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ORIGINAL RESEARCH PAPER

Organic matter from biofilter nitrification by high performance size exclusion chromatography and fluorescence excitation-emission matrix

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ABSTRACT

A combination of high performance size exclusion chromatography with organic carbon detector and ultraviolet detector coupled with peak-fitting technique and fluorescence excitation-emission matrix spectrometry applied fluorescence regional integration method was conducted to determine the characteristics of organic matter during nitrification. The batch scale of bionet nitrification without organic carbon substrate under aerobic conditions was operated for around 150 minutes. Bulk organic parameters and NH_4^+-N concentration were analyzed. Five different molecular weights of organic matter were identified by using chromatography, and five different groups of fluorophores organic fractions detected by fluorescence. According to chromatography with carbon and ultraviolet detector, the main characteristics of organic matter shifted from building blocks aromatic compounds with percentage peak area of carbon/ultraviolet detector: 31%/53% to 14%/27.5% to humic-like substances with percentage peak area of carbon/ultraviolet detector 21%/17% to 27%/46.5% during nitrification. Those former compounds are biodegradable as well as properties of microbial products released during substrate utilization and endogenous phase, which are mainly identified as humic-like substances, thus underwent further biodegradation. However, there was significant change in the fluorophores organic fractions, which exhibited humic acid-like with percentage fluorescence regional index area 53% into 68%, as shown by fluorescence excitation-emission matrix analysis. A combination of these methods indicated that the organic matter released during nitrification mainly consists of humic compounds. These results conjecture that a combination of high performance size exclusion chromatography with carbon and ultraviolet detector and fluorescence excitation-emission matrix can be used to determine the characteristic of organic matter and water quality change during nitrification.

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INTRODUCTION

Biological treatment processes, such as bio-filter processes, nitrification, and membrane bioreactor (MBR) release microbial products (Tian *et al.*, 2011; Xie *et al.*, 2016). Although the three types of microbial products, soluble microbial products (SMPs), extracellular polymeric substances (EPS) and inert biomass, are commonly discussed together from a theoretical perspective (Laspidou and Rittman 2002), it is important to distinguish between them because the content of generated organic compound indicated different characteristic then its quality and quality will be vary. The coexistence of microbial products with natural organic matter (NOM) means that NOM exhibits different qualities during biological treatment, and also increases in quantity (Ni *et al.*, 2011; Liu *et al.*, 2014). After treatment effluent organic matter (EfOM) has been found to contain DBPs. Therefore EfOM in raw water could lead to the formation of disinfection by-products (DBPs) (Liu *et al.*, 2014; Shon *et al.*, 2012). DBPs, which have been found to be carcinogenic and mutagenic, will be formed if EfOM reacts with chlorine or disinfectant during water treatment (Zeng and Mitch 2016). Because the coexistence of heterotrophic bacteria with autotrophs, which increases the organic compound, is potentially problematic, a considerable amount of research has been conducted into the effect of microbial products on the bio-filter nitrification process. Nitrifying bacteria and heterotrophs both need organic substrates to grow, but heterotroph can grow even without a supply of external organic carbon, because they utilize microbial products of nitrifying bacteria, derive from biomass decay and substrate metabolism (Ni *et al.*, 2011; Matsumoto *et al.*, 2010). It is important to understand the characteristics of organic matter in bio-filter nitrification because the variety of organic matter will change due to the effect of microbial products which have been released and/or utilized by the bacteria community in the system, or existing organic carbon (Xie *et al.*, 2016; Ni *et al.*, 2011). Although several studies have investigated the characteristics of microbial products in the form of SMPs and EPS as they undergo biological processes such as activated sludge (Tian *et al.*, 2011; Ni *et al.*, 2011; Kim and Dempsey 2012; Liu *et al.*, 2016; Zhiji *et al.*, 2017), not much work has been conducted in relation to bio-filter nitrification. Organic matter is complex mixture of heterogeneous compound, which can be classified based on their properties, such as based on molecular weight, aromatic, aliphatic, hydrophilic/hydrophobic, humic/non-humic, etc. Therefore, a number of characterization techniques have been employed to obtain a better understanding of the types of NOM present in source water, and their subsequent removal or transformation through the water treatment process train (Matilainen *et al.*, 2011). High performance size exclusion chromatography (HPSEC) with an on-line organic carbon detector (OCD) and ultraviolet detector (UVD) can detect any type of organic carbon bonded species, this method is based on the molecular size of organic matter. The separation technique by HPSEC is based on differential permeation of molecules of various size into a porous matrix (Jiao *et al.*, 2014; Lai *et al.*, 2015). Fluorescence spectroscopy, by using fluorescence excitation emission matrices (FEEM) is another option for organic characterization; it can provide insights into the chemical characteristics of NOM because the results are based on both molecular structure and composition (Hidayah *et al.*, 2017). More rigid aromatic molecular structure and highly conjugated molecules are more likely to fluoresce than aliphatic, alicyclic molecules, and less conjugated systems. Because those molecular structure have smaller energy gaps between the excited and ground states, therefore it will fluoresce at longer wavelength (Murphy *et al.*, 2013). Fluorescence spectroscopy can provide qualitative information to supplement HPSEC, which has limitations as far as the detection of non-chromophores is concerned, and the identification of the chemical and physical properties of the particular molecular size of organic components (Hidayah *et al.*, 2017; Chen *et al.*, 2003). Previous studies have characterized the released organic matter during biological activities by using fluorescent spectroscopy methods only (Liu *et al.*, 2016; Lai *et al.*,2007; Moradi *et al.*, 2018; Ho *et al.*, 2019; Hidayah and Cahyonugroho 2019). Lack of studies using both chromatography and fluorescent methods at the same time for characterizing the released organic matter during biological activities, therefore using these two methods to characterize organic matter quantitatively and qualitatively seems to have several potential advantages. The objective of this study was to combine the advantages of both the chromatography and the spectrophotometry methods in order to determine the characteristics of

organic matter during nitrification and to correlate the effect of ammonia degradation and released organic carbon. In addition, this study was able to quantify the changes in different organic and microbial group compounds in water caused by the nitrification process. This study was carried out in laboratory batch scale at Department of Environmental Engineering, National Cheng Kung University, Taiwan in 2016.

MATERIALS AND METHODS

Bionet filter and sample interval

About 20 pieces small bionet (2 x 4 x 2 cm in size) was put into the sack, it was known as a set of bionet. Bionet is acclimated and taken from nitrification unit in Feng San pilot plant water treatment, then bionet was used as biomedia for nitrifier growth in batch scale process. Bionet is biomass support media with film strips made of UV resistant polyethylene (PEHD) with specific growth surface 100-250 m^2/m^3. Random media of bionet in has different profiles and large surfaces area allowing the growth and attachment of microorganism. Bionet can be easily removed for replacement or cleaning and bionet sack will not stretch or deteriorate when immersed in water for many years (Jacome *et al.*, 2013). The batch scale of bionet filter made of cylindrical glass was fulfilled with a volume of 2 L synthetic water without organic carbon substrate under aerobic conditions. Bionet was taken from nitrification unit process in Feng-San pilot plant. The batch reactor was operated around 150 minutes in the condition of 30°C. Sampling time was taken from 0 minutes as initial time to interval of 20 minutes for the first hour then interval of 30 minutes for the next hours. Around 20 mL of sample volume were filtered through a 0.45 µm membrane filter to remove particulate matter.

Synthetic water

Synthetic water contained of ammonia nitrogen of 5 mg-N/L as substrate for autotrophs growth; however, carbon source for heterotrophs growth was not provided in order to characterize released microbial products during nitrification. Bacterial growth required inorganic nutrient and the composition was as follows: K_2HPO_4 1.3 g/L; KH_2PO_4 g/L; $MgSO_4 \cdot 7H_2O$ 200 mg/L; $CaCl_2 \cdot 2H_2O$ 20 mg/L; $Na_2MoO_4 \cdot 2H_2O$ 0.1 mg/L; $MnCl_2 \cdot 4H_2O$ 0.2 mg/L; $ZnSO_4 \cdot 7H_2O$ 0.1 mg/L; $CuSO_4 \cdot 5H_2O$ 0.02 mg/L; $CoCl_2 \cdot 6H_2O$ 0.002 mg/L; 38.4% Na_2CO_3 0.1152 g/L; Fe-EDTA solution 1 ml/L (Liu, 2013)

HPSEC, FEEM, NPDOC and NH_4^+-N analysis

High performance liquid chromatography (HPLC, LC-20 ATV, Shimadzu, Japan) size exclusion chromatography (SEC) was conducted with sequential on-line detectors consisting of a UVD (254 nm, SPD-20A, UV-Vis detector, Shimadzu) and OCD (modified Sievers Total Organic Carbon Analyzer 900 Turbo, General Electric Water and Process Technologies). Fluorescence measurements were undertaken using a Perkin Elmer LS-55 luminescence spectrometer with a xenon lamp and 1.0 cm quartz cell. Excitation emission matrix (EEM) were generated for each sample by scanning over excitation (Ex) wavelengths between 230 and 400 nm at interval of 10 nm and emission (Em) wavelengths between 160 and 547.5 nm at intervals of 0.5 nm (Murphy *et al.*, 2013; Hidayah *et al.*, 2017). Dissolved organic carbon concentration is represented by non-purgeable dissolved organic carbon (NPDOC) (TOC-5000, Shimadzu, Japan). NH_4^+-N concentration was measured based on Standard Methods (APHA 2005). Peak fitting technique, PeakFit Version 4.12, Systat Software Inc., USA, was applied to resolve the overlapping peaks of HPSEC chromatogram and to determine the area under each peak. The procedure of peak-fitting technique was described in previous study (Chow *et al.*, 2008; Lai *et al.*, 2015; Hidayah *et al.*, 2017). Calculation of fluorescence regional integration (FRI) analysis was used with integration beneath EEMs within selected regions to present the cumulative fluorescence response of organic matter with similar properties. The procedure of FRI analysis was described by Chen for analyzing drinking water and wastewater samples on 2003 in Arizona, USA (Chen *et al.*, 2003).

RESULTS AND DISCUSSION

NH_4^+-N degradation and released organic carbon

As shown in Fig. 1, the nitrification process in bench-scale bionet filter could be divided into two-stage pattern. During the first stage from starting to 40 minutes (min), 90% of 5 mg-NH_3-N/L was utilized by autotrophic microorganisms, indicating that the growth of autotrophic bacteria plays an important role. At the same stage, the percentage of NPDOC increased to 57.5% from initial value of 2.55 mg-C/L

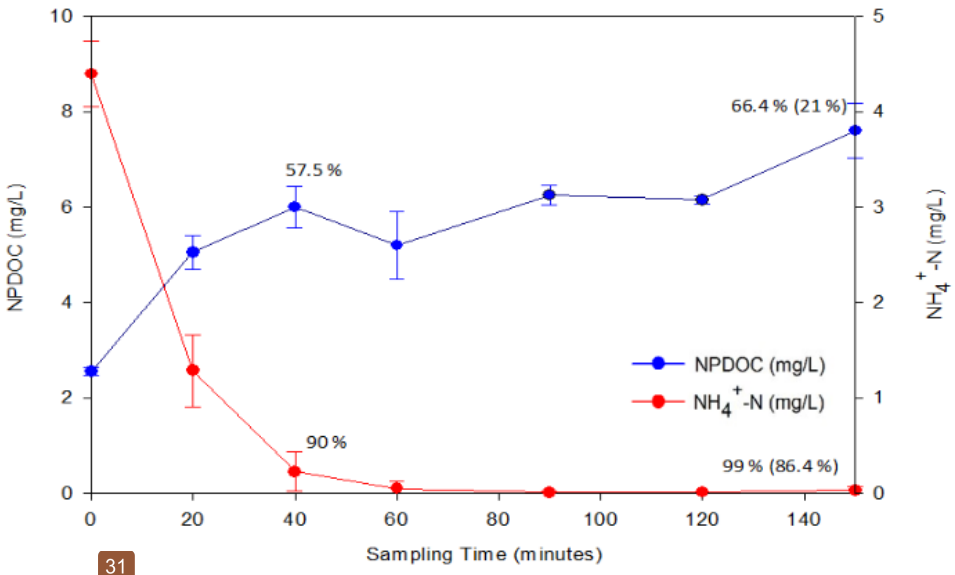

Fig. 1: The variations of NH_4^+-N and NPDOC concentrations during the nitrification operation of bionet filter (Initial ammonium concentration =5 mg/L)

to 6.20 mg-C/L, proving that microbial metabolic products, expressed by NPDOC, was produced in processes of major autotrophs growth or minor heterotrophic metabolism. In addition, carbon source may be related with the diffusion existed in bionet filter taken from Feng-San pilot plant while organic carbon concentration in the bulk phase and was far less than that in bionet filter (Edzwald and Tobiason 2011). Observed from second stage with the slow decomposition of ammonium (40-150 minutes), the accumulation percentage of ammonia removal was close to 99%. The removal ability of autotrophic bacteria on ammonium became slower in comparison with NPDOC removal of 66.4% with extra removal of 8.5%. The released organic carbon was mainly from attributed to microbial products while NH_4^+-N substrate utilization was decomposed by autotrophic bacteria. Afterwards, marginal increase of organic carbon was happened while the removal of NH_4^+-N slowed down. Ammonia substrate consumption by nitrifier bacteria could lead to microbial products production during in different operational process (Krasner *et al.*, 2013; Xie *et al.*, 2016; Zhiji *et al.*, 2017). Soluble microbial by-products has been released during biological process, including during substrate-utilization associated (microbial growth) and biomass-associated product (during endogenous phase). In general microbial by-product compound is known as humic and fulvic acids, polysaccharides, proteins, nucleic acids, organic acids, amino acids, antibiotics, steroids, exocellular enzymes, siderophores, structural components of cells and products of energy metabolism (Barker and Stuckey 1999). According to Ni *et al.* (2010), it was found that humic-like substances were mainly substrate-utilization associated, while fulvic acid-like substance were non-growth associated, as detected by FEEM. In addition, protein-like substances has been released during microbial growth and polysaccharide-like material has been released during endogenous phase, detected by Fourier Transform Infra-Red (FTIR). Urbain *et al.* (1998) found that utilization-associate products are mainly carbon compounds generated from the original substrate and that biomass-associated products are cellular macromolecules containing carbon and nitrogen. Briefly, it can be concluded that microbial by-products is composed of different organic compounds.

Area percentage of different organic properties from bionet filter

Fig. 2, one of the example HPSEC chromatograms from raw water sample, shows a distribution of organic fractions as detected by HPSEC with OCD

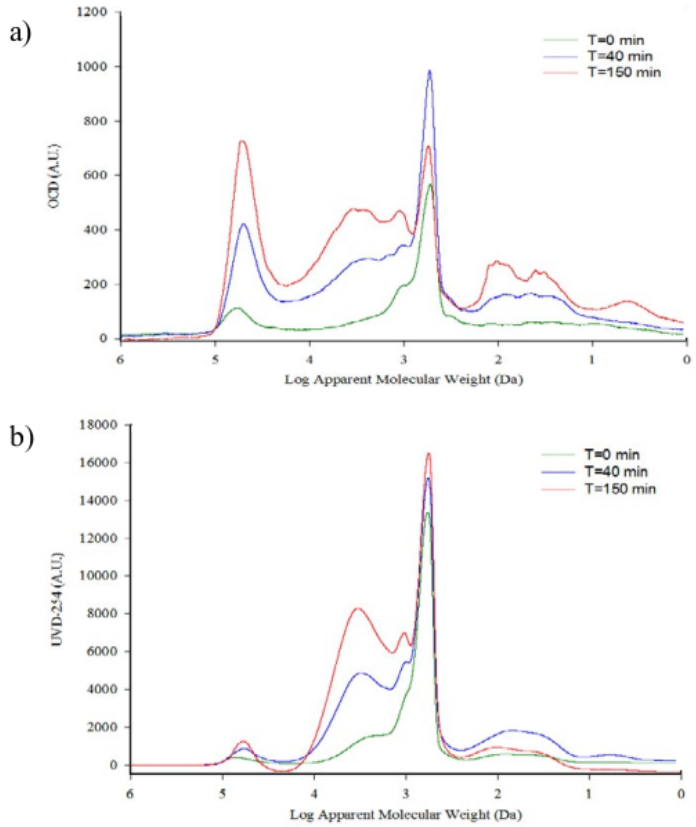

Fig. 2: Distribution of organic fractions as detected by HPSEC with (a) OCD and (b) UVD in water contained of ammonia nitrogen of 5 mg-N/L

and UVD. Peak fitting model of area percentage was applied to chromatogram of each organic fraction from respective measurement by HPSEC with OCD and UVD during the operation of bionet filter, as shown in Fig. 3. According to the description of previous paragraph, the variation of organic fraction distributions were compared with two-stage growth. Regarding to first stage belonging to rapid decomposition of ammonium or rapid increase of organic carbon, it reveals that the organic fractions were dominated by small molecular weight (MW) of Peak D measured by OCD and UVD, with 250 Da<MW<500 Da, regarded as low molecular weight acid (Lai *et al.*, 2015; Hidayah *et al.*, 2017). This substance measured by HPSEC-OCD decreased from 31% to 29%, as shown in Fig. 3a, and that measured by UVD descended from 53% to 35.5%, as shown in Fig. 3b. Observed from in Fig. 3a, the increase of biopolymers (Peak A with average molecular weight (AMW) >20 kDa) was from 12% to 20% and low molecular weight neutrals (Peak E with AMW < 250 Da) from 13% to 17.5%. Due to the impurity of EDTA with MW=292, it may be attributed to one of major resource of low molecular weight acid at starting operation. Other organic fractions should be possibly derived from diffusion of organic carbon attached in bionet filter. Release organic matter, including low molecular weight a high molecular weight larger than 10 kDa had been also reported previously (Tian *et al.*, 2011; Laspidou and Rittman 2002; Ni *et al.*, 2011). However, the decrease of building blocks or Peak C from 23% to 14.5% measured by HPSEC-OCD was attributed to the breakdown products from humic substances-like (Huber *et al.*, 2011). As for the second stage, belonging to slow decomposition of ammonium or marginal increase of organic

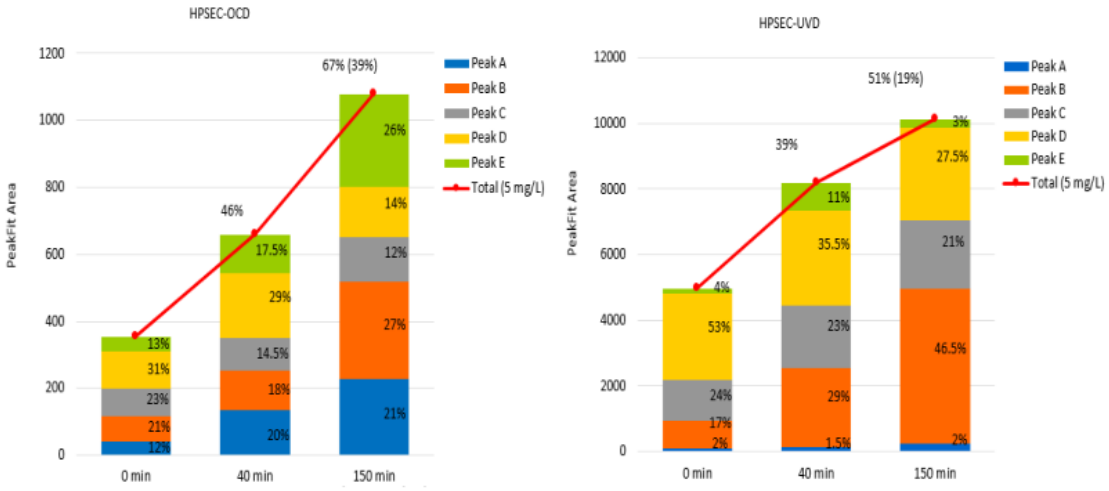

Fig. 3: Area percentage of organic fraction distribution during the bionet expressed by (a) HPSEC-OCD and (b) HPSEC-UVD with peakfitting software

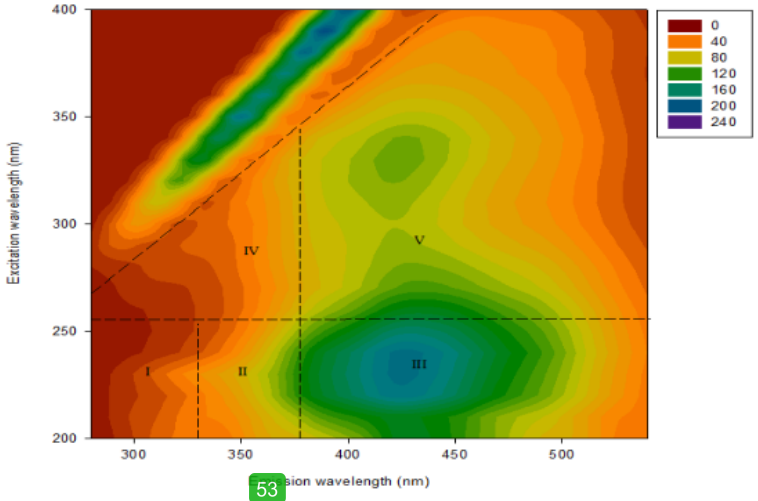

Fig. 4: Distribution of organic fractions as detected by fluorescence excitation-emission matrix (FEEM) spectroscopy

carbon, dominant component was shifted from low molecular weight of acid substance (Peak D) to humic substances-like (Peak B with 500 Da<AMW<20 kDa). Humic-like substances detected with HPSEC-OCD increased from 18% (40 minutes) to 27% (150 minutes). The similar ascending trend, 29% to 46.5%, was observed by HPSEC-UVD.

The variations of Peak B substance measured by both HPSEC were opposite to those of Peak D. For Peak E substance, the increase of 17.5% to 26 % measured by HPSEC-OCD showed inconsistent with the decrease of HPSEC-UVD from 11% to 3% owing to different organic structure to affect the measurement by HPSEC-OCD and HPSEC-UVD. Several reports demonstrated that HPSEC-OCD could detect organic matter fraction with low absorptivity, such as protein and even fraction with no absorptivity, such as polysaccharide; however, HPSEC-UVD detection is available for the measurement aromatic structure (Huber *et al.*, 2011; Lai *et al.*, 2015; Hidayah *et al.*,

2017). The close increase of 20% to 21% for Peak A by OCD in relative with UVD showed same pattern with Peak E, indicating that the organic structures of Peak E and Peak A contained less aromatic or unsaturated function group. Moradi *et al.* (2017) used HPSEC with UVD at wavelength 230 nm to investigate the nitrification occurrence in two drinking water distribution systems in Australia, and the results shows that formation of soluble microbial products and/or the release of extracellular polymeric substances (EPS) during nitrification. A peak of EPS has appeared at apparent molecular weight < 500 Da, which is similar peak at this present study. The peak less than 500 Da are identified as humic substances-like (Peak B) and building block (Peak C) in this present study. Regarding to the processes of nitrification, the transformation of organic compounds fraction was significantly affected by nitrifer to utilize ammonia (Xie *et al.*, 2016; Ni *et al.*, 2011; Matsumoto *et al.*, 2010). Briefly, HPSEC with OCD and UVD has been identified 5 fractions of organic matter in water sampel and during nitrification. It can concluded that humic substances-like has been generated during nitrification process, as the initial stage showed 21% area of OCD and 17% area of UVD become increased into 27% area of OCD and 46.5% area of UVD.

Variations of organic FEEM

Fig. 4, one of the example FEEM spectra from raw water sample, shows a distribution of organic fractions as detected by fluorescence excitation-emission matrix (FEEM) spectroscopy. FEEM was applied to characterize the properties of organic matter through bionet system. Five organics classification were divided from the FEEM contour according to the previous research (Chen *et al.*, 2003). Region I, located at shorter excitation/emission wavelengths (Ex/Em nm), <250/<330 nm, is known as simple aromatic proteins such as tyrosine. Region II, located at <250/330-380 nm, is related to simple aromatic protein such as trypthophan. Region III, located at 200-250/>380 nm, is identified as fulvic like substances. Excitation/emission wavelengths of 250-280/<380 nm related to soluble microbial by product-like material is classified as Region IV. Peak at longer excitation/emission wavelengths of >280/>380 nm, named as Region V, is represented to humic acid-like substances. Average fluorescent intensities of five regions of organic fractions were calculated by FRI methods according to the method proposed in previous study (Chen *et al.*, 2003; Guo *et al.*, 2015).

As shown in Fig. 5, the FEEM classification of organic matter from during nitrification was happened in batch-scale bionet filter. For the starting operation, 53% of humic acid-like proved a fact that bionet filter taken from on-site biofilter contains the accumulated refractory organic matter. Of course, EDTA with aminocarboxylic acid, belonging to humic like polycarboxylate-type, was one of contributions (Liu *et al.*, 2014; Liu *et al.*, 2016) however, the dominant

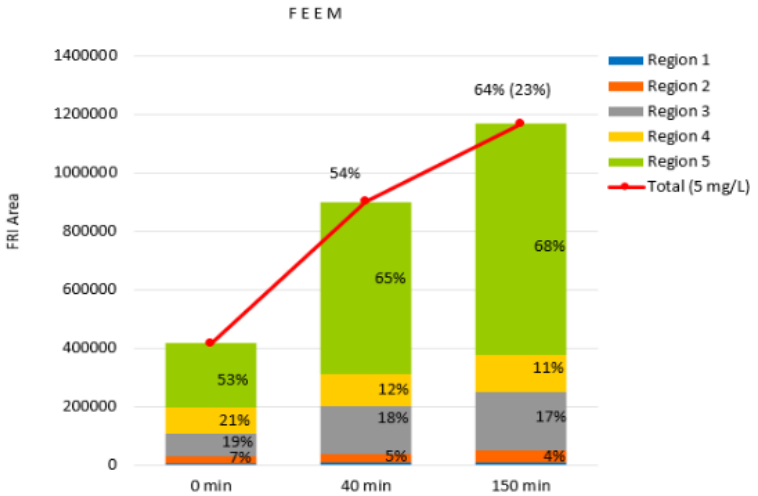

Fig. 5: Variations of average FRI values and percentages for different organic fraction distribution during bionet operation

fractions may be related with the diffusion from exhausted bionet filter (Han *et al.*, 2013; Greenstein *et al.*, 2018). After the operation of 40 minutes, fluorescent characteristic released from bionet filter was mainly occupied by humic like substance of 65%, soluble microbial-products like tryptophan of 12%, fulvic-like substance of 18% and aromatic protein of 7%. This FRI percentage of different organic property was kept constant at 150 minutes of operation. Compared starting operation with 40 minutes, the decrease of fulvic-like substance was opposite to the increase of humic-like substance, revealing that humic-like substance is one of accumulated material while biodegradable process is operated in the bionet filter. Similar reports were demonstrated during the substrate consumption by microorganisms (Ni *et al.*, 2011; Liu *et al.*, 2014; Liu *et al.*, 2016). Briefly, FEEM with FRI analysis has been identified 5 fractions of organic matter in water sampel and during nitrification. It can concluded that humic acid-like has been generated during nitrification process, as the initial stage showed 53% area of FRI increased become 68% area of FRI. The results is consistent with increasing of humic substances-like as detected by HPSEC-OCD and-UVD with peak-fitting technique.

Accumulation of humic-like substance as long-term operation of bionet

According to the current results, the increase of organic carbon was not only related with diffusion of organic originated from bionet media but also with metabolic matter from heterotrophic bacteria located outside of the bionet media while bionet filter was initially operated. In the nitrification process, autotrophic bacteria had gradually grown and eventually present in outer part of the bionet media (Matsumoto *et al.*, 2010; Chang *et al.*, 2019), implying that in this study, fast and slow ammonium decomposition may be attributed to the competition between ammonium nitrifying bacteria and heterotrophic bacteria. Furthermore, released organic fractions could be affected by substrate utilization, transformation to cell mass and metabolic product by autotrophic and heterotrophic microorganism (Laspidou and Rittman 2002; Ni *et al.*, 2011). Previous studies (Liu *et al.*, 2014; Lai *et al.*, 2007; Moradi *et al.*, 2018) also used FEEM to characterize organic fractions and found protein, polyaromatic humic acid-like and polycarboxylate humic acid-like substances were produced and accumulated while the operation time was continuously conducted in the biofilter. Those previous studies had identified humic-acid-like organics as shown a significant peak at Ex/Em wavelength 330/435 nm (Liu *et al.*, 2014), 325/ 420 nm (Lai *et al.*, 2007), 325/425 nm (Moradi *et al.*, 2018), which are similar peak with this present study. In slow ammonium removal, increased NPDOC removal was higher than ammonium removal. The microbial products released in the later phase of biodegradation has high molecular weight because the substrates have been exhausted or depleted, resulting that microorganisms were in decay phase (Ni *et al.*, 2011; Liu *et al.*, 2016). Low molecular weight acid (Peak D) and building blocks (Peak C) decrease during slow ammonium decomposition because those compounds are biodegradable as well as properties of microbial products released during substrate utilization and thus underwent further biodegradation. Heterotrophs could utilized and degraded some released microbial by-products including low molecular weight acid component (Ni *et al.*, 2011; Matsumoto *et al.*, 2010), since heterotrophs existed in bionet filter and survived by background organic carbon from preparation of water sample and diffusion from bionet filter. According to Ni *et al.* (2010), it was found that humic-like substances were mainly substrate-utilization associated, while fulvic acid-like substance were non-growth associated, and both fractions is characterized as humic substances-like.

Table 1 lists the variations of released organic properties from bionet filter with nitrification expressed by HPSEC-OCD, HPSEC-UVD and FEEM. At fast ammonium decomposition, HPSEC-OCD showed that biopolymers (i.e., protein) or Peak A, low molecular weight acids or Peak D, and Peak E of low molecular weight neutrals respectively had the increase of 14%, 12.5% and 10.5%, indicating that the dominant substances were Peak A, D and E, however, the increase of 19% for humic substances-like became more prominent by the measurement of HPSEC-UVD. This discrepancy was due to UV sensible for the detection of aromatic unsaturated groups (Lai *et al.*, 2015; Hidayah *et al.*, 2017) in opposite to difficult combustion by OCD. Observed from FEEM during fast ammonium decomposition, the increase of humic like substance was 41% higher than other organic fraction (i.e., protein and soluble

Table 1: Variation of five organic fractions expressed by HPSEC-OCD, HPSEC-UVD and FEEM from bionet filter operation with nitrification processes

Methods	Operation time (minutes)	Increase (%)*					
		Peak A	Peak B	Peak C	Peak D	Peak E	Total
HPSEC-OCD	0 to 40	14	7	2.2	12.5	10.5	46.2
	40 to 150	17.3	21.5	4.5	3.4	20.5	67.2
HPSEC-UVD	0 to 40	0.3	19	8.4	3.3	8.3	39.3
	40 to 150	4	38.1	8.8	1.5	1.1	50.9
		Region 1	Region 2	Region 3	Region 4	Region 5	
FEEM	0 to 40	0.2	1	9.3	2.6	41	54.1
	40 to 150	0.3	1.6	10.1	3.2	49	64.2

*For HPSEC = $[(\text{Peak Area}_{\text{operation time}} - \text{Peak Area}_{\text{starting}})/\text{Peak Area}_{\text{operation time}}] \times 100\%$
For FEEM = $[(\text{Region}_{\text{operation time}} - \text{Region}_{\text{starting}})/\text{Region}_{\text{operation time}}] \times 100\%$

microbial products as microbial by-product). Overall, the increase of whole organic compounds during 40 minutes was in the order of 54% for FEEM, 46% from HPSEC-OCD, and 39% for HPSEC-UVD. Regarding the characteristic of released organic matter at slow ammonium decomposition, Peak B owned more important role than Peak A and E whatever HPSEC-OCD or HPSEC-UVD was applied. Peak B as microbial by-products is hard to be degraded and will be accumulated in the system. Overall, the whole organic compounds during 110 minutes was in the order, 67% of HPSEC-OCD, 51% of HPSEC-UVD, and 64% of FEEM. Obviously, organic fractions increased marginally around 19% - 39% by HPSEC and 23% by FEEM during 110 minutes. Listed in Table 1, during substrate depletion, polycarboxylate humic acid-like and protein kept increasing trend with consistence (Li *et al.*, 2013; Liu *et al.*, 2014). Those humic-like is non-biodegradable compounds (Ni *et al.*, 2011; Zhiji *et al.*, 2017), therefore, being a dominant component in the second stage is reasonable. FEEM and HPSEC with both detectors have characterized that all organic fractions increased and identified the same fractions during fast and slow ammonium decomposition. In this study, combining the derived fraction obtained from HPSEC-OCD and -UVD with peak-fitting techniques and the derived component from F-EEM with FRI analysis, on the same sample simultaneously, gave a reinforced information and had confirmed the presence of humic substances-like (Peak B)/ humic acid-like (Region 5) as the main organic in nitrification processes. The study indicates that a combination of HPSEC-OCD/UVD and FEEM can be used to determine the characteristics of organic matter and water quality change during nitrification, though FEEM seemed like having more sensitivity than the other instruments.

CONCLUSION

Application of HPSEC-OCD/UVD with peak-fitting method and FEEM with FRI method identified the characteristics of organic compounds released during nitrification. HPSEC-OCD classified five different types of organic matter based on average molecular weight, including biopolymers, humic substances, building blocks, low molecular weight acid, and low molecular weight neutral. HPSEC-UVD showed that these organic compounds have an aromatic structure. FEEM with FRI technique characterized five different organic fluorophores: aromatic protein 1, aromatic protein 2, fulvic acid-like, humic acid-like, and soluble microbial products. During nitrification, the major component of aromatic organic matter changed from low molecular weight of building blocks into high molecular weight of humic substances. This is consistent with the dominant compounds humic acid-like substances in FEEM analysis. In addition, batch biofilter nitrification revealed that simultaneous removing the NH_4^+-N concentration and increasing organic carbon at the same time revealed microbial products also released by autotrophs during substrate utilization or in the stage of ammonium decomposition by autotrophs during substrate utilization or in the stage of ammonium decomposition. These results lead to the conjecture that the organic matter released during nitrification is mainly characterized by humic substances compounds. The study indicates that a combination of HPSEC-OCD/UVD and FEEM can be used to determine the characteristics of organic matter and water quality change during nitrification.

AUTHORS CONTRIBUTIONS

Throughout the study performance; E.N. Hidayah performed the experimental design, analyzed the data, prepared the manuscript text and the literature review. W.L. Lai interpreted the data and helped in manuscript preparation. O.H. Cahyonugroho arranged data into tables and figures, literature review and helped in manuscript preparation. F. Rizqa compiled the data and manuscript edition.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

%	Percentage
µm	Micrometer
AMW	Average molecular weight
C	Carbon
cm	centimeter
$CaCl_2 \cdot 2H_2O$	Calcium chloride dihydrate
$CoCl_2 \cdot 6H_2O$	Cobaltous chloride hexahydrate
$CuSO_4 \cdot 5H_2O$	Copper sulfate pentahydrate
Da	Dalton
DBPs	Disinfection by-products
EDTA	Ethylene diamine tetra acetic acid
EEM	Excitation-emission matrix
EfOM	Effluent organic matter
Em	Emission
EPS	Extracellular polymeric substances
Ex	Excitation
Fe	Ferric
Fe-EDTA	Ferric ethylene diamine tetra acetic acid
FEEM	Fluorescence excitation-emission matrix
FRI	Fluorescence regional integration
FTIR	Fourier transform infra red
g/L	Gram per-liter
H_2O	Hydrogen dioxygen
HPSEC	High performance size exclusion chromatography
HPSEC-OCD	High performance size exclusion chromatography organic carbon detector
HPSEC-UVD	High performance size exclusion chromatography ultra violet detector
K_2HPO_4	Dipotassium phosphate
KH_2PO_4	Potassium dihydrogen phosphate
kDa	Kilo dalton
L	Liter
m^2/m^3	Square meter per-cubic meter
MBR	Membrane bioreactor
$MgSO_4 \cdot 7H_2O$	Magnesium sulfate heptahydrate
mg/L	Milligram per-liter
mg-C/L	Miligram carbon per-liter
mg-NH_3-N/L	Miligram ammonia nitrogen per-liter
min	Minutes
mL	Mililiter
ml/L	Milliliter per-liter
$MnCl_2 \cdot 4H_2O$	Manganese (II) chloride tetrahydrate
MW	Molecular weight
Na_2CO_3	Sodium carbonate
$Na_2MoO_4 \cdot 2H_2O$	Sodium molybdate dihydrate
NH_3-*N*	Ammonia-nitrogen
NH_4^+-*N*	Ammonium-nitrogen
nm	Nanometer
NOM	Natural organic matter
NPDOC	Non-purgeable dissolved organic carbon

PEHD	Polyethylene high density
OCD	Organic carbon detector
SMPs	Soluble microbial products
TOC	Total organic carbon
USA	United states of america
UV	Ultraviolet
UV-Vis	Ultra violet visible
UVD	Ultraviolet detector
$ZnSO_4 \cdot 7H_2O$	Zinc sulfate heptahydrate

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