

## PAPER

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# Integration of aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> processes in a continuous flow system for the degradation of sulfolane in contaminated water†

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Sulfolane contamination has increasingly become a major environmental concern around the world with emerging reports of groundwater, drinking water wells and soil contamination. In this study, a novel approach of integrating aerobic granulation technology with UV/H<sub>2</sub>O<sub>2</sub> process in a continuous flow-through operation sequence was investigated to remediate sulfolane contaminated waters. The new hybrid technology was able to eliminate more than 99.99% of sulfolane in less than 6.3 h of combined retention time. The degradation kinetics of sulfolane were evaluated in batch and continuous flow operation which showed zero and first order for aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> processes, respectively. In addition, the flow-through system was able to generate and maintain a healthy aerobic granular system characterized by a stable MLVSS/MLSS ratio as well as elemental and bacterial community compositions within the granules. H<sub>2</sub>O<sub>2</sub> concentration was a crucial element for sulfolane degradation in UV/H<sub>2</sub>O<sub>2</sub> process and various key factors were also identified that govern residual H<sub>2</sub>O<sub>2</sub> concentration in UV/H<sub>2</sub>O<sub>2</sub> effluents.

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## Water impact

Conventional treatments have proved inefficient in removing emerging contaminants from our water resources. This study demonstrated a novel technology to degrade emerging contaminants, such as sulfolane, by integrating aerobic granulation with UV/H<sub>2</sub>O<sub>2</sub> and provides in-depth knowledge for future field applications. Offering a smaller footprint, this new technology is more economical and efficient in eliminating a wide range of water contaminants.

## 1. Introduction

With industrial advancements, various anthropogenic chemicals are being engineered everyday throughout the world. Many of these chemicals are intended for specific industrial purposes and some of them are designed to be persistent. However, problems arise when these new chemicals that are not regulated for environmental impact are released into our water bodies. One such industrial chemical, sulfolane (C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>S), which is commonly used in oil and natural gas refineries was reported to cause contamination of groundwaters in many places including Alberta,<sup>1</sup> Brisbane,<sup>2</sup> and hundreds of drinking water wells in interior Alaska.<sup>3</sup> Sulfolane is an organo-sulfur compound

having high miscibility in water combined with low affinity for aquifer materials.<sup>4</sup> These properties make sulfolane a good candidate for offsite migration and a persistent groundwater contaminant, once released. Toxicological data on sulfolane is limited to a few studies on exposure to rats and guinea pigs<sup>5</sup> while no chronic effects have been reported on humans yet.<sup>6</sup>

Over the years, various biological and advanced oxidation process (AOP) treatment studies have been conducted in response to the remediation of sulfolane impacted soil and groundwaters. Some of the early work on the biodegradation of sulfolane was conducted by Greene *et al.* who reported that biodegradation of sulfolane was enhanced when mixed bacterial cultures were utilized including indigenous bacteria from contaminated sites in the presence of abundant nutrients.<sup>7–9</sup> Similarly, an intensive study conducted on subarctic aquifers in Alaska by Kasanke and Leigh, also found aerobic biodegradation to prevail over anaerobic.<sup>3</sup> Lately aerobic granulation has emerged as a promising technology compared to conventional biological processes.<sup>10</sup> Consequently, in our

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previous study coaggregation of indigenous microbes from sulfolane contaminated site with aerobic granules generated stable robust granules that were able to degrade sulfolane effectively.<sup>11</sup> This technology is ideal for application because it benefits from having a consortium of millions of microorganisms harboring within a single granule with the potential to treat the most recalcitrant contaminants present in wastewaters.<sup>12</sup> On the other hand, one of the first in-depth AOP studies on treatment of sulfolane in aqueous medium was conducted by Yu *et al.*, who reported the combination of UVC/H<sub>2</sub>O<sub>2</sub>, UVC/O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> and UVC/O<sub>3</sub> to have significant effect on sulfolane degradation.<sup>13</sup> Similarly, UVC/O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>, calcium peroxide/ozone and calcium oxide/ozone have also shown to effectively degrade and mineralize 100 mg L<sup>-1</sup> of sulfolane to carbon dioxide, water and sulfate in around 4 h.<sup>14,15</sup> Izadifard *et al.*, evaluated persulfate as another water-soluble oxidant under UVC irradiation and noticed that bubbling ozone in the presence of persulfate significantly reduced reaction time for sulfolane degradation.<sup>16</sup> It is understandable that the above mentioned AOPs are effective in the degradation of sulfolane, however, higher contaminant concentrations seem to prolong the reaction time for complete elimination which could eventually end up as an expensive treatment.

Both biological treatment and AOPs are effective options for degrading sulfolane in contaminated waters. However, sequential treatment of these two technologies may prove to be more cost effective. Biological treatments require longer retention times and may not meet the very low discharge limits for some recalcitrant micropollutants whereas AOPs can be expensive when contaminant concentrations are high.<sup>17</sup> It is reasonable to argue that an integration of these two technologies can be a way forward for a more cost-effective option. Only few studies have highlighted the significance of integrating a biological process with downstream AOP in which some degree of disinfection can also be achieved along with contaminant removal.<sup>18,19</sup> Of the various AOPs available, UV/H<sub>2</sub>O<sub>2</sub> holds an attractive edge over other processes. For example, treatment of wastewaters by ozone-based AOPs have the potential of forming hazardous by-products as well as bromate in the treated water which is considered to be a human carcinogen.<sup>20,21</sup> Likewise, Fenton-based AOPs require the disposal of iron sludge and produces high concentration of anions in the treated water.<sup>22</sup> In comparison to Fenton-based AOP, Giannakis *et al.*, reported UV/H<sub>2</sub>O<sub>2</sub> as a more efficient option in combination with biological treatment to achieve the highest elimination of micropollutants.<sup>23</sup> Similarly, in our previous study an integration of activated sludge with UV/H<sub>2</sub>O<sub>2</sub> achieved significant sulfolane degradation and disinfection simultaneously.<sup>19</sup> However, it is perceived that aerobic granulation might prevail over activated sludge technology in the future and an investigation of integrating aerobic granulation with AOP is needed.

This study reports for the first time a sequential integration of aerobic granulation with UV/H<sub>2</sub>O<sub>2</sub> in a continuous flow system to study the synergistic effect of these

technologies in removing sulfolane from contaminated waters. To the best of our knowledge this is a novel approach for sulfolane treatment and has never been investigated before. Aerobic granulation has already been shown to be more effective than conventional activated sludge and UV/H<sub>2</sub>O<sub>2</sub> benefits outweigh other AOPs. This study evaluated the performance of the integrated technology and characterized the aerobic granules during continuous operation mode including the granule community. It is reported that residual H<sub>2</sub>O<sub>2</sub> from AOPs has the potential to cause negative effects to the ecosystem of the receiving waters.<sup>24</sup> Furthermore, it can react with chlorine, if chlorine is used as disinfectant in downstream process, compromising disinfection before distribution and increasing costs.<sup>25</sup> Therefore, management of H<sub>2</sub>O<sub>2</sub> residual was also investigated in order to establish a more efficient and effective integrated technology for potential field application of sulfolane groundwater remediations.

## 2. Materials and methods

### 2.1 Chemicals

Sulfolane with 99% purity, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, A.R. grade, ≥30%), ammonium chloride (NH<sub>4</sub>Cl, 99.99%), potassium phosphate dibasic (K<sub>2</sub>HPO<sub>4</sub>, ≥98%), potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>, 99.99%), sodium acetate anhydrous (>99%), dichloromethane (DCM), and titanium(IV) oxy sulfate solution (1.9–2.1%) for H<sub>2</sub>O<sub>2</sub> determination were purchased from Sigma Aldrich, Oakville, Canada. Deionized water was used for all analytical procedures.

### 2.2 Experimental setup

**2.2.1 Aerobic granules and UV/H<sub>2</sub>O<sub>2</sub> system.** Aerobic granules used in this study were cultivated as described in our previous work.<sup>11</sup> A schematic of the continuous operation of integrated aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> processes is presented in Fig. 1. The treatment of wastewater by aerobic granulation was conducted in a 12 liter column reactor with 11 liter working volume. Fine air bubbles at 1–3 mm in size were produced *via* polyethylene air diffusers (Model: AS4, pore size: 140 μm, pentair Aquatic Eco-Systems, Inc., British Columbia, Canada). The diffusers were installed at the bottom of the granule reactor and air was supplied continuously through two Fluval® Q2 air pumps (Hagen Inc., Montréal, Canada) with a combined flow rate of 480 L h<sup>-1</sup>. The granule column reactor was fitted with a mesh (size 60) at the top to contain the granules within the reactor during continuous operation. Synthetic wastewater was prepared using tap water with a biochemical oxygen demand (BOD) representing that of typical municipal wastewater treatment plants in the range of 190–350 mg L<sup>-1</sup>.<sup>26</sup> The water quality parameters of the synthetic wastewater are presented in Table 1. The composition of the wastewater was as follows (concentrations, mg L<sup>-1</sup>): sodium acetate 450, sulfolane 20, NH<sub>4</sub>Cl 28, K<sub>2</sub>HPO<sub>4</sub> 4.11, KH<sub>2</sub>PO<sub>4</sub> 3.21, and microelement solution of 1 mL L<sup>-1</sup> giving a chemical oxygen demand (COD) concentration in the range of 431 ± 2 mg



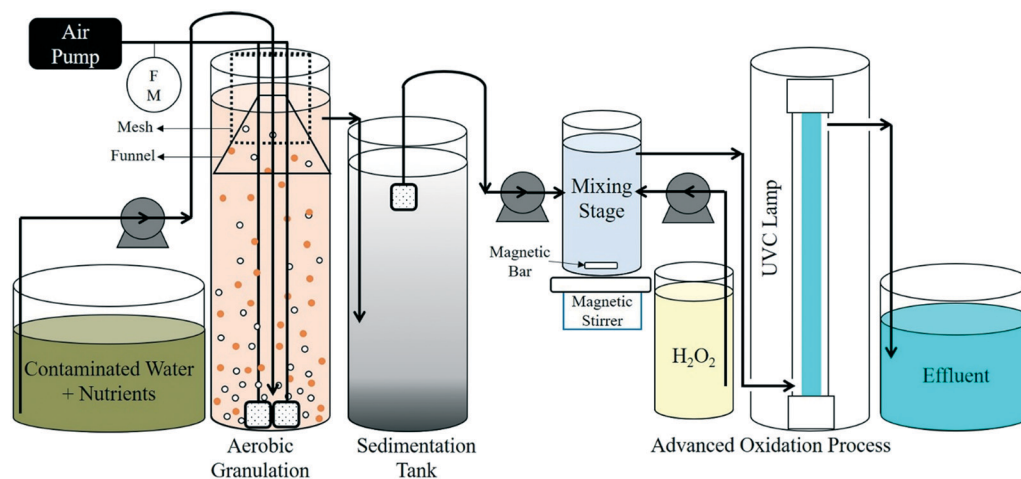


Fig. 1 Schematic of a continuous flow integrated aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> setup.

L<sup>-1</sup>. The microelement solution composition was as described previously.<sup>19</sup>

The UV/H<sub>2</sub>O<sub>2</sub> process was carried out using an annular vertical flow-through photoreactor with a low pressure UVC mercury lamp installed in the center. The 254 nm UVC lamp was placed inside a quartz sleeve with an outer diameter of 23.8 mm. The contaminated water flowed from the bottom of the photoreactor to the top (36 cm), filling the space between the quartz sleeve and the outer glass layer of the reactor (diameter of 44.5 mm) with a volume of approximately 300 mL. The radiation intensity by the UVC lamp entering the reaction vessel was determined to be  $1.17 \times 10^{18} \pm 0.07$  photons per s using ferrioxalate actinometry.<sup>27</sup>

**2.2.2 Continuous flow experiments.** Wastewater was pumped using a peristaltic pump (Model: BT100S, Head: YZ15, Golander Pump, USA) to the bottom of aerobic granulation reactor at flow rates of  $30 \pm 1$  and  $15 \pm 0.5$  mL min<sup>-1</sup> giving an organic loading rate (OLR) of  $1.69 \pm 0.6$  and  $0.85 \pm 0.02$  kg COD m<sup>-3</sup> d<sup>-1</sup>, respectively. The biotreated wastewater was discharged from the top port of the bioreactor into a sedimentation tank at below mid-level. The sedimentation tank allowed the settling of any biomass that crossed over from the bioreactor. The post-bio treated water was driven into a mixing tank where 100 mg L<sup>-1</sup> of H<sub>2</sub>O<sub>2</sub> at  $0.3 \pm 0.02$  mL min<sup>-1</sup> was continuously pumped and mixed with the aid of a magnetic stirrer. A diffuser was installed at

the water intake point in the sedimentation tank to allow filtration. Water in the mixing tank moved from the top port to the bottom of the UV reactor and travelled upwards to be discharged from the top port. The reactor was operated in continuous flow mode for five days and samples were collected at specific intervals. On the fifth day, the continuous flow was discontinued, and the aeration was stopped for reactor cleanup in an anaerobic state. After cleanup, the reactor was aerated for the next two days in batch mode to induce famine period. Subsequently, this process was repeated weekly.

**2.2.3 Batch experiments.** Batch experiments were conducted separately to compare the degradation kinetics with the continuous flow system. For aerobic granulation, 10 liters of synthetic wastewater was prepared as described in section 2.2.1 and introduced into the granular reactor as shown in Fig. 1. Samples were withdrawn at specific time intervals, filtered using 0.45 µm filters and analyzed for sulfolane. Batch experiments for UV/H<sub>2</sub>O<sub>2</sub> were conducted using aerobic granule treated water. Approximately 2 liters of biotreated water was collected in a beaker and pumped at 30 mL min<sup>-1</sup> into the annular vertical flow-through photoreactor as shown in Fig. 1. A diffuser was installed at the water intake point to allow filtration. The outlet from the reactor was redirected back into the beaker and the water in the beaker was continuously stirred using a magnetic stirrer to have a homogenous solution. Initially, the biotreated water used for UV/H<sub>2</sub>O<sub>2</sub> batch experiment was spiked with 0.5 mg L<sup>-1</sup> of sulfolane to mimic the same conditions that were observed during the continuous flow. Samples were collected from the beaker at specific time intervals, filtered using 0.45 µm filters and analyzed for sulfolane.

### 2.3 H<sub>2</sub>O<sub>2</sub> residual test

High concentrations of residual H<sub>2</sub>O<sub>2</sub> can have negative impacts on the environment. Therefore, evaluation of residual H<sub>2</sub>O<sub>2</sub> concentrations, post UV/H<sub>2</sub>O<sub>2</sub> were conducted using the

Table 1 Water quality parameters for the synthetic wastewater

Parameter	Units	Value
pH	—	7.7 ± 0.3
COD	mg L <sup>-1</sup>	431 ± 2
Total alkalinity	mg L <sup>-1</sup> (CaCO <sub>3</sub> )	118 ± 21
Bicarbonate	mg L <sup>-1</sup> (CaCO <sub>3</sub> )	0.033 ± 0.01
Calcium	mg L <sup>-1</sup>	40 ± 10
Total dissolved solids	mg L <sup>-1</sup>	194 ± 23
Conductivity	µS cm <sup>-1</sup>	339 ± 42
Hardness	mg L <sup>-1</sup> (CaCO <sub>3</sub> )	168 ± 32
Sulphate	mg L <sup>-1</sup>	51 ± 8



**Table 2** Experimental design to evaluate the impact of various parameters on residual H<sub>2</sub>O<sub>2</sub> concentration

Sample I.D.	Water matrix	Flow rate (mL min <sup>-1</sup> )	Sulfolane (mg L <sup>-1</sup> )	H <sub>2</sub> O <sub>2</sub> (mg L <sup>-1</sup> )
F1	D.I.	120	0.5	100
F2	D.I.	60	0.5	100
F3	D.I.	30	0.5	100
F4	D.I.	10	0.5	100
S1	D.I.	30	5	100
S2	D.I.	30	2	100
S3	D.I.	30	0.5	100
H1	D.I.	10	0.5	100
H2	D.I.	10	0.5	30
H3	D.I.	10	0.5	10
W1	B.W.	30	0.5	100
W2	B.W.	10	0.5	100

D.I. = deionized water spiked with sulfolane. B.W. = biotreated synthetic wastewater.

above-mentioned flow-through photoreactor. A series of experiments were conducted with varying flow rates, sulfolane and H<sub>2</sub>O<sub>2</sub> concentrations, and water matrix as shown in Table 2. Samples were collected at specified time intervals, filtered using 0.45 µm filters before sulfolane and H<sub>2</sub>O<sub>2</sub> analysis. For biotreated water, a diffuser was installed at the influent to allow filtration of the water before entering the photoreactor.

## 2.4 Analytical methods

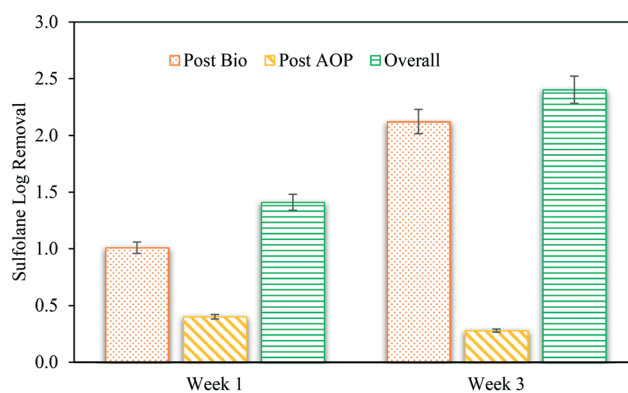
Turbidity measurements were conducted using 2100Q HACH Turbidimeter and UV Transmittance at 254 nm (UVT<sub>254</sub>) was measured using DR/4000 UV-vis spectrophotometer, HACH. H<sub>2</sub>O<sub>2</sub> concentration in water was determined according to DIN 38409 H15.<sup>28</sup> The concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured using Standard Methods.<sup>29</sup> Sulfolane concentration was analyzed using gas chromatography flame ionization detector (GC-FID) and mass spectrometry (GC-MS) as described previously.<sup>19</sup> Surface images, elemental characterization were obtained using scanning electron microscopy (SEM) whereas granule microbial community were analyzed through 16S rRNA gene extraction using DNeasy® PowerSoil® kit as described previously.<sup>30</sup>

## 3. Results and discussion

### 3.1 Evaluation of continuous flow integrated technology

A sequential integration of aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> processes was investigated to treat sulfolane contaminated water in a continuous flow setup. Pre-grown aerobic granules were harvested from a sequencing batch reactor and were inoculated in a continuous flow bioreactor. The system was initially operated over two weeks to reach steady state before experimental studies. At 30 mL min<sup>-1</sup>, corresponding to a residence time of ~6.3 h, the integrated technology had significant improvement over the three weeks of continuous operation. As shown in Fig. 2, sulfolane degradation after aerobic granulation treatment had increased by more than

one log over the span of three weeks leading to an improvement in the overall sulfolane degradation (>99%). However, the sulfolane residual in the effluent was still above the guideline value of 90 µg L<sup>-1</sup>.<sup>31</sup> When the flow rate was decreased to 15 mL min<sup>-1</sup>, sulfolane (20 mg L<sup>-1</sup>) degradation by aerobic granules was observed to be greater than 4.0 log in the bioreactor as sulfolane effluent concentration were below detection limit of 10 µg L<sup>-1</sup>. This was due to the slow flow rate that allowed granules enough time to degrade the contaminant. However, longer retention time require larger space and potentially increase operating costs associated with biological treatment. It is understandable to have a faster optimum flow rate where majority of the contaminant can be removed by the biological process, thereby, leaving AOP as a polishing step and disinfecting purposes. AOP as post treatment to biological processes almost certainly guarantees a complete mineralization of the organics in the wastewater.<sup>32</sup> It should be noted in a continuous flow reactor, limited availability of substrate (low sulfolane concentration and other carbon source) can result in outgrowth of loose flocculent bacteria and ultimate collapse of granule.<sup>33</sup> Therefore, efficient functioning of aerobic granulation system is dependent on higher substrate concentration. The reverse is true for AOP, where higher concentrations can make the



**Fig. 2** Evaluation of integrating aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> in a continuous flow mode (30 mL min<sup>-1</sup>) for sulfolane degradation.





entire system inefficient with incomplete removal due to oxidant limitations.<sup>17</sup>

In a previous study, a batch mode integration of activated sludge with UV/H<sub>2</sub>O<sub>2</sub> was able to achieve 2.8 log degradation of sulfolane (~28 mg L<sup>-1</sup> sulfolane concentration) effectively in less than 24 h.<sup>19</sup> Although the initial concentration used in that study was much higher, nevertheless, ~28 mg L<sup>-1</sup> of sulfolane was degraded by sequential integration of 20 h activated sludge and 30 min UV/H<sub>2</sub>O<sub>2</sub>. In comparison, in this study an integration of aerobic granulation (6 h) and UV/H<sub>2</sub>O<sub>2</sub> (10 min) in a continuous flow-through operation was able to achieve ~2.5 log degradation (20 mg L<sup>-1</sup> sulfolane concentration). This illustrates the potential and efficacy of aerobic granulation over activated sludge technology as a pretreatment for sulfolane degradation. It should be noted that Khan *et al.*, used sulfolane as the sole carbon source for the activated sludge treatment<sup>19</sup> whereas, a combination of acetate and sulfolane was provided as the substrate for aerobic granulation. Here the activated sludge biomass comprised of indigenous microbes from the sulfolane contaminated site whereas the aerobic granules were cultivated by coaggregating two different bacterial communities including the indigenous microbes.<sup>11</sup> This has been reported by Greene *et al.*, as well where the inoculation of mixed cultures provided a better sulfolane degradation compared to single isolates.<sup>8</sup>

The continuous flow operation of aerobic granulation was effective in removing more than 97% sulfolane and was consistent throughout the duration of the experiment as shown in Fig. 3a. Previous continuous flow aerobic granular reactor studies have also reported more than 90% COD removal efficiencies.<sup>34,35</sup> The efficacy of the aerobic granular reactor is largely due to the dense granular biomass making it more efficient than the activated sludge process. A sedimentation tank was kept inline after the bioreactor and before the photoreactor. The purpose of the tank was to allow settling of any new biomass that might escape out of the mesh at the top of the bioreactor, thereby, avoiding the presence of biomass in the photoreactor. Fig. 3c shows the turbidity level overtime near the outlet of the bioreactor and the top of the sedimentation tank. It was observed that after 24 h, there was significant change in the effluent turbidity level of the bioreactor from new biomass growth. However, as seen from Fig. 3c, the turbidity level of the sedimentation tank at the top was consistently below 20 NTU throughout the duration of the experiment. There was accumulation of biomass at the bottom of the sedimentation tank as shown in Fig. S1† indicating the significance of the tank. Subsequently, in Fig. 3b the continuous flow-through UV/H<sub>2</sub>O<sub>2</sub> process was also consistently effective in removing ~50% sulfolane with 10 min of reaction time. Both Fig. 3a and b show data after the reactors reaching steady state.

The degradation kinetics of sulfolane was also compared between batch and continuous mode as shown in Table 3. It was observed that aerobic granulation strictly follows zero order kinetics irrespective of reactor mode. However, the rate constant was approximately 79% faster in continuous mode.

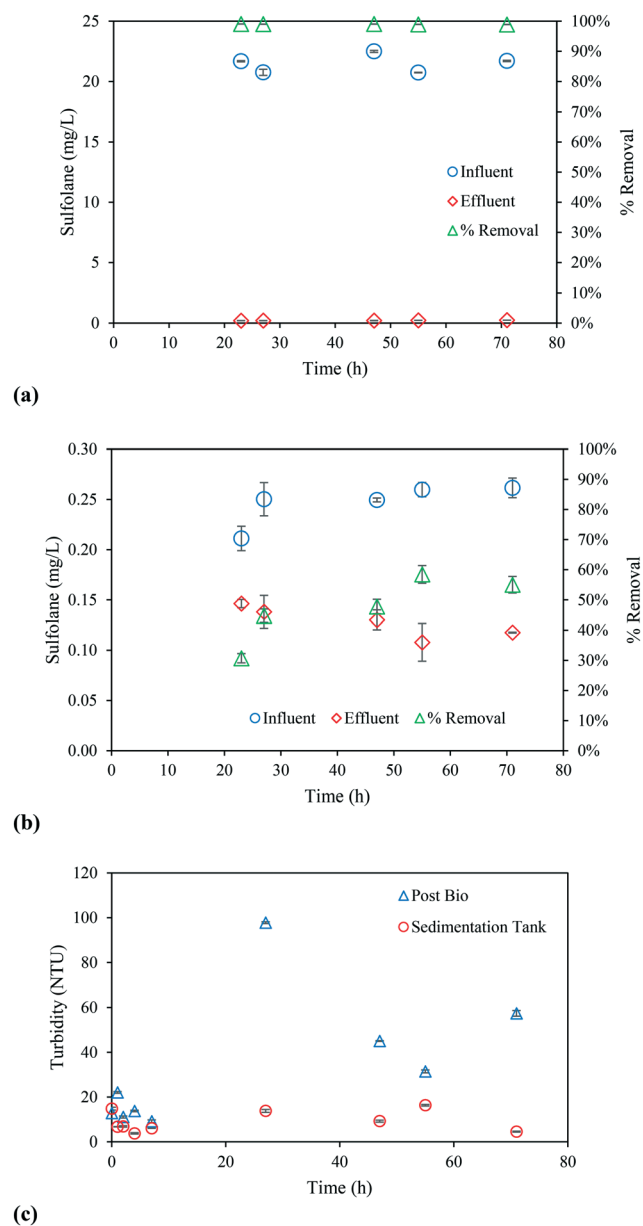


Fig. 3 Degradation of sulfolane in a continuous flow reactor by (a) aerobic granulation, (b) UV/H<sub>2</sub>O<sub>2</sub> and (c) changes in turbidity overtime in sedimentation tank and post-bio (before outlet of bioreactor).

This shows that application of aerobic granulation for the degradation of sulfolane is more efficient when applied in continuous flow reactor. Previous studies have also shown aerobic granulation to follow zero order kinetics for contaminant degradation.<sup>11,36</sup> However, it is understood that the zero-order kinetics for biodegradation holds true for high concentration of contaminants. As the concentration depletes to a lower range, the zero-order kinetics no longer becomes valid due to substrate limitation for biomass (when sulfolane is the only carbon source) and the kinetics switch to first order. This characteristic relates to Michaelis-Menten kinetics which states that at low substrate concentrations, reaction rates are linearly proportional to substrate



**Table 3** Comparison of rate order and rate constant for batch and continuous mode

Operation mode	Technology	Rate order	Rate constant
Batch	Aerobic granulation	Zero order	$0.0125 \pm 0.002 \text{ (mg L}^{-1} \text{ min}^{-1}\text{)}$
	UV/H <sub>2</sub> O <sub>2</sub>	First order	$0.0529 \pm 0.001 \text{ (1/min)}$
Continuous	Aerobic granulation	Zero order	$0.0592 \pm 0.002 \text{ (mg L}^{-1} \text{ min}^{-1}\text{)}$
	UV/H <sub>2</sub> O <sub>2</sub>	First order	$0.0566 \pm 0.009 \text{ (1/min)}$

Note that the rate constants are calculated from data presented in Fig. S2–S5 in ESI.†

concentration, thus following first order kinetics. However, at high substrate concentration the rates are independent of substrate concentration, thereby, a zero order kinetics prevails.<sup>37</sup> UV/H<sub>2</sub>O<sub>2</sub> process followed first order kinetics for sulfolane degradation as shown in Table 3. Different from the granular reactor, the continuous mode yielded similar degradation kinetics as batch conditions in UV/H<sub>2</sub>O<sub>2</sub> reactor which clearly demonstrates that the kinetics obtained from batch study can be used to predict the performance of continuous flow-through reactor for sulfolane treatment. In a previous study, Khan *et al.*, had also reported a first order kinetics for sulfolane degradation by UV/H<sub>2</sub>O<sub>2</sub> in batch study for activated sludge treated wastewater.<sup>19</sup>

### 3.2 Management of hydrogen peroxide in a continuous flow integrated treatment system

The presence of H<sub>2</sub>O<sub>2</sub> in treated effluent waters can be harmful to the ecosystem of the receiving waters. The hydroxyl radicals produced from H<sub>2</sub>O<sub>2</sub> can damage cells of microorganisms in rivers, lakes or ponds by oxidation of cellular proteins and DNA.<sup>63,64</sup> Previous studies have shown around 50 mg L<sup>-1</sup> of H<sub>2</sub>O<sub>2</sub> concentrations can affect zooplanktons and phytoplankton in lakes.<sup>65</sup> Therefore, it is a necessity to consider the discharge of residual H<sub>2</sub>O<sub>2</sub> post UV/H<sub>2</sub>O<sub>2</sub> treatment of contaminated waters. A series of experiments were conducted to evaluate the fate of residual H<sub>2</sub>O<sub>2</sub> based on flow rates, water matrix, initial sulfolane and H<sub>2</sub>O<sub>2</sub> concentrations and the results are shown in Table 4. All experiments were conducted with initial sulfolane and H<sub>2</sub>O<sub>2</sub> concentrations of 0.5 and 100

mg L<sup>-1</sup>, respectively, unless otherwise stated. During the integrated technology experiments, more than 96% of the initial sulfolane concentration had been degraded by the aerobic granulation process leaving behind about 0.5 mg L<sup>-1</sup> of sulfolane for the UV/H<sub>2</sub>O<sub>2</sub> process at 30 mL min<sup>-1</sup>. Therefore, to replicate the same scenario, 0.5 mg L<sup>-1</sup> of sulfolane concentration was selected to study the management of H<sub>2</sub>O<sub>2</sub> in the UV/H<sub>2</sub>O<sub>2</sub> process as part of the integrated technology.

It was observed that faster flow rates had higher H<sub>2</sub>O<sub>2</sub> residual in the effluents. This was due to less retention time in the UV reactor for H<sub>2</sub>O<sub>2</sub> cleavage by photons. Consequently, the degradation of sulfolane was also incomplete at flow rates of 60 and 120 mL min<sup>-1</sup>. At 30 mL min<sup>-1</sup>, 100% sulfolane was removed leaving behind approximately 30 mg L<sup>-1</sup> of residual H<sub>2</sub>O<sub>2</sub>. The least amount of residual H<sub>2</sub>O<sub>2</sub> in effluents was found at 10 mL min<sup>-1</sup>. Varying the concentrations of H<sub>2</sub>O<sub>2</sub> at 10 mL min<sup>-1</sup> showed that 100% sulfolane could be eliminated even with 10 mg L<sup>-1</sup> of H<sub>2</sub>O<sub>2</sub> leaving a residual of 2% in a clean water matrix. Interestingly, increasing sulfolane concentrations gave unexpected results. The decomposition of H<sub>2</sub>O<sub>2</sub> in water is determined by the light intensity/wavelength, the presence of reductants and catalysts such as ferric iron. In our previous findings, Yu *et al.*, showed that sulfolane in aqueous solution did not react with hydroperoxide but its degradation byproduct or itself might compete with H<sub>2</sub>O<sub>2</sub> in absorbing photons, thus reducing the decomposition of H<sub>2</sub>O<sub>2</sub>.<sup>13</sup>

The impact of water matrix was quite significant when experiments were conducted with biotreated water as shown

**Table 4** Sulfolane degradation and H<sub>2</sub>O<sub>2</sub> residual under different experimental conditions

Variables		Sample I.D.	Sulfolane degradation (%)	H <sub>2</sub> O <sub>2</sub> residual (mg L <sup>-1</sup> )
Flow rate (mL min <sup>-1</sup> )	120 ± 1	F1	86 ± 1	74.7 ± 2.0
	60 ± 1	F2	96 ± 1	55.4 ± 1.2
	30 ± 1	F3	100 ± 0	30.6 ± 1.8
	10 ± 0.5	F4	100 ± 0	12.2 ± 0.9
Sulfolane (mg L <sup>-1</sup> )	5 ± 0.3	S1	96 ± 1	38.5 ± 1.1
	2 ± 0.1	S2	100 ± 0	35.5 ± 0.8
	0.5 ± 0.01	S3	100 ± 0	30.6 ± 0.9
	100 ± 0.6	H1	100 ± 0	12.8 ± 0.4
H <sub>2</sub> O <sub>2</sub> (mg L <sup>-1</sup> )	30 ± 0.5	H2	100 ± 0	5.0 ± 0.5
	10 ± 0.3	H3	100 ± 0	2.0 ± 0.2
	10 ± 0.3	H3	100 ± 0	2.0 ± 0.2
Water matrix	B.W.	W1	57 ± 8	9.7 ± 0.9
	B.W.	W2	65 ± 8	3.5 ± 0.7

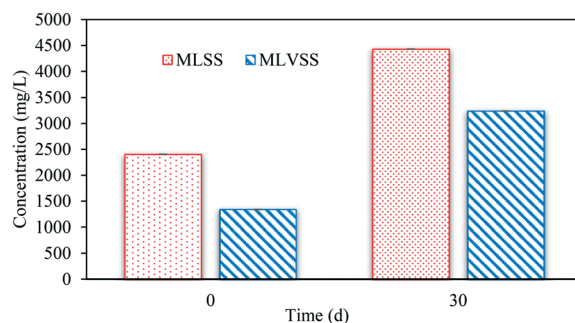
B.W. = biotreated synthetic wastewater. All experiments were conducted using deionized water, except sample W1 (30 ± 1 mL min<sup>-1</sup>) and W2 (10 ± 0.5 mL min<sup>-1</sup>).



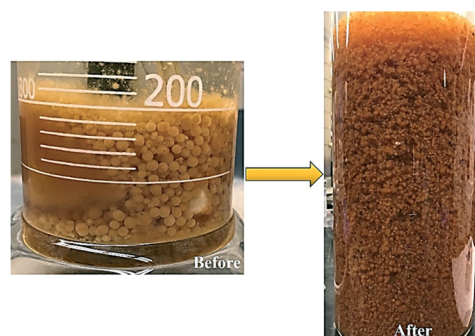
in Table 4 for samples W1 and W2. More than 90 and 96% of  $\text{H}_2\text{O}_2$  had been consumed at 30 and 10  $\text{mL min}^{-1}$ , respectively. Although the residual concentration was much lower compared to the experiments with deionized water, nevertheless, the degradation of sulfolane in the effluent was not efficient. More than 35% sulfolane was detected in the effluent at 10  $\text{mL min}^{-1}$  and even higher at 30  $\text{mL min}^{-1}$ . This is because majority of  $\text{H}_2\text{O}_2$  was being consumed *via* oxidation reaction with microbial intracellular constituents such as DNA, proteins and lipids in the biotreated water.<sup>66</sup> Even though 10  $\text{mL min}^{-1}$  (sample W2) showed more than 64% sulfolane degradation with less than 4%  $\text{H}_2\text{O}_2$  residual, it does not conclude that this flow rate is ideal in managing  $\text{H}_2\text{O}_2$  concentration in a UV/ $\text{H}_2\text{O}_2$  process effluents. Degradation of sulfolane in the contaminated waters is also a priority along with managing minimal  $\text{H}_2\text{O}_2$  discharge concentrations. It is understood that  $\text{H}_2\text{O}_2$  continues to get consumed in biotreated water even without exposure to UV/ $\text{H}_2\text{O}_2$  process. As shown in Table S1† there was immediate reduction of  $\text{H}_2\text{O}_2$  upon injection into biotreated water as observed concentrations were lower than theoretical concentrations. The biotreated water had an indirect correlation with  $\text{H}_2\text{O}_2$  observed concentrations. The observed values were much closer to the theoretical values as the percentage of biotreated water reduced. Furthermore, the applied  $\text{H}_2\text{O}_2$  dose had been completely consumed by the biotreated water after 24 h of injection indicating that  $\text{H}_2\text{O}_2$  continuous to deplete with time in a biotreated water. It should be noted in Table S1† that the sample with clean water had the same concentration of  $\text{H}_2\text{O}_2$  even after 24 h. Therefore, it is the quality of the biotreated water that defines the residual  $\text{H}_2\text{O}_2$  concentration. A lower flow rate might not be ideal for sulfolane treatment by UV/ $\text{H}_2\text{O}_2$  process as most of the peroxide will be consumed for disinfection much faster than for contaminant degradation. A flow rate of 30  $\text{mL min}^{-1}$  can be used, however, conditions should be suitable for the applied  $\text{H}_2\text{O}_2$  dosage depending on the quality of the biotreated water. By optimizing the applied dose of  $\text{H}_2\text{O}_2$ , a dual function of degrading sulfolane and performing disinfection can be achieved.

### 3.3 Characterization of aerobic granules during continuous flow operation

A healthy granular system can be predicted from the amount of biomass contained within the granule. MLVSS analysis in Fig. 4a show the concentration of organic proportion of aerobic granules increased by ~60% over the course of 30 d. Furthermore, Fig. 4b shows the physical appearance of granule quantity which increased significantly in the continuous flow reactor. This indicates that the design and operation of the continuous flow reactor in this study enhanced the growth of granules. A similar concentration of MLVSS was observed by Zou *et al.*, in a continuous flow reactor with a side stream sedimentation tank and kept stable for more than 80 d.<sup>38</sup> The ratio of MLVSS/MLSS in



(a)



(b)

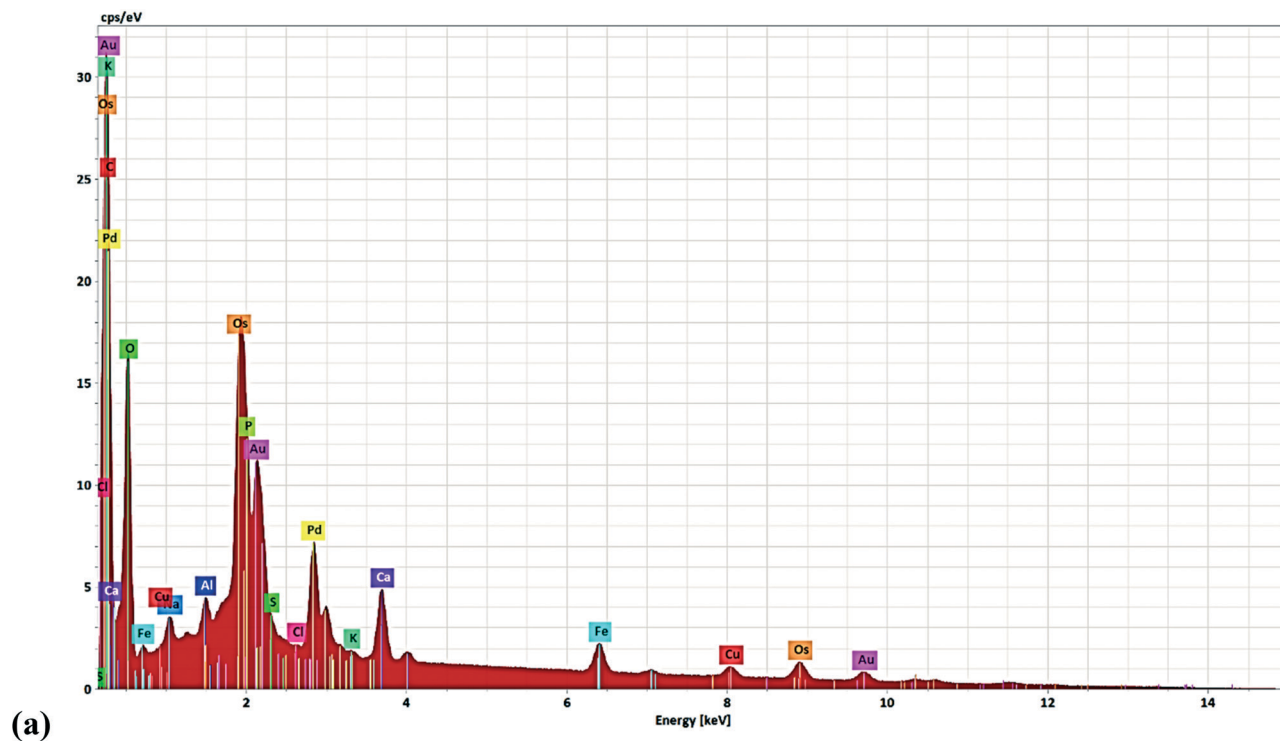
Fig. 4 Transformative changes in aerobic granules during continuous flow operation, a) MLVSS concentration, and b) quantity of granules (before = day 0, after = day 30).

aerobic granules was observed to increase by 30% in the continuous flow reactor and was stable at around 0.7 from day 20 to 30 (Fig. S6†) which further illustrates a healthy granular sludge system. Chen *et al.*,<sup>39</sup> operated a continuous flow membrane bioreactor and reported a similar ratio that kept constant in the range of 0.7–0.75 from day 30 to 120.

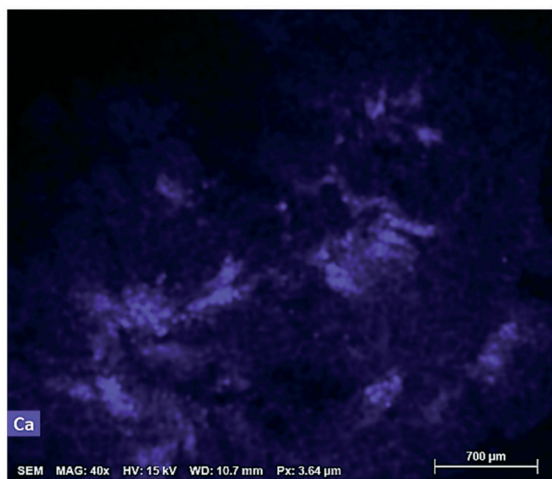
The increase in MLSS concentration (Fig. 4a) can be an indication of inorganic element accumulation within the granule. Fig. 5a shows elemental composition map on the granule surface with Fig. 5b–e showing energy dispersive X-ray spectroscopy (EDX) analysis of the notable elements such as calcium ( $\text{Ca}^{2+}$ ), phosphorus (P), potassium ( $\text{K}^+$ ) and iron (Fe). Other elements were also observed such as sodium ( $\text{Na}^+$ ), carbon (C), oxygen (O) with low peaks of chloride ( $\text{Cl}^-$ ) and sulphur (S) as well. Gold (Au) and osmium (Os) peaks also appeared as they were used as coating reagents during SEM-EDX analysis.  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ , and  $\text{K}^+$  both had two peaks which indicates a higher proportion of precipitates on the granule. These divalent and multivalent elements are predominantly linked with extracellular polymeric substance (EPS) secretion in bacteria that governs the structural stability of granules.  $\text{Ca}^{2+}$  is reported to form the structural backbone of aerobic granules and can bind with other proteins to allow adherence of bacteria to surfaces.<sup>40</sup> Similarly,  $\text{Fe}^{3+}$  is also linked to EPS binding as well as in bio-coagulation allowing other organic and inorganic molecules to aggregate giving granules a compact structure and increased settleability.<sup>41</sup> It is noted that although  $\text{Ca}^{2+}$  was not added in the synthetic



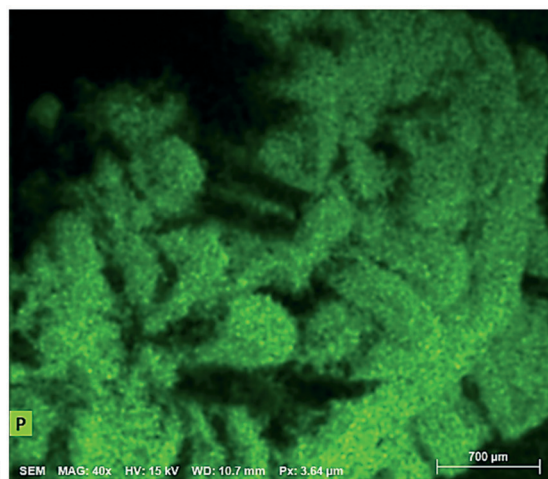




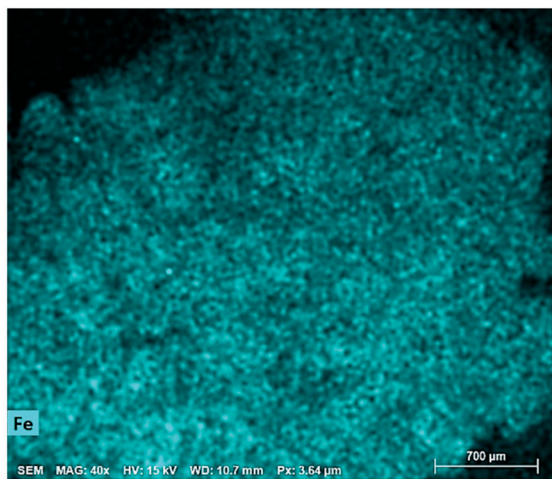
(a)



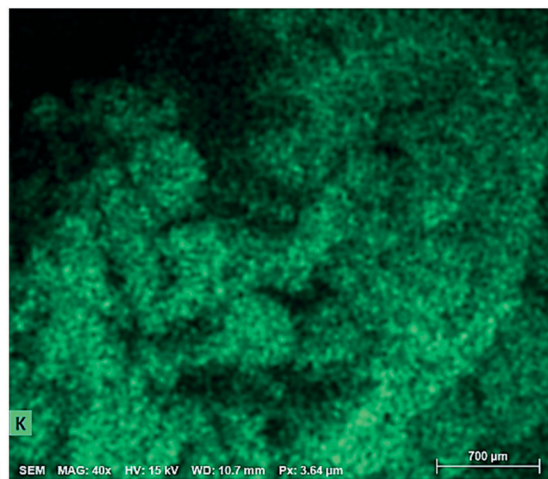
(b)



(c)



(d)



(e)

Fig. 5 EDX analysis of aerobic granule for a) elemental composition map, b) calcium, c) phosphorus, d) iron and e) potassium precipitates.





wastewater, but approximately  $40 \text{ mg L}^{-1}$  of  $\text{Ca}^{2+}$  was present in the local tap water used to prepare the synthetic feed.

In addition, precipitates of P were also detected on granular surface as shown in Fig. 5c. P can form salt precipitates with  $\text{Ca}^{2+}$  and can be biologically removed from the aqueous media by aerobic granules as previously reported.<sup>42</sup> Therefore, the presence of P precipitates on the surface of granules is an indication of phosphorous removal from wastewater. Previous research has shown that majority of  $\text{Ca}^{2+}$ , P and  $\text{Fe}^{3+}$  precipitates occur in the core of the granules compared to the surface.<sup>43,44</sup> Therefore, although EDX analysis were not conducted for the inside of the core but it is hypothesized that there would be a higher proportion of these multivalent ion precipitates. EPS also plays a key role in the accumulation and transferring of these elements from the aqueous phase into phosphate accumulating organisms (PAOs).<sup>45</sup> Consequently, it is suspected that PAOs must have been inhabited within the granules that aided in the removal of P through EPS formation.

In a real-life full-scale wastewater treatment plant, it is suggested that granular reactor on an optimized continuous

flow can be intermittently operated under anaerobic phase at low flow rate, thus, allowing selective pressure. This step can potentially ensure removal of P through anaerobic/aerobic phase while any surplus contaminant bypass during this period can be eliminated in the subsequent AOP stage due to increased retention time by low flow rate. An anaerobic feeding condition has also been linked to the formation of stable aerobic granules during a continuous flow reactor operation.<sup>46,47</sup>

The morphology of aerobic granules used in this study was analyzed through SEM and the images are shown in Fig. 6a. It is evident that filamentous bacteria were dominant on the surface of the granules. A similar formation of filamentous aerobic granules was reported by feeding sucrose at an OLR of  $6 \text{ kg COD m}^{-3} \text{ d}^{-1}$ .<sup>48</sup> However, the appearance of filamentous bacteria was linked to the disintegration of granules due to mass transfer limitations. Furthermore, irregular shaped granules with filamentous outgrowth was also observed and labelled as the reason for poor settleability of granules, disintegration and subsequent washout of biomass.<sup>49,50</sup> On the contrary, the filamentous granules observed in this study appeared more rigid and compact with

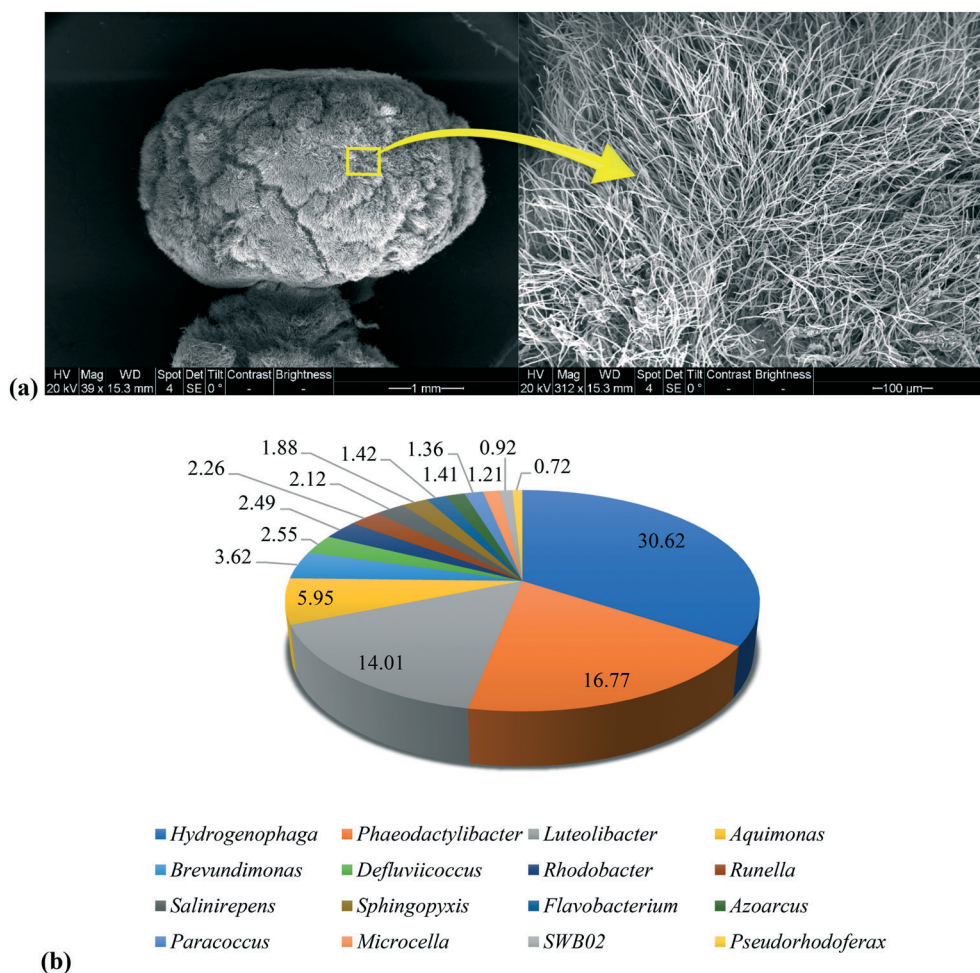


Fig. 6 Microbial analysis on the a) morphology of granules through SEM and b) granule microbial community composition (top 16) at genus level through 16S rRNA gene sequencing analysis.



a symmetrical distribution of filamentous bacteria on the surface and no indication of disintegration. It is understood that these granules did not suffer from mass transfer limitation because of a lower OLR which might have kept the growth of flocculant filamentous bacteria in control. Additionally, these filamentous bacteria appear to provide more stability for the granules by providing protection from toxic contaminants.<sup>51</sup> Our previous study also reported the long-term stability of these granules to be consistent over prolonged operation.<sup>11</sup>

A vast variety of diverse microbial communities' harbor within the aerobic granules. DNA analysis showed *Hydrogenophaga* to be the dominant bacterial genus as shown in Fig. 6b. This genus and *Pseudorhodoferrax* belong to *Burkholderiaceae* family which are known aromatic hydrocarbon pollutant degraders.<sup>52–54</sup> More importantly, another classification of this genus is *Pseudomonas*<sup>55</sup> which is considered as the main degrader of aromatic sulfonic acids such as sulfolane.<sup>56</sup> Additionally, *Paracoccus* species have also been reported to degrade sulfolane.<sup>57</sup> *Phaeodactylibacter*, *Paracoccus* and *Azoarcus* are considered as denitrifiers with the ability to use both oxygen and nitrogen as electron acceptors, thus allowing them to survive in aerobic and anaerobic conditions effectively.<sup>58–60</sup> *Paracoccus* and *Rhodobacter* both belonging to *Rhodobacteraceae* family have also been detected with *Burkholderiaceae* in ecological wastewater treatment plants targeting pharmaceuticals removal.<sup>61</sup> Therefore, it is understood that these genera can coexist together within the aerobic granules with the ability to degrade microcontaminants as observed in this study. *Flavobacterium* and *Sphingopyxis* genus were also identified in the 16S rRNA gene sequencing analysis of the mature aerobic granules. These microorganisms are important part of the granular community that aid in the production of EPS for granule stability and phosphorus accumulation.<sup>62</sup> As indicated earlier, there was evidence of PAOs being inhabited within the aerobic granules in this study. *Aquimonas* and *Luteolibacter* genus were present in higher abundance as well.

## 4. Conclusion

In this paper, an integration of aerobic granulation and UV/H<sub>2</sub>O<sub>2</sub> treatment has been shown as an effective approach to treat sulfolane in water. Aerobic granule process along with UV/H<sub>2</sub>O<sub>2</sub> as a post biological treatment process can achieve more than 99.99% of sulfolane degradation within 6 h and 10 min. The adaption of aerobic granule in wastewater treatment can potentially boost the performance of biological treatment, while the addition of UV/H<sub>2</sub>O<sub>2</sub> as a post-biological treatment can save space requirement in biological treatment to achieve the stringent discharge water guideline. The kinetics study revealed aerobic granulation process to follow zero order while UV/H<sub>2</sub>O<sub>2</sub> process was strictly first order. Furthermore, it was found that the kinetics obtained from batch studies of the UV/H<sub>2</sub>O<sub>2</sub> system can be used to predict sulfolane degradation kinetics in a continuous flow-through

operation. H<sub>2</sub>O<sub>2</sub> is one of the key operational parameters and one of the major costs for UV/H<sub>2</sub>O<sub>2</sub>. The H<sub>2</sub>O<sub>2</sub> management study in this paper showed that the presence of hydrogen peroxide is necessary for fast degradation and high percentage degradation of sulfolane but the excess of H<sub>2</sub>O<sub>2</sub> pose a potential threat to the discharge water receiving body. The selection of H<sub>2</sub>O<sub>2</sub> concentration ultimately depends on the contaminant concentration, water matrix and the retention time. The continuous flow-through system also allowed a healthy granular system to flourish which was evidenced by the identification of multivalent elemental ions, such as Ca<sup>2+</sup>, Fe<sup>3+</sup> and P, and diverse bacterial communities that aided in stable granular structure.

## Conflicts of interest

There are no conflicts to declare.

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