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Importance of nutrient availability for soluble microbial products formation during a famine period of activated sludge: Evidence from multiple analyses

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ABSTRACT

Much remains unknown about compositional variations in soluble microbial products (SMP) with the shift of the substrate condition from a feast to a famine phase in biological treatment systems. This study demonstrated that the formation of SMP could be suppressed by up to 75% during the famine phase with the addition of essential nutrients. In contrast, presence of electron acceptor did not play any significant role during the stress condition, showing the similar amounts of SMP ($r = 0.98$, $p < 0.05$) formation between the bioreactors supplied with air and N_2. The SMP formed in the famine phase was more bio-refractory in the famine versus the feast phase with a linear correlation shown between the production and their aromatic structures in the composition ($R^2 > 0.95$). The fluorescence excitation–emission matrix coupled with parallel factor analysis (EEM-PARAFAC) revealed the presence of four different fluorescent components, including two protein-like (C1 and C4), fulvic-like (C2), and humic-like (C3) components, in the SMP and bEPS formed at different conditions. Both C1 and C4 showed increasing trends ($R^2 > 0.95$) with the length of starvation in the bioreactors without essential nutrients. Nutrient availability was found to be a key factor to quench the production of large-sized biopolymers. This study provides a wealth of information on operation conditions of activated sludge treatment systems to minimize large sized SMP molecules (particularly proteins), which typically exert many environmental concerns to effluent organic matter quality.

Introduction

Biological treatment is highly recognized for its effective degradation of organic matter and nutrient removal from wastewater, ensuring the compliance to regulations and safe discharge into receiving water (Ahmed et al., 2017; Lee et al., 2015). Activated sludge (AS) is the main player for conventional and the advanced biological wastewater treatment systems such as sequencing batch reactor (SBR) and membrane bioreactor (MBR). In the systems, many organic compounds in wastewater are mineralized into inorganics while a part of them are transformed into new cells and consumed for cell maintenances, the formation of extracellular polymeric substances (EPS) and soluble microbial products

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(SMP) (Jo et al., 2016; Kunacheva and Stuckey, 2014; Mutamim et al., 2013). The forms of EPS can be subdivided into bound EPS and soluble EPS. The former is closely bound with the cells while the latter is loosely attached or dissolved into the solutions (Sheng et al., 2010), The soluble EPS constitutes a small part of SMP. SMP can be defined as a pool of organic compounds that are produced from substrate metabolism (usually with biomass growth) and biomass decay (Shin and Kang, 2003).

SMP discharge with treated water as effluent organic matter (EfOM) (Qian et al., 2019), which contains several soluble heterogeneous compounds with varying molecular weights. Proteins, polysaccharides, and humic substances are known to be predominant in SMP (Li et al., 2018). SMP exert several operational and environmental concerns, acting as major contributors to membrane fouling in MBRs and as the extreme precursors of disinfectant by-products (DBPs) formation in wastewater effluent (Liu et al., 2014; Yao et al., 2011). Multiple operational conditions and environmental factors govern the composition of SMP (Yang et al., 2017). For example, a wide range of the differences in SMP composition was observed with growth phases of AS (Maqbool et al., 2017). The diversity of the SMP pool may also be influenced by the composition of influent wastewater, different operation conditions, the presence of trace heavy metals, nanoparticles, and inhibitory organic carbon (Li et al., 2016; Ly et al., 2018; Zhang et al., 2017).

SMP have been classified into two groups based on the phases from which they are derived such as utilization associated products (UAPs) from the substrate consumption during microbial growth, and biomass associated products (BAPs) formed during endogenous phase and the hydrolysis of bEPS (Laspidou and Rittmann, 2002; Ni et al., 2010; Ramdani et al., 2010). The substrate stress condition tends to compel microorganisms to release more extracellular hydrolysis enzymes for dissolution of bEPS as BAPs in mixed liquor (Villain and Marrot, 2013). BAPs cause more environmental problems than UAPs because they have shown superior potential for DBPs formation and the stronger refractory nature to bioavailability (Liu et al., 2014).

The AS in SBR exposes to substantial amounts of external feed for relatively a short period of time. During this feast phase, microorganisms tend to store the substrate internally, and these internal storage products are consumed in the subsequent famine phase (Ni et al., 2009). It was previously reported that the feast and famine cycling in SBR might modify the metabolic potential of microorganisms and improved their performance in the removal of organic pollutants from wastewater (Doble and Kumar, 2005). The internal storage products are mostly polyhydroxyalkanoates (PHAs) and glycogen, which can be used as internal carbon and energy sources for microbial growth after external carbon sources become depleted (Quillaguamán et al., 2006). The external nutrients (ammonia and phosphorus) should be supplemented to the SBRs treating the nutrient-deficient wastewater from the industries of paper, textile manufacturing, and molasses (Freedman et al., 2005; Johnson et al., 2010; O-Thong et al., 2007). The addition of these nutrients was reported to have positive impacts on the performance of the bioreactors. There was no previous study to elucidate the influences of these nutrients (such as ammonia or phosphate) availability on the SMP during the famine phase of AS. Moreover, the role of electron acceptor (i.e., oxygen, O_2) during this famine phase remains unexplored on the production of SMP. The related studies are available, in which the SMP formation was compared between feast and famine periods and the kinetics were modeled with some equations (Ni et al., 2012), or the impact of the SMP production was estimated for engineering systems and natural bodies (Jiang et al., 2010; Ma et al., 2015). However, these studes did not explore the major factors to control the SMP production in the famine phase.

Due to the heterogeneous characteristics of dissolved organic matter (DOM) in SMP, several analytical tools, including ultraviolet–visible (UV–Vis) and fluorescence spectroscopy, and size exclusion chromatography (SEC), have been employed (Jarusutthirak and Amy, 2007; Kimura et al., 2009; Jang and Lee, 2018). In particular, optical methods have presented the advantages of rapid, sensitive, and chemical-free measurements. The advent of a mathematical model, parallel factor analysis (PARAFAC), greatly enhanced the applicability of fluorescence spectroscopy for DOM studies by resolving the complex fluorescence excitation emission matrix (EEM) spectra into the combinations of several independent fluorescent components. It is believed that using both EEM-PARAFAC and SEC successfully describe the change and formation of SMP in biological wastewater systems (Ly et al., 2018; Yu et al., 2015).

The specific objective of the study was to examine the production of SMP in the famine phase and the factors controlling their production. For this, the roles of electron acceptor and nutrients on SMP production were explored during the time period of substrate depletion in batch activated sludge bioreactors. Impacts of the two factors were assessed with the changes in the chemical composition of SMP detected by EEM-PARAFAC and SEC–OCD–OND.

1. Material and methods

1.1. Batch activated sludge bioreactors

A batch AS bioreactor, which has a working volume of 2.5 L, was operated with the synthetic wastewater comprised of glucose (400 mg C/L) as a sole carbon source, and NH_4Cl, and KH_2PO_4, as nitrogen and phosphorus sources, respectively, along with trace amounts of micro-nutrients (i.e., $MgSO_4$, $FeCl_3$, and $CaCl_2$). The detail composition of synthetic wastewater is presented in Table S1. The AS sample for this experiment was collected from an aeration tank of a full-scale wastewater treatment plant, located in Seoul, South Korea, adopting A2O process. It was then acclimated to the synthetic feed wastewater. Before the start of operation, AS was settled down and the supernatant was removed and replaced with the synthetic wastewater. The initial biomass concentration in the bioreactor was 3 g/L. The bioreactor was operated in a continuous oxic mode with an aeration intensity of 2 L/min. All the experiments were conducted in 2.5 L at room temperature with the initial pH to 7.0 using sodium bicarbonate.

After the initial 4 hr of operation, in which more than 80% of the substrate was depleted, the bioreactor was equally divided into four sub-bioreactors to compare the changes in SMP during the famine phase under different conditions.

The experiments for the famine phase were performed in 500 mL for each bioreactor. After transferring to the new bioreactors, AS was allowed to settle for several minutes to remove the supernatant, which was then replaced with de-ionized (DI) water to understand the dynamics of SMP at different working conditions in the famine phase.

Samples from the individual bioreactors were taken at one hour-intervals of operation. In detail, the four bioreactors consisted of (1) a control-bioreactor working on the same supernatant as in the feast phase, (2) an air-bioreactor filled with DI water as the supernatant with continuous air supply rate of 1 L/min, (3) a N_2-bioreactor with DI water as the supernatant at N_2 supply rate of 1 L/min (dissolved oxygen was 0 mg O_2/L), and (4) a nutrient-bioreactor with the DI water containing nutrients (the same as the feed except for the absence of glucose) as the supernatant at an air supply rate of 1 L/min. These divisions are considered to understand the role of multiple factors including electron acceptor and nutrient availability. The purpose of N_2-bioreactor was to eliminate any possibility of SMP degradation in the famine phase, which clarified the major routes for the production and the consumption of SMP in comparison with the air-bioreactor. The replacement of air with nitrogen helped to elucidate the role of the presence of electron acceptor (oxygen) in the famine phase. On the other hand, the nutrient-bioreactor was operated to understand the roles of nutrient availability in the fate of SMP during the famine period.

1.2. Extraction of SMP and bEPS

The samples for bEPS and SMP were extracted based on a previously established method (Frolund et al., 1995). Briefly, 15 mL (occasionally, 50 mL) of mixed liquor samples were collected in a conical tube and centrifuged at 4000 rpm and 4°C for 15 min to obtain the supernatant containing SMP. AS pellets were re-suspended in phosphorus buffer solution (PBS, pH = 7) with cation exchange resin (CER, 70 g per g MLVSS) (DOWEX sodium resin, Sigma Aldrich), stirred at 180 rpm for 24 hr, and centrifuged to collect bEPS. Both SMP and bEPS samples were filtered through a 0.45 μm cellulose triacetate (CTA) membrane filter and stored for further analyses. Dissolved organic carbon (DOC) of the filtered samples was quantified using a TOC-analyzer (TOC-L, Shimadzu, Japan).

1.3. Spectroscopic measurements

Absorption spectra of the filtered samples at 200–800 nm were measured by a UV–visible spectrophotometer (Shimadzu, Japan) with a 1 cm quartz cuvette. When the absorption coefficients at 254 nm were above 0.05 cm^{-1}, the samples were diluted enough to avoid the inner-filter correction prior to fluorescence measurements.

Fluorescence spectroscopy (F-7000, Hitachi, Japan) was employed for the measurements of EEM at the excitation (Ex) and emission (Em) wavelengths of 250–500 and 280–550 nm with a step of 5 and 1 nm, respectively. The scan speed was set at 12,000 nm/min, and the Ex/Em slit widths were adjusted to 10 nm. A 290 nm cut-off filter was placed in front of Ex lamp to remove second order Raleigh scattering (Wang et al., 2018). EEM of DI was subtracted as blank from SMP and bEPS samples using the installed FL solutions software.

1.4. PARAFAC modeling

PARAFAC modeling was performed on combined EEMs (n = 43) of bEPS and SMP samples collected from all bioreactors via DOMFLuor from a toolbox in Matlab 13.0 based on the established procedure of the tutorial (Stedmon and Bro, 2008). The following steps were conducted for the data handling and modeling – scattering removal by data cut, non-negativity constraints, loading leverage, outlier removal, and split half analysis. A suitable number of fluorescent components were selected by split-half validation. The maximum fluorescence (i.e., F_{max}) was used to represent the relative quantity of the individual components. All the EEM-PARAFAC results were normalized on the basis of Raman integrated area (Lawaetz and Stedmon, 2009).

1.5. Size exclusion chromatography analysis

Molecular size distributions of the bulk SMP and bEPS were measured by SEC–OCD–OND (DOC-Labor, Germany). The detail of the instrument can be found elsewhere (Huber et al., 2011). Samples were diluted below 2 mg C/L to avoid column contamination. The injection volume and the flow rate were fixed to 1 mL and 1.1 mL/min, respectively, with the maximum retention time of 130 min. SEC–OCD–OND provides the quantities of four different size fractions including biopolymers (BP, >10 kDa), humic substances (HS, 1 kDa), building blocks (BB, 300–500 Da) and low molecular weight acid/neutral (LMW A/N, <350 Da). The BP fraction in biological treatment systems refers to the DOM constituents mostly consisting of polysaccharides and proteins. The OND detector allows estimating the relative contribution of proteins to BP fraction through the ratio of dissolved organic nitrogen (DON) to DOC (N/C) and the following equation (Huber et al., 2011; Jacquin et al., 2017).

$$\text{Proteins in biopolymers} = \frac{\text{DON (Biopolymers)} \times 300}{\text{DOC (Biopolymers)}} \times 100\% \quad (1)$$

2. Results and discussion

2.1. Dynamics of SMP in control-bioreactor at feast and famine phases

During two phase-operation of the control-bioreactor, the DOC concentrations of the supernatant showed a declining trend with time (Fig. 1), indicative of the substrate (glucose) degradation. While the absorption coefficient (cm^{-1}) at 254 nm (UV_{254}) presented a generally increasing trend

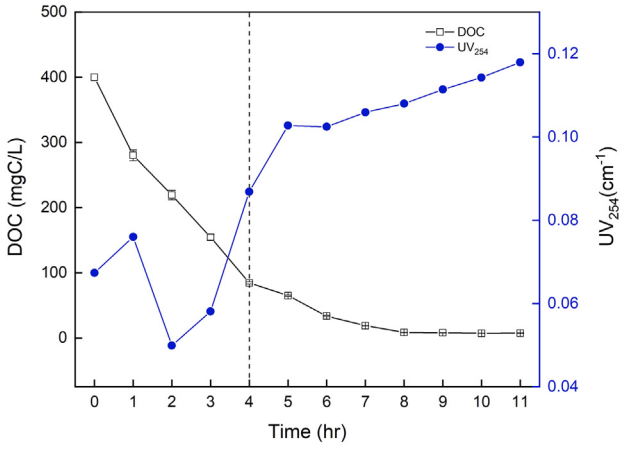

Fig. 1 – Dynamic variations in the DOC concentrations and UV_{254} values of SMP with the operation of the control-bioreactor with a shift of the feast phase to a famine phase.

(Fig. 1), suggesting the formation of aromatic SMP structures. The initial fluctuations of UV_{254} before 4 hr shows the slight formation of aromatic SMP and its subsequent degradation. The increased aromatic SMP indicates the cumulative production of proteins and humic substances (HS) (Liu et al., 2014; Tsai et al., 2008). During the initial 4 hr of operation, the substrate was depleted linearly with time and microorganisms experienced a substrate-rich condition. In contrast, extensive production of SMP as depicted by UV_{254} and less availability of easily biodegradable substrate were the main features of the famine condition after the 4 hr-operation (Fig. 1). The UAPs produced during a feast phase are composed of mostly non-aromatic structures while the SMP (as BAPs) formed during a famine phase are enriched with aromatic structures (Ni et al., 2011).

The fast degradation of the substrate but the substantial decline of the UV_{254} value (i.e., depletion of aromatic SMP) in the initial feast phase (to 2 hr of operation) might have supported the cometabolism under the high abundance of the easily degradable substrate (i.e., glucose). It is also possible that SMP formed in substrate rich conditions are easily biodegradable. Such a cometabolism of AS has been frequently proposed in previous studies to explain the degradation of slowly biodegradable organic compounds (e.g., pharmaceuticals), in which readily biodegradable substrate was intentionally added to provide an energy source for heterotrophic biomass growth and to stimulate the microbial activities to decompose organic pollutants (Batt et al., 2006; Müller et al., 2013; Wen et al., 2010). The SMP produced in the feast phase is mostly a type of UAPs which are less aromatic and more degradable compared to the BAPs formed in a famine phase (Dong and Jiang, 2009). Microorganisms may thus easily utilize UAPs in the absence of external substrates. By contrast, a very low biodegradability has been assumed for BAPs. For example, biochemical oxygen demand (BOD_5) of BAP samples corresponded to only 14% (Ni et al., 2011) or even 2.8% of chemical oxygen demand (COD) (Jiang et al., 2010). Xie et al. (2013) have also reported a higher production of UAPs in initial hours of operation followed by their degradation afterwards.

The linearly increasing trend of UV_{254} values in the famine phase suggests the continuous production of aromatic SMP. The overall trends in the famine phase can be described by the production from continuous hydrolysis of bEPS and cell lysis, followed by the formation of BAPs through their dissolution. BAPs are reported to be more aromatic in nature than UAPs (Ni et al., 2011). During the famine phase, as limited carbon available to microorganisms, stress condition compelled the microorganisms to hydrolyze the bEPS and the BAPs tend to be steadily accumulated in this period of time (Ni et al., 2009). Moreover, it is not likely that microorganisms easily degraded the produced BAPs during a short period of time (Jiang et al.,

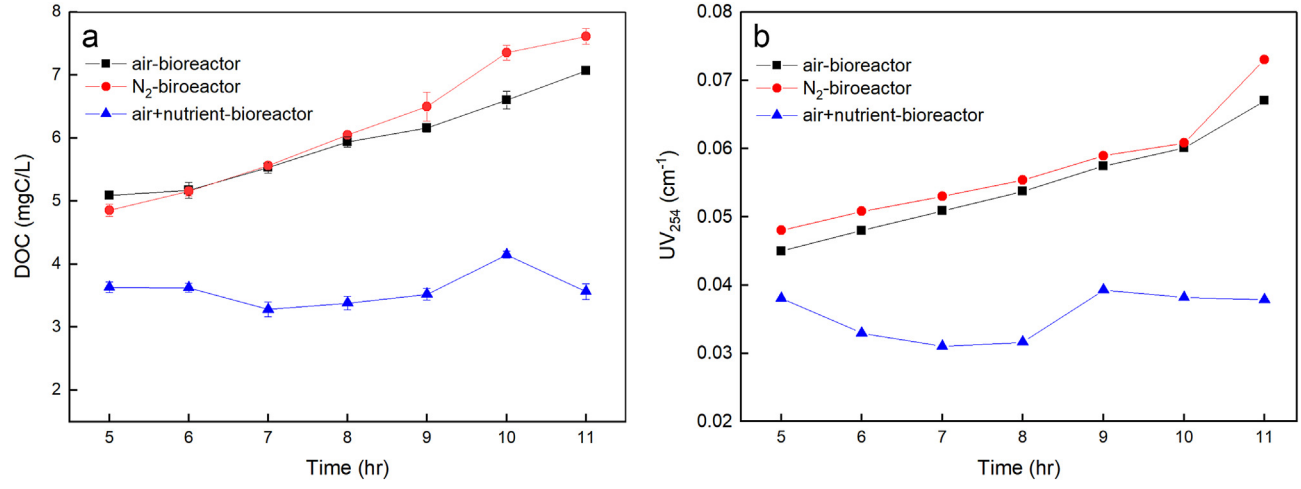

Fig. 2 – Changes of SMP in famine phase under different operation conditions. Please see the text for the detail of the different bioreactors. The initial differences in the DOC and UV_{254} values among the three bioreactors can be attributed to the results of the first hour of operation, during which SMP in nutrient-bioreactor remained the same while it increased linearly with time in air- and N_2-bioreactors.

2008). It was previously reported that a substantial portion of UAPs could be present with a bio-refractory nature in this phase (Ni et al., 2012).

Similar increasing trends in the DOC concentrations and the UV_{254} values are shown between air- and N_2-bioreactors (Fig. 2). The dynamics of UV_{254} in both bioreactors are matched with the increasing tendency of SMP in famine period of control-bioreactor (Fig. 1). The comparable results of the air- versus the N_2-bioreactors suggest that the addition of the electron acceptor (O_2) did not considerably affect the rates of SMP formation during the famine phase. It can be inferred that oxygen played an insignificant role in the generation of the BAPs with bio-refractory nature. At the end of the operation (to 11 hr), the aromatic SMP produced in both the air-bioreactor and the N_2-bioreactor was nearly two times higher in the concentrations than those of the nutrient-bioreactor. This difference suggests that the added nutrients might stimulate microorganisms to consume cell internal storage products for their survival instead of taking a route of the hydrolysis of bEPS, which seems to result in a small production of SMP at the end of the operation (Fig. 2). The review by Ni et al. (2015) has also presented an insignificant role of O_2 in the consumption of internal storage products. The combined results signify the importance of nutrient availability in the generation of SMP during the substrate depletion condition.

Available ammonium ions are fully depleted in the famine phase. For example, ammonium concentrations were declined from 15 to 0.3 mg/L after 4 hr of operation in the control-bioreactor (Appendix A Fig. S1). According to a review article Ni and Yu (2012), a shortage of essential nutrients is the main trigger of producing substantial amounts of SMP through hydrolysis, consistent with our results. The lack of ammonium at 4 hr of operation was followed by a linear increase of SMP in the control-bioreactor, while the variation of SMP remained relatively stable in the nutrient-bioreactor at the famine phase. The total phosphorus (TP) did not exhibit such a marked difference between the two bioreactors because TP was not fully consumed in the feast phase (Appendix A Fig. S1). However, the higher concentration of TP was maintained at the substrate-deficiency. Our results imply that essential nutrients, rather than carbon sources, might play more critical roles in accelerating or decelerating the bEPS hydrolysis. Therefore, it can be recommended that the operation of AS bioreactors should be targeted to ensure nutrient availability to avoid an undesirable increase of SMP levels in famine conditions. It should be noted, however, that although the dynamics of UV_{254} illustrate the production or degradation behaviors of aromatic SMP, the information is still limited to discriminate between proteins and humic substances.

2.2. Dynamics of different fluorescent components in SMP

Four fluorescent components were validated on the combined EEM dataset of SMP and bEPS samples collected from all the bioreactors at different operation conditions. Their peak positions are displayed in Fig. 3 (Appendix A Fig. S2). The peaks of the C1 (tryptophan-like) and the C4 (tyrosine-like) components are placed at an excitation wavelength (Ex) of 225

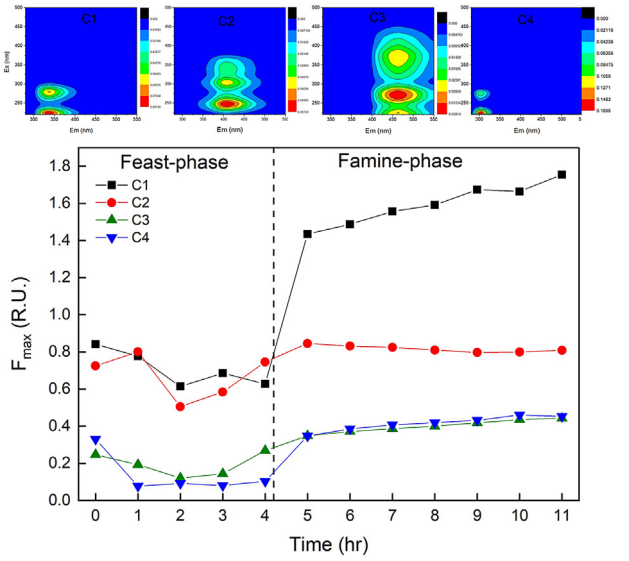

Fig. 3 – Dynamic variations in four different fluorescent components (C1–C4) of SMP with the operation of the control-bioreactor with the shifting condition from the feast phase to a famine phase. The upper panel shows EEM-PARAFAC components with the peak positions (C1: tryptophan-like, C2: fulvic-like, C3: humic-like, C4: tyrosine-like).

(275) nm and the emission wavelengths (Em) of 335 and 305 nm, respectively. Both components are representative of aromatic amino acids or proteins. The C2 and C3 components were matched with the typical fulvic-like and humic-like fluorescence features with the maxima shown at 240/410 nm (Ex/Em) and 265/460 nm (Ex/Em), respectively. The assignments of the components were based on the previous reports using EEM-PARAFAC for biological wastewater treatment systems (An et al., 2017; Cai and Liu, 2018; Li et al., 2014; Zhou et al., 2014). The protein-like (C1 and C4) components in AS systems may represent the proteins-associated biopolymers and extracellular enzymes, which are commonly found in bEPS and SMP samples. Although the two components are indicative of proteinaceous materials, they have exhibited a distinguishable degree of the degradability toward chemical or biological treatment (Mangalgiri et al., 2017). Microbial transformation of such protein-like components could be a source of fulvic-like and humic-like components (Li et al., 2014; Maqbool et al., 2017). The humic-like fluorescence is associated with a more condensed aromatic structure and a more bio-refractory nature than the fulvic-like counterpart. The relative abundances of these components in SMP and bEPS depends upon original wastewater composition, microbial growth stages, and different operational conditions (Ly et al., 2018; Maqbool et al., 2018, 2017).

The dynamics of the four different fluorescent components in SMP extracted from control-bioreactor are shown in Fig. 3. The background fluorescent components present in the initial supernatant were slightly degraded until two hours of operation probably due to the cometabolism initiated upon the addition of glucose (Fig. 3). All the four fluorescent components substantially increased during the operation from 2 to 11 hr with the enhancement ratios of 2.55, 1.39,

3.09, and 5.61 for C1, C2, C3, and C4, respectively. C2 and C3 also have shown significant shares in SMP at the end of the operation (23.4% and 12.8%, respectively). Both humic-like components (C2 and C3), however, do not appear to be the major contributors to the composition of bEPS throughout the operation, as shown by their relative abundances below 5% in bEPS (Appendix A Fig. S3). Several previous studies have also presented that protein-like fluorescence was dominant over humic-like fluorescence in bEPS (Sheng et al., 2013; Zhang et al., 2019).

Strong positive relationships ($r > 0.86$; $p < 0.001$) were found among the three components of C1, C3, and C4, while C2 exhibited a negative correlation with any of the three (Appendix A Fig. S4). Distinct from the other components, C2 showed a slight degradation trend with operation at the famine phase (Fig. 3). It infers that C2 component is likely to be slightly degradable in the famine phase or transformed to others with a characteristic of humic-like fluorescence. Previous studies have described the C2 component as less condensed aromatic substance compared to the humic-like component peaked at a longer emission wavelength. The ratio of humic-like to fulvic-like fluorescence has been used as the extent of microbial humification (Hunt and Ohno, 2007; Hur et al., 2009; Jurado et al., 2015). The observed variation in C2 was generally matched with that of UAPs previously reported in Ni et al. (2012), implying the close association of C2 with UAPs in 314 AS treatment systems.

For the bioreactors without nutrients (i.e., air- and N_2-bioreactors), the components, C1, C3, and C4 of SMP showed steadily increasing trends with time as shown in Fig. 4, indicating a potential association of these components with the hydrolysis of bEPS followed by the formation of BAPs. In contrast, C2 was nearly invariant with time for the two bioreactors, suggesting the production of UAPs might be limited in the absence of nutrients. It was interesting to observe the increasing trend of C3 because this component constitutes only a small proportion of bEPS. For example, the relative abundances of C3 in SMP were 3.3 and 3.9 times higher than those of bEPS at 11 hr of operation for the air-bioreactor and the N_2-bioreactor, respectively. These results may indicate a strong propensity of the component toward the hydrolysis of bEPS, which contrasts with the lower presence of the two protein-like components (C1 and C4) in SMP versus bEPS.

The nutrient-bioreactor showed completely different dynamics in the fluorescent components. The fulvic (C2) exhibited increasing trends in the famine phase (Fig. 4). Meanwhile, the components of C1 and C4 displayed slowly decreasing trends with time. Considering that protein-like components are the primary constituents of bEPS, the trends of C1 and C4 indicate no significant occurrence of bEPS hydrolysis under the condition. Since C2 was present in substantial amounts in the control-bioreactor in the famine phase, it is inferred that the fulvic-like component could be produced via the consumption of bEPS or internal storage products in the presence of nutrients. The non-increasing behavior of C2 during 5–7 hr of operation might because of acclimation of a microorganism to new feed containing only nutrients without carbon source. There is a possibility that, during the initial hours of operation in famine phase, microorganisms might use internal storage compounds before producing the SMP as fluorescence DOM in the supernatant. The results may imply that maintaining minimal amounts of nutrients in operating reactors might be a possible solution to prevent the extensive hydrolysis of bEPS to form BAPs, which is significant in that BAPs can cause more environmental problems compared to UAPs because of its bio-refractory nature and acting as a strong precursor for DBPs (Liu et al., 2014).

2.3. Dynamic changes of different size fractions in SMP

Although four different size fractions were identified, relatively large size fractions (BP and HS) were more dominantly present in SMP than the two smaller sized fractions (i.e., BB and LMW N/A). A slight increase in BP concentration was observed during the early feast (0–1 hr) period. The BP was consumed along with substrate later during one to four hours of operation. The initially produced BP fraction for the first one hour of operation appears to be enriched with polysaccharides as indicated by the decline of the protein's abundance in BP (Fig. 5). Meanwhile, the consumed BP in the later feast phase is likely to be in the form of polysaccharides rather than proteins because of the increased proteins in BP (Fig. 5). Combining these results with EEM-PARAFAC it can be deduced that, although both of contents (protein and polysaccharides) are degraded the polysaccharide has a higher extent of reduction.

At the famine phase of control-bioreactor, BP fraction showed a continuous increasing trend with the relative abundance of proteins ranging between 65% and 71%. DON concentrations in BP had a linear relationship ($R^2 > 0.86$; $p < 0.001$, Appendix A Fig. S4) with both protein-like components (C1 and C4), supporting the potential of these components as the surrogate of nitrogen-containing compounds (mostly proteins) in BP. Meanwhile, HS fraction showed a steadily increasing trend throughout the operation except for the initial decline for the first one hour of operation (Fig. 5). The production of HS fractions in the famine phase can be attributed to the hydrolysis of bEPS. A substantial difference was observed in the relative distributions of BP and HS between SMP and bEPS with the averaged ratios of BP to HS being 1.29 ± 0.05 and 0.52 ± 0.17 in bEPS and SMP, respectively, throughout the operation. Therefore, the HS fraction in bEPS could be more susceptible to the hydrolysis to form SMP compared to the BP fraction in bEPS.

The dynamics of BP and HS fractions in the famine phase were much affected by the operating conditions. The BP fractions showed the similar increasing tends with time in both air- and N_2-bioreactors (Fig. 6), suggesting that the presence of oxygen did not act as a limiting factor for the continuous formation of BAPs under the substrate depletion condition. No discrimination of the protein content in BP (77%–80%) between the two bioreactors can be indirect evidence on the major contribution of bEPS hydrolysis to the formation of BAPs and the minor roles of microbial degradation. For the nutrient-bioreactor, however, no increasing or decreasing trend was observed for BP fraction with relatively a

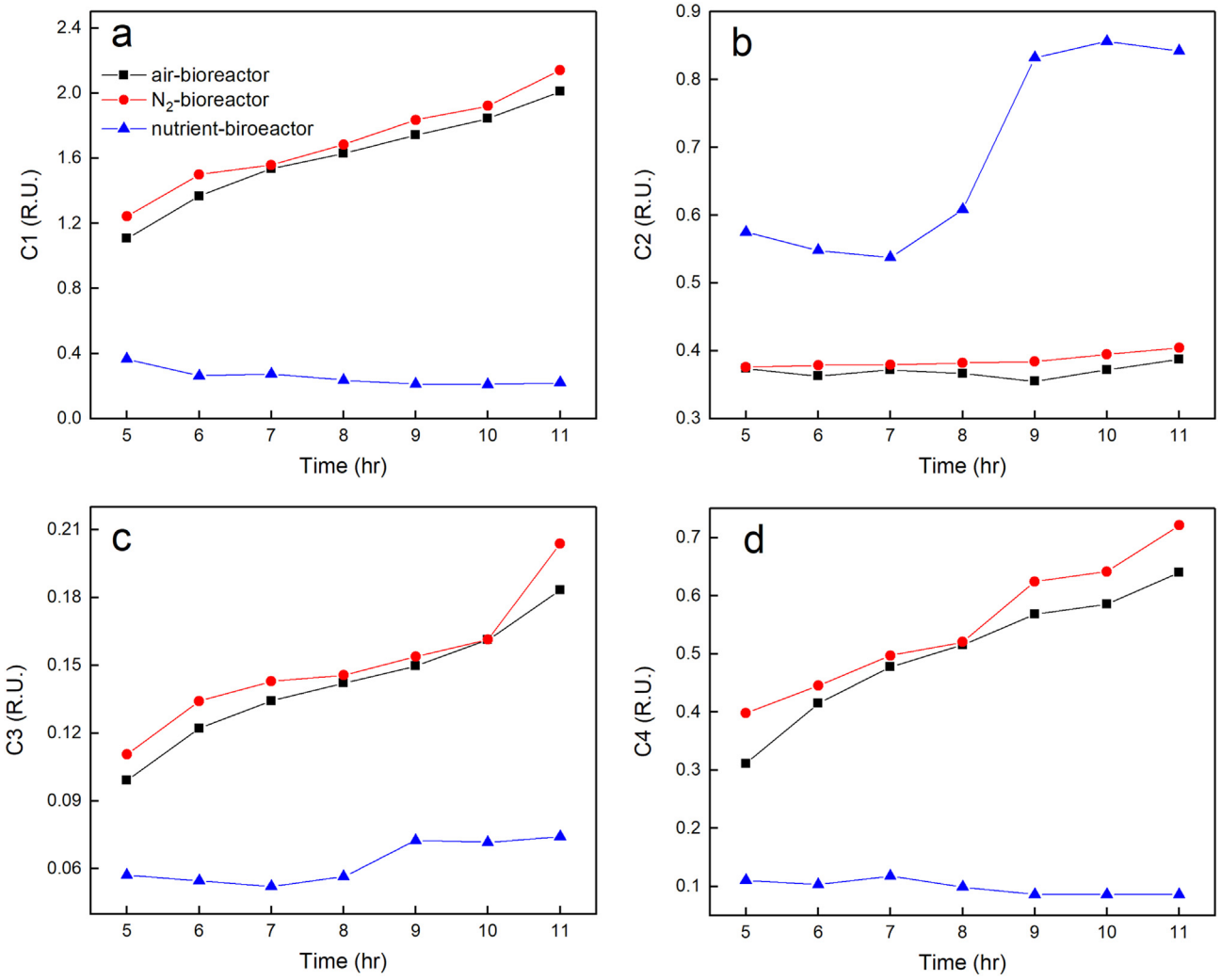

Fig. 4 – Changes of four different fluorescent components in SMP in the famine phase under different operation conditions. Please see the text for the detail of the different bio-reactors. (a) C1, (b) C2, (3) C3, and (4) C4.

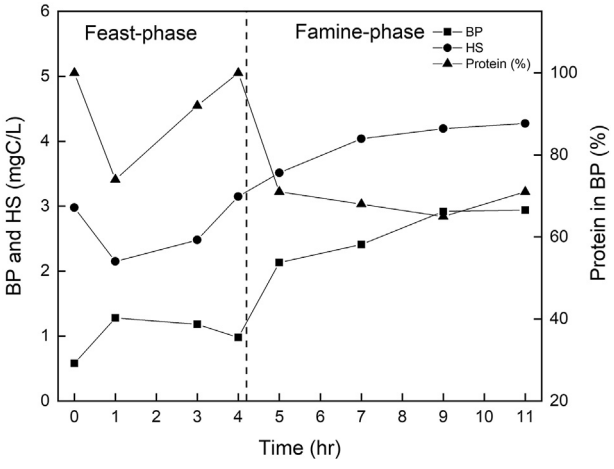

Fig. 5 – Variations of HS and BP concentrations and the relative abundance of proteins in BP fraction upon the operation of the control-bioreactor.

stable variation with time (Fig. 6). The relative abundance of proteins in BP (54%–59%) was much lower than those in the air- and N_2-bioreactors, suggesting the relatively higher contribution of polysaccharides versus proteins to BP for the nutrient-bioreactor. The result may reflect the differences in the origins of BP in bioreactors with versus without nutrients. During such a substrate depletion condition, AS microorganisms tend to utilize the internal carbon source stored in the forms of polyhydroxyalkanoates (PHA), lipids, and polysaccharides (Pratt et al., 2004). Nutrient availability seems to act as a trigger for the microbial function. In contrast to BP, the HS fraction presented similar steadily increasing trends with the operation for all three bioreactors. However, the chemical composition of HS appears to be affected by the availability of electron acceptor (O_2) as the increase of DON concentrations in HS was more pronounced for N_2-bioreactor versus the two others. This result may imply the role of O_2 presence in incorporating nitrogen-containing compounds into complex HS structures in the famine phase although it needs further investigation to show concrete evidence.

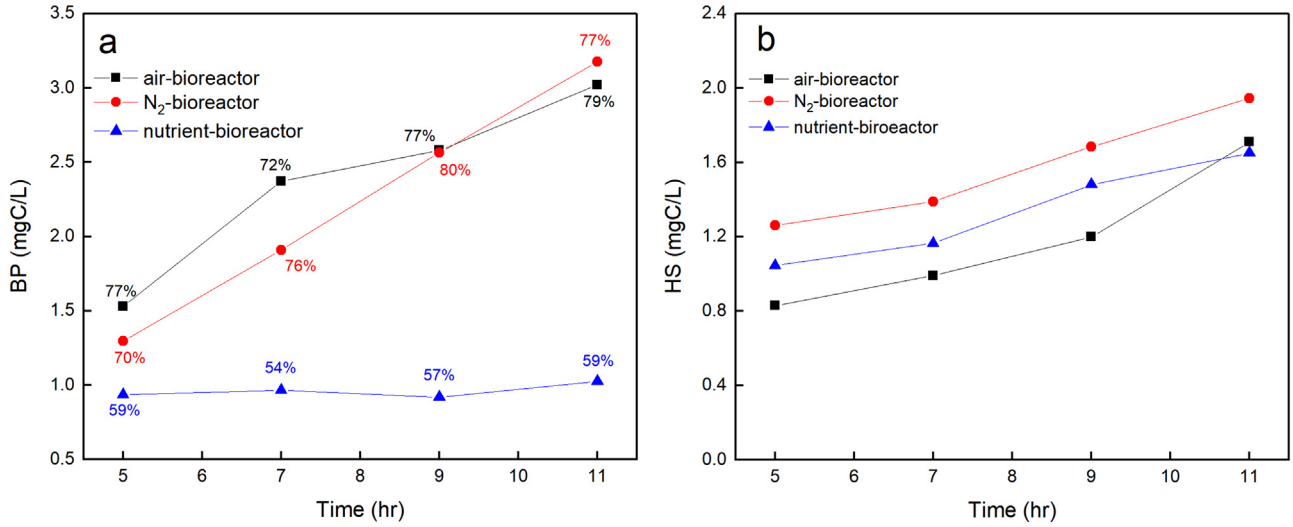

Fig. 6 – Changes of the BP and HS fractions in SMP in the famine phase under different operation conditions. Please see the text for the detail of the different bioreactors. (a) BP, and (b) HS. The relative distributions of proteins in BP are indicated in the numbers with the percentages.

2.4. Engineering implications

The results of this work suggest that a famine phase can be considered to maintain the performance of bioreactor in the biological wastewater systems such as SBR. The storage products formed in the feast phase are further consumed in a famine phase, allowing microorganisms to balance their growth. This study also imples that biological wastewater system should be designed in a way to maintain a minimal level of nutrients throughout the operation against the shortage of carbon supply. In particular, nutrient availability could be a critical factor to control the production of large-sized SMP molecules (mostly BAPs). The finding of this study is important for the operation of biological treatment systems considering that such biopolymers likely cause more environmental problems, such as a high membrane fouling potential and the impediment to AS dewaterability/settleability, than small sized SMP do (Al-Halbouni et al., 2008; Jiang et al., 2010; Xiao et al., 2016). However, the excessive amounts of nutrients (N and P) in effluent are undesirable because of their adverse impacts on receiving water (e.g., algal bloom).

3. Conclusions

The following conclusions can be drawn from the dynamics of SMP composition in a famine phase under different operation conditions regarding the availability of O_2 and nutrients, which were successfully tracked by the measurements of EEM-PARAFAC and SEC–OCD–OND in this study. (1) Linear increment in UV_{254} values ($R^2 > 0.96$) in supernatant during famine phase inferred that the SMP produced in the substrate depletion condition consisted of aromatic structures and biorefractory in nature. (2) A similar increase in SMP ($r = 0.987$, $p < 0.05$) between air- and N_2-bioreactors showed the minimal role of electron acceptor during the famine phase. (3) The availability of essential nutrient during the famine phase reduced the SMP production by up to 75% compared to the biore$_a$ctor without nutrients under famine condition. (4) Protein-like components (C1 and C4) behaved as a signature of bEPS hydrolysis as shown by their steadily increasing trends ($R^2 > 0.95$) in the famine phase in the absence of nutrients. The fulvic-like component (C2) seems to be produced from microbial transformation, which operated as an indicator for microbial activity, as it became more abundant in SMP when nutrients were available. (5) The more enrichment of BP and the lower relative abundance of proteins in BP (54%–59%) in bioreactors with versus without nutrients (70%–79%) signifies the importance of nutrient availability in the formation sources/pathways of large-sized SMP molecules as well as in the chemical composition (proteins or polysaccharides).

Conflicts of interest

There are no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.04.021.

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