Characterization of dissolved organic matter extracted from water treatment sludge

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ABSTRACT

The characteristics of dissolved organic matter (DOM) that formed during the aerobic and anaerobic incubation of drinking water treatment sludge stored at different temperatures (5 °C, 20 °C, 40 °C) for long periods (7, 14, and 21 days) were investigated. Anaerobic incubation at high temperatures with prolonged storage was found to result in higher organic content than aerobic incubation (3.6–6.8 times at 40 °C). The high temperatures caused changes in the DOM fractions, with humic-like substances mainly formed in aerobic incubation and protein-like substances in anaerobic incubation. Results showed that the fluorescence intensity of humic-like and protein-like substances increased by 45% and 22%, respectively, at the end of the anaerobic incubation period. The UV-absorbing DOM constituents in aerobic incubation had lower molecular weights and were more heterogeneous than those in anaerobic incubation.

Key words | aerobic incubation, anaerobic incubation, dissolved organic matter, drinking water treatment sludge

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HIGHLIGHTS

- DOM transformation in drinking water treatment sludge was investigated during prolonged aerobic and anaerobic incubation.
- High temperature accelerated the humification process in the sludge during aerobic incubation.
- More protein-like substances and less humic-like substances were produced in anaerobic incubation.

INTRODUCTION

A drinking water treatment plant generates large amounts of drinking water treatment sludge (DWTS) as a by-product of drinking water. DWTS is primarily composed of amorphous Fe/Al hydroxides and is produced during flocculation–sedimentation or floatation, as Fe- and Al-based coagulants are commonly used (Zhou *et al.* 2016). Large volumes of DWTS are generated during the production of drinking water, equivalent to 4%–7% of the total drinking water produced (Sun *et al.* 2015). DWTS also contains organic fractions that can be attributed to the presence of bacteria, humic-like materials derived from the decay of plants, and

small quantities of extracellular polymeric substances (EPS) produced by bacteria (Zhou *et al.* 2018). The properties of DWTS can vary according to the quality of raw water and the treatment method applied. Groundwater tends to be stable in quality, and the DWTS that forms during the treatment of groundwater fluctuates very little in quantity or quality. In contrast, the treatment of surface water sometimes results in noticeable changes in sludge quality and quantity (Ahmad *et al.* 2016).

Increasing urbanization and industrialization have resulted in the dramatic growth of drinking water volumes

generated, which has led to large amounts of DWTS being generated by drinking water treatment plants. Also, environmental restrictions have been placed on disposal methods that require sludge recycling, which is an important pathway for realizing the reduction and reclamation of total waste residues (Zhou *et al.* 2016). In recent years, DWTS dewatering has become more common, and the extracted water is recycled back into the stream of drinking water treatment, achieving zero waste discharge from drinking water treatment plants. However, the main water quality issue associated with the recycling process is the release of dissolved organic matter (DOM) from extracellular or intracellular organics that originate from DWTS (Zhou *et al.* 2015), a release that potentially causes problems for water recycling in drinking water treatment plants.

In some drinking water treatment plants in Japan that rely on rapid sand filtration, sludge from the sedimentation basin is discharged to the thickener tank. The sludge is usually dewatered using various methods, and in some plants, the water that is extracted in dewatering along with other wastewater, including backwash water, is pumped back to the raw water receiving well. In a water supply system located in Central Japan, water extracted from DWTS was found to contain many substances, including DOM, that may affect the quality of the purified water and that may result in a rapid decrease in the residual chlorine concentration of the purified water at some supply points (Suzuki 2017).

Various factors such as temperature can affect DWTS when it is settling in the thickener tank, and these factors can lead to cumulative increases in levels of organic matter and other substances such as ammonia. Many studies have indicated that temperature substantially increases the accumulation of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), and the microbial abundance in the wastewater activated sludge associated with aerobic and anaerobic processes (Shao *et al.* 2013; Jin *et al.* 2016; Du & Li 2017; Komatsu *et al.* 2020). However, little information is available on how the storage condition influences the transformation of DOM at different temperatures, since temperature and retention time of DWTS in the thickener tank are the dominant factors affecting the transformation of DOM in DWTS. Our study found that, after DWTS was stored under different temperature conditions,

the characteristics of DOM released in water extracted from DWTS were distinct from those of freshwater extracted from DWTS and those of raw water. The main objectives of this study were as follows: (1) to characterize the DOM in extracted water obtained after the dewatering of DWTS, and (2) to investigate how different storage conditions influenced the water quality. The characteristics of DOM in water extracted from DWTS can guide the selection of treatment processes and establish grounds for the assessment of quality risks associated with water extracted from DWTS during drinking water production at drinking water treatment plants.

MATERIALS AND METHODS

Raw DWTS used in the experiment

The DWTS sample was collected from the thickener tank in a drinking water treatment plant located in Aichi Prefecture, Japan, that has a treatment capacity of 200,000 $m^3\,day^{-1}$. The plant uses conventional treatment processes, including coagulation, flocculation, sedimentation, and filtration. Poly-aluminium chloride is used as the coagulant. The sludge that forms during water treatment settles in the sedimentation basin and is discharged to the thickener tank. The DWTS sample was transferred immediately to the laboratory after sampling.

The obtained DWTS was measured for volatile solids (VS) and total solids (TS). Initial extracted water was obtained by centrifuging the DWTS at 2,500 rpm for 15 min, and the supernatant was filtered through a 0.2 μm membrane filter for the measurement of pH, electrical conductivity (EC), UV absorbance at 260 nm (UV_{260}), DOC, DON, dissolved inorganic nitrogen (DIN), fluorescence excitation–emission matrix (EEM), and molecular weight distribution in the initial extracted water.

Incubation experiment

Six parallel incubation systems, each with a volume of 3 L, were used for the experiment. Incubation was performed at different incubation temperatures: 5 °C, 20 °C, and 40 °C. Air pumps were used to supply the air at a flow rate

of 2.5 L min^{-1} for the aerobic condition. The anaerobic condition was performed in a tightly sealed reactor. During the incubation experiment, samples were collected from each reactor at designated time points (7, 14, and 21 days). After filtration through a 0.2 μm membrane filter, the filtrates were subjected to quality analysis for DOM. This analysis mainly focused on DOC, UV$_{260}$, fluorescence EEM, and molecular weight distribution, and the details of each are described below. In addition to these measurements, dissolved oxygen (DO), pH, and EC inside all the reactors were monitored, and DIN (NO$_2^-$-N, NO$_3^-$-N and NH$_4^+$-N) was analysed using the filtrates of all samples with an ion chromatography system.

DOM quality analysis

DOC and SUVA analysis

DOC was quantified with a TOC analyser (TOC-V$_{wet}$, Shimadzu, Japan). UV$_{260}$ was measured by a UV-vis spectrophotometer (UV-1600, Shimadzu, Japan). The specific UV absorbance (SUVA) was calculated as the ratio of UV$_{260}$ to DOC.

Fluorescence EEM spectroscopy

The EEM spectra of the extracted water were measured by spectrofluorometer (RF-5300, Shimadzu, Japan). The excitation and emission scans showed wavelengths between 220 nm and 550 nm at 5 nm increments. The obtained fluorescence intensities of the samples were normalized using the quinine sulphate unit (QSU) by dividing the fluorescence intensity values of all extracted water by that of 10 ppb quinine sulphate (in a 0.05 M H$_2$SO$_4$ solution) at the designated excitation wavelength (Ex) of 350 nm and the emission wavelength (Em) of 450 nm. The humification index (HIX), which provides insight into the degree of DOM humification, was calculated as the area under the emission spectra of 435–480 nm divided by the peak area under the emission spectra of 300 to 345 nm + 435 to 480 nm, at an excitation wavelength of 254 nm (Ohno 2002). The fluorescence index (FIX), an indicator of DOM quality, was calculated as the fluorescence intensity ratio of the emission

wavelengths of 450–500 nm at the excitation wavelength of 370 nm (McKnight et al. 2001).

The molecular weight distribution of DOM

The molecular weight characteristics of DOM were evaluated at a wavelength of 260 nm using a high-pressure size-exclusion chromatography (HPSEC) system that consisted of a silica chromatographic column (GL-W250-X, 10.7 × 450 nm, Hitachi) and a UV detector (LC-10AV, Shimadzu). Pure water containing 0.02 M of Na$_2$HPO$_4$ and 0.02 M of KH$_2$PO$_4$ was used as the eluent and was introduced to the column at a constant flow rate of 0.5 mL min^{-1}. Calibration was made with a standard solution composed of polystyrene sulfonate (PSS) with three different molecular weights of 1,430, 4,950, and 6,530 g mol^{-1}. Weight-averaged molecular weight (M_w) and number-averaged molecular weight (M_n) were calculated according to the following equations (Karanfil et al. 1996; Li et al. 2003):

$$M_w = \sum_{i=1}^{N} MW_i(t)h_i(t)\Delta t / \sum_{i=1}^{N} h_i(t)\Delta t$$

$$M_n = \sum_{i=1}^{N} h_i(t)\Delta t / \left(\sum_{i=1}^{N} h_i(t)\Delta t / MW_i \right)$$

where $MW_i(t)$ is the molecular weight as a function of the eluent time t, $h_i(t)$ is the detector response, and Δt is the time interval. The polydispersity index, a parameter defined as the ratio of M_w/M_n, was adopted to evaluate the heterogeneity of DOM and the changes in DOM molecular weight distribution that occurred during sludge incubation (Karanfil et al. 1996; Li et al. 2003).

RESULTS AND DISCUSSION

Physical characteristics of raw DWTS and raw extracted water

Volatile solids (VS) were found in DWTS at 0.23 g L^{-1} and total solids (TS) were found at 2.28 g L^{-1}. The low ratio of VS to TS (0.1008) indicates an abundance of inorganic matter. Raw extracted water was obtained by dewatering

raw DWTS, and that extracted water had a pH of 6.57 and an EC of 21.6 mS m^{-1}. The UV$_{260}$, DOC, DON and NH$_4^+$-N were 2.51 m^{-1}, 8.26 mg L^{-1}, 0.27 mg L^{-1}, 4.81 mg L^{-1}, respectively. The concentrations of NO$_2^-$-N and NO$_3^-$-N were not detected in the initial extracted water. The SUVA of the raw extracted water was 0.31 L mg^{-1} m^{-1}, indicating that the organic matter was rich in hydrophilic and low-molecular-weight components (Edzwald & Tobiason 1999).

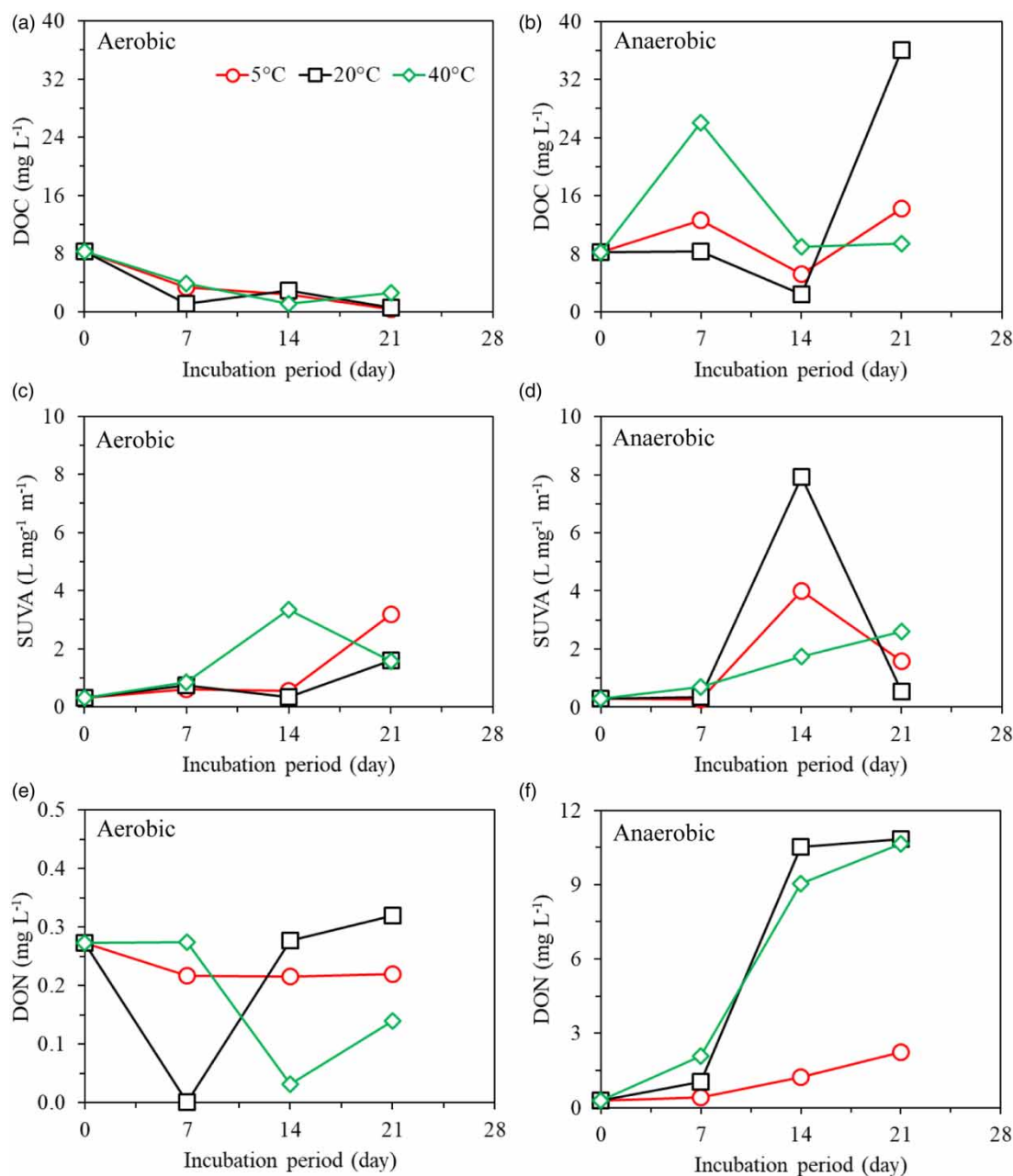## Variations in DOC, SUVA and DON in the extracted water

The trend of DOC during the 21-day incubation period varied among the three incubation temperatures under the aerobic condition and anaerobic condition. The average DOC concentrations in water extracted from DWTS during the incubation period at 21 days of incubation for aerobic and anaerobic incubation were 2.02 mg L^{-1} and 13.7 mg L^{-1}, respectively. The decrease in DOC concentration from the initial concentration (8.26 mg L^{-1}) to the range of 0.38–3.84 mg L^{-1} at the end of the incubation period for each aerobic incubation (Figure 1(a)) was probably due to the endogenous metabolism of microorganisms, and some organic compounds were oxidized to carbon dioxide (He *et al.* 2011). The higher DOC concentration under anaerobic incubation (Figure 1(b)) reflected the increase of biodegradable compounds under that condition due to DOM degradation in the sludge. The DOC concentration clearly increased under anaerobic incubation from day 7, resulting in a release of intracellular materials that exceeded the amount consumed in biodegradation by bacteria. The decline might also have been due to biodegradation by bacteria, which was greater than the rate of the release of intracellular materials (Xu *et al.* 2017).

SUVA is a good parameter for understanding aromatic content and the humic fraction of DOM (Hua *et al.* 2015). Figure 1(c) and 1(d) show the increase of SUVA until day 14 in all incubation conditions except that under aerobic 20 °C. This may be due to its association with the transformation of non-aromatic compounds, which led to an apparent enrichment in aromatics (Vinceslas-Akpa & Loquet 1997). Furthermore, the SUVA for water extracted from DWTS stored at the lower incubation temperatures was higher under anaerobic incubation than under aerobic incubation,

suggesting that the extracted water was high in aromatic compounds. SUVA values were significantly higher under anaerobic incubation at 20 °C at day 14 of incubation than under the other conditions of aerobic and anaerobic incubation, suggesting that greater amounts of aromatic substances formed and remained under anaerobic incubation than under aerobic incubation. In the latter period of incubation, the decrease of SUVA may be attributed to the enrichment production of non-aromatic compounds in the incubated DWTS, which were derived from transformation upon microbial metabolism that may be enriched with aliphatic structure (Ly *et al.* 2019). The relatively low SUVA value for water extracted from DWTS also suggests that those non-aromatic components of the sludge could be efficiently extracted – components that might be attributable to the abundance of non-aromatic carbon chains, polysaccharides, and fatty acids within the sludge organic matter (Wilson & Novak 2009).

Under anaerobic incubation, the SUVA value was higher at 20 °C than at 5 °C or 40 °C. The low incubation temperature could have caused DOM with high aromaticity to be produced during anaerobic incubation through biodegradation under prolonged storage. The high SUVA value under 20 °C incubation is consistent with the findings of fluorophore intensity assessed by fluorescence EEM. Based on the FIX results, which found that DOM mainly originated from extracellular release by microorganisms, the incubation treatment employed for anaerobic DWTS should produce higher SUVA of DOM than that of aerobic DWTS. The SUVA values in all incubated DWTS exhibited a common relationship: anaerobic SUVA > aerobic SUVA.

The changes of DON in the water extracted from DWTS after incubation treatment are shown in Figure 1(e) and 1(f). The average DON concentrations in the extracted water after aerobic incubation and anaerobic incubation were 0.19 mg L^{-1} and 5.34 mg L^{-1}, respectively. DON concentration rose continuously in all conditions after anaerobic incubation. During incubation at high temperature, abundant nitrogen was released to the supernatant due to the degradation of protein in the extracellular polymeric substances, resulting in an increase of DON in the initial 7 days. The gradual increase of DON during anaerobic incubation in all temperature treatments until the end of the incubation period may be due to the mineralization

Figure 1 | Variations in (a, b) DOC, (c, d) SUVA, and (e, f) DON in the water extracted from DWTS under aerobic incubation and anaerobic incubation at 7, 14, and 21 days of incubation.

of DON accompanied by increases in temperature and pH (Chan *et al.* 2016). The concentration of DON was decreased after 7 days of aerobic incubation at 5 °C and 20 °C. The declining trend of DON concentration at 20 °C and 40 °C was due to the depletion of DON. The decreases of DON at 20 °C and 40 °C suggest that DON was transformed to DIN by degradation (Li *et al.* 2019) and also could have

been due to the utilization and decomposition of sludge by microorganisms, as the sludge includes DON. NH_4^+-N concentration was in the range of 1.12–17.8 mg L^{-1} during aerobic and anaerobic incubation (shown in Table S1, Supplementary Material), while NO_2^--N was not detected in most of the incubation conditions. The undetected NO_2^--N may indicate complete nitrification (i.e. ammonia oxidation

or nitrite oxidation) that would reach a balance after bacteria adapted to the environment (Zou *et al.* 2016).

Incubation treatment changed the self-assembly of DOM, which can play a role in the dynamic variations of DOM, whereas microbial degradation became the main cause of DOM degradation. Some DOM fractions that formed during incubation treatment have lower bioavailability under aerobic incubation than under anaerobic incubation. This study found that aerobic treatment was able to reduce the accumulation of DOM and some refractory DOM that might have led to a high chlorine demand during chlorination. In the extracted water, DOM degraded with prolonged storage and DOM concentration increased, especially after 14 days of incubation.

Fluorescence component of DOM in the extracted water

he EEM spectra of the DOM within the entire incubation profile are shown in Figure 2. The EEM spectra of the initial extracted water (Figure 2(a)) show an apparent peak of humic-like substances (Peak 3) at Ex/Em of about 200–250/380–550 nm (Chen *et al.* 2003; He *et al.* 2013). The presence of this peak indicates that humic-like substances were the major DOM fraction found in the water extracted from the DWTS. The DOM fractions in the initial extracted water were mainly produced from extracellular substances by bacteria during the agglomeration of flocs after coagulation–flocculation. Additionally, the presence of
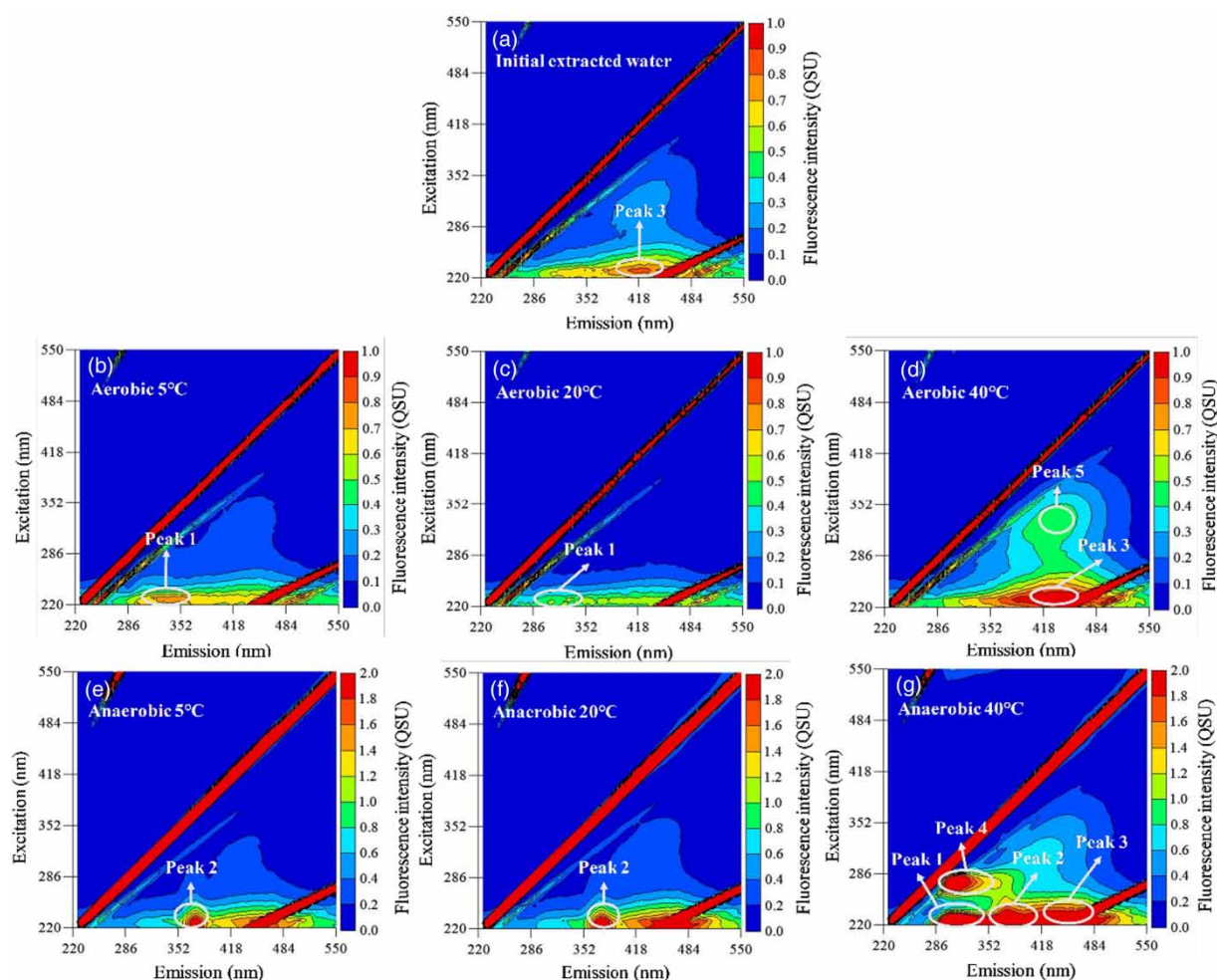
Figure 2 | EEM contour plot for water extracted from DWTS (initial value) and for water extracted from DWTS at 7 days of aerobic and anaerobic incubation. Peak 1: tyrosine-like substances; Peaks 2 and 4: tryptophan-like substances; Peaks 3 and 5: humic-like substances.

Peak 3 arose from raw water that showed humic-like substances as the major DOM fractions (data not shown).

Peaks 3 and 5 were present in all of the samples incubated aerobically at 40 °C (Figure 2(d)), at Ex/Em of 200 to 250 nm/380 to 550 nm and Ex/Em of 225 to 240/420 to 440 nm, respectively, coinciding with peaks typical of humic-like substances (Chen *et al.* 2003; He *et al.* 2013). The presence of these peaks indicates that humic-like substances that are non-biodegradable or that have low biodegradability primarily formed from the hydrolysis of the sludge (Pang *et al.* 2014) and remained in the liquids after incubation probably because of the slower degradation. Peaks in this region were previously cited by Pang *et al.* (2014), Du & Li (2017) and Xu *et al.* (2017), who reported peaks characterized by an Ex/Em wavelength pair centred around Ex/Em of 340/428 and Ex/Em of 240/436 related to humic acid during the incubation and composting of drinking water sludge and wastewater activated sludge. Peaks 1, 2, and 4 were observed in the extracted water after anaerobic incubation at 40 °C (Figure 2(g)). These three peaks can be attributed to aromatic protein-like substances in the DOM fraction, such as tryptophan-like substances and tyrosine-like substances. Peak 4, at Ex/Em of 250 to 450 nm/250 to 380 nm (Chen *et al.* 2003; He *et al.* 2013) may have arisen from the presence of phenol-like materials that originated from lignin degradation. The presence of Peaks 1, 2 and 4 suggested that the sludge incubated anaerobically might consist of large amounts of aromatic proteinaceous material and small amounts in the aerobic condition that could be bonding with, or physically encapsulated within, the humified centres of DOM (Hassouna *et al.* 2010).

Based on the above analysis, the majority of DOM is thought to consist of humic-like materials and protein-like substances presented in the samples. The relatively high fluorophore intensity of Peak 2 under anaerobic incubation at all temperature ranges was mainly attributed to the abundance of aromatic substances produced during the incubation of DWTS (Chen *et al.* 2003). The high fluorophore intensity of Peak 2 is consistent with the high SUVA value under anaerobic incubation.

The variations in the levels of all components in the water extracted from DWTS during the 21-day incubation period were characterized by fluorescence intensity values (Figure 3). Peak 1, described as tyrosine-like substance DOM, might be an indication of some activity of microbial DOM during DWTS incubation and remained in the extracted water (Baghoth *et al.* 2011). The lower intensity of Peak 1 under 5 °C and 20 °C indicated that the DOM was easily degraded by bacteria under the low-temperature conditions compared with that under 40 °C. Meanwhile, Peak 2 and Peak 3 have higher fluorescence intensity than the other three components in the extracted water, indicating that tryptophan-like substances and humic-like substances were completely non-biodegradable and the major contributors to the fluorescent DOM during sludge incubation and were more likely to be accumulated and remain in the extracted water (Jia *et al.* 2013). The variations in Peak 2 averaged 0.67 QSU and 1.82 QSU in aerobic incubation and anaerobic incubation, respectively, and in Peak 3 averaged 0.74 QSU and 1.69 QSU in aerobic incubation and anaerobic incubation, respectively (Figure 3).

With increases in the incubation period, Peak 2 and Peak 3 in the incubation system increased from day 0 to day 7 of incubation, and thereafter slightly declined for 14 days of incubation. The declining trend of DOM fractions is consistent with DOC concentrations under anaerobic incubation. The trend of variation in Peak 2 tended to track the variations in DOC under anaerobic incubation. The highest fluorescence intensity was observed under anaerobic incubation at 40 °C, but Peak 1 at 5 °C and 20 °C showed insignificant changes during the incubation periods under both aerobic and anaerobic incubation.

Variations in HIX and FIX fluorescence indices in the extracted water

HIX and FIX are widely applied to describe the origin and transformation of DOM from various sources (Huguet *et al.* 2009; Hansen *et al.* 2016). The changes in HIX and FIX for the six systems during the experiment are shown in Figure 4. The HIX values ranged from 0.51 to 0.73 in the incubation system. HIX values typically indicate the extent of humification, with higher values corresponding to greater humification (Hansen *et al.* 2016). All HIX values in the six systems were <0.8, indicative of low humic levels. The average HIX at 21 days of incubation was higher for anaerobic incubation than for aerobic incubation, which indicates greater humification under anaerobic incubation than under aerobic incubation.
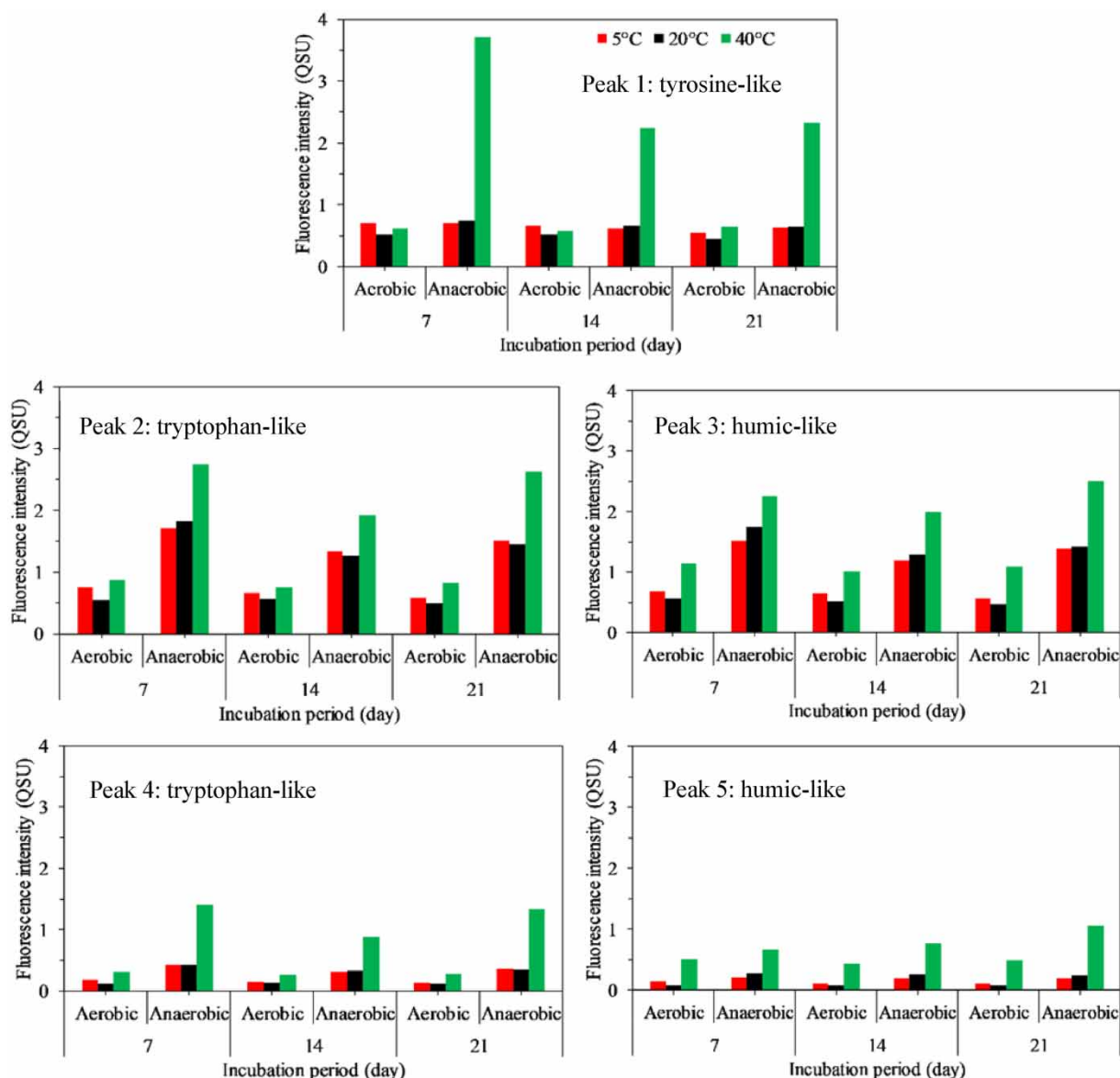
Figure 3 | Variations of DOM fractions in the water extracted from DWTS under aerobic and anaerobic incubation at 7, 14, and 21 days of incubation.

During the 7-day incubation, aromatic protein-like substances were present in all samples under anaerobic incubation. Anaerobic incubation may promote and accelerate the production of protein materials derived from extracellular lysis and lignin degradation. Greater humification is associated with more complex, condensed aromatic structures and/or more conjugation in aliphatic chains, which are resistant to degradation (He et al. 2011). The increase of HIX during aerobic incubation at 40 °C may have been caused by the degradation and accumulation of recalcitrant DOM. On the other hand, anaerobic incubation led more readily to the oxidation process than under aerobic incubation at temperatures of 5 °C and 20 °C. The presence of humic substances in anaerobic incubation can be utilized for microbial respiration and oxidizing DOM in the systems (Shao et al. 2013).

FIX is also an indicator of DOM source, with higher values (~1.8) indicating DOM from extracellular release and leachate from bacteria and algae, and lower values (~1.2) indicating terrestrial DOM sources (McKnight et al.
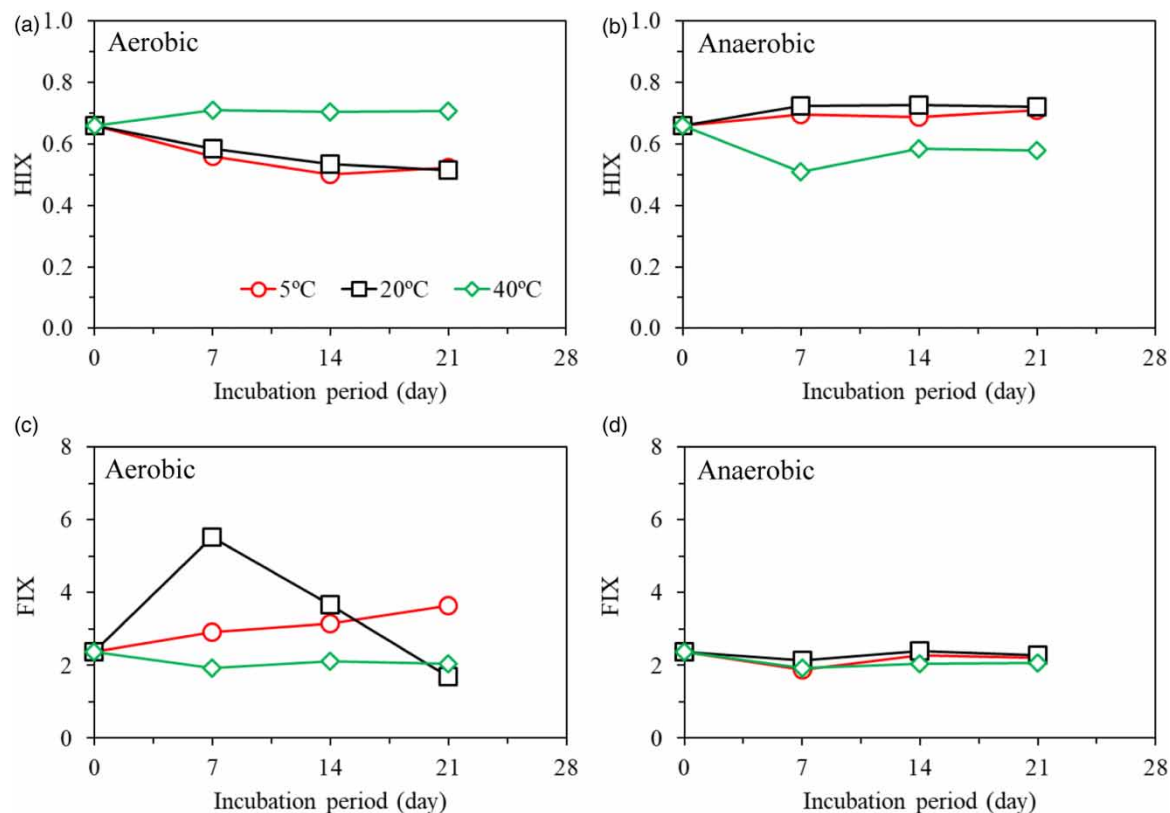
Figure 4 │ (a), (b) HIX and (c), (d) FIX for water extracted from DWTS at 7, 14, and 21 days of aerobic and anaerobic incubation.

2001). The high FIX values in all six incubation systems suggested that DOM was mainly produced from the extra-cellular release of microorganisms as shown in Figure 4(c) and 4(d). FIX values that are greater than the microbial end-member range of 1.7–2.0 (McKnight *et al.* 2001) can be employed for characterizing humic and fulvic acids.

The molecular weight distribution of DOM in the extracted water

The molecular weight distribution of DOM in the water extracted from DWTS was measured by HPSEC and is given in terms of UV absorbance at 260 nm (Figure 5).
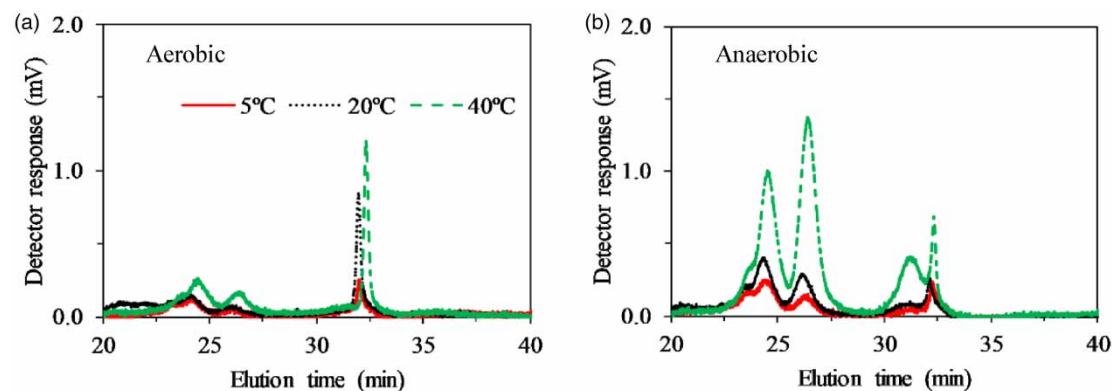
Figure 5 │ The molecular weight distributions for water extracted from DWTS at 7 days of aerobic and anaerobic incubation.

The DOM for anaerobic incubation shows a high molecular weight shown in a narrower distribution. Aerobic incubation resulted in a broader distribution which is positioned on the left side of the molecular weight distribution chromatogram, reflecting low molecular weight (Figure 5(a)). The molecular weights are lower for water extracted from DWTS under aerobic incubation than under anaerobic incubation, indicating the presence of humic-like substances. The distribution of molecular weight in extracted water under anaerobic incubation at a temperature of 40 °C is distinct from that for the same incubation under the two other temperature conditions (5 °C and 20 °C). Under those two conditions, protein-like substances with high molecular weights are associated with high SUVA values.

Table 1 shows that M_w is greater for the DOM that formed and remained after anaerobic incubation. The large weight-averaged molecular weight seen under anaerobic incubation at 20 °C indicates that a large protein-like substance was produced during incubation. This result is consistent with the fluorescence EEM under those conditions at 7 days of incubation, where the protein-like fraction is the main substance in the water extracted from DWTS under anaerobic conditions. The polydispersity index also shows that the DOM which formed and remained after aerobic incubation is more heterogeneous than that which formed after anaerobic incubation. The large fraction of DOM assessed by molecular weight distribution observed in the anaerobic incubation of DWTS is consistent with the results assessed by fluorescence EEM, suggesting that more protein-like substances with relatively high fluorophore intensities formed under anaerobic incubation than under aerobic incubation.

The M_w of the DOM in the water extracted from DWTS was found to be high under anaerobic incubation at 20 °C and mainly consisted of protein-like substances with a high degree of aromaticity. In this study, anaerobic incubation at a moderate temperature (20 °C) could release DOM with high molecular weights. However, prolonged incubation until day 21 did not result in any significant changes in M_w (data not shown). DOM formed under anaerobic incubation at a temperature of 5 °C had a higher polydispersity than those at 20 °C and 40 °C. The high temperature of incubation could present less dispersed DOM under anaerobic incubation than that under aerobic incubation.

CONCLUSIONS

Prolonged aerobic and anaerobic incubation of DWTS (7, 14, and 21 days) at different temperatures (5 °C, 20 °C, 40 °C) and DOM transformation in sludge were investigated. Anaerobic incubation at high temperatures was found to promote high DOC concentrations with low SUVA values, which indicates that non-aromatic DOM formed during incubation. Compositional analysis by fluorescence EEM indicated that more humic-like substances formed during aerobic incubation than during anaerobic incubation and that more protein-like substances formed during anaerobic incubation than during aerobic incubation. With increases in DOM, high-temperature incubation was able to accelerate the humification process for aerobic incubation, suggesting that the bulk DOM was more humified under those conditions. Moreover, compositional analysis based on the molecular weight distribution found that the UV-absorbing DOM constituents that formed in anaerobic incubation had higher molecular weights at high temperatures than the molecular weights seen with aerobic incubation.

This study indicated that incubation treatment under aerobic conditions may decrease the DOM produced from DWTS. The operation of aerobic incubation under alternate aeration also may maintain lower production of refractory organics during the incubation and achieve nitrogen

Table 1 │ Molecular weight and polydispersity of initial extracted water of DWTS and after 7 days incubation period

Sample name	M_w (g/mol as PSS)	M_n (g/mol as PSS)	Polydispersity (–)
Initial extracted water	5,079	4,767	1.06
Aerobic 5 °C	2,349	4,751	0.49
Aerobic 20 °C	6,073	4,279	1.42
Aerobic 40 °C	5,461	4,892	1.12
Anaerobic 5 °C	6,232	4,534	1.37
Anaerobic 20 °C	6,915	5,831	1.18
Anaerobic 40 °C	5,785	5,674	1.02

removal through the nitrification–denitrification process, and at the same time may possibly improve dewaterability properties (Tomei *et al.* 2016). Further work is recommended to provide detailed evidence on the effects of water extracted from DWTS added in the drinking water treatment plant process with respect to rapid chlorine consumption during drinking water distribution.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/ws.2020.120.

REFERENCES

Ahmad, T., Ahmad, K. & Alam, M. 2016 Sustainable management of water treatment sludge through 3'R' concept. *Journal of Cleaner Production* **124**, 1–13.

Baghoth, S. A., Sharma, S. K. & Amy, G. L. 2011 Tracking natural organic matter (NOM) in a drinking water treatment plant using fluorescence excitation–emission matrices and PARAFAC. *Water Research* **45** (2), 797–809.

Chan, M. T., Selvam, A. & Wong, J. W. C. 2016 Reducing nitrogen loss and salinity during 'struvite' food waste composting by zeolite amendment. *Bioresource Technology* **200**, 838–844.

Chen, W., Westerhoff, P., Leenheer, J. A. & Booksh, K. 2003 Fluorescence excitation–emission matrix regional integration to quantify spectra for dissolved organic matter. *Environmental Science and Technology* **37**, 5701–5710.

Du, H. & Li, F. 2017 Characteristics of dissolved organic matter formed in aerobic and anaerobic digestion of excess activated sludge. *Chemosphere* **168**, 1022–1031.

Edzwald, J. K. & Tobiason, J. E. 1999 Enhanced coagulation: US requirements and a broader view. *Water Science and Technology* **40** (9), 63–70.

Hansen, A. M., Kraus, T. E. C., Pellerin, B. A., Fleck, J. A., Downing, B. D. & Bergamaschi, B. A. 2016 Optical properties of dissolved organic matter (DOM): effects of biological and photolytic degradation. *Limnology and Oceanography* **61**, 1015–1032.

Hassouna, M., Massiani, C., Dudal, Y., Pech, N. & Theraulaz, F. 2010 Changes in water extractable organic matter (WEOM) in a calcareous soil under field conditions with time and soil depth. *Geoderma* **155**, 75–85.

He, X., Xi, B., Wei, Z., Jiang, Y., Geng, C., Yang, Y., Yuan, Y. & Liu, H. 2011 Physicochemical and spectroscopic characteristics of dissolved organic matter extracted from municipal solid waste (MSW) and their influence on the landfill biological stability. *Bioresource Technology* **102**, 2322–2327.

He, X., Xi, B., Li, X., Pan, H., An, D., Bai, S., Li, D. & Cui, D. 2013 Fluorescence excitation–emission matrix spectra coupled with parallel factor and regional integration analysis to characterize organic matter humification. *Chemosphere* **93**, 2208–2215.

Hua, G., Reckhow, D. A. & Abusallout, I. 2015 Correlation between SUVA and DBP formation during chlorination and chloramination of NOM fractions from different sources. *Chemosphere* **130**, 82–89.

Huguet, A., Vacher, L., Relexans, S., Saubusse, S., Froidefond, J. M. & Parlanti, E. 2009 Properties of fluorescent dissolved organic matter in the Gironde estuary. *Organic Geochemistry* **40**, 706–719.

Jia, X., Zhu, C. W., Li, M. X., Xi, B. D., Wang, L. J., Yang, X., Xia, X. F. & Su, J. 2013 A comparison of treatment techniques to enhance fermentative hydrogen production from piggery anaerobic digested residues. *International Journal of Hydrogen Energy* **38**, 8691–8698.

Jin, B., Wang, S., Xing, L., Li, B. & Peng, Y. 2016 Long term effect of alkali types on waste activated sludge hydrolytic acidification and microbial community at low temperature. *Bioresource Technology* **200**, 587–597.

Karanfil, T., Kilduff, J. E., Schlautman, M. A. & Weber Jr., W. J. 1996 Adsorption of organic macromolecules by granular activated carbon: 1. Influence of molecular properties under anoxic solution conditions. *Environmental Science and Technology* **30**, 2187–2194.

Komatsu, K., Onodera, T., Kohzu, A., Syutsubo, K. & Imai, A. 2020 Characterization of dissolved organic matter in wastewater during aerobic, anaerobic, and anoxic treatment processes by molecular size and fluorescence analyses. *Water Research* **171**, 115459.

Li, F., Yuasa, A., Chiharada, H. & Matsui, Y. 2003 Polydisperse adsorbability composition of several natural and synthetic organic matrices. *Journal of Colloid and Interface Science* **265**, 265–275.

Li, K., Liu, C., Ma, Y. & Wang, X. 2019 Land-based dissolved organic nitrogen dynamics and bioavailability in Jiaozhou Bay, China. *Estuarine, Coastal and Shelf Science* **220**, 13–24.

Ly, Q. V., Nghiem, L. D., Cho, J., Maqbool, T. & Hur, J. 2019 Organic carbon source-dependent properties of soluble microbial products in sequencing batch reactors and its effects on membrane fouling. *Journal of Environmental Management* **244**, 40–47.

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T. & Andersen, D. T. 2001 Spectrofluorometric characterization of dissolved organic matter for indication of

precursor organic material and aromaticity. *Limnology and Oceanography* **46**, 38–48.

Ohno, T. 2002 Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science and Technology* **36**, 742–746.

Pang, L., Ni, J. & Tang, X. 2014 Fast characterization of soluble organic intermediates and integrity of microbial cells in the process of alkaline anaerobic fermentation of waste activated sludge. *Biochemical Engineering Journal* **86**, 49–56.

Shao, L., Wang, T., Li, T., Lü, F. & He, P. 2013 Comparison of sludge digestion under aerobic and anaerobic conditions with a focus on the degradation of proteins at mesophilic temperature. *Bioresource Technology* **140**, 131–137.

Sun, F., Hu, W., Pei, H., Li, X., Xu, X. & Ma, C. 2015 Evaluation on the dewatering process of cyanobacteria-containing $AlCl_3$ and PACl drinking water sludge. *Separation and Purification Technology* **150**, 52–62.

Suzuki, Y. 2017 *Characteristics of Dissolved Organic Matters (DOMs) in Water Supply System and the Relationships between the DOMs and Chlorine Decay in Water of Sending Pipes*. Master thesis, Graduate School of Engineering, Gifu University, Gifu, Japan (in Japanese).

Tomei, M. C., Angelucci, D. M. & Levantesi, C. 2016 Two-stage anaerobic and post-aerobic mesophilic digestion of sewage sludge: analysis of process performance and hygienization potential. *Science of the Total Environment* **545–546**, 453–464.

Vinceslas-Akpa, M. & Loquet, M. 1997 Organic matter transformation in lignocellulosic waste products composted or vermicomposted (*Eisenia fetida andrei*): chemical analysis and ^{13}C CPMAS NMR spectroscopy. *Soil Biology and Biochemistry* **29** (3–4), 751–758.

Wilson, C. A. & Novak, J. T. 2009 Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pre-treatment. *Water Research* **43** (18), 4489–4498.

Xu, H., Pei, H., Jin, Y., Xiao, H., Ma, C., Sun, J. & Li, H. 2017 Characteristics of water obtained by dewatering cyanobacteria-containing sludge formed during drinking water treatment, including C-, N-disinfection byproduct formation. *Water Research* **111**, 382–392.

Zou, Y., Hu, Z., Zhang, J., Guimbaud, C., Wang, Q. & Yang, F. 2016 Effect of seasonal variation on nitrogen transformations in aquaponics of northern China. *Ecological Engineering* **94**, 30–36.

Zhou, S., Zhu, S., Shao, Y. & Gao, N. 2015 Characteristics of C-, N-DBPs formation from algal organic matter: role of molecular weight fractions and impacts of pre-ozonation. *Water Research* **72**, 381–390.

Zhou, Z., Yang, Y., Li, X., Ji, S., Zhang, H., Wang, S., Zeng, Q. & Han, X. 2016 The removal characteristics of natural organic matter in the recycling of drinking water treatment sludge: role of solubilized organics. *Ultrasonics Sonochemistry* **28**, 259–268.

Zhou, Z., Yang, Y., Li, X., Li, P., Zhang, T., Lv, X., Liu, L., Dong, J. & Zheng, D. 2018 Optimized removal of natural organic matter by ultrasound-assisted coagulation of recycling drinking water treatment sludge. *Ultrasonics Sonochemistry* **48**, 171–180.