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# Promoting waste activated sludge reduction by linear alkylbenzene sulfonates: Surfactant dose control extracellular polymeric substances solubilization and microbial community succession

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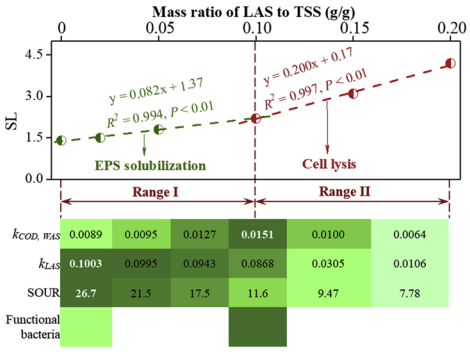
## GRAPHICAL ABSTRACT

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## ABSTRACT

Short-time aerobic digestion (STAD) was proved to promote the reduction of waste activated sludge (WAS). This study systematically disclosed the influential characteristics and mechanisms of linear alkylbenzene sulfonates (LAS) dosage on the reduction of WAS in STAD system. Flow cytometer (FC) combined with SYTOX Green (SG) dye was used to differentiate extracellular polymeric substances (EPS) release and cell lysis of WAS during STAD process. LAS lower than 0.10 g/g total suspended solids (TSS) brought about EPS solubilization and the decrease of sludge floc size, and the accumulated soluble microbial products (SMP) could be biodegraded by heterotrophs. Moreover, the activity of microorganisms (denoted as specific oxygen uptake rate (SOUR)) and proportion of bacteria functional for LAS and SMP biodegradation dramatically increased, leading to a high LAS biodegradation rate ($k_{LAS}$) and increased WAS biodegradation rate ($k_{COD,\ WAS}$). Even more LAS (> 0.10 g/g TSS) caused cell lysis, leading to the decreased $k_{TCOD}$ and $k_{LAS}$, and therefore inhibit the reduction of WAS. High WAS

*Abbreviations:* $COD_{WAS}$, chemical oxygen demand of WAS; DO, dissolved oxygen; EEM, excitation-emission matrices; EPS, extracellular polymeric substances; FC, Flow cytometry; FI, fluorescent intensity; FSC, forward scatter; HS, humic-like substances; IPS, intracellular polymeric substances; *kCOD WAS*, biodegradation constant of WAS; *kLAS*, biodegradation constant of LAS; LAS, linear alkylbenzene sulfonates; NA, nucleic acid; $SCOD_{SMP}$, soluble chemical oxygen demand of SMP; SG, SYTOX Green; SL, slope; SMP, soluble microbial products; SSC, side scatter; STAD, short-time aerobic digestion; TPS, tryptophan protein-like substances; TSS, total suspend solids; WAS, waste activated sludge; WWTPs, wastewater treatment plants

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reduction and LAS biodegradation rate were achieved at the LAS dosage of 0.10 g/g TSS in STAD system. This study lays the foundation for improving WAS reduction by optimizing surfactant dose in STAD system.

## 1. Introduction

As a novel technique for the reduction of waste activated sludge (WAS), short-time aerobic digestion (STAD) attract much attention nowadays due to its low capital investment, few operational problems, short sludge retention time, efficient pathogen inactivation, and fast degradation rate [1,2]. During the STAD process, aerobic microorganisms hydrolyze biodegradable organic solids, resulting in the reduction of WAS solids [3]. Aerobic respiration also leads to a decline in biomass concentration. Moreover, STAD could affects the production of extracellular polymeric substances (EPS), floc size, settleability, flocculability, and dewaterability of WAS [4].

As a commercial mixture of anionic surfactant, linear alkylbenzene sulfonates (LAS) are widely used as industrial and institutional cleaners and household detergents due to the relatively low cost, excellent detergent properties, and generally good biodegradability under aerobic conditions [5–7]. Because of the negatively charged functional groups and hydrophobic characteristics [8,9], LAS could easily bind with WAS as which contains positive charged groups including carboxyl, phosphoryl, and quaternary ammonium [10,11]. While, LAS may also further influences the sludge characteristics due to its special physicochemical properties. For example, LAS has a long linear hydrocarbon group which can form micelles and increase aqueous solubility, thus accelerate the solubilization of EPS from WAS [12]. Zhou et al [13] also mentioned that LAS could be used to effectively promote the extraction of biopolymers from WAS. However, even more LAS leads to too much micelle formation and then causes the release of intracellular polymeric substances (IPS) [13,14].

STAD combined with surfactant could be a unique method for improving the reduction of WAS. Zhou et al. [14] found that after adding 0.10 g/g TSS of cocoamidopropyl betaine, the VSS removal rate was about 28.5% after treated for 1-day in STAD system, which is much higher than the previous published results of 10–16% [1,2]. In order to further shorten digestion time and reduce the cost of WAS reduction, optimizing surfactant dose should be particularly important. Low dose of surfactant could promote the release of EPS from WAS, but too much surfactant could cause cell lysis and may reduce the reduction efficiency of WAS. Thus, the key point should be able to differentiate EPS solubilization from cell lysis when optimize the application of surfactant in WAS reduction by STAD system.

Flow cytometer (FC) combined with SYTOX Green (SG) dye is normally used to determine the characteristics of bacteria cell [15,16]. SG can easily bind with extracellular nucleic acid ($NA_{ex}$) [17] and the complex of SG + $NA_{ex}$ only emit low intensity of fluorescence due to the relatively low concentration of $NA_{ex}$, but which can't directly bind with intracellular NA ($NA_{in}$) in bacteria with an intact cell membrane because of the large molecular size of SG dye size [18]. Surfactant has the ability to improve the release of EPS, increase the permeability of cell membrane and even cause cell lysis [19]. Therefore, SG could pass into the cell and bind with the high concentration of $NA_{in}$, and the high fluorescence intensity emitted from SG + $NA_{in}$ could be easily detect by FC [16]. Thus, FC + SG can be used to distinguish $NA_{ex}$ from $NA_{in}$ sensitively, a distinction that could be used to differentiate EPS releasing from cell lysis during WAS reduction by the combination process of STAD + LAS. For example, previous study [19] successfully used FC + SG to differentiate EPS solubilization from cell lysis of *Synechocystis* sp. PCC 6803 after treatment by various doses of myristyltrimethylammonium bromide (MTAB); an MTAB dose lower than 0.4 g/g dry cell led to the solubilization of EPS, but a higher dose of MTAB led to cell lysis.Although LAS could affect the properties of WAS, but how LAS dosage affects the WAS reduction in STAD system has not been systematically evaluated. In this study, FC combined with SG was used to differentiate EPS solubilization from cell lysis when treated by various doses of LAS. Dynamics of excitation-emission matrices (EEM) of soluble microbial products (SMP), residual LAS, chemical oxygen demand of WAS ($COD_{WAS}$) and soluble chemical oxygen demand (SCOD), combined with the variation of specific oxygen uptake rate (SOUR) and microbial community succession were performed to comprehensively monitor how LAS dosage affects the WAS reduction in STAD system.

## 2. Materials and methods

### 2.1. Chemicals and sludge samples

Linear alkylbenzene sulfonate ($C_{18}H_{29}NaO_3S$, LAS), an anionic surfactant with a molecular weight of 348.48 g/mol, was obtained from Shanghai Chem. Co. Ltd., China. Sludge samples were obtained from the secondary settling tank of the Quyang municipal WWTP in Shanghai, China. The grit in sludge was firstly removed by screening through a 1.2 mm sieve. Sludge samples were concentrated by sedimentation at 4 °C for 2 h. The concentrated sludge had a pH of 6.9 ± 0.2, total suspended solids (TSS) of 9.12 ± 0.25 g/L, $COD_{WAS}$ of 12,400 ± 140 mg/L, and total residual LAS of 5.3 ± 1.2 mg/L. Concentrated sludge was stored at 4°C and used within 48 h.

### 2.2. Activated sludge and sample collection

STAD experiments were carried out in six identical reactors with the LAS dosages of 0.02, 0.05. 0.10, 0.15, and 0.20 g /g TSS (hereafter g/g), respectively, and reactor without adding LAS was used as the control. The configuration of aerobic digestion reactor was detailly described in our published studies [14,20,21]. The work volume of reactor is 5.0-L, and the solution pH, temperature and dissolved oxygen (DO) were controlled at 6.9 ± 0.2, 24 ± 2°C and 2–3 mg/L, respectively. Digested sludge samples were collected at the noted digestion times and then subjected to immediate characterization without storage. Part of the sample was centrifuged at 4 °C with the speed of 4000 × *g* for 20 min in a high-speed freezing centrifuge (Heraeus Multifuge X1R, Thermo Scientific, Germany). Supernatant was further centrifuged at 12,000 × *g* and 4°C for 10 min to remove particles. The collected supernatant contains SMP, which was stored at 4°C for analysis of soluble chemical oxygen demand (SCOD) and excitation-emission matrices (EEM). Part of digested sludge was stained with SYTOX Green dye and analyzed using a FACSAria FC (BD Biosciences, CA, USA). Part of sample was stored at -20 °C prior to the total residual LAS and microbial community analyses.

### 2.3. SG stain with WAS and flow cytometry

FC combined with SG was used to evaluate the solubilization of EPS and cell lysis of WAS after treated by various dosages of LAS in STAD system [19]. We stained WAS by SG dye according to the guidelines (Invitrogen, Carlsbad, CA). 1.0-mL digested sludge was mixed with 1.0-μL SG, and the mixture was immediately shocked on a rocker mixer (Lab-Line, TX, U.S.) under dark condition for 15 min. WAS without adding LAS and aerobic digestion was stained with SG and used as the control.

### 2.4. Determination of LAS

Residual LAS in sludge was extracted according to previous study [8]. Samples were dried at 103°C for 8 h and then powdered with a mortar and pestle. After we add 50 mL impregnating solution (the detailedly prepared method of impregnating solution was shown in Supporting Information), we treated the mixture by ultrasound for 2 min in an ultrasonic cleaner (DS-5510DT, KUDOS, China), adjusted the pH to 8 ~ 9 by adding 1 M NaOH, and placed the mixture in drying cabinet at 50°C for 10 min. The treated culture were filtered two more times and the impregnating solution extract, now containing the extracted LAS, was evaporated to dryness, and then the LAS was redissolved into 50 ml of deionized water.

The concentration of LAS in the aqueous phase was determined by methylene blue spectrophotometric method [22]. 50 ml of aqueous sample was added into the separating funnel with the volume of 200 ml. Using phenolphthalein as indicator, 4% NaOH solution was added to the separating funnel drop by drop until the solution color changed to purple red, and then 0.5 M $H_2SO_4$ was added drop by drop until the purple red color disappeared. After addition of 12.5 mL of methylene blue (300 mg/L) and 20 ml chloroform successively, the mixture solution was allowed to react with hand shaking for 3 min and then let sit for 15 min. The chloroform at the bottom of the separating funnel was transferred into a 50-ml volumetric flask without water. The extraction process was repeated once with 20 ml of chloroform. All of the extracted chloroform was collected in a volumetric flask and diluted to 50 mL using chloroform. A UV–vis spectrophotometry (UV2600, Shimadzu, Japan) with the wavelength of 652 nm was used to determine residual LAS in aqueous phase. A seven-point calibration curve was made from LAS solutions at concentration levels between 0 and 10 mg/L (Figure S1). The recoveries of LAS ranged between 88% and 96%. For the control experiment, 50 ml of deionized water also was added into the separating funnel and then use the same method from step I to IV as shown above.

### 2.5. Chemical analyses

The DO and pH of the mixed culture were measured by a DO & pH meter (HQ40d, Hatch, USA). TSS, TCOD, and SCOD were analyzed following *Standard Methods* [23]. The fluorescence spectra of SMP were determined by an EEM fluorescence spectrophotometer (Varian Cary Eclipse, Agilent, Australia) equipped with a Xenon lamp as the excitation source. Deionized water was used as the control, and data analysis was performed with OriginLab 8.5 (OriginLab Corp, Northampton, USA).

A 100-mL sealed glass bottle equipped with aerator and DO probe was used to determine the oxygen uptake rate (OUR) of WAS. Sludge samples were firstly aerated for 5 min to make the oxygen saturated, and DO concentration of the mixture was measured and continuously monitored by a computer. The OUR of treated sludge was calculated by a linear regression analysis and then quantified to SOUR based on the concentration of digested sludge [24].

As the residual LAS could be part of the mixture TCOD and SCOD, COD of WAS ($COD_{WAS}$) and SCOD of SMP ($SCOD_{SMP}$) could be calculated using the following equations:

$$COD_{WAS} = COD_{Total} - (COD_{LAS,\ aqueous} + COD_{LAS,\ sludge}) \quad (1)$$

$$SCOD_{SMP} = SCOD_{Total} - COD_{LAS,\ SMP} \quad (2)$$

where $COD_{WAS}$ and $SCOD_{SMP}$ are COD concentration for WAS and SMP, respectively; $COD_{Total}$ and $SCOD_{Total}$ are mixture COD and SCOD concentration, respectively; $COD_{LAS,\ aqueous}$ and $COD_{LAS,\ sludge}$ are residual LAS concentration present in COD in aqueous and sludge phase, respectively. The conversion factor of LAS equivalents to COD is 2.25 mg COD/mg LAS, which is derived in the Supporting Information.

The biodegradation rate of organic components containing LAS and $COD_{WAS}$ are approximated by first-order kinetics [25]:

$$ds/dt = -ks \quad (3)$$

where $s$ is the organic components concentration (mg/L), $t$ is the aerobic digestion time (h), and $k$ is the biodegradation rate constant ($h^{-1}$).

### 2.6. Microbial community analyses

10 mL WAS samples in reactors with LAS doses of 0 and 0.10 g/g TSS were collected after digested for 0, 8, and 12 h. Samples were centrifuged at 12,000 × $g$ and 4 °C for 20 min. The pellets were resuspended using phosphate buffered solution (PBS, molar ratio of $Na_3PO_4$, $Na_2HPO_4$, NaCl, and KCl is 2:4:9:1) [26], and genomic DNA was extracted using the Fast DNA® Spin Kit for Soil (MP Biomedicals, USA) following the instructions. DNA samples were sent to Beijing Genewiz biological technology (Beijing, China) to perform sequencing library preparations and standard Illumina MiSeq sequencing protocols. The overall community was evaluated by unweighted UniFrac distance matrix [27]; Cytoscape and principle coordinate analysis (PCoA) were used to investigate the relationships among various samples [28].

### 2.7. Statistical analysis

We withdrew three samples from each reactor and then assayed for TCOD, SCOD, and residual LAS for each sample at the same time. Results are expressed as the mean and standard deviation of the three measured samples (mean ± SD). SMP samples were assayed one time for EEM, light scattering and the spectra from FC. Software of Origin 8.1.5 (Origin Lab Inc., USA) and Pearson's correlation coefficient ($R^2$) were used to identify and estimate the correlation between two parameters. Correlations were considered statistically significance when the confidence interval is higher than 95% ($P < 0.05$).

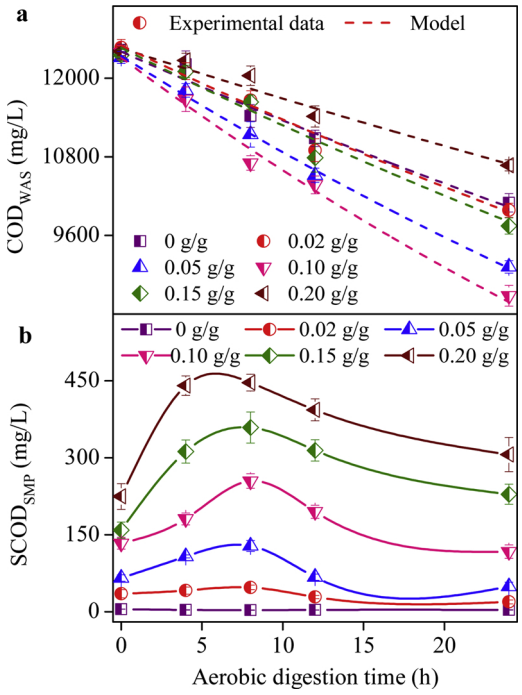


**Fig. 1.** Changes of $COD_{WAS}$ and $SCOD_{SMP}$ after adding LAS with dosages ranging from 0 to 0.2 g LAS/g VSS over time.

**Table 1**
First-order rate constants for CODWAS ($k_{COD,\ WAS}$) for all dosages of LAS during the STAD experiments[a].

| Dosage (g/g) | 0 | 0.02 | 0.05 | 0.10 | 0.15 | 0.20 |
|---|---|---|---|---|---|---|
| $k_{COD,\ WAS}$ ($h^{-1}$) | 0.0089 | 0.0095 | 0.0127 | 0.0151 | 0.0100 | 0.0064 |
| $R^2$ | 0.983 | 0.982 | 0.997 | 0.983 | 0.974 | 0.981 |

[a] The first order biochemical degradation kinetics is $S = S_0 e^{-kt}$. $S$ and $S_0$ are the concentration of TCOD at the aerobic digestion time of t and 0 h, respectively; $k_{TCOD}$ is the biodegradation rate of TCOD.

## 3. Results and discussion

### 3.1. Influences of LAS on $COD_{WAS}$ and $SCOD_{SMP}$ in STAD system

Fig. 1 shows the influence of LAS dose on $COD_{WAS}$ and $SCOD_{SMP}$, and Table 1 presents the corresponding first-order rate constants for $COD_{WAS}$ biodegradation ($k_{COD,\ WAS}$). $COD_{WAS}$ gradually decreased whether or not LAS was added, and first-order kinetics fit the $COD_{WAS}$ removal well. $k_{COD,\ WAS}$ gradually increased from 0.0089 $h^{-1}$ to 0.0151 $h^{-1}$ as LAS dose increased from 0 to 0.10 g/g, but which declined for a higher LAS dose. The increase in $k_{COD,\ WAS}$ under low LAS doses might be related to the increased proportion of functional bacterial responsible for organic matters biodegradation [20]. However, higher LAS caused cell lysis and the loss of active bacteria [13], leading to the decreased $k_{COD,\ WAS}$. With the LAS dosage at 0.10 g/g, the reduction rate of WAS could reach about 30% after 24 h in this system, which is lower than the guideline stipulated by GB18918-2002 [29] that the degradation of organic substances should be higher than 40% by applying aerobic digestion for the stabilization of sewage sludge from WWTPs. However, the reduction rate of WAS could be higher than 40% only after 33.8 h after adding 0.10 g/g LAS into our system.

In the case of $SCOD_{SMP}$, LAS improved the solubilization of EPS from WAS, $SCOD_{SMP}$ in all reactors increased in the initial 8 h, but gradually decreased in the later stage. The long alkyl chain in LAS can form micelles that are effective for solubilizing EPS from WAS [14,19,20]. As the excellent carbon sources and electron donor, $SCOD_{SMP}$ could be consumed by heterotrophs [30].

### 3.2. Influences of LAS on EEM spectra of SMP

Fig. 2 shows the EEM fluorescence spectra of SMP after adding 0.20 g/g LAS and the fluorescence intensity (FI) variations of peaks A and B after adding LAS at the noted digestion times. Table S1 shows the parameters of the linear relationship between LAS dose and FI of peaks A and B at the noted digestion times. Two main peaks were identified from the fluorescence spectra: Peaks A and B, with the Excitation/Emission (Ex/Em) of 280/330 nm and 340/430 nm, are associate with tryptophan protein-like substances (TPS) and humic-like substances

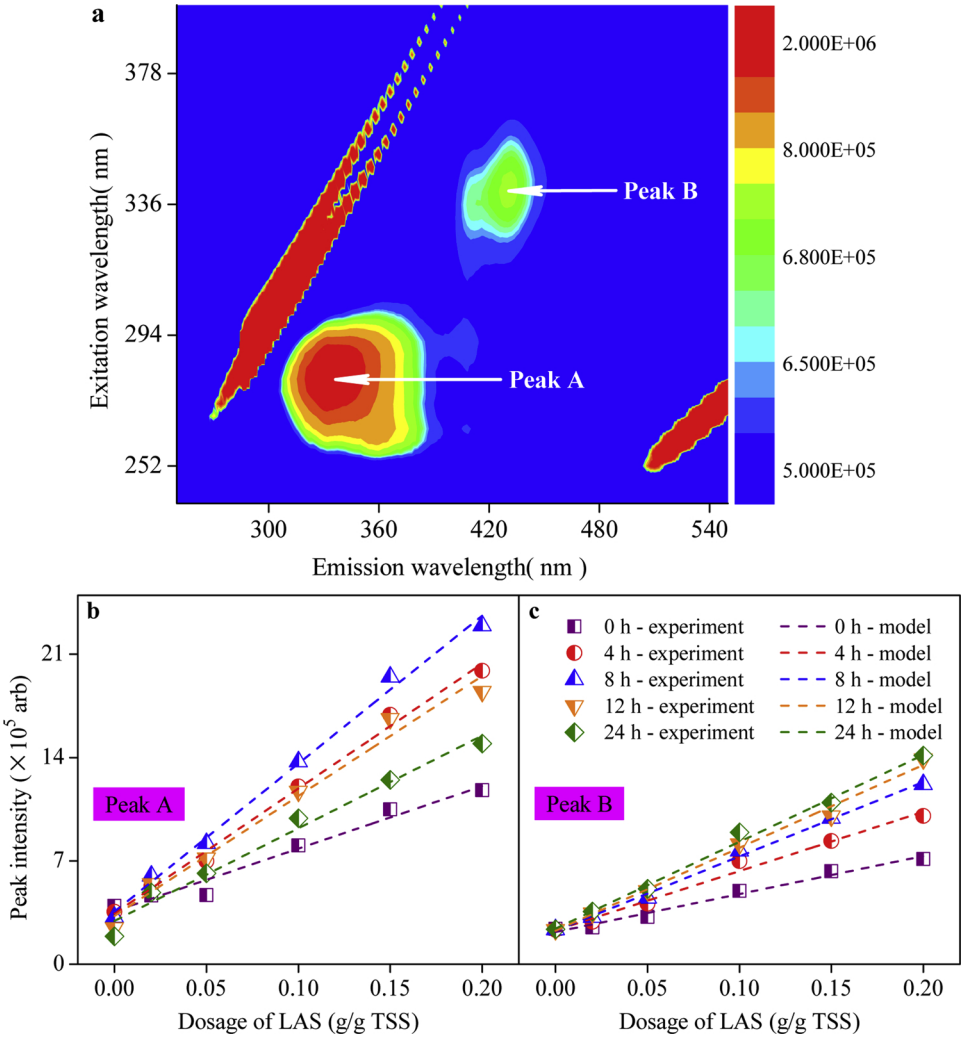


**Fig. 2.** (a) Fluorescence EEM spectra of SMP after adding 0.20 g/g of LAS, and the variations of fluorescence intensity of (b) peak A and (c) peak B after adding various dosage of LAS at the noted STAD times. Peak A and B represent the tryptophan protein-like substances (TPS) and humic-like substances (HS), respectively.

(HS), respectively [31–33].

LAS dramatically improved the FI for both peaks A and B, meaning the increased concentrations of both TPS and HS in SMP, respectively. Moreover, LAS dose had a strong and nearly linear relationship with the FI for both peaks A and B as all of the correlation coefficients ($R^2$) were above 0.97 (Table S1). During the STAD process, $K$ meaning the release of organic matters dramatically increased for peak A in the initial 8 h, but which gradually decreased in the later stage. $K$ for peak B continuously increased throughout the STAD process. Results showed that LAS could enhance the solubilization of TPS and HS from WAS. HS keep increasing but TPS gradually decreased in the later stage, suggesting TPS are more readily biodegraded compared with HS in STAD process [34].

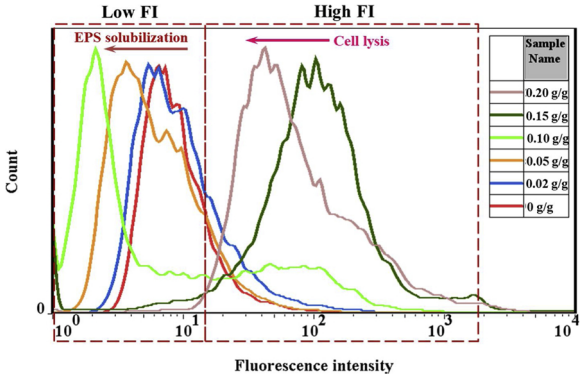


**Fig. 4.** Fluorescence spectra of WAS after adding various doses of LAS and aerobic digested for 8 h.

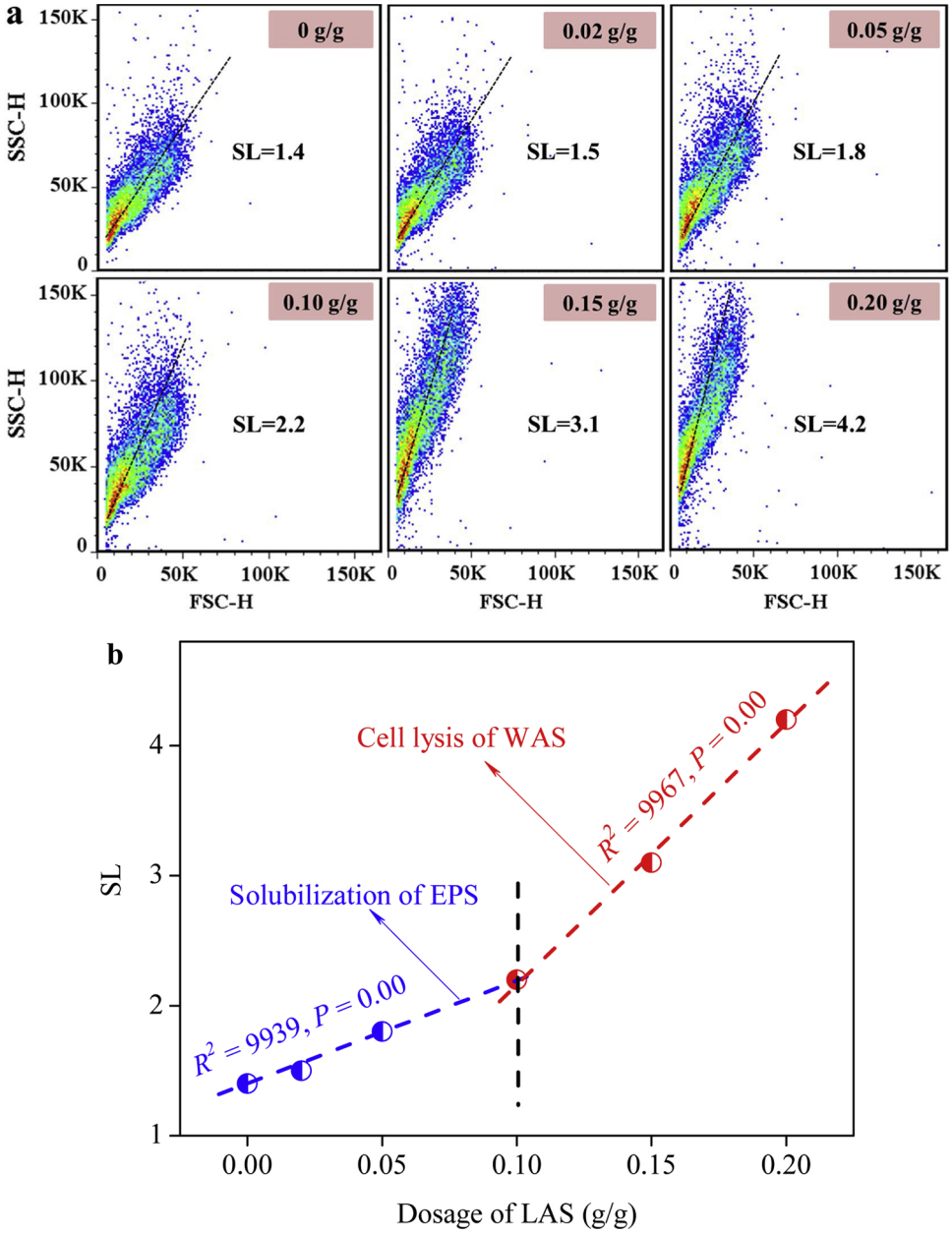


**Fig. 3.** (a) FC results for light scattering of WAS after treatment by various doses of LAS and aerobic treated for 8 h, and (b) the relationship between LAS dose and the slope (SL) between the forward scatter (FSC, horizontal axis) and side scatter (SSC, vertical axis) in the region with the highest density of points. SL, the slope of the linear relationship between FSC and SSC, is inversely related to sludge floc size.

### 3.3. FC analysis of LAS-treated WAS

Fig. 3(a) presents FC results for light scattering of WAS after adding various doses of LAS and aerobic digested for 8 h. Fig. 3(b) presents the relationship between LAS dose and the slope (SL) between side scatter (SSC) and forward scatter (FSC). SL is inversely related to sludge floc size [16,19], and the increase in SL means the reduced sludge floc size [16,35].

Fig. 3(b) indicated that LAS has a significantly impact on the floc size of WAS as SL continuously increased when increasing LAS dose. The variations of LAS could be divided into two ranges. In Range I (from 0 to 0.10 g/g), SL values slowly increased and showed a strong linear relationship ($R^2$ = 0.994, $P < 0.01$) with LAS dose, meaning the decreased floc size of WAS due to the solubilization of EPS via its surfactant action [19]. In Range II (from 0.10 to 0.20 g/g), SL increased sharply and showed a strong linear relationship ($R^2$ = 0.997, $P < 0.01$) with LAS dose, and the slope of the straight line was higher than that of in phase I, indicating a dramatic decrease in WAS floc size due to cell lysis [16,19].

Fig. 4 shows the fluorescence spectra of WAS after adding LAS and aerobic digested for 8 h, which was correspond to the FC results in Fig. 3(a), and both of them were used to ascertain the variations in permeability of bacterial cell membrane [19]. When increasing LAS dose from 0 to 0.10 g/g, the fluorescence peak with low FI on the left of the dashed line continuously shifted to left and even almost disappeared as LAS dose higher than 0.10 g/g. The left shift of fluorescence peak should be resulted from the EPS solubilization due to the low FI fluorescence was emitted from the mixture of SG + $NA_{ex}$ [16,19]. Meanwhile, the new fluorescence peak with high FI on the right of the dashed line was formed when increase LAS dose from 0.10 to 0.15 g/g and more WAS flocs accumulated in this region. Results indicated that LAS could increase the permeability of cell membrane and even cause cell lysis, and SG can pass into the cell and bind with $NA_{in}$ [36,37]. Nevertheless, the fluorescence peak with high FI continuously shifted to left when adding more LAS, indicating the improved cell lysis and release of $NA_{in}$ [18,19,38]. Results showed that LAS with the dosage of 0 ~ 0.10 g/g could cause the release of EPS from WAS, but cell lysis and $NA_{in}$ release occur when adding more LAS.

### 3.4. LAS Biodegradation and microorganisms activity in STAD system

Fig. 5 (a) shows the variations of total residual LAS in the mixture and Table 2 presents the corresponded biodegradation rate constants of LAS using the first order biochemical degradation kinetics. Total residual LAS in all the reactors gradually decreased and the first order biochemical degradation kinetics also fitting well with the LAS removal as all the correlation coefficients ($R^2$) were higher than 0.96. Moreover, the biodegradation rate of LAS ($k_{LAS}$) only slightly decreased from 0.1003 $h^{-1}$ to 0.0868 $h^{-1}$ as LAS dose increased from 0 to 0.10 g/g, but which dramatically decreased for higher LAS does and lower to 0.0106 $h^{-1}$ after adding 0.20 g/g LAS. Results confirmed that low dose of LAS only has minimal effect on the biodegradation of LAS. However, high dose of LAS caused cell lysis, which corroborates the FC results (from Figs. 3 and 4), leading to the decrease of $k_{LAS}$.

Fig. 5(b) shows the SOUR of WAS after adding LAS at the noted STAD times. SOUR gradually increased with the increasing of LAS dosage from 0 to 0.10 g/g, but which decreased for a higher dose of LAS. During STAD process, SOUR of WAS at all the LAS dosages gradually decreased, indicating the decreased activity of aerobic bacteria. Results showed that LAS with the dose from 0 to 0.10 g/g could effectively improve the microorganism activity and then improve the biodegradation of organic matters. However, high dosage of LAS caused the cell lysis (Fig. 4) and reduced the aerobic microorganism activities, which further reduce the biodegradation rate of $COD_{WAS}$ and LAS (Figs. 1(a) and 5 (b)).

### 3.5. Succession of microbial community of WAS

Fig. 6(a) and (b) show the microbial community structure at the class level (relative abundances of dominant microbial phylotypes) and the predominant bacterial genera, respectively. Fig. 6(c) presents the clustering based on the unweighted UniFrac analyses of the microbial community structure in WAS in STAD system. For the untreated WAS, the phylotypes almost remained stable in the first 8 h, and the main bacteria were *Sphingobacteria* (˜36%), *α-Proteobacteria* (˜6.6%), *γ-Proteobacteria* (˜6.9%) and *β-Proteobacteria* (˜9.3%). The phylotypes closely related to *Sphingobacteria* rose to 41% after digested for 12 h. While, microbial community structure of WAS significantly changed after adding 0.10 g/g LAS (Fig. 6(a) and (c)): *α-Proteobacteria*, *Planctomycetacia*, *Caldilineae* and *Actinobacteria* became the dominant phylotypes, and the abundance dramatically increased to about 20%, 18.5%, 13% and 9.1%, respectively. However, *γ-Proteobacteria* abundance dramatically reduced, *Sphingobacteria* and *β-Proteobacteria* almost disappeared. In the case of genus level of microbial community (Fig. 6(b)), *Planctomyces*, *Clostridium*, *Pseudomonas*, *Aeromonas* and *Acinetobacter* were the dominant genera with the proportions of 2.5%, 2.4%, 1.6%, 1.0% and 0.7% in raw WAS, respectively, and all of them increased after adding LAS. Especially, *Pseudomonas* and *Planctomyces* reached to about 12% and 9.1% after digested for 12 h, respectively.

Most of the microbial belongs to *Sphingobacteria* and *Proteobacteria* could be responsible for the biodegradation of organic pollutants [39]. *Planctomycetales* and *Acinetobacter* were identified as the bacterium responsible for the biological nitrogen and phosphate removal [40]. Biodegradation of LAS has been attributed mainly to bacterial species in the genera of *Pseudomonas*, *Aeromonas* and *Clostridium* [41,42], and phylotypes in all of these genera were present in our STAD systems. In STAD system, LAS could significantly improve the proportions of functional microorganisms responsible for the biodegradation of LAS and organic matters.

Fig. 6(d) shows the unweighted PCoA based on the unweighted

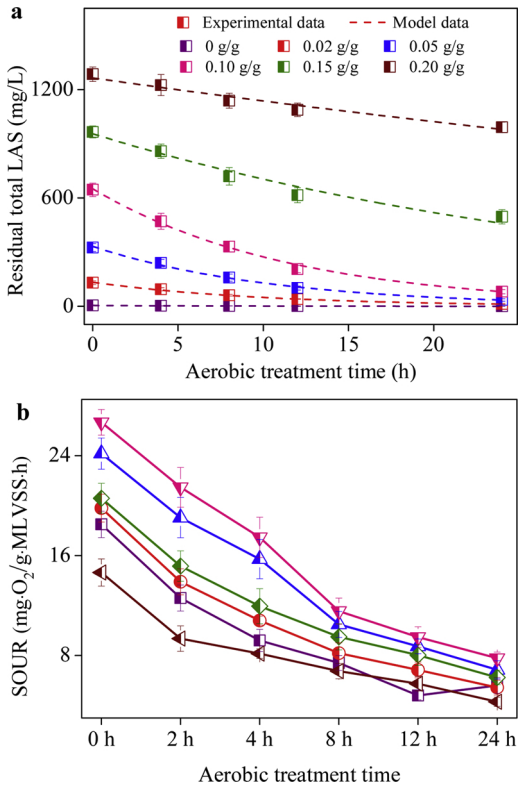


**Fig. 5.** (a) Total residual LAS and (b) SOUR of WAS after adding various doses of LAS at the noted STAD times.

**Table 2**
Biodegradation rate constants of LAS using first-order kinetics during the STAD process[a].

| Dosage (g/g) | 0 | 0.02 | 0.05 | 0.10 | 0.15 | 0.20 |
|---|---|---|---|---|---|---|
| $k_{LAS}$ ($h^{-1}$) | 0.10 | 0.10 | 0.096 | 0.092 | 0.031 | 0.011 |
| $R^2$ | 0.993 | 0.996 | 0.996 | 0.998 | 0.975 | 0.970 |

[a] The first order biochemical degradation kinetics is $S = S_0 e^{-kt}$. $S$ and $S_0$ are the residual total concentration of LAS at the aerobic treatment time of t and 0 h, respectively; $k_{LAS}$ is the biodegradation rate of LAS.

UniFrac analyses showing the microbial community grouping. Digested sludge samples for S1-S3 (without adding LAS) together have much higher PC1 values compared to those for S4-S5 (adding LAS), and samples for S4-S5 were also different from each other, all of those further confirmed that LAS could significantly influence the microbial community structure during the STAD process.

### 3.6. Synthesizing the results

Fig. 7 synthesizes the results in terms of how LAS affects the reduction of WAS in STAD system:

- Low dose of LAS (≤ 0.1 g/g TSS) mainly brought about EPS solubilization (Fig. 3(b) and 4) via its surfactant action, leading to the decreased sludge floc size (denoted by SL) (Fig. 3) and increased concentrations of $SCOD_{SMP}$, TPS and HS (Fig. 1(b) and 2), all of them could be biodegraded in the aerobic digestion process. Meanwhile, SOUR of WAS (Fig. 5(b)) and the proportion of functional microorganisms (Fig. 6) dramatically increased, leading to the fast biodegradation of LAS (Fig. 5(a)) and improved $k_{COD,\ WAS}$ (Fig. 1(a) and Table 1).
- Even more LAS (> 0.1 g/g TSS) led to cell lysis (Fig. 3(b) and 4), caused a large increase in SL, $SCOD_{SMP}$, TPS and HS (Fig. 1(b) and 2). However, cell lysis also reduced the activity and proportion of functional microorganisms, leading to the decreased $k_{COD,\ WAS}$ and $k_{LAS}$ (Fig. 5(a) and Table 2).

For environmentally sustainable development of both environment and society, surfactant-containing wastewater after WAS treatment could seriously threaten the environment and human beings when discharged arbitrarily. A biodegradable anionic surfactant-LAS [43] was selected in this work, and 92.1% of LAS with the initial dose of 0.10 g/g could be biodegraded in STAD system and the total residual LAS is about 118 mg/L after 24 h in this system, which is still unacceptable for the direct discharge. However, the residual LAS could be lower than 5.0 mg/L (Class-A discharge standards for LAS in China) only after 56.5 h, which should be acceptable for the aerobic digestion system; a previous study [44] confirmed that a set of surfactants could be continuous biodegraded by an oxygen-based membrane biofilm reactor ($O_2$-MBfR). Thus, biodegradation of LAS could prevent its discharge to aquatic environments.

## 4. Conclusions

STAD combined with LAS could be a unique method for WAS reduction. LAS dose lower than 0.10 g/g brought about the solubilization of EPS from WAS, and the accumulated SMP could be biodegraded by aerobic microorganisms in STAD system. Both of SOUR of WAS and the proportion of functional microorganisms dramatically increased, leading to a high $k_{LAS}$ and increased $k_{COD,\ WAS}$. Even more LAS (> 0.10 g/g) led cell lysis, resulting to the decline of functional bacteria activity and proportion, which caused the decreased $k_{COD,\ WAS}$ and $k_{LAS}$.

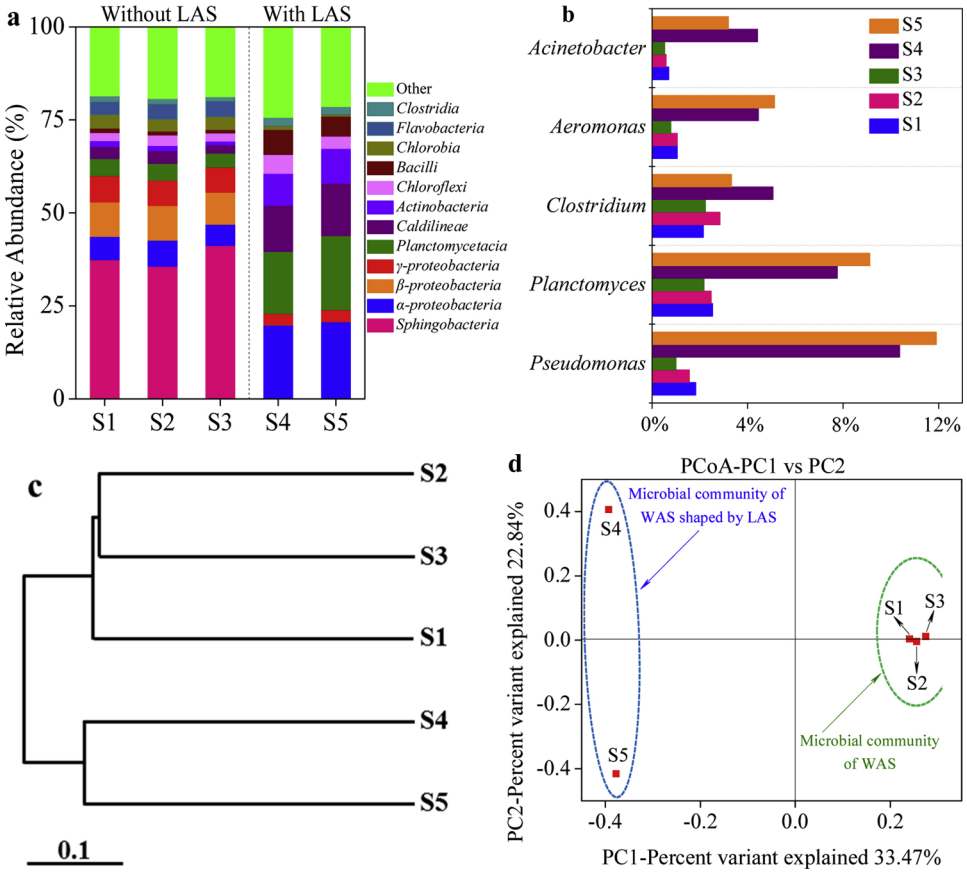


**Fig. 6.** (a) Microbial community structure at the class level (relative abundances of dominant microbial phylotypes) and (b) the predominant bacterial genera, (c) clustering based on the unweighted UniFrac analyses of the microbial community structure, and (d) PCoA based on the unweighted UniFrac analyses showing the microbial community grouping in WAS during the STAD process. S1-S3 are the WAS without adding LAS and aerobic digested for 0 h, 8 h, and 12 h, respectively; S4-S5 are the WAS with 0.10 g/g LAS and aerobic digested for 8 h and 12 h, respectively.

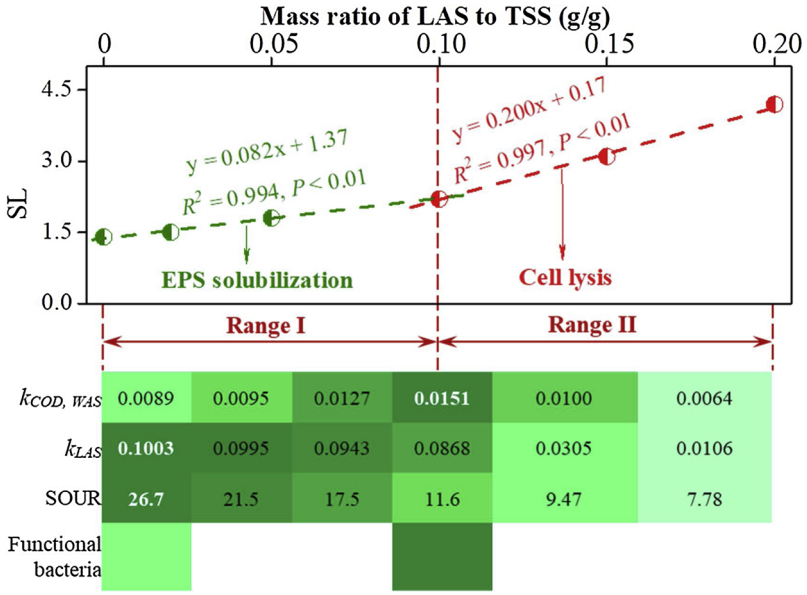


**Fig. 7.** Synthesis of mechanisms about how LAS affects the STAD of WAS. The deeper color and white color in a bar signify the high and low value of the parameters, respectively.

High WAS reduction and LAS biodegradation rate were achieved at the LAS dosage of 0.10 g/g in STAD system. This study lays the foundation for improving WAS reduction by optimizing surfactant dose in STAD system.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.04.024.

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