Norwegian University of Life Sciences
Faculty of Environmental Sciences and Technology
Department of Mathematical Sciences and Technology (IMT)

Master Thesis 2016
30 credits

The Fate of Faecal Indicator Bacteria in Water

Elinga Silva

The Fate of Faecal Indicator Bacteria in Water

Acknowledgements

To my supervisor Ingun Tryland and co-supervisor Fasil Eregno, I am very grateful for the honour to participate in the research project and laboratory study. Your valuable understanding and patience were the reasons I manage to conclude the process of writing this thesis. So, thank you very much.

To the librarians at Sørhellinga library, Anne-Helen Ombustvedt and Atle Hårkau, thank you for all the help and services you provided.

Thank you for the many good writing tips and editing given by the advisers from Writing center help me to shape this thesis. Professor William Warner you have been an inspiration in writing better and better.

I express my gratitude to Norwegian University of Life Sciences (NMBU); I have been fortunate for the education. Thanks to all my lectures, that let me express my self in the classes and through my work. To my colleagues, your advices and support during the Master degree help me with success I see today.

To Lånekassen, your loan gave me a financial stability and decided the success in my studies.

I am very grateful to all my friends, whom I respect and consider, thank you very much for being there for me. Many thanks to Elizabeth Joy, your unique help and friendship transformed me. To Susana Leirdal, a good friend and confident, thanks.

To Elin Gjems Børresen, I would like to thank you for all the support you gave to me.

Many thanks to my parents, Simão António Da Silva e Maria Amélia Caetano, for their economic and emotional support. This gratitude also extends to my brothers, Etanelo, Osmany, and my sisters Valércia and Onima.

I am thankful to Børre-Erik Børresen for taking care of our children while I was busy writing.

To my lovely children, Henrik and Livanda Børresen, for your patience and understanding when mummy was busy. Even though, it was difficult for you to see at I was trying my best to be with you. I love you very much.

I also thank God for keeping sane and give me faith during this period.

Ås, 15 February 2016

Table of Contents

Abstract

Background:

The research experiments provide data on decay rate of faecal indicator bacteria in water. Such data are useful input to transport models to estimate the concentrations of faecal indicator bacteria at beaches in the days after sewage discharges. Sample data come from laboratory experiments where sewage was added to seawater at different environmental conditions. This research is a part of the project "Impact of changing weather patterns on bathing water and seafood quality from the Inner Oslofjord" financed by Regionale Forskningsfond Hovedstaden.

Purpose: This study is aimed to decide what was the best input to the models, to describe the decay when we put sewage into the Oslofjord when it is raining and how long time after this events can the beaches be used. Then main finding was to know how quickly they died by doing experiments and researching the literature.

Methods:

Hipsey provided a process-based model of microbial pollution used on fresh and seawaters. (Hipsey et al. 2008). According to the international requirements and standards for water quality, it is necessary to apply the appropriate indicators or parameters to a specific location of water. The raw wastewater from Drøbak waste treatment plant used as a typical sewage. We selected surface seawater with high biological activity and deep seawater (with probably lower biological activity) because the discharges from large CSOs are often at deep water.

Aim of preliminary experiments: Learn the methods for enumeration of FIB. The Collilert-18 rapid method enumerated *Escherichia coli (E. coli)* by using IDEXX tables (MPN/100mL). Positive colonies interpreted as (CFU/100mL) counted *Intestinal Enterococci (I. enterococci) using membrane filtration method*. The Main experiment aims in enumerate the faecal indicators bacteria (FIB) to evaluate the possibility of contaminants in surface and deep seawater. New suggestions were made to use faster and modern methods to get results at faster pace. The selected methods were Collilert-18 for *E. coli* and Enterolert-E for *I. enterococci.*

Results and discussion:

The results showed that 1-3 log reduction in fecal indicator bacteria from the day of collection to the 3rd to 5th day of the laboratory experiment. The deep seawater curve in *Escherichia coli*,

which was stored at 22^0C, revealed a much better fit to the first order linear model equation. While the *Intestinal enterococci,* at 22^0C deep seawater was the less fitted curve. The results also indicated that the decay rate (k_t) of the deep seawater and surface seawater are not significantly different when stored at same temperature, but the same types of seawater (deep or surface) showed a slightly difference when kept at different temperature. It indicates that the temperature is an importance factor in decay of fecal indicator bacteria. The graphs showed that the decay of FIB decreased after three days with similar behaviors as in the preliminary experiments but using deep and surface water at 4 and 22 degree Celsius (^{0}C). In general, the bacteria decay with temperature of 22^0C was much faster than in 4^0C, which shows the influence of temperature in fate of FIB. The results showed that *E. coli* in seawater declined faster than those in freshwater. And the same occurs with values for *I. enterococci.* Those decay rates show a difference for values between the seawater and freshwater just in 3 to 5 days at room temperature and this correlates with studies where FIB in seawater.

Conclusion:

Measurements of indicator bacteria *E. coli* and *I. enterococci* in water using Colilert-18, Enterolert-E and membrane Filtration methods to predict the decay rate of faecal indicator bacteria in water that was polluted with combined sewage water are acceptable and the linearity of the curves for the decay rate were a good fit with model equation. FIB decay rate results for only 1-2 days to reduce to 1 $\log_{10}$ at warmer temperature (*E. coli)* and more and this was considered useful to show the fate of the bacteria and more that 100 days in cold temperature (*I. enterococci)*. Regardless if whether or not we could have used more days to test the FIB. However, the faecal indicator bacteria may behave different at cold and harmer temperatures and also at different levels of water.

Acronyms

CFU – Colony Forming Units

CWA – Clean Water Act

EU – European Union

EPA – Environmental Protection Agency

FIB – Fecal Indicator Bacteria

ISO – International Standard Organization

NIVA – Norwegian Institute for Water research

NMBU – Norwegian University of Life Sciences

MPN – Most Probable Number

RWQC – Recreational Water Quality Criteria

WBDOs – Waterborne Diseases Outbreaks

WWTP – Wastewater Treatment Plant

WQNRW – Water Quality Norms for Recreational Water

List of Figures

List of Tables

1. Introduction

Clean and safe water is an important issue to all humans. Health problems and direct and indirect costs of using contaminated water are broad societal issues. Faecal contamination of bathing waters creates twelve billion dollars per year of total economic loss globally through impacts to the health of water users (Wade et al. 2006; WHO 2015). Freshwater and coastal water areas continue to be popular destinations for recreational activities and tourism. Still many risks involve the use of those waters because infectious diseases can be contracted there if fecal contaminants are present (Pond 2005; Shuval 2003)

Tourists expose themselves to a diversity of hazardous microorganisms that may cause waterborne diseases (Pond 2005). Types of exposures are swimming or other recreational activities and water sports. The costs associated with waterborne diseases are high. For example, in Orange County and Huntington Beach, California, the cumulative public health cost is approximately 3.3 million dollars per year (Dwight et al. 2005).

1.1 Global health challenges and Waterborne Diseases Outbreaks(Snozzi)

Developing countries are the most affected by waterborne disease outbreaks (WBDO) in the world. Yet, WBDO also targets developed countries, especially when strict hygienic standards are not maintained (Karanis 2006). Statics related to disease outbreaks and improvements still underreport on WBDO cases because cases are often left unregistered. The actual global health challenge, however, is to prevent all cases of water quality-related diseases. One emerging challenge is the increasing use of wastewater in agriculture. Resulting agricultural discharge carries large amounts of pesticides and contaminants from domestic animals' faeces. In this way, the agricultural sector can adversely affect livelihoods and lead to public health risks downstream (WHO 2015).

Waterborne diseases outbreak, infections and illnesses affect large human populations and can cause illness or death for other animals and organisms. Humans, who can be carriers for parasitic or infectious microorganisms, can excrete more disease-causing organisms that can survive residence time in natural water bodies. These organisms include the virus, bacteria, protozoan parasites and Helminthes (Ova).

More than 4% of waterborne diseases cases are caused by pathogen transmission via the fecal-oral route (WHO 2015). The pathogenic strains of *Escherichia coli (E. coli)*, for example, pollute the water where related gastrointestinal diseases occur. Diarrheal disease is caused by unsafe water due to poor sanitation and hygiene (WHO 2015).

Dufour (1984), using Bacterial Indicators of Recreational Water Quality, demonstrates the differences on the gastrointestinal infection rates for waterborne pathogens. He compared illness in seawater swimmers versus freshwater, finding that seawater swimmers contracted illnesses twice as often as those using freshwater. Kay et al (1994) used different methods than Dufour but reached the same results.

Through fecal-oral transmission, the pathogens attack immune system of human receptor; sometimes causing acute symptoms to occur once the infection has spread. A common symptom in humans with bacterial or viral infections is acute diarrhea, although they may also cause infections in ear, nose, throat, eye and skin. The symptoms leading to infections cause major costs to the responsible health sector. Consequent costs involve the treatment as well some extreme cases the hospitalizations costs. There are other permanent economic costs because of the deficiencies to the bodies, for example damage of the kidneys (Pond 2005). Major pathogens responsible for these costs include salmonella, norovirus and cryptosporidium.

1.1.1 Connecting faecal pollution to water borne diseases and outbreaks

Waterborne diseases pose a serious public health concern in both developed and developing countries. There is a high risk factor of obtaining disease infections due to faecal contamination when wastewater is released into water bodies without proper treatment. Most of the pathogens that may cause waterborne diseases have a faecal-oral transmission route, with their transmission stages occurring in human or animal faeces (Pond 2005). Faecal indicator bacteria present in an environment indicate the possibility that water has been contaminated by untreated fecal waste. The contamination affects the part of the population most vulnerable to illness, for example children, people suffering from immune deficiency, and the elderly (Pond 2005). When WBDOs occur, faecal indicators can be used to explain the presence of diseases. Some strains of the indicator bacteria *E. coli* may be pathogenic themselves; including strain O157- H7, but most of the *E. coli* bacteria are harmless and

useful members of human gut flora. More than 99.9999% of all *E. coli* in the world are harmless.

The literature documents major cases of disease outbreaks, showing their most serious effects, including acute diarrhea, bloody diarrhea, gastric infection and kidney infection (Pond 2005). Much statistical information related to disease outbreaks is available, yet many improvements still need to reduce the incidence of these illnesses. The number of cases of waterborne disease left unregistered means that our knowledge of the true extent and effects of these outbreaks is still incomplete and requires further research.

1.1.2 The importance of making of this study

Microorganisms such as *E. coli* and *salmonella* released from untreated wastewater can cause and escalate WBDOs. Differences in bacterial exposure can have mild to fatal consequences, depending on the health conditions of the affected person (Pepper et al. 2015). Pepper (2015, Table 1.2) describes emerging environmentally transmitted microbial pathogens and biological agents. He explains the danger of toxigenic *E. coli*, which can have a virulent increase to cause diseases such as enterohemorrhagic fever and kidney failure. Table 1.1 lists the most common waterborne diseases and the pathogens that cause them..

Table 1.1 List of microorganisms, diseases and their effects due to faecal contamination of water (Arnone & Walling 2007; Pond 2005; Tchobanoglous et al. 2014; WHO 2003).

Microorganism	Disease	Disease symptoms and effects
Bacteria		
Campylobacter ssp.	Campylobacteriosis	Acute diarrhea, occasionally bloody and severe.
Escherichia coli 0157:H7	Gastroenteritis*	Vomiting, diarrhea, Severe bloody diarrhea and abdominal cramps,
Leptospira interrogans	Leptospirosis (Weil's disease)	High fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash.

Salmonella typhi	Typhoid fever	Fever, malaise, aches, abdominal pain, diarrhea or constipation, delirium
Salmonella (1700 serotypes)	Salmonellois*	
Shigella (4 spp.)	Shigellosis*	Severe abdominal pain, watery diarrhea or stools containing blood.
Vibrio cholerae	Cholera*	Acute diarrhea and lost of fluids
Yersinia entercolitica	Yersinosis	Diarrhea
Virus		
Rotavirus		Vomiting, diarrhea
Hepatitis A	Hepatitis	
Adenoviruses	Respiratory disease, gastroenteritis	
Norwalk viruses		Vomiting, diarrhea

Footnote: * human source is one of the main sources of the disease.

1.2 WHO Guidelines for Safe Recreational Water Environments, EU Directive and Water Quality Norms for Recreational Water

The guidelines for safe recreational water environment in WHO (2003) describes the present state of knowledge regarding "the impact of recreational use of coastal and freshwater environments upon the health of users" (WHO 2003). Their main purposes are to maintain safe recreational water environments, while also maximizing their benefits to users. The information in those guidelines can be used at international, national and local levels, although application practices depend on the standards, regulations and norms of each country. This publication provides several useful guidelines for controlling potential health risks of waterborne pathogens in recreational waters (WHO 2003).

Implementing the guidelines depends on economic, environmental and other factors that define management areas. The coastal and freshwater bathing zones of countries, for example, are often regulated differently, but the WHO guidelines can still be used worldwide to target contaminated areas with corrective legislation. In 2003, the WHO reported extreme pollution on California's beaches in the United States of America. Measuring against the WHO (2006) guidelines, Volume 2, the beaches there had poor water quality. The unsafe beaches prompt

the inclusion microbial criteria for recreational water pollution and safety. "**The Clean Water Act (CWA)** requires **EPA** to develop criteria for water ", which "The criteria are designed to protect the public from exposure to harmful levels of pathogens while participating in water-contact activities such as swimming, wading, and surfing in all waters designated for such recreational uses" (EPA 2012) as part of the 2012 **Recreational Water Quality Criteria (RWQC).** Conversely, Scandinavian beaches generally have good water quality, but with extreme rainfall events and Combined Sewage Overflows (CSOs), water can still become contaminated and unsafe for recreational activities.

To comply with the **EU directive** from 2006 and **Water Quality Norms for Recreational Water** (**WQNRW) "Vannkvalitetsnormer for friluftsbade"** must have goals towards achieving safer bathing water (Union 2006). The **EU Union** (2006) is responsible for standardizing the information needed for the improvement of the quality bathing waters, while the Norwegian norms give directions for evaluating recreational water quality. Water quality has been the long-time focal point for the assessment and evaluation for the fate of fecal indicators in water use and recreation in general (Union 2006). Because humans often use water bodies for recreation purposes in inland and ocean zones during warm months of the year, the quality of water must be consistently monitored to respond to contamination by pollutants or outbreaks of waterborne diseases.

The water quality norms for recreational bathing in Norway are laid out in **WQNRW** (*Vannkvalitetsnormer for friluftsbade*), which gives guidelines for the owners of the water bodies or bathing area and institutions in Norway, which make them responsible for the use and maintenance of recreational waters. Measures of maintaining good water quality include inspections, analyses and sampling in case of sewage spills, extreme rainfall episodes or appearance of pollutants in the water. *E. coli* and *I. enterococci* are a priority indicator in selecting analysis parameters and the choices in the Norwegian water norms. These bacteria are also representative fecal indicators recommended in the **New EU Bathing Directive** for testing the quality of bathing waters (Folkehelseinstitutt 2004). The norm suggests taking water samples at least 14 days before the bathing season begins and as close to recreational use areas as possible (Folkehelseinstitutt 2004).

Currently, the **EU directive** (2006) is widely applied in Norway. The Directive suggests specific guidelines for bathing water quality to reduce the risk of infections and associated waterborne illnesses (Tryland et al. 2014). In the **EU Directive**, they are even stricter

although they are based on WHO recommendations themselves. Recreational water management and water quality criteria are still governed by the standard WHO guidelines, with fecal contamination being assessed by using concentrations fecal indicator bacteria (WHO 2003). These WHO guidelines are only recommendations to help countries to set their own directives.

Management approaches such as beach profiling and inspections, risk assessment and public health protection are used to control the outbreaks of infections and diseases (WHO 2003). The organization promotes counts of E. coli and intestinal enterococci to assess recreational waters (Table 1.2) and (Table 1.3). From these basic guidelines, Kay et al. (2004) have developed a basic equation to evaluate the state of a water contamination event, incorporating an assessment of fecal indicator bacteria (FIB) concentrations and the vulnerability of ecosystems to fecal contamination (Kay et al. 2004).

Though, fecal indicators can identify potential contaminants in the water, they do not account for all the microbes living in water or provide information about their persistence in natural water systems. *E. coli and* intestinal enterococci parameters are used to define water quality, of the beaches, which can be classified as poor, sufficient or good depending on the percentile values for microbial enumeration using Colony Forming Units (CFU) per 100ml (Mansilha et al. 2009; Tryland et al. 2014; Union 2006). The levels of fecal indicator parameters for different quality groups used for classification of beaches are shown in Table 1.1and Table 1.2.

Table 1.2 Faecal indicator parameters for inland waters. Derived from EU Bathing Water Directive, Annex I and II (Union 2006).

Parameter	Excellent quality	Good quality	Sufficient	Reference methods of analysis
Intestinal enterococci (cfu/100 ml)	200	400	330	ISO 7899-1
Escherichia coli (cfu/100 ml)	500	1000	900	ISO 9308

Table 1.3 Faecal indicator parameters for coastal and transitional waters. Derived from EU Bathing Water Directive, Annex I and II (Union 2006).

Parameter	Excellent quality	Good quality	Sufficient	Reference methods of analysis
Intestinal enterococci (cfu/100 ml)	100	200	185	ISO 7899-1
Escherichia coli (cfu/100 ml)	250	500	500	ISO 9308

The **EU Directive** (2006) in mentioned in article 6, bathing water profiles that it may be covered at least one bathing water, be reviewed and updated. In addition, have good use of the data from monitoring and assessing those waters. It is described in Annex III. In the Annex III, In Point 1, a) the bathing water profile should include relevant the physical, geographical and hydrological characteristics of the possible polluted water (Union 2006). In Point 1, b), the causes of pollution are identified and assessed. Also in 1.c) and 1.d) the assessment of potential for growth of cyanobacteria and macro-algae are made, respectively. 1.e) If there is risk or an effect from short-term pollution, then it should describe the cause, type and how often and how long time it occurs. Yet, modeling results will give concentrations disregard these high values as long do you warn the people against swimming in specific sites. How long time is it needed to say, precaution and elimination period, or to warn against contamination? In point 1.f) refers to the location of the monitoring point (Union 2006).

The bathing water classification can interfere with subsections in Point 1, so in this instance the profile needs to be updated accordingly. The nature and severity of the pollution defines the scope of the contamination event and how often the profile needs to be reviewed. Table 1.4 indicates the frequency for these reviews.

Table 1.4 Classification of bathing water. As seen in Annex III, the bathing water profile (Union 2006).

Bathing water classification	Good	Sufficient	Poor
Reviews are to take place at least every	Four years	Three years	Two years
Aspects to be reviewed (points of paragraph 1)	(a) to (f)	(a) to (f)	(a) to (f)

Bathing water should be monitored at least four times during bathing season. If pollution occurs, then additional sampling is required to confirm reduced hazard at the end of the incident. Annex IV (WHO, 2006). The EU Bathing Directive (2006) outlines the concentrations of faecal bacteria indicators for monitoring water contamination by pathogenic microorganisms. Bacterial indicators are used to model the behavior of harmful pathogens (Hipsey et al. 2008).

1.3 Norwegian Bathing water and Overview of Oslofjord

A thorough understanding of processes affecting the Norwegian water within aquatic environments is essential for modeling the transport of microorganisms in surface water. When pathogens from a faecal source are discharged to surface water, due to CSOs, their fate and transport may differ (Tryland et al. 2014). Though some microorganisms may thrive, other viruses, parasites and enteric bacteria often die-off. Accounting to their death rate, it will depend on species, water temperature, sunlight and other variables. Pathogens' fate and transport may be affected by sedimentation, association with other particles, or consumption and/or inactivation of enteric organisms by autochthonous. Pathogenic bacteria causing problems in Norwegian recreational waters include *Salmonella spp., Shigella spp., Vibrio spp. Clostridium spp., and Staphylococcus*. The bacteria used as faecal indicator for Norwegian bathing waters are *E. coli* and *I. enterococci* (Union 2006). Detecting faecal indicator bacteria in these waters is a reliable and simple process (Hipsey et al. 2008).

The Oslofjord is a narrow and long channel stretching 100 km, dividing on its landward end into the inner and outer Oslofjord. Inner Oslofjord, traditionally, has an extensive and many areas for recreational bathing waters, but recently large development and construction projects in harbor areas have prompted new efforts to make sure that the seawater in inner Oslofjord is safe to use (Arnesen 2001).

Since the 1900s, pollution has spread from the coastal waters surrounding Oslo city to other parts of the fjord (Arnesen 2001). Arnesen (2001) discussed relevant information on water pollution and protection of Oslofjord. Pollution of water problems in the Oslofjord is largely tied to the increase of the discharge of poorly treated sewage, and the geographical positions with interface of inner and outer fjords. According to Arnesen (2011), many experts have perceived the problems related to the elevated organic matter, nutrients, high levels of

bacteria and hydrogen sulfide in seawater. He suggests addressing water pollution issues through agreements between technological and scientific institutions that improve the efficacy of wastewater treatment plants (WWTPs), including mechanical, chemical and biological treatment processes. Arnesen (2011) redefines the goals for sewage treatment according to the actual need for safe water.

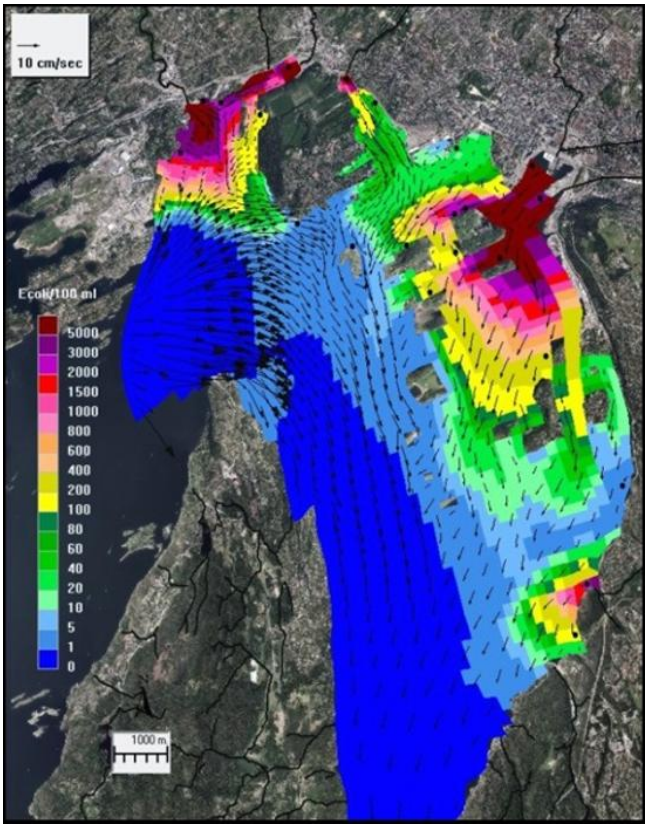


Table 1.5: Simulated concentrations of *E. coli* for bathing zones in Oslofjord during heavy rainfall and CSOs, focusing on the Lysaker and Bekkelaget areas. Blue indicates no *E. coli* while dark red areas have maximum contamination with *E. coli.* Source: http://www.niva.no/waterqualitytools.

Through the "Quality water tools project: Impact of changing weather patterns on bathing water and seafood quality from the Inner Oslofjord (2012-2015)", faecal contaminants were measured and calculated during different weather conditions. The project also investigated the fate of the faecal indicator bacteria and pathogenic contaminants using process models and included them into hydrographic model. The concentrations of the microorganisms were simulated in an analysis for Oslofjord beaches (Table 1.5), considering climate change and different type of discharges in the modeled future scenarios (Tryland et al. 2013). An extensive gap exists relating the fate and transport of faecal pathogens originated from CSOs,

and poorly treated sewage discharges into the rivers and other point sources on the Oslofjord. Most of the faecal pathogenic water pollution in Oslofjord occurs due to CSOs in a event of heavy rainfall (Tryland et al. 2014).

So far, there is not enough information related to total discharges of pollutants in the inner Oslofjord. However, few results were determined from related researches (Berge, et al 2011). Oslofjord is a recipient for untreated wastewater during stormwater overflows and sewage discharge emergency situations. Such heavy rainfalls making it difficult for wastewater treatment plants to carter for all the water discharged into the Fjord. There is an increase of circa 2 $\log_{10}$ in FIB after heavy rainfall episodes in inner Oslofjord (Tryland et al. 2014). When these situations occur, CSOs can cause discharge of large amounts of fecal contaminators in the water (Tryland et al. 2014). Andersen and Mounce (2013) have also been investigating the impact of CSOs, and how they affect the quality of water recipient during rainfall events (Andersen et al. 2013).

Prioritizing comprehensive water management strategies and applying bathing water quality tools to monitor heath risks related to sewage discharges are important steps, along with tracking environmental parameters such as sunlight, temperature and different weather patterns. In recent studies (Staalstrøm 2014), concerning the VEAS tunnel, a description of how the fecal indicator monitoring should be approached in Oslofjord.

1.4 Thesis statements

Raw sewage collected in Drøbak WWTP, water samples are mixed, and afterwards diluted with distilled water to investigate the selected FIB. Because laboratory experiments can only mimic few important environmental factors that affect FIB, realistic results for different events of faecal pollution can be difficult to achieve. The results obtained can be skewed because experimental procedure can't account for the realistic conditions, however, they are still used as the main input for process modeling using FIB bacteria.

In investigating the fate of FIBs, finding to the fate of harmful bacteria in water and fecal contamination at different environmental conditions becomes difficult to assess. This creates a problem in how significant can be the laboratory results. Spatial and temporal variations also make it challenging to effectively predict the effects of environmental factors on FIB.

Consequently, laboratory results and subsequently modeling cannot accurately predict the risk posed by CSOs events.

Water analysis, furthermore, only gives a snapshot of overall water quality. Hygienic water quality at beaches exposed to COs may fluctuate over >1000 for a factor of FIB (i.e. water transitions from good to poor quality after heavy rainfall) very quickly(Union 2006). Results from samples taken the day or week before may not be representative of the water quality after rains. For "real time" evaluation of bathing water quality of beaches exposed for short-term pollution, mathematical models representing the decay rate may therefore be useful.

Models for predicting bathing water quality after pollutant discharges close to beaches and recreation waters (e.g. from CSOs and WWTP) require lots of input data. For example, many models must incorporate the decay rate of fecal indicator bacteria (*E. coli* and *I. enterococci*). Once a greater understanding about the fate of contaminants in water is established, it may be easier to predict how quickly the fecal bacteria die off in natural waters under different conditions. In this thesis, the fate of FIB due to CSOs is analyzed for better understanding of the behavior and how the bacteria die in water at different temperatures and depths.

1.5 Research questions

Is there a difference between rate of decay of faecal indicator bacteria in deep and surface seawater?

Is the rate of decay of faecal indicators from own laboratory experiments in accordance with those values reported in the primary literature (peer-reviewed research)?

Which decay rates/process models should be used to model the decay of fecal indicator bacteria to predict bathing water quality after discharges from CSOs?

1.6 Objectives

Main objective: Determine the fate and persistence of faecal indicator bacteria in seawater as input to models for predicting bathing water quality.

Specific objectives: 1- Review the literature to get information about the fate of faecal indicator bacteria in water, i.e. describe decay constants/models of E.

coli and Intestinal enterococci under different environmental conditions.

2- Present the fate/persistence of Escherichia coli and Intestinal Enterococci in the laboratory experiments.

3- Compare laboratory results of the decay of *E. coli* and Intestinal enterococci in seawater with values from the literature review.

2. Literature Review

Norwegian municipalities surrounding the Oslofjord use the enumeration of indicator bacteria to check faecal contamination (Sen & Ashbolt 2011). The two main faecal indicators are *Escherichia coli* and *Intestinal Enterococci.* If fecal indicators were detected in water, there could be a risk of presence of human pathogens. Then, high levels of fecal indicators show bigger risk of presence of human pathogens. However, the correlation between fecal indicator bacteria and human pathogens is not always good. For example, when the faecal indicators to the pathogenic bacteria originate from harmless sources (e. g. faeces from healthy people or animals) or their persistence time is different to the human pathogen (Romero et al. 2006). Once the indicators and faecal pathogens enter the surface water, their fate and transport may differ. Some bacteria may grow, but viruses, parasites and enteric bacteria will die in general. With varying death rates depending on species, temperature, pH, sunlight and other environmental variables.

The layout for the literature review is review of information, description and significance of FIB. Reviewing on major factors affecting FIB in water. Then, the rate peer reviews and their factors highlight the subtopics bellow. The review also updates the models of faecal indicator bacteria in aquatic systems.

2.1 General Information and Importance of Faecal Indicator Bacteria

Measurement of microbial water quality uses levels of different microorganisms indicators to determine the safety of drinking water. The three types are: general microbial indicators, fecal indicators and index organisms. From last century, the testing for water pollution water uses Escherichia coli and coliform bacteria as FIB for water polluted by animals and human waste (WHO 2001). Testing of FIB is important when fecal contamination occurs in water, which may be infected with harmful microorganisms (Paruch & Mæhlum 2012). Faecal water indicators assess the level of fecal contamination and pollution on surface water. Using indicators is challenging because it has been proved that the some pathogenic microorganisms have low survival period when living away from their hosts, making it difficult to predict their fate on time (Pommepuy et al. 2005). Fate of FIB can be expressed by the decay rate (k_t) or Log reduction (T_{90}) (Barcina et al. 1990; Pommepuy et al. 2005; Rozen & Belkin 2001).

Major contributions to the presence of fecal microorganisms in coastal zones are (Pommepuy et al. 2005):

- The influence of the environmental conditions they face;
- Some bacteria have their unique characteristics. For example, they are physiologically active, absorption onto organic mater. This allow them to be more resistant than others and can survive for longer period of time;
- Pollution outbreaks and COS due to extreme rainfall events;

According to Griffin (2001), good indicator should have the following characteristics (The list of the characteristics of ideal water quality indicator) must have (Griffin et al. 2001):

1. " It must occur where the pathogen does live;
2. It is not able to grow in the environment outside their host;
3. Disinfection is more efficient in pathogen than the indicator;
4. It should be easy to isolate and count;
5. It is found in fecal contaminated waters;
6. It should show higher numbers than the pathogen;
7. Its concentration values should be connected to the degree of contamination and health hazard."

Faecal indicator bacteria such as E. coli and intestinal enterococci are used as indicators to estimate water contamination due to hazardous pathogens. They may be good indicators for several pathogenic bacteria and some viruses. Primary studies have shown that E. coli is a much better and preferred indicator of disease risk than others in the Faecal Coliform group (American Public Health et al. 2005; Odonkor & Ampofo 2013). As reported in several studies, there is a significant mutual relation or correlation between the faecal indicator bacteria or parameters of fecal pollution with the pathogens and gastrointestinal bacteria in water due to CSOs (Byamukama et al. 2000; Cabral 2010; Charriere et al. 1994; Ferguson et al. 1996).

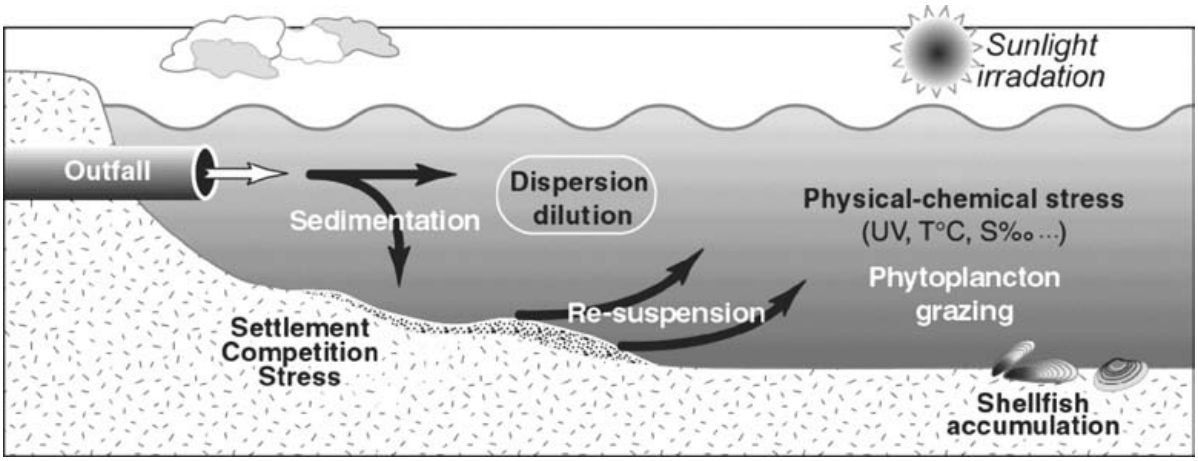


Table 2.1 Drawing of sewage effluent entering into the coastal water showing the dilution process and environmental factors involved in microbial behavior (Pommepuy et al. 2005).

Pommepuy made a description of how the FIB is introduced into water by outfall in deep water using a diagram in Table 2.1. When the microorganisms are discharged into the coastal zone, their concentration is reduced by physical dillution and dispersion, as well as dilution and other seawater factors, such as nutrient competition, chemical reaction, salinity, sunlight, pH and temperature. The combination of many of those factors affects the biological activity of the bacteria.

Escherichia coli is an *Enterobacteriaceae,* (are oxidase-negative catalase-positive straight rods that ferment lactose. *E. coli* is a natural and essential part of the bacterial flora in the gut of humans and animals) (Cabral 2010). Studies reported that *E. coli* appears in most of the species. (Cabral 2010; Gordon & FitzGibbon 1999). E. coli belongs to the faecal coliform group and because it originates from the faeces of humans and animals, making it a leading indicator for faecal pollution (Odonkor & Ampofo 2013). *E. coli* is a valuable faecal contamination indicator because of the differentiation of non-faecal originated faecal coliform bacteria from the ones originated from faeces of humans and animals. Other importance is the appearance of modern and rapid methods for the detection of FIB.

The presence of *I. enterococci* in water indicates fecal water pollution, simply because water is not a normal habitat for them. In particular, enterococci are made of gram-positive and non-spore forming cells which grow at 37°C and in some cases exceed up to 45°C (Pommepuy et al. 2005). The most common enterococci bacteria in humans are *E. faecalis and E. faecium* encountered in urban pollution. Fecal enterococcus survives for longer period than *E. coli* in water ((WHO) 2011; Cabral 2010; Payment et al. 2003). *I. enterococci* are a preferred FIB because

of their use in detecting the interaction between human faeces and the seawater, especially in urban areas (Boehm 2007). Models predicting enterococci concentration not only reduce the impact of contamination and safety of swimmers, but also affect the management, decision-making and economic improvement of the beaches (Hou et al. 2006).

Table 2.2 List of some (Faecal) indicators, and their concentration in raw sewage or effluent of CSOs (Arnone & Walling 2007; Ashbolt 2004; Gerba 2015; Pond 2005; Rechenburg et al. 2006; Tchobanoglous et al. 2014; WHO 2003).

Microorganism	**Concentration in Raw sewage (Number /100ml)**
Escherichia coli	10^5- 10^7
Intestinal enterococci	$4.7 \times 10^3 – 4 \times 10^5$
Clostridium perfringens spores	$6 \times 10^4 – 8 \times 10^4$
Polioviruses	$1.8 \times 10^{2-} 5 \times 10^5$

2.2 Source, transport and fate of faecal indicator bacteria in water

Hipsey (2008) states that the sedimentation and association with particles of enteric organisms affects the transport and the fate of pathogenic bacteria. Decay rate of enteric bacteria varies with their species and parameters like: temperature, sunlight, salinity, turbidity and depth of the water.

2.2.1 Source of FIB in Water

The origins of water pollution and fecal contamination in waters are divided into point source and non-point source. Point sources are the discharges of raw sewage and effluents from domestic and industrial WWTPs, stormwater, rainfall overflows, CSOs, agricultural runoff, urban runoff and settlements in mountains or rural areas (De Brauwere et al. 2014; Gagliardi & Karns 2000). Discharges of CSOs into a receiving freshwater and seawater water sources such as rivers, lakes, estuaries and local beaches may significantly reduce the hygienic water quality of the water source during the discharge and the following days (Tryland et al. 2014). Point sources of microbial contamination in urban areas are mostly raw water from municipal WWTPs. In cities, large numbers of fecal contamination occur mainly from faeces of infected humans and animals. Even though some individuals do not show any symptoms of infection

for long period of time (Pepper et al. 2006). Non-point sources are infiltrated or seepage sewage, drains and leakages from sewer networks and scattered rural areas. In cities, the faecal contamination is from point sources to the water bodies (Servais et al. 2007).

Geographical location for point sources of is well considered and assessed before deciding in an actual spot as a source for discharging of wastewater. However, in many cases, this does not happen. Then, the faecal contamination occurs in places where water as source of drinking water, or popular place for water recreational activities.

2.2.2 Transport of FIB in Water

The transport of FIB in water is done differently from pathogenic microorganisms. The transport of pathogens in water can be horizontal or vertical. The horizontal transport is controlled or defined by inflow, circulation and dispersion or mix of sewage water with seawater, wind currents and the internal waves (Brookes et al. 2004). Vertical transport of pathogens is the settling to the bottom of the sea. Generally, the pathogens tends to survive longer in water the by itself (without a host) than FIB at same environment condition, which give them the opportunity to multiply prior parasitizing to their future host. During rainfall events, it allows pathogens to faster transport over longer distances comparing to FIB (Bradford et al. 2013). In contrary to, FIB are not able to live long outside their host and do not survive well in the environment. Their ability to move is limited to their survival in water function.

In a study for the transport and fate of microbial pathogens in agricultural settings, the speed for the bacteria larger than 400 μms^{-1} was recorded. However, at closer look, they move much slower than the records registered. (Bradford et al. 2013). This information is relevant. It means that, we can know how much the bacteria travel and how long it will take them to enter the waters, WWTP in relation to the coastal zone, before dispersion or dillution occurs.

2.2.3 Fate of FIB in Water

One reason that the fate of faecal indicators in water has been studied in recent years is their ability to predict detrimental effects on bathing waters and other uses. It also has received considerable attention in relation to water contamination and waterborne disease outbreaks. The study of the fate of faecal indicators in water is relevant for the investigation of waterborne diseases in surface and recreational waters. Faecal indicators for pathogenic bacteria, protozoa and viruses can be used to measure the quality of both surface and

recreational (bathing) water. The rates of infections due to faecal pollution vary seasonally, and one of the many reasons could be the ability of the microorganisms to survive at particular season in relation to the other in the year. However, those reasons are not very well understood, so far. Many factors affect this variability (Pepper et al. 2006). They play a part on the differentiation of excretion and its exposure to contaminated water.

The fate of FIB depends on bacteria themselves and the decay rate depends on the environment they live in (Gourmelon et al. 2010). To investigate the fate of FIB is important for evaluating their ability to survive in their environment (Vergine et al. 2005). Faecal bacteria can die quickly in water due to their own mortality or when they are exposed to factors such as temperature, nutrient scarcity and deprivation of natural light, (Brookes et al. 2004; Servais et al. 2007). Chemical and physical characteristics, atmospheric conditions and biotic factors also influence the survival of FIB (Gourmelon et al. 2010).

The fate of faecal indicator bacteria in our Master Thesis can be studied using laboratory experiments, which is affected by biological, chemical and physical factors. Laboratory test can be performed to simulate the discharges from the CSOs and urban runoff from rainfall episodes. The tests can also check how the fecal indicator bacteria survive in the receiving seawater after the discharge. However, these experiments will not fully mimic the real conditions, however they are still useful.

2.3 Factors influencing the faecal indicator bacteria

Describing factors of bacteria is important because it can explain how is the fate of the bacteria in relation to the factors affecting their own survival. Some of the factors can be more influential in the FIB than others at a certain point. Thus, the extent and how they are affecting the bacteria is spatial and temporal variable. In addition to the factors affecting the survival or decay rate of FIB, seasonal variations and habitat of the bacteria seems to have large effects on the decay rate of FIB. These characteristics contribute to different results in transport and source tracking models of the FIB. The models also vary with time because of the effects of night and day in different parts of the World (Crane & Moore 1986). All the parameters listed in the Table 2.3 are responsible for the decrease in number of bacteria in water.

Table 2.3 Factors affecting the survival of FIB and other microorganisms in the water environment are summarized in Table 2.3 (Crane & Moore 1986)

List of factors	Factors Parameters
Nature and characteristic of the microorganism itself	
Atmospheric conditions	Sunlight/ solar radiation Temperature Moisture (humidity and precipitation)
Physical and chemical effects of the environment	pH Temperature Organic mater content Availability of nutrients Elemental composition
Biological interaction with other organisms	Antibiotic Toxic substances Competition with indigenous microflora
Application method	Frequency of discharge Technique

Most relevant factors contributing for enteric bacteria survival can be divided into biotic and abiotic. Examples of abiotic processes are environmental factors such as temperature, salinity, sunlight and pH (Sinton 2005). The effects of temperature, salinity, pH, dissolved Oxygen, sunlight, nutrients and turbidity on the mortality of enteric organisms are used to obtain the decay rate parameters (Belkin & Colwell 2006; Hipsey et al. 2008). The scarcity of assailable nutrients also influences the decay rate.

By disregarding the limitations of their models, several scholars have tried to dismiss the influence of temperature, salinity and pH on mortality rate (k_d) values (Mancini 1978; Mayo 1995; McCorquodale et al. 2004). One of the limitations of such models is that they can only be applied in the natural environment of the microorganism being studied. Another is that those models studies are considered empirical because there is not specific parameters in the equation that do not depend on particular characteristics of the microorganism. (Hipsey et al. 2008). Hipsey (2008) developed a process-based model (Equation 2.1) to describe the influences of temperature (T), salinity (S) and pH (potential hydrogen) on the mortality rate (k_d). He ignored all other environmental effects on microorganisms and highlighted the relationships between these influences and mortality rate. So, the dark decay rate can de expresses as a function of temperature, salinity and pH.

$$k_d = k_d(T, S, pH) \qquad \text{Equation 2.1}$$

2.3.1 Temperature

In this thesis experiments (described in chapter 3), temperature is a controlling factor for the fate of FIB. The research checks whether the bacteria concentration of the FIB increases or decreases in seawater. It is expected to be different for the different temperature and (4^0C and room temperature 22^0C).

Temperature is considered the most important environmental factor affecting the fate of FIB, and can consistently predict the fate of the bacteria. The lower the temperature, the longer survival time the bacteria have (Crane & Moore 1986; Pepper et al. 2006). But, if it reaches freezing state, the intestinal bacteria will die. The relationship between the temperature and the number of nutrients in mix of sewage water effluent and seawater and decay rate of coliform bacteria was observed (Chamberlin & Mitchell 1978; Hipsey et al. 2008). In both studies it was concluded that *E. coli* did not grow as much in comparison to the other pathogenic bacteria under the same conditions. In another study, Lopez-Torres et al. (1998) used tropical seawater and detected *E. coli* in water. It which shows a decrease in its activity, and considered *E. coli* a viable FIB for tropical environments (Lopez-Torres et al. 1988). In contrary to the assumption that bacteria start decreasing as soon, it is stored in closed environment, as closed bottles. Hendricks and Morrison in 1967 observed growth of indicators and pathogenic enteric bacteria in fresh and seawater samples (Hendricks & Morrison 1967). They also claimed that the multiplication of bacteria in low nutrient and low temperature water environment could be quite large. Bradford (2013) agreed that the die-off rates of *E. coli* increase as the temperature rises. The mortality rate (k_d) of *E. coli* and *enterococc*i was graphically represented (Table 2.4) by several researchers and summarized from data available in their literature (Hipsey et al. 2008).

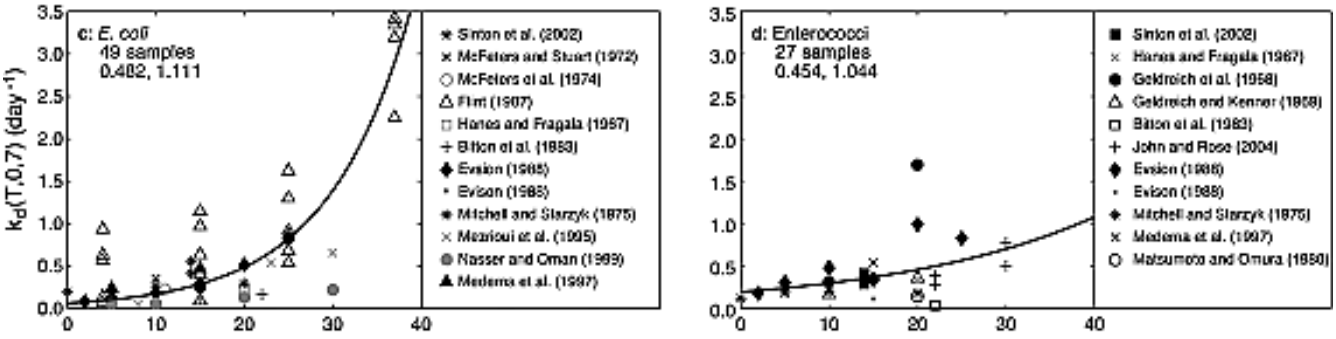


Table 2.4 Relationship of natural mortality rate and temperature for E. coli and enterococci. The salinity of 3% and ph values of 6-8 were used in these analyses. The equations were based on least-square linear regression (Hipsey et al. 2008).

Once all other factors are kept constant and only the temperature variations are used, the function of decay rate (k_T) is the factor of reference temperature (20^0C) and empirical coefficient can be represented in a model equation. The decay rate at temperature T of FBI is expressed in Equation 2.2 (De Brauwere et al. 2014b).

$$\boldsymbol{k_T = k_{20} \times \theta^{T-20}} \qquad \textbf{Equation 2.2}$$

Where: k_{20} is the decay rate at temperature of 20^0C; θ is an empirical temperature coefficient and varies (1.013-1.19); T is the temperature.

2.3.2 Sunlight

The general consensus on the faecal indicator studies is that sunlight is the most effective contributor to inactivation or death of faecal indicator bacteria and other microorganisms in both freshwater and seawater (Auer & Niehaus 1993; Davies & Evison 1991; De Brauwere et al. 2014; Fujioka et al. 1981; Hipsey et al. 2008; Johnson et al. 1997; King et al. 2008; Mancini 1978; Mitchell & Chamberlin 1978; Noble et al. 2004; Sinton et al. 1994; Sinton et al. 2002; Sinton 2005). Sunlight is an important parameter and is used in researching and laboratory experiment for monitoring and enumeration of FIB (Crane & Moore 1986). the thesis research, which takes sunlight as a limiting factor in the environment of the FIB, the research is expected to explain how their fate of in this thesis.

The studies for the effect of sunlight on survival of indicator bacteria in seawater, confirmed that the presence or absence of sunlight largely affected fecal coliforms and faecal streptococci. It also demonstrated that up to 90% of the bacteria were dead after 3 hours after

being exposed to sunlight. And in cases where it was no sunlight they could survive for many days (Fujioka et al. 1981). *E. coli* in faecal coliform group has 70% inactivation from the UV-B wavelength spectrum (280-320 nm). This wavelength has the ability to destroy the DNA of microorganisms in cellular level. While Enterococci is more sensitive to the UV-A wavelength (320-400 nm) of circa 51%, which has weaker effect on microbes than UV-B (Harm 1980; Hipsey et al. 2008; Sinton et al. 1999). Mancini in 1978, made estimates for the influence of light on coliforms and concluded that the rate of mortality of coliform reduced with increase of time, and he also reported a decline in bacteria population.

In seawater the rate of mortality tends to be higher because the light extinction is greater in lethal portion of spectrum in relation to the total spectrum of light (Mancini 1978). In sunlight and survival of enteric bacteria in natural waters, Davies and Evison (1991) found that there was a difference on the count of number of bacteria in seawater and freshwater. In the seawater decay rates were significant faster than the freshwater, once suffer the effect of sunlight (Davies & Evison 1991). Other researchers not only research the simulation of solar radiation in marine waters but also used other environmental factors to test the presence of enteric bacteria (Alkan et al. 1995). Salinity is more accentuated in the culturable microorganism when applied UV radiation and in reverse sunlight effect on bacteria is greater at higher salinity levels. (Davies & Evison 1991; Hipsey et al. 2008; Sinton et al. 2002).

In contrary, Beaudeau (2001), in his research, did not see any improvements in solar inactivation and the same occurred for Rippy in 2013. He realized that the effects of solar radiation is considered temporary n relation to temporally and spatial variations. Therefore, the decay rate is a dominant factor in inactivation due to sunlight (Beaudeau et al. 2001; De Brauwere et al. 2014b).

FIB exposure to sunlight contributes in different quality and types of water (Korajkic et al. 2013). By ignoring the sunlight as function of temperature and using equation 2.3 below, this judgment provides a first estimate of the coliform bacteria. The equation 2.3 can guide calculations of mortality rates in water bodies (Mancini 1978). Mancini suggests that Decay rate in Seawater is affected by the solar radiation but could not prove the sunlight effect in fresh water at that period. In 1979, Mancini publicizes a paper on influence of light in both freshwater and seawater. He concluded that sunlight increases the die off of bacteria in seawater but did not reach a conclusion for freshwater (Chojnowski et al. 1979; Mancini 1978).

$$k_t = [0.8 + 0.006\ (\%seawater)] \times 1.07^{(t-20)} + \frac{I_A}{k_e H}\left[1 - e^{-k_e H}\right] \qquad \textbf{Equation 2.3}$$

Where: k_t: First estimate mortality rate or Decay rate at temperature t, k_e: light extension coefficient, t: temperature, H: completed mixed depth of water, I_A: Average daily surface solar radiation, (Mancini 1978).

Many models for inactivation of pathogenic bacteria were developed in the microbial pollution in aquatic systems. In modeling for sunlight, some factors such as wavelength, bandwidth regions for the solar spectrum, the presence of dissolved oxygen as well suspended material, the effect of pH and lastly the salinity effect of water Hipsey et al. 2008). By assuming, that the bacteria population decay is exponential and ignores the lag and recovery phases on the decay rate graphs (Harm 1980; Hipsey et al. 2008; Sinton et al. 1994). Hipsey (2008) develop an equation (equation 2.4) for a generic model for inactivation of sunlight (Hipsey et al. 2008) and suggests the dissolved oxygen and pH to consider the photo-oxidative effect (De Brauwere et al. 2014b):

$$K_l = \sum_{b=1}^{N_B} [\varphi(k_b + c_{s_b} S) I_b f_b^{LIM}(DO)\, f_b^{LIM}(pH)] \qquad \textbf{Equation 2.4}$$

Where: N_B: number of discrete solar bandwidths to be modeled, b: bandwidth class from 1 to N_B, φ: constant for conversion of units, c_s: coefficient of enhancement of light in seawater, k_b: freshwater inactivation rate coefficient, I_b: intensity of bandwidth class, f_b^{LIM} (DO): dissolved oxygen depended function of light inactivation coefficient, f_b^{LIM} (pH): pH depended function of light inactivation coefficient and DO: dissolved oxygen

Even thought natural sunlight inactivation of bacteria such as *E. coli* and *I. enterococci* is not costly, it is not enough for the treatment of these bacteria in both fresh and seawater. Since there are other factors influencing the survival rate of FIB in water (Byappanahalli et al. 2012). The Equation 2.2 results maybe limited if they consider only on bandwidth for their parameters. However, it needs large field data and parameters values which introduces more uncertainty to the equation (De Brauwere et al. 2014b). They could loose the dynamic of the model that is important fro the different organisms behavior (Hipsey et al. 2008). High clarity water means good inactivation mechanism Different species have different dynamics of sunlight inactivation. Therefore, to consider the processes helping on the inactivation of the

microorganisms is fundamental. Besides there still a gap on modeling the dynamic of sunlight (Hipsey et al. 2008).

2.3.3 Salinity

Bacteria react differently in the presence of salt. *E. coli* and *I. enterococci* have distinct behavior in presence of ions salt. IE can reproduce it self in salt water better than *E. coli* and other coliform bacteria. Studies have been shown controversial results for the relationship of salinity and fecal bacteria. (Byappanahalli et al. 2012) Effect of salinity in the culturable bacteria is more intensified when exposed to sunlight (Davies & Evison 1991). Hipsey (2008) reported results on influence of salinity in dark death rate for *I. enterococci*. The results were not very significant, while *E. coli,* results seemed hardly significant.

In monitoring of *E. coli* survival in water, It used different salinity values to check and confirm that *E. coli* survival increased as salinity decreased. Other studies reported that decay rates of FIB are higher when salinity is also high and lower with lower salinity (Bordalo et al. 2002; Canteras et al. 1995; De Brauwere et al. 2014b). Equation 2.5 showed the linear relationship between the salinity ranging from 0 to 35‰ for *I. enterococci* and "for *E. coli* the salinity effect appears to be hardly significant", and the decay rate of microorganisms. This is a simple way to model (Equation 2.5) the salinity effect on the decay rate due to salinity of FIB in water (De Brauwere et al. 2014b).

$$k_s = \beta \times S \qquad \textbf{Equation 2.5}$$

Where: β is the salinity influence factor and S is the salinity (‰) (De Brauwere et al. 2014b).

At 6.5% NaCl, *enterococci* will grow very well and this is typical of the *genus enterococcus* (Byappanahalli et al. 2012). The characteristic of enterococci to survive successfully in saline environment may increase its performance as faecal indicator used in marine recreational waters (Byappanahalli et al. 2012). *E. coli* is less efficient than *enterococci* in saline waters. But other studies obtained different conclusion on relation of salinity and FIB.

2.3.4 pH

If the water environment is kept with neutral pH, the bacteria tend to survive longer and extend their life. Despite the environmental conditions, this could also cause their rapid death

at same time. “The effects of ionic strength and pH on survival are dependent on the microbial species” (Bradford et al. 2013).

Research articles showed that pH has detrimental effect on bacteria in general. (Crane & Moore 1986).

Researches in seawater and freshwater concluded that alkaline environment was the preferred and better environment for inactivation of the microorganisms (Hipsey et al. 2008). While Crane et al, 1986, preferred acid conditions as better choice for die-off of bacteria.

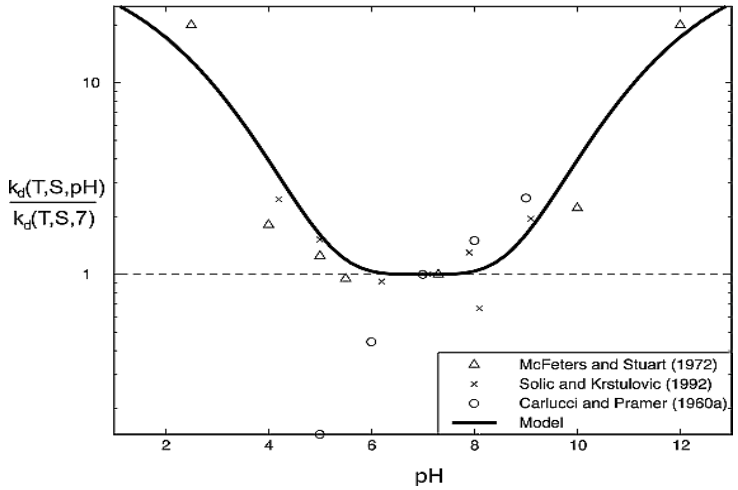


Table 2.5 Model parameters of coliform using data from different mortality rate and pH. The graph shows the variation of behavior of bacteria at neutral pH and the extremes. (Hipsey et al. 2008, Figure 6).

During studies of coliform groups, some problems were raised in finding out the optimum pH value (Figure 2.2). However, the mortality rate increased significantly in the values lower and higher than 7. The more acidic the water (pH<7) was, the faster the bacteria died. (Hipsey et al. 2008). Alkaline water with pH larger than 7 also accelerates the death of the FIB (Crane & Moore 1986). Bradford et al, (2013) agreed that pH values of 6-8 also have lower effect on the inactivation of microorganisms (Bradford et al. 2013); whereas, higher effect was shown for both acid and alkaline water environments (Table 2.5).

2.3.5 Turbidity

The clearer the water, the less debris it can be found in it. Turbidity may influence the quality of water because some of the water that seems to be dirty with suspended sediments can be judged mistaken for water contamination. It is difficult for the light to penetrate deeper in bottom of the water basin, where normally the sewage from WWTP is positioned. Therefore,

this will affect the decay rate of bacteria entering the water. (Gourmelon et al. 2010; Kay et al. 2004).

2.3.6 Growth of bacteria

Literature review on the growth of microorganisms in water bodies has covered little information on faecal bacteria in relation to the rate of decay (Hipsey et al. 2008). Hipsey (2008) assumed that surface water has limited nutrients for the multiplication of nutrients for the multiplication of microorganisms. However, with polluted water originating from the receiving waters from nearby WWTP it is possible that the growth of pathogenic microorganisms does increase. (Hipsey et al. 2008). This increase can happen because the effluents from WWTPs are rich in nutrients and also bacteria can be attached to other microorganisms (Tchobanoglous et al. 2014). Using fresh and seawater, the growth of enteric bacteria was simulated only 2.5-25% by the presence of sewage (Morgan et al. 1990).

2.4 Models for FIB in water

Numerical models are used to narrow the gap in microbial science. It can help in issues concerning the management of water quality (Hipsey et al. 2008). Many models are used to simulate microbial pollution in different water environments, but it difficult to use them because they are specific to a particular analytic system (Hipsey et al. 2008). These models are also used to forecast the concentration of pathogen in sediments due to settling of microorganism in the bottom of the water (Romero et al. 2006).

Microbial pollution analysis in different water environments use to simulate many other microbial models (transport, source tracking and survival) (Brookes et al. 2005). Hipsey made a process-based model of microbial pollution used on fresh and seawaters (Hipsey et al. 2008). The model designs monitoring programs and check the difference between microbial species from various literature articles. Model simulations showed that differences in the fate (for example growth or decay rate) strongly influenced the concentrations of different species in water in the days after the discharge (Hipsey et al. 2008). Therefore, process models are important in assessing the level of contamination of pathogenic bacteria in fresh and seawater. In this thesis, we adopt regression-based models and basic statistics in Excel as stated in De Brauwere (2014) review.

With notion of obtaining and linear fitting, a first order linear equation model and coefficient of determination (R^2) are used to interpret and validate the results of bacteria logarithm FIB concentration as the output. The values of decay rate are simple the linear function of the Logarithm FIB concentration versus time frame which is limited by the experiment budget available.

2.4.1 Decay rate (k_t) of faecal indicator bacteria in water

k_t, Decay rate or mortality rate (k_d) or die-off rate or dark-death rate is a parameter, which shows the dynamic process of microorganisms in surface and coastal waters. Large variations of in the values of mortality rate (Auer & Niehaus 1993; Mayo 1995) are due to the difference in environmental conditions where those results came from. Decay rate calculations have been used for modeling of FIB because it is important for predicting the fate of FIB in the environment.

Harriete Chick (1908) postulated that first-order kinetics equation that considers mortality of bacteria versus time data can be represented as a straight line on a semi-logarithmic graph (Chick 1908). The decay rate coefficient is analyzed by using the data and results from the laboratory experiments. Light and temperature affects the decay rate in biological model processes (Gourmelon et al. 2010).

Variation on decay rates values have to do with the difference environmental conditions the waters are subjected to. Hipsey (2008) makes a collation of several literature data sources and gives the best estimates of decay values. The decay rate of FIB in coastal waters is normally expressed T_{90} which is the time the concentration of bacteria takes to decrease by one log unit (Gourmelon et al. 2010). First order kinetic model developed by Chick (1908) uses integration of decay rate equation to explain the decay model of FIB. By suggesting the use of first order inactivation model of virus, Friborg used equation 2.5 to 2.9 to show the decrease of concentration by 1 $\log_{10}$ (Friborg 2015).

Decay rate values and equations for *E. coli* and intestinal enterococci of different researches are shown in Table 2.6. Values of faecal indicators below were removed from several articles. (Hipsey et al. 2008; Johnson et al. 1997; Kim & Hur 2010; Walker & Stedinger 1999)

Table 2.6 Summary of the decay rate of faecal indicator bacteria in water.

Type of water	*E. coli*	*I. enterococci*	References	Remarks
	k_d (day^{-1})	k_d (day^{-1})		
Freshwater	0.48	0.45	(Walker & Stedinger 1999) and (King et al. 2005)	Temperature used 20^0C.
Seawater	1.09	0.28	(Johnson et al. 1997)	Temperature used in experiment 20^0C.
	0.258	0.276	(Kim & Hur 2010)	Originated from domestic sewage at 20^0C.

2.4.2 Equations for decay rate, log reduction and half life for estimation fate of FIB

There are man method to estimate the rate at which a bacteria die. Some of the most common methods are describe in equations bellow. These equations were adapted from literature are based in rate law in which the various experiment of the same sample and same temperature for each decay rate is used (Aus-e-Tute 1997; Chick 1908; De Brauwere et al. 2014b; Friborg 2015)

2.4.2.1 Equation for first order decay rate: Based on Concentration:

$$C_t/C_0 = 10^{-kt} \qquad \textbf{Equation 2.6}$$

Where: C_t: concentration of the bacteria in time t, C_0: concentration of bacteria at time 0 and t: number of days, t: is the time interval and k: First order decay rate,

2.4.2.1.1 Graphical Method:

$$Slope\ of\ the\ the\ graph = \frac{log_{10}(C_t) - log_{10}(C_0)}{t_f - t_0} \qquad \textbf{Equation 2.7}$$

The plot of mean log FIB concentration against time interval gives a straight line and this is the slope of the graph is the gradient of that line.

2.4.2.1.2 Decay Rate Method:

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \qquad \textbf{Equation 2.8}$$

$$log_{10}\frac{C_t}{C_0} = -\frac{kt}{\ln 10} \qquad \textbf{Equation 2.9}$$

$$-k = \frac{2.303}{t} * (log_{10}(C_t) - log_{10}(C_0)) \qquad \textbf{Equation 2.10}$$

2.4.2.1.3 Log-Reduction Method

$$T_{90} = \frac{ln10}{k} = \frac{2.303}{k} \qquad \textbf{Equation 2.11}$$

Friborg in his master thesis described T_{90} is the time needed to reduce 90% of microorganisms or "the time necessary for the bacteria counts to decrease by a factor of ten" (De Brauwere et al. 2014b). Whereby, T_{90} = 1 $\log_{10}$ reduction is equivalent to $C_t/C_0 = 0.1$

2.4.2.1.4 Half-Life Method:

$$t_{1/2} = \frac{0.693}{k} \qquad \textbf{Equation 2.12}$$

Where: Half-life, $t_{½}$: is the time taken for half the quantity of bacteria to be consumed. Half-life of a first order reaction is a constant, i.e., half-life is independent of initial concentration. k is the specific decay rate constant.

The aim of the literature review is to show evidence of the stances and theories evolving the study of fate of the fecal bacteria; Descriptions of fecal indicator bacteria and factors affecting the rate of decay of fecal bacteria; As well models for decay rate used to evaluates the fate.

3. Materials and Methods

This thesis used laboratory experiments to analyze the decay of FIB in water samples collected from different depths. Experimental methods included the standard membrane filtration method and the Colilert-18 and Enterolert-E tests.

Two phases of the laboratory work were performed in water laboratory Fløy V at NMBU. Preliminary experiments began in February 2015 to collect and analyze seawater and freshwater samples for FIB using the Colilert-18 for coliform and *E. coli,* and membrane filtration methods intestinal enterococci respectively. The main laboratory experiment was performed in June 2015 on seawater samples from different depths using Colilert-18 and Enterolert-E tests. There we used the lower temperature (4^0C) to check how is the decay rate of FIB so we can compare to the decay rate for the same bacteria at room temperature (22^0C).

3.1 Study Area

Seawater was collected in Drøbak, Norway because of its location along the Oslofjord, which extends 20Km south of Oslo. Although away from the main city of Oslo, Drøbak is a popular recreation area among local residents and is one of the 22 cities on the coast of Oslofjord (Figure 3.1). The geographical position of Drøbak beach allows road runoff – including road pollutants – to be washed down to the beach when it rains. The sewage pollution from Drøbak WWTP decreases the quality of bathing water, increases the risk of waterborne illness, and harms local marine life. During the summer season, large and small boats, swimmers, and aquatic bird species heavily use Drøbak harbor.

For the preliminary phase of laboratory testing, seawater was collected in early 2015 from Drøbak beach. Glomma River sample water was collected and used as practice with freshwater in the preliminary experiments. Freshwater samples have been stored in cold temperature at NMBU laboratory because it is part of a University ongoing project. Deep seawater (from 60m) and surface seawater (from 0.3m) samples were collected in June 2015 from NIVA's Marine Research Station in Solbergstrand to be used for the main experiments.

Additional water samples were taken of non-treated, combined sewage from Drøbak WWTP because the treatment facility is positioned very close to the recreation area. In the case of CSOs during heavy rainfall event and the WWTP gets overwhelmed, and the water could then be discharged directly to the beaches and surrounding water bodies. The experiments using

water from Drøbak were aimed at studying the concentrations of *E. coli* and intestinal enterococci bacteria found in sewage effluent.

Table 3.1 Inner Oslo Fjord. Area for collection of water and sewage Samples in Drøbak beach. Sources: https://no.wikipedia.org/wiki/Oslofjorden and http://kart.gulesider.no.

3.2 Water Samples

On February 12, 2015, samples of seawater and combined sewage were collected in Drøbak for later evaluating the fate of faecal bacteria in the laboratory. Raw wastewater was sampled at Drøbak WWTP and seawater was sampled from Drøbak beach. Both locations could be easily accessed for sampling. A swimmer's ramp at Drøbak beach allowed samples to be taken almost two meters beyond the shoreline in clear water without debris. At the WWTP, there was a collection point from which untreated influent sewage could be sampled. The water samples collected in Drøbak and the stored water from the Glomma River had temperatures of 7 and 10^0C, respectively. There was 4.6 l of water from each sample site available for testing (**Error! Reference source not found.**).

Table 3.2 Quantities and temperatures of water samples collect in Drøbak and Glomma.

Type of water	Quantity of water (L)	Temperature (C^0)
Freshwater	4.6	7
Seawater	4.6	10

Table 3.3 The seawater sampling point source for preliminary experiments on FIB. This is Drøbak beach in early spring. (Picture: Silva, 2015).

Drøbak beach (Table 3.3) is a very popular recreation area with local residents, children, and tourists who use it as a place to swim and recreate in Oslofjord. It can be considered contaminated, however, because effluent from boats and stormwater discharges end up in bathing zones.

Deep and surface seawater samples were collected at Solbergstrand on June 8, 2015 because conditions were more similar to those during the summer bathing season. A surface water sample from 0.3 m depth and a deep-water sample from 60 m depth were pumped from the same water tank at Solbergstrand. Both seawater samples were taken immediately to the NMBU (Fløy V) water laboratory for the biological and chemical analyses. Analyzing samples from both deep and surface seawater was needed to measure the decay of FIB at different temperatures and depths.

3.3 Methodology for Analyze of Water in Laboratory

Quantitative methods in water analysis have been developed to quantify the posing effects of diseases and oxygen depletion in receiving water bodies Wastewater treatment plants discharge infectious microorganisms into lakes, river and oceans which affect the quality of water. The methods are also used to evaluate the demand of oxygen in water and degree of treatment needed for sewage water before entering water bodies' recipients.

Biological, chemical and physical analyses can be used in the laboratory to evaluate the decay rate of bacteria over time (Pepper et al. 2006). In this thesis, the effects of sunlight were excluded by storing the water samples inside dark bottles.

The established results and the decay rate calculations were assumed by the following (Crane & Moore 1986; Davies et al. 1995; Hipsey et al. 2008): Some growth or a stationary period in the bacteria population may occur before they begin to die. Day 3 of the main experiment was used as the end of the experiment, but in reality, there was or could have expected some activity in water until all the process is completed. If not that, at least it may have been some residual bacterial population, which could have been calculated, in more strict research. The decay rate coefficient is the slope of the logarithm concentration of FIB against the time in days. For the results, it was important to separate de decay into lag phase and differential decrease in bacterial population.

3.4 Methods for enumerating *E. coli* and *I. enterococci* in water

When pollution affects the water, the same bacteriological methodologies described in Standard methods for the examination of water and wastewater can be applied to both freshwater and seawater. Although bacteriological methods in Part 900 of Standard methods for the examination of water and wastewater (Rice & Bridgewater 2012) were mainly developed for efficient and rapid testing, they are also valuable techniques in water treatment and sanitation research. Analyses of both freshwater and seawater samples can be made using membrane filtration, Enterolert-E, and Colilert-18. Colilert-18 is a rapid test that requires only few hours to get results. Membrane filtration is a longer process because it requires more than 48 hours to indicate the presence of (only) *enterococci* bacteria and an additional two hours to confirm *Intestinal enterococci* bacteria colonies. Enterolert-E gives faster results; 24 hours are required to check for the presence of intestinal enterococci (IDEXX-QC enterococci). All of these methods provide the independent variable or parameters that are the basis for evaluating the decay rate of bacteria populations.

Enumerations of bacteria obtained from the above-discussed methods are used as indicators to confirm the presence of pathogenic microorganisms in water samples and to demonstrate their decay over time. Once test results are documented, graphs can be made to help analyze and interpret the results. Even with these proven tests, however, accurately quantifying bacterial populations can be difficult because of errors in measurements and sample contamination.

3.4.1 Membrane Filtration

Membrane filtration (NS–EN ISO7899-2: 2000) was the methodology used for *I. enterococci*. Based on the Laboratory Handbook (NMBU 2015), the research on biological factors inside a water laboratory was permitted so long as safety measures are taken while handling potentially harmful microorganisms, media, and reagents used in experiments. No risk assessments were performed for these methodologies, but a Level 1 risk was assumed based on contamination or infection hazard for dealing with FIB. Other working standards for health and safety were considered based on the same handbook.

3.4.2 Enterolert-E

IDEXX laboratories explains:

> "Enterolert-E uses a Defined Substrate Technology® (DST®) nutrient indicator to detect enterococci. This nutrient indicator fluoresces when metabolized by

enterococci. DST improves accuracy and avoids the need for hazardous sodium azide suppressants used in traditional media. The Enterolert®-E Test was developed for the European market and correlates with the EU Bathing Water Directive standard method for enterococci, ISO 7899-1" (IDEXX Laboratories 2015).

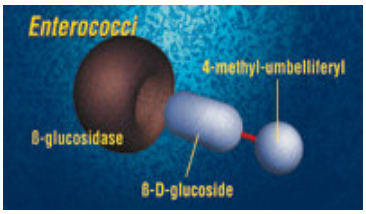


Figure 3.3a

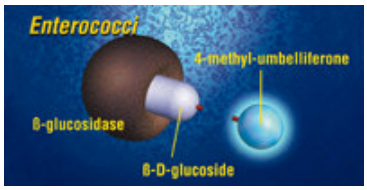


Figure 3.3b

Table 3.4 a and b Diagram showing how Enterolert-E works.
Source:https://www.idexx.com/water/products/colilert.html

These enumerated bacteria obtained from the above-discussed methods are used as indicator organisms to show decay rate in a given number of days and to confirm the presence of pathogenic microorganisms in the actual water sample. Once the experiments are complete and data taken, test results are documented; calculations and graphs are made; and finally, the results are discussed accordingly. But there are some limitations to consider, for example that it is difficult to quantify the bacteria in general even though there have been many studies in relation to fecal bacteria and the authenticity of the test is well proved.

3.4.3 Colilert-18

Colilert-18 is a multi-well enzyme substrate test made for testing the presence of total coliform bacteria and *E. coli* bacteria. The Quanti-tray wells fluoresce under UV light because of the chemical reaction between the test's growth medium and *E. coli* enzymes. Colilert-18 gives results after Quanti-trays have been incubated for 18 to 22 hours at 36 to 37^0C, making it a rapid method for detecting Total coliform (yellow counts) and *E. coli* (fluorescence counts) bacteria in water.

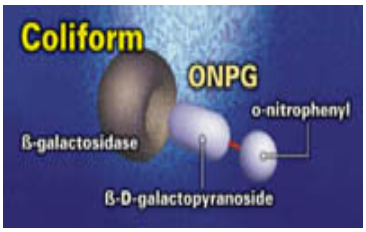


Figure 3.4a

Figure 3.4b

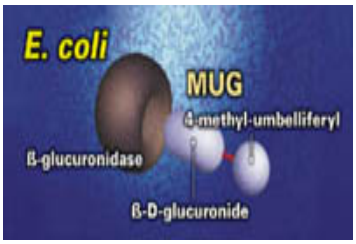


Figure 3.4c

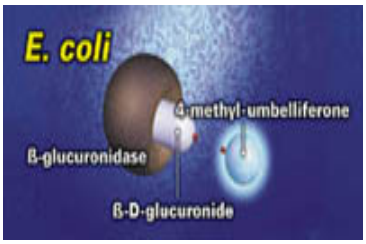


Figure 3.4d

Table 3.5 a, b, c and d Diagrams showing how colilert-18 works.
Source:https://www.idexx.com/water/products/colilert.html

In Figures 3.5 a to b above, the transition of the coliform from colorless to yellow is demonstrated. This colour change occurs when β-galactosidase enzymes metabolize the ONPG nutrient indicator, changing the coliform to a yellow colour. Figures 3.5 c and d show how *E. coli* transitions from colourless to florescent. This colour change occurs because *E. coli* uses β-galactosidase enzymes to metabolize the MUG nutrient indicator, resulting in fluorescence when UV light hits the sample (IDEXX Laboratories 2015). In practice, the bacteria from water samples react noticeably and significantly with the Colilert-18 test medium.

3.5 Preliminary Laboratory Experiments and Data Analysis

For this experiment, six mixed-water samples were analyzed for the presence of transmissible pathogens. Different pathogens and bacteria were expected in these samples because of the variety of wastewater entering the WWTP.

The Standard Methods Most Probable Number (MPN) model and tables were used to determine the values of coliform faecal units (CFU) in 100 mL of water sample (American Public Health et al. 2005). The aim of this biological process was to find the concentration of CFUs using counts of *E. coli* and intestinal enterococci in the Quanti-trays and plate dishes for both freshwater and seawater water samples. Colitert-18 method and Membrane filtration as prescribed in Standard methods for examination of water and wastewater (Rice & Bridgewater 2012), and methodologies based on IDEXX-Colilert and NS-EN ISO 7899-2:2000.

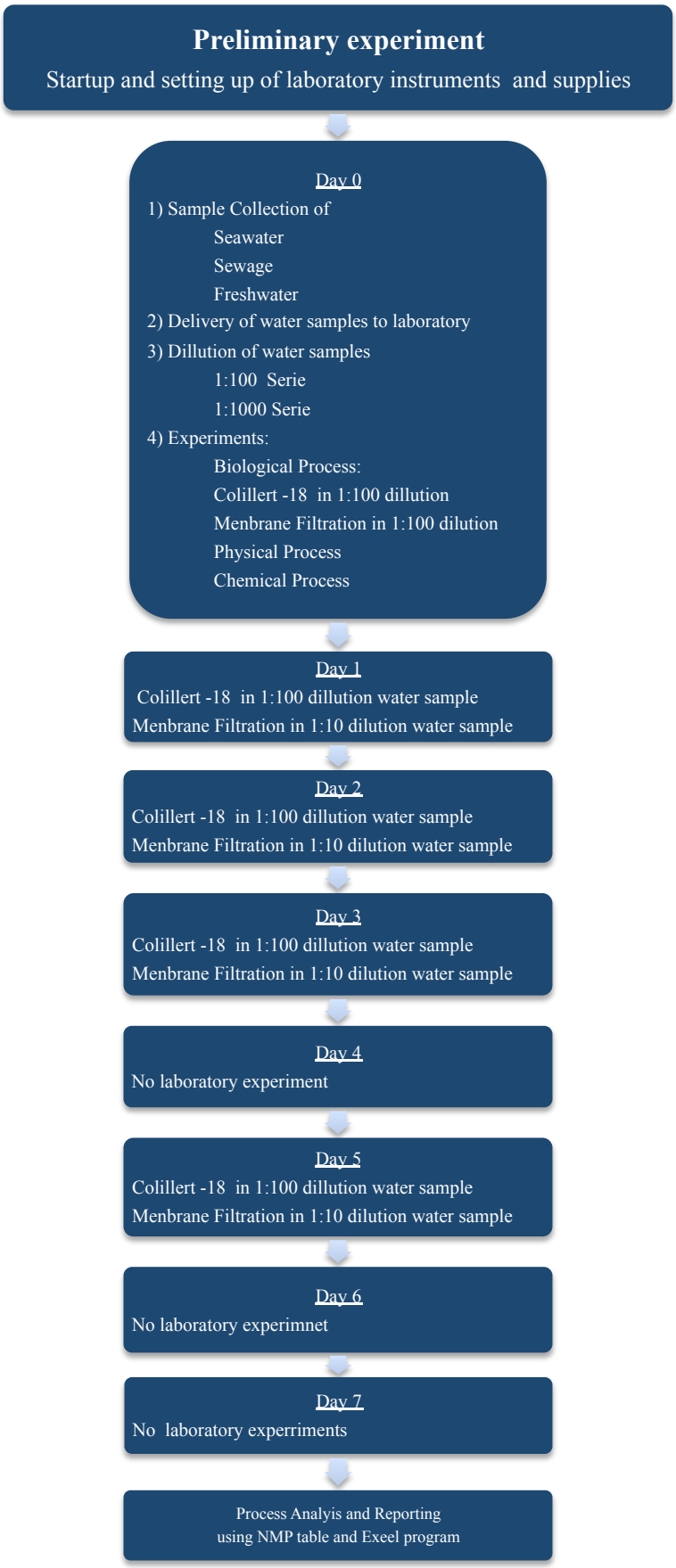


Table **3.6** Preliminary experiment flow chart include experiment start up, collection, dilution and laboratory experiment levels. (Silva, 2015)

Table 3.6 illustrates the methodology applied, including sample collection, preparation, and dilution as well as the experiments and data recording processes. Before analysis was started, the laboratory instruments and supplies had to be set up. Preliminary work included checking for calibration, sanitizing the study area, and sterilizing glassware needed in the experiments.

3.5.1 Collection, Preparation and Dilution Process of Water Samples

Materials used for the collection and preparation of water samples included two 5 L plastic bottles, one 2 L plastic bottle, two 5 L plastic buckets, and 8 2 L brown glass bottles.

The following water samples were used in the experiment:

1. Freshwater collected from Glomma River
2. Seawater collected from Drøbak beach
3. Wastewater collected water from Drøbak WWTP

The dilution process, however, used raw (untreated) wastewater from Drøbak WWTP, Glomma freshwater, seawater from Drøbak beach, and distilled water. The same procedure is used for the seawater samples were prepared by mixing 900 mL of seawater with 100 mL inlet wastewater in two 2 L brown glass bottles. One bottle was kept in cold environment at 4^0C and the other was kept at room temperature (20^0C). These water samples were kept in dark glass bottles away from light and at a stable temperatures (4^0C and 22^0C). The same routine was repeated for three Glomma freshwater samples.

Using the samples from both cold and room temperature environments, each sample was diluted into four 100 mL sub-samples for use in the *E. coli* experiment. The dilution series method was used to dilute two samples each of 1 mL and 10 mL sample water with four 90 mL units of distilled water. These four diluted samples waters were kept for the *E. coli* counting experiment. The remaining water samples remained in two 1000 mL glass bottles to be analyzed in the following days.

The diluted samples containing mixtures with inlet wastewater, freshwater, and seawater were used for each day of the experiment. To obtain 1:10 dilution samples, 10 mL of each water sample type was poured into 90 mL of distilled water. Using the mixed water samples, the 1:100 dilutions was performed by removing 10 mL of this sample from the sample bottle and transfer to the dilution tube containing already 90 ml of sterilized water. The same procedure

was applied to the rest of five samples, after the dilution was done, the solutions were transferred to the 100ml small bottles and filled the rest with distilled water.

3.5.2 Detection and enumeration of *E. coli* in freshwater and seawater using Colilert-18 test

For this experiment purposes, six water samples were analyzed for the presence of transmissible pathogens. Different pathogens and bacteria are expected in these water mixes because of the variety of wastewater entering the Drøbak WWTP.

The Standard Methods Most Probable Number (MPN) model and tables are used to determine the values of *E. coli*. This method requires a 100 ml of water sample and MPN tables followed by (American Public Health et al. 2005).

3.5.2.1 Materials

The list of materials used in the Colilert-18 test includes:

1. IDEXX Quanti Sealer Model 2X instruments (sealing machine)
2. IDEXX Quanti Sealer rubber board
3. UV lamp
4. Quanti tray: Plastic Pockets for incubation of water sample
5. Sterile disposable 100 ml bottles with white lids
6. Incubator at 35±0.5^{o}C (to keep the Quanti tray for 18 to 22 hours)
7. Reagents or nutrient substrates (ampoules – Colitert-18)
8. Distilled water
9. Mixed water samples
10. Pipette
11. Tubes for dilution
12. Two tables for conversion of number of bacteria to concentration values.

3.5.2.2 Procedure

This methodology was based on the Colilert-18 test kit method (IDEXX Laboratories 2015) All the control freshwater and seawater samples were kept at 4^{0}C and rest of the two each freshwater and seawater samples were kept at 22^{0}C .

a) Obtaining two type of diluted water samples

The two types of diluted water samples were used only for five days of testing. The 3 samples contained Glomma Freshwater plus inlet sewage from Drøbak WWTP and other 3 samples were made of seawater plus inlet sewage from the WWTP.

b) Mixing the Colilert-18 ampoules into water samples and sealing the Quanti-trays

One ampoule containing the chemical reagent Colilert-18 was added to a disposable bottle of diluted water sample. *E. coli* nutrient powder reagents for each Colilert-18 test were used in 100 ml of mixed water sample. Each bottle was gently shaken to combine the powder and water inside a small container. Afterwards, the mixtures were transferred to the plastic pocket of the Quanti-trays and placed in a rubber board on the IDEXX Quanti Sealer, before ensuring, the liquid was distributed evenly to all the pockets. In the IDEXX Quanti Sealer machine, the trays were sealed; this process only took a few seconds.

These trays were put in the incubator for 18 hours. The samples were removed from the incubator the next day to count the number of yellow-coloured and fluorescent (under UV light) pockets. Using the count and a reference table, the concentration values for all the samples could be interpolated.

The steps for the Colillert-18 and Quanti-tray enumeration for *E. coli* proceeded as follows:

Day 0 – Water samples were collected and diluted. For the first preliminary experiment, only one parallel for 1/100 dilution mixed sample and 1/1000 sample were done. According to the Colilert-18 test kit instructions, each water sample was poured into Quanti-trays and incubated at $35 \pm 0.5^{o}C$ until the next day.

Day 1 – Large and small wells exhibiting yellow pockets (for total coliform) or UV florescence (for *E. coli* bacteria) were counted and recorded using the method based on MPN/100 mL A new Colillert-18 test was performed to one control and two parallel groups for fresh and seawater using a 1:100 dilution. All trays were then incubated at $36 \pm 0.5^{o}C$ for 18 to 22 hours.

Day 2 – New laboratory experiments were conducted using the Colilert-18 method on the freshwater and seawater control groups and 2 of each samples each using 1:100 dilutions. The Quanti-trays from Day 1 were quantified and recorded.

Day 3 – The same procedure as Days 1 and 2 was followed and Quanti-trays from Day 2 were quantified and given MPN/100 mL values.

Day 4 – No new experiments were performed during this day, but the large and small wells from Quanti-trays prepared on Day 3 were counted and registered.

Day 5 – New Colilert-18 tests were performed in freshwater and seawater using 1:100 dilutions.

Day 6 – No experiments were performed this day**,** but Quanti-tray wells from the Day 5 experiment were counted and registered.

Day 7 – No experiments were performed and the no membrane filtration readings.

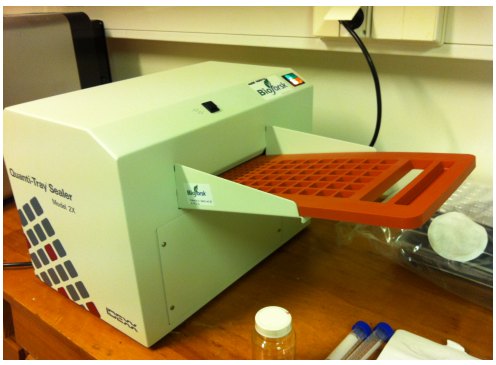


Table 3.7 IDEXX Quanti Sealer model 2X and rubber insert for Quanti-tray. (Picture: Silva, 2015)

Table 3.8 Quanti-trays, Colilert-18 ampoule, and disposable bottles are the materials used for the Colilert-18 test. Source:http://www2.idexx.com/view/xhtml/en_us/water/newsletter/201103.jsf

Table 3.9 Electronic Incubator set at 36±05 ^{o}C. (Picture: Silva, 2015)

Equipment and materials used for the Colillert-18 method are shown in Figures 3.2, 3.3 and 3.4 above. Figure 3.3 shows the rubber that holds the Quanti-tray in the IDEXX Quanti Sealer, while Figure 3.4 shows the material used to perform the test using the ampoules and 100 mL mixed water samples. These samples are then poured into Quanti-trays and sealed to stay in incubator at 36 ± 05^{o}C (Table 3.9) for 18 to 22 hours.

3.5.3 Detection and enumeration of *I. enterococci* in freshwater and seawater using Membrane filtration method

To determine the number of coliform faecal units (CFU) in 100mL of water sample, the colonies, which turned dark purple to confirm the presence of intestinal enterococci, in 100 mL water samples were put through a membrane filter with 0.45 µm pores. The composition of the membrane filters was mainly to remove contaminants in water. Filter materials are made of organic materials that are used to trap the microorganisms such living in the water. Filter paper mesh can be used to remove large suspended solids but membrane filters are very efficient into removing much smaller sizes smaller than 45µm. Such fine filters are able to retain even the smaller of microorganisms and organic mater (Rice & Bridgewater 2012). Once incubated the bacteria are inoculated onto the media and grow in the petri plates for two days. Afterwards they a re changed into a new media and incubated for another two hours in which should reduces significantly the number of pathogens in the water registered by the change in darker color of the colonies in the petri-dishes. This method required 48 hours of incubation at 36 ± 2^{0}C to check enterococci bacteria before transferring the membrane filter into a petri dish containing m-HPC agar and incubated to 44±0.5^{0}C to positive *I. enterococci.*

3.5.3.1 Materials

1. Three freshwater samples and three seawater samples
2. Distilled water
3. Membrane filter apparatus according to ISO8199
4. Membrane filters gridded at 0.45μm
5. Autoclave keeping a temperature of 121 ± 3^0C
6. Thermostatic Incubator keeping a temperature of 36 ± 2^0C and 44 ± 0.5^0C
7. Membrane filter instrument
8. Vacuum pump
9. Sterile medium petri dishes
10. m-HPC agar plates dishes
11. Sterile tweezers

3.5.3.2 Procedure

According to NS EU ISO 7899, Part 2 enumerating and isolating intestinal enterococci can be done using membrane filtration that removes micro-contaminants in water. Filters are made of organic materials that trap microorganisms and organic particles that cannot permeate the small pores of the filter media. Filter paper mesh can be used to remove large suspended solids, but membrane filters can efficiently remove particle sizes smaller than 0.45 μm. The filtered material can be placed into petri plates with m-HPC agar media using sterilized tweezers. In this experiment, the plate dishes containing bacterial colonies were put upside down inside the incubator for approximately two days. Afterwards, they were transferred into new petri dishes with the same media and incubated for another two hours. During this time period, a darker colour of the colonies in the petri dishes indicated a significant increase in the number of pathogens in the water. All procedures for preparation, filtration of water samples, and autoclave the membrane filtration instruments were according to the instructions in ISO 8199 and NS-EN ISO 6887-1.

Day 0 – All six water samples were prepared and diluted two samples, one at 1:100 and the other at 1:1000. Thereafter, the samples were tested and incubated at 36 ± 2^0C for 48 hours.

Day 1 – Three freshwater and three seawater samples with a 1:100 dilution were tested and incubated at 36 ± 2^0C for 48 hours.

Day 2 – The first samples (from Day 0) were removed from incubator after 48 hours and Counted of the dark purple colonies of bacteria from the Day 0. After removing the filter membrane from the petri dishes using the tweezers, the colonies were transferred to a new agar dishes. The dishes were inverted to incubate at 44 ± 0.5^0C for 2 hours to obtain the number of positive colonies for intestinal enterococci. New experiment was performed and expected from incubator in the day 4.

Day 3 – *Enterococci* bacteria from the Day 1 were removed, incubated again, and the confirmed *I. enterococci* dark purple colonies were counted. And in this day the new, bacteriological experiments were performed the same as the other days.

Day 4 – No experiment were performed. Only analyze of confirmation of *I. enterococci* from waters samples of the day 2.

Day 5 – Because fewer than 10 colonies had developed since Day 3, the experiment was stopped after day 3. *I. enterococci* colonies from Day 3 were counted and registered.

Day 6 – No experiment were performed**.**
Day 7 – No experiment were performed**.**.

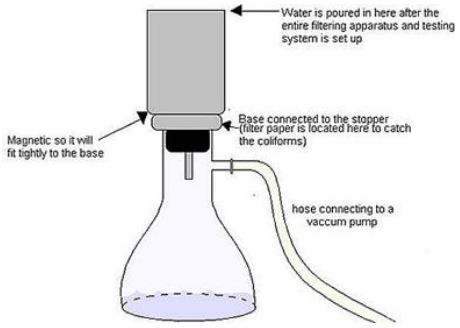


Table 3.10 Labeled drawing of membrane filtration apparatus.
Source:http://water.me.vccs.edu/courses/env211/lab10_print.htm

3.5.4 Chemical and Physical material and methods for freshwater and seawater

The COD test is used to measure microorganism contamination in water. Simple and rapid methods perform to the test for the chemical and physical parameters we used. Spectrometer and pH meters were used for this laboratory experiment.

3.6 Main Experiment

This experiment involved combined wastewater and seawater samples to evaluate the decay rate of *E. coli* and intestinal enterococci bacteria from Day 0 to Day 3. Because the wastewater was a potential pathogen contamination hazard, protective equipment was used during the experiment and NMBU laboratory regulations guided health and safety practices (NMBU 2015).

The hypothesis was that the number of bacteria in the mixed water samples would decline significantly after 5 days, making the water safe to use for bathing purposes. Deep and surface seawater results were expected to give different curves for the decay rate of bacterial populations. The water samples were stored at 4 and 22^0C.

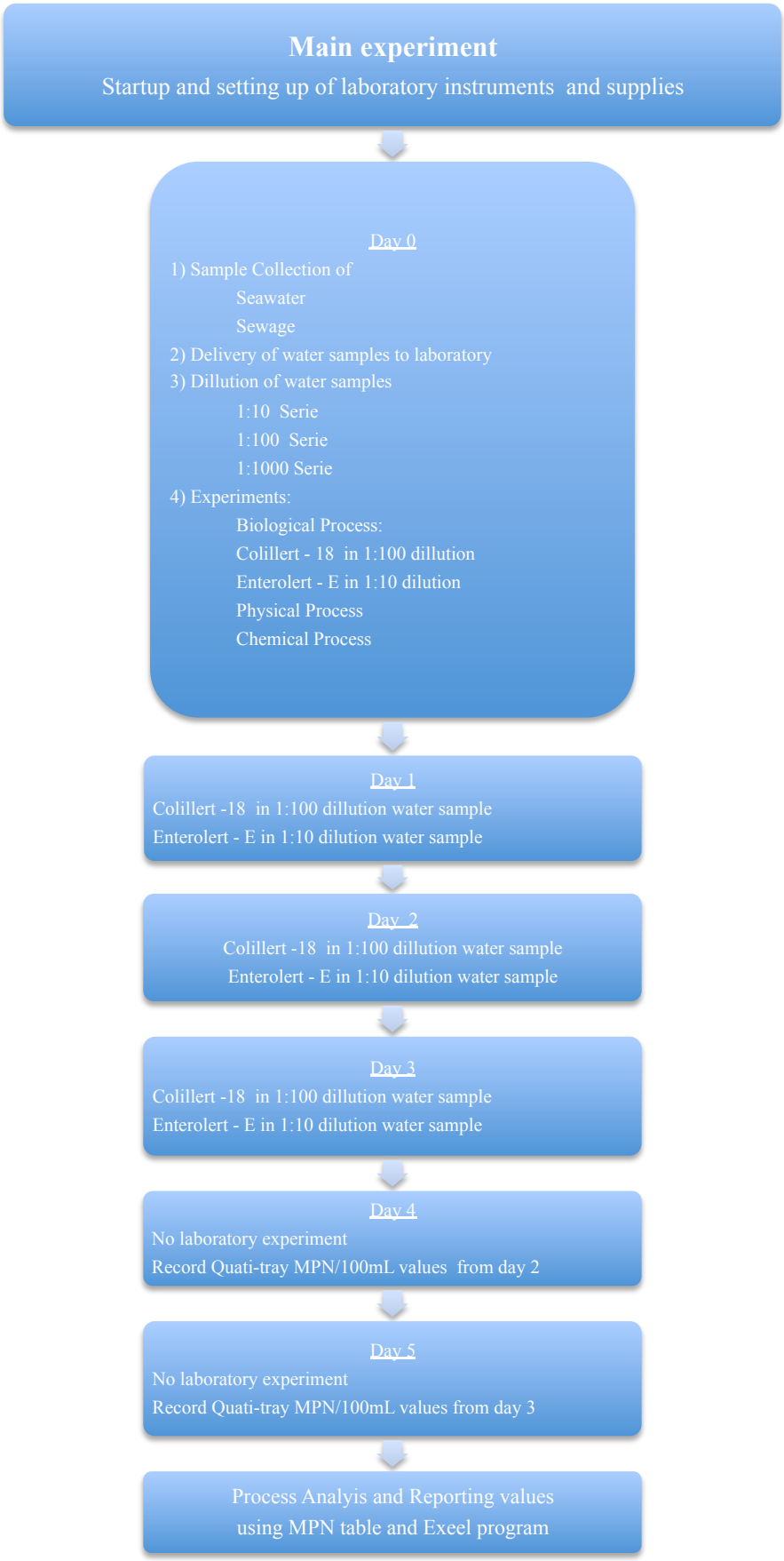


Table 3.11 Designed Main experiment flow chart. From the Start-up to end of the Laboratory experiment levels. (Silva, 2015)

3.6.1 Materials and methods main experiment

3.6.1.1 Preparation and dilution process of water samples

Seawater samples, in 2 10 L containers (one for deep water and other for surface water), were taken immediately to the NMBU Fløy V Laboratory. 1.5 L of raw wastewater from Drøbak WWTP was collected and transported as well.

3.4.3 Enumeration of *E coli* in Deep and surface seawater at 4^oC and 22^oC

Colilert -18 method

In the main experiment, 6 bottles of mixed water samples were analyzed for the decay of faecal bacteria in seawater. Two types of mixed water samples were used for three days of testing. The first samples contained deep seawater plus raw wastewater from Drøbak WWTP, and the other samples were made of surface seawater plus raw sewage from the Drøbak WWTP. The aim of this method was to enumerate *E. coli* in the mixed samples of deep seawater and surface seawater using the Colilert-18 method. These counts were to be used to analyze the relationship between bacteria survival at deep and surface seawater at different temperatures.

3.6.1.2 Materials

Materials used in the main experiment were the same as in Section 3.3.1 for Colilert-18 test.

- 6 dark bottles for the experiment
- 48 Colilert
- 48 Quanti trays
- 48 small plastic bottles for mixing samples

3.6.1.3 Procedure

During Days 0, 1, 2, and 3, twelve parallels of water samples for deep water and surface seawater were analyzed and enumerated as per (IDEXX Laboratories 2015). The same procedure for the Colilert-18 test (Section 3.3.2) was used for the microbiological analysis:

Day 0 – 3 samples with deep seawater and 3 with surface seawater mixed samples were analyzed and incubated at 36 ± 0.5^0C for 18 to 22 hours.

Days 1, 2 and 3 – All 12 samples, as described in in Section 3.3.2, were analyzed.

3.6.2 Enumeration of *I. enterococci* in deep and surface seawater at 4°C and 22°C

3.6.2.1 Materials

6 dark bottles

48 ampoules of Enterolert-E

48 Sterile disposable 100mL bottles

Incubator set at 41 ± 0.5^{0}C

3.6.2.2 Procedure

After the mixing and dilution procedure, the samples were placed in Quanti-tray. These trays were taken to the incubator for 18 to 22 hours, and then the samples were removed from the incubator so the number of pockets that were yellow or fluoresced under UV light could be counted. Using these numbers and a table, the values for the concentration of all the samples could be interpolated.

Day 0 – 1:10, 1:100 and 1:1000 dilution samples were tested and incubated.

Day 1 – One sample of deep seawater control sample plus two parallels of the same water. One sample of surface seawater for control sample plus two surface seawater.

Day 2 – All six samples from Day 1 were removed from the incubators and counted for florescence in Quanti-tray pockets. New samples were tested and incubated at 41 ± 0.5^{0}C for the next 18 to 22 hours to obtain the number of wells testing positive for intestinal enterococci.

Day 3 – Reading bacteria from the Day 2 sample group were counted. And the same procedure as the days before.

Day 4 –Reading of results from day 3.

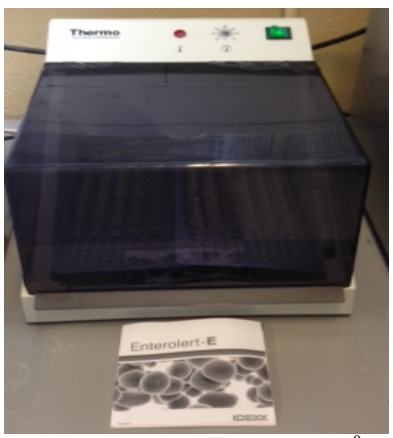


Table 3.12 Incubator machine with Quanti-trays at 41 ± 0.5^{0}C for 18 to 22 hours.

E. coli and *I enterococci* bacteria results from the figures below indicate concentrations that corresponded to the number of Quanti-tray pockets with yellow coloring or UV fluorescence.

3.6.3 Statistics method

The logarithm average method (Equation 3.2) was used to calculate the geometric mean of the number of bacterial colonies. The data for each of the experiments was tabulated and the mean for each of the values was taken.

$$\boldsymbol{Mean\ log_{10} = \frac{\sum log_{10\ of\ sample\ parallel}}{n}} \qquad \textbf{Equation 3.1}$$

Where: n is the number of sample parallels for each day of the experiment.

The calculations for mean (Equation 3.2) and standard deviation (Equation 3.3) apply to the Enterolert-E method.

$$\boldsymbol{SD = \sqrt{\frac{\sum(x - x_{avg})^2}{n}}} \qquad \textbf{Equation 3.2}$$

Where SD is the standard deviation in each day of the experiment using sample parallels; x is the $\log_{10}$ of MPN/100 mL value; X_{avg} is the mean $\log_{10}$ of MPN/100 mL; n is the number of sample parallels for each experiment.

3.6.4 Chemical and physical equipment for deep and surface seawater and freshwater

Table 3.13 Instrument to measure temperature and pH measurements in pH meter. (Elinga, 2015)

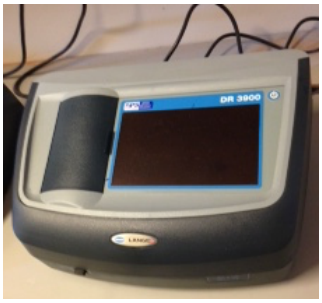


Table 3.14 Spectrometer is a rapid and simple instruments used to measure Total-p, Total-N, COD and of water samples mixed with respective reagents. (Picture: Elinga, 2015)

3.7 Ethical Considerations

The first location for seawater sampling in Drøbak was a public beach with no constraints to access, even in winter season. Local residents near the beach live a good distance from the collection point, so they were not likely to be impacted by sampling. Permission was not required to use this water taken from this public beach. Freshwater from the Glomma River was available in the laboratory, and permission was granted to the thesis co-supervisor for use in these experiments.

The research area in Solbergstrand is used for scientific purposes and is owned by NIVA. Supervisor Ingun Tryland arranged all the permissions needed for obtaining samples for this thesis project.

3.8 Limitations

Freshwater samples from the Glomma River were used for practice in the preliminary experiment and could not be relied upon for acceptable results in the main experiment since the water have been stored for more than six months.

The limitations in the lack of a biochemical marker to separate non-pathogenic microorganisms from pathogenic strains and the relationship between serotype and pathogenicity are still questionable for both seawater and freshwater. However, primarily it is because studies have shown that E. coli was a much better indicator of disease risk than was Fecal Coliform, EPA (1986) has recommended that E. coli be used as criteria for classifying waters for fresh water contact recreation (Rice & Bridgewater 2012)

4. Results

In this section, all the results are presented and interpreted to express the decay rate of *E. coli* and *I. enterococci* bacteria. First, I will present the graphical results of the slope of logarithm MPN or CFU versus time for preliminary and Main experiments. Then, the results the decay rate were selected as average of logarithm values of the concentration, based on linear regression. For deep and surface seawater the results are recorded in tables 4.2 and 4.3. Finally, the results of the Chemical and physical test of water samples are recorded in table 4.1 and 4.4. The equation below (Equation 4.1) is used to analyze the constant decrease in the graphs; we used the fit of the model curve and coefficient of determination to explain the behavior of data of the FIB. We can use these equations for the phases of the graphs. For all the stages of the curve slope for both *E. coli* and *I. enterococci*,

$$\textit{Slope of the graph} = -(\log_{10} Ct - \log_{10} Co)/(t_0 - t_1) \qquad \textbf{Equation 4.1}$$

Where: t_1: time at any point after the lag period and t_2: time at the end of the experiment. C_0 and C_t is the concentration of bacteria at initial time any time t and they are represented as Log_{10} (MPN/ 100mL).

The main values of the concentration of the FIB are given in logarithmic scale. Colillert-18 method Mean values of *E. coli* are presented in logarithmic scale in MPN/100mL versus the number of days used for the experiments. *I. enterococci* bacteria counts from the membrane filtration method are estimated as CFU/ mL in days and MPN/100mL for Enterolert-E test.

4.1 Preliminary Results – Freshwater and seawater at 22^0C

4.1.1 Total coliform for freshwater and seawater

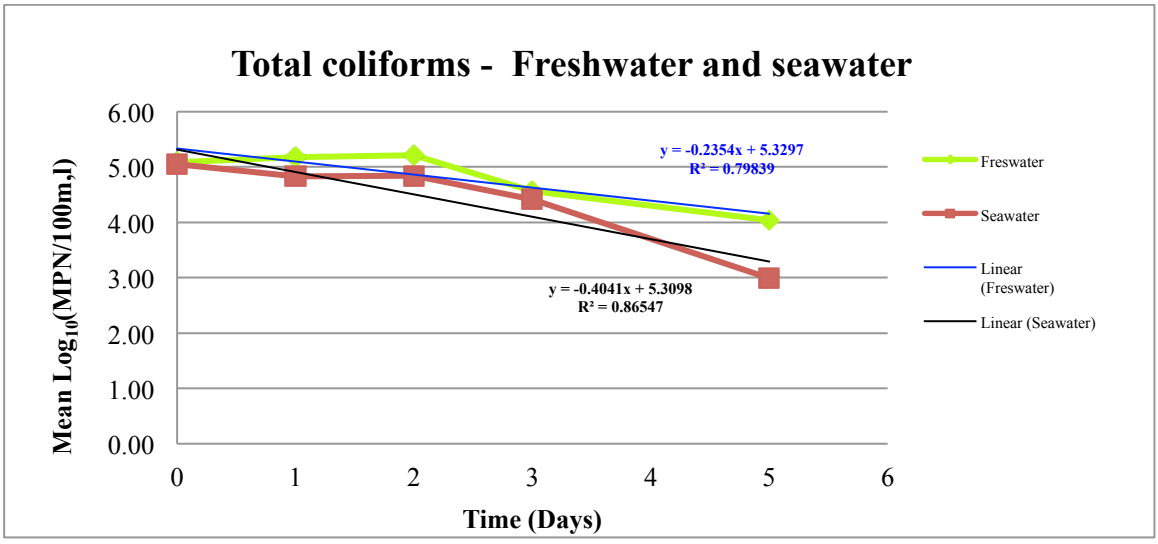


Table 4.1 Logarithm concentrations of *Total coliforms* in freshwater and seawater at room temperature 20 ^{0}C for preliminary experiment.

Results for water samples (Kept at 22^{0}C) were plotted in MPN/100mL of total coliforms per ml against the time for the bacteria to die-off over 5 days.

In total coliform counts, the mean logarithm initial concentration of bacteria was 5.08 log reduction (log_{10}) and decreased to 4.03 log_{10} after 5 days for freshwater sample. The freshwater sample linear graph did not show much change from day 0 to day 2. In fact, it seemed to increase suddenly by 0.2 log reduction for those two days. Thereafter, it declined another 0.7 log_{10} until day 5 (Table 4.1). Day 5 Quanti-trays had less than 20 large and 5 small positive yellow wells. With exception of sample 2, this did not show any changes in the results of large wells in Quanti-tray from day 1 (Table 8.1).

The seawater samples started with a mean value of 5.05 log_{10} (MPN/100mL). It only decreased 0.5 log reduction in the first three days, and in the next two days reduced 1.5 log_{10} The total reduction in concentration of the seawater bacteria is of 2 log reduction (Table 4.1). For the seawater day 5 results, total coliform yellow positive Quanti-tray wells were below 20 for large wells and below 10 for small wells (Table 8.2). No standard deviation was applied to these graphical analyses because the yellow count of total bacteria was only used for reference purposes.

4.1.2 *E. coli* in freshwater and seawater

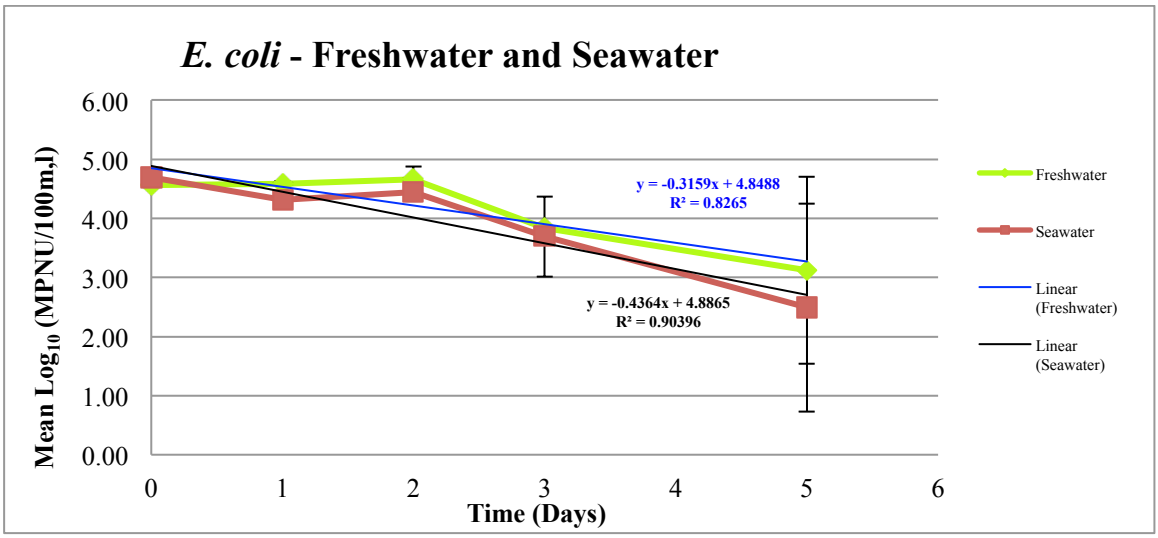


Table 4.2 Graph of logarithm concentrations of *Escherichia coli* in freshwater and seawater for preliminary experiment.

The number of positive wells, which shined when a UV lamp was reflected upon them, resulted into *E. coli* mean logarithm concentrations versus Time (days) (Table 8.2). The

number of freshwater bacteria rose slightly for 2 days before they began to die-off. During the following 3 days, they decreased to the mean value 3.12 $\log_{10}$ (Table 8.2). Seawater samples decayed more than 2 $\log_{10}$ during the five days. At first glance, the standard deviations are quite large, therefore this result is a poor representation of water sample values.

4.1.3 *Intestinal enterococci* using membrane filtration method

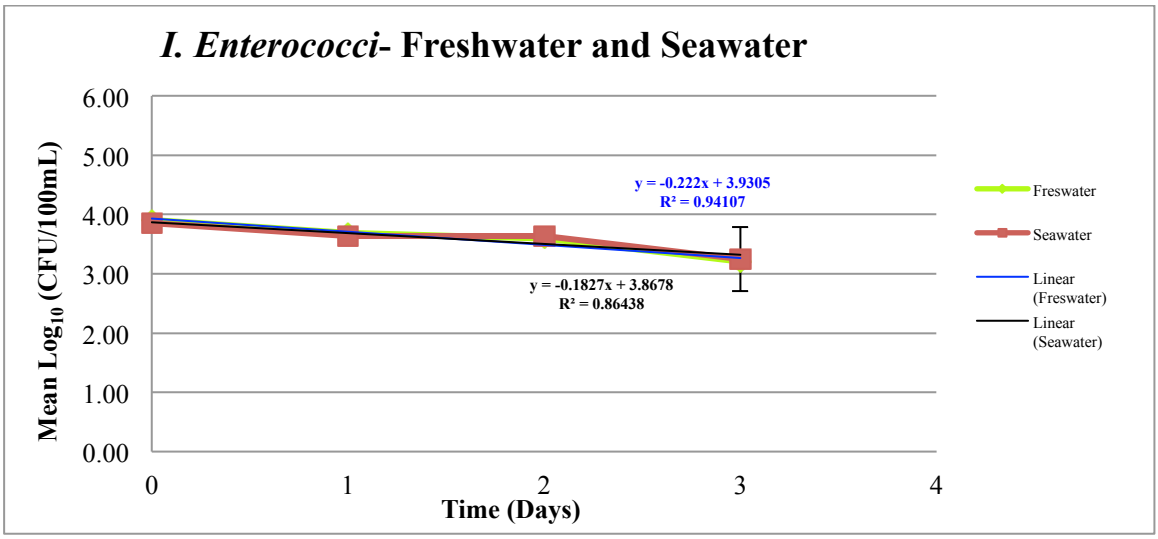


Table 4.3 Graph of logarithm concentrations of *Intestinal enterococci* bacteria in fresh and seawater water samples using membrane filtration method during three days of laboratory analysis**.**

The linear graphs in the Table 4.3 show the *I. Enterococci* bacteria results obtained by the membrane filtration method. The bacteria counts represent are the number of colonies faecal unit (CFU) per 100 mL of water sample. The intestinal bacteria colonies turned purple after the second incubation of two hours at 44±0.5^{0}C, as described in membrane filtration subsection in chapter 3. Freshwater starting value was 3.91 $\log_{10}$ and seawater was 3.96 $\log_{10}$. Freshwater and seawater samples for the *I. Enterococci* showed similar rates of decay and their slopes were almost parallel. This indicates that there was not much difference between the fates of the bacteria for the different types of water.

4.1.4 Results for chemical and physical analysis of freshwater and seawater

The chemical and physical properties of water samples used during the laboratory analysis were used to obtain readings of temperature, chemical oxygen demand (COD), total Phosphorous, total Nitrogen, electro-conductivity (EC), pH, turbidity and colour of both fresh and seawater samples (Table 4.4). Freshwater and seawater results are presented as concentrations of milligram per liter for COD, total Nitrogen and total Phosphorous in columns three, four and five. In those columns, the seawater values are larger than freshwater values.

Table 4.4 Chemical and physical parameters for freshwater and seawater measured in the first day of laboratory data collection.

Type of Waster sample	T	COD	Total N	Total P	EC	pH	Turbidity	Colour
	^{0}C	mg/l	mg/l	mg/l			FNU	
Freshwater	**7**	9,27	0,658	0,052	23 MS/cm	7,41	2,03	26mg/lpt
Seawater	**10**	165	0,997	0,073	24,81 mS/cm	7,84	1,59	17mg/lpt

COD: Chemical Oxygen Demand

EC: Electro Conductivity

T: Temperature

4.2 Main Experiment in Deep and Surface seawater

In 1908, Chick used Log-linear models to estimate the decay rate of FIB (Chick 1908). The bacteria experiments and analysis used different temperatures, namely 4°C and 22°C, for both deep and surface water samples. Decay rate results were compared and adjusted to the models at the two different temperatures and they showed a similar pattern between the deep and surface waters 24 hours detention methods for Colllilert-18 were used to test E. coli and Enterolert-E for *I. enterococci* using the both types of seawater samples (IDEXX Laboratories 2015; Rice & Bridgewater 2012; WHO 2006). Wastewater used for the mixture of water samples came from sewage from Drøbak WWTP.

4.2.1 Escherichia coli in deep and surface seawater at 4°C

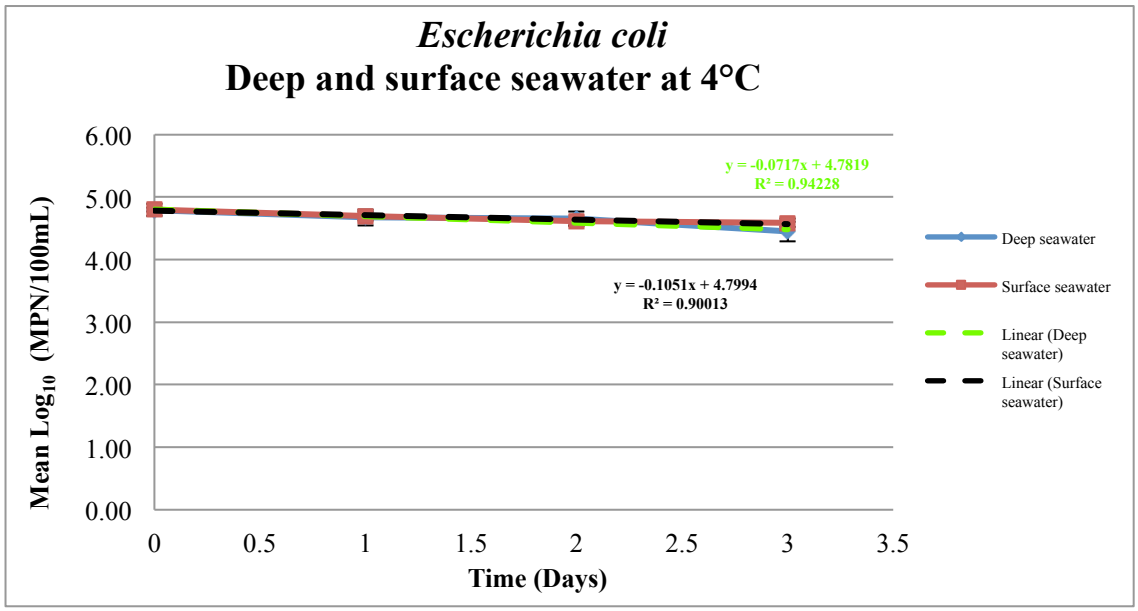


Table 4.5 Results of the fate of *E. coli* bacteria in deep and surface seawater at 4^{0}C

From day 0 to day1, the graph of *E. coli* experience a lag phase, which means that the bacteria seem to be stationary for the first day. The *E. coli* in surface seawater was less than 0.1 log reduction. The decay rate equal 0.162 day^{-1} for the *E. coli* surface water samples at 4°C temperature. While at same temperature, the deep seawater sample decay rate for *E. coli* was with 0.265 day^{-1} (Table 4.7). The graph shows that the numbers of *E. coli* bacteria dying were not significant at this low temperature during the experiment. Overall, *E. coli* in deep seawater was inactivated with 0.32 log_{10}. The main reasons for these discrepancies in the *E. coli* graph curves were not enough parallel samples to make a mean value with better-fit model and the accumulation of error in the experiment. However, with the number of bacteria decreasing faster, steeper decline in the slope graph for both deep and surface seawater was also implied. With an approximately linear graph compared to the deep seawater, the surface seawater samples of *E. coli* decayed circa 0.21 log_{10} for the tree days experiment (Table 4.5). This is a case of very small change between the *E. coli* initial concentration and the concentration at the end of the experiment.

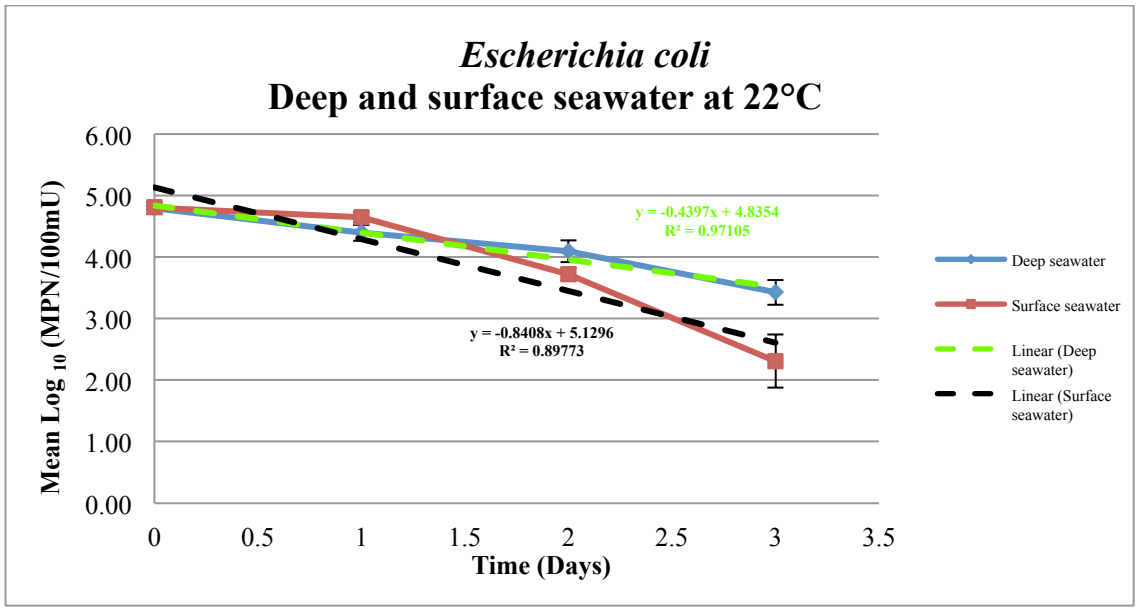


Table 4.6 Results of the fate of *E. coli* bacteria in deep and surface water at 22^0C during tree day's experiment using Solbergstrand sample seawater.

The *E. coli* behave differently at a temperature of 22°C, then at 4°C. For the *E. coli* in deep seawater, the value was of 1.46 log_{10} in just 3 days. While the surface samples started reducing as much as 2.49 log_{10} during the experiment. The highest *E. coli* in deep seawater decay rate was 1.049 day^{-1}, and the *E. coli* in surface decay rate was 1.914 day^{-1} (Table 4.7).

Results of the same type of water sample were not agreed if the storage temperature changes. In case of *E. coli* in the deep seawater slope graph at 4°C declined less than the *E. coli* at 22°C. This suggests that temperature played a role in decreasing the bacteria population. In the *E. coli* of the surface water sample at 4^0C showed little difference in decay rate from day 0 to day 3 of the experiment. When the water sample kept at 22^0C, the indicator bacteria decreased rapidly after two days at 2.5 $\log_{10}$.

Table 4.7 Results for the *E. coli* for the deep and surface seawater.

Type of water	Slope of the graph	Decay rate	Half time	Log reduction
	Mean Log reduction/day	k_t (day-1)	$T_{1/2}$ (days)	T_{90} (days)
Deep seawater 4°C	0,11	0,265	2,62	9
Surface seawater 4°C	0,07	0,162	4,27	14
Deep seawater 22°C	0,46	1,049	0,66	2,2
Surface seawater 22°C	0,83	1,914	0,36	1,2

4.2.2 Intestinal enterococci in deep and surface seawater

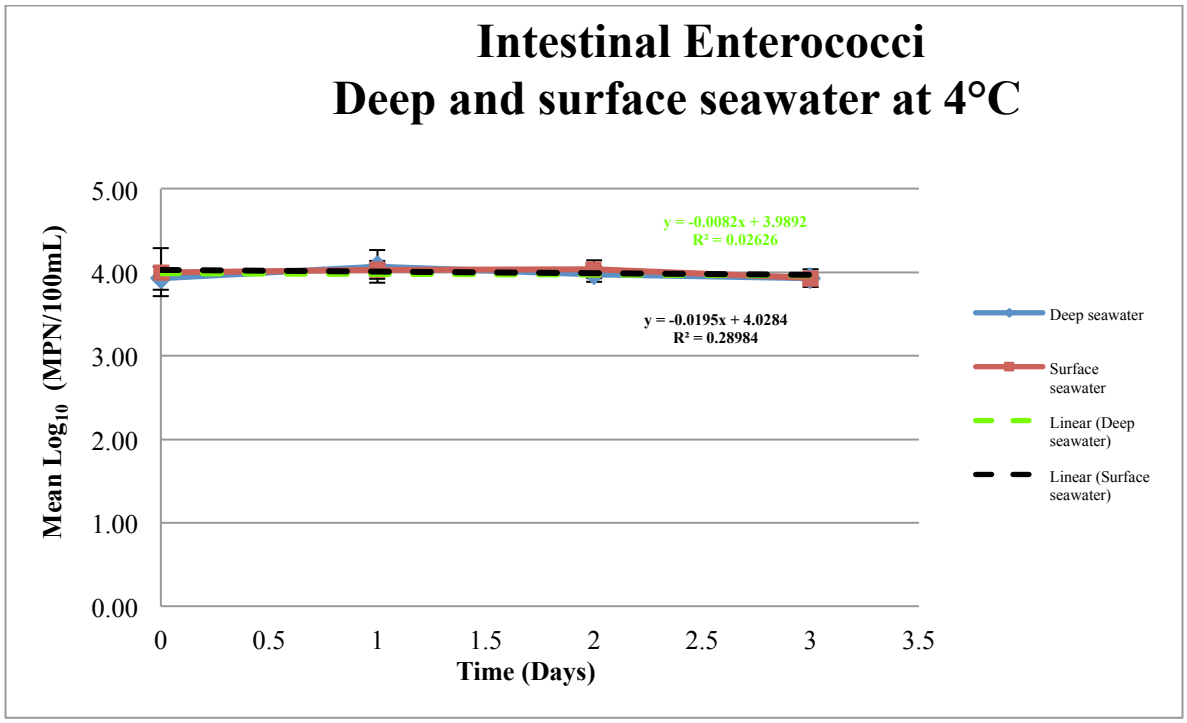


Table 4.8 Results of fate of *Intestinal enterococci* bacteria in deep and surface water at 4^0C.

Results for *I. enterococci* of deep and surface seawater graphs at 4^0C have similar pattern. They superimpose onto each other. They also both have very small decay rate values and to

be stationary during the whole experiment. This may be because the cold environment did not allow *I. enterococci* to die faster. A very small difference in the *I. enterococci* slope showed from the first day of the experiment to day 3, it was less than 0.1 $\log_{10}$. Standard deviations in the graph are very small, except in the beginning of the experiment.

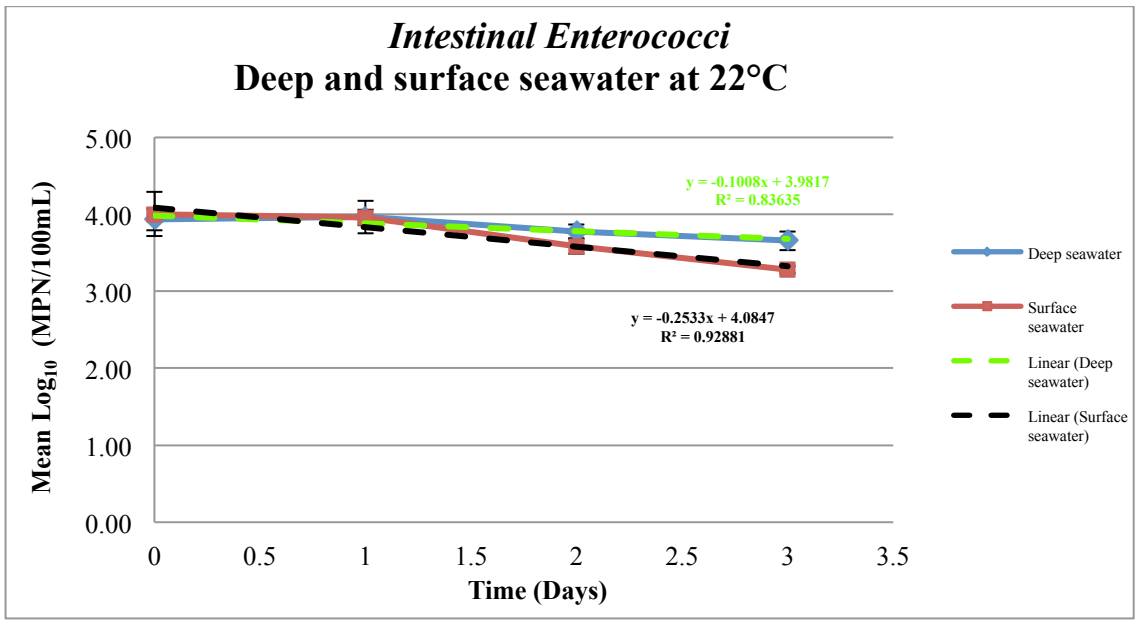


Table 4.9 Results of fate of *Intestinal enterococci* bacteria in deep and surface seawater at 22^0C

From day 0 to day 1, the bacteria experienced a lag phase and could have increase instead of decay. At 22^0C, the *I. enterococci* in deep and surface seawater samples, as showed in Table 4.9, reduced 0.2 $\log_{10}$ and 0.4 $\log_{10}$ after the first day of the experiment, respectively. Deep seawater at different temperatures (4 and 22^0C) showed small changes in the decay of the *I. enterococci*. While in the bacteria of the surface seawater samples, at some extent, only experience decay of *I. enterococci* after the day 1 of the experiment.

Table 4.10 Results for *I enterococci* in deep and surface seawater.

Type of water	Slope of the graph	Decay rate	Half time	Log reduction
	Mean Log reduction/day	k_t (day-1)	$T_{1/2}$ (days)	T_{90} (days)
Deep seawater 4°C	0,0	0	>100	>100
Surface seawater 4°C	0,023	0,052	13,37	44
Deep seawater 22°C	0,091	0,210	3,29	11
Surface seawater 22°C	0,240	0,553	1,25	4,2

4.2.3 Chemical and physical characteristics of seawater

Chemical characteristics of water samples for deep and surface seawater are shown in (Table 4.11). The test analysis results for COD would be within the range of 100 to 200 mg/L. Even though more stable water samples were used, still all the results were above 200 mg/L COD.

Table 4.11 Chemicals and physical parameters of deep and surface untreated seawater analyzed in first day of experiment.

Type of Waster sample	**COD**	**Total N**	**Total P**	**EC**	**pH**	**Turbidity**
	mg /l	**mg /l**	**mg /l**	**mS /cm**		**FNU**
Surface ocean water	909	N/A	N/A	19,72	7,2	1,92
Deep (60m) ocean water	2407	N/A	N/A	29,41	7,4	3,02
Surface ocean water with sewage	905	N/A	N/A	19,18	7,7	2,95
Deep (60m) ocean water with sewage	2460	N/A	N/A	28,67	7,7	3,1

NB: Surface Deep water is bellow 60m.water ranges (0-0.3m).

EC: Electro conductivity

Total P was out of the range so that the results are negative.

The main results and findings found above an the theory related to this thesis can be discussed in the chapter 5.

5. Discussion

In the thesis of the fate of faecal indicator bacteria, he more specific and stronger results given in the chapter 4 are discussed and thereafter, are also discussed. Therefore, the general implications of those results, the factors affecting these experiments are discussed. Whether they agree or not with peers review from previous studies cited in the introduction and Literature review.

5.1 Factors influencing FIB Results

The literature review discussed the main factors influencing fate of faecal indicator bacteria,, however many aspects of the research still remain to be discussed in this chapter. As shown in the literature review, when considering the results obtained from experiments the temperature, sunlight and salinity of the FIB seem most relevant for analyzing the fate of FIB in coastal waters. The selected factors were examined in the results and confirmed previous statements in literature review. Lower temperatures increased the survival period of the bacteria while higher temperatures seemed to increase the rate of death of the bacteria (Crane & Moore 1986). Temperature, in fact, is used as the controlling parameter and for storing the water samples (chapter 3). The results for *E. coli* and *I. enterococci* in colder temperature (4°C) demonstrated that the faecal bacteria can lived longer than the number of days of the experiments. While the FIB in seawater kept at warmer temperature (22°C) died faster. If we look at the cold temperature at Norwegian coastal waters in most of the year, it is possible to say that if FIB can survive longer, then maybe the pathogenic microorganisms also are affected by the cold temperatures living longer, which affect their fate in environmental water or at their host.

From the light deprived experimental conditions, the decay of the bacteria decreased at a more rapid rate for seawater than freshwater. This could have been because of the influence of other factors such as: salinity of seawater and temperature at which the water samples were kept. This was in favor of the s the experiment to avoid the die-off of the bacteria. From earlier studies, it is well proved that the presence of sunlight does increase the mortality of bacteria (De Brauwere et al. 2014a; Hipsey et al. 2008). Keeping the samples away from sunlight allowed us some time to analyze the decay of the bacteria.

Not enough data was gathered in this thesis to evaluate the optimum pH for the FIB. Instead they use pH=7 to incorporate in decay rate function by depriving the bacteria from solar

radiation. Almost any data is available to analyze pH and the only data used in those experiment form figure 6 were the ones calculated by the scientist themselves (Hipsey et al. 2008, Figure 6). pH values, for both experiments, varies from 7 to 8 which will give decay rate fraction close to 1 by adapting those values in Table 2.5.

5.2 Rapid Methods and their Effectiveness

Although many studies have revealed that changes in season affect the rate of decay of FIB (Crane & Moore 1986). The same was not shown, but not so significant, in the results of the decay rate of *E. coli* done from preliminary and main experiments. Reflection on effectiveness of different methods used has some impact on the trustfulness of the results.

In our study with FIB, the results of concentration of the bacteria in 100mL indicated that there is no significant difference between the results from deep and surface seawater. However, there was not enough funding to continue the experiments for more days and explore further the behavior or death of all faecal bacteria in water. After the preliminary results, with very few bacteria in the water sample, the supervisors decided to limit the test for three days. The agreed assumption was that after 3 days there was not enough data to calculate the concentration of the FIB in water for both the seawater and the freshwater samples. The purpose of the design of this study was to investigate the fate of faecal indicator bacteria in the Oslofjord. The main study used the decay rate values of *E. coli* and *I. Enterococci* at two different temperatures and water surfaces. Several other studies have also used similar approaches to evaluate the mortality of faecal indicator bacteria (Hipsey et al. 2008).

The sewage discharges into the freshwater bodies and ocean affect the quality of water at deep and surface levels, contrary to the statement that "the sewage system and size of the combined sewage overflows do not affect the microbiological quality of the discharged water during storm water events" (Rechenburg et al. 2006). What it is really important to focus on is how polluted or contaminated the CSOs entering the water bodies are. Boehm (2007) investigated how the variability of FIB can affect their concentration. Errors occurred in the calculations of FIB because it varies over a 24-hour period. To solve this problem, one could use rapid detection methods to reduce the presence of errors (Boehm 2007). Confirmation of the presence of *E. coli* and *I. enterococci* in water using Colilert-18 and Enterolert-E, respectively, is beneficial to the experiments described in chapter 3 Those are simple and fast

methods to enumerate bacteria in both fresh and seawater, since results can be obtained just after 20 hours.

5.3 Statistical analyze

Standard deviation (SD) of the experimental data can interpret the data and provide a form to check the results. So, "the smaller the SD the lower the uncertainty, thus providing more confidence in the experiment, and this increases the reliability of the experiment'' (Mendenhall & Sincich 2012). The standard deviations found in this experiment were less than 0.5 and the highest was the last experiment for surface water at 22^0C with SD = 0.4, that is only circa 40% of the value falling within the mean value and is fairly uniform, even though there were just two parallels used in the experiment in each day. A reason for these discrepancies could be that in the graph the curves did not to make a good fit. The accumulation of error in the experiment, may also contribute to such an effect. Besides this SD, the rest of the experiments gave smaller SD and we can conclude that the mean values of the experiments are viable. For all the experiments except *I. enterococci* at 4^0C, the log reduction was quicker in the end of the experiment than the days before and leads us to agree with the hypothesis that as the nutrient decreases this compromises the bacterial survival.

E. coli at 4^0C did not reduce in concentration since the log reduction after 3 days was less than 0.5. Deep seawater R^2= 0.942 and surface seawater R^2= 0.90 had both a good fit and linearity. Decay rate was: for deep seawater, k_4= 0.265 $day^{-1}$and surface seawater, k_4=0.162 day^{-1}. It was difficult to find information on decay rate at cold temperatures in the Literature from other peers. It may be because and like in our experiments, the decay rate at 4^0C did not chance much for the 3 days of experiment, therefore not very significant results.

Throughout the experiment, the concentration of the deep and surface seawater *E. coli* at 22^0C for deep and surface seawater decreased faster linearly than the waters at 4^0C. This implicates a stronger evidence of influence of temperature in fate of FIB. Deep seawater had a slightly better fit with model equation than E. coli kept at colder temperature (R^2= 0.97105) , but the opposite in surface seawater (R^2= 0.8977). Deep seawater, k_{22}= 1.049 day^{-1} and surface seawater, k_{22} =1.914 day^{-1}. Comparing those values with literature review (k= 1.09 and 0.258 at 20^0C) (Johnson et al. 1997; Kim & Hur 2010) we can see that one is close to our decay rate but the other is very low. This just confirms that results are variable depending where and when they obtained, regardless whether they had both use 20^0C in their experiments. As the

temperature increased (4 to 22^0C), we also saw an increase in the decay rate values of the E. coli curves in both deep and surface water.

For *I. enterococci* at 4^0C, it showed almost same decay rate during the main experiment. Deep seawater with k_4= 0 day^{-1} and R^2= 0.02626, which is close to zero. This value indicates poor fit and a curve with a very low linear relationship with the model equation. Surface seawater also had a small linearity and very small decay rate value, R^2= 0.28 and k_4 = 0.052$day^{-1,}$ respectively. *I. enterococci* at 22^0C. Deep seawater showed R^2= 0.864 and k_{22}= 0.210 day^{-1}, and Surface seawater R^2=0.929 with k_{22} =0.553 day^{-1}. Both of them indicate linearity and a good fit (R^2 close to 1) and much better decay rates than at colder temperature.

5.4 Implications of the results

In the study with faecal bacteria indicators, we want to use decay rates values into the models when there is discharges of sewage into the Oslofjord and using them to decide how long time, it would take for the recover of the water in fjord. Then the decay will be different depending on whether we put in cold or warm water closed environment (water samples flasks). This would have an effect in experiment for surface seawater if there was sunlight inactivation and increase of the temperature. But if we put in the deep seawater at cold temperature, the FIB will have long survival time, 9 days 2.5 days to reduce by 50%. If we put at surface water, which has high biological activity, then the decay is more rapid, only 1 day is need to get 1 $\log_{10}$. Therefore, the temperature effect is stronger when there is a combination of high temperature and surface, where the microorganisms also participate. But our results for the deep water did not show a significant difference at warmer temperature for *E. coli* , which led us to believe that maybe for the same bacteria there can be similar in the fate regardless I the sewage is discharged in deep or surface. Looking at decay rate values, there is a significant difference on the values for surface water at temperature of 4 and 22^0C, i.e. for 1 day they decay much faster that the same water at 4^0C that took 14 days, which is not entirely bad. *I. enterococci* followed the same, but it could take many days for the bacteria to die at cold temperature. This could not be so exact, since the values in the literature varies largely depend in theit experiments.

Results (Table 4.2 and 4.3) indicated that there was no significant difference between the deep and surface seawater at 4^0C for both *E. coli* and *I. Enterococci,* but a significant difference with more than 1 $\log_{10}$ for indicators at 22^0C. Unfortunately, due to lack of funds to extend the number of days and continue the experiments for more days and see further the behavior

of Faecal bacteria in water. The curve for *I. enterococci* at 4^0C did show a low value for R^2, which represents a poor fit of the slope of the curve to the linear model equation. The lag phase, which compromises from the start of the experiment with no change in rate of the bacteria, and the position where and the steep decline of the curve begins. The horizontal behavior of the curve is more evident for *I. Enterococci* that *E. coli.* The same was seen in other decay rate graphs where they seemed to have two distinguished phases (Crane & Moore 1986).

One of the main particularities in this study was to compare the results of the decay rate from FIB and the decay rate of same indicators of similar peer research. In the analysis of the decay rate of seawater samples we adopted the notion of first order decay rate as first designed by Chick (Chick 1908). The results reveal that the concentration of the fecal indicator bacteria are a function of time and can be modified by difference in temperature. From those results, it is possible to confirm decrease of FIB and get knowledge about environmental conditions, such as temperature affecting the fate FIB due to faecal pollution (Schulz & Childers 2011).

Shellenbarger proved in his 2008 article that during the summer season FIB concentration levels increase in water (Shellenbarger et al. 2008). Our results did not show seasonal variation in the quality of water, or significant differences at initial stages in the decay rate curves of the bacteria. They showed different behavior two days after having been put under the same conditions (warm or cold room). The results of different fate of FIB confirms that they can be largely different from each other (Shibata et al. 2004), and for this reason it is difficult to predict accurately how particular detrimental bacteria will behave in a natural environment.

Information still lacking in regard to how FIB die in water. A general acceptance that indicator bacteria and in particular, *E. coli* and *I. enterococci* will die-off even under the most polluted and variable environmental conditions (Nemerow 1991). Therefore, in the experiment to analyze the fate of FIB, it was expected to explain how they die. As well researched indicators, they also inform of the existence of other pathogens in the water. As discussed previously in chapter 1, Health problems due to poor water quality, poor sanitation and hygiene, and water contamination and pollution are extensive in many parts of the world. The faecal contamination of bathing waters and exposure to detrimental microorganisms lead to infectious diseases (Pond 2005; Shuval 2003). Those global issues impact recreational waters in many parts of the world. (WHO 2014).

In general, the *I. enterococci* persisted/survived longer in warmer water than *E. coli* in both preliminary and main experiments. No other factors were considered in besides temperature, sunlight deprivation and time during experiments. Biological microorganisms may compete the nutrients existent in water, and this may influence the fate of *E. coli* even though other environmental parameters are kept constant. For these reasons, like other studies, some of the results in our experiments could be untruthful (Korajkic et al. 2013). The presence of *I. enterococci* bacteria in water can be the explained by high probability of getting faeces from toilet visits, runoff from roads and animal droppings, and contaminated sewage water.

Large concentration values for the *E. coli* and *I. enterococci* indicated that the waters were highly contaminated during the laboratory procedure. The bacteria indicators of faecal bacteria are highly represented in all the water samples. Yellow colour and florescence had showed from all the Quanti trays until day 5, even though very few were confirmed, maybe because they wee so few that the test could not detect them. Mixed freshwater samples gave values above 10^4 in concentrations for each 100 ml for the first two days for dilution of 1/100 (Table 8.1 and Table 8.2), while the results of seawater samples by the same dilution showed mostly lower levels of presence faecal bacteria. So, both methods were quite effective and controlled the levels of bacterial decay. Bacterial concentration per 100 ml should have been reduced the amount of faecal bacteria in water considerably once the raw water samples were diluted several times. Instead, the opposite was observed with FIB around 5 $\log_{10}$ as starting point. Maybe due to the fact humans' faeces from the water collected in Drøbak WWTP are highly contaminated with bacteria and other pathogens, one continues to find intestinal bacteria in water. This is quite peculiar, because these bacteria should have been dissipated when diluted in water. My interpretation of the results and additional information from the laboratory analyzes and results should help to explain the fate of the bacteria from the beginning and the throughout the experiment. As seen from the results for both preliminary and main experiments, *E. coli* and *I. Enterococci* are very small number of MPN values just after three days and for the preliminary laboratory there almost no signs of positive results for *Escherichia coli* or *Intestinal Enterococci.* These results in the thesis can provide evidence to support my interpretation of researched fate of indicator bacteria in water.

5.5 Limitations

Errors in measurement and reading of bacterial colonies for *I. enterococci* and contamination of the Quanti-trays during the laboratory sections are blamed for any type of over or

underestimation in reading the positive wells. And therefore affecting the results obtained from the laboratory experiment**.** Standard deviation values were very large due to the fact that just two parallels were used for the mean and they enabled elimination of large errors encountered in laboratory procedure

5.6 Recommendations

Future research studies could use the results and information of this thesis to expand the knowledge on FIB in seawater due to CSOs. Modern, efficient and rapid methods open the possibility to explore Fib at the atomic level and get results even more viable.

6. Conclusion

The presence of bacteria indicators indicates the presence of faecal microorganisms in sea and fresh water. This was confirmed by the tests made in laboratory. The fate of FIB was interpreted by the results as $\log_{10}$, or decay rate, or half time, and same time as T_{90} that also shows how much reduction of FIB occurs in water samples after a number of days.

Preliminary experiments

After completing the laboratory analysis and report the results, it is possible to conclude, from reading the graphs, that most of *E. coli* bacteria were reduced by 2 $\log_{10}$ in freshwater and even above 2 $\log_{10}$ for seawater samples from Drøbak beach. *I. enterococci* concentration values declined very slowly. The values of *I. Enterococci* ranged from 0.5 $\log_{10}$ for both freshwater and seawater samples in both experiments.

Although there was a visible difference in the results between the seawater and freshwater, the correlation between the results found in laboratory analysis and the literature reviewed are the following:

1. The decay rate of *Escherichia coli* bacteria in seawater is faster declining than in freshwater;
2. The fate of *Intestinal enterococci* is faster in seawater than in freshwater
3. The comparison of laboratory experiments will also indicate that *I. Enterococci* and *E. coli* did not reduce at same rate.
4. Temperature does have an influence in the survival rate of FIB.
5. Most of FIB will die-off after 3 days if the samples are left at room temperature.

The above correlations cannot be applied to all situations because the source of the FIB varies spatially and with time. In addition, there are also others environmental factors that must be considered when fate of FIB are being studied. It means that the knowledge on fate of FIB can be used to draw conclusions about possible outcomes in similar conditions in Oslofjord and therefore be applied to modeling processes such as transport of FIB in coastal waters and track sourcing of FIB in those waters.

Main Experiments

The fate of FIB in in Oslo fjord was confirmed that it will take at least 14 days before it reaches of 1-2 $\log_{10}$ in surface water FIB in relation to 9 days, which is the same as a halftime of 2.6 before we can reach 1 $\log_{10}$ in the deep seawater at 4^0C, while water samples kept at room temperature (22^0C) reached reduction up to 3 $\log_{10}$ after 2 to 3 days (Table 4.7 and Table 4.10).

Some of the gaps encountered in the peer journals are:

- Microbial hazard in recreational waters has not been a priority in most scientific articles in most of scientific articles related to water contamination from sewage and faeces (Pond 2005).
- Information regarding the microbiological quality of water is available to the public but much still needs to be done to spread awareness of the monitoring practices even further (Pond 2005).

The results of this thesis are not enough to evaluate the overall situation of the FIB in the coastal Drøbak area due to the simple fact that one grab of sample is not sufficient to explain the behavior of the waters and assess the microbial quality of the water in relation to health risks associated to the water. It would be wiser to take several samples after the occurrence of CSOs and test them as soon as possible with methods that are even more rapid.

The above correlations cannot be applied to all situations because the source of the FIB varies spatially and with time. In addition, there are also others environmental factors that must be considered when fate of FIB are being studied. It means that the knowledge on fate of FIB alone can be used to draw conclusions about possible outcomes in similar water conditions in Oslofjord. Maybe we can use results of the decay rate to get a more significant and scientific information about their fate in addition with modeling processes such as transport of FIB in coastal waters and track sourcing many other models requiring decay rate of FIB. Where it is possible

7. Bibliography

Abdelzaher, A. M., Wright, M. E., Ortega, C., Solo-Gabriele, H. M., Miller, G., Elmir, S., Newman, X., Shih, P., Bonilla, J. A. & Bonilla, T. D. (2010). Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. *Applied and environmental microbiology*, 76 (3): 724-732.

Alexander, M., Andow, D. A. & Gillett, J. w. (1986). Fate and movement of microorganisms in the environment. *Environmental Management*, 10 (4): 463-493.

Alkan, U., Elliott, D. J. & Evison, L. M. (1995). Survival of enteric bacteria in relation to simulated solar radiation and other environmental factors in marine waters. *Water Research*, 29 (9): 2071-2080.

American Public Health, A., Water Environment, F. & American Water Works, A. (2005). *Standard methods for the examination of water and wastewater*. 21st ed. ed. Washington: American Public Health Association.

Amisah, S. & Nuamah, P. A. (2014). Spatial and Temporal Variations in Microbiological Water Quality of the River Wiwi in Kumasi, Ghana. *Water Quality Exposure and Health*, 6 (4): 217-224.

Andersen, S. T., Erichsen, A., Mark, O. & Albrechtsen, H.-J. (2013). Effects of a 20 year rain event: a quantitative microbial risk assessment of a case of contaminated bathing water in Copenhagen, Denmark. *Journal of water and Health*, 11 (4): 636-646.

Anderson, K. L., Whitlock, J. E. & Harwood, V. J. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Applied and environmental microbiology*, 71 (6): 3041-8.

Arnesen, V. (2001). The pollution and protection of the inner Oslofjord: redefining the goals of wastewater treatment policy in the 20th century. *AMBIO: A Journal of the Human Environment*, 30 (4): 282-286.

Arnone, R. & Walling, J. (2007). Waterborne pathogens in urban watersheds. *Journal of Water and Health*, 5 (1): 149-162.

Ashbolt, N. J. (2004). Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*, 198 (1–3): 229-238.

Ashbolt, N. J., Schoen, M. E., Soller, J. A. & Roser, D. J. (2010). Predicting pathogen risks to aid beach management: The real value of quantitative microbial risk assessment (QMRA). *Water Research*, 44 (16): 4692-4703.

Aus-e-Tute. (1997). *Determining Rate Law* Available at: http://www.ausetute.com.au/ratelcal.html (accessed: 16, December 2015).

Bai, S. & Lung, W.-S. (2005). Modeling sediment impact on the transport of fecal bacteria. *Water Research*, 39 (20): 5232-5240.

Barcina, I., Gonzalez, J., Iriberri, J. & Egea, L. (1990). Survival strategy of Escherichia coli and Enterococcus faecalis in illuminated fresh and marine systems. *Journal of applied bacteriology. Oxford*, 68 (2): 189-198.

Beaudeau, P., Tousset, N., Bruchon, F., Lefèvre, A. & Taylor, H. D. (2001). In situ measurement and statistical modelling of Escherichia coli decay in small rivers. *Water Research*, 35 (13): 3168-3178.

Belkin, S. & Colwell, R. R. (2006). *Oceans and health: pathogens in the marine environment*: Springer.

Berge, J. A. (2011). *Overvåking av forurensningssituasjonen i Indre Oslofjord 2010*. Rapport (Fagrådet for vann- og avløpsteknisk samarbeid i Indre Oslofjord), vol. nr. 109. Oslo: Norsk institutt for vannforskning.

Bernacky, B. J., Gibson, S. V., Keeling, M. E. & Abee, C. R. (2002). Chapter 16 - Nonhuman Primates. In Quimby, J. G. F. C. A. M. L. W. (ed.) *Laboratory Animal Medicine (Second Edition)*, pp. 675-791. Burlington: Academic Press.

Blaustein, R. A., Pachepsky, Y., Hill, R. L., Shelton, D. R. & Whelan, G. (2013). Escherichia coli survival in waters: Temperature dependence. *Water Research*, 47 (2): 569-578.

Boehm, A. B. (2003). Model of microbial transport and inactivation in the surf zone and application to field measurements of total coliform in Northern Orange County, California. *Environmental Science & Technology*, 37 (24): 5511-5517.

Boehm, A. B. (2007). Enterococci concentrations in diverse coastal environments exhibit extreme variability. *Environmental Science & Technology*, 41 (24): 8227-8232.

Boehm, A. B., Griffith, J., McGee, C., Edge, T. A., Solo-Gabriele, H. M., Whitman, R., Cao, Y., Getrich, M., Jay, J. A., Ferguson, D., et al. (2009). Faecal indicator bacteria enumeration in beach sand: a comparison study of extraction methods in medium to coarse sands. *Journal of Applied Microbiology*, 107 (5): 1740-1750.

Bordalo, A. A., Onrassami, R. & Dechsakulwatana, C. (2002). Survival of faecal indicator bacteria in tropical estuarine waters (Bangpakong River, Thailand). *Journal of Applied Microbiology*, 93 (5): 864-871.

Borts, I. H. (1949). Water-borne Diseases. *American Journal of Public Health and the Nations Health*, 39 (8): 974-978.

Bradford, S. A., Morales, V. L., Zhang, W., Harvey, R. W., Packman, A. L., Mohanram, A. & Welty, C. (2013). Transport and fate of microbial pathogens in agricultural settings. . *Critical Reviews in Environmental Science and Technology*, 43: 777-893.

Bridle, H. (2014). Chapter Five - Optical Detection Technologies for Waterborne Pathogens. In Bridle, H. (ed.) *Waterborne Pathogens*, pp. 119-145. Amsterdam: Academic Press.

Brookes, J. D., Antenucci, J., Hipsey, M., Burch, M. D., Ashbolt, N. J. & Ferguson, C. (2004). Fate and transport of pathogens in lakes and reservoirs. *Environment International*, 30 (5): 741-759.

Brookes, J. D., Hipsey, M. R., Burch, M. D., Regel, R. H., Linden, L. G., Ferguson, C. M. & Antenucci, J. P. (2005). Relative Value of Surrogate Indicators for Detecting Pathogens in Lakes and Reservoirs. *Environmental Science & Technology*, 39 (22): 8614-8621.

Bui, Y. N. (2014). *How to write a master's thesis*. 2nd ed. ed. Los Angeles: Sage.

Burton, G. A., Gunnison, D. & Lanza, G. R. (1987). Survival of pathogenic bacteria in various freshwater sediments. *Applied and Environmental Microbiology*, 53 (4): 633-638.

Byamukama, D., Kansiime, F., Mach, R. L. & Farnleitner, A. H. (2000). Determination of Escherichia coli contamination with chromocult coliform agar showed a high level of discrimination efficiency for differing fecal pollution levels in tropical waters of kampala, Uganda.(Statistical Data Included). *Applied and Environmental Microbiology*, 66 (2): 864.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R. & Harwood, V. J. (2012). Enterococci in the environment *Microbiology and Molecular Biology Reviews*, 76 (4): 685-706.

Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International journal of environmental research and public health*, 7 (10): 3657-3703.

Calderon, R. L., Mood, E. W. & Dufour, A. P. (1991). Health effects of swimmers and nonpoint sources of contaminated water. *International journal of environmental health research*, 1 (1): 21-31.

CANN, K. F., THOMAS, D. R., SALMON, R. L., WYN-JONES, A. P. & KAY, D. (2013). Extreme water-related weather events and waterborne disease. *Epidemiology & Infection*, 141 (04): 671-686.

Canteras, J. C., Juanes, J. A., Pérez, L. & Koev, K. N. (1995). Modelling the coliforms inactivation rates in the Cantabrian Sea (Bay of Biscay) from in situ and laboratory determinations of T 90. *Water Science and Technology*, 32 (2): 37-44.

Chamberlin, C. E. & Mitchell, R. (1978). A decay model for enteric bacteria in natural waters. *Water pollution microbiology*, 2: 325-348.

Charles, K. J., Souter, F. C., Baker, D. L., Davies, C. M., Schijven, J. F., Roser, D. J., Deere, D. A., Priscott, P. K. & Ashbolt, N. J. (2008). Fate and transport of viruses during sewage treatment in a mound system. *Water Research*, 42 (12): 3047-3056.

Charriere, G., Mossel, D., Beaudeau, P. & Leclerc, H. (1994). Assessment of the marker value of various components of the coli‐aerogenes group of Enterobacteriaceae and of a selection of Enterococcus spp. for the official monitoring of drinking water supplies. *Journal of applied bacteriology*, 76 (4): 336-344.

Chick, H. (1908). An investigation of the laws of disinfection. *Journal of Hygiene*, 8 (01): 92-158.

Chojnowski, K. J., Mancini, J. L. & Jeris, J. S. (1979). INFLUENCE OF LIGHT ON COLIFORM MORTALITY-RATES IN FRESH-WATER AND SEA-WATER. *Journal Water Pollution Control Federation*, 51 (10): 2538-2539.

CM, D., JA, L., M, D. & NJ., A. (1995). Survival of fecal microorganisms in marine and freshwater sediments. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY,*, 65 (5): 1888-1896.

Colford, J. M. J., Wade, T. J., Schiff, K. C., Wright, C. C., Griffith, J. F., Sandhu, S. K., Burns, S., Sobsey, M., Lovelace, G. & Weisberg, S. B. (2007). Water Quality Indicators and the Risk of Illness at Beaches With Nonpoint Sources of Fecal Contamination. *Epidemiology*, 18 (1): 27-35.

Colford Jr, J. M., Schiff, K. C., Griffith, J. F., Yau, V., Arnold, B. F., Wright, C. C., Gruber, J. S., Wade, T. J., Burns, S., Hayes, J., et al. (2012). Using rapid indicators for Enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Research*, 46 (7): 2176-2186.

Crane, S. & Moore, J. (1986). Modeling enteric bacterial die-off: A review. *Water, Air, and Soil Pollution*, 27 (3): 411-439.

Crowther, J., Kay, D. & Wyer, M. D. (2001). Relationships between microbial water quality and environmental conditions in coastal recreational waters: the fylde coast, UK. *Water Research*, 35 (17): 4029-4038.

Dale, K., Kirk, M., Sinclair, M., Hall, R. & Leder, K. (2010). Reported waterborne outbreaks of gastrointestinal disease in Australia are predominantly associated with recreational exposure. *Australian and New Zealand Journal of Public Health*, 34 (5): 527-530.

Dalton, C. F. (1930). Health Aspects of Summer Recreation. *American Journal of Public Health and the Nations Health*, 20 (7): 697-704.

Darakas, E. (2002). E. Coli Kinetics - Effect of Temperature on the Maintenance and Respectively the Decay Phase. *Environmental Monitoring and Assessment*, 78 (2): 101-110.

Davies, C. M. & Evison, L. M. (1991). Sunlight and the survival of enteric bacteria in natural waters. *Journal of Applied Bacteriology*, 70 (3): 265-274.

Davies, C. M., Long, J., Donald, M. & Ashbolt, N. J. (1995). Survival of fecal microorganisms in marine and freshwater sediments. *Applied and Environmental Microbiology*, 61 (5): 1888-1896.

Davies-Colley, R. J., Bell, R. G. & Donnison, A. M. (1994). Sunlight inactivation of enterococci and fecal coliforms in sewage effluent diluted in seawater. *Applied and environmental microbiology*, 60 (6): 2049-2058.

Davis, J., Pickering, A. J., Rogers, K., Mamuya, S. & Boehm, A. B. (2011). The Effects of Informational Interventions on Household Water Management, Hygiene Behaviors, Stored Drinking Water Quality,

and Hand Contamination in Peri-Urban Tanzania. *American Journal of Tropical Medicine and Hygiene*, 84 (2): 184-191.

de Brauwere, A., de Brye, B., Servais, P., Passerat, J. & Deleersnijder, E. (2011). Modelling Escherichia coli concentrations in the tidal Scheldt river and estuary. *Water Research*, 45 (9): 2724-2738.

De Brauwere, A., Gourgue, O., de Brye, B., Servais, P., Ouattara, N. K. & Deleersnijder, E. (2014a). Integrated modelling of faecal contamination in a densely populated river–sea continuum (Scheldt River and Estuary). *Science of the Total Environment*, 468-469: 31-45.

De Brauwere, A., Ouattara, N. K. & Servais, P. (2014b). Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review. *Critical Reviews in Environmental Science and Technology*, 44 (21): 2380-2453.

de Man, H., Heederik, D. D. J., Leenen, E., Husman, A. M. D., Spithoven, J. J. G. & van Knapen, F. (2014). Human exposure to endotoxins and fecal indicators originating from water features. *Water Research*, 51: 198-205.

Dufour, A. P. (1984). Bacterial Indicators of Recreational Water Quality. *Canadian Journal of Public Health / Revue Canadienne de Sante'e Publique*, 75 (1): 49-56.

Dufour, A. P. (1984). Health effects criteria for fresh recreational waters.

Dwight, R. H., Baker, D. B., Semenza, J. C. & Olson, B. H. (2004). Health Effects Associated With Recreational Coastal Water Use: Urban Versus Rural California. *American Journal of Public Health*, 94 (4): 565-567.

Dwight, R. H., Fernandez, L. M., Baker, D. B., Semenza, J. C. & Olson, B. H. (2005). Estimating the economic burden from illnesses associated with recreational coastal water pollution—a case study in Orange County, California. *Journal of Environmental Management*, 76 (2): 95-103.

Dziuban, E. J., Liang, J. L., Craun, G. F., Hill, V., Yu, P. A., Painter, J., Moore, M. R., Calderon, R. L., Roy, S. L., Beach, M. J., et al. (2006). Surveillance for waterborne disease and outbreaks associated with recreational water--United States, 2003-2004. *Morbidity and mortality weekly report. Surveillance summaries (Washington, D.C. : 2002)*, 55 (12): 1-30.

Ebentier, D. L., Hanley, K. T., Cao, Y. P., Badgley, B. D., Boehm, A. B., Ervin, J. S., Goodwin, K. D., Gourmelon, M., Griffith, J. F., Holden, P. A., et al. (2013). Evaluation of the repeatability and reproducibility of a suite of qPCR-based microbial source tracking methods. *Water Research*, 47 (18): 6839-6848.

Ekramul Hoque, M., Hope, V. T., Kjellström, T., Scragg, R. & Lay-Yee, R. (2002). Risk of giardiasis in Aucklanders: a case—control study. *International Journal of Infectious Diseases*, 6 (3): 191-197.

Elliot, E. L. & Colwell, R. R. (1985). Indicator organisms for estuarine and marine waters. *FEMS Microbiology Letters*, 32 (2): 61-79.

Engström, E., Thunvik, R., Kulabako, R. & Balfors, B. (2015). Water Transport, Retention, and Survival of Escherichia coli in Unsaturated Porous Media: A Comprehensive Review of Processes, Models, and Factors. *Critical Reviews in Environmental Science and Technology*, 45 (1): 1-100.

EPA, U. S. E. P. A. (2012). Recretional Water Quality Criteria , US Environmental Protection Agency. Office of water 820-F-12-058. Washington DC.

Eregno, F. E., Chong, Y. X. & Kitterød, N.-Ø. (2013). Modeling Hydrological impact of climate change. *International Journal of Climate*, 5 (3): 21.

Eregno, F. E., Nilsen, V., Seidu, R. & Heistad, A. (2014). Evaluating the Trend and Extreme Values of Faecal Indicator Organisms in a Raw Water Source: A Potential Approach for Watershed Management and Optimizing Water Treatment Practice. *Environmental Processes*, 1 (3): 287-309.

Feitosa, R. C., Rosman, P. C., Carvalho, J. L., Côrtes, M. B. & Wasserman, J. C. (2013). Comparative study of fecal bacterial decay models for the simulation of plumes of submarine sewage outfalls. *Water Science & Technology*, 68 (3): 622-631.

Ferguson, C., Husman, A. M. d. R., Altavilla, N., Deere, D. & Ashbolt, N. (2003). Fate and transport of surface water pathogens in watersheds.

Ferguson, C. M., Coote, B. G., Ashbolt, N. J. & Stevenson, I. M. (1996). Relationships between indicators, pathogens and water quality in an estuarine system. *Water Research*, 30 (9): 2045-2054.

Ferguson, D., Moore, D., Getrich, M. & Zhowandai, M. (2005). Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. *Journal of Applied Microbiology*, 99 (3): 598-608.

Flint, K. P. (1987). The long-term survival of Escherichia coli in river water. *Journal of Applied Bacteriology*, 63 (3): 261-270.

Folkehelseinstitutt. (2004). Vannkvalitetsnormer for friluftsbad. FHI Nasjonalt Folkehelseinstitutt. FRILUFTSBAD - BADEVANN: Vedlegg til Rundskriv IK-21/94. Erstatter "Kvalitetskrav til vann - badevann" revidert utgave fra nov. 1976. Gjeldende fra 1. juli 1994.

Food, U. & Administration, D. (2012). *Kinetics of microbial inactivation for alternative food processing technologies—overarching principles: kinetics and pathogens of concern for all technologies*.

Fox, J. G., Newcomer, C. E. & Rozmiarek, H. (1984). Chapter 22 - Selected Zoonoses and Other Health Hazards. In Loew, J. G. F. J. C. M. (ed.) *Laboratory Animal Medicine*, pp. 613-648: Academic Press.

Friborg, T. (2015). *Inaktivering av virus ved pasteurisering av svartvann fra Røde Kors' feltsykehus ; Inactivation of Viruses by Pasteurisation of Blackwater from Red Cross' Field Hospitals*: Norwegian University of Life Science, Ås.

Frommel, U., Bohm, A., Nitschke, J., Weinreich, J., Gross, J., Rodiger, S., Wex, T., Ansorge, H., Zinke, O., Schroder, C., et al. (2013a). Adhesion patterns of commensal and pathogenic Escherichia coli from humans and wild animals on human and porcine epithelial cell lines. *Gut Pathogens*, 5.

Frommel, U., Lehmann, W., Rodiger, S., Bohm, A., Nitschke, J., Weinreich, J., Gross, J., Roggenbuck, D., Zinke, O., Ansorge, H., et al. (2013b). Adhesion of Human and Animal Escherichia coli Strains in Association with Their Virulence-Associated Genes and Phylogenetic Origins. *Applied and Environmental Microbiology*, 79 (19): 5814-5829.

Fujioka, R., Sian‐Denton, C., Borja, M., Castro, J. & Morphew, K. (1998). Soil: the environmental source of Escherichia coli and enterococci in Guam's streams. *Journal of applied microbiology*, 85 (S1).

Fujioka, R. S., Hashimoto, H. H., Siwak, E. B. & Young, R. H. (1981). Effect of sunlight on survival of indicator bacteria in seawater. *Applied and environmental microbiology*, 41 (3): 690-696.

Gagliardi, J. V. & Karns, J. S. (2000). Leaching of Escherichia coli O157: H7 in diverse soils under various agricultural management practices. *Applied and environmental microbiology*, 66 (3): 877-883.

GARGANO, J. W., FREELAND, A. L., MORRISON, M. A., STEVENS, K., ZAJAC, L., WOLKON, A., HIGHTOWER, A., MILLER, M. D. & BRUNKARD, J. M. (2015). Acute gastrointestinal illness following a prolonged community-wide water emergency. *Epidemiology & Infection*, 143 (13): 2766-2776.

Gerba, C. P. & Pepper, I. L. (2004). 17 - Microbial Contaminants. In Brusseau, J. F. A. L. P. L. (ed.) *Environmental Monitoring and Characterization*, pp. 313-333. Burlington: Academic Press.

Gerba, C. P. (2009). Chapter 22 - Environmentally Transmitted Pathogens. In Gerba, R. M. M. L. P. P. (ed.) *Environmental Microbiology (Second Edition)*, pp. 445-484. San Diego: Academic Press.

Gerba, C. P. (2015a). Chapter 22 - Environmentally Transmitted Pathogens. In Pepper, I. L., Gerba, C. P. & Gentry, T. J. (eds) *Environmental Microbiology (Third edition)*, pp. 509-550. San Diego: Academic Press.

Gerba, C. P. (2015b). Chapter 23 - Indicator Microorganisms. In Pepper, I. L., Gerba, C. P. & Gentry, T. J. (eds) *Environmental Microbiology (Third edition)*, pp. 551-564. San Diego: Academic Press.

Gessner, K., Wichmann, G., Boehm, A., Reiche, A., Bertolini, J., Brus, J., Sterker, I., Dietzsch, S. & Dietz, A. (2011). Therapeutic options for treatment of Merkel cell carcinoma. *European Archives of Oto-Rhino-Laryngology*, 268 (3): 443-448.

Given, S., Pendleton, L. H. & Boehm, A. B. (2006). Regional public health cost estimates of contaminated coastal waters: A case study of gastroenteritis at southern california beaches. *Environmental Science & Technology*, 40 (16): 4851-4858.

Gizewski, E. R., Gasser, T., de Greiff, A., Boehm, A. & Forsting, M. (2003). Cross-modal plasticity for sensory and motor activation patterns in blind subjects. *Neuroimage*, 19 (3): 968-975.

González, J. (1995). Modelling enteric bacteria survival in aquatic systems. *Hydrobiologia*, 316 (2): 109-116.

Gordon, D. M. & FitzGibbon, F. (1999). The distribution of enteric bacteria from Australian mammals: host and geographical effects. *Microbiology*, 145 (10): 2663-2671.

Gourmelon, M., Lazure, P., Hervio-Heath, D., J.C. Le Saux, J. C., Caprais, M. P., Le Guyader, F. S., Catherine, M. & Pommepuy, M. (2010). Chapter: 16 Microbial modelling in coastal environments and early warning systems: useful tools to limit shellfish microbial contamination. 22.

Gray, N. F. (2010). Chapter 12 - Pathogens and Their Removal. In Gray, N. F. (ed.) *Water Technology (Third Edition)*, pp. 360-400. Oxford: Butterworth-Heinemann.

Gray, N. F. (2014). Chapter Thirty-Five - Filtration Methods. In Gray, S. L. P. V. Y. W. W. M. C. F. (ed.) *Microbiology of Waterborne Diseases (Second Edition)*, pp. 631-650. London: Academic Press.

Grebel, J. E., Mohanty, S. K., Torkelson, A. A., Boehm, A. B., Higgins, C. P., Maxwell, R. M., Nelson, K. L. & Sedlak, D. L. (2013). Engineered Infiltration Systems for Urban Stormwater Reclamation. *Environmental Engineering Science*, 30 (8): 437-454.

Green, N. W. S., M.;Øxnevad, S.,Ruus, A., Høgåsen,T., Beylich, B., Håvardstun, J., Rogne, Å.K.G.; Tveiten, L: . (2010). Long-term monitoring of environmental quality in Norwegian coastal waters. 287.

Griffin, D. W., Lipp, E. K., McLAUGHLIN, M. R. & Rose, J. B. (2001). Marine Recreation and Public Health Microbiology: Quest for the Ideal Indicator This article addresses the historic, recent, and future directions in microbiological water quality indicator research. *Bioscience*, 51 (10): 817-825.

Gronewold, A. D., Stow, C. A., Vijayavel, K., Moynihan, M. A. & Kashian, D. R. (2013). Differentiating Enterococcus concentration spatial, temporal, and analytical variability in recreational waters. *Water Research*, 47 (7): 2141-2152.

Hamilton, A. J., Stagnitti, F., Kumarage, S. C. & Premier, R. R. (2007). RIRA: A tool for conducting health risk assessments for irrigation of edible crops with recycled water. *Computers and Electronics in Agriculture*, 57 (1): 80-87.

Harm, W. (1980). *Biological effects of ultraviolet radiation*, vol. 12. United Kingdom: Cambridge University Press. 229 pp.

Heistad, A. (2012).

Hendricks, C. W. & Morrison, S. (1967). Multiplication and growth of selected enteric bacteria in clear mountain stream water. *Water Research*, 1 (8): 567-576.

Henry, A., Scherpereel, G., Brown, R. S., Baudart, J., Servais, P. & Charni Ben Tabassi, N. (2012). Comparison of Rapid Methods for Active Bathing Water Quality Monitoring. In *The Significance of Faecal Indicators in Water: A Global Perspective*, pp. 72-83: The Royal Society of Chemistry.

Hipsey, M., Romero, J., Antenucci, J. & Hamilton, D. (2004). Computational aquatic ecosystem dynamics model: CAEDYM v2. *Computer program manual). Centre for Water Research, University of Western Australia*.

Hipsey, M., Antenucci, J., Brookes, J. D., Hipsey, M., Antenucci, J. & Brookes, J. D. (2008). A Generic, process-based model of microbial pollution in aquatic systems. *Water Resources Research*, 44 (W07408): online - approx 5-20pp.

Hou, D., Rabinovici, S. J. M. & Boehm, A. B. (2006). Enterococci predictions from partial least squares regression models in conjunction with a single-sample standard improve the efficacy of beach management advisories. *Environmental Science & Technology*, 40 (6): 1737-1743.

Huffman, D. E., Quinter-Betancourt, W. & Rose, J. (2003). 12 - Emerging waterborne pathogens. In Mara, D. & Horan, N. (eds) *Handbook of Water and Wastewater Microbiology*, pp. 193-208. London: Academic Press.

Hunter, P. (1997). *Waterborne disease: epidemiology and ecology*: John Wiley & Sons.

IDEXX Laboratories, I. (2015). *Water Testing Solutions*. Available at: https://www.idexx.com/water/water-testing-solutions.html.

Imamura, G. J., Thompson, R. S., Boehm, A. B. & Jay, J. A. (2011). Wrack promotes the persistence of fecal indicator bacteria in marine sands and seawater. *Fems Microbiology Ecology*, 77 (1): 40-49.

Ismail, N. S., Dodd, H., Sassoubre, L. M., Horne, A. J., Boehm, A. B. & Luthy, R. G. (2015). Improvement of Urban Lake Water Quality by Removal of Escherichia coli through the Action of the Bivalve Anodonta califomiensis. *Environmental Science & Technology*, 49 (3): 1664-1672.

ISO 7899-2. ISO 7899-2, 2000. Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method. In: water quality. International Organization for Standardization, Geneva, Switzerland.

ISO 7899-2. ISO 7899-2, 2012. Enumeration of Escherichia coli and coliform bacteria – Part 2: Most probable number method. In: water quality. International Organization for Standardization, Geneva, Switzerland.

Janda, K. (2015). 6.13 - PETROLEUM PRODUCTS. In Falkiewicz-Dulik, M., Janda, K. & Wypych, G. (eds) *Handbook of Material Biodegradation, Biodeterioration, and Biostablization (Second Edition)*, pp. 257-375: ChemTec Publishing.

Johnson, D. C., Enriquez, C. E., Pepper, I. L., Davis, T. L., Gerba, C. P. & Rose, J. B. (1997). Survival of Giardia, Cryptosporidium, poliovirus and Salmonella in marine waters. *Water Science and Technology*, 35 (11–12): 261-268.

Johnston, K. K., Dorsey, J. H. & Saez, J. A. (2015). Stratification and loading of fecal indicator bacteria (FIB) in a tidally muted urban salt marsh. *Environmental monitoring and assessment*, 187 (3): 4314-4314.

Jones, S. (2009). 1 - Microbial contamination and shellfish safety. In Shumway, S. E. & Rodrick, G. E. (eds) *Shellfish Safety and Quality*, pp. 3-42: Woodhead Publishing.

Julian, T. R., Pickering, A. J., Leckie, J. O. & Boehm, A. B. (2013). Enterococcus spp on fomites and hands indicate increased risk of respiratory illness in child care centers. *American Journal of Infection Control*, 41 (8): 728-733.

Karanis, P. (2006). A review of an emerging waterborne medical important parasitic protozoan. *Jpn J Protozool*, 39 (1).

Karim, M. R., Manshadi, F. D., Karpiscak, M. M. & Gerba, C. P. (2004). The persistence and removal of enteric pathogens in constructed wetlands. *Water Research*, 38 (7): 1831-1837.

Kay, D., Jones, F., Wyer, M., Fleisher, J., Salmon, R., Godfree, A., Zelenauch-Jacquotte, A. & Shore, R. (1994). Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. *The Lancet*, 344 (8927): 905-909.

Kay, D., Bartram, J., Prüss, A., Ashbolt, N., Wyer, M. D., Fleisher, J. M., Fewtrell, L., Rogers, A. & Rees, G. (2004). Derivation of numerical values for the World Health Organization guidelines for recreational waters. *Water Research*, 38 (5): 1296-1304.

Kay, D., Stapleton, C. M., Wyer, M. D., McDonald, A. T., Crowther, J., Paul, N., Jones, K., Francis, C., Watkins, J., Wilkinson, J., et al. (2005). Decay of intestinal enterococci concentrations in high-energy estuarine and coastal waters: towards real-time T90 values for modelling faecal indicators in recreational waters. *Water Research*, 39 (4): 655-667.

Kay, D., Crowther, J., Davies, C., Edwards, A., Fewtrell, L., Francis, C., Kay, C., McDonald, A., Stapleton, C., Watkins, J., et al. (2012). Faecal Indicators and Pathogens: Expanding Opportunities for the Microbiology Community. In *The Significance of Faecal Indicators in Water: A Global Perspective*, pp. 1-17: The Royal Society of Chemistry.

Kay, D. & Fricker, C. (2012). *Significance of faecal indicators in water: a global perspective*. Cambridge, U.K.: Royal Society of Chemistry. 1 online resource (vii, 180 s.) : ill pp.

Kim, G. & Hur, J. (2010). Mortality rates of pathogen indicator microorganisms discharged from point and non-point sources in an urban area. *Journal of Environmental Sciences*, 22 (6): 929-933.

King, B. J., Keegan, A. R., Monis, P. T. & Saint, C. P. (2005). Environmental temperature controls Cryptosporidium oocyst metabolic rate and associated retention of infectivity. *Applied and environmental microbiology*, 71 (7): 3848-3857.

King, B. J., Hoefel, D., Daminato, D. P., Fanok, S. & Monis, P. T. (2008). Solar UV reduces Cryptosporidium parvum oocyst infectivity in environmental waters. *Journal of Applied Microbiology*, 104 (5): 1311-1323.

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: a review. *Environment International*, 30 (1): 47-55.

Knee, K. L., Layton, B. A., Street, J. H., Boehm, A. B. & Paytan, A. (2008). Sources of nutrients and fecal indicator bacteria to nearshore waters on the north shore of Kauai (Hawaii, USA). *Estuaries and Coasts*, 31 (4): 607-622.

Knee, K. L., Street, J. H., Grossman, E. E., Boehm, A. B. & Paytan, A. (2010). Nutrient inputs to the coastal ocean from submarine groundwater discharge in a groundwater-dominated system: Relation to land use (Kona coast, Hawaii, USA). *Limnology and Oceanography*, 55 (3): 1105-1122.

Kong, R., Lee, S., Law, T. & Law, S. (2002). Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. *Water Research*, 36 (11): 2802-2812.

Korajkic, A., McMinn, B. R., Harwood, V. J., Shanks, O. C., Fout, G. S. & Ashbolt, N. J. (2013). Differential Decay of Enterococci and Escherichia coli Originating from Two Fecal Pollution Sources. *Applied and Environmental Microbiology*, 79 (7): 2488-2492.

Lalancette, C., Papineau, I., Payment, P., Dorner, S., Servais, P., Barbeau, B., Di Giovanni, G. D. & Prevost, M. (2014). Changes in Escherichia coli to Cryptosporidium ratios for various fecal pollution sources and drinking water intakes. *Water Research*, 55: 150-161.

Layton, B. A., Walters, S. P., Lam, L. H. & Boehm, A. B. (2010). Enterococcus species distribution among human and animal hosts using multiplex PCR. *Journal of Applied Microbiology*, 109 (2): 539-547.

Lazarova, V., Savoye, P., Janex, M. L., Blatchley Iii, E. R. & Pommepuy, M. (1999). Advanced wastewater disinfection technologies: State of the art and perspectives. *Water Science and Technology*, 40 (4–5): 203-213.

Leiendecker, L. (2007). Evaluation Of Current Indicators Of Water Safety For Coastal Recreational Waters. Duke University.

Lipp, E. K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. R. & Rose, J. B. (2001). The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. *Estuaries*, 24 (2): 266-276.

Lipp, E. K., Lukasik, J. & Rose, J. B. (2001). Human enteric viruses and parasites in the marine environment. In vol. Volume 30 *Methods in Microbiology*, pp. 559-588: Academic Press.

Liu, L., Phanikumar, M. S., Molloy, S. L., Whitman, R. L., Shively, D. A., Nevers, M. B., Schwab, D. J. & Rose, J. B. (2006). Modeling the Transport and Inactivation of E. coli and Enterococci in the Near-Shore Region of Lake Michigan. *Environmental Science & Technology*, 40 (16): 5022-5028.

Lopez-Torres, A. J., Prieto, L. & Hazen, T. C. (1988). Comparison of the in situ survival and activity ofKlebsiella pneumoniae andEscherichia coli in tropical marine environments. *Microbial ecology*, 15 (1): 41-57.

Lugg, R. S. W., Cook, A. & Devine, B. (2012). Estimating 95th Percentiles from Microbial Sampling: A Novel Approach to Standardising their Application to Recreational Waters. In *The Significance of Faecal Indicators in Water: A Global Perspective*, pp. 62-71: The Royal Society of Chemistry.

Maier, R. M. & Pepper, I. L. (2015). Chapter 3 - Bacterial Growth. In Pepper, I. L., Gerba, C. P. & Gentry, T. J. (eds) *Environmental Microbiology (Third edition)*, pp. 37-56. San Diego: Academic Press.

Mallin, M. A., Williams, K. E., Esham, E. C. & Lowe, R. P. (2000). Effect of human development on bacteriological water quality in coastal watersheds. *Ecological applications*, 10 (4): 1047-1056.

Mancini, J. L. (1978). NUMERICAL ESTIMATES OF COLIFORM MORTALITY-RATES UNDER VARIOUS CONDITIONS. *Journal Water Pollution Control Federation*, 50 (11): 2477-2484.

Mancini, J. L. (1979). Influence of Light on Coliform Mortality Rates in Fresh Water and Sea Water.

Mansilha, C. R., Coelho, C. A., Heitor, A. M., Amado, J., Martins, J. P. & Gameiro, P. (2009). Bathing waters: New directive, new standards, new quality approach. *Marine Pollution Bulletin*, 58 (10): 1562-1565.

Maraccini, P. A., Ferguson, D. M. & Boehm, A. B. (2012). Diurnal Variation in Enterococcus Species Composition in Polluted Ocean Water and a Potential Role for the Enterococcal Carotenoid in Protection against Photoinactivation. *Applied and Environmental Microbiology*, 78 (2): 305-310.

Maraccini, P. A., Wang, D., McClary, J. S. & Boehm, A. B. (2015). Growth-dependent photoinactivation kinetics of Enterococcus faecalis. *Journal of Applied Microbiology*, 118 (5): 1226-1237.

Marino, R. P. & Gannon, J. J. (1991). Survival of fecal coliforms and fecal streptococci in storm drain sediment. *Water Research*, 25 (9): 1089-1098.

Masters, G. M. & Ela, W. P. (2014). *Introduction to environmental engineering and science*. Harlow: Pearson Education. II, 700 s. : ill. pp.

Mattioli, M. C., Pickering, A. J., Gilsdorf, R. J., Davis, J. & Boehm, A. B. (2013). Hands and Water as Vectors of Diarrhea! Pathogens in Bagannoyo, Tanzania. *Environmental Science & Technology*, 47 (1): 355-363.

Mattioli, M. C., Boehm, A. B., Davis, J., Harris, A. R., Mrisho, M. & Pickering, A. J. (2014). Enteric Pathogens in Stored Drinking Water and on Caregiver's Hands in Tanzanian Households with and without Reported Cases of Child Diarrhea. *Plos One*, 9 (1).

Mattioli, M. C. M., Davis, J. & Boehm, A. B. (2015). Hand-to-Mouth Contacts Result in Greater Ingestion of Feces than Dietary Water Consumption in Tanzania: A Quantitative Fecal Exposure Assessment Model. *Environmental Science & Technology*, 49 (3): 1912-1920.

Mattioli, M. C. M., Davis, J. & Boehm, A. B. (2015). Hand-to-Mouth Contacts Result in Greater Ingestion of Feces than Dietary Water Consumption in Tanzania: A Quantitative Fecal Exposure Assessment Model. *Environmental science & technology*, 49 (3): 1912-20.

Mawdsley, J. L., Bardgett, R. D., Merry, R. J., Pain, B. F. & Theodorou, M. K. (1995). Pathogens in livestock waste, their potential for movement through soil and environmental pollution. *Applied Soil Ecology*, 2 (1): 1-15.

McGuinness, M. (2012). Faecal Indicators in Drinking Water - Is It Time to Move On? In *The Significance of Faecal Indicators in Water: A Global Perspective*, pp. 18-26: The Royal Society of Chemistry.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, 5 (5): 607-625.

Meays, C. L., Broersma, K., Nordin, R. & Mazumder, A. (2004). Source tracking fecal bacteria in water: a critical review of current methods. *Journal of environmental management*, 73 (1): 71-79.

Mendenhall, W. & Sincich, T. (2012). *A second course in statistics : regression analysis*. 7th ed. ed. Upper Saddle River, N.J: Pearson/Prentice Hall.

Miettinen, I. T., Lepisto, O., Pitkanen, T., Kuusi, M., Maunula, L., Laine, J., Ikonen, J. & Hanninen, M. L. (2012). A Waterborne Outbreak Caused by a Severe Faecal Contamination of Distribution Network: Nokia Case. In *The Significance of Faecal Indicators in Water: A Global Perspective*, pp. 34-37: The Royal Society of Chemistry.

Mitchell, R. & Chamberlin, C. (1978). Survival of indicator organisms. In *Indicators of Viruses in Water and Food*, pp. 15-35: Ann Arbor Science Ann Arbor, Mich.

Mohanty, S. K., Torkelson, A. A., Dodd, H., Nelson, K. L. & Boehm, A. B. (2013). Engineering Solutions to Improve the Removal of Fecal Indicator Bacteria by Bioinfiltration Systems during Intermittent Flow of Stormwater. *Environmental Science & Technology*, 47 (19): 10791-10798.

Mohanty, S. K. & Boehm, A. B. (2014). Escherichia coli Removal in Biochar-Augmented Biofilter: Effect of Infiltration Rate, Initial Bacterial Concentration, Biochar Particle Size, and Presence of Compost. *Environmental Science & Technology*, 48 (19): 11535-11542.

Mohanty, S. K., Cantrell, K. B., Nelson, K. L. & Boehm, A. B. (2014). Efficacy of biochar to remove Escherichia coli from stormwater under steady and intermittent flow. *Water Research*, 61: 288-296.

Mohanty, S. K., Bulicek, M. C. D., Metge, D. W., Harvey, R. W., Ryan, J. N. & Boehm, A. B. (2015). Mobilization of Microspheres from a Fractured Soil during Intermittent Infiltration Events. *Vadose Zone Journal*, 14 (1).

Moratö, J., Mir, J., Codony, F., Mas‡, J. & Ribas, F. (2003). 39 - Microbial response to disinfectants. In Mara, D. & Horan, N. (eds) *Handbook of Water and Wastewater Microbiology*, pp. 657-693. London: Academic Press.

Morgan, J., Forster, C. & Evison, L. (1990). A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Research*, 24 (6): 743-750.

Mounce, S., Shepherd, W., Sailor, G., Shucksmith, J. & Saul, A. (2014). Predicting combined sewer overflows chamber depth using artificial neural networks with rainfall radar data. *Water Science and Technology*, 69 (6): 1326-1333.

Muela, A., García-Bringas, J. M., Arana, I. & Barcina, I. (2000). The Effect of Simulated Solar Radiation on Escherichia coli: The Relative Roles of UV-B, UV-A, and Photosynthetically Active Radiation. *Microbial Ecology*, 39 (1): 65-71.

Nemerow, N. L. (1991). *Stream, lake, estuary, and ocean pollution*. 2nd ed. ed. Environmental engineering series. New York: Van Nostrand Reinhold.

Niemela, S. I., Lee, J. V. & Fricker, C. R. (2003). A comparison of the International Standards Organisation reference method for the detection of coliforms and Escherichia coli in water with a defined substrate procedure. *Journal of Applied Microbiology*, 95 (6): 1285-1292.

NMBU. (2015). *HMS-LAB-H0 LABORATORIEHÅNDBOK: Rutiner for ansatte og studenter som arbeider i laboratorium ved NMBU*. Ås, Norge: NMBU. 45 pp.

Noble, R. & Weisberg, R. (2005). A review of technologies for rapid detection of bacteria in recreational waters. *J Water Health*, 3: 381-392.

Noble, R. T. & Fuhrman, J. A. (2001). Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. *Hydrobiologia*, 460 (1-3): 175-184.

Noble, R. T., Leecaster, M. K., McGee, C. D., Weisberg, S. B. & Ritter, K. (2004). Comparison of bacterial indicator analysis methods in stormwater-affected coastal waters. *Water research*, 38 (5): 1183-1188.

Nocker, A., Burr, M. & Camper, A. (2014). Chapter One - Pathogens in Water and Biofilms. In Gray, S. L. P. V. Y. W. W. M. C. F. (ed.) *Microbiology of Waterborne Diseases (Second Edition)*, pp. 3-32. London: Academic Press.

Norton, J. F. (1943). The Examination of Waters and Water Supplies (5th ed.). *American Journal of Public Health and the Nations Health*, 33 (11): 1370-1371.

O'Shea, M. L. & Field, R. (1992). An Evaluation of Bacterial Standards and Disinfection Practices Used for the Assessment and Treatment of Stormwater. In Saul, L. N. & Allen, I. L. (eds) vol. Volume 37 *Advances in Applied Microbiology*, pp. 21-40: Academic Press.

Odonkor, S. T. & Ampofo, J. K. (2013). Escherichia coli as an indicator of bacteriological quality of water: an overview. *Microbiology research*, 4 (1): 2.

Pachepsky, Y. A., Sadeghi, A. M., Bradford, S. A., Shelton, D. R., Guber, A. K. & Dao, T. (2006). Transport and fate of manure-borne pathogens: Modeling perspective. *Agricultural Water Management*, 86 (1–2): 81-92.

Palumbo, S. A., Rajkowski, K. T. & Miller, A. J. (1997). Current approaches for reconditioning process water and its use in food manufacturing operations. *Trends in Food Science & Technology*, 8 (3): 69-74.

Paruch, A. M. & Mæhlum, T. (2012). Specific features of Escherichia coli that distinguish it from coliform and thermotolerant coliform bacteria and define it as the most accurate indicator of faecal contamination in the environment. *Ecological Indicators*, 23: 140-142.

Payment, P., Waite, M. & Dufour, A. (2003). Introducing parameters for the assessment of drinking water quality. *Assessing microbial safety of drinking water*, 4: 47-77.

Paytan, A., Boehm, A. B. & Shellenbarger, G. G. (2004). Bacterial Contamination and Submarine Groundwater Discharge-A Possible Link. *Environmental Chemistry*, 1 (1): 29-30.

Pepper, I. L., Gerba, C. P. & Brusseau, M. L. (2006). *Environmental & pollution science*. Amsterdam: Elsevier/ Academic Press. 1 online resource (xx, 532 s.) : ill. (chiefly col.), maps (chiefly col.) pp.

Pepper, I. L. & Gentry, T. J. (2015). Chapter 2 - Microorganisms Found in the Environment. In Pepper, I. L., Gerba, C. P. & Gentry, T. J. (eds) *Environmental Microbiology (Third edition)*, pp. 9-36. San Diego: Academic Press.

Pepper, I. L., Gerba, C. P. & Gentry, T. J. (2015). Chapter 1 - Introduction to Environmental Microbiology. In Pepper, I. L., Gerba, C. P. & Gentry, T. J. (eds) *Environmental Microbiology (Third edition)*, pp. 3-8. San Diego: Academic Press.

Percival, S., Chalmers, R., Embrey, M., Hunter, P., Sellwood, J. & Wyn-Jones, P. (2004). 25 - The survival and persistence of viruses in water. In Wyn-Jones, S. P. C. E. H. S. (ed.) *Microbiology of Waterborne Diseases*, pp. 345-348. London: Academic Press.

Pickering, A. J., Julian, T. R., Mamuya, S., Boehm, A. B. & Davis, J. (2010). Effect of Daily Activities on Hand Fecal Contamination among Tanzanian Mothers: Implications for Measuring Handwashing Behavior. *American Journal of Tropical Medicine and Hygiene*, 83 (5): 9-9.

Pickering, A. J., Julian, T. R., Mamuya, S., Boehm, A. B. & Davis, J. (2011). Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Tropical Medicine & International Health*, 16 (2): 233-239.

Pickering, A. J., Julian, T. R., Marks, S. J., Mattioli, M. C., Boehm, A. B., Schwab, K. J. & Davis, J. (2012). Fecal Contamination and Diarrheal Pathogens on Surfaces and in Soils among Tanzanian Households with and without Improved Sanitation. *Environmental Science & Technology*, 46 (11): 5736-5743.

Pommepuy, M., Hervio-Heath, D., Caprais, M.-P., Gourmelon, M., Le Saux, J.-C. & Le Guyader, F. (2005). Fecal contamination in coastal areas: an engineering approach. In *Oceans and Health: Pathogens in the marine environment*, pp. 331-359: Springer.

Pond, K. (2005). *Water Recreation and disease*. London, UK: published on behalf of the World Health Organization by IWA Publishing

Praveena, S. M., Chen, K. S. & Ismail, S. N. S. (2013). Indicators of microbial beach water quality: Preliminary findings from Teluk Kemang beach, Port Dickson (Malaysia). *Marine Pollution Bulletin*, 76 (1-2): 417-419.

Rajeshwar, K. & Ibanez, J. G. (1997). Chapter Seven. In Ibanez, K. R. G. (ed.) *Environmental Electrochemistry*, pp. 625-685. San Diego: Academic Press.

Ratnayaka, D. D., Brandt, M. J. & Johnson, K. M. (2009). CHAPTER 6 - Chemistry, Microbiology and Biology of Water. In Johnson, D. D. R. J. B. M. (ed.) *Water Supply (Sixth Edition)*, pp. 195-266. Boston: Butterworth-Heinemann.

Rechenburg, A., Koch, C., Classen, T. & Kistemann, T. (2006). Impact of sewage treatment plants and combined sewer overflow basins on the microbiological quality of surface water. *Water Science & Technology*, 54 (3): 95-99.

Rice, E. W. & Bridgewater, L. (2012). *Standard methods for the examination of water and wastewater*. Washington, D.C.: American Public Health Association. 1 b. (flere pag.) : ill. pp.

Riedel, T. E., Thulsiraj, V., Zimmer-Faust, A. G., Dagit, R., Krug, J., Hanley, K. T., Adamek, K., Ebentier, D. L., Torres, R., Cobian, U., et al. (2015). Long-term monitoring of molecular markers can distinguish different seasonal patterns of fecal indicating bacteria sources. *Water research*, 71: 227-43.

Rippy, M. A., Franks, P. J. S., Feddersen, F., Guza, R. T. & Moore, D. F. (2013). Factors controlling variability in nearshore fecal pollution: The effects of mortality. *Marine Pollution Bulletin*, 66 (1–2): 191-198.

Romero, J. R., Imberger, J., Antenucci, J. P. & Dallimore, C. J. (2006). *ARMS (Aquatic Real-time Management System): An automated decision support system for lakes, estuaries and coastal zones*. Proc. 2nd Intl. Symp. Mangt, Engin. Informatics (MEI), Orlando, USA.

Rozen, Y. & Belkin, S. (2001). Survival of enteric bacteria in seawater. *FEMS microbiology reviews*, 25 (5): 513-529.

Russell, T. L., Yamahara, K. M. & Boehm, A. B. (2012). Mobilization and Transport of Naturally Occurring Enterococci in Beach Sands Subject to Transient Infiltration of Seawater. *Environmental Science & Technology*, 46 (11): 5988-5996.

Russell, T. L., Sassoubre, L. M., Wang, D., Masuda, S., Chen, H. L., Soetjipto, C., Hassaballah, A. & Boehm, A. B. (2013). A Coupled Modeling and Molecular Biology Approach to Microbial Source Tracking at Cowell Beach, Santa Cruz, CA, United States. *Environmental Science & Technology*, 47 (18): 10231-10239.

Russell, T. L., Sassoubre, L. M., Zhou, C., French-Owen, D., Hassaballah, A. & Boehm, A. B. (2014). Impacts of Beach Wrack Removal via Grooming on Surf Zone Water Quality. *Environmental Science & Technology*, 48 (4): 2203-2211.

Ruud, J. (1968). Introduction to the studies of pollution in the Oslofjord. *Helgoländer wissenschaftliche Meeresuntersuchungen*, 17 (1-4): 455-461.

Rzeżutka, A. & Cook, N. (2004). Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews*, 28 (4): 441-453.

Sadowsky, M. J. & Whitman, R. L. (2011). *The Fecal bacteria*. Washington, D.C.: ASM Press. XII, 315 s. : ill pp.

Sales-Ortells, H., Agostini, G. & Medema, G. (2015). Quantification of Waterborne Pathogens and Associated Health Risks in Urban Water. *Environmental Science & Technology*, 49 (11): 6943-6952.

Samadpour, M., Evans, P. S., Everett, K. D. E., Ghatpande, P., Ma, G. J. & Miksch, R. R. (2005). Chapter 54 - Occupational and Environmental Exposures to Waterborne Microbial Diseases. In Redlich, L. R. R. C. A. B. A. (ed.) *Textbook of Clinical Occupational and Environmental Medicine (Second Edition)*, pp. 1195-1221. Edinburgh: W.B. Saunders.

Sassoubre, L. M., Walters, S. P., Russell, T. L. & Boehm, A. B. (2011). Sources and fate of Salmonella and fecal indicator bacteria in an urban creek. *Journal of Environmental Monitoring*, 13 (8): 2206-2212.

Sassoubre, L. M., Love, D. C., Silverman, A. I., Nelson, K. L. & Boehm, A. B. (2012a). Comparison of enterovirus and adenovirus concentration and enumeration methods in seawater from Southern California, USA and Baja Malibu, Mexico. *Journal of Water and Health*, 10 (3): 419-430.

Sassoubre, L. M., Nelson, K. L. & Boehm, A. B. (2012b). Mechanisms for Photoinactivation of Enterococcus faecalis in Seawater. *Applied and Environmental Microbiology*, 78 (21): 7776-7785.

Sassoubre, L. M., Nelson, K. L. & Boehm, A. B. (2014a). Mechanisms for Photoinactivation of Enterococcus faecalis in Seawater (vol 78, pg 7776, 2012). *Applied and Environmental Microbiology*, 80 (9): 2964-2964.

Sassoubre, L. M., Ramsey, M. M., Gilmore, M. S. & Boehm, A. B. (2014b). Transcriptional response of Enterococcus faecalis to sunlight. *Journal of Photochemistry and Photobiology B-Biology*, 130: 349-356.

Sassoubre, L. M., Yamahara, K. M. & Boehm, A. B. (2015). Temporal Stability of the Microbial Community in Sewage-Polluted Seawater Exposed to Natural Sunlight Cycles and Marine Microbiota. *Applied and Environmental Microbiology*, 81 (6): 2107-2116.

Savichtcheva, O. & Okabe, S. (2006). Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*, 40 (13): 2463-2476.

Schets, F. A., van Wijnen, J. H., Schijven, J. F., Schoon, A. & Husmant, A. (2008). Monitoring of waterborne pathogens in surface waters in Amsterdam, The Netherlands, and the potential health risk associated with exposure to Cryptosporidium and Giardia in these waters. *Applied and Environmental Microbiology*, 74 (7): 2069-2078.

Schoen, M. E., Soller, J. A. & Ashbolt, N. J. (2011). Evaluating the importance of faecal sources in human-impacted waters. *Water Research*, 45 (8): 2670-2680.

Schulz, C. J. & Childers, G. W. (2011). Fecal Bacteroidales diversity and decay in response to variations in temperature and salinity. *Applied and environmental microbiology*, 77 (8): 2563-2572.

Sen, K. & Ashbolt, N. J. (2011). *Environmental microbiology: current technology and water applications*. Norfolk, UK: Caister Academic. x, 316 s. + vedl. pp.

Servais, P., Garcia-Armisen, T., George, I. & Billen, G. (2007). Fecal bacteria in the rivers of the Seine drainage network (France): Sources, fate and modelling. *Science of The Total Environment*, 375 (1–3): 152-167.

Shellenbarger, G. G., Athearn, N. D., Takekawa, J. Y. & Boehm, A. B. (2008). Fecal indicator bacteria and Salmonella in ponds managed as bird habitat, San Francisco Bay, California, USA. *Water Research*, 42 (12): 2921-2930.

Shibata, T., Solo-Gabriele, H. M., Fleming, L. E. & Elmir, S. (2004). Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. *Water Research*, 38 (13): 3119-3131.

Shuval, H. (2003). Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. *J Water Health*, 1: 53-64.

Silverman, A. I., Peterson, B. M., Boehm, A. B., McNeill, K. & Nelson, K. L. (2013). Sunlight Inactivation of Human Viruses and Bacteriophages in Coastal Waters Containing Natural Photosensitizers. *Environmental Science & Technology*, 47 (4): 1870-1878.

Sinton, L. W., Davies-Colley, R. J. & Bell, R. G. (1994). Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in seawater chambers. *Applied and Environmental microbiology*, 60 (6): 2040-2048.

Sinton, L. W., Finlay, R. K. & Lynch, P. A. (1999). Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Applied and Environmental Microbiology*, 65 (8): 3605-3613.

Sinton, L. W., Hall, C. H., Lynch, P. A. & Davies-Colley, R. J. (2002). Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Applied and environmental microbiology*, 68 (3): 1122-1131.

Sjølander, I. & Universitetet for miljø- og biovitenskap Institutt for matematiske realfag og, t. (2012). *Modeling the decay of E. coli and Ascaris suum in wastewater irrigated vegetables : implications for microbial health risk reduction*. Ås: I. Sjølander.

Soller, J. A., Bartrand, T., Ashbolt, N. J., Ravenscroft, J. & Wade, T. J. (2010a). Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Research*, 44 (16): 4736-4747.

Soller, J. A., Schoen, M. E., Bartrand, T., Ravenscroft, J. E. & Ashbolt, N. J. (2010b). Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research*, 44 (16): 4674-4691.

Soller, J. A., Schoen, M. E., Varghese, A., Ichida, A. M., Boehm, A. B., Eftim, S., Ashbolt, N. J. & Ravenscroft, J. E. (2014). Human health risk implications of multiple sources of faecal indicator bacteria in a recreational waterbody. *Water Research*, 66: 254-264.

Souza, D. S. M., Ramos, A. P. D., Nunes, F. F., Moresco, V., Taniguchi, S., Guiguet Leal, D. A., Sasaki, S. T., Bícego, M. C., Montone, R. C., Durigan, M., et al. (2012). Evaluation of tropical water sources and mollusks in southern Brazil using microbiological, biochemical, and chemical parameters. *Ecotoxicology and Environmental Safety*, 76: 153-161.

Staalstrøm, A. T., Torulv ; Tryland, Ingun ; Kempa, Magdalena. (2014). Vurdering av VEAS sitt dykkede overløp av overløpsvann i Lysakerfjorden.

Staley, Z. R., Rohr, J. R. & Harwood, V. J. (2011). Test of Direct and Indirect Effects of Agrochemicals on the Survival of Fecal Indicator Bacteria. *Applied and Environmental Microbiology*, 77 (24): 8765-8774.

Sterling, C. R. & Arrowood, M. J. (1993). CHAPTER 2 - Cryptosporidia. In Kreier, J. P. (ed.) *Parasitic Protozoa (Second Edition)*, pp. 159-225. San Diego: Academic Press.

Stewart, J. R., Boehm, A. B., Dubinsky, E. A., Fong, T. T., Goodwin, K. D., Griffith, J. F., Noble, R. T., Shanks, O. C., Vijayavel, K. & Weisberg, S. B. (2013). Recommendations following a multi-laboratory comparison of microbial source tracking methods. *Water Research*, 47 (18): 6829-6838.

Sunger, N. & Haas, C. N. (2015). Quantitative Microbial Risk Assessment for Recreational Exposure to Water Bodies in Philadelphia. *Water Environment Research*, 87 (3): 211-222.

Tallon, P., Magajna, B., Lofranco, C. & Leung, K. T. (2005). Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution*, 166 (1-4): 139-166.

Tchobanoglous, G., Abu-Orf, M., Bowden, G. & Pfrang, W. (2014). *Wastewater engineering: treatment and resource recovery*. New York: McGraw-Hill Education. XXIX, 2018 s. : ill. pp.

Teklehaimanot, G. Z., Coetzee, M. A. A. & Momba, M. N. B. (2014). Faecal pollution loads in the wastewater effluents and receiving water bodies: a potential threat to the health of Sedibeng and Soshanguve communities, South Africa. *Environmental Science and Pollution Research*, 21 (16): 9589-9603.

Terai, N., Schlotzer-Schrehardt, U., Lampel, J., Bohm, A. G., Rummelt, C., Schmidt, E. & Pillunat, L. E. (2009). Effect of latanoprost and timolol on the histopathology of the human conjunctiva. *British Journal of Ophthalmology*, 93 (2): 219-224.

Terai, N., Spoerl, E., Pillunat, L. E., Kuhlisch, E., Schmidt, E. & Boehm, A. G. (2011). The relationship between central corneal thickness and optic disc size in patients with primary open-angle glaucoma in a hospital-based population. *Acta Ophthalmologica*, 89 (6): 556-559.

Thoe, W., Gold, M., Griesbach, A., Grimmer, M., Taggart, M. L. & Boehm, A. B. (2014). Predicting water quality at Santa Monica Beach: Evaluation of five different models for public notification of unsafe swimming conditions. *Water Research*, 67: 105-117.

Todd, E. C. D. (2014). Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. In Motarjemi, Y. (ed.) *Encyclopedia of Food Safety*, pp. 221-242. Waltham: Academic Press.

Toze, S. (1999). PCR and the detection of microbial pathogens in water and wastewater. *Water Research*, 33 (17): 3545-3556.

Toze, S. (2006). Water reuse and health risks — real vs. perceived. *Desalination*, 187 (1–3): 41-51.

Tryland, I., Muthanna, T. & Liltved, H. (2013). *Impact of changing weather patterns on bathing water and seafood quality from the Inner Oslofjord*. 6th International Conference on Water Resources and Environment Research (ICWRER), Koblenz, Germany: Norsk institutt for vannforskning.

Tryland, I., Myrmel, M., Østensvik, Ø., Wennberg, A. C. & Robertson, L. J. (2014). Impact of rainfall on the hygienic quality of blue mussels and water in urban areas in the Inner Oslofjord, Norway. *Marine Pollution Bulletin*, 85 (1): 42-49.

Tryland, I., Eregno, F. E., Braathen, H., Khalaf, G., Sjølander, I. & Fossum, M. (2015). On-Line Monitoring of Escherichia coli in Raw Water at Oset Drinking Water Treatment Plant, Oslo (Norway). *International Journal of Environmental Research and Public Health*, 12 (2): 1788-1802.

Turbow, D. J., Kent, E. E. & Jiang, S. C. (2008). Web-based investigation of water associated illness in marine bathers. *Environmental Research*, 106 (1): 101-109.

Union, E. (2006). EU Directive, 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union, L64/37, 4.3.2006.

Van Der Linden, I. C., Bart; Uyttendaele, Mieke. (2014). Enteric survival varies substancially in Irrigation water from belgian lettuce producers. *International Journal of Environmental Research and public Health* (11).

Van Donsel, D. J., Geldreich, E. E. & Clarke, N. A. (1967). Seasonal Variations in Survival of Indicator Bacteria in Soil and Their Contribution to Storm-water Pollution. *Applied Microbiology*, 15 (6): 1362-1370.

Vergine, P., Saliba, R., Salerno, C., Laera, G., Berardi, G. & Pollice, A. (2005). Fate of the fecal indicator Escherichia coli in irrigation with partially treated wastewater. *Water Research*.

Viau, E. J. & Boehm, A. B. (2011). Quantitative PCR-based detection of pathogenic Leptospira in Hawai'ian coastal streams. *Journal of Water and Health*, 9 (4): 637-646.

Viau, E. J., Goodwin, K. D., Yamahara, K. M., Layton, B. A., Sassoubre, L. M., Burns, S. L., Tong, H. I., Wong, S. H. C., Lu, Y. A. & Boehm, A. B. (2011a). Bacterial pathogens in Hawaiian coastal streams-Associations with fecal indicators, land cover, and water quality. *Water Research*, 45 (11): 3279-3290.

Viau, E. J., Lee, D. & Boehm, A. B. (2011b). Swimmer Risk of Gastrointestinal Illness from Exposure to Tropical Coastal Waters Impacted by Terrestrial Dry-Weather Runoff. *Environmental Science & Technology*, 45 (17): 7158-7165.

Wade, T. J., Calderon, R. L., Sams, E., Beach, M., Brenner, K. P., Williams, A. H. & Dufour, A. P. (2006). Rapidly Measured Indicators of Recreational Water Quality Are Predictive of Swimming-Associated Gastrointestinal Illness. *Environmental Health Perspectives*, 114 (1): 24-28.

Wade, T. J., Calderon, R. L., Brenner, K. P., Sams, E., Beach, M., Haugland, R., Wymer, L. & Dufour, A. P. (2008). High Sensitivity of Children to Swimming-Associated Gastrointestinal Illness: Results Using a Rapid Assay of Recreational Water Quality. *Epidemiology*, 19 (3): 375-383.

Walker, J., F Russell & Stedinger, J. R. (1999). Fate and transport model of Cryptosporidium. *Journal of Environmental Engineering*, 125 (4): 325-333.

Walters, E., Schwarzwälder, K., Rutschmann, P., Müller, E. & Horn, H. (2014). Influence of resuspension on the fate of fecal indicator bacteria in large-scale flumes mimicking an oligotrophic river. *Water Research*, 48 (0): 466-477.

Walters, S. P., Yamahara, K. M. & Boehm, A. B. (2009). Persistence of nucleic acid markers of health-relevant organisms in seawater microcosms: Implications for their use in assessing risk in recreational waters. *Water Research*, 43 (19): 4929-4939.

Walters, S. P., Thebo, A. L. & Boehm, A. B. (2011). Impact of urbanization and agriculture on the occurrence of bacterial pathogens and stx genes in coastal waterbodies of central California. *Water Research*, 45 (4): 1752-1762.

Walters, S. P., Gonzalez-Escalona, N., Son, I., Melka, D. C., Sassoubre, L. M. & Boehm, A. B. (2013). Salmonella enterica Diversity in Central Californian Coastal Waterways. *Applied and Environmental Microbiology*, 79 (14): 4199-4209.

Wang, D., Farnleitner, A. H., Field, K. G., Green, H. C., Shanks, O. C. & Boehm, A. B. (2013). Enterococcus and Escherichia coli fecal source apportionment with microbial source tracking genetic markers - Is it feasible? *Water Research*, 47 (18): 6849-6861.

Wang, D., Green, H. C., Shanks, O. C. & Boehm, A. B. (2014). New Performance Metrics for Quantitative Polymerase Chain Reaction-Based Microbial Source Tracking Methods. *Environmental Science & Technology Letters*, 1 (1): 20-25.

Weintraub, A. & Romero, C. (2006). Operations research models and the management of agricultural and forestry resources: a review and comparison. *Interfaces*, 36 (5): 446-457.

Whitman, R. L., Ge, Z. F., Nevers, M. B., Boehm, A. B., Chern, E. C., Haugland, R. A., Lukasik, A. M., Molina, M., Przybyla-Kelly, K., Shively, D. A., et al. (2010). Relationship and Variation of qPCR and Culturable Enterococci Estimates in Ambient Surface Waters Are Predictable. *Environmental Science & Technology*, 44 (13): 5049-5054.

WHO, W. H. O. (2000). Monitoring Bathing Waters - A Practical Guide to the Design and Implementation of Assessments and Monitoring Programmes.

WHO, W. H. O. (2001). *Water quality: Guidelines, standards and health - Assessment of risk and risk management for water-related infectious disease.*: IWA Publishing. 289-316. pp.

WHO, W. H. O. (2003). *Guidelines for safe recreational water environments : Volume 1 : Coastal and fresh waters*, 1. Geneva: World Health Organization.

WHO, W. H. O. (2006). *Guidelines for safe recreational water environments : Volume 2 : Swimming pools and similar environments*. 4th ed., 2. Geneva: World Health Organization. p. 564.

WHO, W. H. O. (2008). *Guidelines for drinking-water quality (electronic resource), incorporating 1st and 2nd addenda. WHO press. Geneva, Switerland*: Volume.

WHO, W. H. O. (2011). Guidelines for Drinking-Water Quality. 564.

WHO, W. H. O. (2014). Preventing diarrhoea through better water, sanitation and hygiene: exposures and impacts in low- and middle-income countries, 978 92 4 156482 3. 48 pp.

WHO, W. H. O. (2015). World Health statistic 2015. WHO Press, World Health Organization. Geneva, Switzerland. 164p. http://www.who.int/gho/publications/world_health_statistics/2015/en/.

Wong, S. H. C., Santoro, A. E., Nidzieko, N. J., Hench, J. L. & Boehm, A. B. (2012). Coupled physical, chemical, and microbiological measurements suggest a connection between internal waves and surf zone water quality in the Southern California Bight. *Continental Shelf Research*, 34: 64-78.

Wong, S. H. C., Monismith, S. G. & Boehm, A. B. (2013). Simple Estimate of Entrainment Rate of Pollutants from a Coastal Discharge into the Surf Zone. *Environmental Science & Technology*, 47 (20): 11554-11561.

Wu, J., Long, S., Das, D. & Dorner, S. (2011). Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. *Journal of water and health*, 9 (2): 265-278.

Yamahara, K. M., Walters, S. P. & Boehm, A. B. (2009). Growth of Enterococci in Unaltered, Unseeded Beach Sands Subjected to Tidal Wetting (vol 75, pg 1517, 2009). *Applied and Environmental Microbiology*, 75 (9): 2997-2997.

Yamahara, K. M., Sassoubre, L. M., Goodwin, K. D. & Boehm, A. B. (2012). Occurrence and Persistence of Bacterial Pathogens and Indicator Organisms in Beach Sand along the California Coast. *Applied and Environmental Microbiology*, 78 (6): 1733-1745.

Yang, P. Q., Hippler, S., Deen, C. P., Boehm, A., Brandner, W., Henning, T., Huber, A., Kendrew, S., Lenzen, R., Rohloff, R. R., et al. (2012). Optimizing the transmission of the GRAVITY/VLTI near-infrared wavefront sensor. *Optical and Infrared Interferometry Iii*, 8445.

Yates, M. V. (2007). Classical Indicators in the 21st Century—Far and Beyond the Coliform. *Water Environment Research*, 79 (3): 279-286.

Yau, V. M., Schiff, K. C., Arnold, B. F., Griffith, J. F., Gruber, J. S., Wright, C. C., Wade, T. J., Burns, S., Hayes, J. M., McGee, C., et al. (2014). Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. *Water Research*, 59: 23-36.

Yeh, R. C., Hyun, J. K., Boehm, A. K., Lis, J. T. & Franck, C. (2005). Improving slide-based assays by stirring: Application of liquid-on-liquid mixing to immunofluorescence staining of polytene chromosomes. *Journal of Biochemical and Biophysical Methods*, 64 (1): 59-68.

Yoder, J. S., Hlavsa, M. C., Craun, G. F., Hill, V., Roberts, V., Yu, P. A., Hicks, L. A., Alexander, N. T., Calderon, R. L., Roy, S. L., et al. (2008). Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events--United States, 2005-2006. *Morbidity and mortality weekly report. Surveillance summaries (Washington, D.C. : 2002)*, 57 (9): 1-29.

Zuckerman, J. N., Rombo, L. & Fisch, A. (2007). The true burden and risk of cholera: implications for prevention and control. *The Lancet Infectious Diseases*, 7 (8): 521-530.

8. Appendices

8.1 Appendix A1 - Laboratory Log

8.2 Appendix B: Quanti-tray with yellow wells and Florescence wells

8.3 Appendix C: Preliminary experiment data and calculations

Table 8.1: Freshwater samples data and calculation for Total coliform and *E coli* using Colillert-18 Quanti-Tray method at 22^0C and control at 4^0C

Table 8.2: Seawater samples data and calculation for Total coliform and *E coli* using Colillert-18 Quanti-Tray methods at 22^0C and control at 4^0C

Table 8.3: Freshwater samples data and calculations for *I. enterococci* using Membrane filtration method

Table 8.4: Seawater samples data and calculations for *I. enterococci* using Membrane filtration method

8.4 Appendix D: Main experiment data and calculations

Table 8.5: Deep seawater samples for Total coliform and *E coli* using Colillert-18 Quanti-Tray method at 4 ^{0}C

Table 8.6: Deep seawater sample for Total coliforms and *E. coli* using Colilert-18 test at 22^0C

Table 8.7: Surface seawater samples for Total coliform and *E coli* using Colillert-18 test at 4^sC

Table 8.8: Surface seawater samples for Total coliform and *E coli* using Colilert Quanti-Tray method at 22 ^{0}C

Table 8.9: Deep seawater samples for *I. enterococci* using Enterolert-E Quanti-Tray method at 4 0C

Table 8.10: Deep seawater samples for I. *enterococci* using Enterolert-E Quanti-Tray method at 22^0C

Table 8.11: Surface seawater samples for Enterococci using enterolert Quanti-Tray method at 4 0C

Table 8.12: Surface seawater samples for Enterococci using enterolert Quanti-Tray method at 22 0C

Appendix C: Preliminary experiment data and calculations

Table 8.1: Freshwater samples data and calculation for Total coliform and *E coli* using Colilert- 18 test sat 22^0C and control at 4^0C

Time	Sample number	Sample Dilution	Total coliforms (TC) as Yellow counts					*E. coli* (UV Fluorescence counts)						
			Wells		MPN/mL	MPN/100 mL		Wells		MPN/mL	MPN/100 mL	Log_{10}	Mean Log_{10}	Standard deviation
Days			Large	Small	Diluted	Undiluted	Log_{10} MPN/100 mL	Large	Small	Diluted	Undiluted	(MPN/ 100mL)	(MPN/100mL)	
0	Control 1	1:100	49	41	1203,3	1,20E+05	5,08	49	21	365,4	3,65E+04	4,56		
	Control 2	1:1000	44	10	125,9	1,26E+05	5,10	29	6	51,2	5,12E+04	4,71	4,56	0
1	Control	1:100	49	43	1413,6	1,41E+05	5,15	49	26	488,4	4,88E+04	4,69		
	Sample 1	1:100	49	45	1732,0	1,73E+05	5,24	49	21	365,4	3,65E+04	4,56		
	Sample 2	1:100	49	42	1299,7	1,30E+05	5,11	49	23	410,6	4,11E+04	4,61		
					Mean =	**1,52E+05**	5,18				3,88E+04	4,59	4,59	0,04
2	Control	1:100	49	40	1119,9	1,12E+05	5,05	49	22	387,3	3,87E+04	4,59		
	Sample 1	1:100	49	42	1299,7	1,30E+05	5,11	49	19	325,5	3,26E+04	4,51		
	Sample 2	1:100	49	46	1986,3	1,99E+05	5,30	49	31	648,8	6,49E+04	4,81		
					Mean =	**1,64E+05**	5,22				4,87E+04	4,69	4,69	0,21
3	Control	1:100	49	43	1413,6	1,41E+05	5,15	49	28	547,5	5,48E+04	4,74		
	Sample 1	1:100	0	0	0,0	0	0	0	0	0	0	0		
	Sample 2	1:100	49	21	365,4	3,65E+04	4,56	34	8	68,9	6,89E+03	3,84		
					Mean =	**1,83E+04**	4,56				6,89E+03	3,84	3.84	0
4	Control	1:100	0	0	0	0	0	0	0	0	0	0		
	Sample 1	1:100	0	0	0	0	0	0	0	0	0	0		
	Sample 2	1:100	0	0	0	0	0	0	0	0	0	0	0	0
5	Control	1:100	49	38	980,4	9,80E+04	4,99	49	25	461,1	4,61E+04	4,66		
	Sample 1	1:100	18	4	26,9	2,69E+03	3,43	1	0	1,0	1,00E+02	2,00		
	Sample 2	1:100	49	24	435,2	4,35E+04	4,64	47	12	172,3	1,72E+04	4,24		
					Mean =	**2,31E+04**	4,36				8,67E+03		3,12	1,58

Footnotes: All control samples were kept at 4^0C

Day 5, sample 2 was a possible contamination during the experiment

Control 2 sample were diluted at 1:1000

Table 8.2: Seawater samples data and calculation for Total coliform and *E coli* using Colilert – 18 tests at 22^0C and control at 4^0C

Time	Sample number	Sample Dilution	Total coliforms (Yellow counts)					*E. coli* (UV Fluorescence counts)						
			Wells		MPN/mL	MPN/100 mL		Wells		MPN/mL	MPN/100 mL	Log10 (MPN/ 100mL)	Mean Log_{10}	Standard deviation
Days			Large	Small	Diluted	Undiluted	Log_{10} MPN/100 mL	Large	Small	Diluted	Undiluted		(MPN/100m)	
0	Control 1	1:100	49	40	1119,9	1,12E+05	5,05	49	26	488,4	4,88E+04	4,69		
	Control 2	1:1000	39	8	88,4	8,84E+04	4,95	29	2	44,8	4,48E+04	4,65	4,69	0
1	Control	1:100	49	33	727,0	7,27E+04	4,86	49	16	275,5	2,76E+04	4,44		
	Sample 1	1:100	49	32	686,7	6,87E+04	4,84	49	12	224,7	2,25E+04	4,35		
	Sample 2	1:100	49	31	648,8	6,49E+04	4,81	47	15	191,8	1,92E+04	4,28		
						6,68E+04	4,82				2,08E+04	4,32	4,32	0,05
2	Control	1:100	49	31	648,8	6,49E+04	4,81	49	19	325,5	3,26E+04	4,51		
	Sample 1	1:100	49	31	648,8	6,49E+04	4,81	46	21	210,5	2,11E+04	4,32		
	Sample 2	1:100	49	33	727,0	7,27E+04	4,86	49	20	344,8	3,45E+04	4,54		
						6,88E+04	4,84				2,78E+04	4,44	4,44	0,15
3	Control	1:100	49	27	517,2	5,17E+04	4,71	47	16	198,9	1,99E+04	4,30		
	Sample 1	1:100	49	25	461,1	4,61E+04	4,66	39	8	88,4	8,84E+03	3,95		
	Sample 2	1:100	27	8	49,6	4,96E+03	3,70	8	1	9,7	9,70E+02	2,99		
						2,55E+04	4,41				4,91E+03	3,69	3,69	0,68
4	Control	1:100	0	0	0	0	0	0	0	0	0	0		
	Sample 1	1:100	0	0	0	0	0	0	0	0	0	0		
	Sample 2	1:100	0	0	0	0	0	0	0	0	0	0	0	0
5	Control	1:100	17	2	22,8	2,28E+01	1,36	3	0	3,1	3,10E+02	2,49		
	Sample 1	1:100	11	6	19,1	1,91E+03	3,28	0	0	<1	<1	0,00		
	Sample 2	1:100	17	2	22,8	2,28E+01	1,36	3	0	3,1	3,10E+02	2,49		
						9,66E+02	2,99				3,10E+02	2,49	2,49	1,76

Footnotes: All control samples were kept at 4 degree celsius.
Control 2 sample were diluted at 1:1000
Dy3, sample 1 was a possipble contamination

Table 8.3: Freshwater samples data and calculations for intestinal Enterococci using Membrane filtration method

Time	Sample number	Sample Dilution	Number of Colonies (CFU)		Intestinal enterococci		Mean Log_{10}	Standard deviation
			Enterococci	Intestinal enterococci				
Days			CFU/mL	CFU/ mL	CFU/100mL	Log_{10} (CFU/100mL)	(CFU/100mL)	
0	Control 1	1:100	81	81	8,10E+03	3,91		
	Control 2	1:1000	7	7	8,10E+04	4,91	3,91	0
1	Control	1:100	52	52	5,20E+03	3,72		
	Sample 1	1:100	53	51	5,10E+03	3,71		
	Sample 2	1:100	48	47	4,70E+03	3,67		
				Mean =	4,90E+03	3,69	3,69	0,03
2	Control	1:100	59	54	5,40E+03	3,73		
	Sample 1	1:100	47	42	4,20E+03	3,62		
	Sample 2	1:100	41	36	3,60E+03	3,56		
				Mean =	3,90E+03	3,59	3,59	0,05
3	Control	1:100	52	31	3,10E+03	3,49		
	Sample 1	1:100	16	10	1,00E+03	3,00		
	Sample 2	1:100	15	13	1,30E+03	3,11		
				Mean =	1,15E+03	3,20	3,06	0,08

Footnotes: All control samples were kept at 4 degree Celsius.

Control 2 sample were diluted at 1:1000

CFU are the number of colonies forming units obtained by the total numbers of positive colonies formed in pettry-dishes

Table 8.4: Seawater samples data and calculations for intestinal Enterococci using Membrane filtration method

Time	Sample number	Sample Dilution	Number of Colonies		Intestinal enterococci		Mean log_{10}	Standard deviation
			Enterococci	Intestinal enterococci				
Days			CFU/100 mL	CFU/100 mL	CFU/100mL	Log_{10} (CFU/100mL	CFU/100mL	
0	Control 1	1:100	75	72	7,20E+03	7,20E+03		
	Control 2	1:1000	6	6	6,00E+03	6,00E+03		0
					-	-	3,86	
					-	-	-	
1	Control	1:100	52	50	5,20E+03	5,00E+03		
	Sample 1	1:100	45	43	5,10E+03	4,30E+03		
	Sample 2	1:100	44	43	4,70E+03	4,30E+03		
					4,90E+03	4,30E+03	3,63	0,00
2	Control	1:100	38	34	5,40E+03	3,40E+03		
	Sample 1	1:100	49	47	4,20E+03	4,70E+03		
	Sample 2	1:100	43	40	3,60E+03	4,00E+03		
					3,90E+03	4,35E+03	3,64	0,05
3	Control	1:100	54	38	3,10E+03	3,80E+03		
	Sample 1	1:100	41	29	1,00E+03	2,90E+03		
	Sample 2	1:100	11	5	1,30E+03	5,00E+02		
					1,15E+03	1,70E+03	3,25	0,54

Footnotes: All control samples were kept at 4 degree Celsius.

Control 2 sample were diluted at 1:1000

CFU are the number of colonies forming units obtained by the total numbers of positive colonies formed in preti--dishes

Appendix D: Main experiment data and calculations for Deep seawater and surface seawater

Table 8.5: Deep seawater samples for Total coliform and *E coli* using Colillert-18 Quanti-Tray method at 4 ^{0}C

Time	Water sample	Sample Dilution	Total coliforms (Yellow counts)					*E coli* (UV Fluorescence counts)						
			Wells		Diluted MPN/ mL	Undiluted MPN/ 100 mL	Log_{10} (MPN/ 100 mL)	Wells		Diluted MPN/ 100 mL	Undlluted MPN/ 100 mL	Log_{10} (Ecoli/ 100 mL)	Mean Log_{10} (Ecoli/ 100 mL)	SD
Days			Large	Small				Large	Small					
0	Control*	1:10	1	1	2,0	2,00E+01	1,30	0	0	<1	N/A	N/A		
	Sample 1	1:100	49	42	1299,7	4,20E+03	3,62	49	28	547,5	5,48E+04	4,74		
	Sample 2	1:100	48	46	913,9	4,60E+03	3,66	49	28	547,5	5,48E+04	4,74		
	Sample 3	1:1000	46	10	146,7	1,00E+04	4,00	37	8	79,8	7,98E+04	4,90		
						Mean =	3,80			Mean =	6,31E+04		4,79	0,09
1	Sample 1	1:100	49	33	727,0	7,27E+04	4,86	49	20	344,8	3,45E+04	4,54		
	Sample 2	1:100	49	40	1119,9	1,12E+05	5,05	8	30	613,1	6,13E+04	4,79		
	Sample 3	1:100	49	42	1299,7	1,30E+05	5,11	49	26	488,4	4,88E+04	4,69		
						Mean ==	5,02			Mean =	4,82E+04	4,68	4,67	0,13
2	Sample 1	1:100	49	40	1119,9	1,12E+05	5,05	49	30	613,1	6,13E+04	4,79		
	Sample 2	1:100	49	33	727,0	7,27E+04	4,86	49	22	387,3	3,87E+04	4,59		
	Sample 3	1:100	49	37	920,8	9,21E+04	4,96	49	22	387,3	3,87E+04	4,59		
						Mean =	4,96			Mean ==	4,63E+04	4,67	4,65	0,12
3	Sample 1	1:100	49	29	579,4	5,79E+04	4,76	49	19	325,5	3,26E+04	4,51		
	Sample 2	1:100	49	30	613,1	6,13E+04	4,79	49	21	365,4	3,65E+04	4,56		
	Sample 3	1:100	49	30	613,1	6,13E+04	4,79	48	11	186	1,86E+04	4,27		
						Mean =	4,78			Mean ==	2,92E+04	4,47	4,45	0,16

Footnotes: SD is Standard deviation

*Control sample in day zero did not add sewage

Table 8.6 Deep seawater sample for Total coliforms and *E. coli* using Colilert-18 test at 22^0C

Time	Water sample	Sample Dilution	Total coliforms (Yellow counts)				*E coli* (UV Fluorescence counts)							
			Wells		Diluted	Undiluted	Log_{10}	Wells		Diluted	Undiluted	Log_{10}	Mean Log_{10}	SD
Days			Large	Small	MPN/mL	MPN/ 100 mL	(MPN/ 100 mL)	Large	Small	MPN/mL)	MPN/ 100 mL	MPN/100 mL	MPN/100mL)	
0	Control*	1:10	1	1	2,0	2,00E+01	1,30	0	0	<1	N/A	N/A		
	Sample 1	1:100	49	42	1299,7	4,20E+03	3,62	49	28	547,5	5,48E+04	4,74		
	Sample 2	1:100	48	46	913,9	4,60E+03	3,66	49	28	547,5	5,48E+04	4,74		
	Sample 3	1:1000	46	10	146,7	1,00E+04	4,00	37	8	79,8	7,98E+04	4,90	4,79	0,09
1	Sample 1	1:100	49	34	770,1	7,70E+04	4,89	47	18	214,2	2,14E+04	4,33		
	Sample 2	1:100	49	33	727,0	7,27E+04	4,86	49	20	344,8	3,45E+04	4,54		
	Sample 3	1:100	49	27	517,2	5,17E+04	4,71	48	13	201,4	2,01E+04	4,30	4,39	0,13
2	Sample 1	1:100	47	11	166,4	1,66E+04	4,22	44	4	105,4	1,05E+04	4,02		
	Sample 2	1:100	49	27	517,2	5,17E+04	4,71	47	16	198,9	1,99E+04	4,30		
	Sample 3	1:100	48	17	238,2	2,38E+04	4,38	38	11	91,0	9,10E+03	3,96	4,09	0,18
3	Sample 1	1:100	35	2	60,5	6,05E+03	3,78	18	1	23,1	2,31E+03	3,36		
	Sample 2	1:100	42	11	113,7	1,14E+04	4,06	27	5	45,0	4,50E+03	3,65		
	Sample 3	1:100	24	6	40,2	4,02E+03	3,60	13	3	18,3	1,83E+03	3,26	3,43	0,20

Table 8.7: Surface seawater samples for Total coliform and *E coli* using Colillert-18 test at 4°C

Time	Water sample	Sample Dilution	Total coliforms (Yellow counts)					Ecoli (UV Fluorescence counts)						
			Wells		Diluted	Undiluted	Log_{10}	Wells		Diluted	Undiluted	Log_{10}	Mean Log_{10}	S
Days			Large	Small	(MPN/ mL)	(MPN/ 100 mL)	(MPN/ 100 mL)	Large	Small	(MPN/ 100 mL)	(MPNi/ 100 mL)	(MPN/100 mL)	(MPNi/ 100 mL)	
0	Control*	1:10	20	1	26,2	2,62E+02	2,42	0	0	<1	N/A	N/A		
	Sample 1	1:100	49	43	1413,6	1,41E+04	4,15	49	32	686,7	6,87E+04	4,84		
	Sample 2	1:100	49	46	1986,3	1,99E+04	4,30	49	30	613,1	6,13E+04	4,79		
	Sample 3	1:1000	47	10	160,7	1,61E+05	5,21	34	3	59,4	5,94E+04	4,77	4,80	0,
1	Sample 1	1:100	49	43	1413,6	1,41E+05	5,15	49	27	517,2	5,17E+04	4,71		
	Sample 2	1:100	49	42	1299,7	1,30E+05	5,11	49	29	579,4	5,79E+04	4,76		
	Sample 3	1:100	49	41	1203,3	1,20E+05	5,08	49	23	410,6	4,11E+04	4,61	4,70	0,
2	Sample 1	1:100	49	37	920,8	9,21E+04	4,96	49	20	344,8	3,45E+04	4,54		
	Sample 2	1:100	49	39	1046,2	1,05E+05	5,02	49	26	488,4	4,88E+04	4,69		
	Sample 3	1:100	49	37	920,8	9,21E+04	4,96	49	23	410,6	4,11E+04	4,61	4,61	0,
3	Sample 1	1:100	49	37	920,8	9,21E+04	4,96	49	25	461,1	4,61E+04	4,66		
	Sample 2	1:100	49	27	517,2	5,17E+04	4,71	49	21	365,4	3,65E+04	4,56		
	Sample 3	1:100	49	31	648,8	6,49E+04	4,81	49	20	344,8	3,45E+04	4,54	4,59	0,

Table 8.8: Surface seawater samples for Total coliform and *E coli* using Colillert Quanti-Tray method at 22 ^{0}C

Time	Water sample	Sample Dillution	Total coliforms (Yellow counts)					Ecoli (UV Fluorescence counts)						
			Wells		Dilluted	Undilluted	Log10	Wells		Dilluted	Undilluted	Log10	Mean Log10	SD
Days			Large	Small	(MPN/ mL)	(MPN/ 100 mL)	(MPN/ 100 mL)	Large	Small	MPN/ mL	(Ecoli/ 100 mL)	(Ecoli/ 100 mL)	(Ecoli/ 100 mL)	
0	Control*	1:10	20	1	26,2	2,62E+02	2,42	0	0	<1	N/A	N/A		
	Sample 1	1:100	49	43	1413,6	1,41E+05	5,15	49	32	686,7	6,87E+04	4,84		
	Sample 2	1:100	49	46	1986,3	1,99E+05	5,30	49	30	613,1	6,13E+04	4,79		
	Sample 3	1:1000	47	10	160,7	1,61E+05	5,21	34	3	59,4	5,94E+04	4,77	4,80	0,03
1	Sample 1	1:100	49	40	1119,9	1,12E+05	5,05	49	28	547,5	5,48E+04	4,74		
	Sample 2	1:100	48	39	658,6	6,59E+04	4,82	48	29	416,0	4,16E+04	4,62		
	Sample 3	1:100	49	38	980,4	9,80E+04	4,99	49	22	387,3	3,87E+04	4,59	4,65	0,08
2	Sample 1	1:100	47	12	172,3	1,72E+04	4,24	27	3	42,0	4,20E+03	3,62		
	Sample 2	1:100	49	27	517,2	5,17E+04	4,71	29	6	51,2	5,12E+03	3,71		
	Sample 3	1:100	46	21	210,5	2,11E+04	4,32	34	7	67,0	6,70E+03	3,83	3,72	0,10
3	Sample 1	1:100	8	1	9,7	9,70E+02	2,99	1	0	1,0	1,00E+02	2,00		
	Sample 2	1:100	8	0	8,6	8,60E+02	2,93	0	0	<1	N/A	N/A		
	Sample 3	1:100	13	3	5,2	5,20E+02	2,72	4	1	4,1	4,10E+02	2,61	2,31	0,43

Table 8.9: Deep seawater samples for *I.enterococci* using Enterollert-E Quanti-Tray method at 4 0C

Time	Water sample	Sample Dillution	Wells	Intestinal Enterococci (UV Fluorescence counts)					
Days			Large	Small	Dilluted MPN/ mL	Undilluted MPN/ 100mL	Log10 (MPN/100mL)	Mean Log10 (MPN/100mL)	SD
0	Control*	1:10	27	7	48,1	4,81E+02	2,68		
	Sample 1	1:10	49	40	1119,9	1,12E+04	4,05		
	Sample 2	1:10	49	37	920,8	9,21E+03	3,96		
	Sample 3	1:100	34	3	59,4	5,94E+03	3,77	3,93	0,14
1	Sample 1	1:10	49	42	1299,7	1,30E+04	4,11		
	Sample 2	1:10	49	45	1732,9	1,73E+04	4,24		
	Sample 3	1:100	36	6	71,7	7,17E+03	3,86	4,07	0,20
2	Sample 1	1:10	49	41	1203,3	1,20E+04	4,08		
	Sample 2	1:10	49	35	816,4	8,16E+03	3,91		
	Sample 3	1:10	49	36	866,4	8,66E+03	3,94	3,98	0,09
3	Sample 1	1:10	49	35	816,4	8,16E+03	3,91		
	Sample 2	1:10	49	35	816,4	8,16E+03	3,91		
	Sample 3	1:10	49	35	816,4	8,16E+03	3,91	3,91	0,00

Table 8.10: Deep seawater samples for I. *enterococci* using Enterolert-E Quanti-Tray method at 22^0C

Time	Water sample	Sample Dilution		Intestinal Enterococci (UV Fluorescence counts)					
			Wells		Diluted	Undiluted	Log10	Mean Log10	SD
Days			Large	Small	MPN/ mL	MPN/ 100mL	(MPN/100mL)	(MPN/100mL)	
0	Control*	1:10	27	7	48,1	4,81E+02	2,68		
	Sample 1	1:10	49	40	1119,9	1,12E+04	4,05		
	Sample 2	1:10	49	37	920,8	9,21E+03	3,96		
	Sample 3	1:100	34	3	59,4	5,94E+03	3,77	3,93	0,14
1	Sample 1	1:10	49	40	1119,9	1,12E+04	4,05		
	Sample 2	1:10	49	42	1299,7	1,30E+04	4,11		
	Sample 3	1:100	29	7	52,8	5,28E+03	3,72	3,96	0,21
2	Sample 1	1:10	49	30	613,1	6,13E+03	3,79		
	Sample 2	1:10	48	32	478,6	4,79E+03	3,68		
	Sample 3	1:10	49	33	727,0	7,27E+03	3,86	3,78	0,09
3	Sample 1	1:10	49	27	517,2	5,17E+03	3,71		
	Sample 2	1:10	49	19	325,5	3,26E+03	3,51		
	Sample 3	1:10	49	28	547,5	5,48E+03	3,74	3,65	0,12

Table 8.11: Surface seawater samples for Enterococci using enterolert Quanti-Tray method at 4 0C

Time	Water sample	Sample Dillution	Wells	Intestinal Enterococci (UV Fluorescence counts)					
Days			Large	Small	Dilluted MPN/mL	Undilluted MPN/ 100mL	Log10 (MNP/100mL)	Mean Log10 (MPN/100mL)	SD
0	Control*	1:10	48	15	218,7	2,19E+03	3,34		
	Sample 1	1:10	49	45	1732,9	1,73E+04	4,24		
	Sample 2	1:10	49	41	1203,3	1,20E+04	4,08		
	Sample 3	1:100	29	4	48,0	4,80E+03	3,68	4,00	0,29
1	Sample 1	1:10	49	37	920,8	9,21E+03	3,96		
	Sample 2	1:10	49	43	1413,6	1,41E+04	4,15		
	Sample 3	1:100	41	6	93,3	9,33E+03	3,97	4,03	0,11
2	Sample 1	1:10	49	42	1299,7	1,30E+04	4,11		
	Sample 2	1:10	49	35	816,4	8,16E+03	3,91		
	Sample 3	1:10	49	41	1203,3	1,20E+04	4,08	4,04	0,11
3	Sample 1	1:10	49	35	816,4	8,16E+03	3,91		
	Sample 2	1:10	49	40	1119,9	1,12E+04	4,05		
	Sample 3	1:10	49	32	686,7	6,87E+03	3,84	3,93	0,11

Time	Water sample	Sample Dillution	Wells	Intestinal Enterococci (UV Fluorescence counts)					
					Dilluted MPN/ 100mL	Undilluted MPN/ 100mL	Log10 (MPN/100mL)	Mean Log10 (MPN/100mL)	SD
Days			Large	Small					
0	Control*	1:10	48	15	218,7	2,19E+03	3,34		
	Sample 1	1:10	49	45	1732,9	1,73E+04	4,24		
	Sample 2	1:10	49	41	1203,3	1,20E+04	4,08		
	Sample 3	1:100	29	4	48,0	4,80E+03	3,68	4,00	0,29
1	Sample 1	1:10	49	37	920,8	9,21E+03	3,96		
	Sample 2	1:10	49	40	1119,9	1,12E+04	4,05		
	Sample 3	1:100	37	4	71,2	7,12E+03	3,85	3,96	0,10
2	Sample 1	1:10	49	23	410,6	4,11E+03	3,61		
	Sample 2	1:10	49	25	461,1	4,61E+03	3,66		
	Sample 3	1:10	48	22	298,7	2,99E+03	3,48	3,58	0,10
3	Sample 1	1:10	49	7	179,3	1,79E+03	3,25		
	Sample 2	1:10	45	7	179,3	1,79E+03	3,25		
	Sample 3	1:10	45	11	214,3	2,14E+03	3,33	3,28	0,04

Appendix A - Laboratory Log

Preliminary experiment

Laboratory analysis: Colillert- Quanti-tray 2000 and Membrane Filtration Methods

Site Name 1: Drøbak Beach and WWTP

Collection sample date: 12/02/2015

Site Name 2: Drøbak Wastewater Treatment Plant
Time: 10:30
Analyzed by: Fasil Eregon
First analysis Date: 12/02/2015
Read by: EKC Da Silva

Main experiment

Laboratory analysis: Colillert-18 and Enterolert- E Quanti-tray 2000

Site Name 1: Solbergstrand Research facility

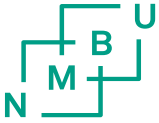
Collection sample date: 08/06/2015

Site Name 2: Drøbak Wastewater Treatment Plant
Time: 11:00
Laboratory analysis:
Analyzed by: EKC Da Silva
First analysis Date:08/02/2015
Read by: EKC Da Silva

Appendix B: Quanti-tray with yellow wells and Florescence wells

Figure 8.1 Quanti trays showing positive large and small well yellow pockets for coliform bacteria from day o laboratory experiments. (Silva 2015)

Figure 8.2 Example of Confirmation of *E coli* bacteria by the UV florescence. (Silva, 2015)



Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no