Reduction of soluble microbial products in activated sludge system with pure oxygen aeration under toxic stress condition of phenol

Dong Wang [a,b,*], Zhihua Sun [a,b], Chunsheng Qiu [a,b], Shaopo Wang [a,b]

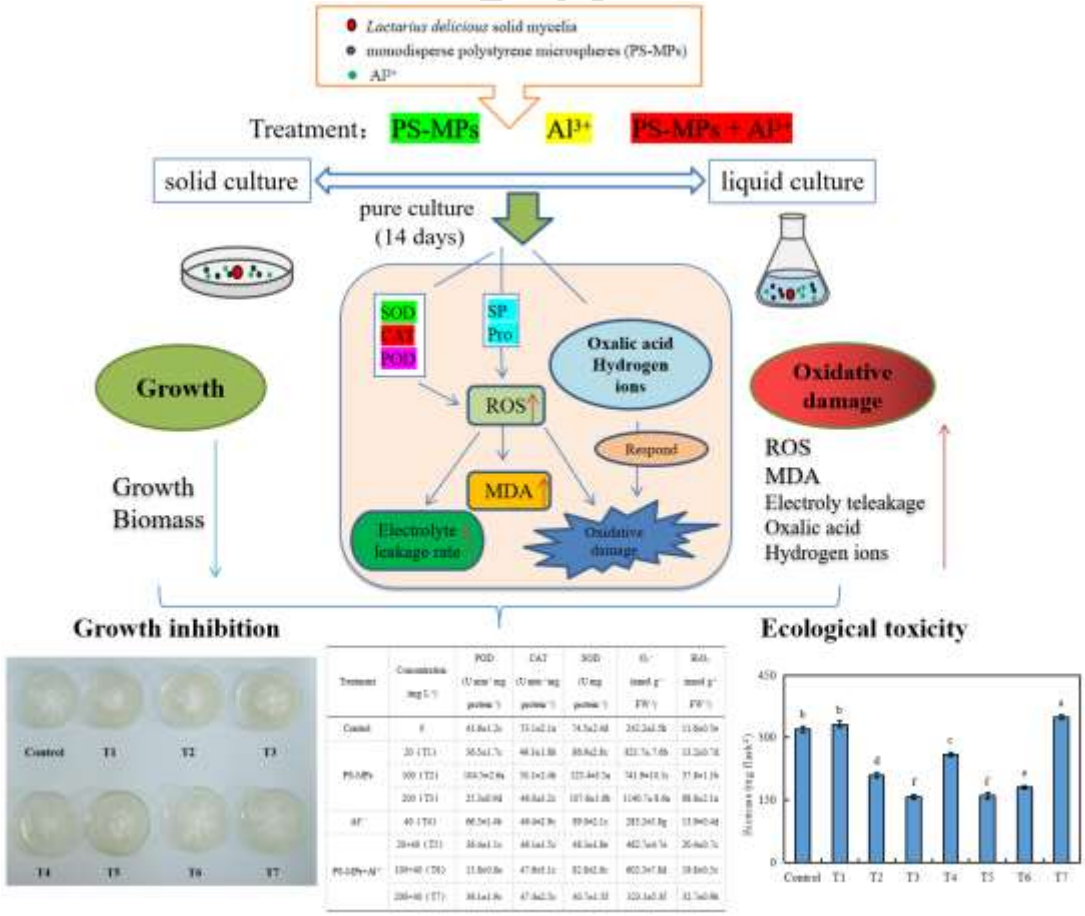
[a] Tianjin Key Laboratory of Aquatic Science and Technology, Tianjin 300384, China

[b] School of Environmental and Municipal Engineering, Tianjin Chengjian University, Tianjin 300384, China

(E-mail: wangdong06@tcu.edu.cn; 3020369577@qq.com; qcs254@163.com; wspfr@sina.com)

[*] Corresponding author

GRAPHICAL ABSTRACT



Abstract

This study aimed to explore the effect of pure oxygen aeration on the generation of soluble microbial products (SMP) when activated sludge was under toxic stress condition of phenol. Two parallel sequencing batch reactors, one with air and the other with pure oxygen aeration, were used to investigate the relationships among effluent chemical oxygen demand (COD), N-acyl-homoserine lactones (AHLs), microbial community, extracellular polymeric substances (EPS), and SMP. The results showed that pure oxygen aeration caused rapid recovery of the activated sludge and low effluent COD concentration in steady condition when the two reactors were fed with phenol. In contrast, these results were not observed with sodium acetate as substrate. A strong correlation was found among C4-HSL, loosely bound EPS (LB-EPS), and SMP. The genera *Zoogloea*, *Bacteroides*, and *Flavobacterium* were also related to effluent COD, SMP, and LB-EPS production under toxic stress condition of phenol. Pure oxygen aeration reduced AHL production and decreased the relative abundance of specific genera (*Zoogloea* and *Flavobacterium*). These changes caused the reduction of SMP generation in the pure oxygen-aerated activated sludge systems. These results provide insights into the effect of pure oxygen aeration on SMP generation of activated sludge with toxic substance existence.

Keywords

extracellular polymeric substances, N-acyl-homoserine lactones, pure oxygen aeration, quorum sensing, soluble microbial products

INTRODUCTION

Activated sludge processes are widely applied not only in municipal wastewater but also in industrial wastewater treatment. In these processes, oxygen plays an essential role in contaminant biodegradation by microbes. However, some characteristics of industrial wastewater, such as high concentration of pollutants, high salinity, and oil content, generally have an adverse effect on oxygen transfer (Zhuang *et al.* 2016; Hu *et al. 2019; Wang et al.* 2021). Moreover, industrial wastewater also probably contains toxic and refractory pollutants. Some researchers promoted oxygen transfer and improved the removal of toxic and refractory compounds through pure oxygen-aerated activated sludge processes (Zhuang *et al.* 2016).

Pure oxygen aeration is not always indispensable. The pure oxygen-aerated activated sludge processes exhibit several advantages: improved oxygen transfer, fast organic removal rate, high enzyme activity, and less electricity consumption during aeration (Skouteris *et al.* 2020). Although oxygen production was considered as an additional energy-consuming process, the oxygen source can be replaced by oxygen waste gas obtained from industrial manufacturing processes (Wang *et al.* 2021). Thus, pure oxygen-aerated activated sludge systems can be advisable to treat industrial wastewater.

Soluble microbial products (SMP) are mainly derived from soluble organic substances arising from substrate degradation, metabolism, and microbial decay (Soh *et al.* 2020). SMP is generally the main component of secondary effluent organic matter (Yu *et al.* 2015). The contribution of SMP is also non-negligible in the effluent of biological treatment processes for industrial wastewater(Yu *et al.* 2015). Moreover,

SMP is still considered as the potential precursor of disinfection by-products and the foremost reason for membrane fouling(Yu *et al.* 2015).

Pure oxygen aeration affected the production of SMP and extracellular polymeric substances (EPS) (Skouteris *et al.* 2020). The content of EPS was less under pure oxygen aeration because of its decomposition (Zhang *et al.* 2019). The production of SMP under pure oxygen aeration was affected by the food-to-microorganism (F/M) ratio (Zhang *et al.* 2019). There was no significant difference in SMP contents between air and pure oxygen aeration under low F/M ratio conditions. In contrast, SMP with pure oxygen aeration increased in the conditions with high F/M ratios. These batch experiments were operated with readily biodegradable substances as substrate.

The effect of aeration type on EPS and SMP production was complex when the activated sludge was under stress conditions. However, limited research has focused on it (Hu *et al.* 2019). Pure oxygen aeration caused more EPS production than air aeration under stress conditions of salinity (Hu *et al.* 2019). The increase in EPS was related to the self-protection of microorganisms against stress conditions. However, the production of SMP under pure oxygen aeration depended on the extent of stress conditions (Hu *et al.* 2019). Pure oxygen aeration promoted higher content of SMP production than air aeration at low stress conditions (salinity), whereas the opposite was observed under high stress conditions. The researchers believed that the high content of SMP was related to EPS release to bulk solution and a high rate of substrate utilization under pure oxygen aeration under stress conditions of salinity. However, the stress effect of industrial wastewater on the activated sludge frequently originated from

toxic and refractory compounds. Thus, it is more important to investigate the effect of pure oxygen aeration on SMP production when the activated sludge was under stress conditions of the toxic substances.

The generation of EPS and SMP is related to the microbial community, which can be affected by the type of aeration (Skouteris *et al.* 2020). Moreover, EPS excretion also depends on quorum sensing (QS), and stress conditions provoke signal molecule excretion by microbes(Shi *et al.* 2017). Thus, microbial community structure and signal molecule variation may reveal the effect of pure oxygen aeration on EPS and SMP production under stress conditions. The objective of this study was to investigate the effect of oxygen aeration on EPS and SMP production under stress condition of phenol. Two parallel SBRs with different aeration types (air and pure oxygen) were operated under shock loading of phenol. The relationships among the type of aeration, signal molecule, microbial community, EPS, and SMP were investigated during the non-steady and steady states of the two SBR systems.

MATERIALS AND METHODS

Reactor set up, operation, experimental design, and sampling

Two identical sequencing batch reactors, A-SBR and O-SBR (with a working volume of 4.5 L), were used and operated parallel using air and pure oxygen aeration, respectively (Fig. S1). An oxygen generator supplied pure oxygen in O-SBR, while the air was aerated to the A-SBR system by a pump. The flow rates of aeration in A- and O-SBRs were kept at 1 L/min during aeration. The SBR systems run at room temperature and were operated as follows: 10 min of influent filling, 240 min of

aeration, 30 min of settling, 10 min of effluent withdrawal, and 70 min of resting.

The characteristics of the synthetic wastewater were as follows: chemical oxygen demand (COD) of 500 mg/L, NH_4Cl of 95 mg/L, and KH_2PO_4 of 22.4 mg/L. Then, Ca^{2+}, Mg^{2+}, and trace elements were also added to the synthetic wastewater. The sludge was obtained from the secondary sedimentation tank at Xianyang Road Wastewater Treatment Plant in Tianjin, China. The details of the experimental design and system operation parameters are shown in Table S1.

The water samples of influent and effluent from both reactors were collected from the two SBRs and then passed through 0.45 μm membrane filters for water quality analysis. The parameters of COD, phenol, acetate, dissolved oxygen (DO), total phosphorus, ammonia nitrogen, and total nitrogen were determined by the standard methods (SEPA, 2002). The acetate concentration was detected by a gas chromatograph with a flame ionization detector. The phenol concentration was measured using 4-Aminoantipyrine and spectrophotometry. The sludge samples (approximately 200 mg in dry weight) were obtained from the two reactors with replication. These replications were mixed into single samples and frozen at −20 °C for DNA extraction.

Extraction and analysis of EPS and SMP

A modified heat extraction method was used to separate EPS from activated sludge (Li & Yang, 2007). The total EPS included tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS). Then, 50 mL of the sample with sludge and water mixture was centrifuged and dewatered at 4,000 g for 5 min. The residual sludge was resuspended and diluted with a heated NaCl solution (0.05 %, 70 ℃) to the original volume (50 mL).

The sludge mixture could reach a temperature of approximately 50 ℃. The mixture was shaken by a vortex mixer (VM-370, INTLLAB) for 1 min and centrifuged at 4,000 g for 10 min. The supernatant was collected and passed through a 0.45 μm membrane filter. The collection was considered as the LB-EPS of the biomass. The NaCl solution was added to the residual solid again (50 mL). The supernatant containing TB-EPS was extracted from the resuspended mixture at 4,000 g centrifuged (15 min) after a water bath at 60 ℃ for 30 min.

The SMP was extracted from the effluents of the two SBR systems. The effluent samples were passed through a 0.45 μm membrane filter, and the SMP was involved in the effluent samples.

The compositions and concentration of the EPS and SMP extractions were determined by humic-like substances (HS), polysaccharides (PS), and proteins (PN). The PN and HS were analyzed through the modified Lowry method, and the PS was determined with the phenol-sulphuric acid method.

Sampling, extraction, and analysis of AHLs

N-acyl-homoserine lactones (AHLs) were extracted from the supernatant, and 400 mL of supernatant was harvested. The method of AHL extraction was introduced by Tang *et al.* (2015). The supernatant was passed through a cellulose filter (0.22 μm) and extracted with an equal volume of ethyl acetate in triple. The solvent was collected and mixed in a conical flask. The collected solvent was dried with anhydrous sodium sulfate and then evaporated at 40 ºC via a rotary evaporator (Yarong, China). The extract was resoluted by 2 mL methanol and stored at –20 ºC for further analysis.

The AHLs in the samples were identified and determined via high-performance liquid chromatography-mass spectrometry (Agilent, USA) with an XDB-C18 column (5 μm, 4.6 mm × 180 mm, Agilent, USA). In the high-performance liquid chromatography condition, the mobile phase consisted of solvent A (ultrapure water with 0.1% of formic acid in v/v and 2 mmol/L ammonium acetate) and solvent B (methanol with 0.1% of formic acid in v/v and 2 mmol/L ammonium acetate) for gradient elution. The column temperature was kept at 40 ºC, and the flow rate was set at 0.2 mL/min.

In the mass spectrometry condition, electric spray ion scanning and multi-reaction ion monitoring mode were used. The voltage was set at 4 kV, and the heated capillary was maintained at 350 ºC.

In this study, N-Butanoyl-L-homoserine lactone (C4-HSL), N-hexanoyl-L-homoserine lactone (C6-HSL), N-octanoyl-L-homoserine lactone (C8-HSL), and N-Decanoyl-L-homoserine lactone (C10-HSL) were continuously found and determined in the two reactors. The details of the standard curves are shown in Supplementary Materials.

High-throughput sequencing analysis

High-throughput sequencing analysis was implemented by Novogene (Beijing, China). The processes of DNA extraction were followed according to our previous study (Wang *et al.* 2021).

First, DNA from the sludge samples was extracted via CTAB method. In this process, 1% agarose gel was used to monitor the DNA concentration and purity. Then,

the DNA of the samples was diluted to 1 ng/μL. The V4 region of the 16S rDNA gene with the forward primer 515F and reverse primer 806R was amplified. Quantification of amplicons was detected through electrophoresis with 1X loading buffer and 2% agarose gel. Then, the amplicons were mixed in equidensity ratios and purified with a Qiagen Gel Extraction Kit (Qiagen, Germany). The sequencing was processed through the Illumina NovaSeq platform. Data split, sequence assembly, and filtration were also completed via FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) and QIIME (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html). Canonical correspondence analysis (CCA) was conducted from the online platform (Novogene) and regenerated through Origin 2021.

Statistical analyses

SPSS 22 was used in statistical analyses. The differences in effluent COD, SMP and EPS contents between the two SBR systems were obtained via paired t-test analysis. The relationships among AHL contents and EPS and SMP components were obtained via Pearson correlation analysis. The negative coefficients (R) of the Pearson correlation (−1-0) indicated a negative correlation. The positive R values (0-1) show a positive correlation. R = 0 represents noncorrelation. The coefficient matrixes were shown by heatmap via Origin 2021.

RESULTS AND DISCUSSION

Organic removal performance

The COD removal performances of A- and O-SBR are depicted in Fig. 1. When the substrate was altered from sodium acetate to phenol, the effluent COD of both reactors

189 increased and gradually decreased. Phenol cannot be degraded by non-incubation
190 activated sludge in the initial period. The toxic effect of phenol also caused biomass
191 decay and cell lysis, which built up SMP (Wu *et al.* 2016). The residual phenol and
192 SMP caused the increase of effluent COD.

193 The changes of effluent COD from A- and O-SBRs showed a significant difference
194 with phenol as substrate. Pure oxygen aeration accelerated the recovery of COD
195 removal performance compared with air aeration in the non-steady state of the phenol
196 shock loading stage. This condition indicates that pure oxygen aeration alleviated the
197 toxic effect of phenol on the activated sludge due to the high enzyme activity during
198 pure oxygen aeration (Calderon *et al.* 2012).

199 In the steady state (Fig. 1b), the effluent COD concentration of A- and O-SBRs
200 showed no significant difference when the two reactors were fed with sodium acetate.
201 In contrast, the effluent COD of O-SBR was lower than that of A-SBR with phenol as
202 substrate via t-test analysis ($p < 0.001$). This finding implies that pure oxygen aeration
203 can polish the effluent quality with toxic carbon source feeding rather than a readily
204 biodegradable carbon source. Both sodium acetate and phenol were in low
205 concentration because of their removal by incubation activated sludge (Table S2)
206 (Zhuang *et al.* 2016). The residual COD mainly consisted of SMP in the two reactors.
207 The relatively low COD concentration in O-SBR may be related to reducing SMP by
208 pure oxygen aeration.

209 **Content variations of EPS and SMP**

210 The changes of EPS and SMP contents were investigated to elucidate the effect of pure

oxygen aeration on the SMP production of activated sludge with toxic substrate feeding (Fig. 2). As shown in Fig. 2a, the type of aeration seemed not to affect the SMP production when the systems were fed with sodium acetate. This result coincides with the findings of Zhang *et al.* (2019b). The reason is that the substrate plays a more critical role in the SMP generation of activated sludge compared with other factors such as oxygen supply (Xu *et al.* 2011). Sodium acetate, a readily biodegradable substrate, has no adverse influence on the SMP generation of the activated sludge. Hence, the SMP contents had no difference between air and pure oxygen aeration in this study.

When the substrate was altered to phenol, the SMP contents initially increased significantly and gradually decreased in the two reactors. The activated sludge in O-SBR released less SMP than that in A-SBR (Fig. 2a). The increase of SMP was related to cell lysis and EPS release into the bulk solution with toxic substrate existence (Aquino & Stuckey, 2004). This result also represents that pure oxygen aeration reduced the SMP production of activated sludge under stress conditions, which was different from previous findings (Hu *et al.* 2019). Hu *et al.* (2019) found that pure oxygen aeration increased the SMP generation of the activated sludge under stress conditions of low salinity due to the high organic removal rate. The reason is that the high content of SMP release was related to biomass-associated product generation of improved microbial metabolism and utilization-associated products from rapid organic degradation (Hu *et al.* 2019). In this study, a high organic removal rate was also obtained (Table 1). The reduced SMP production by oxygen-aerated sludge under toxic stress condition of phenol may be related to rapid substrate removal rate and toxicity

alleviation.

One of the SMP production sources originates from EPS hydrolysis (Ni *et al.* 2010) The changes of EPS content are shown in Figs. 2b, 2c, and 2d. When the substrate was altered to phenol, the total and LB-EPS contents increased in both the reactors and gradually decreased. However, the TB-EPS content increased insignificantly in the two reactors with phenol feeding. The contents of the total, LB-, and TB-EPS in the presence of phenol were higher than those in the absence of phenol. The increase of EPS excretion is beneficial in protecting microbes from toxic substrates (Zhang *et al.* 2021b). In this study, the changing pattern of LB-EPS was similar to that of SMP in the two reactors. It indicates that LB-EPS had the potential to be released into the effluent as SMP due to their loose structure.

The type of aeration caused a difference in EPS content between O- and A-SBR. The TB-EPS content in O-SBR were higher than those in A-SBR (Fig. 2c). These results showed that pure oxygen aeration caused more TB-EPS excretion of activated sludge than air aeration. Some researchers found that pure oxygen aeration made less EPS generation than air aeration due to EPS consumption as substrate at the end of the aeration stage (Zhuang *et al.* 2016; Zhang *et al.* 2019b). However, the opposite conclusion was found under stress conditions (Hu *et al.* 2019). In this study, high content of TB-EPS was excreted to protect microbes and contain extracellular enzymes with pure oxygen aeration.

The variations of EPS components are shown in Fig. 3. The PS and PN contents of LB-EPS in O-SBR were lower than those in A-SBR except the samples in Day 20 (Figs.

3a and 3b), while the PS and PN contents of TB-EPS in O-SBR were higher than those in A-SBR (Figs. 3c and 3d). The low PS and PN contents of LB-EPS in O-SBR may be related to toxicity alleviation of toxic substrate removal (Shi *et al.* 2021). The high PS and PN contents of TB-EPS in O-SBR may contain more abundant extracellular redox-active components to promote pollutant reduction than those in A-SBR, such as reducing saccharides and various extracellular enzymes (Yu 2020; Zhou *et al.* 2020). Substrate bioavailability and its removal rate may be promoted by the high content of TB-EPS in O-SBR. The higher efficiency of toxic substrate removal may cause less SMP and LB-EPS generation in O-SBR. Meanwhile, pure oxygen aeration influenced the signal molecule of QS and microbial community structure, which also played an essential role in EPS generation.

Variations of AHLs

The QS plays a vital role in regulating EPS secretion, which further influences SMP production. The variations of AHLs (C4-, C6-, C8-, and C10-HSL) were investigated and shown in Fig. 4. In A-SBR, when the substrate was altered from sodium acetate to phenol, the concentration of C4-HSL in A-SBR increased initially with phenol as substrate and then decreased gradually. The content of C4-HSL in A-SBR was correlated with SMP and LB-EPS (Fig. 5a). The concentrations of C6-, C8-, and C10-HSL in A-SBR increased until day 20 and then decreased gradually.

In the activated sludge systems with air aeration, various studies found that AHLs played an essential role in EPS and SMP generation (Zhang *et al.* 2019e; Zhang *et al.* 2019d; Xiong *et al.* 2020). However, the effect of AHLs on SMP and EPS generation

277 cannot draw a uniform conclusion. Xiong *et al.* (2020) believed that C6-HSL and
278 3OC6-HSL controlled EPS and SMP production. Some researchers also found that the
279 C8- and 3OHC8-HSL might be necessary for EPS secretion (Zhang *et al.* 2019d; Zhang
280 *et al.* 2019e). In this study, C4-HSL played a critical role in SMP and LB-EPS
281 production. This result is different from previous findings because the experiment was
282 under the stress condition of toxic compound in the present study. In contrast, the
283 former experiments were based on no stress conditions. Ding *et al.* (2016) found that
284 C4-HSL was related to LB-EPS production under oligotrophic nitrogen supply
285 conditions, which is similar to the result of our study. Thus, C4-HSL may be related to
286 LB-EPS and SMP production in stress conditions with air aeration. Meanwhile, Zhang
287 *et al.* (2019c) found that the concentrations of C6- and C8-HSL increased to maintain
288 bacterial activity after substrate shock loading. In our study, C6-, C8-, and C10-HSL
289 increased after phenol shock loading, which could be related to the self-defense
290 behavior of microbes and maintained bacterial activity under stress conditions (Zhang
291 *et al.* 2019c).

292 However, the four AHLs contents in O-SBR were lower than those in A-SBR
293 under shock loading of phenol (Fig. 4). This result indicates that pure oxygen aeration
294 or DO significantly affected AHL generation. Recent research found that facultative
295 anaerobe can generate AHLs only in low DO conditions (DO < 2 mg/L) (Huang *et al.*
296 2021). In this study, the production of AHLs by the facultative bacteria may be inhibited
297 during pure oxygen aeration. Thus, the concentrations of AHLs in O-SBR were lower
298 than those in A-SBR. The lower C4-HSL concentrations with pure oxygen aeration

caused less SMP and LB-EPS generation. The content of C4-HSL also showed no correlation with LB-EPS and SMP production (Fig. 5b). The lower concentrations of C6-, C8-, and C10-HSL in O-SBR showed non-significant effects on organic removal performance.

Changes in microbial community

The microbial diversity and richness indices are shown in Table 2. The estimators of Chao 1 and ACE indicate the richness of the microbial community. The results of Chao 1 and ACE illustrate that the O-SBR had a lower richness of microbial community than A-SBR with sodium acetate as substrate. In contrast, the richness of microbial community in O-SBR was higher than that in A-SBR when the substrate was altered with phenol. The estimators of Shannon and Simpson indicate the diversity of the microbial community. The values of Shannon and Simpson showed that O-SBR had a higher diversity of microbial community than A-SBR with sodium acetate and phenol as substrate. These results demonstrate that the diversity and richness of the microbial community with pure oxygen aeration were higher than those with air aeration with phenol as substrate. The differences in diversity and richness between A- and O-SBR were related to the type of aeration (Zhuang *et al.* 2016). The high diversity and richness of the microbial community with pure oxygen aeration was beneficial to the rapid recovery under toxic stress condition of phenol.

The notable changes in microbial communities at the phylum level are considered in Fig. 6a. The predominant phyla in all samples contained *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*. *Proteobacteria* was always dominant in both reactors,

with sodium acetate and phenol as substrates. However, the relative abundance of *Proteobacteria* decreased gradually when the substrate was altered from sodium acetate to phenol in both two reactors. On the contrary, the phyla of *Bacteroidetes* and *Firmicutes* increased in both reactors with phenol as substrate. *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were usually the dominant phyla in activated sludge samples from municipal and industrial wastewater treatment plants (Sebastian *et al.* 2020). The relative abundance of *Bacteroidetes* and *Firmicutes* indicates that these phyla contained the functional species related to phenol degradation.

The variations of the top 20 genera in the samples are shown in Fig. 6b. The dominant genera in the two reactors contained *Zoogloea*, *Bacteroides*, *Flavobacterium*, *Competibacter*, *Rhizobium*, and *Luteococcus*. The changes in the microbial community in both the SBRs were different when the substrate was altered from sodium acetate to phenol. In the A-SBR system, the genera of *Zoogloea*, *Bacteroides*, and *Flavobacterium* increased significantly with phenol as substrate. These genera were related to EPS generation against toxic conditions (Zhang *et al.* 2019a; Zhang *et al.* 2021a). In O-SBR, the genera *Bacteroides*, *Competibacter*, *Rhizobium*, and *Luteococcus* were found to increase and dominate with phenol as substrate. *Rhizobium* and *Luteococcus* were related to phenol degradation (Tong *et al.* 2018; He *et al.* 2021; Zhang *et al.* 2021a;). The genera *Competibacter* and *Luteococcus* were also considered as EPS producers (Wei *et al.* 2020; He *et al.* 2021). However, *Zoogloea* and *Flavobacterium* changed insignificantly in O-SBR. The specific dominant genera in O-SBR, such as *Rhizobium*, preferred high DO concentration (Yadav et al. 2020). Thus,

343 the dominant and functional microbes difference between A- and O-SBR was due to
344 the type of aeration and DO concentration difference.

345 The relationships among system performances, EPS, SMP, AHLs, and microbial
346 community are further illustrated via CCA. In Fig. 6c, the acute angles among effluent
347 COD, SMP, LB-EPS, and AHLs indicated a positive correlation. *Zoogloea*,
348 *Bacteroides*, and *Flavobacterium* were also related to effluent COD, AHLs, SMP, and
349 LB-EPS content. It should also be noted that TB-EPS production was correlated with
350 *Luteococcus*. In previous studies, *Zoogloea* and *Flavobacterium* correlated with EPS
351 generation under toxic conditions (Zhang *et al.* 2019a; Zhang *et al.* 2021a).
352 *Flavobacterium* was also proved to be the AHL producer (Zhang *et al.* 2018). In this
353 study, AHL content and microbial community composition affected LB-EPS and SMP
354 production. Compared with A-SBR, the lower relative abundance of *Zoogloea* and
355 *Flavobacterium* in O-SBR caused less LB-EPS and SMP production (Fig. 6b).
356 Furthermore, the high content of TB-EPS in O-SBR was related to the high relative
357 abundance of *Luteococcus*.

358 According to the results above, pure oxygen aeration influenced AHL excretion
359 and microbial community structure. The reduced AHL contents and low abundance of
360 functional bacteria for LB-EPS generation in the reactor with pure oxygen aeration
361 tended to excrete less LB-EPS and SMP. This result caused lower effluent COD with
362 oxygen aeration than with air aeration. These results imply that high dissolved oxygen
363 concentration could reduce the SMP production of activated sludge under stress
364 conditions of the toxic substrates from industrial wastewater.

CONCLUSIONS

In this study, the effect of pure oxygen aeration on activated sludge with phenol as a toxic substrate was investigated via two parallel SBRs using pure oxygen and air aeration. Compared with air aeration, pure oxygen aeration promoted the recovery of activated sludge under shock loading of phenol. Pure oxygen aeration also caused the reduction of AHL excretion and decreased the abundance of functional bacteria for LB-EPS production under stress condition of toxic substrate. This reduced LB-EPS and SMP production of activated sludge in the system with pure oxygen aeration and finally decreased the effluent COD. These results may provide insights into the effect of pure oxygen aeration on SMP generation under stress conditions of toxic compounds.

FUNDING

This study was supported by National Natural Science Foundation of China (Grant No. 51608350).

Author Contributions

All the co-authors read and approved the final manuscript. Z.H.S. carried out the experiments and data. D. W. conceptualized and supervised the project, acquired funding, provided resources for the experiments, wrote the original draft and contributed to the final revision of the manuscript. C.S.Q. and S.P.W. co-supervised the student and reviewed the written manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aquino S. and Stuckey D. (2004), Soluble microbial products formation in anaerobic chemostats in the presence of toxic compounds. *Water Research,* **38**(2), 255–266.

Calderon K., Gonzalez-Martinez A., Montero-Puente C., Reboleiro-Rivas P., Poyatos J. M., Juarez-Jimenez B., Victoria Martinez-Toledo M. and Rodelas B. (2012), Bacterial community structure and enzyme activities in a membrane bioreactor (MBR) using pure oxygen as an aeration source. *Bioresource Technology*. **103**(1), 87–94.

Ding Y., Feng H., Zhao Z., Shen D., Wang M. and Zhou Y. (2016), The Effect of Quorum Sensing on Mature Anaerobic Granular Sludge in Unbalanced Nitrogen Supply. *Water Air & Soil Pollution*, **227**(9), 334.

He Q., Xie Z., Fu Z., Wang H., Chen L., Gao S., Zhang W., Song J., Xu P., Yu J. and Ma J. (2021), Effects of phenol on extracellular polymeric substances and microbial communities from aerobic granular sludge treating low strength and salinity wastewater. *Science of Total Environment*, **752**, 141785.

Hu Y. Q., Wei W., Gao M., Zhou Y., Wang G. X. and Zhang Y. (2019), Effect of pure oxygen aeration on extracellular polymeric substances (EPS) of activated sludge treating saline wastewater. *Process Safety and Environmental Protection*, **123**(B), 344–350.

Huang Y., Lu Z., Jiang T., Zeng Y., Zeng Y. and Chen, B. (2021), Oxygen availability affects the synthesis of quorum sensing signal in the facultative anaerobe Novosphingobium pentaromativorans US6-1. *Applied Microbiology and Biotechnology*, **105**(3), 1191–1201.

Li X. Y. and Yang S. F. (2007), Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research,* **41**(5), 1022–1030.

Ni B. J., Zeng R. J., Fang F., Xie W. M., Sheng G. P. and Yu H. Q. (2010), Fractionating soluble microbial products in the activated sludge process. *Water Research,* **44**(7), 2292–2302.

Sebastian N., Monika H., Slawomir C., Ewa K. and Adriana O. (2020), Environmental fate of Bacteroidetes, with particular emphasis on Bacteroides fragilis group bacteria and their specific antibiotic resistance genes, in activated sludge wastewater treatment plants. *Journal Of Hazardous Materials*, **394**, 122544.

SEPA (2002), Water and Wastewater Monitoring Methods, Fourth Edition, Environmental Science Publishing House, Beijing.

Shi Y., Huang J., Zeng G., Gu Y., Chen Y., Hu Y., Tang B., Zhou J., Yang Y. and Shi L. (2017), Exploiting extracellular polymeric substances (EPS) controlling strategies for performance

enhancement of biological wastewater treatments: An overview. *Chemosphere*, **180**, 396–411.

Shi Z., Xu H., Wang Z., Du H. and Fu X. (2021), Effects of co-exposure to copper and humic acids on microalga Chlorella vulgaris: growth inhibition, oxidative stress, and extracellular secretion. *Environmental Pollutants and Bioavailability*, **33**(1), 415–424.

Skouteris G., Rodriguez-Garcia G., Reinecke S. F. and Hampel U. (2020), The use of pure oxygen for aeration in aerobic wastewater treatment: A review of its potential and limitations. *Bioresource Technology*,. **312**, 123595.

Soh Y. N. A., Kunacheva C., Webster R. D. and Stuckey D. C. (2020), Identification of the production and biotransformational changes of soluble microbial products (SMP) in wastewater treatment processes: A short review. *Chemosphere*, **251**, 126391.

Tang X., Liu S., Zhang Z., and Zhuang G. (2015), Identification of the release and effects of AHLs in anammox culture for bacteria communication. *Chemical Engineering Journal*, **273**, 184–191.

Tong K., Zhang Z., Lin A., Song Q., Ji G., Wang D. and Zhang A. (2018), Treatment of super heavy oil wastewater by a combined process of lignite-activated coke adsorption and immobilized biological filter degradation: performance and the relevant microbial community analysis. *Journal of Chemical Technology and Biotechnology*, **93**(10), 2942–2951.

Wang D., Sun D., Tian X., Liu N., Wang C., Yu J., Qiu C. and Wang S. (2021), Role of microbial communities on organic removal during petrochemical wastewater biological treatment with pure oxygen aeration. *Journal of Water Process Engineering*, **42**, 102151.

Wei S. P., Stensel H. D., Quoc B. N., Stahl D. A., Huang X., Lee P.-H. and Winkler M.-K. H. (2020), Flocs in disguise? High granule abundance found in continuous-flow activated sludge treatment plants. *Water Research*, **179**, 115865.

Wu C., Zhou Y., and Song J. (2016), The activated sludge metabolic characteristics changing sole carbon source from readily biodegradable acetate to toxic phenol. *Water Science & Technology*, **73**(10), 2324–2331.

Xiong F., Zhao X., Wen D., and Li Q. (2020), Effects of N-acyl-homoserine lactones-based quorum sensing on biofilm formation, sludge characteristics, and bacterial community during the start-up of bioaugmented reactors. *Science of Total Environment*, **735**, 139449.

Xu J., Sheng G.-P., Luo H.-W., Fang F., Li W.-W., Zeng R. J., Tong Z.-H., and Yu H.-Q. (2011), Evaluating the influence of process parameters on soluble microbial products formation using response surface methodology coupled with grey relational analysis. *Water Research*, **45**(2), 674–680.

Yadav T. C., Jadeja N. B. and Kapley A. (2020), Metagenomic Insights in Activated Biomass Treating Industrial Wastewater at Different DO Levels. *Applied Microbiology and Biotechnology*, **192**(2), 544–556.

Yu H., Qu F., Sun L., Liang H., Han Z., Chang H., Shao S. and Li G. (2015), Relationship between soluble microbial products (SMP) and effluent organic matter (EfOM): Characterized by fluorescence excitation emission matrix coupled with parallel factor analysis. *Chemosphere*, **121**, 101–109.

Yu H.-Q. (2020), Molecular Insights into Extracellular Polymeric Substances in Activated Sludge. *Environment. Science & Technology*, **54**(13), 7742–7750.

Zhang B., Lens P., Shi W., Zhang R., Zhang Z., Guo Y., Bao X. and Cui F. (2018), The attachment potential and N-acyl-homoserine lactone-based quorum sensing in aerobic granular sludge and algal-bacterial granular sludge. *Applied Microbiology and Biotechnology*, **102**(12), 5343–5353.

Zhang C., Cheng F., Zhao H., and Li J. (2021a), Enhanced phenol degradation under different shock-stress in LAC/AS system: The combination effects of LAC toxicity mitigation and microbial community shift. *Journal of Water Process Engineering*, **40**, 101824.

Zhang H., Song S., Jia Y., Wu D. and Lu H. (2019a), Stress-responses of activated sludge and anaerobic sulfate-reducing bacteria sludge under long-term ciprofloxacin exposure. *Water Research*, **164**, 114964–114975.

Zhang H.-L., Jiang W.-L., Liu R., Zhou Y., and Zhang Y. (2019b), Organic degradation and extracellular products of pure oxygen aerated activated sludge under different F/M conditions. *Bioresource Technology*, **279**, 189–194.

Zhang J., Zhang Y., Wang X., Li J., Zhou R., Wei J., Liang D. and Zhang K. (2019c), Effects of substrate shock on release of AHL signals in ANAMMOX granules and properties of granules. *Environmental Science-water Research & Technology*, **5**(4), 756–768.

Zhang Z., Cao R., Jin L., Zhu W., Ji Y., Xu X., and Zhu L. (2019d), The regulation of N-acyl-homoserine lactones (AHLs)-based quorum sensing on EPS secretion via ATP synthetic for the stability of aerobic granular sludge. *Science of Total Environment*, **673**, 83–91.

Zhang Z., Yu Y., Xi H. and Zhou Y. (2021b), Single and joint inhibitory effect of nitrophenols on activated sludge. *Journal of Environmental Management* **294**, 112945.

Zhang Z., Yu Z., Wang Z., Ma K., Xu X., Alvarezc P. and Zhu L. (2019e) Understanding of aerobic sludge granulation enhanced by sludge retention time in the aspect of quorum sensing. *Bioresource Technology*, **272**, 226–234.

Zhou X., Kang F., Qu X., Fu H., Alvarez P. J. J., Tao S. and Zhu D. (2020), Role of Extracellular Polymeric Substances in Microbial Reduction of Arsenate to Arsenite by Escherichia coli and Bacillus subtilis. *Environ. Sci. Technol.* **54**(10), 6185–6193.

Zhuang H., Hong X., Han H. and Shan S. (2016), Effect of pure oxygen fine bubbles on the organic matter removal and bacterial community evolution treating coal gasification wastewater by membrane bioreactor. *Bioresource Technol.* **221**, 262–269.

Table and Figure captions

Table 1 COD changes in batch assays of different aeration types with phenol as substrate

Table 2 Bacterial richness and diversity estimators of both reactors with different substrates

Fig. 1 Organic removal perfromance of two SBR systems. (a) COD removal performance and (b) Effluent COD comparison of the two SBR systems in steady state via t-test analysis

Fig. 2 Content variations of (a) SMPs, (b) total EPS, (c) TB-, and (d) LB-EPS. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Fig. 3 Content variations of PN in (a) LB-EPS, (b) PS in LB-EPS, (c) PN in TB-EPS, and (d) PS in TB-EPS. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Fig. 4 Changes of (a) C4-HSL, (b) C6-HSL, (c) C8-HSL, and (d) C10-HSL concentration in the reactors

Fig. 5 Pearson correlation among AHLs, SMP, and EPS components in (a) A-SBR and (b) O-SBR (*: $p < 0.05$, **: $p < 0.01$)

Fig. 6 Relative abundance changes of microbial communities at (a) phylum and (b) genus level in the samples, and (c) CCA on correlation of genus with various factors

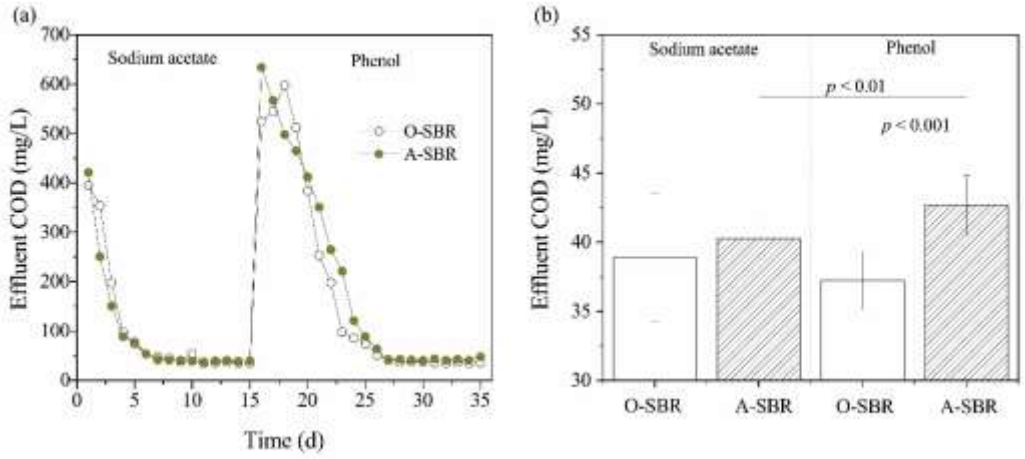
Table 1. COD changes in batch assays of different aeration types with phenol as substrate.

Reactor	Effluent COD (mg/L)					
	0 h	0.5 h	1 h	2 h	3 h	4 h
A-SBR	240±12	153±8	67±5	46±7	43±3	45±7
O-SBR	235±14	48±5	40±3	41±2	35±5	36±9

515 **Table 2.** Bacterial richness and diversity estimators of both reactors with different
516 substrates.

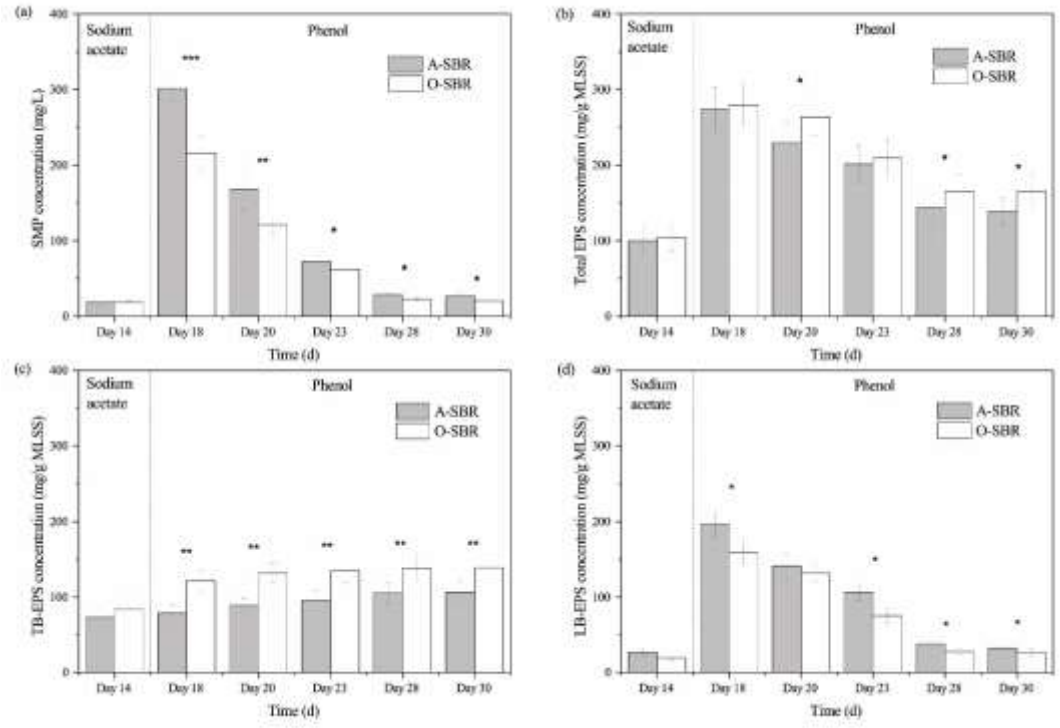
Reactor	Substrate	OTUs	Shannon	Simpson	Chao1	ACE	Goods coverage (%)
A-SBR	Sodium acetate	1407	7.141	0.955	1497.004	1545.389	99.5
	Phenol	848	6.674	0.968	914.275	929.731	99.7
O-SBR	Sodium acetate	1229	7.686	0.987	1332.147	1359.238	99.5
	Phenol	1209	7.491	0.9845	1330.921	1341.0245	99.4

517



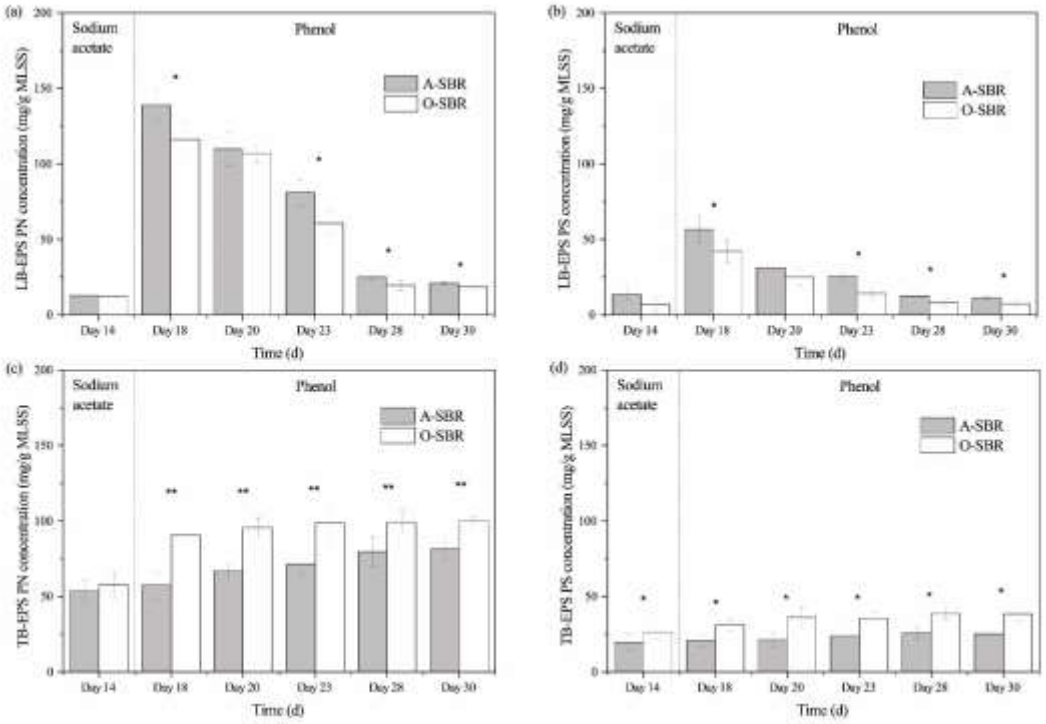
518

519 Figure 1.



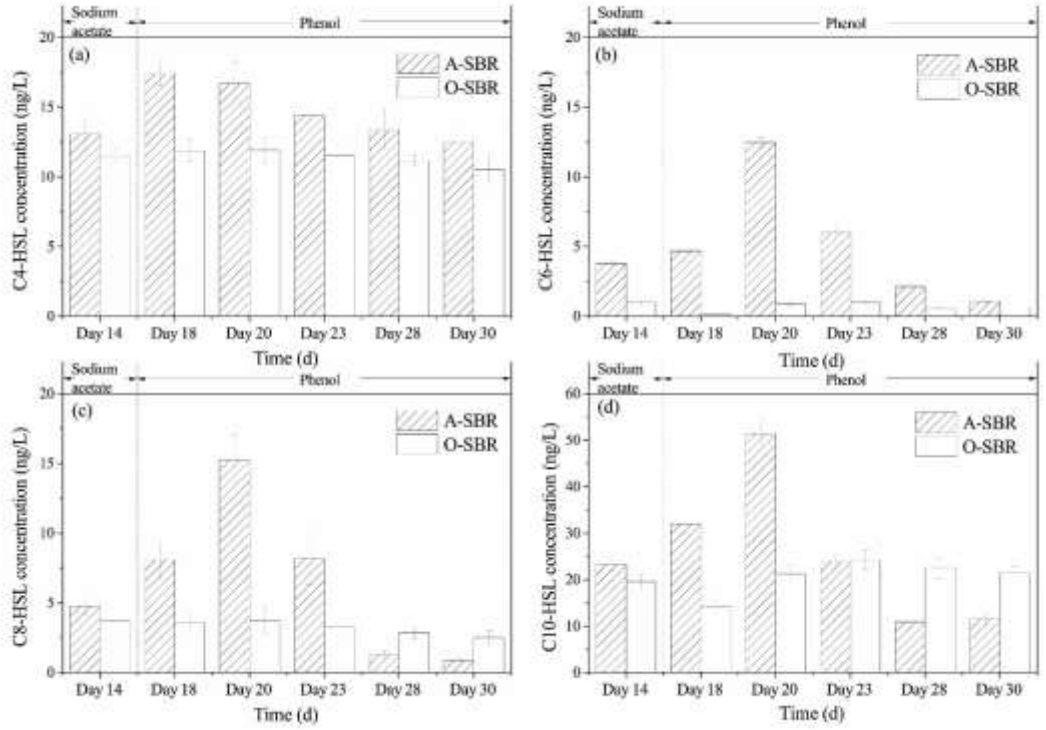
520

521 Figure 2.



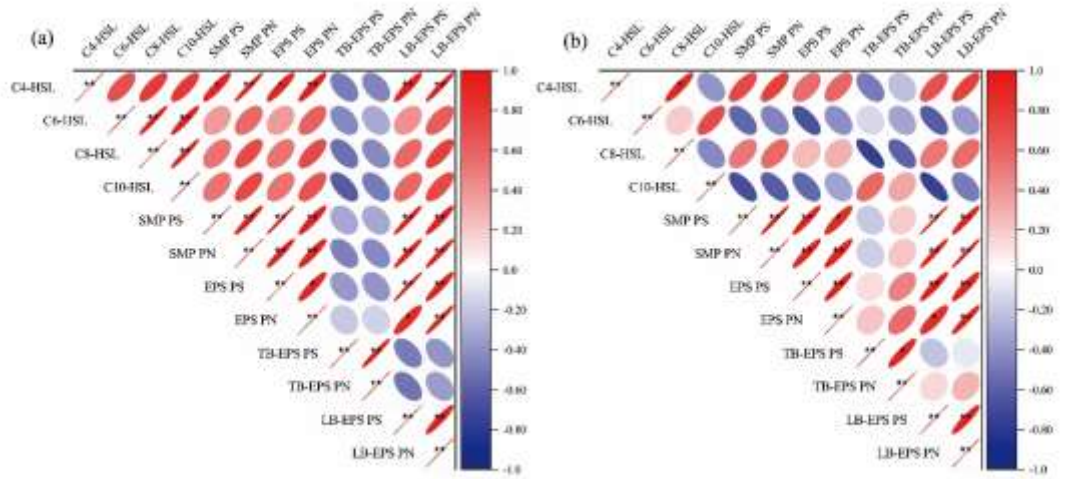
522

523 Figure 3.



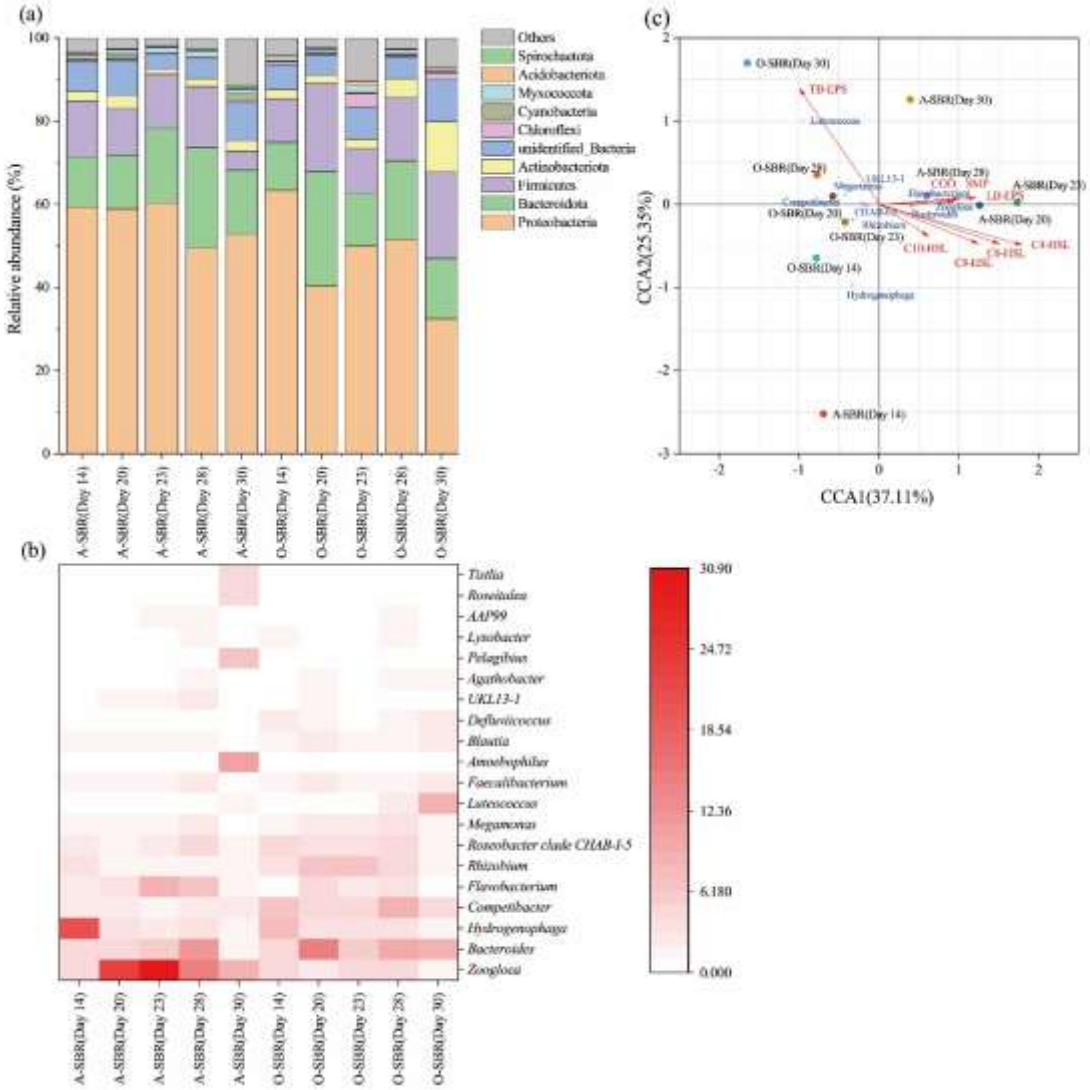
524

525 Figure 4.



526

527 Figure 5.



528

529 Figure 6.

530

Supplementary Materials

Reduction of soluble microbial products in activated sludge system with pure oxygen aeration under toxic stress condition of phenol

Dong Wang [1,2*], Zhihua Sun[1,2], Chunsheng Qiu[1,2], Shaopo Wang[1,2]

[1] *Tianjin Key Laboratory of Aquatic Science and Technology, Tianjin 300384, China*

[2] *School of Environmental and Municipal Engineering, Tianjin Chengjian University, Tianjin 300384, China*

[*] Corresponding author

Wangdong06@tcu.edu.cn

Tel: +86 022-23085117 Fax: +86 022-23085117

EXPERIMENT:

Determination of AHLs

In this study, the AHLs standards (C4-HSL, C6-HSL, C8-HSL and C10-HSL) were purchased from Sigma Aldrich. AHLs standards were configured into standard solutions of AHLs with concentration gradients of 2, 4, 6, 8, 10, 20 and 40μg/L. The standard curves were generated with the peak area corresponding to quantitative ion of m/z 102 via HPLC-MS.

AHL	Standard curve		Recovery (%)
C4-HSL	Y = 163.29X+576.93	R^2 = 0.9952	86.43
C6-HSL	Y = 221.76X+224.82	R^2 = 0.9935	89.85
C8-HSL	Y =279.07X-37.78	R^2 = 0.9943	95.77
C10-HSL	Y =250.29X+7.16	R^2 = 0.9911	83.64

* Corresponding author.

E-mail address: wangdong06@tcu.edu.cn (D Wang).

FIGURE:

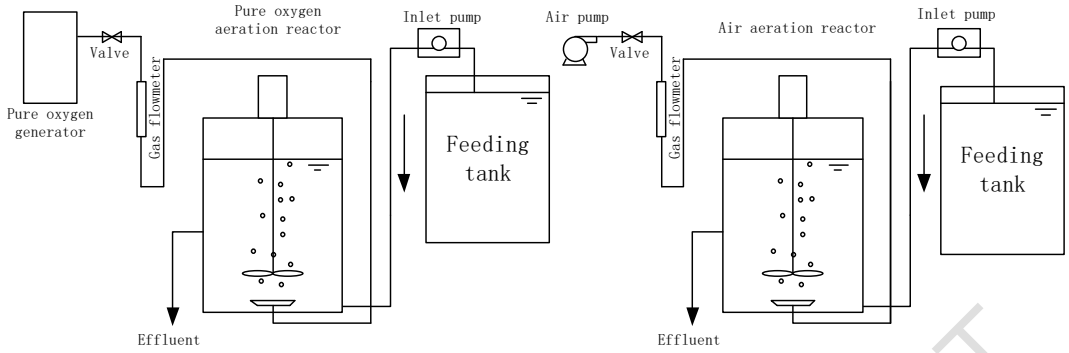


Figure S1. Schematic diagram of the SBR systems with pure oxygen and air aeration.

TABLE:

Table S1. Details of system operation parameters and sampling.

Reactor	Carbon source	Operation time	State	Sampling time	COD_{Inf}（mg/L）	HRT (h)	MLSS (g/L)	SRT (d)
A-SBR	Sodium acetate	Day 1–15	Sludge cultivation	——	500	4	4.1±0.5	10-20
		Day 7–15	Steady state	Day 14	500	4	4.0±0.2	
	Phenol	Day 16–26	Shock loading	Day 18, 20 and 23	500	4	4.0±0.6	
		Day 27–33	Steady state	Day 28 and 30	500	4	3.9±0.1	
O-SBR	Sodium acetate	Day 1–6	Sludge cultivation	——	500	4	4.0±0.3	10-20
		Day 7–15	Steady state	Day 14	500	4	4.1±0.1	
	Phenol	Day 16–26	Shock loading	Day 18, 20 and 23	500	4	3.9±0.4	
		Day 27–33	Steady state	Day 28 and 30	500	4	4.0±0.1	

Table S2. Substrate removal performance of the two SBRs in steady state

Reactor	Carbon source	Sampling time	Concentration (mg/L)
A-SBR	Sodium acetate	Day 10–15	5.2±2.3
	Phenol	Day 30–33	3.2±0.3
O-SBR	Sodium acetate	Day 10–15	4.7±1.5
	Phenol	Day 30–33	3.7±0.6