Performance of photobioreactor, constructed wetland and anaerobic membrane bioreactor in treating antibiotic resistant bacteria in the Barapullah drain, New Delhi, India

CIE5050-09 Additional Graduation Work

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Contents

Abstract

Limitless usage of antibiotics has led antibiotic resistance to be one of the largest threats to world health and development. In this study, the concentrations of Extended spectrum beta-lactamase Escherichia coli and carbapenem resistant Escherichia Coli was assessed in a major drain in New Delhi, India. The performance of Anaerobic membrane bioreactor, photobioreactor and constructed wetlands in treating ESBL-E.coli and CRE-E.coli was evaluated. The results showed ESBL-E.coli and CRE-E.coli removal efficiencies of 99.82% and 99.69% for AnMBR, 99.62% and 99.86% for PBR and 98.1-99.3% for constructed wetlands respectively. Log_{10} reduction values of 2.7-3 for AnMBR, 2.8-3.2 for PBR and 1.8-2.3 for CWs was achieved in this study. Coupling micro-aeration with AnMBR improved the removal efficiency by 36-46%. A quantitative microbial risk assessment showed probability of infection by Enterotoxigenic E.coli (ETEC O55) post treatment to be reduced below 10-18% for AnMBR and PBR and below 20-35% for CWs. Treated effluents accounted for a high reduction in the total DALYs pppy by 63% for PBR, followed by 41.6% for AnMBR and 12.5% for CWS. ESBL-E. coli and CRE-E.coli counts decreased below the monitoring level of 10^3-10^5 for unrestricted irrigation and 10^4-10^5 for restricted irrigation as declared by WHO. Treated water was not recommended for direct consumption due to higher risk above 10%. This study exhibits the potential of these efficient and sustainable technologies in treating antibiotic resistant bacteria.

1.Introduction

1.1 Water, health and sanitation in India

Well-managed hygienic water and adequate sanitation are the fundamentals for a better health and socio-economic development. Consumption of unregulated, contaminated, inadequate quantity and quality of water is causing the death of millions of poor people each year due to preventable diseases in developing countries. Insufficient sanitation costed $54 billion or 6.4% of India's GDP in 2006, of which more than 70% of this economic impact was due to diarrhoea followed by acute respiratory infections (Kumar et al.,2011). Water, sanitation and health are linked in many ways. Consumption of water contaminated with pathogens can cause several waterborne diseases like typhoid, cholera, pneumonia which is amplified by poor hygiene. Inadequate supply of clean water for personal hygiene can increase the spread of infections. Water based diseases like malaria, lymphatic filariasis can spread through stagnant waters which are breeding grounds for mosquitos, flies, snails. As per the census of 2011, household access to safe drinking water in India has increased from 38% in 1981 to 85.5% in 2011. Though 25 per cent households in nine states like Assam, Mizoram, Tripura, Odisha, Jharkhand, Nagaland, Manipur, Meghalaya and Kerala lacked access to safe drinking water (Kumar and Das, 2014). In terms of sanitation, 69.3% households in rural and 18.6% in urban still have no access to latrines within the premises and practice open defecation (Kumar and Das, 2014). Only 3.2% of households use public toilets even on implementation of various sanitation programs (Census of India, 2011).

Improvement in water quality does not make any difference if the sanitation conditions are still poor. Mortality rates due to waterborne diseases have not reduced with increasing availability of potable water. India loses around 0.4-0.5 million children below 5 years due to diarrhoea (Hamadani et al., 2014). Though infant mortality has been dropped over the years in the country, there are many states where the numbers are stagnant due to lack of improvements in personal and public hygiene concerning young children and new born (Liu et al., 2015). As per the findings, improvements in both sanitation and water quality is needed for the betterment of infant health in developing countries. The Millennium development goals target to provide sufficient access to water and sanitation to at-risk populations (Kumar et al.,2011). Intervention of effective and feasible approaches to provide low cost, simple and locally acceptable water and sanitation is the need of the hour.

1.2 Barapullah drain – A major drain in New Delhi, India

Figure 1 View of the Barapullah drain, New Delhi, India

India's still growing population has caused a major crisis for fresh water. More than half of the rivers in the country are polluted due to rapid urbanization, improper sanitation and water management. Since last five years, the number of polluted rivers has increased from 121 to 275 due to rising sewage flows into the water bodies (Burke, 2015). According to the study by Central Pollution Control Board in 2015, the urban sewage treatment plant capacities in India were designed to treat only 38% of the total 61,948MLD of sewage produced in the urban regions of the country (Central Pollution Control Board, 2015). An ill effect of this is the flow of untreated sewage into major rivers, water bodies and percolation underground. Such is the case of Barapullah drain in New Delhi, which was once a major storm water drain. The drain now carries one-third of the wastewater, discharging 125,000 kilolitres/day of domestic sewage into river Yamuna, one of the major rivers in the country. Yamuna is one of the sacred rivers of India and is widely worshipped by devotees. The Mughals built the magnificent Taj Mahal on its bank few centuries ago, but today it has been lowered to a pale stinking drain (Misra,2010). The drain is located at Sarai Kale Kahn, in the central part of New Delhi. Barapullah is a key drain with a total catchment area of 376.27 square km and a width of 100 meters, flowing over 16 kilometres. Being the second largest drain, it discharges around 80% of the storm water into Yamuna river (Bhaduri, 2017). Over the years, the drain flows through densely populated built-up areas of south-central Delhi. The drain is dumped with garbage, sewage dumps, slum wastes, construction debris and is characterised by complex sewage with

emerging heavy metals, micropollutants, antibiotic resistant bacteria, untreated industrial effluents and pesticides residues (Malik et al., 2014). During wet weather, there is excessive flow due to increased runoff whereas dry weather flow constitutes polluted discharged from unsewered areas near the drain. This discharge is due to poor maintenance of sewer lines and lack of understanding the need to maintain the integrity of the drain. During the construction of Barapullah flyover, the drain was clogged by construction debris which created a backflow causing sewage to enter the houses in low-lying residential areas. The heaps of garbage dumped has turned the drain into a breeding ground for diseases. According to the UNESCO report, more than 70 per cent pollution in river Yamuna is caused due to the sewage discharged by the local drains (UNESCO, 2012).

The drain water is anaerobic, with a pH range of 6-8, temperature of 26-32^{0}C and contains significant concentration of faecal coliforms, antibiotic resistant bacteria, heavy metals, organic micropollutants and other conventional pollutants. The presence of Escherichia coli, a coliform bacterium prevailing in faecal waste is considered as an important indicator in monitoring bacteriological quality of potable water (Gopal and Pathak, 2008). E coli is a high diversity bacterial specie which ranges from intestinal commensal strain to intestinal pathogen causing urinary tract infection (Liang et al., 2017). Contamination of water sources with faecal waste is a vital public health risk for waterborne infectious diseases. Furthermore, treating infections caused by certain E. coli strains is more complex with the developing antibiotic resistance. There are various mechanisms such as antibiotics inactivation, altering target regions that produce antibiotic resistance (Rao et al.,2014). Production of extended-spectrum beta-lactamase (ESBL) enzyme and Carbapenem resistant Enterobacteriaceae genes are two such mechanisms developed by the gram-negative bacteria (Logan and Weinstein,2017). The drain water is polluted with E coli producing beta lactamase and carbapenemase enzymes. The measured ESBL-E.coli and CRE-E.coli concentration in the drain varied from $1x10^6$ CFU/L-$4.1x10^7$ CFU/L and $3x10^6$-$8x10^7$ for wet and dry weather flows respectively. The CRE-E.coli concentration exceeded ESBL-E.coli concentration indicating the severity of the situation.

1.3 Origin of antibiotics and antibiotic resistant bacteria in India

Antimicrobial resistance is a major global issue. Antimicrobial resistance is the ability of microorganisms that include bacteria, virus, fungi, parasites to overcome antimicrobials and grow rapidly. Whereas antibiotic resistance includes the ability of bacteria to overcome antibiotic dosages and multiply (Gandra et al.,2017). The soaring burden of bacterial diseases

due to unsafe water, lack of hygiene and sanitation has led to the consumption of antibiotics and similar drugs since 1940 in India for treating illness and reducing deaths from diseases (Prevention, 2018). Since then the consumption of antibiotics in India has increased by 103% from 2000 to 2015 (DTE, 2018). According to the survey in 2014, India consumed the highest amount of antibiotics in terms of sales volume followed by China and the United States (Gandra et al.,2017). Due to the extensive usage of these antibiotics, bacteria have adapted to these drugs and gained resistance making them ineffective, increasing medication costs, increasing mortality and the duration of treatment. WHO has declared Antibiotic resistance as one of the biggest dangers concerning global health, food security and development (WHO, 2018). Approximately, 0.7 million people die of antibacterial resistance each year all over the world. Persistence of this trend can cause death numbers by ARBs to exceed the number due to cancer by middle of the century (Singh, 2017).

Conventional water treatment systems determine the fate, transport and removal of antibiotic resistant bacteria. Unless the system is adapted from time to time for the removal of emerging pollutants, treatment systems serve as a hotspot in enhancing the population of ARBs through horizontal gene transfer (Dires et al.,2018). Contamination of surrounding land, waterbodies and environment with human waste, sewage and hospital wastewaters has led to the spread of ARBs and ARGs in large numbers. According to the studies conducted on major rivers, River Yamuna consisted of 17.4% of isolates of different groups of gram negative bacteria which produced ESBL, along with ARBs resistant to different antibiotics (Gandra et al.,2017). Many other social and cultural factors lead to inappropriate usage of antibiotics in India. Some factors include self-medication, procuring antibiotics without a prescription, lack of knowledge as to when to consume them. In healthcare centres it can be perceived demand by patients, fear of losing customers/patients, lack of medical education and many more. One major cultural reason for the spread of ARBs and ARGs include religious practices of taking a dip or mass bathing in the rivers. Places like the Ganges river is an active hotspot for the spread of ARGs especially during the pilgrimage season (Ahammad et al.,2014).

1.4 Local Treatment of Urban Sewage Streams for Healthy Reuse – A wastewater management approach

Worldwide water scarcity has sparked efficient water production and reuse. Apart from the risk of polluted water bodies, there is also rising water demand with decreasing water availability (Cosgrove and Loucks, 2015). A beneficial move in this regard is to treat wastewaters and use it for recreational purposes. Efficient and economical technologies are need of the hour for

countries with developing economies like India. The Local Treatment of Urban Sewage Streams for Healthy Reuse is one such initiative that focuses on reuse and wastewater management. The aim of the project is to reuse the treated drain water for agriculture, industrial uses along with energy and nutrient recovery from wastewater. The focus is on the removal of both conventional and emerging pollutants. Furthermore, the selection of treatment technologies must be practical and potent in terms of both treatment and the cost.

The proposed treatment consists of anaerobic membrane bioreactor, photobioreactor and constructed wetlands which are tested for good water quality.

1.4.1 Anaerobic membrane bioreactor

Anaerobic membrane bioreactor has a significant potential in improving wastewater treatment process efficiency and sustainability. AnMBRs function on low energy without aeration and in turn produces methane on degrading organic compounds. Other advantages include low sludge production, reduced operational costs, low nutritional requirements, small reactor footprint, potential to handle high strength wastewaters and treat emerging pollutants like ARBs and ARGs in sewage. They also function as a consolidated system by eliminating needless treatment units such as activated sludge, secondary clarification and anaerobic digestor used in conventional wastewater treatment (Harb and Hong, 2017).

1.4.2 Photobioreactor

Photobioreactor is a specially designed system which depends on photosynthetic organisms like algae to achieve a good treatment. The interactions between algae and bacterial communities assist water treatment to reduce pathogenic concentration and break down organic compounds (Krustok, 2016). Over the last ten years, microalgae technology has moved from tertiary treatment to secondary treatment by replacing old existing technology like activated sludge process.

1.4.3 Constructed wetland

Constructed wetland is a low energy, low cost decentralised system that improves the effluent water quality. Plants and microorganisms are the main drivers in wetlands that uptake nutrients either aerobically or anaerobically (Hazra et al., 2011). As this green system reaches out to soil/sediments, plants and microorganisms, it is a potential solution for treating antibiotic resistant bacteria and other pathogens. Though wetland is an efficient technology in terms of operation, maintenance, cost and effluent quality, there is still a lack of understanding

concerning the impact of design, effect of environmental factors on the removal efficiency and risk of toxicity (Boto et al.,2016). The performance depends on the type of vegetation, seasonal variations and composition of water which does not provide a standard removal pattern.

1.5 Study objectives

To overcome the threat of antibiotic resistant bacteria, it is necessary to adopt technologies that deviate from the existing conventional treatment. The aim of this study was to evaluate the performance of Anaerobic membrane bioreactor, constructed wetland and photobioreactor in terms of removal efficiency and log reduction value of ESBL-E.coli and carbapenem resistant E coli. This study also aimed at conducting a Quantitative Microbial Risk Assessment to ensure sufficient microbial quality for recreational purposes. The research aimed at the following research questions:

1. What is the ESBL-E.coli and CRE-E.coli concentration in the Barapullah drain?
2. Can AnMBR, photobioreactor and constructed wetland effectively reduce the concentration of antibiotic resistant bacteria?
3. What are the considerable log reduction values achieved by these technologies?
4. Is the treated water suitable for reuse in terms of the bacteriological water quality?

2. Materials and Methodology

2.1 Experiment site

To analyse the existing concentration of CRE-E.coli and ESBL-E.coli, the wastewater was directly pumped from the location opposite to the pilot plant. The sample was pumped from 3-5 inches below the water surface into the storage containers directly. The raw water stored in the tanks was pumped by peristaltic pump into the reactors based on their flow rates. Before analysis, the sample was filtered with Whatman filter paper of 2µm size. The filtered samples were stored in centrifuge tubes and used for microbial analysis.

Figure 2 Sample collection from the drain

2.2 Experimental setup

2.2.1 Anaerobic membrane bioreactor

AnMBR used in this study was a submerged membrane bioreactor with a flow rate of 8L/day. The volume of continuous stirred-tank reactor and filtration unit was 4.6L and 4L respectively. The filtration unit consisted of a single filter module with dimensions 0.108m x 0.098m x 0.015m with a volume of 0.317L. A total flux of 10L/m2h was maintained. The filter unit was connected to a continuous stirred tank reactor to ensure complete mixing of wastewater. The setup of AnMBR is shown in figure 3.

2.2.2 Photobioreactor

The photobioreactor was a cylindrical column of height 1.5m and a capacity of 100 L. The raw water was collected in storage tanks and pumped into the reactors at a flow rate of 50.4L/day. The influent was dosed from top and effluent was collected at the bottom of the column. The reactor was exposed to sunlight to ensure sufficient energy for algal growth. A hydraulic retention time of 48 hours was maintained throughout the study period. The speciation of algae present in the reactor was not considered for this study. The setup of PBR is shown in figure 4.

2.2.3 Constructed wetland

The wetlands were set up in 5 different plastic tubs with dimensions 0.8m x 0.25m x 0.28m and were exposed to sunlight to support plant growth. The base of the wetland was filled with drain block which behaved as a filter material. Drain block is a mineral wool, which is a fibrous material made by spinning molten material or rock materials like slag (Snow, 2003). Three different types of plants-Rose Periwinkle, Cana Lily and Canna Pretoria were planted. Therefore, there were two sets of the same plant. A flow rate of 10 L/day was maintained in the reactor with a retention time of 24 hours. Figure 5 and 6 shows the setup of CW and figure 7 shows a tub filled with mineral wool.

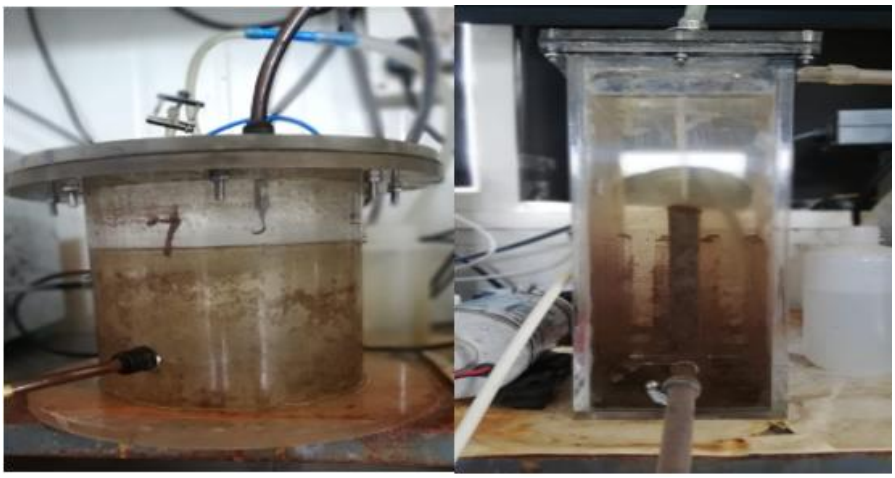

Figure 3 Setup of AnMBR a) CSTR b) Filter unit

Figure 4 Setup of Photobioreactor

Figure 5 Set-up of constructed wetlands

a) b) c)

Figure 6 Plants in constructed wetlands a) Canna Pretoria b) Cana Lily c) Rose Periwinkle

Figure 7 Mineral wool

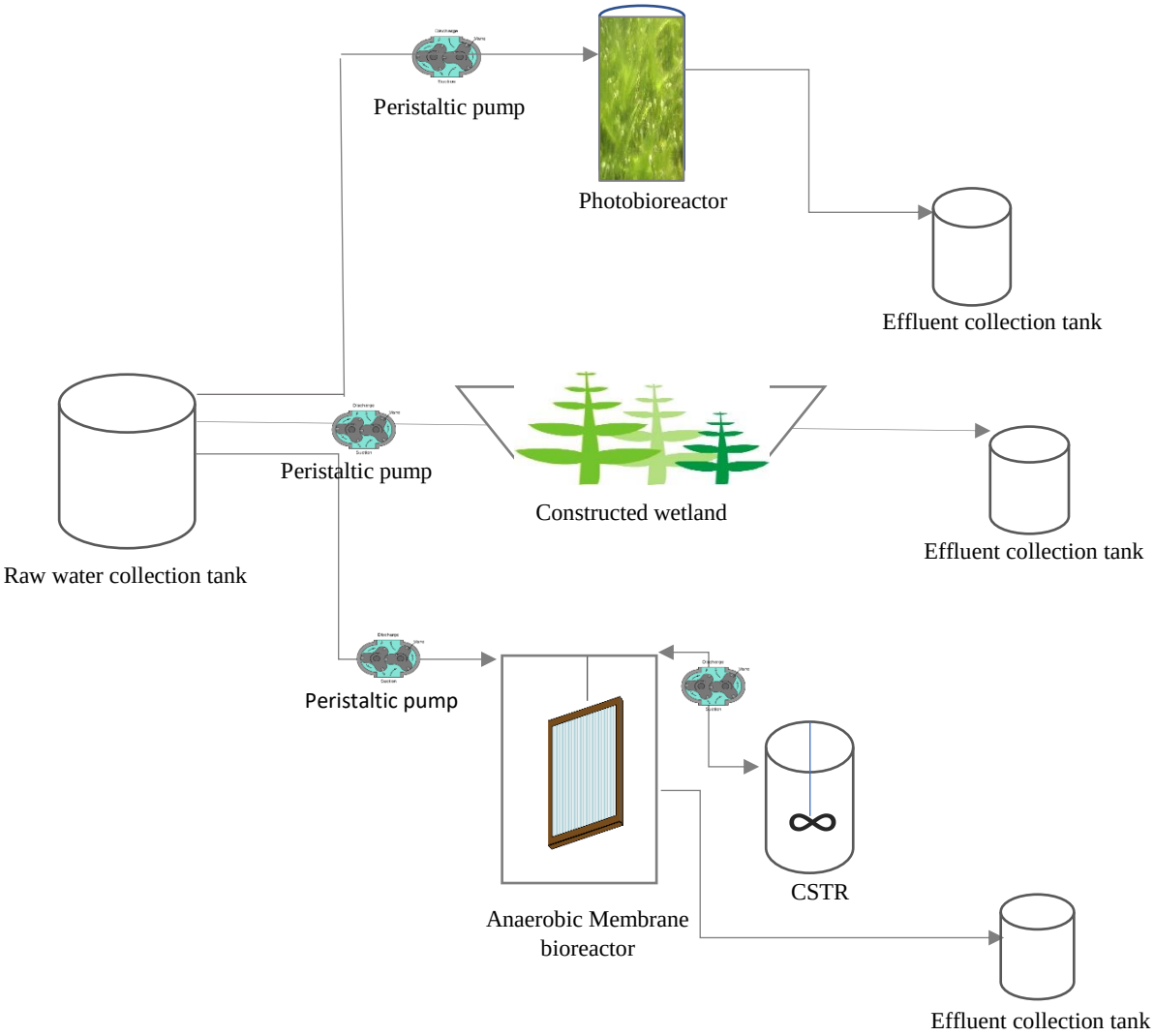

Figure 8 Schematic representation of the experimental setup

2.3 Microbial Enumeration and Analysis

The concentration of ESBL-E.coli and CRE-E.coli was analysed using HiCrome ESBL Agar Base, a selective media for isolation of ESBL producing Enterobacteriaeceae and HiCrome KPC Agar Base, a selective media for detecting gram negative bacteria with a reduced susceptibility to carbapenem agents respectively. Pre-poured media plates from Biomerieux was used for inoculation of samples. The raw influent was filtered using 2µm filter to remove any suspended particles or sludge material. Both raw and filtered samples were plated to check the effect of filtration on the removal of ARBs. Before starting the experiment, the work area was cleaned with ethanol to eliminate any surrounding bacterial contamination. Due to the high strength of wastewater, the influent was diluted to 10^{-2} with distilled water. For inoculation, 0.1 ml of diluted sample was pipetted out and spread on the agar plates using an L shaped spreader to ensure even distribution of the sample. This step was carried out in a laminar hood to avoid contamination from the surrounding air. Similarly, effluents from three reactors were collected in falcons and concentrated to 5ml and 10ml (for wet weather flows). The concentrated samples were filtered using membrane filtration technique with 0.45µm filters. The used filters were placed in plates using forceps and incubated for 24 hours between 35-37 degree centigrade for bacterial growth. Inoculation was done in duplicates. After 24 hours, the number of colonies formed was enumerated by colony forming unit (CFU/L) which indicates the number of colonies that remain feasible to grow rapidly and form colonies (Sankaranarayanan et al., 2014). The formula to calculate CFU/L is expressed in equation 1.

$$\frac{Colony\ forming\ unit}{ml} = \frac{(number\ of\ colony \times dilution\ factor)}{volume\ plated\ in\ ml} \quad \text{- Equation 1}$$

On determining the counts of the bacterial colonies, the removal efficieny was calculated for filtered influent and effluent in comparision with raw influent. The removal efficiency was calculated using the formula expressed in equation 2

$$\text{Percentage removal (\%)} = \frac{Cin - Cout}{Cin} \times 100 \quad \text{- Equation 2}$$

Log_{10} reduction was calculated to determine the reduction in the relative number of microbes. It refers to a 10-fold reduction in the quantitative microbial population. It is calculated as shown in equation 3

$$\text{Log reduction} = \log\left(\frac{Cin}{Cout}\right)$$ - Equation 3

Where,

Cin = CFU/L of influent sample

Cout = CFU/L of effluent sample

2.4 Quantitative microbial risk assessment

Reusing water can expose users to wide range of pathogens including those which are naturally present in the water. These pathogens are a result of faecal pollution by sewage, surface runoff. It is necessary to assess and manage microbial risks caused during reuse to avoid disease burden. A Quantitative microbial risk assessment can be a useful tool to manage hazards and estimate risks from exposure to pathogenic bacteria (Barbagallo et al., 2012). QMRA includes four steps, Hazard identification, Exposure assessment, Dose-response and risk characterization.

In this study, Enterotoxigenic E.coli (ETEC O55) is selected for QMRA. The ETEC O55 is not measured directly in the drain. The concentration is derived from E.coli concentration assessed in a recent study in the Barapullah drain. Hazard identification, exposure assessment and dose response relationship will be carried out for ETEC O55 to assess transmission pathways, exposed population, ingestion volumes. Finally, risk characterization is performed.

2.4.1 Hazard identification

Firstly, the hazards associated with Enterotoxigenic E.coli (ETEC O55) is identified by literature research. Also, the threat of ETEC gaining antibiotic resistance is discussed.

2.4.3 Exposure assessment

In this section, water sources, exposure pathways, affected population, ingestion volumes and concentration of ETEC O55 will be analysed.

2.4.2 Dose-response

Quantitative relationship between the possible adverse effects and the level of microbial exposure is analysed. Dose response defines the relation between microbial dose and rate of infection in humans depending on the incubation period (Mayer et al.,2011). The dose response relationship is formulated using a mathematical model

2.4.5 Risk characterization

Considering the dose response model discussed in the previous section and dose exposure from exposure assessment, the probability of infection is calculated at different doses in function with ingested volumes.

3. Results

3.1 ARB concentration in the feed water

The concentration of ESBL-E.coli and CRE-E.coli in the feed was calculated using equation 1. ESBL-E.coli concentration varied from $4.1x10^7$ to $1x10^6$ and CRE-E.coli varied from $8x10^7$ to $3x10^6$ for dry and wet weather flows respectively. Figure 9 indicates ESBL-E.coli and CRE-E.coli concentrations in raw feed measured over 4 weeks. It is evident from the graph that CRE-E.coli concentration is higher than that of ESBL-E.coli. There was sudden decrease in the concentration from 27th July which indicates wet weather flows. The heavy rains increased the water level in the drain thus diluting ARB concentration. Initial sample collected before rain shows a higher concentration. Figure 10 indicates the concentration in raw and filtered samples. Upon filtering the raw sample, 46.9% reduction in ESBL-E.coli and 34.7% reduction in CRE-E.coli concentration was observed. The reductions might have been due to the removal of suspended solids in the raw feed as pathogens tend to adhere to these particles (Stenmark et al., 2012). Furthermore, the effluent results of AnMBR, PBR and CWs were compared with the filtered influent samples. Figure 11 shows the concentration of ESBL-E.coli and CRE-E.coli in feed water.

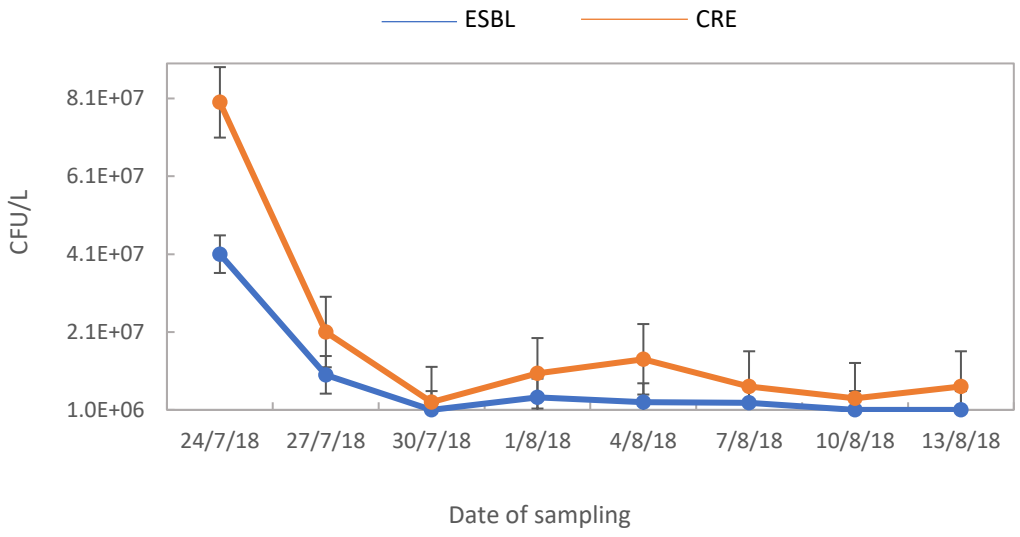

Figure 9 Concentrations in feed water

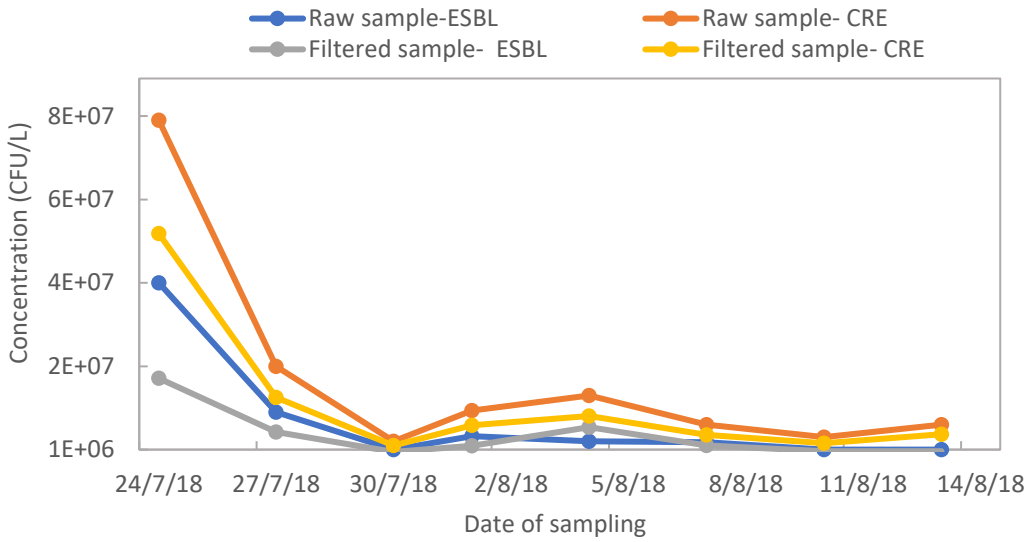

Figure 10 Concentrations in raw and filtered sample

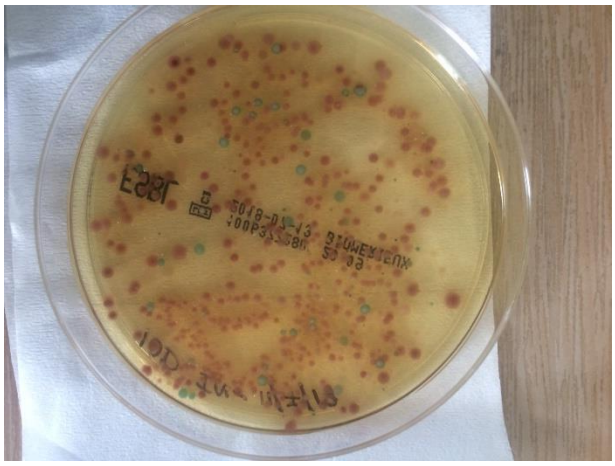

Figure 11 Microbial growth in feed water sample

3.2 Log reduction and removal percentage of ARB

3.2.1 Log reduction and removal removal percentage in constrcuted wetlands

The log reduction in three constructed wetlands with different plants is shown Figure 12. Wetland 1 corresponds to the tub with Rose Periwinkle, wetland 2 to Cana Lily and wetland 3 to Canna Pretoria. An average log reduction value of 2 and 1.8 for wetland 1, 2.1 and 2 for wetland 2 and 2.2 and 2.1 for wetland 3 was achieved for ESBL-E.coli and CRE-E.coli respectively. Reduction in wetlands can depend on various mechanisms within the wetland system such as natural die-off of bacteria, oxidation, filtration, adherence to biofilm, exposure to UV rays from the sun and other variables like plant variety, microbial activity in the system

and interaction with the biofilm. Weber and Legge (2008) reported natural die-off to be a prominent mechanism of pathogen removal in wetlands. They also estimated the die-off rate of faecal coliforms to be 0.256 $\log_{10}$/day in water. The presence of plants boosted the removal in the systems, as per the study conducted by Karathanasis et al., (2003). Vegetated systems were observed to perform the best in warmer months with a removal efficiency of 98.5%. The study was conducted during summer which might have benefited ARB reduction. As the plants discharge oxygen from their roots into the rhizosphere space, the DO concentration in the wetland system was elevated facilitating E coli removal (Green et al.,1997). This rhizosphere region with a higher oxygen level boosted aerobic microenvironment in a horizontal subsurface flow wetland system (Batty et al., 2000). Mineral wool used as a physical filter might have adsorbed ARBs as it has a large surface area. Garcia et al.,2003 reported in their study that coarse filter material (5-25mm) allowed a microbial inactivation ratio of around 0.1-2.7 log units for faecal coliforms. The square drain blocks used in this study had a dimension of around 25-30mm and hence might have benefited bacterial inactivation.

The percentage removal of ARBs in three wetlands is shown in Figure 13. The efficiency varied between 99.1-99.4% for ESBL-E.coli and 98-99.34% for CRE-E.coli. The observed pH of 7.5 might have contributed to the reduction of ARBs as neutral and alkaline pH benefit antibiotic resistant E. coli inactivation in wetland systems (Kipasika et al.,2016).

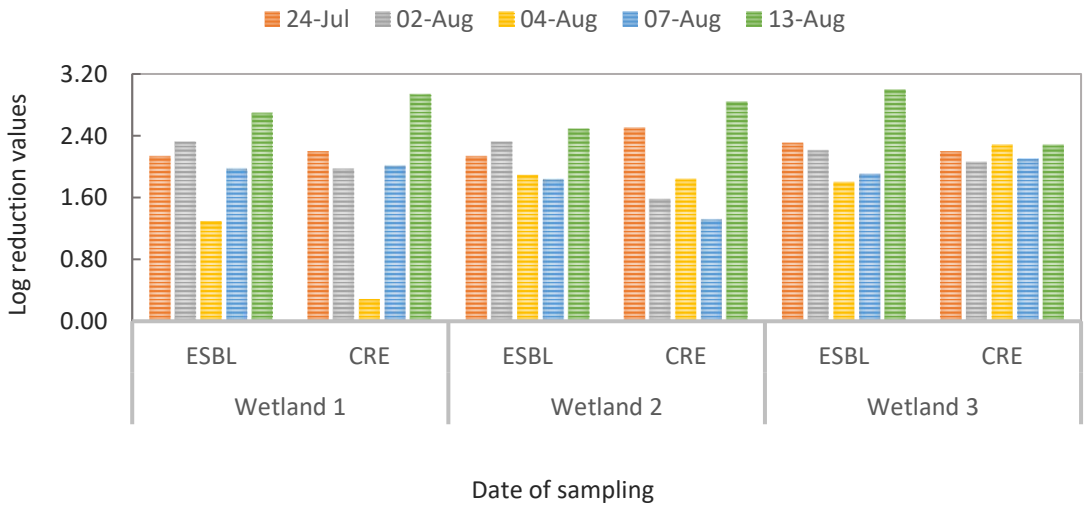

Figure 12 Log reduction values of wetland effluents

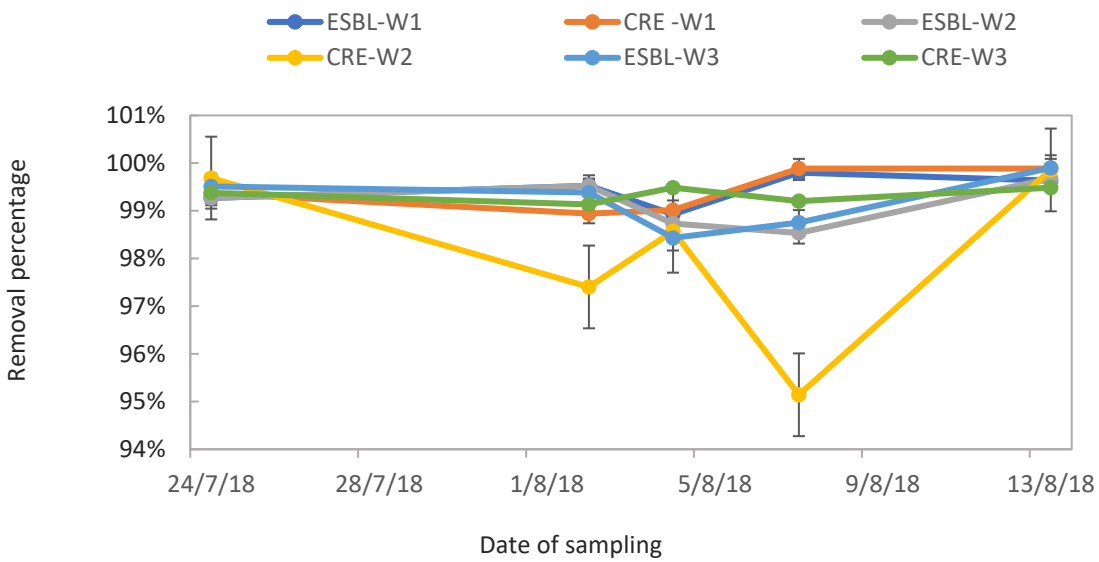

Figure 13 Percentage removal in CWs

3.2.2 Log reduction and removal percentage in photobioreactor

Log reduction achieved by a photobioreactor is shown in Figure 14. An average log reduction value of 2.8 and 3.2 was achieved for ESBL-E.coli and CRE-E.coli. The bacterial inactivation might have been due to various types of antibacterial substances produced by specific microalgae (Mezzari et al.,2017). Though in this study, analysis of different algal species present in the reactor was not conducted. An increase in dissolved oxygen concentration and pH due to algae resulted in the inactivation of E. coli (Ansa et al., 2010). Presence of algae increased the pH and high pH values tended to be bactericidal even at low oxygen concentration. An average pH of 9.8 was observed during the study. High pH was the effect of increased chlorophyll concentration. Therefore, the bacterial inactivation was due to the chlorophyll concentration which directly altered the rate of decay of E. coli (Ansa et al.,2010).

Percentage removal efficiency of PBR is shown in figure 15. An average removal efficiency of 99.62% and 99.86 was obtained for ESBL-E.coli and CRE-E.coli respectively. It is evident from both the graphs that PBR was efficient in achieving a high log reduction value and removal efficiency.

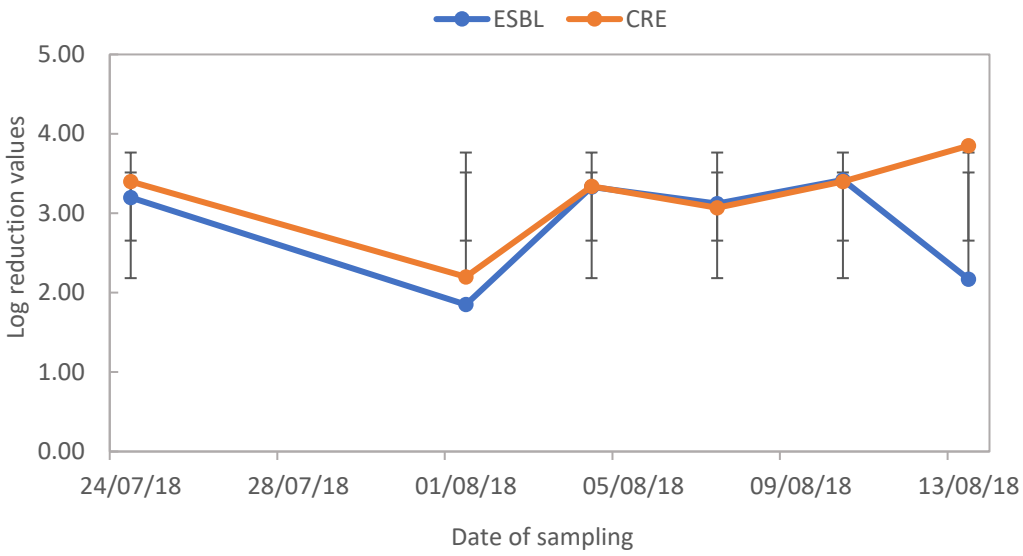

Figure 14 Log reduction values of PBR effluent

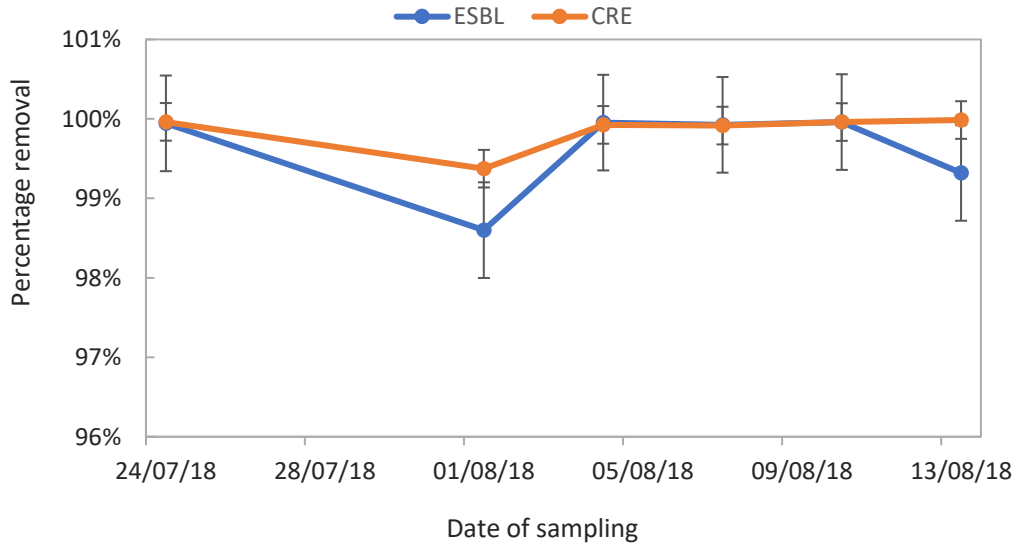

Figure 15 Percentage removal in Photobioreactor

3.2.3 Log reduction and removal percentage in Anaerobic membrane bioreactor

The LRV of a regular AnMBR varied between 1.9-3.9 (Chen and Hong, 2017). In this study, AnMBR achieved an average log removal of 3 and 2.7 for ESBL-E.coli and CRE-E.coli respectively. Figure 16 indicates LRV values for ESBL-E.coli and CRE-E.coli. Micro-aeration was started in the existing setup from 3 August with oxygen concentration of 0.4L/day. Studies conducted by Calderon and Hong, 2017 with E coli P17 isolates showed that the decay of this

model bacterium was higher by one magnitude in aerobic environment when compared to anaerobic environment. Oxygen is a key driving factor for decay of E coli as they develop oxidative stress that decreases its fitness and hence decay faster (Hong et al.,2017). From the results it is evident that micro-aeration improved ARB inactivation by 46% for ESBL E.coli and 36% for CRE-E.coli. The start of micro-aeration is indicated by dashed line which shows a higher log reduction for both ESBL-E.coli and CRE-E.coli. The hydrophobic cell nature of E. coli might have benefited its attachment to hydrophobic anaerobic membranes. The lower negative charge on E. coli results in a weak charge repulsion causing a stronger adsorption onto the membrane. Fouled membranes claimed a higher attachment of cells compared to new ones (Chen and Hong, 2017). Since the experiment was run for 4 weeks, excess fouling was not observed. This study can further be continued for extended period to study the effect of fouling on ARB inactivation.

The removal efficiency of AnMBR treating raw influent is shown in figure 17. ESBL-E.coliand CRE-E.coli achieved an average removal efficiency of 99.82% and 99.69% respectively. AnMBR achieved a high removal efficiency and log reduction for both ESBL-E.coli and CRE-E.coli.

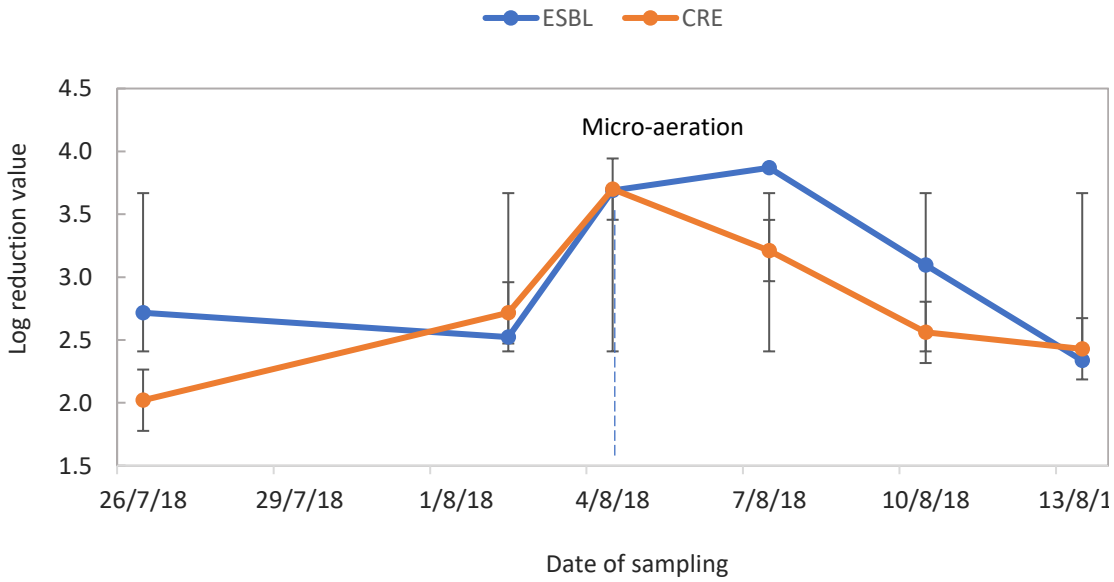

Figure 16: Log reduction values of AnMBR effluent

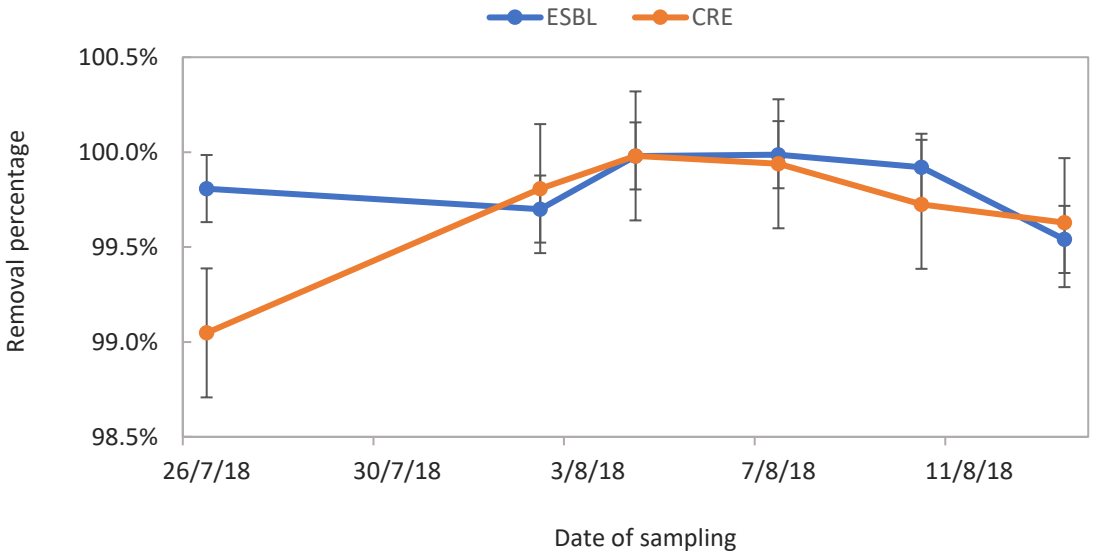

Figure 17: Percentage removal in AnMBR

3.3 ARB balance in AnMBR sludge

Anaerobic membrane bioreactor was monitored for a period of 4 weeks. CSTR was fed with feed water with an initial ESBL-E.coli and CRE-E.coli concentration of $1x10^7$ and $2.1x10^7$ CFU/L respectively. With a hydraulic retention time of 1-day, average effluent concentrations of $6.36x10^3$ CFU/L and $4.40x10^4$ CFU/L were obtained for ESBL-E.coli and CRE-E.coli respectively. The remaining concentration from the influent was accumulated on the filter module in the filter unit and the rest circulated from CSTR to the filter unit. The concentrations in CFU/L is shown in Table 1. The effluent carried 0.17% and 0.42% of the influent ESBL-E.coli and CRE-E.coli concentrations respectively. The CSTR had 7.27% and 1.14% and the filter unit had 1.23% and 0.71% of influent ESBL-E.coli and CRE-E.coli concentrations respectively. The remaining percentage might have decayed due to increase in oxygen concentration during micro-aeration and washed out in the effluent. ARB balance in the reactor has been depicted in the Figure 18. Figure 19 shows a graph of ESBL-E.coli and CRE-E.coli concentrations in AnMBR effluent and the sludge of CSTR and filter unit. ESBL-E.coli percentage is lesser in the effluent compared to CRE-E.coli whereas in the sludge, ESBL-E.coli percentage is high as it is retained in the CSTR or attached to biofilm on the filter. Likewise, for CRE-E.coli the percentage in the sludge is less and the rest is washed out in the effluent. Figure 20 shows cultured bacterial colonies on the agar medium. Pink colonies formed

indicates ESBL-E.coli and CRE-E.coli. Green colonies indicate the growth of a different species of no relevance to this study.

Table 1 ARB concentration in Filter unit and CSTR

ARB type	AnMBR influent (CFU/L)	AnMBR effluent (CFU/L)	Filter unit (CFU/L)	CSTR (CFU/L)
ESBL-E.coli	$2.20x10^7$	$3.82x10^4$	$2.70x10^5$	$1.60x10^6$
CRE-E.coli	$6.34x10^7$	$2.64x10^5$	$4.50x10^5$	$7.20x10^5$

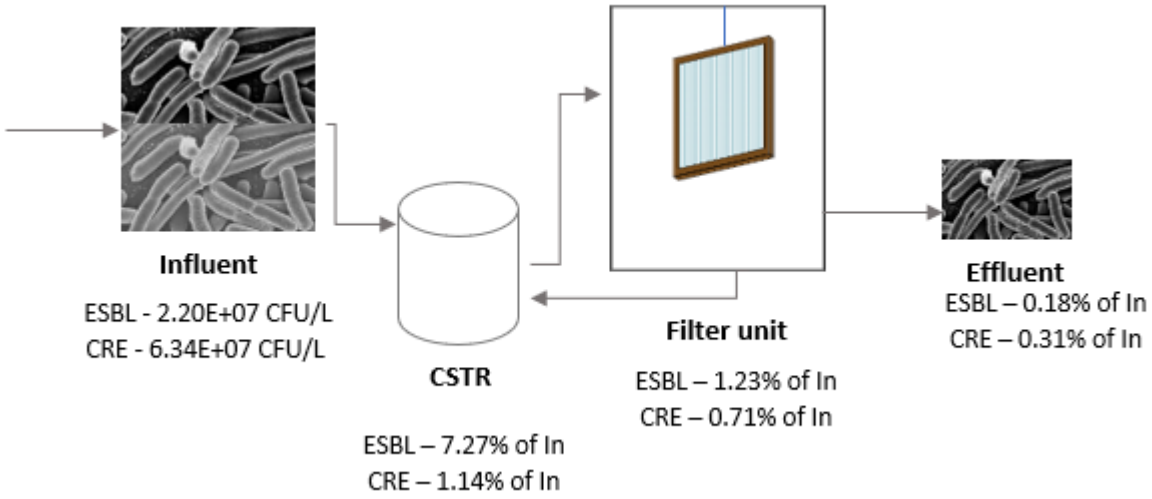

Figure 18 ARB balance in sludge

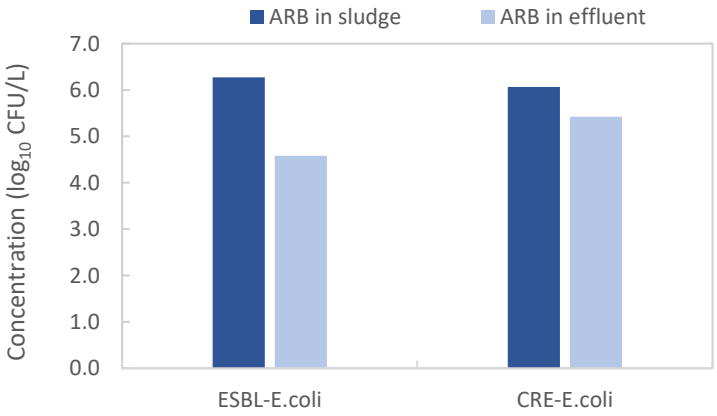

Figure 19 ARB percentage in effluent and sludge

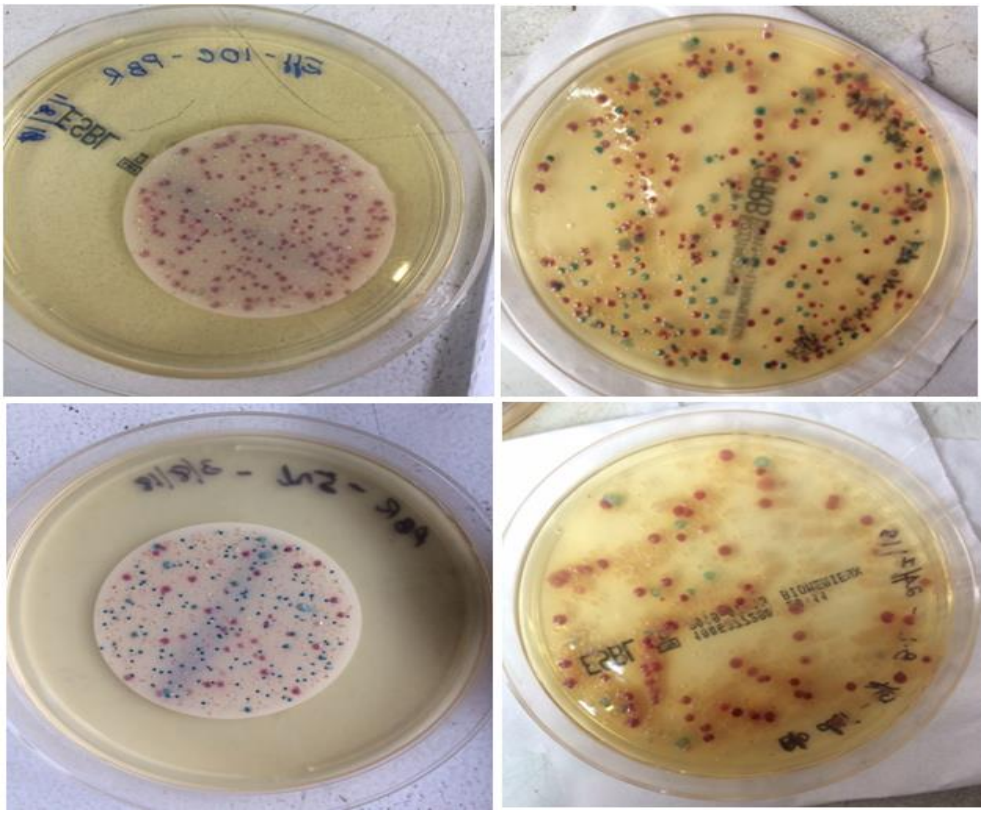

Figure 20 Bacterial colonies formed on agar medium

3.4 Quantitative microbial risk assessment

3.4.1 Hazard identification

In this section, the possible hazards associated with Enterotoxigenic E.coli (ETEC O55) will be discussed. The drain mainly carries domestic sewage, runoff and poses potential risks from faecal coliforms which are indicator organisms for many other species. Enterobacteriaceae which includes Escherichia coli is a reference pathogen with a large diversity varying from intestinal commensal strains to intestinal pathogenic strains and is the leading cause of societal infection (Liang et al., 2017). Reusing this water after improper treatment can compromise human and environmental health. The main transmission route for E. coli is by faecal-oral pathways. As per epidemiological studies, reuse of water relates to several health impacts like gastrointestinal illness, eye, ear, nose, throat infections, skin irritations (Kay et al.,2015). Of many groups of E.coli, Enterotoxigenic E.coli is known for producing certain toxins which stimulate intestinal linings causing them to secrete excess fluid, and thus causing diarrhea E. coli (ETEC), 2014). Virulence factors such as enterotoxins and colonization separate ETEC from other forms of diarrheagenic E.coli (Qadri et al., 2005).

Being regarded as traveller's diarrhea, it is a major cause of illness, especially among children in developing countries like India (Enterotoxigenic E. coli (ETEC), 2014). ETEC being the second leading cause of death in children below 5 years has caused over 200 million cases of diarrhea and over 0.38 million deaths, of mostly children in developing countries (WHO, 2010). The infection is associated with malnutrition, stunned growth and cognitive defects in children. Of all major etiological agents including ETEC, rotavirus, Vibrio Cholerae and Shigella spp, ETEC is difficult to recognize, hence is not regarded as a major cause of infant diarrhea or cholera like disease among all age groups (Qadri et al., 2005).

The main transmission route for ETEC O55 is through food and water contaminated with human or animal feces. Infection results in excess watery diarrhea and abdominal cramping. Other symptoms include fever, vomiting, headache, muscle cramps and bloating (Enterotoxigenic E. coli (ETEC), 2017). Major threat from ETEC O55 is due to development of high resistance to major antibiotics including quinolone and fluoroquinolone groups (ciprofloxacin) which increases the severity of the disease. Qadri et al., (2005) reported the presence of ciprofloxacin resistant ETEC O55 in water sources in Bangladesh and India. 71.4% of ETEC strains were resistant to ampicillin, 57.1% to chloramphenicol and 7.1% to ciprofloxacin (Nguyen et al., 2005). Emergence of resistant-enteropathogens indicates a situation where available strong antibiotics are ineffective.

3.4.2 Exposure Assessment

Exposure pathway is identified from source of ETEC O55 to its exposure to the population. As per the study conducted by (Anand et al., 2004), the average sewage discharge from Barapullah drain is 156 MLD from approximately 520,000 inhabitants living around the drain. The population exposed to these unhygienic practises include all age groups, adults and children being the larger part. On using conventionally treated drain water for households, irrigation and industries, the threat to population from ETEC cannot be controlled (Barancheshme and Munir,2018).

The treated drain water through AnMBR, photobioreactor/constructed wetlands can be safely reused, provided it complies with water safety standards. A good quality reuse water would gain social acceptance and meet water demand. For this study, the treated water is considered to be used for irrigation. The farmers irrigating the lands manually are at a higher risk from exposure to various bacterial species. Agricultural workers exposed to untreated wastewater witness skin diseases such as dermatitis and rashes. In urban and semi-urban areas

of developing regions, farmers are dependent on wastewater for irrigation in spite of knowing the significant contamination which contributes to a large burden of water borne diseases.

Exposure pathway includes unintentional ingestion by farmers during spray, drip and surface irrigation. This exposure can spread among workers and their families due to lack of hygienic practices. In the case of high level of mechanization, exposure can vary based on irrigation methods like plating, weeding, ponding. Runoff of wastewater on to the adjacent lands can lead to exposure during playing, commuting (Dickin et al., 2016). Children in particular are vulnerable by playing in contaminated areas, which rises the frequency and duration of exposure. To reuse the treated drain water or any wastewater for irrigation, the bacterial concentration and log reduction values must comply with the prescribed standards. The exposure due to consumption of crops grown using contaminated water is not considered in this study. Table 2 shows the log reduction values and required concentration for safe reuse for E. coli. As the probability of infection is dependent on volume of ingested water, the ingestion volume is considered between 1 ml to 25ml considering the above-mentioned exposure pathways.

Table 2: Log reduction values of E coli for irrigation (Shuval et al., 1997)

Type of irrigation	Option (Figure 2.1)	Required pathogen reduction by treatment (log units)	Verification monitoring level (*E. coli* per 100 ml)	Notes
Unrestricted	A	4	$\leq 10^3$	Root crops
	B	3	$\leq 10^4$	Leaf crops
	C	2	$\leq 10^5$	Drip irrigation of high-growing crops
	D	4	$\leq 10^3$	Drip irrigation of low-growing crops
	E	6 or 7	$\leq 10^1$ or $\leq 10^0$	Verification level depends on the requirements of the local regulatory agency[b]
Restricted	F	3	$\leq 10^4$	Labour-intensive agriculture (protective of adults and children under 15 years of age)
	G	2	$\leq 10^5$	Highly mechanized agriculture
	H	0.5	$\leq 10^6$	Pathogen removal in a septic tank

[a] "Verification monitoring" refers to what has previously been referred to as "effluent standards" or "effluent guideline" levels.

[b] For example, for secondary treatment, filtration and disinfection: five-day biochemical oxygen demand (BOD_5), <10 mg/l; turbidity, <2 nephelometric turbidity units (NTU); chlorine residual, 1 mg/l; pH, 6–9; and faecal coliforms, not detectable in 100 ml (State of California, 2001).

3.4.3 Dose response

In this section, β-Poisson model is used to relate infected and unaffected individuals with governed dose and the probability of infection. The β-Poisson model considers infection and survival probabilities of the infected person. The ETEC O55 concentration was not measured directly in the drain due to unforeseen complications in the field. The concentration of E.coli in the drain was adopted from a study conducted by Bruno Bicudo (unpublished raw data). An average E.coli concentration of 1.58×10^8 CFU/L was obtained. However, the obtained E.coli concentration cannot be entirely pathogenic. Based on a study by Harada et al., (2018) in Bangladesh, 18.6% of the total E.coli isolates obtained from toilet wastewater was regarded as pathogenic, of which 16.3% included Stlb-positive ETEC O55, which was the dominant pathotype. An average ETEC O55 concentration of 1.06×10^7 CFU/L was obtained by applying the above relation. Figure 21 shows the concentration range of E.coli, ETEC O55 in the drain.

The estimated concentration was applied to dose response model to estimate the probability of infection. Probability of infection is calculated using β-Poisson model as given in equation 4.

$$P_{inf} = 1 - [1 + \frac{d}{N50}(2^{1/\alpha} - 1)]^{-\alpha} \qquad \text{- Equation 4}$$

Where,

P_{inf} = Probability of infection based on the dose of pathogen consumed in a single exposure

N50 = Dose at which 50% of the population is expected to be affected

α = pathogen specific equation parameter

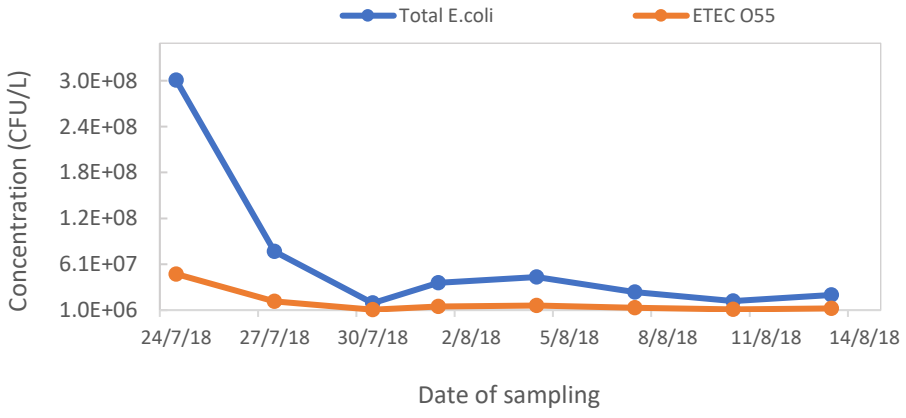

Figure 21 Estimated E.coli, ETEC O55 concentration in the drain

3.4.4 Risk characterization

Risk characterization involves combining the data of previous steps of hazard identification, exposure assessment and dose-response relationship to compare the magnitude of risk to the current admissible health targets (Maimom et al., 2010). Quantitative measurement of risk includes the measure of probability of risk and the impact of the risk.

3.4.4.1 Probability of infection

The dose response was calculated for ETEC O55 using the beta-Poisson model shown in equation 4, with $\alpha = 8.70x10^{-2}$ and $N50 = 2.05x10^{5}$ (Enger, 2011) and dose was calculated using d = concentration * volume ingested. Volume ingested was considered from 1ml - 25ml. The figures 22,23,24 show the probability of infection of ETEC O55 from direct exposure to raw drain water and treated effluents from PBR, CWs and AnMBR respectively as a function of ingestion volume. The graphs show a notable difference in the probability of infection from the influent and effluent of treatment units. The effluent from PBR resulted in least probability of infection of less than 10% compared to AnMBR which had Pinf below 15-18%. The wetland effluents resulted in a P_{inf} up to 15-22% which is slightly higher than PBR and AnMBR treated water.

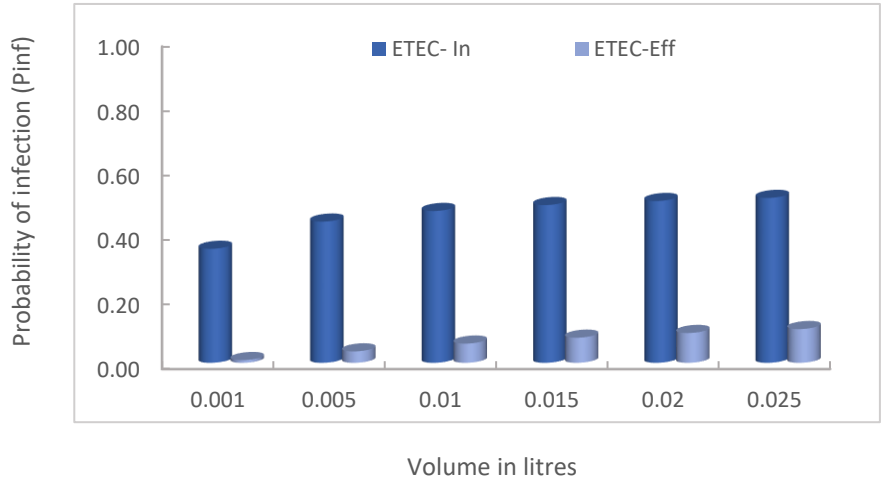

Figure 22 Probability of infection from ETEC O55 in influent and PBR effluent

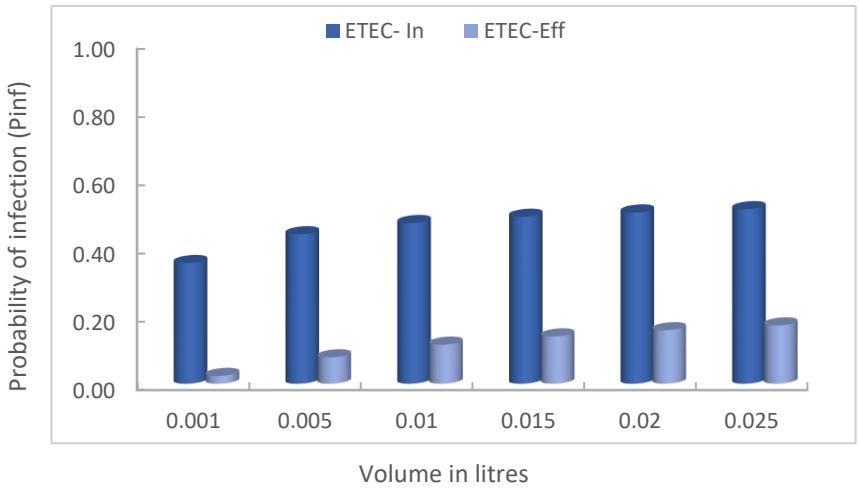

Figure 23 Probability of infection from ETEC O55 in influent and AnMBR effluent

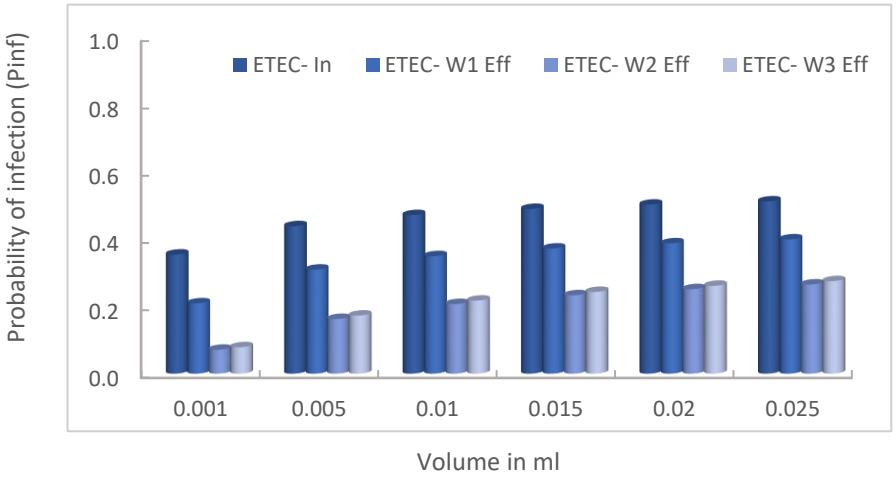

Figure 24 Probability of infection from ETEC O55 in influent and wetland effluent

3.4.4.2 Probability of illness

After estimating the probability of infection, the next step is to estimate the probability of illness. The probability of illness of an individual is calculated considering that the individual is infected (WHO, 2016). Qadri et al., (2005) reported risk of diarrheal disease given infection to be in the range of 80-100%. Hence risk of 80% is chosen for the calculation. The model shown in equation 5 is used for estimating P_{ill} (Thomas et al., (2015).

$$\text{Pill} = P_{inf,y} \times P_{ill\ I\ inf} \qquad \text{- Equation 5}$$

Where,

$P_{inf,y}$ = Annual Probability of infection

$P_{ill\ I\ inf}$ = Risk of diarrheal disease given infection

Annual probability of infection is calculated using the formula shown in equation 6. Overall annual exposure is taken as 8 days (2times/year x 4days/time = 8days/year) (Hora et al., 2017).

$$P_{inf,y} = 1 - (1 - P_{inf})^t \quad \text{- Equation 6}$$

Where,

P_{inf} = Probability of infection

t = number of exposures in one year

Figures 25,26 and 27 show the probability of illness from ETEC O55 in influent and effluent of PBR, AnMBR and CWs respectively. A significant difference is observed from influent to effluent, with PBR effluent having the least and CW effluents having the highest risk of illness.

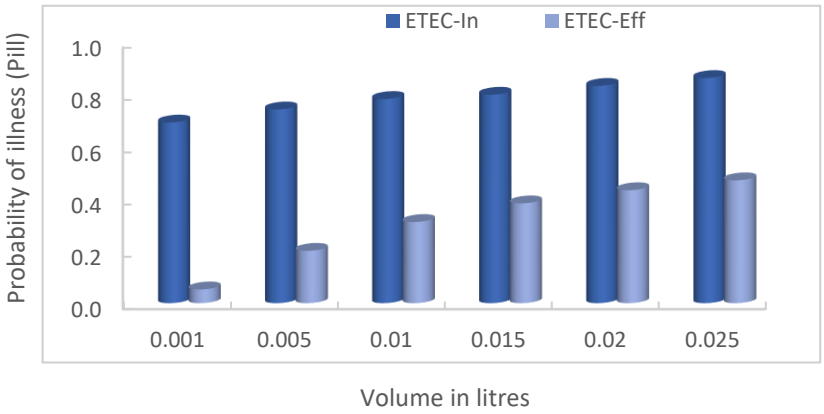

Figure 25 Probability of illness from ETEC in influent and PBR effluent

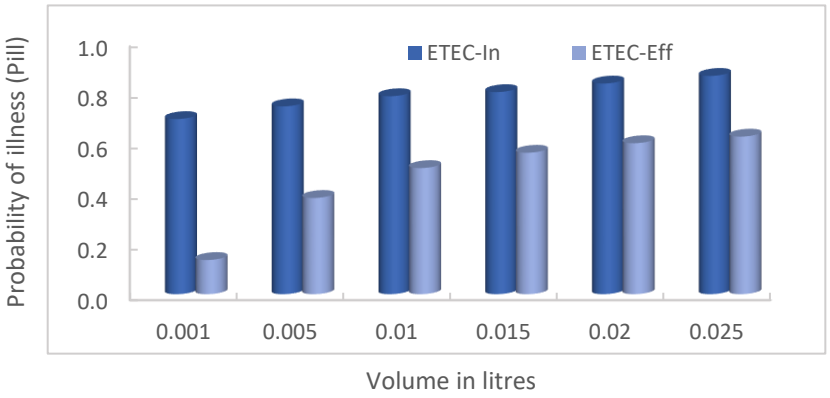

Figure 26 Probability of illness from ETEC in influent and AnMBR effluent

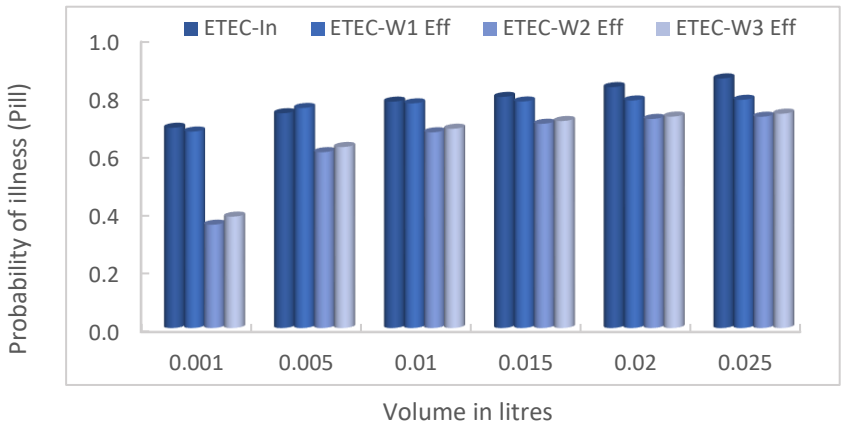

Figure 27 Probability of illness from ETEC in influent and CW effluent

Based on probability of illness, the result of disease cases was transformed to DALY loss per person per year (pppy). Disability Adjusted Life Years (DALYs) is a recommended metric in estimating the gap between population's health status and an ideal level of health and survival. DALY uses the term disability to relate to any acute or chronic illness that reduces the status of physical or mental health in a short or long term (Chen et al.,2015). As per WHO, the recommended health target of any water intervention is 10^{-6} DALYs per person per year (World Health Organization, 2011). The methodology proposed by Thomas et al.,(2015) was followed for the DALY calculation. Equation 7 was used for further estimation.

$$DALY = P_{ill} * \text{mdb} * fs \qquad \text{- Equation 7}$$

Where,

P_{ill} = Probability of illness

mdb = maximum disease burden

fs = Susceptible fraction.

A maximum disease burden of 0.601 and a susceptible fraction of 10% was considered following the study by Thomas et al., (2015). Table 3 shows DALY values for influent and treated effluents. Raw influent attributed to 0.048 DALYs pppy is reduced to 0.018 DALYs pppy for PBR effluent, 0.028 DALYs pppy for AnMBR effluent and 0.038-0.046 DALYs pppy for CW effluents. PBR effluent accounted for a high reduction in the total DALYs of upto 63%, followed by AnMBR-41.6% and CWs-12.5%.

Table 3 DALYs pppy for influent and treated effluents in order of rank

Water source	**DALY(pppy)**
Raw influent	0.048
CW1 effluent	0.046
CW3 effluent	0.039
CW2 effluent	0.038
AnMBR effluent	0.028
PBR effluent	0.018

4.Discussion

The widespread use of antibiotics has resulted in the development of resistant microorganisms and their genetic elements which are pervasive in humans, food, animals and environment. Itwas declared by the World Health organisation in their 2014 Global report on surveillance that “antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, virus, parasites and fungi” (WHO, 2014). Specifically, wastewater acts as a breeding ground for the growth, fate and transport of these bacteria and genes which hampers the subsequent efforts to reuse water. Effective treatment processes which perform better than the conventional activated sludge process is needed to remove ARBs from the wastewater before discharging. Though this study focussed on ARBs, specifically antibiotic resistant E. coli there is a wide potential to expand this study to antibiotic resistant genes and other species.

This study aimed at assessing the concentrations of Extended spectrum beta-lactamase and carbapenemase producing E. coli in the Barapullah drain. The drain is a reservoir of antibiotic resistant bacteria and fostered high concentration of ESBL-E.coli and CRE-E.coli ranging from 10^6 to 10^7 CFU/L. The focus was only on these enzymes producing E. coli because of its scope to cause potential hazards to the exposed population. Furthermore, assessment of Enterobacteriaceae species including Klebsiella pneumonia and E coli is important to estimate the potential risks from other infections.

Membrane bioreactor was considered as an effective treatment unit for the removal of ARBs. High removal efficiency of 99.82% for ESBL-E.coli and 99.69% for CRE-E.coli was achieved in this study. A prior study by Harb et al., (2017) concluded that the concentration of ARBs in a full-scale membrane bioreactor (Aerobic/Anaerobic) permeate stream was 1-3 log units less than concentration achieved in activated sludge process making it an effective treatment choice (Harb and Hong, 2017). The log reduction values achieved in this study with a single filter AnMBR was 3 for ESBL-E.coli and 2.7 for CRE-E.coli. In future studies, additional filters could be installed in the filter module to achieve even higher removal efficiency. Combining micro-aeration with anaerobic condition boosted the performance of ARB removal. The LRVs were 1- 1.5 log units higher than in the anaerobic condition. Further experiments could be carried out with varying oxygen concentrations to check the decay rates of the cells at different oxygen concentrations.

The sludge balance indicated the flow of ARBs in the influent and effluent. A total concentration of $2.01x10^7$ and $6.20x10^7$ of ESBL-E.coli and CRE-E.coli was lost either by inactivation during micro-aeration or decay of cells. A longer evaluation of the reactor would give a better understanding on sludge balance as there would be excess fouling on the filter and in the CSTR.

Phycoremediation in a photobioreactor proved to be an effective treatment system. A log reduction of 2.9 for ESBL-E.coli and 3.2 for CRE-E.coli was achieved. This reduction in ARB concentration might be an effect of the antibacterial substance released by specific algal species and exposure to sunlight which is proved to be an important bactericidal agent. PBR resulted in a higher log reduction of CRE-E.coli than ESBL-E.coli, when compared with AnMBR and CW. A detailed study on specific algal species in the PBR would have benefited a better understanding of antibacterial substances and ARB removal mechanism. Higher pH value of 9 and increased oxygen level due to algae improved ARB inactivation at a 48h HRT. The performance assessment with longer HRTs can be considered for future study.

Constructed wetlands are efficient and economical treatment systems for wastewater. The system uses a combination of plants and microorganisms to further degrade and remove emerging pollutants. In this study, the performance of CWs with Rose Periwinkle, Canna Lily and Cana Pretoria was investigated. The log reduction obtained in this system for ESBL-E.coli and CRE-E.coli was 2.1 and 1.9 for Rose Periwinkle, 2.1 and 2 for Cana Lily and 2.3 and 2.2 for Cana Pretoria respectively. Due to lack of information on the reduction of ESBL-E.coli and CRE-E.coli in CWs, the reduction in this analysis was based on E. coli. The LRVs obtained in this study fit through the values measured by Sidrach-Cardona and Becares (2013), where a log reduction of 1.9 – 2.6 was achieved for E. coli. A total removal efficiency of 95.2 – 99.9% was achieved in this study which was better than the study conducted by S.Dires et al., 2018 for hospital wastewater, where ARB abundance reduced by 80.8-93.2% in planted wetlands. A pH of 7-7.5 was observed in the wetlands. The growth of Cana Pretoria was better than the other two plants which relates to the study conducted by Konnerup et al., (2009). They observed that Cana had higher growth rate and six times higher biomass production than Heliconia. This agreed with the results obtained in this study as the performance of wetland 3 was better compared to other 2 wetlands.

QMRA

The health risk assessment of Barapullah drain water in New Delhi, India was conducted following the guidelines of Quantitative microbial risk assessment. A microbial risk assessment was necessary for treated drain water as it would be reused which involves human contact. This study was focused on Enterotoxigenic E.coli (ETEC O55), a harmful pathogenic group of E.coli due to its potential to cause various hazards to humans exposed to contaminated water. The evaluation focused only on parts of India as the asymptomatic rates differ with region and the risk of infection from ETEC depends if the country is developing or developed (Havelaar et al., 2009) (Adedayo and Kirkpatrick, 2008). This study was addressed to the population surrounding the Barapullah drain area, where adults could be exposed through irrigation, gardening and domestic purposes and children through playing in contaminated areas (soil).

Hazard identification was done for Enterotoxigenic E.coli (ETEC O55) as it was the most relevant pathogen to be evaluated in waters polluted with human and animal feces. Evaluation of other pathogens is crucial to have a complete overview of the potential hazards. For future studies, assessment of resistant genes, Enterococcus, Enterobacter and Pseudomonas species would be interesting as their antibiotic-resistant isolates prevail even after post treatment, especially in chlorinated effluent (Al-Jassim et al., 2015). The risk from these emerging pollutants might be enhanced due to increasing water crisis and water reuse practices. The dose response was calculated using beta Poisson model as they are used to extrapolate experimental dose response data at low doses (Teunis and Havelaar, 2000). Probability of infection and illness from ETEC O55 was estimated. The P_{inf} and P_{ill} reduced drastically for the effluent of all treatment units which followed the reduction in concentration. The P_{inf} and P_{ill} from PBR effluent was the least and highest for CW effluents. Hence, it can be inferred that antimicrobial substances produced by algae might be one of the main reason causing drastic reduction of ARB concentrations and leading to low probability of infection and illness. The probability of death decreased from 0.048 DALYs pppy for raw influent to 0.018 DALYs pppy for PBR effluent, 0.028 DALYs pppy for AnMBR effluent and 0.038-0.046 DALYs pppy for CW effluents. Study by Shuval et al., (1997) reported a monitoring level of E. coli concentration less that 10^3-10^5 for unrestricted irrigation and 10^4-10^5 for restricted irrigation. The concentrations obtained in this study, varied from 10^2-10^4 which complied with these monitoring levels. Hence it is safe to reuse the treated water for gardening, irrigation. The estimated risk of gastrointestinal illness from enterococci was higher than 500/100ml which

indicated high level of illness transmission (WHO, 2013). The concentration obtained in this study after post treatment was in the range of 10^2-10^3 per 100ml which could be classified under a risk higher than 10%. Hence it is not recommended for direct consumption or washing purposes. The crops grown with this water must be washed with clean water to ensure safe consumption.

5. Conclusion and Recommendations

Barapullah drain served as a reservoir of antibiotic resistant bacteria. Concentration of ESBL-E.coli and carbapenem resistant E.coli was observed to be $8x10^6$ CFU/L and $1.8x10^7$ CFU/L respectively. Assessment of emerging technologies like Anaerobic membrane bioreactor, photobioreactor and constructed wetlands resulted in effective removal of these antibiotic resistant bacteria. Average log reduction values of 3 and 2.7 for anaerobic membrane bioreactor, 2.8 and 3.2 for photobioreactor, 2 and 1.8 for Rose Periwinkle, 2.1 and 2 for Cana Lily and 2.2 and 2.1 for Cana Pretoria in constructed wetlands were achieved for ESBL E.coli and CRE E.coli respectively. Inclusion of micro-aeration in AnMBR improved the removal efficiency by 46% for ESBl-E.coli and 36% for CRE-E.coli. Sludge balance in AnMBR showed a higher concentration of ARBs in the CSTR and filter unit. The use of mineral wool in wetlands enhanced adsorption of ARBs on its surface due to its high surface area. Bacterial reduction in PBR was promoted by algal species which released antibacterial substances and through sunlight which proved to be bactericidal. Quantitative microbial risk assessment of ETEC O55 allowed the evaluation and management of microbial risks, caused during reuse to avoid disease burden. Upon treatment the probability of infection dropped from 40-50% to below 15% for PBR, below 18% for AnMBR and between 15-25% for CWs. The probability of illness was the lowest in PBR effluent and highest in the CW effluents. PBR effluent accounted for a high reduction in the total DALYs of upto 63%, followed by AnMBR-41.6% and CWs-12.5%. The monitoring level of E.coli was within the range of 10^3-10^5 for unrestricted irrigation and 10^4-10^5 for restricted irrigation as declared by WHO which makes the treated drain water safe for reuse in irrigation. In terms of comparison, PBR and AnMBR performed exceptionally well. The performance of CWs could be improved by proper maintenance, hence can be considered as a feasible post treatment option.

Recommendations

Based on the discussion above, few recommendations are given for this study:

- Performing experiments in clean laboratory environments to testify these results.
- Maintenance of reactors in good working condition to ensure stable performance.
- Investigating microbial contamination in the mineral wool for a microbial balance in the system.

- Investigating algal species in PBR to improve performance and better understanding of ARB removal mechanisms.
- Paying more attention to AnMBR membrane fouling.

Study experience

The above study was conducted for an additional thesis course work as a part of my graduation study. It was a great pleasure to work in my own country and apply the knowledge gained during my Master's in TU Delft. I would like to thank my supervisors Bruno Bicudo Perez and Doris Van Halem for constantly supporting and guiding me throughout the study. This project gave me an insight to work in a real field and handle practical problems. As it was my first field study, it was a great learning experience. Constant support and help from Mr Susant Kumar Padhi at TERI and other colleagues working at the pilot plant helped me overcome any problems faced in the site. Mr Theo Den Bieman was very kind and helpful in providing all the necessary things needed for my experiments.

Few facilities in the site could have been better for a better evaluation. It was slightly difficult to procure materials like autoclave, microbial plates needed for the assay. As my study was concerned with microbial analysis, sampling, plating and handling was difficult due to lack of clean space and environment. Though the site was cleaned once a day, the space wasn't clean enough to ensure no interference of contamination. I feel the results would have been much better if the same analysis was performed in a laboratory environment. The reactors at times offered varied results due to sudden power cuts, as there were no invertors to run the reactors during power cut.

Overall, it was great to be a part of this project and I thoroughly enjoyed working.

References

Adedayo, O., & Kirkpatrick, B. D. (2008). Campylobacter jejuni infections: update on presentation, diagnosis, and management. *Hospital Physician*, *44*(7), 9.

Ahammad, Z. S., Sreekrishnan, T. R., Hands, C. L., Knapp, C. W., & Graham, D. W. (2014). Increased waterborne bla NDM-1 resistance gene abundances associated with seasonal human pilgrimages to the Upper Ganges River. *Environmental science & technology*, *48*(5), 3014-3020

Akiba, M., Sekizuka, T., Yamashita, A., Kuroda, M., Fujii, Y., Murata, M., ... & Subramanian, K. (2016). Distribution and relationships of antimicrobial resistance determinants among extended-spectrum-cephalosporin-resistant or carbapenem-resistant Escherichia coli isolates from rivers and sewage treatment plants in India. *Antimicrobial agents and chemotherapy*, *60*(5), 2972-2980.

Al-Jassim, N., Ansari, M. I., Harb, M., & Hong, P. Y. (2015). Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: is the treated wastewater safe to reuse for agricultural irrigation? *Water research*, *73*, 277-290.

Anand, C., Akolkar, P., & Chakrabarti, R. (2006). Bacteriological water quality status of river Yamuna in Delhi. *Journal of environmental biology*, *27*(1), 97-101

Antibiotic / Antimicrobial Resistance. (2018, March 29). Retrieved July 11, 2018, from Centres for Disease control and Prevention: https://www.cdc.gov/drugresistance/index.html

Barancheshme, F., & Munir, M. (2018). Strategies to Combat Antibiotic Resistance in the Wastewater Treatment Plants. *Frontiers in microbiology*, *8*, 2603.

Barbagallo, S., Cirelli, G. L., Consoli, S., Licciardello, F., Marzo, A., & Toscano, A. (2012). Analysis of treated wastewater reuse potential for irrigation in Sicily. *Water Science and Technology*, *65*(11), 2024-2033.

Batty, L. C., Baker, A. J. M., Wheeler, B. D., & Curtis, C. D. (2000). The effect of pH and plaque on the uptake of Cu and Mn in Phragmites australis (Cav.) Trin ex. Steudel. *Annals of Botany*, *86*(3), 647-653.

Bhaduri, A. (2016, July 5). *Once a drain, now a sewer*. Retrieved August 23, 2018, from India water portal: http://www.indiawaterportal.org/articles/once-drain-now-sewer

Bhaduri, A. (2017, October 17). How the Barapullah drain turned into a sewer. *Civic Responsibility*. Retrieved from https://yourstory.com/2017/10/barapullah-drain-turned-sewer/

Board, C. P. (2015, October 9). *Directions issued under Section 5 of EPA Act, 1986 regarding Treatment and Utilization of Sewage for Restoration of Water Quality of River to Metropolitan Cities and States/UT Capitals*. Retrieved July 13, 2018, from India Environmental Portal: http://www.indiaenvironmentportal.org.in/content/420116/directions-issued-under-section-5-of-epa-act-1986-regarding-treatment-and-utilization-of-sewage-for-restoration-of-water-quality-of-river-to-metropolitan-cities-and-statesut-capitals/

Bôto, M., Almeida, C. M. R., & Mucha, A. P. (2016). Potential of constructed wetlands for removal of antibiotics from saline aquaculture effluents. *Water*, *8*(10), 465.

Burke, J. (2015, April 7). *Half of India's rivers are polluted, says government report.* Retrieved July 16, 2018, from The Guardian: https://www.theguardian.com/world/2015/apr/07/half-india-rivers-polluted-new-government-report

Cosgrove, W. J., & Loucks, D. P. (2015). Water management: Current and future challenges and research directions. *Water Resources Research*, *51*(6), 4823-4839

Cheng, H., & Hong, P. Y. (2017). Removal of antibiotic-resistant bacteria and antibiotic resistance genes affected by varying degrees of fouling on anaerobic microfiltration membranes. *Environmental science & technology*, *51*(21), 12200-12209.

Dickin, S. K., Schuster-Wallace, C. J., Qadir, M., & Pizzacalla, K. (2016). A review of health risks and pathways for exposure to wastewater use in agriculture. *Environmental health perspectives*, *124*(7), 900-909.

Dires, S., Birhanu, T., Ambelu, A., & Sahilu, G. (2018). Antibiotic resistant bacteria removal of subsurface flow constructed wetlands from hospital wastewater. *Journal of Environmental Chemical Engineering*, *6*(4), 4265-4272.

DTE. (2018, March 27). *India's antibiotics consumption shot up by 103% since 2000: study*. Retrieved July 18, 2018, from Down to Earth: https://www.downtoearth.org.in/news/india-s-antibiotics-consumption-shot-by-103-since-2000-study-60037

Enerhall, C., & Stenmark, E. (2012). Disc Filters to Reduce Wastewater Pathogen Levels in Raw Water Sources.

Enger, K. S. (2011, November 11). *Dose response models for Pathogenic Escherichia coli*. Retrieved November 9, 2018, from QMRA wiki: http://qmrawiki.canr.msu.edu/index.php/Dose_response_models_for_Pathogenic_Escherichia_coli

Enterotoxigenic E. coli (ETEC). (2014, December 1). Retrieved November 16, 2018, from Centres for Disease Control and Prevention: https://www.cdc.gov/ecoli/etec.html

Enterotoxigenic E. coli (ETEC). (2017). Retrieved November 16, 2018, from Global Health Primer: http://www.globalhealthprimer.emory.edu/diseases/enterotoxigenic-e-coli.html

Fewtrell, L., & Kay, D. (2015). Recreational water and infection: a review of recent findings. *Current environmental health reports*, *2*(1), 85-94.

Franz, E., Veenman, C., Van Hoek, A. H., de Roda Husman, A., & Blaak, H. (2015). Pathogenic Escherichia coli producing Extended-Spectrum β-Lactamases isolated from surface water and wastewater. *Scientific reports*, *5*, 14372.

Gandra, S., Joshi, J., Trett, A., & Sankhil, L. A. (2017). Scoping report on antimicrobial resistance in India. Washington, DC: Center for Disease Dynamics. *Economics & Policy*.

García, J., Vivar, J., Aromir, M., & Mujeriego, R. (2003). Role of hydraulic retention time and granular medium in microbial removal in tertiary treatment reed beds. *Water Research*, *37*(11), 2645-2653.

Green, M. B., Griffin, P., Seabridge, J. K., & Dhobie, D. (1997). Removal of bacteria in subsurface flow wetlands. *Water science and technology*, *35*(5), 109-116.

Harb, M., & Hong, P. Y. (2017). Molecular-based detection of potentially pathogenic bacteria in membrane bioreactor (MBR) systems treating municipal wastewater: a case study. *Environmental Science and Pollution Research*, *24*(6), 5370-5380.

Harada, H., Fujimori, Y., Gomi, R., Ahsan, M. N., Fujii, S., Sakai, A., & Matsuda, T. (2018). Pathotyping of Escherichia coli isolated from community toilet wastewater and stored drinking water in a slum in Bangladesh. *Letters in applied microbiology*, *66*(6), 542-548.

Hamadani, J. D., Tofail, F., Huda, S. N., Alam, D. S., Ridout, D. A., Attanasio, O., & Grantham-McGregor, S. M. (2014). Cognitive deficit and poverty in the first 5 years of childhood in Bangladesh. *Pediatrics*, peds-2014.

Hazra, M., Avishek, K., & Pathak, G. (2011). Developing an artificial wetland system for wastewater treatment: a designing perspective. *International Journal of Environmental Protection*.

Havelaar, A. H., van Pelt, W., Ang, C. W., Wagenaar, J. A., van Putten, J. P., Gross, U., & Newell, D. G. (2009). Immunity to Campylobacter: its role in risk assessment and epidemiology. *Critical reviews in microbiology*, *35*(1), 1-22.

Hazra, M., Avishek, K., & Pathak, G. (2011). Developing an artificial wetland system for wastewater treatment: a designing perspective. *International Journal of Environmental Protection*.

India, C. o. (2011). *Houses, Household Amenities and Assets; House-listing and Housing Census Data Highlights – 2011*. Goverment of India.

Kappell, Anthony & Kimbell, Lee & D Seib, Matthew & E. Carey, Daniel & Choi, Melinda & Kalayil, Tino & Fujimoto, Masanori & H Zitomer, Daniel & Joseph McNamara, Patrick. (2018). Removal of Antibiotic Resistance Genes in an Anaerobic Membrane Bioreactor Treating Primary Clarifier Effluent at 20 ○ C. Environmental Science: Water Research & Technology. 10.1039/C8EW00270C.

Karathanasis, A. D., Potter, C. L., & Coyne, M. S. (2003). Vegetation effects on fecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. *Ecological engineering*, *20*(2), 157-169.

Kipasika, H. J., Buza, J., Smith, W. A., & Njau, K. N. (2016). Removal capacity of faecal pathogens from wastewater by four wetland vegetation: Typha latifolia, Cyperus papyrus, Cyperus alternifolius and Phragmites australis. *African Journal of Microbiology Research*, *10*(19), 654-661.

Konnerup, D., Koottatep, T., & Brix, H. (2009). Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with Canna and Heliconia. *Ecological engineering*, *35*(2), 248-257.

Krustok, I. (2016). *Microbiological analysis of municipal wastewater treating photobioreactors* (Doctoral dissertation, Mälardalen University).

Kumar, A., & Das, K. C. (2014). Drinking water and sanitation facility in India and its linkages with diarrhoea among children under five: evidences from recent data. *Int J Humanit Soc Sci Invent*, *3*, 50-60.

Kumar, G. S., Kar, S. S., & Jain, A. (2011). Health and environmental sanitation in India: Issues for prioritizing control strategies. *Indian journal of occupational and environmental medicine*, *15*(3), 93.

Liang, W. J., Liu, H. Y., Duan, G. C., Zhao, Y. X., Chen, S. Y., Yang, H. Y., & Xi, Y. L. (2018). Emergence and mechanism of carbapenem-resistant Escherichia coli in Henan, China, 2014. *Journal of infection and public health*, *11*(3), 347-351.

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., ... & Black, R. E. (2015). Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *The Lancet*, *385*(9966), 430-440.

Logan, L. K., & Weinstein, R. A. (2017). The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *The Journal of infectious diseases*, *215*(suppl_1), S28-S36.

Maimon, A., Tal, A., Friedler, E., & Gross, A. (2010). Safe on-site reuse of greywater for irrigation-a critical review of current guidelines. *Environmental science & technology*, *44*(9), 3213-3220.

Malik, D., Singh, S., Thakur, J., Singh, R. K., Kaur, A., & Nijhawan, S. (2014). Heavy metal pollution of the Yamuna River: An introspection. *Int. J. Curr. Microbiol. App. Sci*, *3*(10), 856-863.

Mantilla-Calderon, D., & Hong, P. Y. (2017). Fate and persistence of a pathogenic NDM-1-positive Escherichia coli strain in anaerobic and aerobic sludge microcosms. *Applied and environmental microbiology*, AEM-00640.

Mariappan, S., Sekar, U., & Kamalanathan, A. (2017). Carbapenemase-producing Enterobacteriaceae: risk factors for infection and impact of resistance on outcomes. *International Journal of Applied and Basic Medical Research*, *7*(1), 32.

Mayer, B. T., Koopman, J. S., Ionides, E. L., Pujol, J. M., & Eisenberg, J. N. (2011). A dynamic dose–response model to account for exposure patterns in risk assessment: a case study in inhalation anthrax. *Journal of The Royal Society Interface*, *8*(57), 506-517.

Mezzari, M. P., Prandini, J. M., Kich, J. D., & Da Silva, M. L. B. (2017). Elimination of Antibiotic Multi-Resistant Salmonella Typhimurium from Swine Wastewater by Microalgae-Induced Antibacterial Mechanisms. *J Bioremediat Biodegrad*, *8*(379), 2.

Misra, A. K. (2010). A river about to die: Yamuna. *Journal of water resource and protection*, *2*(05), 489.

Nguyen, T. V., Van Le, P., Le, C. H., & Weintraub, A. (2005). Antibiotic resistance in diarrheagenic Escherichia coli and Shigella strains isolated from children in Hanoi, Vietnam. *Antimicrobial Agents and Chemotherapy*, *49*(2), 816-819.

Pathak, S. P., & Gopal, K. (2008). Prevalence of bacterial contamination with antibiotic resistant and enterotoxigenic fecal coliforms in treated drinking water. *Journal of Toxicology and Environmental Health, Part A*, *71*(7), 427-433.

Qadri, F., Svennerholm, A. M., Faruque, A. S. G., & Sack, R. B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical microbiology reviews*, *18*(3), 465-483.

Rao, S. P., Rama, P. S., Gurushanthappa, V., Manipura, R., & Srinivasan, K. (2014). Extended-spectrum beta-lactamases producing Escherichia coli and Klebsiella pneumoniae: A multi-centric study across Karnataka. *Journal of laboratory physicians*, *6*(1), 7.

Reinthaler, F. F., Posch, J., Feierl, G., Wüst, G., Haas, D., Ruckenbauer, G., ... & Marth, E. (2003). Antibiotic resistance of E. coli in sewage and sludge. *Water research*, *37*(8), 1685-1690.

Rendón, M. A., Saldaña, Z., Erdem, A. L., Monteiro-Neto, V., Vázquez, A., Kaper, J. B., ... & Girón, J. A. (2007). Commensal and pathogenic Escherichia coli use a common pilus adherence factor for epithelial cell colonization. *Proceedings of the National Academy of Sciences*, *104*(25), 10637-10642.

Sales-Ortells, H., & Medema, G. (2014). Screening-level microbial risk assessment of urban water locations: a tool for prioritization. *Environmental science & technology*, *48*(16), 9780-9789.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi journal of biological sciences*, *22*(1), 90-101.

Shuval, H., Lampert, Y., & Fattal, B. (1997). Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Water Science and Technology*, *35*(11-12), 15-20.

Skouteris, G., Hermosilla, D., López, P., Negro, C., & Blanco, Á. (2012). Anaerobic membrane bioreactors for wastewater treatment: a review. *Chemical Engineering Journal*, *198*, 138-148.

Sidrach-Cardona, R., & Bécares, E. (2013). Fecal indicator bacteria resistance to antibiotics in experimental constructed wetlands. *Ecological engineering*, *50*, 107-111

Singh, P. K. (2017). *Water, Sanitation and Hygiene: An essential ally in a superbug age.* World Health Organisation

Snow, D. A. (Ed.). (2001). *Plant engineer's reference book*. Elsevier.

Sumanth Gandra, Jyoti Joshi, Anna Trett, Anjana Sankhil Lamkang, and Ramanan Laxminarayan. 2017. Scoping Report on Antimicrobial Resistance in India. Washington, DC: Center for Disease Dynamics, Economics & Policy

Teunis, P. F. M., & Havelaar, A. H. (2000). The beta Poisson dose-response model is not a single-hit model. *Risk Analysis*, *20*(4), 513-520.

Thomas, K., McBean, E., Shantz, A., & Murphy, H. M. (2015). Comparing the microbial risks associated with household drinking water supplies used in peri-urban communities of Phnom Penh, Cambodia. *Journal of water and health*, *13*(1), 243-258.

UNESCO. (2012, May 22). *Delhi - A Heritage City*. Retrieved 11 8, 2018, from UNESCO: https://whc.unesco.org/en/tentativelists/5743/

Wankhade, Kavita & Balakrishnan, Krishnachandran & M.J, Vishnu. (2014). Urban Water and Sanitation: Sustaining Policy Momentum.

Weber, K. P., Gehder, M., & Legge, R. L. (2008). Assessment of changes in the microbial community of constructed wetland mesocosms in response to acid mine drainage exposure. *Water research*, *42*(1-2), 180-188.

World Health Organization. (2006). *Guidelines for the safe use of wastewater, excreta and greywater* (Vol. 1). World Health Organization.

World Health Organization. (2014). *Antimicrobial resistance: global report on surveillance*. World Health Organization.

WHO. (2018, February 5). *Antibiotic resistance*. Retrieved July 11, 2018, from World Health Organisation: http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

World Health Organization. (2003). *Guidelines for safe recreational water environments: Coastal and fresh waters* (Vol. 1). World Health Organization.

World Health Organization. (2010, December 8). *Enterotoxigenic "Escherichia coli" (ETEC)".* Retrieved November 15, 2018, from World Health Organisation: http://www.who.int/immunization/research/en/