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Master's Thesis

Resilience of Microbial Activity in Enhanced Sewer Self-Purification against Salinity Shock and Thermal Shock

(下水道内自己浄化機能強化における塩分ショックと熱ショックに対する

微生物活動の耐性と回復力)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOB: Ammonium-oxidizing bacteria

EC: Electrical conductivity

HSR: Heat shock response

HSP: Heat shock proteins

ICOP: Intermittent Contact Oxidation Process

NOB: Nitrite-oxidizing bacteria

OCR: Oxygen consumption rate

OUR: Oxygen uptake rate

PVC: Polyvinyl chloride

SMP: Soluble microbial product

SS: Suspended solid

TOC: Total organic carbon

TDS: Total dissolved solid

VSS: Volatile suspended solid

WWTP: Wastewater treatment plant

LIST OF UNIT MEASUREMENT

°C: Degree Celsius

g: gram

g/L: gram per litre

$gO_2/m^2/hr$: gram of oxygen per metre square per hour

hr: hour

hPa: hectopascal

J: Joule

K: Kelvin

L: liter

mm: millimetre

mg/L: milligram per liter

ml/day: millilitre per day

m^2: square meter

m^3: cubic meter

min: minute

ml/min: millilitre per minute

Pa: pascal

S/m: siemens per meter

%: percentage

Abstract

This study investigates the resilience of an enhanced sewer self-purification system to salinity and thermal shocks, focusing on two specific objectives. The first objective is to quantify the impact of short-term salinity and thermal shocks on microbial performance. The second objective is to compare the relative effects of salinity and thermal shocks on microbial activity.

The literature review explains previous studies on sewer processes, enhanced sewer self-purification, and the effects of salinity and thermal shocks on microorganisms and the wastewater treatment process.

To achieve the study's objectives, the material and method section describes the procedures for observing the effects of salinity and thermal shocks on microbial activity in an enhanced sewer self-purification system. These two shocks experiments were conducted separately. In this study, a lab-scale ICOP self-purification reactor was operated to prepare sponges with biomass, then salinity or thermal shock was applied to the sponges with biomass. The extent of shock was determined by measuring OCR before and after shock to evaluate the microbial response.

Separate experiments were conducted to study the effects of salinity shock (0.5%, 1%, 2%, 3% concentrations of sodium chloride with durations of 3-30 minutes) under two conditions (rinse with tap water and no rinse). Rinse condition of salinity shock experiment was conducted two series. Thermal shock (40˚C, 50˚C, 55˚C, 60˚C, 65˚C, 70˚C with durations of 3-30 minutes) on OCR. OCR was monitored before and after shocks to evaluate the microbial response.

The results indicated that higher salinity levels led to greater OCR reduction under the no rinse condition, having a more pronounced impact compared to two series of rinse condition experiment. The highest negative impact was found in 3 % shock for 30 minutes was shown moderate reduction of OCR around 40% reduction. Under the two series of rinse conditions, the first series with 0.5 %, 1% and 2% salinity shocks cause very little reduction of OCR, while the second series observed more reduction with slow recovery, due to the growth of higher organism likely tubifex and nematoda.

The thermal shock results indicated that temperatures above 40˚C caused a significant immediate reduction in OCR, but a rapid recovery was observed within a few hours.

A comparison of both thermal and salinity shocks implied that the sponge with biomass were resilient to both types of shocks. Thermal shocks led to a rapid increase in OCR after the shock, while salinity shocks (up to 3%) had a minor effect.

This study emphasizes the resilience of the enhanced sewer self-purification system against salinity and thermal shocks which are potential common shocks in sewer environments. The findings demonstrate that the system’s sponge with biomass are resilient to both types of shocks, suggesting that the system can be effectively implemented in upstream sewers to enhance sewage treatment processes. The resilience mechanisms identified offer potential opportunities for future research on other shock loads in sewer self-purification system.

Chapter 1

INTRODUCTION

1.1 Research background

The sewer is not just a conduit for wastewater but also a place for chemical and microbial transformations. It can affect the quality of the wastewater. The activity of microorganisms in sewer networks can actually become treatment systems by breaking down the pollutants in the wastewater before it goes to a larger treatment facility, acting as a kind of pre-treatment step[1][2]. As the concept of "pipe-and-plant treatment" is introduced by Hvitved-Jacobsen *et al.* (2002), sewer treatment should start at the source (the sink) and be integrated with the sewer processes for integrated urban wastewater management [1]. Y. Tanji et al (2006) stated that voided materials like porous material improved the conversion rates of dissolved oxygen, total organic carbon, and nitrogen compounds, indicating enhanced self-purification capacity [3]. By arising from this concept, the enhanced sewer self-purification system had developed. Shoji *et al.* (2015) demonstrated that the potential of porous media in sewer pipes to improve the efficiency of wastewater treatment processes [3].

The system has potential advantages, by consuming low energy, less space requirement and allowing for the reuse of wastewater on a small scale[3]. The system works by installing sponge media inside sewer pipes. As sewage flows intermittently through the pipes, these microorganisms living on the sponges remove organic matter from the wastewater. Sewage containing organic matter is passing to sponge media during flow, and oxygen is supplied through surface aeration during no-flow conditions, promoting aerobic degradation[3]. Microorganisms in the sponge media take up soluble organic matter and store it in their cells, which is then gradually oxidized, especially during non-flow periods[3].

The system intends to install not only in downstream of sewer, but further upstream in the sewer network since a longer sewer network can enhance the overall quality of sewage treatment. In sewer upstream areas with higher fluctuations in flow rate [4], the concentrations of various components in the sewage are also likely to fluctuate significantly. Various shocks load can suddenly discharge and change the conditions in the upstream sewer network, such as salinity, temperature, pH, lipids, and even emerging contaminants. If the sewage from each different sources have high load of substances that damage to microorganisms, it can impact in-sewer purification. Two such commonly encountered shocks are salinity shocks, originating from household kitchen sinks, and thermal shocks from sources like hot showers, dishwashers, and washing machines.

Understanding how salinity and thermal shocks affect the microbial activity within the system is crucial for successful upstream implementation. Both shocks can impact microorganisms, but through different mechanisms. Salinity shocks, often caused by discharges of leftover salty foods, soy sauce, or pickles, can harm microorganisms through osmotic stress, membrane disruption, protein denaturation, and ionic imbalance within the cells[5]. In contrast, thermal shocks, associated with increased water temperature, can damage cell membranes, leading to leakage of proteins and other vital molecules, ultimately causing cell death[6] [7]. Furthermore, thermal shocks can disrupt cellular functions by misfolding protein[8].

In this study, salinity shock is defined as a brief and sudden increase in the salinity levels within the sewer network, caused by the discharge of saline water. Previous research has primarily focused on the effects of longer-duration shocks (hours to days), with limited understanding of the impact from brief, short-term shocks lasting only 3 to 30 minutes. These shorter events are more common in real-world scenarios, such as when salty kitchen wastewater (1-3% salt concentration) is discharged. It's particularly important to investigate how these short-term saline discharges, potentially followed by rinsing with tap water, affect microbial communities within the system.

While both salinity and thermal shocks can temporarily disrupt wastewater treatment processes, microorganisms possess inherent resilience mechanisms that allow them to recover over time. This study investigates the impact of these brief shocks on enhanced sewer self-purification system, designed for implementation within sewer networks. By examining the system's response to these shocks, this study targets to assess its sensitivity and resilient capacity. By comparing the effects of salinity and thermal shocks on microbial activity, this study intends to comprehensively evaluate the resilience of enhanced sewer self-purification system in upstream sewer environments.

1.2 Research Objectives

This study aims to investigate the resilience of microbial activity in enhanced sewer self-purification against salinity shock and thermal shock for real sewer application.

Specific Objectives:

The first objective is to quantify the impact of short-term salinity and thermal shocks on microbial performance: This objective investigates how both salinity and thermal shocks affect microbial activity in the system. To evaluate this, by measuring the oxygen consumption rate (OCR) of sponge with biomass before and after exposure to each type of shock will be assessed. Separate experiments will be conducted for salinity and thermal shocks to isolate the effects of each shock.

> Salinity Shock: Sponge with biomass will be exposed to varying concentrations of saline water for durations ranging from 3 to 30 minutes. Then, the OCR of shocked sponges with and without a subsequent rinse using tap water will be compared.
>
> Thermal Shock: Sponge with biomass will be exposed to different hot water temperatures from 3 to 30 minutes. Similar to the salinity experiment, the OCR after shock will be measured.

The second objective is to compare the relative effects of salinity and thermal shocks on microbial activity: By analysing the changes in OCR across to all shock conditions (salinity with/without rinse, and thermal shocks at various temperatures), the sensitivity of the microbial community within the system to different types of shocks will be compared. This comparison will allow to determine the shock type (salinity or thermal) that has a more detrimental impact on system performance and resilience.

1.3 Thesis Structure

This thesis is divided into five chapters as follows:

Chapter 1 is the general introduction of the study, including the research background, objectives, and thesis structure.

Chapter 2 covers the literature review about enhanced sewer self-purification system, effect of salinity shock on microorganism and wastewater treatment process.

Chapter 3 describes the detailed methodology of this study. This section explains the experimental setup, preparation of sponge with biomass, monitoring oxygen consumption rate, salinity shock application of no rinse, two series of rinse condition, and thermal shock application.

Chapter 4 explains the results and discussions observed in this study.

Chapter 5 summarises findings, limitations, and recommendations.

Chapter 2

LITERATURE REVIEW

This chapter describe a review of previous studies on sewer processes and enhanced sewer self-purification. Additionally, it reviews the effects of salinity and thermal shocks on microorganisms and the wastewater treatment process.

2.1 Sewer process

A sewer system is a network that collects wastewater from various sources such as residential areas, commercial areas and industries, channelling it from small upstream sewers through gradually into larger downstream ones until it reaches wastewater treatment plant [9]. The sewer is not just a conduit for wastewater but also a site for significant chemical and biological reactions, affecting the quality of the wastewater and the environment. The transformations within the sewer system can impact the performance of downstream treatment plants and the quality of the receiving water bodies[1]. Microorganisms in sewers engage in complex biochemical processes, which can offer potential for engineered solutions. There's potential to engineer biofilms to enhance sewer sustainability and improve wastewater pre-treatment [2]. The enhanced sewer self-purification system which agrees to the potential of sewer improvements will be discussed in section 2.2.

Hvitved-Jacobsen *et al.* (2013) described the sewer system as a complex environment where microbial and chemical processes occur across various phases[9] . These phases include the water phase, which contains the wastewater and its suspended solids, and the biofilm phase which consists of microbial communities attached to submerged solid parts of the sewer. Additionally, there are sewer sediments, also known as deposits, and the sewer atmosphere or headspace, which is the gaseous environment above the water. The sewer walls, which are exposed to the sewer atmosphere, have a moisture layer with adhered substances. Interactions among these phases, such as the exchange of volatile organic compounds from the water phase into the sewer atmosphere, influence on the overall performance of in-sewer processes. A photograph of a sewer environment is shown in Figure 1.

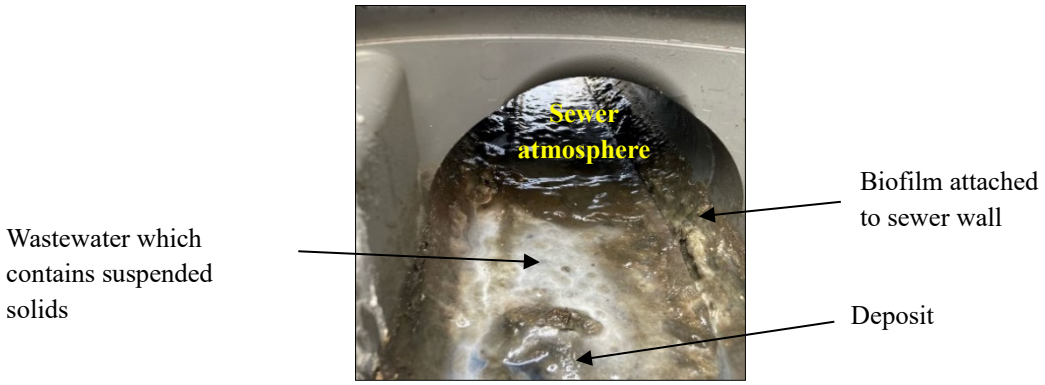


Figure 1. A sewer where the microbial activity and chemical reaction occur

2.2 Enhanced sewer self-purification

Previous research suggests that sewer systems can play a more active role in wastewater treatment, alongside traditional treatment plants. This concept, "pipe-and-plant treatment" by Hvitved-Jacobsen *et al.* (2002), emphasizes treating wastewater within the sewer network itself, integrated with urban wastewater management [1]. Studies by Tanji *et al.* (2006) and Shoji *et al.* (2015) support this idea. They found that using a medium, like porous material, in sewer pipes can improve the breakdown of pollutants and increase the sewer's natural ability to clean itself (self-purification)[3], [10].

While the self-purification process in sewers removes some pollutants, its efficiency is generally low [3]. However, it is possible to enhance self-purification by installing sponge media inside sewer pipes as a habitat for microorganisms[3]. As sewage intermittently flows through the pipes, the microorganisms feed on the organic material. During dry periods (no flow), oxygen from the air dissolves into sewage through the surface of the sponge, promoting aerobic degradation by the microorganisms. These microbes in the sponge take up the dissolved organic matter and store it within their cells. Then they gradually oxidized, even during periods without sewage flow[11] . However, it has a significant advantage that it doesn't require additional energy sources. This makes self-purification a valuable technology in areas with limited or no wastewater treatment infrastructure.

Several research have been done to investigate the capacity of enhanced sewer self-purification. Shoji *et al.* (2015) developed a double-layered sewer pipe system to improve self-purification within the sewer [3]. This system has two compartments. The lower deck contains a sponge material that provides a habitat for microorganisms, promoting the organic matter degradation. The upper deck allows for smooth sewage flow. The water falls between the two decks provide interaction with air, which helps dissolve oxygen from the air into the wastewater. This system is more applicable for downstream sewer where high sewage flow prevails. However, the double-layer structure may not be as effective in sewers with low flow. In these cases, setting the sponge media directly at the bottom of the sewer might be more practical. This allows oxygen to reach the microorganisms through the sponge during periods of intermittent flow. Other research by Sotelo *et al.* [12], [13], [14], [15] and Lyu *et al.*(2020) [4] investigated enhanced sewer self-purification using single-layer pipes, as illustrated in Figure 2.

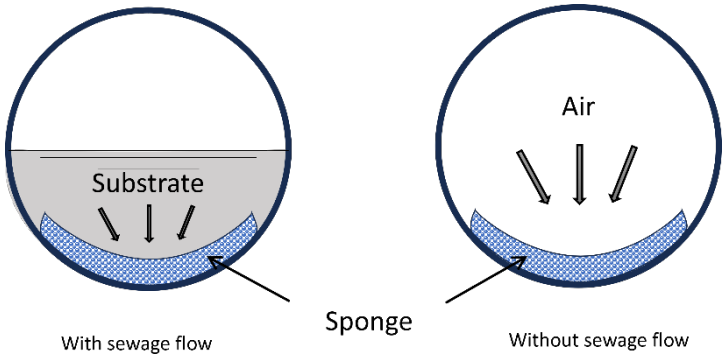


Figure 2. Enhanced sewer self-purification system

2.2.1 Self-purification in sewer upstream

The effectiveness of enhanced sewer self-purification technologies depends on the available sewer pipe length per capita, influenced by population density [16]. The system is intended to install not only in downstream of sewer, but further upstream in the sewer network since a longer sewer network can enhance the overall quality of sewage treatment in sewer.

Sewer upstream is the starting point of the wastewater's passage through the sewer system before it reaches downstream sections such as trunk or intercepting sewers [9]. Upstream sewer systems are described by low sewage flow, extended pipe lengths, long hydraulic retention times, and a high proportion of biodegradable substrates. The sewage flow in these areas is intermittent, with frequent dry conditions and hydraulic instability [17].

Sewer upstream has intermittent flow with high shock loads and is mostly dry, while sewer downstream has a more continuous flow pattern with smaller chance of shock load due to the dilution effect of the sewer network. Upstream sewers encounter highly irregular flow patterns due to household activities[17], resulting in very brief contact times between sewage and biomass. This contact duration can impact the performance of enhanced sewer self-purification[18].

Upstream sewer sections with fluctuating flow rates can also experience significant variations in wastewater composition. These fluctuations, caused by sudden changes in the discharge of various materials, can impact factors like salinity, temperature, pH, lipids, and even the presence of emerging contaminants. High concentrations of substances harmful to microorganisms, from different sources within the sewer network, can affect in-sewer purification.

2.3 Effect of salinity shock on microorganisms

The term salinity shock in this study refers to a sudden change in the salinity of the environment to which microorganisms are exposed. This present study focuses on the effects of high salinity shock on wastewater treatment, since low salinity environments are less common in sewer systems. Salinity shock can have a significant impact on survival of microorganisms and cell's function.

In the context of microorganism, high salinity environments create a hypertonic (higher concentration) condition outside the microorganism's cell. The difference in concentration between high and low salt solutions creates a driving force called osmotic pressure[19]. This pressure is the force exerted by water as it moves across a semipermeable membrane due to differences in solute concentration[19]. Osmotic pressure balance is essential for cell growth, shape, and division[20]. Generally, bacterial cell walls are to be strong enough to withstand high pressure[20]. Bacteria need to maintain a higher internal pressure adjust to their surroundings [20]. If a cell is in a lower saline solution (hypotonic), it might swell up because water will be absorbed by the cell. But if a cell is in a stronger saline solution (hypertonic), it will shrink because the water moves out from inside the cell.

Still, microorganisms can slowly adapt to high saline environments through physiological and genetic mechanisms, such as regulating intracellular ionic concentrations and accumulating compatible solutes to balance external salinity stress [21]. Microorganisms can live across a large range of salinity levels due to diverse adaptations for osmotic balance. Halotolerant microbes like some bacteria and fungi can survive in a wide range of salt concentrations, while halophiles require high salinity for growth around [5].These microorganisms use various mechanisms to resist dehydrating effects of high salt. One common adaptation is the accumulation of compatible solutes which are highly water-soluble organic molecules and include sugars, alcohols, and amino acid derivatives in their cell[5]. These small organic molecules balance the external pressure of microbial cell without disrupting cellular processes [22] . On the other hand, halophilic archaea, may utilize inorganic ions like potassium to maintain osmotic equilibrium, while some eukaryotes like algae use glycerol and other compatible solutes for osmotic balance [23]. These adaptations allow microorganisms to not only survive but grow in environments ranging from freshwater to extreme saline water.

While microorganisms can slowly adapt to high salinity environments through various mechanisms like accumulating compatible solutes or using inorganic ions, a rapid increase in salinity creates an osmotic pressure imbalance. Even if the microorganism survives at the initial shock, its growth and metabolic activity can be significantly reduced. This is because they need to utilize energy to restore and maintain their internal osmotic balance[24]. The stress caused by salinity shock can also disrupt essential cellular processes such as protein synthesis and enzyme activity, that make further delaying growth and function [5]. This means that even if the microorganism survives at the initial shock, its growth and metabolic activity can be significantly reduced. Microbial function inhibition and biodiversity reduction may occur alongside with increasing salinity[25]. H. Chen *et al*. (2022) reported that that high salt concentration generally suppressed the abundance of most major bacterial types in wetland ecosystems [25]. Another study by Lew *et al*. observed a significant decrease in total bacterial numbers in nine coastal lakes along the southern Baltic Sea coast, stating that increased salinity can negatively impact bacterial populations[26]. Therefore, understanding the detrimental effects of salinity shock is crucial for predicting the performance microorganisms in environments with fluctuating salinity.

2.4 Effect of salinity shock on wastewater treatment process

Saline water can enter wastewater treatment plants through various ways. In coastal areas, rising sea levels or storm surges can cause seawater intrusion into sewer systems that will suddenly change of salinity concentration. During winter, de-icing salts applied to roads can be washed away by snowmelt or rain, eventually reaching the wastewater treatment plant through storm drains[27]. This seasonal increase in salinity can damage the microbial community. This drainage water can flow to the wastewater treatment system through collection channels, impacting the biological treatment processes.

Biological wastewater treatment relies heavily on the activity of microorganisms to break down organic pollutants. A sudden increase in salinity can significantly disrupt these biological processes. Salinity shock disrupts the osmotic balance of microorganisms in the treatment system, that can lead to dehydration and reduced metabolic activity. This means that high salinity can decrease their ability to degrade organic pollutants.

Researchers stated that the performance of activated sludge systems in treating domestic wastewater is influenced by the concentration of NaCl shock loading. Wang *et al.* 2005 reported that beyond 5 g/L of NaCl, there is a notable decrease in both (TOC) removal efficiency over 30% and oxygen uptake rate (OUR) more than 35% in activated sludge systems [28]. Reduced microbial activity directly impacts the removal efficiency of organic matter, nutrients, and other pollutants from the wastewater. This can lead to a decline in the overall treatment performance. In addition, Li *et al.* (2013) demonstrated that substrate removal efficiency was significantly decreased when NaCl level reached above 1% in activated sludge system by accumulation of soluble microbial product (SMP), especially proteins[29]. Another study by Zhang *et al*. (2017), reported that low salinity shocks (0-1% salinity) improved substrate removal rates, while high salinity shocks (2.5-3% salinity) had detrimental effects in unacclimated activated sludge with pressurized aeration[30].Higher salinity levels significantly delay biological wastewater treatment by increasing osmotic pressure, which adversely affects microbial activity and substrate removal rates.

It is important to understand how salinity shocks will affect upstream sewer system if the enhanced sewer self-purification system. Compared to conventional wastewater treatment plants, salinity shocks in enhanced sewer self-purification systems have a distinct characteristic. While coastal areas might experience salinity incidents due to storm surges or road de-icing practices, these events typically last for hours or even days. Similarly, intentional treatment of high-saline wastewater can also involve prolonged exposure. In contrast, salinity shock within enhanced sewer self-purification system is much shorter-lived, lasting mere minutes due to brief discharges from kitchen sinks.

Above mention studies are primarily focused on salinity shocks lasting for hours or even days, neglecting the impact of shorter-term events (3 to 30 minutes). These brief salinity shocks are more common in real sewer applications, such as discharges of salty wastewater from kitchen sinks (containing 1% to 3% salt concentration). However, the frequency of these short-term salinity events is significantly higher compared to the infrequent occurrences in conventional systems. These shocks can happen for various reasons, such as discharge leftover of salty foods, soy sauce or pickled. Kitchen wastewater, which can be salty, might flow through the sewer sometimes. Then, rinse water from washing dishes, might follow, making it complicated to the treatment process. This unique feature requires a more understanding of how these brief salinity shocks impact microbial activity within self-purification sewer system.

2.5 Effect of thermal shock on microorganisms

Thermal shock refers to a shock caused by a rapid and significant change in the surrounding temperature. This sudden temperature fluctuation can have a detrimental effect on microbial cells, potentially leading to death or reduced functionality. Cell membranes are composed of phospholipids and proteins that maintain a specific fluidity for proper function[5]. Thermal shock can change this fluidity, causing the membrane to

become rigid or leaky [5], [31]This leakage disrupts the movement of essential molecules and ions in and out of the cell, interrupting cellular processes.

Many of bacteria, the dominant single-celled microorganisms in wastewater treatment plants, grow on dissolved organic matter. Each bacterial species has a preferred temperature range for optimal growth. Psychrophiles grow best below 20°C, mesophiles between 25°C and 40°C, thermophiles between 45°C and 60°C, and hyperthermophiles near boiling [32]. Facultative thermophiles extend their range from thermophilic to mesophilic. These categories are not limited but bacteria can grow more slowly outside their ideal range and even survive in extreme temperatures. For example, *Escherichia coli* (a mesophile) can grow from 20°C to 50°C, but it can even reproduce slowly at 0°C. Rapidly frozen, E. coli and many other bacteria can survive for years. However, exceeding the optimal temperature range rapidly reduces growth due to protein denaturation.

Proteins play a critical role in various cellular functions. When exposed to extreme temperatures, proteins can lose their structure, rendering them dysfunctional[8]. This denaturation can inactivate enzymes, disrupt transport processes, and finally lead to damage cell. Moreover, thermal shock can disrupt enzymes' activity, leading to imbalance in metabolic activities. These imbalances can prevent cells from generating energy, synthesizing essential molecules, and maintaining proper cellular functions[8].

Even though thermal shock can be harmful to microorganisms, they possess a defence system known as the heat shock response (HSR)[8]**.**This response includes the rapid production of a specific group of proteins called heat shock proteins (HSPs)[33]. With HSPs during and after thermal shock, cells can refold denatured proteins back into their functional form and prevent the formation of harmful protein. Also, they can target damaged proteins for degradation and removal. HSPs help cells to recover from thermal shock and maintain cellular function. Although thermal shock can be a significant threat to microorganisms, the production of HSPs is an important defence system for cellular repair and survival. However, it is important that the performance of the HSR is limited and may not be able to prevent cell damage, if the thermal shock is too severe or prolonged. Additionally, different kinds of microorganisms to produce HSR are not the same.

While exposure to high temperatures can disrupt their growth and activity, studies have shown their ability to recover after a period of heat shock. Ji *et al.* (2018) investigated the impact of mild heat shock on the microbial communities in water distribution systems [34]. Their findings showed minimal long-term effects on microbial diversity or abundance on thermal shock. They observed reduction in total bacterial numbers following thermal shock appeared to be temporary, since the microbes demonstrated a significant ability to recover to pre-shock levels within two months. This suggests that while thermal shock can cause a temporary setback, microorganisms possess mechanisms to adapt and resume normal activity. While sudden temperature changes from hot water discharges can indeed cause a temporary decline in microbial activity, the resilience of these populations implies a return to normal function within a reasonable timeframe.

2.6 Effect of thermal shock on wastewater treatment processes

Temperature plays an important role in wastewater treatment efficiency. Favourable temperatures can reduce the amount of land needed for treatment facilities, improve the effectiveness of conversion processes, enhance removal efficiencies of pollutants, and even enable the use of specific treatment methods [35]. Furthermore, the solubility of oxygen in water decreases as the temperature increases [36].This means that warm water holds less dissolved oxygen compared to cold water.

Maintaining stable temperatures is critical for optimal wastewater treatment efficiency. A study by T. Liu *et al.* (2022) described this by demonstrating that temperature shocks can negatively impact effluent quality in aerobic activated sludge systems. Their research found that sudden temperature changes began an increase in the production of soluble microbial products (SMPs)[37]. This can negatively affect subsequent

treatment processes. Furthermore, the study reported that the system required more than 24 hours to adapt to these temperature fluctuations, suggesting a potential delay in performance after thermal shock events.

However, within specific wastewater treatment processes, thermal shock can be a tool for manipulating microbial populations. J. Chen *et al.* (2019) demonstrated this in anammox reactors, where heat shock selectively suppressed certain microbes, allowing others to take over [38]. Their study showed that both ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were negatively affected by the heat shock. However, AOB showed a faster recovery rate, leading to their dominance and successful nitritation in sewage treatment. This finding suggests that thermal shock can be strategically employed to target specific microbial populations, and this allowed for the selective growth of desired species that contribute to efficient wastewater treatment.

The previous studies discussed above explained the complex effects of thermal shock on wastewater treatment. While sudden temperature fluctuations can disrupt microbial activity and increase the production of unwanted byproducts[37], other research suggests microbial resilience[39]. In the case of anammox reactors, thermal shock can even be employed to desired microbial populations [38]. These findings offer various perceptions for understanding the potential impact of thermal shock. While temporary disruptions in performance are likely, the resilience of microbial communities suggests a recovery period followed by returning to normal function.

Above studies on thermal shocks in sewer systems has primarily focused on long-lasting events (hours), neglecting the impact of shorter-term shocks lasting only 3 to 30 minutes. These brief shocks are far more common in real-world sewer environments. For example, hot water discharges from baths and laundry machines typically reach temperatures between 40°C and 50°C. While these individual events may be less severe than longer shocks, their frequency is significantly higher in upstream sewer systems. Common household appliances, such as washing machines, dishwashers, and hot water from kitchens and bathtubs, can discharge wastewater at temperatures exceeding normal sewer temperatures. Therefore, studying thermal shock on enhanced sewer self-purification system can provide the information for the system's performance in sewer upstream.

2.7 Thermal shock on enhanced sewer self-purification system

A previous researcher, Ban (2023) investigated the impact of thermal shock on microbial activity and organic matter degradation within an Intermittent Contact Oxidation Process (ICOP) system[40] .She constructed a lab-scale enhance sewer self-purification reactor to prepare the sponge with biomass and tested thermal shock. Her research aimed to quantify two key aspects:

> Reduction in microbial activity: The study measured the decrease oxygen consumption rate (OCR) after thermal shock. OCR displays the rate at which microbes consume oxygen while degrading organic matter. A decrease in OCR indicates a reduction in microbial activity.
>
> Recovery time: The research also examined how long it took for microbial activity (as measured by OCR) to recover after a thermal shock.

2.7.1 Outline of thermal shock experiment by Ban (2023)

The study employed a lap-scale ICOP reactor with biofilm-covered sponges. She tested these sponges to various thermal shock temperatures (50°C to 65°C) for different durations (3 and 10 minutes). Her study employed controlled experiments to assess the impact of thermal shock on the microbial activity within sponge with biomass used in the system. Each experiment involved the following steps:

(a) Pre-Shock Measurement: Oxygen Consumption Rate (OCR) was measured to each sponge which was placed in an airtight container with an oxygen sensor and monitored for 10 hours in an incubator at 25°C. This allowed for the determination of initial OCR before the thermal shock.

After the initial OCR measurement, the nine sponges were divided into three groups (control, 3-minute shock, and 10-minute shock) with three sponges in each group.

(b) Dewatering: Sponges were dewatered by placing them on a tissue for 3 minutes.
(c) Thermal shock application: Hot water (ambient temperature, 50°C, 55°C, 60°C, 65°C) was applied in a downward flow at 200 mL/minute for either 3 or 10 minutes, depending on the designated group. The control group was applied room temperature water flow for 10 minutes.
(d) Cooling: After thermal shock application, all sponges were cooled in a water flow of 250 ml/minute for 2 minutes.
(e) After-shock measurement: Following thermal shock, all nine sponges were placed back in their airtight sensor's containers and monitored for 24 hours in the incubator to assess their after-shock OCR.

Her findings indicated that the higher the degree of shock in temperature, the more OCR reduction occurs. In addition, she observed that oxygen consumption rate (OCR) immediately after thermal shock was reduced and then recovered within a few hours. However, she encountered unstable OCR of sponge with biomass before thermal shock in her study, so the effect of thermal shock was uncertain in some cases.

2.7.2 Building upon previous work for better understanding of thermal shock effects

This study builds upon the research conducted by Ban (2023) who investigated the impact of thermal shock on microbial activity and organic matter degradation within an Intermittent Contact Oxidation Process (ICOP). While her study provided original methodology, this current research takes a further step to refine the methodology and improve the understanding of thermal shock effects on enhanced sewer self-purification system. The improvements in this study include:

(a) Achieving stable initial OCR: The initial OCR of sponge with biomass before shock was stabilized by aerobic incubation in order to observe the effect of thermal shock clearer
(b) Minimizing biomass washout: The shock application was optimized to minimize the loss of biomass during the shock event by wrapping tissue to sponge with biomass, potentially leading to a more accurate assessment of the impact on microbial activity within the system.

This current thesis will continue to study the effect of thermal shock on enhanced sewer self-purification system and compared with salinity shock response on the system.

2.8 Chapter summary

This chapter reviews previous studies on sewer processes and enhanced sewer self-purification, the effects of salinity and thermal on microorganisms and the wastewater treatment process. The summaries are described as follows:

1. Sewer system is a network for wastewater collection and a reactor for significant chemical and biological reactions, that has impacts on water quality and wastewater treatment process.
2. Previous studies reported that using porous materials in sewers can improve pollutant removal and increase self-purification capacity.
3. Enhanced sewer self-purification technologies in upstream sewer, was characterized by intermittent flow and high shock loads.
4. Salinity and thermal shocks have impacts on microorganisms and the wastewater treatment process, that emphasize the need for understanding their effects on enhanced sewer self-purification system.
5. This thesis methodology was built upon a previous study by Ban (2023) that investigated the effects of thermal shock on enhanced sewer self-purification system. The current study was adapted and improved the methodology used in Ban (2023) to further examine the impact of thermal shock. Additionally, this research will compare the system's response to salinity shock and thermal shock.

Chapter 3

METHODOLOGY

This chapter describes the material and methods to achieve the objectives of the study, to observe salinity and thermal shock effects on microbial activity in enhanced sewer self-purification to apply in sewer system. These two shocks experiments were conducted separately. In this study, a lab-scale ICOP reactor was operated to prepare sponges with biomass, then salinity or thermal shock was applied to the sponges with biomass. The extent of shock was determined by measuring OCR before and after shock to evaluate the microbial response.

This chapter provides detailed procedures for applying shocks and subsequent monitoring. The general outline of each shock experiment is as follow:

1. Reactor operation to prepare sponges with biomass
2. Shock application experiment
    a. Preliminary (preparatory) incubation
    b. OCR monitoring before shock
    c. Shock application
    d. OCR monitoring after shock

3.1. Preparation of sponge with biomass

A laboratory-scale ICOP reactor, illustrated in Figure 3, was used for the experiment. It consists of channels installed with sponges, a recirculation tank, and a recirculation pump. The reactor had three parallel channels, each 90 cm length, 7 cm width, and 6 cm depth, with a 2% slope. Pieces of polyurethane sponge media, each measuring 4 cm length, 7 cm width, and 1 cm thickness (CFH-13, Inoac Corporation, Nagoya, Japan), were placed inside the channels. The reason behind for installing three channels to operate the reactor was to prepare enough sponges with biomass for testing different shock levels in multiplicate. After applying a shock to the sponges, they require time to fully recover before being tested again. To avoid delays and ensure the integrity of the testing conditions, 21 sponge with biomass pieces were prepared across the three channels.

The outlets of the channels led to a 7-litre recirculation tank commonly used for the three channels, where diluted return activated sludge was filled as the feed for the reactor. Return activated sludge was used in a wish to stabilize OCR of biomass, as activated sludge biomass consists of mostly living microorganisms and slowly biodegradable. Feed was replaced typically every three days. Details of the feed are shown in Table 1. Return activated sludge was obtained from the Sugano Sewage Terminal Treatment Plant in Ichikawa City, Chiba, Japan.

To apply the feed to the sponges in the channels, a recirculation pump (Masterflex Model 77201-60, USA) with three pump heads each connected to an individual channel, were employed with an Arduino UNO-compatible microcontroller to have intermittent feeding of 10 min every 30 min. During flow, the flow rate to each channel was adjusted to 200 ml/min. The reactor operated in an air-conditioned room maintained at 25±1˚C throughout the study.

Sponges with biomass prepared were used for thermal shock experiments (October 2023), salinity shock experiments with rinse (November 2023 and May 2024), and salinity shock experiments without rinse (January to February 2024), as tabulated in Table 1. Determination methods of suspended solids and volatile suspended solids are described in 3.4.2.

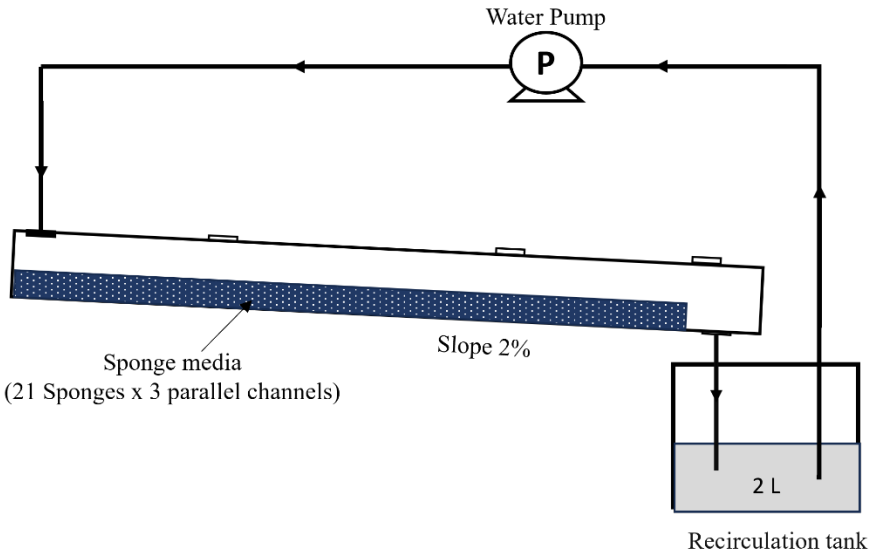


Figure 3. Enhanced sewer self-purification ICOP reactor

Table 1. Concentration of total suspended solids and volatile suspended solids in feed return activated sludge to the reactor

No.	Date of feed (yy-mm-dd)	Collected date from Sugano WWTP	Suspended Solid (mg/l)	Volatile Suspended Solid (mg/l)	Amount feed		Remark
					Return sludge (ml)	Tap water (ml)	
1	2023-08-16	2023-08-07	14000	10000	500	2000	
2	2023-08-19	2023-08-07	14000	10000	500	2000	
3	2023-08-20	2023-08-07	14000	10000	500	2000	
4	2023-08-23	2023-08-07	14000	10000	500	2000	
5	2023-08-26	2023-08-08	14000	10000	500	2000	
6	2023-08-29	2023-08-09	14000	10000	500	2000	
7	2023-09-01	2023-08-16	8000	6120	500	2000	
8	2023-09-04	2023-08-16	8000	6120	500	2000	
9	2023-09-07	2023-08-16	8000	6120	500	2000	
10	2023-09-11	2023-08-16	8000	6120	500	2000	
11	2023-09-14	2023-08-16	8000	6120	500	2000	
12	2023-09-17	2023-08-16	8000	6120	500	2000	
13	2023-09-20	2023-08-16	8000	6120	500	2000	
14	2023-09-23	2023-09-05	8160	6340	500	2000	
15	2023-09-26	2023-09-05	8160	6340	500	2000	
16	2023-09-29	2023-09-05	8160	6340	500	2000	
17	2023-10-02	2023-09-05	8160	6340	500	2000	Thermal shock
18	2023-10-05	2023-09-22	10320	8060	500	2000	
19	2023-10-07	2023-09-22	10320	8060	500	2000	
20	2023-10-10	2023-09-22	10320	8060	500	2000	
21	2023-10-13	2023-09-23	10320	8060	500	2000	
22	2023-10-16	2023-09-24	10320	8060	500	2000	
23	2023-10-19	2023-09-25	10320	8060	500	2000	
24	2023-10-22	2023-10-03	8080	6240	500	2000	
25	2023-10-26	2023-10-03	8080	6240	500	2000	
26	2023-10-28	2023-10-03	8080	6240	500	2000	
27	2023-10-31	2023-10-03	8080	6240	500	2000	
28	2023-11-04	2023-10-03	8080	6240	1000	1000	

29	2023-11-07	2023-10-03	8080	6240	1000	1000	
30	2023-11-10	2023-10-03	8080	6240	1000	1000	
31	2023-11-11	2023-10-03	8080	6240	1000	1000	
32	2023-11-12	2023-10-03	8080	6240	1000	1000	
33	2023-11-14	2023-10-03	8080	6240	1000	1000	
34	2023-11-17	2023-10-03	8080	6240	500	1500	Salinity shock: 1st series under rinse condition
35	2023-11-20	2023-11-16	9120	7080	500	1500	
36	2023-11-24	2023-11-16	9120	7080	500	1500	
37	2023-11-27	2023-11-16	9120	7080	500	1500	
38	2023-11-30	2023-11-16	9120	7080	500	1500	
39	2023-12-04	2023-11-16	9120	7080	500	1500	
40	2023-12-07	2023-11-16	9120	7080	500	1500	
41	2023-12-10	2023-11-16	9120	7080	500	1500	
42	2023-12-13	2023-11-24	9140	7160	500	1500	
43	2023-12-16	2023-11-24	9140	7160	500	2000	
44	2023-12-20	2023-11-24	9140	7160	500	2000	
45	2023-12-23	2023-12-13	8620	6980	500	2000	
46	2023-12-27	2023-12-13	8620	6980	500	2000	
47	2023-12-30	2023-12-13	8620	6980	500	2000	
48	2024-01-01	2023-12-13	8620	6980	500	2000	
49	2024-01-04	2023-12-13	8620	6980	500	2000	
50	2024-01-07	2023-12-13	8620	6980	500	1500	Salinity shock: under no rinse condition
51	2024-01-10	2023-12-13	8620	6980	500	1500	
52	2024-01-13	2023-12-13	8620	6980	500	1500	
53	2024-01-16	2023-12-27	8740	7060	500	1500	
54	2024-01-19	2023-12-27	8740	7060	500	1500	
55	2024-01-22	2023-12-27	8740	7060	500	1500	
56	2024-01-23	2024-01-12	7740	6340	1000	0	
57	2024-01-26	2024-01-12	7740	6340	1000	1000	
58	2024-01-27	2024-01-12	7740	6340	1000	1000	
59	2024-01-28	2024-01-12	7740	6340	1000	1000	
60	2024-01-31	2024-01-12	7740	6340	500	1500	
61	2024-02-04	2024-02-01	6760	5340	500	1500	
62	2024-02-07	2024-02-01	6760	5340	500	1500	
63	2024-02-10	2024-02-01	6760	5340	500	1500	
64	2024-02-14	2024-02-01	6760	5340	500	1500	
65	2024-02-17	2024-02-01	6760	5340	500	1500	
66	2024-02-21	2024-02-01	6760	5340	500	1500	
67	2024-03-09	2024-02-01	6760	5340	500	1500	
68	2024-03-14	2024-02-14	8400	6720	500	1500	
69	2024-03-18	2024-02-14	8400	6720	500	1500	
70	2024-03-22	2024-02-14	8400	6720	500	1500	
71	2024-03-26	2024-02-14	8400	6720	500	1500	
72	2024-04-02	2024-03-11	7100	5300	500	1500	
73	2024-04-08	2024-03-11	7100	5300	500	1500	
74	2024-04-22	2024-03-11	7100	5300	500	1500	
75	2024-04-23	2024-04-03	2220	1460	500	1500	
76	2024-04-24	2024-04-03	2220	1460	500	1500	
77	2024-04-25	2024-04-03	2220	1460	500	1500	
78	2024-04-26	2024-04-23	6000	4600	500	1500	
79	2024-04-29	2024-04-23	6000	4600	500	1500	
80	2024-05-02	2024-04-23	6000	4600	500	1500	
81	2024-05-05	2024-04-23	6000	4600	500	1500	Salinity shock: 2nd series under rinse condition
82	2024-05-08	2024-04-23	6000	4600	500	1500	
83	2024-05-12	2024-04-23	6000	4600	500	1500	
84	2024-05-16	2024-04-23	6000	4600	500	1500	
85	2024-05-24	2024-05-08	6060	4580	500	1500	
86	2024-05-28	2024-05-08	6060	4580	500	1500	

3.2 Salinity shock experiment

The study involved preparation of sponges with biomass, conditioning of sponges with biomass to stabilize OCR, OCR measurement before thermal shock, salinity shock application, and OCR measurement after salinity shock as shown in Figure 4. The extent of salinity shock was 3 to 30 minutes in duration and 0.5%, 1%, 2%, 3% in concentration. This extent of shock was selected to resemble the conditions that can happen in the enhanced sewer self-purification system.

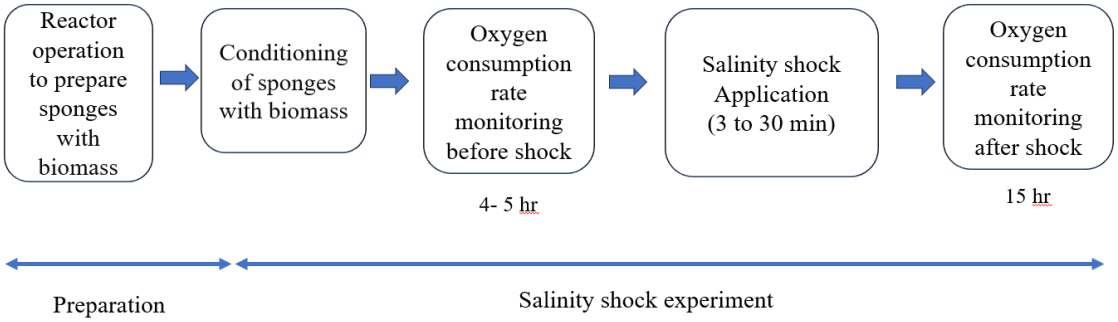
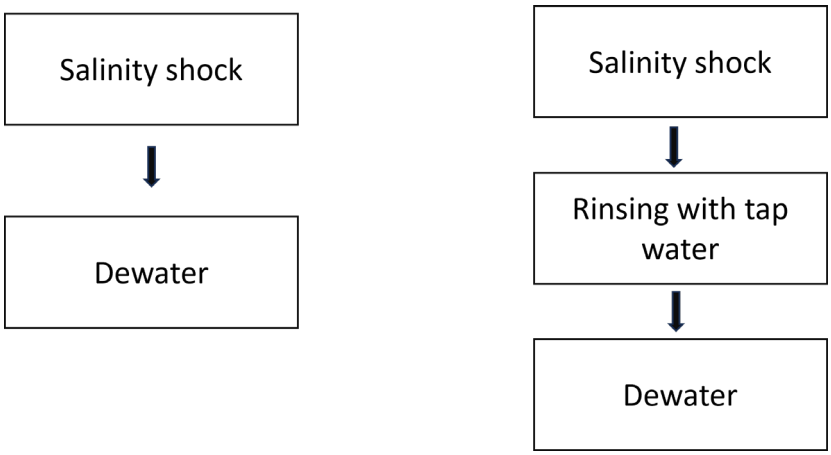


Figure 4. Outline of salinity shock experiment

In the salinity shock experiment, sodium chloride (NaCl) was utilized to simulate salinity shock encountered by microbial communities within the system. Two scenarios as shown in Figure 5 were tested. In the first scenario, a salinity shock was applied, and the saline water was retained in sponge (without rinse scenario, or no rinse scenario). In the second scenario, after the brief salinity shock, rinse water was applied to remove salinity remaining in sponge (with rinse scenario). The latter is for the case when saline wastewater flow is followed by freshwater flow such as rinse of dish washing.



(a) No Rinse Scenario (b) With Rinse Scenario

Figure 5. Procedures of without rinsing and with rinsing with tap water after salinity shock

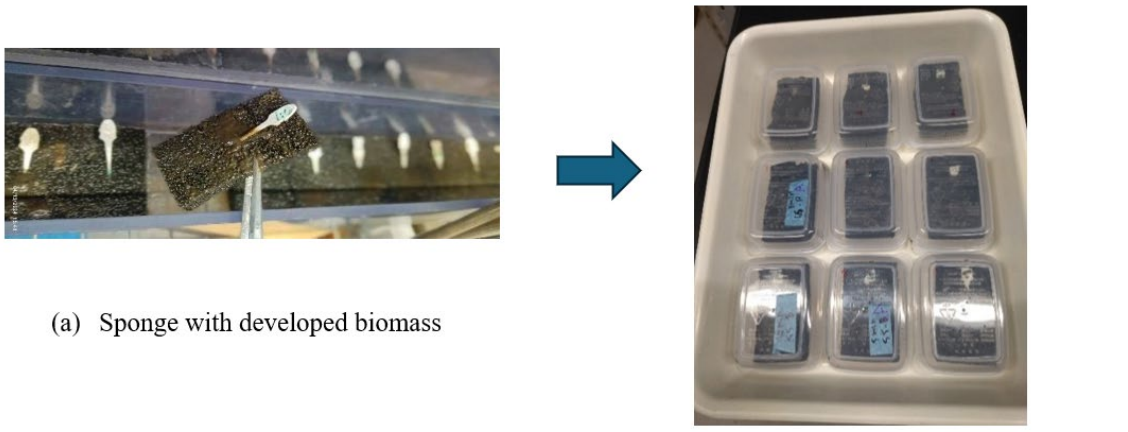
In the experimental procedure, sponges were dewatered repeatedly. Here describes the terms and procedure of dewatering in this thesis.

3.2.1 Conditioning of sponges with biomass

Nine sponges with biomass developed in the ICOP reactor mentioned in section 3.1 were collected from the channels had higher OCR after replacement of feed. Thus, sponges with biomass for the shock experiments were taken from the ICOP reactor after typically three days from replacement of feed. Further to stabilize OCR, these sponges with biomass were subjected to conditioning as explained here.

The sponges with biomass contain a high amount of water, which delays effective oxygen transfer. Each sponge collected from the ICOP reactor was gently placed between two pairs of folded paper towels (Cresia Slim EF Hand Towel, Nippon Paper Cresia, Co. Ltd., Tokyo, Japan) to dewater for 3 minutes. Following dewatering, the sponges with biomass were placed in 100 ml plastic boxes and incubated under aerobic condition for 24 hours as the preparatory incubation at an air-conditioned room temperature of 25±1˚C, as shown in Figure 6. The covers of the plastic boxes were slightly opened to allow air with oxygen gas to enter.

The biomass accumulated on sponge surface differed within each channel although the sponge pieces are the same size. In the upper part of the channel, the biomass accumulated on the sponges was thinner. In the middle, the biomass accumulated became thicker, and at the end of the channel, the biomass accumulated was the thickest. Based on the eyes' observation, sponges with biomass with medium level of accumulation were carefully selected from the reactor for the experiments. Thick layers were avoided as they could block oxygen transfer within the sponge, while thin layers were not used due to being underdeveloped and having little biomass attached. The wet weight of sponges with biomass were measured every time before aerobic incubation started. Generally, sponges with biomass weighed around 22 ± 3 g were chosen for testing. These sponges with biomass weighed around 13 ± 3 g after dewatering. The growth of higher organisms, most likely worms or tubifex, were observed inside the sponges with biomass during second series rinse condition experiments. Many of these worms were found outside sponge at the end of conditioning. Those higher organisms were not found by eyes observation during the first series rinse condition and no rinse condition experiment.



(a) Sponge with developed biomass

(b) Aerobic incubation to stabilize biomass

Figure 6. Preparatory incubation

3.2.2 Monitoring oxygen consumption rate (OCR) before shock

After conditioning, each sponge with biomass was placed in an airtight sensor box with an oxygen gas sensor, and OCR was monitored for around 240-300 minutes (4-5 hours) in an air-conditioned room at 25 ±1˚C. When stability of OCR before thermal shock was confirmed, then, the experiment proceeded to salinity shock application. If OCR was found not stable, the experiment was cancelled at this step. Detailed procedure of OCR monitoring is explained in section 3.4.1.

3.2.3 Salinity shock application

Following the OCR measurement before shock, nine sponges were divided into three groups. Each group containing three sponges was assigned to each different shock duration. Subsequently, each group of sponges were taken out from the airtight sensor boxes. Each sponge with biomass was wrapped with one sheet of wiper tissue (Kimwipe, size 120mmx215mm, Nippon Paper Cresia, Co.Ltd., Tokyo, Japan) to avoid biomass detachment by water flow during the salinity shock application. The tested sponges were placed on a spacer in the tray where saline water was filled at a depth of around 1cm. Then, salinity shock was applied by applying saline water flow at 100 mL/min till the sponges were totally submerged as illustrated in Figure 7. The tested sponges were placed under saline water for their designated shock duration. The temperature of the saline water was 20 ±1˚C. In each experiment, only one salinity level was applied.

The salt concentration levels, and duration of salinity shock are tabulated in Table 2. Experiments with rinse were conducted twice, as can be seen in Table 2. The first and second series of experiments with rinse were conducted during 2023 November to December and 2024 May respectively. The second series experiment was conducted to have control runs.

Table 2. Salinity levels and shock duration of each experiment

Salinity shock level	1st series under rinse condition	No rinse condition	2nd series under Rinse condition
0.5%	3 min	Control	Control
	10 min	3 min	3 min
	30 min	30 min	30 min
1%	3 min	Control	Control
	10 min	3 min	3 min
	30 min	30 min	30 min
2%	3 min	Control	Control
	10 min	3 min	3 min
	30 min	30 min	30 min
3%	3 min	Control	Control
	10 min	3 min	3 min
	30 min	30 min	30 min

In the case of experiment without rinse, sponge with biomass was taken out from the wrap tissue, held with a tweezer while dripping water, and further dewatered for 3 minutes on paper towel. On the other hand, in salinity shock experiment with rinse, each sponge with biomass was taken out from the saline water, gently dewatered by vertically holding it for several seconds, and placed in a container containing 300ml of tap water left for 3-minutes as shown in Figure 8. During soaking in tap water, occasionally the sponge was gently agitated. After rinsing, the sponge with biomass was taken out from the wrap tissue, held with a tweezer while dripping water, and further dewatered for 3 minutes on paper towel. Then, the sponge with biomass was placed again in the airtight sensor box to monitor OCR after salinity shock.

In control experiments shown in Table 2, instead of saline water, sponges were applied with tap water for 3 min.

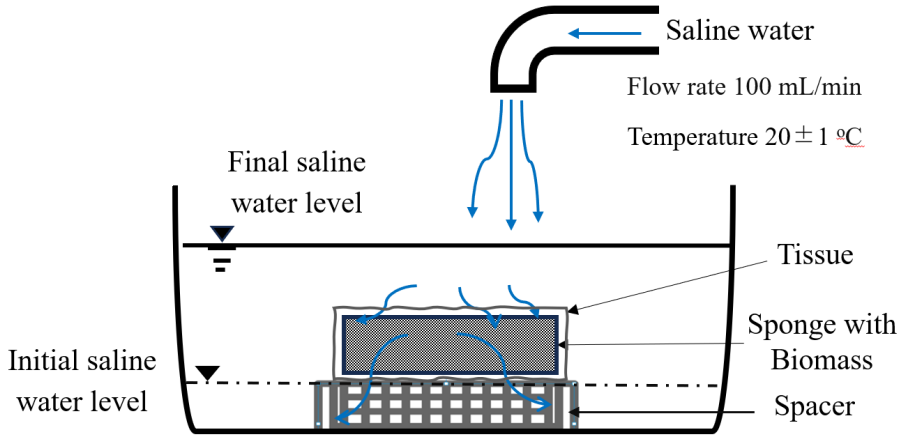


Figure 7. Applying saline water to sponge with biomass

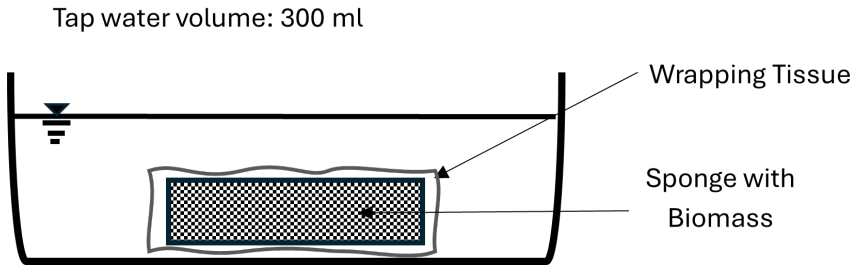


Figure 8. Rinse with tap water after salinity shock

3.2.4 Monitoring oxygen consumption rate (OCR) after shock

After salinity shock application, the dewatered sponges with biomass were placed inside the same oxygen sensor boxes immediately and started monitoring oxygen consumption rate (OCR). The consumption of oxygen inside the sensor box was monitored for another 900 minutes (15 hours) in an air-conditioned room at 25 ±1˚C. Detail OCR measurement after shock can be seen section 3.4.1.

3.3 Thermal shock experiment

Sponges with biomass were exposed to thermal shocks of different durations (3, 10 and 30 minutes) and hot water temperature (40˚C,50˚C,55˚C, 60˚C, 65˚C,70˚C) to assess the impact on Oxygen consumption rate (OCR). The study involved preparation of sponges with biomass, reactor operation, thermal shock application, and monitoring of OCR before and after shocks to evaluate microbial response as shown in Figure 9. The method of thermal shock experiment was mostly the same as salinity shock experiment section 3.2 aside from salinity shock application section 3.2.3.

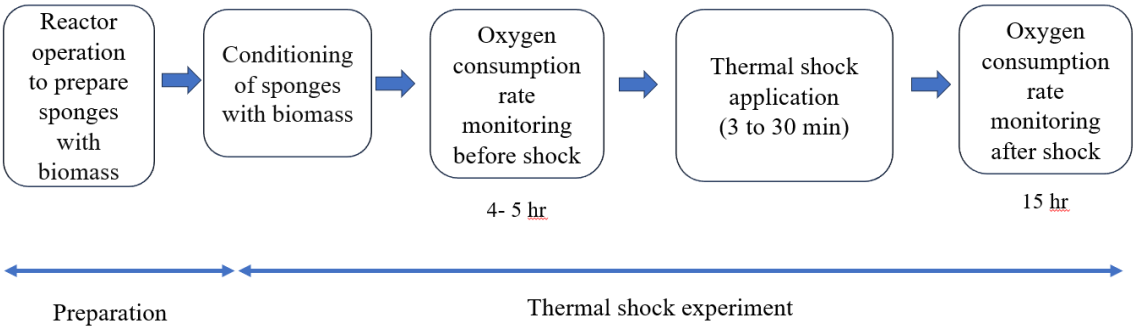


Figure 9. Outline of thermal shock experiment

Nine sponges with biomass taken from the ICOP reactor were conditioned, and then, OCR of the sponges with biomass before thermal shock was monitored in the same way as in salinity shock experiments. The detailed methods are described in 3.2.1. for conditioning and 3.2.2 and 3.4.1 for OCR measurement.

Following the measurement of OCR before thermal shock, nine sponges were divided into three groups each with 3 sponges. Each group was assigned to either 3-minute, 10-minute or 30-minute thermal shock. Subsequently, each group of sponges were taken out from the airtight sensor boxes. Each sponge with biomass were wrapped with one sheet of wiper tissue (Kimwipe, size 120mmx215mm, Nippon Paper Cresia, Co.Ltd., Tokyo, Japan) to reduce biomass washout by hot water flow during the thermal shock application. The hot water was initially filled in a water bath (TAITEC, Thermo Minder SDN-B) and stainless-steel tray was placed in the water bath to warm up its temperature. The tested sponges were placed in the tray where initial low level of hot water was filled. The hot water flow rate was 100 ml/min.

The hot water was flowed down above the sponge with biomass till the sponge was totally submerged in water, as illustrated in Figure 10. Then, the tray was covered with polypropylene cover at the top to reduce the heat transfer out of the tray. The tray was kept in the hot water with the same temperature during the application to stabilize the temperature of the sponge. The tested sponges were shocked for their designated shock duration. After applying thermal shock, the sponges with biomass were taken out from the wrap tissue, held with a tweezer while dripping water, and further dewatered by placing the sponge on two sheet pairs of folded paper (Cresia Slim EF Hand Towel, Nippon Paper Cresia, Co.Ltd., Tokyo, Japan) for 3 minutes. During dewatering on paper, the sponges with biomass were cooled down by air flow by a fan for 3 minutes.

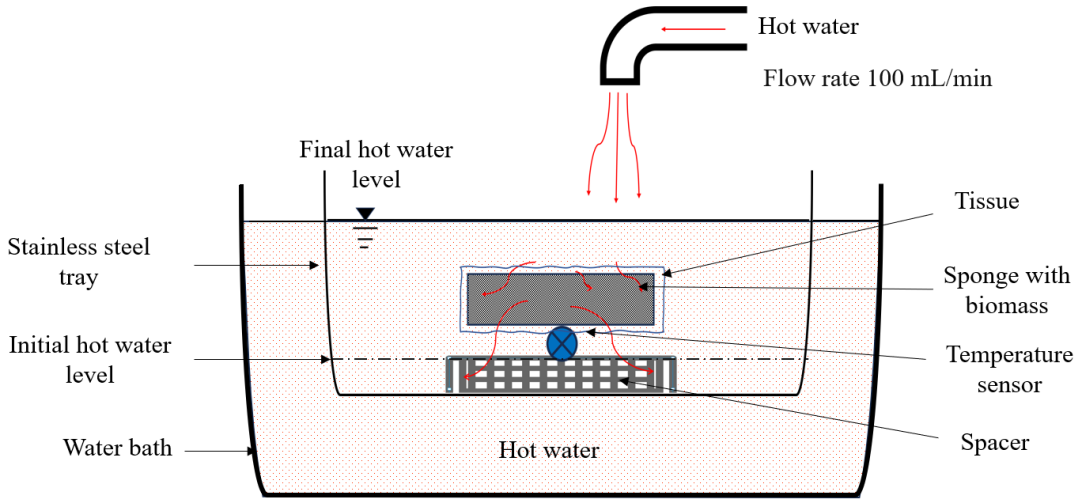


Figure 10. Applying thermal shock to sponge with biomass

After cooling down the sponges with biomass from thermal shock application, the sponges with biomass were placed inside the same oxygen sensor boxes immediately and started monitoring oxygen consumption rate (OCR). Detail OCR measurement after thermal shock is the same as salinity shock. (See in section 3.2.4 and section 3.4.1)

3.4 Analytical methods

3.4.1 Oxygen consumption rate (OCR)

Oxygen consumption rate (OCR) was monitored by utilizing an oxygen sensor (LuminOX-S, SST sensing, UK). The detected oxygen concentration was collected by an Arduino UNO compatible micro controller processors board and the data was collected every 5 min in the unit of hectopascal. The sensor box was made of plastic boxes (K202, Nakaya, Higashi-Osaka, Japan) with an inner volume of 100 ml as shown in Figure 11. Each sensor box was placed upside down with its lid at the bottom and a sponge with biomass on the lid and placed half-submerged under the water in a bat in order to prevent entering air via the gap between the box and the lid.

Since 9 sponges with biomass were monitored under 3 groups, each 3 oxygen sensors were connected to one Arduino processor via three software serial communication setups. The data from 3 Arduino processors was further sent to the parent Arduino processor via wireless UART transmitters (Twelite Blue UART, Mono Wireless Inc., Zama, Kanagawa, Japan), and the collected data was further transferred to the main computer. Oxygen concentration data was organized and stored automatically in the computer together with the temperature data in the airtight sensor boxes every 5 minutes.

Through the partial pressure of oxygen, the mass of oxygen in the airtight sensor box was calculated as follows:

Through the partial pressure of oxygen, the mass of oxygen in the airtight sensor box was calculated as follows:

Mass of O_2 = 32 × n (mol) = $32 \times \frac{PV}{TR}$

Where;

Mass of O_2: Mass of oxygen in the airtight sensor box (gO_2)

P: Partial pressure in (Pa), (1 hPa = 100 Pa)

V: gas volume in the airtight sensor box (m^3) (inner volume of the box – volume of O_2 sensor inside box – volume of sponge)

Sensor volume here is about half of the total volume of O_2 sensor.

Box size ($1x10^{-4}$ m^3), sponge size (0.04 m, 0.07m, 0.01m), sensor volume inside the box (2.12×10^{-6} m^3)

R: Gas constant, 8.314 (J/K/mol)

T: Absolute Temperature (K =temperature (˚C) + 273)

32: Mass of 1 mole of oxygen (g)

The decrease rate of oxygen in terms of mass in gram was evaluated every 5 minutes. After mass of oxygen data retrieved, the oxygen consumption rate (OCR) was calculated as the slope of the reduction of oxygen mass in the container standardized by the surface area of sponge (28 cm^2 = 0.0028 m^2).

OCR = Slope x $\frac{1}{\text{Area}}$

Where;

OCR : Oxygen consumption rate per unit surface area of sponge with biomass ($gO_2/m^2/hr$)

Slope : Slope of oxygen reduction in the airtight sensor box (gO_2/hr)

Area : Surface area of sponges with biomass (m^2)

Finally, OCR profile along time for a given shock condition was obtained as the of the average of OCR profiles from three sponges exposed to the same shock condition.

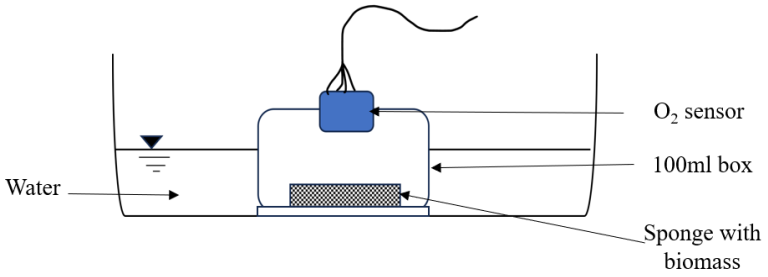


Figure 11. Airtight oxygen monitoring before and after shock

3.4.2 Suspended solids and volatile suspended solids concentrations in feed activated sludge mixture

Suspended solid (SS) is the dry mass of solids suspended in water sample. To measure suspended solids, usually dissolved solids are removed by filtration or centrifugation. However, here, the step to remove dissolved solids was omitted, as salt concentrations in samples were negligible when compared with suspended solids concentrations. To measure suspended solids, dry dishes with a known permanent mass (M_0 mg) were prepared, and 5 ml of each return activated sludge sample were placed in them. The dishes were then placed in a drying oven (DVS 602, Yamato, Tokyo, Japan) at 105°C overnight. The mass of the dish with the dried solids was measured (M_1 mg), subtracted by the permanent mass of the dish, and divided by 0.005 litres to obtain the suspended solids concentrations in mg/L. That is,

Suspended solids concentration (mg/L) = $(M_1 – M_0)/0.005$

Volatile suspended solids represent the organic portion of suspended solids in a water sample. It is derived from the loss on ignition of suspended solids. To measure volatile suspended solids, the dried dish already measured for suspended solids was placed in a muffle furnace (FO310, Yamato, Tokyo, Japan) at 600 °C for 30 min. The mass of the dish with ash was determined (M_2 mg), and the mass of volatile suspended solids in the sample was determined by the calculation below.

Volatile suspended solids concentration (mg/L) = $(M_1 – M_2) / 0.005$

Chapter 4

RESULTS AND DISCUSSIONS

In this chapter, results from salinity shock and thermal shock experiments are explained.

4.1 Salinity shock

4.1.1 OCR calculation

Here, the way how OCR was calculated is explained using oxygen concentration data obtained in a set of salinity shock experiment (3% salinity shock).

Figure 12 shows the oxygen partial pressure profiles of 9 sensor boxes over 1440 minutes (24 hours). The observed decrease in oxygen concentration within these boxes indicates oxygen consumption by microorganisms. The nine sensor boxes were divided into three groups as follows: three for control sponges (O1, O2, O3), three for 3-minute shock sponges (O4, O5, O6), and three for 10-minute shock sponges (O7, O8, O9). The average OCR for each condition was calculated from the oxygen concentration data of the three corresponding sponges, as shown in Figures 13 to 15. Here, OCR was standardized per unit surface area of a sponge with biomass (0.07 m × 0.04 m = 0.0028 m^2) expressed by means of the mass of oxygen consumed per surface area of sponge per hour. The OCR result of three sponges (Figure 13 to 15) was averaged for each experimental condition shown in Figure 16.

In Figure 16, the green line represents the average OCR for the control condition, the red line for the 3-minute shock, and the brown line for the 30-minute shock. The time when the shock was applied to the sponge with biomass is indicated as the red vertical line. Note that lower OCR in the latter half of monitoring may have been caused by the lowered oxygen concentrations in the airtight sensor box.

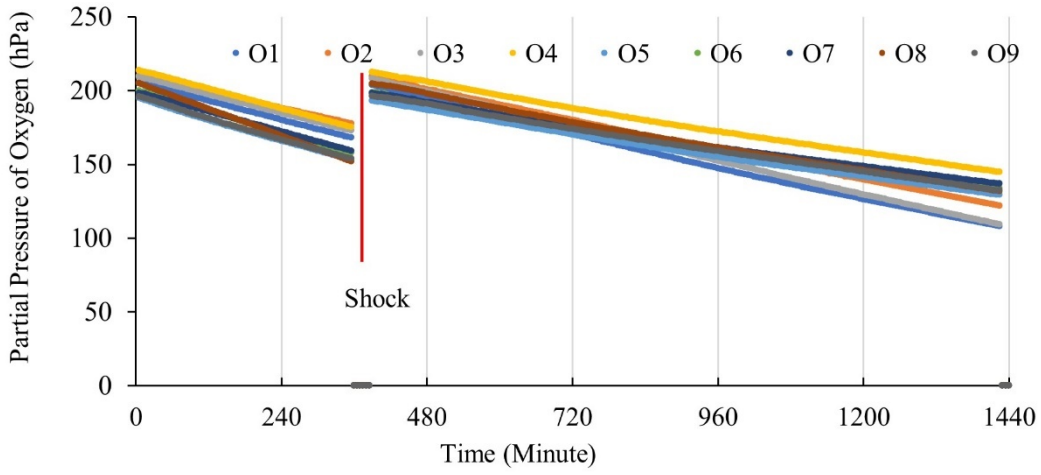


Figure 12. An example of oxygen concentration profile in airtight sensor boxes over time (Specify experiment ID or condition, for Figure. 13 ~ 16 also.)

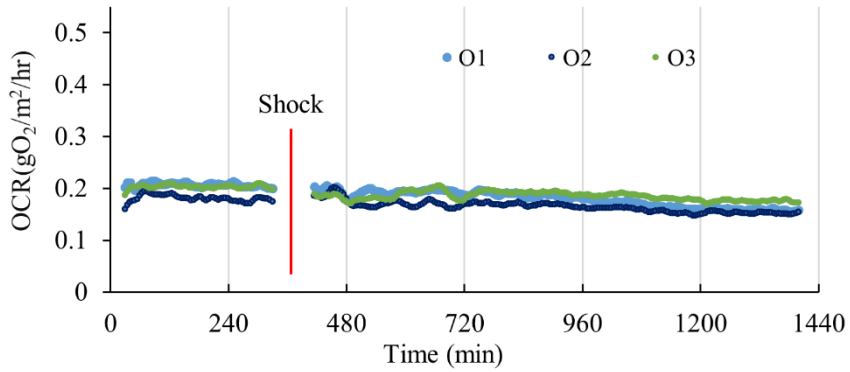


Figure 13. OCR for 3% salinity shock for control

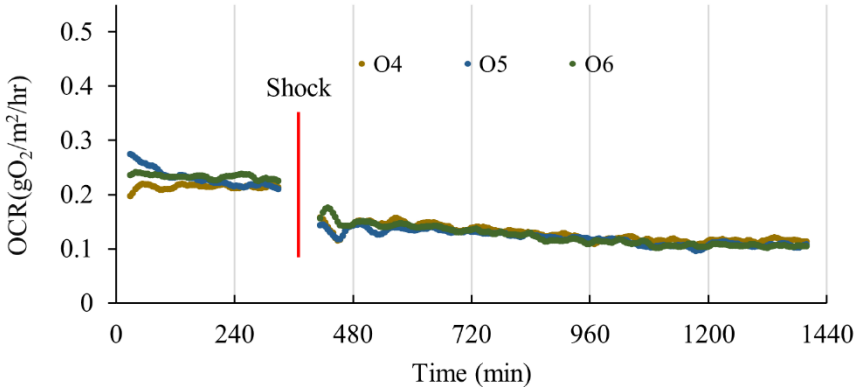


Figure 14. OCR for 3% salinity shock for 3 min

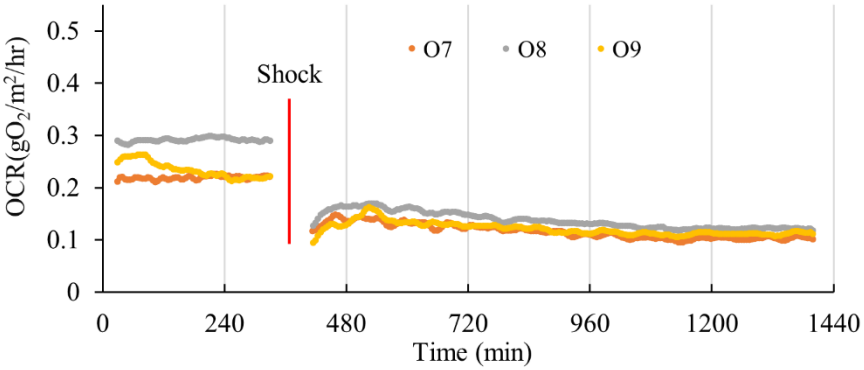


Figure 15. OCR for 3% salinity shock for 30 min

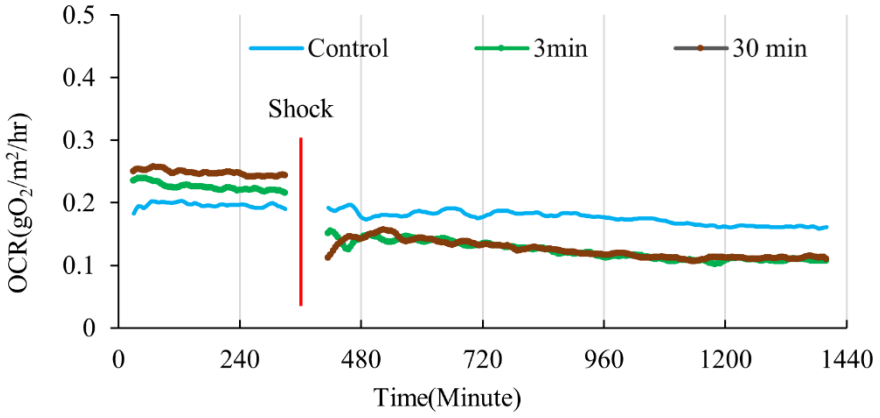


Figure 16. An example of average oxygen consumption rates for 3% salinity shock

4.1.2 OCR profile under no rinse condition

Salinity shock experiments under no rinse condition were conducted in January to February 2024. The changes in OCR after salinity shock on the no rinse condition can be seen in Figure 17 (a), (b), (c), (d). In Figure 17 (a), 0.5% salinity shock had minimal impact on OCR, which remained stable after the shock. In Figure 17 (b), 1% salinity shock had slight reduction in OCR after the shock, with a gradual decrease until stabilization at 480 minutes. In Figure 17 (c), 2% salinity shock showed a little noticeable effect on OCR, with a gradual decline until 960 minutes. In Figure 17 (d), 3% salinity shock had the most significant impact on OCR, with a reduction and a gentle decline lasting up to 960 minutes.

It can be observed that the no-rinse condition impacted OCR gradually for long hours, except for the 0.5% shock. Longer ongoing decrease in OCR in lower salinity (1% and 2%) suggests that salinity hadn't impacted immediately but OCR was impacted overtime. Compared to lower salinity shocks without rinsing, the 3% shock displayed a bigger immediate reduction but a slower decline in OCR. This suggests that the immediate impact of salinity shock might depend on a combination of the concentration of salt influx and exposure duration. The ongoing reduction could be attributable to salt remaining on the surface of the sponge with biomass, affecting them over time.

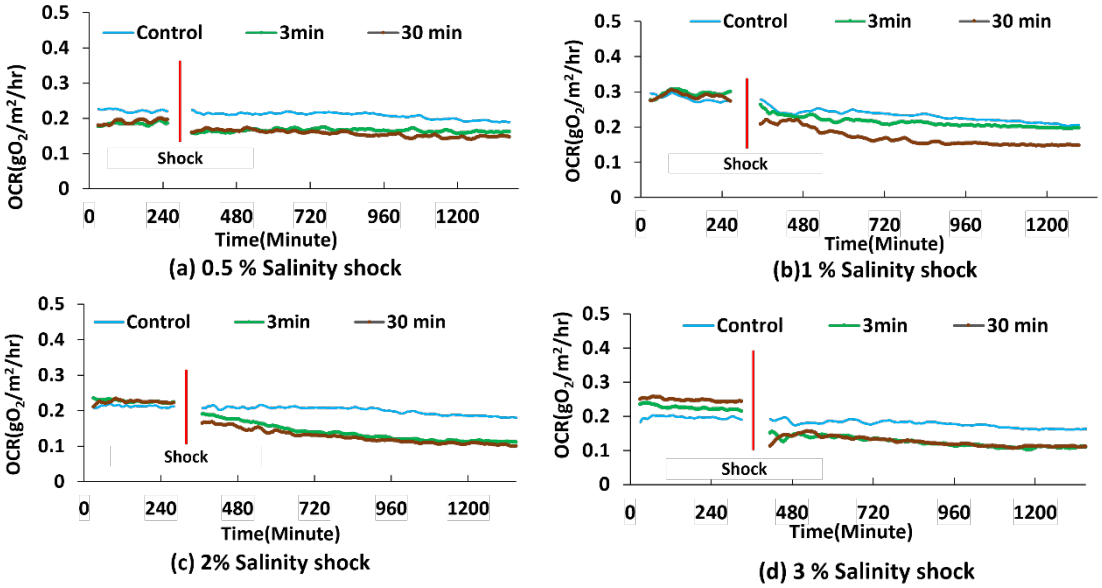


Figure 17.Oxygen consumption rate (OCR) of no rinse condition

4.1.3.1 OCR profile under rinse condition

First series

First series of salinity shock experiments under rinse condition was conducted in November to December 2023. Changes in OCR under salinity shocks with rinse was as shown in Figure 18. In Figure 18 (a), it is observed that 0.5% salinity shock had no impact. In Figure 18 (b), the 1% salinity shock also showed minimal negative impact on OCR. In Figure 18 (c), the 2% salinity shock showed no immediate impact on the OCRs of sponge with biomass, but a gradual decrease in OCR was observed until around 960 minutes. In Figure 18 (d), the 3% salinity shock shows a more noticeable impact, particularly for the 30- minute shock. After salinity shock, all three 3% shock conditions exhibited a gradual decrease in OCR until around 960 minutes.

The rinse condition generally did not cause a significant immediate drop in OCR, except for the 30-minute 3% shock. This may be because the rinse process washed away the salt retained on the sponges. Additionally, OCR reduction continued till around 960 minutes for both of 2% and 3% shocks. One of the possible reasons for this gradual reduction might be due to loss of cytoplasmic solutes from cells when water was extracted from cells under high salinity. Even with rinsing, these solutes could not re-enter into the cells, which may have caused initial stability followed by damage over time. However, this hypothesis lacks proof. Another possible cause may be the reduced oxygen level in the box. However, this possibility is less because experiments with higher OCR (0.5% shock experiment, Figure 18 (a) which resulted in lower remaining oxygen concentrations did not show significant reduction of OCR.

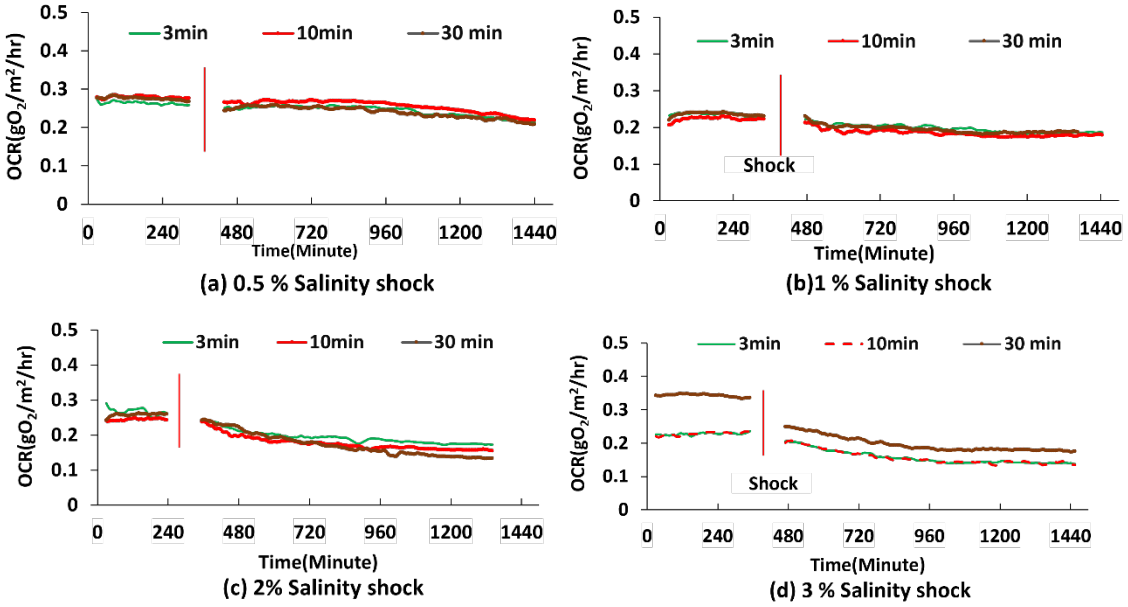


Figure 18. Oxygen consumption rate (OCR) of rinse condition

Second series

Second series of salinity shock experiments under rinse condition was conducted in May 2024. Figures 19 (a) and (b) show that 0.5% and 1% salinity shocks caused an initial increase in OCR for both the control and shocked sponges, followed by a gradual decrease until the 720-minute. In contrast, Figures 19 (c) and (d) demonstrate a negative effect on OCR for both 2% and 3% shocks, even after rinsing with tap water. Interestingly, OCR recovery trend appeared around 720 minutes for the 2% shock (30 minutes) and 960 minutes for both durations (30 and 3 minutes) of the 3% shock.

The cause for the initial OCR increased in the 0.5% and 1% salinity shocks remains unclear. Similarly, the mechanism behind OCR recovery in the 2% and 3% salinity shocks is unknown. However, visual

observations showed the presence of higher organisms, like tubifex and nematoda worms in the sponge during the period when the experiments were performed. These higher organisms were not observed in the first series experiments. These higher organisms may have been more susceptible to salinity shock.

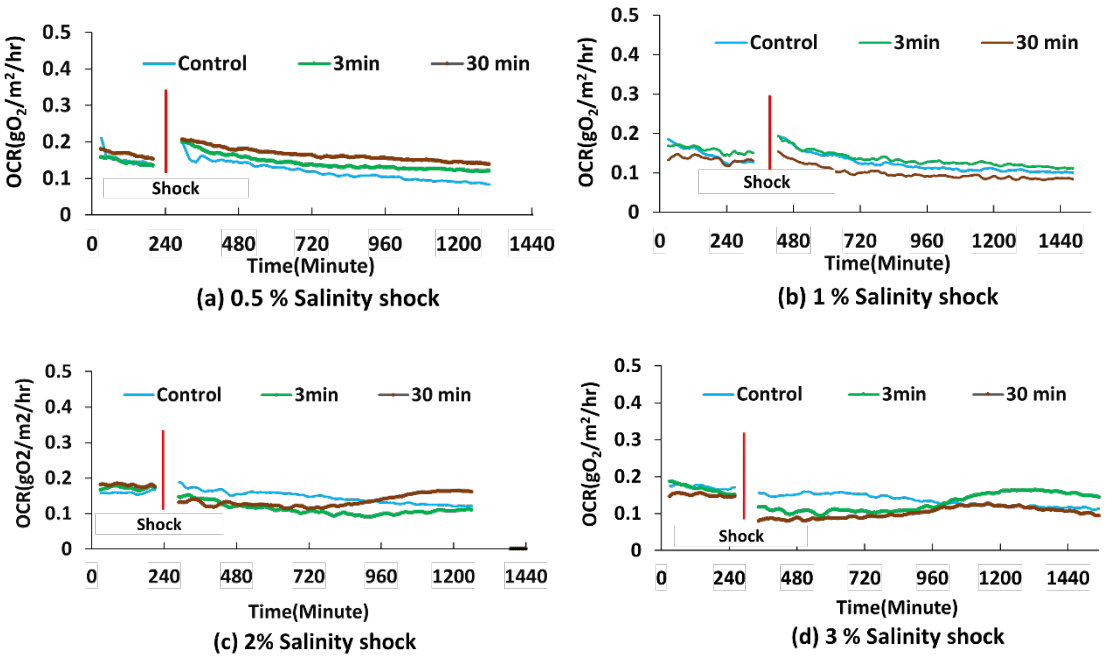


Figure 19. Oxygen Consumption Rate (OCR) of rinse condition

4.1.4 Immediate impact of salinity shock

Extent of immediate salinity shock was calculated as the reduction of OCR right after salinity shock by the equation below [40]. As shown in Figure 20, OCR_{before} works as the baseline. Here, OCR_{before} was determined as the average of OCR during 1 hr before salinity shock. On the other hand, OCR_{after} representing average OCR during the initial 1 hr after salinity shock.

Reduction of OCR by salinity shock was calculated by the equation below.

$$\%\ \text{Reduction of OCR} = \frac{OCR_{before} - OCR_{after}}{OCR_{before}} \times 100\% \quad (\text{Ban}, 2024)$$

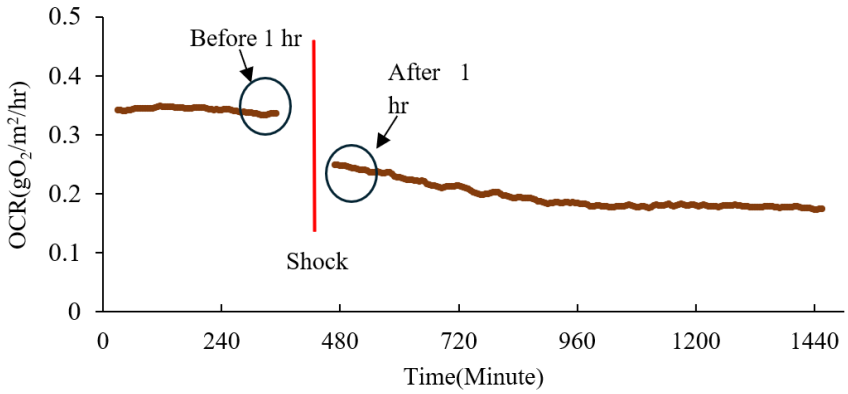


Figure 20. Demonstration of % OCR reduction

4.1.4.1 1 Reduction of OCR by salinity shock: under no rinse condition

Figure 22 shows the reduction of OCR under no rinse condition. Higher salinity levels lead to greater OCR reduction due to increase in osmotic pressure. For both the 3-minute and 30-minute shocks at 0.5% salinity, OCR reduction was around 13%. OCR reduction increased as salinity increased. At 3% salinity, the OCR reduction reached 35% for the 3-minute shock and 43% for 30-minute shock, demonstrating that higher salinity level, the greater OCR reduction.

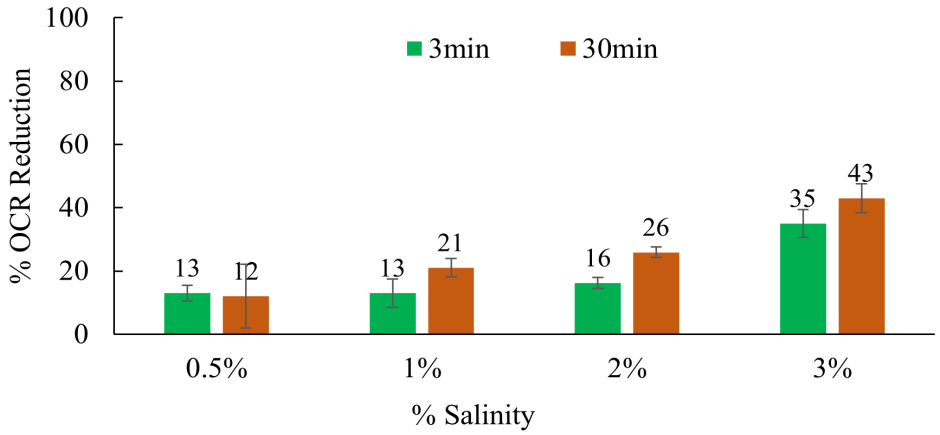


Figure 21. % OCR Reduction (No rinse experiment)

4.1.4.2 Reduction of OCR by salinity shock: under rinse condition

First series

Figure 22 shows the reduction of OCR under rinse condition in the first series experiment. After rinsing with tap water, 0.5%, 1%, and 2% salinity shocks caused very little OCR reduction. Only the 3% shock for 30 minutes showed a more significant reduction in OCR. At 0.5% salinity, both the 3-minute and 10-minute shocks resulted into similar OCR reduction of around 4%, while the 30-minute shock resulted in 8% reduction. At 1% salinity, 3-minute shock led to 5% reduction, and the 10-minute and 30-minute shocks both resulted in a 7% reduction. At 2% salinity, the 3-minute shock increased slightly to 8%, the 10-minute shock remained at 7%, and the 30-minute shock increased to 9%. At 3% salinity, the OCR reduction reached 11% for the 3-minute and 10-minute shocks and 27% for the 30-minute shock.

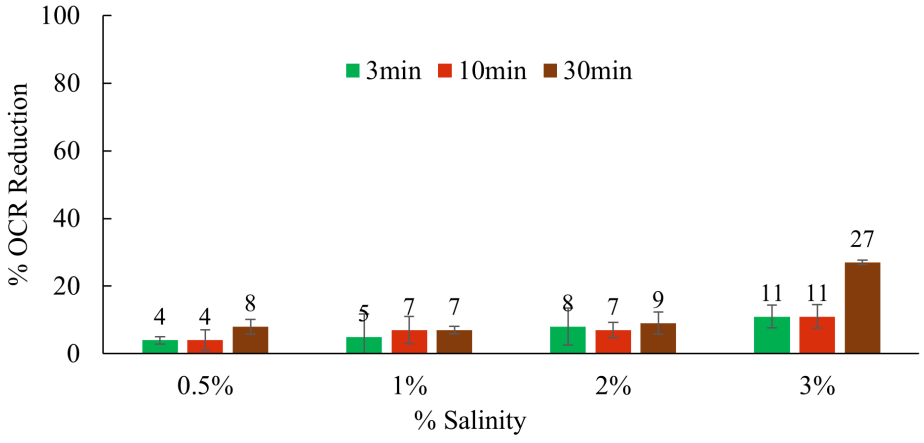


Figure 22. % OCR Reduction (1st series rinse experiment)

Second series

Figure 23 shows the OCR profile in the second series experiment conducted in May 2024. Higher negative impact was observed in 2nd series experiment than in 1st series experiment for 1%, 2%, and 3% shocks. Both 3-minute and 30-minute displays at these higher concentrations resulted in similar OCR reductions (around 20±3%), while the 30-minute exposure led to a slightly more pronounced reduction (30±3%). Notably, the 2nd series rinse experiment appears to have a more pronounced effect on OCR compared to the 1st series experiment, but less impact overall than the no-rinse condition. Interestingly, rinsing with tap water after a 0.5% shock led to a temporary increase in OCR, with a 25% rise for the 3-minute shock and a 13% increase for the 30-minute shock.

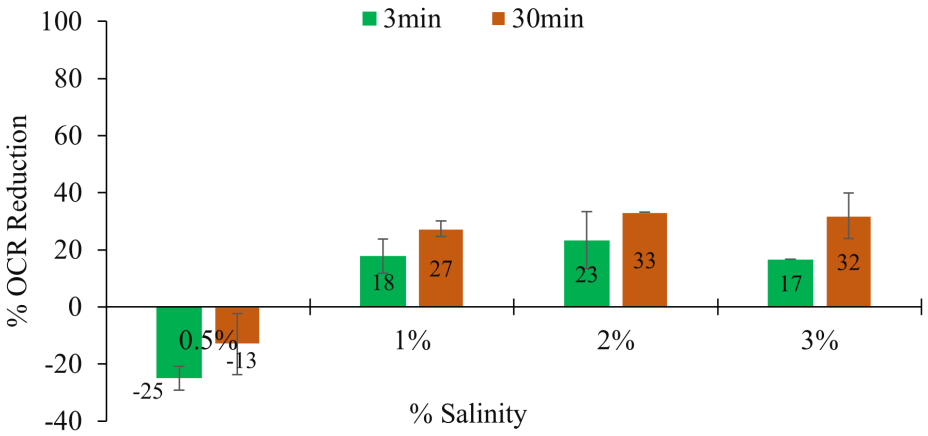


Figure 23. % OCR Reduction (2nd series rinse experiment)

4.1.5 Relationship between OCR reduction and salinity shock

4.1.5.1 Under no rinse condition

OCR reduction was found to be related to salinity shock under no rinse condition. Figure 24 (a) shows an upward trend: as salinity shock increased, OCR reduction also increased. Higher linearity was found with 30 min shock than 3 min shock under no rinse condition. This suggests that longer exposure to high salinity levels is more stressful for the system. With a correlation between increasing salinity shock levels and a decrease in OCR, salinity shock reduced microbial activity. Longer exposure to high salinity is more affecting. Under shorter shock duration, microorganisms were resilient up to 2% salt concentration.

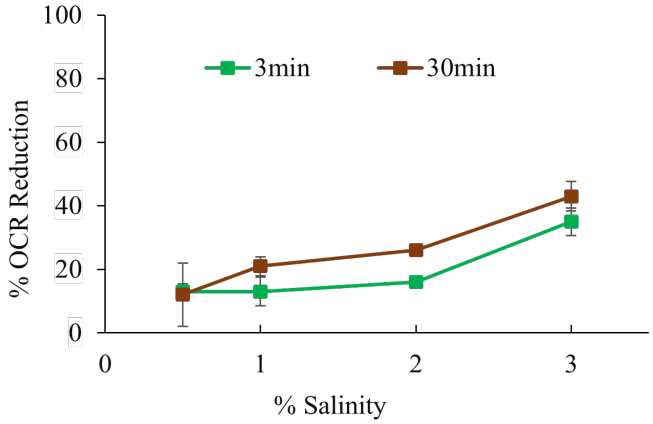
4.1.5.2 Under rinse condition

In the first series experiments, Figure 24 (b) shows that OCRs for shock durations from 3 and 30 minutes are not much affected by salinity shocks from 0.5% to 2%. Noticeable OCR reduction is observed only when 30-minute shock reached 3% salinity. It appears that the immediate impact of salinity shock had been washed away by rinse water. Compared to no rinse condition, 1st series experiment shows minimum OCR reduction to all shock durations (3 to 30 minutes) for salinity over 2%. A noticeable OCR decreased occurs at 3% salinity for 30-minute shocks, even with rinsing. This indicates that rinsing may not completely protect to microorganisms from 3% or higher salinity shock with longer duration. This suggests that rinsing with tap water to salinity shock, can reduce the negative impact in the system. However, high salinity (3%) remains a bit risky. OCR reduction is minimal until salinity reaches 3% for 30 minutes shock. This implies a possible threshold effect where 3% salinity below 30 minutes shock is less harmful, regardless of rinsing. Still, rinsing effectively decreased OCR reduction by approximately half across all shock conditions.

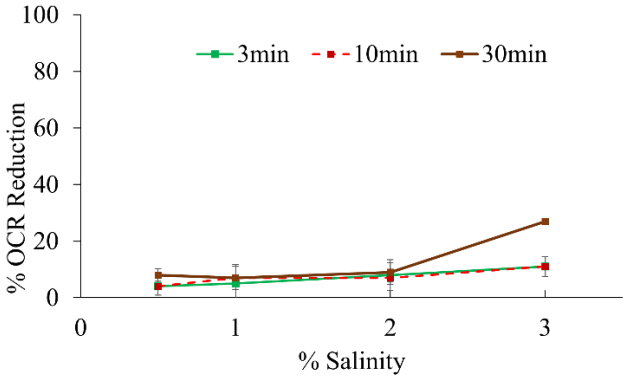
Figure 24 (c) shows the reduction of OCR in the 2nd series experiments on salinity shock experiment in rinse condition. 0.5% salinity shock followed by rinsing resulted in a positive impact on OCR. Conversely,

shocks ranging from 1% to 3% showed negative effects, with no significant difference in the %OCR reduction observed between these higher concentrations. This suggests a potential effect, where higher organisms may play a role in the impact of lower salinity shocks (0.5%). Their activity might be reduced at higher shock levels (1% to 3%).

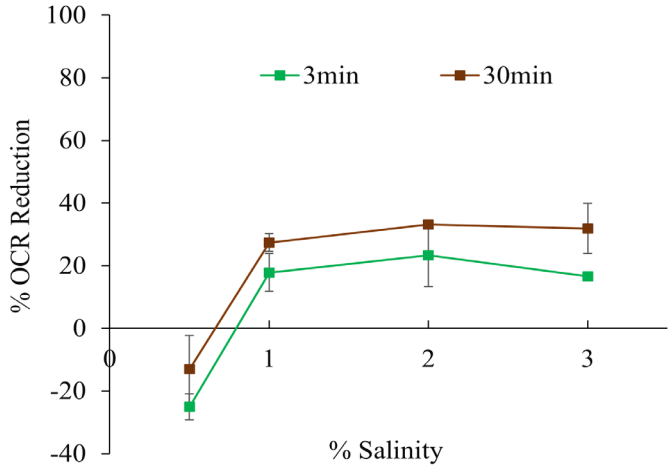
Compared to the 1st series, under 2nd series experiment salinity shock impacted OCR more that does not appear significant protection for sponges exposed to shocks exceeding 0.5%. However, the overall impact on OCR remains less severe compared to the no rinse condition.



(a) No rinse experiment



(b) 1st series rinse experiment



(c) 2nd series rinse experiment

Figure 24. Relationship between OCR reduction and salinity shock

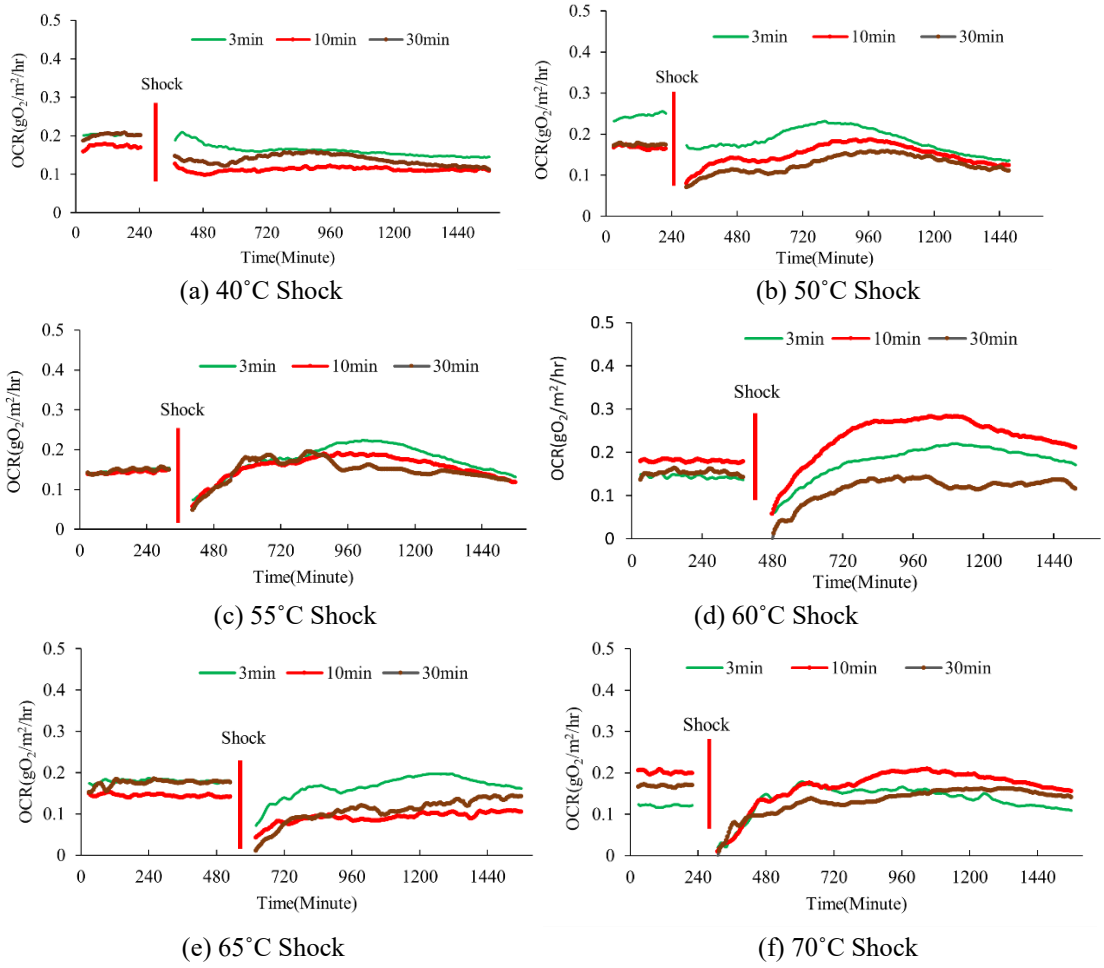
4.2 Thermal shock

The estimation of average OCR for thermal shock from the oxygen concentration in the sensor boxes is the same as sanity shock. Detail can be seen in section 4.1.1.

4.2.1 OCR profile

The immediate changes in OCR following thermal shocks at 40°C, 50°C, 55°C, 60°C, 65°C, and 70°C can be seen in Figures 25 (a), (b), (c), (d), (e) and (f).

The effect of 40°C shocks, illustrated in Figure 25 (a) shows that the OCR of sponge with biomass experienced a slightly negative impact for both the 10-minute and 30-minute shocks. OCRs remained stable during the monitoring period compared to higher temperature shocks. In Figure 25 (b), the 50°C shocks began to show a negative effect on OCR. In Figure 25 (d), the 60°C shocks showed a significantly negative effect on OCR. However, OCR recovered sharply within 240 minutes (4 hours) after the shock. The 10-minute shock have been observed OCR increase, even higher than the initial OCR. In Figure 25 (e), the 65°C shocks showed a larger negative effect on OCR. In Figure 25 (f), the 70°C shocks had the most substantial negative impact on OCR. For all degree of thermal shocks, OCR dramatically bounced back immediately after the shocks. In fact, the maximum surplus OCR higher than initial condition was observed at 60°C. Overall, thermal shocks significantly impacted the OCR of sponge with biomass. As the temperature increased, the OCR's drop was deeper, but immediate recovery trends were also observed.



(a) 40˚C Shock (b) 50˚C Shock

(c) 55˚C Shock (d) 60˚C Shock

(e) 65˚C Shock (f) 70˚C Shock

Figure 25. OCR profile of thermal shock

4.2.2 Quantitative analysis of thermal shock effect

4.2.2.1 Reduction of OCR immediate after thermal shock

Extent of thermal shock was evaluated based on the reduction of OCR in the same calculation explained in 4.1.4. The results are shown in Figure 26. At 40°C shock, no significant OCR reduction was observed for the 3-minute duration. However, longer durations (10 and 30 minutes) lead to a reduction of around 30%. As the shock temperature increased, the OCR reduction of all durations became more significant. % OCR Reduction of 40°C shock to 70°C shock are ranging from around 0 to over 90%. Reduction of OCR increased when the duration and temperature increased. This indicates that the higher the temperature and the longer the shock duration, the greater the negative effect on OCR.

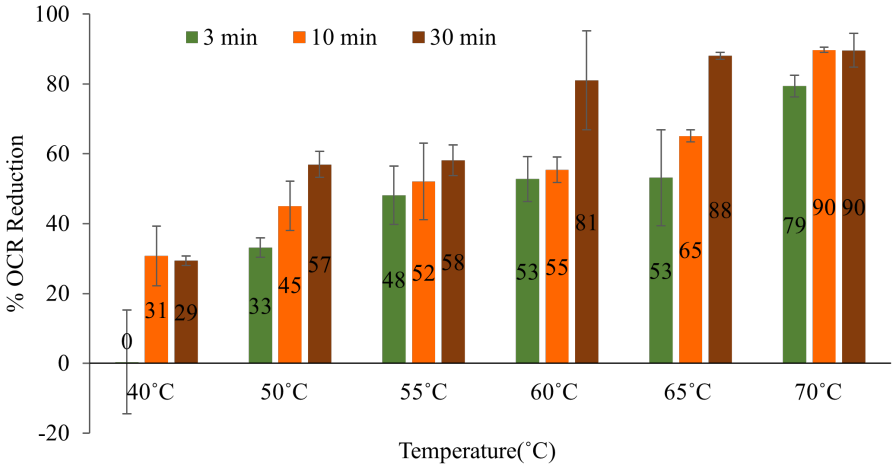


Figure 26. % OCR Reduction

4.2.2.2 Linear regression model of OCR reduction against temperature and time

A clear correlation existed between the increase in thermal shock intensity and increase in OCR reduction as shown in Figure 27. A multiple linear regression was run to explain OCR reduction against temperature and time shown in Table 7.

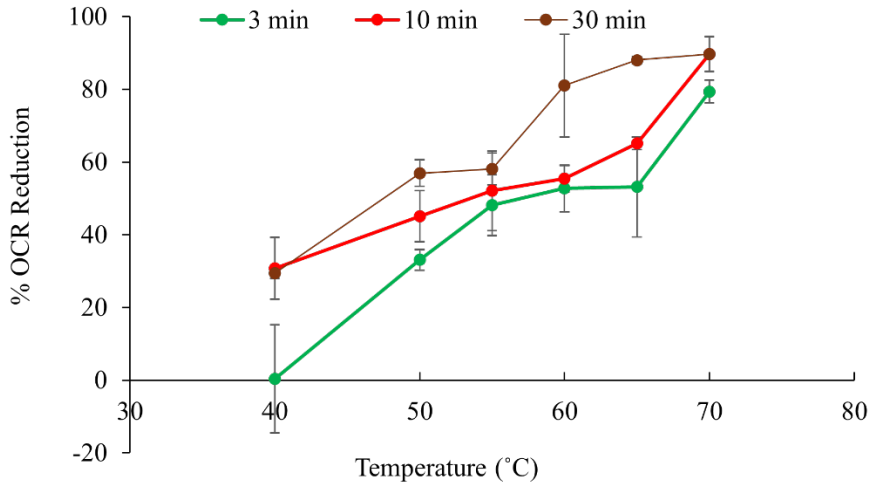


Figure 27. Thermal Shock Intensity and OCR Reduction

Table 3 Regression model of OCR reduction against temperature and time

	Coefficient	**Standard error**	**T value**
Intercept	-72.5541	10.3118	-7.036***
Temperature	2.0774	0.1753	11.850***
Shock duration	0.7771	0.1511	5.143***

R^2 = 0.9175, Adjusted R^2 = 0.9065, F (2, 15)= 83.44, $p < 0.001$

Residual standard error: 7.333

*** Significance at 0.001 level

As a result from Table 4-1, the independent variables (temperature and shock time) had significant relationships with the reduction of OCR, with an adjusted R^2 value of 0.9665.

From the result above, a model below is obtained:

% OCR Reduction = -72.5541 + 2.0774 Temperature (˚C) + 0.7771 Shock time (min) ± 7.333

It is important to note that the regression model is only appropriate for the current data analysis, for temperature range between 40˚C to 70˚C.

Although thermal shocks above 40˚C had significant negative impact on OCR, the immediate recovery of OCR was observed. This study showed resilience of sponge with biomass for enhanced sewer self-purification is resilient on thermal shock. Moreover, surplus OCR which was even higher than the initial OCR of sponge with biomass can be found afterwards for over 50˚C shocks. While OCR reduction is an indicator, further investigation into microbial activity might provide a more comprehensive understanding of the thermal shock's effect.

4.3 Comparison between effect of salinity shock and thermal shock

The system of enhanced sewer self-purification may be encountered by both thermal and salinity shocks in a different situation. Based on the results in 4.1, salinity shock by brief exposure from kitchen sink is thought to have mild negative effect. Even under high salinity (3% NaCl for 30 minutes without rinsing), OCR reduction remained moderate around 40%. But these OCR reductions may continue for many hours. If rinsed water follows, reduction of OCR was significantly reduced the impact by over half across all shock durations and concentrations examined here. However, the impact is more complicated if higher organisms are present in the sponges with biomass for rinse condition. The activity of sponges with biomass reduced but the slow recovery of OCR trend observed at several hours after 2% and 3% shock. But still there was a minor OCR reduction which stayed for longer hours even with rinse. The possible reason behind this might be residual salt was still retaining on the sponge with biomass. Nevertheless, the results suggest that salinity shock may cause a minimal threat to overall performance of the enhanced sewer self-purification system. Additionally, the brief nature of salinity shock and rinsing with just tap water further reduced the impact in the system.

In contrast, thermal shock by hot water had a significant impact on microorganisms. Reduction of OCR ranged from 33% to over 90% for temperatures over 50°C for durations of 3 to 30 minutes. However, significant differences were found after applying these two kinds of shocks. Unlike salinity shock, thermal

shock caused a rapid and dramatic increase in OCR within a few hours. This might be due to heat was lost from sponge with biomass after a brief thermal shock. Thermal shock causes the immediate reduction, but over a short time, the OCR increased even surpassing pre-shock levels in some cases. This suggests a potential in adaptation or an increase in microbial activity after thermal shock.

In short, both salinity and thermal shocks have affected microorganisms in the system, but their impacts differed significantly. Although there was a more dramatic immediate reduction in activity, thermal shock showed a significant recovery trend. On the other hand, salinity shock had a milder effect and it further weakened by rinsing with tap water. Both shocks have different responses in terms of the resilient of enhanced sewer self-purification system.

4.4 Managing salinity shock and thermal shock in expected real-world scenarios

Enhanced sewer self-purification system may be faced by brief salinity shock from kitchen sinks and thermal shocks from sources such as warm shower water.

While both types of shock have undeniable negative effects on microorganisms. Salinity shock is less severe below 2% concentration, but prolonged exposure can have lasting effects. Fortunately, rinse water following salinity shock, such as from washing dishes, can wash away the salt and reduce the impact. However, in actual situation, there will be the growth of higher organisms such as worms in the system. They might be affected more to salinity shock, even with rinse water followed but the system's performance will recover slowly after a period of time. Reducing salinity shock effect can happen easily through less frequent salty water discharges and dilution with rinse water flow. Under the thermal shock, the system continued its performance after the initial high negative impact, since the system shows rapid recovery and even increased activity. The system assumes that it can handle both thermal shock with increasing in its activity and salinity shock with generally small and brief nature of the impact.

4.5 Chapter summary

The summary for this chapter is described as follows:

1. The result of salinity shocks at 0.5%, 1%, 2%, 3% that higher salinity levels led to greater OCR reduction, with the no rinse condition having a more pronounced impact compared to two rinse conditions. The highest negative impact was found in 3 % shock for 30 minutes was shown moderate reduction of OCR around 40% reduction. Under the two series of rinse experiment, the 1st series rinse experiment, 0.5 %, 1% and 2% salinity shocks cause very little reduction of OCR, while the 2nd series rinse experiment, observed more reduction with slow recovery trend, than the 1st one. The reason might be due to the growth of higher organism in the 2nd one.
2. The result of thermal shocks at temperatures 40^{o}C, 50^{o}C, 55^{o}C, 60^{o}C, 65^{o}C, 70^{o}C on oxygen consumption rate (OCR) of sponges with biomass showed that higher temperatures (over 40^{o}C) caused a significant immediate reduction in OCR, but a rapid recovery trend was also observed within a few hours.
3. The result of both thermal shock and salinity shock were compared. The result implied that the sponges with biomass showed resilience on both shocks. Thermal shocks lead to rapid increased in OCR after shock, while salinity shocks (up to 3%) had minor effect.

Chapter 5

CONCLUSIONS

5.1 Key findings

The general objective of this study was to investigate the resilience of microbial activity in enhanced sewer self-purification against salinity shock and thermal shock for real sewer application. Salinity shocks had a milder effect, when followed by rinsing with tap water, which suggests minimal threat to the system's overall performance. Thermal shocks led to a rapid increase in oxygen consumption rate (OCR) after the initial shock, indicating a strong recovery and potential adaptation of the microorganisms.

The first specific objective was to quantify the impact of short-term salinity and thermal shocks on microbial performance. For salinity shocks, even at high concentrations (3% NaCl for 30 minutes without rinsing), the OCR reduction was moderate around 40 % reduction, and the impact was significantly reduced around half by subsequent rinsing. However, the presence of higher organisms like worms within the system might cause a complexity. While rinsing with tap water can remove the negative effects of salinity shock for lower concentrations (e.g., 0.5%), the shock level (1% to 3%) might impact these organisms, even with rinsing. This could lead to OCR reduction (around 30%), with the system's performance recovering slowly over time. For thermal shock, higher temperatures (over 40^{o}C) caused a significant immediate reduction in OCR, but a rapid recovery trend was also observed. The system showed significant resilience to thermal shocks, with OCR not only recovering but also surpassing pre-shock levels in some cases.

The second specific objective was to compare the relative effects of salinity and thermal shocks on microbial activity. Thermal shocks lead to rapid increased in OCR after immediate impact of shock, while salinity shocks (up to 3%) had immediate minor effect which was likely to resume for many hours. Both of these shocks have different responses by the system.

This study emphasizes the resilience of the enhanced sewer self-purification system against salinity and salinity which are common shocks in sewer environments. The results demonstrated that the system's sponges with biomass are resilient to both types of shocks. The findings suggest that the enhanced sewer self-purification can be effectively implemented upstream sewer to enhance sewage treatment processes. The resilience mechanisms offer potential opportunities for future research in other shock loads in self-purification system.

5.2 Limitations and suggestions

This study didn't investigate the mechanisms that enable microorganisms to withstand and recover from salinity and thermal shocks. This includes studying cell leakage, osmotic balance, and the role of heat shock proteins during shock events.

Additionally, this study didn't examine how the diversity and abundance of microorganisms in the sponge media contribute to the system's resilience. Assessing changes in microbial community composition before and after shock application should be studied further to determine which microbial groups are most affected by shocks, which continued the system's treatment efficiency for salinity shock, and which contribute to rapid recovery for thermal shock. Besides, the present study didn't evaluate the long-term effects which last hours to days after shocks application. This study could only demonstrate the results of immediate impact of shocks on sponge with biomass. Moreover, actual salinity level and temperature degree in the real sewer need to be investigated.

Another suggestion is that there are other shock loads that can affect the enhanced sewer self-purification system, such as lipids, pH and emerging contaminants, which should be studied to observe the system's resilience.

References

[1] T. Hvitved-Jacobsen, J. Vollertsen, and J. S. Matos, “The sewer as a bioreactor-a dry weather approach,” 2002. [Online]. Available: https://iwaponline.com/wst/article-pdf/45/3/11/425081/11.pdf

[2] A. Augustyniak et al., “Biofilms in the gravity sewer interfaces: making a friend from a foe,” Sep. 01, 2021, Springer Science and Business Media B.V. doi: 10.1007/s11157-021-09582-0.

[3] T. Shoji, Y. Matsubara, S. Tamaki, K. Matsuzaka, H. Satoh, and T. Mino, “In-sewer Treatment System of Enhancing Self-Purification: Performance and Oxygen Balance in Pilot Tests,” 2015.

[4] R. Lyu, T. J. Sotelo, H. Satoh, and T. Mino, “Effect of contact time on the performance of the intermittent contact oxidation process for enhanced in-sewer purification,” J Water Environ Technol, vol. 18, no. 3, pp. 166–174, 2020, doi: 10.2965/jwet.20-002.

[5] Bender, Madigan, Buckley, Sattley, and Stahl, Brock Biology of Microorganisms, 16th ed. PEARSON, 2021.

[6] A. D. Russell and D. Harries, “Some Aspects of Thermal Injury in Escherichia coli,” APPLIED MICROBTOLOGY, vol. 15, no. 2, pp. 407–410, 1967, [Online]. Available: https://journals.asm.org/journal/am

[7] A. Heitzer, N. Ai-Awadhi, and G. Hamer, “Some effects of heat shocks on bacterial growth,” Microbiology Biotechnology, vol. 30, pp. 408–414, 1989.

[8] G. Thibault and D. T. W. Ng, “Heat/Stress Responses,” Encyclopedia of Biological Chemistry: Second Edition, pp. 522–525, Jan. 2013, doi: 10.1016/B978-0-12-378630-2.00427-8.

[9] T. Hvitved, J. Jes, V. Asbjørn, and H. Nielsen, SEWER PROCESSES: Microbial and Chemical Process Engineering of Sewer Networks, Second Edition. CRC Press Taylor & Francis Group, 2013.

[10] Y. Tanji, R. Sakai, K. Miyanaga, and H. Unno, “Estimation of the self-purification capacity of biofilm formed in domestic sewer pipes,” Biochem Eng J, vol. 31, no. 1, pp. 96–101, Aug. 2006, doi: 10.1016/j.bej.2006.05.021.

[11] T. J. Sotelo, H. Satoh, and T. Mino, “Lipid degradation behavior for the in-sewer application of the intermittent contact oxidation process,” J Water Environ Technol, vol. 16, no. 5, pp. 211–219, 2018, doi: 10.2965/jwet.18-009.

[12] T. J. Sotelo and H. Satoh, “Accumulated Organic Matter Degradation and the Function of Porous Media during Enhanced Sewer Self-purification,” J Water Environ Technol, vol. 18, no. 6, pp. 415–424, 2020, doi: 10.2965/JWET.20-060.

[13] T. J. Sotelo and H. Satoh, “Enhanced sewer self-purification with porous media: Performance evaluation with various organic loading rates and flow intermittency,” Biochem Eng J, vol. 168, Apr. 2021, doi: 10.1016/j.bej.2021.107932.

[14] T. J. Sotelo, H. Satoh, and T. Mino, “Effect of sponge media structure on the performance of the intermittent contact oxidation process for in-sewer purification,” Biochem Eng J, vol. 149, Sep. 2019, doi: 10.1016/j.bej.2019.107254.

[15] T. J. Sotelo, H. Satoh, and T. Mino, “Effect of Flow Intermittency on Lipid Degradation Behavior during In-sewer Purification by the Intermittent Contact Oxidation Process,” Biochem Eng J, vol. 154, Feb. 2020, doi: 10.1016/j.bej.2019.107430.

[16] T. J. Sotelo, G. B. Sioen, and H. Satoh, “Circling the drain: A systems analysis of opportunities for enhanced sewer self-purification technologies in wastewater management,” Jun. 15, 2021, Academic Press. doi: 10.1016/j.jenvman.2021.112451.

[17] D. Butler and N. J. D. Graham, “MODELING DRY WEATHER WASTEWATER FLOW IN SEWER NETWORKS,” J. Environ. Eng, pp. 161–173, 1995.

[18] R. Lyu, T. J. Sotelo, H. Satoh, and T. Mino, “Effect of contact time on the performance of the intermittent contact oxidation process for enhanced in-sewer purification,” J Water Environ Technol, vol. 18, no. 3, pp. 166–174, 2020, doi: 10.2965/jwet.20-002.

[19] R. Chang, Chemistry/Raymond Chang-7th ed. McGraw Hill, 2002.

[20] L. N. Csonka, “Physiological and Genetic Responses of Bacteria to Osmotic Stress,” Microbiol Rev, vol. 53, no. 1, pp. 121–147, 1989.

[21] Nina. Gunde-Cimerman, A. Oren, and Ana. Plemenitaš, Adaptation to life at high salt concentrations in archaea, bacteria, and eukarya, vol. 9. Springer, 2005.

[22] A. D. Brown, “Microbial Water Stress,” American Society for Microbiology, vol. 40, no. 4, pp. 803–846, 1976.

[23] N. Gunde-Cimerman, A. Plemenitaš, and A. Oren, “Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations,” May 01, 2018, Oxford University Press. doi: 10.1093/femsre/fuy009.

[24] R. D. Sleator and C. Hill, “Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence,” FEMS Microbiol Rev, vol. 26, no. 1, pp. 49–71, Mar. 2002, doi: 10.1111/j.1574-6976.2002.tb00598.x.

[25] H. Chen, K. Ma, Y. Huang, Q. Fu, Y. Qiu, and Z. Yao, “Significant response of microbial community to increased salinity across wetland ecosystems,” Geoderma, vol. 415, Jun. 2022, doi: 10.1016/j.geoderma.2022.115778.

[26] S. Lew, K. Glińska-Lewczuk, P. Burandt, K. Kulesza, S. Kobus, and K. Obolewski, “Salinity as a Determinant Structuring Microbial Communities in Coastal Lakes,” Int J Environ Res Public Health, vol. 19, no. 8, Apr. 2022, doi: 10.3390/ijerph19084592.

[27] Mostafa Hashad, Surabhi Sharmi, Loring F. Nies, and James E. Alleman, “STUDY OF SALT WASH WATER TOXICITY ON WASTEWATER TREATMENT,” Aug. 2006, Final Report, Purdue University.

[28] J.-L. Wang, X.-M. Zhan, Y.-C. Feng, and Y. I. Qian, “Effect of Salinity Variations on the Performance of Activated Sludge System 1,” BIOMEDICAL AND ENVIRONMENTAL SCIENCES, vol. 18, pp. 5–8, 2005.

[29] Y. Li, A. M. Li, J. Xu, W. W. Li, and H. Q. Yu, “Formation of soluble microbial products (SMP) by activated sludge at various salinities,” Biodegradation, vol. 24, no. 1, pp. 69–78, Feb. 2013, doi: 10.1007/s10532-012-9558-5.

[30] Y. Zhang, W. L. Jiang, R. X. Xu, G. X. Wang, and B. Xie, “Effect of short-term salinity shock on unacclimated activated sludge with pressurized aeration in a sequencing batch reactor,” Sep Purif Technol, vol. 178, pp. 200–206, 2017, doi: 10.1016/j.seppur.2017.01.048.

[31] A Tissières, H K Mitchell, and U M Tracy, “Protein Synthesis in Salivary Glands of Drosophila melanogaster : Relation to Chromosome Puffs,” J. Mol. Biol, vol. 84, pp. 389–398, 1974.

[32] Mackenzie L. Davis, WATER AND WASTEWATER ENGINEERING, Professional Edition. The McGraw-Hill Companies, Inc, 2010.

[33] M. Ponomarenko, I. Stepanenko, and N. Kolchanov, “Heat Shock Proteins,” in Brenner’s Encyclopedia of Genetics: Second Edition, Elsevier Inc., 2013, pp. 402–405. doi: 10.1016/B978-0-12-374984-0.00685-9.

[34] P. Ji, W. J. Rhoads, M. A. Edwards, and A. Pruden, “Effect of heat shock on hot water plumbing microbiota and Legionella pneumophila control,” Microbiome, vol. 6, no. 1, 2018, doi: 10.1186/s40168-018-0406-7.

[35] H. A. O. Alisawi, “Performance of wastewater treatment during variable temperature,” Apr. 01, 2020, Springer Science and Business Media Deutschland GmbH. doi: 10.1007/s13201-020-1171-x.

[36] Metcalf L & Eddy HP, Wastewater Engineering Treatment and Reuse, Fourth Edition. McGraw Hill, 2004.

[37] T. Liu et al., “Effects of temperature shocks on the formation and characteristics of soluble microbial products in an aerobic activated sludge system,” Process Safety and Environmental Protection, vol. 158, pp. 231–241, Feb. 2022, doi: 10.1016/j.psep.2021.12.010.

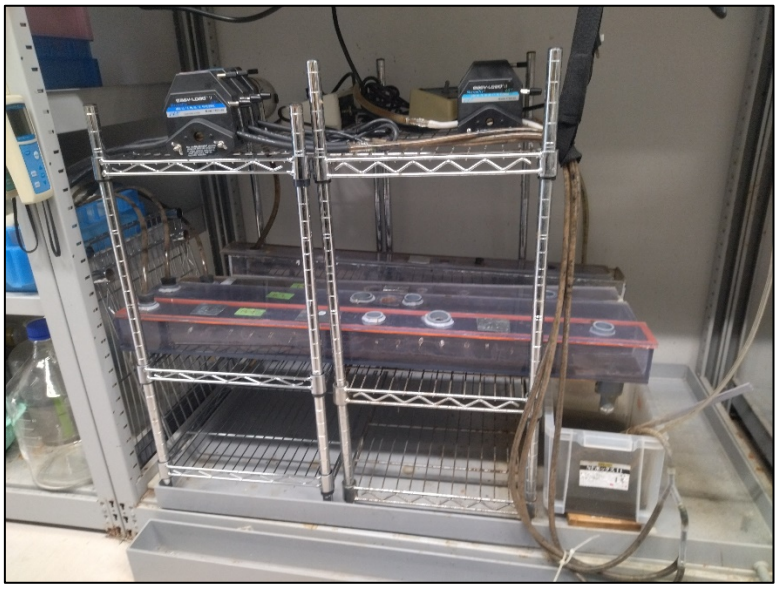
[38] J. Chen, S. Zhang, X. Han, L. Zhang, and Y. Peng, “Nitritation of real sewage: Start-up and maintenance by the side-stream heat-shock treatment,” Water Science and Technology, vol. 79, no. 4, pp. 753–758, 2019, doi: 10.2166/wst.2019.095.

[39] P. Ji, W. J. Rhoads, M. A. Edwards, and A. Pruden, “Effect of heat shock on hot water plumbing microbiota and Legionella pneumophila control,” Microbiome, vol. 6, no. 1, 2018, doi: 10.1186/s40168-018-0406-7.

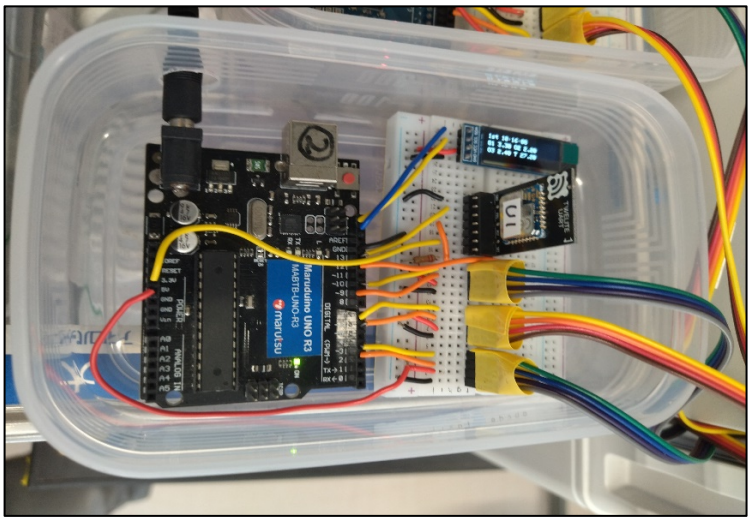
[40] BAN Teav, “Effect of Thermal Shock on Microbial Activity on Organic Matter Degradation in Intermittent Contact Oxidation Process (ICOP),” Master Thesis, Graduate School of Frontier Sciences, The University of Tokyo , 2023.

Appendix

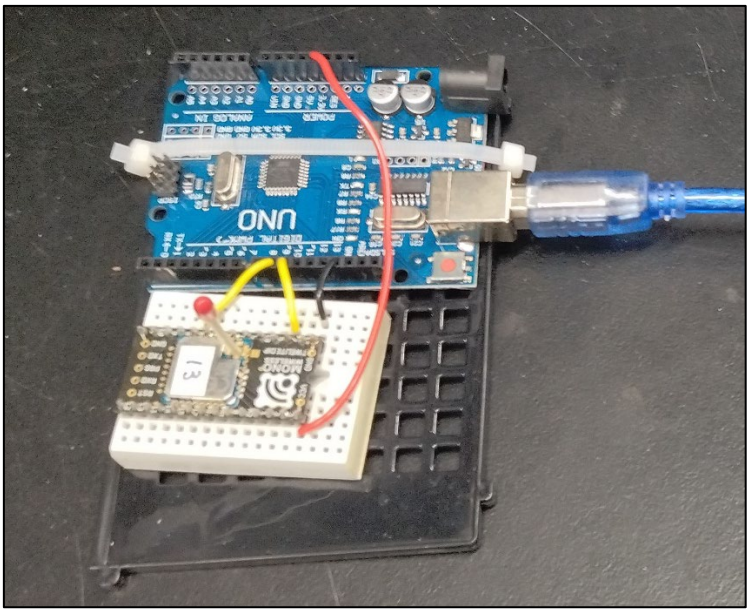
1. Documentations



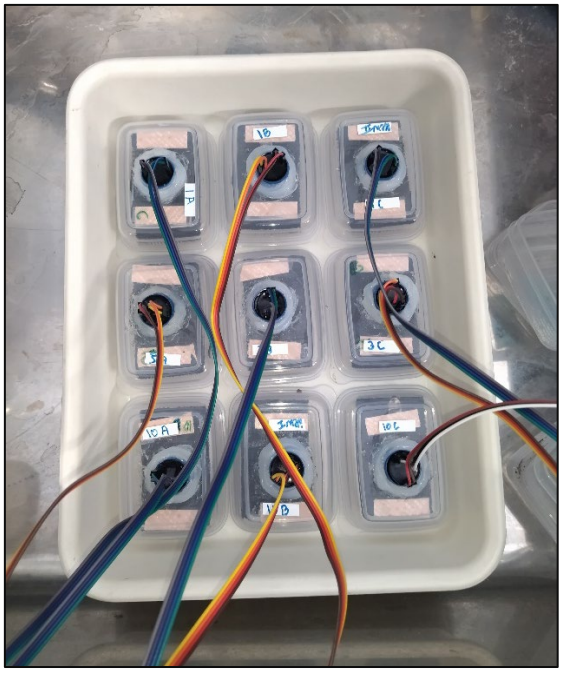
ICOP reactor arrangement



An Arduino connection to bread board for 3 Oxygen gas sensors

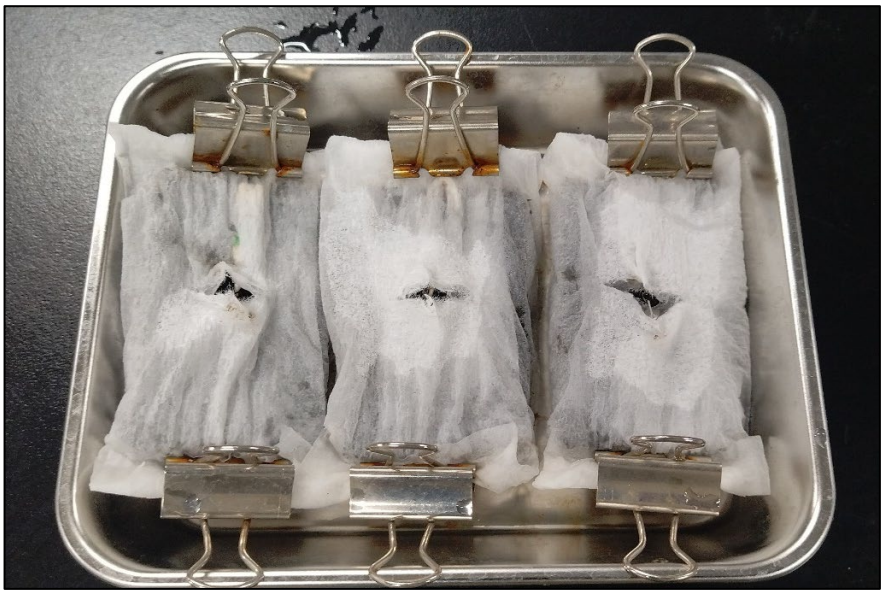


Parent Arduino to connect to computer

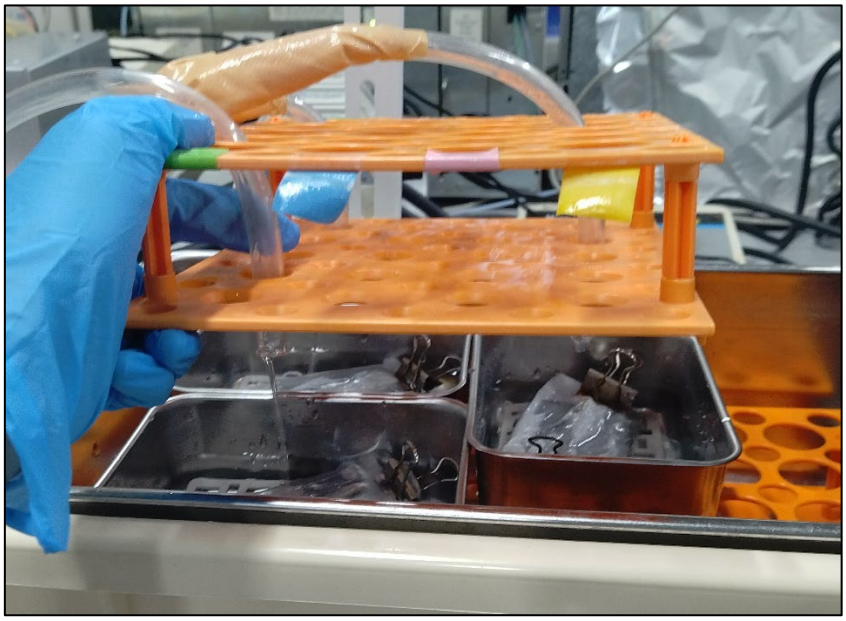


9 oxygen sensor’s boxes

9 sponges with biomass

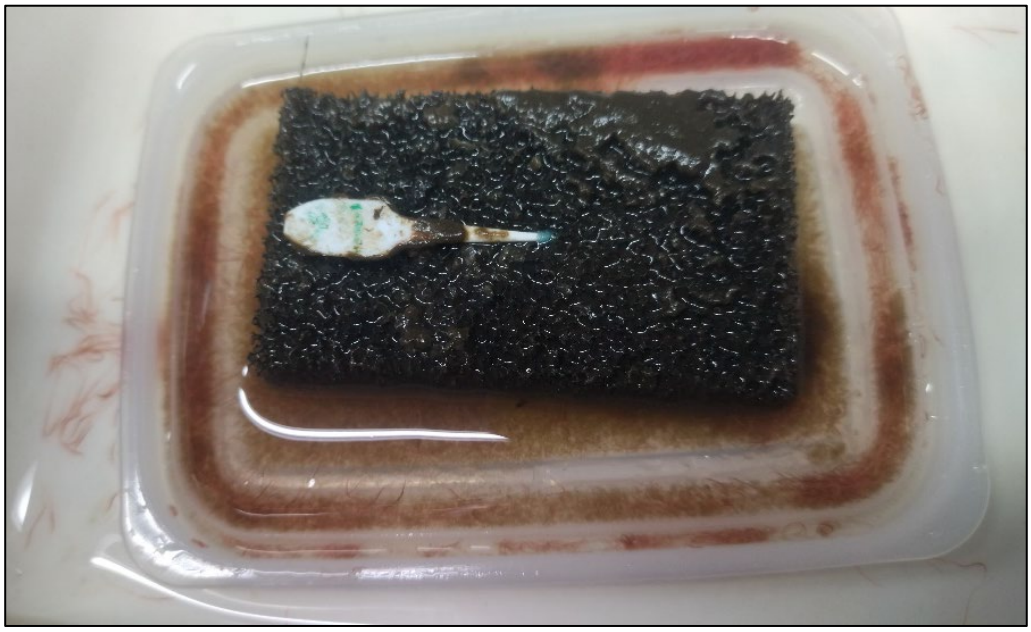


Sponge wrapping with tissue to reduce biomass washout

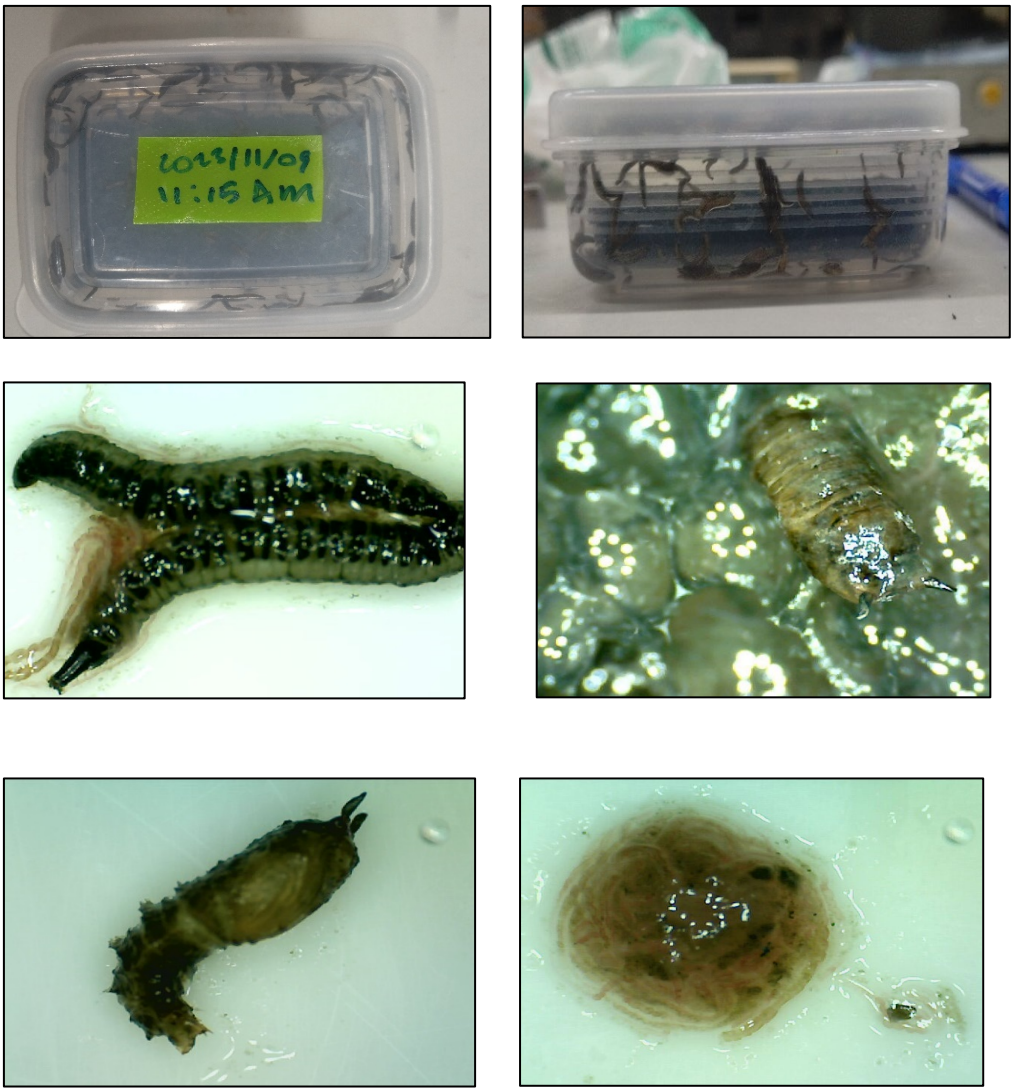


Shock applies to sponges with biomass

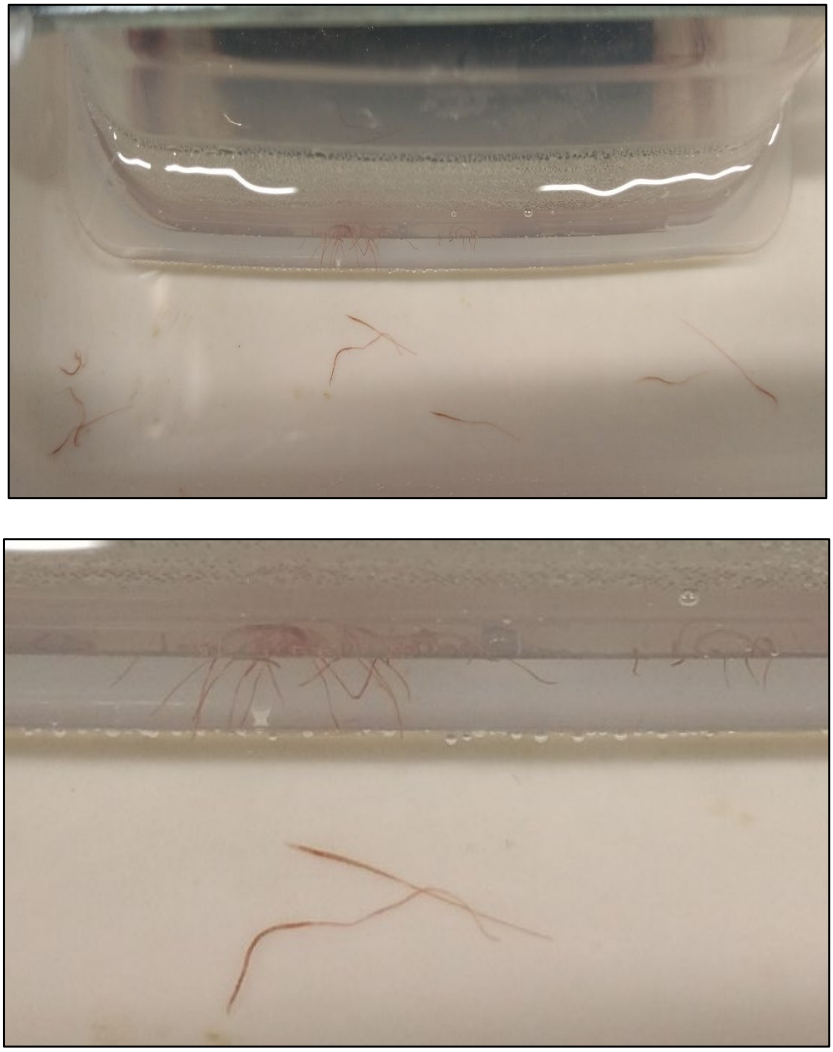
Before the shock's experiments were started, the reactor was thoroughly cleaned with hot water (85˚C) and the sponges were soaked in hot water (85˚C) for 30 minutes a week. Over time, filter flies, larvae and worms were growing in the reactor. If filter flies, larvae and worms were growing over time in the reactor, the experiments results would be different. After initial OCR measurement just before shock application started, the sensor boxes were opened, larvae become flies in some sponges and flew away. But some larvae and worms remained inside the sponges, they tried to be escaped when oxygen concentration is low inside the sensor's boxes. Some of them escaped outside the sensor boxes. The results were not consistent with the expectation if they present in the sponge with biomass.



Photos of worms growing in sponge with biomass



Photos of larvae and worms in the sponge with biomass while preparatory incubating



Photos of worms escaping outside of the sensor boxes during OCR measurement

2. Arduino's code

```
# For Parent Arduino

9 : mySerial3 LuminOx3 rx
   10: mySerial3 LuminOx3 tx

 Memo: NineOxygen: collect data every around 10 sec, then send it to parent.
      NineOxygenParent:
         Receive data from end point Arduinos and transmit to Processing.
         Every 20 sec format the received data and send to Processing.
      Processing (NineOxygenPlotter):
         Receive formatted data from NineOxygenParent.
          Update values every time, but not the chart.
          Chart is updated only every 5 min.
          Data is saved only every 5 min.

*/

#include <SoftwareSerial.h>
#include <TimeLib.h>


SoftwareSerial mySerial =  SoftwareSerial(9, 10);//rx,tx,

String text;
String v1stO1;
String v1stO2;
String v1stO3;
String v2ndO1;
String v2ndO2;
String v2ndO3;
String v3rdO1;
String v3rdO2;
String v3rdO3;
String v1stT1;
String v1stT2;
String v1stT3;
String v2ndT1;
String v2ndT2;
String v2ndT3;
String v3rdT1;
String v3rdT2;
String v3rdT3;
int interval = 8;  // send data every 60 sec.
boolean flag = false;

void setup() {
```

```
  pinMode(13, OUTPUT);
  digitalWrite(13, HIGH);
  delay(10);
  Serial.begin (9600);
  mySerial.begin(38400);
  Serial.println("start");
  setTime(0, 0, 0, 1, 1, 2021);  //set time
  mySerial.println("4th started");
}

// the loop routine runs over and over again forever:
void loop() {
  readSerialText();
  delay(10);
  //Serial.print(minute());
  if (second() % interval == 0 && flag == false) {
    //Serial.println("now report");
    report();
    vinitialize();
    flag = true;
  } else if (second() % interval > 0) {
    flag = false;
  }
}

void  readSerialText() {
  if (mySerial.available()) {
    text = mySerial.readStringUntil('\n');
//    Serial.print("this is received:");
      Serial.println(text);
    if (text.length() == 28) {
//      Serial.println("length OK");
//      Serial.println(text.substring(0, 3));
      if (text.substring(0, 3) == "1st") {
        v1stO1 = text.substring(3, 7);
        v1stT1 = text.substring(7, 11);
        v1stO2 = text.substring(11, 15);
        v1stT2 = text.substring(15, 19);
        v1stO3 = text.substring(19, 23);
        v1stT3 = text.substring(23, 27);
      }    else if (text.substring(0, 3) == "2nd") {
        v2ndO1 = text.substring(3, 7);
        v2ndT1 = text.substring(7, 11);
        v2ndO2 = text.substring(11, 15);
        v2ndT2 = text.substring(15, 19);
        v2ndO3 = text.substring(19, 23);
        v2ndT3 = text.substring(23, 27);
      }    else if (text.substring(0, 3) == "3rd") {
        v3rdO1 = text.substring(3, 7);
        v3rdT1 = text.substring(7, 11);
```

```
        v3rdO2 = text.substring(11, 15);
        v3rdT2 = text.substring(15, 19);
        v3rdO3 = text.substring(19, 23);
        v3rdT3 = text.substring(23, 27);
      }
    }
  }
}

void report() {
  String tmptext = "";
  tmptext += "DATA;";
  tmptext += v1stO1;
  tmptext += ",";
  tmptext += v1stO2;
  tmptext += ",";
  tmptext += v1stO3;
  tmptext += ",";
  tmptext += v2ndO1;
  tmptext += ",";
  tmptext += v2ndO2;
  tmptext += ",";
  tmptext += v2ndO3;
  tmptext += ",";
  tmptext += v3rdO1;
  tmptext += ",";
  tmptext += v3rdO2;
  tmptext += ",";
  tmptext += v3rdO3;
  tmptext += ";";
  tmptext += v1stT1;
  tmptext += ",";
  tmptext += v1stT2;
  tmptext += ",";
  tmptext += v1stT3;
  tmptext += ",";
  tmptext += v2ndT1;
  tmptext += ",";
  tmptext += v2ndT2;
  tmptext += ",";
  tmptext += v2ndT3;
  tmptext += ",";
  tmptext += v3rdT1;
  tmptext += ",";
  tmptext += v3rdT2;
  tmptext += ",";
  tmptext += v3rdT3;
  Serial.println(tmptext);
}
```

```
void vinitialize() {
  v1stO1 = "    ";
  v1stT1 = "    ";
  v1stO2 = "    ";
  v1stT2 = "    ";
  v1stO3 = "    ";
  v1stT3 = "    ";
  v2ndO1 = "    ";
  v2ndT1 = "    ";
  v2ndO2 = "    ";
  v2ndT2 = "    ";
  v2ndO3 = "    ";
  v2ndT3 = "    ";
  v3rdO1 = "    ";
  v3rdT1 = "    ";
  v3rdO2 = "    ";
  v3rdT2 = "    ";
  v3rdO3 = "    ";
  v3rdT3 = "    ";
}

# For Nine Oxygen
/*
   Pin assignment
   0 :
   1 :
   2 :
   3 : mySerial1 LuminOx1 tx
   4 : mySerial1 LuminOx1 rx
   5 :
   6 : mySerial2 LuminOx2 tx
   7 : mySerial2 LuminOx2 rx
   8 :
   9 : mySerial3 LuminOx3 tx
   10: mySerial3 LuminOx3 rx
   11:
   12: mySerial4 Twelite  tx
   13: mySerial4 Twelite  rx
   GND:

   A5:
   A4:
   A3:
   A2:
   A1:
   A0:

   Vin:
   GND: GND to LuminOx
   GND: GND to OLED
```

```
   5V: power supply to LuminOx
   3.3V: power supply to OLED
   Reset:
   IORef:
   -:

   You need to install following libraries.
    TimeLib....by PaulStoffregen
    Adafruit_FeatherOLED.h ... by MacroSchwartz

*/

#include <Wire.h>
#include <TimeLib.h>
#include <SoftwareSerial.h>
#include <Adafruit_GFX.h>
#include <Adafruit_SSD1306.h>
#include <Adafruit_FeatherOLED.h>


Adafruit_FeatherOLED oled = Adafruit_FeatherOLED();
SoftwareSerial mySerial4 =  SoftwareSerial(13, 12);//rx,tx,
SoftwareSerial mySerial3 =  SoftwareSerial(9, 10);//rx,tx,
SoftwareSerial mySerial2 =  SoftwareSerial(6, 7);//rx,tx,
SoftwareSerial mySerial1 =  SoftwareSerial(3, 4);//rx,tx,

float sensorValueO1;
float sensorValueO2;
float sensorValueO3;
float sensorValueT1;
float sensorValueT2;
float sensorValueT3;
boolean flag;
boolean sendflag;
boolean Dmode;  // Dmode=true to collect data.
String myName = "3rd";  //either 1st, 2nd, or 3rd
boolean first = true;

void setup() {
  Serial.begin (9600);
  Serial.println("start");
  pinMode(13, INPUT_PULLUP);
  oled.init();                    // initialize the lcd
  delay(10);
  // initialize serial communication at 9600 bits per second:
  mySerial1.begin(9600);
  delay(10);
  mySerial2.begin(9600);
  delay(10);
  mySerial3.begin(9600);
```

```
  delay(10);
  mySerial4.begin(38400);
  // Print a message to the LCD.
  delay(10);
  mySerial1.listen();
  delay(10);
  Serial.println("OK0");
  luminoxInitialize1();
  Serial.println("OK1");
  delay(10);
  mySerial2.listen();
  luminoxInitialize2();
  Serial.println("OK2");
  delay(10);
  mySerial3.listen();
  luminoxInitialize3();
  Serial.println("OK3");
  setTime(0, 0, 0, 1, 1, 2021);  //set time
  mySerial1.listen();
  delay(10);
  Serial.print("Temp:");
  Serial.print(luminoxT1Measure());
  Serial.print("\tO");
  Serial.println(luminoxO1Measure());
  mySerial2.listen();
  delay(10);
  Serial.print("Temp:");
  Serial.print(luminoxT2Measure());
  Serial.print("\tO");
  Serial.println(luminoxO2Measure());
  mySerial3.listen();
  delay(10);
  Serial.print("Temp:");
  Serial.print(luminoxT3Measure());
  Serial.print("\tO");
  Serial.println(luminoxO3Measure());
  flag = false;
  sendflag = false;
}

// the loop routine runs over and over again forever:
void loop() {
  int elapsedTime = hour() * 60 + minute();  //calculate elapsed time within a day (after start of
operation)
  mySerial1.listen();
  delay(10);
  sensorValueT1 = luminoxT1Measure();
  sensorValueO1 = luminoxO1Measure();
  mySerial2.listen();
  delay(10);
```

```
sensorValueT2 = luminoxT2Measure();
sensorValueO2 = luminoxO2Measure();
mySerial3.listen();
delay(10);
sensorValueT3 = luminoxT3Measure();
sensorValueO3 = luminoxO3Measure();
int currentSecond = second();
//if ((currentSecond % 10 == 0 and flag == false) || first == true) {
Serial.print(myName);
Serial.print("\t[1]:O\t");
Serial.print(sensorValueO1);
Serial.print("\tT\t");
Serial.print(sensorValueT1);
Serial.print("\t[2]:O\t");
Serial.print(sensorValueO2);
Serial.print("\tT\t");
Serial.print(sensorValueT2);
Serial.print("\t[3]:O\t");
Serial.print(sensorValueO3);
Serial.print("\tT\t");
Serial.println(sensorValueT3);
int convertedValueT1 = sensorValueT1 * 10;
int convertedValueO1 = sensorValueO1 * 10;
int convertedValueT2 = sensorValueT2 * 10;
int convertedValueO2 = sensorValueO2 * 10;
mySerial4.listen();
delay(10);
mySerial4.print(myName);
mySerial4.print(printFourDigits(sensorValueO1 * 10));
mySerial4.print(printFourDigits(sensorValueT1 * 10));
mySerial4.print(printFourDigits(sensorValueO2 * 10));
mySerial4.print(printFourDigits(sensorValueT2 * 10));
mySerial4.print(printFourDigits(sensorValueO3 * 10));
mySerial4.println(printFourDigits(sensorValueT3 * 10));
//    mySerial4.println(minute());
first = false;
flag = true;
//} else if (currentSecond > 0) {
flag = false;
//}

oled.clearDisplay();
oled.setCursor(0, 0);
oled.print(myName);
oled.print(" ");
oled.print(printDigits(hour()));
oled.print(":");
oled.print(printDigits(minute()));
oled.print(":");
oled.print(printDigits(second()));
```

```
  oled.setCursor(0, 12);
  oled.print("O1 ");
  oled.print(sensorValueO1);
  oled.print(" O2 ");
  oled.print(sensorValueO2);
  oled.setCursor(0, 24);
  oled.print("O3 ");
  oled.print(sensorValueO3);
  oled.print(" T ");
  oled.print(sensorValueT1);
  oled.display();
  delay(200);
}


String printDigits(int digits) {
  String result = "";
  if (digits < 10)
    result += "0";
  result += digits;
  return result;
}

String printFourDigits(int digits) {
  String text = "";
  if (digits == "") {
    text = "xxxx";
  } else {
    if (digits < 10) {
      text += ("000");
      text += digits;
    }
    else if (digits < 100) {
      text += ("00");
      text += digits;
    }
    else if (digits < 1000) {
      text += ("0");
      text += digits;
    }
    else {
      text += digits;
    }
  }
  return text;
}

void luminoxInitialize1() {
  mySerial1.print("M 1");
  mySerial1.print("\r\n");
```

```
  mySerial1.println("O");    //"O" for ppO2 [mbar], "%" for O2 in [%], "A" for all data
  int i = 0;
  while (mySerial1.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.clearDisplay();
      oled.setCursor(0, 0);
      oled.print("Oxygen Sensor 1 Error");
      oled.display();
      break;
    }
  }
  while (mySerial1.available() > 0) {
    mySerial1.readString();
  }
}

void luminoxInitialize2() {
  mySerial2.print("M 1");
  mySerial2.print("\r\n");
  mySerial2.println("O");    //"O" for ppO2 [mbar], "%" for O2 in [%], "A" for all data
  int i = 0;
  while (mySerial2.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.clearDisplay();
      oled.setCursor(0, 0);
      oled.print("Oxygen Sensor 2 Error");
      oled.display();
      break;
    }
  }
  while (mySerial2.available() > 0) {
    mySerial2.readString();
  }
}

void luminoxInitialize3() {
  mySerial3.print("M 1");
  mySerial3.print("\r\n");
  mySerial3.println("O");    //"O" for ppO2 [mbar], "%" for O2 in [%], "A" for all data
  int i = 0;
  while (mySerial3.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.clearDisplay();
      oled.setCursor(0, 0);
```

```
      oled.print("Oxygen Sensor 3 Error");
      oled.display();
      break;
    }
   }
   while (mySerial3.available() > 0) {
    mySerial3.readString();
   }
}

float luminoxO1Measure() {
  String text;
  float result;
  mySerial1.flush();
  mySerial1.println("O");    //"O" for ppO2 [mbar], "%" for O2 in [%], "A" for all data
  int i = 0;
  while (mySerial1.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.setCursor(0, 0);
      // oled.print("Oxygen Sensor 1 Error in measurement");
      oled.display();
      return 1.0;
      break;
    }
  }
  while (mySerial1.available() > 0) {
    text = mySerial1.readString();
    text = text.substring(3, 8);
    result = text.toFloat();
    return result;
  }
}

float luminoxT1Measure() {
  String text;
  float result;
  mySerial1.flush();
  mySerial1.println("T");    //"T" for temperature
  int i = 0;
  while (mySerial1.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.setCursor(0, 0);
      // oled.print("Oxygen Sensor 1 Error in measurement");
      oled.display();
      return 10.0;
    }
```

```
  }
  while (mySerial1.available() > 0) {
   text = mySerial1.readString();
   text = text.substring(3, 7);
   result = text.toFloat();
   return result;
  }
}

float luminoxO2Measure() {
  String text;
  float result;
  mySerial2.flush();
  mySerial2.println("O");    //"O" for ppO2 [mbar], "%" for O2 in [%], "A" for all data
  int i = 0;
  while (mySerial2.available() == 0) {
   delay(10);
   i++;  // i=i+1
   if (i >= 100) {
    oled.setCursor(0, 0);
    // oled.print("Oxygen 2 Sensor Error in O measurement");
    oled.display();
    return 2.0;
   }
  }
  while (mySerial2.available() > 0) {
   text = mySerial2.readString();
   text = text.substring(3, 8);
   result = text.toFloat();
   return result;
  }
}
float luminoxT2Measure() {
  String text;
  float result;
  mySerial2.flush();
  mySerial2.println("T");    //"T" for temperature
  int i = 0;
  while (mySerial2.available() == 0) {
   delay(10);
   i++;  // i=i+1
   if (i >= 100) {
    oled.setCursor(0, 0);
    // oled.print("Oxygen 2 Sensor Error in T measurement");
    oled.display();
    return 20.0;
   }
  }
  while (mySerial2.available() > 0) {
   text = mySerial2.readString();
```

```
    text = text.substring(3, 7);
    result = text.toFloat();
    return result;
  }
}
float luminoxO3Measure() {
  String text;
  float result;
  mySerial3.flush();
  mySerial3.println("O");    //"O" for ppO2 [mbar], "%" for O2 in [%], "A" for all data
  int i = 0;
  while (mySerial3.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.setCursor(0, 0);
      // oled.print("Oxygen 3 Sensor Error in O measurement");
      oled.display();
      return 3.0;
    }
  }
  while (mySerial3.available() > 0) {
    text = mySerial3.readString();
    text = text.substring(3, 8);
    result = text.toFloat();
    return result;
  }
}
float luminoxT3Measure() {
  String text;
  float result;
  mySerial3.flush();
  mySerial3.println("T");    //"T" for temperature
  int i = 0;
  while (mySerial3.available() == 0) {
    delay(10);
    i++;  // i=i+1
    if (i >= 100) {
      oled.setCursor(0, 0);
      // oled.print("Oxygen 3 Sensor Error in T measurement");
      oled.display();
      return 30.0;
    }
  }
  while (mySerial3.available() > 0) {
    text = mySerial3.readString();
    text = text.substring(3, 7);
    result = text.toFloat();
    return result;
  }
```