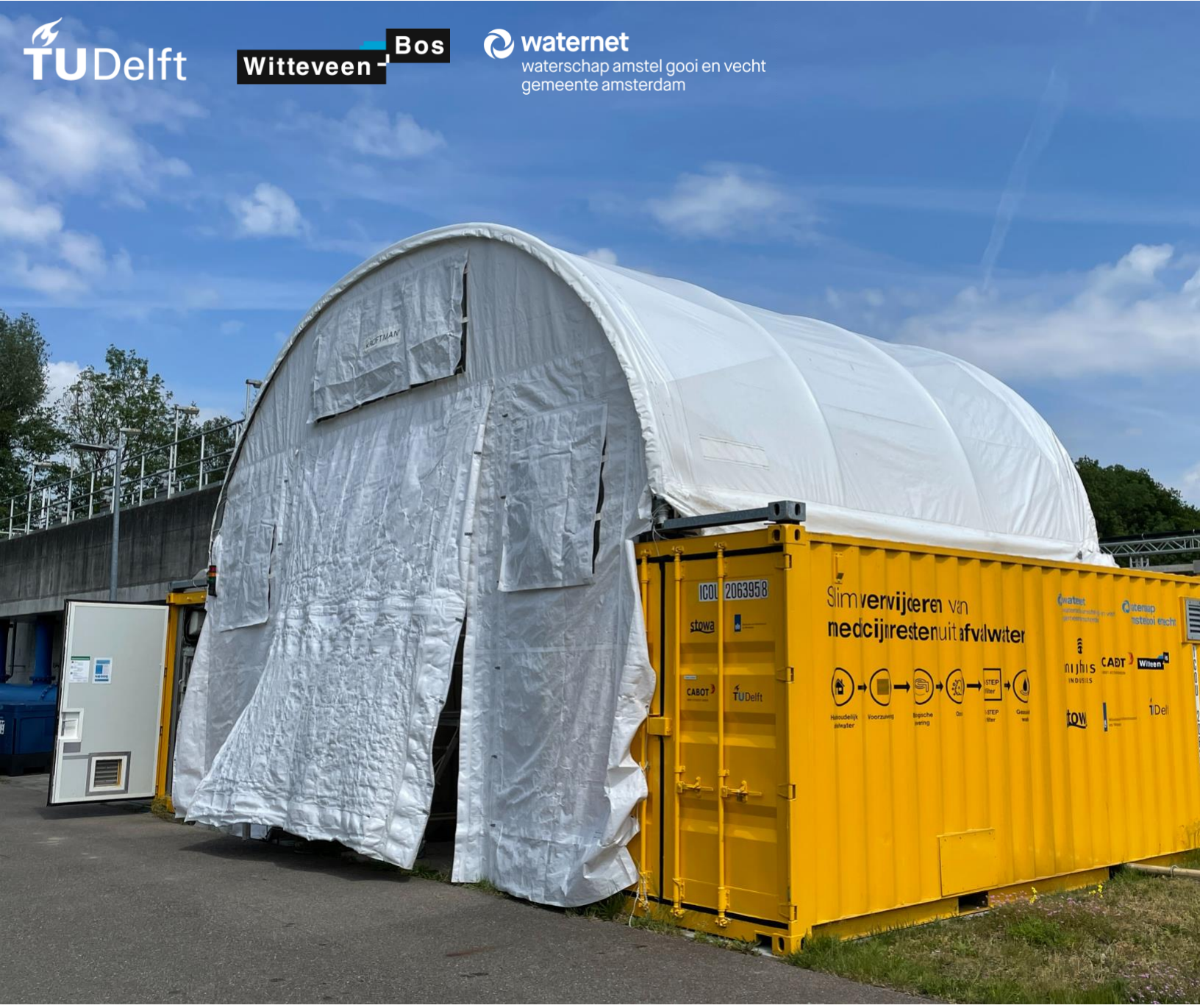

Biological Bromate Reduction in the O_3-STEP Filter: Mechanism and Relationship with Denitrification

A MASTER THESIS REPORT BY TIANYI DENG

Biological Bromate Reduction in the O_3-STEP Filter: Mechanism and Relationship with Denitrification

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This report marks not only the end of the hard work I put into the thesis over the past ten months but also the end of my fruitful two-and-a-half years at TU Delft as a master's student. The feelings are mixed as I am writing down these words.

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Tianyi (Paul) Deng
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Abstract

Bromate is a possible human carcinogen that does not naturally exist in surface or groundwater bodies. Its formation mostly results from ozonating bromide-containing water during wastewater treatment or drinking water production. Bromate is difficult to remove from water due to its high solubility and low reactivity in the aqueous environment; however, some bacteria showed the ability to reduce bromate to bromide, but the mechanism is unknown. Previous studies postulated a cometabolic pathway of biological bromate reduction, a side reaction of denitrification using the same enzymes. This study investigates the biological bromate reduction mechanism with a focus on its relationship with the denitrification process.

Wastewater and biologically active granular activated carbon (BAC) from a methanol-supplemented pilot filter called O_3-STEP in the wastewater treatment plant (WWTP) Horstermeer, the Netherlands, were used for research. This study first measured the crucial water quality parameters at eight different heights in the filter to investigate the redox condition's influence on biological bromate removal. After that, batch experiments were conducted to validate the findings. The filter showed the ability to remove bromate as it lowered the bromate concentration from 2.7 to 0.9 µg/L. Bromate reduction happened at all depths including the supernatant, although the redox conditions significantly changed. Decreasing nitrate and dissolved oxygen (DO) concentrations did not change the bromate reduction rate in the filter. The batch experiments confirmed that nitrate did not affect bromate reduction. However, a DO concentration of 8 mg/L led to a 50% reduced bromate reduction rate compared to anoxic conditions. Experiments with varying chemical oxygen demand (COD, in the form of methanol) concentrations showed an extensive accelerating effect on bromate reduction. This explained why the bromate reduction rate was not lower at high DO levels in the filter, as the high COD concentration promoted bromate reduction. Nitrate reduction was found to have a high positive correlation with bromate reduction in both filter and batch experiments, indicating similarities in their mechanisms. Nitrate reduction happened under highly oxic conditions. The intensive mixing of the granules in the filter may have provided alternating aeration and anoxic conditions for the enrichment of aerobic denitrifiers.

This study is the first study to observe simultaneous nitrate and bromate reduction under oxic conditions. Taken together, biological bromate reduction is likely to be a synergetic cometabolic process of aerobic denitrification. The robustness of the biological bromate reduction under high DO and nitrate conditions enables the O_3-STEP® filter to steadily produce bromate-free effluents under more extreme influent conditions.

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Abbreviations

TrOC	Trace organic compound
OMP	Organic micropollutant
WWTP	Wastewater treatment plant
WFD	European Water Framework Directive
DBP	Disinfection byproduct
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
GAC	Granular activated carbon
BAC	Biologically active activated carbon
MAR	Managed aquifer recharge
DO	Dissolved oxygen
SS	Suspended solids
WHO	World Health Organization
NOM	Natural organic matter
DOC	Dissolved organic carbon
DNRA	Dissimilatory nitrate reduction to ammonium
HRT	Hydraulic retention time
MBfR	Membrane film reactor
IEMR	Ion-exchange membrane bioreactor
COD	Chemical oxygen demand
IC	Ion chromatography
VSS	Volatile suspended solids
OUR	Oxygen uptake rate
SEM	Scanning electron microscope
EBCT	Empty bed contact time
EPS	Extracellular polymeric substance

Chapter 1: Introduction

1.1 Background

Trace organic compounds (TrOCs), also known as emerging or organic micropollutants (OMPs), have raised increasing public concerns because of their toxicity, bioaccumulation, and persistence to degradation. As a hotspot for OMPs, wastewater acts as the media to convey OMPs to the aquatic ecosystem. Drinking water may be contaminated by OMPs if wastewater is discharged to surface water bodies that are used as drinking water sources. Although the impacts of OMPs in aquatic environments are not well known, studies have indicated that they are likely to have acute and chronic effects on the ecosystem and human health (Dehdashti et al., 2020; Grandclément et al., 2017). Wastewater treatment plants (WWTPs) are OMPs' last stop before entering the ecosystem. They are thus essential in preventing the spread of OMPs to the ecosystem. However, conventional WWTPs have no or very limited removal ability of OMPs, rendering them a major source of OMPs discharge to the aquatic system (Choi et al., 2022b). The European Water Framework Directive (WFD) published in 2000 listed 45 priority substances or groups of substances whose loads should be reduced in surface waters. A variety of OMPs was included in this list. The directive prompted many investigations on OMPs removal technologies, including adsorption (Reungoat et al., 2010; Tong et al., 2019), filtration (Reungoat et al., 2010; J. Wang et al., 2020), advanced oxidation (Choi et al., 2022b; Hollender et al., 2009; Yang et al., 2014), biodegradation (J. Wang et al., 2022, 2020) and combined processes (Choi et al., 2022a; Echevarría et al., 2019). Ozonation has been proven to be an effective method to break down OMPs and many methods combine ozonation with a filtration step to achieve high OMP removal (Echevarría et al., 2019; Hollender et al., 2009; Reungoat et al., 2010). Eliminating more resistant OMPs by a full-scale post-ozonation followed by sand filtration installation was found to be more than 85% at a medium ozone dose (~0.6 g O_3 / g DOC) (Hollender et al., 2009). Another full-scale installation using ozonation and activated carbon filtration with an ozone dosage of 0.5 g O_3 / g DOC achieved more than a 90% reduction of 50 OMPs among 54 that were quantified (Reungoat et al., 2010).

1.2 Problem Statement: Bromate Removal

A significant disadvantage of ozonation is bromate (BrO_3^-) formation, a major disinfection byproduct (DBP) of ozonation, and a possible human carcinogen in drinking water. Bromate formation occurs when the water treated with ozonation contains bromide (Br^-). Discharging industrial wastewater treated by ozonation was suspected to primarily contribute to the bromate concentration in surface water (Butler et al., 2005).

Bromate is very stable in aqueous environments due to its high solubility and low reactivity in water. Conventional water treatment technologies such as filtration or chlorination cannot remove it from water (Assunção et al., 2011). Therefore, WWTPs should control bromate formation better during ozonation to limit bromate concentration in aquatic environments. Meanwhile, it is also essential to develop novel and effective bromate-removal methods for WWTPs to ensure low bromate concentrations in their effluent if they fail to limit the bromate formation during ozonation. Otherwise, bromide levels in some catchment areas will effectively preclude ozonation as an option to remove OMPs based on a higher-than-acceptable level of bromate production. Current bromate removal technologies can be classified into three categories, physical, chemical, and biological methods. Physical methods include adsorption (Kirisits et al., 2000), filtration (Lin et al., 2020), and electrodialysis (Wiśniewski et al., 2011); while dosing coagulant or reductant represents standard chemical methods (Gordon et al., 2002). Although these methods are proven to remove bromate from water, their disadvantages are more worrying and thus render them not cost-effective for full-scale implementation in WWTPs. Filtration has frequent clogging problems and adsorption technologies face quick breakthroughs of pollutants, while limited operational conditions and low treatment efficiency are common problems of coagulant dosing (Jahan et al., 2021).

In comparison, biofilm reactors have shown much potential for full-scale implementation to remove bromate. These reactors are mostly designed as denitrifying units for nitrogen removal in wastewater or drinking water. Later studies have found many of them able to remove bromate too. Various denitrifying biofilm reactors have demonstrated the ability to remove bromate, including biologically active activated carbon filters (BAC), fixed film

filters, membrane reactors, and managed aquifer recharge (MAR) (Hijnen et al., 1999; Jahan et al., 2021; Wang et al., 2018a). The removal efficiency of those methods can reach up to 100% and stabilize throughout the operational period due to microbes' ability to remove bromate. However, a lot is still unknown about the mechanism of biological bromate removal in these reactors. Understanding its mechanism will help find the optimal operation conditions for those reactors to limit bromate emissions to the environment.

Chapter 2: Theories and Literature Review

Relevant concepts and theories about the formation and removal of bromate are discussed in this chapter.

2.1 O_3-STEP filter

The O_3-STEP filter concept is designed as a polishing step to remove OMPs and nutrients from WWTP effluent. It combines two readily available technologies – ozonation and GAC filtration (1-STEP® filter) (Figure 2-1).

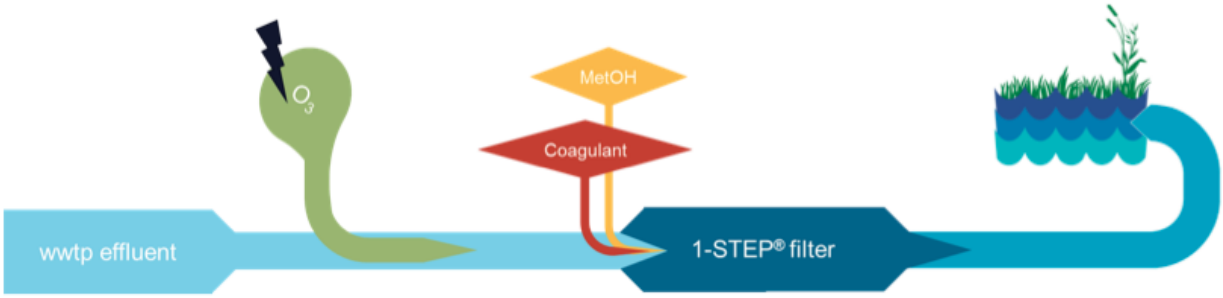

Figure 2-1 Schematic overview of the O3-STEP filter concept (STOWA, 2020)

The initial intention of designing the O_3-STEP filter is to extend the lifetime of the 1-STEP® filter because it needs frequent regeneration every 4 to 6 months as it reaches the adsorption capacity (STOWA, 2020). Standard methods for GAC regeneration include thermal, chemical, and electrochemical approaches (Narbaitz and McEwen, 2012). They require high energy input or chemical dosage, meaning high cost and less sustainable. The O_3-STEP filter is proven to effectively extend the lifetime of the filter bed to 12 to 30 months (STOWA, 2020) due to the addition of ozonation.

Figure 2-2 Illustration of the functions of the 1-STEP® filter

In Horstermeer, the Netherlands, the filter concept was tested in a pilot setup. The pilot received wastewater effluent from WWTP Horstermeer. In the ozonation tank, OMPs are broken down into smaller molecules. Later in the 1-STEP® filter, suspended solids (SS), residues, transformation products, and ozone-resistant OMPs were removed by adsorption. A coagulant was dosed to remove phosphorous (P), and methanol (CH_3OH) was dosed as the electron donor for heterotrophic denitrification, where nitrogen (N) is removed. Heterotrophic denitrification is an anoxic process conducted by a group of bacteria – heterotrophic denitrifiers, who use nitrate as the electron acceptor to

ultimately oxidize carbon sources and produce nitrogen gas when oxygen concentration is low (<0.5 g/L). The denitrification process is very sensitive to oxygen. Therefore, it is essential to keep an anoxic condition for denitrification in the filter.

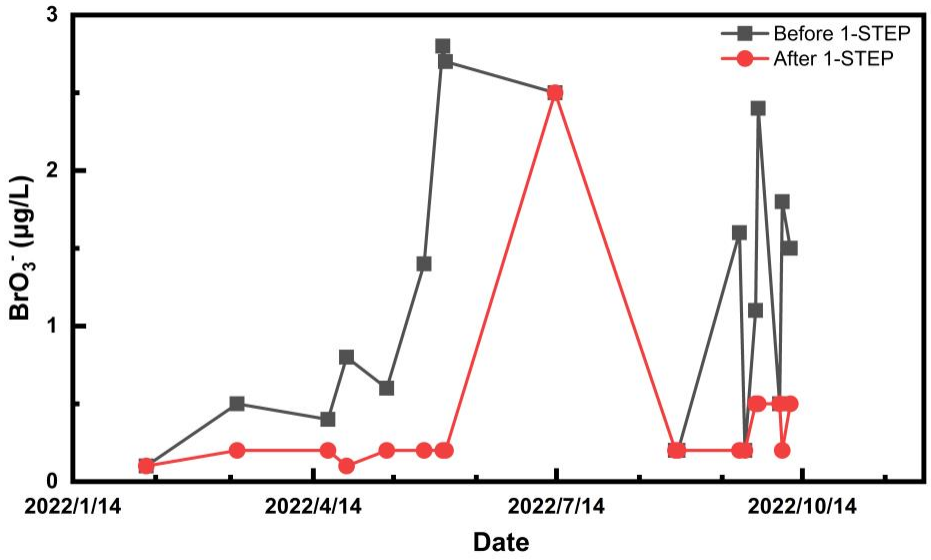

Figure 2-3 Bromate and bromide concentrations in O3-STEP filter

Although not designed for bromate removal, measurements showed an apparent constant reduction of bromate concentration in the 1-STEP® filter over the period of operation (Figure 2-3). In June 2022, a bromide-spiking experiment was undertaken at the pilot by the water company Waternet. 2.7 µg/L bromate was present in the water before the 1-STEP® filter, which was later reduced to lower than 0.2 µg/L in the effluent. It is obvious that the 1-STEP® filter is removing bromate, but the mechanism is unknown.

Table 2-1 Bromate concentrations before and after the 1-STEP® filter, Waternet, May 2022.

Exp nr.	Bromate before GAC (µg/L)	Bromate after GAC (µg/L)
1	1.4	0.2
2	2.8	0.2
3	2.7	0.2

2.2 Toxicity of Bromate

Although bromate's influence on human health is still unknown, it has been proven to cause renal cell tumors in rats and male mice that ingest aqueous bromate, which might indicate similar effects on humans (DeAngelo et al., 1998; Kurokawa et al., 1990, 1986). The World Health Organization (WHO) thus classified bromate as a Group 2B carcinogen or "possible human carcinogen" and recommended a provisional guideline concentration value of 10 μg/l in drinking water (WHO, 2005). The presence of bromate in surface water has also attracted attention because surface water is being used as the primary drinking water source in many areas around the world, and current drinking water treatment plants are not designed for bromate removal. To regulate bromate discharge, the Dutch National Institute for Public Health and the Environment, Rijksinstituut voor Volksgezondheid en Milieu (RIVM) recently proposed a bromate standard of 1 μg/L for the surface waterbodies near drinking water intake points in the Netherlands. In addition to human health concerns, the ecotoxicity of bromate also received attention. Limited studies showed that fish eggs exposed to bromate developed chronic, pathological disorders, particularly in the brain and spine (Burton and Richardson, 1981), and a study on marine phytoplankton discovered that 13.6 mg/L bromate increased cell division in at least two of the four species (Hutchinson et al., 1997). Above all, bromate is potentially harmful in aquatic systems and precautionary guidelines should be made to lower its risk to human health and the ecosystem.

2.3 Presence of Bromate in Water Systems

Bromate does not exist naturally in either surface waters or aquifers (Soltermann et al., 2016), meaning that its source in natural water systems is anthropogenic. The industries use several bromate salts commercially; the most prevalent are sodium bromate ($NaBrO_3$) and potassium bromate ($KBrO_3$), colorless and odorless soluble crystals in water. Potassium bromate is a powerful oxidant once widely employed as a food additive, especially as a bread dough conditioner. Nonetheless, in Europe and many other parts of the world, it is no longer legal to be used in the food sector due to health concerns surrounding bromate. In the United States, it is still used in malting barley (Health

Canada, 2015; RIVM, 2021). Because bromate salts are very stable in water, industry wastewaters may contribute to the bromate concentration in natural water systems once discharged even though it was not a common contaminant that was detected in waterbodies until recently. The DBP formation during the treatment of bromide-containing wastewater is believed to have caused the increasing bromate concentrations in natural waterbodies (Butler et al., 2005). As public concern about OMPs grows, WWTPs are implementing ozonation as a quaternary treatment to reduce OMP concentrations in their effluents. It contributes to the increasing bromate concentrations in natural waterbodies because of the prevalence of bromide in most water systems.

2.4 Environmental Characteristics of Bromate

The most prevalent bromate salts, potassium bromate, and sodium bromate are both crystalline solids readily dissolvable in water (potassium bromate solubility is 75 g/L at 25 ℃) (Butler et al., 2005). In the aquatic environment, bromate solutes are highly stable at room temperature – they do not volatilize and cannot be removed by boiling. Moreover, the abiotic degradation rate of bromate is relatively insignificant in a natural context, although it is thermodynamically a powerful oxidant (RODGERS, 1980). The high solubility of bromate salts increases the chance of waterbody contamination following any industrial spill, and the low reactivity rate indicates its conservative character in surface and groundwaters.

2.5 Presence of Bromide in Water Systems

Contrary to bromate, bromide is a prevalent ion in most water systems, and it is the precursor of bromate formation. Bromide sources in aquatic environments are complex and can be traced back to both natural existence and anthropogenic activities. Oceans are the most prominent natural reservoir of the bromine element, where the element exists in the form of bromide. Because of seawater intrusion, bromide concentrations in near-coast groundwater can be high. Bromide can end up in wastewater when drinking water production uses groundwater because drinking water brings bromide to the urban water system. In terrestrial water systems, the primary natural source of bromide is seaborne aerosols which are transported through the wind (Soltermann et al., 2016). Therefore,

bromide concentrations in precipitations, groundwater, and soil decrease further inshore, making its natural background concentration in European natural soft waters vary in an extensive range from approximately 30 to 200 μg/L (Butler et al., 2005; Legube, 2008). This natural distribution, however, is frequently influenced by anthropogenic sources (Soltermann et al., 2016). Bromine is widely used in industries, and those industrial processes often generate bromide as the ultimate product, which ends up in the industrial wastewater effluents. For example, an estimated 52% of the bromide load in the catchment of Weil am Rhein in Switzerland is from the chemical industry (Soltermann et al., 2016). Before the mid-1980s, up to 70% of the global bromine production was used as an additive to leaded gasoline. Later, it was also utilized in pesticide production, de-icing salt for roads, biocides in pool water, and cooling water for industries (Soltermann et al., 2016). In many WWTPs, ozonation is used for disinfection (Metcalf et al., 2004). Therefore, the ozone dosage is often decided by its efficiency in bacteria removal, mostly E-coli as the indicator bacterium. The determination of E-coli removal efficiency is usually very conservative, leading to an ozone overdose in most WWTPs. A bromide concentration level of 50-100 μg/l may already be problematic regarding the excessive bromate formation during the ozonation process. For waters containing more than 100 μg/l bromide, bromate formation can become a serious problem if the WWTP has high pathogen removal standards and minimal competition substances for bromide (von Gunten, 2003).

2.6 Bromate Formation during Ozonation of Wastewater

2.6.1 The reaction of Bromide with Ozone

The mechanism of bromate formation in ozonated water is complicated and highly non-linear (von Gunten, 2003). Two complex oxidation pathways can be differentiated based on the intermediates and oxidants involved. The two essential oxidants during the oxidation process are molecular ozone (O_3) and hydroxyl radical ($^{\bullet}OH$). In aquatic environments, ozone is naturally unstable, so it decomposes into molecular oxygen (O_2) and hydroxyl radicals (Metcalf et al., 2004). As a result, hydroxyl radicals are common in ozonated water. They are even more powerful oxidants than molecular ozone and can convert bromide ions to bromide radicals (Br·) (Jarvis et al., 2007). Bromide radicals can

then be oxidized to bromate ions by molecular ozone or hydroxyl radicals or a combination of both through complex oxidation processes (Pinkernell and von Gunten, 2001). The other oxidation pathway from bromide to bromate only includes molecular ozone as the sole oxidant and hypobromite and bromite are the intermediates. The simplified illustration of the oxidation process is shown in Figure 2-4.

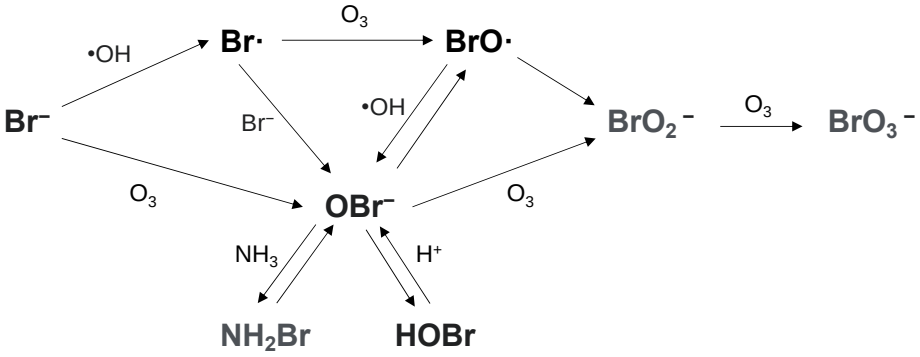

Figure 2-4 Oxidation of bromide to bromate, revised from (Jarvis et al., 2007)

2.6.2 Factors that Influence Bromate Formation during Ozonation

Bromate yield from bromide oxidation largely depends on the ozone dosage and water matrix. A study used wastewater with different water qualities from seven Swiss WWTPs to test their suitability for ozonation. Different wastewaters treated by the same ozone dosage showed a wide range of bromate yield from 0.3 to 31.7% mg BrO_3^- / mg Br^- (0.2-19.8% mol BrO_3^- / mol Br^-); In addition, increasing specific ozone dosages[1] resulted in increasing bromate formation in all wastewaters (Schindler Wildhaber et al., 2015). In the second oxidation pathway introduced in section 2.6.1, hypobromite is a vital intermediate that influences the bromate yield. As is shown in Figure 2-4, hypobromous acid can be formed from hypobromite in certain pH and temperature ranges. However, the reaction rate of hypobromous acid with molecular ozone is slower than that of hypobromite, meaning that the production of bromate can be largely influenced by pH and temperature (Jahan et al., 2021). Generally, higher pH and temperature favor bromate formation during ozonation (Siddiqui and Amy, 1993). Moreover, the concentration of ammonia (NH_3) also affects the yield of bromate because the oxidation of bromide and hypobromite

[1] To help compare different ozone dosages, specific ozone dosage is often used. It is expressed as grams of ozone per gram of dissolved organic carbon (DOC).

ions is relatively slow compared to the reaction between hypobromite and ammonia. Therefore, more hypobromite can be more easily used to oxidize ammonia than be oxidized by ozone. Natural organic matter (NOM) and nitrite (NO_2^-) are also proven to scavenge the ozone concentration in treated water, thus impeding the oxidation of bromide to bromate (Legube et al., 2004). Generally, Soltermann et al. found in Swiss waterworks that typical ozone dosages below 0.4~0.5 g O_3/g DOC caused minimal bromate formation in bromide-containing waters, although the tested water varied in their water qualities. Because bromate formation is a slow process while other substances in water like the NOMs consume ozone quickly (Soltermann et al., 2016). When ozone dosage is low, it can become the limiting factor of bromate formation.

2.7 Biological Bromate Degradation

Previous studies on the biological reduction of bromate have mainly focused on the characterization of bromate-reducing bacteria. So far, no precise mechanisms or reduction pathways have been identified (Jahan et al., 2021). Most postulations of the mechanism encompass the analogy to the denitrification process, and the suggested bromate reduction pathways mainly surround the theory of co-metabolism. Recently, studies have shown the potential existence of specific bromate reduction pathways. In the O_3-STEP pilot, it is unknown if these pathways exist. It is, therefore, crucial to investigate the mechanisms for better control of bromate concentrations in wastewater treatment processes. This section summarizes the current knowledge of biological bromate reduction mechanisms and the characterization of bromate-reducing bacteria.

2.7.1 Natural Respiration of Bacteria

Classification of Microorganisms

All microorganisms need sources of carbon and energy to synthesize new cells, grow, and maintain the existing cells. Classification of microorganisms can be made based on their carbon and energy sources. In the natural environment, depending on the carbon source, microorganisms can be classified as heterotrophs who take up organic carbon or autotrophs who use inorganic carbon, e.g., carbon dioxide, as their carbon source. Organisms that obtain their energy from solar radiation or oxidation are called

phototrophs or chemotrophs, respectively (Metcalf et al., 2004). The biodegradation process where bacteria gain energy from oxidation reactions is called bacterial respiration. Natural bacterial respiration is well understood and widely utilized in wastewater and drinking water treatment processes to remove DOC and nutrients. Microorganisms in biological wastewater treatment processes are all chemotrophs. The partition between heterotrophs and autotrophs depends on the wastewater quality and operating conditions (Metcalf et al., 2004).

Selectivity of E-acceptors

Chemotrophs need an electron donor (e-donor) and an electron acceptor (e-acceptor) for their bacterial respiration. In a natural aquatic setting, there is often more than one e-donor and acceptor. As a result, bacteria tend to favor the reactions that generate more energy for their respiration. Thus, e-donors and e-acceptors that can provide more energy for bacteria's anabolic activities are usually used for respiration. Jørgensen (Jørgensen, 1989) made a schematic illustration demonstrating the fate of the most common e-donor in nature - organic matter when various e-acceptors are present in the saturated zone (Figure 2-5). Thermodynamically, oxygen (O_2) is the preferred e-acceptor as it has the highest energy yield for bacteria. Therefore, aerobic bacteria use oxygen in the saturated zone to oxidize organic carbon until almost depletion. When oxygen becomes limited, facultative anaerobes switch to using both oxygen and nitrate (NO_3^-) as e-acceptors. As oxygen concentration decreases, obligate anaerobes begin to use nonoxygenous e-acceptors starting from nitrate. Other compounds with lower energy yields, namely manganese (Mn), ferric iron (Fe^{2+}), and sulfate (SO_4^{2-}), will be utilized as e-acceptor by bacterial species able to metabolize them when the previous one depletes (Butler et al., 2005; Dell, 2020; Korom, 1992).

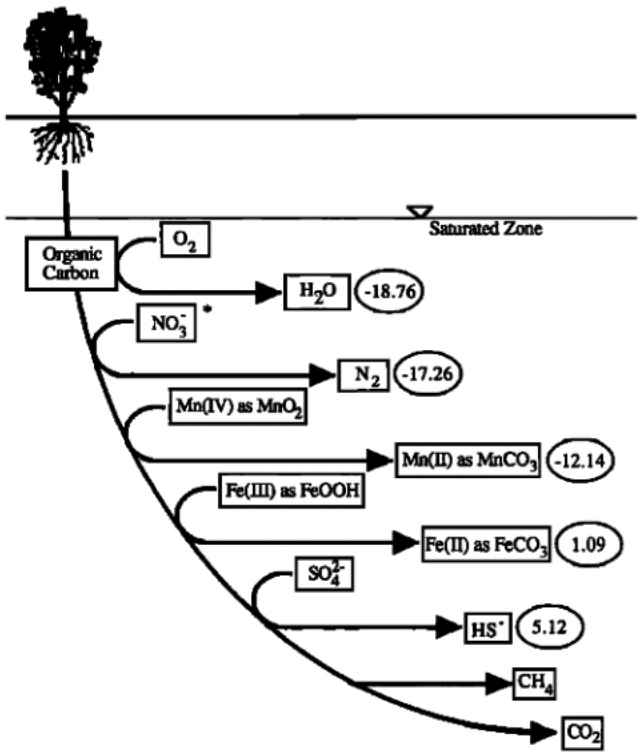

Figure 2-5 Illustration of the degradation of organic carbon in the soil environment. Redox potentials are noted in circles after the substances (Jørgensen, 1989) .

Denitrification

Specifically, bacterial respiration reactions where nitrate is reduced to nitrous oxide (N_2O) and dinitrogen gas (N_2) are referred to as denitrification. The physiological property of denitrification belongs exclusively to bacteria; however, not all bacteria can denitrify. The ones that can denitrify are referred to as denitrifiers, and they are a very diverse group of bacteria that includes more than 27 genera (Dell, 2020; Korom, 1992). Most denitrifiers are heterotrophic and facultative anaerobes, meaning they can survive with or without oxygen. *Pseudomonas* is, for example, one typical species of this kind. Few denitrifiers are autotrophic and use inorganic compounds as e-acceptors, e.g., manganese, ferrous iron, and sulfides. This process is called autotrophic denitrification. Some denitrifiers can only survive by utilizing nitrate as their e-acceptor in an anoxic environment, and they are called obligate anaerobes. Although denitrifiers are somewhat diverse, they all contribute to nitrate reduction in the same reduction pathway, which is shown in steps with the corresponding oxidation states of nitrogen in each molecule in Figure 2-6.

$$NO_3^- (+5) \rightarrow NO_2^- (+3) \rightarrow NO (+2) \rightarrow N_2O (+1) \rightarrow N_2 (0)$$

Figure 2-6 Nitrate reduction pathway during denitrification (Korom, 1992)

Each step in the pathway is realized by a discrete enzyme system (Butler et al., 2005). Some denitrifiers can perform the entire nitrate reduction pathway from nitrate to dinitrogen gas, depending on the enzymes they produce. Meanwhile, others are only able to facilitate individual processes (Dell, 2020). Therefore, the overall denitrifying microflora can be regarded as a consortium composed of complementary bacterial strains that only when the function of each strain is combined can convert nitrate to dinitrogen gas (Butler et al., 2005).

Dissimilatory Nitrate Reduction to Ammonium (DNRA)

DNRA is a very similar anaerobic process to denitrification. It provides bacteria with the energy for cell growth and maintenance. The difference between DNRA and denitrification is the end product. In WWTPs, denitrification converts nitrate into dinitrogen gas. In contrast, DNRA converts nitrate to ammonium, which may increase the risk of ammonium being oxidized to nitrate by nitrification when the effluent enters surface water. Wang et al.'s study showed that DNRA bacteria widely occurred in WWTPs in China with abundance levels lower than those of denitrifiers (S. Wang et al., 2020). Because DNRA and denitrification share the same substrates, the DNRA process competes with denitrification. Wang et al.'s investigation indicated that in WWTPs, denitrifiers ARE more advantageous in the competition. However, DNRA could be favored over denitrification in nitrate-limiting conditions (S. Wang et al., 2020). Other studies argued that the importance of DNRA in N removal was positively correlated with a more reduced environment, e.g., lower redox potential and higher C/N ratio (Chen et al., 2015; Shan et al., 2016).

2.7.2 Natural Bromate Biodegradation

Bromate is not a common e-acceptor in natural environments. Studies on other possible electron acceptors than the ones shown in Figure 2-5 included chlorate, perchlorate,

selenate, chromate, iodate, and bromate. They show that biological bromate reduction would be expected to happen prior to all but denitrification and aerobic respiration, based on thermodynamics (Butler et al., 2005). This was confirmed by Hijnen et al. (Hijnen et al., 1995)'s research, where bromate was enzymatically reduced to bromide in a mixed denitrifying bacteria culture after nitrate was utterly degraded. However, it is unknown whether this process happens in natural aquifers as degradation patterns are highly site-specific. The availability and concentration of e-donors, and the heterogeneous spatial distribution of other e-acceptors, i.e., oxygen and nitrate, will significantly impact natural bromate biodegradation. Natural biological bromate reduction may happen in small and specific pockets in natural aquifers. However, investigating its patterns has been limited in a generally oxic and organic matter-limiting environment (Butler et al., 2005).

2.7.3 Mechanisms of Biological Bromate Reduction

The understanding of the biological bromate reduction mechanism is limited due to the lack of research in this area. However, research suggests that the oxyanion reduction pathways are more interlinked than previously known (Butler et al., 2005). Therefore, postulations about bromate reduction pathways mainly encompass the analogy to the well-studied denitrification process. In addition, studies on (per)chlorate reduction pathways have received more attention in recent years due to rising issues surrounding groundwater (per)chlorate contamination. Those studies, in combination with the extensive knowledge of denitrification, may provide more insights into bromate reduction pathways.

Early studies of biological bromate reduction suggest the bromate reduction pathway to be more of a cometabolic process that relies on denitrification enzymes. In a co-metabolic process, bromate reduction happens fortuitously as a side reaction of denitrification, utilizing the same enzymes that catalyze the denitrification process. Bacteria cannot harness the generated energy from the cometabolic process for growth or maintenance. In a mixed microbial community that was mainly composed of denitrifiers, near stoichiometric anaerobic bromate reduction to bromide during which ethanol was the energy source was observed (Hijnen et al., 1995). Bromate has been known to be a

substrate for purified nitrate reductase and Hijnen et al. suggested that nitrate reductase might be involved in the biological bromate reduction process (Hijnen et al., 1995; Morpeth and Boxer, 1985; Yamamoto et al., 1986). Moreover, Zhong et al. found that the denitrifying microbial community that can reduce bromate was phylogenetically diverse at the phylum level (Zhong et al., 2018). Nevertheless, bromate reduction is not a functional characteristic shared by all denitrifiers, as Downing and Nerenberg illustrated that denitrifier *Ralstonia eutropha* was incapable of bromate reduction (Downing and Nerenberg, 2007).

Bacteria with functions other than denitrification are also possible to reduce bromate. Nitrate-respiring but non-denitrifying isolates whose growth was linked to nitrate reduction without dinitrogen gas production were also found capable of reducing bromate (Hijnen et al., 1995). In addition to the potential role of nitrate reductase on co-metabolic bromate reduction, other reductases also demonstrated the ability to catalyze bromate reduction. Purified (per)chlorate reductase was observed to reduce bromate by Kengen et al. (Kengen et al., 1999), and Martin et al. demonstrated bromate reduction without measurable cell growth by the perchlorate reducing *Dechloromonas sp. PC1* (Martin et al., 2009a). Furthermore, the selenate reductase of *Enterobacter cloacae* expressed aerobically was found capable of reducing bromate at low rates, specifying the possibility of bromate reduction by aerobic bacteria (Ridley et al., 2006).

In contrast to cometabolic reduction pathways, a specific reduction pathway is catalyzed by its specific enzyme. Whether the energy can be utilized by cell growth is not a detrimental factor in identifying this process. Van Ginkel demonstrated bromate as a terminal electron acceptor for the anaerobic growth of an enrichment culture adapted to bromate. In addition, no other electron donors, including perchlorate, chlorate, and nitrate, were reduced by the same culture, which is promising evidence for a specific bromate reduction pathway (van Ginkel et al., 2005). Furthermore, the abundance of perchlorate-reducing *Dechloromonas* increased by 17% in a mixed denitrifying culture without nitrate and (per)chlorate after adding bromate (Luo et al., 2017). Overall, current research suggests diversity in biological bromate reduction mechanisms, with co-

metabolism of bromate through nitrate or (per)chlorate reductase and specific bromate reduction pathways that can be both dissimilatory and non-dissimilatory.

2.7.4 Influencing Factors of Biological Bromate Reduction

Presence of E-acceptors

Theoretically, high DO levels affect the formation and activity of nitrate reductase for the co-metabolic nitrate and bromate reduction process (Kirisits and Snoeyink, 1999). Furthermore, since the enzyme used by the co-metabolic process is non-specific, competitive inhibition of the less-preferred substrate is expected. Kirisits et al. calculated and compared nitrate, bromate, and oxygen reduction potentials, showing that bromate was the most potent oxidant (Kirisits and Snoeyink, 1999). However, in most studies, the bromate reduction only happened in the anoxic environment and when nitrate was depleted, indicating a solid inhibition of bromate reduction by the presence of oxygen and nitrate. Other oxyanions, e.g. (per)chlorate, sulfate, and phosphate have not yet been found to inhibit biological bromate reduction.

Nonetheless, the degree of competitive inhibition in co-metabolism is highly dependent on the bacterial species, the type of enzyme involved, the ions' diffusivity, etc. (Sharp et al., 2010). It is thus not definitive that co-metabolic bromate reduction only happens in the absence of oxygen and nitrate. Recently, Zhong et al. showed a microbial community where 99.1% were denitrifiers capable of degrading bromate when 25 mg N/L nitrate was present in a rotating biofilm-electrode reactor (Zhong et al., 2018). Liu et al. demonstrated a complete reduction of bromate in a biologically active activated granular activated carbon filter (BAC) by denitrifying and chlorate-reducing bacteria at a DO level of 8 mg/L (Liu et al., 2012). These phenomena may indicate a species that can reduce bromate when relatively high concentrations of oxygen or nitrate are present. Further studies of the species are needed as nitrate contamination is becoming a severe issue in many parts of the world; thus, inventing effective technologies for a broader range of water qualities is pivotal.

Gradients in the biofilm

Nitrate and oxygen gradients have been found in microbial flocs. The gradients create local differences in nitrate and oxygen concentrations within the biofilm. Theoretically, the concentrations drop from the water-microbial interface to deeper into the biofilm. It is possible that bromate can be reduced when oxygen and nitrate reach a level at which bromate becomes thermodynamically more advantageous in the competition.

Water Quality Factors

In addition to competing oxyanions, other water quality factors also influence bromate reduction. It was reported that an increase in influent pH from 6.8 to 8.2 caused a decrease in the bromate removal rate in a lab-scale BAC reactor by around 20%. The optimum pH for biological bromate reduction in the same study was suggested from 6.8 to 7.2 (Kirisits et al., 2001). Additionally, more basic environments tend to be more inhibitory to biological bromate reduction than acid environments (Downing and Nerenberg, 2007). Moreover, a self-inhibition of bromate reduction by an extensive influent bromate concentration of 5 mg/L was observed (Martin et al., 2009b). The inhibition was likely due to the toxicity of the intermediate product – bromite (BrO_2^-), which was not seen to accumulate during the reduction of bromate to bromide in all reported studies (van Ginkel et al., 2005).

2.8 Bromate Reduction by Biological Methods

A few wastewater treatment technologies have been studied for biological bromate reduction to date. Currently, major units in the wastewater treatment process that include biological treatment mainly utilize either aerobic or anaerobic biological respiration to remove COD and nutrients in wastewater streams. Those units' oxygen and nitrate concentrations are typically too high for effective biological bromate reduction. Operational parameters must be modified to achieve bromate removal, e.g., hydraulic retention time, which may increase the operational cost. Hijnen et al. demonstrated bromate removal from groundwater in a denitrifying bioreactor where ethanol was supplemented as the carbon source. Restrained by the need for excessive ethanol dosage

and extensive post-treatment of the effluent, a denitrifying reactor presented little potential for bromate remediation in wastewater treatment (Hijnen et al., 1999).

Following the increasing attention on OMP removal, more WWTPs are implementing quaternary treatment technologies, which are more advantageous to couple bromate removal, because nitrate concentrations are much lower in quaternary treatment units compared to that in primary treatment units where high concentrations of ammonium are oxidized to nitrate, simultaneous bromate and nitrate removal can thus be achieved under certain operational conditions. Kirstis et al. achieved 86% bromate removal in a lab-scale BAC filter with an Empty Bed Contact Time (EBCT) of 25 minutes when the influent contains 0.2 mg/L nitrate and two mg/L DO (Kirisits and Snoeyink, 1999). In a later study by Liu et al., effective bromate removal in an acetate-supplemented lab-scale BAC reactor was observed at a DO level of 8 mg/L (Liu et al., 2012). It was believed that nitrate and oxygen gradients could form in the biofilm, allowing bromate and nitrate reduction to happen in different layers of the biofilm. Similar results were also found in studies using biofilm reactors. At a hydraulic retention time (HRT) of 24 hours, a membrane biofilm reactor (MBfR) supplied by methane removed all bromate in the influent when DO was 7-9 mg/L (Luo et al., 2017). However, compared to BAC filtration, most lab or bench-scale biofilm reactors require a much longer HRT to achieve bromate removal. To address this problem, Cristina et al. tested the ability of an Ion-exchange Membrane Bioreactor (IEMR) to remove bromate. The ion-exchange membrane allows bromate ions to pass through and eventually be biodegraded in the biofilm attached to the membrane. This way, bromate can stay in the biofilm for enough time to be reduced entirely without having to set long HRT for the bioreactor. Furthermore, no additional chemicals need to be added to the water stream so that secondary water pollution can be avoided (Matos et al., 2008).

Above all, the bromate reduction rates demonstrated in studies to date are similar regardless of the technology used, indicating that biological bromate reduction may be promising for implementation in WWTPs.

Chapter 3: Research Questions and Hypotheses

The cause and mechanism of bromate removal in the 1-STEP® filter were unknown. However, due to their limited adsorption capacity, GAC filters are unlikely to maintain effective bromate adsorption consistently for over one year, according to previous research on bromate adsorption in GAC filters (Bao et al., 1999; Huang and Chen, 2010; Zhang et al., 2015). Therefore, bromate reduction in the 1-STEP® filter was hypothesized to be a biological process related to denitrification.

This study aims to investigate the mechanism of biological bromate reduction in the 1-STEP® filter, especially its possible interconnection with the denitrification process. Complying with the research objective, the main research question of this study is:

How is bromate biologically removed, and what is the relationship between biological bromate reduction and denitrification?

The following sub-questions are formulated to help address the main research question:

1. *Where do nitrification and denitrification happen in the filter, respectively?*
2. *Does DO inhibit nitrate reduction, and is there another factor that influences nitrate reduction?*
3. *In which area does bromate removal take place in the filter?*
4. *Does the presence of nitrate and oxygen inhibit bromate removal in the filter?*
5. *Are there other water quality parameters that influence bromate reduction ?*
6. *Is bromate removal cometabolic in the filter?*

The hypotheses of this research are:

1. The redox conditions in the filter should be crucial to the biological processes. The filter column should be oxic at the top and anoxic or anaerobic at the bottom. Nitrification, denitrification, and bromate reduction should happen in sequence and at different depths. Nitrification occurs at the top part of the filter; denitrification starts when oxygen is depleted by nitrification; bromate reduction

happens at the bottom part of the filter when nitrate concentration becomes limited.

2. The presence of nitrate in the wastewater inhibits biological bromate reduction because denitrification and bromate reduction use the same enzymes. Since nitrate ions are thermodynamically more favorable for microbial catabolism than bromate ions, the presence of nitrate inhibits bromate reduction.
3. The backwash does not essentially influence the water qualities in the filter because the microbial composition is homogeneous in the filter column.
4. Biological bromate reduction is a cometabolic process of denitrification.

Chapter 4: Materials and Methods

4.1 Pilot Setup

This study is based on the research of the pilot O_3-STEP filter in WWTP Horstermeer, the Netherlands. Figure 4-1 shows the overview of the O_3-STEP pilot setup. The effluent from the secondary clarifiers in the main treatment plant is pumped into the ozone contactor, where ozone is dosed for the oxidation of OMPs. After the ozone contactor, the water enters a buffer tank and is introduced to the top of the 1-STEP® under gravity. Before the filter bed, methanol is dosed to the water through a pipe for oxygen depletion by aerobic respiration and subsequently denitrification. Due to the low P-level in the WWTP effluent, the coagulant was not dosed for P removal during this research. In the filter bed of the 1-STEP® filter, nitrate is converted to nitrogen gas by the biofilm attached to the carbon granules. The filter filtrate is collected in the filtrate buffer tank and discharged to the river De Vecht.

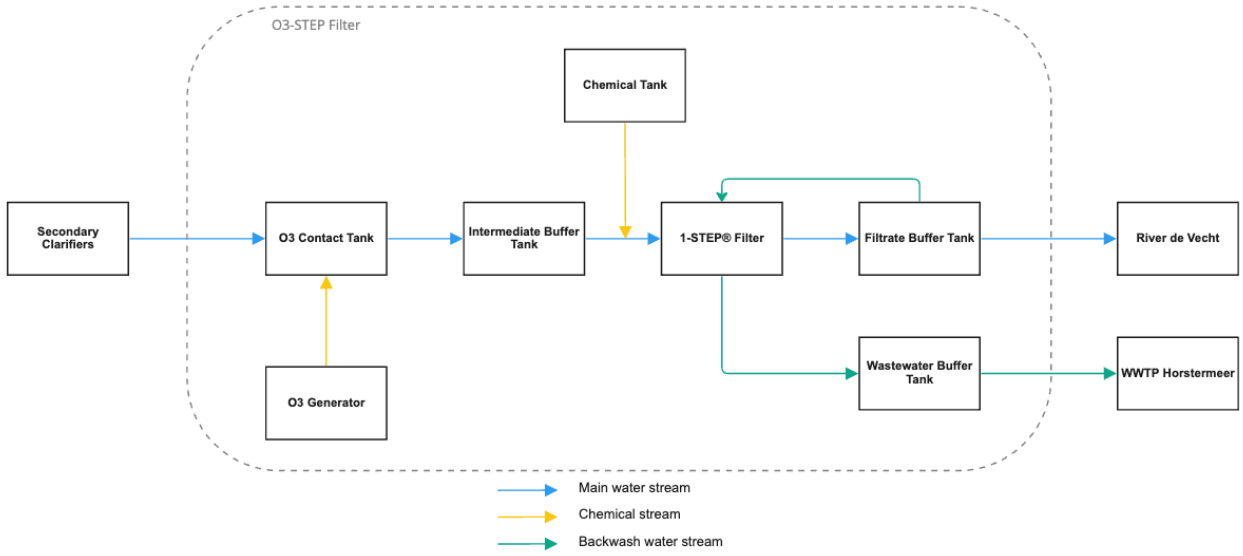

Figure 4-1 Process overview of O_3-STEP pilot

The 1-STEP® filter column had a height of 4 meters and the filter bed was 2 meters tall with 1.1 meters of supernatant above. The filter column's inner volume was 0.87 m^3 and was operated in a downflow mode with a flow rate of 3 m^3/h and a filtration rate of 13.8 m/h. The empty bed contact time (EBCT) was 17.4 minutes. The filter column was

backwashed by upflow every 260 minutes with water from the filtrate buffer tank. A more intensive backwash happened once every three backwashes, where the air was flushed from the bottom of the filter in addition to water. Each backwash by water lasted 400 seconds, and 120 seconds by air. The flow rate of the backwash was 11.3 m^3/h. Substantial filter bed expansion could be seen during backwashing. The filter column had seven designed sampling ports distributed throughout the column for water sampling, the heights of which are indicated in Figure 4-2.

Design Parameter	Value
Inner volume (m3)	0.87
EBCT (min)	17.4
GAC Height (cm)	200
Column Height (cm)	400

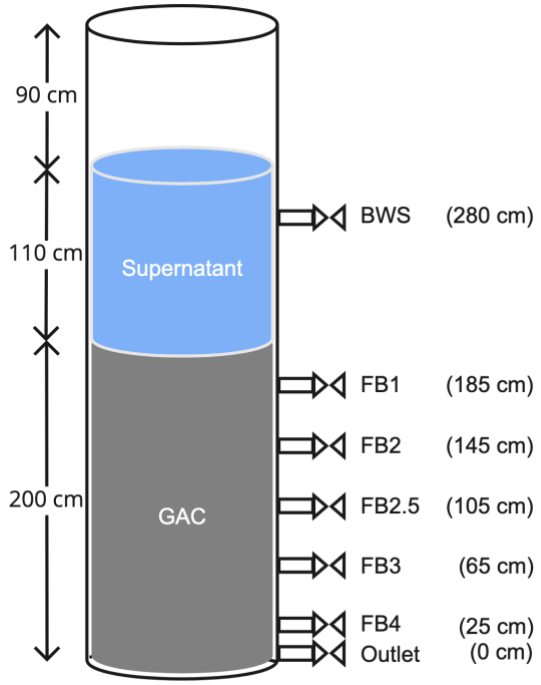

Figure 4-2 Simplified illustration of the pilot 1-STEP® filter column

4.2 Materials

4.2.1 Wastewater and GAC

The wastewater and GAC samples for the batch experiments were collected at WWTP Horstermeer in September and October 2022. The wastewater used in this study was collected from the sampling ports shown in Figure 4-2. GAC was collected from the top 50 centimeters of the filter bed using a scoop sampler. Originally the virgin GAC granules used in the pilot filter were Norit GAC 612 WFD (Norit Nederland B.V., Amersfoort, the Netherlands). Their grain sizes were 1.70-3.35 mm (6-12 mesh). Carbon collected from the filter had sizes of 4.60~9.15 mm due to biofilm growth on the granules. To minimize

microbiological activity, all wastewater and GAC samples were stored in a cold cell with the temperature controlled at 4°C. In principle, all water samples collected during this study should be used for analyses within 24 hours after collection if no specification was made.

4.2.2 Chemicals

Sodium bromate ($NaBrO_3$) and potassium nitrate (KNO_3) reagents were used in this study. Both sodium bromate and potassium nitrate powder were purchased from Sigma Aldrich (St Louis, MO, United States). All solutions were prepared using ultrapure water produced by a Milli-Q Gradient water purification system (18 MΩ·cm, Veolia). All chemicals used in this study were of analytical grade. Pure dinitrogen gas (N_2) was used for flushing the wastewater to reduce the DO level to 0.1 mg/L and lower.

4.3 Overview of Research Strategy

The experiments were divided into two phases, namely the filter characterization phase and the validation phase, as shown in Figure 4-3. The aims of phase I were first to investigate the change of redox conditions in the filter column and second to postulate possible mechanism(s) of bromate removal based on how redox conditions influenced the bromate removal. These were done by multi-element measurements on wastewater samples collected at eight locations in the filter column. Experiments in phase II aimed to validate the findings of phase I by conducting batch experiments that represented the redox conditions in some regions of the pilot filter. The influence of DO, NO_3^-, and COD on bromate removal was investigated in this phase.

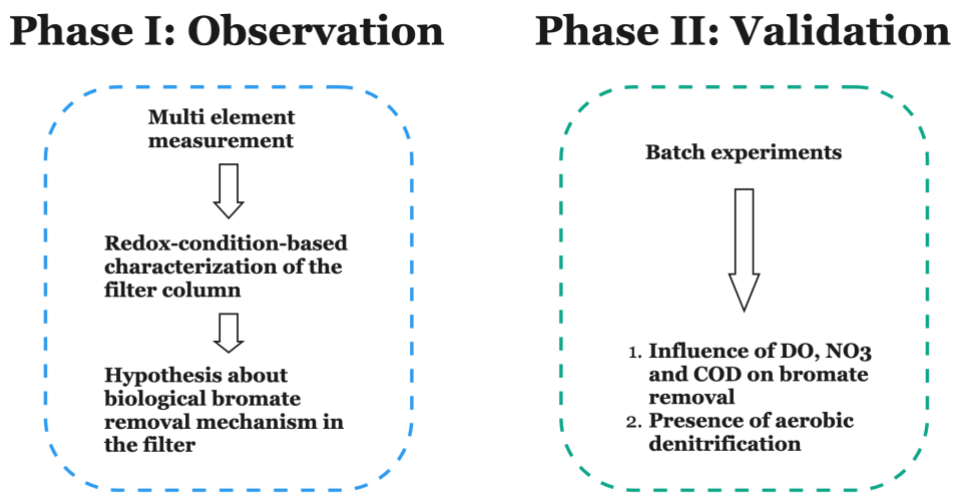

Figure 4-3 Overview of experimental strategy

4.4 Experiments

4.4.1 Characterization of the 1-STEP® filter

Redox conditions and microbial composition of the biofilm are two crucial factors that influence the microbiological behaviors of BAC filters. The redox condition is represented by the concentrations of ions that can give out or receive electrons. It changes as the wastewater flows through the filter and is likely to influence the bromate removal rate. To investigate bromate removal throughout the whole pilot 1-STEP® filter and its relationship with specific water quality parameters, water samples were taken at eight different locations in the filter. Besides the seven installed sampling ports shown in Figure 4-2, water samples were taken at the filter's inlet. Multi-element measurements were conducted within 24 hours of the sample collection. Samples were collected on the 29th of September 2022, 30 minutes before backwashing. The ozone dosage was around 1.1 g O_3/g DOC, higher than normal levels (0.4 g O_3/g DOC). 1 mg/L bromide was dosed to the influent of the O_3-STEP filter during the sampling period.

On the other hand, the author hypothesized that the microbial composition in the filter was consistent throughout the depths because of the frequent and intensive mixing of carbon granules caused by backwashing. Therefore, it was assumed that any possible difference in bromate removal rates in the filter column should not be the result of

microbial composition difference, and the sampling location of GAC should not influence the results acquired from this study. As such, the scope of this study only included the relationship between the redox condition and bromate removal. To prove the assumption, water samples were taken at 7 locations (FB2.5 not included) in the filter 20 minutes after backwashing on the 29th of September 2022. Analyses were conducted in triplicates. The parameters measured include dissolved oxygen (DO), bromate (BrO_3^-), bromide (Br^-), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), redox potential, dissolved organic carbon (DOC) and chemical oxygen demand (COD).

- Bromate and bromide are the main parameters to be analyzed. The variation of bromate concentration in the filter provides the most direct information on the bromate removal rate. The variation of bromide concentration gives insight into the bromate reduction mechanism. According to previous studies, bromate can be stoichiometrically reduced to bromide by biological activity as well as reduction by surface functional groups. In contrast, bromate removal by adsorption should, in principle, not affect bromide concentration.
- DO refers to the amount of oxygen that is dissolved in the solution. Lower DO levels were found more favorable to bromate removal (Kirisits and Snoeyink, 1999). Since the pilot 1-STEP® filter received ozonated water, a high DO was expected at the top, likely inhibiting denitrification and bromate reduction.
- Nitrate, nitrite, and ammonium concentrations are the indicators of the nitrification and denitrification process. As discussed in chapter 2, biological bromate reduction was found to be primarily affected by the denitrification process. The presence of nitrate can inhibit the biological reduction of bromate.
- Phosphate and sulfate are common oxyanions in water. Their influence on the biological bromate reduction process is still to be studied. Nevertheless, they are both potential competitors against bromate, therefore, were measured in this study.
- DOC refers to the fraction of organic carbon that can pass through a filter with a pore size between 0.22 and 0.7 μm. As biomass takes up the biodegradable fraction of DOC for their metabolism, the variation of DOC can indicate the metabolic rate of the biomass in the filter.

- Redox potential shows the tendency of a chemical species to either acquire or lose electrons. It is expressed in volts (V)—the more positive the redox potential, the greater the species' ability to acquire electrons. Every chemical species has its intrinsic redox potential. In aqueous solutions, redox potential is a measure of the tendency of the solution to either gain or lose electrons when it is subjected to change by introducing a new species. A solution with a higher (more positive) reduction potential than the new species will tend to gain electrons from the new species (i.e., to be reduced by oxidizing the new species) and vice versa. The redox potential of an aqueous solution is determined by the types of chemical species present and their concentrations. It is difficult to determine each species' redox potential in wastewater samples; thus, aqueous redox potential of the water samples was measured.

4.4.2 Batch Experiments

Figure 4-4 shows the batch configurations and the procedure of the batch experiments. Fifty-four batches were evenly divided into three groups (A, B, and C) based on the water matrix to investigate the relationship between nitrate concentration, DO and bromate removal. Before being distributed to the batches, the water was prepared in one 2L or 4L bottle to ensure a unified starting condition of the water matrix in every batch. The water used in Group A and B was taken from sampling port FB4 (bottom of the filter column) and was supplemented with certain chemicals. Water from sampling port FB1 (top of the filter column) was used for group C with a chemical addition. Bromate concentrations in both source water were below the detection limit (<1 μg/L). Therefore, all batches were spiked with 100 μg/L bromate using a 100 mg BrO_3/L $NaBrO_3$ solution. KNO_3 reagent was directly dosed to the prepared water in the 2L and 4L bottles; the specific dosage is mentioned in more detail in the following paragraphs. In groups A and B, the DO concentration was lowered to below 0.5 mg/L by sparging the prepared water with pure nitrogen gas and immediately sealing the bottles with caps and rubber stoppers to prevent any gas transfer between the bottle and the atmosphere. For group C, a high DO concentration (>8 mg/L) was maintained by the aeration stones in the batches connected to aerators. To increase the mixing condition in the batches, all batches were incubated at

150 rpm and room temperature (22-25 °C) in an incubator (New Brunswick™ Innova® 44). All analyses in this experiment were conducted in duplicates.

Groups A and B were used to investigate the influence of nitrates' presence on bromate removal under a low DO concentration. The GAC dosages (wet weight, w/v) were 120 g/L and 200 g/L in groups A and B, respectively. 12 mg/L nitrate as KNO_3 reagent was added in group A only. Two batches were taken out from the shaker every hour in the first 8 hours, and water samples were taken for Br^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, NH_4^+, and COD analyses. The last two batches were taken out and sampled at the 72nd hour.

Group C was used to investigate high DO concentration's influence on nitrate removal by the pilot GAC and its influence on bromate removal. A GAC dosage (wet weight, w/v) of 120 g/L was adopted for this group. 9 mg/L nitrate was added as KNO_3 reagent. Bottles were capped with two-port caps. One port was connected to aeration stones on one end, the aerators on the other, and the other was connected to the atmosphere by a tube to balance the air pressure in the batches. The DO concentrations were above 8 mg/L in all batches throughout the experiment. However, individual differences in DO concentrations among batches existed due to the aerator difference. Two batches were taken out from the shaker every hour in the first 8 hours, and water samples were taken for Br^-, NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, NH_4^+, and COD analyses. The last two batches were taken out and sampled at the 24th hour.

Group D aimed to investigate the influence of COD on nitrate and bromate removal under aerobic conditions. The batch in group D was prepared in the same configuration as group C. Water collected from FB4 (the bottom of the filter) was used for group D, whose COD concentration was significantly lower than FB1. Two 2L batches were used for this experiment. 10 ml samples were taken every 15 minutes for water quality analyses until the 120th minute. The aeration method was the same as group C and the DO concentrations were kept above 8 mg/L.

Table 4-1 Batch configuration

Group Name	GAC Dosage	NO_3^- Dosage	BrO_3^- Dosage	DO	Water source
A	120 g/L	12 mg/L	0.1 mg/L	<0.5 mg/L	FB4
B	200 g/L	-	0.1 mg/L	<0.5 mg/L	FB4
C	120 g/L	9 mg/L	0.1 mg/L	>8 mg/L	FB1
D	120 g/L	9 mg/L	0.1 mg/L	>8 mg/L	FB4

Bromide concentration was used as the indicator for bromate reduction. It was suggested by previous studies that bromide can be produced almost stoichiometrically from bromate reduction by both surface functional groups and bacteria (Bao et al., 1999; Kirisits and Snoeyink, 1999). To verify this hypothesis, bromate concentration was measured for group A.

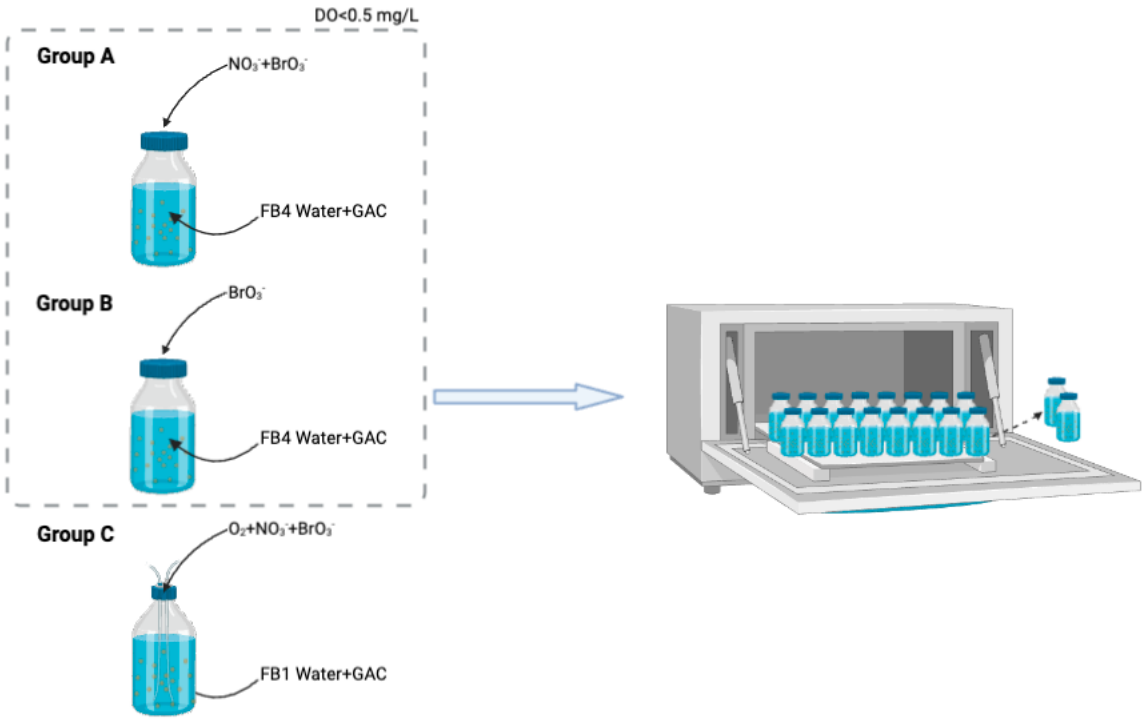

Figure 4-4 Schematic diagram of the experiment configuration

4.5 Analytical Methods

All DO and redox measurements in this study were conducted in aqueous phases and were analyzed onsite or directly from the samples taken from the batches with a multimeter (Multi 340i WTW, Germany). DO in the filter column was measured in a way that would prevent the aeration created by the water flow from the sampling port to the containers. The DO probe was placed diagonally in a beaker and measure continuously for 10 minutes while the water is flowing to the beaker. Values were recorded after 10 minutes. Bromate concentration was analyzed at Het Waterlaboratorium (Haarlem, the Netherlands) using ion chromatography (IC), conforming to the operation procedure suggested by NEN-EN-ISO 11206. The detection limit was 0.2 µg/L. Ammonium and COD concentrations were analyzed by HACH Lange DR3900 spectrophotometer using HACH kits. Nitrate, nitrite, phosphate, sulfate, and bromide concentrations were analyzed with a ProfIC 15 - AnCat ion chromatography (Metrohm 881 anion (suppressed) system) (Metrohm, Switzerland) in the Waterlab of the faculty of Civil Engineering and Geosciences, TU Delft. The A Supp 150/4.0 anion column was used for the anion measurement with 3.2 mM Na_2CO_3 and 1mM $NaHCO_3$ eluent (runs at 0.7 mL/min). The suppressor was fed with 50 mM H_2SO_4 reagent. A 100 µL sample loop was used. All samples analyzed with the IC were filtered through 0.45 µm filters (Whatman, Germany) before analyzation. The conductivity data were translated to concentration by the MagIC Net software.

Chapter 5: Results

5.1 Change of Water Quality in the 1-STEP® Filter

Multi-element measurements were conducted before and after a backwash to demonstrate the water quality change throughout the 1-STEP® filter. Water quality data from eight different depths were collected. Due to technical issues, water quality data from sampling port FB2.5 after the backwash were missing.

In this section, the changes in the selected key water quality parameters were demonstrated and analyzed to help identify or distinguish the biological and abiotic processes in the filter column.

5.1.1 Nitrification and Denitrification

DO, ammonium, nitrite, and nitrate are key representative water quality parameters for nitrification and denitrification processes. The concentration profiles of these four parameters before and after the backwash are shown in Figure 5-2.

As is shown in Figure 5-2, based on DO concentration, the filter bed can be divided into two parts: the section from 145 centimeters to 200 centimeters represented an aerobic zone where much oxygen was present and decreased to 1.65 mg/L. The water in the filter bed below the aerobic zone had nearly no oxygen and was thus anoxic. The supernatant from 200 to 300 cm was highly oxic. Besides the biofilm on the granules in the filter bed, biomass also existed in the supernatant, which can be seen in Figure 5-1.

Figure 5-1 Photograph of the supernatant in the 1-STEP filter. The brown granules are biomass.

What stands out in the figure is that the nitrate concentration decreased not only in the anoxic zone after the depletion of DO and ammonium but also in the aerobic zone and the supernatant, simultaneously with the decrease in DO and ammonium concentrations. On average, 59% of the influent nitrate was removed in the aerobic zone and the supernatant, where DO concentration reached up to 22 mg/L. Within the aerobic zone, more nitrate decrease was seen when DO was lower than 10 mg/L.

Ammonium oxidation, on the other hand, happened only in the aerobic zone and the supernatant, contributing to the decrease of DO. As for nitrite concentration, it first

increased in the aerobic zone and supernatant and then decreased in the anoxic zone until depletion.

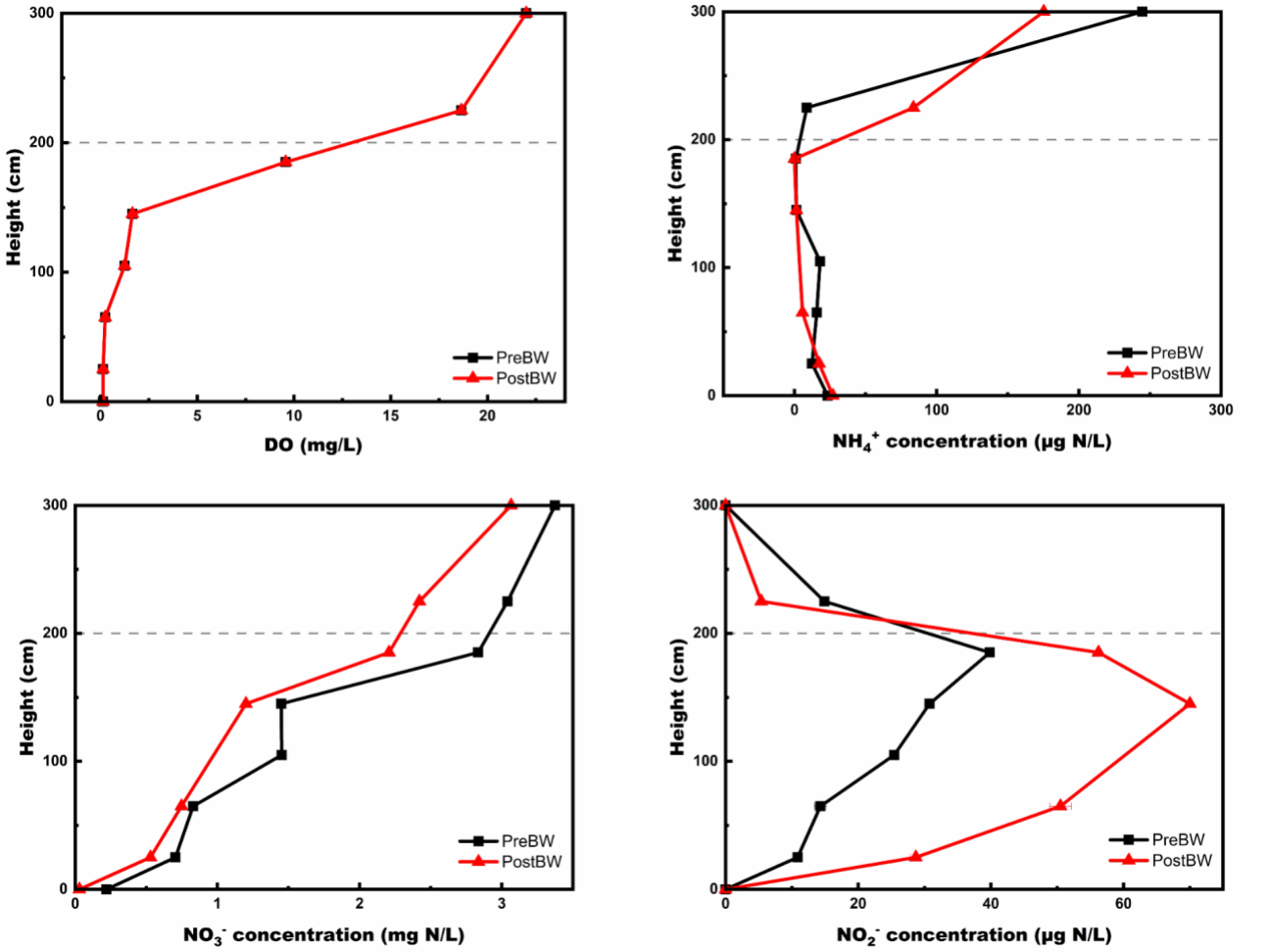

Figure 5-2 DO (upper left), ammonium (upper right), nitrate (lower left), and nitrite (lower right) concentrations over the filter column before and after backwash. The grey dash lines represent the height of the top surface of the GAC filter bed.

5.1.2 Bromate Removal

Bromate and bromide concentrations in the filter column are demonstrated in Figure 5-3. Like nitrate concentration, except at 195-centimeter height, bromate concentration kept decreasing over the filter column, and an average of 65% bromate was removed in the filter column. Bromide concentration in the filter column also decreased, by an average of 10%.

As stated in section 5.1.1, the nitrate concentration decreased throughout the filter. However, it did not affect the rate of nitrate decrease. When comparing the bromate profile with the nitrate one in Figure 5-2, significant similarities in their trends were found, especially in the filter bed. The reduction rates of both ions were lower in the anoxic zone than in the aerobic zone after backwashing. Both profiles before backwashing had a 45-centimeter zone where their concentrations remained unchanged. This observation suggests a possible connection between denitrification and biological bromate reduction.

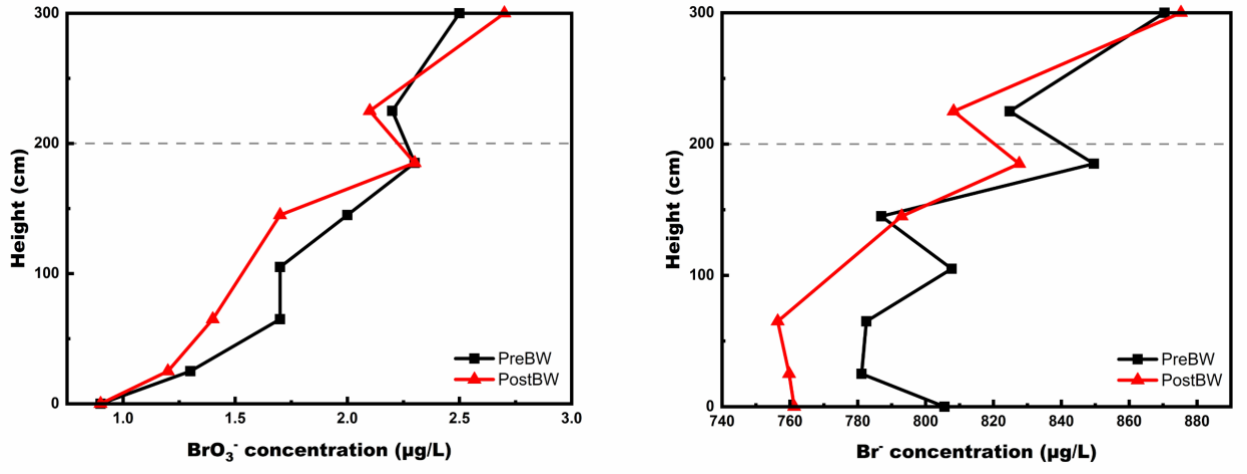

Figure 5-3 Bromate (left) and bromide (right) concentrations over the filter column before and after backwash. The grey dash lines represent the height of the top surface of the GAC filter bed.

5.1.3 Other Oxyanions

Phosphate and sulfate concentrations in the filter column are shown in Figure 5-4. Phosphate is almost depleted in the aerobic zone both before and after backwashing. In the anoxic zone, its concentration remained stable at around 8 µg/L. Its trend was similar to ammonium shown in Figure 5-2. The profile of sulfate concentration fluctuated at around 6 mg S/L at all depths. In contrast, the effluent concentrations did not see significant change compared to the influent concentrations, suggesting that no biological process was able to influence the sulfate concentration.

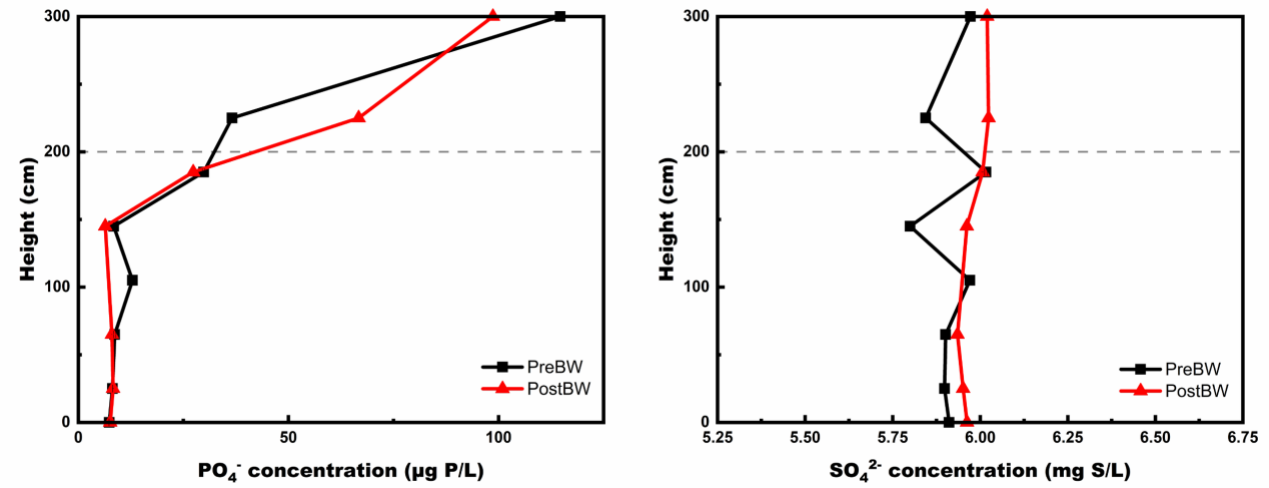

Figure 5-4 Phosphate (left) and sulfate (right) concentrations over the filter column before and after backwash. The grey dash lines represent the height of the top surface of the GAC filter bed.

5.1.4 COD consumption and Granule Morphology

The central part of the COD in the influent of the filter column was in the form of methanol (CH_3OH) dosed before the filter column to prompt the microbial activity. It is apparent in Figure 5-5 that most COD was consumed in the aerobic zone and the supernatant, below which its concentration slightly decreased (by 2.034 mg/L before backwash) or remained unchanged (after backwash).

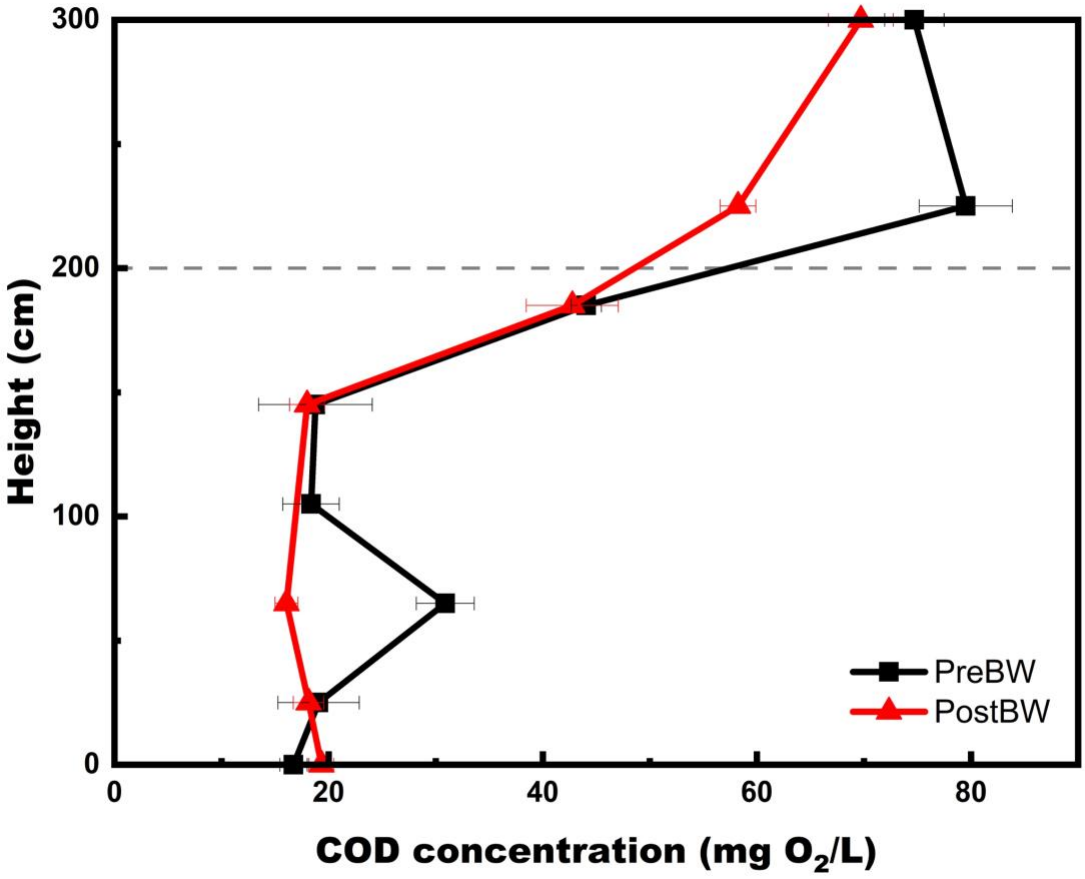

Figure 5-5 COD concentrations over the filter column before and after backwash. The grey dash lines represent the height of the top surface of the GAC filter bed.

The microscopic photograph (Figure 5-6) of an activated carbon granule from the aerobic zone showed extensive immobilized bacteria growth on the carbon surface. The thickness of the biofilm was up to 2.9 millimeters in the granule demonstrated in the photograph. Further sampling of granules in the aerobic zone revealed similar granular sizes at different areas and depths, as seen in Figure 5-7 (left). Figure 5-7 (right) shows the granules collected at the bottom of the filter column. Lower COD concentration in the anoxic zone caused less immobilized bacteria growth on the carbon surface.

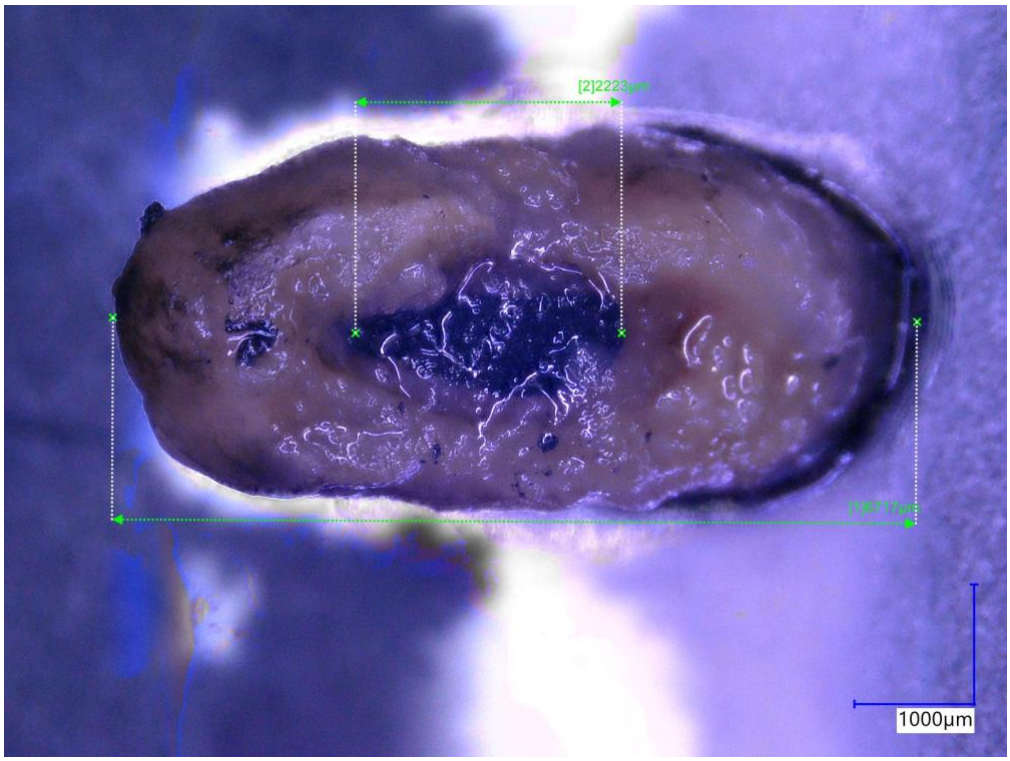

Figure 5-6 Microscopic photograph of the cross-section of a middle-sized activated carbon granule in the aerobic zone of the filter column. The black core of the granule was activated carbon with a diameter of around 2 millimeters. The pink-brownish layer surrounding the black core is the biofilm formed, with a thickness of around 2 millimeters.

Figure 5-7 Biofilm morphology and size distribution of the granules from the aerobic zone (left) and anoxic zone (right).

5.1.5 Effect of Backwash on the Water Quality in the 1-STEP® Filter

Comparing the profiles before and after backwashing, it can be seen that the backwash only had a limited impact on most water quality parameters shown in Figure 5-2, Figure 5-3, Figure 5-4, and Figure 5-5. It did not change the trend of all water quality parameters shown. For nitrate, ammonium, bromate, bromide, and COD, their profiles slightly shifted to the left after the backwash. The backwash had the most influence on nitrite concentration. A more significant increase was seen in the aerobic zone after the backwash.

5.1.6 Correlations Between Bromate Concentration and the Measured Water Quality Parameters

Correlation analyses of the measured water quality parameters were conducted using the Pearson correlation coefficient, and the results are illustrated in heat maps in 0. The result showed that the bromate concentrations are most correlated to nitrate concentrations, represented by positive correlation coefficients of 0.93 and 0.99 before and after backwashing, respectively, meaning that the decrease of the two ions may have similar

mechanisms. Phosphate (r=0.82) and sulfate (r=0.83) concentrations also showed strong correlations with bromate concentration after the backwash. However, these strong correlations were not seen in the dataset obtained before the backwash (r=0.68 for phosphate and 0.18 for sulfate). Therefore, batch experiments were conducted to investigate the relationship between denitrification and biological bromate reduction.

5.2 Batch Experiments

5.2.1 Bromide Production by Biological Bromate Reduction

Previous research has found that bromide was the end product of biological bromate reduction (Kirisits and Snoeyink, 1999), so the increase in bromide concentration would be representative of the biological consumption of bromate. To verify this assumption, bromate analysis was done on batch group A where bromide concentration increased.

As expected, a simultaneous increase in bromide concentration was observed while bromate concentration decreased. However, the increased bromide concentration (30 μg/L) only accounted for 54% of the decreased bromate concentration (56 μg Br/L).

5.2.2 Group A

Batches in group A were prepared using water from the bottom of the 1-STEP® filter with bromate and nitrate addition. The DO concentration was kept below 0.5 mg/L. The bromide and nitrate concentrations in group A are demonstrated in Figure 5-8. It can be seen that the bromide concentration drastically increased in the first hour by 19 μg/L. Then the increasing rate became lower with 17 μg/L bromide produced from the 1st to 5th hour, after which bromide concentration did not further increase. The average bromide increasing rate was 11.2 μg/(L•h) in the first five hours. A similar pattern was observed in the nitrate concentration; nitrate concentration decreased by 7 mg/L in the first hour which was followed by a 5.7 mg/L decrease from the 1st to 5th hour. No change in nitrate concentration was seen after the 5th hour.

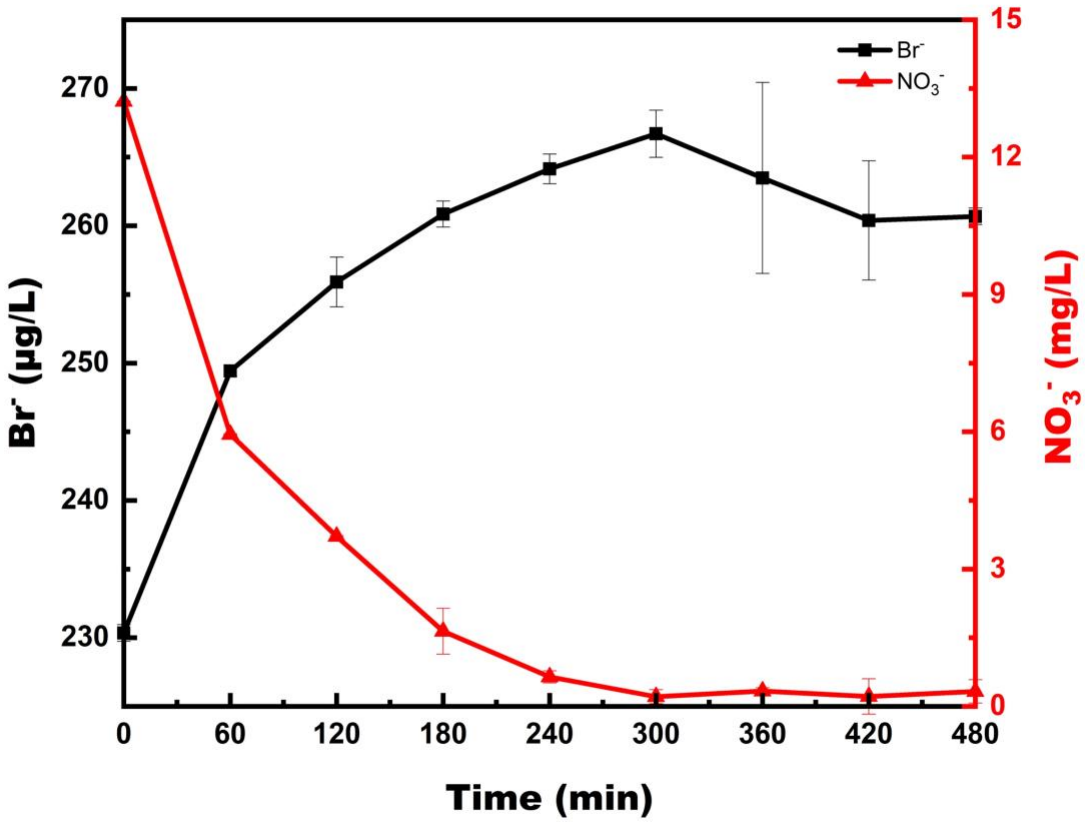

Figure 5-8 Bromide and nitrate concentrations of group A. Water taken from the bottom of the 1-STEP® filter was used with nitrate and bromate addition for this group. DO concentration was below 0.5 mg/L in the batches.

5.2.3 Group B

Batches in group B used the same water from the filter as group A however, nitrate was not added, and a 200 g/L GAC dosage was applied in this group. The bromide concentration during the experiment is shown in Figure 5-9. It is similar to group A that the most significant increase in bromide concentration was seen in the first hour, increasing from 247 µg/L to 284 µg/L. It kept increasing until the 3rd hour and reached 299 µg/L. A decrease in bromide concentration was seen from the 5th to the 7th hour. The average increasing rate of bromide concentration during the first 3 hours was 17.3 µg/L•h.

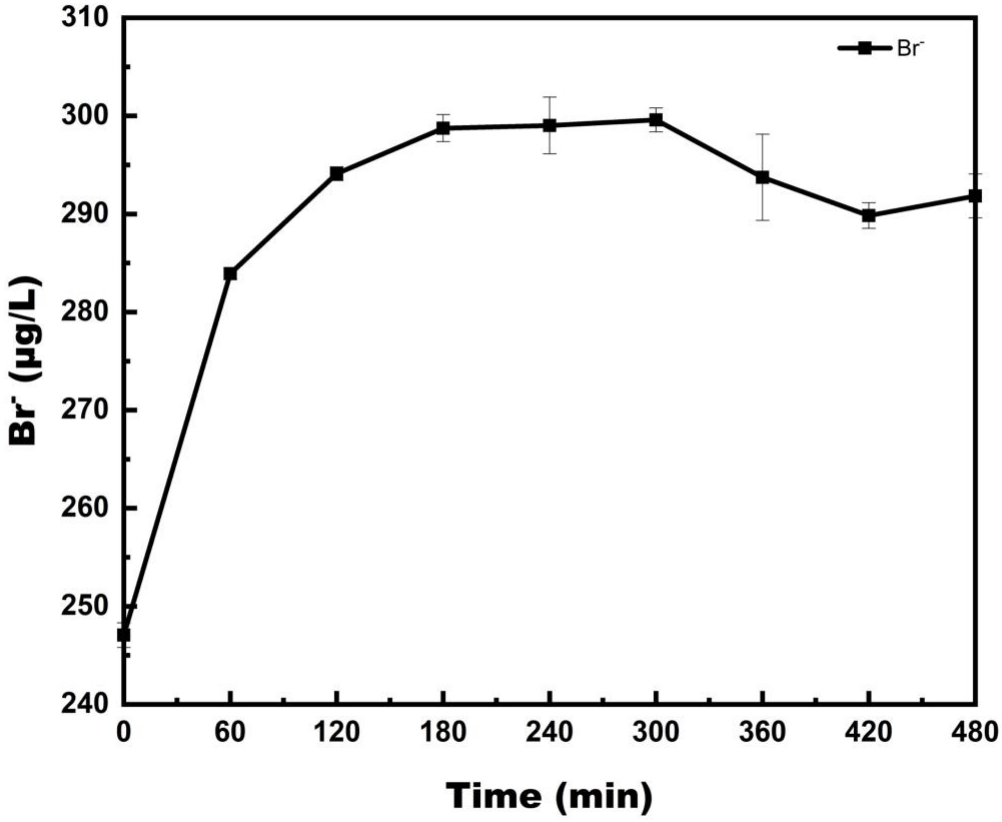

Figure 5-9 Bromide concentration of group B. Water taken from the bottom of the 1-STEP® filter was used with bromate addition for this group. DO concentration was below 0.5 mg/L in the batches.

5.2.4 Group C

In group C, water from the top of the 1-STEP® filter was used. Nitrate and bromate were added to the water with constant aeration that kept the DO above 8 mg/L throughout the experimental period. The bromide and nitrate concentrations were shown in Figure 5-10. Within the first hour, bromide concentration increased from 231 µg/L to 288 µg/L. It kept increasing until 303 µg/L in the 4th hour. The average increasing rate of bromide within the first four hours was 18 µg/(L•h). 87% of the initial nitrate concentration was reduced in the first hour, from 10.6 mg/L to 1.3 mg/L. 1 mg/L nitrate was reduced from the 1st to the 3rd hour and stabilized. An increase was seen within the 7th hour.

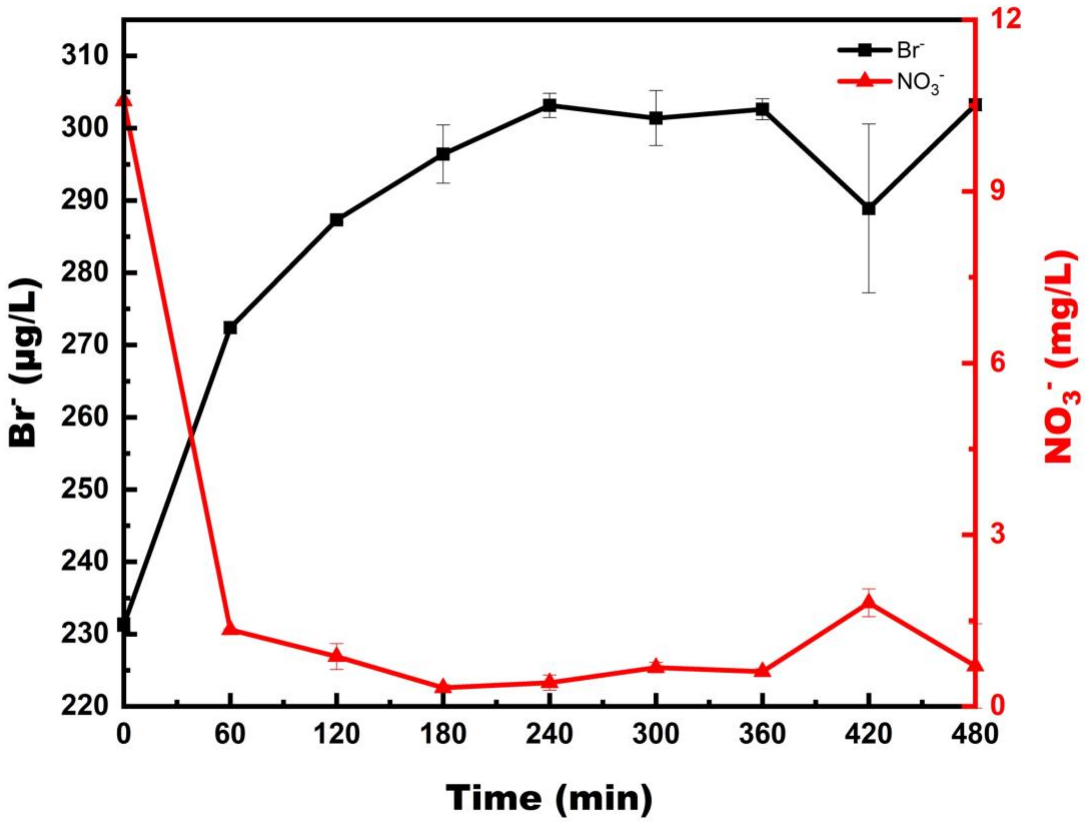

Figure 5-10 Bromide and nitrate concentrations of group C. Water taken from the top of the 1-STEP® filter was used with bromate and nitrate addition for this group. DO concentration was above 8 mg/L in the batches.

5.2.5 Group D

Group D used water from the bottom of the 1-STEP® filter with constant aeration that kept the DO above 8 mg/L. Nitrate and bromate were supplemented in the water. Figure 5-11 illustrates the nitrate and bromide concentrations during the two-hour experimental period. Both bromate and nitrate concentrations showed increasing trends. The increasing rate of bromide concentration gradually decreased while the nitrate concentration showed a linear decreasing trend.

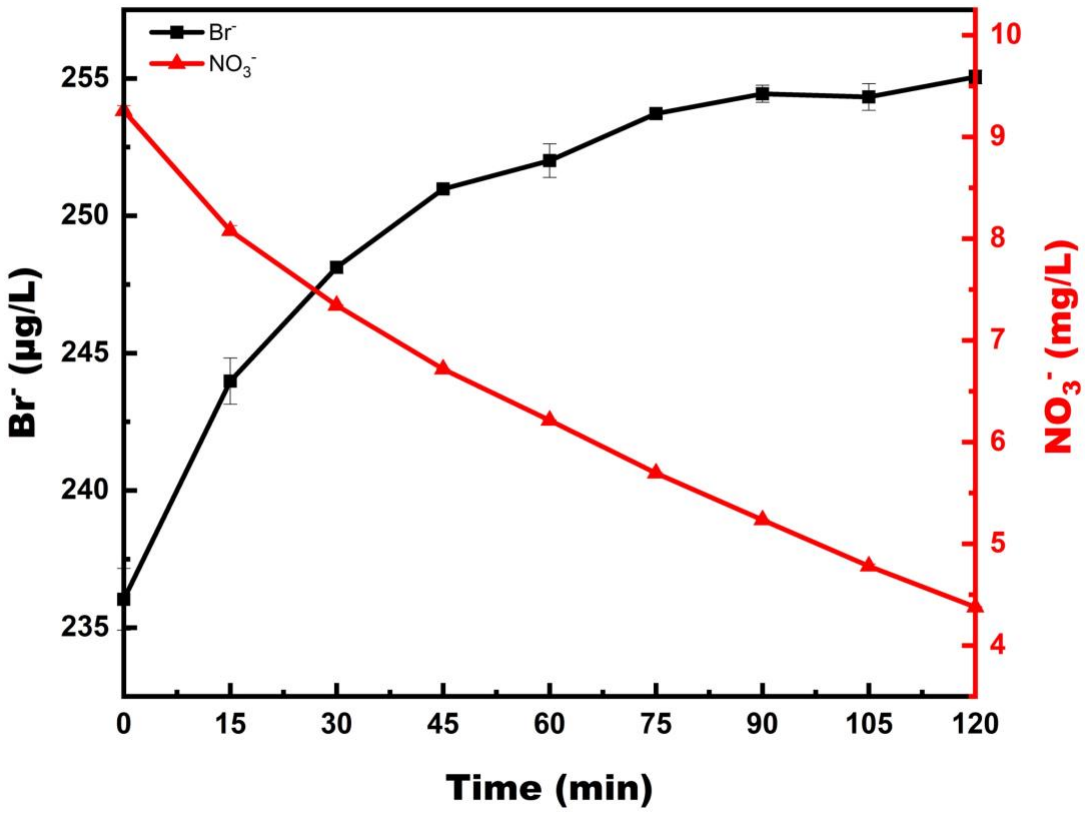

Figure 5-11 Bromide and nitrate concentrations of group D. Water taken from the bottom of the 1-STEP® filter was used with bromate and nitrate addition for this group. DO concentration was above 8 mg/L in the batches. The duration of this experiment was 120 minutes.

Chapter 6: Discussion

Although biological bromate reduction was discovered more than 20 years ago (Liu et al., 2012), current knowledge about its mechanism is still minimal. The pilot O_3-STEP filter in WWTP Horstermeer was found likely to be capable of reducing bromate biologically. The redox condition in the filter column was measured using multi-element measurements, and batch experiments were conducted for validation. Research questions in Chapter 3: are answered in this chapter with the results of this study and previous ones.

6.1 Redox Zones of the 1-STEP® Filter

Based on the redox condition in the liquid phase, the 1-STEP® filter column can be divided into three zones: the aerobic supernatant from 200 to 300 centimeters, the aerobic zone from 145 to 200 centimeters, and the anoxic zone from 0 to 145 centimeters. The changes in water quality parameters are summarized in Figure 6-1.

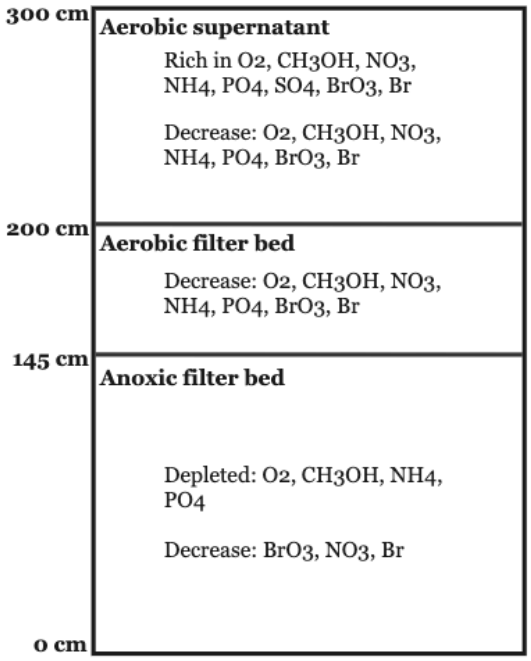

Figure 6-1 Illustration of the three redox zones in the 1-STEP® filter.

The supernatant was highly oxic, with DO concentration ranging from more than 10 mg/L to 22 mg/L because the supernatant received wastewater from the ozonation tank, where the water was rich in oxygen as a result of ozone decomposition. The supernatant had the

highest redox potential in the filter column, as its DO, nitrate, phosphate, and sulfate concentrations were high. As can be seen in the plots in section 5.1, all the parameters measured in the supernatant saw changes. Changes in supernatant As(III) and NH_4^+ concentrations were seen in pilot-scale rapid sand filters before (Gude et al., 2018). Through the experimental period, the removal of most As(III) and NH_4^+ gradually shifted to the top of the filters, which was believed to result from the ripening of the bacteria in the filter (Gude et al., 2018). Therefore, bacteria may also exist in the supernatant of the 1-STEP® filter and cause the change in redox conditions. In addition, many air bubbles were seen in the supernatant. The bubbles may create turbulence that carries bacteria from the filter bed to the supernatant, causing a change in water quality.

The aerobic zone in the filter bed expanded from 145 to 200 cm high. DO, ammonium, phosphate, and methanol were almost completely removed. In this zone, methanol was dosed for heterotrophic denitrification and aerobic respiration to deplete oxygen so that denitrification could happen anoxically. However, nitrate concentration already started to decrease in the aerobic zone as well as bromate.

The anoxic zone in the filter bed was from 0 to 145 cm high. In the anoxic zone, DO, ammonium, and phosphate were depleted, and COD concentration was low. Nitrate and bromate kept decreasing in this zone. Theoretically, the filter was designed in a way that denitrification happens in this zone. In addition, it was hypothesized in this research that bromate reduction happens when nitrate concentration is low (Hijnen et al., 1995; Jahan et al., 2021; Wang et al., 2018b), which should be in the anoxic zone.

In addition, the granules in the aerobic zone did not seem to travel deeper into the filter (anoxic zone) as can be seen in Figure 5-7 that the biofilm growth in the anoxic zone was much less extensive compared to that in the aerobic zone. As a result, backwashing possibly did not contribute to the stratification of redox conditions in the filter.

6.2 N-Removal in the 1-STEP® Filter: Nitrification and Denitrification

Previous studies on biological bromate reduction suggested a strong connection with denitrification processes (Korom, 1992; Luo et al., 2017; Ridley et al., 2006; Wang et al., 2018b; Zhong et al., 2018). To investigate this connection, it is crucial to understand the mechanism of denitrification in the filter, as it may share significant similarities with biological bromate removal.

6.2.1 Location of Nitrification and Denitrification in the Filter

In Figure 5-2, complete ammonium removal was seen in the aerobic supernatant, indicating that nitrification happened in the supernatant. Meanwhile, nitrate concentration decreased at all sampled depths, suggesting denitrification happened at all depths in the filter. However, it was hypothesized that ammonium oxidation happens at the top of the filter, whereas nitrate reduction only happens in the anoxic zone (STOWA, 2020) as ammonium oxidation preferably uses oxygen while nitrate reduction normally happens under anoxic conditions (Seitzinger et al., 2006). Therefore, the result of this study is partially in contradiction to the hypothesis. Nitrate removal under high DO conditions also happened in batch experiments C and D, where GAC and water from the 1-STEP® filter were used (Figure 5-10 & Figure 5-11). Complete nitrate removal of 10.6 mg/L in aerated waters within eight hours was observed. It is possible that nitrate was used as an N source for cell growth aerobically, instead of denitrification, especially in the absence of ammonium (Robertson and Kuenen, 1984). However, in the aerobic zone of the filter, using nitrate solely for cell growth requires an unrealistic bacterial growth rate of 355 mg VSS/(L • h), which is in apparent conflict with the actual bacterial growth rate observed in the filter (Appendix C Mass Balance on the Aerobic Zone of 1-STEP® Filter). Therefore, nitrate must have been partially removed by denitrification in the filter under aerobic conditions.

6.2.2 Denitrification under Aerobic Conditions: Oxygen Gradient in the Biofilm

Denitrification in the aerobic zone and the supernatant was unexpected because conventionally, denitrification mostly happens under oxygen-limiting conditions

(DO<0.5 mg/L) (Seitzinger et al., 2006) and the filter was designed to let denitrification happen in the anoxic zone as mentioned earlier. Nevertheless, denitrification in aerobic environments was observed in several studies before, offering two possible explanations. The first explanation is that the depletion of oxygen in the biofilm occurs and allows denitrification to take place. An anoxic layer is created after oxygen depletion, which favors nitrate utilization by denitrifying bacteria. This mechanism has already been utilized in a GAC filter by Liang et al., where the immobilized microbial community could perform denitrification when DO was higher than 6 mg/L (Liang et al., 2019). In a draft tube spouted bed reactor with GAC dosage as supporting material for microbial growth, denitrification under aerobic conditions was also observed (Joshi et al., 2017). A cubic equation enables one to calculate the oxygen penetration depth within biofilms with the bacteria's maximum oxygen uptake rate (OUR) (Appendix A Oxygen Penetration Depth in the Biofilm). The calculation was made using the typical OUR value of autotrophic nitrifying bacteria and the granular density of aerobic granular sludge. Albeit one of the solutions of the cubic equation does fall into the thickness of the actual biofilm (3300 μm), it may be an overestimation as the OUR was considered constant in the biofilm for the calculation. The COD and ammonium concentrations in the 1-STEP® filter (around 70 mg O_2/L and 0.26 mg/L on average, respectively) were much lower than the typical values for aerobic granular sludge. For example, the influent COD and ammonium concentrations were 500 mg/L and 2 mg/L, respectively in the study of Liang et al. (Liang et al., 2019). Therefore, the biofilm is more likely to have a COD limitation rather than an oxygen limitation. The insufficient COD concentration in the filter was unable to maintain a high microbial activity going deeper in the biofilm and the OUR is thus much lower than the maximum OUR used in the calculation (Garcia-Ochoa et al., 2010), possibly leading to a larger-than-the-biofilm-thickness oxygen penetration depth that prevents the formation of an anoxic zone. Nevertheless, oxygen limitation is still possible even if COD limitation exists since the biofilm structure strongly influences the oxygen distribution within biofilms (de Beer et al., 1994). Because biofilm structure parameters including density, porosity, pore size, convection, and type of extracellular polymeric substances (EPSs) all influence oxygen's diffusion coefficient in the biofilm (Hibiya et al., 2004), yet the current study did not investigate the carbon granules' biofilm structures.

6.2.3 Denitrification under Aerobic Conditions: Aerobic Denitrification

The second possible explanation for nitrate degradation under aerobic conditions is aerobic denitrification. Proposed by Robertson and Kuenen in 1984 (Robertson and Kuenen, 1984), aerobic denitrification represents a specific group of bacteria able to perform denitrification under oxic conditions. A few aerobic denitrifiers are from the genus *Pseudomonas,* which was also found to have species capable of bromate reduction (Ji et al., 2015; Liu et al., 2012). It was concluded that intermittent aeration was required to enrich aerobic denitrifiers (Ji et al., 2015, 2014; Robertson and Kuenen, 1984). Despite alternating anoxic and aerobic phases, the production of N_2O gas and the expression of the *NapA* gene are other important factors that would help identify aerobic denitrification. To confirm the occurrence of aerobic denitrification, additional studies on these two factors will hence be needed.

6.2.4 Influence of DO and COD on Denitrification

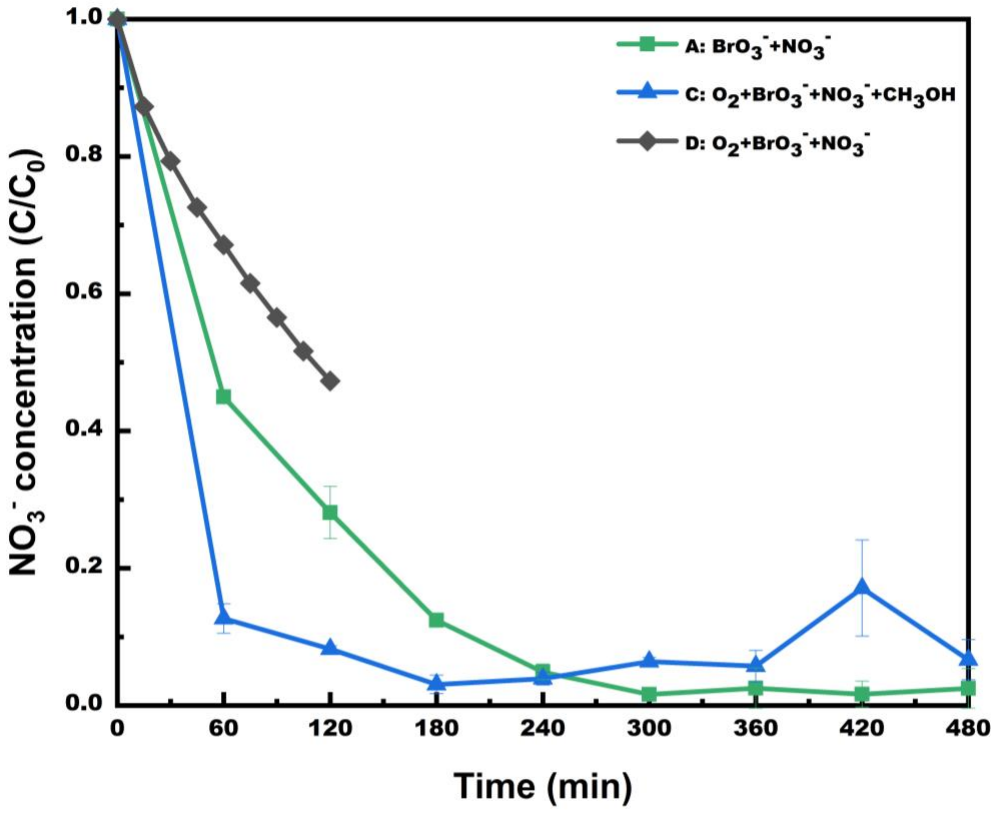

Figure 6-2 Nitrate concentrations in groups A, C, and D. The experimental duration was 480 minutes for groups A and C, and 120 minutes for group D.

In Figure 6-2, the comparison between the anoxic group A and the aerated group D shows that nitrate reduction was slower in the aerated group A. This indicates a partial inhibition of DO on the denitrification process. Comparing the methanol-supplemented group C with group D, an accelerating effect of methanol dosage can be seen as the nitrate reduction rate was higher in group C. Therefore, it can be concluded from the batch experiments that DO partially inhibits denitrification whereas COD (in the form of methanol) accelerates denitrification. However, the multi-element measurements revealed that 57% of the nitrate in the influent of the 1-STEP® filter was reduced in the aerobic zone and the supernatant, indicating that more nitrate was reduced aerobically than anoxically. This difference might be ascribed to the higher COD consumption in the aerobic zone than in the anoxic zone, as 76% of the COD in the influent was consumed in the aerobic zone. Therefore, the accelerating effect of COD compensated for the negative effect of oxygen in the aerobic zone and supernatant. This postulation is in line with the findings of the batch experiments, where the aerated group C had a higher nitrate reduction rate (18 µg/(L•h)) than the anoxic group A (11.2 µg/(L•h)) due to higher COD concentrations. It seems that the availability of e-donors in the filter is more likely to be limiting the respiration than e-acceptors. In addition, the high COD concentration possibly led to higher microbial activity and more extensive immobilized bacterial growth in the aerobic zone than in the anoxic zone (Hanaki et al., 1990; Wang et al., 2018a) Therefore, more nitrate degradation was observed in the aerobic zone of the filter column.

6.3 Bromate Reduction and Its Influencing Factors

6.3.1 Location of Bromate Reduction

It was hypothesized that only when nitrate concentration becomes limited at the bottom of the 1-STEP® filter would bromate reduction start because the competition between nitrate and bromate reduction by the same enzyme would favor the reduction of nitrate so that nitrate has an inhibitory effect on bromate reduction (Hijnen et al., 1995; Jahan et al., 2021; Wang et al., 2018b). However, this hypothesis is rejected by the result of the multi-element measurements and batch experiments. In Figure 5-3 bromate

concentration decreased at all sampled depths, indicating that bromate reduction happened throughout the whole filter column.

6.3.2 Influence of NO_3^- on Bromate Reduction

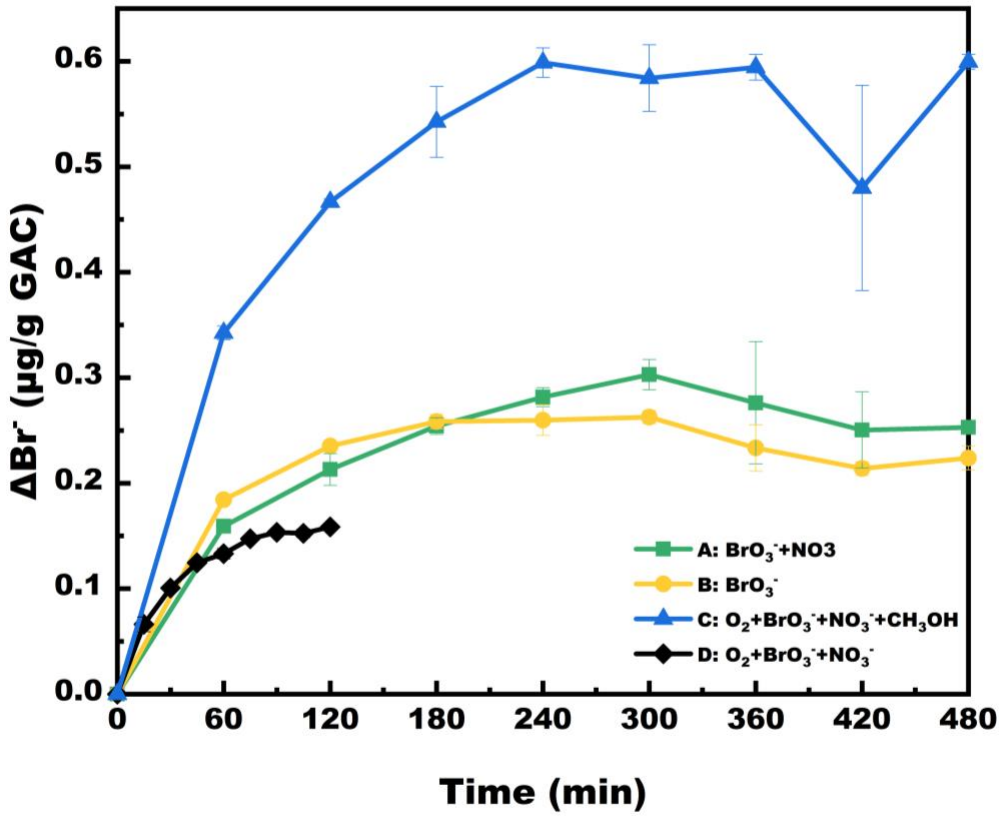

Figure 6-3 Normalized change of bromide concentrations in groups A, B, C, and D. Since group B had a different GAC dosage from the other groups, the change in bromide concentration was normalized based on the GAC dosage of each group and expressed as the change of bromide concentration per gram of GAC.

The presence of nitrate did not show any inhibitory effect on bromate reduction in the current research, which contradicts the hypothesis that nitrate has an inhibitory effect on bromate reduction. Firstly, in the 1-STEP® filter, the bromate reduction rate did not change significantly as nitrate concentration decreased. Secondly, the batch experiments suggested no inhibition of bromate reduction by the presence of 10 mg/L of nitrate since the bromide's increasing rate of group A is similar to the nitrate-supplied group B as can

be seen in Figure 6-3. Moreover, in Figure 5-8, Figure 5-10, and Figure 5-11, bromide concentrations drastically increased when nitrate concentrations were relatively high, indicating that nitrate did not inhibit bromate reduction. To explain the discrepancy from the hypothesis, one could argue that the adsorption of bromate by GAC may not be affected by the presence of nitrate (Kirisits et al., 2000). However, the GAC in the 1-STEP® filter was in use for two years. Wang et al. compared the scanning electron microscope (SEM) photographs of virgin GAC surface and GAC surface after one and eight years of running, showing that only few pores seen on the virgin GAC surface remained after one and eight years of use. Biodegradation was thus suggested to be dominant for water quality purification after one year of running (F. Wang et al., 2022). The three-millimeter-thick biofilm shown in Figure 5-6 is, therefore, likely to block the pores on GAC surfaces, eliminating the adsorption capacity of GAC granules. Nevertheless, one should be cautious about attributing all the bromate removal to biological reduction as only 54% of the decreased bromate concentration was accounted for by the increased bromide concentration in batch group A, meaning that biological bromate reduction might not be the only process that removed bromate in the filter.

In Liu et al.'s study, inhibition of bromate reduction by nitrate was not observed either. Almost complete bromate removal (60 μg Br/L) was achieved when nitrate concentration was 1.5 to 2.4 mg/L in the tap-water-supplied aerobic BAC column (Liu et al., 2012). However, contrary to the current study, no nitrate was reduced in Liu et al's BAC column. A possible explanation can be that the COD concentration was too low in Liu et al.'s study for denitrification, as only 1 mg/L sodium acetate (CH_3COONa) was dosed. Nevertheless, Liu et al. isolated the bromate-reducing strains from the BAC and investigated their performance. Consistent with their findings in the BAC, no nitrate reduction was seen while bromate was removed when 200 mg/L sodium acetate was present, indicating that nitrate is not a competitor of bromate as an electron acceptor (Liu et al., 2012). However, the isolated strains did not reduce bromate until DO was depleted, which was contradictory to the findings in their BAC column. A DO limitation in the BAC biofilm was thus postulated to explain the bromate reduction in the aerobic BAC column (Liu et al., 2012). Similarly, bromate reduction happened in Wang et al.'s oxic MAR column,

where no nitrate reduction was seen. It was suggested by Wang et al. that the bromate might be reduced by aerobic bacteria (Wang et al., 2018b). Overall, both studies suggested the existence of specific bromate-reducing bacteria that cannot remove nitrate. However, this possibility is low in this study because the average bromate concentration in the 1-STEP® filter was deficient (<0.5 μg/L). The need for the microbial community to adapt to such low bromate concentration is likely to be limited. Furthermore, it is difficult to infer if the bromate-reducing bacteria in the top part of the 1-STEP® filter are anaerobes or aerobes because the existence of DO limitation in the biofilm is still unknown, as stated in section 6.2.2.

6.3.3 Influence of DO and COD on Bromate Reduction

In Figure 6-3, the bromide increasing rate of the aerated group D was evidently lower than the anoxic group A. This comparison suggests a partial inhibitory effect of DO on bromate reduction. Additionally, COD (in the form of methanol) accelerated bromate reduction, suggested by the fact that the methanol-supplemented group C had a higher bromide increasing rate than group D. The finding about DO's influence on bromate reduction is in line with Kirisits et al.'s research on an aerobic BAC filter treating drinking water, where the bromate removal dropped from 40% to 11% when the DO was increased from 2.1 to 13.6 mg/L (Kirisits et al., 2001). An accelerating effect of COD was observed before in Wang et al.'s study, where the acetate-supplied water induced higher bromate reduction rate in a pilot MAR column (Wang et al., 2018a). The mechanism of the DO and COD's influence on bromate reduction is discussed in section 6.4.3.

6.4 Mechanism of Bromate Reduction: Cometabolism?

6.4.1 Existence of Denitrifying Bacteria

In the 1-STEP® filter and batch experiments, extensive nitrate removal was achieved under both anoxic and aerobic conditions, indicating that the microbial community in the filter includes denitrifying bacteria. In addition, the bromate reduction started immediately after the batch experiments began. The absence of any lag period indicates that bromate reduction is an inherited ability of the bacteria in the 1-STEP® filter. As introduced in section 2.7.3, it has been widely reported that denitrifying bacteria can also

reduce bromate (Jahan et al., 2021; Ridley et al., 2006). In addition, it has been discussed in section 6.3.2 that specific bromate-reducing bacteria are unlikely to exist in the 1-STEP® filter. Therefore, denitrifying bacteria are possibly responsible for the decrease in bromate concentrations in this study.

6.4.2 Simultaneous Nitrate and Bromate Reduction

In the 1-STEP® filter, both nitrate and bromate decreased throughout the filter column. This finding was confirmed by the batch experiments: in Figure 5-8, nitrate concentration decreased whilst bromide concentration was increasing under anoxic conditions. In Figure 5-10 and Figure 5-11, an increase in bromide concentration was also observed when nitrate concentration was decreasing at the same time, but under aerobic conditions. These findings indicate that bromate was reduced simultaneously with denitrification by the microbial community in the GAC filter. Simultaneous bromate and nitrate reduction by denitrifiers has been reported in anoxic reactors before. A fixed bed column reactor was operated in autotrophic conditions and then mixotrophic (autotrophic+heterotrophic) conditions. 45 mg/L NO_3–N and 100~500 μg/L bromate in the influent were mostly reduced to under-detection limit levels (Demirel et al., 2014). Although in that study, no measurement of bromate and nitrate concentration in the column was conducted to confirm if their reduction happened at the same depth, evidence of it was shown that when a temporarily high effluent nitrate concentration (5 mg/L NO_3^--N) emerged, the bromate was still reduced to below the detection limit. Another study achieved simultaneous nitrate and bromate removal in a rotating biofilm-electrode reactor using hydrogen as the sole e-donor (Zhong et al., 2018). Bromate reduction was believed to be a cometabolic process of denitrification in those studies (Demirel et al., 2014; Zhong et al., 2018).

6.4.3 Nitrate Reductase in Bromate Reduction

In sections 6.3.3 and 6.2.4, DO and COD's influence on bromate and nitrate reduction are discussed. The comparisons in these two sections indicate that COD concentrations and the presence of oxygen had the same effect on denitrification and bromate reduction: higher COD concentration accelerates denitrification and bromate reduction, whereas the

presence of high DO partially inhibits the two processes. This finding is consistent with that of Wang et al., who observed an accelerating effect of COD and an inhibitory effect of DO's presence on both bromate reduction and denitrification in a pilot MAR column (Wang et al., 2018a). As introduced in section 2.7.3, bromate was found to be a substrate of nitrate reductase, and Hijnen et al. suggested that nitrate reductase might be involved in the biological bromate reduction process (Hijnen et al., 1995; Morpeth and Boxer, 1985; Yamamoto et al., 1986). The similar effect of the presence of DO on denitrification and bromate reduction can thus be potentially explained by the activity and the synthesis of dissimilatory nitrate reductase (Kirisits et al., 2001). Since it was found for some bacteria that increasing DO levels partially or completely repressed the synthesis of nitrate reductase (Krul and Veeningen, 1977), the bacteria's synthesis of nitrate reductase in the 1-STEP® filter was possibly repressed by the presence of oxygen, leading to a lower nitrate and bromate reduction rate. On the other hand, the accelerating effect of COD on denitrification and bromate reduction may be explained by the availability of e-donors in the 1-STEP® filter. When more methanol is present, more e-donors are thus available so that more e-acceptors, in this case, bromate and nitrate can be reduced (Kirisits et al., 2001).

Although simultaneous bromate and nitrate reduction was observed before, it was only reported to happen under anoxic conditions. The current study is the first to record simultaneous bromate and nitrate reduction under aerobic conditions. In section 6.2, the author argues that aerobic denitrifiers are likely to be responsible for denitrification in the supernatant and aerobic zone of the 1-STEP® filter. Therefore, it is possible that aerobic denitrifiers can potentially reduce bromate if aerobic denitrifiers are proven to exist in the filter (Kirisits et al., 2001).

6.4.4 NO_3^- in Cometabolic Bromate Reduction

In most studies of biological bromate reduction by denitrifiers, nitrate was found to be inhibitory for cometabolic bromate reduction (Assunção et al., 2011; Davidson et al., 2011; Hijnen et al., 1995; Jahan et al., 2021; Wang et al., 2018a). However, the presence of 9 mg/L nitrate did not display any inhibitory effect on the biological bromate reduction in

the 1-STEP® filter. A study on the general mechanism of cometabolism indicated that the substrate concentration that can cause competition with the other substrate is highly dependent on the type of bacteria (Bouchez et al., 1995). It is possible that the bacteria in the 1-STEP® filter need relatively high nitrate concentrations (>11 mg/L) to cause competition for the use of limited enzymes. Because the nitrate concentrations used in this research are close to that the bacteria are used to in the 1-STEP® filter, and it was already seen in the filter that the bacteria can reduce bromate under these nitrate concentrations. Besides competitional behavior in the cometabolic process, synergetic interactions between growth substrates and non-growth substrates were observed before, even though not prevalent (Bouchez et al., 1995). This means that the two substrates' consumption has a synergy that requires the presence of both. As a result, a positive correlation between substrates is more likely to exist than the competition. In addition, in Wang et al.'s research, nitrate was suggested to be a prerequisite for bromate reduction because the bromate reduction rate gradually decreased within 75 days' absence of nitrate (Wang et al., 2018a).

6.5 Practical Implications for the Operation of the O_3-STEP Filter

Bromate formation in the O_3-STEP filter resulted from the ozonation of bromide-containing influent. The risk of bromate formation is positively linked to the bromide concentration in the influent and the ozone concentration in the ozone contact tank. Up to 3 µg/L bromate was produced by ozonation and ended up in the feedwater for the pilot 1-STEP® filter during the study period, which was double the RIVM target for drinking water intake points.

The pilot 1-STEP® filter, on the other hand, showed the ability to partially or entirely remove the bromate produced by ozonation. Up to 2.8 µg/L bromate was removed to a concentration lower than the detection limit of 0.2 µg/L. A dip in bromate removal efficiency was seen after the pilot restarted from a temporary stop for two and a half months. 2.7 µg/L bromate in the feedwater was reduced to 0.9 µg/L during the dip. Nonetheless, the RIVM regulation was still met. In conclusion, the O_3-STEP filter was

consistent in bromate removal and could ensure a safe effluent quality that meets the RIVM regulation.

Among the water quality parameters tested in this study, dissolved oxygen (DO) and methanol concentrations were found to influence the biological bromate removal. The removal efficiency decreased as DO increased whereas increasing methanol concentration significantly increased the efficiency. For the operation of the O_3-STEP filter, an ideal ozone dosage is crucial to optimize the filter for the most efficient removal of nutrients, OMPs, and a lower-than-regulation effluent bromate concentration when biofilm is well developed. For bromate concentration control, the ideal ozone dosage should be as low as possible. It means not only less formation of bromate but also a higher bromate removal efficiency. Meanwhile, the ozone dosage should also be sufficient to achieve enough OMP removal as it is a fundamental goal of the technology. Increasing the methanol dosage can be used as an optional measure to improve bromate removal, only to be used when the effluent bromate concentration becomes high. Cautious determination of methanol dosage should be made to ensure both low COD and bromate concentrations in the effluent.

As mentioned in section 6.2, the availability of e-donors may be the rate-limiting factor for microbial respiration, particularly in the anoxic zone of the 1-STEP® filter where COD concentration was low. It can be seen in the COD profile in Figure 5-5 that the filter only removed COD in the top part, meaning that the rest of the filter bed can still be used to remove COD if more COD is present in the influent. The filter had already shown the ability to effectively remove nutrients, COD and OMPs as a post-treatment step; the author believes that it also has the potential to be used as a biological treatment step for domestic wastewater if more methanol is dosed. Because more methanol will likely increase the microbial activity in the filter and enable more extensive nutrient removal in the lower part of the filter where currently the denitrification is limited by the low COD concentration. The system's compatibility offers many advantages over the traditional three-or-two-tank treatment facilities, including lower CAPEX and less floor area needed. The removal of OMPs could also be achieved without introducing post-treatment steps.

Further tests on the filter's total capacity for nutrient removal would help develop its potential as a biological wastewater treatment step.

Chapter 7: Conclusions and Recommendations

7.1 Conclusions

This research aimed to investigate the mechanism of bromate reduction in the pilot 1-STEP® filter. Five research questions were formulated in Chapter 3:, and their answers are as follows:

1. *Where do nitrification and denitrification happen in the filter, respectively?*

In the pilot 1-STEP® filter, nitrification happened in the supernatant and the aerobic zone of the filter bed (top 65 cm of the filter bed). It was hypothesized that denitrification happens after oxygen depletion, however, this is not the case as denitrification happened in the whole filter column, including the aerobic zone. Due to the relatively low COD concentration in the filter compared with wastewater, DO limitation is less likely to be the cause of denitrification under aerobic conditions. Instead, the existence of aerobic denitrifiers in the filter is suggested.

2. *Does DO inhibit nitrate reduction, and is there another factor that influences nitrate reduction?*

DO showed a partial inhibitory effect on nitrate reduction in the batch experiments using GAC from the top part of the 1-STEP® filter. Other than DO, COD (in the form of methanol) was also shown to have an impact on nitrate reduction. Increased COD concentration significantly accelerated nitrate reduction under aerobic conditions during the batch experiments.

3. *In which area does bromate removal take place in the filter?*

Bromate removal took place in the whole filter column, which differs from the hypothesis. Aerobic and anoxic bromate reduction was also seen in the batch experiments where GAC and water from the pilot 1-STEP® filter were used. It is the first research to report simultaneous bromate and nitrate reduction under aerobic conditions.

4. *Does the presence of nitrate and oxygen inhibit bromate removal in the filter?*

From the multi-element measurement results and the batch experiments, no inhibitory effect of nitrate on bromate reduction was observed. The bromate reduction rate was rather consistent in the filter while nitrate concentration was dropping. In the batch

experiments, the increase of bromide concentration in the nitrate-supplied group was similar to the nitrate-free group, which indicates no inhibitory effect on bromate reduction by nitrate.

In the batch experiments, a partial inhibitory effect of DO on bromate reduction was observed as >8 mg/L of DO slowed the bromate reduction rate compared with the anoxic group. This phenomenon was explained by the repression of nitrate reductase by oxygen.

5. *Are there other water quality parameters that influence bromate reduction ?*

COD concentration was found to have an accelerating effect on bromate reduction.

6. *Is bromate removal cometabolic in the filter?*

Bromate removal is likely to be cometabolic in the filter because first of all, bromate was removed simultaneously both in the filter and during the batch experiments; secondly, COD and DO showed the same influence on bromate and nitrate reduction, which may indicate that both processes share the same enzymes.

7.2 Limitations and Recommendations

During this study, grab samples were taken from the pilot O_3-STEP filter in WWTP Horstermeer. Analysis of grab samples only reveals the momentary water quality of the samples when they are taken. It may not represent the overall characteristics of the pilot's redox conditions. Especially for identifying different redox conditions in the filter, composite sampling during at least 24 hours is more likely to reflect the general situation in the filter. Therefore, it is recommended to carry out composite sampling in further research.

Another limitation of the multi-element measurement is that the O_3-STEP pilot was partially off for two and a half months due to the malfunctioning of an ozone sensor. During that period, the ozone generator, the backwashing program, and the methanol dosage were turned off. Albeit the water and GAC samples were taken two weeks after the restart of the pilot to allow the filter to stabilize, the microbial composition in the pilot 1-

STEP® filter may have changed due to the two-month stop. The decrease in bromate removal efficiency can result from this temporary stop.

The batch experiments also have some limitations. The batch experiments were conducted in multiple batches to reduce the effect of feedwater volume change by sampling. However, doing so also brings the uncertainty of the biomass quantity. Since the amount of GAC placed in each batch was decided by measuring the wet weight of the granules, due to the individual difference in the sizes of the virgin carbon granules and the biofilm, it is impossible to ensure the biomass concentration is the same in each batch. The possible difference in biomass concentration may have contributed to the deviations in each batch's water quality. To lower this effect, the author thrived on selecting granules that had similar sizes for the batch experiments. However, the limitation can be overcome by separating the biofilms from the carbon granules and measuring the wet and dry weight of the biomass after the batch experiments. This enables one to calculate the biomass-specific change rate of each water quality parameter, which will not only help eliminate the error caused by the difference in biomass concentration but also offer the possibility of calculating and comparing the kinetics of the biomass with previous studies. Further studies are thus suggested to investigate the kinetics of both the denitrification and bromate reduction of the bacteria in the pilot 1-STEP® filter.

Another limitation of the batch experiments lies in the groups where aerators were used. The aerators used in this study are designed for aquariums and did not allow precise control of the DO concentration in water. This may cause unstable aeration conditions and different DO concentrations among batches. Further studies are recommended to establish a calibration curve of each aerator to allow more precise control of the DO concentrations. Meanwhile, continuous monitoring of each batch's DO values would be beneficial to ensure a stable aeration condition.

Based on experimental results, postulations about the microbial composition in the pilot 1-STEP® filter were brought up in Chapter 6:. It is postulated that aerobic denitrifiers are likely to exist in the filter and are responsible for the simultaneous bromate and nitrate

reduction. To confirm this hypothesis, it is imperative to conduct a phylogenic analysis of the microbial community and compare it with previously found aerobic denitrifying strains. Moreover, the isolation of bromate-reducing strain(s) will also be beneficial, as it allows one to study the effect of oxygen and nitrate on bromate reduction. This will greatly help verify the aforementioned hypothesis.

Feifei et al. conducted a column experiment to test nitrate's impact on bromate reduction. The bromate reduction rate gradually decreased within 75 days' absence of nitrate, indicating nitrate to be a prerequisite for bromate reduction by denitrifying bacteria (Wang et al., 2018a). Although the absence of the growth substrate - nitrate did not have an immediate effect on bromate reduction in the current study, it is still possible that a longer absence of nitrate might affect bromate reduction. Therefore, more extended batch experiments are needed. Furthermore, since the non-growth substrate in cometabolic processes does not contribute to microbial growth, which bromate is suspected to be in this study, the microbial growth rate of the bacteria in the 1-STEP® filter should be measured when bromate is dosed as the only e-acceptor, to see if the reduction of bromate contributes to bacterial growth.

As shown in section 6.2, although the low COD concentration in the influent to the 1-STEP® filter does not favor the depletion of oxygen within the biofilm, to validate the hypothesis that the oxygen did not deplete in the biofilm, it is necessary to measure the oxygen concentrations in the biofilm using microelectrodes. More importantly, microelectrodes could also allow one to measure the presence of nitrate ions in the biofilm, which will show where nitrate is consumed. With these two pieces of information, one can conclude that the nitrate degradation was due to the oxygen limitation in the biofilm. Meanwhile, one can investigate the presence of aerobic denitrifiers by measuring N_2O gas and the *NapA* gene, as mentioned in section 6.2.

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Appendix

Appendix A Oxygen Penetration Depth in the Biofilm

$$\frac{q_{max}C_X(R^3-(R-\delta)^3)}{3R^2}=\frac{D_{O_2,aq}(C_{SB}-C_{Si})}{d_{MTBL}}$$

δ : penetration depth (m)

R: granule radius (m)

d_{MTBL}: thickness of mass transfer boundary layer (m)

$D_{O2,aq}$: diffusion coefficient of oxygen in the liquid (m^2/s)

$D_{O2,g}$: diffusion coefficient of oxygen in the granule (m^2/s)

C_{SB}: concentration in the bulk liquid (mgO_2/L)

C_{Si}: concentration at the granule surface (mgO_2/L)

q_{max}: maximum uptake rate ($mgO_2/gVSS/h$)

C_X: biomass concentration in the granule (gVSS/L)

Simplify by $C_{SB}=C_{Si}$:

$$3\delta^2-\frac{2\delta^3}{R}=\frac{6D_{O_2,g}C_{Si}}{q_{max}C_X}$$

In this case:

R=0.0033 m (3300 μm)

$D_{O2,g}$=1.4E-9 m^2/s

C_{Si}=9.57 mgO_2/L

q_{max}=4.9/3600 $mgO_2/mgVSS/h$

C_x=70 mgVSS/L

Solutions are:

δ_1=-0.43 mm

δ_2=480 μm

δ_3=4910 μm

Appendix B Correlation Coefficient

Pearson correlation coefficient.

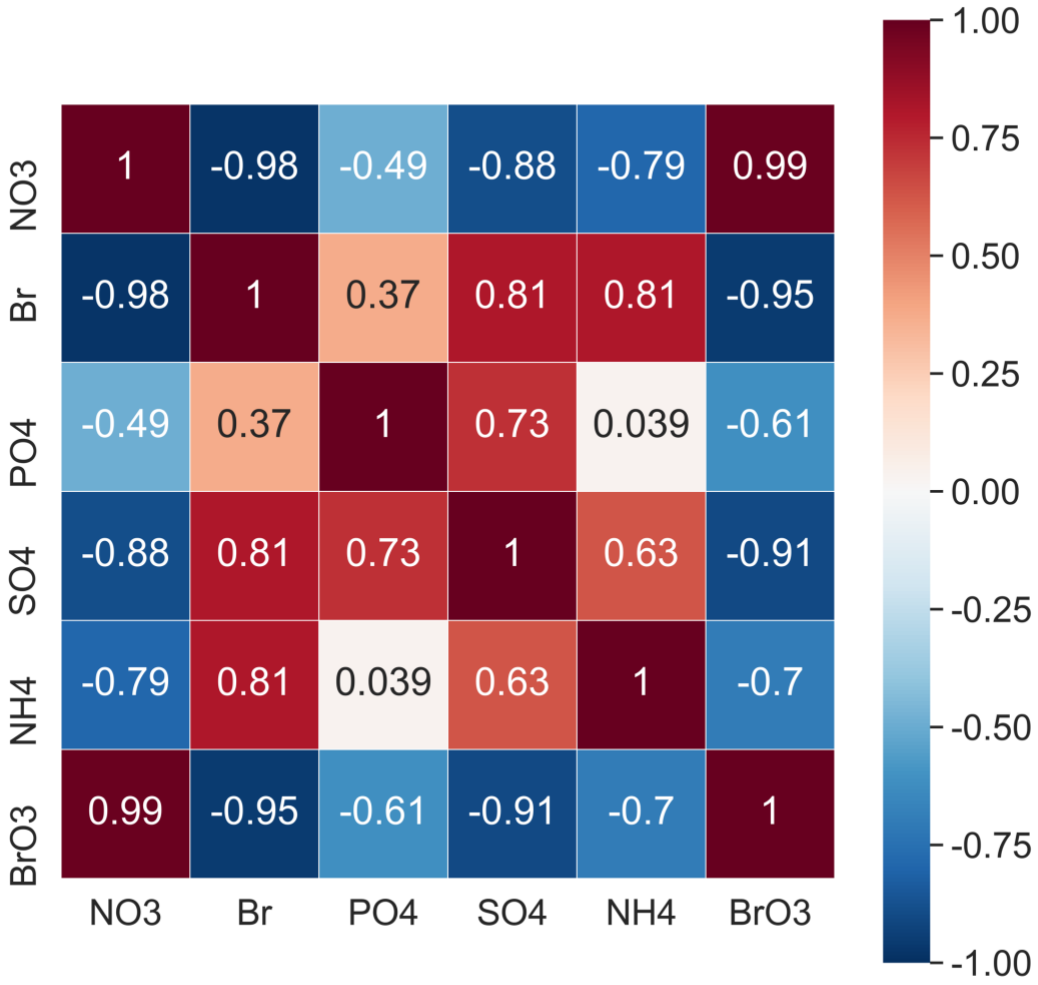

Figure 0-1 Pearson correlation coefficient heat map of group A

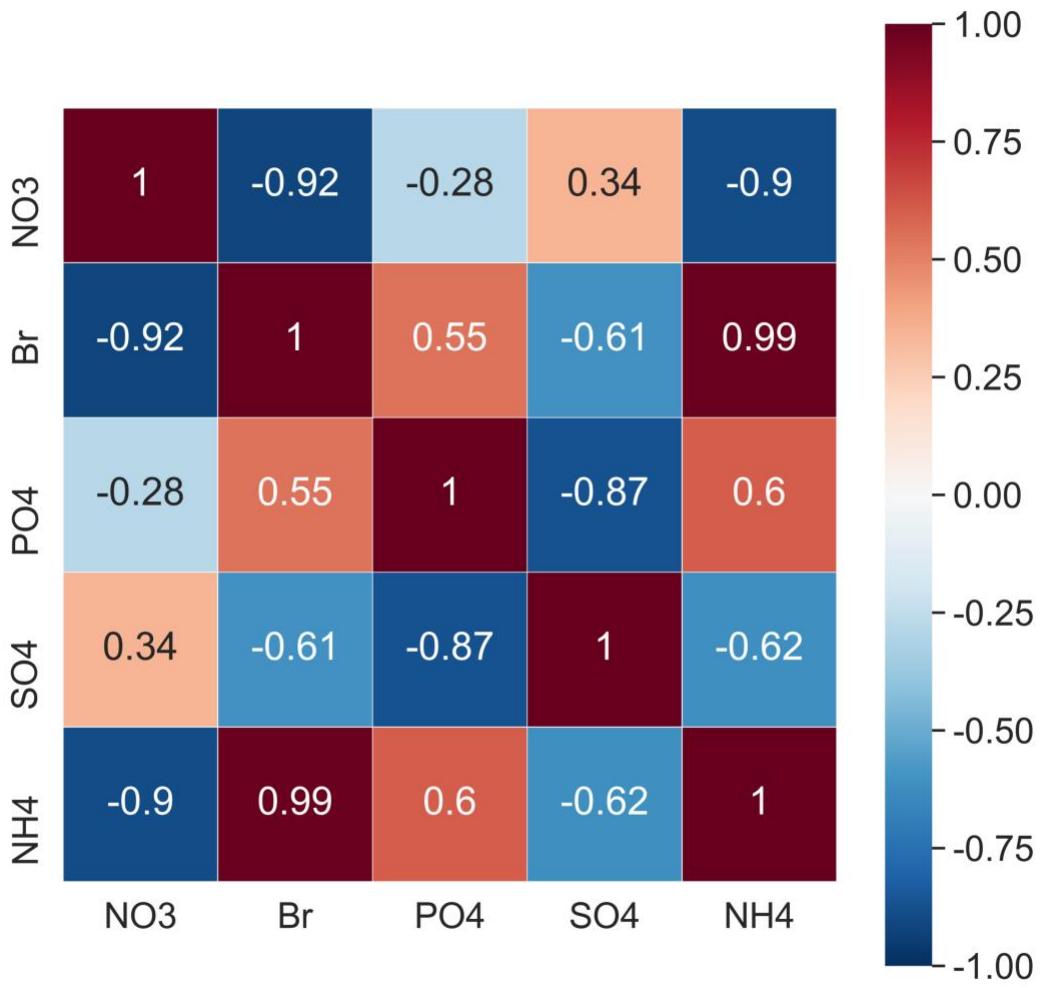

Figure 0-2 Pearson correlation coefficient heat map of group A2

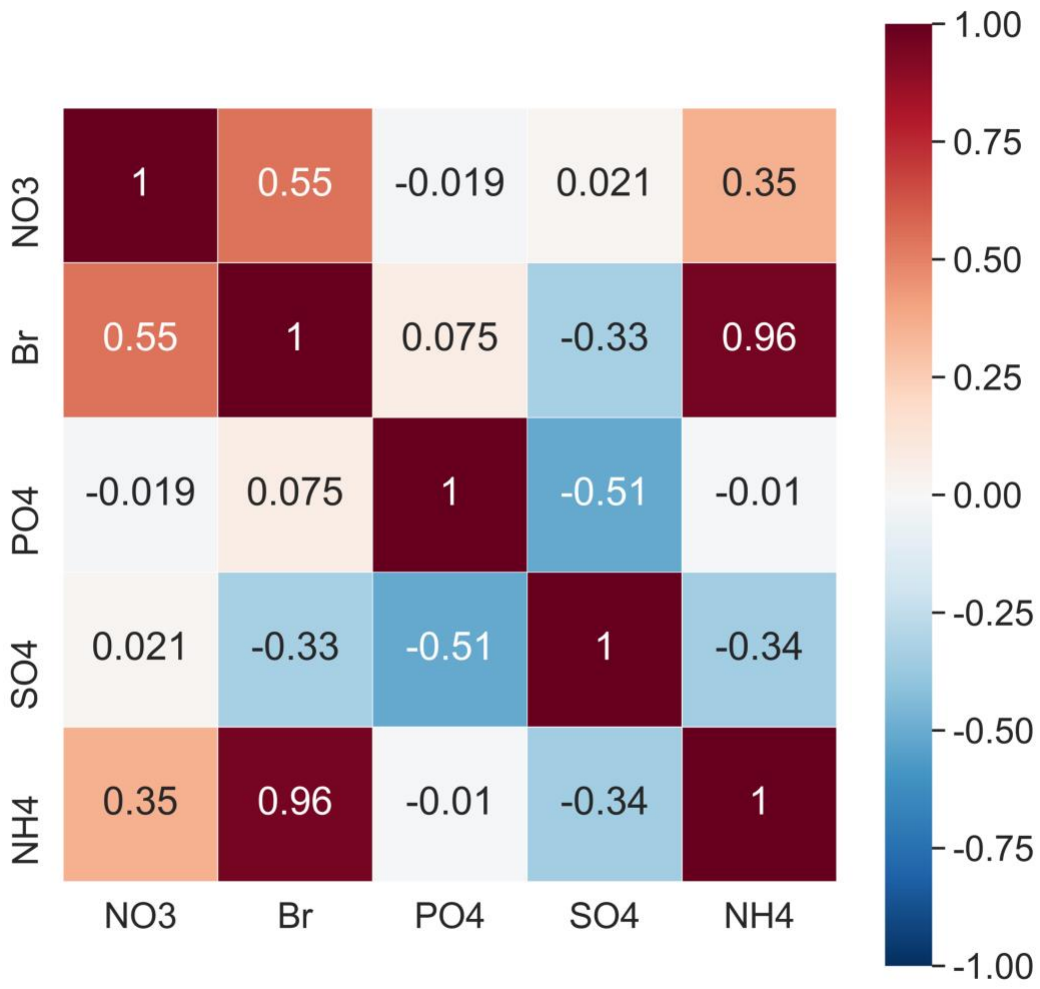

Figure 0-3 Pearson correlation coefficient heat map of group B

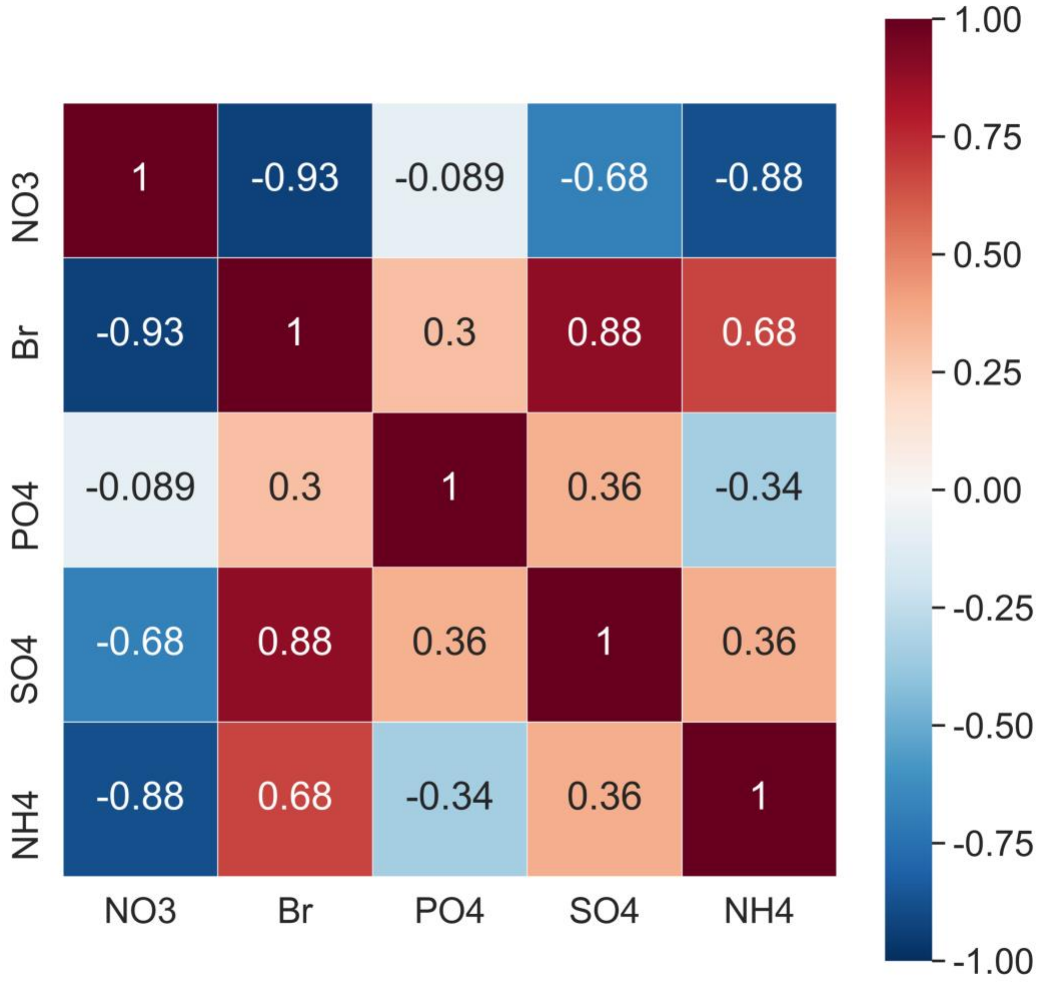

Figure 0-4 Pearson correlation coefficient heat map of group C

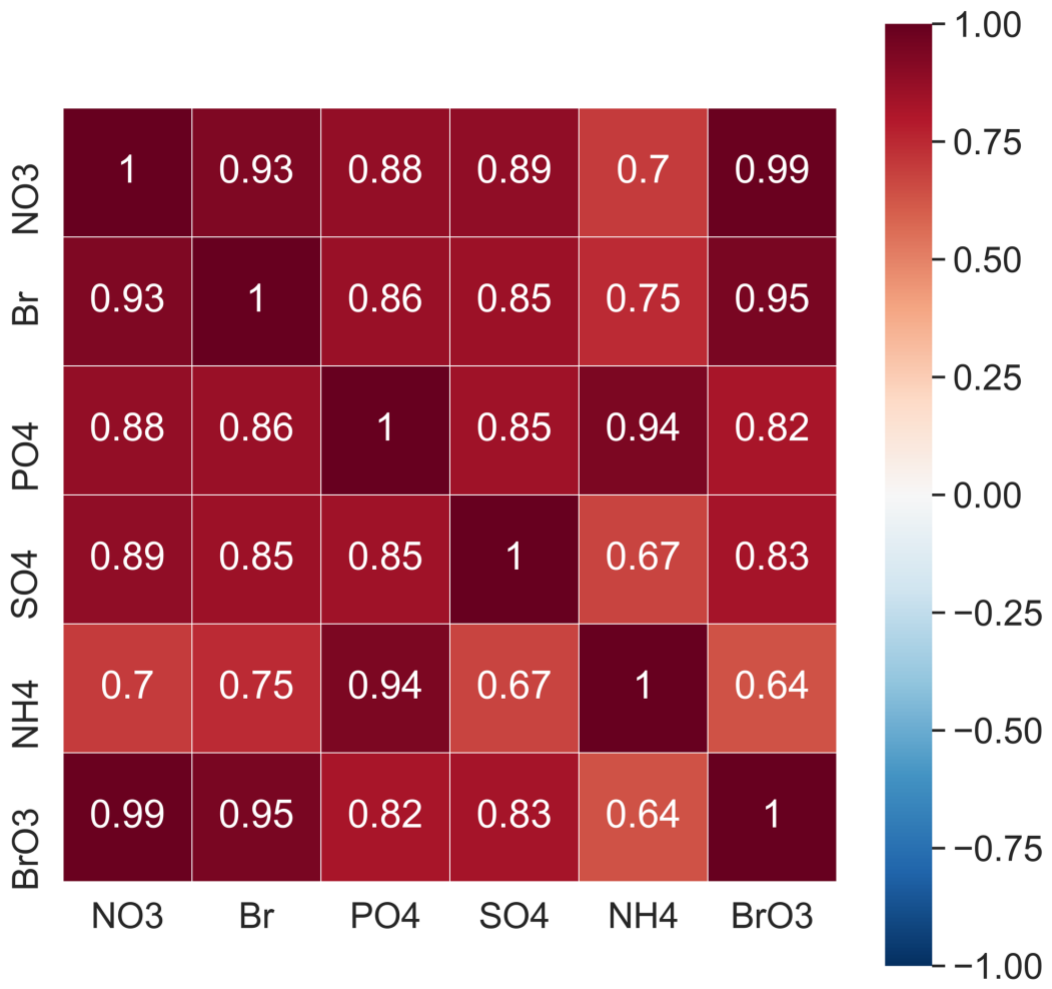

Figure 0-5 Pearson correlation coefficient heat map of water quality parameters in the profile after backwash

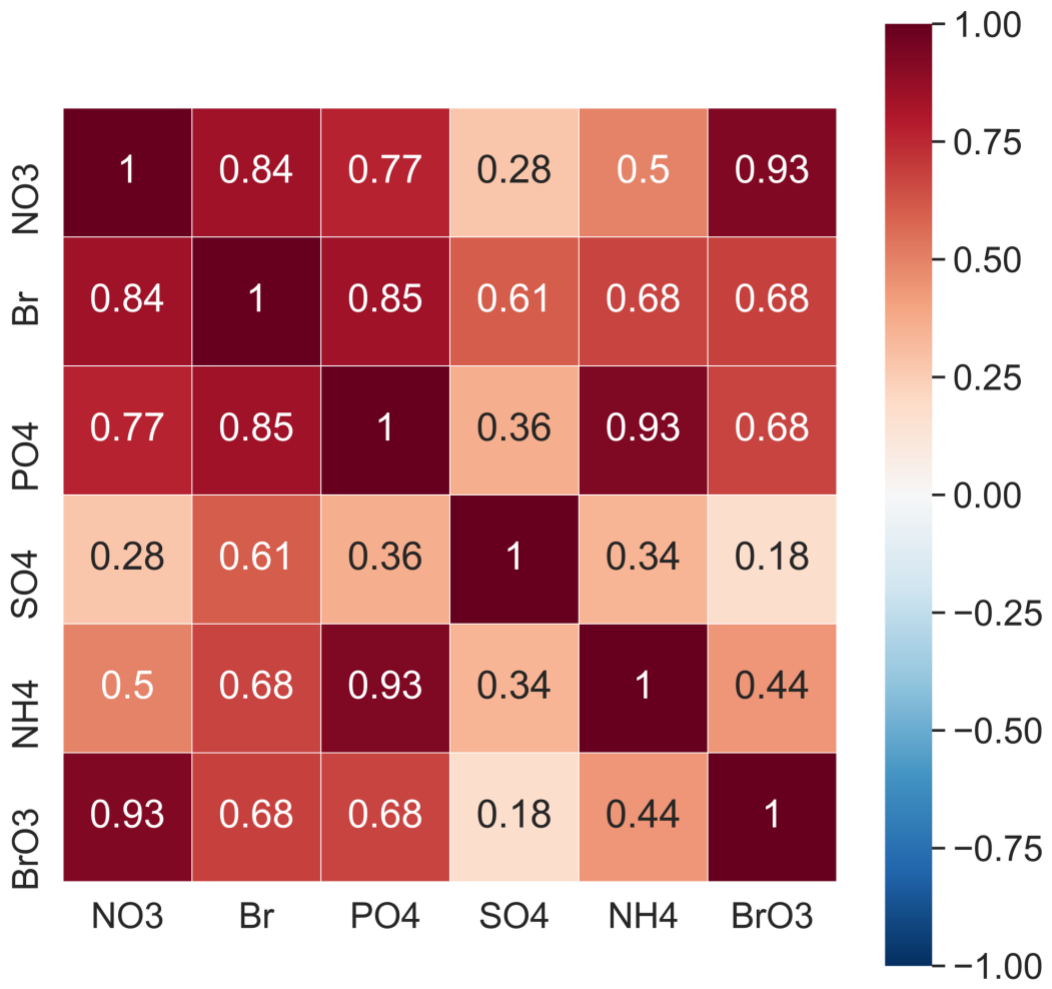

Figure 0-6 Pearson correlation coefficient heat map of water quality parameters in the profile before backwash

Appendix C Mass Balance on the Aerobic Zone of 1-STEP® Filter

This mass balance is established based on the data acquired on 29th of September 2022 before backwashing.

Parameter	Concentration at 200 cm	Concentration at 145 cm
NO_3^-	3.041 mg N/L	1.453 mg N/L
COD	79.5 mg/L	18.367 mg/L
DO	18.65 mg/L	1.25 mg/L

This scenario assumes nitrate consumption solely for cell synthesis and calculates the respective theoretical microbial growth rate. Since NH_4^+ concentration was too low (around 10 μg N/L), nitrification and cell synthesis using NH_4^+ were neglected.

To calculate the nitrate consumption rate, a constant flow rate in the filter was assumed. Therefore, the hydraulic retention time (HRT) of the aerobic zone is:

HRT = 17.4 min * (200 cm – 145 cm) / 300 cm = 3.19 min

The nitrate consumption rate in the aerobic zone is therefore:

$r_{NO3} = (c_{NO3} – c_0)/HRT$ = (3.041 – 1.453) mg N/L / 3.19 min = 30 mg N/(L • h)

Assume the chemical composition of VSS is $C_5H_7NO_2$

Then N% (w/w) =12%

The net microbial growth rate is therefore:

$r_{gn} = r_{NO3}/N\%$ = 30 / 12% mg VSS/(L • h) = 250 mg VSS/(L • h)

Respective COD consumption rate for cell growth is:

$r_{COD} = 1.42 \times r_{gn}$ = 1.42 * 250 mg O_2/(L • h) = 355 mg O_2/(L • h)

Translating into COD consumption for cell growth in the aerobic zone:

$\triangle c_{COD}$ = HRT * r_{COD} =3.19 /60 h * 355 mg O_2/(L • h) = 18.9 mg O_2/h

The total COD consumption requires knowledge of the existing biomass concentration, which was not collected in this study.

Appendix D Concentrations of All Ions Tested in the Batch Experiment

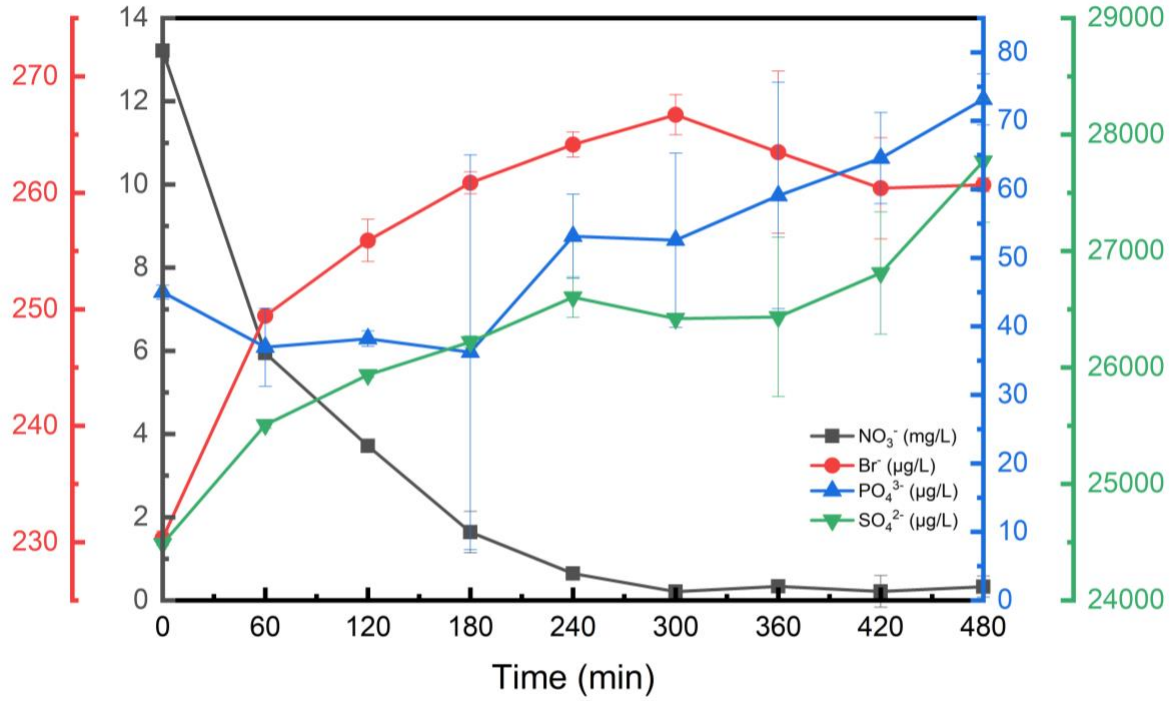

Figure 0-7 Concentrations of nitrate, bromide, phosphate and sulfate during the batch experiment on group A. The experimental duration was 480 minutes.

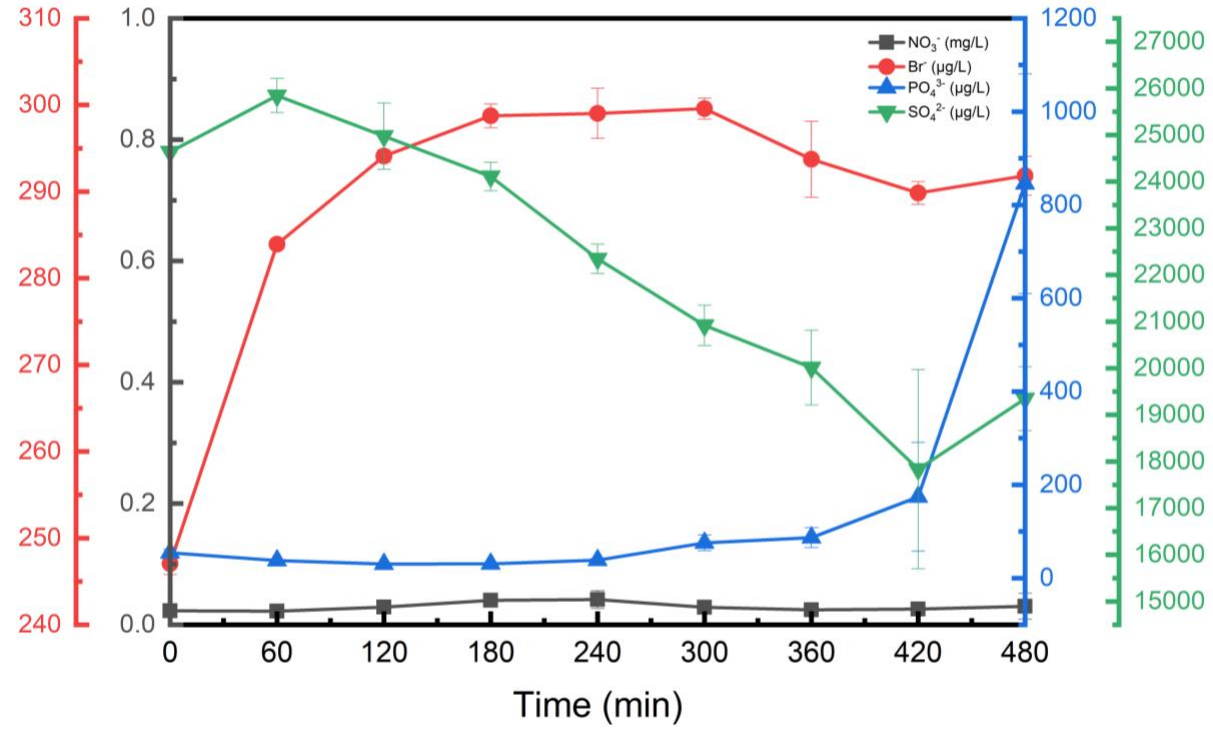

Figure 0-8 Concentrations of nitrate, bromide, phosphate and sulfate during the batch experiment on group B. The experimental duration was 480 minutes.

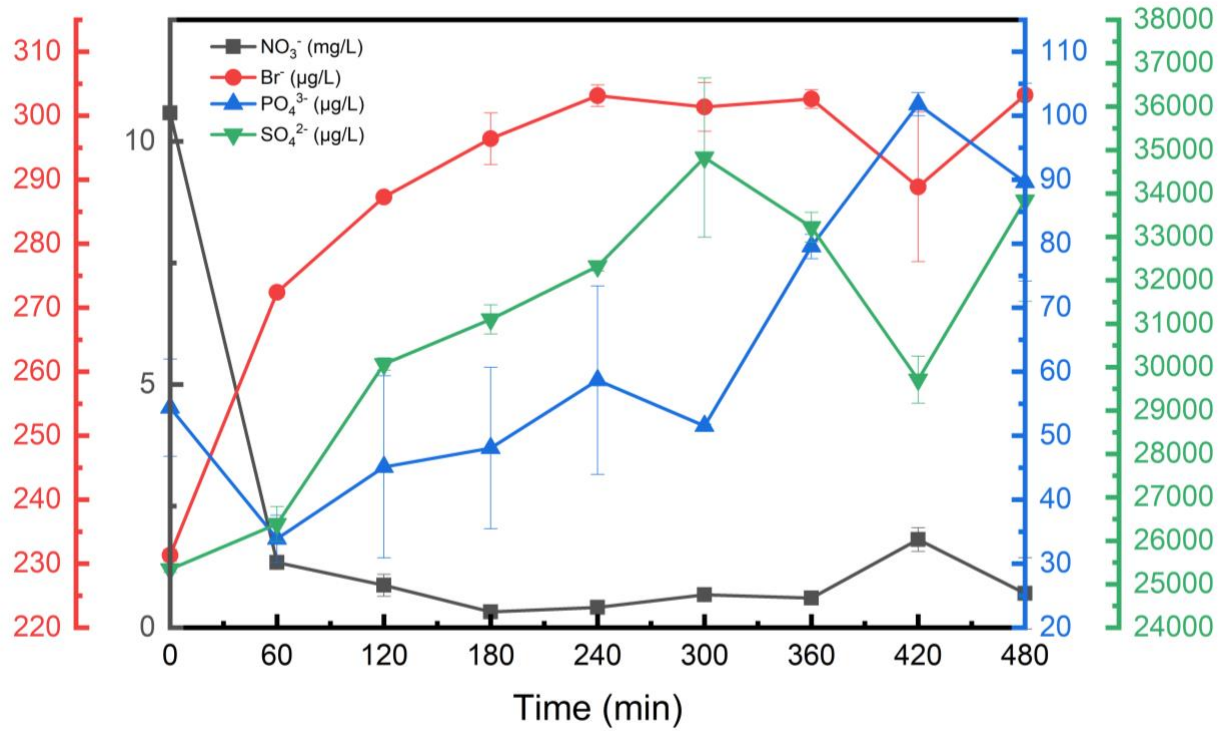

Figure 0-9 Concentrations of nitrate, bromide, phosphate and sulfate during the batch experiment on group C. The experimental duration was 480 minutes.

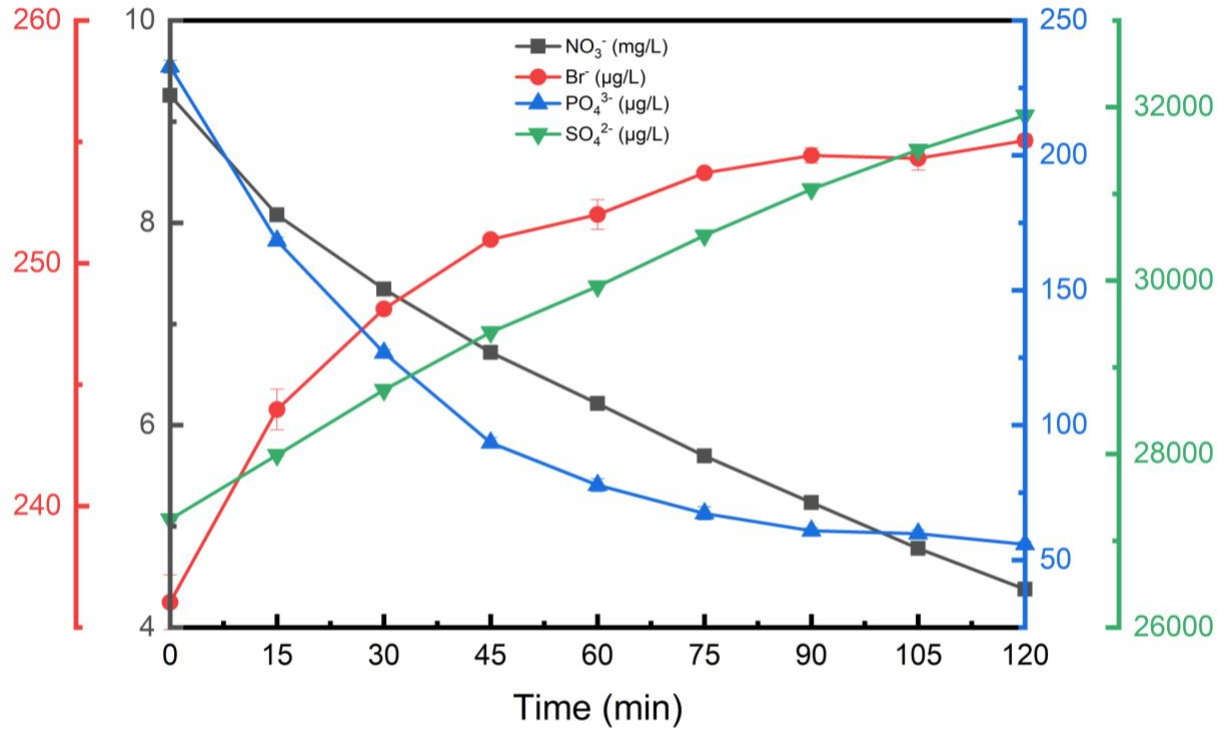

Figure 0-10 Concentrations of nitrate, bromide, phosphate and sulfate during the batch experiment on group D. The experimental duration was 120 minutes.