東京大学大学院新領域創成科学研究科
社会文化環境学専攻

2021 年度

修士論文

Effect of Dodecylbenzene Sulfonate on Organic Pollutants Removal in the Intermittent Contact Oxidation Process

間欠接触酸化法における有機性汚濁物質除去へのドデシルベンゼンスルホン酸の影響

2021 年 7 月 9 日提出

Advisor: Professor Hiroyasu Satoh

Co-Advisor: Professor Jun Sasaki

Regina Mardatillah

レギナ マルダティラ

ACKNOWLEDGMENT

First and foremost, praise and thank God, the Almighty, for blessings throughout my studies.

I would like to express my deep and sincere gratitude to my advisor, Prof. Hiroyasu Satoh, for allowing me to research and provide invaluable guidance throughout this research. Thank you for your patience in teaching and evaluating my works during my studies in the past two years.

I would also like to thank my co-advisor, Prof. Jun Sasaki, for his valuable suggestions during my research.

I am gratefully acknowledging the funding that I received towards my master's degree from the Asian Development Bank – Japan Scholarship Program for allowing me to pursue my education.

My thanks also go out to the support I received from all the members of Satoh Laboratory.

I would also like to say a heartfelt thank you to my family and friends for supporting and encouraging me until now.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SDBS	Sodium dodecylbenzene sulfonate
DBS	Dodecylbenzene sulfonate
MBAS	Methylene blue active substances
COD	Chemical oxygen demand
BOD	Biochemical oxygen demand
TLC	Thin layer chromatography
ICOP	Intermittent contact oxidation process
OCR	Oxygen consumption rate
ω-oxidation	Omega oxidation
β-oxidation	Betha oxidation
UASB	Up-flow anaerobic sludge blanket
ABS	Alkylbenzene sulfonate
LAS	Linear alkylbenzene sulfonate
EC_{50}	Median effective concentration
NOEC	No observed effect concentration

LIST OF UNIT MEASUREMENTS

°C	degree Celsius
d	day
h	hour
g	gram
L	litre
mL	millilitre
min	minute
m	meter

ABSTRACT

This study explored the effect of dodecylbenzene sulfonate (DBS) during the intermittent contact oxidation process (ICOP) for enhanced in-sewer purification. The discussion is divided into five parts: introduction, literature review related to the study followed by experimental methods, results and discussion from the experiment, and conclusions.

The literature review explains the surfactants and their classification, toxicity of linear alkylbenzene sulfonate (LAS) on microorganisms, biodegradation pathway of LAS, surfactant market, and potential contribution of chemical oxygen demand (COD) load to the sewer system, surfactant in sewage and LAS in sewage treatment plants. Finally, the potential of sewer processes for enhanced self-purification in the sewer is discussed.

Furthermore, the effect of DBS on organic pollutants removal during the ICOP for enhanced sewer self-purification was studied. The ICOP is a process that uses sponge media installed in sewer pipes to retain water and supply habitat for microorganisms. To do so, synthetic sewage containing 0 – 74.7 mg L^{-1} of DBS as a model of surfactant was supplied to a channel installed with sponge retaining microorganisms. First, the organic pollutants removal was evaluated by the removal of COD and by oxygen consumption. Then, the potential of ICOP to remove DBS was examined.

While in the initial series of experiments, COD removal was negatively affected by 17-32% when DBS concentration was higher than 56 mg L^{-1}, after microorganisms were acclimatised, adverse effect on COD removal performance was not observed under the existence of DBS at least up to 74.7 mg L^{-1}.

The removal of DBS evaluated by the reduction of methylene blue active surfactant remained less. However, by thin-layer chromatography analysis, DBS was found to be degraded to other forms after 24 hours of treatment.

Chapter 1

INTRODUCTION

1.1 Research Background

The most critical role of sewer pipe is to transfer sewage from its sources to the destination, often sewage treatment plants. However, Warith et al. [1], Marjaka et al. [2], and Jacobsen et al. [3] suggested that sewer pipes can act as a reactor for the pre-treatment of sewage. That is when sewage flows through the sewer system, chemical, physical, and biological processes can change components in sewage. The term "sewer self-purification" means the ability of water to purify itself of contaminants in sewer pipes. Tanji et al.[4] reported that installing porous media in sewer pipes could enhance the ability for sewer self-purification.

One of the promising methods to enhance sewer self-purification is the intermittent contact oxidation process (ICOP). The ICOP promotes the retention of microorganisms in sewer pipes by installing sponge media and exposes the media to the intermittent flow of sewage [5]– [10]. When sewage flows, it is the feeding time for the microorganism as the organic pollutants in the sewage is their substrates. Furthermore, organic pollutants removed by microorganisms will be oxidized when the sewage flow is stopped, and sponge media is exposed to the air. One of the target applications of in-sewer purification with the ICOP is sewer upstream because flow intermittency is bigger in sewer upstream [6].

In sewer upstream with higher fluctuation of flow rate, fluctuation of concentrations of the component in sewage is also expected to be high. If sewage contains substances toxic to microorganisms, they may negatively affect in-sewer purification by ICOP. One of the possibly toxic substances which exist in sewage is surfactants which are commonly used in household cleaning activities. Surfactants are usually contained in grey wastewater, sewage from households' activities except for human excreta. It derives from bathroom, laundries, kitchen sink, showers, and accounts for 50% – 80% of the sewage volume [11]– [15]. The composition of greywater produced in a household can vary depending on lifestyle, fixtures, and climatic conditions [11], [12], [16]. In terms of chemical contaminants, surfactants have been found as the primary chemical used in cleaning or washing activities. The surfactant can be classified into four main types: anionic, cationic, amphoteric, and non-ionic [17]. In this study, anionic surfactant dodecylbenzene sulfonate (DBS) is focused on as a surfactant model. It belongs to the linear alkylbenzene sulfonate (LAS) group, a synthetic anionic surfactant used in laundry detergent [18]. The typical greywater contains anionic surfactants determined by the methylene blue method in the range of 3 – 70 mg L^{-1} [19].

Surfactants discharged into the sewer system may negatively affect microbial activities for sewer self-purification and thus purification performance due to their toxicity. Therefore, to clarify the potential of sewer pipes as a reactor for pre-treatment of sewage, this study assessed the possible adverse effect of DBS on the microorganisms in the ICOP.

1.2 Research Objectives

This study assessed the effect of DBS, one of the most widely used anionic surfactants, in the ICOP for enhanced sewer self-purification. This study's specific objectives are directed toward the following two points:

The first objective is to examine the potential of the ICOP to remove organic pollutants under exposure to DBS. This experiment can be done by monitoring the organic pollutants removal using synthetic sewage containing DBS at different concentrations. The range of surfactants concentration was selected based on the typical concentration found in domestic sewage [20].

The second objective is to examine the capability of the ICOP for DBS removal. This second objective can be achieved firstly by monitoring the removal rate of methylene blue active surfactant concentration in sewage in the ICOP. Also, if necessary, generation of surfactants from biomass and formation of intermediate degradation products from DBS will be studied upon necessity.

1.3 Thesis Structure

This thesis is divided into five chapters as below:

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

Chapter 2 is the literature review about surfactants, including surfactants in sewage, classification of surfactants, surfactant market, biodegradation pathway of LAS, and LAS in sewage treatment plants. Furthermore, sewer processes and enhanced in-sewer purification are also explained. This section synthesizes information found in journal articles, proceedings, and textbooks.

Chapter 3 describes the detailed methodology of this study. This section explains the experimental setup, including the reactor setup, inoculation, synthetic sewage composition, performance evaluation, and analytical methods.

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

Chapter 5 summarises findings, limitations, and recommendations.

Chapter 2

LITERATURE REVIEW

This chapter provides a review of studies about surfactants, including surfactant and their classification, the toxicity of DBS and other surfactants on the microorganism, biodegradation pathway of LAS, surfactant market and its potential contribution for COD load to the sewer system, surfactant in sewage and LAS in sewage treatment plants. Furthermore, sewer processes and enhanced in-sewer purification are explained.

2.1 Surfactants

In this section, the different aspects of surfactants and their relation to the sewer system are reviewed.

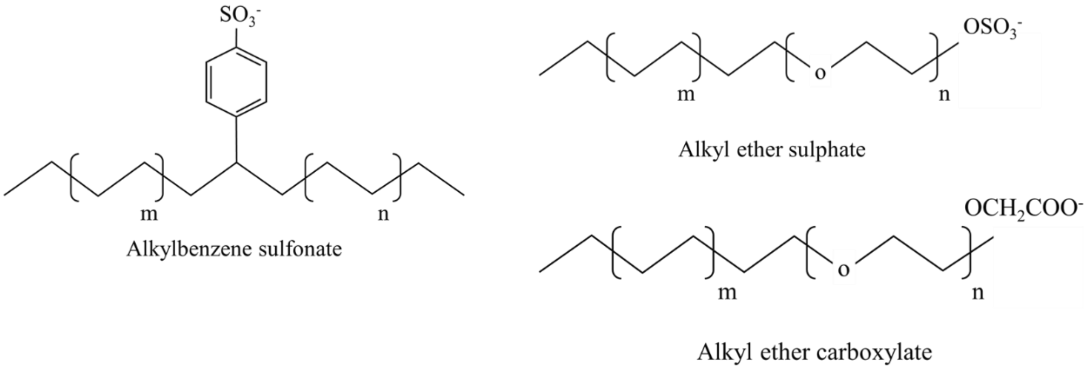
2.1.1 Classifications

Surfactants are amphiphiles which means a molecule that has both a hydrophobic tail (insoluble in water) and a hydrophilic head group (soluble in water) [16]. The model surfactant used in this study is Dodecylbenzene Sulfonate (DBS). Dodecylbenzene sulfonate is categorised as an anionic surfactant, linear alkylbenzene sulfonate (LAS), used in laundry detergent as cleansing agents [17]-[19].

According to the dissociation properties of polar groups, surfactants are classified as follows:

1. Anionic surfactants

   Anionic surfactants dissociate into anions in aqueous solutions. Anionic surfactants are commonly used in cleaning, such as detergent and dishwashing [14],[18]. Anionic surfactants can be classified according to their polar group and include such chemicals as alkylbenzene sulfonate (detergents), soap (fatty acid salt), lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent) [27]. Examples of anionic surfactant structures are shown in **Figure 2-1** [28], [29].



Alkylbenzene sulfonate

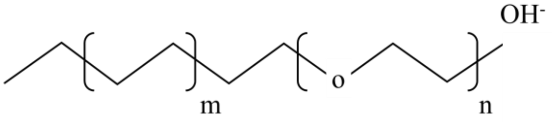
Alkyl ether sulphate

Alkyl ether carboxylate

Figure 2-1 Examples of anionic surfactants structures

2. Non-Ionic Surfactants

   The classifications of non-ionic surfactants depend on the type of their hydrophilic groups, which do not dissociate into ions in aqueous solutions. An example of a non-ionic surfactant structure is shown in **Figure 2-2**.

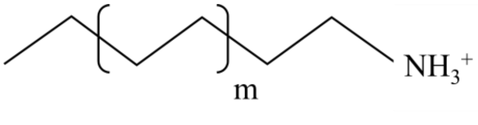


Fatty alcohol ethoxylate

Figure 2-2 An example of non-ionic surfactants structures

3. Cationic surfactants

   Cationic surfactants dissociate into a cation. They are primarily amine-based surfactants and are applied in fabric softening [29]. Cationic surfactants are generally more expensive than anionic surfactants due to their difficulty in synthesis. An example of cationic surfactant structure is shown in **Figure 2-3**.

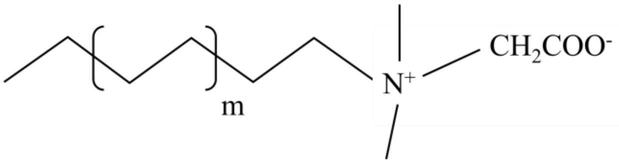


Fatty amine salt

Figure 2-3 An example of cationic surfactants structures

4. Amphoteric surfactants/ zwitterionic

   This type of surfactant dissociates with both anions and cations, depending on the pH because it contains at least one negative and one positive charge in the molecules simultaneously [14],[18]. An example of the amphoteric surfactant structure is shown in **Figure 2-4**.



Betaine

Figure 2-4 An example of amphoteric surfactants structures

2.1.2 Toxicity of LAS on Microorganisms

The toxicity of LAS on the microorganisms depends on the concentration of bioavailable LAS homologues. Garcia et al. [30] reported that the toxicity of LAS with an effective concentration (EC_{50}) of 14 mg L^{-1} for methanogenic biomass. It means that LAS in the concentration of 14 mg L^{-1} affects 50% of the microorganisms. Wang et al. [31] reported that a high concentration of LAS (20 mg L^{-1}) inhibited the growth of *Microcystis aeruginosa*, a blue, green alga.

Different homologues and isomers have different toxicity. Human and Environmental Risk Assessment [32] suggested that shorter chain homologues have less toxic effects. For instance, EC_{50} of C_{14} LAS (LAS with C_{11} alkyl chain) on *Daphnia magna* is 1.5 mg L^{-1} mg L^{-1} while that of C_{10} LAS is 16.7 mg L^{-1}.

No observed effect concentration (NOEC) is the highest concentration used in a toxicity test that does not cause a toxic effect that is significantly [33]. Brandt et al. [34] reported that ammonia-oxidising bacteria isolated from the soil are inhibited by 5-9 mg L^{-1} of LAS, which was shown by its effect on specific growth rate and CO_2 fixation rate.

2.1.3 Biodegradation of LAS

Biodegradations is a critical process to handle LAS either in the sewer or sewage treatment plants. Microorganisms can utilise surfactants either as substrates for energy production or resources for their cell growth [35].

Biodegradation is divided into three types as following [36], [37]:

1. *Primary biodegradation* means the surface-active properties are lost due to degradation of the parent substance due to structural changes (transformations) by microorganisms.
2. *Ultimate biodegradation* happens when the surfactant is used by microorganisms resulting in its breakdown to inorganic end-products such as carbon dioxide, water, mineral salts of any other elements present (mineralisation), and new microbial cellular constituents (biomass).

Biodegradation is influence by various factors [36], such as:

1. The capability of microorganisms to metabolise the organic compound.
2. The growth factors (temperature, pH, oxygen availability, nutrients, etc.).
3. The availability of the other organic substrate.

Co-metabolism of LAS with other organic substrates produces shorter-chain homologues under aerobic conditions [35]. Biodegradation of LAS starts with ω-oxidation of an alkyl chain, followed by β-oxidation for successive cleavage of C2 fragments. The reaction between ω-oxidation and β-oxidation produces sulpho phenyl carboxylates (SPC). As a result, the interfacial activity as surfactant and toxicity is less [38]– [41].

The biodegradation pathway of LAS is illustrated in **Figure 2-5** [43].

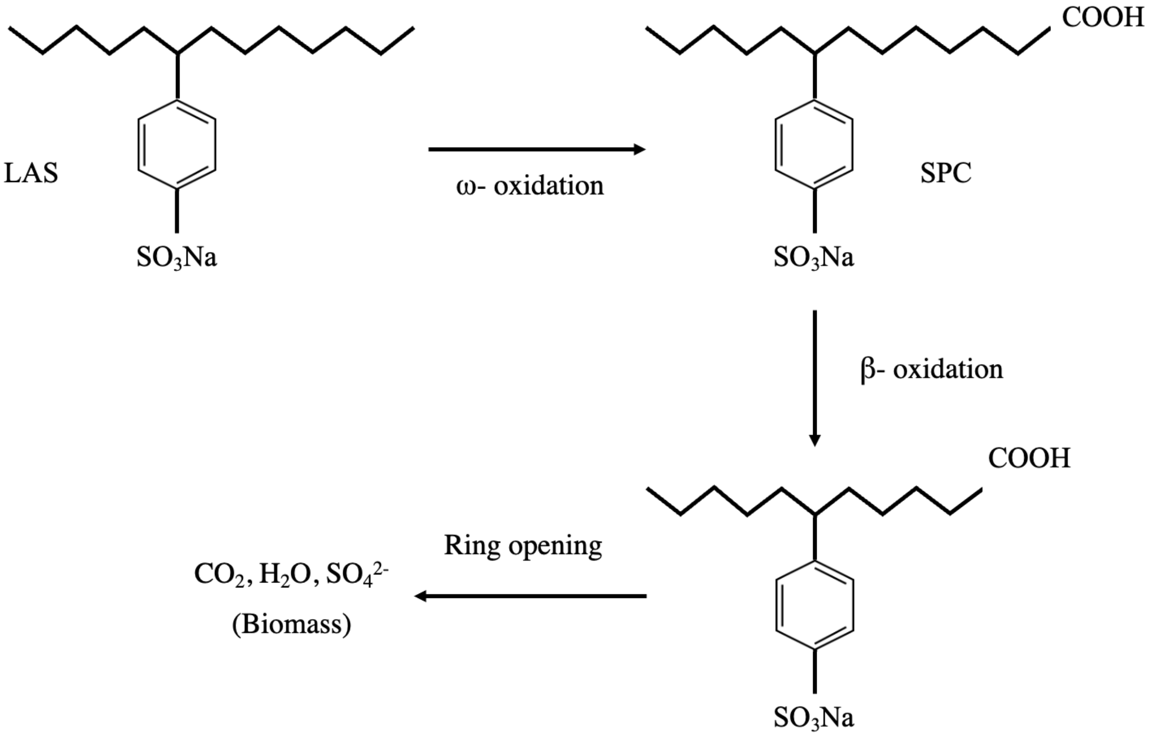


Figure 2-5 Biodegradation Pathway of LAS

Several studies regarding LAS biodegradation under anaerobic condition has been conducted [36], [41], [44], [45]. Different electron acceptors such as sulphate, nitrate or carbonate can be used for anaerobic LAS biodegradation [35]. Anaerobic bacteria can utilise surfactant as a carbon source in the presence and absence of additional carbon sources [44].

2.1.4 Surfactant Market and Its Potential Contribution for COD Load to Sewer System

The introduction of LAS in 1964 was to intend to substitute the poorly biodegradable alkylbenzene sulfonate (ABS) [23],[46]. The poor biodegradability of ABS was attributed to the branched-chain alkyl group. Thus, to improve its biodegradability, LAS was introduced.

In Japan, the surfactant production in 2016 was 1,109,750 tons, with a sale

of 890,663 tons [47]. Anionic surfactants contributed 37.1% of the total production. When production of anionic surfactant is expressed per capita in Japan (population in Japan in 2016 is 127million), 9 g capita-1 day^{-1} is obtained. As surfactants are mostly used for washing different things with water, a significant part of anionic surfactants goes to sewage after use. When typical water consumption per capita per day is around 200 L, 9 (g capita^{-1} day^{-1})/ 200 (L capita^{-1} day^{-1}) = 45 mg L^{-1}. This value is an overestimate, as 9 g capita^{-1} day$^{-1 \text{ is}}$ production, not consumption, and some part of consumption may not come into sewage. However, this calculation gives an approximate estimation of anionic surfactant concentration in sewage.

Anionic surfactants are also one of the causes of increased COD in sewage. One gram of sodium salt of DBS (SDBS) is equivalent to about 2.4 gCOD, as the calculation is shown below.

$$4\ C_{18}H_{29}NaSO_3 + 103\ O_2 \rightarrow 72\ CO_2 + 58\ H_2O + 4\ NaSO_4$$

$$\text{The COD equivalent } = \frac{\Delta O_2}{\Delta C_{18}\,H_{29}Na_2SO_3} = \frac{103\times 32}{4\times 348.5} = 2.364\text{ g } O_2$$

Based on this, the LAS production of 9 g capita^{-1} day^{-1} is equivalent to 21.6 gCOD capita^{-1} day^{-1}. According to Metcalf & Eddy [48], COD discharge per capita per day is reported to be 110-295 g capita^{-1} day^{-1}. Considering this, the contribution of surfactant to COD is expected to be high.

2.1.2 Surfactants in Sewage

Surfactant contributes to a significant fraction of organic pollutants in greywater due to household activities such as cleaning, dishwashing, etc. [11], [20]. Greywater contains anionic surfactants in the range of 3-70 mg L^{-1} [19]. This concentration is higher than the concentration in sewage treatment facilities which ranges between 1 – 10 mg L^{-1} [49].

The ratio of biochemical oxygen demand to chemical oxygen demand (BOD_5: COD) indicates the biodegradability of contaminants in greywater [50]. Mostly, almost half of the organic pollutants in greywater are biodegradable, with an average BOD_5: COD ratio in the range of 0.31 - 0.71 [51]. However, the COD: BOD ratio of surfactants can be as high as 4:1, indicating lower biodegradability of surfactants [11], [52].

2.1.6 LAS in Sewage Treatment Plants

The removal efficiency of anionic surfactants removal in sewage treatment has been reviewed by Stamatelatou et al. (2011) [46], [53]. The surfactant can be removed by biological degradation with activated sludge in aerobic conditions than anaerobic conditions [38], [40], [43], [54], [55]. In Activated sludge, LAS can be eliminated in the range 95% - 99% [39], [46], [49], [56]– [58]. Regarding trickling filter processes, McAvoy et al. [59] reported 82% removal, and in the lagoon Marcomini, et al. [60] reported LAS removal was 90%. Moreover, Mungray et al. [61] reported that LAS removal is 2-18% in up-flow anaerobic sludge blankets.

The treatment of municipal sewage includes mechanical, biological, and chemical methods. Surfactants have strong absorbing properties, and their molecules are absorbed by activated sludge flocks. Because of its absorption and toxicity, which affect the microorganisms, causing decreased sewage treatments efficiency [62], particularly during the aeration phase and or/final clarifier [63]– [65].

Foams are formed when the concentration of surfactant is high. Here, "foam" means "a set of stable bubbles produced when air or other gases are introduced below the surface of the liquid that expands to enclose the gas with a liquid film", as stated by Collivignarelli et al. (2020) [66]. The foam presence is causing some problems: 1) Reduction in oxygen transfer 2) Biomass decreased in the biological reactor [67], [68]. Furthermore, surfactants can also damage the microbial cell and inhibit microbial growth [69].

2.2 Sewer processes

Sewage flows from various sources to the sewage treatment plant through sewer pipes. Organic pollutants are transformed by chemical and biological processes that take place in a sewer. Sewer pipes are not always filled with water. Rather, sewage flow, in many cases, has a free water surface. The wet periphery of the sewer pipe is covered with biofilm, and at the bottom, there can be sediments. Biofilm is even observed on the canopy surface of pipe where it does not usually contact with sewage. Chemical and biological processes proceed in these different phases. Further, the mass transfer of substances between different phases occurs [3]. The chemical and biological processes in water and sediment phases proceed in aerobic, anoxic, and anaerobic conditions [3].

2.2.1 Enhanced in-sewer purification

The concept of utilising sewer pipe for organic pollutants removal emerges as an alternative or pre-treatment technology for end-of-pipe sewage treatment [1], [3], [70]– [72]. Here, "pre-treatment" means that the chemical and biological reactions as natural processes in the sewer pipes can result in considerable reductions in the organic pollutants load to an end-of-pipe treatment plant. The removal efficiency is usually small, but the removal does not require external energy resources. In addition, natural self-purification can ease environmental sanitation problems, particularly when inadequate or no sewage treatment plants exist [73].

The ICOP is one of the technologies to enhance in-sewer self-purification. In the ICOP, a porous media, a sponge, is installed in sewer pipes as a habitat for microorganisms. When the media is installed in sewer upstream, the sewer flow is intermittent, and thus the media is exposed to sewage intermittently. Moreover, when sewage flow is stopped, water in the pipe is discharged, and the media is exposed to air. Thus, biomass that grows on the sponge media will be exposed to sewage containing organic substrates and air containing oxygen. Thus, microorganisms can aerobically utilise organic pollutants accumulated during sewage flow.

Several studies have been done to investigate the capability of ICOP for enhanced sewer self-purification. Shoji et al. [74] utilised a double layer pipe to enhance self-purification in-sewer. In their study, a sponge media was installed in the lower deck providing habitat for the microorganisms and enhance self-purification, while the upper deck was used for smooth transportation of sewage. The drop formed between the upper and lower decks facilitates turbulence and oxygen dissolution in the air to the sewage. The double-layer ICOP is illustrated in **Figure 2-6**.

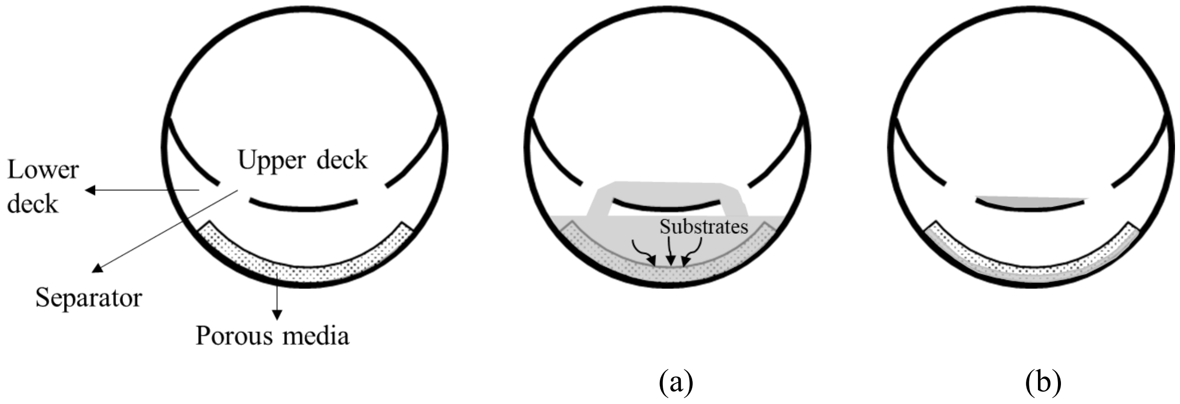


Figure 2-6 Cross-section of double layer's ICOP (a) High flow condition (b) Low flow condition

Other studies conducted by Sotelo et al. [5], [7]– [10], [75] and Lyu et al. [6] focused on ICOP using single-layer pipe. For both single-and-double-layer types, the organic pollutants were removed even when sewage flow is stopped. The single-layer ICOP is illustrated in **Figure 2-7**.

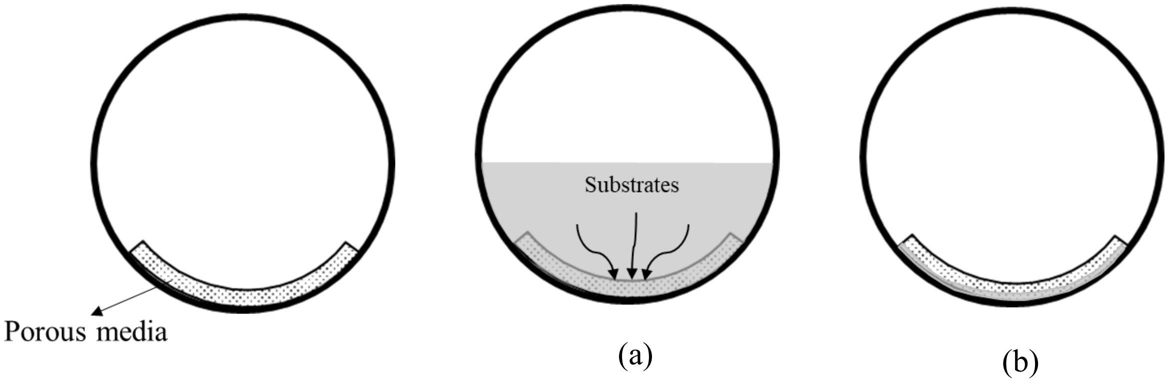


Figure 2-7 Cross-section of the single layer's ICOP (a) High flow condition (b) Low flow condition

2.3 Chapter Summary

In this chapter, the role and fate of surfactants were explained, and the potential of sewer processes as a pre-treatment for end-of-pipe sewage treatment. The summaries are described as follows:

1. Surfactants are amphiphiles which means a molecule that has both a hydrophobic tail (insoluble in water) and a hydrophilic head group (soluble in water).
2. Surfactants are classified into four parts: anionic surfactants, non-ionic surfactants, cationic surfactants, amphoteric surfactants/ zwitterionic.
3. The toxicity of LAS on microorganisms depends on the concentration of bioavailable LAS homologues.
4. LAS biodegradation starts with ω-oxidation of an alkyl chain, followed by β-oxidation for successive cleavage of C2 fragments. The reaction between ω-oxidation and β-oxidation produces sulpho phenyl carboxylates (SPC), resulting the interfacial activity as surfactant and toxicity are less.
5. The contribution of surfactants to COD is expected to be high, with approximately 21.6 gCOD capita^{-1} day^{-1}.
6. The presence of surfactants has the potential to reduce the efficiency of sewage treatment plants due to their strong absorbing properties and toxicity.
7. Installing porous media in the sewer pipes could enhance in-sewer purification.
8. Surfactants used in household activities for cleaning, dishwashing, etc., contribute to a significant fraction of organic pollutants in greywater potentially and potentially negative to microorganisms due to their toxicity.

Chapter 3

METHODOLOGY

This chapter aims to assess the possible adverse effect of DBS on the microorganisms in the ICOP. A lab-scale ICOP reactor was operated with synthetic sewage containing readily biodegradable organic matter and DBS. This chapter is organised as follows: First, the experimental section explains reactor setup for the experiment, reactor operation, inoculating a new sponge media, stock solution for synthetic sewage, and feeding strategy during the experiment. Secondly, the performance evaluation is explained. Furthermore, the last additional experiment on the biological conversion of DBS in the ICOP is described.

Note that in this study, the objective is to assess the effect of DBS on ICOP and the degradation of DBS in ICOP. However, the concentration of DBS is often expressed as SDBS. In the experiment in this study, the concentration of DBS in synthetic feed sewage was designed as SDBS concentration, and the measurement of methylene blue active substances (MBAS) utilised SDBS as the standard. Thus, SDBS and DBS will be used in a compatible way, where designed or measured DBS concentrations are concerned "SDBS" will be used, while degradation of chemical structure is of concern, "DBS" will be used.

3.1 Experimental Section

3.1.1 Reactor Setup

A lab-scale ICOP reactor, as shown in **Figure 3-1,** was prepared. The channel simulating sewer pipe had an inner dimension of 48.5 cm (length) × 7 cm (width) × 6 cm (depth), giving a volume of 2.06 L and had a slope of around 2% in the length direction. A piece of inoculated sponge media made of polyurethane sized 44 cm x 7 cm x 1 cm (Model BCD-2, pore cell density 6 cell/cm, Achilles Corporation Tokyo, Japan) was placed on the bottom of the channel as a habitat for microorganisms. The channel's outlet was led to the synthetic sewage tank with a working volume of 1L. A recirculation pump (Masterflex Model 77201-60, USA) recirculated synthetic sewage in the synthetic sewage tank to the channel. An air pump (Iwaki Air Pump, APN-085V-1 with maximum capacity 5/6 L/min, Tokyo Japan) was used to refresh air (headspace gas) in the channel. The operation of these pumps was controlled by an Arduino UNO compatible microcontroller connected to a relay. The whole reactor was placed in an air-conditioned room at 20 ± 1 °C during the study.

The channel was installed with an oxygen sensor (ME2-O2-φ20, Winsen Electronics Technology, Zhengzhou, China). The channel was made airtight to ensure reliable oxygen consumption measurement during the experiment. To do so, the reactor lid was fitted with a silicone rubber gasket and installed onto the channel with screws. The lines connected to the channel, such as the inflow line and oxygen sensor signal line, were fixed with a rubber plug. Silicon sealant and the channel's outlet to the synthetic sewage tank was made water sealed by fully submerging the outlet under water level in the synthetic sewage tank. The Arduino UNO compatible microcontroller was used to collect data from the oxygen sensor.

The operation of the reactor was basically as follows. Every day, 1 L synthetic sewage described in 3.1.3 was freshly prepared and placed in the synthetic sewage tank. The recirculation pump was operated for 5 minutes every 30 minutes repeatedly at a 200 mL/min flow rate. The air pump was operated for 5 minutes every 6 hours to refresh the air inside the channel.

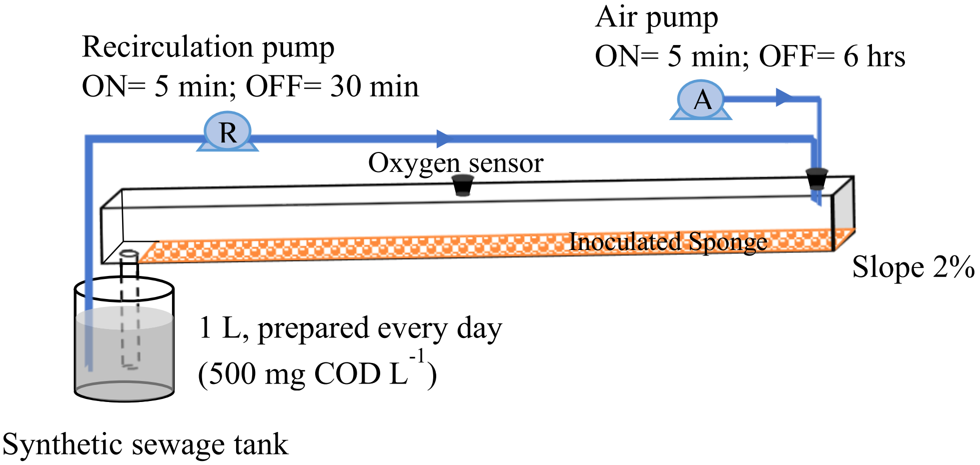


Figure 3-1 Airtight Reactor Setup

3.1.2 Reactor Operation

The reactor's operation, together with monitored parameters and additional experiments, are summarised in **Table 3.1**. The initial 37 days was for inoculation and to develop biof omass in the sponge. During this period, synthetic feed without DBS (described in 3.1.3) was fed to the reactor.

The main experiments are Run 1 and Run 3 shown in the table, together with Run 2. In these periods, synthetic sewage with different concentrations of DBS to be described in section 3.1.3 was fed to the reactor.

Additional experiments are mentioned in the comment column of Table 3.1: monitoring conducted on days 62, 63, 68-77 were to observe biosurfactant generation from biomass, and the sampling on day 103 was for TLC analysis of surfactant identity in water treated for 24 h.

Table 3-1 Rector operation summary

Run No.	Period (day)	SDBS (mg L^{-1})	Comments
	1-7	0	Inoculation
	7-37	0	Biomass development
Run 1	38-43	20	COD, methylene blue active substances (MBAS), and OCR were measured.
	44-48	40	
	49-54	60	
	55-60	80	
Run 2	61-77	0	On days 62, 63, 68 – 77, biosurfactant generation was monitored.
Run 3	78-83	20	Only OCR was measured in Run 2. On day 103 effluent sample was obtained analysed by TLC.
	84-89	40	
	90-95	60	
	96-104	80	

Before starting the experiment, the sponge media was inoculated with microorganisms from sponge media used in a reactor similar to the one described in 3.1.1 operated with sewage.

First, the fresh sponge sheet and the used sponge sheet were put together in the reactor channel described in 3.1.1. The base sponge was removed when biofilms grew on the new sponge media, which took about a week. Then, biomass in the inoculated sponge was further developed by incubation with synthetic sewage without SDBS for another month. The inoculation was done when the ICOP performance showed stable results evaluated by measuring the COD influent and effluent after 24 h. The composition of the synthetic sewage used during inoculation and biomass development is described in 3.1.3.

3.1.3 Synthetic Sewage

Synthetic sewage was prepared by mixing stock synthetic sewage solution with a prescribed amount of SDBS in ultrapure water (MilliporeSigma^{TM} Milli-Q^{TM} Advantage A10, Fisher Scientific, New Hampshire, USA)

Stockfeed solution was prepared per litre of ultrapure water as tabulated in **Table 3-2**.

Table 3-2 Preparation for stockfeed solution

No.	Amount	Unit	Chemicals	Manufacture
1	15.3	g	Peptone	Kyokuto Pharmaceutical Industrial Co, Ltd, Tokyo
2	5.1	g	Yeast extract	
3	52.6	g	$CH_3COONa\cdot3H2O$	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan
4	5.1	g	KCl	
5	11.6	g	NH_4Cl	
6	25.6	g	$MgSO_4\cdot7H_2O$	
7	8.4	g	K_2HPO_4	Kishida Chemical Co. Ltd, Osaka, Japan
8	3.1	g	$CaCl_2\cdot2H_2O$	
9	34.9	mL	Trace metal	

The trace metal stock solution was prepared per litre of ultrapure water reported by Smolders et al. [76] as tabulated in **Table 3-3**.

Table 3-3 Preparation for trace metal stock solution

No.	Amount (g)	Chemicals	Manufacture
1	3.75	$FeCl_2\cdot6H_2O$	Wako Pure Chemical Corporation, Osaka, Japan
2	0.375	$CoCl_2\cdot6H_2O$	
3	0.075	$CuSO_4\cdot5H_2O$	Kanto Chemical Co., Inc., Tokyo, Japan
4	0.15	$Na_2MoO_4\cdot2H_2O$	
5	0.3	$ZnSO_4\cdot7H_2O$	
6	0.3	$MnCl_2\cdot4H_2O$	

No.	Amount (g)	Chemicals	Manufacture
7	25	EDTA	Kishida Chemical Co. Ltd, Osaka, Japan
8	0.45	KI	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan
9	0.375	H_3BO_3	SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany

The COD concentration of stock feed solution was 167,240 mg L^{-1}. The synthetic sewage containing various SDBS concentrations was prepared by mixing stock feed solution and a weighed amount of SDBS (Kanto Chemical Co. Inc, Tokyo, Japan) as tabulated in **Table 3-4**. The COD concentration was set at 500 mg L^{-1} COD as typical COD in sewage [48].

Table 3-4 The synthetic sewage preparation by mixing stock feed solution and SDBS

Target SDBS concentration (mg L^{-1})	0	20	40	60	80
Target concentration as DBS (mg L^{-1})	0	18.7	37.4	56.0	74.7
Stock synthetic sewage solution (mL)	10	9	8.2	7.2	6.7
SDBS (mg L^{-1})	0	20	40	60	80
Ultrapure water(mL)	990	991	992	992	993
COD from synthetic sewage (mg L^{-1})	506	456	411.7	364.3	316.5
COD from SDBS (mg L^{-1})	-	47	94.6	141.8	189.1
Total COD (mg L^{-1})	506	503	506	506	505

3.2 Performance evaluation

For each monitoring day, the samples were taken eight times at 0, 30, 60, 120, 240, 360, 480, and 1440 minutes after synthetic sewage was replaced with a new one and the operation was resumed. The time 0 sample is meant for the synthetic sewage filled in the recirculation tank right before recirculation of flow was started. For each sample and each series of samples, the following parameters were measured:

1. The organic pollutants removal

   Organic pollutants removal performance was evaluated by two measures: removal of the COD and oxygen consumption in the headspace gas.

   The measurement of COD was conducted by the mercury-free dichromate method, where the digestion condition is reported by Kishimoto and Okumura [77] in combination with titration. While their method quantifies remaining dichromate after digestion by colourimetry, unconsumed dichromate was quantified by titration with ferrous ammonium sulphate (FAS) solution. For each sample, 1 mL aliquot was placed in a screw cap tube containing 0.6 mL of 0.00695 M $K_2Cr_2O_7$ mixed with 1.4 mL of 0.06125 M Ag_2SO_4 in conc. H_2SO_4. The mixture was vortexed and heated at 150°C for 2 hours, then cooled to room temperature. After cooling, 5 µL ferroin indicator solution was added to each of the tubes. After that, the solution was titrated with 0.01 M FAS until the colour changes. Solutions of potassium hydrogen phthalate at 62.5 mgCOD L^{-1} and 125 mgCOD L^{-1} were used as a standard for calibration.

On the other hand, the consumption of oxygen in the headspace gas was monitored continuously for 24 hours, and the detected oxygen concentration was recorded every minute. Mass of oxygen decreased in the headspace gas was considered as the mass of COD oxidised.

The decrease rate of oxygen concentration in terms of per cent of atmospheric pressure was evaluated every hour and was averaged for a day. Mass of oxygen consumed per day was calculated by the equation below [8].

$$\mathrm{OCR} = \frac{\mathrm{slope}}{100} \times \rho_{\mathrm{oxygen,\ 20\ ^\circ C}} \times V_{\mathrm{reactor}}$$

Where:

OCR = mass of oxygen consumed per day (g day^{-1})

Slope = slope of oxygen reduction ($\frac{\%\ \mathrm{oxygen}}{\mathrm{day}}$)

$\rho_{\mathrm{oxygen,\ 20^oC}}$ = oxygen density at 100% oxygen at 20^0C, 1 atm, 1.33 g L^{-1}

V_{reactor} = void volume in the reactor (L)

Further, the OCR value was divided by the footprint area of the sponge in the ICOP reactor to obtain COD removal (oxidation) performance per unit area of sponge per day.

2. DBS degradation

The degradation of DBS was estimated by monitoring methylene blue active substances (MBAS). In MBAS analysis, not only DBS but also anionic surfactants are detected.

There are different protocols for MBAS measurement, some are laborious but suitable for samples with a more complicated matrix, and others are easy to implement but can be applied to samples with a simpler matrix.

Here, I employed the protocol by E. Jurado et al. [78] for its easiness. In a 10mL screw cap glass tube containing 200 µL of sodium tetraborate buffer solution (50 mM at pH 10.5) and 100 µL of methylene blue (3.13 mM at pH 5-6), 5 mL aliquot of a sample was added, homogenised, then 4 mL of chloroform was added. After vigorous stirring, the tube was left for 5 min, and the absorbance at 650 nm in the chloroform phase was determined against air. A spectrophotometer (HACH Company, Mod: DR 3900, Germany) was used for absorbance.

The concentration of surfactant is calculated from the calibration curve established with SDBS. Therefore, the concentration of DBS determined by the method above described should be called MBAS concentration with SDBS as the standard.

3.3 Identity of Remaining MBAS

As a result, which will be explained in 4.1.2, the removal of anionic surfactant by ICOP was low. However, it was suspected that the chemical structure of SDBS might have changed to another form. That is, there is a possibility that anionic surfactant activity remains while the chemical structure is changed by microbial conversion. Therefore, to explore this possibility, samples were analysed by thin-layer chromatography (TLC). Here, TLC is a chromatography technique used to separate a mixture that depends on the relative affinity of compounds towards the stationary and the mobile phases. The mobile phase travels over the stationary phase's surface.

The analytical work was conducted by modifying the method by Fernandes et al. [79]. First, the water samples, freshly prepared synthetic sewage solution and the sample after treatment by ICOP for 24 hours were filtrated using 0.45 µm membrane filters (Ireland, Rev 07/04). Then, they were frozen and lyophilised. After lyophilisation, dried samples in powder form were collected, and their masses were measured. For each sample, about 28 mg influent sample and 57 mg effluent sample after 24 h was dissolved in 2.52 mL and 5.13 mL mixture of chloroform and methanol (2:1 v/v). Then, the solutions were vigorously shaken for a minute using a vortex mixer, and 0.2 µL of the solution was spotted on a TLC plate (Glass TLC plate, silica gel coated with fluorescent indicator F254, 5×10 cm, Merck, NJ, USA). The solvent system used for the development of TLC was a mixture of Chloroform: Methanol (80:20 v/v). Spots were visualised by spraying with rhodamine B 0.25% w/v in absolute ethanol to detect lipids presence under UV light at 254 nm (UV lamp, SLUV-4, AS ONE, Osaka, Japan).

Chapter 4

RESULTS AND DISCUSSIONS

This section describes the results and discussions of the experiments.

4.1 Results

4.1.1 The Organic Pollutant Removal

4.1.1.1 COD Removal

Changes in COD concentration of the water in the synthetic sewage tank with different SDBS concentrations during Run-1 and Run-2 are shown in **Figure 4-1**.

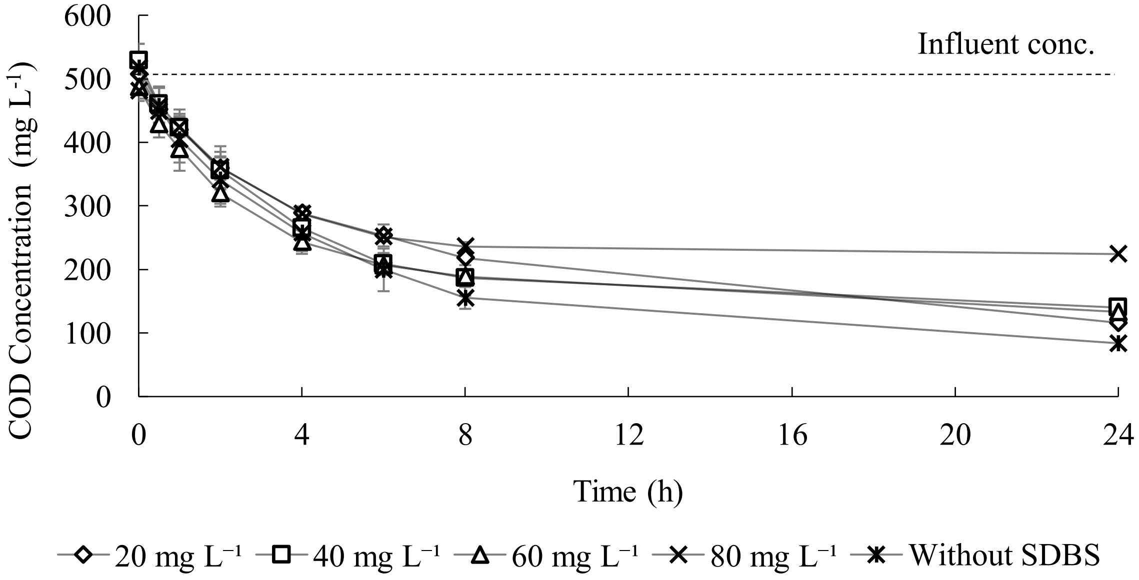


Figure 4-1 Changes in COD concentration in each SDBS condition in Run-1 and Run-2. Influent COD concentration was approximately 500 mg L^{-1} (16.2 g O m^{-2} d^{-1}). Each of the plots is from 4 days except for 80 mg L^{-1} (5 days) and without SDBS (10 days) monitoring results

Figure 4-1 shows that the concentration of COD decreased dramatically in the initial 4 hours after the synthetic sewage was introduced, then slowly decreased afterwards. The SDBS affected the COD removal, as shown in **Figure 4-1**, where with increasing SDBS concentration in synthetic sewage, COD removal decreased. Compared to the condition without SDBS, the ICOP performance decreased by 8%, 14%, 12%, 34% for 20, 40, 60, 80 mg L^{-1} SDBS containing in synthetic sewage, respectively.

4.1.1.2 Oxygen Consumption Rate

Figure 4-2 shows an example of the oxygen concentration profile in the headspace gas of the airtight channel.

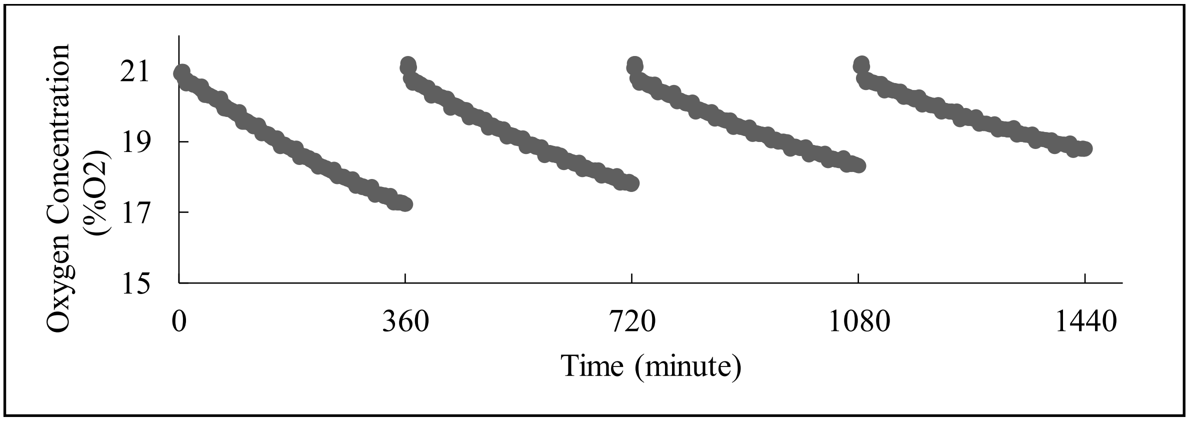


Figure 4-2 An example of oxygen concentration profile over time in the headspace gas of the airtight channel (taken from the 104th day of the experiment)

In the figure, 0 min is at 10 a.m. when synthetic sewage in the synthetic feed tank was replaced with a new one, and 1440 minutes is the next day at 10 a.m. At 0, 360, 720, 1440 min, fresh air was supplied into the channel. Thus, the headspace gas oxygen concentration reached close to 21% O_2. Oxygen concentration in the headspace-gas decreased over time during the experiment.

Changes in the average OCR for each day for the whole experiment during Run-1 to Run-3 are shown in **Figures 4-3**.

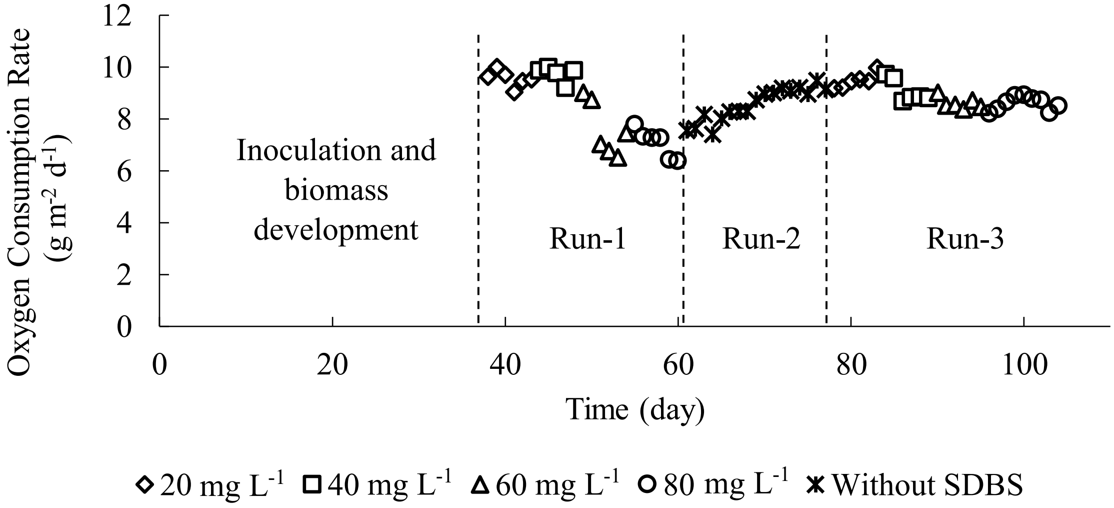


Figure 4-3 Oxygen consumption rate during Run-1, Run-2, and Run-3

As is shown in **Figure 4-3**, during Run-1 and Run-3, feed SDBS concentration was gradually increased from 20 mg L^{-1} to 80 mg L^{-1}. On the other hand, during Run-2, SDBS was omitted from the synthetic sewage.

In Run-1, OCR was around 9.5 to 9.7 g O m^{-2} d^{-1} (average 9.6 g O m^{-2} d^{-1}) during SDBS 20 mg L^{-1} and 40 mg L^{-1}. After SDBS concentration was increased to 60 mg L^{-1}, OCR remained similar for two days, then suddenly dropped to around 7 g O m^{-2} d^{-1}. During days 51 to 60, OCR dropped about 27 % of days 38 to 49 with SDBS 20 mg L^{-1} and 40 mg L^{-1}. During Run-2 without SDBS, OCR gradually increased and came back to its original level. During Run-3, SDBS concentration in feed was increased from 20 mg L^{-1} to 80 mg L^{-1}. However, in Run 3, the reduction of OCR was not significant: OCR during the period with SDBS concentration 60 mg L^{-1} or 80 mg L^{-1} was only 7.5% less than that of the period with SDBS concentration 20 mg L^{-1} or 40 mg L^{-1}.

4.1.2 The Removal of MBAS

The concentration profiles of MBAS were collected almost every day in Run-1 and on selected days in Run-2. The data are shown in **Figures 4-4 and 4-5**, where **Figure 4-4** is for the data during Run 1 possibly showing degradation of SDBS, and **Figure 4-5** for during Run 2 when SDBS was omitted in synthetic feed.

Figure 4-4 shows that the MBAS removal rates showed a consistent trend for all the concentrations applied. The MBAS concentration was observed to decrease during the initial 8 hours and then maintained at a relatively lower concentration. The removal rates of each MBAS concentrations during 24 h were: 0.17 g m^2 d^{-1} for 20 mg L^{-1}, 0.31 g m^2 d^{-1} for 40 mg L^{-1}, 0.51 g m^2 d^{-1} for 60 mg L^{-1}, 0.43 g m^2 d^{-1} for 80 mg L^{-1}.

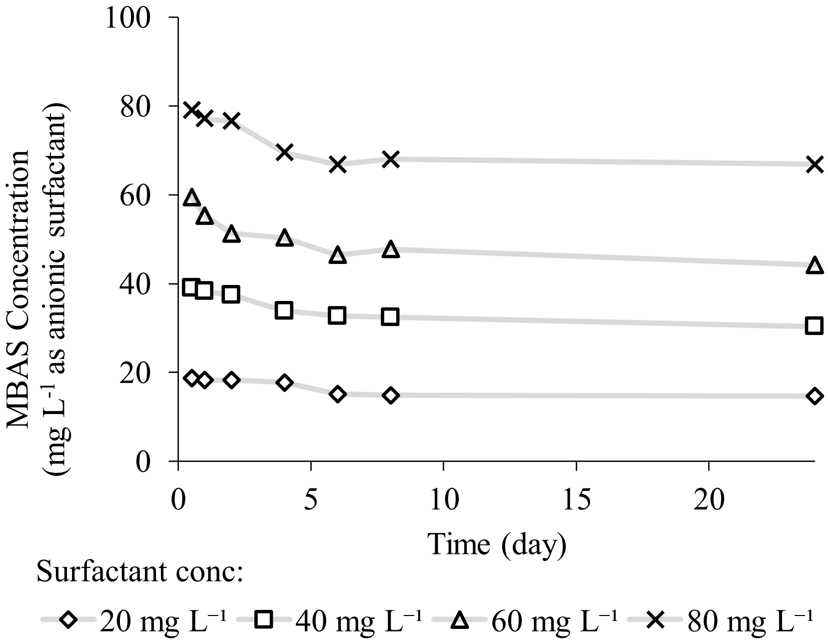


Figure 4-4 SDBS removal rate of various concentration. The error bar represents the standard deviation forn = 4, except for 40 mgL^{-1} n = 5

To examine the identity of MBAS remaining after 24-hour treatment, two additional experiments were conducted.

In the first additional experiment, a possibility that surfactant is generated by biomass was considered. To examine the possibility, on days 62, 63, 69, 70, 73, 74, and 77 without supplying SDBS, the MBAS concentration was observed. The result was as shown in **Figure 4-5**.

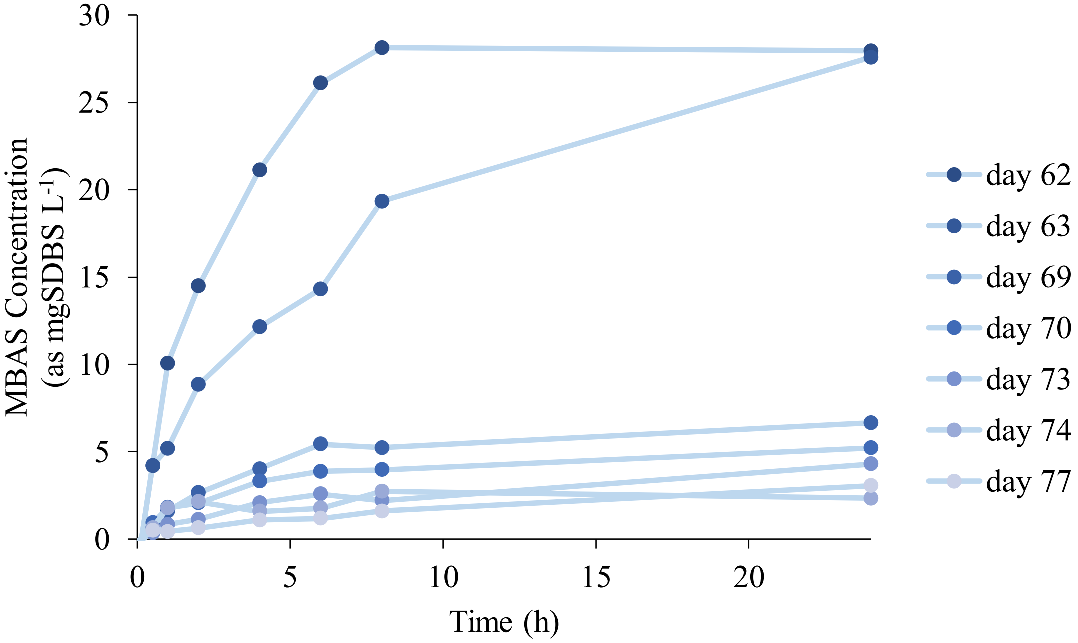
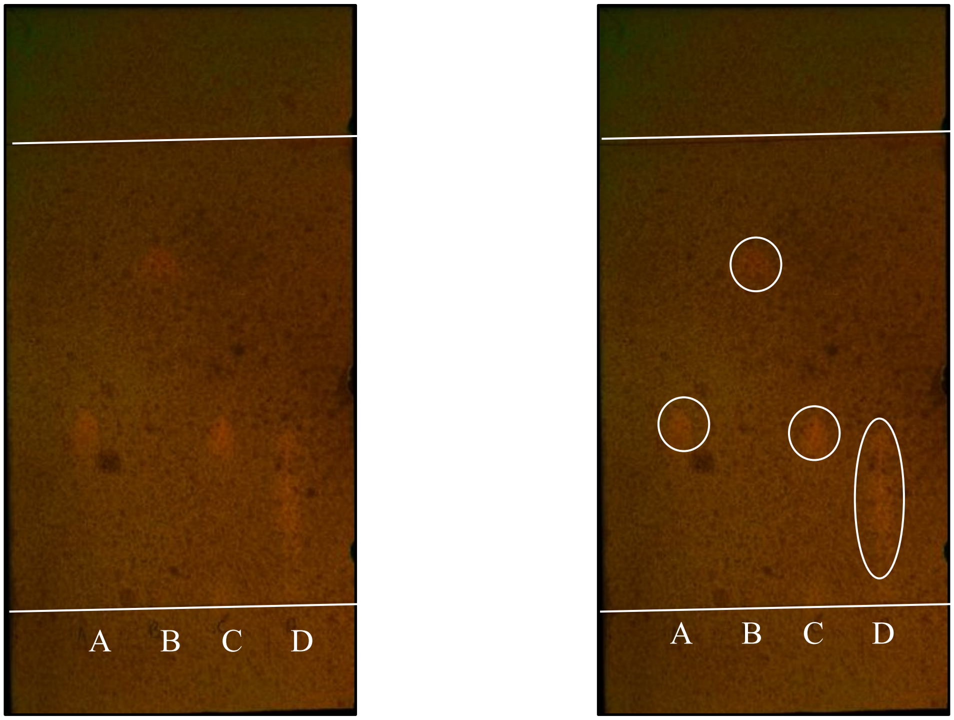


Figure 4-5 MBAS concentration in synthetic sewage without introducing SDBS

The feed SDBS was set at 80 m/L until day 60, and after day 61, SDBS was omitted from the feed. On days 62 and 63, MBAS concentration gradually increased and reached around 27 mg L^{-1} after 24hours. The final MBAS concentration decreased as the day without SDBS feeding went on, and on day 77, the final MBAS concentration went as low as 3 mg L^{-1}.

In the second additional experiment, the chemical components in the synthetic sewage with an initial SDBS concentration of 80 mg L^{-1} before and after treatment was analysed by TLC. The result was as shown in **Figure 4-6,** taken by a smartphone camera (iPhone 11, Apple Inc., California, America).



(a) (b)

Figure 4-6 TLC analysis result for conforming to the purity of the compound.
A: SDBS, B: Oleic Acid, C: synthetic sewage before treatment (SDBS concentration 80 mg L^{-1}), and D: synthetic sewage after treatment for 24 hours. (a) Original image of TLC visualised after staining with Rhodamine B under UV irradiation @254nm. (b) The white circle indicates the detected white spot in the picture '(a')

As shown in **Figure 4-6**, the position of the spot in the sample after treatment (D) was not identical to that of the sample before treatment (C). This means that MBAS in the effluent was not SDBS.

4.2 Discussions

The discussions are on two issues. First, in 4.2.1, the effect of SDBS on organic pollutant removal is discussed. Then, in 4.2.2, the removal of SDBS by ICOP is discussed.

4.2.1 Organic Pollutants Removal under Exposure to SDBS

There are two ways to evaluate the organic pollutants removal performance during the ICOP. The first is based on the difference between COD influent and COD effluent. The second way is to examine the rate of COD oxidation, which is represented by OCR.

The removal rate of COD per unit area of sponge during Run-1 was as shown in **Figure 4-1**. From the result of COD measurement, the removal rate per unit footprint area of a sponge was calculated as follows:

$$\text{COD removal rate } (\text{gO m}^{-2}\ \text{d}^{-1}) = \frac{\frac{\text{Volume x } (\text{COD}_{\text{influent}} - \text{COD}_{\text{effluent}})}{\text{A}}}{1000}$$

Where,

COD influent = the COD concentration before treatment 0 h (mg L^{-1})
COD effluent = the COD concentration after 24 h (mg L^{-1})
Volume = volume of synthetic sewage supplied (L d^{-1})
A = surface area of sponge (m^2)

The results of the calculation were as shown in **Figures 4-7**. A decreasing trend of COD removal was observed with an increase in feed SDBS concentration. The COD removal rate per day based on COD discharged were 13.5 g O m^{-2} d^{-1} without SDBS, 12.5 g O m^{-2} d^{-1} for 20 mg L^{-1} of SDBS, 12 g O m^{-2} d^{-1} for 40 and 60 mg L^{-1} of SDBS, and 9 g O m^{-2} d^{-1} for 80 mg L^{-1}. The decrease of COD removal rate was 7.5%, 13.5%, 12%, and 33.5% for the condition 20, 40, 60, and 80 mg L^{-1} of SDBS applied to compare with the condition without SDBS, respectively. Note that COD was measured only during Run-1.

The removal performance of COD can also be evaluated from OCR in the channel. As was explained in the previous section using **Figure 4-3**, OCR was negatively affected by 27% in Run-1 when SDBS concentration was increased to 60 mg L^{-1} and higher. In Run 2, when Welch's T-test was applied between days 78 to 89 (SDBS 20 mg L^{-1} and 40 mg L^{-1}) and days 90 to 104 (SDBS 60 mg L^{-1} and 80 mg L^{-1}), the difference was statistically significant ($p < 0.05$). These mean that SDBS negatively affected microbial activity to remove organic pollutant. However, in Run-2, the negative effect of SDBS up to 80 mg L^{-1} was not as apparent as that during Run 1.

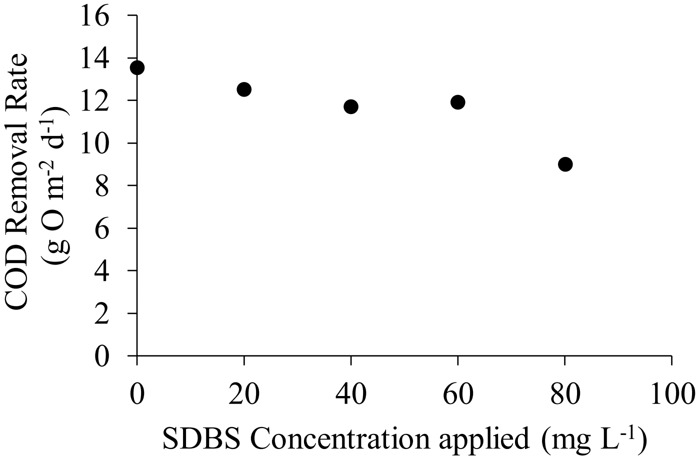


Figure 4-7 The COD removal rate estimated by COD influent and COD effluent from Run-1 and Run-2

Here, I review the negative impact of DBS on COD removal. Brandt et al. [80] and Flores et al. [81] speculated that decreased microbial activity was probably related to damage of microbial cells caused by the direct interaction between DBS and microorganisms. Flores et al. [81] evaluated the LAS concentration ranging 0.16, 0.8, 1.6, 16, and 160 mg L^{-}1of LAS. Yin et al. [82] reported that the toxicity of DBS caused by reduced surface tension on the cells could inhibit microbial activity with the concentration applied 0, 10, 50, 100, and 200 mg L^{-1}. The decline of the cellular membrane (due to the adsorption of surfactants on it) results in its malfunction; substrates cannot enter the cell, and toxic substances inside cells, if any, cannot be removed from the cell. Both cases cause cellular decay.

Foaming was observed when 80 mg L^{-1} SDBS was applied. While DBS can directly affect microbial activities, as discussed in the previous paragraph, a high concentration of surfactants causing foams can affect microbial activity in other ways. The formation of foaming has been claimed to reduce oxygen transfer, thus reducing aerobic microbial activity. In a bioreactor with suspended biomass, biomass concentration is reduced as biomass is accumulated in foam [66], [68]. Furthermore, the adsorption and accumulation of anionic surfactant in activated sludge flocs has been reported to inhibit some enzymes in AS [67]. However, in the present study, the negative effect of forming was not observed. Foaming in my reactor was ceased within several hours, and after 24 h the foam was mostly removed.

In general, the effect of SDBS up to 80 mg L^{-1} on the COD removal was limited, and after microorganisms are acclimatised, the effect became much less. It means that the ICOP can show its COD removal performance under the existence of SDBS at least up to 80 mg L^{-1} (74.7 mg L^{-1} as DBS).

4.2.2 Removal of DBS

Findings of the MBAS removal (**Figure 4-4**) showed that the trend of MBAS concentration in each condition applied was similar. The MBAS removal rate remained less, ranging from 17 – 32% in a day operation. Interestingly, MBAS concentration decreased in the initial 8 hours in the range of 17 – 27%, and then the removal was almost stopped. Note that MBAS concentration is not identical to SDBS concentration. In freshly prepared synthetic feed, all MBAS is SDBS. However, during treatment, SDBS (or DBS) may be degraded and converted into an intermediate degradation product which is still an anionic surfactant. In addition, studies are reporting the generation of biosurfactants by microorganisms. Thus, MBAS detected might contain degradation products from DBS and biosurfactants [83].

The generation of biosurfactant was studied, as shown in **Figure 4-5**. The concentration of MBAS after 24-hour treatment was as high as 27 mg L^{-1} on days 62 and 63 but later decreased to around 3 mg L^{-1}. While the 24-hour concentration (27 mg L^{-1}) observed on days 62 and 63 were high enough to explain the poor removal of MBAS shown in **Figure 4-4**, it may also be possible that the increase of MBAS on days 62 and 63 was coming from MBAS originated from DBS which had been fed to the reactor until day 60. That is, DBS or its partly degraded product was absorbed on sponge and biomass in sponge and was slowly leaked into water.

Therefore, the TLC analysis was conducted to examine the identity of MBAS after 24 h treatment (**Figure 4-6**). Findings suggested that the position of the white spots found in the synthetic wastewater samples before and after treatment were not identical. In addition, the after-treatment sample showed smear spots. It means that the compounds detected were not SDBS. It was converted to other forms by microbial activity.

To find out more about the presence of anionic surfactants, the spots found on the TLC plate were recovered and analysed using MBAS. The white spot from the sample after treatment before spraying with Rhodamine B was recovered into three parts (upper, middle, and lower parts). After that, 1.5 mL (2:1 v/v) of chloroform and methanol was added and mixed until it evaporated. After the chloroform and methanol had evaporated, 1 mL of ultrapure water was added to the sample. Finally, MBAS was analysed by the method described in section 3.2, point 2. The results showed that the treated water after 24 h contained anionic surfactant with the concentration 6 mg L^{-1}, 6.7 mg L^{-1} and 2 mg L^{-1} for upper, middle, and lower parts, respectively. This experiment was not done in quantitative way, but the results support that chemical in the white smear spots from the sample after treatment are MBAS other than DBS.

Overall, the DBS removal using the ICOP was less, but the remained surfactants after 24 h treatment was not fully DBS. There is a possibility of surfactant produced by microbes and DBS that was converted to other forms. The specific composition of the compound was not further focused on.

Chapter 5

CONCLUSIONS

5.1 Key Findings

The general objective of this study was to examine the effect of DBS on organic pollutants removal in ICOP for enhanced sewer self-purification. Findings are provided as follow:

The first objective was to examine the potential of the ICOP to remove organic pollutants exposure to DBS. Increasing DBS concentration in sewage decreased the ICOP performance. However, after microorganisms were acclimatised, the effect became much less. It means that the ICOP can show its COD removal performance under the existence of DBS at least up to 80 mg L^{-1} as SDBS.

The second objective was to examine the capability of ICOP for DBS removal. The removal of MBAS, not DBS, after 24-hour treatment remained less: 17 – 32%. However, generation of surfactant by microorganisms was observed, and the identity of MBAS after 24 h treatment was found not to be SDBS. Thus, although the removal of MBAS was not so high, SDBS was indeed degraded by ICOP.

5.2 Limitations and suggestions

Limitations and suggestions of this study are described as follows.

In my study, the DBS concentration in the synthetic sewage was the same every day during each condition, and a day with the same synthetic sewage, MBAS concentration was almost the same. However, in the actual situation, anionic surfactant concentration in the sewage will fluctuate over time.

For the final discussion, the present study could not investigate the degradation pathway of DBS. However, understanding this mechanism would be an interesting target for the future study.

ICOP shows promising performance as a technology for enhanced in sewer self-purification as a pre-treatment to reduce the organic pollutants load to an end-of-pipe treatment plant. Hence, the possibility of ICOP applied in the sewer pipe should be further studied.

REFERENCES

[1] M. A. Warith, K. Kennedy, and R. Reitsma, "Use of sanitary sewers as wastewater pre-treatment systems," *Waste Management*, vol. 18, no. 4, pp. 235–247, 1998, doi: 10.1016/S0956-053X(98)00023-3.

[2] I. W. Marjaka, K. Miyanaga, K. Hori, Y. Tanji, and H. Unno, "Augmentation of self-purification capacity of sewer pipe by immobilising microbes on the pipe surface," *Biochemical Engineering Journal*, vol. 15, no. 1, pp. 69–75, 2003, doi: 10.1016/S1369-703X(02)00182-1.

[3] T. Hvitved-Jacobsen, J. Vollertsen, and J. S. Matos, "The sewer as a bioreactor - A dry weather approach," *Water Science and Technology*, vol. 45, no. 3, pp. 11–24, 2002, doi: 10.2166/wst.2002.0044.

[4] Y. Tanji, R. Sakai, K. Miyanaga, and H. Unno, "Estimation of the self-purification capacity of biofilm formed in domestic sewer pipes," *Biochemical Engineering Journal*, vol. 31, no. 1, pp. 96–101, 2006, doi: 10.1016/j.bej.2006.05.021.

[5] T. J. Sotelo and H. Satoh, "Enhanced sewer self-purification with porous media: Performance evaluation with various organic loading rates and flow intermittency," *Biochemical Engineering Journal*, vol. 168, p. 107932, 2021, doi: 10.1016/j.bej.2021.107932.

[6] 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," *Journal of Water and Environment Technology*, vol. 18, no. 3, pp. 166–174, 2020, doi: 10.2965/jwet.20-002.

[7] T. J. Sotelo and H. Satoh, "Accumulated Organic Matter Degradation and the Function of Porous Media during Enhanced Sewer Self-purification," *Journal of Water and Environment Technology*, vol. 18, no. 6, pp. 415–424, 2020, doi: 10.2965/JWET.20-060.

[8] T. J. Sotelo, H. Satoh, and T. Mino, "Lipid degradation behaviour for the in-sewer application of the intermittent contact oxidation process," *Journal of Water and Environment Technology*, vol. 16, no. 5, pp. 211–219, 2018, doi: 10.2965/jwet.18-009.

[9] 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," *Biochemical Engineering Journal*, vol. 149, no. May, pp. 1–7, 2019, doi: 10.1016/j.bej.2019.107254.

[10] 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,” *Biochemical Engineering Journal*, vol. 154, no. July 2019, 2020, doi: 10.1016/j.bej.2019.107430.

[11] M. Oteng-Peprah, M. A. Acheampong, and N. K. deVries, “Greywater Characteristics, Treatment Systems, Reuse Strategies and User Perception—a Review,” *Water, Air, and Soil Pollution*, vol. 229, no. 8, 2018, doi: 10.1007/s11270-018-3909-8.

[12] E. D. A. Do Couto, M. L. Calijuri, P. P. Assemany, A. D. F. Santiago, and I. D. C. Carvalho, “Greywater production in airports: Qualitative and quantitative assessment,” *Resources, Conservation and Recycling*, vol. 77, pp. 44–51, 2013, doi: 10.1016/j.resconrec.2013.05.004.

[13] M. Firdayati, A. Indiyani, M. Prihandrijanti, and R. Otterpohl, “Greywater in Indonesia: Characteristic and Treatment Systems,” *Jurnal Tehnik Lingkungan*, vol. 21, no. 2, pp. 98–114, 2015, doi: 10.5614/jtl.2015.21.2.1.

[14] O. R. Al-Jayyousi, “Greywater reuse: Towards sustainable water management,” *Desalination*, vol. 156, no. 1–3, pp. 181–192, 2003, doi: 10.1016/S0011-9164(03)00340-0.

[15] E. Eriksson, K. Auffarth, M. Henze, and A. Ledin, *Characteristics of grey wastewater*, vol. 4, no. 1. Elsevier, 2002, pp. 85–104.

[16] A. Y. Katukiza, M. Ronteltap, C. B. Niwagaba, F. Kansiime, and P. N. L. Lens, “Greywater characterisation and pollutant loads in an urban slum,” *International Journal of Environmental Science and Technology*, vol. 12, no. 2, pp. 423–436, 2015, doi: 10.1007/s13762-013-0451-5.

[17] N. M. Hanif, S. N. N. Adnan, M. T. Latif, Z. Zakaria, M. P. Abdullahand, and M. R. Othman, “The composition of surfactants in river water and its influence to the amount of surfactants in drinking water,” *World Applied Sciences Journal*, vol. 17, no. 8, pp. 970–975, 2012.

[18] A. Fachini, M. A. Mendes, I. Joekes, and M. N. Eberlin, “Oxidation of sodium dodecylbenzenesulfonate with chrysotile: on-line monitoring by membrane introduction mass spectrometry,” *Journal of Surfactants and Detergents*, vol. 10, no. 4, pp. 207–210, 2007, doi: 10.1007/s11743-007-1032-8.

[19] A. Wiel-Shafran, Z. Ronen, N. Weisbrod, E. Adar, and A. Gross, “Potential changes in soil properties following irrigation with surfactant-rich greywater,” *Ecological Engineering*, vol. 26, no. 4, pp. 348–354, 2006, doi: 10.1016/j.ecoleng.2005.12.008.

[20] A. W. Shafran, A. Gross, Z. Ronen, N. Weisbrod, and E. Adar, “Effects of surfactants originating from reuse of greywater on the capillary rise in the soil,” *Water Science and Technology*, vol. 52, no. 10–11, pp. 157–166, 2005, doi: 10.2166/wst.2005.0690.

[21] G. G. Ying, “Fate, behaviour and effects of surfactants and their degradation products in the environment,” *Environment International*, vol. 32, no. 3, pp. 417–431, 2006, doi: 10.1016/j.envint.2005.07.004.

[22] A. Klaus, “Solubility of triglycerides in water using an extended surfactant,” 2011.

[23] F. J. Beltrán, J. F. García-Araya, and P. M. Álvarez, “Sodium dodecylbenzenesulfonate removal from water and wastewater. 1. Kinetics of decomposition by ozonation,” *Industrial and Engineering Chemistry Research*, vol. 39, no. 7, pp. 2214–2220, 2000, doi: 10.1021/ie990721a.

[24] F. Bergfeld *et al.*, “Final Report of the Cosmetic Ingredient Review Expert Panel - Amended Safety Assessment of Dodecylbenzenesulfonate, Decylbenzenesulfonate, and Tridecylbenzenesulfonate Salts as Used In Cosmetics,” 2009.

[25] M. Nazari and B. Ayati, “ARTICLE ORIGINAL Removing Sodium Dodecyl Benzene Sulfonate Using a Hybrid Electrocoagulation / Flotation and Photocatalytic System,” vol. 4, no. 3, pp. 236–243, 2019, doi: 10.22090/jwent.2019.03.006.

[26] D. Paola and O. Gonzalez, *by*. 2009.

[27] A. K. Asok *et al.*, *Surfactants: Chemistry, Toxicity and Remediation Authors Sharrel Rebello , Chapter 5 Surfactants : Chemistry , Toxicity and Remediation*, no. January 2013. 2014.

[28] E. Olkowska, M. Ruman, and Z. Polkowska, “Occurrence of surface-active agents in the environment,” *Journal of Analytical Methods in Chemistry*, vol. 2014, 2014, doi: 10.1155/2014/769708.

[29] J. Salager, Louis, “SURFACTANTS Types and Uses,” vol. 2, 2002.

[30] M. T. Garcia, E. Campos, M. Dalmau, P. Illán, and J. Sánchez-Leal, “Inhibition of biogas production by alkyl benzene sulfonates (LAS) in a screening test for anaerobic biodegradability,” *biodegradation*, vol. 17, no. 1, pp. 39–46, 2006, doi: 10.1007/s10532-005-2798-x.

[31] Z. Wang, J. Zhang, L. Song, E. Li, X. Wang, and B. Xiao, “Effects of linear alkylbenzene sulfonate on the growth and toxin production of *Microcystis aeruginosa* isolated from Lake Dianchi,” *Environmental Science and Pollution Research*, vol. 22, no. 7, pp. 5491–5499, 2015, doi: 10.1007/s11356-014-3784-9.

[32] Human and Environmental Risk Assessment, “Linear Alkylbenzene Sulphonate (CAS No. 68411-30-3),” no. 68411, pp. 1–101, 2013.

[33] M. Warne and R. van Dam, “NOEC and LOEC data should no longer be generated or used,” *Australasian Journal of Ecotoxicology*, vol. 14, no. 1, pp. 1–5, 2008.

[34] K. K. Brandt, M. Hesselsøe, P. Roslev, K. Henriksen, and J. Sørensen, “Toxic Effects of Linear Alkylbenzene Sulfonate on Metabolic Activity, Growth Rate, and Microcolony Formation of Nitrosomonas and Nitrosospira Strains,” *Applied and Environmental Microbiology*, vol. 67, no. 6, pp. 2489–2498, 2001, doi: 10.1128/AEM.67.6.2489-2498.2001.

[35] A. K. Mungray and P. Kumar, “Fate of linear alkylbenzene sulfonates in the environment: A review,” *International Biodeterioration and Biodegradation*, vol. 63, no. 8, pp. 981–987, 2009, doi: 10.1016/j.ibiod.2009.03.012.

[36] U. Merrettig-Bruns and E. Jelen, “Anaerobic biodegradation of detergent surfactants,” *Materials*, vol. 2, no. 1, pp. 181–206, 2009, doi: 10.3390/ma2010181.

[37] European Commission, “Regulamento nº648/2004,” *Official Journal of the European Communities*, vol. L 269, no. September 2000, pp. 1–15, 2000.

[38] R. A. Kimerle and R. D. Swisher, “Reduction of aquatic toxicity of linear alkylbenzene sulfonate (LAS) by biodegradation,” *Water Research*, vol. 11, no. 1, pp. 31–37, 1977, doi: 10.1016/0043-1354(77)90178-6.

[39] L. Cavalli, A. Gellera, and A. Landone, “LAS removal and biodegradation in a wastewater treatment plant,” *Environmental Toxicology and Chemistry*, vol. 12, no. 10, pp. 1777–1788, 1993, doi: 10.1002/etc.5620121004.

[40] M. J. Scott and M. N. Jones, “The biodegradation of surfactants in the environment,” *Biochimica et Biophysica Acta - Biomembranes*, vol. 1508, no. 1–2, pp. 235–251, 2000, doi: 10.1016/S0304-4157(00)00013-7.

[41] F. Haggensen, A. S. Mogensen, I. Angelidaki, and B. K. Ahring, “Anaerobic treatment of sludge: Focusing on reduction of LAS concentration in sludge,” *Water Science and Technology*, vol. 46, no. 10, pp. 159–165, 2002, doi: 10.2166/wst.2002.0318.

[42] A. K. Mungray and P. Kumar, “Occurrence of anionic surfactants in treated sewage: Risk assessment to an aquatic environment,” *Journal of Hazardous Materials*, vol. 160, no. 2–3, pp. 362–370, 2008, doi: 10.1016/j.jhazmat.2008.03.025.

[43] M. M. Aonyas *et al.*, “Degradation of anionic surfactants using the reactor based on dielectric barrier discharge,” *Journal of the Serbian Chemical Society*, vol. 81, no. 9, pp. 1097–1107, 2016, doi: 10.2298/JSC160303056A.

[44] J. L. Sanz, E. Culubret, J. De Ferrer, A. Moreno, and J. L. Berna, “Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in up-flow anaerobic sludge blanket (UASB) reactors,” *biodegradation*, vol. 14, no. 1, pp. 57–64, 2003, doi: 10.1023/A:1023557915653.

[45] V. Mezzanotte, F. Castiglioni, R. Todeschini, and M. Pavan, “Study on anaerobic and aerobic degradation of different non-ionic surfactants,” *Bioresource Technology*, vol. 87, no. 1, pp. 87–91, 2003, doi: 10.1016/S0960-8524(02)00211-0.

[46] K. Stamatelatou, C. Pakou, and G. Lyberatos, *Occurrence, Toxicity, and Biodegradation of Selected Emerging Priority Pollutants in Municipal Sewage Sludge*, Second Ed., vol. 6. Elsevier B.V., 2011.

[47] Japan Surfactant Industry Association, “Japan surfactant industry association. [internet]. Available from: https://jpsurfactant.jp/surfactant/history/ index. Html [Accessed on 2021 June 28].”

[48] Metcalf and Eddy, Inc., Tchobanoglous G, Burton F, Stensel HD. Wastewater Engineering: Treatment and Reuse. 4th Ed. New York: McGraw-Hill; 2004. p.186

[49] H. De Henau, E. Mathijs, and W. D. Hopping, “Linear Alkylbenzene Sulfonates (LAS) in Sewage Sludges, Soils and Sediments: Analytical Determination and Environmental Safety Considerations,” *International Journal of Environmental Analytical Chemistry*, vol. 26, no. 3–4, pp. 279–293, 1986, doi: 10.1080/03067318608077120.

[50] F. Li, K. Wichmann, and R. Otterpohl, “Review of the technological approaches for greywater treatment and reuses,” *Science of the Total Environment*, vol. 407, no. 11, pp. 3439–3449, 2009, doi: 10.1016/j.scitotenv.2009.02.004.

[51] M. Halalsheh, S. Dalahmeh, M. Sayed, W. Suleiman, M. Shareef, M. Mansour, and M. Safi., “Grey water characteristics and treatment options for rural areas in Jordan,” *Bioresource Technology*, vol. 99, no. 14, pp. 6635–6641, 2008, doi: 10.1016/j.biortech.2007.12.029.

[52] Y. Boyjoo, V. K. Pareek, and M. Ang, “A review of greywater characteristics and treatment processes,” *Water Science and Technology*, vol. 67, no. 7, pp. 1403–1424, 2013, doi: 10.2166/wst.2013.675.

[53] A. Ferradj and M. Idouhar, "Determination of anionic surfactants in a wastewater treatment plant in Algiers City," *Desalination and Water Treatment*, vol. 57, no. 53, pp. 25677–25685, 2016, doi: 10.1080/19443994.2016.1157038.

[54] M. I. Bautista-Toledo, J. Rivera-Utrilla, J. D. Méndez-Díaz, M. Sánchez-Polo, and F. Carrasco-Marín, "Removal of the surfactant sodium dodecylbenzenesulfonate from water by processes based on adsorption/bioadsorption and biodegradation," *Journal of Colloid and Interface Science*, vol. 418, pp. 113–119, 2014, doi: 10.1016/j.jcis.2013.12.001.

[55] P. A. Lara-Martín, A. Gómez-Parra, T. Köchling, J. L. Sanz, R. Amils, and E. González-Mazo, "Anaerobic degradation of linear alkylbenzene sulfonates in coastal marine sediments," *Environmental Science and Technology*, vol. 41, no. 10, pp. 3573–3579, 2007, doi: 10.1021/es062372z.

[56] E. Olkowska, Z. Polkowska, and J. Namieśnik, "Analytics of surfactants in the environment: Problems and challenges," *Chemical Reviews*, vol. 111, no. 9, pp. 5667–5700, 2011, doi: 10.1021/cr100107g.

[57] J. Waters and T. C. J. Feijte, "AIS+/CESIO+ Environmental surfactant monitoring programme: Outcome of five national pilot studies on linear alkylbenzene sulphonate (LAS)," *Chemosphere*, vol. 30, no. 10, pp. 1939–1956, 1995, doi: 10.1016/0045-6535(95)00074-I.

[58] T. C. J. Feijtel, E. Matthijs, A. Rottiers, G. B. J. Rijs, A. Kiewiet, and A. de Nijs, "AIS/CESIO environmental surfactant monitoring programme. Part 1: LAS monitoring study in 'de Meern' sewage treatment plant and receiving river 'Leidsche Rijn,'" *Chemosphere*, vol. 30, no. 6, pp. 1053–1066, 1995, doi: 10.1016/0045-6535(95)00003-Q.

[59] D. C. Mcavoy, S. D. Dyer, N. J. Fendinger, W. S. Eckhoff, D. L. Lawrence, and W. M. Begley, "Removal of alcohol ethoxylates, alkyl ethoxylate sulfates, and linear alkylbenzene sulfonates in wastewater treatment," *Environmental Toxicology and Chemistry*, vol. 17, no. 9, pp. 1705–1711, 1998, doi: 10.1002/etc.5620170909.

[60] A. Marcomini, G. Pojana, A. Sfriso, and J. M. Quiroga Alonso, "Behavior of anionic and nonionic surfactants and their persistent metabolites in the Venice Lagoon, Italy," *Environmental Toxicology and Chemistry*, vol. 19, no. 8, pp. 2000–2007, 2000, doi: 10.1002/etc.5620190807.

[61] A. K. Mungray and P. Kumar, "Anionic surfactants in treated sewage and sludges: Risk assessment to aquatic and terrestrial environments," *Bioresource Technology*, vol. 99, no. 8, pp. 2919–2929, 2008, doi: 10.1016/j.biortech.2007.06.025.

[62] E. Liwarska-Bizukojc and M. Bizukojc, “Digital image analysis to estimate the influence of sodium dodecyl sulphate on activated sludge flocs,” *Process Biochemistry*, vol. 40, no. 6, pp. 2067–2072, 2005, doi: 10.1016/j.procbio.2004.07.020.

[63] H. Li, S. Wu, and C. Yang, “Performance and biomass characteristics of SBRs treating high-salinity wastewater at the presence of anionic surfactants,” *International Journal of Environmental Research and Public Health*, vol. 17, no. 8, 2020, doi: 10.3390/ijerph17082689.

[64] R. Pujol, P. Duchene, S. Schetrite, and J. P. Canler, “Biological foams in activated sludge plants: Characterisation and situation,” *Water Research*, vol. 25, no. 11, pp. 1399–1404, 1991, doi: 10.1016/0043-1354(91)90118-A.

[65] F. Guo, Z. P. Wang, K. Yu, and T. Zhang, “Detailed investigation of the microbial community in foaming activated sludge reveals novel foam formers,” *Scientific Reports*, vol. 5, no. January 2015, doi: 10.1038/srep07637.

[66] M. C. Collivignarelli *et al.*, “Foams in wastewater treatment plants: From causes to control methods,” *Applied Sciences (Switzerland)*, vol. 10, no. 8, 2020, doi: 10.3390/APP10082716.

[67] R. Tomczak-wandzel, A. Dereszewska, S. Cytawa, C. Faculty, W. Treatment, and P. Swarzewo, “The effect of surfactants on activated sludge process,” *Journal of Water Resources Engineering*, vol. 5, no. 2, pp. 1–8, 2009.

[68] M. Fryer, E. O’Flaherty, and N. F. Gray, “Evaluating the measurement of activated sludge foam potential,” *Water (Switzerland)*, vol. 3, no. 1, pp. 424–444, 2011, doi: 10.3390/w3010424.

[69] K. K. Brandt, N. O. G. Jørgensen, T. H. Nielsen, and A. Winding, “Microbial community-level toxicity testing of linear alkylbenzene sulfonates in aquatic microcosms,” *FEMS Microbiology Ecology*, vol. 49, no. 2, pp. 229–241, 2004, doi: 10.1016/j.femsec.2004.03.006.

[70] M. Green, G. Shelef, and A. Messing, “Using the sewerage system main conduits for biological treatment. Greater Tel-Aviv as a conceptual model,” *Water Research*, vol. 19, no. 8, pp. 1023–1028, 1985, doi: 10.1016/0043-1354(85)90371-9.

[71] L. Coulibaly, S. N. Agathos, and G. Gourène, “Enhancement of domestic wastewater treatment under long sewer line condition in a laboratory set-up by Aspergillus niger bioaugmentation,” *Journal of Water and Environment Technology*, vol. 4, no. 1, pp. 1–7, 2006, doi: 10.2965/jwet.2006.1.

[72] S. Shahvi, E. Orsi, R. Canziani, E. Larcan, and G. Becciu, "Aerobic transformations of organic matters in sewer wastewater, a case study of Milan, Italy," *Urban Water Journal*, vol. 14, no. 10, pp. 1058–1064, 2017, doi: 10.1080/1573062X.2017.1363246.

[73] E. Matthijs, "The Fate of Detergent Surfactants in Sewer System," pp. 321–328, 2005.

[74] 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," *Journal of Water and Environment Technology*, vol. 13, no. 6, pp. 427–439, 2015, doi: 10.2965/jwet.2015.427.

[75] T. J. Sotelo, H. Satoh, and T. Mino, "Assessing wastewater management in the developing countries of Southeast Asia: Underlining flexibility inappropriateness," *Journal of Water and Environment Technology*, vol. 17, no. 5, pp. 287–301, 2019, doi: 10.2965/jwet.19-006.

[76] G. J. F. Smolders, J. Van Der Meij, M. C. M. Van Loosdrecht, and J. J. Heijnen, "Model of the Anaerobic Metabolism of the Biological Phosphorus Removal Process: Stoichiometry and pH Influence," 1993.

[77] N. Kishimoto and M. Okumura, "Feasibility of mercury-free chemical oxygen demand (COD) test with excessive addition of silver sulfate," *Journal of Water and Environment Technology*, vol. 16, no. 6, pp. 221–232, 2018, doi: 10.2965/jwet.18-016.

[78] E. Jurado, M. Fernández-Serrano, J. Núñez-Olea, G. Luzón, and M. Lechuga, "Simplified spectrophotometric method using methylene blue for determining anionic surfactants: Applications to the study of primary biodegradation in aerobic screening tests," *Chemosphere*, vol. 65, no. 2, pp. 278–285, 2006, doi: 10.1016/j.chemosphere.2006.02.044.

[79] P. A. V. Fernandes, I. R. De Arruda, A. F. A. B. Dos Santos, A. A. De Araújo, A. M. S. Maior, and E. A. Ximenes, "Antimicrobial activity of surfactants produced by Bacillus subtilis R14 against multidrug-resistant bacteria," *Brazilian Journal of Microbiology*, vol. 38, no. 4, pp. 704–709, 2007, doi: 10.1590/S1517-83822007000400022.

[80] K. K. Brandt, A. Pedersen, and J. Sørensen, "Solid-phase contact assay that uses a lux-marked *Nitrosomonas europaea* reporter strain to estimate the toxicity of bioavailable linear alkylbenzene sulfonate in soil," *Applied and Environmental Microbiology*, vol. 68, no. 7, pp. 3502–3508, 2002, doi: 10.1128/AEM.68.7.3502-3508.2002.

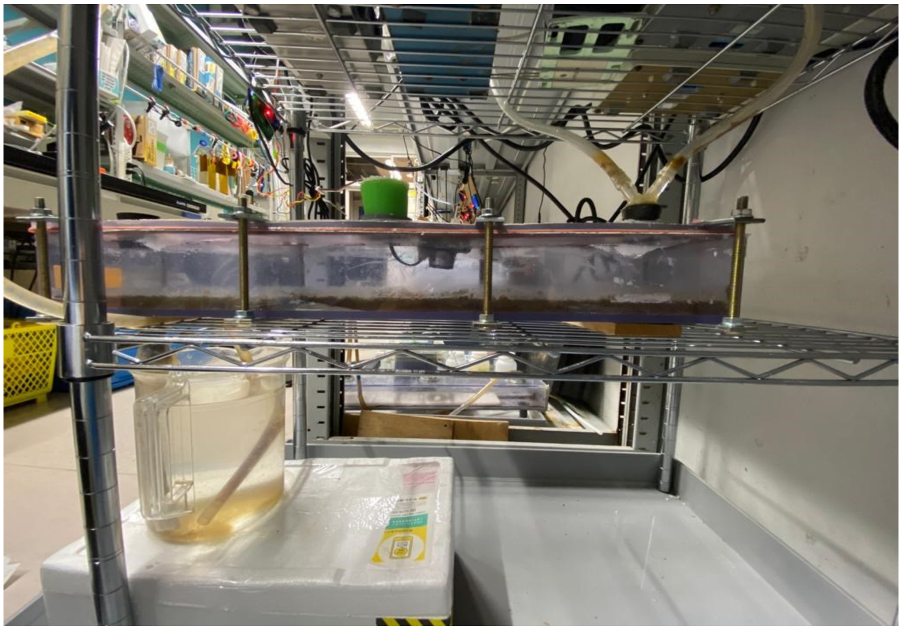
[81] P. Flores *et al.*, “Toxic effects of Linear Alkylbenzene Sulfonate, Athracene and Their Mixture on Growth of Microbial Consortium Isolated from Polluted Sediment,” *Rev. Int. Contam. Ambient*, vol. 26, pp. 39–46, 2010.

[82] C. Yin, Y. Li, T. Zhang, J. Liu, Y. Yuan, and M. Huang, “Effects of exposure to anionic surfactants (SDBS and SDS) on nitrogen removal of aerobic denitrifier,” *Water Environment Research*, vol. 92, no. 12, pp. 2129–2139, 2020, doi: 10.1002/wer.1384.

[83] P. G. Deo, N. K. Veenanadig, and N.G.K. Karanth, “Microbial production of biosurfactants and their importance,” *Studies in Environmental Science*, vol. 66, no. C, pp. 179–195, 1997, doi: 10.1016/S0166-1116(97)80043-X.

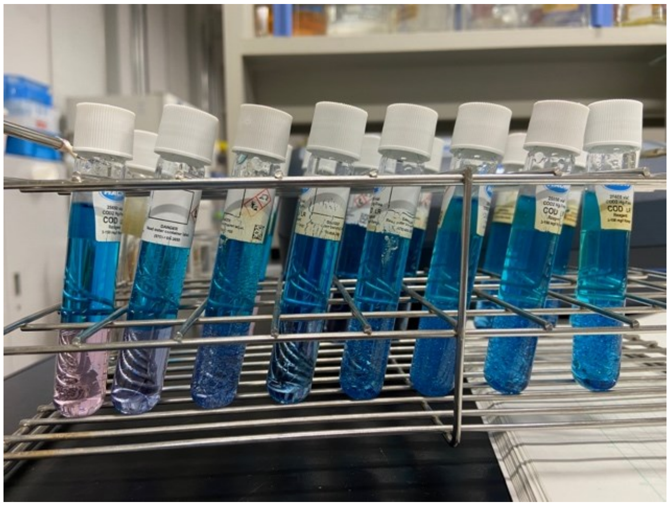
APPENDIX

1. Documentations



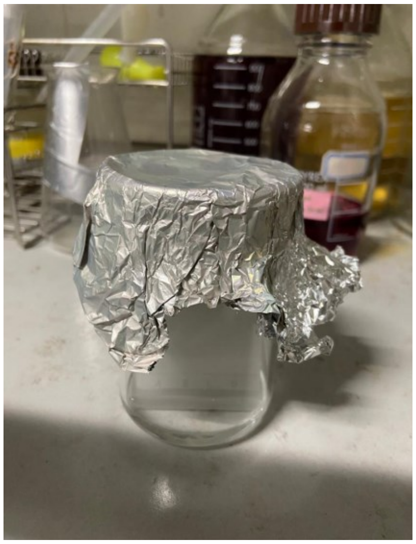
Airtight reactor arrangement

COD test: After 2h heating



MBAS test: After adding chloroform and mixing (No. 1-3 from the left side are the standard solution)


Lyophilised processes


TLC test

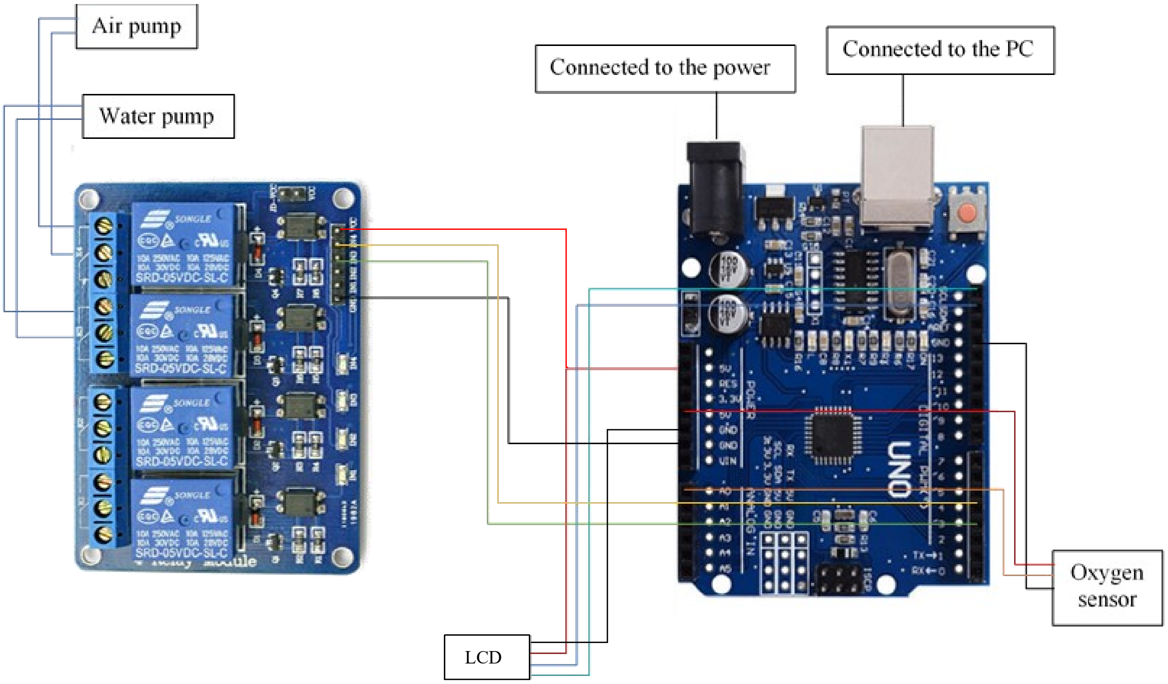
2. Arduino’s Code

```
#include <Wire.h>
#include <TimeLib.h>
#include <LiquidCrystal_I2C.h>

LiquidCrystal_I2C lcd(0x27, 20, 4); // set the LCD address to 0x27 for a 16 chars and 2 line
display

int airpump = 4;
int recpump = 3;
int airpumpend = 5; //air pump is operated for 5 min at the beginning of an aeration cycle
int airpumpcycle = 360; //duration of an aeration cycle
int recpumpon = 5; // recirculation pump is operated for 5 min at the beginning of a
recirculation cycle
int reccycle = 30; //duration of an recirculation cycle
int relayairpump;
int relayrecpump;
int relay4;

void setup() {
  // initialize serial communication at 9600 bits per second:
  Serial.begin (9600);
  pinMode (airpump, OUTPUT);
  pinMode (recpump, OUTPUT);
  relayairpump = 0;
  relayrecpump = 0;
```

```
  relay4 = 0;
  digitalWrite(airpump, HIGH);
  digitalWrite(recpump, HIGH);
  analogReference(INTERNAL);
  setTime(0, 0, 0, 1, 1, 2021);  //set time
  lcd.init();                      // initialize the lcd
  lcd.init();
  // Print a message to the LCD.
  lcd.backlight();
}

// 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)

  if (elapsedTime % airpumpcycle < airpumpend) {
    digitalWrite(airpump, LOW);  //air pump is turned on.
    digitalWrite(recpump, HIGH);  //air pump is turned off.
    relayairpump = 1;
    relayrecpump = 0;
  }
  else {
    digitalWrite(airpump, HIGH);  //air pump is turned off.
    relayairpump = 0;
    if ((elapsedTime - airpumpend) % reccycle < recpumpon) {
      digitalWrite(recpump, LOW);  //recirculation pump is turned on.
      relayrecpump = 1;
    }
    else {
      digitalWrite(recpump, HIGH);  //recirculation pump is turned off.
      relayrecpump = 0;
    }
  }

/*
 *  Once a minute, the operation status and the detected O2 value are reported to PC.
 *  This is done by the following lines.
 */

  if (second() == 2) {
    int sum = 0;
    for (int i = 0; i < 10; i++) {
      sum += Measure();
```

```
      delay(10);
    }
    int sensorValue = sum / 10;
    Serial.print(elapsedTime);
    Serial.print("\t");
    Serial.print("AP");
    Serial.print(relayairpump);
    Serial.print("\tWP");
    Serial.print(relayrecpump);
    Serial.print("\t");
    Serial.println(sensorValue);
    delay(800);
  }

/*
 *  Time displayed in LCD is updated every around 0.2sec.
 */

  lcd.setCursor(0, 0);
  lcd.print(modifyDigits(hour()));
  lcd.print(":");
  lcd.print(modifyDigits(minute()));
  lcd.print(":");
  lcd.print(modifyDigits(second()));

  delay(200);
}

int Measure() {
  int result;
  result = analogRead(A0);
  return result;
}

String modifyDigits(int digits) {
  // utility function for digital clock display: prints preceding colon and leading 0
  String result = "";
  if (digits < 10)
    result += "0";
  result += digits;
  return result;
}
```

3. Arduino arrangements



Sources:
https://fut688.en.made-in-china.com/product/NCnQsABcLbkX/China-Factory-Outlet-CH340g-Chip-Improved-Version-Uno-R3-for-Arduino-with-Cable-Without-Logo.html
http://arduinolearning.com/code/5-volt-4-channel-arduino-relay-module-example.php