©2021 This manuscript version is made available under the CC-BY-NC-ND 4.0 license
https://creativecommons.org/licenses/by-nc-nd/4.0/

The definitive publisher version is available online at
https://doi.org/10.1016/j.biortech.2021.126125

Non-submerged attached growth process for domestic wastewater treatment: Influence of media types and internal recirculation ratios

Bao-Trong Dang[1], Thanh-Tin Nguyen[2,3], Xuan-Thanh Bui[3,4,*], Huu Hao Ngo[5], Kun-Yi Andrew Lin[6], Itayama Tomoaki[7], Todd Saunders[8], Tan-Nhut Huynh[3,4], Thanh Ngoc-Dan Cao[3], Chettiyappan Visvanathan[9], Sunita Varjani[10], Eldon R. Rene[11]

[1] Ho Chi Minh City University of Technology (HUTECH), 475A Dien Bien Phu, Ward 25, Binh Thanh District, Ho Chi Minh City, Vietnam.

[2] School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology, 123 Cheomdangwagi-ro, Buk-gu, Gwangju 61005, Republic of Korea

[3] Key Laboratory of Advanced Waste Treatment Technology, Ho Chi Minh City University of Technology (HCMUT), Vietnam National University Ho Chi Minh (VNU-HCM), Thu Duc city, Vietnam.

[4] Faculty of Environment and Natural Resources, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet str., district 10, Ho Chi Minh City 700000, Vietnam.

[5] School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NWS 2007, Australia.

[6] Department of Environmental Engineering & Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung, Taiwan.

[7] Graduate School of Engineering, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.

[8] Graduate School of Biomedical Science, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.

[9] Department of Energy, Environment and Climate Change, School of Environment, Resources and Development, Asian Institute of Technology, P.O. Box 4, Klong Luang, Pathumthani 12120, Thailand.

[10] Gujarat Pollution Control Board, Gandhinagar, Gujarat 382010, India.

[11] Department of Environmental Engineering and Water Technology, IHE – Delft, Institute of Water Education 2601 DA, Delft, the Netherlands

*Corresponding author

Xuan-Thanh Bui, Ho Chi Minh City University of Technology (HCMUT), Viet Nam National University Ho Chi Minh, Viet Nam. Email: bxthanh@hcmut.edu.vn

Journal Pre-proofs

Abstract

This study is aimed to comprehend the treatment of non-submerged attached growth systems using bio-sponge, bio-cord, and bio-cloth media. Three reactors were set up with internal recirculation ratio of 1 (IR = 1) and similar media surface area. Bio-sponge and bio-cloth reactors showed removal of COD (79 vs. 76%) and NH_4^+–N (78 vs. 73%). While bio-cord treatment was deteriorated due to time-dependent process. Multiple linear regression revealed that alkalinity governed the formation degree of the anaerobic zone in bio-sponges, partially affecting nitrification. Increasing IR from 1 to 3 caused sludging of the attached biomass and was positively correlated with effluent nitrite nitrogen concentration, indicating the sensitivity of nitrification to spatial distribution effects. In addition, bio-sponge system obtained superior performance at IR of 2 while bio-cloth one might be also an effective media for wastewater treatment if having good durability.

Keywords: *Nitrite accumulation; Attached growth; Multiple linear regression; Down-flow hanging media; Domestic wastewater.*

1. Introduction

In low-income countries, setting up centralized wastewater treatment systems to assist rural areas is costly and underfunded. This is due to scattered populations and geographic, hydrologic, economic, and social constraints (Asano et al., 2007). Hence, the adoption of small-scale treatment systems has been recognized as a vitally alternative solution for decentralized treatment to prevent multidimensional consequences from eutrophication (Itayama et al., 2006; Ushijima et al., 2015). In this light, many eco-friendly methods have been proposed for wastewater treatment, such as bioremediation, non-submerged attached growth, microalgae-activated sludge process, anaerobic osmosis membrane bioreactor, modified constructed wetlands, or biochar (Varjani and Upasani, 2016; Watari et al., 2017;

Nguyen et al., 2020; Nguyen et al., 2020; Gao et al., 2021; Dang et al., 2021).Among them, the non-submerged attached growth process has been paid much attention to thanks to its cutoff of aeration requirement. This process is similar to trickling filter integrated with a packing media (bio-carrier) to enrich biofilm under natural ventilation (Tra et al., 2021). This configuration allows extending the food chain from aerobic to anaerobic bacteria contributing to energy savings, which could be a green technology towards sustainability.

Media, the core of the trickling filter, has been widely studied over the past twelve decades. The first trickling filter, derived from a rock, was applied to sewage treatment in the UK in 1893 (Bressani-Ribeiro et al., 2018). By the 1970s, plastic was explored as an effective alternative media due to its light weight. Currently, the characteristics of plastic media allow it to fulfill dozens of commercial needs. Such characteristics include a sufficient surface area of 200 m^{-2} m^{-3} or more (Rittmann and McCarty, 2020; Budgen and Le-Clech, 2020), a high porosity of 95% to avoid clogging (Hayder et al., 2017), good pore size (Machdar et al., 2018), allowance for dissolved oxygen (DO) transfer (Araki et al., 1999; Bundy et al., 2017), maintaining a proper biofilm thickness (Arabgol et al., 2020), and a high durability and cost performance (Loupasaki and Diamadopoulos, 2013). Several media (i.e., polystyrene, polyurethane, polypropylene, cotton, polyvinyl chloride, and geotextile) have been subsequently evaluated on their treatment efficiencies and the mechanism of pollutant removal (Loupasaki and Diamadopoulos, 2013; Husein et al., 2019). However, it is essential to note that some materials often did not attain optimal interaction between media types, pollutants, and native microorganisms interactions. Critical issues found in this process are odor, filter clogging events, and the treated water sometimes cannot comply with effluent standard limits (Rittmann and McCarty, 2020).

Currently, polyurethane (bio-sponge) has emerged as a potential candidate for trickling filter applications thanks to its high specific surface area up to 2400 m^{-2} m^{-3} (Tra et

Journal Pre-proofs

al., 2021). This feature allows it to operate for a long time without hindrance, caused mainly by clogging or media durability (Onodera et al., 2014). In addition, Miyaoka et al. (2017) reported that bio-sponge enhanced biomass growth up to 20–35 g L^{-1}, 10–20 times higher than the activated sludge process. The unique structure of bio-sponge favors improved nitrogen removal via nitrogen dissimilation pathways such as shortcut nitrification and denitrification, partial nitrification, and single-stage mainstream partial nitritation–anammox process (Chuang et al., 2007; Matsubayashi et al., 2016, Watari et al., 2020). Additionally, the complex physico-chemical interactions occurring inside sponge carriers might improve pathogen bacteria removal of 1.8 $\times 10^5$ MPN 100 ml^{-1} and 1.5 – 3.7 log of virus (Tawfik et al., 2006; Kobayashi et al., 2017). The concentration of biomass trapped inside the media pores was found to be 1.3 – 4.3 times higher than that of outside sponge surface (Onodera et al., 2016). This is a unique feature of sponge carriers in preventing clogging. However, like other biological treatment methods, certain adverse conditions can affect nitrogen removal (e.g., increasing internal circulation (IR), salt accumulation, or decreasing natural ventilation). For example, switching IR from 0 to 1.5 and 2.0 resulted in an increase of 39.5%, then an 8% decrease in the denitrification, respectively (Ikeda et al., 2013). In addition, reducing dissolved oxygen in bulk liquid or aerobic biomass exposed to anaerobic biological products such as volatile fatty acids can inhibit nitrification and thereby induce nitrite accumulation (Chuang et al., 2007; Matsubayashi et al., 2016, Le et al., 2020).

Contemporaneously, researchers have recently focused on polypropylene media in biofilm reactors, denoted here as bio-cord (Yuan et al., 2012; Tian et al., 2019; Aqeel and Liss, 2020). This concept originated from a farmer in Japan who realized that the ropes cleaned water in oyster beds by branch strands submerged in the seawater. The commercial bio-cord consisting of the core thread with several polymer fibers is looped to facilitate microorganisms proliferation (i.e., vertical distribution). This media exhibited good performance by removing 87% TSS (Yuan et al., 2012) and 92 % NH_4^+–N (Tian et al., 2019). Around 98 % NH_4^+–N removal was found in a bench-scale bio-cord for wastewater treatment from lagoon treatment system (Skoyles et al., 2020). These results highlighted that bio-cord could be a potential alternative or supplement to treat agricultural wastewater (Zhou et al., 2018).

Another idea is to use flat cotton or synthetic fabrics (bio-cloth). The cellulose content in cotton quickly accelerates biodegradation caused by bacterial enzymes (Varjani, 2017), reducing its durability for application to biological processes (Chauhan et al., 2019). To overcome this issue, bio-cloth modified using the hydrosulphuric acid improved its durability and removal performance (i.e., COD and NH_4^+–N at 98.34% and 85.44%, respectively) (Husein et al., 2019).

Currently, either bio-cloth or bio-cord has been extensively studied in submerged biofilters by means of intensive aeration. However, lack of studies used these media in the non-submerged attached growth processes (based on recent Scopus publications). In essence, each material has distinct durability under certain operating conditions (Loupasaki and Diamadopoulos, 2013), while differences in media shape and distribution (i.e., packaging or vertical distribution) are suspected to influence treatment activity. Despite the availability and abundance of attached growth materials, media comparisons have rarely been explored. Whether potential materials possess proper activity treatment and value replacement is still, moreover, questionable.

Looking for a suitable media for the low-cost treatment process, this work aimed to comprehend the practical treatment of bio-sponge, bio-cord, and bio-cloth media. The internal recirculation (IR = 1) was applied for three reactors corresponding to the hydraulic loading rate (HLR) of 4.4 $m^3\ m^{-2}\ d^{-1}$. Subsequently, the effect of IR ratios was investigated

Journal Pre-proofs

for the best media under IRs of 2 (HLR = 6.6 m^3 m^{-2} d^{-1}) and 3 (HLR = 8.7 m^3 m^{-2} d^{-1}) to diminish the feed concentration of nitrogen species (Ikeda et al., 2013; Bundy et al., 2017; Tra et al., 2021). Further, multiple linear regressions were used to explore interaction mechanisms for each media type and potential applications to practical aspects. Such outcomes can bring a basis for selecting an ideal bio-media for wastewater treatment in rural areas.

2. Materials and methods

2.1. Wastewater source

The wastewater was taken from a sewage drain located inside campus of Ho Chi Minh City University of Technology. The influent parameters were monitored during experiment (See E-supplement). The average concentrations (mg L^{-1}) are shown: chemical oxygen demand (COD) of 161 ± 66 (n = 64); soluble chemical oxygen demand (sCOD) of 125 ± 57 (n = 60), suspended solids (SS) of 42 ± 39 (n = 48), biochemical oxygen demand (BOD_5) of 102 ± 43 (n = 48), total Kjeldahl nitrogen (TKN) of 47 ±1 9 (n = 58), ammonia (NH_4^+-N) of 39 ± 18 (n = 58), nitrite (NO_2^--N) of 0.13 ± 0.38 (n = 58), nitrate (NO_3^--N) of 0.13 ± 0.24 (n = 58), total phosphorus (TP) of 6.9 ± 4.2 (n = 51), dissolved oxygen (DO) of 0.25 ± 0.22 (n = 61) and alkalinity of 208 ± 95 mg$CaCO_3$ L^{-1} (n = 55). Moreover, the pH was stable at the range of 7.0 ± 0.3, a total coliform (TC) of $2.3\times10^5 – 1.1\times10^7$ MPN 100 mL^{-1}. The system was operated at ambient temperature (26 – 32°C), and all samples were taken in the morning to avoid fluctuations.

2.2. Reactor set-up

Three Plexiglas reactors have different configurations (Fig. 1). The length, width, and height (mm) of the bio-sponge (R1), bio-cord (R2), and bio-cloth (R3) reactor were 107×107×800, 60×60×800, and 107×40×800, respectively. Selecting reactor size was to

Journal Pre-proofs

ensure a fixed media/reactor ratio at 36% (v/v). Reactors allowed passive ventilation through the holes (34 mm-diameter) from the bottom to the top reactor (total in 12 holes). A settling tank of 5.4 L collected excess sludge and sloughed biomass (height × length × width dimension, i.e., 230 ×170×170 mm). The wastewater was fed from the influent tank (120 L) and then pumped to reactors by peristaltic pumps (i.e., PULSAtron® (Q = 0–18.9 L h^{-1}, Pulsafeeder, Inc, USA), Chem-Feed® (Q = 0 – 13.6 L h^{-1}, Blue-White Industries, USA), Iwaki Es pump (Q = 0 – 12 L h^{-1}, Iwaki Co., Ltd., Japan) (See E-supplement).

2.3. Media types and sludge inoculation

The bio-sponge, bio-cord, and bio-cloth media were compared due to their distinct structure, materials, and distribution properties. Specifically, the bio-sponge was the third-generation downflow hanging sponge (DHS G3) (Maruei Co. Ltd, MSC-E16). The bio-sponge was cylindrical with a dimension of 30 mm in length and diameter. It was made from polyurethane, covered by a polyethylene plastic mesh, and distributed randomly into the system. The specific surface area, void volume, and specific density of bio-sponge were 600 m^2 m^{-3}, 98.4%, and 2.5 kg m^{-3}, respectively. A total of 145 sponge cubes was used in the bio-sponge reactor with a total surface area of 1.8 m^2.

The material of bio-cord was made from polypropylene (PP-45, TBR Co. Ltd). Its configuration consists of an elongated central core and multiple fiber loops placed along body parts to collect organisms from the water. The bio-cord media can be distributed vertically for non-submerged conditions (Haley III, John W., 2007) and submerged conditions to establish nitrifying biofilters (Sesuk et al., 2009). The specific surface area and density of bio-cord were 2.8 m^2 m^{-1} and 3.4 kg 100 m^{-1}, respectively. The central core has a dimension of length and diameter (700 mm and 50 mm). Polymer fiber loops of 450 mm cover the outer diameters. A total of 0.7 m of bio-cord was used in the bio-cord reactor with a total surface area of 1.8 m^2.

The bio-cloth was a manufactured mix of cotton and polyester to improve durability (Icotext, Japan). The presence of polyester in bio-cloth helps improve acid resistance and possesses high tensile strength and permeability in the long term. Bio-cloth was threaded through stainless steel bars, distributed vertically, and arranged by the author into parallel sheets. The specific surface area was 2 m^2 m^{-2}, and its particular density was 6.2 kg 100 m^{-2}. A total of 15 pieces of bio-cloth was used in the bio-cloth reactor with a total surface area of 1.8 m^2.

The three media were inoculated with diluted activated sludge (AS) to indulge a microbial community before conducting tests with wastewater. The AS was collected from a recycled sludge line of the secondary sedimentation tank with an initial MLSS value of 12,000 mg L^{-1}. Subsequently, the sludge was diluted with domestic wastewater to reach the MLSS of 1500 mg L^{-1}. In the inoculation process, three media were separately submerged in the diluted sludge for 3 days.

2.4. Experimental designs

The first experiments aimed to access the potential efficacy treatment of different media. Three independent reactors were operated with the same organic loading rate from Eq.(1) (OLR = 2.2 ± 0.9 g COD $m^{-2}{}_{media}$ d^{-1}), the total surface area of media (S_{media} = 1.82 m^2), hydraulic biofilm loading rate (HLR_{bf} = 0.027 m^3 $m^{-2}{}_{media}$ d^{-1}) and internal recirculation (IR = 1, e.g., Q_r = 25 L d^{-1}). Each system was operated in the continuous-feeding mode for biomass acclimation for 22 d. Then, the experiment was continued for 60 d. The feed flowrate (Q) of wastewater was set at 25 L d^{-1} (total flow rate: $Q+Q_r$ =50 L d^{-1}). The best systems were evaluated by considering performance treatment such as removal rate Eq.(2), media durability, and biomass stability.

In the second experiment, the best bioreactor from the first study was operated in the continuous-feeding mode for 50 d. The IR ratios were alternated to IR = 2 (25 d) and IR = 3 (25 d). It means that the total feed flowrate were 75 L d^{-1} and 100 L d^{-1}, respectively. The HLR_{bf} estimated from Eq.(3) was 0.027, 0.041, and 0.055 $m^3\ m^{-2}{}_{media}\ d^{-1}$, corresponded with IR = 1, 2, and 3. Next, the hydraulic loading rate (HLR, $m^3\ m^{-2}\ d^{-1}$) was calculated using Eq. (4), retaining 4.4, 6.6, and 8.7 $m^3\ m^{-2}\ d^{-1}$, in that order. The sludge retention times of the systems were not controlled except for sampling, and the biomass is self-released into the wastewater. The sludge retention time was estimated for each reactor based on Eq.(5).

The organic loading rate (OLR, g $m^{-2}{}_{media}\ d^{-1}$) and removal rate (R, g $m^{-2}{}_{media}\ d^{-1}$) were calculated based on surface media as follows:

$$OLR = \frac{[C_{inf}] \times Q}{A_{media}} \tag{1}$$

$$R = \frac{([C_{inf}] - [C_{eff}]) \times Q}{A_{media}} \tag{2}$$

The hydraulic biofilm loading rate (HLR_{bf}, kg $m^{-3}{}_{media}\ d^{-1}$) and hydraulic loading rate (HLR, $m^3\ m^{-2}$ d) was expressed as Eq. (3) and Eq. (4):

$$HLR_{bf} = \frac{Q + Q_r}{A_{media}} \tag{3}$$

$$HLR = \frac{Q + Q_r}{\textit{Cross - sectional area} \text{ of reactor}} \tag{4}$$

The sludge retention time (SRT, d) in the systems were calculated based on mass balance of VSS as follows :

$$SRT = \frac{V_{media} \times [X]}{Q^w \times [X^w] + (Q - Q^w) \times [X_{eff}]} \tag{5}$$

Where:

V_{media} = media volume in each reactor (m^3); [X] = Average sludge concentration (g VSS $m^{-3}{}_{media}$); Q_w = Excess sludge from the reactor (m^3 d^{-1}); [X_w] = Concentration of the sludge flow rate discharged (g TSS $m^{-3}{}_{mixed\ liquor}$); Q = Influent flowrate wastewater (m^3 d^{-1}); Q_r = Effluent recirculation flowrate (m^3 d^{-1}); X_{eff} = Effluent suspended solids discharged (g SS $m^{-3}{}_{mixed\ liquor}$); [C] = Concentration of individual of parameters such as COD or TN, TP, etc., (g m^{-3}); [COD_r] = Removed COD concentration (g m^{-3}) and A = total surface area of media used (m^2).

2.5. Analytical methods

The influent and effluent samples were collected and analyzed with the frequency of three times per week. Wastewater characteristics were analyzed following standard methods, 20th edition (APHA, 1999), including COD (5220C), BOD (5210B), TSS and VSS (2540D), TKN ($4500N_{org}B$), NH_4^+-N ($4500N_{org}B$), NO_2^-N ($4500NO_2^-B$), NO_3^-N ($4500NO_3^-B$), TP (4500PD), Alkalinity (2320B), total coliform (9221E). pH Dissolved oxygen (DO) values were measured using a pH meter (Hanna pH21, Hanna Instruments, Inc., Canada) and a DO meter (Hanna Hi 9143). For sludge samples, TSS and VSS (2540D) were analyzed.

2.6. Statistical analyses

Statistical analysis was performed using R studio 1.3.959 (https://www.R-project.org/). A *tidyverse* package was used to visualization the raw data. Data were subjected to analysis of variance (ANOVA) and the probability *p-value* was set at 0.05 for all analyses. Moreover, Multiple linear regression was used to analyze the experimental results. Pearson's correlation was used to analyze the relationships between influent and effluent

Journal Pre-proofs

parameters. The effluent parameters of sCOD, NH_4^+-N, NO_3^--N, TKN are defined as dependent variable (*Y*) and is expressed as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon$$

where $\beta_0, \beta_1, \beta_2 \ldots, \beta_n$ are the regression coefficients, ε are the random errors due to other factors not included in the study. The regression was done using $X_1, X_2 \ldots, X_n$ (independent variables) as the factors affecting *Y*. The distribution of errors between observed and predicted values (i.e., the residuals of the regression) was subjected to check normality by using the Kolmogorov-Smirnov test (ks. test() function) with significance values of $p > 0.05$. Based on the change of removal performance during the 82 days of operation, the independent variable was divided into the early period (from day 23rd to 58th) and the final period (day 59th to 82nd). The time effect is taken into account as an independent variable. Each model was selected by reducing the number of independent variables and satisfied $p > 0.01$. Finally, the second-order Akaike Information Criterion (*AICc*) was used as model selection. The Akaike Information Criterion (*AIC*) is expressed as follows:

$$AIC = 2K - 2\,ln\,(\hat{L})$$

$$AICc = AIC + \frac{2K\,(K+1)}{n-K-1}$$

Where, *K* is the number of estimable parameters, *n* is the sample size, and ln $(\hat{L})$ is the maximum log-likelihood of the model estimated. The "best" model can be determined by AIC or AICc score in which AICc taking into account for sample size, essentially increases the relative penalty for model complexity with data sets (n/K < 40).

Results and discussion

3.1. Effect of media type on the non-submerged attached growth process

3.1.1. Organics, nutrient, and coliform removal

Journal Pre-proofs

The first experiments evaluated the treatment behavior of different media under identical conditions: OLR of 2.2 ± 0.9 g COD $m^{-2}{}_{media}$ d^{-1}, S_{media} of 1.82 m^2, HLR_{bf} of 0.027 m^3 $m^{-2}{}_{media}$ d^{-1}, and IR of 1. The dissimilarity of media structures is doubted to alter the organic and nitrogen biotransformation. However, no significant difference in removal was found (ANOVA, $p > 0.05$) (81 ± 9% of $COD^{bio\text{-}sponge}$, 79 ± 7% of $COD^{bio\text{-}cloth}$, and 75 ± 16% $COD^{bio\text{-}cord}$) (Table 1). As a result, the effluent $BOD^{bio\text{-}sponge}$ and $BOD^{bio\text{-}cloth}$ achieved below 30 mg L^{-1}. Bio-sponge and bio-cloth systems showed a well-executed organic treatment. The NH_4^+-$N^{bio\text{-}sponge}$ conversion achieved 78 ± 21%, followed by 73 ± 20% of NH_4^+-$N^{bio\text{-}cloth}$, suggesting high nitrification occurred in both media. Besides, bio-sponge exhibited higher stability than bio-cloth in terms of SS treatment (effluent $SS^{bio\text{-}sponge}$ of 5 ± 3 mg L^{-1} vs. effluent $SS^{bio\text{-}cloth}$ of 8 ± 8 mg L^{-1}). These results implied that the packed distribution facilitated improve SS removal via physical filtration. In contrast, due to the longitudinally distributed media, the physical filtration of the bio-cloth could be lower than the bio-sponge, so it fluctuates slightly.

For bio-cord system, NH_4^+-$N^{bio\text{-}cord}$ conversion showed high fluctuation (61 ± 30%) (Table 1). Beside, the organic and nitrogen removal has been degraded at the final stage (Fig 2, 3). In detail, the effluent $DO^{bio\text{-}cord}$ gradually decreased from day 59 to 82 (Fig. 2A). This result has increased effluent $COD^{bio\text{-}cord}$ (Fig. 2B), $pH^{bio\text{-}cord}$, and $SS^{bio\text{-}cord}$ for the last 24 d (Fig. 2C and 2D). Together with the resultants in low removal of $TKN^{bio\text{-}cord}$ and NH_4^+–$N^{bio\text{-}cord}$ (Fig. 3A and 3B), these outcomes suggested that nitrification of bio-cord did not occur adequately. Moreover, a low effluent concentration of NO_3^-–$N^{bio\text{-}cord}$ could be observed due to reduced nitrification from days 59 to 82 (Fig. 3D). Such result implied that simultaneous nitrification and denitrification in bio-cord might hardly be achieved at the final stage. This issue will be discussed further in *section 3.1.3*

The treatment trends of bio-cloth were found to be quite similar to bio-cord in several parameters (such as decreased NO_3^-–N, increased pH, SS). However, the fact that processing is not inhibited but instead indicates an increased treatment activity (Fig. 2A, 2C, 3A, 3B). High NH_4^+–N removal and low concentration of effluent NO_3^-–N was noticed (Fig. 3D), indicating bio-cloth system is a feasible process for nitrogen removal. The average $TP^{bio\text{-}cloth}$ removal was comparable with $TP^{bio\text{-}sponge}$ (effluent TP of 5 vs. 6 mg L^{-1}) (Table 1). Furthermore, although the ability to remove COD and SS in the bio-cloth is lower than that of the bio-sponge, both effluent concentrations complied with the national discharge standard (COD < 75 mg l^{-1}, SS < 50 mg l^{-1}). Such findings highlighted that bio-cloth could be also a promising candidate for the non-submerged attached growth process

The influent coliform could range from 3.6×10^5 to 1.1×10^6 MPN 100 ml^{-1}. The effluent are summarized in Table 1 (n = 8). The effluent was 251 ± 324, 1670 ± 3100, and 773 ± 767 MPN 100 ml^{-1} for bio-sponge, bio-cord, and bio-cloth systems, respectively. The removal of total coliform in bio-sponge and bio-cloth systems were less than 3000 MPN 100 mL^{-1}, complying with Vietnam's national technical regulations for domestic wastewater QCVN14:2008/BTNMT (A). The removal achieved a 4.1 and 3.5 log reduction for bio-sponge and bio-cloth systems. The high biomass attached in the media could eliminate coliform through various mechanisms, i.e., biofilm adsorption, oxygen stress, natural die-off, predation, and competition (Tandukar et al., 2005). Moreover, Tawfik et al. (2006) suggested that the pathways of coliform elimination in the down-flow hanging sponge system are adsorption followed by predation. It should be noted that the less removal of coliforms in the bio-cord could be due to the improper distribution of the biomass on the media *(see section 3.1.3)*, reducing interaction between predator and foreign coliforms. Furthermore, DO decreases in the bio-cord reactor at the end period, which is ascribed to cause a decline in the ability of pathogenic bacteria elimination.

3.1.2. Biomass growth

The harvested biomass was determined based on two features (e.g., biomass/volume media used as TSS_{vol} and biomass/effective surface area as TSS_{area}). As shown in Fig. 4A, the $TSS_{Vol}^{bio\text{-}sponge}$, $TSS_{Vol}^{bio\text{-}cord}$ and $TSS_{Vol}^{bio\text{-}cloth}$ were 21.0 ± 3.4 gTSS L^{-1}, 21.4 ± 0.6 g TSS L^{-1} and 113.6 ± 5.2 g TSS L^{-1}, respectively. The $TSS_{Vol}^{bio\text{-}sponge}$ was comparable with the previous studies (from 20 to 46 gTSS L^{-1}) (Onodera et al., 2016; Miyaoka et al., 2017). Meanwhile, bio-cloth possessed a three-fold higher TSS yield than the others. This fact can be theoretically explained by the difference in media volume used (e.g., $V^{bio\text{-}cloth} = 0.182$ L, $V^{bio\text{-}sponge} = 3.045$ L and $V^{bio\text{-}cord} = 1.113$ L).

Fig. 4B showed the $TSS_{Area}^{bio\text{-}sponge}$ was 36.2 ± 5.8 gTSS m^{-2}, being three times higher than the others (i.e.,12.5 ± 0.4 g TSS m^{-2} of $TSS_{Area}^{bio\text{-}cord}$ and 11.3 ± 0.5 g TSS m^{-2} of $TSS_{Area}^{bio\text{-}cloth}$). This is theoretically achievable due to the highly porous structure of bio-sponge (surface area of 600 m^2 m^{-3}) which enhances biomass retention. As estimated, the SRT of the bio-sponge (168 days) was significantly higher than that of the bio-cord (117 days) and the bio-cloth (98 days). Interestingly, although bio-sponges contain higher biomass per surface area of media used, the highly porous structure proved effective in preventing clogging (Onodera et al., 2016). For bio-cloth, this media offered a sufficient treatment in which the removal rate is comparable to bio-sponge such as COD (0.31 vs. 0.35 g m^2 d^{-1}), SS (1.75 vs. 1.78 g m^2 d^{-1}), and NH_4^+–N (0.24 vs. 0.24 g m^2 d^{-1}) (Table 1). Since SRT of the bio-cloth is the lowest, such a high removal rate implied that the bio-cloth structure could facilitate oxygen and substrate diffusion into the biofilm to increase the treatment activity. The bio-cloth media possessed a simple effective structure allowing a more uniform distribution of biomass from top to bottom. Pristine hydrophilic groups of OH– on cotton media favor water attraction and bacteria growth (Chauhan et al., 2019). A separate study indicated that under submerged conditions, the biomass densities of bio-cloth media could be

Journal Pre-proofs

retained up to 47.33 ± 1.2 g L^{-1} (Husein et al., 2019). Herein, for operating with a trickling filter, the biomass was 2.4 times greater (i.e., 113.6 ± 5.2 g L^{-1}). Although commercial cotton has a high filtration efficiency, its durability is an obstacle compared to synthetic fibers. Some properties such as tensile strength, fabric resilience began to wane after 30 days of testing (Husein et al., 2019). In this work, polyester-cotton-made bio-cloth allowed to extend durability up to 80 days, which figured out an important practical aspect of bio-cloth media (See E-supplement). Bio-cloth configuration allowed a longitudinal distribution to potentially limit the colonization and proliferation of certain macrofauna species by gravity effect. In addition to bio-sponge, overall findings highlighted that bio-cloth might be considered a potential candidate for utilization in the attached growth. Practical aspects of bio-sponge and bio-cloth will be discussed in detail in *section 3.3.*

3.1.3. Multiple linear regression analysis: Exploring critical factors affecting bio-media performance

Multiple linear regression (MLR) was implemented for predicting the effluent concentration of NH_4^+-N, TKN, NO_3-N, and sCOD. The best model was determined for each explanatory variable by selecting the lowest AICc (Table 2). This section is not only to explore critical factors affecting bio-media performance but also to propose empirical models to predict the effluent of parameters (i.e., sCOD, NH_4^+–N, NO_3^-–N, and TKN).

For bio-sponge, the effluent NH_4^+–N correlated with both influent alkalinity ($p < 0.01$) and effluent DO ("***" as $p\text{-value} = 0$). Both DO and alkalinity are well known to control the activity of ammonia-oxidizing bacteria (AOB) to metabolize NH_4^+–N. Thus, the predictive models are considered to be reasonable. In addition, the models for TKN_{eff} (Table 2) showed a positive correlation between TKN and NO_2^-–N concentrations ($p<0.001$) (e.g., simultaneously either increase or decrease in effluent concentrations of TKN and NO_2^-–N). It can be theoretically explained that TKN is the sum of ammonia-nitrogen plus organically bound nitrogen. The organic nitrogen (NH_2 groups) can be converted to ammonia (NH_4^+) by heterotrophs microorganisms (i.e., ammonification). Thus, the variation of complex environmental factors likely governs the activity of NOB and heterotrophs microorganisms. Similarly, the effluent sCOD also correlated with effluent NO_2^-–N ($p < 0.001$). These two phenomena raise the question of whether the effluent of TKN and sCOD values are relevant to effluent NO_2^-–N. To explain this correlation, the discussion preliminarily considers the formation of the anaerobic zone. Under an extended SRT operation, biomass is gradually accumulated from outside to inside the sponges. The anaerobic zone could then be developed below the surface of a 10 mm sponge, in which DO drop to 0 mg/L (Araki et al., 1999). This fact therefore induced lysis of dead cells and consequently released sCOD (Kim et al., 2016), a rich carbon source for the growth of acclimatized anaerobic microorganisms (Onodera et al., 2016; Hatamoto et al., 2018). As demonstrated, anaerobic reactions and their bio-product caused profound inhibition of NOB activity, thereby led to nitrite accumulation (Eilersen et al.,1994; Philips et al., 2002; Qian et al., 2017). Such findings confirm the results of positive correlations above (i.e., between effluent concentrations of NO_2^-–N and sCOD; between effluent concentrations of NO_2^-–N and TKN). Moreover, the effluent sCOD concentrations also positively correlated with influent alkalinity concentrations based on Pearson's correlation ($r = 0.66$). This fact indicated that alkalinity consumption is a major driver of anaerobic reactions. It was reported that alkalinity-influenced sCOD and VFA release are commonly measured in anaerobic digestion (Li et al., 2017). These results emphasized that there was undoubtedly competition for alkalinity consumption between anaerobic and aerobic reactions in bio-sponges.

In bio-cord system, the effluent sCOD was significantly governed by operating time (p = 0) (Table 2). The biomass has been degraded from day 69th to 82nd (e.g., color of biomass changes from brown to black) (E-supplement). This fact indicated that the incomplete removal of COD and TN was due to improper accumulation of biomass in certain area leading to self-destruction. The biomass has arisen in a small area of media surface instead of being distributed evenly. Such localized accumulation of biomass could reduce the diffusion of oxygen and substrates into the biofilm. Although bio-cord performed sufficiently in the submerged mode (Yuan et al., 2012; Zhou et al., 2018; Tian et al., 2019; Skoyles et al., 2020), its applicability for trickling filters might be limited due to time-dependent performance."

In bio-cloth system, the effluent sCOD has a positive correlation with TP ($p < 0.001$) and TKN ($p < 0.01$). In addition, the effluent NH_4^+-N strongly correlated to effluents of pH, COD, and TP value ($p = 0$). This result implied that the removal of organic matter and nutrients is likely dependent on the stability of the attached biomass. The bio-cloth possessed thinner biomass and a lower biomass yield than the bio-sponge (E-supplement). Under a prolonged SRT of 98 d, the aged biofilm is easily detached from the media surface due to mechanical stress and can be weakened by hydraulic and gravity effects. When these phenomena occur it is perhaps not surprising that the concentrations of organic and nutrients fluctuate to a large extent in effluent. This result is consistent with the less stable treatment of bio-cloth than bio-sponge, especially for COD and SS. (Fig. 2B and 2D). Hence, the biomass of bio-cloth could be sensitive to the hydraulic condition, affecting treatment stability. Overall, multiple regression analyses have been successfully applied for determining the essential interactions of each media type. The explored complex parameters and their relationship to treatment criteria can serve as a guide for subsequent experiments or design studies.

3.2. Organic and nitrogen removal drove by internal circulation ratio: Bio-sponge reactor

3.2.1. Organic removal and biomass properties

Journal Pre-proofs

To investigate the impact of IR (1, 2, 3) on the performance system, the bio-sponge is chosen for evaluation (Table 3 and Figure 5). The COD removal rate achieved 1.78 ± 0.74 g m^{-2}_{media} d^{-1} (IR = 1), 2.44 ± 1.79 g m^{-2}_{media} d^{-1} for IR = 2 and 1.99 ± 0.73 g m^{-2}_{media} d^{-1} (IR = 3) (Table 3). Although feed concentration fluctuated at IR of 2 (Figure 5A), the removal rate still improved around 1.5 times. Increasing IR (e.g., hydraulic loading rate) could slough off the attached biomass from sponge carriers, and this fact favored improving the removal rates. As the hydraulic biofilm loading rate (HLR_{bf}) was increased from 0.027 to 0.055 m^3 m^{-2}_{media} d^{-1}, corresponding IR from 1 to 3, total biomass decreased linearly from 21.0 ± 3.3 g L^{-1}_{media} to 9.2 ± 0.4 g L^{-1}_{media} (R^2 = 0.97) (Figure 4C). For the IR of 2, sponge carriers captured a lower biomass concentration by 32% compared to IR = 1, greatly enhancing by 37% removal rate. It was noted that DO concentration increased to 3.6 mg L^{-1} (IR = 2) compared to 3.0 mg L^{-1} (IR = 1). These outcomes indicated that increasing IR helps reduce excess biomass accumulation and improves natural ventilation. However, increasing IR did not favor enhanced performance under low organic loading rate. A previous study on a pilot-scale DHS indicated the COD removal decreased with increasing IR from 0 to 1 (Tra et al., 2021). This fact is attributed to deterioration in biomass growth induced by increased hydraulic loading and the long-term lack of fed COD loading (fed COD < 80 mg L^{-1}). Therefore, if the organic concentration is sufficiently loaded (COD about 200 mg L^{-1}), an IR of 2 could be the optimal condition for COD removal.

The COD removal rate of IR = 3 was reduced by 0.5 g m^{-2}_{media} d^{-1} compared to IR = 2 (Table 3). The high HLR_{bf} of 0.055 m^3 m^{-2}_{media} d^{-1} (IR = 3) might impact the amount of biomass retained and pollutant absorption capacity. These results, therefore, suggested that the IR of 2 is an ideal condition to favor positive interactions of COD input, hydraulic biofilm loading rate, and biomass activity. An optimal IR value should be selected in relation to influent feeding conditions.

3.2.2. Nitrogen removal

The NH_4^+–N removal rate increased from 0.24 ± 0.09 (IR = 1) to 0.60 ± 0.17 (IR = 2), and 0.59 ± 0.14 g $m^2{}_{media}$ d^{-1} (IR = 3) (Table 3). This finding confirmed that the IR of 2 improved ammonia nitrogen removal. For the TKN effluent the outcomes of regression model showed as follow: log (TKN_{eff}) = 0.47 IR*** + 0.27 log (NO_{2eff})*** + 1.68*** (*** as *p-value = 0*) (Table 4). It means that increasing IR ratio posed a strongly positive correlation with the effluent of TKN and NO_2^-–N, suggesting that overuse of IR could adversely affect the nitrification process. As mentioned in *section 3.2.1*, the changes in both HLR and HLR_{bf} factors impacted the juxta-positioning of microorganisms and reducing attached biomass. This fact could disturb the spatial organization of the synergistic association between AOB and NOB, leading to more aggressive nitrite accumulation (Philips et al., 2002).

For nitrate removal, NO_3^-–N was negatively correlated with the IR value ($p < 0.01$) (Table 4). It means nitrate nitrogen could be removed by increasing IR operation. Moreover, although increasing IR to 2 and 3 reduced biomass up to 2.2 times, the removal rate of TN was at least two times higher than that of IR = 1 (Table 3). This pinpointed that nitrogen removal was due to denitrification rather than biomass assimilation. It is confirmed the fact that IR did trigger the removal of TN via the simultaneous nitrification and denitrification pathway.

Based on overall results, the highest COD removal and nitrification were noticed at IR = 2, which could be considered a critical value governing bio-sponge performance. Operating above a critical value would not only induce an adverse impact on organic removal (i.e., IR = 3) but also increase operating costs. A study by Ikeda et al. (2013) used phenol as a COD substrate (COD > 1440 mg L^{-1}) in a down-flow hanging sponge system. Their study reported denitrification improved from 19.1 to 58.6 % with increasing IR from 0 to 1.5, but it slightly decreased to 50.9% at IR = 2. This fact highlighted that the change in optimal IR value is dependent on the type/substrate concentration. Overall this findings suggested that for domestic wastewater with COD around 200 mg L^{-1}, the IR of 2 is an ideal operating condition for the designed loading rate. Meanwhile, if a low-strength waster is introduced in the reactor, the lower internal recirculation (IR = 1 or less) should be retained to minimize energy cost.

3.3. Implications of this work

The increasing prevalence of drought, salinization, and eutrophication is affecting agricultural production and human health globally. Under such pressure, saving water is vital. This practice can be accomplished by selecting a proper decentralized system for reuse of treated water, such as a slanted soil system (Ushijima et al., 2015) or the down-flow hanging sponge (Tawfik et al., 2006; Onodera et al., 2014). To extend the diversity and feasibility of this idea, the application of bio-treatment systems which were similar to the trickling filters driven by different bio-carriers could be a complementary option for domestic wastewater treatment. This study indicated that bio-sponge system possessed the superior treatment performance in the long term operation, while bio-cord system might be a sound competitive candidate for short-term applications. The bio-cord structure is well suited to submerged conditions since matured and aged biofilm layers can be easily separated without inhibiting the process (Tian et al., 2019; Aqeel and Liss, 2020). Thus, to make bio-cord workable in non-submerged condition, frequent shock hydraulic steps must be applied to remove the matured and aged biofilm layer to avoid ineffective biofilm zones/layers. Besides, bio-cloth has demonstrated treatment versatility in both submerged (Husein et al., 2019) and non-submerged conditions with acceptable durability. Bio-cloth system will be a viable option for wastewater treatment if the durable and effective materials for biofilm growth are used.

Journal Pre-proofs

Although current bio-media may not receive competitive performance as conventional activated sludge processes or membrane technologies (Nguyen et al., 2020), the quality of treatedwastewater could still meet the requirements of the national discharge standards, which can be discharged or possibly reused as a beneficial purpose. Furthermore, the simplicity of reactor design, bio-carriers, and operating condtions are consistent with the concept of on-site wastewater treatment, taking much advantage in rural areas (Itayama et al., 2006). For example, using bio-media available in rural areas may reduce investment costs while the media replacement is easy and convenient. Applications do not necessarily end with using a homogeneous bio-media, but of course, taking advantages of different media types should be considered (i.e., mixed-bio-media). As known, current livestock husbandry methods in tropical rural areas often produce large amounts of antibiotics and pharmaceuticals in wastewater. The combined treatment of degradation and adsorption processes in bio-media for removal of organic matters, nutrient and micropollutants will support practical applications of green technology for sustainable wastewater management."

4. Conclusions

This study proposed bio-sponge and bio-cloth as ideal media for the non-submerged attached growth process. Both bio-carriers exhibited sufficient removal of organic, nitrogen and coliforms. In all aspects, bio-sponge system showed the best performance among media. Bio-cloth system also exhibited good treatment performance without biofilm clogging issue but it required a durable hanging materials for real application. Besides, the effficacy of bio-cord system was detoriated in two months of operation due to the excessive accumulation of biomass. Finally, the internal recirculation ratio (IR) of 2 was found to be the optimal operating condition for bio-sponge system treating domestic wastewater effectively.

Journal Pre-proofs

Acknowledgments

The authors would like to thank for IJL "LECZ-CARE" project for supporting the Equipment for laboratory analysis. We acknowledge the support of time and facilities from Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for this study. In addition, the initial support of Mr. Phong Vo, Ms. Hong Nguyen and other students are highly appreciated.

References

1. APHA, AWWA, WEF, 1999. Standard Methods for the Examination of Water and Wastewater, 20st Edition. American Public Health Association, Washington, D.C., USA.
2. Aqeel, H., Liss, S.N., 2020. Autotrophic Fixed-Film Systems Treating High Strength Ammonia Wastewater. Front. Microbiol. 11, 1–15. https://doi.org/10.3389/fmicb.2020.551925
3. Arabgol, R., Vanrolleghem, P.A., Piculell, M., Delatolla, R., 2020. The impact of biofilm thickness-restraint and carrier type on attached growth system performance, solids characteristics and settleability. Environ. Sci. Water Res. Technol. 6, 2843–2855. https://doi.org/10.1039/d0ew00314j
4. Araki, N., Ohashi, A., Machdar, I., Harada, H., 1999. Behaviors of nitrifiers in a novel biofilm reactor employing hanging sponge-cubes as attachment site. Water Sci. Technol. 39, 23–31. https://doi.org/10.1016/S0273-1223(99)00146-8
5. Asano, T., Burton, F.L., Leverenz, H.L., Tsuchihashi, R., Tchobanoglous, G., 2007. Water Reuse Issues, Technologies, and Applications. McGraw-Hill.
6. Bressani-Ribeiro, T., Almeida, P.G.S., Volcke, E.I.P., Chernicharo, C.A.L., 2018. Trickling filters following anaerobic sewage treatment: state of the art and perspectives. Environ. Sci. Water Res. Technol. 4, 1721–1738. https://doi.org/10.1039/c8ew00330k
7. Budgen, J., Le-Clech, P., 2020. Assessment of brewery wastewater treatment by an attached growth bioreactor. H2Open J. 3, 32–45. https://doi.org/10.2166/h2oj.2020.023
8. Bundy, C.A., Wu, D., Jong, M.C., Edwards, S.R., Ahammad, Z.S., Graham, D.W., 2017. Enhanced denitrification in Downflow Hanging Sponge reactors for decentralised domestic wastewater treatment. Bioresour. Technol. 226, 1–8. https://doi.org/10.1016/j.biortech.2016.11.122
9. Chauhan, P., Kumar, A., Bhushan, B., 2019. Self-cleaning, stain-resistant and anti-

bacterial superhydrophobic cotton fabric prepared by simple immersion technique. J. Colloid Interface Sci. 535, 66–74. https://doi.org/10.1016/j.jcis.2018.09.087

10. Chuang, H.-P., Imachi, H., Tandukar, M., Kawakami, S., Harada, H., Ohashi, A., 2007. Microbial Community that Catalyzes Partial Nitrification at Low Oxygen Atmosphere as Revealed by 16S rRNA and amoA Genes. J. Biosci. Bioeng. 104, 525–528. https://doi.org/10.1263/jbb.104.525
11. Chuang, H.P., Ohashi, A., Imachi, H., Tandukar, M., Harada, H., 2007. Effective partial nitrification to nitrite by down-flow hanging sponge reactor under limited oxygen condition. Water Res. 41, 295–302. https://doi.org/10.1016/j.watres.2006.10.019
12. Cong Nguyen, N., Cong Duong, H., Chen, S.S., Thi Nguyen, H., Hao Ngo, H., Guo, W., Quang Le, H., Cong Duong, C., Thuy Trang, L., Hoang Le, A., Thanh Bui, X., Dan Nguyen, P., 2020. Water and nutrient recovery by a novel moving sponge – Anaerobic osmotic membrane bioreactor – Membrane distillation (AnOMBR-MD) closed-loop system. Bioresour. Technol. 312, 123573. https://doi.org/10.1016/j.biortech.2020.123573
13. Dang, B., Gotore, O., Ramaraj, R., Unpaprom, Y., Whangchai, N., 2021. Sustainability and application of corncob-derived biochar for removal of fluoroquinolones.
14. Eilersen, A.M., Henze, M., Kløft, L., 1994. Effect of volatile fatty acids and trimethylamine on nitrification in activated sludge. Water Res. 28, 1329–1336. https://doi.org/10.1016/0043-1354(94)90298-4
15. Gao, F., Liu, G., She, Z., Ji, J., Gao, M., Zhao, Y., Guo, L., Jin, C., 2021. Effects of salinity on pollutant removal and bacterial community in a partially saturated vertical flow constructed wetland. Bioresour. Technol. 329, 124890. https://doi.org/10.1016/j.biortech.2021.124890
16. Haley III, John W. (Providence, RI, U., 2007. US20070151923A1.pdf. US 2007/0151923 A1.
17. Hatamoto, M., Okubo, T., Kubota, K., Yamaguchi, T., 2018. Characterization of downflow hanging sponge reactors with regard to structure, process function, and microbial community compositions. Appl. Microbiol. Biotechnol. 102, 10345–10352. https://doi.org/10.1007/s00253-018-9406-6
18. Hayder, G., Ahmed, A.N., Fu'ad, N.F.S.M., 2017. A review on media clogging in attached growth system. Int. J. Appl. Eng. Res. 12, 8034–8039.
19. Husein, M., Zhao, R.-J., Zhu, H.-D., Xu, C., Yang, S., Abomohra, A.E.-F., Kaba, P., Yang, Q.-Z., 2019. Assessing the performance of modified waste cotton cloth (MWCC)

Journal Pre-proofs

installed in a biological contact reactor as a biofilm carrier used for domestic wastewater treatment. SN Appl. Sci. 1, 1–15. https://doi.org/10.1007/s42452-019-1414-3

20. Ikeda, N., Natori, T., Okubo, T., Sugo, A., Aoki, M., Kimura, M., Yamaguchi, T., Harada, H., Ohashi, A., Uemura, S., 2013. Enhancement of denitrification in a down-flow hanging sponge reactor by effluent recirculation. Water Sci. Technol. 68, 591–598. https://doi.org/10.2166/wst.2013.235
21. Itayama, T., Kiji, M., Suetsugu, A., Tanaka, N., Saito, T., Iwami, N., Mizuochi, M., Inamori, Y., 2006. On site experiments of the slanted soil treatment systems for domestic gray water. Water Sci. Technol. 53, 193–201. https://doi.org/10.2166/wst.2006.290
22. Kim, N.K., Oh, S., Liu, W.T., 2016. Enrichment and characterization of microbial consortia degrading soluble microbial products discharged from anaerobic methanogenic bioreactors. Water Res. 90, 395–404. https://doi.org/10.1016/j.watres.2015.12.021
23. Kobayashi, N., Oshiki, M., Ito, T., Segawa, T., Hatamoto, M., Kato, T., Yamaguchi, T., Kubota, K., Takahashi, M., Iguchi, A., Tagawa, T., Okubo, T., Uemura, S., Harada, H., Motoyama, T., Araki, N., Sano, D., 2017. Removal of human pathogenic viruses in a down-flow hanging sponge (DHS) reactor treating municipal wastewater and health risks associated with utilization of the effluent for agricultural irrigation. Water Res. 110, 389–398. https://doi.org/10.1016/j.watres.2016.10.054
24. Le, L.T., Lee, S., Bui, X.T., Jahng, D., 2020. Suppression of nitrite-oxidizing bacteria under the combined conditions of high free ammonia and low dissolved oxygen concentrations for mainstream partial nitritation. Environ. Technol. Innov. 20, 101135. https://doi.org/10.1016/j.eti.2020.101135
25. Li, X., Peng, Y., Zhao, Y., Zhang, L., Han, B., 2017. Volatile Fatty Acid Accumulation by Alkaline Control Strategy in Anaerobic Fermentation of Primary Sludge. Environ. Eng. Sci. 34, 703–710. https://doi.org/10.1089/ees.2016.0399
26. Loupasaki, E., Diamadopoulos, E., 2013. Attached growth systems for wastewater treatment in small and rural communities: A review. J. Chem. Technol. Biotechnol. 88, 190–204. https://doi.org/10.1002/jctb.3967
27. Machdar, I., Onodera, T., Syutsubo, K., Ohashi, A., 2018. Effects of sponge pore-size on the performance of a down-flow hanging sponge reactor in post-treatment of effluent from an anaerobic reactor treating domestic wastewater. Sustain. Environ. Res.

Journal Pre-proofs

Journal Pre-proofs
28, 282–288. https://doi.org/10.1016/j.serj.2018.07.001

28. Mahmoud, M., Tawfik, A., El-Gohary, F., 2011. Use of down-flow hanging sponge (DHS) reactor as a promising post-treatment system for municipal wastewater. Chem. Eng. J. 168, 535–543. https://doi.org/10.1016/j.cej.2011.01.019
29. Matsubayashi, M., Harada, H., Okubo, T., Uemura, S., 2016. Recovery of partial nitrification in a down-flow hanging sponge reactor by salt shock loading. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 51, 262–266. https://doi.org/10.1080/10934529.2015.1094348
30. Miyaoka, Y., Yoochatchaval, W., Sumino, H., Banjongproo, P., Yamaguchi, T., Onodera, T., Okadera, T., Syutsubo, K., 2017. Evaluation of the process performance of a down-flow hanging sponge reactor for direct treatment of domestic wastewater in Bangkok, Thailand. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 52, 956–970. https://doi.org/10.1080/10934529.2017.1324708
31. Nguyen, T.T.D., Nguyen, T.T., An Binh, Q., Bui, X.T., Ngo, H.H., Vo, H.N.P., Andrew Lin, K.Y., Vo, T.D.H., Guo, W., Lin, C., Breider, F., 2020. Co-culture of microalgae-activated sludge for wastewater treatment and biomass production: Exploring their role under different inoculation ratios. Bioresour. Technol. 314, 123754. https://doi.org/10.1016/j.biortech.2020.123754
32. Onodera, T., Okubo, T., Uemura, S., Yamaguchi, T., Ohashi, A., Harada, H., 2016. Long-term performance evaluation of down-flow hanging sponge reactor regarding nitrification in a full-scale experiment in India. Bioresour. Technol. 204, 177–184. https://doi.org/10.1016/j.biortech.2016.01.005
33. Onodera, T., Tandukar, M., Sugiyana, D., Uemura, S., Ohashi, A., Harada, H., 2014. Development of a sixth-generation down-flow hanging sponge (DHS) reactor using rigid sponge media for post-treatment of UASB treating municipal sewage. Bioresour. Technol. 152, 93–100. https://doi.org/10.1016/j.biortech.2013.10.106
34. Philips, S., Laanbroek, H.J., Verstraete, W., 2002. Origin, causes and effects of increased nitrite concentrations in aquatic environments. Rev. Environ. Sci. Biotechnol. 1, 115–141. https://doi.org/10.1023/A:1020892826575
35. Qian, W., Peng, Y., Li, X., Zhang, Q., Ma, B., 2017. The inhibitory effects of free ammonia on ammonia oxidizing bacteria and nitrite oxidizing bacteria under anaerobic condition. Bioresour. Technol. 243, 1247–1250. https://doi.org/10.1016/j.biortech.2017.07.119
36. Rittmann, B.E., McCarty, P.L., 2020. Environmental Biotechnology: Principles and

Applications, Second Edi. ed. McGraw-Hill Education.

37. Sesuk, T., Powtongsook, S., Nootong, K., 2009. Inorganic nitrogen control in a novel zero-water exchanged aquaculture system integrated with airlift-submerged fibrous nitrifying biofilters. Bioresour. Technol. 100, 2088–2094. https://doi.org/10.1016/j.biortech.2008.10.027
38. Skoyles, A., Chaganti, S.R., Mundle, S.O.C., Weisener, C.G., 2020. "Nitrification kinetics and microbial community dynamics of attached biofilm in wastewater treatment." Water Sci. Technol. 81, 891–905. https://doi.org/10.2166/wst.2020.170
39. Tandukar, M., Uemura, S., Machdar, I., Ohashi, A., Harada, H., 2005. A low-cost municipal sewage treatment system with a combination of UASB and the "fourth-generation" downflow hanging sponge reactors. Water Sci. Technol. 52, 323–329. https://doi.org/10.2166/wst.2005.0534
40. Tawfik, A., El-Gohary, F., Ohashi, A., Harada, H., 2006. The influence of physical-chemical and biological factors on the removal of faecal coliform through down-flow hanging sponge (DHS) system treating UASB reactor effluent. Water Res. 40, 1877–1883. https://doi.org/10.1016/j.watres.2006.02.038
41. Tian, X., Ahmed, W., Delatolla, R., 2019. Nitrifying bio-cord reactor: performance optimization and effects of substratum and air scouring. Environ. Technol. (United Kingdom) 40, 480–488. https://doi.org/10.1080/09593330.2017.1397760
42. Tra, V., Dang, B., An, Q., Nguyen, Q., 2021. Influence of hydraulic loading rate on performance and energy-efficient of a pilot-scale down-flow hanging sponge reactor treating domestic wastewater. Environ. Technol. Innov. 21, 101273. https://doi.org/10.1016/j.eti.2020.101273
43. Ushijima, K., Tanaka, E., Suzuki, L.Y., Hijikata, N., Funamizu, N., Ito, R., 2015. Grey water treatment by the slanted soil system with unsorted soil media. Environ. Technol. (United Kingdom) 36, 2603–2609. https://doi.org/10.1080/09593330.2015.1040078
44. Varjani, S.J., 2017. Microbial degradation of petroleum hydrocarbons. Bioresour. Technol. 223, 277–286. https://doi.org/10.1016/j.biortech.2016.10.037
45. Varjani, S.J., Upasani, V.N., 2016. Biodegradation of petroleum hydrocarbons by oleophilic strain of Pseudomonas aeruginosa NCIM 5514. Bioresour. Technol. 222, 195–201. https://doi.org/10.1016/j.biortech.2016.10.006
46. Watari, T., Mai, T.C., Tanikawa, D., Hirakata, Y., Hatamoto, M., Syutsubo, K., Fukuda, M., Nguyen, N.B., Yamaguchi, T., 2017. Performance evaluation of the pilot scale upflow anaerobic sludge blanket – Downflow hanging sponge system for natural

Journal Pre-proofs

rubber processing wastewater treatment in South Vietnam. Bioresour. Technol. 237, 204–212. https://doi.org/10.1016/j.biortech.2017.02.058

47. Watari, T., Vazquez, C.L., Hatamoto, M., Yamaguchi, T., van Lier, J.B., 2020. Development of a single-stage mainstream anammox process using a sponge-bed trickling filter. Environ. Technol. (United Kingdom) 0, 1–12. https://doi.org/10.1080/09593330.2020.1720309
48. Yaashikaa, P.R., Senthil Kumar, P., Varjani, S.J., Saravanan, A., 2019. Advances in production and application of biochar from lignocellulosic feedstocks for remediation of environmental pollutants. Bioresour. Technol. 292, 122030. https://doi.org/10.1016/j.biortech.2019.122030
49. Yuan, X., Qian, X., Zhang, R., Ye, R., Hu, W., 2012. Performance and microbial community analysis of a novel bio-cord carrier during treatment of a polluted river. Bioresour. Technol. 117, 33–39. https://doi.org/10.1016/j.biortech.2012.04.058
50. Zhou, L., Bai, C., Cai, J., Hu, Y., Shao, K., Gao, G., Jeppesen, E., Tang, X., 2018. Bio-cord plays a similar role as submerged macrophytes in harboring bacterial assemblages in an eco-ditch. Environ. Sci. Pollut. Res. 25, 26550–26561. https://doi.org/10.1007/s11356-018-2697-4

Journal Pre-proofs

Table 1. Performance treatment with different media types

Parameters (mg L^{-1}, except Coliform and pH)	Bio-Sponge			Bio-Cord			Bio-Cloth			QCVN 14-MT: 2015/BTNMT
	Effluent	Removal (%)	Removal rate (g m^2 d^{-1})	Effluent	Removal (%)	Removal rate (g m^2 d^{-1})	Effluent	Removal (%)	Removal rate (g m^2 d^{-1})	**Vietnam A[a]**
SS	5±3	83±12	0.35±0.32	8±9	72±21	0.28±0.27	8±8	73±22	0.31±0.32	50
COD	27±10	81±9	1.78±0.74	38±30	75±16	1.52±0.62	32±12	79±7	1.75±0.67	75
sCOD	22±10	79±9	1.22±0.58	30±25	73±15	1.03±0.49	26±11	76±7	1.17±0.55	_
BOD	15±6	81±7	0.97±0.36	22±17	74±16	0.82±0.30	17±7	79±6	0.96±0.34	30
TN	19±5	37±18	0.16±0.10	20±6	33±15	0.13±0.10	21±4	31±13	0.14±0.10	30
TKN	9±6	72±18	0.30±0.09	13±9	59±26	0.22±0.11	10±5	66±18	0.28±0.11	_
NH_4^+–N	5±5	78±21	0.24±0.09	9±8	61±30	0.17±0.11	6±5	73±20	0.24±0.10	_
TP	6±4	13±13	0.01±0.02	6±4	7±19	0.01±0.02	5±4	11±24	0.01±0.04	6
pH	6.6±0.5	_	_	6.8±0.7	_	_	6.5±0.6	_	_	6-9
NO_2^-–N	0.7±0.8	_	_	0.3±0.3	_	_	0.4±0.6	_	_	_
NO_3^-–N	9.4±4.5	_	_	7±4.8	_	_	10±5.1	_	_	_
DO	3.0±0.5	_	_	2.4±0.5	_	_	2.3±0.4	_	_	_
Alkalinity	21 ± 24.7	_	_	52 ± 57	_	_	28±44	_	_	_
Coliform (MPN 100ml^{-1})	251±324	_	_	1670 ± 3100	_	_	773±767	_	_	3000

Remarks: [a] Vietnam National effluent standard limits for domestic wastewater treatment, level A.

Journal Pre-proofs

Table 2. Multiple linear regression models for each media

Media	Pollutant	Mutiple linear regression model	AICc	R^2	p-value
Bio-Sponge	$sCOD_{eff}$	$\log(sCOD_{eff}) = -0.29\log(SS_{inf})^{**} + 0.21\log(NO_2^-{-}N_{inf})^{**} + 0.28\log(NO_3^-{-}N_{inf})^{*} + 0.45\log(sCOD_{inf})^{*} - 3.4pH_{inf} - 7.5$	34	0.69	5.55×10^{-4}
	NH_4^+-N_{eff}	$\log(NH_4^+{-}N_{eff}) = -2.78\log(Alkalinity_{inf})^{*} - 5.44\log(DO_{eff})^{***} + 1.20\log(sCOD_{eff}) + 16.88^{**}$	107	0.48	0.001
	NO_3^--N_{eff}	$\log(NO_3^-{-}N_{eff}) = 1.42\log(Alkalinity_{inf})^{**} - 1.67\log(TKN_{inf})^{**} - 0.55\,pH_{eff}^{*} + 4.4$	57	0.52	0.001
	TKN_{eff}	$\log(TKN_{eff}) = 0.31\log(NO_2^-{-}N_{eff})^{**} - 2.08\log(DO_{eff})^{**} + 4.45^{***}$	68	0.46	5.02×10^{-4}
Bio-Cord	$sCOD_{eff}$	$\log(sCOD_{eff}) = -0.34\,pH_{eff}^{*} - 1.77\log(DO_{eff})^{**} + 0.79\log(sCOD_{inf})^{***} + 0.04\,Day^{***} - 0.80\,Period_{Final}^{*} + 1.82$	42	0.73	1.0×10^{-5}
	NH_4^+-N_{eff}	$\log(NH_4^+{-}N_{eff}) = 0.74\,pH_{eff}^{**} - 0.91pH_{inf}^{*} - 2.58\log(DO_{eff})^{**} + 5.25$	69	0.68	4.06×10^{-6}
	NO_3^--N_{eff}	$\log(NO_3^-{-}N_{eff}) = 1.64\log(Alkalinity_{inf})^{***} - 2.09\log(TKN_{inf})^{**} - 0.67pH_{eff}^{***} + 1.44\log(DO_{eff})^{*} + 4.08$	56	0.71	5.86×10^{-6}
	TKN_{eff}	$\log(TKN_{eff}) = 0.45\log(SS_{inf})^{**} - 0.38\log(NO_3^-{-}N_{eff})^{*} + 0.27\log(NO_2^-{-}N_{eff})^{*} + 0.83\log(Alkalinity_{inf}) - 1.29\log(DO_{eff}) - 1.03^{**}$	54	0.78	1.6×10^{-6}
Bio-Cloth	$sCOD_{eff}$	$\log(sCOD_{eff}) = 0.22\log(TP_{eff})^{**} + 0.61\log(sCOD_{inf})^{***} + 0.25\log(TKN_{eff})^{*} - 0.57$	14	0.70	1.30×10^{-6}
	NH_4^+-N_{eff}	$\log(NH_4^+{-}N_{eff}) = 0.96\,pH_{eff}^{***} + 1.30\log(COD_{eff})^{***} - 1.08\log(TP_{inf})^{***} - 7.60^{***}$	65	0.75	1.89×10^{-7}
	NO_3^--N_{eff}	$\log(NO_3^-{-}N_{eff}) = 1.56\log(TP_{inf})^{***} - 1.44\log(TP_{eff})^{***} - 0.5\,pH_{eff}^{***} + 1.58\log(Alkalinity_{inf})^{***} - 1.69\log(TKN_{inf})^{**} - 1.04\log(NH_4^+{-}N_{inf})^{*} - 0.81\log(DO_{eff})^{*} + 6.95^{***}$	27	0.83	2.32×10^{-6}
	TKN_{eff}	$\log(TKN_{eff}) = 0.34\log(SS_{inf})^{*} - 0.64\log(NO_3^-{-}N_{eff})^{**} + 2.44^{**}$	42	0.60	1.12×10^{-5}

Remarks: Significant codes: 0 '***'; 0.001 '**'; and 0.01 '*' indicated that explanatory variables control the response variable. A p-value <0.05 indicates that the multiple linear regression model is statistically significant. All models are selected based on the lowest AICc which are presented in the table.

Journal Pre-proofs

Table 3. Performance treatment with different internal recirculation (IR) conditions

Parameters	IR = 2			IR = 3		
(mg L^{-1}, except Coliform and pH)	Influent	Removal (%)	Removal rate (g $m^2.d^{-1}$)	Influent	Removal (%)	Removal rate (g $m^2.d^{-1}$)
SS	47±24	76±11	0.51±0.34	70±58	80±10	0.82±0.77
COD	238±161	75±16	2.44±1.79	199+54	79±7	1.99±0.73
sCOD	170±110	73±15	1.76±1.19	162±43	76±7	1.70±0.55
BOD	151±63	74±16	1.78±0.79	114±42	79±6	1.35±0.54
TN	65±16	33±15	0.34±0.17	66.8±11	31±13	0.39±0.17
TKN	65±16	59±26	0.65±0.19	66.3±11.2	66±18	0.64±0.17
NH_4^+–N	55±14	79±7	0.60±0.17	56±11	77±6	0.59±0.14
TP	5.9±3	7±19	0.014±0.019	7.9±2.7	11±24	0.007±0.021
pH	6.8±0.7	_	_	6.5±0.6	_	_
	Effluent			Effluent		
NH_4^+–N	11.3±3.6			12.9±3.6		
NO_2^-–N	2.2±2.1			0.8±0.5		
NO_3^-–N	21.3±4.6			18.2±6.7		
DO	3.66±0.8			3.62±0.4		
Coliform (MPN 100 ml^{-1})	$4.35x10^3 \pm 7.69x10^3$ (3.5 ± 1.5 log removal)			553±486 (2.9 ± 1.3 log removal)		

Remarks: TP removal rate value is rounded to 3 numbers

Journal Pre-proofs

Table 4. Multiple linear regression models for the performance of bio-sponge at different internal recirculation conditions

Pollutant	Multiple linear regression model	AICc	R^2	p-value
$NH_4^+\text{–}N_{eff}$	$\log(NH_4^+\text{–}N_{eff}) = 1.17$ IR*** - $2.902\log(DO_{eff})$** + 3.05**	165	0.38	7.661×10^{-06}
$NO_3^-\text{–}N_{eff}$	$\log(NO_3^-\text{–}N_{eff}) = -0.62\ pH_{eff}$**- 0.55 IR* + 0.02 Day** - $1.01\log(TP_{inf})$* + $0.98\log(TP_{eff})$* + $0.13\log(TSS_{inf})$ + 5.66***	87	0.56	1.161×10^{-06}
TKN_{eff}	$\log(TKN_{eff}) =$ 0.47 IR*** + $0.27\log(NO_{2eff})$*** + 1.68***	112	0.44	7.665×10^{-7}

Remarks: Significant codes: 0 '***'; 0.001 '**' and 0.01 '*' indicated that explanatory variables control the response variable. A p-value < 0.05 suggests that the multiple linear regression model is statistically significant.

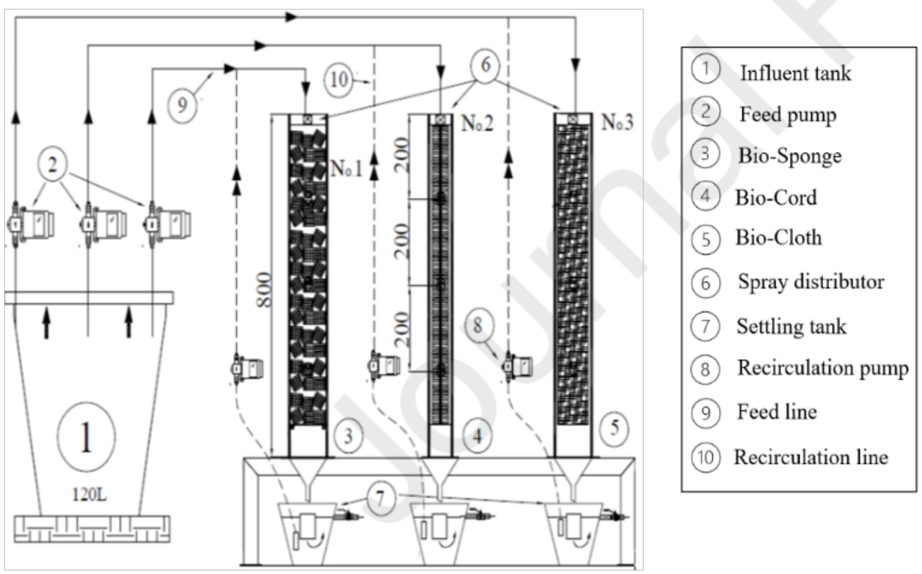

Fig. 1. Schematic diagram of the non-submerged attached growth systems

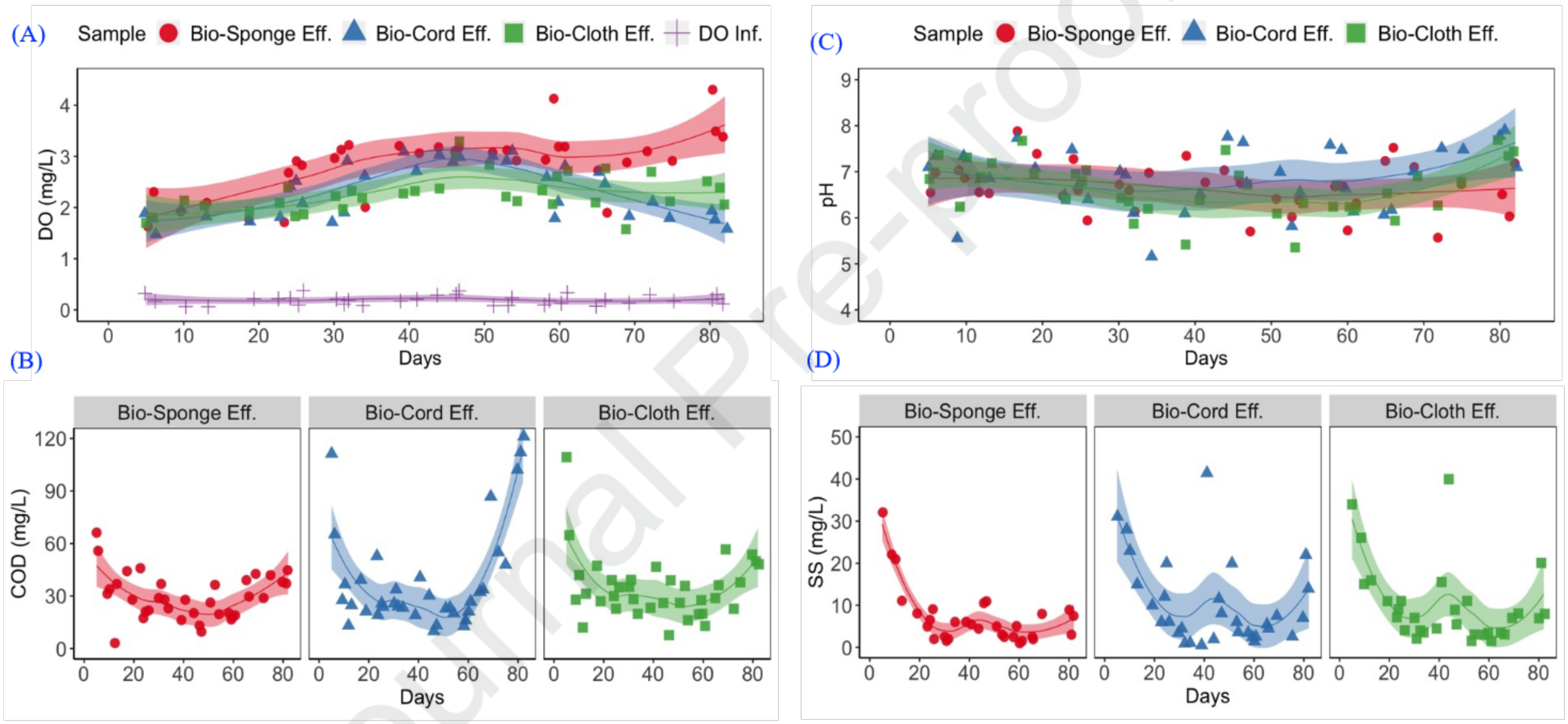

Fig. 2. Time courses of A) DO, B) pH, C) COD, and D) SS for three media under IR=1. The mean line and variance (shaded area) are estimated based on a 95% confidence interval.

Journal Pre-proofs