Effects of wastewater treatment processes on the sludge reduction system with 2,4-dichlorophenol: Sequencing batch reactor and anaerobic-anoxic-oxic process

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ABSTRACT

The effects of two wastewater treatment processes (sequencing batch reactor, SBR; and anaerobic-anoxic-oxic, A2O) on sludge reduction with metabolic uncoupler 2,4-dichlorophenol (DCP) were studied in laboratory. The experimental results showed that the reduction of cumulative excess sludge in SBR and A2O was 43.7% and 44.2%, respectively, during the stable stage of the test. The two processes had similar average sludge yield and sludge yield reduction, i.e., 0.306 and 0.305 mg of SS/mg chemical oxygen demand (COD), and 16.9% and 17.8%, respectively. The effect of DCP on the wastewater treatment efficiencies (namely, removal of COD, total nitrogen, NH\textsubscript{4}\textsuperscript{+}-N, and total phosphorus) of the two processes were also similar. SBR was more likely to slightly retard the increase of activated sludge SVI with lesser increase in extracellular polymeric substances and protein/polysaccharide ratio. Although DCP did not dramatically affect the microbial communities of sludge, SBR was more favorable for increasing the activated sludge SOUR and maintaining the primary microorganisms of sludge than A2O.

1. Introduction

Excess sludge, a significant and undesirable byproduct of biological wastewater treatment, is mass-produced. In activated sludge process, a typical biological wastewater treatment process, the sludge yield is 0.3–0.5 g/g chemical oxygen demand (COD; Grady et al., 2011). For example, in China, over 3.5 × 10\textsuperscript{7} tons of dewatered sludge (with 80% water content) was produced in 2013 (China State EPA, 2014). With more wastewater treatment facilities and increasingly stringent environmental and legislative constraints, the production of excess sludge will continue to increase. Currently, the treatment and disposal of excess sludge have become serious issues for many wastewater treatment plants (WWTPs) because its cost can account for 40–60% of the total operational costs of WWTPs (Guo et al., 2013). Therefore, effective in-situ treatment technologies for reducing excess sludge production from the source (namely, in-situ sludge reduction technologies) should be developed.

To date, the studied in-situ sludge reduction technologies are based on four mechanisms: lysis-cryptic growth, maintenance metabolism, predation on bacteria, and uncoupling metabolism (Guo et al., 2013). Among these, the metabolic uncoupler addition method is promising because of its high efficiency and low effect on wastewater treatment. Moreover, this method does not require the modification needed for conventional wastewater treatment processes or the installation of expensive facilities. Typical metabolic uncouplers studied in previous researches include 2,4-dichlorophenol (DCP; Chen et al., 2006; Li et al., 2016; Song et al., 2010; Xie et al., 2010), 3,3′,4′,5-tetrachlorosalicylanilide (TCS; Feng et al., 2014; Li et al., 2016; Velho et al., 2016), para-nitrophenol (Zuriaga-Agustí et al., 2016), 2,4,6-trichlorophenol (TCP; Feng et al., 2013; Zheng et al., 2008), and tetrakis (hydroxymethyl) phosphonium sulfate (THPS; Guo et al., 2014b; Xiao et al., 2016; Li et al., 2016).

Three biological wastewater treatment processes were used in previous continuous studies on sludge reduction with metabolic uncouplers, as follows: conventional activated sludge (CAS; Romero-Pareja et al., 2017; Xiao et al., 2016; Ye and Li, 2005), sequencing batch reactor (SBR; Feng et al., 2014; Zuriaga-Agustí et al., 2016), and anaerobic-anoxic-oxic (A2O) processes (Guo et al., 2014b; Li et al.,...
Among these three processes, SBR and A2O, being biological nutrient removal technologies, are popular wastewater treatment techniques. Organic components (COD), nutrients (N and P), and other contaminants can be simultaneously removed from wastewater by these two processes (Liu et al., 2014; Lu et al., 2016; Yan et al., 2016). SBR and A2O are widely applied in wastewater treatment plants. For example, among the WWTPs of China, A2O process is the most popular, with a ratio of 31%, while SBR accounts for 10% (Zhang et al., 2016). Thus, studies using SBR or A2O processes are more useful for research purposes than those using CAS in wastewater treatment. However, previous studies only focused on the effects of metabolic uncoupler with almost no research available on the simultaneous performance comparison between SBR or A2O processes as sludge reduction processes with metabolic uncoupler. To promote the application of the metabolic uncoupler addition method, it is necessary to evaluate the sludge reduction with metabolic uncoupler addition in the two wastewater treatment processes.

Therefore, the present research aims to study the effects of the two wastewater treatment processes (SBR and A2O) on sludge reduction with metabolic uncoupler, using DCP as an example.

2. Materials and methods

2.1. Wastewater and metabolic uncoupler

The wastewater used in the test was obtained from a residential area in Beijing, China. The characteristics of the wastewater are summarized in Table 1. The metabolic uncoupler, DCP, used in the test, was bought from Tokyo Chemical Industry Co., Ltd.

2.2. Wastewater treatment processes and their operations

Two laboratory-scale SBRs and two laboratory-scale A2Os were operated in parallel during the test. One SBR and one A2O were operated with 10 mg of DCP/L influent (SBR-DCP and A2O-DCP), while the other two were operated as controls without DCP addition (SBR-Con and A2O-Con). DCP was continuously added in the filling phase of SBR and the oxic tank of A2O. The four processes were operated for 85 d at room temperature (20–28 °C).

The working volumes for each part of the A2O were as follows: anaerobic tanks, 4 L; anoxic tanks, 4 L; oxic tanks, 16 L; and settling tanks, 4 L. The anaerobic and anoxic tanks were mixed via mechanical stirring, while the oxic tanks were aerated to maintain the dissolved oxygen (DO) levels. The hydraulic retention times (HRTs) and DO of the first three tanks were maintained at 2 h and < 0.1 mg/L (anaerobic tanks), 2 h and 0.2–0.5 mg/L (anoxic tanks), and 8 h and 1.5–3 mg/L (oxic tanks). The HRT in the settling tank was 2 h. The internal (mixing liquor) and external (sludge) recycling ratios were 100% and 200%, respectively.

The working volume of SBR was 5 L. It was operated for 6 h per cycle. To stimulate the A2O, each cycle included 15 min for the filling phase, 45 min for the stirring phase, 4 h for aerating and stirring, 45 min for the settling phase, and 15 min for the decanting phase. The DO in the filling and stirring phases was maintained at less than 0.1 mg/L, which could be regarded as the anaerobic stage. The DO in the aerating and stirring phases was maintained at 2–4 mg/L, regarded as the oxic stage. Meanwhile, the DO in the settling and decanting phases was maintained at 0.2–0.5 mg/L, regarded as the anoxic stage. Therefore, the HRTs of the three stages were 1 h (anaerobic stage), 4 h (oxic stage), and 1 h (anoxic stage).

In order to maintain similar sludge concentrations in the oxic tank of A2O and the aeration and stir phase of SBR, activated sludge was regularly discharged as excess sludge from the oxic tank of A2O and the last stage of the aerating and stirring phase of SBR. The sludge concentrations in the oxic tank of A2O and the aeration and stir phases of SBR were maintained at 2–4 g/L. The sludge retention times (SRT) in the two control systems were about 9.6–10.5 d, while those in the two metabolic uncoupler-added processes were 12.6–13.4 d.

2.3. Analysis and calculation

The influent, effluent, and mixed sludge in the aerating and stirring phases of SBR and the oxic tanks of A2O were regularly sampled during the test. Water quality parameters, including chemical oxygen demand (COD), NH4+-N, total nitrogen (TN), total phosphorous (TP), pH, and suspended solid (SS) of both the influent and effluent were analyzed. Mixed sludge characteristics, including sludge concentration (suspended solids [SS] and volatile suspended solids [VSS]), sludge volume index (SVI), extracellular polymeric substances (EPS), and special oxygen uptake rate (SOUR), were analyzed. COD was determined using a COD meter (DR2800, HACH, USA), while the pH was measured using a pH meter (PB-10, Sartorius, Germany). DO was measured using an online DO meter (3310, WTW, Germany). The EPS of the activated sludge samples was extracted by using a cation exchange resin (Dowex Marathon C) technique described by Guo et al. (2014a). The polysaccharide (PS) content of the extracted EPS was determined using the phenol-sulfuric acid method, with glucose as a standard (Dubois et al., 1956), while the protein (PN) content was determined using the Lowry method (Lowry et al., 1951), with bovine serum albumin as a standard. SOUR was measured using the method described by Mancuso et al. (2017). Other parameters were analyzed using standard methods (Rice et al., 2012).

The excess sludge production, the sludge yield (Y(obs)) in the four processes and their reduction in the two DCP-added processes were calculated in accordance with the method of Guo et al. (2014b). The energy uncoupling coefficients for the DCP-added system, Ecps, can be defined by Eq. (1) (Chen et al., 2008).

\[
E_{cp} = \frac{Y_{obs,DCP} - Y_{obs,Con}}{Y_{obs,Con}}
\]

(1)

where \(Y_{obs,DCP}\) is the sludge yield of the control system; \(Y_{obs,Con}\) is the sludge yield of the DCP-added system.

The removal rates for each water quality parameter, R, were defined by Eq. (2) as:

\[
R (\%) = \frac{C_{inf} - C_{eff}}{C_{inf}} \times 100
\]

(2)

where \(C_{inf}\) is the concentration of the water quality parameter in the influent, \(C_{eff}\) is the concentration of the water quality parameter in the effluent.

The relative specific removal rates of each water quality parameter, RSRR, were defined as shown in Eq. (3).

\[
RSRR = \frac{R_{DCP}}{R_{Con}}
\]

(3)

where \(R_{DCP}\) is the removal in the DCP-added system, \(R_{Con}\) is the removal in the control system.

The inhibition coefficients of removal for each water quality parameter, IC, were defined as shown in Eq. (4) (Chen et al., 2006).

| Parameter | Range   | Mean |
|-----------|---------|------|
| pH        | 7.56–8.43 | 7.87 |
| COD (mg/L)| 64–452  | 172  |
| SS (mg/L) | 16–551  | 195  |
| NH4+-N (mg/L) | 24.41–59.54 | 46.11 |
| TN (mg/L) | 37.6–92.4 | 56.93 |
| TP (mg/L) | 3.03–9.45 | 5.86 |
\[ IC = \frac{R_{\text{Con}} - R_{\text{DCP}}}{R_{\text{Con}}} \]  

(4)

2.4. DNA extraction, PCR, and DGGE

The mixed sludge in the aericating and stirring phases of SBR and the oxic tank of A2O were sampled on the 70th day, while the microbial communities in those samples were analyzed using molecular biological methods (DNA extraction, PCR, DGGE, and sequencing). The DNA of the sludge samples was extracted using a nucleic acid automatic extraction system (TANBead Smart LabAssist-16, Taiwan). The extracted DNA was subsequently used as a template for PCR amplification and DGGE.

PCR primers, 341F (5’-CCTACGGGAGGCAGCAG-3’) and 534R (5’-ATTACCGCGGCTGCTGG-3’), were used to amplify a segment of the eubacterial 16S rDNA. A GC-clamp was added to the forward primers to facilitate DGGE. PCR amplification was performed using a C1000 thermal cycler (BioRad, USA) at a final volume of 50 μl of PCR. The reaction mixture contained 1 μl of both the primers (10 μM), 4 μl of each dNTP, 5 μl of 10 × buffer, 50 ng of DNA template, and 1.5 units of Taq DNA polymerase. The temperature cycling conditions were 95 °C for 5 min, followed by 30 cycles at 93 °C for 1 min, at 48 °C for 1 min, and at 72 °C for 1 min. A final extension at 72 °C for 10 min was used. A 5-μl aliquot of the PCR product was separated on a 0.8% (w/v) agarose gel at 100 V for 30 min to verify the amplification prior to DGGE. DGGE of the PCR-amplified 16S rDNA was performed using a D-Code system (BioRad, USA). A total of 30–60% denaturing gradients were used to separate the amplified 16S rDNA. The gel was electrophoresed in 1 × TAE buffer at 70 V and 60 °C for 10 h. The resulting gel was visualized using Gel Red (Biotium, USA). The DGGE gel was analyzed using the Bio-Rad software, Quantity One™ (BioRad, USA).

2.5. Data analysis

Average results and standard errors were reported based on triplicates for each analysis or determination. Statistical significance was tested by the analysis of the means using t-tests, with a threshold P-value of 0.05 declared as significant. All data were analyzed using SPSS 19.0 for Windows (IBM, USA).

3. Results and discussion

3.1. Reduction of excess sludge production and sludge yield

The primary function of a sludge reduction system is to reduce sludge production. Therefore, the cumulative excess sludge production was calculated in the test according to the method of Guo et al. (2014b). The results are summarized in Table 2. At the end of the test, the cumulative excess sludge production in the four processes was 166.8 (SBR-Con), 95.2 (SBR-DCP), 538.2 (A2O-Con), and 304.4 g of SS (A2O-DCP; Fig. 1A). The excess sludge production per unit aerobic volume in the four processes was 33.4 (SBR-Con), 19.0 (SBR-DCP), 33.6 (A2O-Con), and 19.1 g of SS/L (A2O-DCP). The results showed that DCP addition can lower excess sludge production in both the processes. The excess sludge production per unit aerobic volume was similar in the two control processes and the metabolic uncoupler-added processes. Compared to the control processes, the reduction of excess sludge in the two metabolic uncoupler-added processes can be determined through calculations. The results are summarized in Table 2. A reduction of excess sludge rapidly increased during 0–20 d (SBR-DCP) and 0–25 d (A2O-DCP), maintaining relative stability thereafter. In the stable stage, the average reduction of excess sludge in the two processes was 43.7% (SBR-DCP) and 44.2% (A2O-DCP). The excess sludge reduction in the two processes was due to the metabolic uncoupling action of DCP (Chen et al., 2006; Song et al., 2010). Meanwhile, the excess sludge reduction in the test was similar to previous studies (Li et al., 2016; Zheng et al., 2008). For example, Zheng et al. (2008) found that 2 mg/L of TCP could reduce excess sludge generation by about 47% during the treatment of municipal wastewater with SBR under uncoupling metabolic conditions. The aforementioned result indicated that DCP addition led to a slightly higher excess sludge reduction in A2O than in SBR. However, the difference between the two processes was not significant because the P-value of the t-test was higher than 0.05 (Table 2).

The changes in sludge yield (Yobs) among the four processes, which was also calculated according to the method of Guo et al. (2014b), are summarized in Fig. 1B. At 0–30 d, the sludge yields in all the four processes decreased, subsequently achieving stability. The stable mean sludge yields of the four processes were 0.367 (SBR-Con), 0.305 (SBR-DCP), 0.370 (A2O-Con), and 0.306 mg SS/mg-COD. Although the sludge yield in the two SBR was slightly lower than that in the two A2O, statistical analysis (Table 2) suggested the difference between the two processes was not significant because the P-values of their t-test were higher than 0.05. In other words, the sludge yields of the two processes were similar, which is reasonable, since the sludge yield is essentially the same for all suspended growth processes with a given SRT and biochemical environment, regardless of the bioreactor configuration (Grady et al., 2011). The sludge yield reduction in the two DCP-
added processes in the test, calculated according to the method of Guo et al. (2014b), are also summarized in Fig. 1B. The maximum and average sludge yield reductions for the two processes were 27.8% and 16.9% (SBR-DCP) and 28.2% and 17.8% (A2O-DCP). Additionally, the reduction of sludge yield fluctuated with the operation time because of the changes in influent quality. Although DCP addition led to a slightly higher sludge yield reduction in A2O than in SBR, the difference in sludge yield reductions between the two processes was not significant because the P-value of the t-test was higher than 0.05 (Table 2). The reduction of sludge yield in the test was similar to those in some previous studies with other metabolic uncouplers (Guo et al., 2014b; Ye and Li, 2005; Zheng et al., 2008). For example, Ye and Li (2005) found that TCS could reduce sludge yield by approximately 30% at a dosage of 40 mg/day, while Guo et al. (2014b) found that the sludge yield could be reduced by 14.7% with 1.08–1.86 mL THPS/m³ influent. The average energy uncoupling coefficients of the two uncoupler-added processes, calculated according to Eq. (1), were 0.168 ± 0.061 (DCP-SBR) and 0.178 ± 0.065 (DCP-A2O). Although the average energy uncoupling coefficient of DCP-SBR was slightly lower than that of DCP-A2O, they were comparable since the P-value of the t-test was higher than 0.05 (data not shown).

3.2. Wastewater treatment efficiencies

Wastewater treatment efficiency is important for the sludge reduction system. The results of this study are summarized in Table 3. DCP addition in the two processes lowered the average removal of four detected parameters (COD, NH₄⁺-N, TN, and TP). The average COD removal with the two DCP-added processes was 86.4% (SBR-DCP) and 85.3% (A2O-DCP), which was lower by 3.8% (SBR-DCP) and 6.0% (A2O-DCP), compared with the corresponding control processes. The average removal and reduction of NH₄⁺-N for the two DCP-added processes were similar. The average removal of TN for the two DCP-added processes was also similar, with their reduction being 10.3% (SBR-DCP) and 8.6% (A2O-DCP). DCP addition resulted in a slightly higher average TP removal and lower decrease in A2O than in SBR in the test. The relative specific removal rates of each water quality parameter and the inhibition coefficients of their removal for the two uncoupler-added processes were similar (Table 3). Significance analysis of the data suggested the differences in wastewater treatment efficiencies between the two processes were not significant because their P-values of t-test were higher than 0.05 (Table 4). The addition of DCP in the two wastewater treatment processes resulted in low wastewater treatment efficiency, which was consistent with the previous studies (Tian et al., 2013; Zheng et al., 2008).

3.3. Sludge characteristics (SVI, EPS, and SOUR)

The addition of metabolic uncoupler would affect the characteristics of activated sludge, such as settleability, EPS content, and SOUR.

### Table 3

Wastewater treatment efficiencies in the test.

| Item                   | Average COD | Average NH₄⁺-N | Average TN | Average TP |
|------------------------|-------------|----------------|------------|------------|
| Removal in SBR-Con (%) | 96.2 ± 5.4  | 99.1 ± 4.3     | 77.8 ± 2.6 | 55.9 ± 2.9 |
| Removal in SBR-DCP (%) | 86.4 ± 4.7  | 96.4 ± 4.1     | 67.5 ± 2.9 | 51.5 ± 2.2 |
| RSRR of DCP in SBR    | 0.958 ± 0.040 | 0.973 ± 0.036 | 0.868 ± 0.053 | 0.921 ± 0.028 |
| IC of removal in SBR   | 0.042 ± 0.040 | 0.027 ± 0.036 | 0.132 ± 0.053 | 0.079 ± 0.028 |
| Removal in A2O-Con (%) | 91.2 ± 2.6  | 98.9 ± 5.4     | 76.9 ± 3.7 | 58.9 ± 1.8 |
| Removal in A2O-DCP (%) | 85.2 ± 3.0  | 96.1 ± 3.3     | 68.3 ± 2.7 | 55.7 ± 2.5 |
| RSRR of DCP in A2O    | 0.934 ± 0.037 | 0.972 ± 0.042 | 0.888 ± 0.041 | 0.946 ± 0.034 |
| IC of removal in A2O   | 0.066 ± 0.037 | 0.028 ± 0.042 | 0.112 ± 0.041 | 0.054 ± 0.034 |

* RSRR: Relative specific removal rate.
* IC: Inhibition coefficient.

Settleability is an important characteristic of activated sludge, which affects the wastewater treatment efficiency. In the test, SVI was used as an index of sludge settleability, which was measured via standard methods (Rice et al., 2012). Its changes are summarized in Fig. 2. The means of sludge SVIs for the two processes were 76.0 (SBR-Con), 85.7 (A2O-Con), 123.2 (SBR-DCP) and 127.7 mL/g SS (A2O-DCP). The results showed the settleability of activated sludge in the two

### Table 4

P-value of t-test between the removal of water quality parameters in the two processes.

| Item       | Removal of       |       |       |       |
|------------|------------------|-------|-------|-------|
|            | COD              | NH₄⁺-N | TN    | TP    |
| Con system | 0.786 ± 0.991    | 0.305 ± 0.523 | 0.324 ± 0.325 |       |
| DCP system | 0.798 ± 0.933    | 0.324 ± 0.325 | 0.324 ± 0.325 |       |

* P > 0.05 indicates no significant difference; P < 0.05 indicates significant difference.
DCP-added processes changed from good to fair. Although the sludge SVIs for A2O-DCP were slightly higher than those for SBR-DCP, the statistical analysis suggested their differences were not significant (Table 2). Some researchers also reported an increase in sludge SVI in the sludge reduction system with metabolic uncoupler (Fang et al., 2015; Guo et al., 2014b; Ye and Li, 2005; Zheng et al., 2008). For example, Ye and Li (2005) found that 40 mg/d of TCS could increase SVI by 15% in 15-L aeration tank, while Fang et al. (2015) found that the SVI of activated sludge increases by approximately 4%, 25%, and 13%, respectively, with the addition of pCP, oCP, and oNP in the concentration range of 5%–20%.

Metabolic uncoupler is typically a chemical toxin to sludge microorganisms (Detchanamurthy and Gostomski, 2012). Those microorganisms will generate a large amount of EPS to resist the uncoupler toxicity for survival and to adapt to the new environment, when they are placed into an uncoupler-existent environment (Feng et al., 2014; Geyik and Çeçen, 2016). Thus, the sludge EPS, presented by PS + PN, was extracted using a cation exchange resin technique (Guo et al., 2014a), while the changes in sludge EPS were detected through the test (Fig. 3). The EPS of sludge in both the DCP-added processes was higher than their controls. The EPS of sludge in SBR-DCP was 1.96 g/g-SS (mean value) higher compared to its control, while that in A2O-DCP was 2.93 g/g-SS (mean value) higher than in its control, which suggested that DCP addition resulted in higher EPS generation in A2O than in SBR. The average PN/PS of EPS generated by sludge microorganisms in the four processes was 1.67 (SBR-Con), 1.66 (SBR-DCP), 1.61 (A2O-Con), and 1.60 (A2O-DCP). Therefore, the PN/PS of EPS generated by sludge microorganisms in the two SBRs was higher than that in the two A2Os. However, in the DCP-added processes, it was lower than that in the controls. The changes in PN/PS of EPS suggested that sludge microorganisms generated more PS than PN in DCP-existent environment. The changes in EPS and PN/PS were consistent with those of sludge SVIs (Figs. 2 and 3), which is reasonable because an increase of sludge EPS and decrease of PN/PS would result in an increase of sludge SVI (Ren et al., 2016; Sheng et al., 2010).

The changes in SOUR of activated sludge were measured according to the method described by Mancuso et al. (2017), with the results summarized in Fig. 4, which can be used to evaluate the effect of the metabolic uncoupler on microbial metabolic activity of sludge. Among the four processes, the SOURs of activated sludge in SBR-DCP were the highest, with 4.79 mg of O2/g VSS d being the average value, which

![Fig. 4. Change in activated sludge SOUR during the test.](image-url)

![Fig. 5. DGGE profiles and similarities for sludge samples in the four processes. 1: A2O-Con; 2: A2O-DCP; 3: SBR-Con; 4: SBR-DCP.](image-url)

Band similarities: 100% 51.6% 100% 78.2%

The changes in SOUR of activated sludge were measured according to the method described by Mancuso et al. (2017), with the results summarized in Fig. 4, which can be used to evaluate the effect of the metabolic uncoupler on microbial metabolic activity of sludge. Among the four processes, the SOURs of activated sludge in SBR-DCP were the highest, with 4.79 mg of O2/g VSS d being the average value, which
was followed by those in A2O-DCP, with 3.87 mg O2/g VSS being the average value. In addition, the SOURs of activated sludge in SBR-Con were lower than those in A2O-Con, which was consistent with COD removal. Metabolic uncouplers could increase the SOUR of sludge because they can uncouple the oxidative phosphorylation, inhibiting the synthesis of adenosine triphosphate and promoting respiration in sludge microorganisms (Song et al., 2010; Xie et al., 2010). High SOUR means over-consumption of oxygen, which implies a high-level of energy dissipation for metabolic regulation, which could be the cause of sludge growth reduction (Mayhew and Stephenson, 1998). The results indicated that DCP addition could enhance the microbial metabolic activity of sludge in both the wastewater treatment processes, with SBR being more favorable for enhancing SOUR than A2O.

### 3.4. Microbial communities

In this work, the microbial communities of the activated sludge in the four processes, which were sampled on the 70th day, were analyzed by the molecular biological technique. After the total DNA of sludge was extracted, the eubacterial 16S rDNA was amplified using bacteria universal primers (314F/534R), based on the total DNA template. The microbial diversity of sludge was observed through DGGE. The results are summarized in Fig. 5. The similarities between DCP-added processes and their control processes were 78.2% (SBR-DCP) and 51.6% (A2O-DCP). A total of 31 (SBR-Con), 31 (SBR-DCP), 38 (A2O-Con), and 35 (A2O-DCP) bands were observed in the DGGE profiles. The results showed that DCP does not alter the DGGE profiles of the corresponding control reactors dramatically because their band similarities were higher than 50%. Additionally, the dominant bands of sludge samples from the four processes (namely high-brightness bands) were different. The differences in band similarities, band numbers, and banding patterns suggested that microbial communities of activated sludge in the four processes were different. Previous studies have reported a change in microbial communities due to metabolic uncouplers added into SBR and A2O (Zheng et al., 2008; Guo et al., 2014b; Kimura et al., 2016). The greater similarity of SBR-DCP with SBR-Con suggests that SBR could retain more primary microorganisms of activated sludge under metabolic uncouple condition.

### 4. Conclusion

The effects of DCP addition on sludge reduction, wastewater treatment efficiencies, activated sludge characteristics, and microbial communities were compared through two laboratory-scale wastewater treatment processes: SBR and A2O. Experimental results showed that the reduction of excess sludge and sludge yield in the two processes was similar. The effects of DCP on wastewater treatment efficiencies in the two processes (removal of COD, total nitrogen, NH4-N, and total phosphorus) were also similar. SBR was slightly more likely to retard the increase of sludge SVI than A2O by lower increase in EPS and being more favorable to the increase in sludge SOUR, while better maintaining the primary microorganisms of activated sludge under metabolic uncouple condition, than A2O.

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### References

Chen, G.W., Yu, H.Q., Xi, P.G., Xu, D.Q., 2006. Response of activated sludge to the presence of 2,4-dichlorophenol in a batch culture system. Process Biochem. 41, 1758–1763.
Chen, G.-W., Yu, H.-Q., Xi, P.-G., Xu, D.-Q., 2008. Modeling the yield of activated sludge in the presence of 2,4-dinitrophenol. Biochem. Eng. J. 40, 150–156.
China State EPA, 2014. China Environment Yearbook of 2013. China Environmental Science Press, Beijing (in Chinese).
Detchanamurthy, S., Gostomski, P.A., 2012. Metabolic uncouplers in environmental research: a critical review. Rev. Chem. Eng. 28, 309–317.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Fang, F., Hu, H.-L., Qin, M.-M., Xue, Z.-X., Cao, J.-S., Hu, Z.-R., 2015. Effects of metabolic uncouplers on excess sludge reduction and microbial products of activated sludge. Bioresour. Technol. 185, 1–6.
Feng, X.-C., Guo, W.-Q., Yang, S.-S., Zheng, H.-S., Du, J.-S., Wu, Q.-L., Ren, N.-Q., 2014. Possible causes of excess sludge reduction adding metabolic uncoupler, 3,3′,4,5′-tetrachlorosalicylaldehyde (TCS), in sequence batch reactors. Bioresour. Technol. 173, 96–103.
Geyir, A.G., Çeçen, F., 2016. Exposure of activated sludge to nanosilver and silver ions: inhibitory effects and binding to the fractions of extracellular polymeric substances. Bioresour. Technol. 211, 691–697.
Grady Jr., C.P.L., Druigger, G.T., Love, N.F., Filipe, C.D.M., 2011. Biology of Wastewater Treatment, 3rd ed. IWA Publishing, London.
Guo, W.-Q., Yang, S.-S., Xiang, W.-S., Wang, X.-J., Ren, N.-Q., 2013. Minimization of excess sludge production by in-situ activated sludge treatment processes—a comprehensive review. Biotechnol. Adv. 31, 1386–1396.
Guo, W., Liu, J.X., Xiao, B.Y., 2014a. Evaluation of the damage of cell wall and cell membrane for various extracellular polymeric substance extractions of activated sludge. J. Biotechnol. 188, 130–135.
Guo, X.S., Yang, J.M., Liang, Y., Liu, X.J., Xiao, B.Y., 2014b. Evaluation of sludge reduction by an environmentally friendly chemical uncoupler in a pilot-scale anaerobic-anoxic-oxic process. Bioprocess Biosyst. Eng. 37, 553–560.
Kimura, Z., Hirano, Y., Matsuura, Y., Hiraiishi, A., 2016. Effects of 3,5-dichlorophenol on excess biomass reduction and bacterial community dynamics in activated sludge as revealed by a polyphasic approach. J. Biosci. Bioeng. 122, 467–474.
Li, P., Li, H.C., Li, J., Guo, X.S., Liu, J.X., Xiao, B.Y., 2016. Evaluation of sludge reduction of three metabolic uncouplers in laboratory-scale anaerobic-anoxic-oxic process. Bioresour. Technol. 221, 31–36.
Liu, Y., Cheng, X., Sun, D.Z., 2014. CE, emission and conversion from A2O and SBR processes in full-scale wastewater treatment plants. J. Environ. Sci.-China 26, 224–230.
Lowry, O.H., Rosebrugh, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Lu, Q., de Toledo, R.A., Shim, H., 2016. Effect of COD/TP ratio on biological nutrient removal in A2O and SBR processes coupled with microfiltration and effluent reuse potential. Environ. Technol. 37, 1461–1466.
Mancuso, G., Langone, M., Andreottola, G., 2017. A swirling jet-induced cavitation to increase activated sludge solubilisation and aerobic sludge biodegradability. Ultrason. Sonochem. 35, 489–501.
Mayhew, M., Stephenson, T., 1998. Biomass yield reduction: is biochemical manipulation possible without affecting activated sludge process efficiency? Water Sci. Technol. 38, 137–144.
Ren, B., Young, B., Variola, F., Delattola, R., 2016. Protein to polysaccharide ratio in EPS as an indicator of non-optimized operation of tertiary treating MBBR. Water Qual. Res. J. Can. 51 (4), 297–306.
Richardson, B., Baird, R., Eaton, A.D., Clesceri, L.S., 2012. Standard Methods for the Examination of Water and Wastewater, 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation.
Romero-Pareja, P.M., Araoz, C.A., Quiroga, J.M., Coello, M.D., 2017. Evaluation of a biological wastewater treatment system combining an OS process with ultrasonic for sludge reduction. Ultrason. Sonochem. 36, 336–342.
Sheng, G.-P., Yu, H.-Q., Li, X.Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment processes: a review. Biotechnol. Adv. 28, 882–894.
Song, L., Jiang, W.J., Qiang, T., Li, Y.Z., 2010. Impact of a metabolic uncoupler, 2,4-dichlorophenol on minimization of activated sludge production in membrane bioreactor. Water Sci. Technol. 62, 1379–1385.
Tian, Y., Zhang, J., Wu, D., Li, Z.P., Cui, Y.N., 2013. Distribution variation of a metabolic uncoupler, 2,6-dichlorophenol (2,6-DCP) in long-term sludge culture and their effects on sludge reduction and biological inhibition. Water Res. 47, 279–288.
Velho, V.F., Daudt, G.C., Martins, C.L., Filho, P.B., Costa, R.H.R., 2016. Reduction of possible causes of excess sludge production in an activated sludge system based on lysis-cryptic growth, uncoupling metabolism and folic acid addition. Braz. J. Chem. Eng. 33, 47–57.
Xie, D.Q., Li, H.C., Yan, H., Gou, X.S., 2016. Evaluation of the sludge reduction effectiveness of a metabolic uncoupler- tetrakis (hydroxymethyl) phosphonium sulfite in anaerobic/anoxic/oxic process. Desalin. Water Treat. 57, 5772–5780.
Q., Wang, X.C., Ao, D., 2016. Current status of urban wastewater treatment plants in China. Environ. Int. 92–93, 11–22.

Zheng, G.H., Li, M.N., Wang, L., Chen, Z.Y., Qian, Y.F., Zhou, Q., 2008. Feasibility of 2,4,6-trichlorophenol and malonic acid as metabolic uncoupler for sludge reduction in the sequence batch reactor for treating organic wastewater. Appl. Biochem. Biotechnol. 144, 101–109.

Zuriaga-Agustí, E., Mendoza-Roca, J.A., Bes-Piá, A., Alonso-Molina, J.L., Amorós-Muñoz, L., 2016. Sludge reduction by uncoupling metabolism: SBR tests with para-nitrophenol and a commercial uncoupler. J. Environ. Manage. 182, 406–411.