FACULTY OF SCIENCE AND TECHNOLOGY

BACHELOR'S THESIS

Study programme / specialisation: Biological Chemistry/Water Science and Technology	The spring semester, 2023 Open
Author: Shanjai Sivanesan	
Supervisor at UiS: Ilke Pala-Ozkok External supervisor(s):	
Thesis title: Respirometric Assessment of the Chronic impact of Chloroxylenol on a Moving Bed Biofilm Reactor (MBBR)	
Credits (ECTS): 20	
Keywords: Activated Sludge, Chloroxylenol, Disinfectant, MBBR, Respirometry-Wastewater Treatment	Pages: 39 Stavanger, 15/05/23

Abstract

Chloroxylenol (CHL) is a compound we find in disinfectant products such as soap to clean the skin and other surfaces, and as a result, a large amount of CHL is discharged into the wastewater treatment plants (WWTPs). To investigate the effect of CHL on the microorganisms in biological WWTPs, four tests were conducted by using sludge and carriers from the control reactor, namely controls tests with and without nitrogen inhibitor and acute CHL tests with and without nitrogen inhibitor, in which the sludge was exposed to CHL for the first time. Additionally, chronic tests with and without nitrogen inhibitor were conducted using the sludge and carriers from the chronic reactor which was exposed for CHL for 43 days. Respirometry test were conducted to measure the respiration rate of the culture in WWTPs and in that way get a knowledge of how CHL affect substrate removal and biomass growth. Nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and chemical oxygen demand (COD) values were measured regularly to control the behaviour of the microorganisms in the WWTPs. We discovered CHL did not show any sign of COD removal inhibition. However, oxygen utilization rate (OUR) measurement showed microorganisms used more oxygen for growth in the presence of CHL. Chronic test showed a higher oxygen consumption by the microorganisms than acute test which can be acclimation due to time with the presence of CHL. The control reactor operations showed high efficiency in COD and NH_4-N removals. The effluent concentrations in the chronic reactor showed a fluctuating trend.

Acknowledgments

I would like to acknowledge and express my thanks to my supervisor Ilke Pala Ozkok for giving me the opportunity to work on this thesis and helping me during my project with the lab work and the writing. I want to thank you for always taking the time to answer my question and motivating me during stressful times in the lab.

I wish to thank Mohammadreza Ghadirian for helping me with the experimental work in the Laboratory. It has been a true pleasure to work with you in the lab.

Finally, I want to thank my family for supporting me and motivating me through my bachelor´s degree.

List of Abbreviations

bCOD	Biodegradable COD
CHL	Chloroxylenol
CO	Consumed Oxygen
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
IVAR SNJ	IVAR Sentralrenseannlegg Nord-Jæren
MBBRs	Moving Bed Biofilm Reactors
OUR	Oxygen Utilization Rate
OUR end	Endogenous Oxygen Utilization Rate Test
ROS	Reactive Oxygen Species
SolA	Solution A
SOUR	Specific OUR
SRT	Solid Retention Time
SS	Suspended Solids
TSS	Total Suspended Solids
U	COD Utilization Rate
VSS	Volatile Suspended Solids
WWTPs	Wastewater Treatment Plants
q	Specific U

List of Figures

List of Tables

1. Introduction

Disinfectants have been used by people for many centuries in different forms. Egyptians were known for using wine and vinegar as a disinfectant. Later in the history mercury and sulphur were used as disinfectant to detoxify and purify the space. Over the years wine, vinegar, sulphur, and mercury have been replaced by other various chemicals to clean things, including Chloroxylenol (CHL). The idea of creating various disinfectant started a few years after the discovery of the "invisible" microorganism and when people realised that these microorganisms could cause diverse disease. That was the beginning of using chemicals like CHL as a disinfectants[1].

Nowadays disinfectants are associated with the covid- 19 pandemic as the use of disinfectants globally changed extremally during the covid-19 pandemic where people went from not using disinfectant product often to rapidly use it to reduce the probability of getting COVID-19. It has been reported that only in Wuhan in kina where COVID-19 was first discovered that 2000 tons of disinfectant was used. When disinfectant is used in large quantities as mentioned it may lead to adverse effect in the environment and the human health[2]. Disinfectants can enter our body in different form such as dermal absorption, inhalation and ingestion and may lead to asthma or other disease. On the other hand, the overuse of disinfectants will later give rise to wastewater that reaches wastewater treatment plants (WWTPs) and may affect the aquatic ecosystem and the organisms within, accumulation on vegetables, and contamination of the food chain due to sludge application[3]. Chemicals in disinfectants can also react with other chemicals in WWTPs and result with producing other toxic substances that can cause harm to the biomass in the WWTPs and effect their function[4].

Most of the research so far is focusing mainly on how we can use disinfectant to clean and kill microorganisms in the wastewater treatment system and then prevent virus/microorganism transmissions through wastewater but there has not been reported any scientific clue related to how disinfectants affect the beneficial microorganisms that are a part of the wastewater treatment system. Therefore, it is important to know the behaviour of disinfectants in wastewater and get knowledge of how disinfectants can influence the biomass used in the wastewater treatment system to treat the organic compounds.

2.Background

2.1 Biocides

Biocides can be defined as all types of chemicals and biological agents used to stop or control viruses, microorganisms, and fungi by performing chemical or biological action on them. Biocides contains the "active substance", which makes them capable of inhibiting or killing diverse pests as mentioned above[5]. Biocides can be divided into two groups based on if we want to stop or kill the pest. The first group is biostatic, which contain the active substance that inhibit pest growth and the second group is biocidal, which contain the active substance that kill the pest[6]. Some commonly used biocides are disinfectants, wood preservatives, and insect repellents[5].

This study specializes in a biocide of the type disinfectant named Chloroxylenol (CHL) and the possible effects of this disinfectant on the beneficial microorganisms used in wastewater treatment system to degrade organic content.

2.2 Disinfectants

Disinfectants, which can be defined as a compound used for cleanse surfaces by killing microorganisms is another source to contaminations of wastewater. Disinfectants kill microorganisms by destroying their cell walls or by disturbing their metabolisms. Disinfectants are used every day by individuals, in hospitals and dental surgeries[7]. However, it is more correctly to say that disinfectant kill most of the microbes, but it does not kill all the microbes. How fast disinfectants kill or inhibit the microbes varies from disinfectant to disinfectant, but a good disinfectant removes most of the microbes within 20 seconds. Disinfectants attack microorganisms or viruses in different ways. The four main ways are:

1. Nonpolar molecules from disinfectants may dissolve and become more soluble, which gives them the ability to enter inside the cell through the cell membrane.
2. Interaction with components from the transport system and enter inside the cell
3. Components on the disinfectants can interfere with membrane proteins and make them weak or denatured.
4. Disinfectant reacts with the microbe which result in inhibition on the normal metabolic processes[8].

2.2.1 Chloroxylenol as a Disinfectant

Chloroxylenol (CHL) is a halophenolic compound as can be seen in Figure 1 substituted with a methyl group, alcohol group and a chlorine group which is found in disinfectant products such as soap to clean the skin and other surfaces. Frequent use of such products will increase the concentration of disinfectant in aquatic environments and result with ecological risk. The molecular structure of CHL consists of an OH-group also known as an alcohol group, which reacts with cytoplasmatic membrane proteins in the cell membrane which will cause cell interruption and later bacteria death. The aforementioned effect of CHL is part of a larger mechanism that we have not completely understood yet despite its widespread use over the years[9]. Phenolic compounds like CHL are also toxic to cats and other animals since they can't digest it completely[10].

Previous research on CHL has given us the information on how CHL affects the freshwater, the organisms that live in the freshwater after the release from the wastewater treatment plants and how we can remove CHL from the wastewater treatment system but there has not been reported any effect of CHL on the beneficial biomass that is a part of the WWTPs[11]. In short, the research with CHL has not been reported any scientific clues related to kinetic response from the biomass in the wastewater treatment plants or the impact on the biomass.

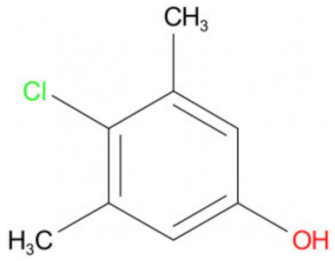

Figure 1. Molecular structure of Chloroxylenol[12].

2.2.2 Impact of Chloroxylenol in Natural Waters

Aquatic pollution is a well-known global problem we find all over the world and are mostly caused by urbanization and discharge of xenobiotics and anthropogenetic pollutants such as disinfectant (CHL) due to human activity. It has been reported that disinfectants and other xenobiotic pollutants have found their way from the WWTPs to the fresh water[13]. It was for example reported in Jakarta in Indonesia that the concentration of CHL was around 60-1200

ng/L. It has also been reported CHL existents with different concentration in different countries. Previous research has reported CHL concentration in surface water of south Wales, UK and Japanese river and the values were around 358ng/L and 17.8 ng/L, respectively[14].

When disinfectants find their way to the aquatic environment it may result in adverse effect for the organisms within. In aquatic environment we find high concentration of dissolved organic content, which allows the disinfectants to produce by-products such as haloacetic acids, which will decrease the pH in the aquatic environment and kill the organisms in the aquatic environment[15]. When wastes like disinfectants end up in natural waters and kill one organism it may lead to a domino effect and affect the entire food chain in the aquatic environment. An example is death of primary consumer like fish caused by the decreased level of pH as an effect of discharge of disinfectant waste also effect the secondary consumer like the sea birds that eats those fishes[16].

2.3 Wastewater

The term wastewater can be defined as contaminated water caused by human activities such as domestic, industrial, and commercial use. Wastewaters consist of 99.9% water and the 0.01% consist of content we want to remove, including organic compounds, microorganism, detergents, cleaning agents and inorganic compounds. The treated wastewater also known as effluent will at the end be released into diverse lakes, ponds, streams, rivers, and oceans and therefore discharge of untreated wastewater into the variety of environments can cause harm to the environment and the human health[17,18]. Treated wastewater can be reused for certain purposes such as agriculture[19]. As mentioned above we have different sources that contribute to wastewater:

- Domestic wastewater: Wastewater generated from non-industrial activities such as households, hotels, restaurants, schools, and shopping malls. We can separate domestic wastewater into grey and black water. Black water consists mainly of organic matter, microorganisms, and suspended matter. Grey water, which is generated from bathtubs, kitchen sinks etc. consist of the same content as black water but the grey water don't consist of urine, faeces, and food waste. When we are treating domestic wastewater, the goal is to remove most of the organic content and the nutrients and not recycle them[20,21].
- Industrial wastewater: Wastewater generated from water used in industrial activities and different industrial activities contributes with different contaminants and substances to

the wastewater which gives the industrial wastewater different quality. Industrial wastewater consist mainly of organic synthetic substances and heavy metals[22].

- Commercial wastewater: Wastewater generated from non- domestic sources. Some examples are beauty salon, instrument cleaning or furniture refinishing[23].

As described, the different types of wastewaters contain different compounds which means the different types of wastewaters will affect the environment in different ways as you can see in Table 2.1.

Table 2.1. Wastewater compounds and the following effects/outcome[24].

Compound	Example	Outcome
Microorganisms	Pathogenic bacteria, virus, and worms eggs	Risk when bathing and eating shellfish
Biodegradable organic materials	Oxygen depletion in rivers, lakes, and fjords	Fish death, odours
Other organic materials	Detergents, pesticides, fat, oil and grease, colouring, solvents, phenols, cyanide	Toxic effect, aesthetic inconveniences, bio accumulation in the food chain
Nutrients	Nitrogen, phosphorus, ammonium	Eutrophication, oxygen depletion, toxic effect
Metals	Hg, Pd, Cd, Cr, Cu, Ni	Toxic effect, bioaccumulation
Other inorganic materials	Acids, for example hydrogen sulphide, bases	Corrosion, toxic effect
Thermal effects	Hot water	Changing living conditions for flora and fauna.
Odour (and taste)	Hydrogen sulphide	Aesthetic inconveniences, toxic effect
Radioactivity		Toxic effect, accumulation

2.3.1. Wastewater Treatment Plants

A wastewater treatment plant receives influent wastewater and converts it to effluent wastewater by performing various treatments on the influent wastewater. That means WWTPs remove waste, grease, floating oil, and sand from the wasted water, and the result is effluent water, also known as treated water. In this way, we can remove organic and inorganic materials from the wastewater and prevent this from entering the environment. The primary purpose of WWTPs is to minimize water pollution and reuse the waste that originates from the WWTPs. WWTPs treat the water in four stages, which are preliminary treatment, primary treatment, secondary treatment, and tertiary treatment[25].

The first stage of WWTPs is preliminary treatment, also known as pre-treatment. This stage involves preparing the water for purification by the other phases by removing big objects such as sticks and grits that can damage the WWTPs or the other equipments used during the cleaning process. The next stage is called primary treatment. In this stage, suspended solids are separated and removed from the water by holding off the water for 1-2 hours in a decanter centrifuge, where we use the power of gravity to separate the particles from the water. In secondary treatment are organic matter, nitrogen, phosphorus, and other nutrients separated and removed from the water by performing biological action in the water. Mainly bacteria and microorganisms are used to utilize and degrade the organic content and the nutrients in the water. One of the most used treatments are activated sludge, where the water is treated in a tank with bacteria and different oxygen condition determent by the removal requirement. The organic content and nutrients in the water act as food for the bacteria in the tank, which means the bacteria will consume and degrade the organic content and the nutrients in the water. The last step is tertiary treatment, also known as the chemical treatment, where the goal is to purify the water as much as possible so it can be released into the environment or reused for human activities. This is performed by removing all pathogen and bacteria by for example using filtration with sand beds and disinfectant[26].

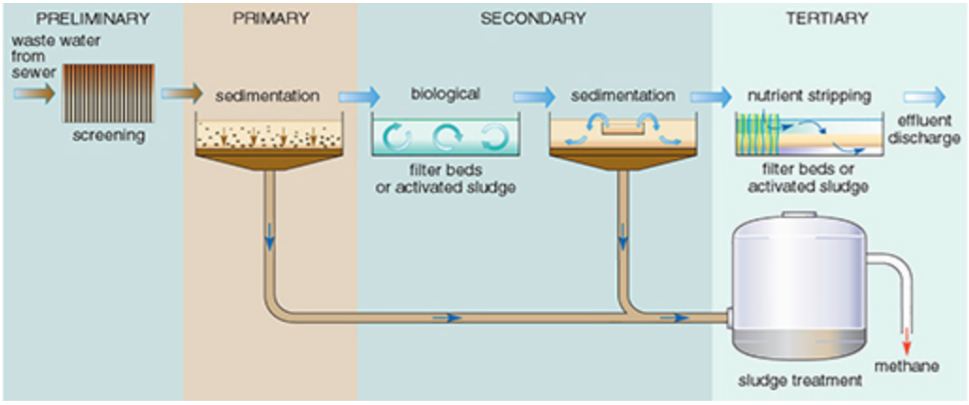

Figure 2. Diagram of different stages of wastewater treatment system[27].

2.3.2 Disinfectants in Wastewater Treatment Systems

It is essential to separate disinfectant as a cleaning agent in wastewater treatment and disinfectant entering the wastewater treatment with the influent. During the covid 19 pandemic, it has been reported an excessive increment of disinfectant contamination in the influent of wastewater treatment plant as a result of the increased use of disinfectant as mentioned earlier[28]. Disinfectant has also been used as a tertiary treatment in wastewater treatment to purify the water, as mentioned earlier. Compounds with calcium hypochlorite, sodium hypochlorite, chlorine gas, and chlorine dioxide has been used as disinfectants. Recently, disinfectant products with chlorine have been replaced by ozone and ultraviolet light due to an increase of disinfectant by- products in the effluent[29].

2.3.3 Impact of Disinfectant on Wastewater Treatment Systems and the Sludge

It has been reported that disinfectant with hypochlorite could inhibit respiration rate and the activated sludge´s functional enzyme activity and result in reduced COD removal efficiency. It has also been reported production of intracellular reactive oxygen species (ROS) in the sludge that has been exposed to disinfectant with hypochlorite[30].

2.4 Activated Sludge System

Primary treatment alone is not able to separate or degrade the organic content in the wastewater. Nowadays, it is common to use a biological treatment like an activated sludge system in secondary treatment to degrade the organic and inorganic content in wastewater. In biological treatment, microorganisms are placed with the wastewater in an aerated tank. Wastewater is then mixed with the pumping air and the microorganism. That increases the connection between the wastewater and the microorganisms and allows the oxidation of the organic content in

wastewater, resulting in production of water, carbon dioxide and new cells as described in Figure 3. Finally, the sludge particles can be separated and removed by gravity settling. The treated water is then released to the environment or for further treatment, and the settled activated sludge is then led back to the aeration tank, as shown in Figure 4. It is essential to provide enough air/oxygen to satisfy the microorganism, to keep the solids in suspension and increase the connection between the microorganisms and the wastewater. Activated sludge system exists in different configuration with their own benefits, and disbenefits and which configuration one should use depends on the different processes[31–33].

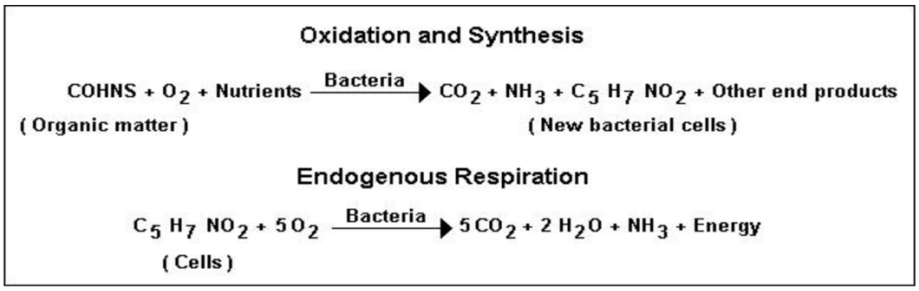

Figure 3. Reaction equation of the process in activated sludge system[34].

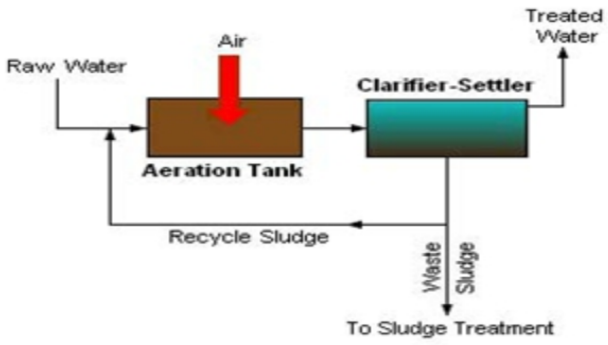

Figure 4. Illustration of activated sludge system[33].

2.4.1 Suspended Growth Systems

An activated sludge system is a type of suspended growth system where naturally occurring microorganisms in the surface water are kept suspended in the wastewater, resulting in bacterial growth and purification of the wastewater. In suspended growth systems microorganisms use organic matter as nutrients and break them into smaller/simpler components. They also convert other components into gases and cell tissue, as mentioned earlier. When the water is purified or ready for further treatment, a purification device separates the microorganism/sludge from the purified water, and then some of the sludge is resent back to the aeration tank where the wastewater purification takes place[35,36]. The suspended growth process may occur under aerobic, anaerobic, and anoxic conditions depending on the quality of the wastewater. For example, wastewater from industrial activities is treated aerobic, but high strength wastewater from industrial wastewater is treated with anaerobic processes[37].

2.4.2 Moving Bed Biofilm Reactors (MBBRs)

Moving bed biofilm reactor, also known as MBBR, has been an important way to treat wastewater since its discovery by professor Hallvard Ødegaard in the 1980s[38]. In MBBR, we use bacteria in controlled environments to treat/degrade and consume the organic materials in wastewater by biological processes[39]. In the MBBR system, recycled carriers are used to place the sludge, and with the help of aeration, the carriers will move perfectly in the tank, which will increase the connection between the microorganism and the wastewater. The carriers also facilitate optimal bacteria or activated sludge grow on the internal surface of the bio-media and the excess sludge will flow with the water[40]. In MBBR, 50-70% of the tank consists of carriers, and they adapt to the wastewater density, which makes them able to be mixed with the wastewater rather than floating or sinking[41].

There are several advantages of MBBR, such as an MBBR system consumes less energy than other wastewater systems, making it cheaper to drift and most of the work is done by microorganism which gives the operator fewer controls to manage. On the other hand, the MBBR system can handle a higher volume of wastewater than other systems and at the same time, offer better treatment efficiency[42]. In the MBBR system, nitrification and denitrification processes may occur if we use nitrifying microorganisms. Nitrification is the process where ammonia is converted to nitrate, and denitrification is the process where nitrate is converted to nitrogen gas. This is illustrated in Figure 5. This means MBBR system can also be used to treat nitrogen components[41].

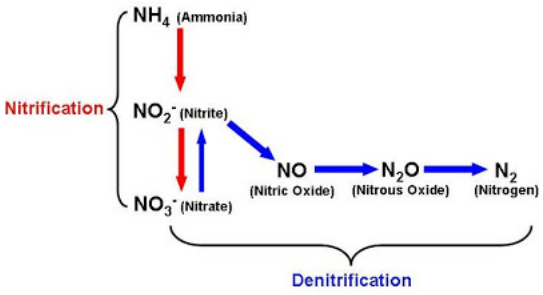

Figure 5. Denitrification and nitrification process[43].

3. Materials and Methods

3.1 Reactor Setup and Operation

The seed sludge was taken from IVAR sentralrenseannlegg Nord-Jæren (IVAR SNJ) aeration tank and acclimated to the peptone-meat extract mixture (peptone mixture), which is a completely biodegradable synthetic domestic wastewater containing different COD fractions. This was conducted to gain a biological decomposition of the wastewater by using microorganisms from the seed sludge. Using seed sludge in WWTPs is a common way to jump-start the biological system[44,45]. For both control and the chronic reactor, a solid retention time (SRT) of 10 days in a 5-liter reactor was maintained by removing 0.5L of suspended sludge every day. The formula for SRT calculation is shown in Equation 1. SRT is the time solids fractions such as bacteria spend in the treatment system before they are wasted out of the system[46].

$$SRT = \frac{Volume\ of\ the\ reactor\ (L)}{The\ amount\ sludge\ \ taken\ out\ every\ day\ (\frac{1L}{days})} = 10\ days \qquad (1)$$

Both control and the chronic reactor were fed with 600 mg COD/L peptone mixture every day. Once a week 1L peptone mixture was prepared and used for the week. 1 L peptone mixture consisted of 32 g casein peptone type 1, 22 g beef extract powder, 6 g urea agar base, 1.4g NaCl, 0.8 g $CaCl_2 \cdot 2\ H_2O$, 0.4 g $MgSO_4 \cdot 7\ H_2O$ and 5.6 g K_2HPO_4. Additionally, for each 1000 mg COD fed to the reactor 20 ml of solution A (macronutrient, SolA), which consisted of 320 g/L K_2HPO_4 and 160 g/L K_2HPO_4, was added every day to the reactor together with the peptone mixture. The chloroxylenol stock solution had the concentration of 0.01g CHL/L.

The reactors were operated in an anoxic and aerobic sequence. After feeding there was a 3 hour anoxic period, which means an environment with the absent of free oxygen and the microorganisms use NO_3^- and NO_2^- as the final electron acceptor. This is important to prevent NO_3^- accumulation because if the system was aerated constantly without stop, then microorganisms would use free oxygen to convert NO_2^- to NO_3^- and caused NO_3^- accumulation[47]. At the end of the anoxic period the aeration was turned on and the reactor was aerated for 19 hours. This is illustrated in Figure 6.

First, 0.5 L sludge was removed while the aerations and mixer were on. Then the sludge was left to settle down by turning off the mixer for 1 hour before pumping out 2 L effluent. Finally, the reactor was fed with peptone mixture, SolA and room-temperature tap water until 5L. In addition to the peptone mixture and the SolA, the chronic reactor was fed with 100 µg/L CHL every day.

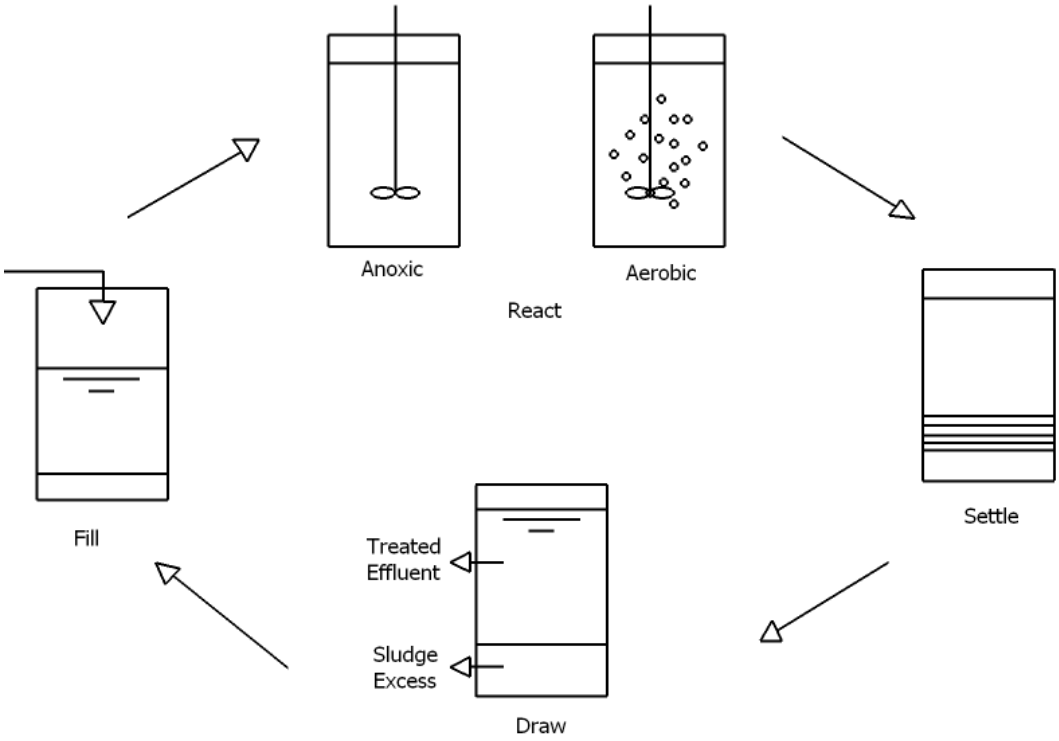

Figure 6. The operation modus for the control and the chronic reactor[48] .

Figure 7. Picture of the chronic reactor in the aerobic phase.

3.2 Experimental Procedures

3.2.1 Suspended Solids (SS) and Volatile Suspended Solids (VSS)

In wastewater, we find different forms of solids. We can separate them into solids contained in raw wastewater and biological solids produced through biological processes. Solids in raw wastewater can be both dissolved and suspended in water[49].

Suspended solids (SS) can be described as tiny particles of sediment that remain in the wastewater and act as a collide and can be removed by a filter[50]. Some examples of suspended solids are soil, biological solids, and decaying organic content. The amount of suspended solid affects the quality of the water. Suspended solids enter water differently and some examples are urbanization and agriculture. An increasement in sedimentation will pollute the water and decrease water clarity, affecting the organisms in the water by decreasing their visibilities[51]. SS value can be measured by taking out a known amount of well-mixed sample, which is then filtered through a weighted glass-fiber filter. Finally, we place the weighted filter with filtered sample into an oven with a constant temperature between 103-105°C for 1 hour for drying. After 1 hour, the filter with the filtered sample is placed in a desiccator for 30 minutes to get

further dried out before we weigh the filter, and the increase of the weight of this filter compared to a new filter is the value of SS[52]. The formula for finding SS value is shown in Equation 2.

$$SS = \frac{Dry-filter}{V\ sample*10^{-3}} \left[\frac{mg}{L} \right] \quad (2)$$

Dry = Weight of the filter with suspended solids after cooling down for 30 min after drying for 1 hour at 105°C [mg].
Filter = The weight of a new dried filter [mg].

Before the SS values were conducted, the glass microfiber filters (GF/C™, diameter 47mm) were washed with distilled water and dried for 24 hours in an oven at 105°C. Then the filters were placed in a desiccator for 30 minutes before they were used. Washing the filters makes the membrane pores in the filter wet, which will destroy the pathways used by contaminants like particles[53].

Volatile suspended solids (VSS) are biological solids produced during the biological treatment, which means they are produced during aerobic/anaerobic processes. One example is bacteria. Measuring VSS to control the biological concentration in the treatment system is important. VSS only contains organic content in contrast to total suspended solids (TSS), which contains inorganic and organic content. VSS is measured by first measuring SS value. After weighing the filter to find SS value, the filter with the sample is placed in a furnace with a temperature of 550°C for 30 minutes and then placed in a desiccator for 30 minutes before we weigh the filter again. The weight loss between the SS value and the last measured filter represents the VSS value[52]. Both SS and VSS are measured in mg/L. The formula for finding VSS value is shown in Equation 3.

$$VSS = \frac{Dry-Ash}{V\ sample*10^{-3}} \left[\frac{mg}{L} \right] \quad (3)$$

Dry = Weight of the filter with suspended solids after cooling down for 30 min after drying for 1 hour at 105°C [mg].
Ash = The weight of the filter with ash fractions after cooling down for 30 minutes after the incineration at 550°C.

3.2.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is often measured to determine the quality of wastewater and to get a knowledge of the efficiency of the WWTPS. COD is the amount of dissolved oxygen (DO) in the water needed to completely oxidize organic content to inorganic end components. High COD values in wastewater indicate that the wastewater is polluted with a great amount of organic content and may lead to environmental and regulatory consequences if this wastewater ends up in the environment. One environmental consequence of wastewater with high COD levels ending up in the environment is that DO in the water will be used to oxidize the organic content from the wastewater, which will result in reduction of DO in the water and that will affect the organisms in the aquatic environment[54,55]. COD values can be measured by adding 2ml of effluent sample filtered through a 0.45 µm cartridge filter into a 1.09772.0001 (10-150 mg/l) or 1.14895.0001 (15-300 mg/l) Spectroquant COD cell test consisting of a hot sulfuric solution of potassium dichromate with silver sulphate as a catalyst, which oxidizes the sample. Chloride is masked with mercury sulphate. Finally, photometrically unused yellow $Cr_2O_7^{2-}$ ion concentration is used to find the COD values. This test takes around 3 hours because the COD kit with the sample needs to be mixed well and placed in the heating block at 148°C for 2 hours and then let it cool down for at least 30 minutes before we measure the values using spectroquant. It is important to know that in these tests both inorganic and organic components are both oxygen demanding components and therefore will be measured as COD[56].

3.2.3 Nitrogen Compounds

Nitrite nitrogen (NO_2^--N) measurement was conducted by adding 5ml of effluent sample filtered through 0.45 µm cartridge filter into a 1.14547.001 Spectroquant nitrite cell test (0.010-0.700 mg/l) and then shaking the sample until the reagent was completely dissolved. The sample was left for 10 minutes before the values were measured using a spectroquant. When the nitrite components from the wastewater come in contact with the acidic solution in the cell test, the nitrite components react with sulfanilic acid to form dihydrochloride, which leads to the formation of a red-violet azo dye which can be analysed photometrically to determine the nitrite values in the sample[57].

Nitrate nitrogen (NO_3^--N) measurement was conducted by adding 500 µl of effluent sample filtered through 0.45 µm cartridge filter into a 1.14764.0001 Spectroquant nitrate cell test (1-50 mg/l) with 1.0ml reagent, and then the test was mixed well. The sample was left for 10

minutes before the values were measured using a spectroquant. In sulfuric and phosphoric solution, nitrate ions react with 2,6 dimethylphenol also known as DMP and this reaction result with the production of 4 – nitro – 2,6 – dimethylphenol, which can be used photometrically to find nitrate values in the sample[58].

Ammonium nitrogen (NH_4^+-N) measurement was conducted by adding 500 µl of effluent sample filtered through 0.45 µm cartridge filter into a 1.14544.0001 Spectroquant ammonium cell test (0.5-16 mg/l) with 1 dose reagent, and then the test was shaken. The sample was left for 15 minutes before the values were measured using a spectroquant. Ammonium nitrogen exists in two forms, which are ammonium ions and ammonia depending on the pH. In an alkaline solution, we find mostly ammonium as ammonia, and ammonia reacts with hypochlorite ions and forms monochloramine. Monochloramine will further react with a substituted phenol and form a blue indophenol derivative which is used to determine the ammonium values photometrically[59].

3.2.4 Respirometry and Oxygen Utilization Rate

A respirometer is a machine that measures the respiration rate of a culture, which means it measures the exchange rate of oxygen and carbon dioxide under aerobic conditions[60]. Since respiration rate is related to substrate removal and biomass growth, we can use data from respirometry tests to monitoring, modelling, and have control of the activated sludge system[61].

In this experiment, a respirometer multi- purpose double respirometer (BM-EVO2) from surcis S.L (Figure 6), which is specifically developed for practical and efficient biological wastewater treatment management, was used for analytical tests. BM-EVO 2 measures oxygen concentration and calculates the following values using the initial information provided to the program; oxygen utilization rate (OUR, mg O_2/L·h), specific OUR (SOUR, mg O_2/mg VSS·h), biodegradable COD (bCOD, mg/L), consumed oxygen (CO, mg O_2/L), COD utilization rate (U, mg bCOD/ L·h) and specific U(q, mg bCOD/mg VSS.d)[62].

Figure 8. Picture of BM–EVO2[63].

Oxygen utilization rate (OUR) is one of the parameters the respirometer calculates. OUR measures an organisms respiration rate based on calculating how much dissolved oxygen that has been utilized by the organisms. This is important to know because how much oxygen microorganisms use is related to the process of removing organic content from the wastewater. Therefore, OUR values give us information about the biodegradability of the wastewater and aeration requirement. OUR values also give us information about the toxicity since toxic substances in the sample will decrease the oxygen utilization rate[64].

The oxygen utilization rate (OUR) measurement was conducted in two parts. First, endogenous oxygen utilization rate test (OUR end) of the activated sludge was conducted after letting the sludge aerated for a certain time and thereby eliminate other diverse degradable substrates. Endogenous respiration state can be observed by looking at the oxygen reading and check if the readings are stable within its oxygen saturation level. A DO base line is determined from the endogenous test, and the R test can be conducted to study OUR profile for organic matter removal[65,66]. In the R tests, the conditions for all tests were fixed to 500 mg/L VSS biomass and 300 mg COD/L peptone mixture to avoid oxygen limitation during the tests.

Calculations were conducted to know how many carriers were needed to perform the test. First five mutag Biochip 30^{TM} carriers from the MBBR tank were placed in a furnace with a constant temperature at 80°C over the night to be dried. Then they were placed in a desiccator for 30 minutes to be cooled down before they were weighed. The same procedure was conducted for 5 new carriers without sludge. The average weight was calculated and compared with the average of 5 new carriers without sludge[67]. In that way, we were able to know the average

amount of sludge in each carrier. To conduct this test VSS value from the carriers needed to be 500mg/L. Calculations were conducted by using the formulas under.

*(Average carrier with sludge – average new carriers) *0.85=amount sludge in one carrier (mg).* (4)

$$500mg = x\ carriers \quad (5)$$

$$Amount\ sludge\ in\ one\ carrier\ (mg) * x = 500mg * 1\ carrier \rightarrow x = \frac{500\ mg * 1\ carrier}{Amount\ sludge\ in\ one\ carrier\ (mg)} \quad (6)$$

The next measurement was about how much suspended sludge that was needed to give a VSS value equal to 500mg/L, which is the requirement for this test. This was calculated by using Equation 7. In this way, we could calculate how much sludge sample that is needed from the reactor. The needed amount of sludge was taken out, and the sludge got to settle down before the top part was poured out. The sludge was then filled with the same amount of tap water as the top part, which was poured out. This was conducted two times, and it was done to wash the sludge and get rid of various contaminants.

$$X\ ml * VSS\ (\frac{mg}{1000ml}) = 500\ mg \rightarrow X\ ml = \frac{500\ mg * 1000\ ml}{VSS\ (mg)} \quad (7)$$

The next calculation that was needed to be calculated for the test was the amount of peptone that was needed to be added for the R test. This was done by first calculating the COD concentration in the peptone mixture and then calculating how much peptone that are needed to gain 300mg COD/L. This is illustrated in Equation 8.

$$X\ ml * peptone\ mixture\ (\frac{COD\ mg}{1000ml}) = 300\ mg \rightarrow X\ ml = \frac{300\ mg * 1000\ ml}{peptonmixture\ COD\ mg} \quad (8)$$

The last calculation that was needed to be calculated for those tests with CHL was the amount of CHL that was going to be added. In five liter reactor, 50 ml CHL was added. That means in a respirometer vessel with a 1L volume, we need to add 10ml CHL. Finally, when the amount of peptone, (CHL), and sol A were known, we could calculate the amount of tap water that are needed to reach 1L after adding peptone, Sol A, and (CHL).

To start up the R test, determined number of carriers from the MBBR system were placed in a container in the chamber. The control and acute test were conducted with 18 carriers, while the chronic tests were conducted with 8 carriers. Suspended sludge sample, SolA, and required amount of tap water were added to the vessel of 1L volume. After the OUR_{End} was determined, the peptone mixture and CHL (when necessary) were added to start the R test. In the R test, all liquid and the suspended sludge circulated continuously in the respirometer vessel with a 1L volume, while the carriers were stable in the chamber[68]. The OUR reactor was aerated throughout the test while samples were taken at specific times to conduct nitrogen and COD tests. The R test was conducted with and without nitrogen inhibitor and CHL to get a knowledge of how diverse microorganisms are affected by CHL. It took approximately 22 hours to complete one R test.

Table 3.1 R test conditions**.**

Test Run	Peptone mixture (mg COD/L)	CHL (ml)	Solution A (ml)	Biomass concentration (mg VSS/L)	Nitrification inhibitor (g)
Control with Nitr. Inh	300	-	6	500	1.00
Control without Nitr. Inh	300	-	6	500	-
Acute with Nitr. Inh	300	10	6	500	1.00
Acute without Nitr. Inh	300	10	6	500	-
Chronic with Nitr. Inh	300	10	6	500	1.00
Chronic without Nitr. Inh	300	10	6	500	-

4. Results and Discussions

4.1 Analysis of Test Results

The control reactor operations showed high efficiency in COD and NH_4-N removals. The concentrations in the effluent were measured to be to be 54±16 mg/L, 1.1±0.4 mg/L, 16±10 mg/L and 40±7 mg/L for COD, NH_4-N, NO_2-N and NO_3-N, respectively. The effluent concentrations in the chronic reactor showed a fluctuating trend, which was possible due to the addition of CHL (Figure 9). Moreover, the biomass concentration in the reactors were 1510±270 and 2225±438 mg VSS/L for control and chronic reactors, respectively.

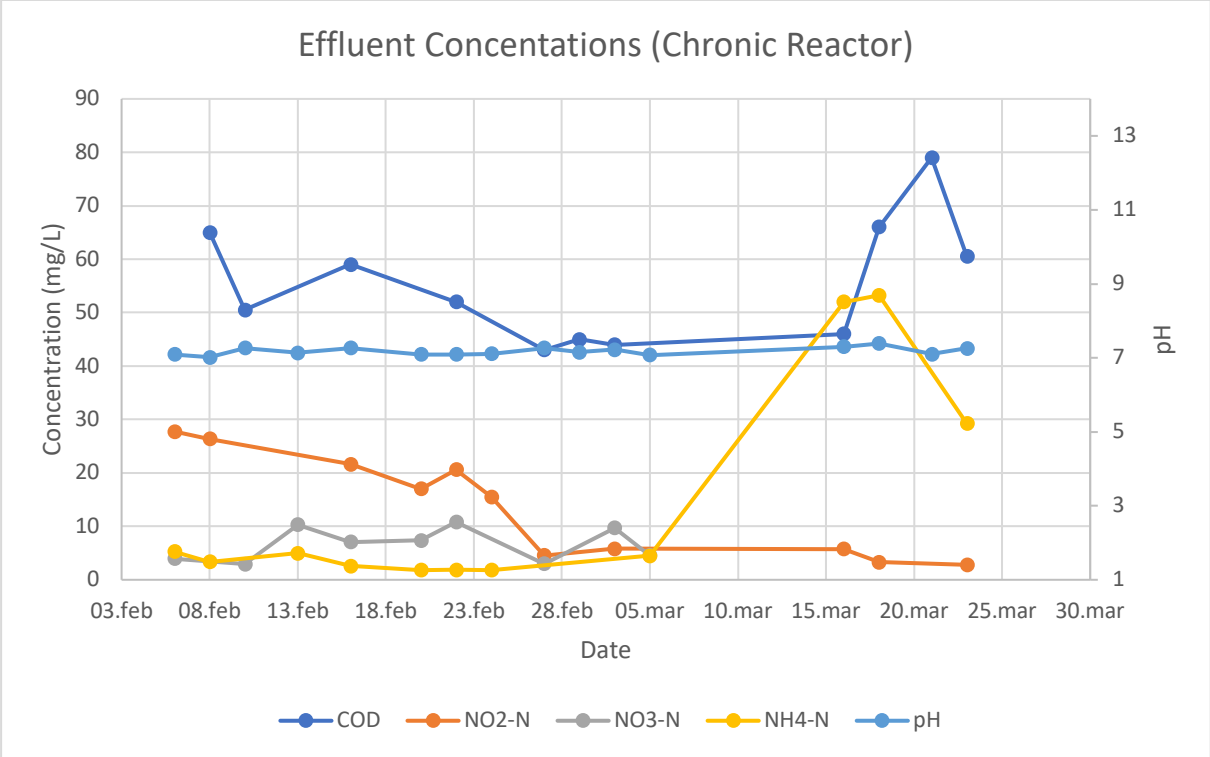

Figure 9. Effluent concentration of COD, Nitrogen av pH in Chronic Reactor**.**

4 tests were conducted by using sludge and carriers from the control reactor, namely controls tests with and without nitrogen inhibitor and acute CHL tests with and without nitrogen inhibitor, in which the sludge was exposed to CHL for the first time. Additionally, chronic tests with and without nitrogen inhibitor were conducted using the sludge and carriers from the chronic reactor which was exposed for CHL for 43 days. Table 4.1 shows the oxygen and COD mass balance for all the experimental tests carried out and a through explanation for each run is provided in the sections below.

Table 4.1. Consumed oxygen and COD calculations for experimental runs (Y_H = 0.55 mg cell COD/mg COD).

Test Run	Area for Growth (ΔO_2) [mg O_2/L]	Area for Nitrification (ΔO_{2A}) [mg O_2/L]	Area for Carbon Removal (ΔO_{2H}) [mg O_2/L]	COD corr. to ΔO_{2H} [mg COD/L]	O2 eq. of NO3-N removed [mg O_2/L]	COD used for Denitrification [mg COD/L]	Rest COD for Aerobic Oxidation [mg COD/L]
Control w Nitr. Inh	91	0	91	201	63	102	198
Control wo Nitr. Inh.	170	66	104	231	43	70	230
Acute w Nitr. Inh.	126	0	126	280	12	20	280
Acute wo Nitr. Inh.	128	32	96	214	47	76	214
Chronic w Nitr. Inh.	153	45	107	239	31	50	238
Chronic wo Nitr. Inh.	165	60	105	233	42	68	232

4.1.1 Control Tests

Control test with nitrification inhibitor showed the oxygen consumption by the heterotrophic microorganisms for the degradation of 300 mg COD/L peptone mixture. The area under the graph and above the endogenous decay level (orange line) represent the amount of oxygen used by the heterotrophic microorganism for growth during the time present in the graph in Figure 10. For the test with nitrogen inhibitor, the area was calculated to be 91 mg O_2 / L. The calculations were done by first finding the total area beneath the graph using trapezoid area calculation approach and then subtraction the rectangular area underneath the endogenous decay level. In Figure 10 it can be seen that the OUR profile of the peptone mixture for the test with inhibitor had a peak at 26 mg O_2/L·h and then the graphs went all the way down until it reached a stable endogenous decay level, which is a result of complete oxidation of all the available carbon source.

Figure 11 shows the NH_4-N, NO_2-N and NO_3-N profiles throughout the experiment. It can be seen in Figure 11 that nitrification was successfully inhibited by the addition of the inhibitor. However, due to oxygen limitation in the biofilm's, denitrification of the available NO_3-N took

place. Using the conversion factor of 2.86 g O_2/g NO_3- N, the oxygen equivalent of 22 mg NO_3-N/L was calculated as 63 mg/L for the test with nitrogen inhibitor. Assuming Y_H as 0.55 mg cell COD/mg COD and $\eta = 0.7$, COD consumption required for the conversion of 22mg NO_3-N/L to N_2 was calculated as 102 mg COD/L. Since the total amount of COD added in the beginning of the test was 300 mg/L and 102 mg COD/L was used for denitrification, the rest which is around 200 mg COD/L was used by the heterotrophs during aerobic conversion of organic carbon to CO_2 and H_2O. The COD utilization curve is shown under in Figure 12. The area calculation done directly from the curve also showed that 201 mg COD/L was utilized for Y_H 0.55 mg cell COD/mg COD (Table 4.1).

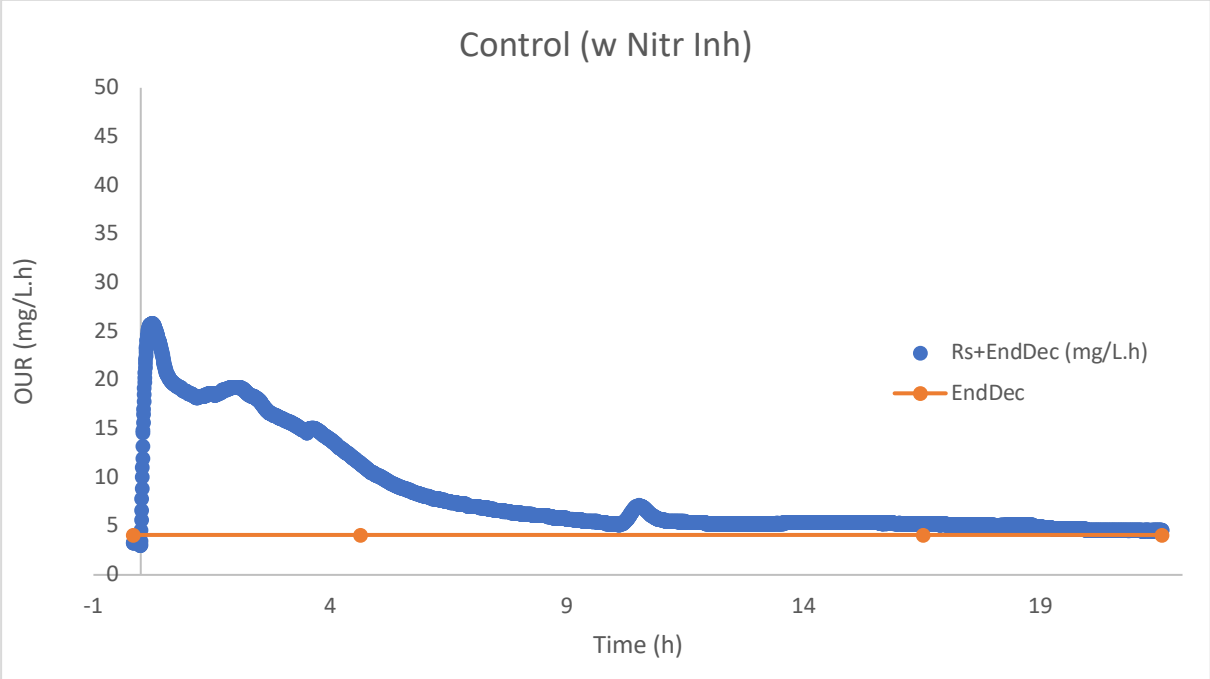

Figure 10. OUR test of the control rector with nitrogen inhibitor.

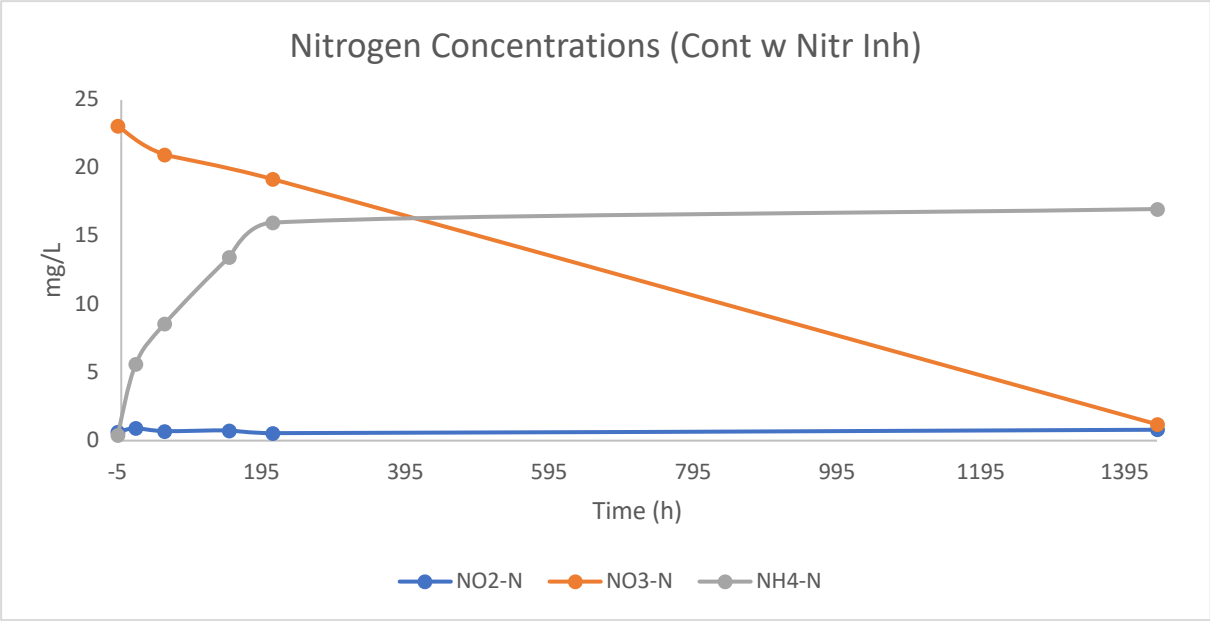

Figure 11. Nitrogen concentration throughout the experiment with nitrogen inhibitor.

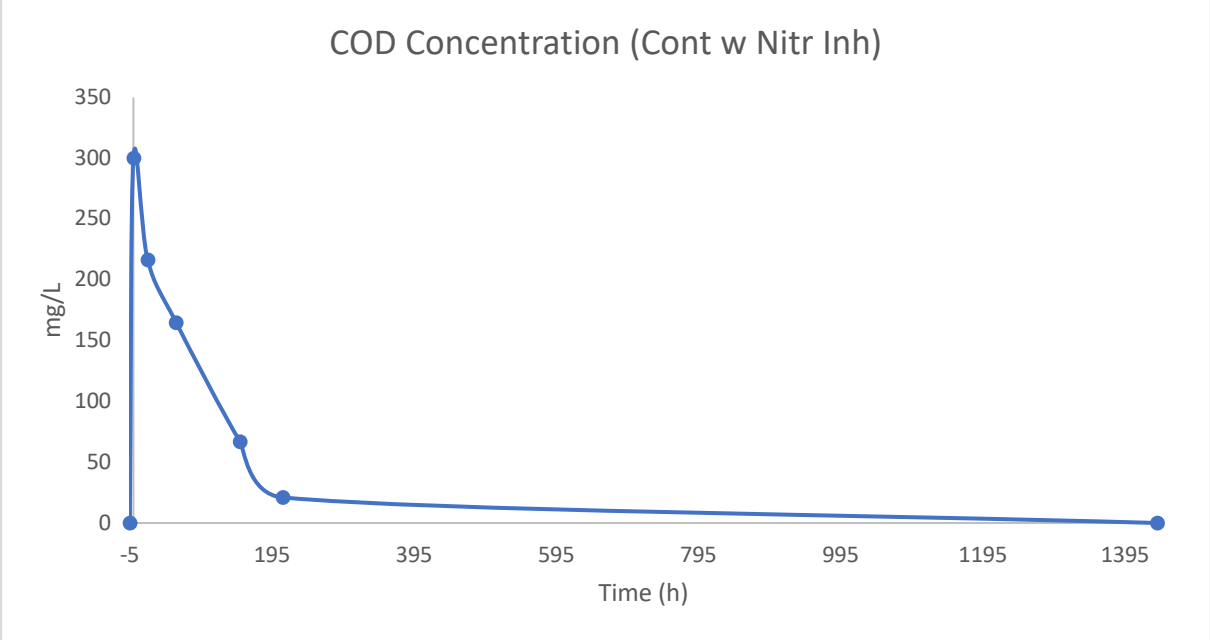

Figure 12. COD concentration during the OUR test with nitrogen inhibitor.

Control test without nitrogen inhibitor was conducted to study the behaviour of the total microbial community. Figure 13 shows the biodegradation profile of the peptone mixture, which had a peak around 47 mg O_2/L·h and then the graphs went all the way down until it reached a stable endogenous decay level, which is a result of complete oxidation of all the available carbon source. In this test, the area was calculated to be 170 mg O_2 / L which is almost the double of the area in the test with nitrogen inhibitor which is a result of the oxygen

consumption of the nitrifying organisms. Based on the nitrogen profiles, it has been found that 9 mg O_2 / L and 57 mg O_2 / L has been utilized for conversion of NH_4-N to NO_2-N and NO_3-N, respectively making 66 mg O_2 / L for nitrification in total. 104 mg O_2 / L was used by heterotopic organisms in aerobic process, which corresponds to 231 mg COD/ L with a heterotrophic yield coefficient equal to 0.55 mg cell COD/mg COD. Moreover, using the same approach as for the other runs, the impact of denitrifications in the carriers biofilm on the COD mass balance has been calculated. Calculations showed that 70 mg COD / L has been used for the conversion of 15mg NO_3-N/L in denitrification, leaving 230 mg COD/L available for the aerobic conversion, which also correspond to number calculated in Table 4.1 The COD utilization curve is shown under in Figure 15.

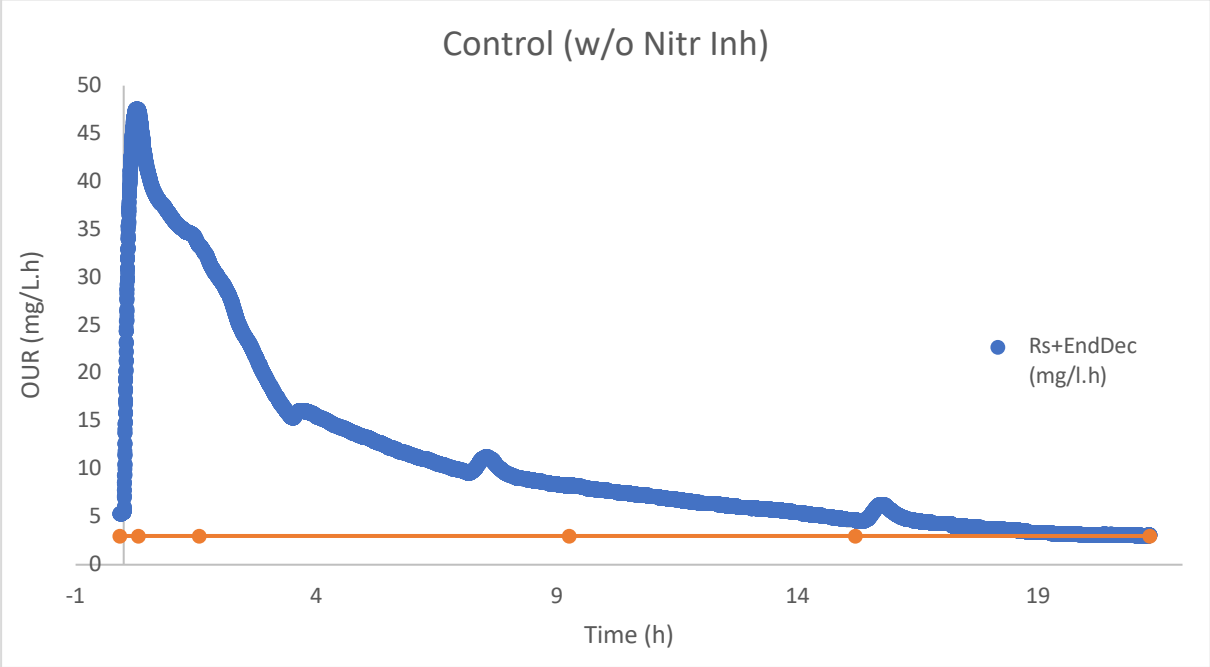

Figure 13. OUR test of the control rector without nitrogen inhibitor.

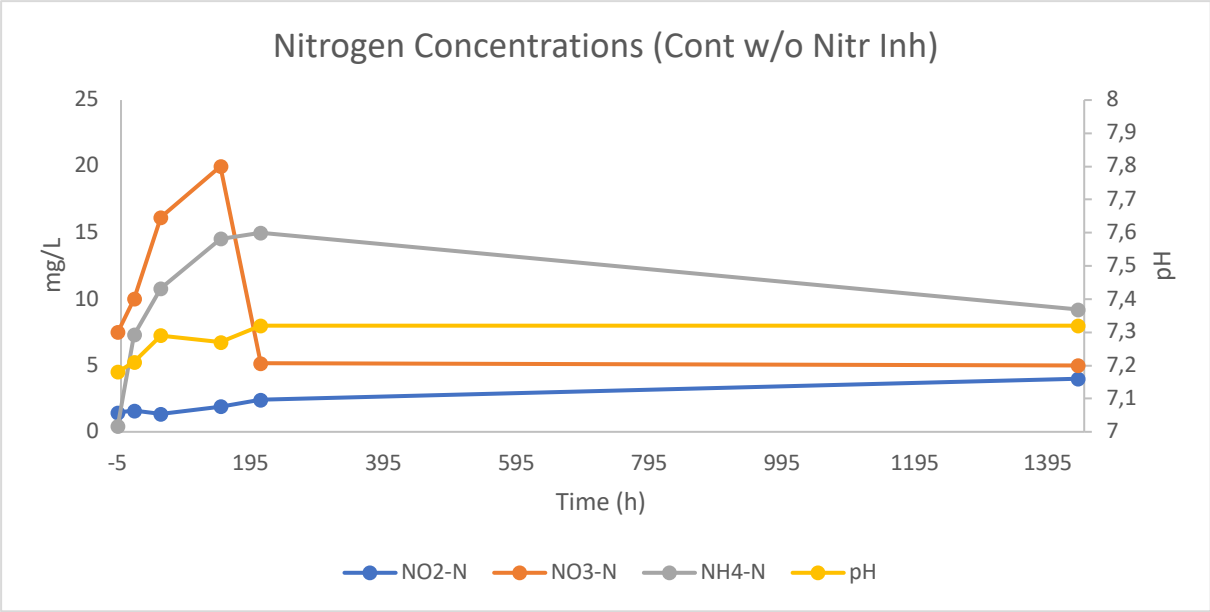

Figure 14. Nitrogen concentration throughout the experiment without nitrogen inhibitor.

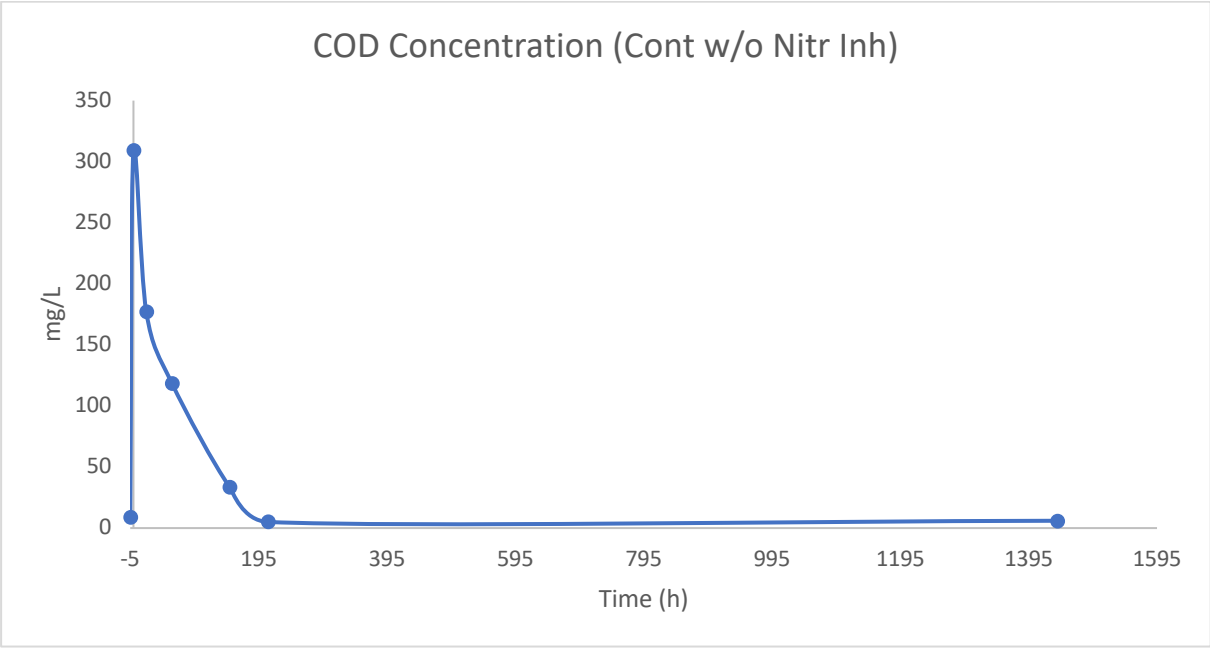

Figure 15. COD concentration during the OUR test without nitrogen inhibitor.

4.1.2 Acute Tests

Acute test with nitrification inhibitor showed the oxygen consumption by the heterotrophic microorganisms for the degradation of 300 mg COD/L peptone mixture under CHL exposure for the first time. For the test with nitrogen inhibitor, the area was calculated to be 126 mg O_2 / L. The same calculations that were used to find the area in the control tests were used here as well. In Figure 16 it can be seen that the OUR profile of the peptone mixture for the test with inhibitor had a peak at 33 mg O_2/L·h and then the graphs went all the way down until it reached a stable endogenous decay level, which is a result of complete oxidation of all the available carbon source.

Figure 17 shows the NH_4-N, NO_2-N and NO_3-N profiles throughout the experiment. However, due to oxygen limitation in the biofilm's, denitrification of the available NO_3-N took place here again. Using the same conversion factor as earlier, the oxygen equivalent of 4.35 mg NO3-N/L was calculated as 12 mg/L for the test with nitrogen inhibitor. Using the same Y_H and η as earlier, COD consumption required for the conversion of 4.35 mg NO_3-N/L to N_2 was calculated as 20 mg COD/L. Since the total amount of COD added in the beginning of the test was 300 mg/L and 20 mg COD/L was used for denitrification, the rest, which is around 280 mg COD/L was used by the heterotrophs during aerobic conversion of organic carbon to CO_2 and H_2O. The area calculation done directly from the curve also showed that 280 mg COD/L was utilized for Y_H 0.55 mg cell COD/mg COD (Table 4.1). The COD utilization curve is shown under in Figure 18.

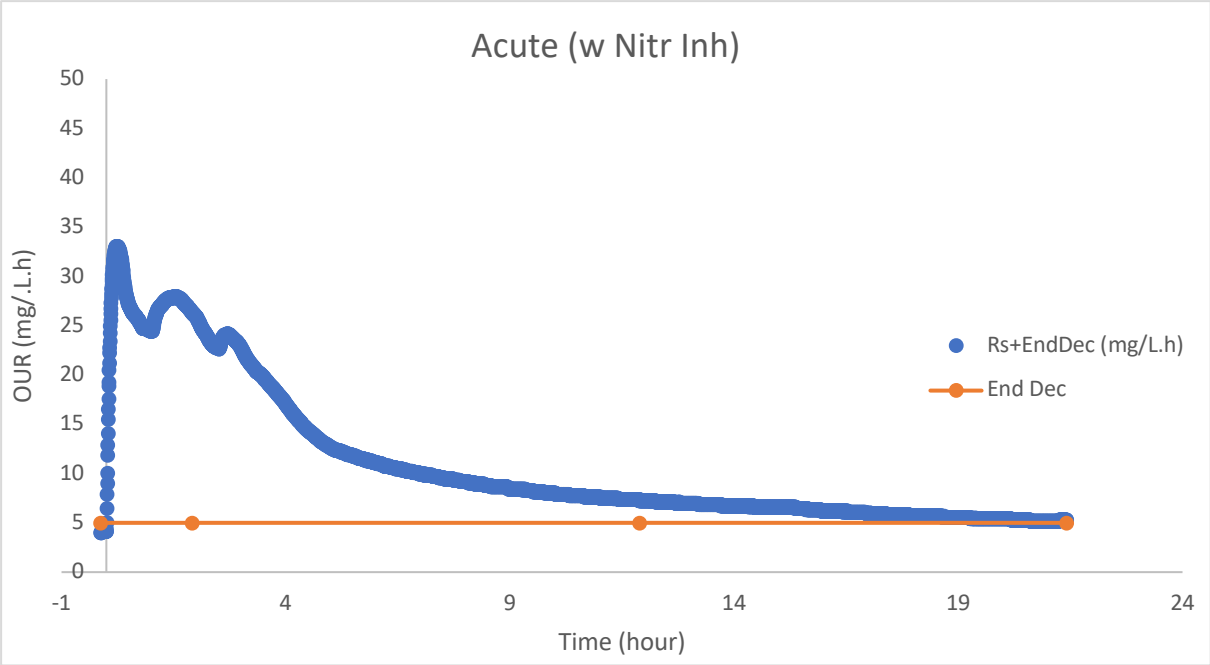

Figure 16. OUR test of the acute test with nitrogen inhibitor.

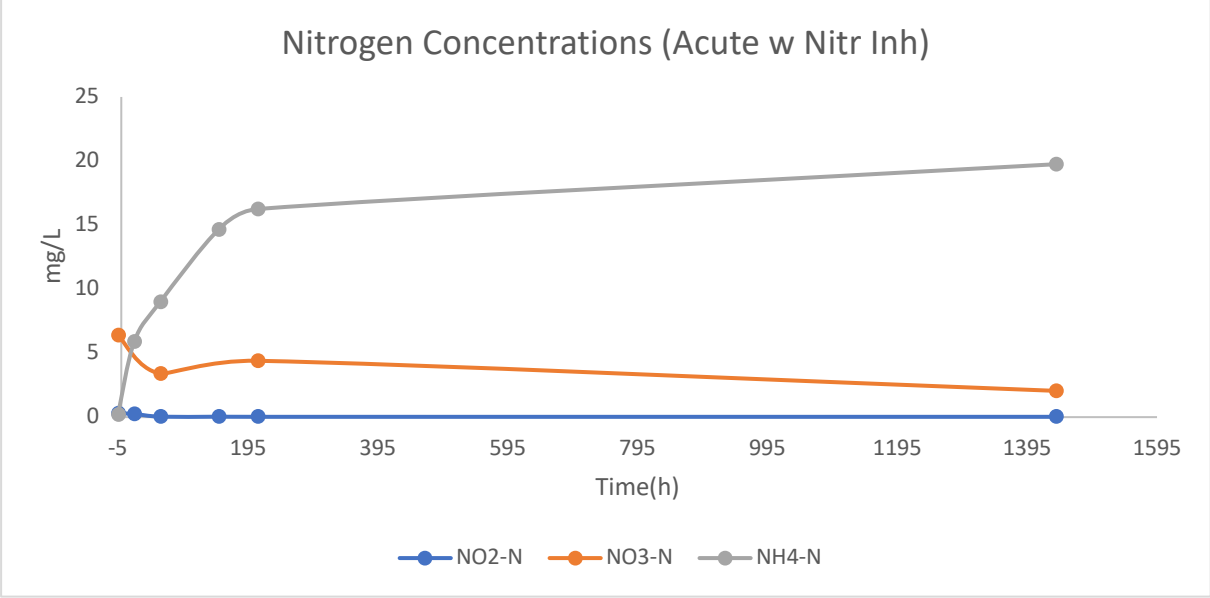

Figure 17. Nitrogen concentration throughout acute test with nitrogen inhibitor.

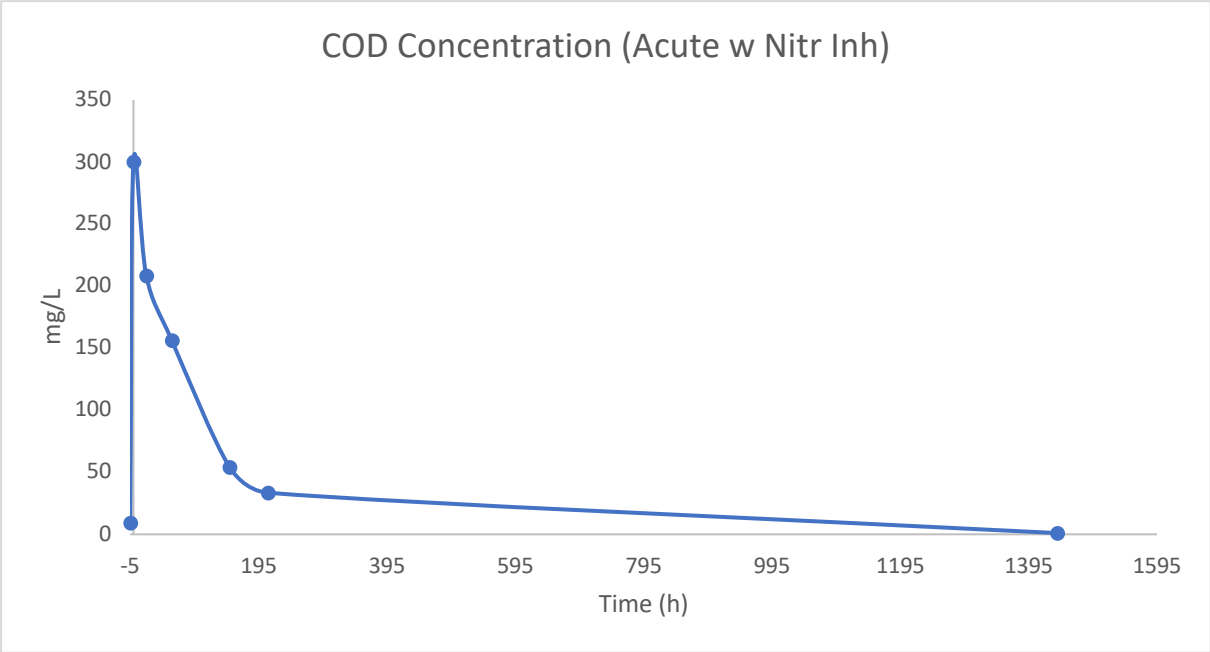

Figure 18. COD concentration during the OUR test for acute test with nitrogen inhibitor.

Acute test without nitrogen inhibitor was conducted to study the total microbial community during their first time meeting with CHL. Figure 19 shows the biodegradation profile of the peptone mixture, which had a peak around 44 mg O_2/L·h and then the graphs went all the way down until it reached a stable endogenous decay level. In this test, the area was calculated to be 128 mg O_2 / L which is almost the same as the test with nitrogen inhibitor. Based on the nitrogen profiles, it has been found that 8 mg O_2 / L and 24 mg O_2 / L has been utilized for conversion of NH_4-N to NO_2-N and NO_3-N, respectively making 32 mg O_2 / L for nitrification in total. 96 mg O_2 / L was used by heterotopic organisms in aerobic process, which corresponds to 214 mg COD/ L with a heterotrophic yield coefficient equal to 0.55 mg cell COD/mg COD. Moreover, using the same approach as for the other runs, the impact of denitrifications in the carriers biofilm on the COD mass balance has been calculated. Calculations showed that 76 mg COD / L has been used for the conversion of 16.5 mg NO_3-N/L in denitrification, leaving 214 mg COD/L available for the aerobic conversion, which also correspond to number calculated in Table 4.1. The COD utilization curve is shown under in Figure 21.

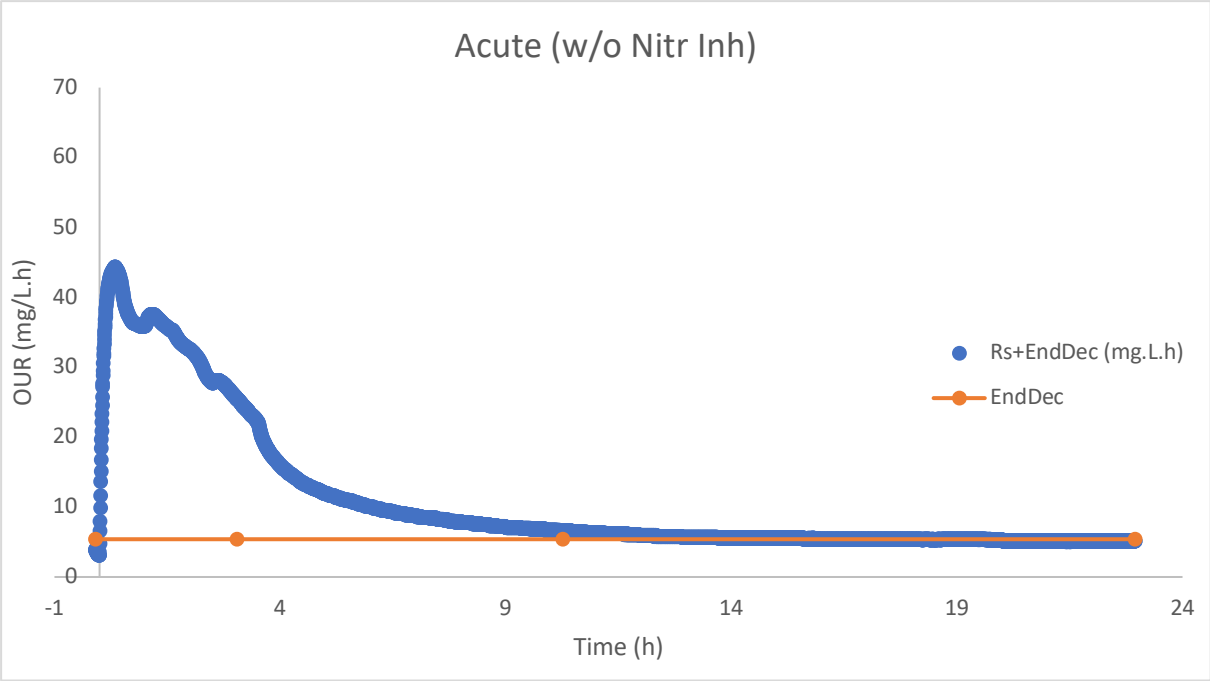

Figure 19. OUR test of the acute test without nitrogen inhibitor.

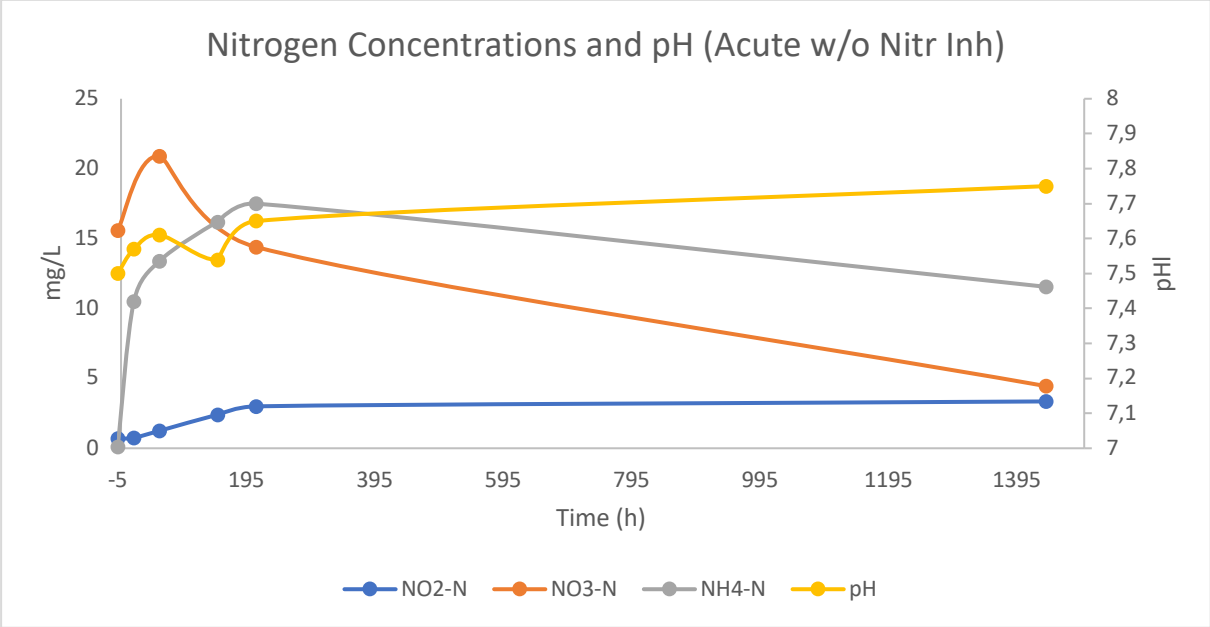

Figure 20. Nitrogen concentration and pH throughout the acute test without nitrogen inhibitor.

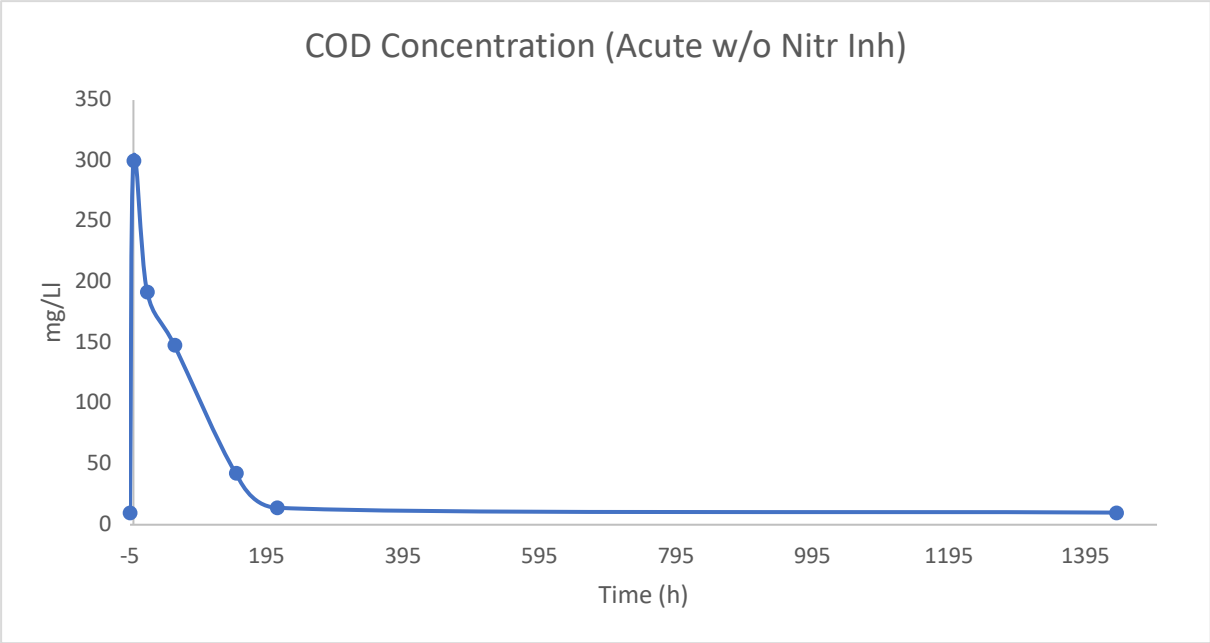

Figure 21. COD concentration during the OUR test for acute test without nitrogen inhibitor.

4.1.3 Chronic Tests

Chronic test with nitrification inhibitor showed the oxygen consumption by the heterotrophic microorganisms for the degradation of 300 mg COD/L peptone mixture. For the test with nitrogen inhibitor, the area was calculated to be 153 mg O_2 / L. The same calculations that were used to find the area in the control test were used here as well. In Figure 22 it can be seen that the OUR profile of the peptone mixture for the test with inhibitor had a peak at 54 mg O_2/L·h and then the graphs went all the way down until it reached a stable endogenous decay level.

Chronic test with nitrogen inhibitor was conducted to study how the heterotrophic microorganisms responded to CHL after they already had been exposed to CHL for 43 days. Based on the nitrogen profiles, it has been found that 1 mg O_2 / L and 44 mg O_2 / L has been utilized for conversion of NH_4-N to NO_2-N and NO_3-N, respectively making 45 mg O_2 / L for nitrification in total. 107 mg O_2 / L was used by heterotopic organisms in aerobic process, which corresponds to 239 mg COD/ L with a heterotrophic yield coefficient equal to 0.55 mg cell COD/mg COD. Moreover, using the same approach as for the other runs, the impact of denitrifications in the carriers biofilm on the COD mass balance has been calculated. Calculations showed that 50 mg COD / L has been used for the conversion of 10.8 mg NO_3-N/L in denitrification, leaving 238 mg COD/L available for the aerobic conversion, which also correspond to number calculated in Table 4.1 The COD utilization curve is shown under in Figure 24.

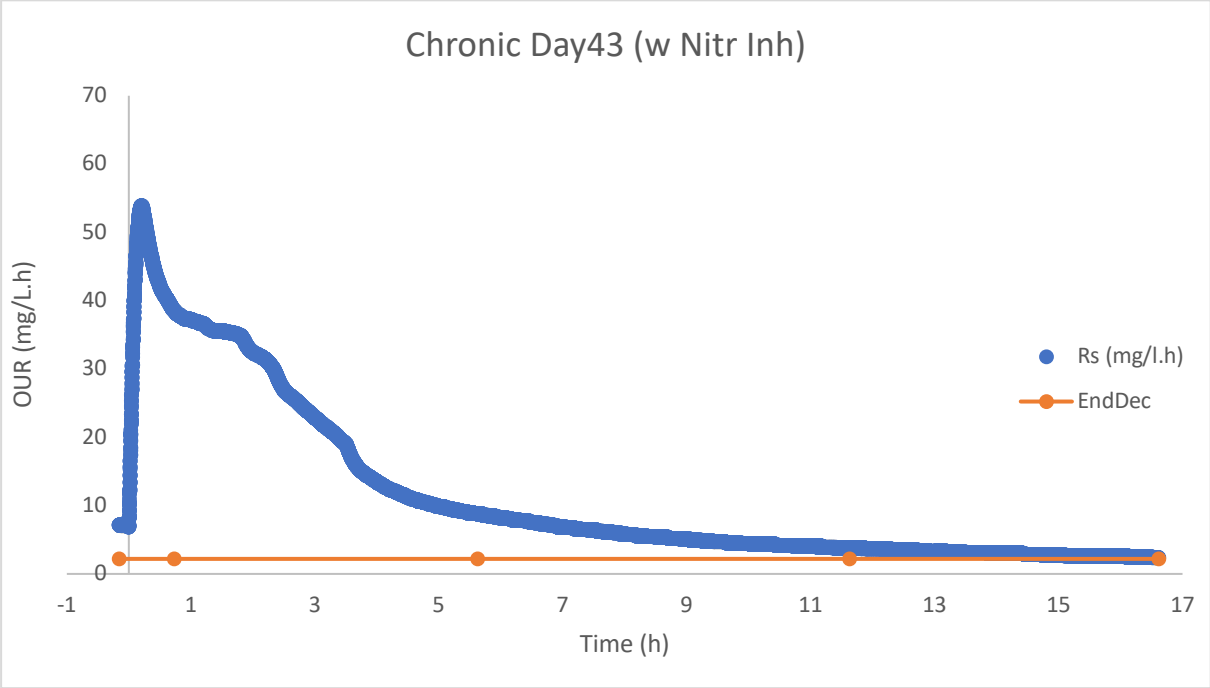

Figure 22. OUR test of the chronic test with nitrogen inhibitor.

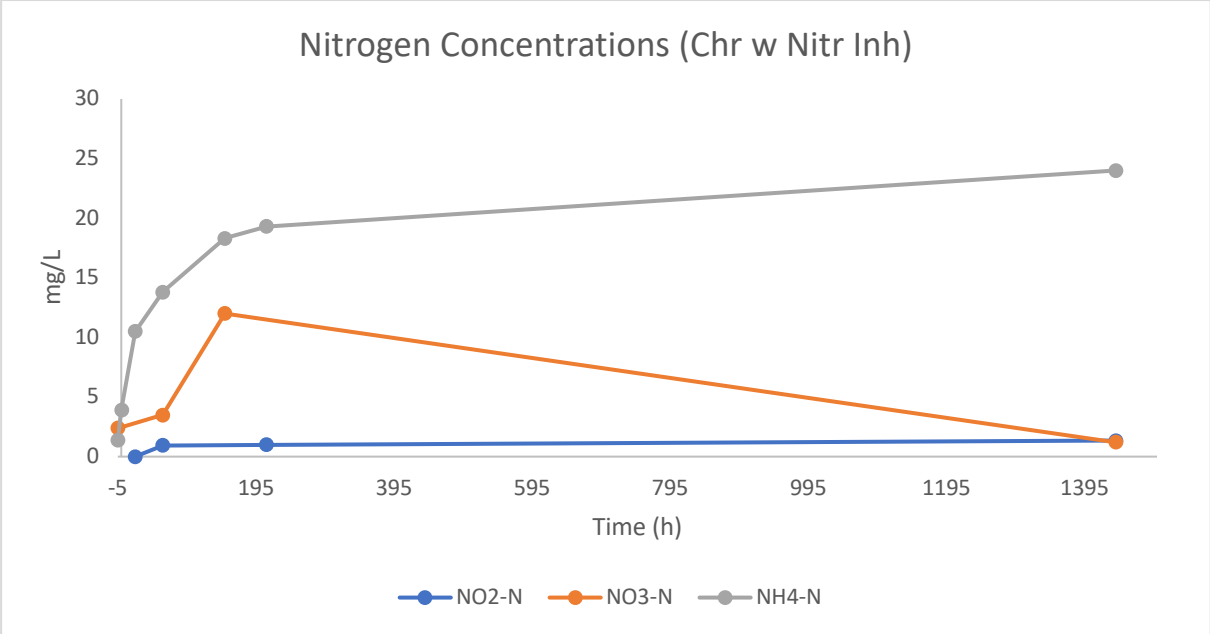

Figure 23. Nitrogen concentration throughout the chronic test with nitrogen inhibitor.

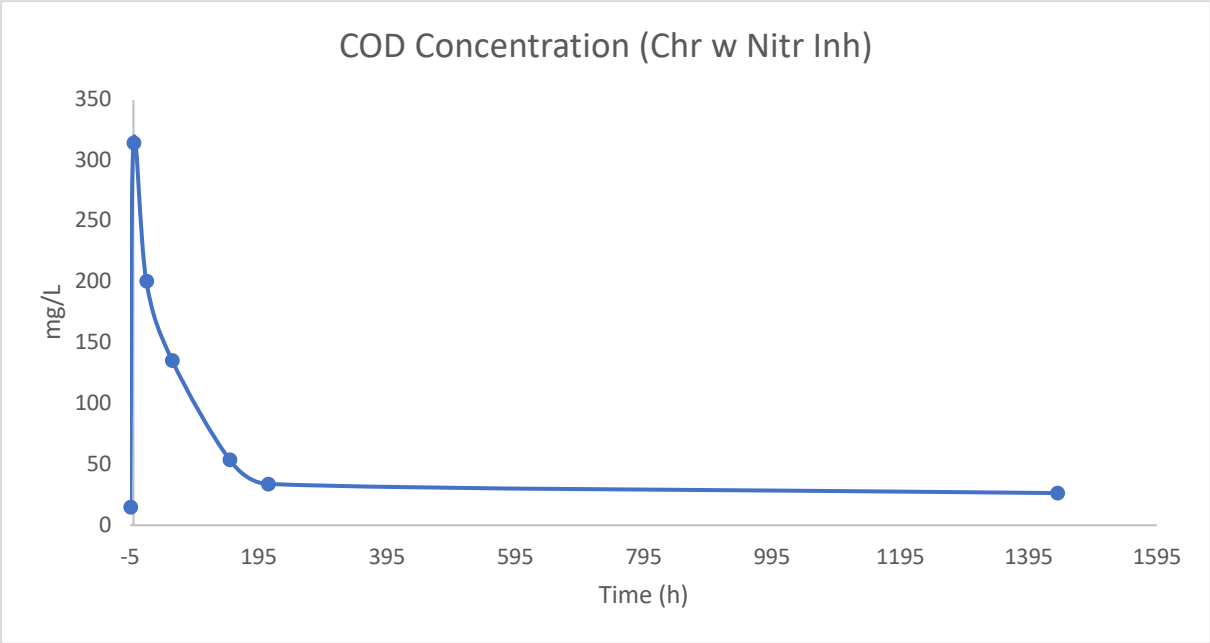

Figure 24. COD concentration during the OUR test for chronic test with nitrogen inhibitor.

Chronic test without nitrification inhibitor showed the oxygen consumption by the total microbial community for the degradation of 300 mg COD/L peptone mixture. For the test without nitrogen inhibitor, the area was calculated to be 165 mg O_2 / L which is approximately the same as for the test with nitrogen inhibitor. The same calculations that were used to find the area in the control test were used here as well. In Figure 25 it can be seen that the OUR profile of the peptone mixture for the test without nitrogen inhibitor had a peak at 64 mg O_2/L·h and then the graphs went all the way down until it reached a stable endogenous decay level.

Chronic test without nitrogen inhibitor was conducted to study how total microbial community responded to CHL after they already had been exposed to CHL for 42 days. Based on the nitrogen profiles, it has been found that 18 mg O_2 / L and 42 mg O_2 / L has been utilized for conversion of NH_4-N to NO_2-N and NO_3-N, respectively making 60 mg O_2 / L for nitrification in total. 105 mg O_2 / L was used by heterotopic organisms in aerobic process, which corresponds to 233 mg COD/ L with a heterotrophic yield coefficient equal to 0.55 mg cell COD/mg COD. Moreover, using the same approach as for the other runs, the impact of denitrifications in the carriers biofilm on the COD mass balance has been calculated. Calculations showed that 68 mg COD / L has been used for the conversion of 14.7 mg NO_3-N/L in denitrification, leaving 232 mg COD/L available for the aerobic conversion, which also correspond to number calculated in Table 4.1 The COD utilization curve is shown under in Figure 27.

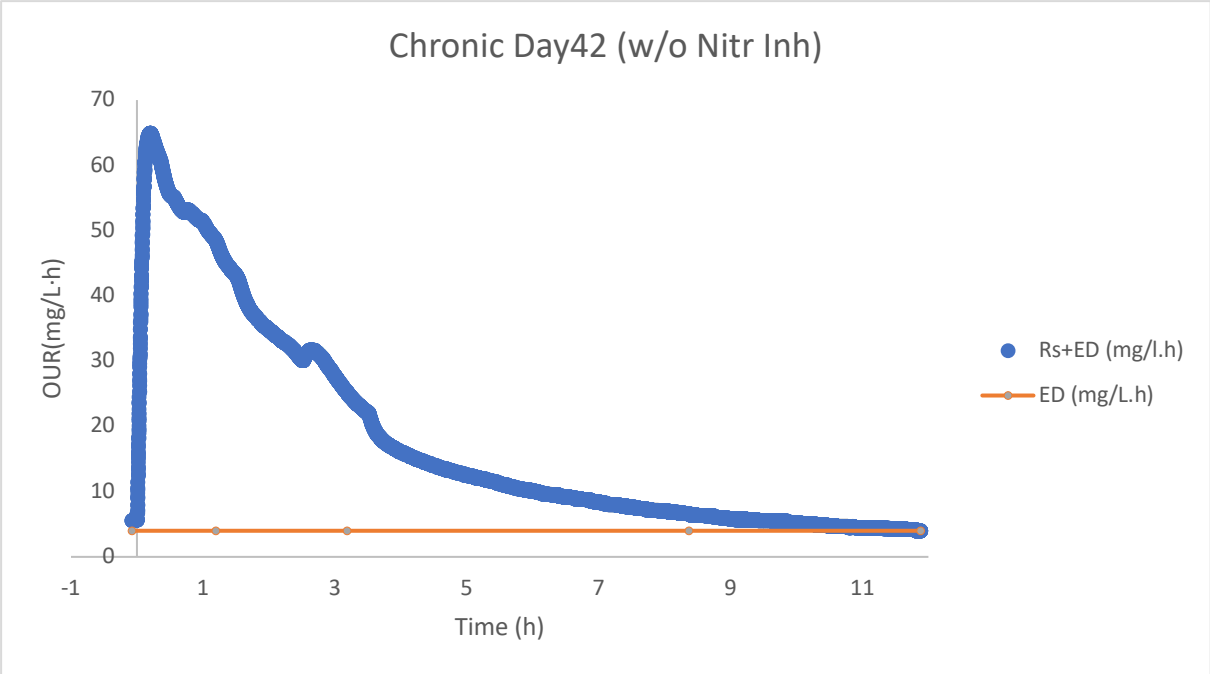

Figure 25. OUR test of the chronic test without nitrogen inhibitor.

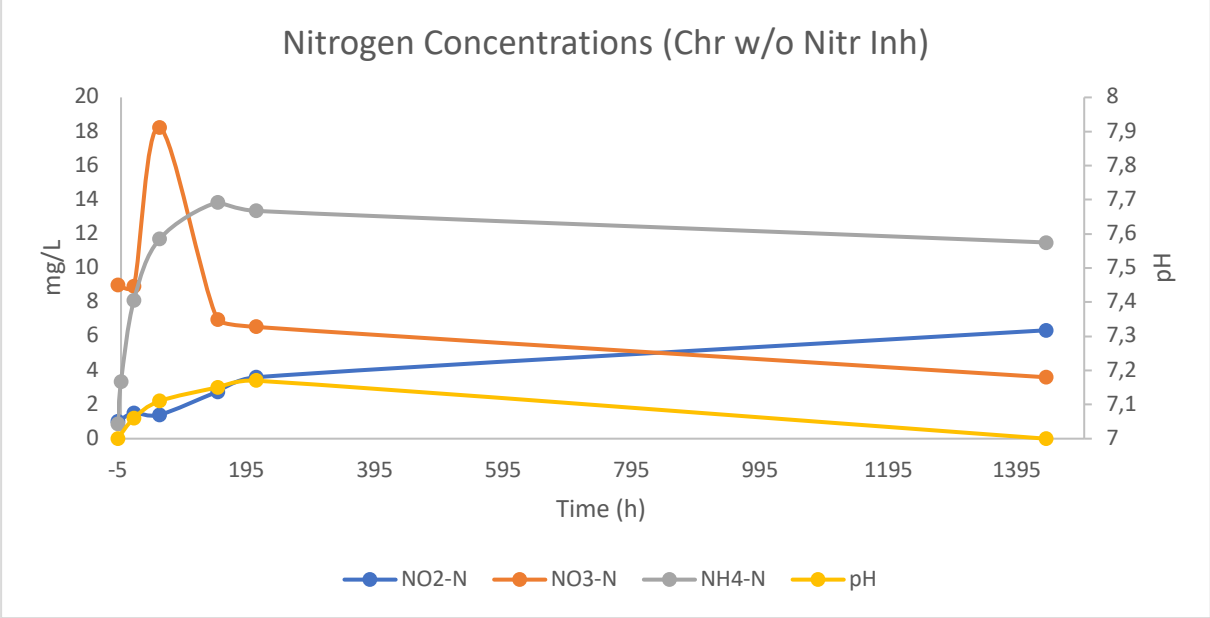

Figure 26. Nitrogen concentration and pH throughout the chronic test without nitrogen inhibitor.

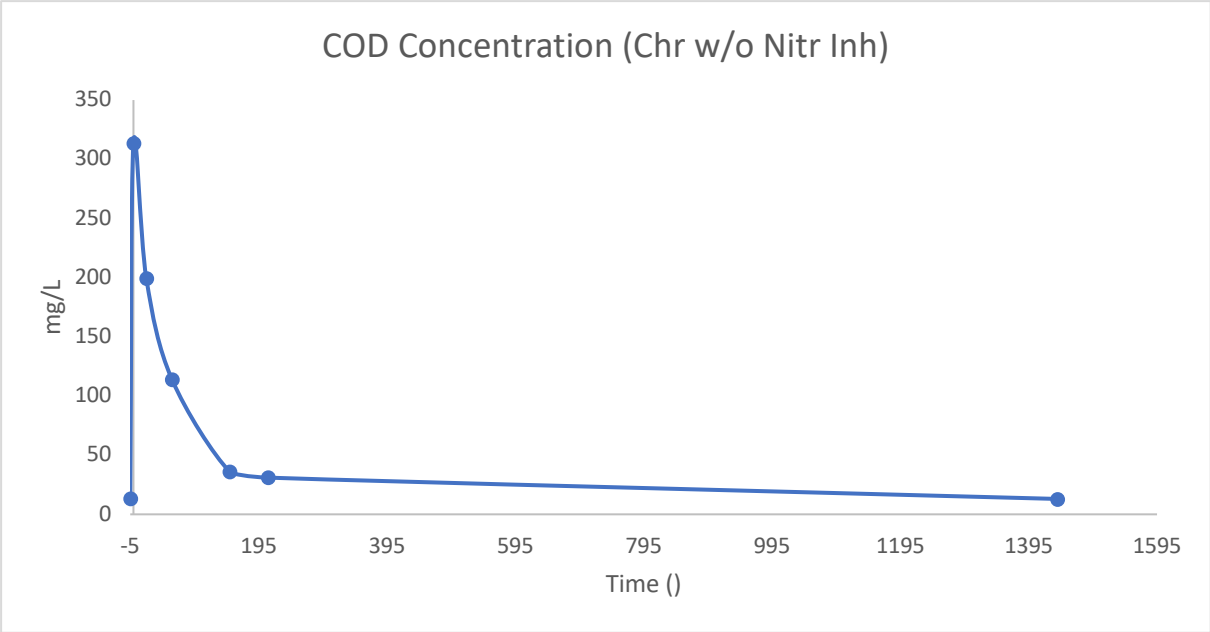

Figure 27. COD concentration during the OUR test for chronic test without nitrogen inhibitor.

5. Conclusions

The objective of the thesis was to determine the chronic impact of CHL on the microorganisms in biological wastewater treatment systems. Based on this objective, a model synthetic substrate called the peptone mixture was used and the performance of the wastewater treatment plant was determined by conducting experimental analysis on the effluent and the sludge itself from the bioreactor. However, it is important to understand in the reactor, COD removal, nitrification and denitrification take place simultaneously. Modelling studies are used to get a clear picture of the process and to conclude. This study should be considered far more significant now that the use of disinfectants has increased considerably.

The results did not suggest any inhibition of COD removal caused by addition of CHL. However, the OUR curves showed that there is an impact. It was observed that in the OUR tests microorganisms used more oxygen for growth in the presence of CHL, which could be a result of microorganisms ability to tolerate the concentration of CHL used in this study or their ability to use CHL as a carbon source. Chronic tests showed a higher oxygen consumption by the microorganisms than the acute test, which can be acclimation due to time with the presence of CHL.

Nitrogen mass balance given in this thesis were only given as an approach. These results will further be analysed by activated sludge modelling. Moreover, the CHL samples collected during the tests will be measured to conclude on the theory of CHL being used as a carbon source.

6. References

(1) *History and Evolution of Surface Disinfectants*. PDI Healthcare. https://pdihc.com/blog/history-and-evolution-of-surface-disinfectants/ (accessed 2023-03-02).

(2) Guo, J.; Liao, M.; He, B.; Liu, J.; Hu, X.; Yan, D.; Wang, J. Impact of the COVID-19 Pandemic on Household Disinfectant Consumption Behaviors and Related Environmental Concerns: A Questionnaire-Based Survey in China. *J. Environ. Chem. Eng.* **2021**, *9* (5), 106168. https://doi.org/10.1016/j.jece.2021.106168.

(3) Dewey, H. M.; Jones, J. M.; Keating, M. R.; Budhathoki-Uprety, J. Increased Use of Disinfectants During the COVID-19 Pandemic and Its Potential Impacts on Health and Safety. *ACS Chem. Health Saf.* **2022**, *29* (1), 27–38. https://doi.org/10.1021/acs.chas.1c00026.

(4) Committee, N. R. C. (US) S. D. W. *The Chemistry of Disinfectants in Water: Reactions and Products*; National Academies Press (US), 1980.

(5) *Biocides: introduction to regulation, supply and use - Biocides - HSE*. https://www.hse.gov.uk/biocides/introduction.htm (accessed 2023-03-02).

(6) McDonnell, G.; Russell, A. D. Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clin. Microbiol. Rev.* **1999**, *12* (1), 147–179.

(7) Disinfectant. *Wikipedia*; 2023.

(8) *How Does Disinfectant Work?* https://www.reagent.co.uk/blog/how-does-disinfectant-work/ (accessed 2023-03-03).

(9) Choi, D.; Oh, S. Removal of Chloroxylenol Disinfectant by an Activated Sludge Microbial Community. *Microbes Environ.* **2019**, *34* (2), 129–135. https://doi.org/10.1264/jsme2.ME18124.

(10) Chloroxylenol. *Wikipedia*; 2022.

(11) Ma, K.; Zhou, L.; Bai, Y.; Xin, Y.; Chen, M.; Li, H.; Bao, C.; Zhou, Y. Degradation and Mechanism Analysis of Chloroxylenol in Aqueous Solution by Gas–Liquid Discharge Plasma Combined with Ozonation. *RSC Adv.* **2021**, *11* (21), 12907–12914. https://doi.org/10.1039/D1RA01886H.

(12) *Chloroxylenol (PCMX), a disinfectant disinfectant.* https://www.myskinrecipes.com/shop/en/disinfectant/7286-chloroxylenol-pcmx-disinfectant-

disinfectant.html (accessed 2023-03-04).

(13) Amusan, B. O.; Koleosho, A. F.; Richard, G. E. *First Report on the Acute Toxicity of Chloroxylenol to the Whirligig Beetle, Orectogyrus Alluaudi (Coleoptera: Gyrinidae)*; preprint; In Review, 2022. https://doi.org/10.21203/rs.3.rs-1493089/v1.

(14) Tan, J.; Kuang, H.; Wang, C.; Liu, J.; Pang, Q.; Xie, Q.; Fan, R. Human Exposure and Health Risk Assessment of an Increasingly Used Antibacterial Alternative in Personal Care Products: Chloroxylenol. *Sci. Total Environ.* **2021**, *786*, 147524. https://doi.org/10.1016/j.scitotenv.2021.147524.

(15) Zhang, H.; Tang, W.; Chen, Y.; Yin, W. Disinfection Threatens Aquatic Ecosystems. *Science* **2020**, *368* (6487), 146–147. https://doi.org/10.1126/science.abb8905.

(16) *Chemical Waste That Impact on Aquatic Life or Water Quality*. http://blog.idrenvironmental.com/chemical-waste-that-impact-on-aquatic-life-or-water-quality (accessed 2023-03-03).

(17) Tuser, C. *What is Wastewater?*. Wastewater Digest. https://www.wwdmag.com/wastewater-treatment/wastewater-treatment/article/10938418/what-is-wastewater (accessed 2023-04-14).

(18) water science school. *Wastewater Treatment Water Use | U.S. Geological Survey*. https://www.usgs.gov/special-topics/water-science-school/science/wastewater-treatment-water-use (accessed 2023-04-14).

(19) *What is Wastewater? - Definition from Trenchlesspedia*. https://www.trenchlesspedia.com/definition/3361/wastewater (accessed 2023-04-14).

(20) Jiao, Y. *Domestic Sewage - an overview | ScienceDirect Topics*. https://www.sciencedirect.com/topics/engineering/domestic-sewage (accessed 2023-04-14).

(21) *What are the different types of domestic wastewater?*. SafeGroup. https://thesafegroup.co.uk/guides/what-are-the-different-types-of-domestic-wastewater/ (accessed 2023-04-14).

(22) thomas, oliver; thomas, marie- florence. *Industrial Wastewater - an overview | ScienceDirect Topics*. https://www.sciencedirect.com/topics/earth-and-planetary-sciences/industrial-wastewater (accessed 2023-04-14).

(23) *Wastewater - What Is It?*. UNL Water. https://water.unl.edu/article/wastewater/wastewater-what-it (accessed 2023-04-14).

(24) henze, morgens; Comeau, Y. *Henze 08Wastewater characterization - © 2008 Mogens Henze. Biological Wastewater Treatment: - Studocu*. https://www.studocu.com/no/document/norges-miljo-og-biovitenskapelige-

universitet/renseteknologi-for-vann-og-avlop-grunnkurs/henze-08wastewater-characterization/34330020 (accessed 2023-04-14).
(25) Novo, C. *What is a WWTP?*. Smart Water Magazine. https://smartwatermagazine.com/q-a/what-a-wwtp (accessed 2023-04-16).
(26) *The four stages of wastewater treatment plants - IDRICA*. https://www.idrica.com/blog/stages-of-wastewater-treatment-plants/ (accessed 2023-04-16).
(27) *Understanding water quality*. Understanding water quality. https://www.open.edu/openlearn/nature-environment/environmental-studies/understanding-water-quality/nature-environment/environmental-studies/understanding-water-quality (accessed 2023-04-16).
(28) Teymoorian, T.; Teymourian, T.; Kowsari, E.; Ramakrishna, S. Direct and Indirect Effects of SARS-CoV-2 on Wastewater Treatment. *J. Water Process Eng.* **2021**, *42*, 102193. https://doi.org/10.1016/j.jwpe.2021.102193.
(29) Feb 01, 2001. *Basics of wastewater disinfection* -. Environmental Protection. https://eponline.com/articles/2001/02/01/basics-of-wastewater-disinfection.aspx (accessed 2023-04-16).
(30) Li, Y.; Ling, J.; Xue, J.; Huang, J.; Zhou, X.; Wang, F.; Hou, W.; Zhao, J.; Xu, Y. Acute Stress of the Typical Disinfectant Glutaraldehyde-Didecyldimethylammonium Bromide (GD) on Sludge Microecology in Livestock Wastewater Treatment Plants: Effect and Its Mechanisms. *Water Res.* **2022**, *227*, 119342. https://doi.org/10.1016/j.watres.2022.119342.
(31) *The Activated Sludge Process | Cropaia*. https://cropaia.com/blog/activated-sludge/ (accessed 2023-04-16).
(32) *How Does Activated Sludge Wastewater Treatment Work? | AOS*. AOS Treatment Solutions. https://aosts.com/how-does-activated-sludge-wastewater-treatment-work/ (accessed 2023-04-16).
(33) *Activated Sludge Process | IWA Publishing*. https://www.iwapublishing.com/news/activated-sludge-process (accessed 2023-04-16).
(34) *Toprak Home Page*. https://web.deu.edu.tr/atiksu/toprak/kinetik.html (accessed 2023-04-16).
(35) *Suspended Growth Treatment Systems Selection Guide: Types, Features, Applications | GlobalSpec*. https://www.globalspec.com/learnmore/manufacturing_process_equipment/environmental_instruments_equipment/suspended_growth_treatment_systems (accessed 2023-04-24).
(36) Ltd, N. W. S. P. *What is Suspended growth treatment process in SBR*. Water Treatment

Company in India - Buy STP, ETP, RO Plant | Netsol Water. https://www.netsolwater.com/suspended-growth-treatment-process-in-sbr.php?blog=1661 (accessed 2023-04-24).

(37) Ghangrekar, M.; Behera, M. Suspended Growth Treatment Processes. In *Comprehensive Water Quality and Purification*; 2014; pp 74–89. https://doi.org/10.1016/B978-0-12-382182-9.00087-6.

(38) Moving Bed Biofilm Reactor. *Wikipedia*; 2023.

(39) Frankel, T. *What Is MBBR Wastewater Treatment & How Does It Work? | SSI*. SSI Aeration. https://www.ssiaeration.com/what-is-mbbr-how-does-it-work/ (accessed 2023-04-24).

(40) *Moving Bed Biofilm Reactor*. https://www.lenntech.com/processes/mbbr.htm (accessed 2023-04-24).

(41) Ltd, N. W. S. P. *How does MBBR wastewater treatment work - Netsol Water*. Water Treatment Company in India - Buy STP, ETP, RO Plant | Netsol Water. https://www.netsolwater.com/understanding-the-process-of-mbbr-via-diagram.php?blog=1381 (accessed 2023-04-24).

(42) *Moving Bed Biofilm Reactor Wastewater Treatment Systems*. SSI Aeration. https://www.ssiaeration.com/mbbr-systems-ssi/ (accessed 2023-04-24).

(43) *Lesson 8: Nitrification and Denitrification*. https://water.mecc.edu/courses/Env149/lesson8_print.htm (accessed 2023-04-24).

(44) *seed sludge*. https://www.owp.csus.edu/glossary/seed-sludge.php (accessed 2023-05-07).

(45) *Sludge Bacterial Seeding for Biological System*. http://www.wastewatersystem.net/2011/05/sludge-bacterial-seeding-for-biological.html (accessed 2023-05-07).

(46) *Solids Retention Time | SSWM - Find tools for sustainable sanitation and water management!* https://sswm.info/content/solids-retention-time (accessed 2023-05-07).

(47) Frankel, T. *Anoxic vs. Anaerobic vs. Aerobic Wastewater Treatment | SSI*. SSI Aeration. https://www.ssiaeration.com/anoxic-vs-anaerobic-vs-aerobic-wastewater-treatment/ (accessed 2023-05-07).

(48) *Sequence Batch Reactor (SBR) | SSWM - Find tools for sustainable sanitation and water management!* https://sswm.info/step-nawatech/module-1-nawatech-basics/appropriate-technologies-0/sequence-batch-reactor-%28sbr%29 (accessed 2023-05-11).

(49) Annunziato, P. *Wastewater Solids (TSS or VSS) | bioprocessH2O*.

https://www.bioprocessh2o.com/blog/waste-water-solids-tss-or-vss (accessed 2023-04-25).
(50) *What are Suspended Solids? - Definition from Corrosionpedia*. Corrosionpedia. http://www.corrosionpedia.com/definition/1063/suspended-solids-ss (accessed 2023-04-24).
(51) *Total Suspended Solids*. Lower Fox Demonstration Farms Network. https://fyi.extension.wisc.edu/foxdemofarms/the-basics/total-suspended-solids/ (accessed 2023-04-24).
(52) ebs. *Total Suspended Solids (TSS) & Volatile Suspended Solids (VSS)*. EBS. https://www.ebsbiowizard.com/articles/total-suspended-solids-tss-volatile-suspended-solids-vss/ (accessed 2023-04-25).
(53) Entegris. *Filtration Fundamentals: Membrane Wetting*. https://blog.entegris.com/filtration-fundamentals-membrane-wetting (accessed 2023-05-01).
(54) *Water for COD - Chemical Oxygen Demand | Application | Water Purification | Merck*. https://www.merckmillipore.com/NO/en/water-purification/learning-centers/applications/environment-water-analysis/cod/CLqb.qB.BIMAAAFAZwsQWTdi,nav?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bd=1 (accessed 2023-04-25).
(55) *Chemical Oxygen Demand (COD) - Water Quality Parameter | Hach*. https://www.hach.com/parameters/chemical-oxygen-demand (accessed 2023-04-25).
(56) 109773_hpk_eng.Pdf. https://mibio.ru/docs/111/109773_hpk_eng.pdf (accessed 2023-04-27).
(57) 114547e.Pdf. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/389/837/114547e.pdf (accessed 2023-04-27).
(58) 114764e.Pdf. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/241/475/114764e.pdf (accessed 2023-04-27).
(59) 114544e.Pdf. https://www.sigmaaldrich.cn/deepweb/assets/sigmaaldrich/product/documents/967/874/114544e.pdf (accessed 2023-04-27).
(60) *Respirometry | BioNinja*. https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/28-cell-respiration/respirometry.html (accessed 2023-04-24).
(61) Bm-Respirometry_21300.Pdf. https://www.surcis.com/en/bm-respirometry_21300.pdf (accessed 2023-04-24).
(62) Bm-Evo2_eng_17167.Pdf. https://www.surcis.com/en/bm-evo2_eng_17167.pdf

(accessed 2023-04-24).
(63) *SURCIS - BM-EVO2 - BM Multi-purpose Respirometry System - Multi-Purpose Double Respirometer System by SURCIS, S.L.* https://www.environmental-expert.com/products/surcis-model-bm-evo2-multi-purpose-double-respirometer-system-617286 (accessed 2023-04-24).
(64) ebs. *Dissolved Oxygen Uptake Rate (DOUR) Test*. EBS. https://www.ebsbiowizard.com/articles/dissolved-oxygen-uptake-rate-dour-test/ (accessed 2023-04-24).
(65) *SURCIS - BM-Advance2 - BM Multi-purpose Respirometry System - Multi-Purpose Double Respirometer by SURCIS, S.L.* https://www.environmental-expert.com/products/surcis-model-bm-advance2-multi-purpose-double-respirometer-649607 (accessed 2023-05-01).
(66) Practical-Bm-Respirometry-for-Asp_15205.Pdf. https://www.surcis.com/es/practical-bm-respirometry-for-asp_15205.pdf (accessed 2023-05-01).
(67) Mutag BioChip 30 Operation Manual_eng.Pdf.
(68) Bm-Evo_eng_19121.Pdf. https://www.surcis.com/en/bm-evo_eng_19121.pdf (accessed 2023-05-01).