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Performance of non-activated carbon biofilter in water treatment

Biofilm

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Abstract:
This study aims to a biological technology called biofilm, which is normally used in water treatment. The non-activated carbon is used as the media of the biofilm while a circular system is operated continuously from the cultivation to a lab-scale treatment while efficiency of flow rate is also experimented.

The biofilm using non-activated carbon as media was cultivated for 2 months before applying for a water lab-scale treatment with water from a lake at the end of an agricultural runoff. The water was normally changed every single week. The measurement of biofilm capacity depends on 6 parameters including pH, UV254, DOC, ammonia nitrogen, nitrite and nitrate, which aims to figure out the biofilm' interference on organic matter content and nitrogen's component content. On average, after one week, at the flowrate of 7.9 ml/min, the UV254 and dissolved organic carbon removal rate is respectively 19,37% and 52.11%, which points out a normal charcoal biofilter has a good removal rate when compared to other normal-material biofilters but it's less practical and effective than activated materials. As for nitrogen removal, the biofilm is good at converting and eradicating ammonia nitrogen and nitrite nitrogen, but it can't lower the nitrate nitrogen content. Furthermore, the flow rate was also changed in order to check how the water velocity can affect the treatment of biofilm as well as determine which flow rate would be the best choice for apply in reality. Beside the velocity used initially, two other flowrates tested were 2.4ml/min and 13.1 ml/min.

Overall, the non-activated carbon biofilter should be used as a supplementary part in nitrogen removal system because of its efficiency in converting ammonia and nitrite except nitrate. Meanwhile, for removing organic matter in drinking water treatment, the activated materials still have their own preeminence so that the use of the activated materials should be dominantly considered compared to non-activated ones. On the other hand, the water velocity can really affect the organic removal and the nitrogen components conversion. Spectacularly, the most stable and efficient flow rate is 2.1 ml/min compared to the 2 other experimented flowrates.

Keywords

Biofilm, carbon biofilter, charcoal, water treatment, biofiltration, thesis

CONTENTS

1 INTRODUCTION

Access to safe sources of water, which participates in the metabolism of nutrients and convey them around body, is the primordial demand for all the creatures. Once water containing toxic substances get into body, it may lead to harmful effects and several serious consequences (Hassimi *et al*. 2020, 1). According to WHO, that downtrend of water quality lead directly to approximately 30% of infectious diseases and 40% of deaths all over the world (Shama & Iffat *et al*. 2016, 1). That's why water must be treated carefully before disposal to environment or any consumptions. Afterall, water treatment is mandatory process that can satisfy the safety level for any uses of human beings while reach a regulated standard on quality set by authorities or any competent agencies.

Biological filtration has demonstrated its efficiency in water treatment, especially drinking water. This method aims to getting a source of water whose biological property can remain steady and not contribute to any growths of microbiology when allocated (Rittmann 1995, 61-87). Biological filtration is proved to relate to the consequent mitigations in organic matters, nitrite, sulfides, which lead to the oscillations in the allocation process. Those oscillations occur biologically and bring about the strange taste as well as scent, stimulate the corrosion, trigger a surge in heterotrophic plate counts, turbidity and regeneration of bacteria (Rittmann and McCarty 2001). And biological filtration is capable of devouring that regeneration even during the allocation and minimize the progress of disinfection by-products after final disinfection (Collins *et al*. 1992, 80-90, Urfer *et al*. 1997, 83-98, Huck *et al*. 1998, 158-168, Weiss *et al*. 2003, 69). On the other hand, the cost for running one biological system is reported to be lower than the counterparts of other methods due to its natural characteristics and ability of simplifying substances (Bhatia *et al*. 2018, 1).

In biological filtration, biofilm is one of the most promising options. Biofilm is an innovative technology which has been tested and applied in water treatment. It shows high efficiencies in removing organic substances, compounds made of

nitrogen and phosphorous in water. Moreover, this technique is sustainable and operated naturally without any interference of toxic substances.

This thesis aims to a usage of biofilm cultured on non-activated carbon (normal charcoal). The biofilm is cultured in 2 months then tested in a lab-scale treatment, which targets the changes in concentration of organic matters and some nitrogen's component. Beside the default flow rate, there are 2 other water velocity used to point out the differences by flow rate. Two questions set out in the thesis are:

1. How efficient is the biofilm using charcoal in removing organic matters and nitrogen components?
2. What is the effect of different flow rates on the performance of biofilm using charcoal?

In this study, the principle and formation of biofilm are explained clearly while the use of charcoal is also mentioned. There are the total 6 parameters measured in this thesis consisting of pH, UV254, DOC, ammonia nitrogen, nitrite and nitrate, which illustrate the changes in organic matter and nitrogen's components. Furthermore, the steps of building the biofilm and the measurement of parameters are described in detail.

2 THEORETICAL FRAMEWORK

2.1 Principle of biofilm

2.1.1 Definition

Biofilm is an innovative technology in water and wastewater purification, in which solid media is utilized in suspended growth reactors due to its attachment characteristic on surface. It aims is to enhance the microbial density along with the degradation rate of biofilms, which lead to versatile applications such as biodegradation, biosorption, biomineralization, bioaccumulation, conversion and removal of contaminants (Pal *et al*. 2010, 1-2). Microbial activities inside biofilms crumble a variety of nutrients, including organic carbon, compounds containing nitrogen and phosphorous, even pathogens in wastewater. Biofilm matrix constituents are proved to do the biosorption effectively for heavy metals (Guibaud

et al. 2006, 1955-1962) and organic solvents (Späth *et al*. 1998, 209) while the use of natural microbial flora has been mentioned with the ability to eliminate compounds like pyrene and phenanthrene (Eriksson *et al*. 2002), n-alkanes (Yamaguchi *et al*. 1999, 167-172), chlorophenols (Chang *et al*. 2004, 989, Kargi *et al*. 2005, 2106; Zilouei *et al*. 2006, 597) or some mixtures of pharmaceutical substances (Rosen *et al*. 1998, 257). The water after going through that biological filtration can be either disposed to the environment or reused for irrigation or run into disinfection for drinking water provision (Shama *et al*. 2016, 124; Ikuma *et al*. 2013, 5).

For drink water treatment specifically, biofilm is mostly driven into the mitigation of the disinfection byproduct (DBPs). Briefly, DBPs is the result of chemical reactions between disinfectants, such as chlorine and natural organic matter (Hua & Reckhow 2007, 3309). Normally, biofilms stick and grow on the surface of media, which is typically granular media filters. Water undergoing those biofilters will have dropped density of natural organic carbon due to the crumbling process of microorganisms. It's linked directly to the reduction of the feasibility of disinfection byproduct (figure 1). (Ikuma *et al*. 2013, 6) Furthermore, throughout deterioration of organic matters, biofilm is said to take effects in deodorization and other flavor-related aspects (Huck *et al*. 2000, 5).

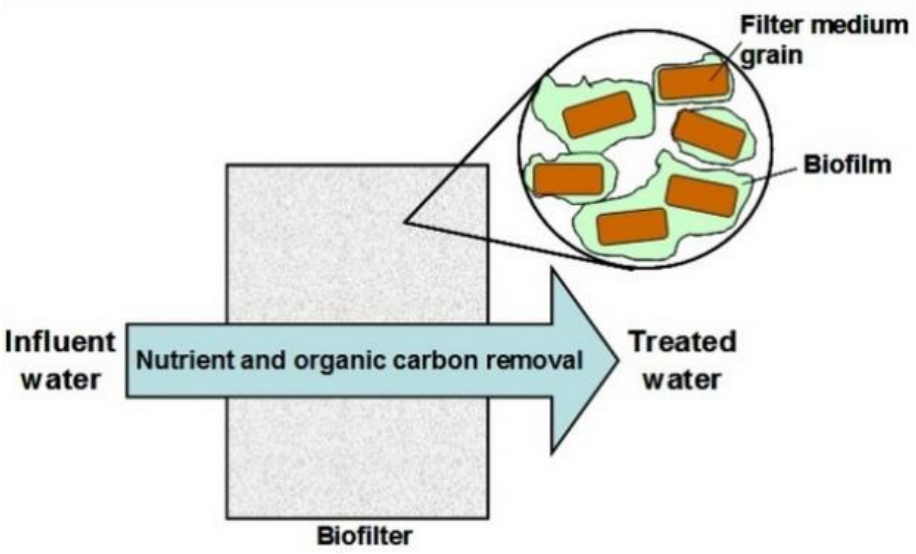

Figure 1: Biofilm's impact on water sample. The graph was cited from Ikuma *et al*. 2013.

As for wastewater, biofilm can be used in secondary treatment phase of the treatment process, where the involvement of microorganisms and biological activities is necessary in break down organic wastes as well as basic nutrients. In particular, basic nutrients in wastewater are phosphorous, nitrogen, ammonia and the removal of those things is crucial to environmental protection from eutrophication and types of pollution (Kloc *et al*. 2012, 5-10). This process is carried out biologically, mostly using activated sludge. Specifically, the activated sludge, known as flocs, is formed completely by the integration of microbial complex. It can fall apart several types of organic compounds or nitrogen. On the other hand, biofilms which are attached to the surface of media also can be applied to wastewater treatment, normally in form of trickling filters (Shama *et al*. 2016, 123).

2.1.2 Formation

Biofilm can form and grow in almost humid environments, and its formation is an intricate process containing many sequence steps which is briefly described in figure 2. The first step is the adsorption of macromolecules (proteins, polysaccharides, humic acids, nucleic acids) and other molecules (lipids, fatty acids and pollutants) onto surfaces that leads to the formation of conditioning films (figure 2, step 1). Those conditioning films take responsibility of modifying the physicochemical features of the surface, motivating the toxic metal ion's disposal off the surface, detoxicating the bulk solutions via adsorption, accumulating nutrients for microorganisms, supplying trace elements and necessary nutrients for biofilms, stimulating the sloughing of biofilm (Lewandowski *et al*. 2011, 530). Then, attachment begins whenever the surface is ready. Bacteria tends to approach by chemotaxis or Brownian motion, which ends up with a momentary connection (figure 2, step 2) supported by interactive forces such as hydrogen bonding, Brownian motion forces, Van der Waals forces and electrostatic forces (Gottenbos *et al*. 1999, 526).

On the surface, the generation of extracellular polymetric substances will bond the cells onto surface. It is an irreversible attachment (figure 2, step 3), which is barely done without chemical and physical impacts. The essence of irreversible attachment is about a synthetic process of exopolysaccharides which take part

predominantly in raising complexes between surface and microorganisms as well as excreting special nutrition adhesives that act intermediately in molecular adherence (Dunne 2002, 155). Specifically, water insoluble amyloid fibrils called β-sheet-rich which take great portion in proteinaceous adhesives are normally found in 5-40% of the strains available in biofilms, including ones for wastewater treatment and freshwater (Larsen *et al*. 2007, 3085). The initial attachment contains the interactive participations of hydrophobic factors, covalent, hydrogen and ionic bonding. Due to electrostatic forces, the stuck cells are not willing to contact the surface but the connecting excreted polymers (Kuma *et al.* 1998, 19). The alternation from reversible to irreversible attachment doesn't last long, reportedly just few minutes (Palmer *et al.* 2007, 579). When the complexes get enough steady, they keep attracting and consuming planktonic bacteria while start separating cell, which end up with the growth and enlargement of the biofilm (figure 2, step 4).

The progress of biofilms occur slowly, requires commonly many days for the structure to be mature (Stoodley *et al*. 2002, 190). Once mature, biofilm becomes energetic and eventually contain a flexible and greedy system which tends to adapt to environment around. It leads to the readiness and secession of bacteria under disadvantage conditions to seek for new sources of nutrients as well as more suitable environment. This is called detachment (figure 2, step 5). The detachment is believed to affected by physical, chemical and biological factors such as lack of nutrition or oxygen, quorum sensing, degeneration of EPS, hydraulic forces, shedding or corrosion. (Chambless 2007, 1574).

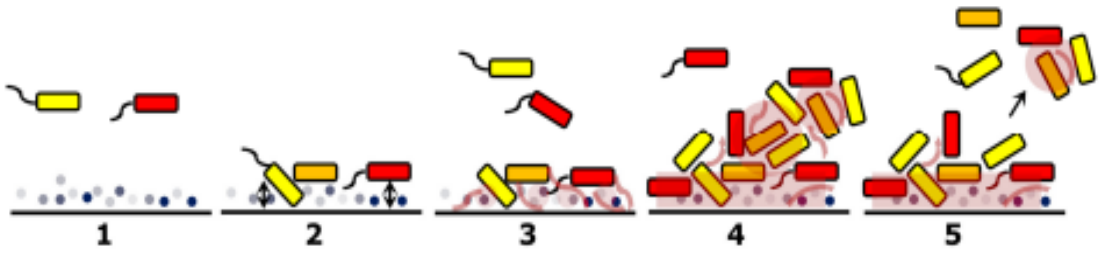

Figure 2: Biofilm life cycle from formation to attachment. The graph was cited from Anderson 2009, 10.

2.2 Characteristics of charcoal

Charcoal has been utilized for purifying water for thousands of years, since the time of ancient Egyptians and Greeks in which charcoal is applied to eliminate strange flavor and scent or basically to avoid some health issues such as chlorosis, anthrax and epilepsy (Bandosz *et al*. 2006, 3). And charcoal can be described as the graphite conformation of primordial carbons, existing in many forms such as wood char, coal char or activated carbon (Kearns 2007, 1; J. A. Bandosz *et al*. 2006, 3). In this study, the term “non-activated carbon” is used to distinguish normal charcoal with other activated charcoal/activated carbon, which has been widely used. In fact, carbon filters are deployed widely in households and wastewater treatment plants these days (Kearns 2007, 1). It is highly acknowledged for its absorption, which plays the critical role in the water treatment or elimination in gas phase (Bandosz *et al*. 2006, 5). Its illustration relies almost on the porosity and high surface area per volume, which make it possible to adsorb volatile and semi-volatile chemicals, comprising monocyclic aromatic compounds, aliphatic amines and aldehydes (Pauly *et al*. 1997, 37, Office of the Surgeon General *et al*. 2004, 43-45; Gaworski *et al*. 2009, 699; Branton *et al*. 2009, 1010). Moreover, carbon filters show a spectacular efficiency in getting rid of inorganic and organic substances such as volatile organic compounds formaldehyde, hydrogen sulphide, besides other molecules like iodine, chlorine and mercury (Kearns 2007, 2).

2.3 Parameters for capacity assessment of biofilm in water treatment

2.3.1 pH

According to Sorenson, pH is defined as -log [H^+] (Sorenson 1909, 131). It is the “intensity” element of acidity. In other word, pH is a standard to determine the acid-base equilibrium in water. The pH of highly dilute solutions is equal to negative common logarithm of hydrogen ion concentration. Normally, pH in natural water is between 4 and 9 and almost water in nature is basic due to the attendance of carbonates and bio-carbonates of the alkali and alkaline earth metal. (American Water Works Association 2017, 454).

In water chemistry, pH is one of the most critical tests and regularly carried out. In fact, every phase of wastewater treatment and water supply including coagulation, disinfection, precipitation, acid-base neutralization, water softening, must use pH as an indispensable element. pH measurement is done in carbon dioxide and alkalinity measurements and other acid-base equilibria. At a certain temperature, pH can indicate the intensity of acidic or basic values of the solution. In this thesis, pH is checked during the filtration prior to the DOC measurement. Besides, pH also determines the state of ammonia nitrogen in the water sample whether it's either ammonia or ammonium.

2.3.2 Organic content

Natural organic matters (organic matters) are problematic in water treatment system, especially the drinking water. They are the results of both degradation occurring naturally in nature and impacts of human. That's why organic pollutants differ between locations, depending on local biosystem, natural characteristics as well as human activities. The chemical characteristics of natural organic matter in potable water supply is specified by its material source and by biogeochemical process at watershed (Huang *et al*. 2004, 1050). In this thesis, the fluctuation of organic matter is reflected via the value of UV254 while one of the most problematic elements of organic matter, which is organic carbon, is also featured via DOC. On the other hand, with just only common water treatment techniques like coagulation, it's impossible to get rid of mainly organic matter, which is associated with its penetration into water treatment system and relevant troubles along the distribution. It could be even worse when handled with chlorine in normal treatment technique, organic matter can be alternated into detrimental disinfection by-products (DBPs) such as haloacetic acids (HAAs), haloacetonitriles (HANs) and trihalomethanes (THMs) (Panyapinyopol *et al*. 2005, 229). The reaction between chlorine and organic matter can be described by following reaction (Marhaba *et al*. 1998, 103-115)

Organic matter + free chlorine → HAAs + HANs + THMs + cyanogen-halides + other DBPs

UV254

UV absorbance is regarded as a measurement to assess chlorinated organic substance precursor content (Serodes *et al*. 2003, 254). The use of UV absorbance is remarkable in water treatment process. It's needed for pointing out cognition of aromatic substance content as well as susceptibility to the removal via unit processes. With the variety of potential coming out from diverse wavelengths, the UV absorbance lead to the replacement of some other progressive analytical methods such as DOC analysis or chromatography (Szerzyna *et al*. 2017, 2). Specifically, UV absorbance at 254 nm wavelength values (UV254) along with dissolved organic carbon (DOC) concentration are used in DBPs determination, where it acts as aromatic substance content (Chang *et al*. 2007, 71). In brief, UV254 is able to indicate the content of organic matter in water (detectronic.org, 2019). UV absorbance measurements is even utilized in many different wavelengths for evaluate the organic's susceptibility in water surface toward the adsorption on granulated activated carbon in South Africa (Lobanga *et al*. 2013, 379). Additionally, UV absorbance can be valuable to point out the content of substances such as fulvic and humic acids, which are linked to water's color in nature, normally yellow brown (Szerzyna *et al*. 2017, 2).

DOC (Dissolved Organic Carbon)

In organic matter, the carbon content is one of the most problematic parts because of its contribution to the disinfection by-products in drinking water treatment. The organic carbon in water bodies contains various diverse organic compounds in various states, some of whom even get oxidized via biological or chemical factors. In general, the total amount of carbon in water consists of many types such as inorganic carbon, total organic carbon, dissolved organic carbon, suspended organic carbon, purgeable organic carbon (or volatile organic carbon), non-purgeable organic carbon (American Water Works Association 2017, 592). Dissolved organic carbon (DOC) is about carbon atoms bonding covalently which pass through 0,45-μm-pore-diameter filter. DOC is a copious source of energy and carbon itself for heterotrophic organisms and participates in motivating the natural metabolism of the entire ecosystem (Kaplan *et al*. 2000, 237-258). It's transported

from environments on land to stream ecosystem via vector made by subsurface water (Fisher & Likens 1973, 422). DOC can have impacts on several stages of drinking water treatment such as chlorination, ozone sterilization and ultraviolet. Also, DOC contributes to the promotion of microorganisms as the main source of nutrients.

In general, DOC is not harmful to human health unless its concentration is high enough to bring about disinfection by products in drinking water. According to Health Canada, the benefits from chlorinated drinking water even overwhelm its chlorination-related health issues. When DOC's concentration is higher than 5 mg/L, it will lead to the formation of disinfection by-products. DOC is able to change color in water at the final phase. Besides, water source in which DOC's concentration is lower than 2 mg/L, can be treated basically for disinfection by-products which avoids the color change (Government of Saskatchewan 2009).

2.3.3 Nitrogen

Nitrogen is a primordial element, which is consumed and resorbed by flora in ecosystem while a small amount is leaked into ground water thought its concentration is low (Vitousek *et al*. 2002, 97). The removal of nitrogen's component, in fact, is a critical part of water treatment, spectacularly wastewater treatment. The content of nitrogen in streams can surge when the amount of nitrogen exceeds the consumption ability of crops or transformation process into the air via denitrification or volatilization. According to USEPA, the main source of nitrogen increase is from agricultural activities such as fertilizers and livestock by-products; the other source is the results of atmospheric deposition and septic processes (USEPA (n.d), 1).

Nitrogen penetrates water bodies in several forms, including organic and inorganic forms. In general, organic-N can be found urea, proteins, amino acids, organisms like algae or bacteria, and decomposing botanical scraps. Organic-N is usually measured by Total Kjeldahl nitrogen (TKN), which points out the combination of organic N and ammonia + ammonium. On the other hand, inorganic-N in waters consists of ammonia, ammonium, nitrate and nitrite. Majority of inorganic nitrogen

is dissolved compounds in waters. Due to the transformability of Nitrogen between forms, it's normally determined in total as total nitrogen (TN). In fact, the form and the amount of Nitrogen are dependent on a lot of factors such as proximity to pollution sources, effects of ground water, availability and type of wetlands, lakes and any other types of water bodies crossing by that is illustrated in figure 3. (Wall 2013, 2)

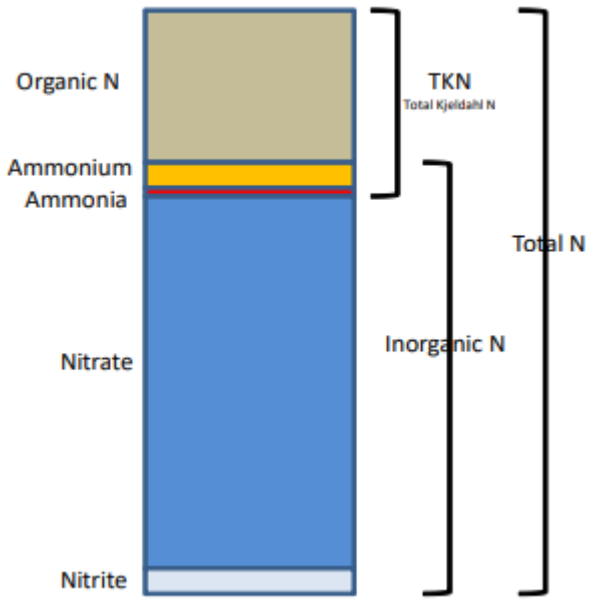

Figure 3. Nitrogen components in water. The graph was cited from Wall 2013, 2.

According to United States Environmental Protection Agency (USEPA), the pH value of drinking water is recommended to be around 6.5 – 8.5, while other parameters for standards and health advisories are listed in the table 1:

Table 1: Nitrogen components standard in water. The table was cited from USEPA 2018, 9-10

Chemicals	Maximum Contaminant Level (mg/L)	Maximum Contaminant Level Goal (mg/L)	Cancer Descriptor	Taste threshold (mg/L)
Ammonia	-	-	Not classifiable as to human carcinogenicity	30.0
Nitrate (as N)	10.0	10.0	-	-

Nitrite (as N)	1.0	1.0	-	-
Nitrate + nitrite (both as N)	10.0	10.0	-	-

Normally, in a drinking water system applying biological nitrification, the conversion between different forms of nitrogen start with ammonium and end with nitrate. Those 2 following equations describe the mutation process of ammonium and existing nitrite to nitrate (Schullehner *et al*. 2017, 2).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Nitrogen removal is also a crucial process in wastewater treatment. The most widely used method to eradicate nitrogen is respectively carrying out an aerobic nitrification and a following anaerobic denitrification. Nitrification is basically utilizing microorganism's activities to perform an aerobic oxidation, which convert ammonia to nitrite, and a subsequent oxidation, which convert nitrite to nitrate (Winograsky 1891, 691-693; Bock & Wagner 2006, 83-118). As for denitrification, it's the process that inorganic nitrogen is reduced gradually from nitrate to respectively nitrite, nitric oxide, nitrous oxide with dinitrogen gas as the final result. Another method is making ammonia and nitrite directly transform into dinitrogen gas through an ammonium oxidation simultaneously in both aerobic and anaerobic conditions (Almstrand 2012, 15-18).

Nitrate and nitrite

Both nitrate and nitrite are ions triggering by nature in nitrogen cycle. The nitrite ion (NO_2-) is mainly nitrogen in an unsteady oxidation state and it's easily mutable when exposed through biological or chemical processes, which end up with nitrate or any other compounds. The nitrate ion (NO_3-) is a stable structure of oxygenated nitrogen and reducible via microbial activities (ICAIR *et al*. 1987).

Nitrite is a medial stage when ammonium is converted to nitrate by actions of microorganisms. Normally, it's rarely upraised in water bodies in a long time. Nitrite

is also the intermediate product when nitrate mutates to Nitrogen gas via denitrification.

Nitrate is a highly water-soluble compound and it's negatively charged, which make it possible to moves easily in the water through the soil profile, ending up at subsurface or groundwater. As long as the groundwater is supplied enough oxygen, nitrate will remain unchanged and keep roaming until it come to the surface. On the other hand, nitrate is also able to precipitate and penetrate waterways which head out to ditches or surface waters. In case of low-oxygen, nitrate will transform to gas state (nitrogen) via denitrification (Wall 2013, 4). Nitrate mostly originates from inorganic fertilizer. It's also utilized in the production line of explosives or glass. Additionally, sodium nitrate is also used as a food preservative, especially in cured meats. Nitrates exist in plants naturally as an important part of the nutrition chain. With mammals and humans, the formation of nitrate and nitrite occurs endogenously (WHO 2011, 1).

In potable water, nitrite and nitrate can pose a threat to human health to an extent meanwhile ammonium doesn't have direct effects on human health. The toxicity of nitrate to human health is mainly from its conversion to nitrite. As for nitrite, it contributes to oxidation of normal hemoglobin to methemoglobin, which can't carry blood to tissues. The decline of oxygen turns into clinically apparent when the concentration of methemoglobin is the 10% as much as the concentration of normal hemoglobin, which leads to cyanosis and even asphyxia at higher concentration. In general, the level of methemoglobin in humans is lower than 2% while in infants the value is lower than 3% (WHO 2011, 10).

On the other hand, because of the essence as precursors of genotoxic N-nitroso compounds in endogenous nitrosation, the presence of nitrate and nitrite in potable water is very suspicious toward their correlation to cancer in urinary and gastrointestinal tract and at other sites (Ward *et al*. 2005, 1608; Villanueva *et al*. 2010, 215). According to the International Agency for Research on Cancer, nitrate and nitrite is mortal carcinogenic in potential conditions containing endogenous nitrosation (International Agency for Research on Cancer 2010, 111). Although the

possibility of carcinogen has never been fully confirmed (Ward *et al*. 2005, 1609, Villanueva *et al*. 2010, 218-219), two recent studies point out the linkage between cancer and nitrate with nitrate in drinking water, specifically bladder cancer (Jones *et al*. 2016, 1757) and colorectal cancer (Espejo-Herrera *et al*. 2016, 345). Furthermore, nitrate and nitrite are mentioned in detail that is relevant to risk of birth defects (Brender *et al*. 2004, 334) and contradictory impregnation outcomes (Stayner 2017, 302).

Ammonia and Ammonium Nitrogen

Ammonia (NH_3) is toxic to aquatic organisms in general, including fish. Meanwhile, with the normal pH level of natural water, ammonium (NH_4^+) is the prevailing form and it's not as toxic as much as NH_3. When pH in natural water upsurge above 8, the amount of ammonia starts rising fast. The fraction of ammonium and the fraction of ammonia can be nearly equal if pH exceeds 9, but in general this case hardly occur with normal water bodies. In general, the term "ammonium" and "ammonia" can be used interchangeably in reports or presentations to stand for concentration of "ammonium plus ammonia-N" measured in laboratory. Particularly, ammonia, also named as unionized ammonia, can be calculated out of the "ammonium plus ammonia-N" reports when the values like pH or temperature of water sample are valid (Wall 2010, 4). Commonly, the ammonium form is used to represent the majority of both ammonia and ammonium due to its predominance in the range of pH and temperature values of drinking water (Schullehner *et al*. 2017, 2).

Ammonia and ammonium mainly come from the agricultural fertilizers and some types of industrial waste and even organism's dregs. Therefore, they often get into the surface waters through drainage systems or overland runoffs. On the other hand, the mineralization of organic matter in soil to inorganic nitrogen also results in an amount of ammonium, which afterward stick to the soil particles like organic matters or clay. That's why ammonium seldom follows the stream upward to surface like nitrate. Ammonium also appears in wells by the concentration of 1 mg/L or more (Rezania 2011, 27). At a certain condition of humidity and temperature in soil, ammonium can transform into nitrate (Wall 2013, 4).

2.4 Effect of flow rate

Water flow velocity is pointed out as one of the main factors which are able to affect the formation of biofilm, besides bacteria itself, nutrition availability, disinfectants or pipe materials (Ollos *et al*. 2003, 87). According to studies, the number of bacteria forming the biofilm is proportional to the velocity of water, particularly with the small amount of degradable organic matter (Ollos *et al*. 2003, 87; Percival *et al*. 1999, 152-153). In fact, the detachment in biofilm is found out that happen when the velocity is 3-4 m/s (Cloete *et al*. 2003, 58) and to avoid the dispersion from stronger fluid shears, a defensive mechanism is utilized (Rupp *et al*. 2005, 2176). The alternation of flow rate is linked to the turbidity throughout the distribution system and apparently spoil the aesthetic aspect of drinking water (Vreeburg *et al*. 2004, 123-124; Lehtola *et al*. 2004a, 601). If there is a certain oscillation in flow rate, it can interfere the biofilm's activity and let some organic matter, phosphorous or metals penetrate and integrate the drinking water (Lehtola *et al*. 2004b, 490-491). The growth in biomass and biofilm's activity was not only due to the increase in water flow rate but also affected by consumption of microbial nutrients in water (Lehtola *et al*. 2006, 2157).

3 MATERIALS AND METHODS

3.1 Chemicals and reagents

In order to cultivate the biofilm, a plastic column (327 mL) cylinder with 2 head attached with plastic ducts (4 mm in diameter); inox nets cut into 3,5-diameter circles; non-activated carbons were grinded into 2-3 mm^3 pieces and washed clearly with DI water before being dried in oven at 105 ºC for 24 hours. Then, the mixture was put in a vacuum desiccator; slender rubber tape was used to strengthen the joints of the column cylinder, aluminum foil was taken to keep the materials and activities inside the column away from the light; while an iron ring stand was applied to hold the column cylinder standing. Besides, a cassette tube pump (SPM-23 EYELA) was employed to recirculate the water flow while a glass tank (4 mL) was used for water sampling.

To evaluate the water quality for the removing capacity assessment of the biofilm, a pH meter (EUTECH instruments pH 510) was used in measuring pH. The organic content and the concentration of ammonia, nitrite and nitrate were calculated based on the UV absorbances in different wavelengths, which was measured by using UV-Vis Spectrophotometer (Hach DR 5000 ™). Moreover, the membrane filters (cellulose acetate, pore size 0.45μm, diameter 47mm) were used in filtration for DOC measurement, while TOC-V_{CSH} Analyzer (Shimadzu), sulfuric acid were used in the measurement of organic carbon and nitrogen gas tank was used to purge volatile organic carbon. On the other hand, chloric acid 1M was employed for the nitrate measurement and a solution containing NO_3- (KNO_3 100 mg/L) was used in making the calibration curve. Chemicals used in the nitrite measurement included coloring agents ($C_{12}H_{16}Cl_{12}N_2$ + H_3PO_4) and a solution containing NO_2- ($NaNO_2$ 250 mg/L), which was for the calibration curve making. To measure ammonia content, a solution containing NH_3- (NH_4CL 1000 mg/L) was used to make calibration curve in advance then sodium hydroxide boric acid buffer solution (NaOH), sulfuric acid absorption solution (H_2SO_4), phenol, sodium nitroprusside, sodium hypochlorite solution and alkaline citrate solution were employed. And a 2100 Kjeltec Distillation Unit (FOSS TECATOR) was used in sample distillation.

3.2 Experimental procedure

3.2.1 Preparation of biofilm cultivation

The experimental set up includes 3 main parts running in a circular system that is illustrated in figure 4 and 5. In general, 4 L of sampled water was poured into the water tank and recirculated through the system with the default level of flow rate (level 6, 7.9 mL/min). The water was changed every 7 days to renew the nutrient source. This was to fill the micro bacteria living inside the water lake into the cylinder, where those organisms would attach to the charcoal surface and form a biomass. This step was repeated weekly in around 2 months until the pores on the charcoal surface were fulfilled by microorganisms. At that point, the filter could stay constant as a biofilm, which was ready for water treatment appliance.

Figure 4. Real image of the lab-scale experimental establishment.

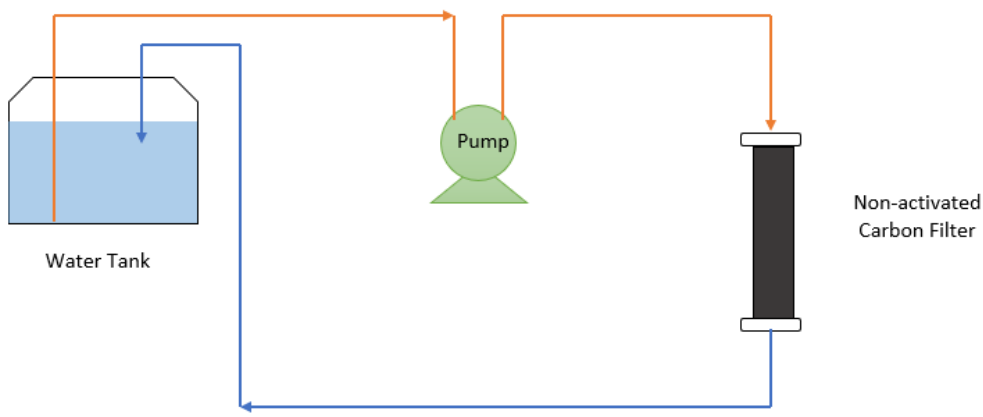

Figure 5: Schematic diagram of lab-scale experimental establishment.

In this study, the sampled water was the wastewater collected Jing-Si lake, located in Pingtung, Taiwan. The sampling site was an intersection of an agricultural drainage system and the natural lake. The characteristics of the raw collected wastewater were summarized in table 2, which was cited from a previous study.

Table 2: Jing-si Lake water quality index. Data was cited from Nguyen *et al*. 2019, 3.

Parameter	Unit	Raw Water
pH	-	7,743 ± 0,766
UV254	cm^{-1}	0,157 ± 0,118
NO_2-N	mg/L	0.145 ± 0.002
NO_3-N	mg/L	2.837 ± 0.963
NH_3-N	mg/L	0.330 ± 0.308

DOC	mg/L	4,165 ± 2.443
sCOD	mg/L	8 ± 2
Alkalinity	mg$CaCO_3$/L	88.750 ± 5.000
PO_4-P	mg/L	0.024 ± 0.011
Tetracycline	mg/L	Not detected

3.2.2 Biofilm application in water treatment

The biofilm cultivation system was continued using for the agriculture wastewater treatment appliance. In this stage, the capacity of the biofilm in removing organic contents and some nitrogen's components was assessed with flow rate 6 (default, 7.9 mL/min). The sampled wastewater was changed every week, and the water quality was measured on raw water and after the 3rd and 7th day of that week-cycle, following these selective parameters: pH, UV254, dissolved organic carbon (DOC), ammoniacal nitrogen (NH_3-N), nitrite (NO_2-), and nitrate (NO_3-). That process was performed in 3 weeks. The following passages describe the detail steps to measure the values of parameters.

pH: The first step was rinsing the pH electrode with DI water then calibrating the pH meter with pH 7 in CAL Mode and clicking Start button. The same procedures were carried out with pH 4 and pH 10. After 3 times of calibration, the pH meter was automatically changed to MEAS Mode and ready for any pH measurement. Consequently, an immersion of the pH electrode into a water sample with a click in Start button was the final step to get the pH result.

UV254: The UV-Vis Spectrophotometer needed to be turned on for about 15 minutes before using and got calibrated with DI water. In this case, the equipment was set up at single wavelength of 254 and 3ml DI water for calibration was put into a 3.5ml cuvette, then pressing the "zero" button start the process. Until the screen appeared the result 0.00, the equipment could be considered as usable. Similarly, 3ml water sample was taken into that plastic cuvette, which was put into the UV-Vis Spectrophotometer at the same wavelength and pressing "read" button was to have the results.

In order to measure dissolved organic carbon, a filtration step was required to eliminate suspended organic carbon (particulate organic carbon). Water sample was poured through into a filter using 0.45μm-pore paper.

DOC: Firstly, sulfuric acid was added into the samples until its pH dropped to 2. Then, 2 20-ml vials were prepared and roughly rinsed with previous acidic solutions before filled up with those solutions. Two vials afterward got purged with Nitrogen (N2) for 3 minutes, which aimed to get rid of volatile organic carbon. Finally, the TOC-V_{CSH} Analyzer was used to measure the data of Dissolved Organic Carbon in those 2 vials. To take a more reliable data, the results coming out were taken with an average.

Ammoniacal nitrogen (NH_3-N):
First of all, a calibration curve on DI water was needed, using Microsoft Excel. The Solution containing NH_3- was diluted and distributed to 7 25ml vials with different concentration: 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/L with 10ml each vial. For each vial, it was added with 0.4 ml sodium nitroprusside, 0.4 ml phenol and 1ml of mixture Alkaline citrate solution and sodium hypochlorite solution (10ml Alkaline citrate solution to 2.5 ml Sodium Hypochlorite solution; proportion of 4:1). Those vials afterward needed to be kept in darkness for at least 1 hour. Using UV-Vis spectrophotometer at single wavelength of 640 with a 3.5ml plastic cuvette, the data in UV format of those vials could be measured and collected, which ended up with a calibration curve between NH_3- concentrations (mg/L) and UV absorbance. Specifically, with the calibration curve, the equation of correlation between NH_3- concentration and UV absorbance was figured out, which was needed to calculate the NH_3- concentration.

The next phase was determining the NH_3- concentration in water sample. But water sample needed to go through a distillation process using the 2100 Kjeltec Distillation Unit since this action could avoid the risks of complex from the interaction between inorganic substances and ammonia (Hach Company, 2017). The method for distillation required 350ml test tubes to operate the machine. After turned on, the machine itself needed calibrating 3 times with DI water,

100ml into the test tube each, with an empty flask in receiver position. After that, 100ml water sample poured in test tube was added with 10 ml boric acid buffer solution and put in distillation position. Receiver position was for a 20 ml sulfuric acid absorption solution in a flask. The distillation process started with red button and took for 3 minutes to finish. After that process, the solution's volume in receiver solution was about 30-40ml, and it's poured into a 100ml empty flask and diluted with DI water until the water reach 100ml mark, of which 25ml solution was taken out afterward then added with 0.4 ml phenol, 0.4 ml sodium nitroprusside and 1 ml mixture of alkaline citrate and sodium hypochlorite (proportion of 4:1). That solution was kept in darkness for at least 1 hour. Next, the solution was measured with UV at wavelength of 640, using UV-Vis spectrophotometer, which leaded to the UV640 absorbance of the solution. That value was applied to the equation in the calibration curve so that the concentration of ammonia of water sample could be figured out.

Nitrite (NO_2-):

For measuring Nitrite, the making of calibration curve was also required. Firstly, solution containing NO_2- was diluted and distributed into 6 25ml vials with different concentration: 0, 0.02, 0.04, 0.06, 0.08, 0.1 mg/L as long as every vial had 10ml diluted solutions. Each vial afterward was added 0.4 ml coloring agent. After 15 minutes, those vials turned into pink, in which clarity of color depended on concentration. Next, UV-Vis spectrophotometer was used to measure UV absorbance at single wavelength of 543. The equipment needed calibrating using UV543 first with DI water in the cuvette to be ready for any measurements. After the calibration, solutions in vials respectively were measured in the UV-Vis spectrophotometer. The data coming out was used to make a calibration curve via Excel about the relation between NO_2- and UV543 absorbance. Normally, that calibration curve and equation from it could be used for the whole week. Secondly, to apply the calibration curve to measure the real NO_2- concentration of water sample, performers only needed to take about 10ml water sample into a vial and drop 0.4 ml coloring agent in then wait for 15 minutes to measure it. It resulted in the value in UV absorbance, which afterward could be exchanged to NO_2- concentration via the equation.

Nitrate (NO_3-):

For measuring Nitrate, the use of calibration curve was also needed. Firstly, solution containing NO_3- was diluted and distributed into 7 25ml vials with different concentration: 0, 1, 2, 3, 4, 5, 6 mg/L so long as every vial had 10ml diluted solutions. Each vial afterward was added 0.2 ml HCL 1M. Next, UV-Vis spectrophotometer was used to measure UV absorbance at multiple wavelength of 220 and 275. The equipment needed calibrating using UV220 and UV 275 first with DI water in the cuvette to be ready for any measurements. After the calibration, solutions in vials respectively were measured in the UV-Vis spectrophotometer. The data coming out was used to make a calibration curve via Excel about the relation between NO_3- and UV220+UV275 absorbance. Normally, that calibration curve and equation from it could be used for the whole week.

In the second step, to apply the calibration curve to measure the real NO_3- concentration of water sample, performers only needed to take about 10ml water sample into a vial and drop 0.2 ml HCL 1M then measured it. It resulted in the value in UV absorbance, which afterward could be exchanged to NO_3- concentration via the equation.

3.2.3 Change of flowrate to test the difference in removal

In this phase, water velocity was changed from flow rate 6 (default) respectively to flow rate 10 and flow rate 3 to test the effects of flow rate on the activity of the biofilm. Each flow rate was tested in 2 weeks.

At flow rate 3: water velocity = 2.4 ml/min

At flow rate 10: water velocity = 14.3 ml/min

The data afterward were converted to removal rate, which facilitated the comparison in capacity between flowrate

3.3 Analytical methods

3.3.1 Evaluation of removal rate

In this study, removal rate plays an important role to make a comparison and determine the capacity of the treatment in biofilm. Specifically, it was employed for

concentration measurement of UV254, DOC, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen. The removal rate was measured according to the following equation:

$$Removal\ rate\ = \frac{(Q_0 - Q_t)}{Q_t} \times 100$$

where	Q_0	initial concentration	[mg/L]
	Q_t	concentration at t time	[mg/L]

3.3.2 Statistical tests

In this study, the Pearson correlation test was used to check the relevance between values of DOC and UV254. Based on that, the data was determined as quite valid and according to the theory. The test is effectuated with Microsoft Excel. The correlation coefficient (R) features the preciseness of correlation with a table of values based on Evans's guide described in table 3:

Table 3: Pearson correlation rendition. Data was taken from Evans (1996, cited in Beldjazia *et al*. 2016, 26-27)

Absolute value of R	State
0.00-0.19	Very weak
0.20-0.39	Weak
0.40-0.59	Moderate
0.60-0.79	Strong
0.80-1.0	Very strong

On the other hand, a t-test was also performed along with the correlation test to confirm the significance of the correlation. In details, if the p-value is lower than 0.05, the values are significantly correlated with each other. On the contrary, the correlation is insignificant.

4 RESULTS AND DISCUSSION

4.1 Evaluation of biofilm efficiency

4.1.1 Organic matter removal

Theoretically, organic matter, specifically organic carbon, is the main source of nutrients for microorganism. With the weekly change of water sample into water tank, the availability of nutrients for bacteria is implemented regularly. In general, the organic matter, including organic carbon, tends to fall due to the bacterial consumption. Since there is no other additional source of nutrients, the data measured normally should drop no matter fast or slow. Figure 6 describes the trend of organic matter content via UV254 and DOC values in a period of 7 days.

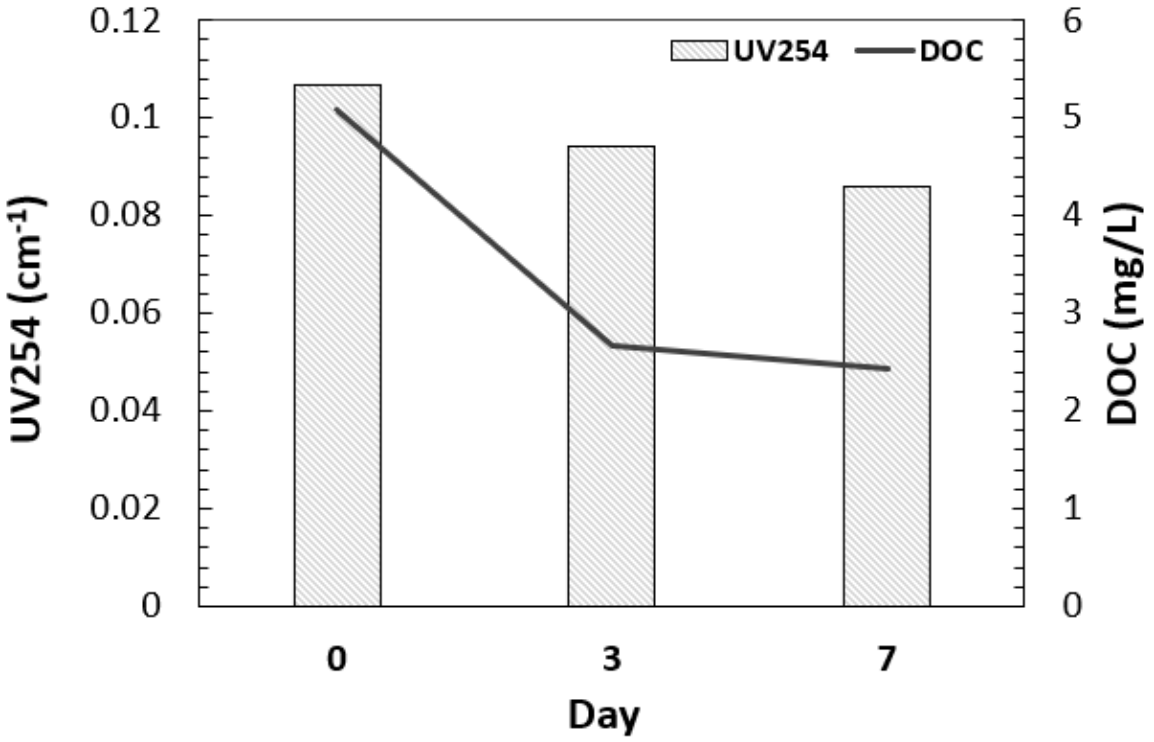

Figure 6: Record of UV254 and DOC values (flowrate 6) in 7 days within 7.08 – 7.14 of pH. There was a strong correlation between UV254 and DOC values that was statically insignificant (R = 0.95, p-value=0.2).

From figure 6, it revealed a downward trend in organic content after a week as predicted. For instance, the UV254 value of the water dropped gradually from 0.107 to 0.086 cm^{-1} after 7 days applying the biofilm. Also, the DOC parameter, which had been above 5 mg/L, halved nearly after 3 days, and dropped slowly to

approximately 2.43 mg/L on the last day. Furthermore, the removal rate of UV254 after 3 days was 11.87% while the whole period of 7 days was 19.37%. Whereas, the removal rate of DOC was 47.55% in the first 3 days while the rate of the whole week was 52.11%. It demonstrated that the removal rate of calculated parameters was in the increment with the decline of factors. The performance was apparent that the microbiological activities were strong on the first 3 days, then became slower on the last 4 days. The organic content fell to a certain level.

A previous study from Su found that the UV254 removal rate of a biofilm using sand could achieve in the range of 19.47 - 34.74% (Su *et al*. 2011, 1870). Comparing to that, on the 7th day, the current biofilter using charcoal reached the minimum capacity of the sand biofilm. Moreover, the pace of the removal representing in UV absorbance showed a stable increase in the current studied biofilter, which suggested that the removal process might require more time. On the other hand, according to a research on activated carbon biofilm, the average UV254 removal in a period of 7 days was 25.2% (Wang *et al*. 2010, 221), which was higher than the capacity of the current non-activated carbon biofilter. It indicated that the current biofilm using charcoal as media might bring less efficient UV254 removal rate compared with the appliance of the biofilter using activated carbon.

About the DOC removal rate within a week, the current biofilter using charcoal was less efficient than the biofilter using activated carbon, which achieved 93% of removal rate (Lohwacharin *et al*. 2015, 1643). However, the DOC removal rate of the sand biofilm was about 25% (Pipe-Martin 2008, 449), which was much lower than the removal rate of the current biofilm using charcoal. Those findings suggested that the DOC removal capacity of biofilter using charcoal might be better than the biofilm using sand. However, compared to an activated carbon biofilm, the DOC removal rate of a charcoal biofilter was much less efficient.

4.1.2 Assessment of nitrogen components

From figure 7, it was observed that there were 2 different trendlines of the nitrogen components within a week: a downward trend in NH_3 and NO_2, and a surge in NO_3

concentration. For instance, the concentration of NH_3 and NO_2 decreased rapidly on the first 3 days before dropped slowly to approximately to 0.0237 mg/L and 0.0023 mg/L respectively on the last day. Moreover, the conversion rate of NH_3 and NO_2 was respectively 93.22% and 96.97% within the first 3 days, while the rate reached 95.54% and 98.48% respectively on the final day. On the contrary, the concentration of NO_3 up surged dramatically to about 4.1043 mg/L with the conversion rate of 53.45% after 3 days, and then increased gradually to 4.4733 mg/L with 67.25% of conversion rate on the last day. The results indicated that the conversion of the nitrogen components might occur with the strongest level in the first 3 days.

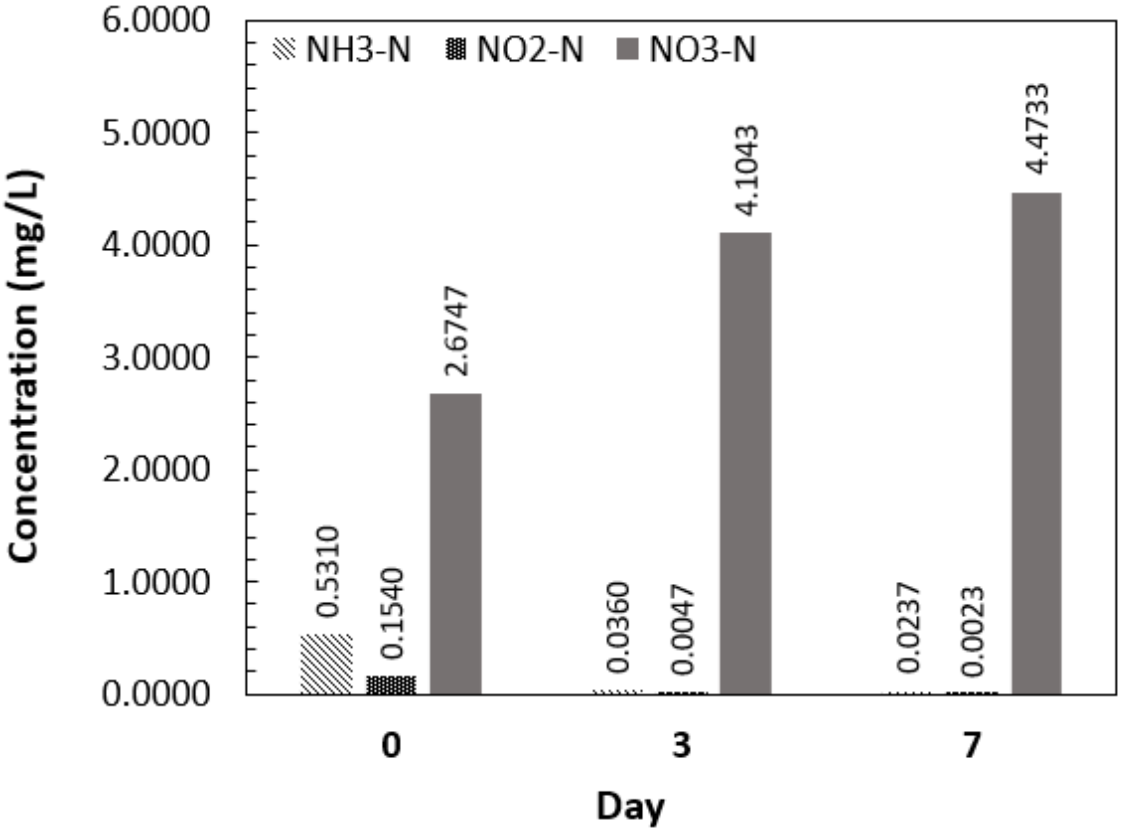

Figure 7. Record of the nitrogen components values (flow rate 6) in 7 days within 7.08 – 7.14 of pH. At this pH level, ammonium and ammonia would exist as ammonia.

In a data synthesis of Santos and Daniel on filtration of biofilter using activated carbon (Santos & Daniel 2019, 602), the conversion rate of NH_3-N could reach 98% as the maximum range (Anderson 2001, 2928), while other study found that the appliance could reach 100% in 4 days (Feng *et al*. 2012, 1589-1591).

Compared to the result of the current study, the ammonia conversion rate of the current biofilter using normal charcoal was a bit lower than the ones using activated carbon.

On the other hand, a research on biofilter using sulfur-limestone found that the NO_2 removal after a week was about 92%, and it could extend to approximately 120% after 140 days (Zhou *et al*. 2011, 1765). Moreover, other study on biofilter using activated sludge revealed that the nitrite concentration in normal condition (pH 7, non-added calcium ions) could be completely removed from sampled media in 7 days (Cyplik *et al*. 2011, 1795-1796). Compared those appliances to the current charcoal biofilm, it was clearly that the charcoal is effective in nitrite removal.

Surprisingly, some studies about activated sludge biofilter brought about a decrease in the concentration of nitrate (Cyplik *et al*. 2011, 1795-1796, Fudala-Ksiazek *et al*. 2014, 7312). Meanwhile, the NO_3 concentration in the current study up surged largely, indicating an unusual performance of the biofilm appliance. It might be due to the nitrification occurring during the process that increases the NO_3 contents. Therefore, extra technological steps to trigger denitrification should be studied and applied in order to enhance the nitrate metabolism to nitrite and then to nitrogen (gases) (Ruiz *et al.* 2006, 330).

Generally, the biofilm using charcoal in the current study is promising in removing ammonia and nitrite with high conversion rate. However, there is a drawback in the nitrate removal with inefficient performance that requires further additional stages to trigger the denitrification. Moreover, as the ammonia and nitrite removal rates were high efficiency in the first 3 days, the required time for ammonia and nitrite conversion might not have to stay for the whole 7 days, which could be utilized for the nitrogen removal process.

4.2 Effect of different flow rates on the biofilm capacity

The figure 8 describes different charcoal biofilter performance in removing UV254, DOC, NH_3, NO_2 and NO_3 by applying 3 different flow rates. It was observed from figure 8 that level 6 of water flow brought the highest DOC removal rate (52% after

a week) in the comparison with 2 other velocities, while the UV254 removal rate only achieved 19% after 7 days which is the lowest value among 3 levels. Interestingly, the DOC removal rate of flow rate 3 and 10 was at the same range of around 40%. On the other hand, the charcoal biofilter achieved the highest UV254 removal rate (29%) after a week by applying flow rate 10, while the flow rate 3 only reached 23% on the last day. Moreover, it could be observed the difference of the organic conversion between 3-day and 7-day phases in every experiment of 3 level of water velocity. In which, the conversion generally was more active in the first 3 days to all the flow rates and might lead to the reduction in dissolved organic carbon removal capacity.

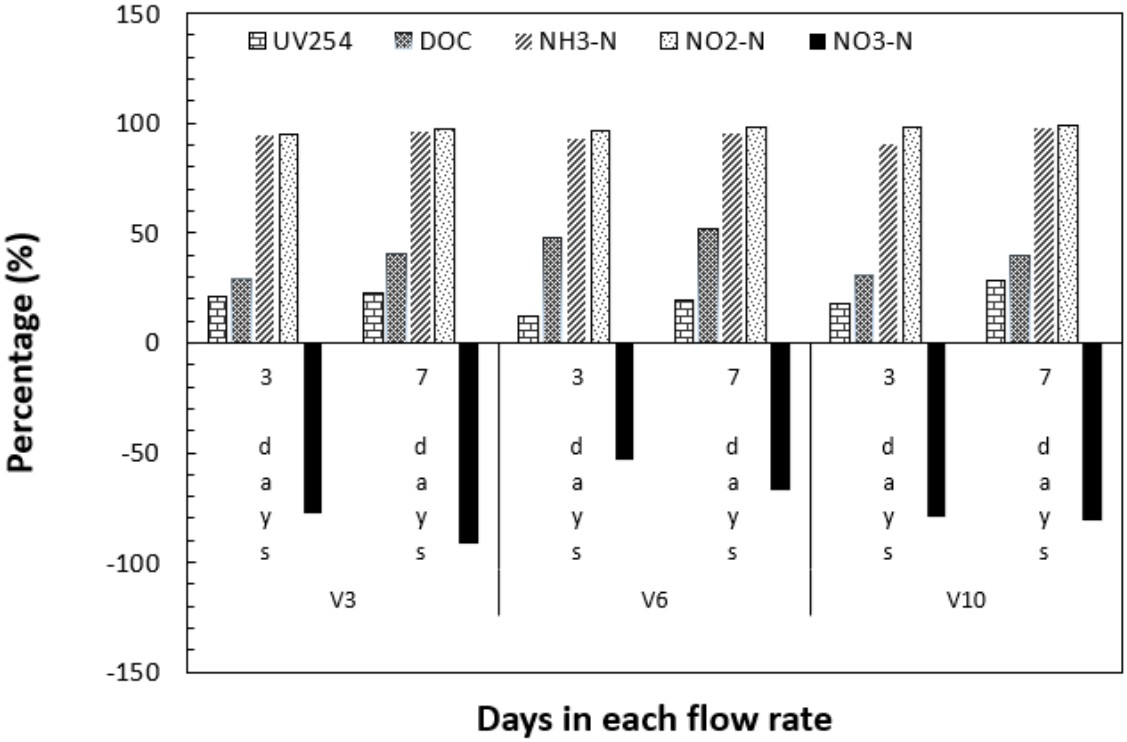

Figure 8. Performance of charcoal biofilter in removing UV254, DOC, NH_3, NO_2 and NO_3 by applying 3 different flow rates: level 3 (V3), level 6 (V6), and level 10 (V10).

The removal rate of ammonia and nitrite was varied around 95-98% after a week, and there were not any apparent differences in these 2 parameters under 3 different levels of water flow. Besides, it was observed that the removal process was a bit more active in the first 3 days, which made the removal rate up to 90%. Meanwhile, the nitrate removal rates had a clear divergence. The level 3 of flow rate caused the highest conversion percentage of NO_3 removal rate, which was

around 78% in the first 3 days before derived about 92% in the final day. Meanwhile, flow rate at level 6 and 10 brought the nitrate conversion of 67% and 80% respectively after 7 days, in which the level 6 was the least efficient velocity on the biofilter. Apparently, for the long-term application, flow rate 3 was the most optimal and effective one, which had significant removal capacity in organic matter and dissolved organic carbon while the conversions in nitrogen components were high after only the first 3 days. Maybe the higher water velocity, the more affected detachment, which undoubtedly affected the treatment of biofilm. Velocity 3 was equal to 2.4 ml/min, at which the attachment nearly couldn't happen so that its capacity was better eventually (Cloete *et al*. 2003, 58).

5 CONCLUSION

The biofilm cultured in 2 months had positive results at a flow rate of 7.9 ml/min, which could be seen via the removal efficiency. The main results showed that the biofilm using normal carbon as media is not totally optimized as much as any activated materials in term of organic removal. That means in drinking water treatment, the use of activated materials should be more considered. For ammonia and nitrite, the biofilm itself work well but for nitrate, it can't trigger the denitrification. Consequently, the charcoal biofilm should be used as a part of nitrogen removal system. On the other hand, the water velocity affected the bio-treatment much, representing in the differences in capacity of organic substances removal and nitrogen conversion. It is apparent that choosing a slow flow rate below the detachment threshold makes the biofilm's activities more stable and efficient.

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