there was some doubt between two integers;

4. The scale was established as follows: a sample of clear water was shown to each volunteer. She/he was told that this hypothetical sample would be assigned a value of 'zero' ('complete clarity'). Then a sample of the same raw sewage used for the experiment (but not treated) was shown to the volunteer, who was told that this hypothetical sample would be assigned a value of 'ten' ('completely opaque');

5. A number from one to six was assigned to each jar: '1' for the first jar from the left representing the control sample and '6' for the last jar from the left, representing the sample subjected to the most extreme treatment;

6. A number from one to six was then randomly selected. The volunteers were not permitted to look at the researcher, as he was 'uncovering' the jar-test rig, removing the jar corresponding to the selected number, whilst always minimising turbulence, which would have involuntarily mixed the jar content. The jar was placed on a bench that was well-lit by natural light (in an attempt to minimise the addition of unnecessary bias, such as different types of artificial light, and to make the experiment easier to reproduce in the field in future) and the jar-test rig was

covered again. After approximately 30 seconds the volunteers were allowed to view the sample jar;

7. Each volunteer was given enough time to look carefully at the jar, assess its clarity and to assign a numerical value to it. The volunteers were not permitted to exchange any information with each other or the researcher regarding the assigned values or any subjective evaluation of the clarity of the sample;

8. The volunteers were then asked to close their eyes, allowing the researcher to replace the jar in the jar-test rig and to cover it. The procedure was then repeated, starting again from step 6.

Following completion of the ‘stand-alone’ (alternatively referred as ‘single comparison’) test, a second ‘all in’ (also referred to as a ‘grouped comparison’) test was immediately performed. The test was structured in a similar way, the main difference being that the participants were allowed to observe all jars inside the rig, one next to the other, and asked to assign numerical values. Also during this test the volunteers were not allowed to exchange information regarding the assigned values or any other evaluation about the clarity of the sample.

4. CHAPTER FOUR: RESULTS OF FULL-SCALE TREATMENT (PHASE I)

In the current chapter, the results of the fieldwork studies are presented. The average treatment results of the full-scale batches are summarised, first in terms of contaminant removal rates, and subsequently in terms of consumption of reagents and levels of residual chemicals. The results of a brief cost-benefit analysis are also provided. Finally, the key findings regarding the sludge production are briefly presented at the end. The results from the final eight treatment batches, which were performed when adequate monitoring equipment had become available in the field, are summarised in Table 4.1.

4.1 THE DESIGN OF A 'MULTIPLE-BARRIER' WASTEWATER MANAGEMENT STRATEGY

Based on the initial estimations of *Vibrio cholerae* levels in the CTC wastewaters and with reference to the available literature [81] and expert advice, the author developed a wastewater management strategy that involved four consecutive and distinct barriers to the transmission of *Vibrio cholerae*, as follows:

1. Initial chlorination of patient faeces within stool buckets immediately following collection by MSF health-care professionals, as already practised according to MSF protocols [99, 152];
2. Storage of pooled CTC wastewaters in open tanks - in practice for up to 12 weeks (estimated average six weeks, minimum estimated storage three weeks) at relatively high ambient temperatures (30 – 40 °C) - resulting in a further reduction in levels of enteric microorganisms as a result of natural biological, chemical and physical processes;
3. The design and operation of a novel batch-operated on-site wastewater treatment and disinfection plant, as described in detail below; and finally
4. Controlled effluent disposal within soil infiltration trenches according to existing MSF protocols [99].

4.2 SHORTLISTED TECHNOLOGIES FOR FULL-SCALE TREATMENT

A shortlist of three potentially promising technologies that might meet the objective of achieving effective, robust and relatively low-cost *in situ* treatment of the CTC wastewaters were initially proposed:

1. High-pH physico-chemical treatment (also referred to as ‘Protocol A’): Coagulation/flocculation and disinfection of the wastewater with hydrated (slaked) lime ($Ca(OH)_2$) at high pH levels, using a treatment system that was based on the previously explained methodology of Taylor et al. [101-104];
2. Low pH physico-chemical treatment (also referred to as ‘Protocol B ’): A novel approach involving disinfection at low pH levels using hydrochloric acid (HCl), followed by pH neutralisation and subsequent coagulation/flocculation, achieved using low-cost coagulant (e.g. aluminium sulphate, or similar); and
3. Septic tank treatment combined with anaerobic filtration (also referred to as ‘Protocol C’).

As previously explained in section 2.6.4: ‘Septic tank faecal waste treatment’ (p. 46), initial desk-based analyses of the septic tank treatment (Protocol C) approach revealed that this treatment option was unsuitable for a number of reasons, and as such should not be pursued further.

4.3 CONTAMINANT REMOVAL RATES

A mean wastewater volume of 12.8 m^3 was treated in each of the batch processes reported here, six of which were executed according to the low-pH procedure (Protocol B), and two of which were executed according to the high-pH procedure (Protocol A).

Table 4.1: Comparison of raw and treated wastewater quality from full-scale treatment (batch volumes ranged from 10 to 14.5 m^3)

Parameter	Raw wastewater		Treated effluent		Removal (%)	
	Mean	Range	Mean	Range	Mean	Range
Protocol A (low pH): mean results from two batches [*1]						
Turbidity (NTU)	805	740-870	15	5-26	98.2	97.01 – 99.32
TTC (CFU per 100 ml)	1.75 x 10^4	1.7 x 10^4 - 1.8 x 10^4	5	5-5	99.97	99.97 – 99.97
COD (mg O_2/l)	17,080[*2]	-	131	108-154	99.2	99.10 – 99.37
TSS (mg/l)	1,155	980-1330	112	81-143	90.5	89.21 – 91.79
Protocol B (high pH): mean results from six batches [*1]						
Turbidity (NTU)	430	1200-120	23	2-40	91.3	84.58 – 99.57
TTC (CFU per 100 ml)	4.98 x 10^4	1.1 x 10^4 - 1.8 x 10^5	106	20-390	99.52	98.05 – 99.95
COD (mg O_2/l)	17,080[*2]	-	149	134-160	99.15	99.1 – 99.2
TSS (mg/l)	1077	280-4350	38	3-95	92.9	79.6 – 99.3
Summary: overall mean from all batches [*1]						
Turbidity (NTU)	520	120-1200	21	2-40	93.0	84.58 – 99.57
TTC (CFU per 100 ml)	4.1 x 10^4	1.1 x 10^4 - 1.8 x 10^5	81	5-390	99.91	98.05 – 99.97
COD (mg O_2/l)	17,080[*2]	-	144	108-160	99.1	99.10 – 99.37
TSS (mg/l)	1,097	280-4350	57	3-143	92.3	79.6 – 99.3

TTC= Thermotolerant Coliforms; COD= Chemical Oxygen Demand; TSS= Total Suspended Solids

[*1] Performed when adequate monitoring equipment had become available in the field

[*2] Calculated with reference to an average value for untreated wastewater in the absence of quantitatively sufficient data

Treatment by both high- and low-pH methods (Protocols A and B) achieved an effectively clarified wastewater effluent, with a turbidity reduction consistently greater than 80% and a mean reduction for all measured samples of 93% (1.1 log). Mean TSS reduction was 92% (1.1 log), while removal of thermotolerant coliforms was consistently greater than 99.8% (2.7 log), with a mean reduction of 99.9% (3 log). The mean removal of COD (calculated with reference to an average value for

untreated wastewater in the absence of sufficient data) was estimated to be consistently in excess of 99% (2 logs).

As previously described, the 'final effluent' was considered adequate to be directly infiltrated into on-site soil trenches, but only provided that the effluent had reached a quality considered to be 'satisfactory' (see Chapter 3 for quality criteria). If the effluent quality failed to meet these quality criteria, the entire treatment procedure was repeated before the final effluent was allowed to be infiltrated into the soil trenches. Interestingly, no single batch required a total or partial repetition of the treatment procedure during the entire fieldwork period.

4.4 CONSUMPTION OF REAGENTS AND LEVELS OF RESIDUAL CHEMICALS

The consumption of chemical reagents during full-scale treatment (when adequate monitoring equipment had become available in the field) is summarised in Table 4.2.

Table 4.2: Comparison of consumption rates of chemical reagents, residual aluminium and volume of sludge resulting from full-scale treatment

Parameter	**High pH treatment (Protocol A)**		**Low pH treatment (Protocol B)**	
	Average	Range	Average	Range
Total volume treated [m³]	25	NA	78	NA
Mean concentration of HCl used [l/m³] = [ml/l]	2.25	1.58 – 2.92	1.30	0.95 – 1.65
Mean concentration of $Ca(OH)_2$ used [kg/m³] = [g/l]	3.96	3.0 – 4.9	0.47	0.1 – 0.9
Mean concentration of $AlSO_4$ used [g/m³] = [mg/l]	-	NA	112.5	75 – 150
Residual aluminum concentration in the effluent [g/m³] = [mg/l]	0.01	0.01 – 0.01	0.07	0.01 – 0.10
Approximate sludge volume [m³]	0.81	0.6 – 1.2	0.71	0.5 – 1.0
Approximate sludge volume [%]	5.6	4.0 – 7.3	6.2	4.9 – 8.8

The low pH treatment method (Protocol B) was shown to require on average 1.30 L of HCl per m³ of raw wastewater, compared with 2.25 L HCl per m³ of raw wastewater for the high pH treatment method (Protocol A). Additionally, a mean dose of 0.47 kg of hydrated lime ($Ca(OH)_2$) was required per m³ of raw wastewater for the low-pH protocol (Protocol B), compared with 3.96 kg $Ca(OH)_2$ per m³ of raw wastewater for the high-pH protocol (Protocol A). In other words, Protocol A (high-pH treatment) required on average 73% more HCl and 740% more $Ca(OH)_2$ (by mass), than Protocol B (low-pH) in order to achieve comparable results.

The mean residual aluminium level in the treated effluent was shown to be 0.05 mg/L and 0.07 mg/L for Protocol A and B, respectively. Levels of residual aluminium were never reported to exceed 0.1 mg/l.

4.5 VOLUME OF SLUDGE PRODUCED COMPARED TO THE VALUES RECORDED IN THE LITERATURE

The recorded average volume of produced sludge, at 6% (vol/vol), was slightly higher than the values reported in the extant literature for coagulation/flocculation systems operating both with hydrated lime and/or aluminium sulphate. This point will is discussed in further detail in Chapter 9.

4.6 COST ANALYSIS

A basic cost analysis was undertaken to compare the costs of treating a single unit of volume (e.g. 1 m^3) of pathogen-laden hospital wastewater through either one of the two studied treatment technologies or through the super-chlorination approach to disinfection. The cost analysis was performed after the total costs of treatment were structured as follows:

1. Capital costs
2. Operating costs:
    i. Labour costs
    ii. Energy costs
    iii. Reagent costs

The capital costs were considered as the sum of:

- the costs to rent the land upon which the CTC was built multiplied by the percentage of space occupied within the CTC by the wastewater treatment plant,
- the legal and permit costs,
- the equipment needed to run the treatment, and
- the cost of construction of the treatment plant.

As the aim of this cost analysis was to compare both studied protocols with the super-chlorination approach, and as all three compared techniques required approximately the same space to be operated within the hospital, it is possible to ignore the land rental and legal and permit costs.

The total estimated cost of the construction of the treatment plant and the equipment required to treat 600 m^3 of faecal waste during the entire duration of the project using either high- and low-pH physico-chemical treatment is 5,500 €. This estimation is based on the field experience of the author, where he had limited access to MSF material purchase catalogues. As explained in Chapter 3, both studied treatment techniques are based on the same physico-chemical principle and thus require exactly the same components: a 30 m^3 treatment reactor, 4 m^3 tanks for the storage of the faecal waste prior to and after the treatment, pumps, and tents for the storage of the material and to be used by the staff members as changing rooms.

There are two options to estimate the costs for the construction of the treatment plant and the equipment required to disinfect the waste through the super-chlorination approach. The mixing of the chlorine could be performed inside the same 30 m^3

treatment reactor and through the same mechanical pumps used during this study or could be performed inside one of the smaller 4 m^3 tanks used for the storage of the same waste. The first option would allow a better mix and would likely produce higher disinfection performance, while the second option would be cheaper (as smaller tanks are significantly cheaper), but less effective. In the first case, the total estimated costs for the construction of the treatment plants and the equipment required to run it would parallel the approximate cost for the high- and low-pH treatment: 5,500 €. In the second case, the total estimated costs for the construction of the treatment plants and the equipment required to run it is approximately 2,000 €. This is the value that was used for the cost analysis (most conservative approach).

The energy costs include electricity and petrol. Electricity was partially made available by a generator and partially provided by the unreliable Haitian national electricity network. The energy costs are difficult to estimate and further study is needed to more accurately assess them, but it is reasonable to assume that costs would be comparable for all treatment options. Energy costs were estimated to be approximately 2.5 € per m^3 of treated faecal waste.

Labour costs for Protocol A and B (high- and low-pH treatment) were estimated to be approximately 5.5 € per m^3 of treated faecal waste. The same costs for disinfection through super-chlorination were estimated to be approximately 3 € per m^3 of treated faecal waste regardless which chlorine-based reagent was used. Both estimations were made for a total volume of faecal waste of 600 m^3 and are based on the information available to the author regarding how much time is required to disinfect the waste through the super-chlorination approach. It is important to point out that labour costs can vary significantly, even within the same country, and are closely related to the level of expertise of the staff. Labour costs decrease as the staff acquires experience on the technique, thereby decreasing as the total volume of treatment increases.

The estimated reagent costs are provided in Table 4.3. and 4.4 which indicate why significant financial savings in relation to reagent costs may be achieved using the protocols presented here. Table 4.5 summarizes the total costs per unit of faecal waste volume for four different treatment options.

Table 4.3: Material and reagent cost analysis for the super-chlorination options

Super-chlorination	Unit	Price per unit [€]	Weight [kg]	Dose required to produce 1 L solution at 2%	Dose required to produce 1 m3 sol. at 2%	Price / unit [€]	Price to produce 1 m3 solution at 2%	Quantity to disinfect/treat 1m3 ww [m3]	Price to disinfect 1m3 wastewater
HTH (Calcium hypochlorite)	Grams	3.237	0.45	30	30,000	0.00719	216	0.1 *	21.6
NaDCC (Klorsept)	Tablet			20	20,000	0.00720	144	0.1 *	14.4

* Based on the assumption that 10% vol/vol of 2% solution was used

Table 4.4: Material and reagent cost analysis for the physico-chemical options

Physico-chemical treatment	Unit	Price per unit [€]	Weight [kg]	Dose required to produce 1 L solution at 2%	Dose required to produce 1 m3 sol. at 2%	Price / unit [€]	Price to produce 1 m3 solution at 2%	Quantity to disinfect/treat 1m3 ww [m3]	Price to disinfect 1m3 wastewater
HCl consumption									
Low pH		0.15	1					1.3	0.2
High pH		0.15	1					2.25	0.3
Hydrated lime consumption									
Low pH								0.47	0.7
High pH								3.96	6.2
AlSO4 consum-ption (field tests)									
Low pH								0.112	0.4
High pH								0	0.0
								Total low pH	1.3
								Total high pH	6.5

Table 4.5: Comparison of total costs to disinfect 1 m^3 of pathogen-laden hospital wastewater [€]

Disinfection method →	Super-chlorination with HTH (calcium hypochlorite)	Super-chlorination with NaDCC (Klorsept)	Protocol A (High pH physico-chemical treatment)	Protocol B (Low pH physico-chemical treatment)
Capital	3.3	3.3	9.1	9.1
Labour	3	3	5.5	5.5
Energy	2.5	2.5	2.5	2.5
Reagent	21.6	14.4	6.5	1.3
Total	30.4	23.2	23.6	18.4

HTH = High Test Hypochlorite; a form of calcium hypochlorite ($Ca(OCl)_2$)
NaDCC = Sodium dichloroisocyanurate ($C_3Cl_2N_3NaO_3$)
Conversion rates: 1 USD = 0.89 Euro = 0.66 GBP

Notably, the main aim of this study was not to assess the costs of treatment per unit of faecal waste volume for all three options, but rather to understand if the two proposed (and demonstrably safer) approaches could be reasonably considered 'low cost' options. It is also important to point out that the efficacy and safety of the super-chlorination approach to disinfection has been widely questioned [106, 107, 111, 112] and therefore, the cost of treatment should not be a priority criterion for selecting the best method to safely disinfect this type of human waste.

5. CHAPTER FIVE: RESULTS OF LABORATORY METHOD DEVELOPMENT

5.1 COMPOSITION OF THE FINAL 'FAECAL WASTE MATRIX'

The initial plan of this laboratory study was to test the performance of the studied physico-chemical treatment technology using municipal wastewater, collected from small-scale and medium-scale wastewater treatment facility located in South East England.

The composition of the faecal waste matrix was determined following evaluation of a range of different types of mixed liquors in order to select one that was deemed to be the most representative (and conservative) substitute for the type of faecal waste that was treated during the field study presented in 'Chapter 4'. After completion of the initial laboratory trials, the results of which are presented below, the original plan was amended a few times during the initial design phase. A few different types of liquor were analysed in detail and considered as possible 'candidates' to be used for the composition of the faecal waste matrix. The main types were:

- The raw wastewater collected from the influent prior to preliminary screening, grit chamber and primary sedimentation at Southern Water's Hailsham wastewater treatment plant in East Sussex, UK;
- The semi-solid waste (or sludge) collected from two stabilisation tanks at the same wastewater treatment works. The untreated sludge was considered as an option because it was microbiologically and physico-chemically more polluted than the municipal wastewater. It presented higher concentrations of faecal coliform and intestinal enterococci, somatic coliphages, COD, suspended solids, higher turbidity and other physico-chemical indicators of pollution;
- Another type of liquor derived from an anaerobic bio-digester located at the small wastewater treatment facility at Cooksbridge in East Sussex was also considered as a potential option in addition to the municipal wastewater, with the aim of maximising the likely levels of pathogenic organisms and organic matter. The approximate location of the Cooksbridge wastewater treatment site, located near the town of Lewes at latitude: +50.90706 and longitude: -0.00922 is shown in the Appendix 2.

The sludge from the Hailsham storage tanks was finally chosen as the best option to be added to the raw wastewater because of its higher levels of faecal indicators and higher concentration of total suspended solids and organic matter.

The final outcome of the decision-making process was, (as mentioned in Chapter 3), to prepare a 'faecal waste matrix' consisting of 80% (vol/vol) municipal wastewater to 20% (vol/vol) highly concentrated sludge from the two stabilisation tanks at Hailsham.

Table 5.1 illustrates the main values for levels of faecal indicators and physico-chemical contamination levels of different mixed liquors from the field study in Haiti and two types of faecal waste collected at the Hailsham treatment facility. The figure briefly elucidates the criteria followed for the selection of the liquor and the proportions of municipal wastewater and sludge added to the final mixed liquor.

Table 5.1: Average concentration of several bacterial and physico-chemical indicators for liquors from the field and the Hailsham wastewater treatment facility. The presented figures guided the rationale for the composition of the faecal-waste matrix to be used during the bench scale tests.

	Phase I		**Phase II**			
	Raw wastewater field tests (Haiti)		**municipal waste-water (Hailsham)**	**sludge (Hailsham)**	**wastewater-sludge mix (80 – 20) (Hailsham)**	
Parameter	Mean	Range	Mean	Mean	Mean	Range
Turbidity (NTU)	520	120 – 1200	34.2	14,000	2,366	1,668 – 3,220
Total suspended solids (mg/l)	1,155	980 – 1330	506	14,260	4,378	3,257 – 5,500
Thermotolerant coliforms (CFU per 100 ml)	$1.75x10^4$	$1.7x10^4$ – $1.8x10^4$	1.4×10^7	2.2×10^8	$2.9x10^7$	$3.3x10^6$– $5.5x10^7$
COD (mg O_2/l)	17,080[*2]	-	1,217	15,600	5,349	2,358 – 6,598

[*2] Calculated with reference to an average value for untreated wastewater in the absence of quantitatively sufficient data.

A more detailed explanation of the rationale for choosing to add 20% (vol/vol) highly concentrated sludge to 80% (vol/vol) municipal wastewater (and the resulting limitations this decision entails) is provided in Chapter 9.

5.2 THE DEGRADATION PROCESSES OCCURRING IN THE MATRIX WITH TIME

As previously mentioned, during the preliminary test, the quality of the liquor mix was monitored over the two week period following municipal wastewater and sludge collection, in order to estimate the reduction of levels of faecal contamination, COD and other physico-chemical parameters resulting from sample/matrix degradation. The variation of these parameters was within expected levels, with a reduction in bacterial and viral indicators generally <1Log_{10} reduction and a reduction of all other physico-chemical parameters significantly lower than a single Log_{10}.

Nevertheless the author eventually opted for the most 'conservative' approach: i.e., always performing jar-tests, the results of which are reported in this study, using 'freshly' collected wastewater. The main difficulty of applying such a protocol was the extra time required and therefore the lower number of repetitions that were possible, given the limited project duration. The advantage, on the other hand, was the opportunity always to monitor the performance of a system tested using the most 'critical' faecal waste matrix (i.e., containing highest possible level of faecal indicator bacteria). This is why the second option was preferred.

5.3 VARIATION IN THE CHARACTERISTICS OF THE MATRIX COMPONENTS FROM ONE CYCLE TO THE NEXT

Preliminary tests, conducted during method development and optimisation highlighted considerable variability in the levels of the physico-chemical and microbiological parameters within the faecal waste matrix. Although the faecal waste matrix was prepared on each occasion according to the proportions outlined in section 5.1 and only involved the use of 'freshly' obtained wastewater, it is important to be aware that the influent characteristics included some natural variability.

Indeed, one of the drawbacks of bench-scale (and full-scale) wastewater treatment experiments performed with 'real' wastewater instead of 'synthetic' wastewater (a standardised mix prepared in the laboratory through the addition of chemicals to distilled water), is that the characteristics of the mixed liquor used to create the faecal waste matrix is constantly changing as a result of seasonality, time of collection and, in particular, proximity to extreme precipitation events. For example the consistency of municipal wastewater tends to be significantly more diluted following rainfall (even a few hours after the event).

Therefore, these fluctuations in the mixed liquor composition were the reason why such a significant variation in concentrations of bacterial, viral indicators, COD, turbidity and suspended solids etc., were observed during the preliminary experiments. However, this is not necessarily a major issue when analysing the treatment results in terms of removal, as Log_{10} (or percentage removal) is always reported as the relationship between influent and effluent value; therefore a certain amount of natural variation in the influent characteristics is taken into account during the calculations and has a limited effect on the final removal. Nevertheless this variation can be problematic when determining the amount of reagent to apply during the coagulation-flocculation experiments. Different mixed liquors present a different alkalinity, pH and organic matter content and therefore possess different buffering capacities. Experiments aimed at validating/optimising physico-chemical treatment should therefore be repeated several times under (ideally) identical conditions. This is unfortunately seldom the case for this type of physico-chemical treatment, even in a carefully controlled laboratory study.

5.4 HIGH pH TREATMENT PROTOCOL

5.4.1 ALTERNATIVES TO THE EXPERIMENTAL DESIGN

As a consequence of the above mentioned fluctuation in faecal matrix composition, two alternative experimental design scenarios for the high pH treatment protocol were identified:

1. Fixed amount of reagent and therefore 'unstable' end-point pH levels: Keeping the amount of reagent (coagulant) added to each jar constant, whilst simultaneously accepting the inevitable fluctuations in the levels of contamination present in the faecal matrix would have also meant accepting significant variation in the final pH reached during the test repetitions. For example, if the experimental design requires the addition of 40 ml of 10% $Ca(OH)_2$ solution to the fourth jar during each test, because of the variation in the influent composition between different test cycles, the pH level reached by this jar could fluctuate between 10.4 and 11.4, with an average pH level of 11.2. (but accompanied by an unacceptably high standard deviation, or level of uncertainty).
2. Variable amount of reagent and therefore 'stable' end-point pH level: Conversely, if the main goal is repeating coagulation-flocculation and

disinfection (which is not a 'dosage-driven', but a 'pH-driven' process) performed always at approximately the same pH, it should be accepted that any faecal waste matrix would in principle require a slightly different amount of $Ca(OH)_2$ in order to reach the same goal, i.e., a pH level defined at the beginning during the design phase. In this case the protocols would be defined not by constant reagent dosage, but by pH 'end-points', in that reagents are added to reach a prescribed pH level, so that variability in wastewater composition ceases to be a factor in treatment efficacy. The advantage here would be that the pH reached by each single jar after coagulant addition would be almost constant. If, for example, the experiment was designed with the aim of using the data from the fourth jar to investigate what happens at pH 11.2, accepting therefore that each experiment repetition would require a slightly different amount of $Ca(OH)_2$ to reach the pH 'target', the result would be a much more limited fluctuation around the target pH level.

The second modification to the experimental design, chosen at the end of the first phase of preliminary experiments, involved coagulation/flocculation and disinfection steps. The main reason is explained as follows. This treatment had two 'components' running 'in parallel':

1. A 'coagulation-flocculation' component, which is responsible for the physical separation of the microorganisms (pathogens), suspended solids and many other pollutants and determines their concentration in the resulting sedimentation sludge. The most important parameters to be monitored for the design of the coagulation-flocculation are: mixing regimes, time and most importantly the coagulant dose. The pH level is one of the aspects to monitor, but not the only one [155]; and
2. A 'disinfection' component. As previously explained, the extreme pH level reached during this type of batch treatment is the main factor responsible for the deactivation of microorganisms (including bacterial and viral pathogens). The further the pH from neutrality (either extreme acid or extreme basic environment), the higher the disinfection capacity of the system. As the explored technique is part of a global public health effort aimed at controlling the spread of a disease in an epidemic context, this study mainly focuses on the disinfection component of this treatment. In other words, it was more important that this work was designed to be a pH driven disinfection process, rather than just a dosage-driven clarification process. Therefore, the main goal was not to monitor how the faecal waste matrix reacted to a certain coagulant

dosage, but how it reacted to a certain pH level. Consequently, a pH target was determined during the experimental design phase; prior to each test cycle, and the amount of reagent required in order to reach the target was determined.

It should be noted that there is an important difference between this study and the majority of the coagulation-flocculation works reported in the literature. Those works aim to optimisee the treatment for a 'chosen', known water matrix or raw sewage: a matrix whose characteristics are defined and for which the assumption can be reasonably made that these characteristics will not significantly change over time. The goal of such studies is therefore, once the matrix is defined, to identify the optimal mixing speed and times, type and amount of coagulant-flocculant added, etc.

However, the aim of the study described here is different, in that the faecal matrix is only an approximation (or surrogate) of what might be expected within the context of an emergency setting. Whilst having to resort to a surrogate matrix-based approach may appear to be a limitation of this study, it more closely reflects the situation in the field, as the equipment required to carry out such Phase II analyses seldom exists in emergency and/or low-resource settings.

5.4.2 RATIONALE FOR THE CHOICE OF THE SPECIFIED REAGENT DOSAGES AND TARGETED pH

The rationale for the choice of the dosages and targeted pH specified in Chapter 3 is here discussed. It is important to make it clear that, although the expression 'incremental addition' was previously used, the addition of varying amounts of coagulant took place simultaneously in all jars.

1. Jar 1: Control jar (no addition of $Ca(OH)_2$). This jar enabled the removal achieved as a result of simple mechanical agitation and overnight sedimentation to be compared with the removal achieved by the other jars, to which different amounts of $Ca(OH)_2$ were added;
2. Jar 2: Sufficient $Ca(OH)_2$ dosage to achieve a final pH level of approximately 8.6, within a range in which none of the discussed reactions had yet started to take place (see section 2.6.4);
3. Jar 3: Sufficient $Ca(OH)_2$ dosage to reach a final pH level of approximately 9.5, at which point reactions (1) and (2) (precipitation of calcium carbonate) have been completed, but reactions (3) and (4) (precipitation of calcium phosphates) and (5) (precipitation of magnesium ions) have not yet begun;

4. Jar 4: Sufficient $Ca(OH)_2$ dosage to reach a final pH level of approximately 10.4, at which point reactions (1) and (2) have been completed, reactions (3) and (4) have not yet taken place (at least not significantly) and reaction (5) (precipitation of magnesium ions) has started to take place, but is still far from completion;
5. Jar 5: Sufficient $Ca(OH)_2$ dosage to reach a final pH level of approximately 11.3, at which point all reactions, i.e., 1-5 are completed;
6. Jar 6: Sufficient $Ca(OH)_2$ dosage to reach a final pH level that is equal to or higher than 12.2. The objective of this last jar experiment was to quantitatively analyse the effect of a further addition of lime after completion of all reactions previously mentioned. According to the chemical theory discussed, at pH higher than 11.3, no more chemical reactions inducing further precipitation should take place. The only precipitation taking place is the one induced by gravity. On the other hand pH-driven disinfection will be greater at higher pH levels.

The limitations of this study related to the specified choice of the dosage and targeted pH are discussed in Chapter 9.

5.4.3 PRELIMINARY TEST

The experimental design led to the addition of a preliminary test to be performed before commencing the jar-test experiment. This was designed to determine the exact amount of $Ca(OH)_2$ to be added to each new batch of faecal waste matrix, in order to reach the desired pH. The test was performed the day that the jar-test commenced, within four hours of the start of the test and consisted of the incremental addition of $Ca(OH)_2$ to a sample of mixed liquor, followed by rapid manual mixing, pH recording and subsequent $Ca(OH)_2$ addition. The aim was to produce a graph like the one in Figure 5.1, which could be used to guide the author in deciding the amount of subsequent reagent to add to each jar in order to reach the target pH level.

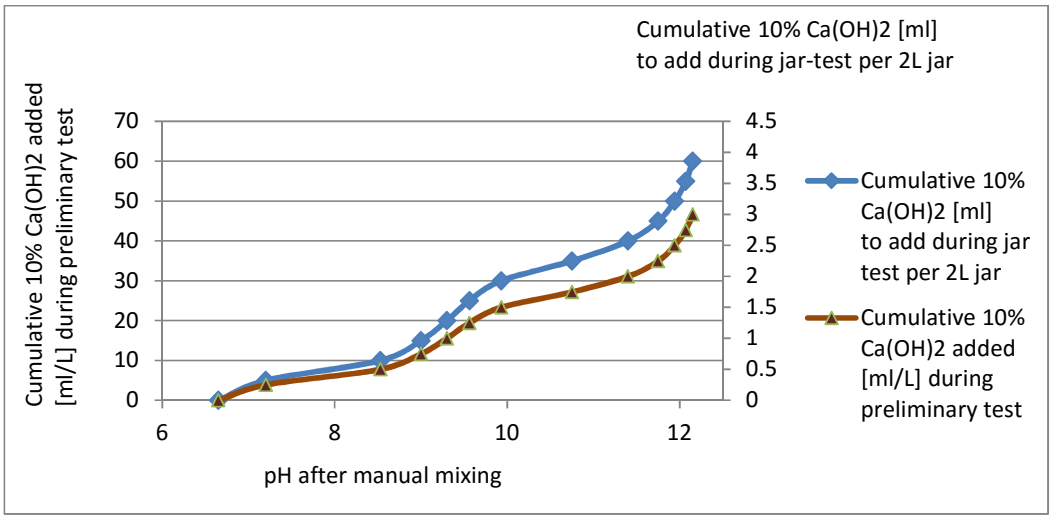


Figure 5.1: Example of graph plotted after a preliminary test, representing the non-linear correlation between addition of hydrated lime and pH level after manual mixing

The choice of the pH-range to target was guided by the theory on hydrated-lime induced coagulation-flocculation, as explained in the previous chapter. The main goal of the laboratory investigations was to understand the role played by the chemical reactions; all started ('driven') as a consequence of the fact that a certain pH level had been reached (rather than the fact that a specific amount of coagulant had been added).

The research objective was the identification, for a hypothetical future full-scale treatment, of the minimum pH level required to be able to reasonably expect that a specific 'target' removal efficiency was achieved. As a result, this would allow the designer of a treatment unit to know which of the discussed reactions would be completed at the pH level necessary to ensure that a certain removal efficiency had been achieved.

In order to achieve a better understand the role played by each group of reactions in the removal of microorganisms (pathogens), suspended solids and other pollutants, and to correlate the pH with the expected removal rate, it was decided to add the coagulant doses required to reach the above mentioned pH levels for each jar (see Chapter 3).

5.4.4 THE ROLE PLAYED BY THE PRESENCE OF MAGNESIUM IONS

During the preliminary phase of the bench-scale experiments, the concentration of magnesium ions present in the matrix before and after physico-chemical treatment with different dosages of coagulant was measured using two sets of jar-tests. The

aim here was to elucidate the role played by the magnesium ions during the lime-induced coagulation-flocculation process, (Eq. 5). This was achieved by means of the following two objectives:

- To establish if the concentration of magnesium ions present in the faecal waste matrix used, contributed to additional formation of precipitate and, therefore, additional removal of suspended solids and indicator organisms;
- To explore the possibility of adding magnesium salts (e.g., magnesium sulphate) to the faecal waste matrix in order to promote further formation of precipitates and, therefore, to enhance the treatment efficiency.

It is important to mention that it is considered beyond the scope of this study to establish a complete correlation between the concentration of magnesium ions (Eq. 5) or any other element involved in the reactions presented in Eq. (1) to (4) (i.e. carbonic acid, calcium bicarbonate and phosphate ions) and the final removal efficiency. This correlation would be applicable only to the specific faecal waste matrix analysed and therefore would not be applicable to different types of waste. The aim here was rather to analyse the importance of the mentioned reactions in the lime induced physico-chemical treatment studied and to determine whether the concentration of magnesium and phosphate ions plays a role in the coagulation and flocculation efficiency.

5.5 LOW-pH TREATMENT PROTOCOL

5.5.1 ALTERNATIVES TO THE EXPERIMENTAL DESIGN

As for the high-pH treatment, two alternatives for the experimental design of the low pH treatment were identified: (1) the addition of a constant amount of acid to each jar during the test repetitions, accepting the inevitable significant variation, from test to test, in the pH range reached by each single jar; or (2) defining the protocol not by constant acid addition, but by pH ‘end-points’, in that the acid is added to reach a prescribed pH level so that variability in wastewater composition ceases to be a factor in treatment efficacy. As for the previously explained treatment, the second alternative was chosen by the author of this study at the end of the first phase of preliminary experiments. The main reason was that this type of treatment has two ‘components’ running ‘in parallel’: a ‘sedimentation’ component (that proved to be more important than had been expected, although the reagent added here is not defined as a coagulant) and a ‘disinfection’ component. The first one is responsible for the physical

separation of the pathogens, the suspended solids and many other pollutants. As previously discussed, the most important parameters regarding this aspect are mixing regimes, time and, particularly, acid dose. The low pH reached during this type of batch treatment is the main factor responsible for the second treatment 'component': the deactivation of bacterial and viral pathogens. As the technique investigated is part of a global public health effort aiming to control the spread of a disease in an epidemic context rather than the reduction of the effluent suspended solids, priority was given to the 'disinfection' component of the treatment. In other words this technique was designed not as a 'dosage-driven', but as a 'pH-driven' disinfection process.

5.5.2 RATIONALE FOR THE CHOICE OF THE SPECIFIED REAGENT DOSAGES AND TARGETED pH

The rationale for the choice of the dosages and targeted pH specified in Chapter 3 is discussed below. Although the expression 'incremental addition' is used here, the addition of different amounts of acid took place simultaneously in all jars.

1. Jar 1: Control jar (no addition of HCl). This jar enabled the removal achieved as a result of simple mechanical agitation and overnight sedimentation to be compared with the removal achieved by the other jars, to which different amounts of HCl were added;
2. Jar 2: Sufficient HCl dosage to achieve a final pH level of approximately 5.8. The following jar experiments were designed to reach a final pH level of approximately 5.0, 4.2, 3.4 and 2.6.

After completion of a set of preliminary jar-tests, performed during the initial design-phase of this study, the previously mentioned 'target pH levels' were defined and kept constant during the entire course of the study. As previously mentioned, the main goal of the study was the repetition of acidification and disinfection process (which is pH-driven rather than dosage-driven) to be performed always at approximately the same pH level. It was therefore accepted that any faecal waste matrix requires variable quantities of reagent to keep the target pH level constant. The limitations of this study related to the specified choice of the dosages and targeted pH are discussed in Chapter 5.

5.5.3 PRELIMINARY TEST

The experimental design as presented led to the addition of a preliminary test to be performed before starting the jar-test experiment, aimed at determining the exact amount of HCl to be added to each new - specific - faecal waste matrix in order to reach the target pH level. The test was performed the same day that the jar-test began, within four hours and consisted of the incremental addition of HCl to a sample of mixed liquor, followed by rapid manual mixing, pH recording and addition of fresh acid. The aim was to plot a graph like the one in Figure 5.2. This graph was plotted to guide the author in deciding the amount of acid to add to each jar in order to reach the target pH level.

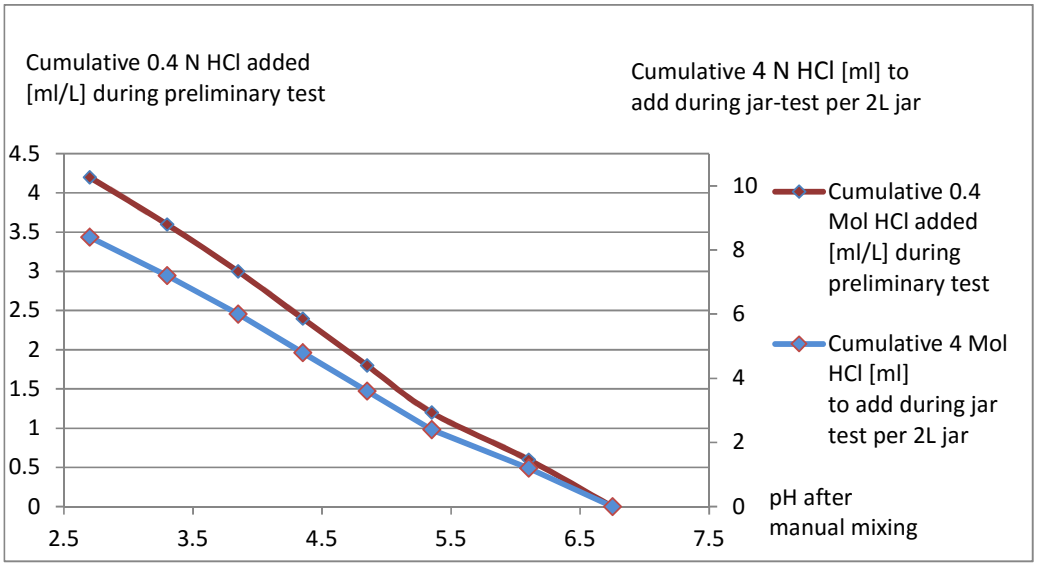


Figure 5.2: Example of graph plotted after a preliminary test, representing the non-linear correlation between hydrochloric acid addition and pH after manual mixing. The correlation is to be followed from the right to the left (from neutrality to acidic conditions).

5.6 ADAPTATION OF THE STANDARD METHOD FOR THE MEASUREMENT OF COD

As mentioned above, the test was performed using reagents prepared in the laboratory, with the aim of keeping costs under control. The samples were digested in a strong solution of sulphuric acid, potassium dichromate and mercury sulphate. During digestion, the dichromate ion oxidises the organic material, which results in the change of chromium from the hexavalent (+6) to the trivalent (+3) state; the two

ions have a different colour, both absorbed in the visible region of the spectrum. Through photometric measurement it is possible to determine indirectly the amount of chromium that has turned to the trivalent (+3) state during digestion and therefore to infer the amount of COD in the original sample.

The applied standard closed reflux colorimetric method is an empirical method based on the assumption that a linear correlation between the absorbance of the digested sample and its COD value can be established, as suggested by standard method [160]. Therefore, at the beginning and end of the study a correlation curve was prepared using six different standards of known COD concentration, prepared by combining different volumes of distilled water and potassium hydrogen phthalate. This curve is shown in Figure 5.3.

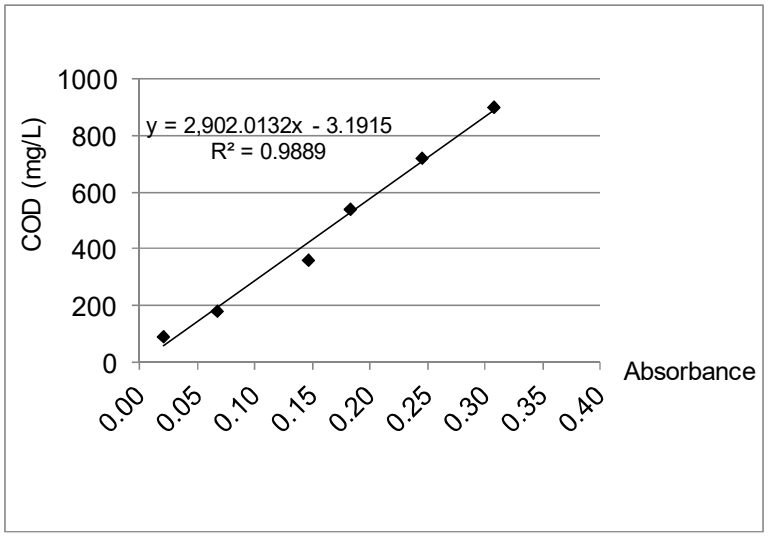


Figure 5.3: Linear correlation between COD and absorbance at 600 nm wavelength

During the preliminary tests it became clear that the type of digester available in the laboratory would perform better when used with a lower sample volume than the one recommended by standard methods, as the recommended volume (7.5 ml in total) was causing some vials to break during digestion. Therefore, the standard method was adapted slightly and the volumes of acid, digestion solution and sample were proportionally reduced, always ensuring that the prescribed proportions were respected.

6. CHAPTER SIX: RESULTS OF LABORATORY-SCALE TREATMENT (PHASE II MICROBIOLOGY)

In this chapter, the results of the laboratory-scale (or 'bench-scale') studies are presented, with a particular focus on the microbiological parameters. Section 6.1 provides a brief comparison of the disinfection efficacy achieved under controlled laboratory conditions for treatment protocols A and B, as they were applied at full-scale in Haiti (during Phase I). The mean removal rates achieved by both methods at the most extreme pH levels are compared in order to determine the removal of key indicators of microbiological pollution (namely, thermotolerant coliforms, intestinal enterococci and somatic coliphage). While this section specifically focuses on the treatment performance under extreme (high and low) pH conditions only (as was the case in Haiti), the subsequent two sections provide further detail of the treatment performance at a range of pH conditions beyond neutrality, with section 6.2 focussing on the high-pH treatment results and section 6.3 focussing on the low-pH treatment.

6.1 VALIDATION OF PHASE I FIELD EXPERIMENTS: OVERVIEW OF MICROBIOLOGICAL REMOVAL RATES UNDER THE MOST EXTREME pH CONDITIONS

The results from the laboratory batch jars tests that were performed under the most extreme pH conditions (i.e., highest concentration of reagent) for both Protocol A and B are summarised in Table 6.1. These results serve to satisfy the first research objective: the validation of Phase I field-experiments results. A wastewater volume of 2 L was treated in each of the 114 batch experiments reported here, approximately half of which were undertaken according to Protocol A (high-pH), and half according to Protocol B (low-pH) .

Protocol A (high-pH), achieved at a pH level that was equal to or higher than 11.7, resulted in an effectively disinfected effluent, with a recorded removal rate of thermotolerant coliforms that was consistently greater than 4.3 Log_{10} (99.995%), with an average reduction of 5.3 log_{10} (99.9986%). For the same treatment protocol, removal of intestinal enterococci was consistently greater than 0.9 Log_{10} (88%), with an average reduction of 3.1 log_{10} (97%). The highest recorded removal rates were 6.2 log_{10} (99.9999%) for thermotolerant coliforms and 5.3 log_{10} (99.9995%) for intestinal enterococci.

It is important to note that the log_{10} of the arithmetic mean (or average) is not equivalent to the arithmetic mean of all log_{10} removal rates. For the purposes of this study, the arithmetic mean of all log_{10} removal rates was used.

For the same treatment, the removal of somatic coliphages was consistently greater than 1.1 Log_{10} (92%), with an average reduction of 2.7 Log_{10} (98%). The highest recorded removal rate was 4.9 Log_{10} (99.999%).

Protocol B (low-pH), achieved at a pH level equal to or lower than 3, resulted in an effectively disinfected effluent, but a less uniform removal rate of thermotolerant coliforms was observed (only 6% on one occasion) and an average removal rate of 2.2 Log_{10} (82%). For the same treatment, removal of intestinal enterococci was more uniform, being consistently observed to be greater than 1.1 log_{10} (91%), with an average reduction of 3.1 Log_{10} (98.8%). The highest recorded removal rates were 3.97 log_{10} (99.99%) for thermotolerant coliforms and 5.1 Log_{10} (99.999%) for intestinal enterococci.

For the same treatment protocol, the recorded removal rate for somatic coliphages was consistently greater than 2.34 log_{10} (99.55%), with an average reduction of 3.27 log_{10} (99.86%). The highest recorded removal rates were 4.95 Log_{10} (99.9989%). The key values for thermotolerant coliforms, intestinal enterococci and somatic coliphages concentrations (mean values and ranges) for raw wastewater and treated effluent are summarised in Table 6.1.

Table 6.1: Comparison of raw and treated wastewater quality from laboratory-scale treatment protocols

Parameter	Raw wastewater		Treated effluent [*1]		Removal [as % and Log10]	
	Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range
High pH treatment						
TTC (CFU per 100 ml)	2.7×10^7	1.7×10^7 – 4.3×10^7	3.2×10^2	0.5 – 7.7×10^2	99.9986% 5.3 Log10*	99.9953% – 99.99989% 4.3 – 6.2 Log10
IE (CFU per 100 ml)	6.2×10^5	1.3×10^5 - 9.8×10^5	5.7×10^3	5 – 2.3×10^4	97% 3.1 Log10*	88% – 99.9995% 0.9 – 5.3 Log10
SC (PFU per 100 ml)	5×10^5	4.2×10^4 – 1.1×10^6	6.75×10^3	10 – 2.2×10^4	98% 2.7 Log10*	92% – 99.9988% 1.1 – 4.9 Log10
Low pH treatment						
TTC (CFU per 100 ml)	1.2×10^7	3.3×10^6 – 4.3×10^7	6.4×10^5	5×10^2 – 3.1×10^6	82% 2.17 Log10*	6% – 99.989% 0.03 – 3.97 Log10
IE (CFU per 100 ml)	7.8×10^5	1.2×10^5 – 1.9×10^6	4.5×10^3	1.4×10^1 – 3.5×10^4	98.8% 3.1 Log10*	91% – 99.9993% 1.1 – 5.1 Log10
SC (PFU per 100 ml)	1.9×10^5	4.4×10^3 – 2.8×10^5	1×10^2	2.5 – 3×10^2	99.86% 3.27 Log10*	99.55% – 99.9989% 2.34 – 4.95 Log10
Combined Summary						
TTC (CFU per 100 ml)	1.76×10^7	3.3×10^6 – 4.3×10^7	4.1×10^5	0.5 – 3.1×10^6	-	-
IE (CFU per 100 ml)	7.2×10^5	1.2×10^5 – 1.9×10^6	4.9×10^3	5 – 3.5×10^4	-	-
SC (PFU per 100 ml)	4.1×10^5	4.4×10^3 – 1.1×10^6	3.4×10^3	10 – 2.2×10^4		

TTC= Thermotolerant Coliforms; COD= Chemical Oxygen Demand; TSS= Total Suspended Solids; CFUs= Colony-Forming Units; PFUs= Plaques-Forming Units

[*] Note: The mean of the Log_{10} reduction is different from the Log_{10} of the mean of all % reductions

6.2 MICROBIOLOGICAL REMOVAL RATES UNDER ALL ALKALINE PH CONDITIONS FOR PROTOCOL A

In this section, results achieved by Protocol A (high pH) are presented in detail.

6.2.1 MICROBIOLOGICAL INDICATOR REMOVAL AS A FUNCTION OF pH LEVEL

Figure 6.1 demonstrates the Log_{10} removal of thermotolerant coliform organisms as a function of the pH level reached within each jar following completion of the coagulation – flocculation process. Here, the data points have been divided into five distinct groups, according to the pH level reached following the addition of hydrated lime. The groups may be defined as follows:

a. pH level corresponding to the first jar, to which no hydrated lime had been added (control jar);
b. pH level ranging from that of the raw sewage with the initial addition of hydrated lime (always higher than pH 7) to pH 8.99 (second jar). These data-points represent the jars in which only minimum flocculation had been induced;
c. pH level from 9 to 9.6. This group represents the jars in which the precipitation of calcium carbonate (see reactions (1) and (2) as outlined in Chapter 2, section 2.6.4 had taken place to full completion, but in which the precipitation of calcium phosphate (reaction (3)), calcium hydrogen phosphate (reaction (4)) and magnesium hydroxide (reaction (5)) had not yet begun;
d. pH level from 9.7 to 11.7. This group represents those jars in which the coagulation of calcium phosphate, calcium hydrogen phosphate and magnesium hydroxide (reaction (3), (4) and (5)) has taken place to completion;
e. pH levels higher than 11.7. These data-points describe what was observed when the aforementioned reactions had all run to completion, but when additional hydrated lime had subsequently been added to increase further the pH level for improved disinfection.

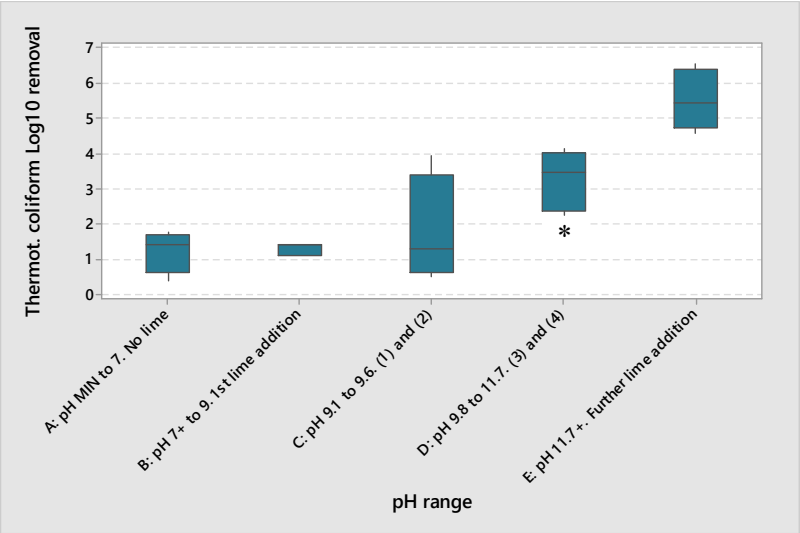


Figure 6.1: Log_{10} removal of thermotolerant coliforms as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 28. Data points deemed to be outliers are indicated by an asterisk; data points defined as outliers in the graphs representing the percentage removal of a certain parameter have been also considered as outliers when representing the same removal as Log_{10}.

Figure 6.2 shows the Log_{10} removal of intestinal enterococci as a function of the pH level. The data points have been divided into the same groups (A-E) as for the thermotolerant coliforms.

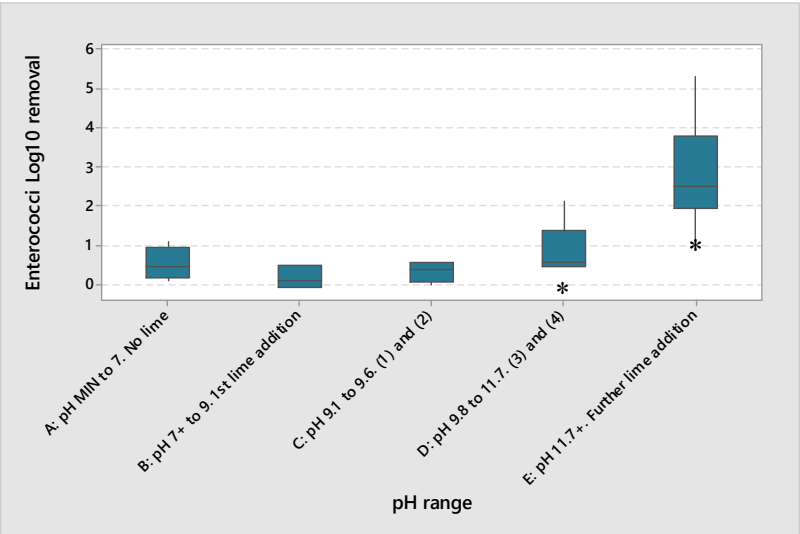


Figure 6.2: Log_{10} removal of intestinal enterococci as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 28. Data points deemed to be outliers are indicated by an asterisk; data points defined as outliers in the graphs representing the percentage removal of a certain parameter have been also considered as outliers when representing the same removal as Log_{10}.

Figure 6.3 shows the Log_{10} removal of somatic coliphages as a function of the pH level. The data points have been divided into the same groups as previously explained.

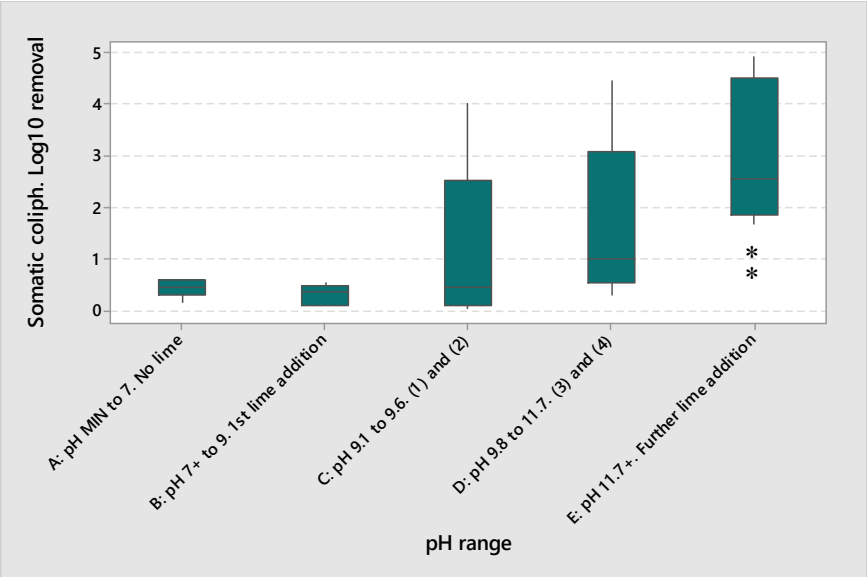
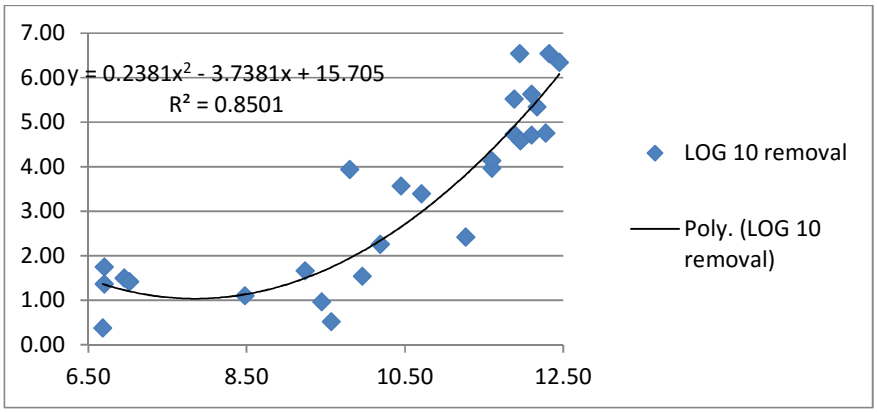


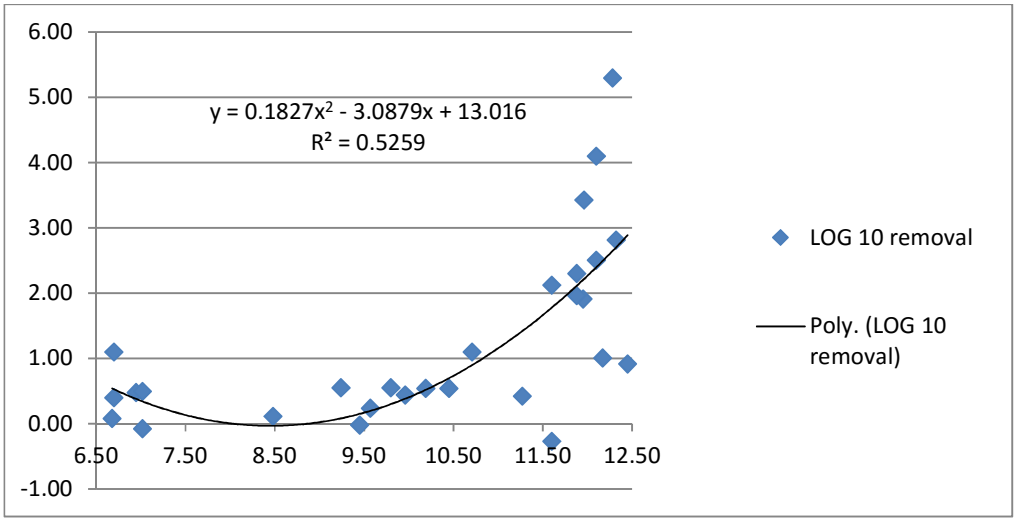
Figure 6.3: Log_{10} removal of somatic coliphages as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 40. Data points deemed to be outliers are indicated by an asterisk; data points defined as outliers in the graphs representing the percentage removal of a certain parameter have been also considered as outliers when representing the same removal as Log_{10}.

Figures 6.4 represents a functional regression of values representing the Log_{10} removal of thermotolerant coliforms as a function of pH level. The regression was performed using a 2nd degree polynomial function (parabolic regression). The reasons for this choice of a non-linear function are discussed in Chapter 9.



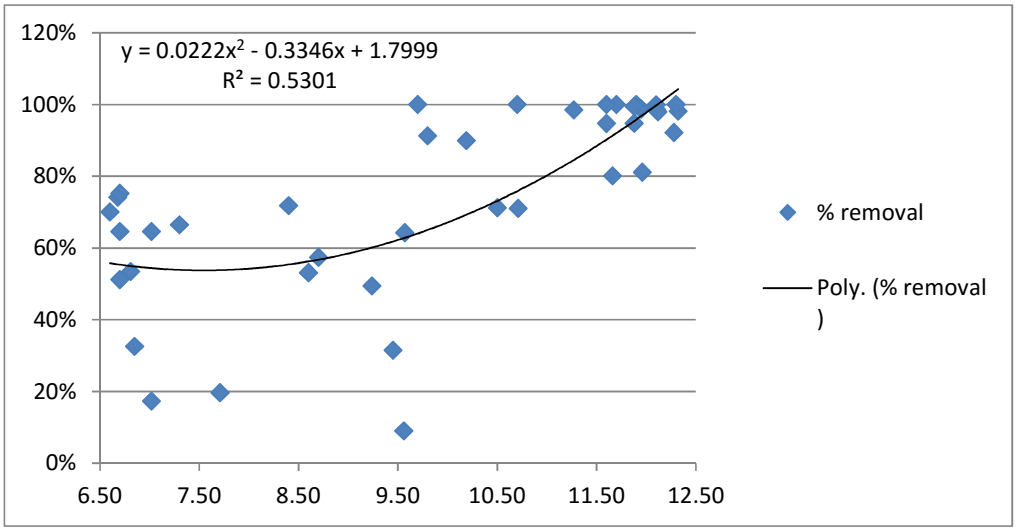
Figures 6.4: 2nd degree polynomial regression for all data-points representing the Log_{10} removal of thermotolerant coliform as a function of the pH. Total number of data points (n): 28.

Figures 6.5 represents a functional regression for the data representing the Log_{10} removal of intestinal enterococci as a function of pH level. As with the thermotolerant coliforms, the regression was performed using a 2nd degree polynomial function (parabolic regression).



Figures 6.5: 2nd degree polynomial regression for all data-points representing the Log_{10} removal of intestinal enterococci as a function of pH. Total number of data points (n): 28.

Figures 6.6 represents a functional regression for the data representing the Log_{10} removal of somatic coliphages as a function of pH level. The regression was performed using a 2nd degree polynomial function (parabolic regression). The reasons for the choice of a non-linear regression through a 2nd degree polynomial instead of a linear regression are discussed in Chapter 9, although for the abovementioned cases the choice of a linear regression may also be considered acceptable.



Figures 6.6: 2nd degree polynomial regression for all data-points representing the percentage removal of somatic coliphages as a function of pH level. Total number of data points (n): 40.

6.2.2 PATHOGEN DEACTIVATION VS. PATHOGEN SEPARATION

As previously explained in Chapter 2, microorganisms are deactivated (or killed) by the direct effect of high pH on the organism's cell wall and internal components (cytotoxic effect). Microorganisms are also physically removed by the coagulation-flocculation sedimentation process (in other words, they are separated from the analysed supernatant and concentrated into the sedimentation sludge). The following terminology is used throughout this thesis: microbial deactivation as a result of high pH is defined as 'disinfection', whereas physical removal caused by physico-chemical processes is defined as 'separation'. The data presented within the current section serve the purpose of quantifying both components. The results are further discussed in Chapter 9.

Figure 6.7 and Figure 6.8 represent the role played by disinfection, and latterly by separation, in the reduction of presumptive thermotolerant coliforms and intestinal enterococci numbers.

The light blue line of Figure 6.7 represents the 2nd degree polynomial function, which approximates the 'log$_{10}$ removal vs. pH' relationship for thermotolerant coliform for samples collected immediately after coagulation-flocculation. The dark blue line represents the function that best approximates the same relationship for samples collected after the coagulation-flocculation process, followed by overnight sedimentation.

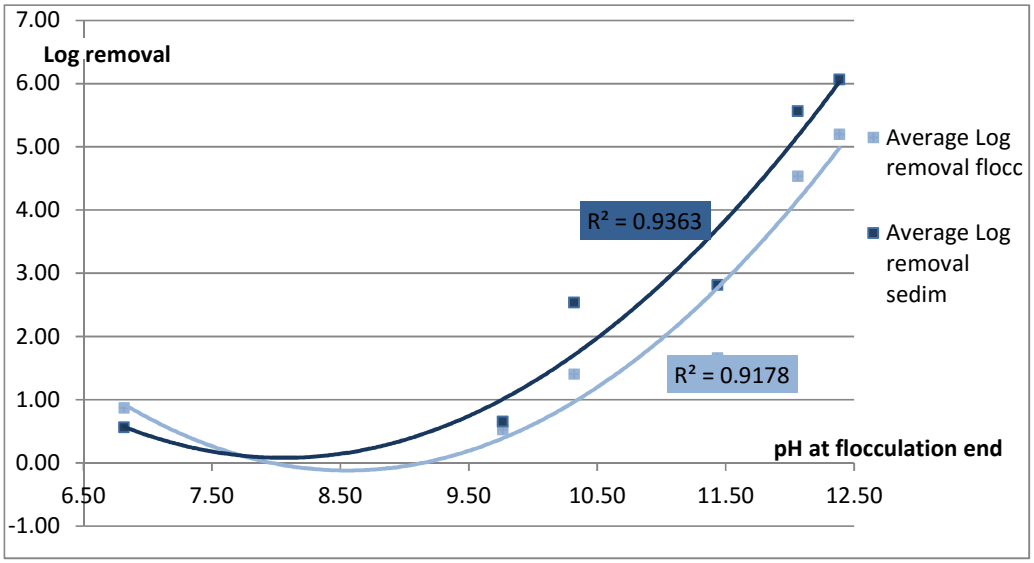


Figure 6.7: 2nd degree polynomial regression of all data-points representing the log$_{10}$ removal of thermotolerant coliform as a function of pH level. Total number of data points (n): 24. The light blue line represents samples collected immediately after coagulation-flocculation; the dark blue line represents the same relationship for samples collected after the coagulation-flocculation process followed by overnight sedimentation.

Similarly, the light blue line of Figure 6.8 represents the 2nd degree polynomial function, which best approximates the 'log removal vs. pH' relationship for presumptive intestinal enterococci samples collected immediately after coagulation-flocculation. The dark blue line represents the function that best approximates the same relationship for effluent samples collected after the coagulation-flocculation process followed by overnight sedimentation.

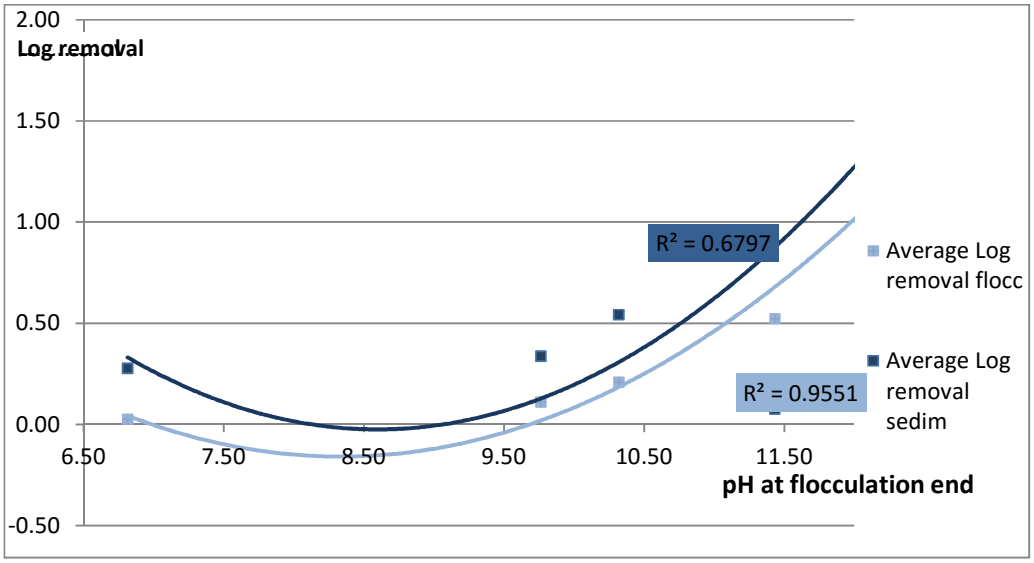


Figure 6.8: 2nd degree polynomial regression of all data-points representing the log_{10} removal of presumptive intestinal enterococci as a function of pH level. Total number of data points (n): 24. The light blue line represents samples collected immediately after coagulation-flocculation; the dark blue line represents the same relationship for effluent samples collected after the coagulation-flocculation process followed by overnight sedimentation.

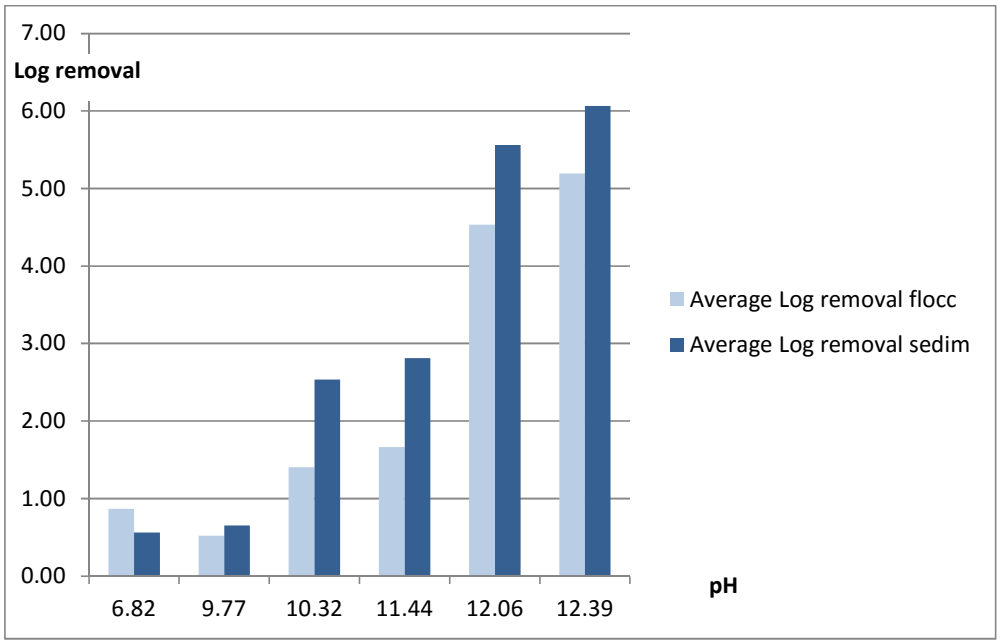


Figure 6.9: Comparison of log_{10} removal of thermotolerant coliforms as a function of pH level. Total number of data points (n): 24. The light blue bars represent samples collected immediately after coagulation-flocculation; the dark blue line represents the same relationship for samples collected after the coagulation-flocculation process followed by overnight sedimentation

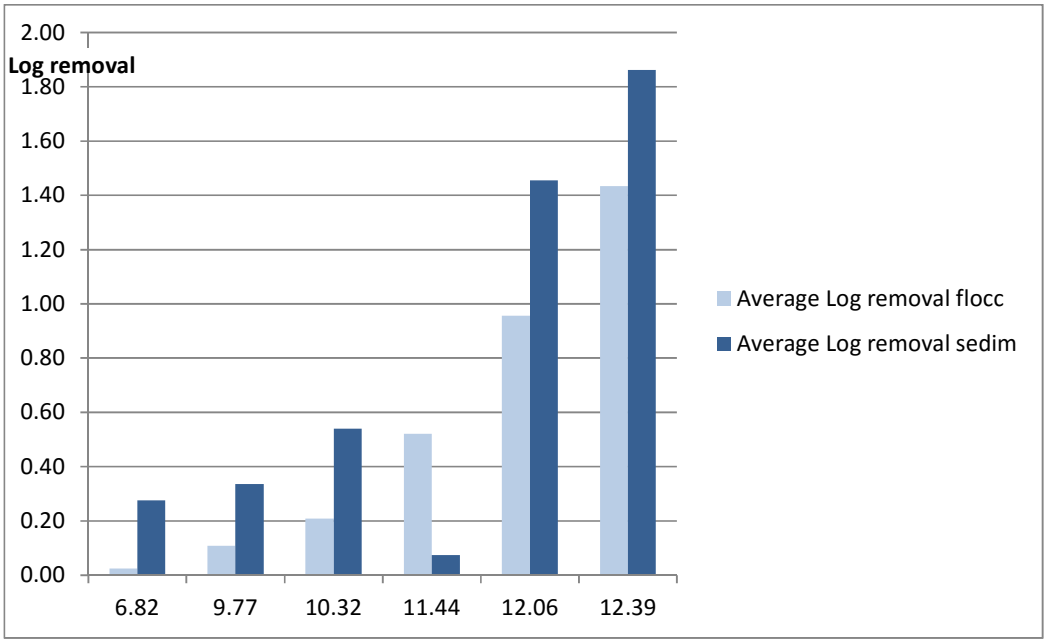


Figure 6.10: Comparison of log_{10} removal of presumptive intestinal enterococci as a function of pH level. Total number of data points (n): 24. The light blue bars represent samples collected immediately after coagulation-flocculation; the dark blue line represents the same relationship for samples collected after the coagulation-flocculation process followed by overnight sedimentation

A comparison of the removal values represented by the light and the dark blue lines in Figure 6.7 and Figure 6.8, and in particular a comparison of the light and the dark blue bars of Figure 6.9 and Figure 6.10, gives an estimation of the role played by pH-induced disinfection only and by the pH-induced disinfection followed by overnight sedimentation (physical separation) respectively in the final (combined) removal of thermotolerant coliform and intestinal enterococci. These specific indicators were chosen to compare the removal values given by the two subsequent processes, but similar considerations could be made for other indicators of microbiological and physico-chemical pollution as well, as explained in Chapter 9.

6.3 MICROBIOLOGICAL REMOVAL RATES UNDER ALL ACIDIC pH CONDITIONS FOR PROTOCOL B

In this section, the results relating to the low-pH treatment protocol are presented in detail.

Figure 6.11 demonstrates the $\log_{10}$ removal of thermotolerant coliform organisms as a function of the pH level reached within each jar following completion of the acidification process. Here, the data points have been divided into five distinct groups, according to the pH level reached following the addition of hydrochloric acid. The groups may be defined as follows:

a. pH level corresponding to the first jar, to which no hydrochloric acid has been added (control jar);
b. pH level ranging from that of the raw sewage with the initial addition of hydrochloric acid (always lower than pH 5.99) to pH 5 (second jar). These data-points represent the jars in which only minimum disinfection had been induced;
c. pH level from 4.99 to 4;
d. pH level from 3.99 to 3; and
e. pH level lower than 3.

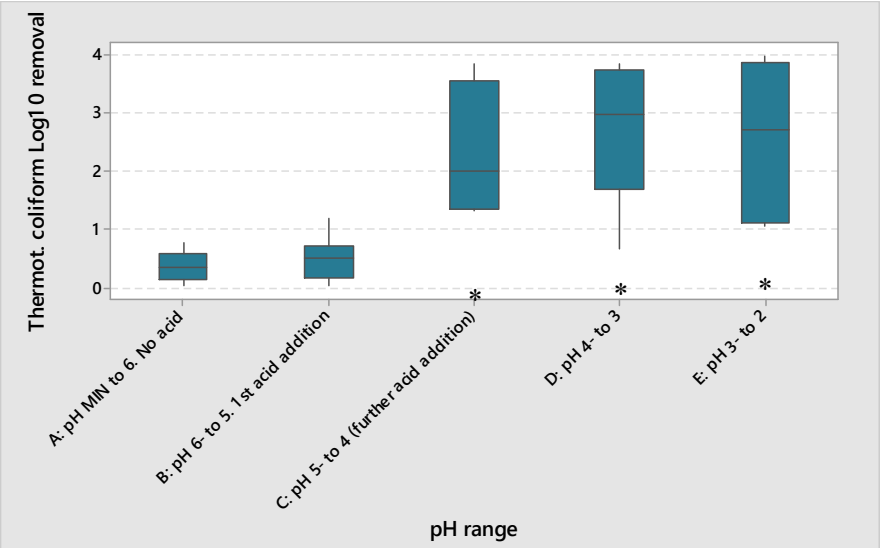


Figure 6.11: Log_{10} removal of thermotolerant coliform organisms as a function of the pH level reached within each jar following completion of the acidification and overnight sedimentation process. Total number of data points (n):51. Data points deemed to be outliers are indicated by an asterisk; outliers are defined consistently in both figures representing percentage and log_{10} removal

Figure 6.12 presents the log_{10} removal of intestinal enterococci as a function of pH level. The data points have been divided into the same groups (A-E) as for the thermotolerant coliform.

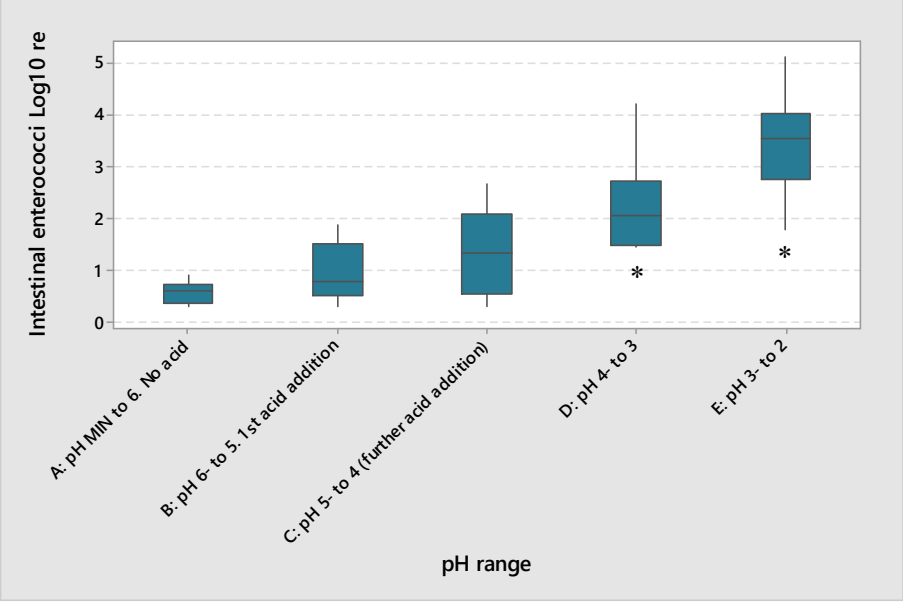


Figure 6.12: Log10 removal of intestinal enterococci as a function of the pH level reached within each jar following completion of the acidification and overnight sedimentation process. Total number of data points (n):51. Data points deemed to be outliers are indicated by an asterisk; outliers are defined consistently in both figures representing percentage and log10 removal

Figure 6.13 presents the log_{10}removal of somatic coliphages as a function of the pH level. The data points have been divided into the same groups (A-E) as previously explained.

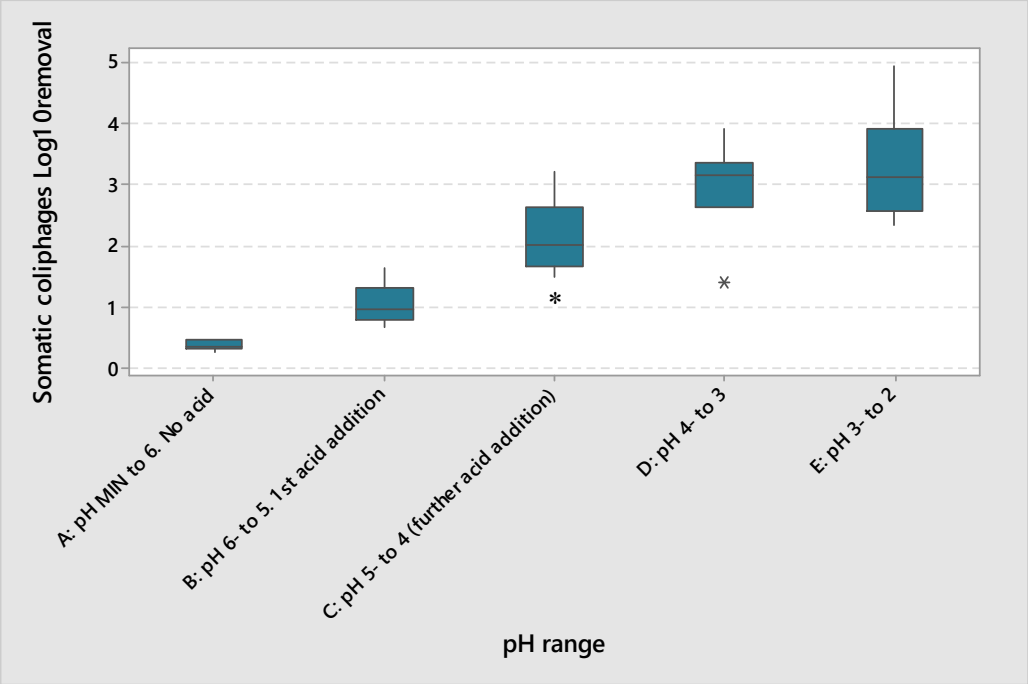
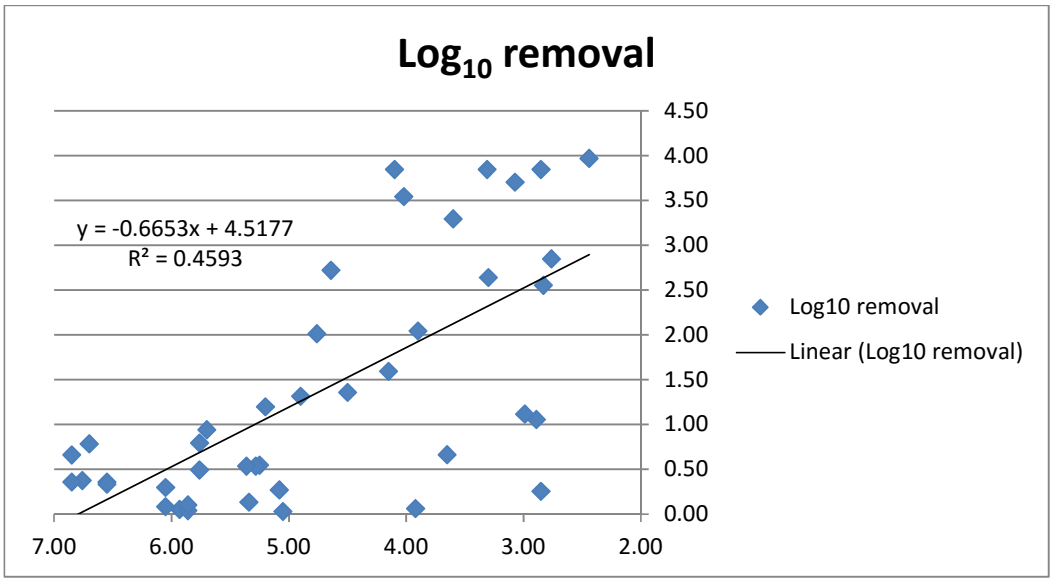


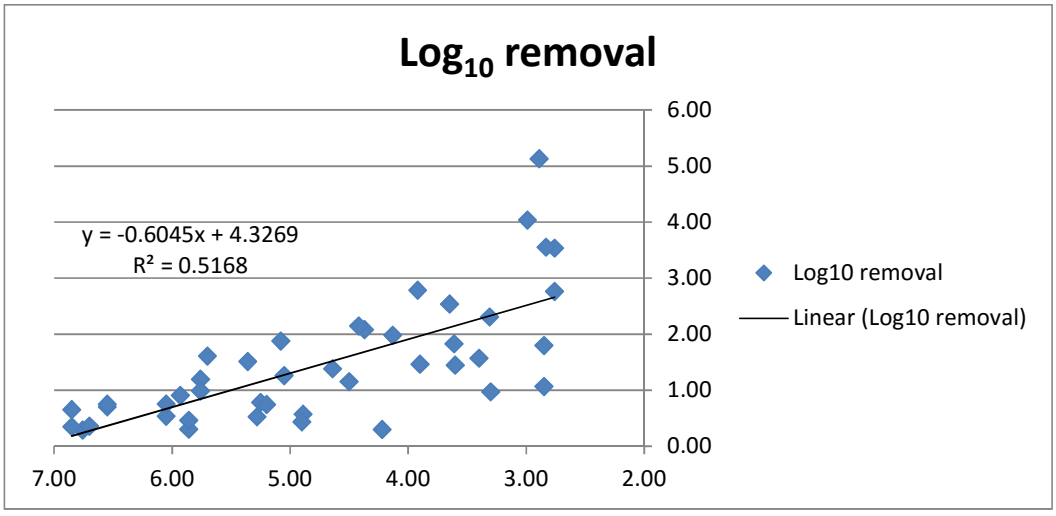
Figure 6.13: Log_{10} removal of somatic coliphages as a function of the pH level reached within each jar following completion of the acidification and overnight sedimentation process. Total number of data points (n):39. Data points deemed to be outliers are indicated by an asterisk; outliers are defined consistently in both figures representing percentage and Log_{10} removal

Figures 6.14 presents a functional regression for the data for log_{10} removal of thermotolerant coliforms as a function of the pH level. The regression was performed using a linear function. The reasons for these choices are discussed in Chapter 9.



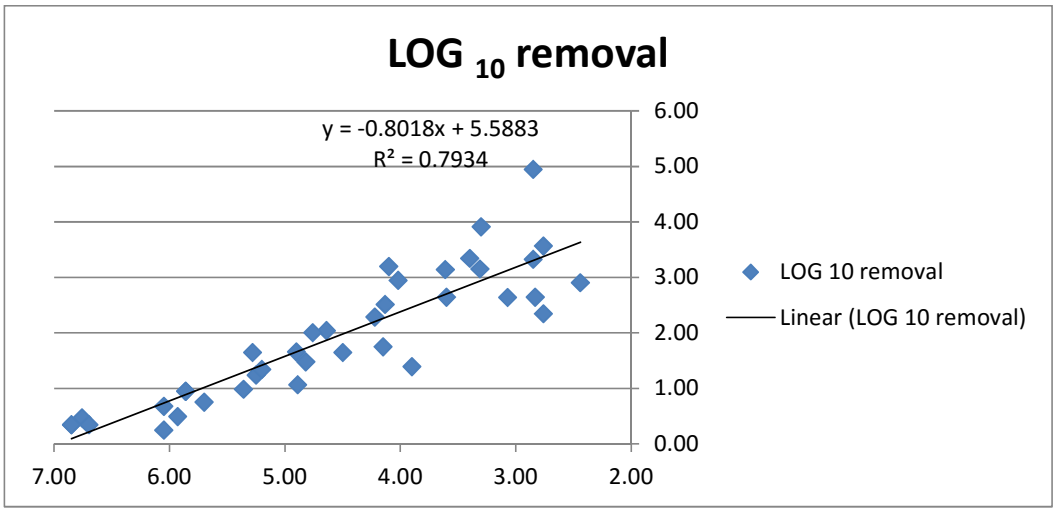
Figures 6.14: Linear regression for all data-points representing the Log_{10} removal of thermotolerant coliforms as a function of the pH reached within each jar following completion of the acidification and overnight sedimentation process. Total number of data points (n): 51.

Figures 6.15 represents a functional regression for the data for the log_{10} removal of intestinal enterococci as a function of the pH level. The regression was performed using a linear function.



Figures 6.15: Linear regression for all data-points representing the log_{10} removal of intestinal enterococci as a function of pH. Total number of data points (n): 51.

Figures 6.16 represents a functional regression for the data for percentage and log_{10} removal of somatic coliphages as a function of the pH level. The regression was performed using a linear function. The reason for the choice of a linear regression for the previous three figures is discussed in Chapter 9, although for these cases the choice of a non-linear regression is also acceptable.



Figures 6.16: Linear regression for all data-points representing the log_{10} removal of somatic coliphages as a function of the pH level. Total number of data points (n):39.

7. CHAPTER SEVEN: RESULTS OF LABORATORY-SCALE TREATMENT: (PHASE II – PHYSICO-CHEMICAL PARAMETERS)

In this chapter, the results of the 'bench-scale' laboratory studies are presented, focusing on the physico-chemical parameters. Section 7.1 provides a brief comparison of physico-chemical removal efficacy under controlled laboratory conditions for both treatment protocols, as applied at full-scale in Haiti (Phase I): the mean treatment results for both methods at the most extreme pH levels are compared, with a focus on the removal of the main indicators of physico-chemical pollution. As in Chapter 6, the first section specifically focuses on the treatment performance under extreme pH conditions only (as in Haiti), whereas the subsequent two sections present a more comprehensive overview of the entire treatment results, not only for the most extreme pH conditions, but more generally for any pH condition beyond neutrality: in sections 7.2, a detailed review of the high-pH treatment results is presented; finally in section 7.3 a detailed review of the low-pH treatment results is presented in a similar way.

7.1 VALIDATION OF PHASE I FIELD-EXPERIMENTS: OVERVIEW OF PHYSICO-CHEMICAL REMOVAL RATES UNDER THE MOST EXTREME pH CONDITIONS

The results of the batch laboratory jars tests performed under the most extreme pH conditions (i.e., highest concentration of reagent), for both treatment methods and for the parameters that were both measured in the field and during the laboratory study, are summarised in Table 7.1.

As in Table 6.1, these results serve to support the first research objective: namely, the validation of phase I field-experiments results. As previously mentioned, a wastewater volume of 2 L was treated in each of the 114 batch experiments reported here, approximately half of which were completed according to Protocol A (high-pH), and half according to Protocol B (low-pH).

Protocol A (achieved at a pH level that was equal to or higher than 11.7) resulted in an effluent with a recorded removal rate of total suspended solids that was consistently greater than 85%, with an arithmetic mean reduction of 90%. For the same treatment protocol, reduction of turbidity was demonstrated to be greater than

87%, with a mean reduction of 92%. The highest recorded levels of removal were 96% for total suspended solids and 98% for turbidity.

For the same treatment, the removal of COD was consistently greater than 80%, with a mean reduction of 84%. The highest recorded removal was 88%.

Protocol B (achieved at a pH level equal to or lower than 3) resulted in an effectively disinfected wastewater, but a less uniform removal rate of total suspended solids and turbidity was observed: it resulted in an effluent with a recorded removal rate of total suspended solids that was consistently greater than 31%, with an average reduction of 48%. For the same treatment protocol, reduction of turbidity was consistently greater than 8% with a mean reduction of 23%. The highest recorded removal rates were 74% for total suspended solids and 49% for turbidity.

For the same treatment, the removal of COD was consistently greater than 77%, with a mean reduction of 83%. The highest recorded removal was 87%.

The most important values for total suspended solids, turbidity and COD (mean values and ranges) for raw and treated wastewater are summarised in Table 7.1.

Table 7.1: Comparison of raw and treated wastewater quality (TSS, turbidity and COD) following laboratory-scale treatment

Parameter	Raw wastewater		Treated wastewater		Removal [as %]	
	Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range
High pH treatment						
Total suspended solids (mg/l)	2,927	1,830 – 4,065	314	111 – 427	90%	85% – 96%
Turbidity (NFU)	2,081	1,668 – 2,800	161	40 – 291	92%	87% – 98%
COD (mg O_2/l)	5,263	2,358 – 8,466	814	283 – 1,027	84%	80% – 88%
Low pH treatment						
Total suspended solids (mg/l)	5,515	5,500 – 5,530	2,873	1,424 – 3,805	48%	31% – 74%
Turbidity (NFU)	2,764	2,309 – 3,220	2,755	1,650 – 2,960	23%	8% – 49%
COD (mg O_2/l)	6,798	6,381 – 7,198	1,144	942 – 1,593	83%	77% – 87%
Summary						
Total suspended solids (mg/l)	3,789	1,830 – 5,530	1,141*	111 – 3,805	71%*	31% – 96%
Turbidity (NFU)	2,309	1,668 – 3,220	1,314*	40 – 2,960	61%*	8% – 98%
COD (mg O_2/l)	5,775	2,358 – 8,466	878	283 – 1,593	84%	77% – 88%

* These mean values should be evaluated with caution, as they represent mean and range of figures related to very different types of treatment (Protocol A and B)

Further results from the batch laboratory jars tests, which were performed under the most extreme pH conditions (i.e., highest concentration of reagent) for both treatment protocols (and presenting the parameters that were only measured during the laboratory study), are summarised in Table 7.2 and Table 7.3.

Protocol A (achieved at a pH level that was equal to or higher than 11.7) resulted in a treated wastewater with a recorded removal of total nitrogen that was consistently greater than 24%, with an average reduction of 51%. For the same treatment protocol, reduction of 'Total Phosphorus' was more variable (no reduction was detected in one batch), but with a mean reduction of 53%. The highest recorded removal rates were 71% for Total Nitrogen and 79% for total phosphorus.

For the same treatment, the removal of ammonia and ammonium ion (recorded as NH_3) was consistently greater than 34%, with a mean reduction of 55%. The highest recorded removal was 87%.

Protocol B (achieved at a pH level equal to or lower than 3) resulted in a treated wastewater with a recorded Total Nitrogen removal that was consistently greater than 29%, with an average reduction of 32%. For the same treatment protocol, reduction of Total Phosphorous was much lower: with 7% being the mean and 3% the minimum reduction recorded. The highest recorded removal levels were 33% for Total Nitrogen and 10% for Total Phosphorous.

For the same treatment, the removal of ammonia and ammonium ion was consistently greater than 31%, with a mean reduction of 33%. The highest recorded removal rate was 35%.

Table 7.2: Comparison of raw and treated wastewater quality for Total Nitrogen, Total Phosphorous and Ammonia – Ammonium (recorded as NH_3) from laboratory-scale treatment

Parameter	Raw wastewater		Treated wastewater		Removal [as %]	
	Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range
High pH treatment						
Total Nitrogen (mg/l)	249	190 – 281	125	69 – 215	51%	24%– 71%
Total Phosphorous (mg/l)	76	54 – 96	29	10 – 58	53%	-7% – 79 %
Ammonia – ammonium (mg/l)	184	60 – 380	45	40 – 50	55%	34% – 87%
Low pH treatment						
Total Nitrogen (mg/l) *	281	281 – 281	191	187 – 200	32%	29% – 33%
Total Phosphorous (mg/l) *	96	96 – 96	90	86 – 94	7%	3% – 10%
Ammonia – ammonium (mg/l) *	88	88 – 88	59	57 – 60	33%	31% – 35%
Summary						
Total Nitrogen (mg/l)	255	190 – 281	136	69 – 215	48%	24 – 71
Total Phosphorous (mg/l)	81	54 – 96	44	10 – 94	41%	-7% – 79 %
Ammonia – ammonium (mg/l)	147	60 – 380	48	40 – 60	50%	31% – 87%

The high-pH treatment required an average addition of 31 ml 10% hydrated lime solution per litre of raw wastewater, the minimum quantity of added lime slurry was 22 ml and the maximum was 39 ml 10% $Ca(OH)_2$ per litre of raw wastewater. The low-pH treatment required an average addition of 3.9 ml 4 molar hydrochloric acid per litre of raw wastewater, the minimum quantity was 3.2 ml and the maximum was 4.6 ml hydrochloric acid per litre of raw wastewater.

Protocol A recorded a mean sludge production volume of 14.3% (v/v sludge / raw wastewater), ranging from a minimum of 8.5% to a maximum of 17.6% (vol/vol). The low-pH treatment recorded an average sludge volume of 25.5%, ranging from 17.7% to 45% (vol/vol).

After drying at 104°C, the high-pH treatment was shown to produce a sludge with a dry weight of 43 g dry matter/L sludge, ranging from a minimum of 26 g to a maximum of 80 g dry matter/L sludge. The low-pH treatment resulted in an average sludge density of 22 g dry matter / L sludge, ranging from a minimum of 12 g to a maximum of 31 g dry matter/L sludge.

The supernatant from the high-pH treatment recorded a mean settleable solids volume of 8.6 ml per L of wastewater, ranging from a minimum of 0.8 ml to a maximum of 35.4 ml settleable solids per L of wastewater. The supernatant from the low-pH treatment recorded a mean settleable solids volume of 64 ml per L of wastewater, ranging from a minimum of 39 ml to a maximum of 108 ml per L of wastewater.

Table 7.3: Comparison of reagent consumption levels and sludge and supernatant characteristics from laboratory-scale treatment

	Arithmetic mean	Range
Protocol A (high pH treatment)		
Concentration 10% hydrated lime solution (ml 10% $Ca(OH)_2$ / L ww)	31	22 – 39
% sludge volume (V sludge / V ww)	14.3%	8.5% – 17.6%
Sludge density after drying at 104°C (g sludge / L sludge)	43	26 – 80
Settleable solids (ml / L effluent)	8.6	0.8 – 35.4
Protocol B (low pH treatment)		
Concentration hydrochloric acid (ml HCl/ L ww)	3.9	3.2 – 4.6
Sludge volume (ml sludge / L ww)	25.5%	17.7% – 45%
Sludge density after drying at 104 C (g sludge / L sludge)	22	12 – 31
Settleable solids (ml / L effluent)	64	39 – 108
Summary		
Concentration 10% hydrated lime solution or hydrochloric acid	–	–
Sludge volume (ml sludge / L ww)	19.9%	8.5% – 45%
Sludge density after drying at 104°C (g sludge / L sludge)	32.3	12 – 80
Settleable solids (ml / L effluent)	36.3	0.8 – 108

7.2 PHYSICO-CHEMICAL REMOVAL RATES FOR ALL ALKALINE pH CONDITIONS FOR THE HIGH-pH TREATMENT

7.2.1 REMOVAL OF PHYSICO-CHEMICAL INDICATORS AS A FUNCTION OF pH

Figure 7.1 shows the removal of COD as a function of the pH level. The data points have been divided into the same groups (A-E) as in Chapter 6 for microbiological indicator behaviour during Protocol A. Further study is needed to more accurately assess if there is a clear correlation between the two values, nevertheless it should be pointed out that there is a little increase and the values are much less 'scattered' and instead closer and instead closer to the (satisfactory) average removal of 83%.

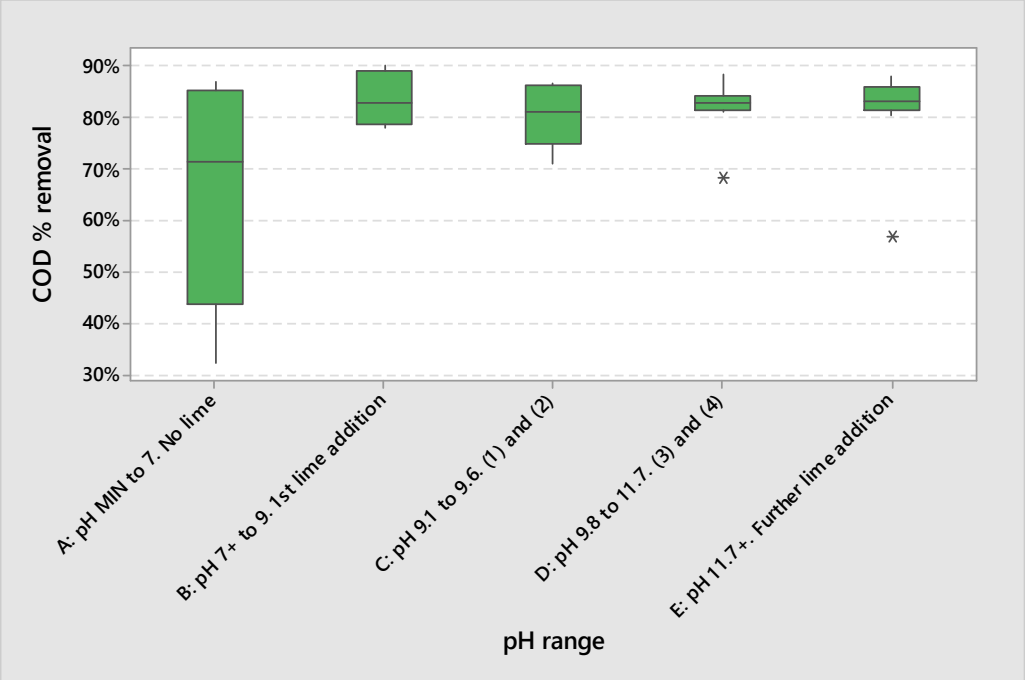


Figure 7.1: Percentage removal of COD as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 42. Data points deemed to be outliers are indicated by an asterisk.

Figure 7.2 presents the removal of total suspended solids as a function of the pH level. The data points have been divided into the same groups (A-E) as previously explained.

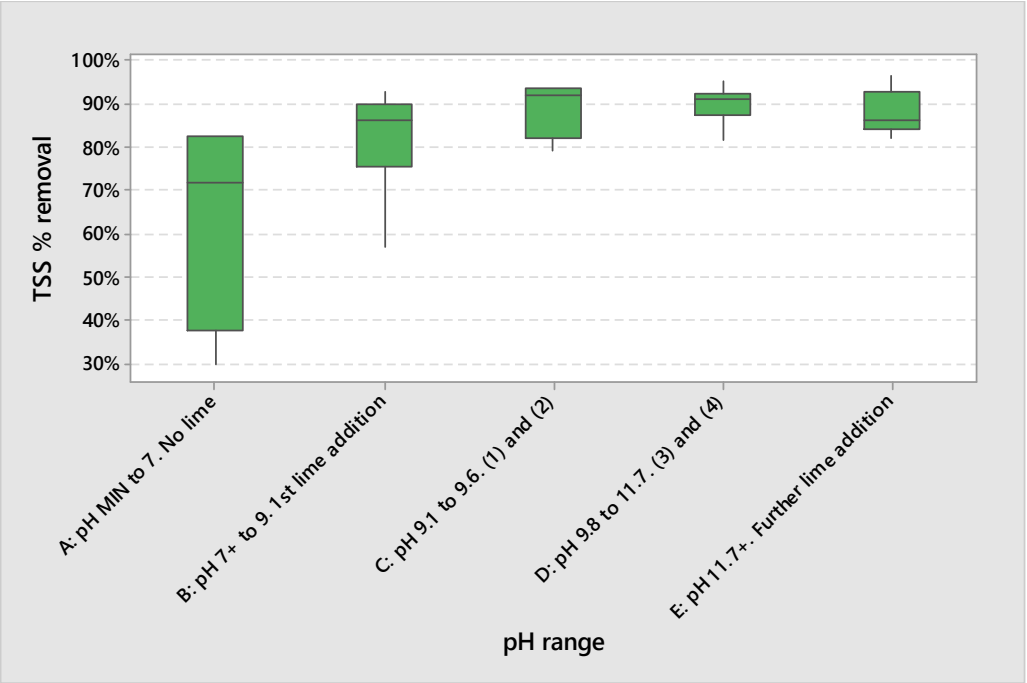


Figure 7.2: Percentage removal of total suspended solids as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 30.

Figure 7.3 presents the removal of turbidity as a function of the pH level. The data points have been divided into the same groups (A-E) as for the total suspended solids.

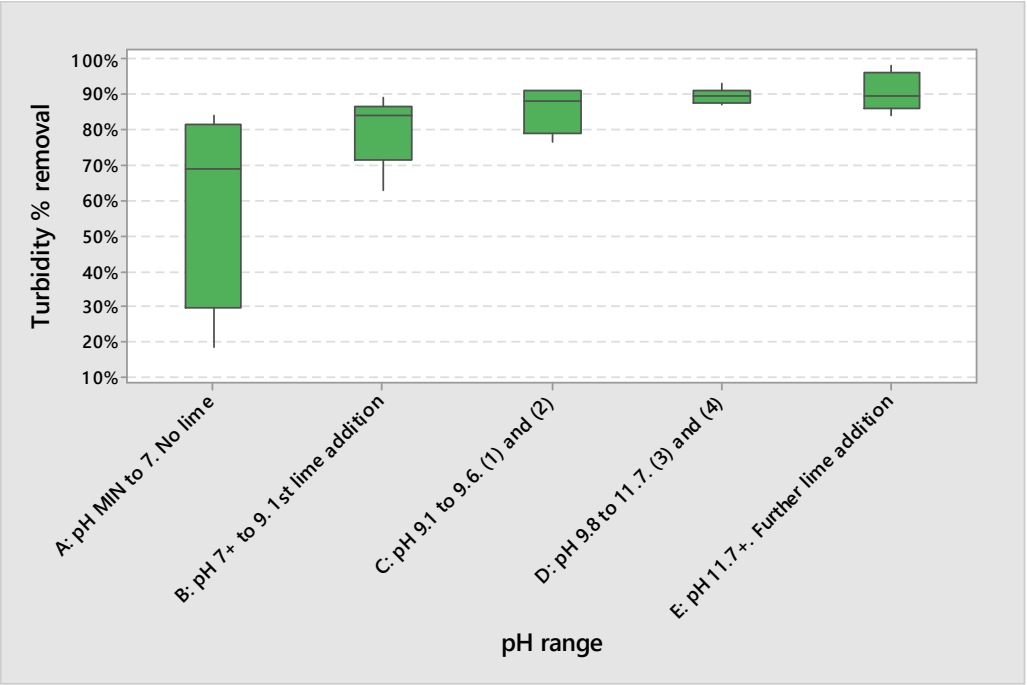


Figure 7.3: Percentage removal of turbidity as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 30.

Figure 7.4 presents the removal of Total Nitrogen as a function of the pH level.

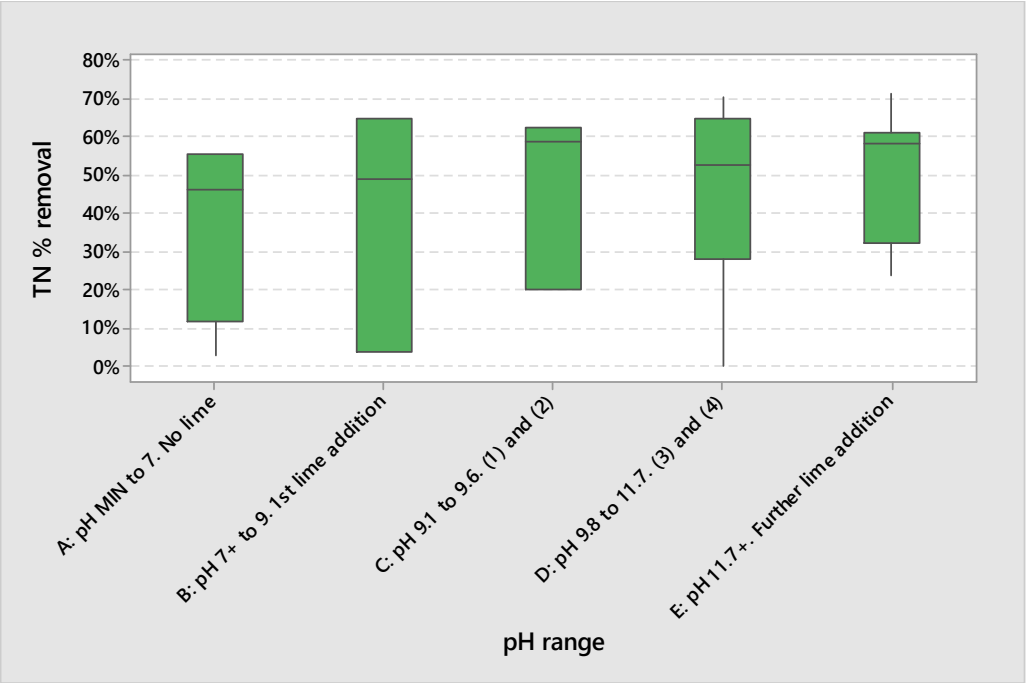


Figure 7.4: Percentage removal of Total Nitrogen as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 30.

Figure 7.5 presents the removal of Total Phosphorous as a function of the pH level.

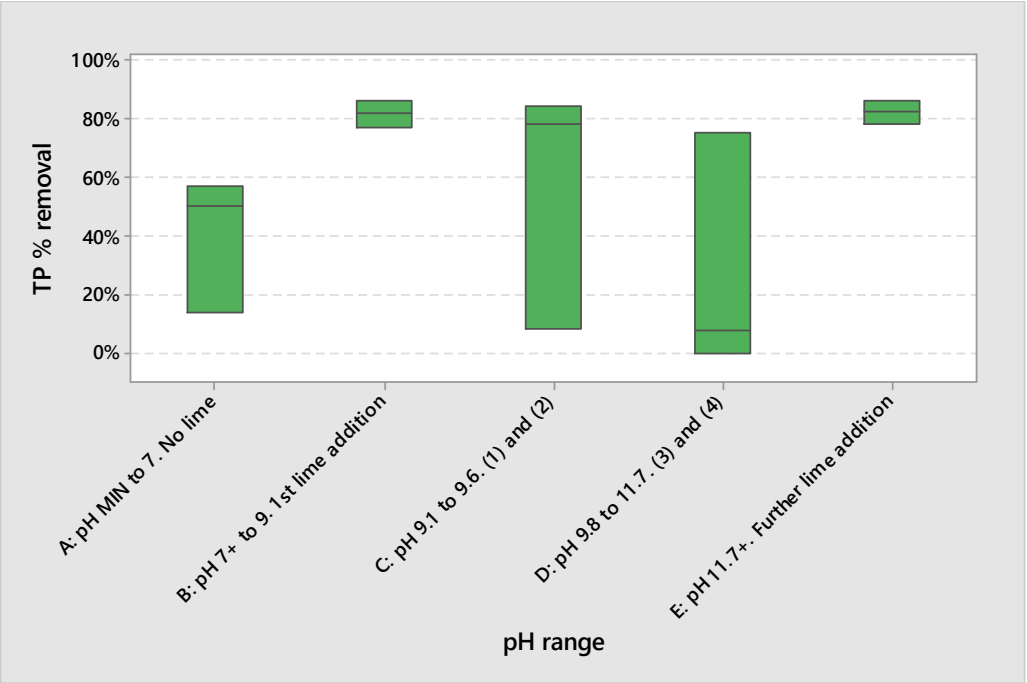


Figure 7.5: Percentage removal of Total Phosphorous as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 16.

Figure 7.6 presents the removal of ammonia and ammonium ion as a function of the pH level.

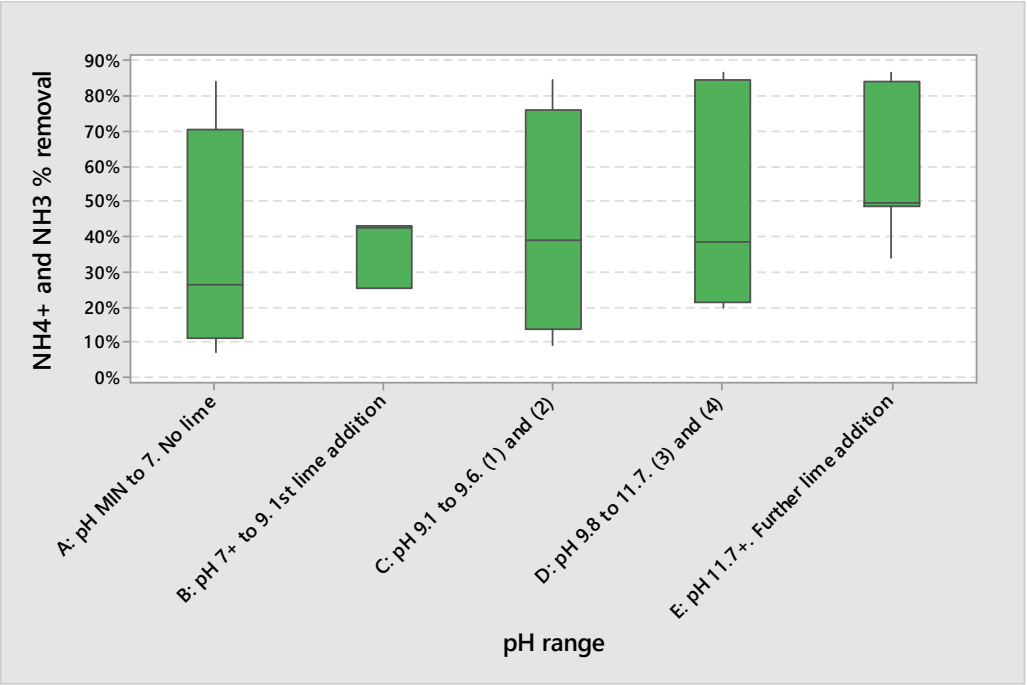


Figure 7.6: Percentage removal of ammonia and ammonium ion as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 24.

In Figure 7.7 and Figure 7.8 (below) the concentration of hydrated lime added to the batch reactor is correlated with the pH level reached at the end of the coagulation-flocculation process. The aim of this approach was to provide a tool to estimate the amount of reagent consumed per unit of faecal waste treated so as to support the practitioner in the field during the design and operation of a full-scale treatment system.

Figure 7.7 demonstrates the amount of hydrated lime added to the reactor as a function of the target pH within each jar following completion of the coagulation – flocculation process.

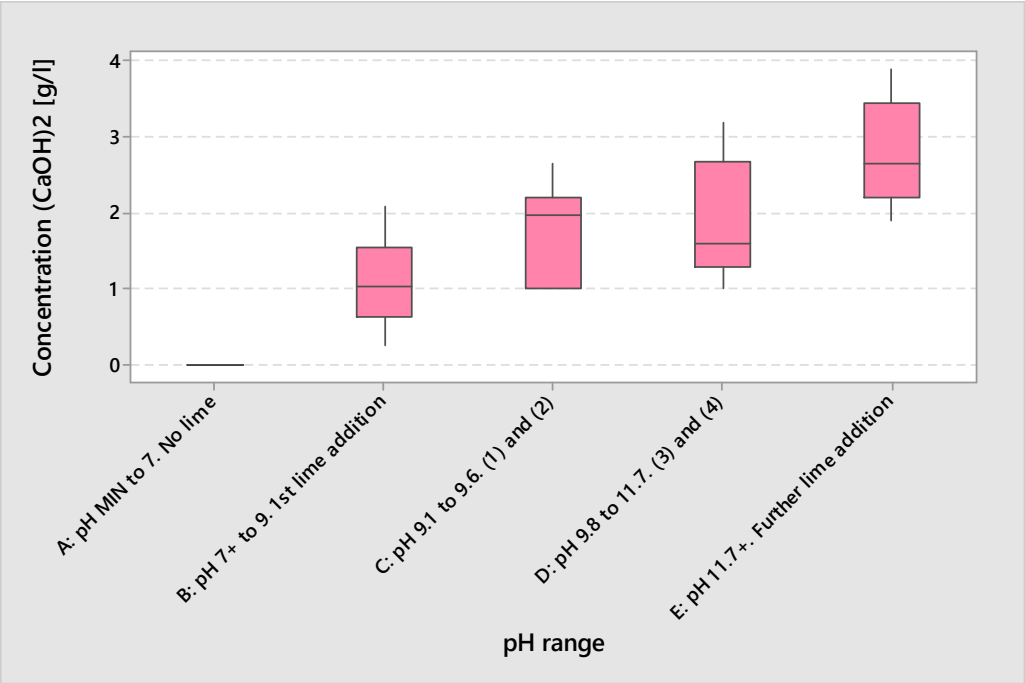


Figure 7.7: Quantity of hydrated lime added to the batch-reactor as a function of the target pH level for each jar following completion of the coagulation – flocculation process. Total number of data points (n): 52.

Conversely, Figure 7.8 provides an estimation, through a linear regression, of the pH reached within each jar following completion of the coagulation – flocculation process for different amounts of added reagent.

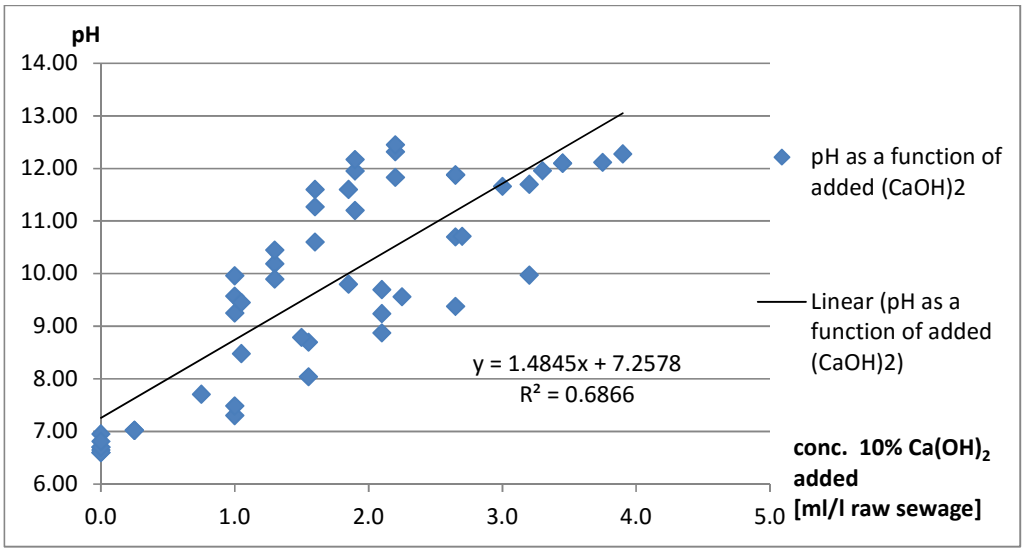


Figure 7.8: Linear regression for all data-points representing the amount of hydrated lime added to the batch-reactor as a function of the target pH level for each jar following completion of the coagulation – flocculation process. Total number of data points (n): 52.

The following two figures (Figure 7.9 and Figure 7.10) present correlations of the sludge characteristics with the pH level reached at the end of the coagulation-flocculation process (and therefore indirectly with the amount of reagent added to the jar). The aim of this approach to the data was to provide a tool to estimate the expected quantity (as volume of sludge per unit of raw wastewater volume) and the concentration (as grammes dry mass per litre of sludge after drying at 104ºC) of the sedimented sludge, so as to provide the practitioner in the field with valuable information during the design and operation of a full scale treatment system.

Figure 7.9 demonstrates the quantity (as volume of sludge) of sedimented sludge per unit volume of raw wastewater, as a function of the pH reached within each jar following completion of the coagulation – flocculation process. Although the sludge volume was on average higher for all treated samples when compared to the control one (no added lime), no clear correlation could be established between sludge volume and treatment pH.

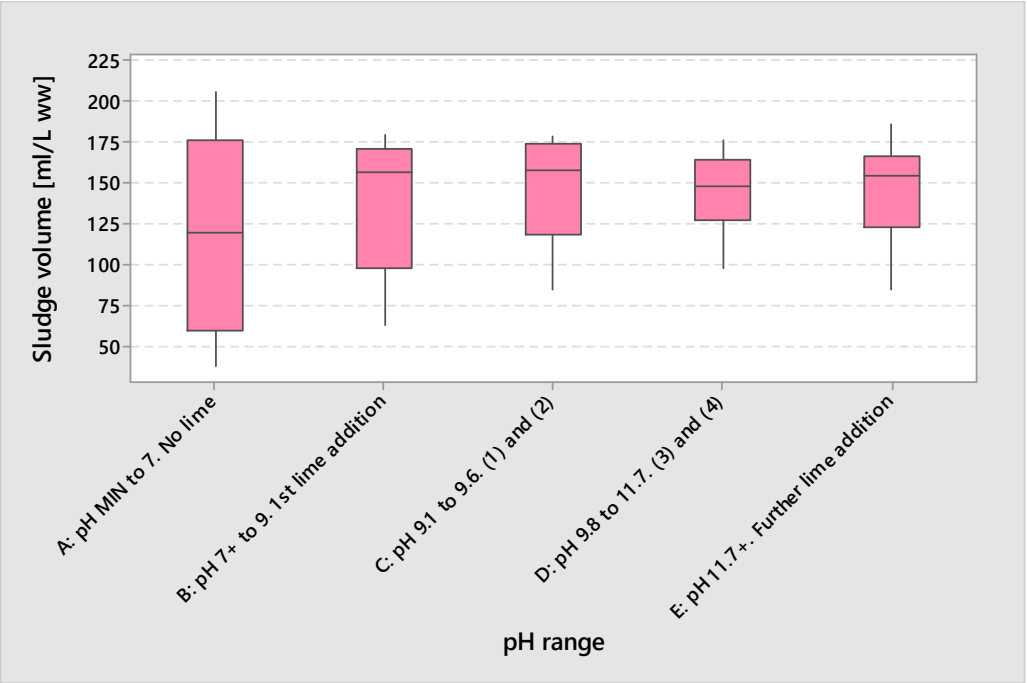


Figure 7.9: Quantity (as volume of sludge expressed in ml) of sedimented sludge per unit volume of raw wastewater (expressed in L) as a function of the pH reached within each jar following completion of the coagulation – flocculation process. Total number of data points (n): 52.

Figure 7.10 demonstrate the density of sedimented sludge (expressed as grammes dry mass per litre of sludge after drying at 104ºC) as a function of the pH reached within each jar following completion of the coagulation – flocculation process. As above mentioned for the sludge volume, a slight increase in sludge density was shown with increase in treatment pH. In other words, a tendency of the sludge to become more 'thickened' for batch treatments with higher amounts of dosed reagent (and therefore higher final pH) was observed.

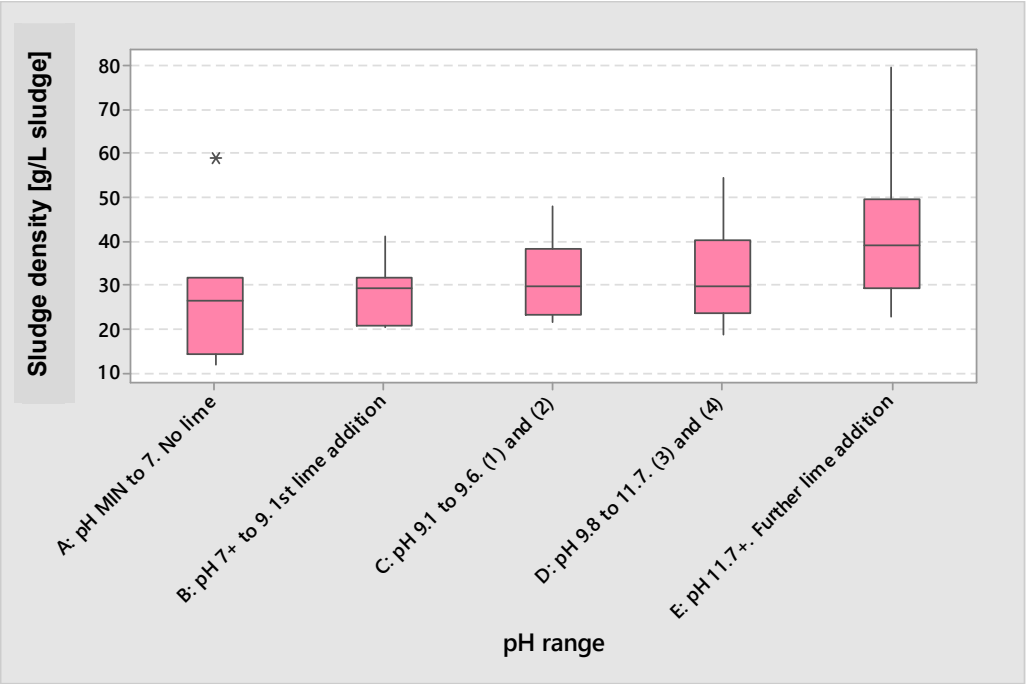


Figure 7.10: Density of sedimented sludge after drying at 104°C (expressed as sludge mass in g per unit of sludge volume in L) as a function of the pH reached within each jar following completion of the coagulation – flocculation process. Total number of data points (n): 52.

Figure 7.11 demonstrates the volume of settled solids per unit volume of supernatant (expressed as ml of settled solids per litre of wastewater) as a function of the pH reached within each jar following completion of the coagulation – flocculation process. No linear regression between volume of settled solids and pH was performed, nor was a regression attempted using a more complex function, as the correlation between the two parameters does not appear to follow a clear pattern.

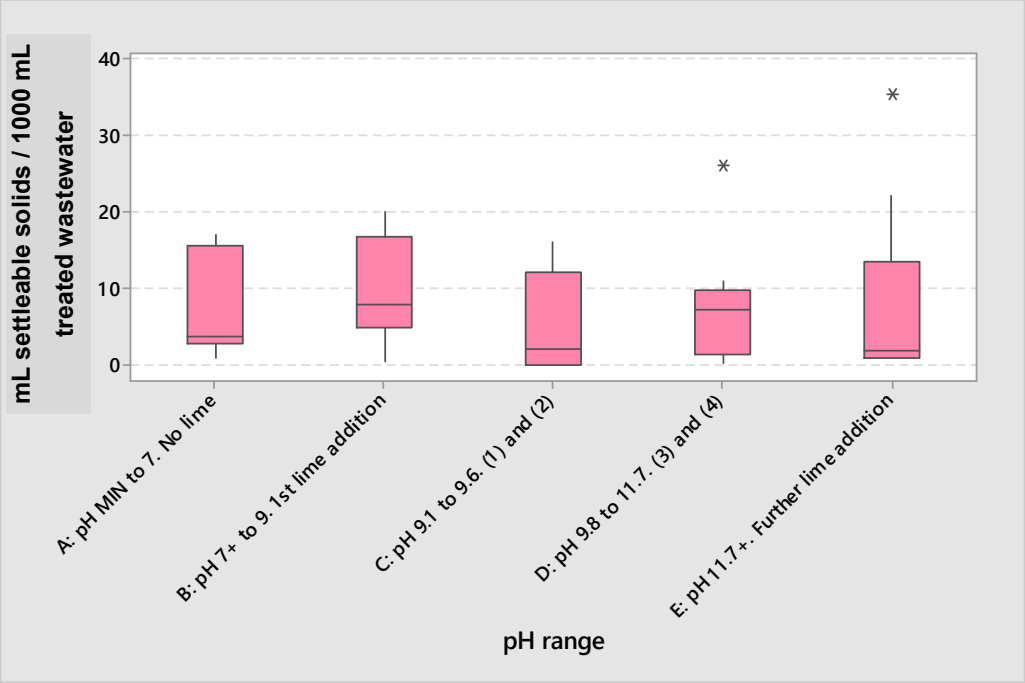


Figure 7.11: Volume of settled solids per unit volume of wastewater (ml of settled solids per litre of effluent) as a function of the pH reached within each jar following completion of the coagulation – flocculation process. Total number of data points (n): 46.

7.2.2 REMOVAL OF PHYSICO-CHEMICAL PARAMETERS AS A RESULT OF COAGULATION-FLOCCULATION VS. REMOVAL RESULTING FROM OVERNIGHT SEDIMENTATION

As previously explained in section 6.2.2, high-pH coagulation-flocculation and subsequent overnight sedimentation each play a distinct role in the removal of microbiological and physico-chemical components of the wastewater. The data presented in this section serve to quantify the contribution made to the removal of physicochemical components by each of these two distinct stages of the treatment process.

Figure 7.12 demonstrates the role played by the coagulation-flocculation process, and subsequently overnight sedimentation, in the reduction of COD, an indicator of the amount of potentially oxidisable organic matter in the wastewater. The light blue line represents the linear function that best approximates the 'percentage removal vs. pH' relationship for wastewater samples collected immediately after coagulation-flocculation alone. The dark blue line represents the linear function that best approximates the 'percentage removal vs. pH' relationship for wastewater samples collected after the coagulation-flocculation process followed by overnight sedimentation. The interpretation of these figures is discussed in Chapter 9.

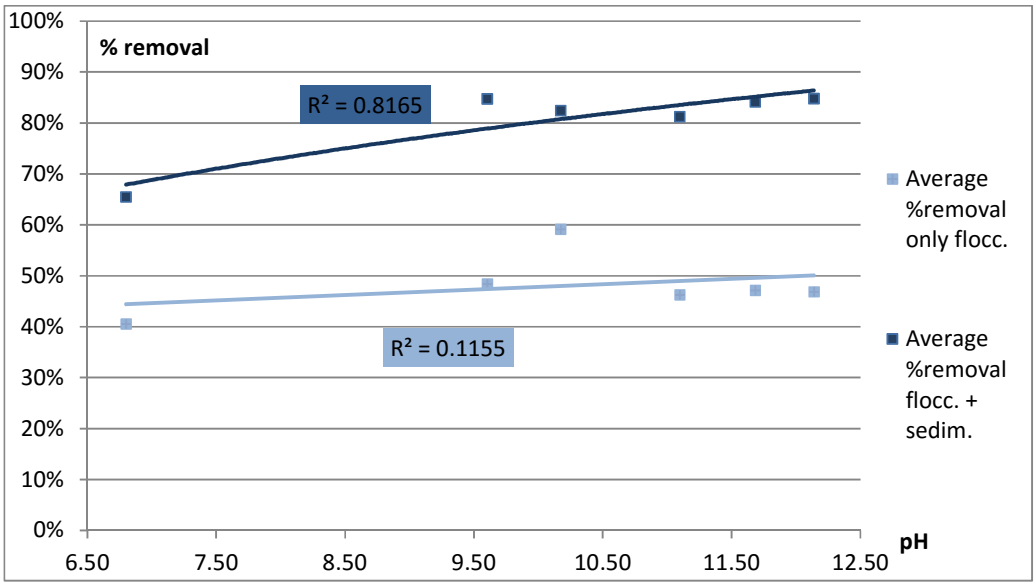


Figure 7.12: linear regression of all data-points representing the percentage removal of COD as a function of the pH level. Total number of data points (n): 24. The light blue line represents effluent samples collected immediately after coagulation-flocculation; the dark blue line represents effluent samples collected after the coagulation-flocculation process followed by overnight sedimentation. Total number of data points (n): 24.

A comparison of the removal values given by the light and the dark blue lines in Figure 7.12, and in particular a comparison of the light and the dark blue bars of Figure 7.13, gives an estimation of the role played by coagulation-flocculation only and by the coagulation-flocculation followed by overnight sedimentation (physical separation) respectively in the final (combined) removal of COD. This specific indicator was chosen to compare the removal values given by the two subsequent processes, but similar considerations could also be made for other physico-chemical parameters, as explained in Chapter 9.

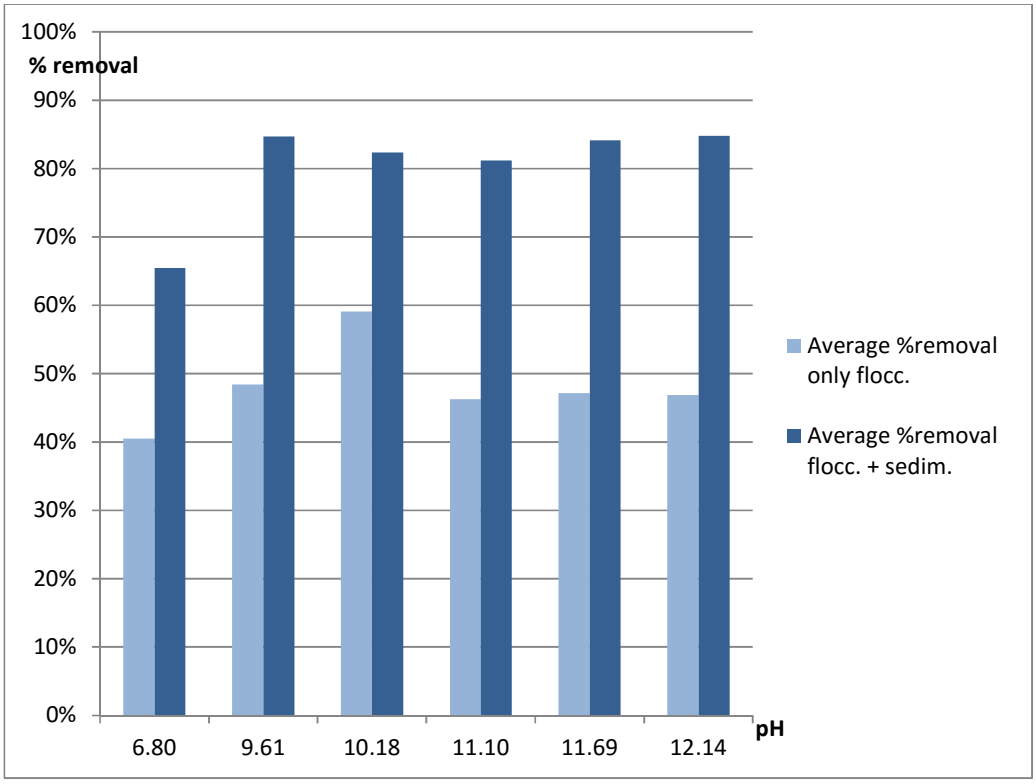


Figure 7.13: Comparison of the percentage removal of COD as a function of the pH level. Total number of data points (n): 24. The light blue bars represent wastewater samples collected immediately after coagulation-flocculation; the dark blue bars represent the same relationship for wastewater samples collected after the coagulation-flocculation process followed by overnight sedimentation.

7.3 THE ROLE OF CALCIUM AND MAGNESIUM IONS IN THE HIGH-PH COAGULATION-FLOCCULATION PROCESS

The role of calcium and magnesium ions in the coagulation-flocculation process, with regard to the chemical reactions discussed on section 2.6, was analysed by measuring the concentration of elemental magnesium and calcium, before and after the coagulation-flocculation process.

The concentrations of elemental magnesium and calcium in both raw wastewater and in the jars following sedimentation were measured using ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectroscopy), as explained in Chapter 3.

7.3.1 FIRST SERIES OF TESTS

These analyses were first performed for three sets, each containing six jars (18 jars in total, duplicate samples taken from each jar resulting in 36 measurements). Varying concentrations of hydrated lime were added to the raw wastewater with no further addition of magnesium ions to improve the coagulation-flocculation process. The most important values from these tests are summarised in Table 7.4 and Figure 7.14. The significance of these values is briefly explained later in this section and further discussed in Chapter 9.

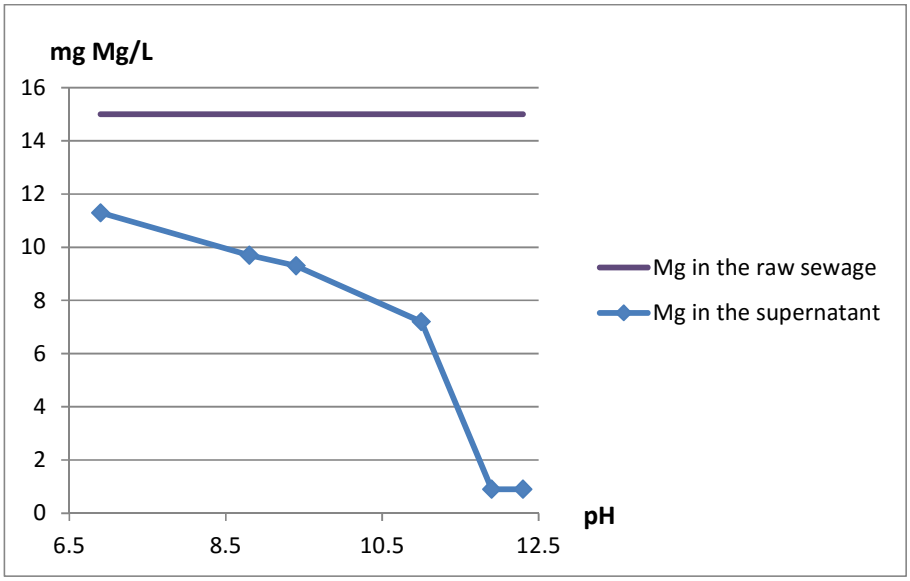


Figure 7.14: average concentration of elemental magnesium for three sets of six jars, where different concentrations of hydrated lime were added to the raw wastewater, with no addition of further magnesium ions. Total number of data points (n): 36.

Table 7.4: average concentration of elemental magnesium for three sets of six jars, where different concentrations of hydrated lime were added to the raw wastewater, with no addition of further magnesium ions. Each value is the average of three samples- measurements, each repeated twice (i.e., average of six values)

Sample → **Parameter ↓**		Raw waste water	A	B	C	D	E	F
1	mg 10% $Ca(OH)_2$/L added	-	0	30	42	54	66	78
2	pH	6.9	6.9	8.8	9.4	11.0	11.9	12.3
3	Mg [mg /L] after sedimenta-tion*	15.0	11.3	9.7	9.3	7.2	0.9	0.9

** elemental magnesium [mg/L]*

The values presented in Table 7.4 and Figure 7.14 support the chemical theory (see Chapter 2) in that a significant amount of the magnesium naturally present in the raw wastewater (15 mg/L elemental magnesium) does not remain in solution, but precipitates as a major component of the floc and subsequently the sedimented sludge, suggesting that that the previously mentioned reaction no. (5) takes place as expected.

7.3.2 SECOND SERIES OF TESTS

At a later stage, two parallel sets of six jars were monitored for the presence of elemental magnesium and calcium. It has been hypothesised that the precipitation of magnesium hydroxide, beginning at pH 9.5 and becoming significant at a pH higher than 10.5, results in a loose floc that can remove colloidal components as it sediments, as explained in Chapter 2. Therefore a series of experiments was undertaken to assess the potential value of adding additional quantities of magnesium sulphate as coagulation aid.

For the first set, varying concentrations of hydrated lime were added to the raw wastewater, with no further addition of magnesium ions to improve the coagulation-flocculation process; the second parallel set was undertaken using a standard addition of hydrated lime (corresponding to the amount added to the last jar from the previous set, the one with the highest lime addition – see Table 7.5, column F) and the later addition of different quantities of magnesium sulphate (dissociating into magnesium ions).

The most important values from the second series of tests, with regard to the concentration of elemental magnesium and calcium, pH and amount of hydrated lime and magnesium sulphate added are presented in Figure 7.15, Figure 7.16 and Table 7.5.

Figure 7.15 shows how the significant amount of the magnesium naturally present in the raw wastewater (16.2 mg/L elemental magnesium in the raw sewage) does not remain in solution, but precipitates as a major component of the floc and subsequently the sedimented sludge, once again suggesting that that the previously mentioned reaction no. (5) takes place as expected.

Figure 7.16 shows how does the system reacts when a constant quantity of hydrated lime and an increasing quantity of magnesium is added to the jars: most of the magnesium added to the raw wastewater (quantity displayed on the x axis) does not remain in solution, but precipitates as a major component of the floc and subsequently the sedimented sludge, suggesting once again that the previously mentioned reaction no. (5) takes place as expected.

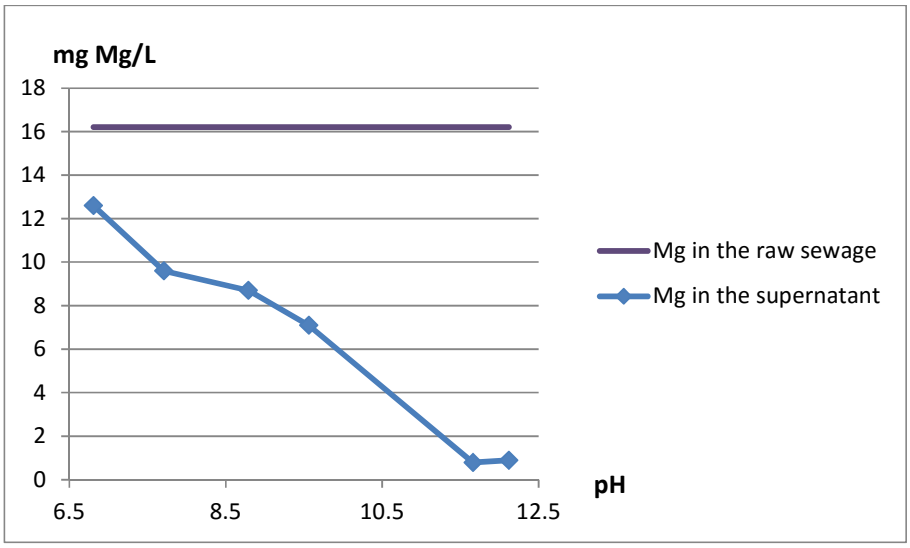


Figure 7.15: average concentration of elemental magnesium in the supernatant, for an experiment in which different quantities of hydrated lime were added to the raw wastewater and no magnesium ions were added. Total number of data points (n): 12.

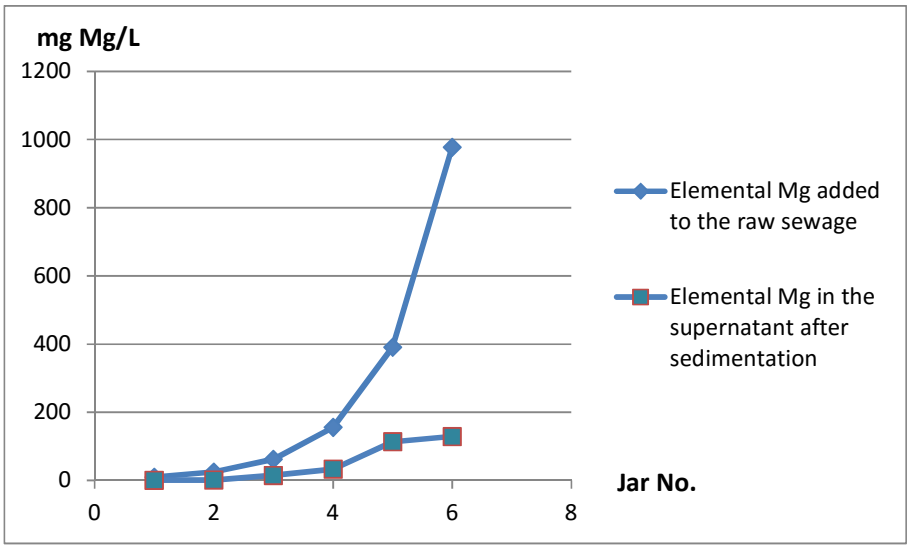


Figure 7.16: average concentration of elemental magnesium in the supernatant, for an experiment in which the same quantity of hydrated lime was added to the raw wastewater [highest quantity from previous experiment] and different amounts of magnesium ions were added to improve the coagulation - flocculation. Total number of data points (n): 12.

Table 7.5: concentration of elemental magnesium and calcium, pH and quantity of hydrated lime and magnesium sulphate added to the two parallel sets of jars

	Sample →		Raw waste water	A	B	C	D	E	F
	Parameter ↓								
With-out Mg addi-tion	1	mg added 10% $Ca(OH)_2$/L	-	0	15	30	45	60	75
	2	pH	6.81	6.81	7.71	8.79	9.56	11.66	12.12
	3	mg Mg/L after sedimenta-tion	16.2	12.6	9.6	8.7	7.1	0.8	0.9
	4	mg Ca/L after sedimenta-tion	137.9	106.3	111.8	127.1	134.3	218.6	210.7
With Mg addi-tion	5	mg added 10% $Ca(OH)_2$/L	-	75	75	75	75	75	75
	6	pH	6.81	11.96	11.64	11.08	10.90	10.10	10.12
	7	mg added elemental Mg/L *		10	25 (10x2.5)	62,5 (25x2.5)	156 (63x2.5)	391 (156 x2.5)	977 (391 x2.5)
	8	mg Mg/L after sedimenta-tion	16,2	1,1	1,6	15,5	33,5	113,5	129,2
	9	mg Ca/L after sedimenta-tion	137,9	251,9	196,8	164,0	183,1	198,0	125,4

** These values have been extrapolated from the amount of magnesium sulphate added to the jars*

The 1st and the 5th rows of Table 7.5 demonstrate the quantity of hydrated lime added to each of jar. The 2nd and the 6th rows describe the pH variation resulting from the varying levels of chemicals added. As it is reasonable to expect, based on the reaction (5) discussed on section 2.6:

$$Mg^{2+} + 2OH^- \leftrightarrow Mg(OH)_2\downarrow \qquad (5)$$

the higher the amount of magnesium added, the more the equilibrium tends towards the right side of the reaction, thereby increasing the 'consumption' of OH- anions and

consequently the concentration of H_3O^+ cations, thus leading to a decrease in the pH level, even though the amount of added $Ca(OH)_2$ is the same. The amount of magnesium added (expressed as concentration of elemental magnesium) is detailed on 7th row. The 3rd and the 8th row present the concentration of elemental magnesium detected in each jar at the end of the sedimentation phase. A comparison of these two rows ('mg Mg/L after sedimentation') with 7th row (mg added Mg/L), demonstrates that it is reasonable to assume that a significant amount of added magnesium does not remain in solution, but precipitates, becoming part of the sedimented sludge, thus suggesting that that the previously mentioned reaction no. (5) takes place as expected. The reaction is triggered to a further level by a higher concentration of magnesium.

An analysis of 4th and 10th rows, particularly a comparison of the calcium concentration for the final jar of the first set (i.e., jar F, in which 210.7 mgCa/L were recorded) with all calcium concentrations for the jars where magnesium ions were added to improve coagulation-flocculation, suggests that, where the amount of hydrated lime added is constant, a further addition of magnesium ions improves the precipitation of suspended matter. Therefore the decrease in levels of calcium ions in the supernatant (and a consequent increase in levels in the sedimented sludge). This is in accordance with chemical theory and was confirmed by the analyses.

The most important values for the same experiments, correlating amount of added magnesium sulphate with the removal of turbidity (used here as physico-chemical indicator to monitor the coagulation-flocculation effectiveness), are reported in Figure 7.17, Figure 7.18 and Table 7.6.

The most important values for the same experiments, correlating amount of added magnesium sulphate with the removal of total suspended solids (also used here as a physico-chemical indicator to monitor the effectiveness of coagulation-flocculation), are reported in Figure 7.19, Figure 7.20 and and Table 7.6.

The values are relatively self-explanatory but it is perhaps worth pointing out that, based on the abovementioned chemical theory, the removal of turbidity and total suspended solids both significantly increase with the amount of added magnesium sulphate and therefore the availability of magnesium ions (see turbidity value for cell F3 compared with all values of row 9 and TSS value for cell F5 compared with all values of row 11).

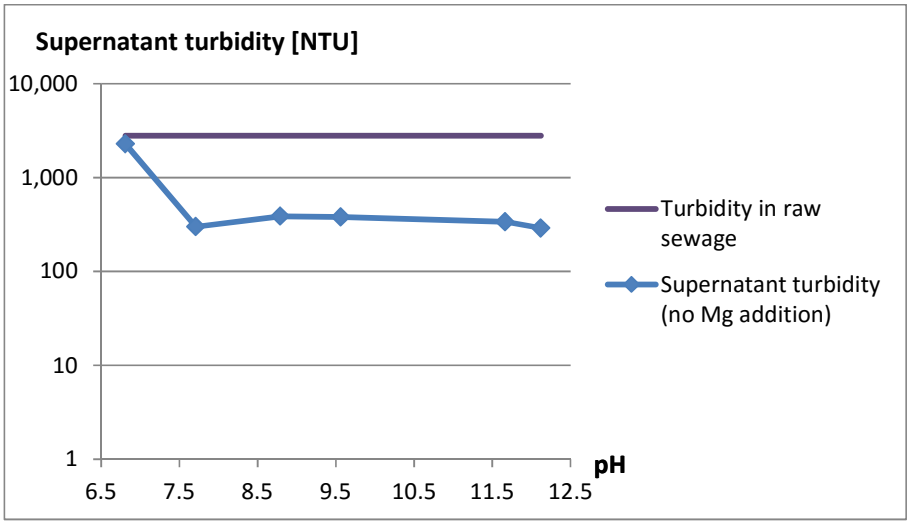


Figure 7.17: supernatant turbidity for an experiment in which different quantities of hydrated lime were added to the raw wastewater and no magnesium ions were added to improve coagulation-flocculation. Total number of data points (n): 6.

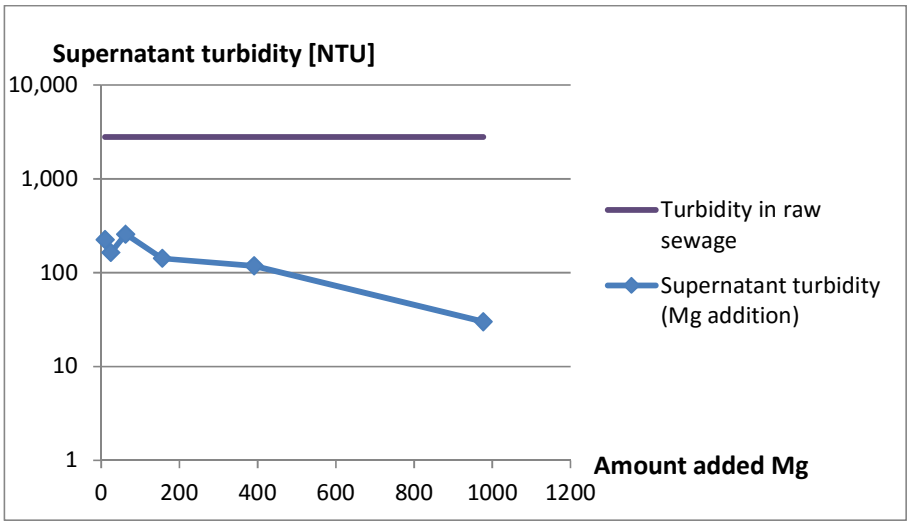


Figure 7.18: supernatant turbidity for an experiment in which the same quantity of hydrated lime was added to the raw wastewater [highest quantity from previous experiment] and magnesium ions were also added to improve coagulation-flocculation. Total number of data points (n): 6.

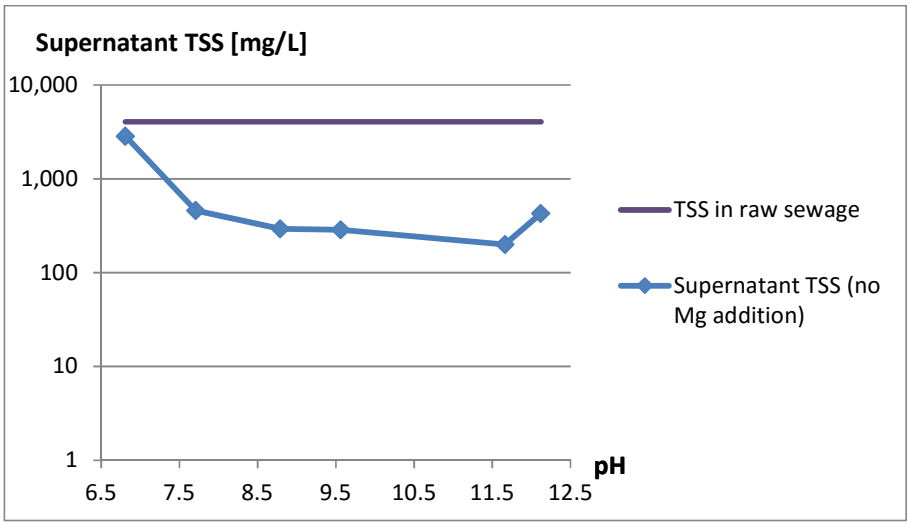


Figure 7.19: supernatant TSS for an experiment in which different quantities of hydrated lime were added to the raw wastewater and no magnesium ions were added to improve coagulation-flocculation. Total number of data points (n): 6.

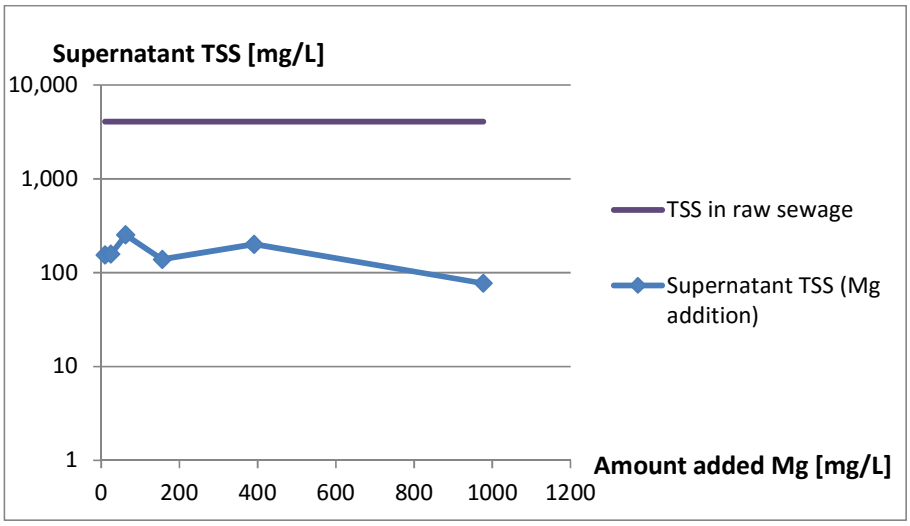


Figure 7.20: supernatant turbidity for an experiment in which the same quantity of hydrated lime was added to the raw wastewater [highest quantity from previous experiment] and magnesium ions were also added to improve coagulation-flocculation. Total number of data points (n): 6.

Table 7.6: correlation between levels of added magnesium sulphate and removal of turbidity and total suspended solids

	Sample →		Raw sew-age	A	B	C	D	E	F
	Parameter ↓								
	1	mg added 10% $Ca(OH)_2$/L	-	0	15	30	45	60	75
With-out Mg addi-tion	2	Turbidity [NFU]	2,800	2,288	300	389	382	340	291
	3	Turbidity removal [%]	-	18%	89%	86%	86%	88%	90%
	4	TSS [mg/L]	4,065	2,860	460	293	287	200	427
	5	TSS removal [%]	-	30%	89%	93%	93%	95%	89%
	6	mg added 10% $Ca(OH)_2$/L	-	75	75	75	75	75	75
	7	mg added elemental Mg/L *		10	25 (10x2.5)	62,5 (25x2.5)	156 (63x2.5)	391 (156 x2.5)	977 (391 x2.5)
With Mg addi-tion	8	Turbidity output [NFU]	2,800	225	164	257	142	118	30
	9	Turbidity removal [%]	-	92%	94%	91%	95%	96%	99%
	10	TSS [mg/L]	4,065	154,0	158,0	252,5	138,3	200,0	77,0
	11	TSS removal [%]	-	96%	96%	94%	97%	95%	98%

The most important values for the same experiments, correlating amount of added magnesium sulphate with the removal of COD (used here as a physico-chemical indicator to monitor the effectiveness of coagulation-flocculation), are reported in Figure 7.21, Figure 7.22, and Table 7.7.

The most important values, correlating amount of added magnesium sulphate with the removal of somatic coliphages (also used here as a microbiological indicator to monitor the effectiveness of coagulation-flocculation), are reported in Figure 7.23, Figure 7.24 and Table 7.7.

Figure 7.23 outlines the assessed level of somatic coliphage in supernatant for an experiment in which different quantities of hydrated lime were added to the raw wastewater and no magnesium ions were added to improve coagulation-flocculation:

the large peak in somatic coliphages at pH 8.5 is difficult to interpret and is probably due to a single point measurement error. The relationship between Mg added and number of somatic coliphages in figure 7.24 is non uniform as there is a slight increase then a decline. It is difficult to recognize a clear behaviour in the removal of COD and somatic coliphages when the concentration of available magnesium ions is 'artificially' increased. Further study is needed to more accurately assess if there is a clear correlation between the two values. Nevertheless it should be pointed out that for very high concentrations of added magnesium ions and the consequent precipitation of magnesium hydroxide the removal of both COD and somatic coliphages significantly improves (see COD removal value for cell F3 compared with all values of row 9 and somatic coliphage removal value for cell F5 compared with all values of row 11).

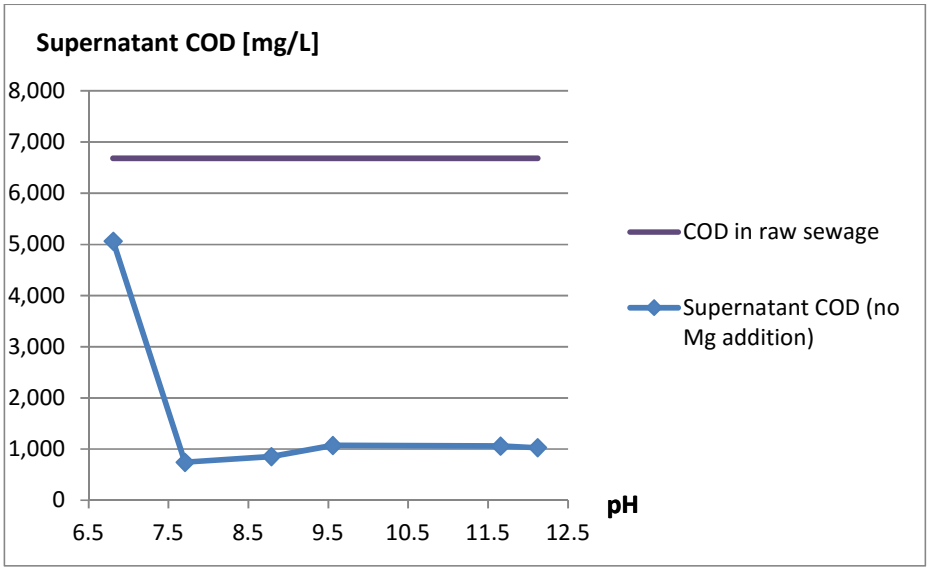


Figure 7.21: supernatant COD for an experiment in which different quantities of hydrated lime were added to the raw wastewater and no magnesium ions were added to improve coagulation-flocculation. Total number of data points (n): 6.

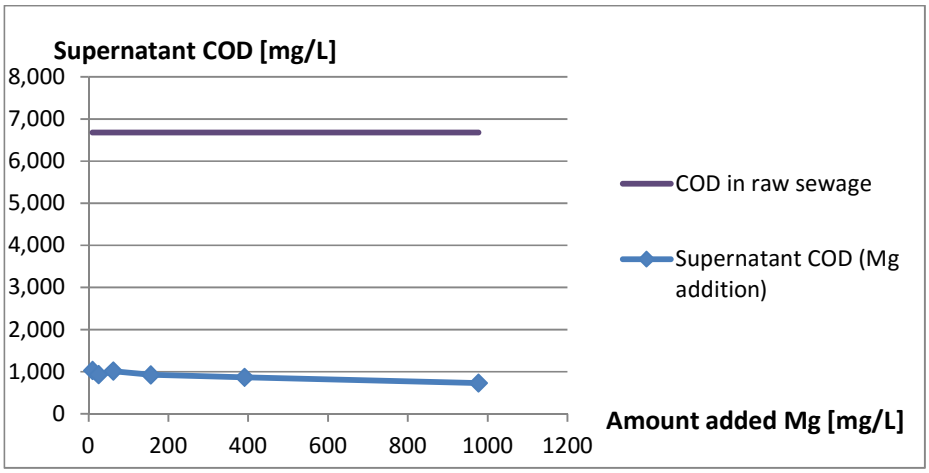


Figure 7.22: supernatant COD for an experiment in which the same quantity of hydrated lime was added to the raw wastewater [highest quantity from previous experiment] and magnesium ions were also added to improve coagulation-flocculation. Total number of data points (n): 6.

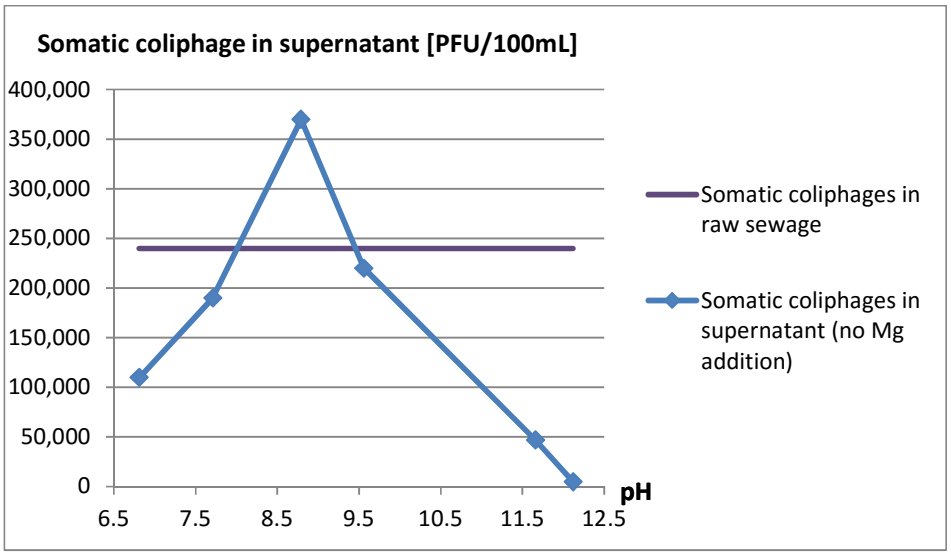


Figure 7.23: Somatic coliphage in supernatant for an experiment in which different quantities of hydrated lime were added to the raw wastewater and no magnesium ions were added to improve coagulation-flocculation. Total number of data points (n): 6.

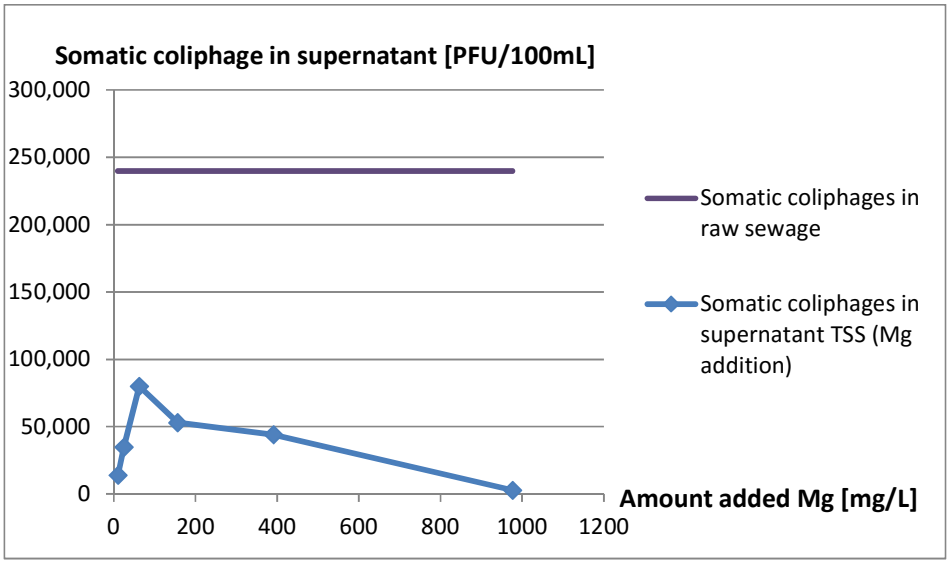


Figure 7.24: Somatic coliphage in supernatant for an experiment in which the same quantity of hydrated lime was added to the raw wastewater [highest quantity from previous experiment] and magnesium ions were also added to improve coagulation-flocculation. Total number of data points (n): 6.

Table 7.7: correlation between added magnesium sulphate and removal of turbidity and total suspended solids.

	Sample →		Raw sew-age	A	B	C	D	E	F
	Parameter ↓								
With-out Mg addi-tion	1	mg added 10% $Ca(OH)_2$/L	-	0	15	30	45	60	75
	2	COD output [g/l]	6,679	5061	742	854	1071	1059	1027
	3	COD removal [%]	-	32%	90%	89%	86%	86%	86%
	4	Somatic coliphage [PFU/100 ml]	2,4E+05	1,1E+05	1,9E+05	3,7E+05	2,2E+05	4,7E+04	4,9E+03
	5	Somatic coliphage removal [%]	-	53%	20%	-57%	9%	80,1%	97,9%
With Mg addi-tion	6	mg added 10% $Ca(OH)_2$/L	-	75	75	75	75	75	75
	7	mg added elemental Mg/L *		10	25 (10x2.5)	62,5 (25x2.5)	156 (63x2.5)	391 (156 x2.5)	977 (391 x2.5)
	8	COD output [g/l]	6,679	1030	929	1016	924	869	733
	9	COD removal [%]	-	85%	86%	85%	86%	87%	89%
	10	Somatic coliphage output [CFU/100 ml]	2,4E+05	1,4E+04	3,5E+04	8,0E+04	5,3E+04	4,4E+04	2,8E+03
	11	Somatic coliphage removal [%]	-	94%	85%	66%	78%	82%	99%

7.4 REMOVAL OF PHYSICO CHEMICAL PARAMETERS UNDER ALL ACIDIC CONDITIONS OF PROTOCOL B (LOW-pH TREATMENT)

Figure 7.25 demonstrates the removal of COD as a function of the pH level for the low pH treatment process. The data points have been divided into the same groups (A-E) as in Chapter 6 for the consideration of microbiological parameters under the same low-pH treatment conditions. Further study is needed to more accurately assess if there is a clear correlation between the two values, but it is worth to point out that there is a little increase in the average COD removal as the pH becomes very high and the values are less 'scattered' and instead closer to the average (fairly good) removal of 85%.

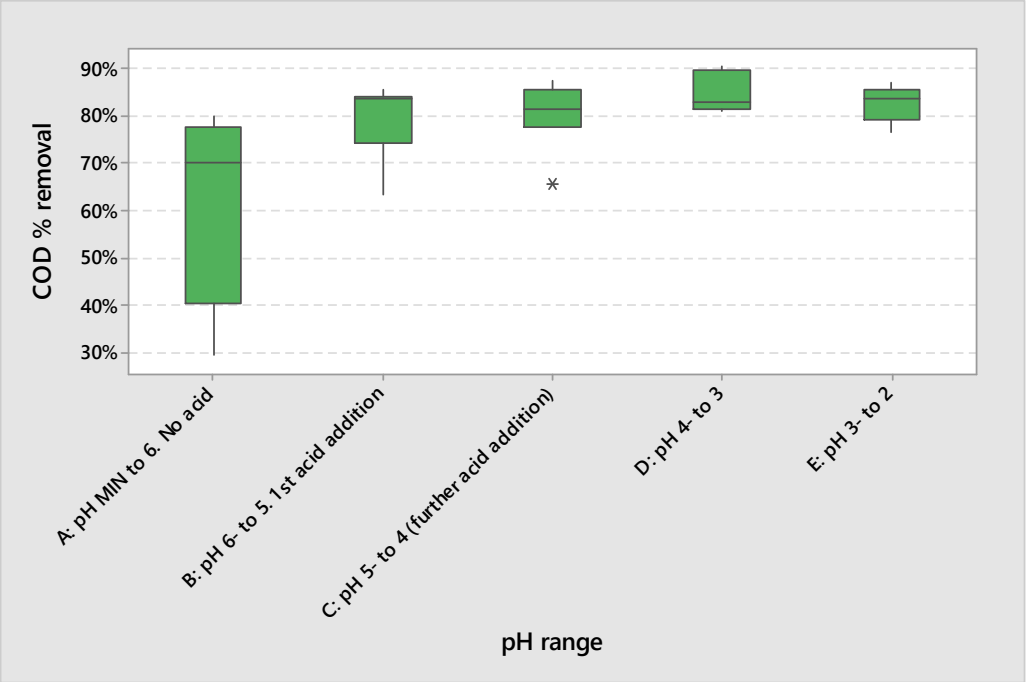


Figure 7.25: Percentage removal of COD as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 30. Data points deemed to be outliers are indicated by an asterisk.

Figure 7.26 demonstrates the removal of total suspended solids as a function of the pH level. The data points have been divided into the same groups (A-E) as explained for COD. Further study is needed to more accurately assess if any correlation between the two values can be established.

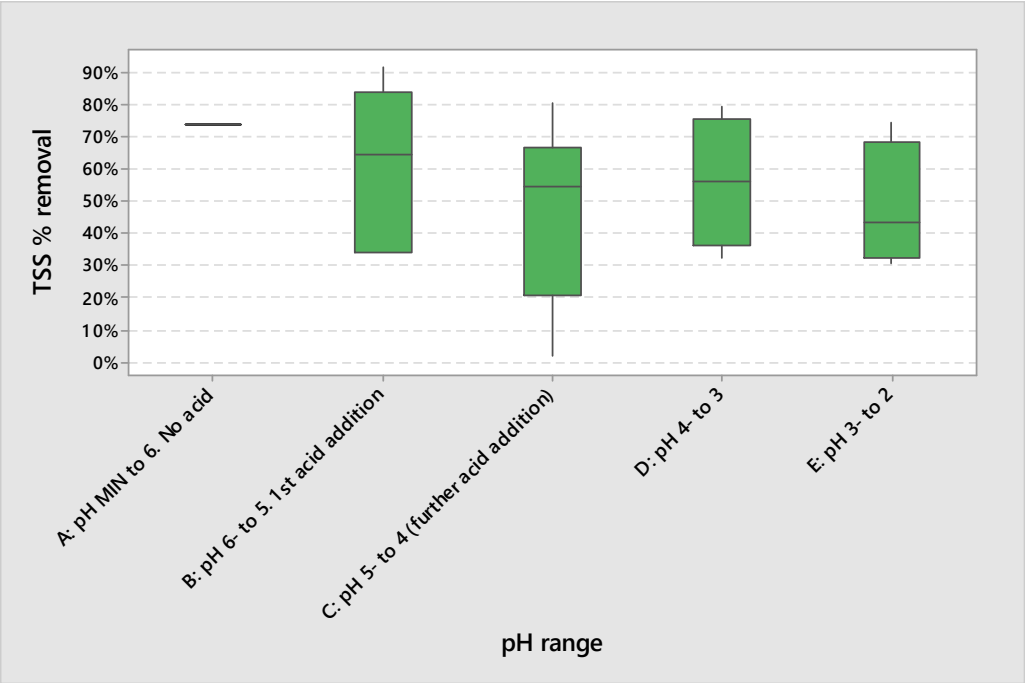


Figure 7.26: Percentage removal of total suspended solids as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 24.

Figure 7.27 demonstrates the removal of turbidity as a function of the pH level. The data points have been divided into the same groups (A-E) as for the total suspended solids. Further study is needed to more accurately assess if any correlation between the two values can be established.

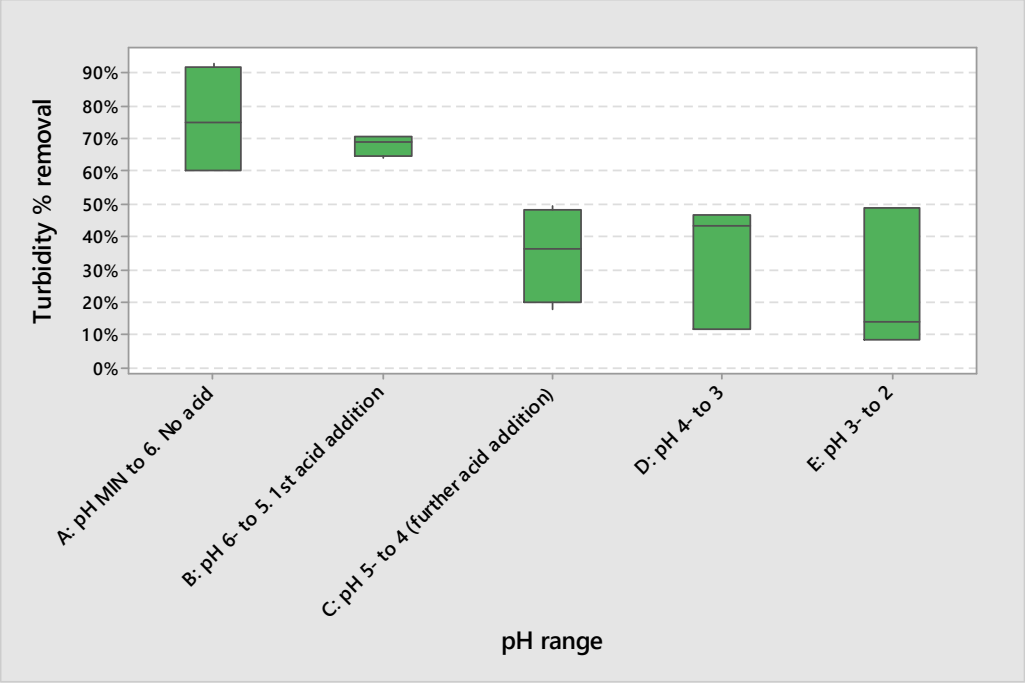


Figure 7.27: Percentage removal of turbidity as a function of the pH level reached within each jar following completion of the coagulation – flocculation and overnight sedimentation process. Total number of data points (n): 18.

Figure 7.28 and Figure 7.29 demonstrate the correlation of concentration of hydrochloric acid added to the batch reactor with the pH level reached at the end of the acidification process. The aim here was to provide a tool to estimate the amount of consumed acid per unit of treated faecal waste, so as to support the practitioner in the field in the design and operation of a full-scale treatment system.

Figure 7.28 demonstrates the amount of hydrochloric acid added to the reactor as a function of the targeted pH following completion of the acidification process.

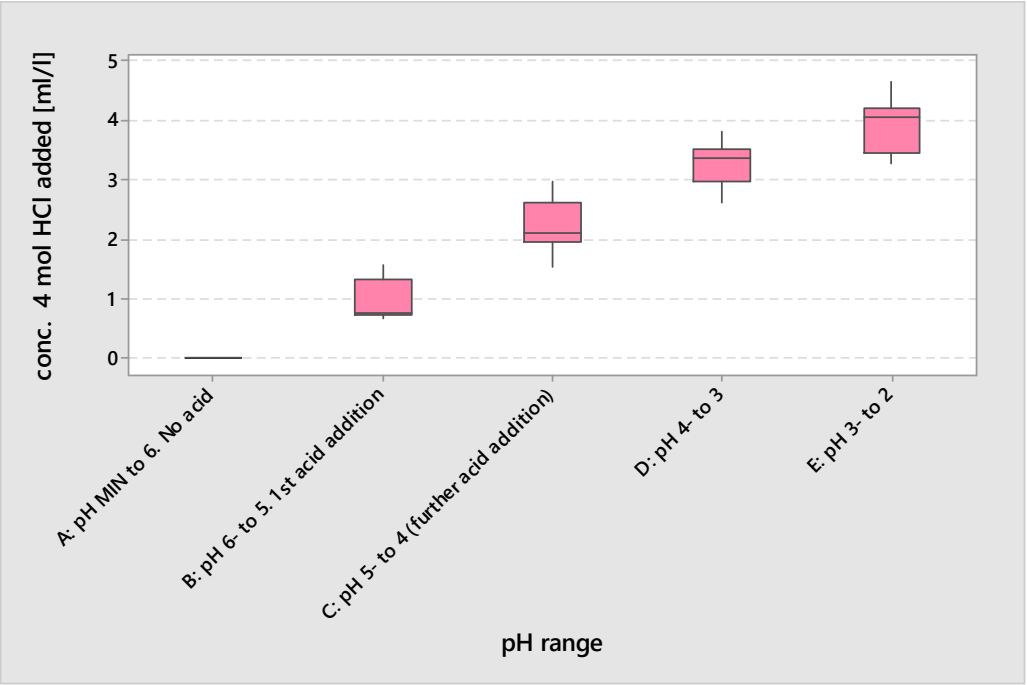


Figure 7.28: amount of hydrochloric acid added to the batch-reactor as a function of the target pH level for each jar following completion of the acidification process. Total number of data points (n): 54.

Conversely, Figure 7.29 provides an estimation, through a linear regression, of the pH reached within each jar following completion of the acidification process for varying amounts of added reagent.

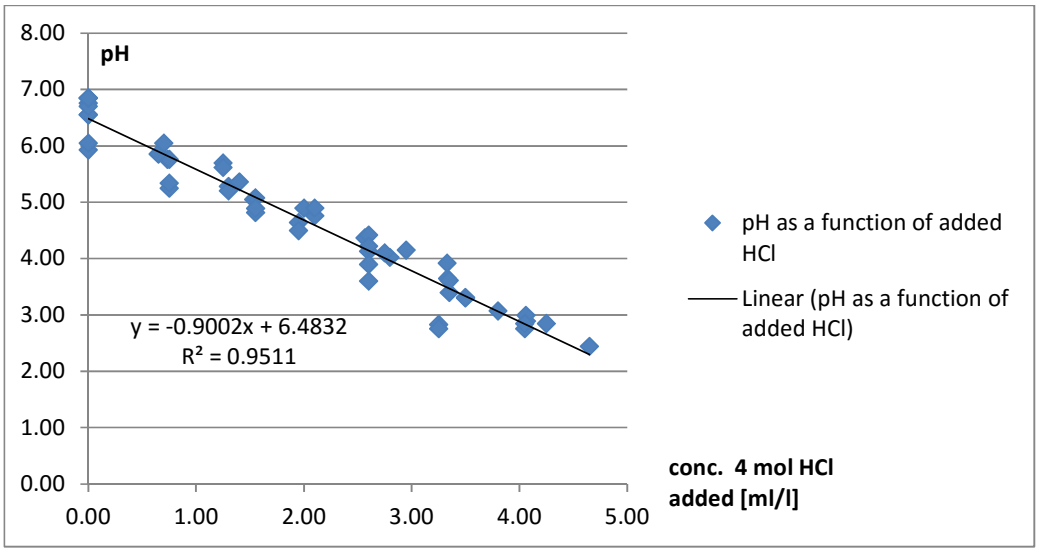


Figure 7.29: Linear regression for all data-points representing the target pH level as a function of the amount of hydrochloric acid added to the batch-reactor for each jar following completion of the acidification process. Total number of data points (n): 54.

Figure 7.30 and Figure 7.311 demonstrate the correlation between the sludge characteristics with the pH level reached at the end of the acidification process (and therefore indirectly with the amount of reagent added to the jar). The aim of this approach was to provide a tool to estimate the expected quantity (as volume of sludge per unit of raw wastewater volume) and the physico-chemical quality (density after drying at 104°C as grammes of dry mass per litre of sludge) of the sedimentation sludge, and therefore to support the practitioner in the field in the design and operation of a full-scale treatment system.

Figure 7.30 demonstrates the quantity (as volume) of sedimented sludge per unit volume of raw wastewater, as a function of the pH level (therefore indirectly the amount of reagent added) reached within each jar following completion of the acidification process. As above mentioned for Protocol A (high pH treatment), a slight increase in sludge volume with higher amounts of dosed acid (and therefore lower final pH) was observed.

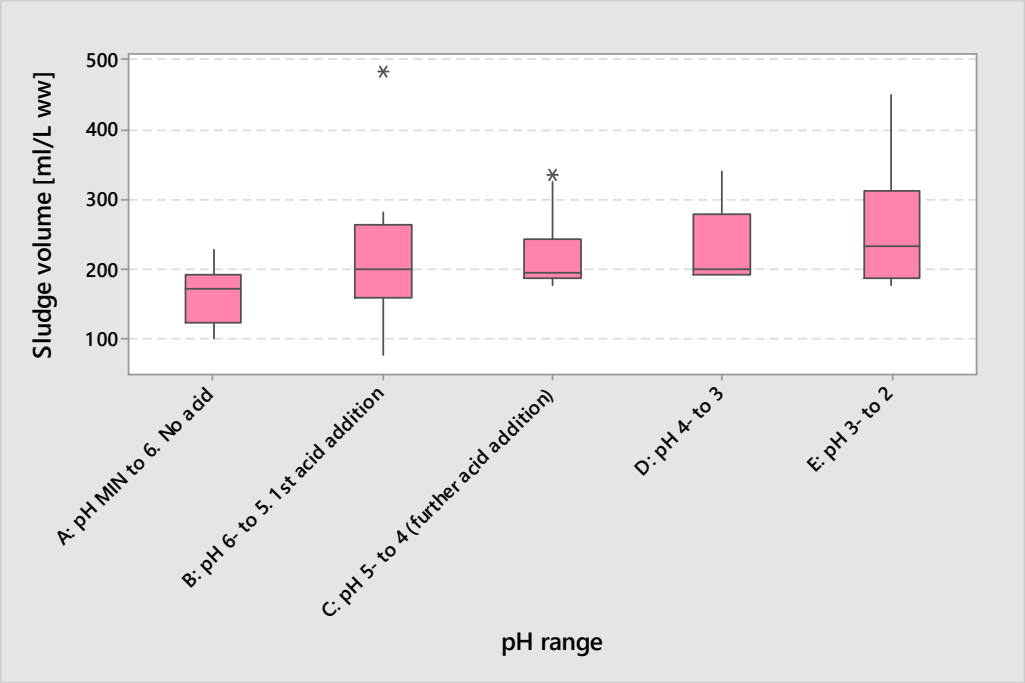


Figure 7.30: Quantity (as volume of sludge expressed in ml) of sedimented sludge per unit volume of raw wastewater (expressed in L) as a function of the pH level reached within each jar following completion of the acidification process. Total number of data points (n): 54.

Figure 7.31 demonstrates the density of sedimented sludge (expressed as grammes (dry weight) per litre of sludge after drying at 104◦C) as a function of the pH level reached within each jar following completion of the acidification process, first as a boxplot, then as linear regression for the same data-points. As mentioned previously for Protocol A, a slight increase in sludge density was shown with decrease in treatment pH. In other words, a tendency of the sludge to become more 'thickened' for batch treatments with higher amounts of dosed acid (and therefore lower final pH) was observed.

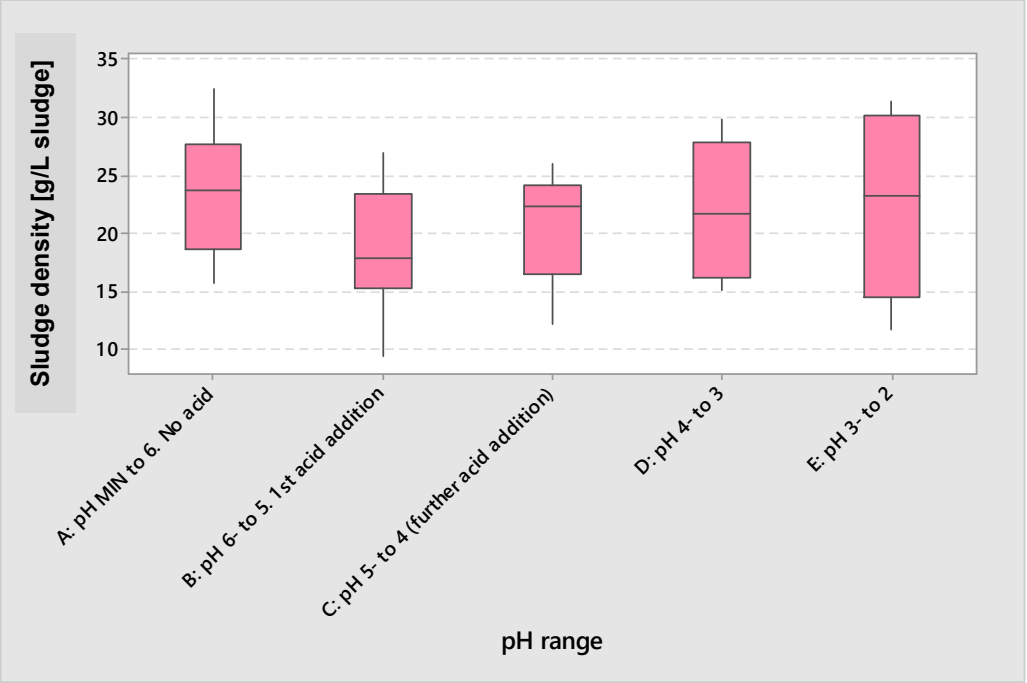


Figure 7.31: sedimentation sludge density expressed as sludge weight (in g) per unit of sludge volume (in L) as a function of the pH reached within each jar following completion of the acidification process. Total number of data points (n): 54.

Figure 7.32 demonstrates the quantity of settled solids per unit volume of supernatant (expressed as ml of settled solids per litre of effluent) as a function of the pH level reached within each jar following completion of the acidification process. As mentioned previously for Protocol A, a slight increase in quantity of settled solids with decrease in treatment pH (and therefore higher amounts of dosed acid) was observed.

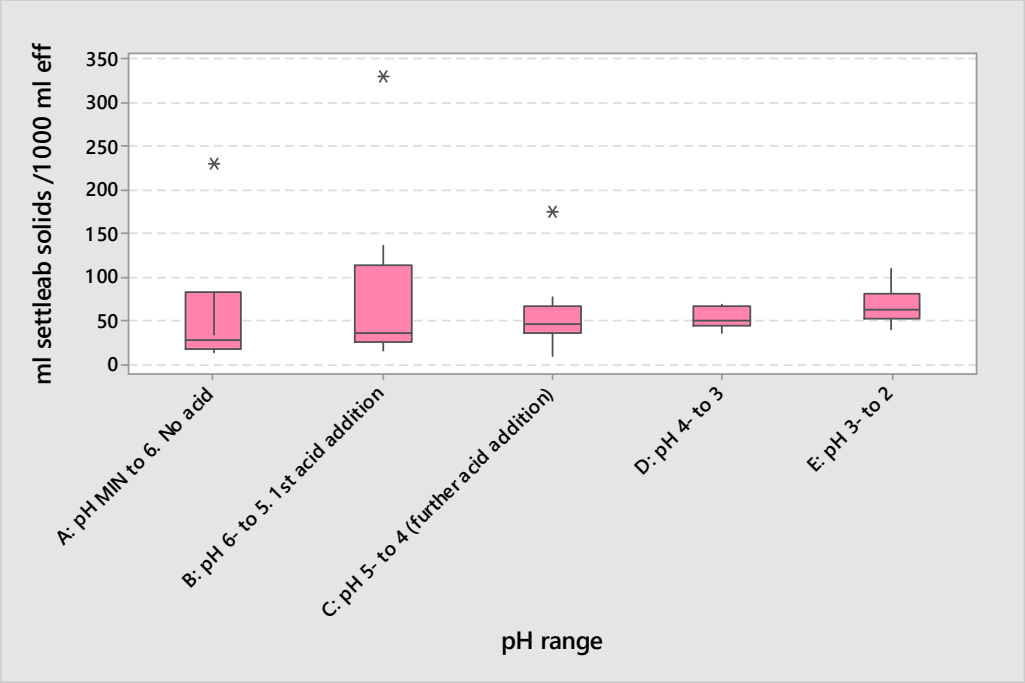


Figure 7.32: Quantity of settled solids per unit volume of wastewater (ml of settlement solids per litre of effluent) as a function of the pH reached within each jar following completion of the coagulation – flocculation process. Total number of data points (n): 46. Data points deemed to be outliers are indicated by an asterisk.

8. CHAPTER EIGHT: SUBJECTIVE VISUAL ASSESSMENT OF TREATMENT PERFORMANCE PERFORMED BY VOLUNTEERS

In this chapter, the results of a visual assessment of treatment performance, undertaken by non-experts and correlated with other more widely-used indicators of microbiological and physico-chemical treatment performance are presented for both treatment protocols (A and B). As previously mentioned in Chapter 3, the aim of this work was to explore the potential of replacing turbidity and TSS with a visual assessment of effluent 'clarity', so that in certain situations (such as under field conditions during an emergency and where full laboratory analysis is either unavailable or unfeasible) clarity could be used as a surrogate of treatment effectiveness in order to rapidly, and simply evaluate the microbiological and physico-chemical treatment performance. Therefore, the following chapter presents the results of a so-called 'stand-alone' (alternatively referred to as a 'single comparison' or quasi 'double blind') and a so-called 'all-in' (also referred as 'grouped comparison', or quasi 'single blind') comparison of monitoring vs. visual assessment for Protocol A and B.

8.1 SUBJECTIVE VISUAL ASSESSMENT OF TREATMENT PERFORMANCE FOR THE HIGH-pH TREATMENT (PROTOCOL A)

Figure 8.1 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of coagulation-flocculation and sedimentation processes involving different amounts of added hydrated lime (for the high-pH treatment) and the different Log_{10} percentage removal levels of thermotolerant coliforms. The results are reported for each 'single' and 'grouped' set of jar test comparisons, following the coagulation-flocculation and sedimentation processes achieved using different amounts of added reagent. The volunteers were asked to assess the level of clarity of different effluent samples, giving a whole value numerical response between zero (for 'completely transparent' samples) to ten (for 'completely opaque' samples). Each data point represents the mean (N=30) of each of the five assessments for subjective determination of clarity.

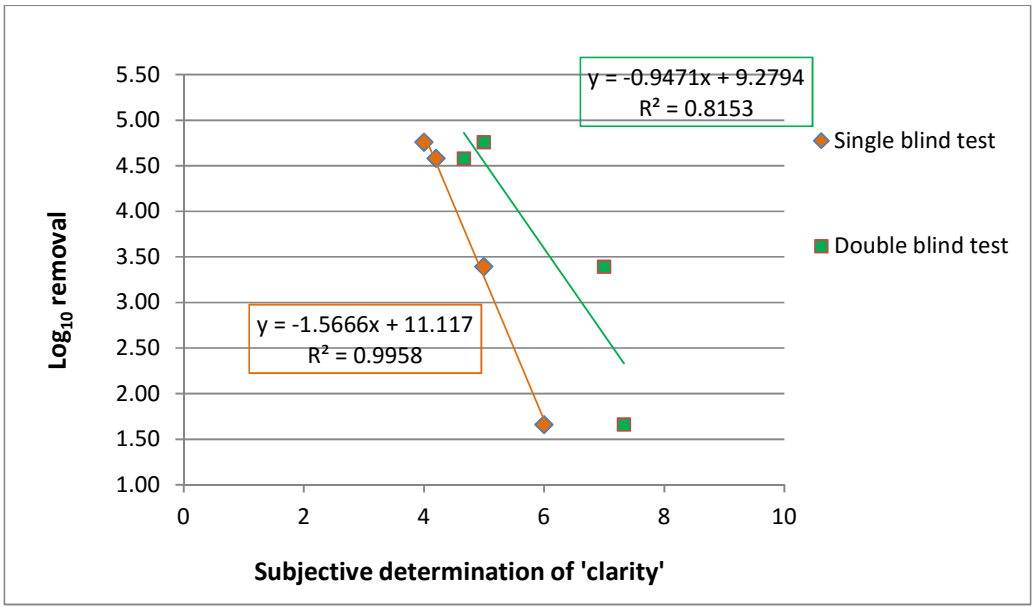


Figure 8.1: Log_{10} removal of thermotolerant coliforms with respect to clarity score (Protocol A)

Figure 8.2 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following completion of coagulation-flocculation and sedimentation processes involving different amounts of added hydrated lime (for the high-pH treatment) and, the different Log_{10} percentage removal levels of intestinal enterococci.

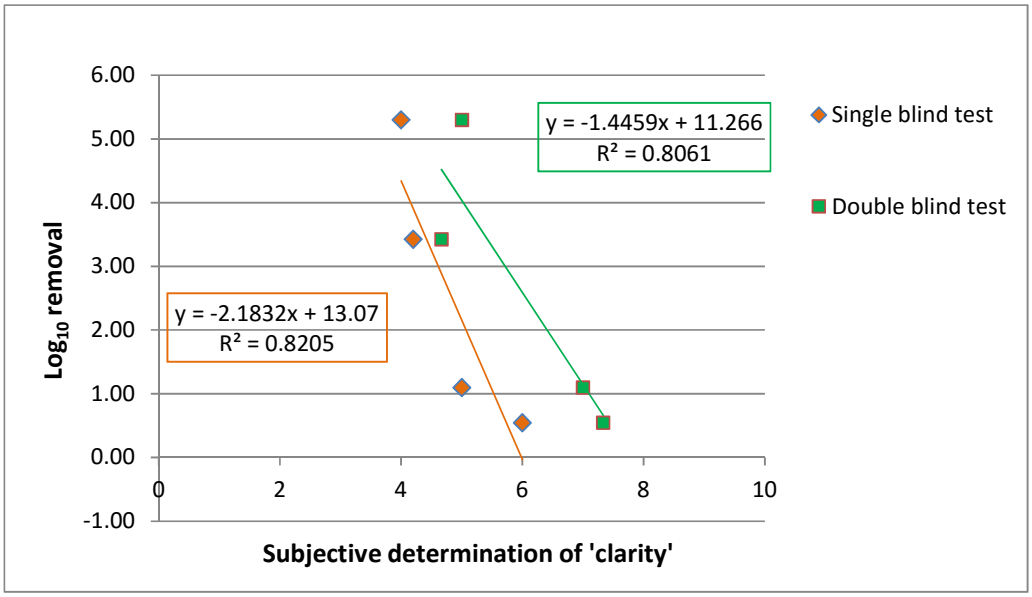


Figure 8.2: Log_{10} removal of intestinal enterococci with respect to clarity score (Protocol A)

Figure 8.3 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each 'single' and 'grouped' comparison tests of jars following completion of coagulation-flocculation and sedimentation processes for different amounts of added hydrated lime (for the high-pH treatment) and, the different Log_{10} percentage removal levels of somatic coliphage .

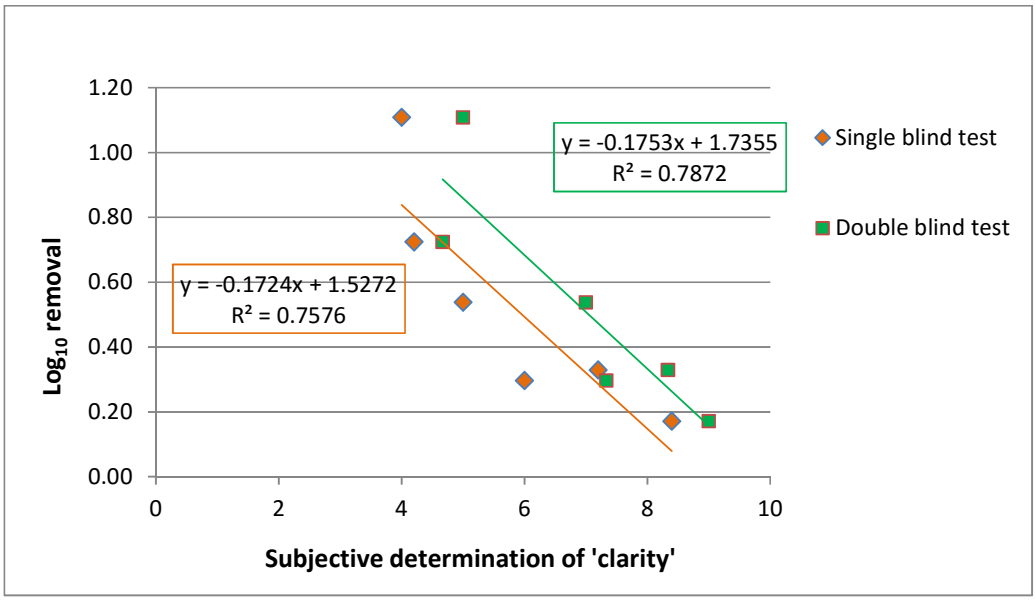


Figure 8.3: Log_{10} removal of somatic coliphage with respect to clarity score (Protocol A)

Figure 8.4 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each 'single' and 'grouped' comparison tests of jars following completion of coagulation-flocculation and sedimentation processes for different amounts of added hydrated lime (for the high-pH treatment) and, the different percentage removal levels of F-RNA phage .

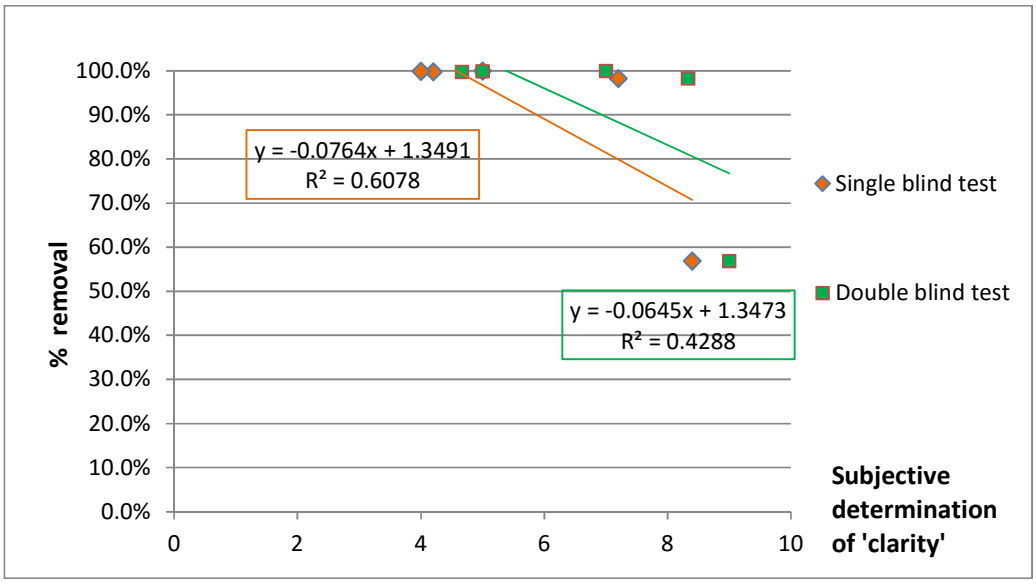


Figure 8.4: Percentage removal of F-RNA phage with respect to clarity (Protocol A)

Figure 8.5 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each 'single' and 'grouped' comparison tests of jars following completion of coagulation-flocculation and sedimentation processes for different amounts of added hydrated lime (for the high-pH treatment) and, the different Log_{10} removal levels of *Vibrio parahaemoliticus*, *vulnificus*, *mimicus* and *proteus* and *pseudomonas* species Log_{10} removal for the low-pH treatment. As mentioned in Chapter 3, the media used for estimating the number of these species only made it possible to make a total count of the five types of bacteria, without any distinction between them.

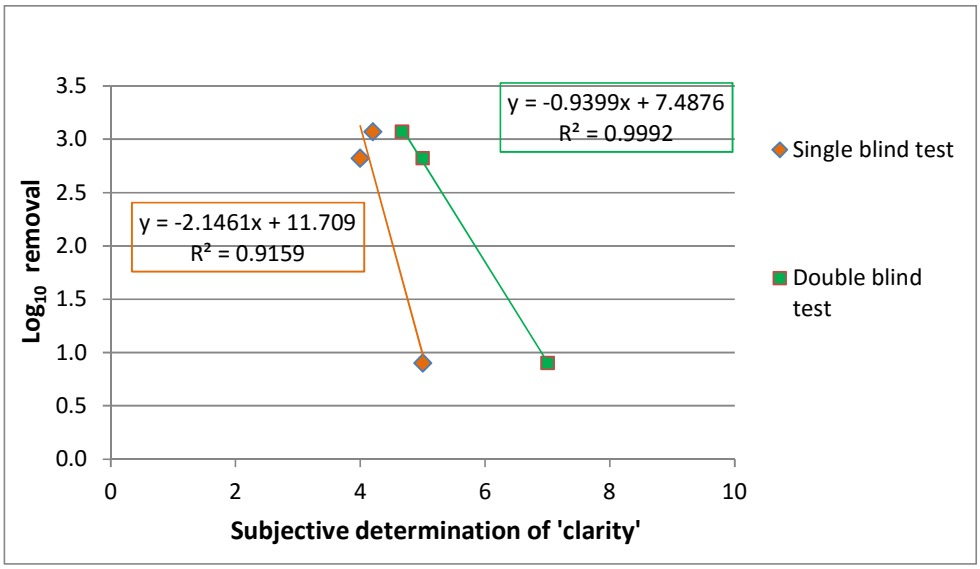


Figure 8.5: Log_{10} removal of Vibrio parahaemoliticus, vulnificus, mimicus *and* Proteus *and* Pseudomonas *species with respect to clarity score (Protocol A)*

Figure 8.6 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each 'single' and 'grouped' comparison tests of jars following completion of coagulation-flocculation and sedimentation processes for different amounts of added hydrated lime (for the high-pH treatment) and, the different Log_{10} percentage removal levels of *Vibrio cholerae*, *alginolyticus*, *metschnikovii*, *fluvialis* and *Enterococcus* species for the low-pH treatment. As mentioned in Chapter 3, the media used for estimating the number of these species only made it possible to make a total count of the five types of bacteria, without any distinction between them.

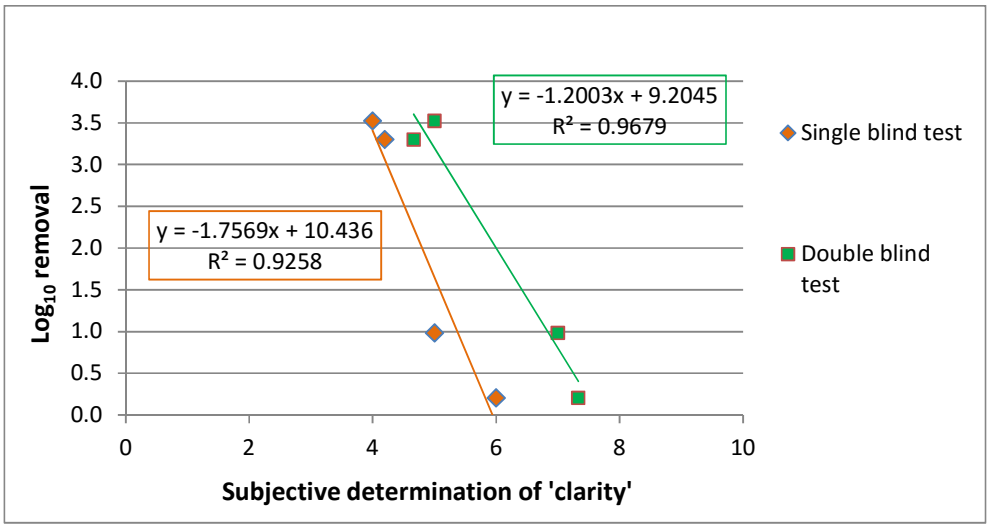


Figure 8.6: Log_{10} removal of Vibrio cholerae, alginolyticus, metschnikovii, fluvialis and *Enterococcus species with respect to clarity score (Protocol A)*

8.2 SUBJECTIVE VISUAL ASSESSMENT OF TREATMENT PERFORMANCE FOR THE LOW-PH TREATMENT (PROTOCOL B)

Figure 8.7 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of acidification and sedimentation processes involving different amounts of acid (for the low-pH treatment) and the different Log_{10} percentage removal levels of thermotolerant coliforms. Each data point represents the mean (N=30) of each of the five assessments for subjective determination of clarity.

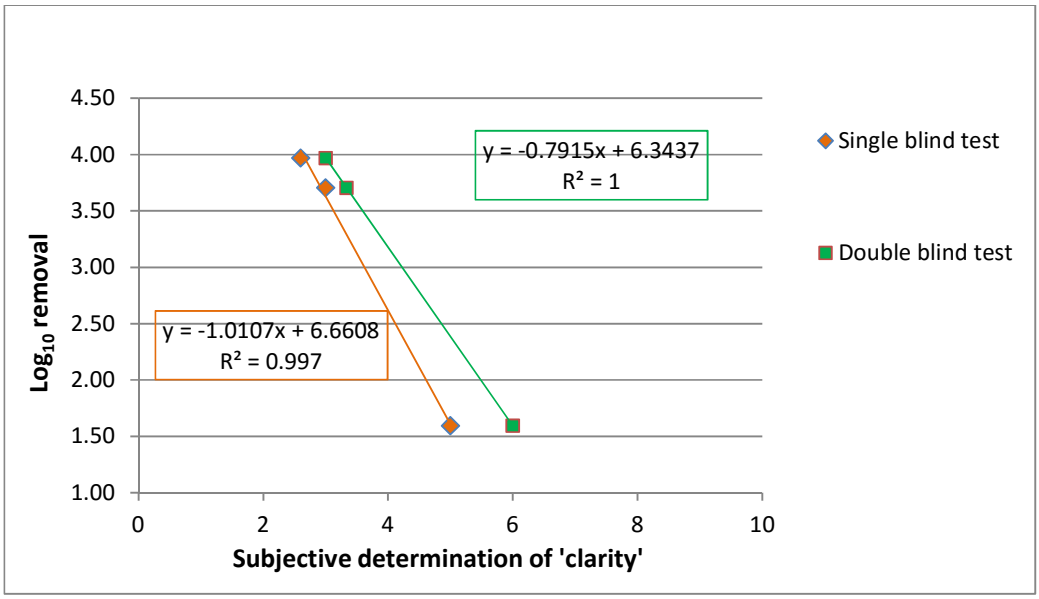


Figure 8.7: Log_{10} removal of thermotolerant coliform with respect to clarity score (Protocol B)

Figure 8.8 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of acidification and sedimentation processes involving different amounts of acid (for the low-pH treatment) and the different Log_{10} percentage removal levels of intestinal enterococci.

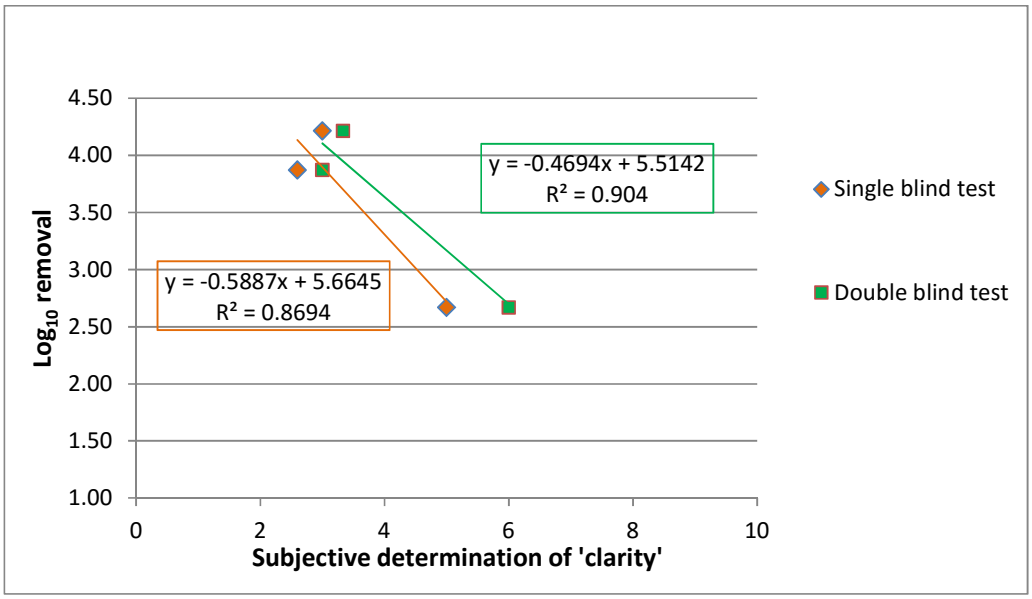


Figure 8.8: Log_{10} removal of enterococci with respect to clarity score (Protocol B)

Figure 8.9 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of acidification and sedimentation processes involving different amounts of acid (for the low-pH treatment) and the different Log_{10} percentage removal levels of somatic coliphage.

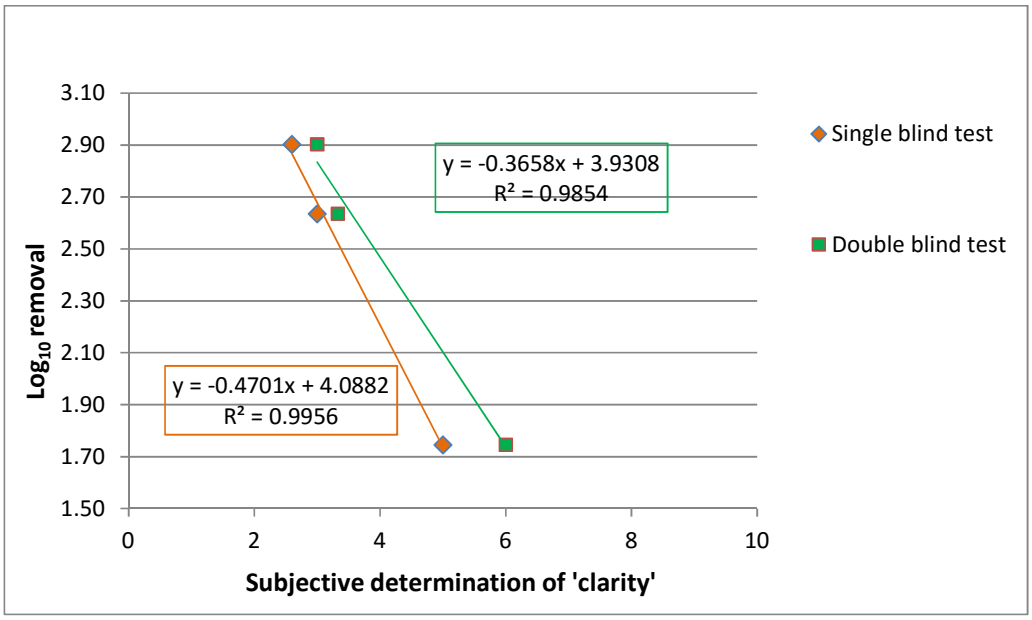


Figure 8.9: Log_{10} removal of somatic coliphage with respect to clarity score (Protocol B)

Figure 8.10 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of acidification and sedimentation processes involving different amounts of acid (for the low-pH treatment) and the different Log_{10} percentage removal levels of F-RNA phages.

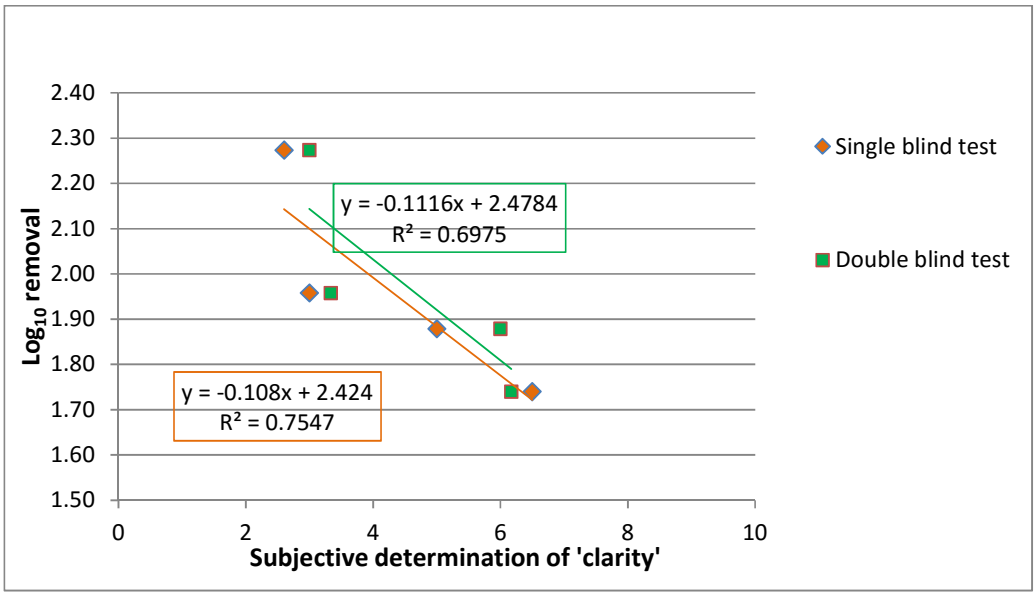


Figure 8.10: Log_{10} removal of F-RNA with respect to clarity score (Protocol B)

Figure 8.11 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of acidification and sedimentation processes involving different amounts of acid (for the low-pH treatment) and the different Log_{10} percentage removal levels of *Vibrio parahaemoliticus*, *vulnificus*, *mimicus* and *proteus* and *pseudomonas* species.

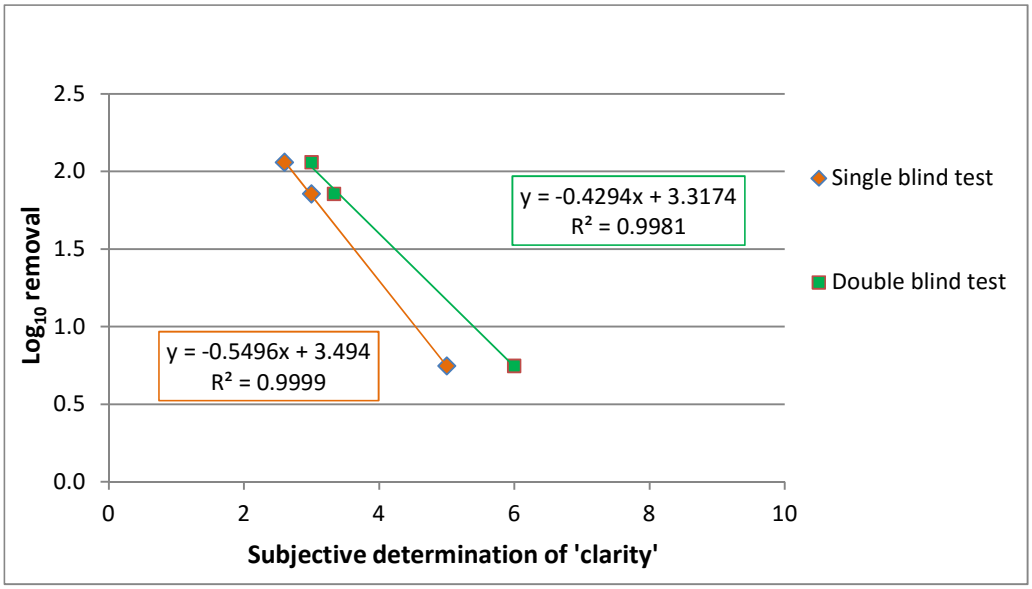


Figure 8.11: Log_{10} removal of Vibrio parahaemoliticus, vulnificus, mimicus *and* Proteus *and* Pseudomonas *species with respect to clarity score (Protocol B)*

Figure 8.12 highlights the relationship between the volunteers' visual assessment of clarity (subjective determination of clarity) for each single and grouped comparison tests of jars following the completion of acidification and sedimentation processes involving different amounts of acid (for the low-pH treatment) and the different Log_{10} percentage removal levels of *Vibrio cholerae*, *alginolyticus*, *metschnikovii*, *fluvialis* and *Enterococcus* species.

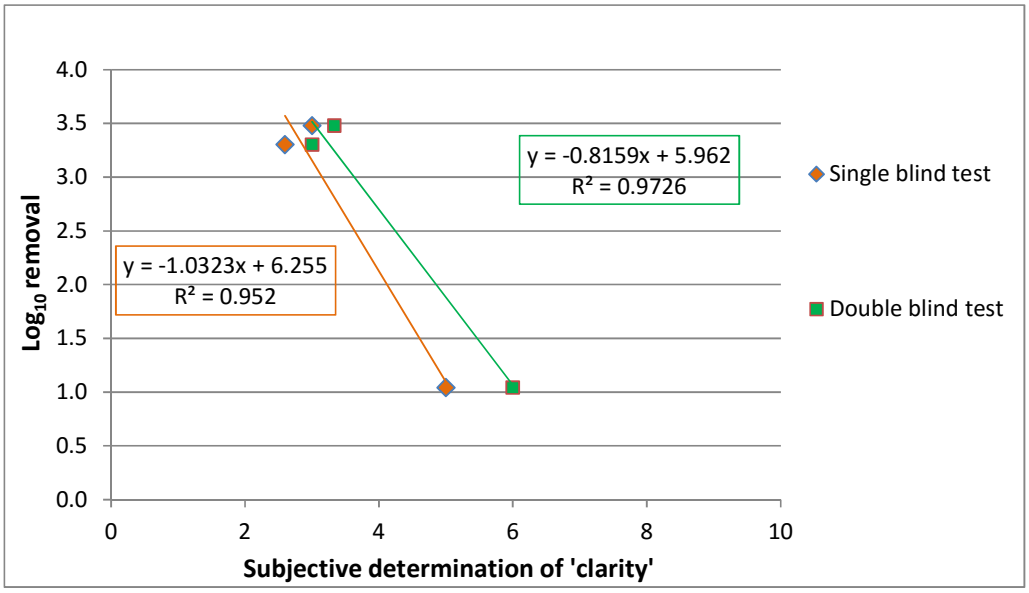


Figure 8.12: Log_{10} removal of Vibrio cholerae, alginolyticus, metschnikovii, fluvialis and *Enterococcus* species with respect to clarity score (Protocol B).

The findings of this initial assessment confirm that a correlation exists between the degree of effluent clarity and the likely levels of the various parameters tested. The results therefore suggest that in certain situations, such as under field conditions during an emergency and where full laboratory analysis is either unavailable or unfeasible, treatment performance (removal) could be assessed, and where necessary optimized by non-experts in the field, based on their assessment of effluent clarity. These findings confirm that the subjective determination of clarity is sufficiently accurate for assisting with performance monitoring and optimization in the absence of more accurate assessments.

9. CHAPTER NINE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The aim of this research programme was to develop an innovative evidence framework that would contribute to the design and operation of a novel, simple and possibly low-cost technique for the *in situ* disinfection of highly contaminated hospital faecal waste. The technique was originally designed to be applied in the context of a cholera outbreak. However, during the course of the study, especially as a consequence of the 2014 West African Ebola outbreak, the scope of the project was modified to take account of the evolving needs of managing disease outbreaks.

With regard to the disinfection of faecal waste from CTCs, it is worth mentioning that the research presented here can rightfully be considered to be timely [176]. A UN report published in 2011 in response to the Haitian cholera outbreak stated that "[…] to prevent introduction of contamination into the local environment, United Nations installations worldwide should treat fecal waste using on-site systems that inactivate pathogens before disposal. These systems should be operated and maintained by trained, qualified […] staff or by local providers with adequate oversight […]" [61]. It is also worth pointing out that in response to the recent Ebola outbreak, the WHO suggested modifications to established WASH practices, potentially including the use of lime to disinfect human excreta [89].

In this chapter, the methods, protocols and findings presented in earlier chapters are critically evaluated, their limitations discussed and recommendations are presented both for future research and for practical application of the methods as they currently stand.

9.1 MICROBOLOGICAL INDICATORS

The high rate of disinfection demonstrated, initially during the full-scale treatment in Haiti and particularly latterly during the laboratory studies of both physico-chemical treatment protocols, clearly suggests that this innovative technology may be an appropriate and potentially valuable option for the onsite disinfection of CTC wastewaters generated in the emergency settings of cholera epidemics and those of other excreta-borne bacterial infections.

The results presented here of studies into the removal of viral surrogates (bacteriophages) clearly suggest that the technology may potentially also offer a

valuable form of wastewater and human excreta disinfection during outbreaks of viral infectious diseases. Although the enveloped Ebola virus is considered to be 'fragile' beyond the environment of bodily fluids (including faeces), its potential presence in large numbers in the faeces of Ebola patients and its very low infective dose [177, 178] present a potent hazard to health care workers. The disinfection options presented here may be readily adapted to provide an important *in situ* excreta disinfection step, as part of an integrated infection control framework during Ebola outbreaks and possibly also during outbreaks of classic excreta-borne viral diseases, particularly hepatitis A and E.

In this study, disinfection efficacy is presented in terms of $\log_{10}$ removal rates as a function of pH level, it being envisaged that these values could subsequently be used by practitioners in the field to support the design of full-scale treatment processes. Once the required percentage (or Log_{10}) removal rate has been determined – ideally following discussions with a multidisciplinary team of public health experts – the presented correlations would enable the engineer designing the system to estimate the pH value required (and therefore the amount of reagent to be added) in order to achieve the required removal rate.

The figures presented in Section 6.2.1 suggest the role played by the alkaline environment in the destruction of indicator bacteria (and therefore, by association, in the destruction of enteric bacterial pathogens) and demonstrate how the removal clearly improves with increasing levels of pH, confirming the findings of the previous field experiments. Similarly, the figures presented in section 6.3 suggest the role played by the acidic environment in the removal of indicator bacteria and how removal clearly improves with decreasing pH levels – once again confirming the results from the field experiments.

9.2 THE STRATEGY FOR THE CHOICE OF A PATHOGEN SURROGATE FOLLOWED DURING THIS STUDY

The choice of a pathogen surrogate (or index) for a novel treatment scenario should (ideally) be based on detailed laboratory experiments to compare the behaviour of the pathogens and candidate surrogate or index organisms. Although currently, given the limited information available in the literature on the issue, it is perhaps fair to suggest that *E. coli* (or alternatively, intestinal enterococci) are currently the best available 'surrogates' for *Vibrio cholerae* in wastewater treatment processes, the correlation between the indicator and the

pathogen of interest (*Vibrio cholerae*) should in principle be explored as part of future laboratory studies. Until the validity of a strong positive correlation between levels of *E. coli* and *V. cholerae* has been demonstrated, it is reasonable to assume that faecal coliform enumeration (or alternatively, intestinal enterococci enumeration), and in particular the observation of its log reduction, is at least the best currently available method to assess the disinfecting potential of the studied experimental treatment protocols.

Moreover, as pointed out in several studies, there are reasons to propose the use of a second indicator of faecal contamination in addition to thermotolerant coliforms when monitoring the efficacy of the studied treatment system. In particular, the parallel enumeration of both thermotolerant coliform and intestinal enterococcal log reduction rates was considered to be the best currently available approach to assessing the disinfecting potential of the two studied experimental treatment protocols and comparing their performance.

Grabow et al. (1969 and 1978) reported that in laboratory studies of the efficacy of lime treatment, the relative sensitivity of Gram-positive and Gram-negative bacteria to hydroxide alkalinity differed extensively. Gram-negative bacteria, such as *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* proved highly sensitive to the high-pH environment, whereas Gram-positive bacteria, such as *Streptococcus faecalis*, *Staphylococcus aureus*, and *Bacillus* spp., and particularly their spores, as well as *Mycobacterium tuberculosis*, were much more resistant. In the same study, coliform organisms were reported to be more sensitive than faecal streptococci when exposed to hydroxide alkalinity [179, 180]. For the purposes of this study, the use of the two commonly used faecal indicator bacteria was thought to indicate potential variability in the response of a variety of enteric bacterial pathogens.

Cotter and Hill (2003) analysed the response of Gram-positive bacteria to low-pH levels and reported that the mechanisms used by these organisms to survive low-pH stress operate in a number of different ways, which include their ability to alter cell membrane composition, extrude protons, protect macromolecules, alter metabolic pathways, and even generate alkalis to compensate for the external pH variation [181]. The considerable alkaline tolerance of the enterococci has been reported by several authors (Davies and Thiel, 1939; Stark and Sherman, 1935; Van den Berghe et al., 2006); this fact may explain why the enterococci shows lower reductions levels at high-pH compared with the low-pH treatment [182-184].

9.3 PATHOGEN DEACTIVATION VS. PATHOGEN SEPARATION

Theory suggests that when the high pH treatment (Protocol A) is performed, microorganisms are deactivated by the cytotoxic effect of high pH on the organism cell walls and internal components (leading to 'disinfection'), but that they are also physically removed ('separation') by coagulation-flocculation that results from the addition of the lime and the subsequent sedimentation of the precipitated flocs. The microorganisms are therefore concentrated into the sedimented sludge. The data presented in Section 6.2.2 quantify both the disinfection and separation components. Figures 6.7 to 6.10 demonstrate the role of pH-induced disinfection only and pH-induced disinfection followed by overnight sedimentation (physical separation) to the total reduction (including destruction by disinfection and physical separation of microorganisms) of thermotolerant coliforms and intestinal enterococci. From the figures presented in that section, it appears that disinfection (i.e. destruction) is the main factor responsible for the reduction of indicator organisms, but the role played by the physical separation is also significant. It can be concluded from the results that one of the main reasons why the low-cost treatment studied here was capable of reaching such a high degree microbiological indicator removal, within a short period of time, was that it combines both mechanisms within a single batch treatment.

9.4 THE DIFFERENT RESPONSE OF A GRAM+ AND A GRAM- INDICATOR OF FAECAL CONTAMINATIONS

As previously discussed in Chapter 2, higher removal rates of thermotolerant coliforms (the majority of which are likely to be the organism *E. coli*) and lower removal rates of intestinal enterococci during the high pH treatment would have been expected from a reading of the extant literature. This is because thermotolerant coliforms, and in particular *E. coli,* have been shown to be highly susceptible to alkaline treatments and to be more capable of adapting to a low pH environment, whereas intestinal enterococci have shown, to a certain extent, the opposite behaviour. Therefore, from a reading of the literature, lower rates of inactivation for thermotolerant coliform for the low pH treatment would be expected than those observed for the high pH treatment. In contrast, higher inactivation rates for intestinal enterococci, compared with the high pH treatment, would be expected under acidic conditions.

The results of the extensive laboratory studies reported here demonstrate a tendency that substantially confirms what was expected from the review of the literature: namely, an average 5.5 log_{10} removal for thermotolerant coliforms and only 2.5 log_{10} removal for presumptive intestinal enterococci under the most extreme alkaline conditions. The results from the most extreme acidic conditions also demonstrated a tendency that again substantially confirmed what would be expected from the literature: namely an average 2.5 log_{10} removal for thermotolerant coliform and a 3.5 Log_{10} removal for presumptive intestinal enterococci. In general, recorded removal rates for faecal coliforms and intestinal enterococci differed, as expected, but the response of these two commonly-used faecal indicator bacteria to the two treatment protocols potentially indicates the range of possible responses by a wide range of enteric bacteria, including bacterial pathogens, in faeces.

Somatic coliphages demonstrated a higher susceptibility to low pH conditions, with an average 2.5 log_{10} (with a maximum of 5 log_{10}) removal for Protocol A and an average 3 log_{10} (with a maximum of 5 log_{10}) removal for Protocol B.

9.5 INDICATORS OF PHYSICO-CHEMICAL POLLUTION

The figures presented in Chapter 7 elucidate the role played by the alkaline (Protocol A) and acidic (Protocol B) environment in the removal of parameters of physico-chemical pollution.

Both Chapters 6 and 7 present results for a broader set of parameters than it was possible to study in the field (Chapter 4), but the general conclusion is the same: for both protocols, contaminant removal is demonstrated clearly to improve as the pH level moves away from neutrality towards more extreme values (either very high, as in Protocol A, or very low, as in Protocol B), confirming the results from the field experiments.

An important distinction needs to be made with regards to turbidity and TSS for the low pH treatment (Protocol B). This is the only case in which the laboratory studies did not confirm the findings of the field experiments, suggesting that both parameters may perhaps not be ideal surrogates of other more reliable (but more difficult to measure) indicators of treatment performance, as will be further discussed later.

9.6 CONSUMPTION OF REAGENTS

Table 9.1 and Table 9.2 present a comparison between the two protocols of consumption rates of reagents used, during both the full-scale field work in Haiti and the laboratory tests in the UK. An evaluation of the consumption of reagents for the full-scale batches in Haiti originally suggested that, overall, the high pH treatment technology demonstrated a greater requirement for chemical reagents than did the low pH method, in terms of total mass of reagents required to achieve the desired treatment outcome. In other words, the low pH method was significantly more resource-efficient in the field.

Table 9.1: average quantity of hydrated lime and hydrochloric acid solution added to the reactor for each treatment protocol during fieldwork

	High pH treatment protocol	**Low pH treatment protocol**
Mean concentration of lime used [kg/m³] = [g/l]	3.96	0.47
Mean concentration of acid used [l/m³] = [ml/l]	2.25	1.30

However, the figures for the consumption of reagents for the same tests performed in the laboratory (Table 9.2) demonstrate markedly different results. Overall, the low pH treatment method demonstrated a marginally greater requirement for chemical reagents than did the high pH method. In other words, the high pH method proved to be slightly more resource-efficient.

Table 9.2: average amount of hydrated lime and hydrochloric acid solution added to the last jar (representing the most extreme pH) for each treatment protocol during the laboratory test

	High	**Low**
Mean concentration of lime used [kg/m³] = [g/l]	2.5	3.9
Mean concentration of acid used [l/m³] = [ml/l]	1.5	4

The requirement for chemical reagents represents the mass of chemicals to be transported into the field per cubic metre of wastewater to be treated. With regard to the first phase of the work (field experiments), it is important to point out here that the

variance in the mass of hydrated lime used per unit of wastewater during the operation of the low pH treatment was higher than had been predicted by the initial laboratory tests. This is probably the result of variations in the characteristics of the wastewater between each batch. Additionally, plant operation was undertaken in conjunction with operator training. During the initial full-scale plant operation (data not reported here), operators were trained to prevent excessive use of reagents that were not required to meet the treatment objectives. By the time that initial plant operation had been completed and the analysed batch treatments had begun, operator training had been completed. Nevertheless, the variance in the mass of hydrated lime used in the field per unit of wastewater could also be partially due to the fact that training and process of 'fine-tuning' continued during the entire project. This resulted in the non-uniform use of lime during some of the reported full-scale low-pH cycles.

Another important point to consider was that the hydrated lime used during the field full-scale treatment reported here was imported, from the UK initially and from Santo Domingo in the Dominican Republic at a later stage. It is reasonable to assume that the level of purity of the lime used in the field was comparable with that used during the laboratory trials. On the other hand, the concentration (in terms of molarity) of the hydrochloric acid used in the field was variable and often not known. Therefore, differences in terms of absolute quantities of reagents used for the same protocol between field and laboratory trials and especially between different batch treatments in the field (different batches of hydrochloric acid) are to be expected. On the other hand, what was not expected was that the low pH treatment (protocol B) would prove to be significantly more efficient during the field full-scale trials, and that the high pH treatment (protocol A) would prove to be slightly more efficient during the laboratory bench scale tests.

Clearly, the laboratory tests were performed in accordance with a more rigorous and controlled protocol, in comparison with those performed during the field work, in that a significantly higher number of repetitions, more controlled working conditions and higher quality reagents were achieved. Therefore, it is reasonable to conclude that the results from Phase II of the project (i.e., the laboratory studies) which suggested the higher efficiency of Protocol A in terms of reagent consumption) more reliably reflect what might be achieved in the field under optimum conditions.

9.7 SLUDGE PRODUCTION

As previously mentioned, the recorded mean volume of sludge produced during Phase I (full-scale treatment in Haiti) was slightly higher than the values suggested in the literature, both for hydrated lime and aluminium sulphate [125, 185]. The recorded average volume of sludge produced during Phase II (laboratory bench-scale experiments in the UK) was higher than the values recorded in the field and significantly higher than the values recorded in the literature, both for high and low pH treatment. Average sludge volume values ranged between 12% and 17% of the initial raw wastewater volume for high pH treatment, and between 20% and 30% for low pH treatment, which suggests that additional measures, to reduce the final volume of sludge to be disposed of, should be considered [125]. In addition to the use of simple drying beds, one option might be the addition of a further aluminium sulphate-based coagulation-flocculation step, as tested during Phase I.

9.8 REMOVAL OF TURBIDITY AND SUSPENDED SOLIDS

The reduction in turbidity and TSS achieved during the laboratory experiments (Phase II) was observed to be slightly lower than that achieved during Phase I for the high-pH treatment and was significantly lower for the low pH treatment.

With regard to the high pH treatment, the difference could be partially related to the variable composition of the faecal waste matrix or possibly to more accurate measurement of test parameters during the laboratory study.

During Phase II, the low pH treatment was performed without the addition of a further aluminium sulphate-based coagulation-flocculation step after the pH based disinfection. This is the main reason why the low-pH treatment achieved no significant reduction in terms of turbidity and TSS.

Turbidity and TSS were selected as parameters to be monitored regularly during both the full-scale treatment of Phase I and the laboratory experiments of Phase II with the aim of exploring the possibility of using one of the two parameters (both of which are relatively simple to measure in the field) as an effective surrogate for other indicators of microbiological and physico-chemical pollution that are considered very reliable in evaluating the treatment performance, but might be more difficult to monitor in emergency settings. Unfortunately, since the correlation for these simple indicators against the suite of microbial indicators tested was weak, it is reasonable to conclude that turbidity and TSS cannot, at this stage, be recommended as acceptable

alternative for monitoring levels of faecal indicator organisms in these treatment systems. In other words turbidity and TSS values cannot be considered as reliable surrogates for other parameters.

Conversely, the findings of this initial assessment of clarity presented in Chapter 8 confirm that a correlation exists between the degree of effluent clarity and the likely levels of the reliable indicators of microbiological and physico-chemical pollution tested. The results are clearly counter-intuitive; it could be suggested that the subjective evaluation of colour change plays also an important role when the volunteers assess the supernatant. These results would benefit from a more detailed investigation to elucidate which components of treatment (beyond reduction in turbidity) are being recorded by visual inspection.

The results therefore suggest that, as the levels of turbidity or TSS removal cannot be considered good surrogates of treatment performance, this simple approach could be evaluated as a better alternative and where necessary optimized by practitioners in the field, based on their assessment of effluent clarity. Performance interventions in the field or in emergency setting based primarily on the assessment of effluent clarity should therefore be possible. These findings suggest that the subjective determination of clarity is sufficiently accurate for assisting with performance monitoring and optimization. In fact, replacing turbidity and TSS measurements with the concept of visually assessed ‘clarity’ proved to be much more reliable as a surrogate for assessing reduction of microbiological and physico-chemical pollution. More detailed investigation should be done to elucidate why the score assigned after visual inspection proved to correlate better to the presented parameters than turbidity and TSS values.

9.9 VISUAL ASSESSMENT OF TREATMENT PERFORMANCE

During the final stage of the laboratory study it became clear that the tentative use of turbidity as a surrogate for other, presumably more reliable, but also time- and resource-consuming indicators of separation efficacy (for example coliphages, thermotolerant coliforms or intestinal enterococci), was unlikely to be successful. Specifically, it appeared difficult to establish a good correlation between turbidity and TSS and any of the microbiological indicators of sedimentation and separation performance. Therefore, it was decided to explore the option of replacing turbidity or TSS removal as a surrogate with a visual assessment of effluent clarity. The tentative idea of establishing a correlation between a numerical value assigned by volunteers

to determine the clarity of different types of effluent and levels of an indicator of separation performance that may be considered to be a surrogate of certain pathogens, proved to be effective. Therefore, it is recommended that the option of using this simple surrogate of separation performance whenever it is not possible to rely on more robust, but also difficult to measure microbial indicators.

9.10 REPRODUCIBILITY OF THE TREATMENT PROTOCOLS

The average velocity gradient (G_{ave}) values mentioned in Chapter 3 are important parameters that should be considered by any practitioner aiming to perform the described batch treatments on a large scale with the goal of achieving performances comparable to the ones from the laboratory studies described here. G_{ave} (see Chapter 2) can be calculated, in an approximate way, for any standard full-scale batch reactor, based on its shape, dimensions and type of mixing regime. The field underlying the calculation of this parameter is known as 'computational fluid dynamics' (CFD). Although the theory briefly elucidated in Chapter 2 may appear to be relatively complex, the steps required to compute G_{ave} and then to scale up the physico-chemical treatment discussed are, for standard batch reactors, relatively straightforward, especially using commercially available software, and require limited technical knowledge of hydraulic and fluid dynamics. As it is beyond the scope of this manuscript to provide detailed guidelines on how to calculate the average velocity gradient, useful guidelines can be found in web resources made available by the 'Osney Thermo-Fluids Laboratory' of Oxford University [186] or in the studies cited by Bridgeman et al. [122].

Given a specific faecal waste matrix, needing to be treated in a certain field location, the average velocity gradient will have to be calculated for all real scale reactors and mixing regimes that are available to the practitioner. It would then be possible to infer which of the different available types of reactor and mixing regime would be the best option to perform the full-scale treatment, in a way that allows results to be maximised in terms of removal rates, reaching values comparable to those presented in Chapters 6, 7 and 8. In other words, and all other things being equal, once G_{ave} has been taken into account, it would be reasonable to expect in full-scale treatment, when performed according to the recommendations presented here, removal rates similar to those presented.

At this stage it is perhaps worth pointing out that, should both treatment protocols be applied at full-scale, it would also be reasonable to assume that, even with an average velocity gradient that is slightly different to the values presented in Chapter 3, the treatment is unlikely to give removal values that are very different from the figures presented in Chapter 6, 7 and 8.

9.11 LIMITATIONS OF THE STUDY AND RECOMMENDATIONS FOR FUTURE WORK

9.11.1 RATIONALE FOR THE FINAL COMPOSITION OF THE 'FAECAL WASTE MATRIX'

The rationale for the final decision to add 20% (vol/vol) of highly concentrated sludge to 80% (vol/vol) municipal wastewater during Phase II is outlined as follows:

1. The initial plan was to test the performance of the physico-chemical treatment process using municipal wastewater as a surrogate for the CTC wastewater encountered in Haiti. This choice would have entailed running both protocols with a matrix having much lower levels of turbidity, COD and total suspended solids than those encountered at the Haiti CTCs (or presumably wastewater from other CTCs or an Ebola care facility).
2. The addition of a more concentrated ('thicker') sludge was therefore considered the best option to provide a model wastewater that most closely reflected the wastewater strength that is likely to be encountered in many emergency settings. In principle, less than 20% (vol/vol) sludge could have been sufficient to reach enteric microorganism, turbidity and TSS levels of the field matrix. In practice, during a preliminary study, the results of which are not reported here, only 2% (vol/vol) sludge was added to the municipal wastewater. Nevertheless, the aim was to test the system under more 'conservative' conditions.
3. In addition, it must be pointed out that, during the fieldwork period (Phase I), the managers of both CTCs decided to mix the greywater with the wastewater from all hospital latrines. Greywater is defined as any wastewater stream generated by the health care facility other than the wastewater from latrines or toilets (blackwater) [187] and is typically less polluted than blackwater [188]. The practice was a precaution because of the obvious presence of faecal waste in the shower effluent - the practice of defecating in the shower being

observed to be quite common (given the nature of diarrhoeal diseases) in CTCs (and possibly in Ebola care units). The addition of greywater to blackwater makes the 'mixed-liquor' to be treated more dilute. It is important to note at this point that there are, in the context of emergency interventions for the control of epidemics, facilities that do not follow this practice. These facilities urgently need to treat a faecal waste matrix exclusively composed by blackwater, therefore more 'polluted' than the faecal waste matrix treated in Haiti during Phase I of the presented work. There are no recorded figures for turbidity, suspended solids, COD and coliform levels within these facilities, but it is reasonable to assume that the values are higher for all these parameters [189]. Therefore, the decision was made to add a high percentage of sludge to achieve a concentration that would better reflect the 'worst case scenario' of blackwaters that have not been diluted by greywater in emergency settings.

4. The choice of target COD value was more complex, as both the wastewater and sludge recorded COD values that were significantly lower than those of the wastewater treated during Phase I. This was probably due to the high level of faeces in the CTC wastewaters. These were excreted by cholera patients, who are recognised to defecate far more frequently than healthy individuals [81, 82]. The option of 'synthetically' increasing the COD levels (for example through the addition of peptone or equivalent products) was considered, but finally not employed, in order to avoid a further increase in the complexity of the laboratory study while maintaining a wastewater matrix more closely related in composition to real world situations. It is also worth noting that the addition of more than 20% sludge would have increased the COD levels, but would also have made the turbidity and suspended solids figures simply too high compared with the field study values, hence the compromise of the 80-20 (wastewater/sludge – vol/vol). The COD levels of the tested matrix in this study, which were high compared with most of the studies recorded in the literature, but still lower than the exceptionally high COD encountered in the field, represent a potential limitation of this research.
5. The values for levels of thermotolerant coliforms were slightly more complex to interpret. The concentration of thermotolerant coliforms present in wastewater without the addition of sludge would, in principle, have been more than sufficient to test the system in a 'conservative' way. In fact, these figures may appear excessively high and could wrongly suggest that a dilution of the municipal wastewater through the addition of a sterile diluent would have been

an option, rather than raising coliform levels through the addition of sludge. At this stage two observations need to be made:

- First, there is no single ideal wastewater-sludge (vol/vol) mix that would make it possible to match the levels of all indicators encountered in the field (Phase I). Changing the proportion in order to adjust one single parameter (e.g., turbidity) would result in another parameter moving out of the target range. The only option was to prioritise the indicators. As the main focus of the study was the development of an evidence-based framework for public health interventions in emergencies, priority was given to the microbiological parameters, specifically the thermotolerant coliform levels, which were the primary means of assessing disinfection efficacy.
- Second, as previously mentioned, the pooled CTC wastewater treated during the field studies (Phase I) was stored in open tanks for up to twelve weeks; resulting in a reduction in levels of enteric microorganisms. The storage became *de facto* an additional treatment stage prior to the physico-chemical treatment processes investigated in this study. This additional barrier to the transmission of pathogens, which was involuntarily added in the field, should not be taken into account when testing the system under controlled laboratory conditions. It was a fortunate circumstance that this extra storage took place, but many health care facilities do not have the capacity to add this step prior to physico-chemical treatment. Even if they do, this may take place sporadically rather than systematically.

In other words, the higher thermotolerant coliform levels of the raw matrix used in the laboratory better correspond to a 'conservative approach', which aims to test a system that can achieve effective treatment within the most difficult scenarios.

The '80-20 wastewater-sludge proportion' (vol/vol) was therefore considered to be the best compromise, allowing acceptable levels of turbidity, TSS and COD to be achieved and at the same time bringing the level of thermotolerant coliform to approximately 2.9×10^7 CFU per 100 ml of sample – a level adequate to assume that the system was being tested under 'critical' conditions.

9.11.2 NON-INCLUSION OF AN ALUMINIUM-BASED COAGULATION-FLOCCULATION STAGE WITHIN THE LABORATORY EXPERIMENTS (PHASE II)

As previously mentioned (see Subsection 3.3.3), the results from the preliminary laboratory tests led to the decision not to include a further aluminium-based coagulation-flocculation stage in the laboratory-based experimental protocol.

The main reasons for this decision were:

1. The primary aim of this study was to evaluate the role played by the pH (and not other factors) in the disinfection performance; therefore the addition of a further step was not considered a priority;
2. During the full-scale fieldwork (Phase I), the addition of an aluminium-based coagulation-flocculation stage in addition to the pH-based physico-chemical treatment was mainly undertaken to add an extra barrier to a technique that was not as yet well-understood. In other words, this further stage was simply an additional precautionary step to ensure adequate clarification of the wastewater, which was a secondary objective of treatment (after disinfection);
3. Most importantly the results of the preliminary laboratory tests in 'Phase II' demonstrated that hydrochloric acid-based disinfection alone was sufficient to achieve a level of disinfection comparable – and most of the time higher – to the high pH hydrated lime-based treatment. Therefore the option of adding an extra coagulation-flocculation step was also not considered to be a priority.

9.11.3 TARGET pH LEVELS

As a consequence of the results presented in Section 5.3.2 and 5.4.2, there appears to be no ideal choice in terms of target pH for each jar (related to hydrated lime or hydrochloric acid dosage). The defined sets of target pH levels were chosen for the two tested protocols as both sets allowed:

- A uniform coverage of the alkaline and acidic pH spectrum;
- A good analysis of the chemistry of lime-based coagulation-flocculation;
- The pH difference between one jar and the next was kept constant (at 0.9 points for the high-pH treatment and at 0.8 points for the low-pH treatment).

It is important to mention at this stage that there is no ideal choice in terms of target pH (and therefore lime dosage) to achieve all desired treatment outcomes. The

identified set of target pH levels represented only one of the many different (and legitimate) combinations considered.

This type of jar test experiment, performed with a combination of municipal wastewater and sludge, can only target pH ranges rather than exact values. Because of differences in the mixing regimes (manual preliminary test mix versus mechanical jar test mix), the composition of the matrix in each jar and the time that elapsed between the preliminary test and actual jar experiment, only rarely was the target pH achieved with any degree of precision at the end of the flocculation phase.

9.11.4 MATHEMATICAL MODELLING

A significant part of the results from the laboratory studies is presented in terms of correlations between pH levels and achieved removal rates of contaminants. These correlations are plotted using first or second degree regressions (i.e., by linear and parabolic functions, respectively). These are only two of the many available functions that might be used to approximate the behaviour of the removal rates in relation to variation in pH level. There is no reason to assume that the represented relationships should necessarily 'demonstrate' a 'first' or 'second' degree behaviour. The correlation between the represented variables could theoretically also be represented by logarithmic, higher degree polynomial relationships or, more probably, none of these, as complex biochemical processes do not necessarily strictly follow rigid mathematical models.

First or second degree polynomial functions were chosen to represent a correlation because of their simplicity and because they were both considered suitable to provide a reasonable representation of the behaviour of the related parameters. The main focus of this study was not the elaboration of a sophisticated mathematical model, involving complex functions to accurately represent the relationship between these parameters and reach the highest possible R2 values. Rather, the main objective was to provide a framework to allow water and sanitation practitioners working in the field to estimate the pH value required and the amount of reagent to be added in order to achieve a targeted removal rate.

9.11.5 PERSONNEL TRAINING DURING PHASE I

As previously mentioned, plant operation during Phase I was undertaken in conjunction with operator training. During the initial full-scale plant operation (data not

reported here) operators were trained to prevent excessive use of reagents that were not required to meet the treatment objectives. By the time the initial plant operation was completed and the analysed batch treatments had begun, operator training had been completed. Nevertheless the variance in the mass of reagents used in the field per unit of wastewater treated could be partially because training and process fine-tuning continued during the entire duration of the project. Moreover, the removal of supernatant was sometimes found not to have been performed under optimal conditions. This was because it took up to one month to train personnel adequately so as to optimize the process and minimize the sludge volume.

9.11.6 RECOMMENDATIONS FOR FUTURE WORK

Future research should focus on answering the following questions:

1. The findings discussed in section 9.4 present some important insights regarding the behaviour of *E. coli*, the most common Gram negative indicator organism, and intestinal enterococci, the most common Gram positive indicator of faecal pollution, under the tested alkaline and acidic conditions. There is little evidence from the literature to conclude that the response of *Escherichia coli* to a high or low pH environment would necessarily reflect that of *V. cholerae* (both being Gram negative bacteria) more than would intestinal enterococci (Gram positive bacteria). The work presented here provides valuable information on the response of enteric viral and bacterial indicator organisms to the treatment protocols but a useful avenue for future research would be a detailed investigation of the response of specific pathogens to the enhanced protocols resulting from this research;
2. The technique was originally designed to be applied in the context of a cholera outbreak. An interesting future study could involve the use of non-toxigenic and, ideally, also toxigenic *Vibrio cholerae*, with the aim of determining which of the two presented disinfection protocols has the better potential in terms of pathogen deactivation;
3. It has been previously mentioned that both disinfection options presented here may be readily adapted to provide an important *in situ* excreta disinfection step as part of an integrated infection control framework during Ebola outbreaks. A study aiming to assess the performance of both the presented techniques in terms of Ebola virus removal would be not feasible for obvious safety reasons [91]. Nevertheless it would be extremely interesting to assess the capacity of the

studied protocols to deactivate surrogates of Ebola viruses, an example being the Phi6 enveloped virus [190];

4. Similar considerations could be made regarding the possibility to adapt both disinfection options presented here to provide an *in situ* excreta disinfection step as part of an integrated infection control framework during outbreaks of hepatitis A and E. Such a study would also require the use of an appropriate surrogate virus [191].

9.12 CONCLUSIONS

The findings of this study are significant in that they offer practical recommendations on how to perform simple and low-cost *in situ* disinfection of highly-contaminated faecal waste of hospital and treatment centre origin. The two recommended techniques can both be applied in the context of a cholera outbreak, but also within the context of other outbreaks of infectious disease, including the two forms of water- and faecal-borne hepatitis, and Ebola.

The principal conclusions from the research can be summarised as follows:

1. There is no single solution to the eradication of an infectious disease outbreak, the control of which requires a combination of interventions and barriers, ranging from water supply, sanitation and hygiene improvements to medical interventions (this principle being referred to as the 'multiple barrier approach');
2. With this in mind, both disinfection options presented here may be readily adapted to provide an important *in situ* excreta disinfection step, as part of an integrated infection control framework, in scenarios in which hygienic management of sludges and wastewaters has to be achieved rapidly and at a relatively low cost;
3. Assessing the reduction of microbiological pollution indicator levels at the end of each batch is the ideal methods to assess disinfection efficacy of a full scale system. For cases in which this is not feasible, especially within the context of an infectious disease outbreak, such assessment can be performed sporadically and the visual assessment of 'clarity' of a faecal waste sample, before and after performing the treatments described here, appears to be an acceptable field-based 'substitute' for the routine operation.

   It is advisable, however, to assess on a regular basis the level of at least one of the four main indicators of microbiological contamination mentioned in this

study. As the assessment of somatic and f-RNA coliphages is probably too complex to be performed in the field, the best advice is therefore to attempt to test levels of thermotolerant coliforms or intestinal enterococci (to assess disinfection efficacy) and COD (to assess the removal of the organic component) at the beginning of the treatment and, if not for every batch, at least on a regular basis. Then a correlation between the visual assessment of 'clarity' and the more difficult to measure indicators should be attempted, as recommended in Chapter 8. The importance of the visual evaluation of 'clarity' to assess (indirectly) the disinfection and separation efficacy should not be underestimated, especially if the practitioner in the field has been adequately trained in applying this effective and powerful technique;

4. A longer-term challenge for microbial ecologists is to develop a better understanding of how toxigenic strains of *V. cholerae*, Ebola virus, Hepatitis A and E virus and other excreta-borne pathogens behave in the environmental niches present in wastewater treatment plants;
5. It is essential that those actively involved in WASH operational research and practical interventions should take a multi-disciplinary approach to the issue of controlling disease transmission from human excreta. Moreover, those responsible for designing and operating new wastewater treatment technologies in emergency settings should always consider the broader and longer-term public health context of their interventions.

If and when these questions are satisfactorily answered and this technique is regularly applied within the context of WASH interventions, the recommended physico-chemical technique for the *in situ* disinfection of highly contaminated human excreta may well offer an important tool to support global efforts to reduce the burden of human water-borne and faecal-borne disease transmission.

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APPENDIX 1 – LOCATION OF COOKSBRIDGE WASTEWATER TREATMENT SITE

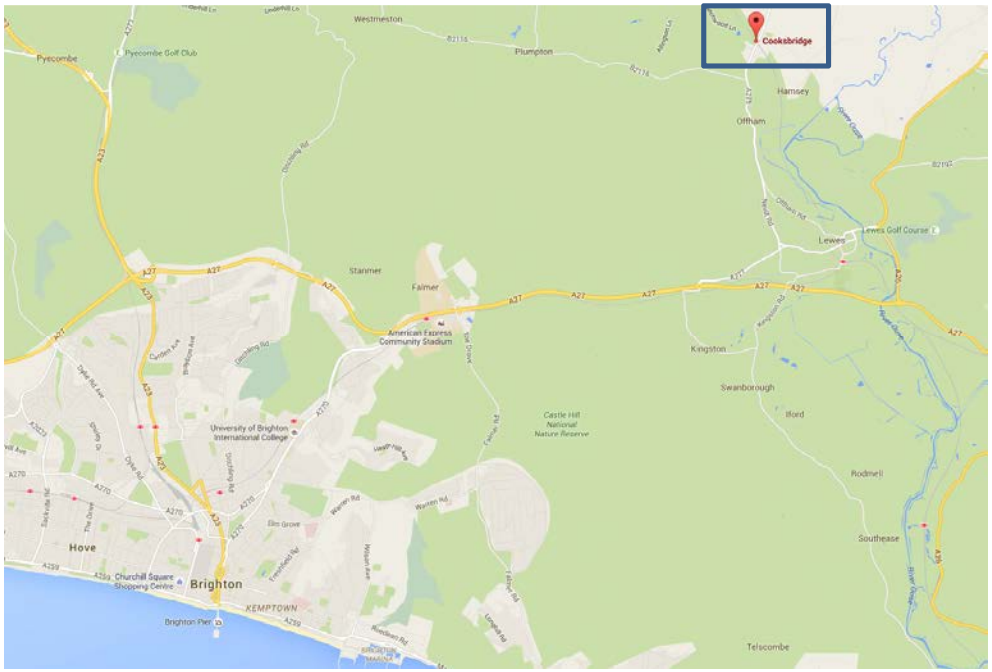

Figure AA.1: Location of the Cooksbridge wastewater treatment site located at latitude: +50.90706 and longitude: -0.00922 (see rectangle)