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Graphical Abstract

Enhanced sludge solubilization and dewaterability by synergistic effects of nitrite and freezing

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Abstract

In this study, nitrite was added into sludge during freezing process to evaluate its role in waste activated sludge (WAS) solubilization and effects on sludge dewatering characteristics. The results showed that the introduction of 100 mg L⁻¹ of nitrite could increase dissolved organic carbon (DOC) concentration from 29.5 to 48.8 mg DOC g⁻¹ VSS under freezing conditions. More DOC was released with the increase of nitrite concentration. Freezing temperature, or freezing speed, also played a role in sludge solubilization. It was found that some readily-biodegradable low molecular weight (LMW) compounds, e.g. LMW protein, LMW polysaccharide, LMW neutrals, building blocks and LMW acids, were mainly released during the freezing process with the presence of nitrite. Interestingly, nitrite could also improve the sludge filterability at the lower nitrite concentration as a result of the increased sludge particle size. However, electrolytes (sodium nitrite) addition effects may mask such enhancement when nitrite concentration was high (800 mg L⁻¹). The rheological characteristics of sludge could be well modeled by Herchel-Bulkley model and the introduction of nitrite into freezing process further increased sludge flowability and decreased sludge viscosity. These results indicated that freezing with the presence of suitable concentration of nitrite could promote sludge solubilization and dewaterability. As such, good liquid and solid separation can be achieved with the recovery of liquid stream as carbon source.

Keywords: Waste activated sludge; Freezing; Dewaterability; Carbon sources; Nitrite

1. Introduction

Waste activated sludge (WAS) dewatering is of major interest to researchers and operators, and it still remains as a bottleneck for the sludge treatment in the wastewater treatment process. Many methods and strategies have been developed to enhance the sludge dewaterability, such as thermal treatment (Guan et al. 2012), freezing (Chu et al. 1997), ultrasonic pretreatment (Murugesan et al. 2014) and chemical oxidation (Xiao et al. 2016b). Among them, freezing has been proven to be an effective physical conditioning method to improve sludge dewaterability that is economically feasible in regions where natural freezing environment is available. It can significantly enhance sludge dewatering characteristics (Chu et al. 1997) by transforming the floc structure into a more compact form (Hu et al. 2011) and inactivating bacteria in the sludge (Gao et al. 2006). Freezing temperature, or freezing speed, was found to be one of the most important parameters affecting sludge dewaterability and settleability (Hung et al. 1996).

Many studies aimed to improve sludge dewaterability using freezing treatment (Chu et al. 1997, Gao 2011, Hu et al. 2011), however, the chemicals released during the freezing process were rarely studied. WAS is rich in organic matter, nitrogen (N) and phosphorous (P). Some of the compounds, e.g. soluble chemical oxygen demand (sCOD), $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$, etc. are often found in sludge dewatering liquor (Peng et al. 2012). Nutrients in the dewatering liquor have to be treated in the mainstream treatment facilities, while sCOD can actually be used as carbon source for nutrient removal processes. In many wastewater treatment plants (WWTPs), the availability of readily degradable organic matter is a limiting factor for a successful biological nutrient removal. Although commercial organic compounds can serve effectively as carbon

sources, use of such external carbon sources results in an increase of the operational costs. Hence, there is a great interest to extract carbon sources through sludge solubilization.

Various methods can be applied to improve the sludge solubilization efficiency, including thermal hydrolysis (Neyens and Baeyens 2003), and chemical treatment using ozone, acids or alkali (Xiao et al. 2017). These methods are very efficient to release sCOD from sludge. However, the higher sludge solubilization efficiency is, the poorer dewaterability of the sludge is due to the increased amount of small size particle following sludge disintegration (Dogan and Sanin 2009). The small particles will bind the porous structure formed by the larger particles during filtration, thus deteriorating the sludge dewaterability (Dogan and Sanin 2009). This can be a major challenge for some WWTPs where an anaerobic digester is not available in the downstream to receive the solids. Therefore, it is essential to develop a method to efficiently extract carbon source from sludge and meantime keep sludge dewaterability reasonably good. As such, this study proposed to combine freezing and chemical treatment methods to achieve the purpose.

Recently, nitrite, or its protonated form free nitrous acid (FNA i.e. HNO_2), has been demonstrated to be a strong biocidal agent, which is effective in enhancing WAS solubilization. Wang et al. (2014) reported that FNA pre-treatment on WAS resulted in increased release of sCOD by 130 mg SCOD g^{-1} VSS at FNA concentrations of 1.43 mg N L^{-1} . Ma et al. (2015) showed that WAS treated with FNA concentration of 2.04 mg N L^{-1} released 66 mg g^{-1} VSS more sCOD after 24 h exposure. Clearly, nitrite-based technique would be beneficial to sludge solubilization. FNA can also be produced in situ in WWTPs by partial nitrification process (Wang et al. 2014). Hence, this study incorporated nitrite into freezing-based sludge treatment method to improve sludge

solubilization.

The objective of this study was to determine the feasibility and potential of using nitrite to improve sludge solubilization and enhance the carbon sources production from WAS with a good separation of liquid and solids via freezing process. It was expected that during freezing process, the added nitrite would be excluded from ice crystals and concentrated into liquid-like ice grain boundaries and form FNA, which may be capable of disrupting extracellular polymeric substances (EPS) and microbial cells further. In this work, the WAS solubilization and sludge filterability with and without nitrite addition during freezing at different temperatures were compared. To elucidate the impact of nitrite addition on the WAS solubilization and filterability, the fraction and characterization of organic compounds in EPS under different conditions were analyzed. Additionally, the sludge physical characteristics, e.g. particle size distribution, sludge settleability and filterability, rheological behavior, and morphology before and after treatment, were revealed.

2. Materials and methods

2.1 Sludge source

WAS was collected from a local municipal wastewater treatment plant in Singapore. Collected sludge was subsequently screened through a 1 mm mesh sieve to remove grit. The characteristics of WAS were as follows: total suspended solids (TSS) $13.3 \pm 0.1 \text{ g L}^{-1}$, volatile suspended solids (VSS) $10.9 \pm 0.1 \text{ g L}^{-1}$, dissolved organic carbon (DOC) $33 \pm 1 \text{ mg L}^{-1}$, pH = 6.5.

2.2 Sludge treatment

Batch experiments were performed to assess and compare the effects of freezing alone and nitrite + freezing treatment on the characteristics of WAS. Experimental conditions are summarized in Table 1. Each serum bottle was fed with 120 mL WAS, and then different volumes of 2.0 M NaNO₂ stock solution were added into these bottles to achieve 0, 100, 200, 400, 800 mg NO₂⁻-N L⁻¹, respectively. Then, the sludge was frozen at -20 °C, -14°C and -5 °C for 24 h in a laboratory freezer and thawed for about 5 h at 20 °C ambient conditions.

2.3 EPS extraction

EPS fractions from WAS before and after treatment were extracted using a modified heat extraction method. Briefly, the sludge samples were centrifuged at 4000 gravitational acceleration (g) for 15 min, and the supernatant was collected as soluble (SB) EPS. The sludge pellet in the tube was then re-suspended to their original volume using 0.05% NaCl solution. The liquor was centrifuged at 4000 g for 10 min and the supernatant was collected as loose bound EPS (LB-EPS). The collected sludge pellet was re-suspended to the original volume with 0.05% NaCl solution, then heated at 60 °C for 30 min, and finally centrifuged at 4000 g for 15 min and the supernatant was collected as tight bound EPS (TB-EPS).

2.4 Analytical methods

Collected samples were filtered through a 0.45 µm membrane filter prior to the analysis of soluble fraction of sludge samples. TSS, VSS, NH₄⁺-N, NO₂⁻-N and pH were determined based on the standard methods (APHA 1998). DOC of organic composition was determined with a total organic carbon (TOC) analyser (Multi N/C® 2100 Analytik Jena, Jena, Germany). Total polysaccharide concentrations and total protein

concentrations were determined as previously described (Xiao et al. 2016a).

The composition of DOC was quantified using a size-exclusion chromatography-organic carbon detection-organic nitrogen detection (SEC-OCD-OND) system (DOC-LABOR, Karlsruhe, Germany). The identified organic fractions can cover biopolymers (high molecular weight (HMW) protein and HMW polysaccharide), building blocks, humic substances, low molecular weight neutrals (LWW neutrals), and low molecular weight acids (LMW acids). The concentrations of these fractions were obtained by a software program (Chrom CALC, DOC-LABOR, Karlsruhe, Germany). Based on the proposed chemical formulae of protein $C_{52.5}H_{6.65}N_{16}O_{21.5}S_2$ and polysaccharide $(C_6H_{10}O_5)_n$, 1 g of protein and polysaccharide equal to 0.497 and 0.444 g of equivalent carbon, respectively. The concentration of LMW protein and LMW polysaccharide were calculated by subtracting HMW protein and HMW polysaccharide from total protein and polysaccharide, respectively.

Sludge settleability was evaluated by measuring sludge volume index (SVI). Sludge filterability was measured in terms of capillary suction time (CST). CST was measured using an apparatus (OFITE Capillary Suction Timer, OFI Testing Equipment, Houston, USA), equipped with an 18-mm diameter funnel and Whatman No. 17 chromatography-grade paper (United Scientific Equipment Pte Ltd, Singapore). The CST values were normalized against VSS concentration, and expressed in unit of seconds liter per gram VSS ($s L g^{-1} VSS$).

The particle size distribution was determined with a laser diffraction particle size analyzer (model SALD-MS30, Shimadzu, Tokyo, Japan). Particle size distributions of sludge samples were characterized in terms of D50 and D90 which are defined as the equivalent diameters in volume of 50% and 90% of the particles, respectively.

Rheological behavior of sludge sample was measured by a rheometer (Physica

MCR101 Modular Compact Rheometer, Anton Paar, Australia), with a bob geometry and a cup (radius: 28.92 ± 0.05 mm, height: 73.10 mm), which was described in previous study (Xiao et al. 2016b). Briefly, 18 mL sludge sample was filled into the cup. A continuous ramp of shear rate increasing from 9.5 to 1000 s^{-1} was applied, and 92 sets of shear rates and shear stress were recorded for the typical rheograms. A rheological model Herschel-Bulkley (Eq. (1)) was used to describe the rheological behavior of a non-Newtonian fluid.

$$\tau = k \times \gamma^n + \mathcal{T}_y \quad (1)$$

Where,

τ = shear stress (Pa)

γ = shear rate (s^{-1})

k = consistency index ($\text{Pa} \cdot \text{s}^n$)

n = flow behavior index (dimensionless)

$\mathcal{T}_y$ = yield stress (Pa)

The morphologies and structures of the sludge samples were observed by a field-emission scanning electron microscope (FESEM, JEOL 6340) equipped with an energy dispersive X-ray spectrometer (EDS) of JOEL 4340 operated at 5 kv. The sludge samples were diluted for 1000 times before observation. The cell viability tests methods and results can be found in supporting document.

2.5. Statistical analyses

Pearson's correlation between two parameters under different conditions was performed using the software SPSS 18.0. The correlations were statistically significant when the probability (p value) was less than 0.05. An analysis of variance (ANOVA) was used to test the significance of results and $p < 0.05$ was considered to be

statistically significant.

3. Results and discussion

3.1 Effects of treatment on sludge solubilization

3.1.1 Sludge solubilization

Fig. 1 shows the changes of soluble DOC, protein and polysaccharide in WAS after 24 h treatment using freezing alone and combined freezing + nitrite under different temperatures (-20 °C, -14 °C and -5 °C). All the treatments resulted in release of DOC. In the control test without freezing or nitrite addition, DOC in SB-EPS only increased by 1.7 mg g⁻¹ VSS from 3.1 mg g⁻¹ VSS after 24 h incubation. In contrast, after freezing treatment, DOC in SB-EPS increased to 27.3, 28.2 and 29.5 mg g⁻¹ VSS at -20 °C, -14 °C and -5 °C, respectively. This was attributed to the freeze-concentration effect that cells might be dehydrated and killed because of the built-up high solute concentration inside the cells, or intracellular and extracellular ice crystals formed (Chu et al. 1997, Gao et al. 2006).

Combined freezing + nitrite seemed to be more effective in WAS solubilization. For example, at -5 °C, the released DOC in SB-EPS rose to 48.8 mg g⁻¹ VSS (equivalent to 130 mg sCOD g⁻¹ VSS) when nitrite concentration was 100 mg L⁻¹. This indicates combined freezing + nitrite was 28.7 times more effective (48.8 versus 1.7 mg DOC g⁻¹ VSS) than that in control, and 1.6 times than (48.8 versus 29.5 mg DOC g⁻¹ VSS) that with freezing treatment alone. Clearly, nitrite led to higher solubilization of particulate organics in WAS. With the increased DOC solubilization, 117 mg sCOD g⁻¹ VSS still remained and could be transferred to downstream as carbon source even after part of DOC was used to reduce the nitrite added. When nitrite concentration increased to 800

mg L⁻¹, DOC concentration further increased to 66.6 mg DOC g⁻¹ VSS (equivalent to 178 mg sCOD g⁻¹ VSS). Pearson's correlation between released DOC in SB-EPS and nitrite concentration indicated that increased released DOC was strongly and positively correlated with the nitrite concentration ($p < 0.05$).

Previous study indicated that FNA could substantially enhance WAS solubilization and 130 - 140 mg sCOD g⁻¹ VSS release was observed at the FNA concentration of 1.4 mg L⁻¹. Further increase of sCOD to 160 mg sCOD g⁻¹ VSS could be obtained at FNA concentration about 2.1 mg L⁻¹ (Wang et al. 2014, Wang et al. 2013b). In this study, FNA concentrations for the nitrite concentration of 100, 200, 400 and 800 mg L⁻¹ at pH 6.5 was 0.17, 0.34, 0.67 and 1.35 mg L⁻¹, respectively. Obviously, nitrite concentration of 100 mg L⁻¹ (FNA of 0.17 mg L⁻¹) under freezing conditions could achieve similar solubilization performance as that obtained with 1.4 mg L⁻¹ FNA at ambient temperature. The results indicate that nitrite was likely concentrated during the freezing process which may create similar or higher FNA concentration in certain part of frigorific mixture.

A similar trend was also observed in the case of -14 °C and -20 °C treatment. However, it should be noted that treatment at -5 °C was most effective for the WAS solubilization in SB-EPS, followed by -14 °C and -20 °C treatment (Fig. 1a) ($p < 0.05$). This result is in agreement with other research on *E. Coli* freezing experiment that higher inactivation was achieved at warmer freezing temperatures (Gao et al. 2006). It implies slower freezing rates (higher freezing temperature) may lead to more solubilization of bacteria and/or EPS during freezing and nitrite enhanced such effects. Increase of DOC concentration was also observed in LB-EPS in both freezing alone and combined freezing + nitrite treatments, which confirmed the floc structure of WAS was effectively destroyed. TB-EPS content, on the other hand, did not change significantly.

It is well-known that protein and polysaccharide are the main constituents of WAS (Yu et al. 2008). In this study, the solubilization of protein and polysaccharide before and after treatment is shown in Fig. 1b and 1c. It is noted that total polysaccharide and protein concentration in SB-EPS increased from 1.5 and 3.9 mg g⁻¹ VSS to 10.1 and 40 mg g⁻¹ VSS, respectively, in freezing alone treatment, and the two compounds peaked at 37.7 and 73.0 mg g⁻¹ VSS, respectively, when WAS was treated with freezing + nitrite (800 mg L⁻¹) at -5 °C. Potassium is a major element of bacterial cells and it is accumulated preferentially to a much higher concentration intracellularly as compared with Na⁺ and Ca²⁺ (Prindle et al. 2015). The integrity of microbial cell wall was indirectly investigated by released potassium concentration in the bulk liquid (Fig. 1d). It was observed that the trend of potassium concentration was similar to those of soluble DOC, protein and polysaccharide, which indicates cell lysis may be partially responsible for the enhanced solubilization.

In summary, the best performance of sludge solubilization was obtained at -5 °C. Nitrite concentrations of 400 – 800 mg L⁻¹ did not significantly increase the DOC concentration as compared to 100 - 200 mg L⁻¹, therefore, from economic point of view, the conditions selected for combined treatment in this study was nitrite concentration of 100 – 200 mg L⁻¹ at -5 °C.

3.1.2 EPS fractionation

Since DOC from WAS solubilization can be potentially utilized as carbon source for biological nutrient removal, it is necessary to fractionize the organic compounds in EPS in order to evaluate their biodegradability. The concentrations of related compounds in SB-, LB- and TB-EPS are described in Fig. 2. Clearly, the hydrophilic DOC concentration (sum of building blocks, LMW neutrals, LMW acids, HMW protein

and HMW polysaccharides) increased significantly after freezing alone and combined freezing + nitrite treatment. With freezing treatment alone, only two types of organic compounds in SB-EPS, that is LMW protein and LMW polysaccharide, increased greatly (160 and 50 mg C L⁻¹, respectively), while in the combined freezing + nitrite treatment (e.g. LFN3), more compounds, such as LMW protein, LMW polysaccharide, LMW neutrals, building blocks and LMW acids, increased 1.9, 2.4, 13.4, 6.7 and 47.8 folds, respectively. Obviously, nitrite could greatly enhance the release of some specific low molecular weight compounds. For LMW neutrals, which mainly compose of LMW alcohols, aldehydes, ketones, sugars and amino acids, were also observed after WAS Fe(II)-oxone treatment (Xiao et al. 2016b). Building blocks may origin from the breakdown products of humic-like substances of lower molecular acids (Huber et al. 2011). In addition, the increase of LMW protein and LMW polysaccharide was more than that of HMW protein and HMW polysaccharide. Previous studies suggested that HMW protein and HMW polysaccharide showed relatively low degradability (Li et al. 2016). This study demonstrated that LMW protein, LMW polysaccharide and LMW acids, which are the easily-biodegradable substrates, were the major compounds released after treatment. Meanwhile, as the release of ammonia after treatment was low (Fig. S1), liquid stream with high carbon to nitrogen ratio could be recovered as carbon source.

A similar trend was also observed in LB-EPS. Combined freezing + nitrite led to higher release of LMW protein, LMW polysaccharide, LMW neutrals, building blocks and LMW acids. However, no obvious changes of the above compounds were observed in the TB-EPS. The unbalanced EPS compounds among the three fractions confirmed that intracellular constituents were released from the cells.

3.2 Effects of treatment on physical characteristics of sludge

Table 2 shows the sludge volume index versus settling time data for the raw and the treated sludge. The freezing treatment enhanced the settleability of sludge as noted by much higher settling speed and the less sediment volume.

The CST values of sludge before and after treatment are shown in Fig. 3. The normalized CST for the control sludge was $3.3 \pm 0.3 \text{ s L g}^{-1} \text{ VSS}$. After freezing treatment, the normalized CST was improved by 2.7 times reaching a CST of $1.2 \text{ s L g}^{-1} \text{ VSS}$. The result was consistent with previous studies that freezing markedly enhanced sludge dewatering characteristics (Chu et al. 1999, Hung et al. 1996). However, it is interesting to note that nitrite could further improve the filterability, while very high nitrite concentration could deteriorate the filterability (Fig. 3).

Above phenomenon could be related to particle size changes. For raw sludge, the average D50 and D90 were 161 and 52 μm , respectively. In contrast, the average D50 and D90 increased to 374 and 161 μm , respectively, after freezing, which may contribute to the improved filterability of the frozen sludge samples. The substantial increase of particle size was attributed to the migration of sludge flocs. During freezing, the growing ice crystals would reject all impurities, and force small sludge particles to merge together and grow into larger particles (Vesilind et al. 1991). Given enough time at a slow freezing rate, sludge can be converted into a matrix of ice crystals and compacted solid particles (Tao et al. 2006). In the case of combined freezing + nitrite, particle size increased as nitrite concentration increased from 100 mg L^{-1} to 200 mg L^{-1} and decreased when nitrite concentration increased further.

It has been reported that addition of electrolytes, such as NaCl, in sludge during freezing process may decrease sludge filterability and particle size (Chu et al. 1997).

The discrepancy obtained in this study may be due to different types and concentrations of electrolytes added. In this study, an increase of DOC including protein and polysaccharide was observed once nitrite was added to WAS (Fig. S2) before freezing, which indicated that EPS were slightly disintegrated and/or the bacterial cells were disrupted. Under this condition, the integrity of cell structure could be destroyed and floc size probably decreased (Wang et al. 2013a) accordingly. Smaller size flocs may thereby easily migrate and be attracted during freezing process (Vesilind et al. 1991). Therefore, after freezing + nitrite treatment, particle size increased and sludge filterability was improved at relatively low nitrite concentration (100 - 200 mg L⁻¹). However, when nitrite concentration increased to a high level (800 mg L⁻¹, LFN4), the electrolytes retarded the flocs migration (Chu et al. 1997), and at the same time, the electrolytes can change ice crystal growth pattern from columnar to dendritic (Martel 2000). As a result, the mean aggregated particle size decreased, thereby unfavourable to filterability.

3.3 Effects of treatment on sludge rheology

Rheological characteristics of sludge before and after treatment are shown in Fig. S2, and the results are summarized in Table 3. The Herchel-Bulkley model was found to be efficient to describe the data generated in this study ($R^2 > 0.99$). The T_y value quantifies the amount of stress that the fluid may experience before it yields and begins to flow (Eshtiaghi et al. 2012). The n value (flow behaviour index) reflects the intensity of sludge. The k value (fluid consistency index) gives an idea of the viscosity of the fluid. In this study, raw sludge showed a good reproducibility of the measurements and presented shear thinning behaviour ($n < 1$), which was in agreement with the results of Baudez et al. (2013). After freezing treatment, n value was above 1, indicating that the

fluid acted as a shear thickening fluid (Eshtiaghi et al. 2012). Overall, n values increased and k value decreased, in other words, sludge flowability increased and viscosity decreased. The trend of n value and k value is typically in consistent with that of sludge filterability ($p < 0.01$), which supported the finding that the filterability after freezing treatment was greatly improved.

3.4 Scanning electron microscopy (SEM)

The SEM images of raw sludge and treated sludge could enhance the understanding of the sludge morphology. As shown in Fig. 5a and Fig. S4, large numbers of angular-shaped rods and smooth bacteria appeared in the raw sludge, and it is important to note that no big floc can be spotted suggesting that there is no obvious bacterial cells aggregation in raw sludge. Moreover, it could be also observed that most of bacteria were viable (Fig. S5). However, after freezing (LF), floc size was much larger and compacted gross floc migration occurred, which supported the increased particle size and improved sludge filterability (Fig. 5b and Fig. S4). Moreover, compared with those cells in the raw sludge which generally kept their intact morphology, slight shrunk and/or damaged cells were spotted in the freezing sample. In the case of freezing + nitrite treatment, similar large flocs were found (Fig. 5c and Fig. S4). More importantly, lots of microbial cells observed were clearly destroyed (Fig. S5). Consequently, such irreversible damage on cells would lead to the leakage of intracellular substances and dissolution of EPS structure. The results are in agreement with observation on DOC and EPS transformation.

3.5 The role of nitrite in the freeze/thaw sludge conditioning and its implications

This study revealed that nitrite had a synergetic role with freezing treatment during

WAS conditioning. Combined freezing + nitrite treatment can achieve much higher carbon source production and better sludge filterability compared to freezing treatment alone.

It has been demonstrated that some electrolytes, such as Na_2SO_4 , KNO_3 , NaNO_3 and NaCl , have little or negative effects on the sludge filterability and settleability regardless of electrolyte species and the dosage (Jean et al. 2000). However, in this study, a different phenomenon was observed after the addition of NaNO_2 in the freezing treatment. In fact, nitrite can affect sludge characteristics in several ways. Firstly, it can destroy the EPS and bacteria cells through its active species FNA. FNA concentration can be concentrated to a much higher level in the liquid-like boundary region upon freezing, thus plays a more effective role compared to liquid phase. Secondly, it can change the electronic potential of the sludge. Studies showed that dissolved ions are distributed unequally between the ice and aqueous phases when freezing occurs. This inequality develops an electric potential (so-called “freezing potential”), which induces OH^- or H_3O^+ to be preferentially accumulated in the ice grain boundary region, resulting in a sharp pH change (Jeong et al. 2015). That is, when solution that contains large anions such as SO_4^{2-} and NO_2^- , and small countercations (e.g. Na^+), undergoes frozen, it would induce the accumulation of H_3O^+ ions (pH decrease) in the liquid-like boundary region through the preferential incorporation of larger anions in the ice matrix (O’Concubhair and Sodeau 2013). Under decreased pH conditions, nitrite/FNA could produce some derivatives (e.g. NO , N_2O_3 and NO_2) (Yoon et al. 2006). FNA and the derivatives can react with the solubilised materials and convert some refractory compounds to readily degradable compounds (Yoon et al. 2006), as evidenced by more release of LMW compounds in this study. Moreover, it could change the structure of

proteins and polysaccharides in cells or EPS through chemical reactions (Wang et al. 2014).

The economic analysis of the proposed freezing + nitrite treatment was constructed based on a full-scale WWTP with a 100,000 population equivalent (PE) capacity (see Table S1). Compared to the conventional sludge dewatering method with belt filter press, the net economic benefit of the proposed method is estimated to be around \$99,207 per year, which arises from the enhanced carbon source recovery, good sludge filterability without chemical polymers addition, as well as sludge reduction due to its pre-solubilization. Therefore, it seems that the proposed combined freezing + nitrite treatment is economically acceptable. However, it should be noted that the economic analysis only describes the preliminary results. To fully estimate the potential economic feasibility of the proposed technology, the benefits and costs need to be performed in the field-scale application.

4. Conclusions

This paper reported a new sludge conditioning technique using combined freezing + nitrite. The results showed that the integration of nitrite into freezing process could enhance WAS solubilization and such effect was higher at slow freezing rates. The dewatered liquor can be then used as carbon source. The results demonstrate that the introduction of 100 mg L⁻¹ of nitrite (0.17 mg L⁻¹ FNA) could increase DOC concentration from 29.5 to 48.8 mg DOC g⁻¹ VSS under freezing treatment at -5 °C. LMW protein, LMW polysaccharide, LMW neutrals, building blocks and LMW acids in SB-EPS mainly contributed to the increased DOC release. Lower nitrite concentrations could also increase the particle size distribution of sludge, and improve the sludge filterability in the freezing process, but sludge filterability was deteriorated at

the nitrite concentration of 800 mg L⁻¹. Sludge rheology could be well modeled by Herchel-Bulkley model and the introduction of nitrite into freezing process increased sludge flowability and decreased sludge viscosity. Changes in cell integrity, EPS compositions, particle sizes, rheology of sludge proved the synergistic effects from combined nitrite and freezing treatment.

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Table 1 Treatment conditions applied in this study

Treatment	Temperature (°C)	NO ₂ ⁻ -N (mg L ⁻¹)	Initial pH
Control			
R	4	0	6.5
Freezing + FNA treatment			
HF	-20	0	6.5
HFN1	-20	100	6.5
HFN2	-20	200	6.5
HFN3	-20	400	6.5
HFN4	-20	800	6.5
MF	-14	0	6.5
MFN1	-14	100	6.5
MFN2	-14	200	6.5
MFN3	-14	400	6.5
MFN4	-14	800	6.5
LF	-5	0	6.5
LFN1	-5	100	6.5
LFN2	-5	200	6.5
LFN3	-5	400	6.5
LFN4	-5	800	6.5

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536 Table 2 Sludge volume index after 5 min (SVI₅) and 10 min (SVI₁₀)

	R	LF	LFN1	LFN2	LFN3	LFN4
SVI ₅ (mL g ⁻¹)	95 ± 0	38 ± 0	36 ± 0.5	37 ± 1	38 ± 0	38 ± 0.5
SVI ₁₀ (mL g ⁻¹)	95 ± 0	36 ± 1	35 ± 1	35 ± 1	37 ± 1	35 ± 1

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541 Table 3 Rheological characteristics of WAS before and after treatment

	R1	R2	LF	LFN1	LFN2	LFN3	LFN4
$\mathcal{T}_y$	0.670	0.674	0.364	0.387	0.411	0.543	0.309
k (×10 ⁻⁴)	73	66	5.50	4.28	4.13	1.92	3.45
n	0.986	1.00	1.309	1.344	1.353	1.458	1.375

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Fig. 1 Soluble DOC (a), polysaccharides (b), protein (c) and potassium (d) after treatment at different frozen temperatures. H: -20 °C, M: -14 °C and L: -5 °C

Fig. 2 Increase in related organic compounds in three EPS fractions after treatment at -5 °C with or without nitrite.

Fig. 3 Variations of sludge filterability before and after treatment. Different letters above the error bar indicate significant differences between different treatments

Fig. 4 Particle size distribution of WAS before and after treatment

Fig. 5 SEM images of WAS before (a) and after treatment (b: LF; c: LF3). The red arrows indicate the cells were destroyed.

Highlights

- ✓ A novel method for WAS conditioning was developed
- ✓ Warmer freezing temperature led to more solubilization of sludge during freezing
- ✓ Nitrite + freezing greatly increased the solubilization of WAS
- ✓ Presence of nitrite enhanced releasing LMW protein, carbohydrate, neutral and acid
- ✓ Low nitrite improved the sludge dewatering characteristics during freezing