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Application of ultrasound-assisted NaClO in membrane bioreactors membrane cleaning

Jianming Cheng[a], Weiye Shi[a,b], Jianan Xiao[c], Yiran Ren[a], Defang Ma[a,*], Yan Wang[a], Baoyu Gao[a]

[a]*Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Qingdao 266237, China, email: defangma@sdu.edu.cn (D. Ma)*
[b]*No. 1 Institute of Geology and Mineral Resources of Shandong Province, Ji'nan 250014, China*
[c]*Shandong Huankeyuan Environmental Testing Co., Ltd., 250013 Shandong, China*

ABSTRACT

Sodium hypochlorite (NaClO) is widely used to remove organic and biological fouling from the membranes in membrane bioreactors (MBR). But using large amounts of NaClO at high concentrations during *in-situ* membrane cleaning adversely affect the activity of activated sludge in the reactor and produce toxic disinfection by-products (DBPs). In this study, ultrasound (US) was used in combination with NaClO to restore the permeability of microfiltration (MF) and ultrafiltration (UF) membranes fouled by activated sludge. We investigated the cleaning efficiency and mechanism of action of US-assisted NaClO, and the influence of US on the activated sludge properties. Low power US (25 kHz, 0.22 W/cm^2) destroyed the structure of polyvinylidene fluoride UF membranes, but did not damage mixed cellulose ester MF membranes. When higher power US (25 kHz, 0.44 W/cm^2) was used in combination with NaClO (100 mg/L), the MF membrane flux recovery was improved by 37.7% compared to NaClO alone. Confocal laser scanning microscopy images indicated that US generated holes in the compact protein areas of biofilm, which would promote the diffusion of NaClO, and enhance the removal of the fouling layer. Moreover, US increased the degradation of membrane foulants (L-tryptophan was chosen as the model substrate) indicated by the reduction in the consumption of NaClO, which might be due to the generation of free radicals. Although US deteriorated the settling performance and increased the extracellular polymeric substances secretion of activated sludge, MBR effluent water quality was not influenced, which makes *in-situ* US-assisted NaClO cleaning application possible.

Keywords: Membrane bioreactors; Membrane fouling; US-assisted NaClO cleaning; Extracellular polymeric substances

1. Introduction

As the demand for water reuse increases, membrane bioreactors (MBR) are being more extensively used due to their stable, high quality effluent and small footprint, especially in municipal wastewater treatment plants [1]. However, membrane fouling is an inevitable problem. Microorganisms, suspended solids and dissolved substances deposit on the membrane surfaces and in the pores, resulting in loss of membrane permeability, primarily due to the formation of biofilm [2]. Biofilm is mainly composed of microorganisms and their extracellular polymeric substances (EPS) secreted during metabolism, which causes the biofilm to wrap tightly onto the solid surface through a complex three-dimensional cross-linked matrix. Since sodium hypochlorite (NaClO) can both sterilize and remove organic matter through oxidation, regular *in-situ* chemical cleaning with NaClO has always been considered as one of the most effective methods to restore the membrane flux [3]. During *in-situ* chemical cleaning, NaClO (100–500 mg/L)

* Corresponding author.

is pumped backwards through the membrane modules. However, when NaClO enters the reactor, it will react with activated sludge and affect the activity of the microorganisms. Recent studies have also shown that NaClO reacts with microorganisms and dissolved organic matter (DOM) in activated sludge to produce large amounts of disinfection by-products (DBPs) [4]. Of note, chlorination of activated sludge is prone to generate more toxic DBPs due to the presence of mass nitrogenous organic matter, aromatic amino acids, etc. [5,6]. On the one hand, these DBPs are generally refractory small molecules that enter MBR effluents, causing deterioration of water quality. On the other hand, the mutagenic DBPs retained in activated sludge may induce or increase development of antibiotic resistance genes in the bacteria [7]. If membrane fouling is serious, a higher NaClO dosage and longer cleaning time are commonly applied. But increased microbial lysis releases more DOM, which promotes the formation of DBPs and decreases the MBR effluent quality [8]. Therefore, it is necessary to develop an efficient and green membrane cleaning method to reduce the NaClO concentration and cleaning time.

Ultrasound (US) has been widely used in many cleaning processes [9], and is considered as a candidate method in polymer membrane cleaning. US cavitation occurs because of the formation, growth and collapse of bubbles [10]. The great turbulence generated in the liquid phase due to the cavitation could efficiently strip pollutants from the liquid–solid interface.

Several studies have shown that the combination of US and chemical agents enhances the membrane cleaning efficiency compared with US or chemical reagents separately, and low frequency US improved membrane cleaning and protected the membrane structures because the bubbles are larger and have a longer lifetime [11]. Thombre et al. [12] used hydrophilic polyamide nanofiltration membranes that were fouled by petroleum refinery effluents and found that after a 4 min combination treatment using 1.0 M aqueous NaOH and US (25 kHz, 135 W), almost 90% of the initial water flux was recovered. Membrane damage was not observed in this study. US-assisted acid cleaning was also found to significantly increase the removal efficiency of inorganic scales on a nanofiltration membrane surface. The membrane cleaning time was also shortened by 1/3–2/3 compared with just acid cleaning [13]. Wang et al. [14] found that US improved the cleaning effect of 0.4% NaClO on membranes when treating polyvinylidene fluoride (PVDF) membranes fouled by fermentation broth. The membrane flux recovery increased from 29.5% to 37.5% after adding US. The enhancement was more obvious when the NaClO concentration was increased to 1.0%. Previous studies have focused on the US-assisted cleaning performance for a single type of membrane fouling, such as inorganic or organic fouling. However, a combination of organic and biofilm fouling is common in MBRs. Application of US-assisted NaClO for MBR membrane cleaning has not been reported and its mechanism of fouling removal is also unknown.

Herein, we aim to evaluate the cleaning effect of US-assisted NaClO in MBR membrane cleaning, and probe the cleaning mechanism by analysis of the fouling layer structure, NaClO mass transfer and NaClO consumption kinetics. The effects of US treatment on the performance of activated sludge were also investigated to evaluate the potential of US for *in-situ* application.

2. Materials and methods

2.1. Reagents

Unless specified, all chemicals used in this study were analytical grade reagents purchased from Sinopharm (Shanghai, China) with purity over 99% and used without further purification. L-tryptophan, anthrone and 2,7-dichlorofluorescein diacetate (DCF-DA) were purchased from Macklin (Shanghai, China). Concanavalin A (ConA) and Calcofluor white (CW) were purchased from Maokangbio (Shanghai, China). Fluorescein isothiocyanate (FITC), bovine serum albumin (BSA), phosphate buffered saline (0.01 mol/L, pH 7.2–7.4) and DOWEX 50 × 8, 20–50 mesh in sodium form were purchased from Shyuanye (Shanghai, China), BioFroxx (Guangzhou, China), Phygene (Fuzhou, China) and Anland (Shanghai, China), respectively.

2.2. Experimental setup

We set up one dead-end microfiltration (MF) and two cross-flow ultrafiltration (UF) plate membrane cells for the membrane fouling experiments. The activated sludge was provided by a laboratory scale submerged MBR, related details were shown in S1 and Table S1. For the MF filtration cell, we chose a mixed cellulose ester membrane with normalized pore size of 0.22 μm and effective membrane area of 4p cm^2 (Shanghai Xinya Purification Device Factory, Shanghai, China), it was fouled by pumping sludge in small quantities but several times. For the UF filtration cell, we used a PVDF plate membrane with normalized pore size of 0.05 μm and effective membrane area of 35 cm^2 (Guochu Technology Co. Ltd., Xiamen, China), it was fouled by 5 d operation continuously.

2.3. Membrane cleaning

After a period of use, the fouled membrane was placed in a polystyrene box with pure water or NaClO which was put into an US cleaner (KH-1000SP, Kunshan, China; Fig. S1). All cleaning processes were conducted at 25°C ± 1°C. Based on preliminary experiments, the US cleaning was performed using a frequency of 25 kHz and in pulsed mode (on for 30 s and off for 2 min). These conditions could achieve efficient membrane cleaning as well as prevent membrane damage and reduce energy consumption [15]. The cleaning efficiencies of US at the intensity of 0.22 and 0.44 W/cm^2 were both investigated. In NaClO alone and US-assisted NaClO cleaning, the chlorine dosage was 100 mg/L.

Membrane flux recovery was used to evaluate the membrane cleaning efficiency of various methods of MF and UF membrane cleaning. Scanning electron microscopy (SEM, Quanta 250 FEG, FEI, USA) was also used to probe the effects of various cleaning methods on the morphology of the fouling layer on the membrane surface. The samples were dried naturally at room temperature for 12 h and sprayed with gold under vacuum for 240 s using an ion sputter to increase their electrical conductivity.

2.4. Multiple fluorescence labeling and confocal laser scanning microscopy analysis

To clarify the effect of US-assisted NaClO cleaning on the structure and components of biofilm EPS on the membrane surface, confocal laser scanning microscopy (CLSM, LSM900, Zeiss, Germany) accompanied with multiple fluorescence labeling was applied to characterize the biofilm after the various treatments [16]. Proteins, α-D-glucopyranose polysaccharides and β-D-glucopyranose polysaccharides were labeled with FITC, ConA and CW; the excitation laser wavelengths used were 488, 543 and 400 nm [4], respectively.

In order to trace the penetration of NaClO in the membrane fouling layer during different cleaning processes, active chlorine was labeled with DCF-DA and analyzed by CLSM, with excitation wavelength of 488 nm and emission wavelength of 510–560 nm [17].

2.5. NaClO decay kinetics

Foulant degradation and NaClO decay kinetics experiments were conducted to analyze the effect of US addition on NaClO oxidizing ability. L-tryptophan (51.6 mg/L) was chosen as the model substrate to simulate the DOM in the sludge supernatant. The concentration of dissolved organic carbon (DOC) in the sludge supernatant and the DOC contribution of the model L-tryptophan solution were the same (33.4 mg/L). First, 400 mL of L-tryptophan working solution was added into a 500 mL brown glass bottle with a PTFE cap. While stirring with a magnetic stirrer, a certain volume of NaClO working solution was added to the bottle to obtain a chlorine dosage of 100 mg/L. Then the bottle was immediately filled with L-tryptophan working solution and mixed evenly. Finally, the solution was rapidly divided into multiple small brown bottles, and subjected to reaction with or without US. Samples were taken from the vials to measure the residual chlorine and L-tryptophan at certain intervals. Residual chlorine concentration was measured according to the DPD method [18], and the L-tryptophan concentration was measured using high-performance liquid chromatography (HPLC).

2.6. Characterization of activated sludge

Mixed liquor suspended solids (MLSS), sludge settling velocity (SV), sludge volume index (SVI) and specific oxygen uptake rate (SOUR) were determined according to standard methods. Sludge supernatant DOC was analyzed using a TOC analyzer (TOC-VCPH, Shimadzu, Japan); the sample was filtered with 0.22 μm MF membrane. An ion-exchange resin [19] was used for the extraction of EPS, more details are in S2. The protein and polysaccharide contents of EPS were determined using the Lowry [20] and sulfate-anthrone methods [21] with BSA and glucose as the standard samples, respectively.

To comprehensively characterize the effects of US on the activity of activated sludge, the DOC removal efficiency of activated sludge after US stimulation in treating synthetic sewage was determined by beaker experiments. The details are in S3.

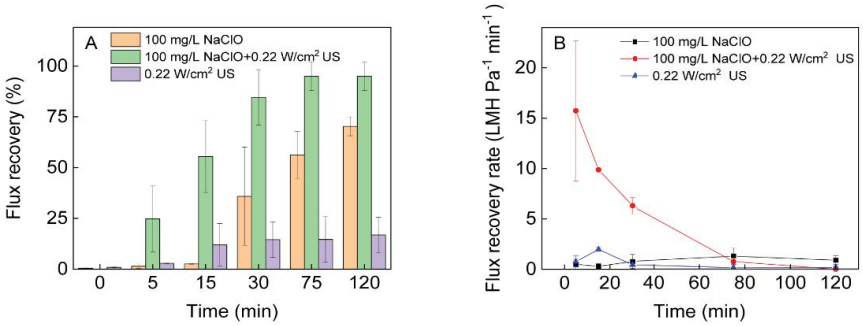
3. Results and discussion

3.1. Comparison of membrane flux recovery after different cleaning methods

3.1.1. Microfiltration membranes

To evaluate the effects of different membrane cleaning methods, we measured the membrane flux recovery and flux recovery rate of fouled MF membranes after cleaning for 5 to 120 min. Despite the variation in cleaning technologies and US power, prolonging the cleaning time generally improved the efficiency in membrane flux recovery. An enhanced effect in membrane fouling removal was observed when US and NaClO were used together. The membrane flux recovery for NaClO was 70.3% while for US alone it was 16.8% after 120 min cleaning. Notably, the membrane flux recovery improved to 84.6% after only 30 min of US-assisted NaClO cleaning (Fig. 1A).

The US-assisted NaClO cleaning process also significantly increased the membrane flux recovery rate (Fig. 1B). The membrane flux recovery rate of US-assisted NaClO was far greater than NaClO alone (8.1–31.1x) or US alone (14.3–20.4x) within the first 30 min. Specifically, when using NaClO alone, the membrane flux recovery rate was slow during the first 15 min, but then increased. This result could be explained by slow diffusion of NaClO in the compact biofilm, where the membrane flux recovery is achieved by a layer-by-layer dissociation of the foulants [17]. At the very beginning of the membrane cleaning process, NaClO could only oxidize the outer layer of foulants, which achieved limited membrane flux recovery. As the surface foulants were detached, NaClO penetrated further and gradually reached and reacted with the deeper layer of the biofilm, which in turn improved the membrane flux recovery rate. In contrast, when using US alone, we observed a



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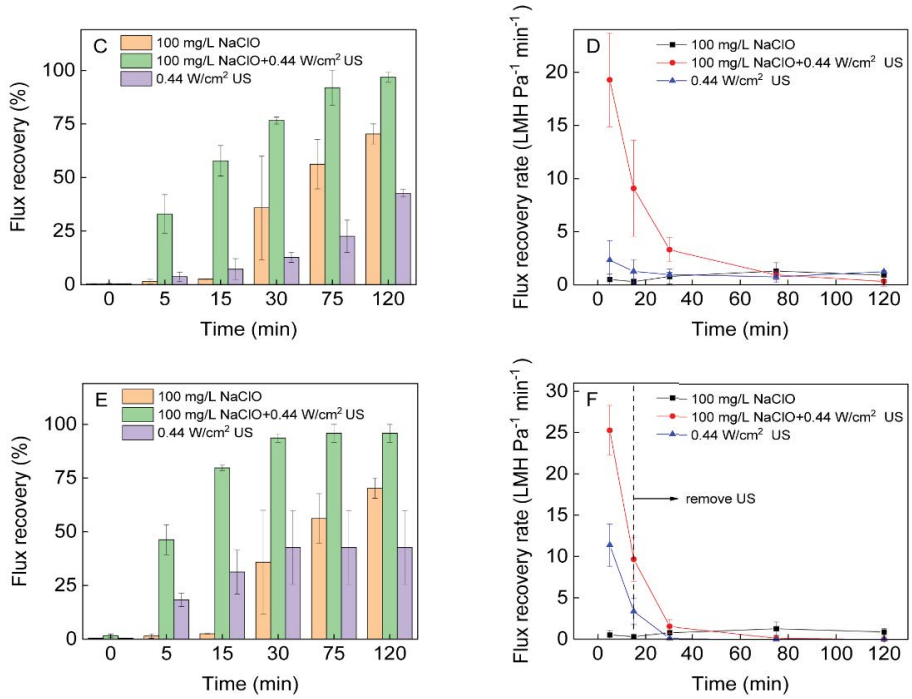

Fig. 1. (A, C, E) membrane flux recovery and (B, D, F) rate following NaClO, US-NaClO and US cleaning for 120 min, respectively. Reaction conditions: [NaClO] = 100 mg/L, (A, B) power intensity of US was 0.22 W/cm², intermittently used for 120 min. (C, D) power intensity of US was 0.44 W/cm², intermittently used for 120 min. (E, F) power intensity of US was 0.44 W/cm², used intermittently for the first 15 min only.

relatively higher membrane flux recovery rate initially that decreased after 15–30 min. Because US plays an important role in the overall relaxing and flaking of the biofilm, the massive removal of the foulants at the beginning may be the reason for the rapid flux recovery. Notably, for US assisted-NaClO, the trend resembled that of US alone, but its initial membrane flux recovery rate was much higher than that of either US or NaClO alone. Hence, US played a vital role in removing membrane foulants.

When the power intensity of US increased to 0.44 W/cm², both membrane flux recovery and flux recovery rate after US-assisted NaClO cleaning were enhanced (Fig. 1C and D), especially in the first 15 min, because more energy is provided by higher power US [11].

Since the flux recovery was so marked in the first 15 min, we tested the effect of a shorter US working time, which would further reduce the energy consumption (Fig. 1E and F). US (0.44 W/cm²) was applied for only the first 15 min, then the membrane was allowed to sit in the solution for different times (up to a total of 120 min) prior to measurement. Under these conditions, the membrane flux recovery increased by 36.3% after 120 min, compared to NaClO alone. The enhancement was only slightly less than that of using US throughout. It is worth noting that after the US treatment was stopped (after 15 min), significant flux recovery was observed for both US alone and US assisted-NaClO, which might come from the weak removal of the biofilm by the action of water for the former and the oxidation of NaClO for the latter. These results imply that US-assisted NaClO cleaning is an efficient and energy-saving strategy.

The effects of various cleaning methods on the structure of MF membrane were studied by SEM imaging (Figs. S2 and S3). US-assisted NaClO had no impact on the original interconnected structures of MF membranes. The overall results demonstrate that US-assisted NaClO cleaning strategy is a potential candidate for MBR membrane cleaning.

3.1.2. Ultrafiltration membranes

To investigate the possibility of using US-assisted NaClO cleaning on UF membranes, we tested the ability of the membrane to withstand short duration, low power US treatment. When US was applied for only 5 min at a power intensity of 0.22 W/cm², the UF membrane was mechanically damaged. The membrane flux recovery exceeded 100% (Fig. S4) and SEM images showed the increase of membrane pore size and loosening of surface structure (Fig. S5). Previous studies on the effects of US on PVDF membrane structure gave contradictory results. Some studies also concluded that US damaged PVDF membranes [22,23], but others did not [24,25]. These contradictory results might arise from the different hydrophilic modification methods used to prepare commercial PVDF UF membranes. Companies use different methods to increase the hydrophilicity of the membrane: (a) surface modification and (b) co-blending modification. The first involves coating or grafting a functional layer on the membrane surface [26]. Hence, the coating on the membrane surface is physically attached to the surface and may be easily damaged by US. However, in co-blending modification, the membrane components are

covalently bonded and homogeneous, which may resist damage by US. As in this study, the method used to prepare the commercial membranes may be proprietary, however, surface coating is widely used due to its cost-efficiency and relative simplicity. Therefore, we conclude that US cannot be freely used for PVDF UF membrane cleaning and MF membranes were used in the following experiments.

3.2. Mechanisms of US-assisted NaClO membrane cleaning

3.2.1. EPS distribution and NaClO diffusion in the biofilm with CLSM

As shown in section 3. 1, US-assisted NaClO treatment showed the same cleaning effect after 120 min even if the US treatment was stopped after 15 min. To investigate the cleaning mechanism, CLSM was used to characterize EPS distribution and NaClO diffusion in the fouling layer after 15 min of US at 0.44 W/cm^2.

It is clear from Fig. 2A that the EPS in biofilm is mostly DOM inated by protein (green). US-assisted NaClO cleaning for 15 min removed both protein and polysaccharides (Figs. 2A and S6). Compared with NaClO cleaning alone, after US-assisted NaClO cleaning, there were great numbers of holes in the compact protein layer (Fig. 2B), showing that US may facilitate the further penetration of NaClO into the biofilm. Like the US-assisted NaClO cleaning, US cleaning alone significantly damaged the compact protein layer (Fig. 2C). This result implies that US induced damage to the EPS matrix structure and played a vital role in removing membrane foulants. However, US alone had limited effects on the α-D-glucopyranose polysaccharides (red) and β-D-glucopyranose polysaccharides (blue) (Fig. 2C). In contrast, NaClO significantly removed the two polysaccharides but left more protein (Fig. 2D).

Moreover, US alone removed more EPS in total than US-assisted NaClO cleaning (Fig. S6), which was mainly attributed to the substantial removal of protein by US alone. However, the removal of protein was reduced in exchange for the enhanced removal of β-D-glucopyranose polysaccharides during US-assisted NaClO cleaning. The membrane flux recovery of US-assisted NaClO was much stronger than that of US alone, which indicated that β-D-glucopyranose

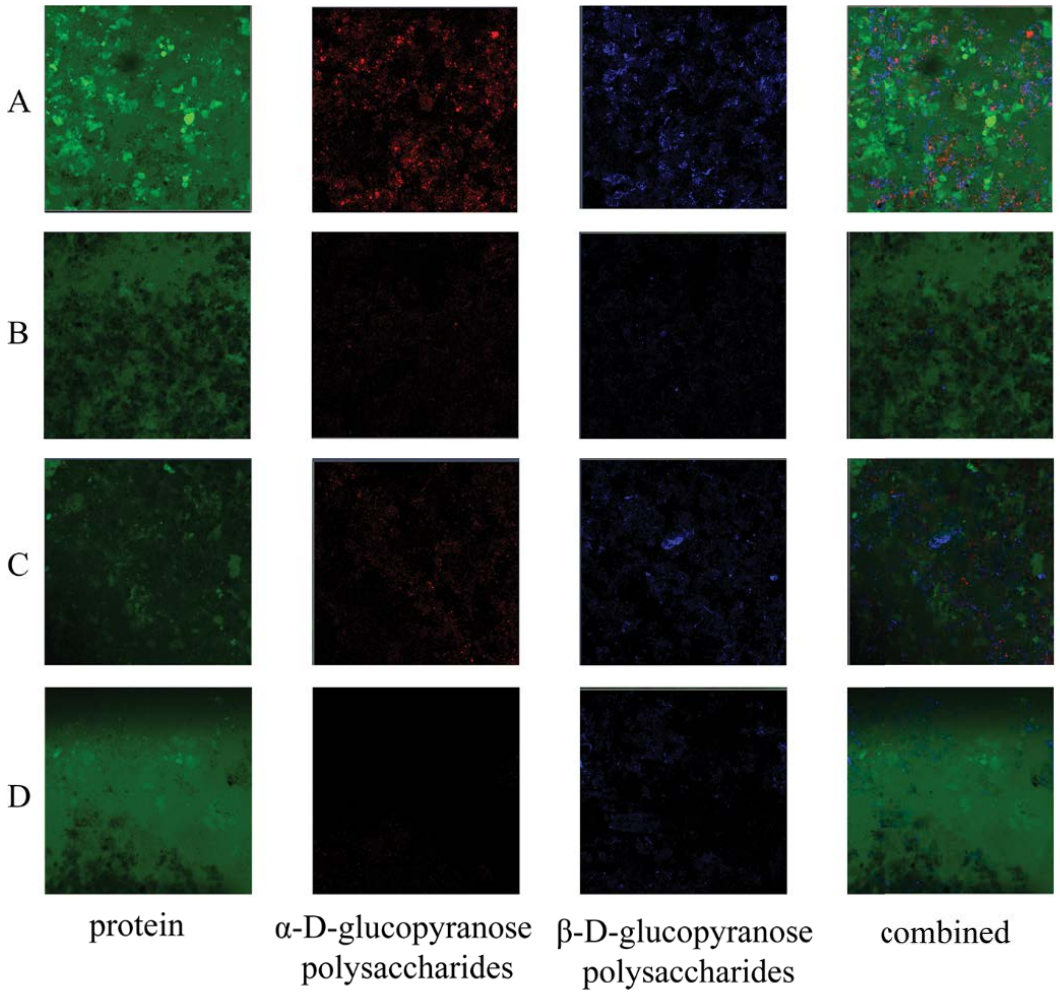

Fig. 2. EPS including protein (green), α-D-glucopyranose polysaccharides (red), β-D-glucopyranose polysaccharides (blue) and combined images of the fouled membrane (A) before cleaning, after (B) US-NaClO, (C) US and (D) NaClO cleaning. Reaction conditions: [NaClO] = 100 mg/L, power intensity of US was 0.44 W/cm^2, used intermittently for 15 min.

polysaccharides were the main reasons for the decrease in membrane flux, despite their small content.

To further characterize the effect of US on NaClO diffusion within the fouling layer, CLSM was also operated to observe the penetration of NaClO (Fig. 3). First, the thickness of the fouling layer after US-assisted cleaning was reduced to about half of that of NaClO alone, which strongly demonstrated again that combined cleaning achieved better cleaning performance in a short period of time. In order to quantify the diffusion of NaClO within the fouling layer, the average fluorescence intensity per unit volume was calculated by dividing the total fluorescence intensity by the volume within the CLSM images. The fluorescence intensity at different positions within each z-stack layer was used to calculate the standard deviation, which represents the uniformity of the NaClO distribution in each intra-layer (error bars in Fig. S7). Here, the average fluorescence intensity per unit volume and penetration uniformity of NaClO in US-assisted NaClO process were 1.68 and 1.42 times higher than that without US. This result implies that NaClO diffuses more easily in the fouling layer with the help of US. The mechanical breakage of the biofilm structure by US allowed NaClO to penetrate rapidly into the bottom of biofilm through the holes generated by US, rather than the layer-by-layer reaction with NaClO alone. This reduced the consumption of NaClO and thus improved the removal of substances that severely reduce membrane flux, such as β-D-glucopyranose polysaccharides. This finding is consistent with our previous hypothesis for the mechanism of slow membrane flux recovery using NaClO alone (Section 3.1 – Comparison of membrane flux recovery after different cleaning methods).

These results indicate that in the US-assisted NaClO cleaning process, US-induced damage to the biofilm protein matrix facilitated the NaClO diffusion in the fouling layer, and NaClO oxidation enhanced the detachment of biofilm polysaccharides, whereas the synergy of US and NaClO improved the removal of foulants from the membrane surface and in turn efficiently restored the membrane flux.

3.2.2. NaClO consumption kinetics under US

To further explore the role of US in the biofilm degradation, we tested NaClO consumption kinetics. We measured the degradation kinetics of L-tryptophan and chlorine decay, where L-tryptophan was chosen as the model substance of biofilm EPS. Since US increased the temperature of reaction system, the initial temperature of the reaction was set at 25°C, and we recorded the temperature changes of the system at different times (Fig. 4A). Based on the results, the temperatures of degradation using NaClO alone were operated at 25°C, 35°C, and 45°C. L-tryptophan was rapidly degraded within 5 min in all systems (Fig. S8). When using NaClO alone, as the temperature increases, the consumption of NaClO also increases (Fig. 4B). However, even the temperature raised to 45°C at the end, US addition significantly decreased the NaClO consumption. Similar results can be found in the study by Zou and Wang [27] which showed the decrease in the NaClO consumption during disinfection when using US and NaClO together. It has been demonstrated that US-induced cavitation produces $^{\bullet}OH$ and H_2O_2 [28–30]. These species exhibited higher oxidative reactivity than NaClO and might contribute to the improved membrane flux recovery efficiency in the US-assisted membrane cleaning process. In addition, the less consumption of NaClO resulted in more chlorine residual, which promoted the diffusion of chlorine in the biofilm and improved the foulant removal, especially the removal of β-D-glucopyranose polysaccharides, and then enhanced the membrane flux recovery.

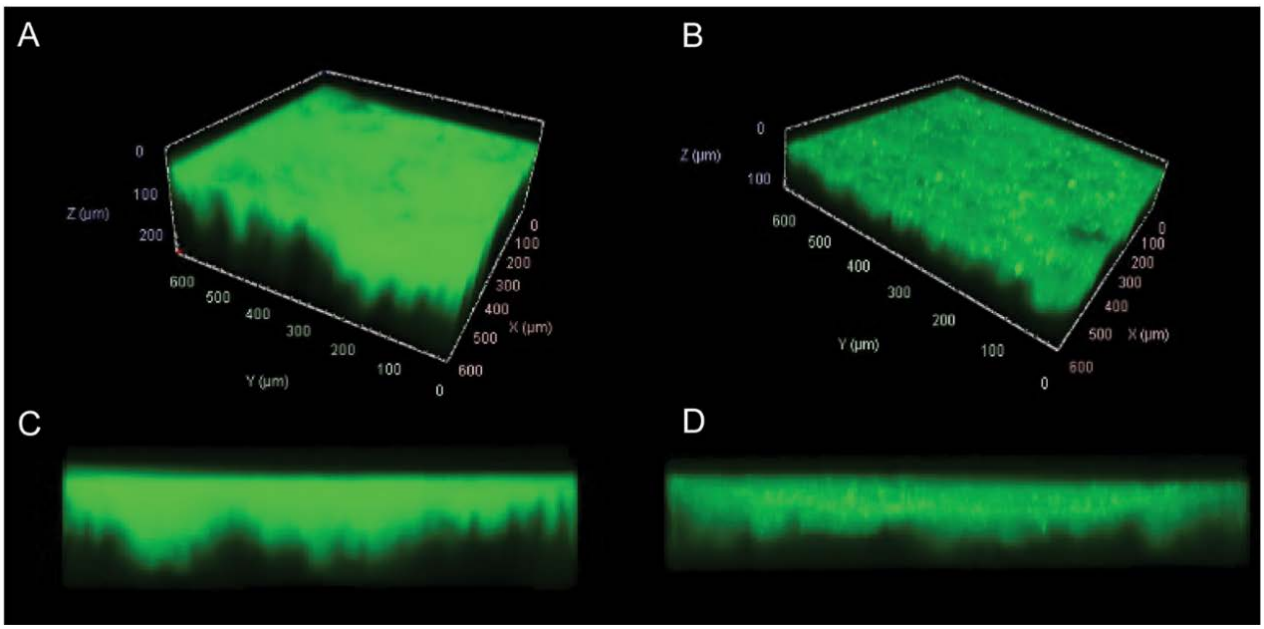

Fig. 3. CLSM (A, B) z-stack 3D and (C, D) vertical section images of NaClO in fouling layer after (A, C) NaClO and (B, D) US-NaClO cleaning. Reaction conditions: [NaClO] = 100 mg/L, power intensity of US was 0.44 W/cm^2, used intermittently for 15 min.

3.3. Effect of US on sludge properties

We evaluated the influences of US on activated sludge from MBR. Standard sludge characteristics (MLSS, SVI, SOUR, supernatant DOC and EPS) were tested after continuous US treatment of 0, 1, 3, 5, 8, and 10 min under the power intensity of 0.22 and 0.44 W/cm^2. Because the properties of activated sludge are different every day, to make the results more comparable, the data of each characteristic are presented as a ratio (e.g., $MLSS_t/MLSS_0$).

The MLSS and settlement performance of activated sludge decreased slightly after US treatment (Fig. S9), which might be due to the sludge disintegration caused by US [31]. US treatment resulted in an increase in SOUR of activated sludge (Fig. 5A), which implies a gain in microbial metabolic activity. This might be due to the fact that US promotes the transport of substances inside and outside the cell and increases the rate of enzymatic reaction [32,33]. This effect rises with time. However, at 0.44 W/cm^2, although SOUR was elevated, it was lower than that at 0.22 W/cm^2. US at

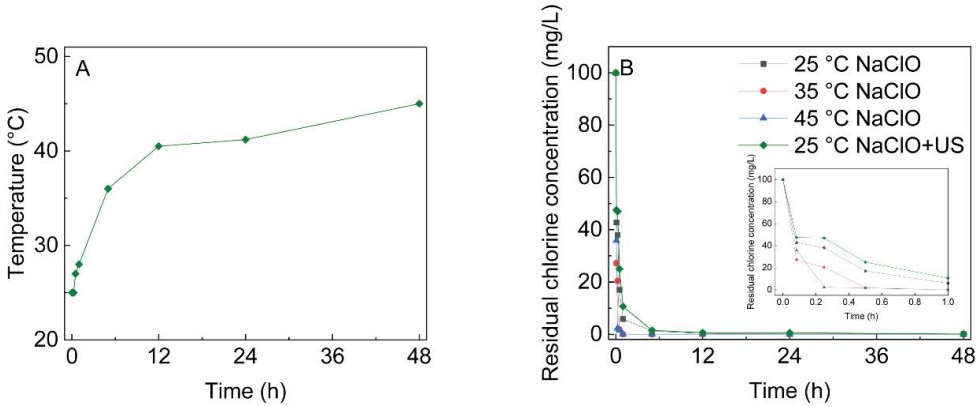

Fig. 4. Changes of (A) temperature in US-NaClO system and (B) residual chlorine concentration under different reaction conditions. The illustration in B shows the residual chlorine concentration during the first 1 h.

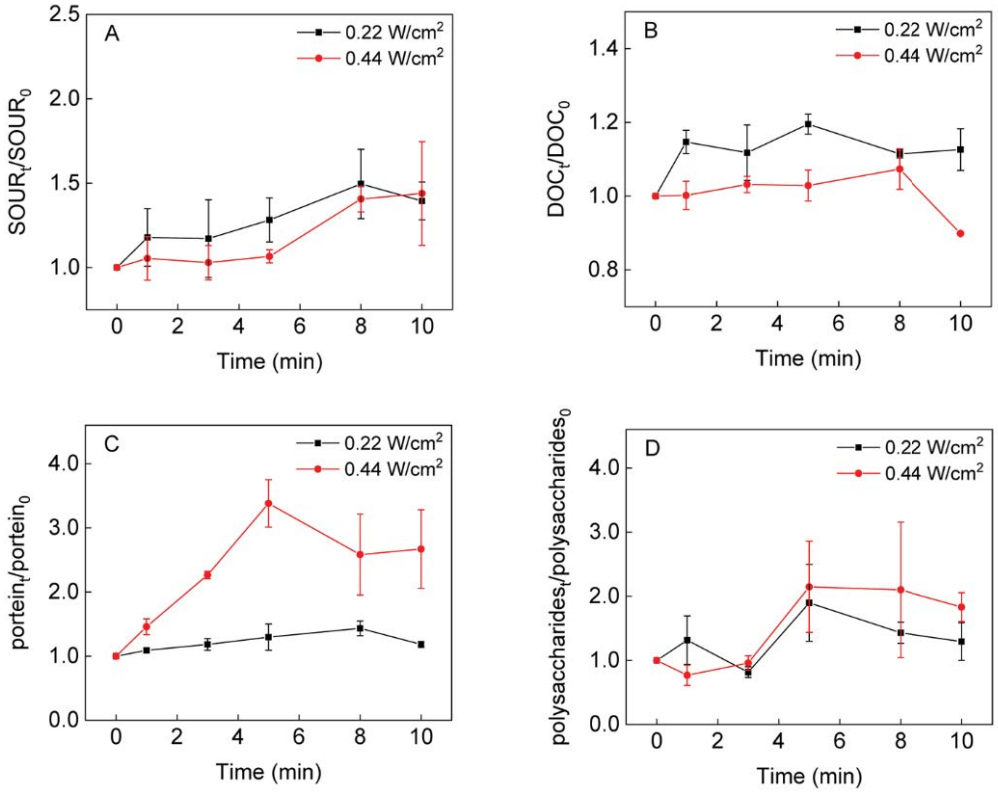

Fig. 5. Effect of US reaction time and power intensity on (A) SOUR, (B) supernatant DOC, (C) protein and (D) polysaccharides.

higher power intensity produces a more pronounced biological stimulation to the activated sludge, then the microorganisms produce a stress response by secreting more EPS and reducing the respiration rate to protect themselves [34] (Fig. 5C and D).

Low power US (0.22 W/cm^2) led to an increase in DOC (Fig. 5B). Loosely bonded EPS on activated sludge surface might be stripped off into the water due to the intense turbulence in the aqueous phase caused by US. In addition, there might be more soluble microbial products (SMP) stimulated by accelerated metabolism and intracellular matter released by cell rupture into supernatant [31]. High power US (0.44 W/cm^2) did not change the DOC significantly until after 8 min, when it decreased sharply. Protein secretion rapidly increased within 5 min (Fig. 5C). The data suggest that the microorganism initiate stress response to prevent higher power intensity of US from destroying them, including reducing their metabolism and enhancing EPS secretion. Massive secreted EPS tightly wrapped outside the cells, which was not easily stripped away by the US turbulence, and it also reduced the contribution of intracellular matter caused by cell rupture. In addition, it has been shown that EPS can absorb DOM [35], which causes supernatant DOC decrease. When the US stimulation was extended to 10 min, due to the high US power, $^{\bullet}OH$ and H_2O_2 were likely to be generated in the system, which degraded DOM into volatile small molecules [36,37], the supernatant DOC also reduced by their volatilization under US stirring and heating.

In conclusion, the effects of US on sludge properties are complex: (a) it enhanced the sludge activity, (b) degraded DOM and (c) promoted the secretion of EPS. To verify whether *in-situ* US can be used in practical MBR membrane cleaning, the performance of US treated activated sludge in degradation of contaminants was investigated by using effluent DOC concentration as an indicator (Fig. 6). There was no significant increase in effluent DOC after 6 h at both 0.22 and 0.44 W/cm^2. This result indicates that US does not ultimately affect the water quality of MBR effluents, which makes *in-situ* US-assisted NaClO cleaning a potential candidate in MBR membrane cleaning.

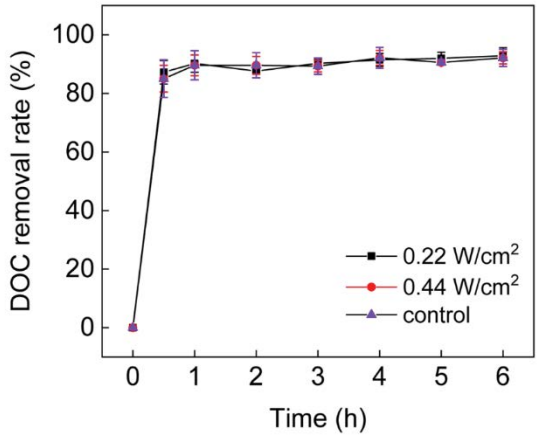

Fig. 6. Effect of US power intensity on sludge degradation capacity of organic matter. Reaction conditions: MLSS = 6 g/L, T = 25°C, DOC_0 = 281 mg/L (same to the MBR influent).

4. Conclusions

US-assisted NaClO was more effective for MF membrane cleaning compared to US or NaClO cleaning alone. US can produce holes in the compact protein matrix of biofilm, then promote the diffusion of NaClO in the fouling layer and reduce NaClO consumption. For activated sludge, US reduced its settlement performance, promoted the secretion of EPS but enhanced the sludge activity and degraded DOM. Overall, it did not have negative impact on effluent water quality. *In-situ* US-assisted NaClO cleaning is a potential candidate in MBR membrane cleaning. Although US caused the damage to PVDF UF membrane, future studies can be devoted to the effects of combined cleaning method on ceramic membrane, which has a stronger mechanical resistance. It is also possible to investigate the role of cleaning methods on rate and degree of membrane recontamination.

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Supplementary information

S1. The setup of laboratory-scale submerged hollow fiber membrane bioreactors

We set up a laboratory-scale submerged hollow fiber membrane bioreactors (MBR) (effective volume was 12 L) for providing samples. The activated sludge was taken from the aeration tank of a wastewater treatment plant. After settling, skimming off the supernatant and aerating for 5 h, the sludge was transferred into the MBR to domestication for 3 weeks. The sludge was fed with simulated sewage, and during the domestication period, the concentration of sewage was increased gradually, so that the activated sludge adapted to the water quality. The final characteristics of simulated sewage are shown in Table S1. The MBR was operated at 25°C ± 1°C with hydraulic retention time (HRT) of 6 h, sludge retention time (SRT) of 30 d and dissolved oxygen (DO) of 4.0 ± 1.0 mg/L. During the operation, we monitored the mixed liquor suspended solids (MLSS), sludge volume index (SVI) and dissolved organic carbon (DOC) every 2 d to ensure the stability of MBR.

Table S1
Characteristics of the simulated sewage

Components	Concentrations (mg/L)	Components	Concentrations (mg/L)
CH_3COONa	760	$CuSO_4{\cdot}5H_2O$	0.39
$(NH_4)_2SO_4$	280	$MnCl_2{\cdot}4H_2O$	0.27
KH_2PO_4	28	$CoCl_2{\cdot}6H_2O$	0.42
$MgSO_4{\cdot}7H_2O$	5.07	$FeCl_3{\cdot}6H_2O$	2.42
$ZnSO_4{\cdot}7H_2O$	0.44	$CaCl_2$	0.20

S2. Extraction of extracellular polymeric substances using ion-exchange resin

Extracellular polymeric substances (EPS) was extracted by ion-exchange resin method. Sludge (30 mL) was put in a centrifuge tube first and centrifuged at 4,000 g for 10 min using a benchtop high-speed frozen centrifuge (TGL-16M, Velcom). After discharge of the supernatant, the precipitate was resuspended with phosphate buffered saline (PBS). The resuspended mixture was diluted to 300 mL with PBS and transferred into a 1 L beaker. Then DOWEX 50 × 8 in the sodium form exchange resins were added into the beaker at the amount of 70 g resin/g

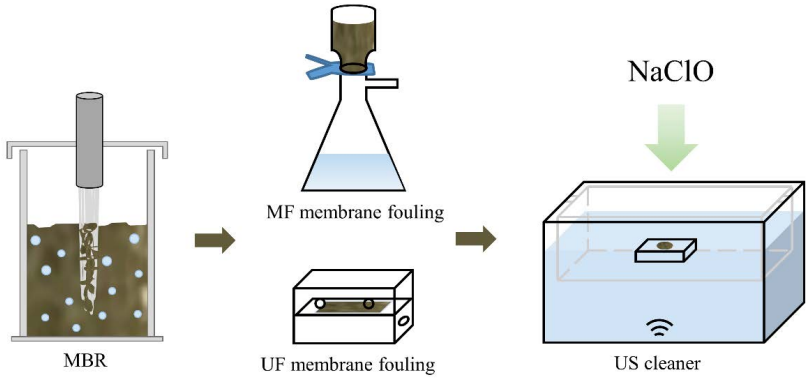

Fig. S1. Diagram of MBR, MF and UF membrane fouling and membrane cleaning.

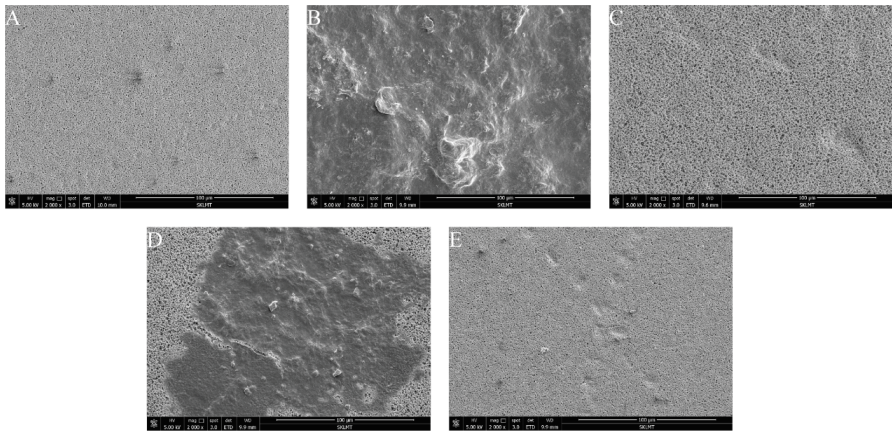

Fig. S2. SEM images (2,000x) of MF membranes (A) without fouling, (B) fouled membrane without cleaning, after (C) NaClO, (D) US and (E) US-NaClO cleaning for 120 min. Reaction conditions: [NaClO] = 100 mg/L, power density of US was 0.44 W/cm^2, intermittently used for the first 15 min only.

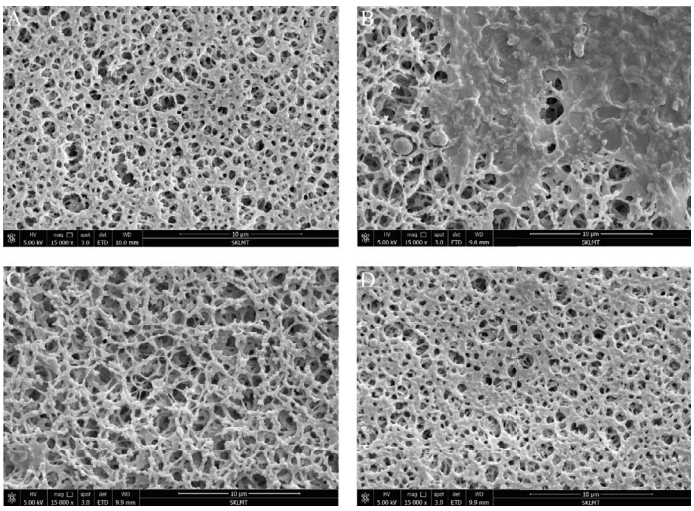

Fig. S3. SEM images (15,000x) of MF membranes (A) without fouling, after (B) NaClO, (C) US and (D) US-NaClO cleaning for 120 min. Reaction conditions: [NaClO] = 100 mg/L, power density of US was 0.44 W/cm^2, intermittently used for the first 15 min only.

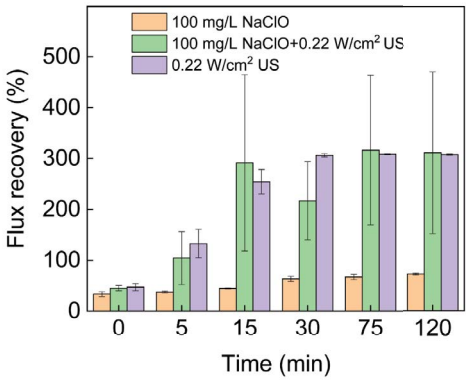

Fig. S4. Water flux recovery following different cleaning methods by 120 min. Reaction conditions: [NaClO] = 100 mg/L, power density of US was 0.22 W/cm^2, intermittently used for the first 30 min only.

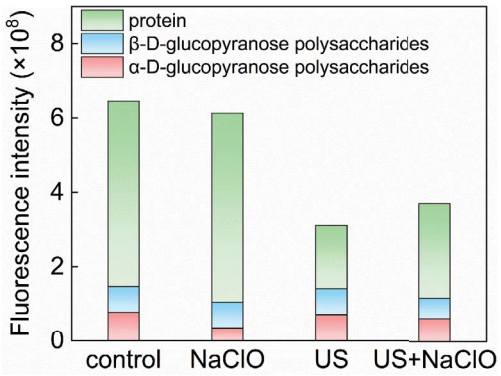

Fig. S6. Contribution of EPS components calculated from CLSM images before cleaning (control), and after NaClO, US and ultrasound-NaClO cleaning for 15 min. Reaction conditions: [NaClO] = 100 mg/L, power density of US was 0.44 W/cm^2, used intermittently.

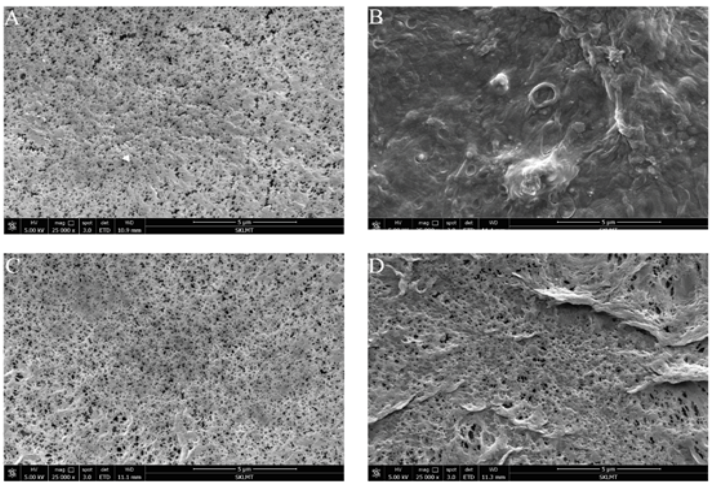

Fig. S5. SEM images (25,000x) of UF membrane (A) before fouling, after (B) NaClO, (C) US and (D) US-NaClO cleaning for 120 min. Reaction conditions: [NaClO] = 100 mg/L, power density of US was 0.22 W/cm^2, intermittently used for the first 30 min only.

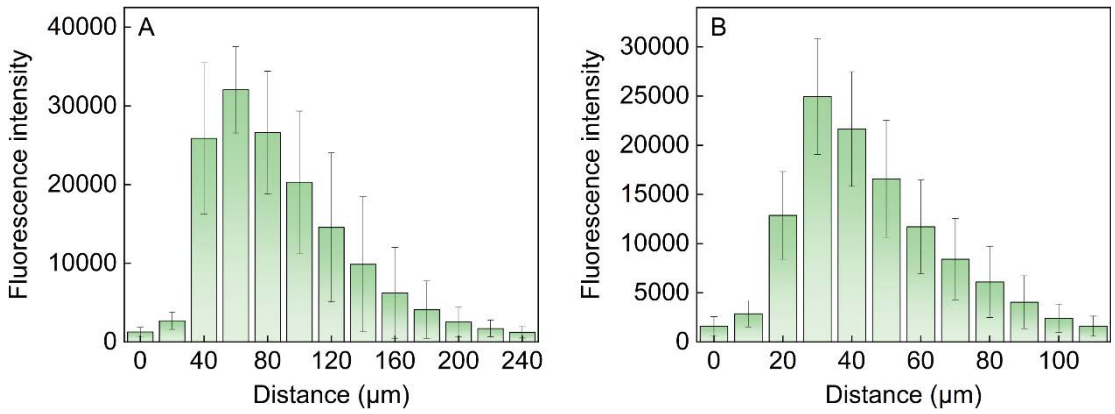

Fig. S7. Depth distribution of fluorescently-labeled NaClO within the biofilm on membrane surface after (A) NaClO and (B) US-NaClO cleaning for 15 min calculated from the CLSM images. The error bar represents the intra-layer uniformity of NaClO concentration at each depth. The distance is calculated from the top of the biofilm. Reaction conditions: [NaClO] = 100 mg/L, power density of US was 0.44 W/cm^2, used intermittently.

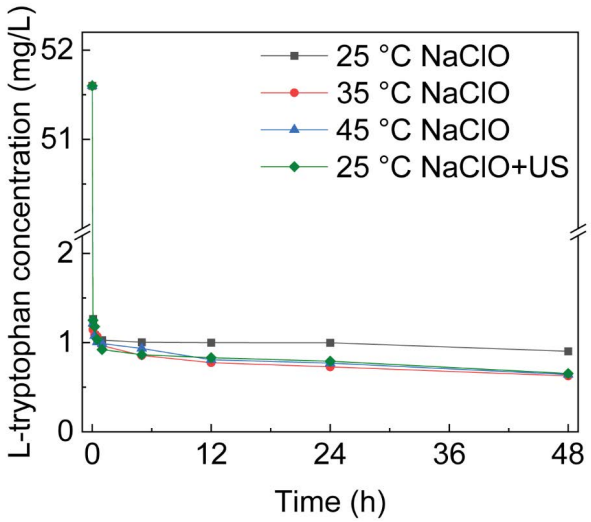

Fig. S8. Degradation kinetics of L-tryptophan under different reaction conditions.

MLVSS. The beaker was placed on a coagulation test mixer (ZR4-6, Zhongrun), stirred at 600 rpm for 1 h. After that, the mixture was centrifuged at 10,000 g for 15 min, and the supernatant filtered through 0.22 μm microfiltration (MF) membrane was EPS solution.

S3. Beaker experiments of US-treated activated sludge treating synthetic sewage

Sludge (720 mL) taken from MBR were mixed well and placed in three 500 mL beakers, and then treated with or without US. After that, the sludge was centrifuged at 4,000 g for 10 min. After discharge of the supernatant, the precipitate was resuspended with PBS and diluted to the original volume. Repeat this proceure to remove the supernate dissolved organic matter. The "cleaned" sludge was placed in beakers that contained the same simulated sewage and under the same operation conditions. The samples were taken every 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h to measure DOC.

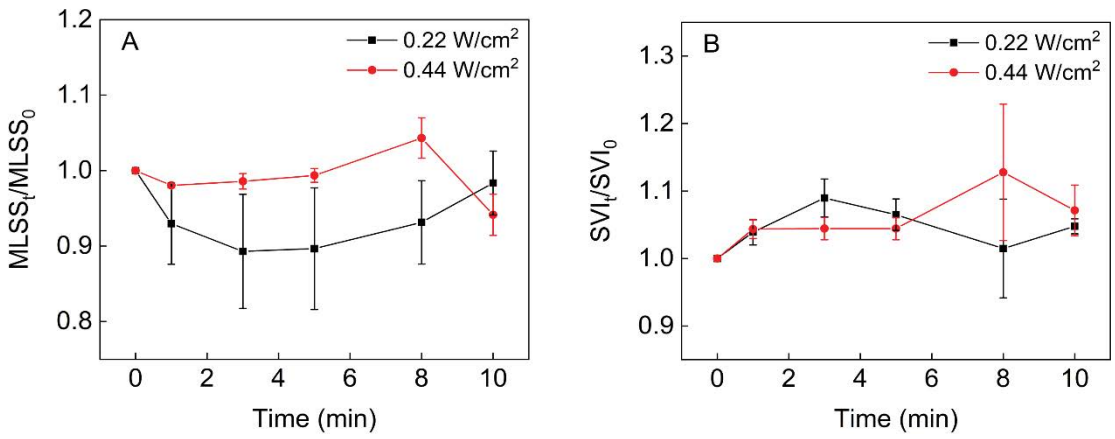

Fig. S9. Effect of US reaction time and power density on (A) MLSS and (B) SVI.