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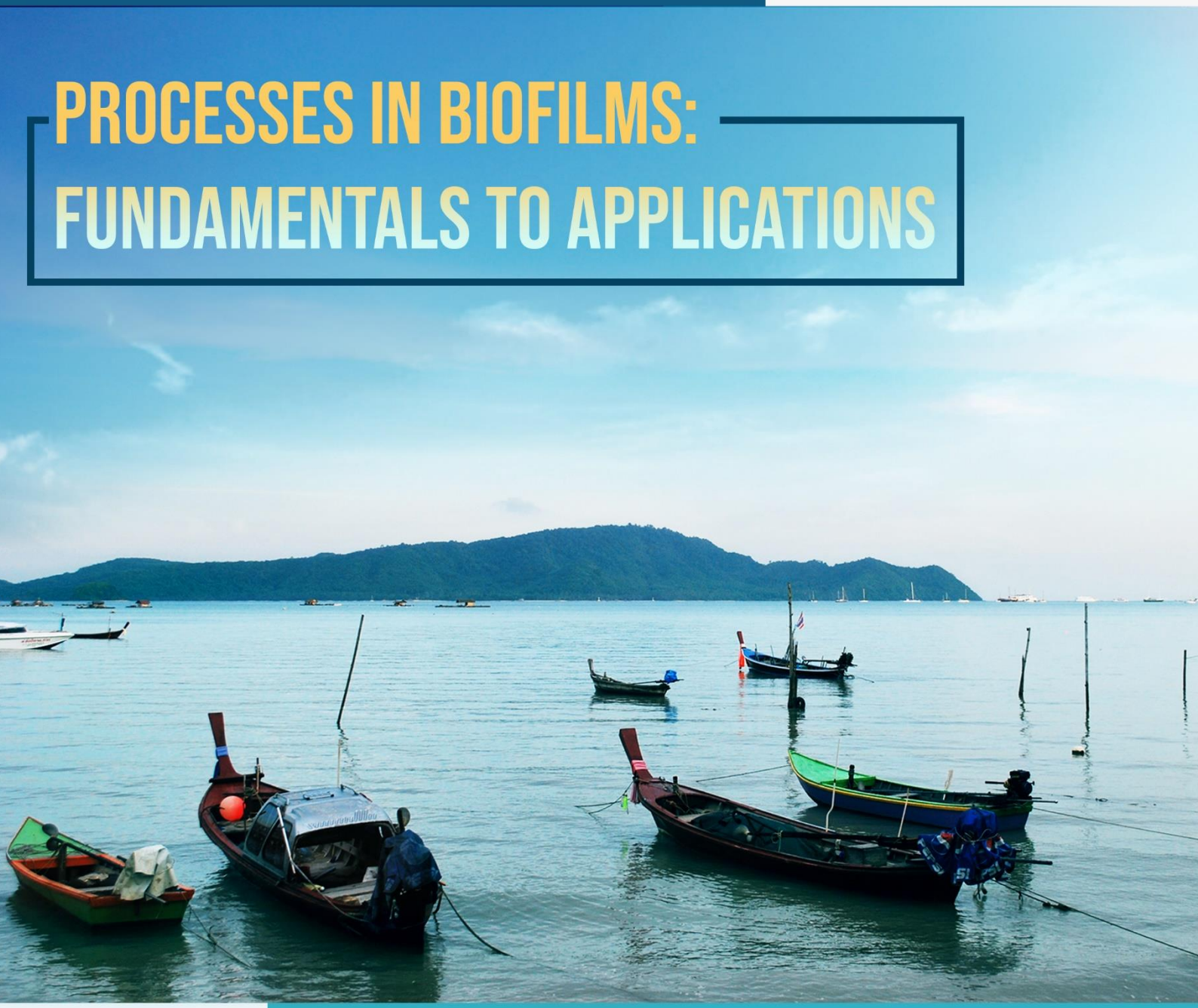

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Determination of six odor compounds and fluorescence components in biofilm from drinking water distribution systems

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Summary: Biofilms can cause many problems in distributed water, including taste and odor. The analyses of biofilms present a significant challenge since traditional evaluation methods for biostability of drinking water and biofilm are limited due to their low sensitivity. There is a wide range of chemical compounds that have been found to cause specific odors and tastes in water but their detection is also challenging since they are found in very low concentrations. Samples of biofilm were collected from 11 water supply pipes across Croatia and analysed by flourescence in order to assess the chemical composition of the biofilm. Furthermore, 6 compounds potentially responsible for odor generation were identified and quantifed by gas chromatography with tandem mass spectrometry detection after solid phase microextraction. The results showed that protein-like components dominate in the biofilm samples, while the key odorants were 2,4-Heptadienal, 2-Methylisoborneol and trans,trans-2,4-Decadienal.

Keywords: Drinking water distribution systems; odor; biofilm

Introduction

Odor and taste are the basic parameters by which consumers assess the quality of water, and if they are present, consumers generally believe that water is not safe for drinking (Krasner et al. 1983). There is a wide range of chemical compounds that have been found to cause specific odors and tastes in water, with characteristic odors that they can cause (earthy / stale /grassy / shady / straw / woody, fish, vegetation, medical, fat-like, cardboard). Unpleasant odors and tastes of water can come from various sources and discovering the specific causes of their presence is a particular challenge because the chemical compounds that can potentially be responsible for their appearance are found in very low concentrations in ng/L (Rogers, 2001), and it would be desirable to determine all of these compounds with a simple analytical method (Wang et al., 2019). Biofilms are widely present in drinking water distribution systems (DWDS) and can cause many problems including taste and odor in the distributed water (Zhou et al., 2017). The analysis of biofilms poses a significant challenge since traditional evaluation methods for biostability of drinking water and biofilm such as heterotrophic plate counts are limited due to their low sensitivity. Different complex organic compounds can be the growth substrate for multispecies biofilms, including humic substances (HS), a terrestrially-derived allochthonous natural organic matter and algal organic matter (AOM), an algae-derived autochthonous natural organic matter (NOM) (Camper, 2004; Li et al., 2019). Fluorescence spectroscopy is one of the most popular methods used to characterize NOM, particularly the excitation emission matrices (EEMs) (Bieroza et al., 2009). Parallel factor analysis (PARAFAC) is a method used to model generated data and to identify components found in EEMs (Murphy et al., 2013). The EEM-PARAFAC technique for monitoring the chemical composition of biofilms was just recently applied (Li et al, 2020).

The aims of this paper were to assess the chemical composition of biofilms with EEM-PARAFAC techniques as well as to identify and quantify the 6 compounds potentially responsible for odor generation using gas chromatography with tandem mass spectrometry detection after solid phase microextraction of biofilms sampled from 11 pipes across different DWDS systems in Croatia.

Materials and methods

The pipes (5 metallic and 6 plastic) were collected during repairs in the corresponding drinking water distribution systems and were transferred in a refrigerated container to the laboratory. Sterilized cotton swabs were used to collect samples from the pipe walls. The swabs were transfered in sterilized tubes containing 8 mL of sterilized deionized water and vortexed for 20 seconds. After sample homogenization, 2 mL of sample was placed in the head-space (HS) vial that contained 0.6 g Na Cl for the determination of odor compounds. For the fluorescence characterization of NOM, 5 mL of sample was taken and filtered

through a 0.45 µm PET filter. Diameter and surafec of pipes were recorded for all pipes in order to calculate the swabbing surface for each sample.

A Horiba Aqualog Jobin Yvonn spectrofluorometer was used to characterize NOM. Excitation-emission matrices (EEMs) were obtained by scanning excitation wavelengths from 240 nm to 600 nm (5 nm increments) and emission wavelengths from 246.62 to 829.14 nm (5 nm increments) with 1.0 s integration times. Analyses were performed in a quartz cuvette with a path length of 1 cm. The blank solution (Milli-Q water) was subtracted from the EEM of samples. CCD gain was set at 'medium' and Saturation Mask Width was 10 nm. Data was corrected for inner filter effects and Rayleigh Masking (1st and 2nd order). After sample normalization, PARAFAC modelling was performed using Eigenvector Solo software (Eigenvector Research Inc.). A three-component model was built for the collected data. Component 1 is a terrestrially derived humic-like substance. Components 2 and 3 represent NOM containing proteins in their structure, and are often referred to as tyrosine-like B1 and B2. Their values were added up together under name tyrosine-like component.

Gas chromatography with tandem mass spectrometry detector (GC-MS/MS) combined with solid phase microextraction (SPME) is a method that allows the detection of a wide range of volatile organic compounds present in water, including those that cause odors (Chen et al., 2013). The following odor compounds were analyzed in the biofilm samples: 2-Ethyl-4-methyl-1,3-dioxolane (2-EMD), 2-Ethyl-5,5-dimethyl-1,3-dioxane (2-EED), 2,4-Heptadienal (2,4-HEP), 2-Methylisoborneol (2-MIB), trans,trans-2,4-Decadienal (t,t-2,4-DEC), and (+/-)-Geosmin (GEO). The analyses of odor compounds were performed at GC-MS/MS TQ8040 (Shimadzu) coupled with *head-space* autosampler AOC-6000. Microextraction was performed using SPME 80 µm DVB/C-RW/PDMS fiber (Shimadzu), preconditioned at 280°C for 10 minutes. Samples were incubated at 45°C for 10 minutes while agitated at 250 rpm, then extracted for 30 minutes and desorbed form the fiber for 10 minutes at injection temperature of 240°C. SPME inlet glass liner (ID 0.75mm) was used to accommodate for SPME injection technique. The analytes were separated by the capillary column HP-5MS UI (Agilent J&W GC Columns I.D.) 0.25mm × 30m × 0.25 µm. Transfer line temperature was 245°C and ion source was kept at 230°C. Detector voltage was set at 1.6 kV.

Results and discussion

Microbial activity that led to the formation of a variety of odor compounds in DWDS contributed to 40% of taste and odor problems (Zhang et al. 2016). Therefore, it is vital to study the role of bioflims in odor production in DWDS. Traditional evaluation methods for biostability of drinking water and biofilm such as heterotrophic plate counts (HPC) are limited due to their low sensitivity.

The results indicated that the chemical composition of biofilms is characterized by humic-like components and protein-like components (tyrosine) (Figure 1). Protein-like components were found to be predominant and were around nine-fold higher than those of humic-like comonents in all the biofilm samples recently studied by Li et al (2020). However, this was a laboratory study and subsequently it was observed that microbial community composition can be shifted in response to the organic matter characteristics in the feed solutions (Li et al., 2019). Thus, those results probably reflect the organic matter characteristic of water distributed through the corresponding DWDS coupled with specific biofilm formation potential at each system.

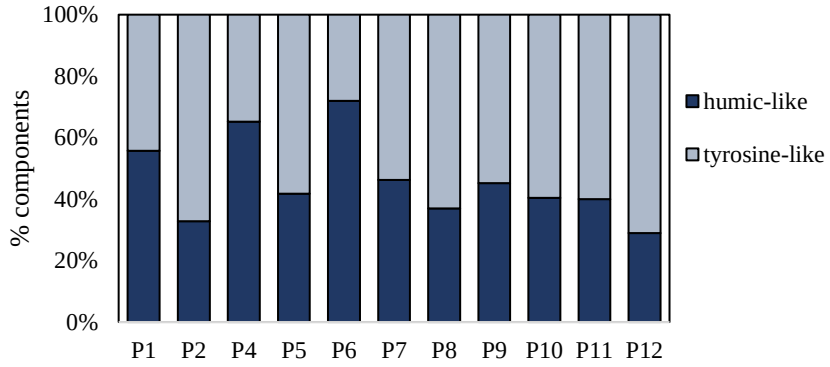

Figure 1 EEM-PARAFAC components within biofilms from drinking water distribution pipes

Tyrosine-like substances and tryptophan-like substances are aromatic proteins (Chen et al., 2003), but compared to tyrosine-like, tryptophan-like substances may indicate more intact proteins or less degraded peptide compounds (Fellman et al., 2010). It is interesting to note that in the studied pipes we did not observe tryptophan-like substances.

Six odorants were detected in the 10 biofilm samples, with concentration ranges of n.d.–86.2 ng/L cm^2. The results indicate that 2,4-HEP, 2-MIB and t,t-2,4-DEC were the key odorants in the analysed samples. 2-MIB and GEO were very often detected as the main odor-causing compounds for musty/earthy odor in DWDS, but in our study GEO was rarely found (only in two samples at very low concentrations). However, 2-MIB was found in 8 samples with concentration range of n.d.–45.5 ng/L cm^2 (Figure 2). The different odor profiles in the studied DWDS could be interpreted by the different raw water types, in which cyanobacteria were the main contributors of odor problems in lake and reservoir sources (Watson et al., 2008). Our results support the previous findings since most odor compounds were found at the pipe 2 (P2) through which treated surface water is distributed, and the highest MIB concentration was observed in the biofilm from this pipe (Figure 2). Concentrations of fishy odorants such as 2,4- Heptadienal and 2,4-Decadienal, with concentration ranges of n.d.–86.2 and n.d.–14.0 ng/L cm^2 respectively, were also found in the pipes (Figure 2).

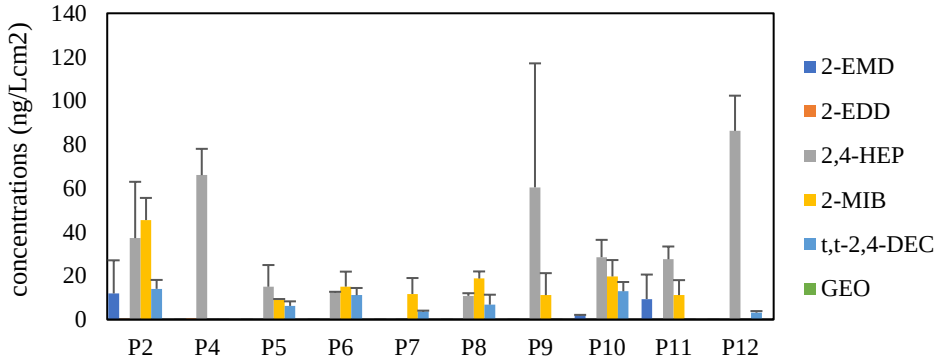

Figure 2 Odor compounds concentrations in biofilm samples from DWDS pipes. Note: Odor compounds from pipe 1 were not analysed.

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