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3 **Use of magnetic powder to effectively improve the performance of sequencing**
4 **batch reactors (SBRs) in municipal wastewater treatment**

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19 **Abstract**

20 This study aims to investigate the effect of adding magnetic powder in the sequencing
21 batch reactor (SBR) on the reactor performance and microbial community. Results
22 indicated that, the magnetic activated sludge sequencing batch reactor (MAS-SBR) had
23 7.76% and 4.76% higher ammonia nitrogen (NH₄⁺-N) and chemical oxygen demand
24 (COD) removal efficiencies than that of the conventional SBR (C-SBR). The
25 MAS-SBR also achieved 6.86% sludge reduction compared with the C-SBR.

26 High-throughput sequencing demonstrated that the dominant phyla of both SBRs
27 (present as ≥1% of the sequence reads) were *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*,
28 *Saccharibacteria*, *Chlorobi*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, *Planctomycetes*
29 and *unclassified_Bacteria*. The relative abundance of *Proteobacteria* and *Bacteroidetes*
30 simultaneously declined whereas the other 8 phyla increased following the addition of

31 magnetic powder. Adding magnetic powder in the SBR significantly affected the
32 microbial diversity and richness of activated sludge, consequently affecting the reactor
33 performance.

34 **Keywords:** Magnetic activated sludge, sequencing batch reactor, sludge reduction,
35 microbial diversity and richness, high-throughput sequencing

36 **1. Introduction**

37 Sequencing batch reactor (SBR) has been widely used in municipal and industrial
38 wastewater treatment, as it provides several advantages such as the flexibility of
39 process, cost effectiveness, and high biodegradation efficiency (Singh and Srivastava,
40 2010). However, the sludge generated in the SBR process is almost 0.3-0.5 g dry
41 biomass produced per g soluble COD removed, and the treatment expense of excessive
42 sludge accounts for as much 60% of the total plant operation costs (Ying et al., 2010). It
43 should be realized currently that finding out the efficient and eco-friendly methods to
44 minimize excess sludge production is of great importance. Recently, researchers (Nazif
45 et al., 2016; Li et al., 2016; Raj et al., 2013) achieved successfully excess sludge
46 reduction (20-70%) using oxic-settling-anoxic (OSA) system, metabolic uncouplers and
47 anaerobic/anoxic/oxic (AAO) system. However, these methods cannot avoid the
48 drawbacks such as additional capital, operating costs, and potentially environmental
49 impact as a part of chemical uncouplers are difficult to metabolic and will cause
50 uncertain effects on human health eventually.

51 Considering its environmentally friendly and positive magnetic bio-effect, the

52 magnetic activated sludge (MAS) process has been used to improve reactor
53 performance, especially sludge reduction. Ji et al. (2010) and Łebkowska et al. (2011)
54 discovered that the static magnetic field exerted a positive effect on activated sludge
55 including biomass growth, dehydrogenase activity and organic matter biodegradation.
56 Ma et al. (2017) reported that 10-60 mg/L Fe_3O_4 nanoparticles improved denitrification,
57 phosphorus removal and enzymatic activity. Liu et al. (2015) compared the
58 conventional activated sludge (CAS) and the MAS processes for the removal of
59 pollutants. Results showed that adding the magnetic powder had no negative influence
60 on the growth of activated sludge, resulting in higher ammonia nitrogen removal
61 efficiency ($88.68 \pm 7.98\%$). Yao et al. (2013) developed a novel magnetic carrier with
62 surface magnetic field of 4 mT to study the magnetic enhanced bio-effect on
63 nitrification in sequencing batch biofilm reactors. They revealed that nitrite and
64 ammonia oxidation activities in biofilm were promoted in the magnetic carrier reactor
65 compared with non-magnetic carrier reactor. Ying et al. (2010) also obtained an average
66 of 91% COD removal efficiency when using the MAS process. Furthermore, they found
67 that the MAS process could possibly maintain high MLVSS concentration and lower
68 the sludge loading rate, and consequently resulted in low sludge production. Zhou et al.
69 (2015) developed a kind of modified Fe_3O_4 nanoparticles as an efficient biomimic
70 catalyst for promoting activated sludge anaerobic hydrolysis,, Their results indicated
71 that sludge reduction efficiency increased from an initial 17.0% to 35.3% under optimal
72 conditions.

73 To the best of our knowledge, the biodegradation process depends on the
74 microorganisms in the bioreactor, thus we should pay more attention to the microbial
75 communities involved for providing insight into how microorganisms influence reactor
76 performance. Especially, with the rapid development of modern molecular biological
77 methods, such as high-throughput sequencing, it is easier to analyze the complex
78 microbial communities. (Neoh et al., 2017; Zhang et al., 2016; Yuan et al., 2016).

79 This study aims to evaluate the use of magnetic powder for enhancing pollutants
80 removal as well as reducing sludge production in the SBR. Further, high-throughput
81 sequencing technology served to analyze the microbial community and composition in
82 the SBR, in order to reveal the impact of added magnetic powder on the microbial
83 communities and how to improve the performance of SBR.

84 **2. Materials and methods**

85 *2.1 SBR and operating conditions*

86 Two SBRs used in this study consisted of two aerobic reactors (each having an
87 effective volume of 4 L). One was a MAS sequencing batch reactor (MAS-SBR) with
88 1g/L added magnetic powder (9.119 μ m, Sinopharm Chemical Regent Co., Ltd, China),
89 and the other was a conventional SBR (C-SBR) without adding magnetic powder.

90 Synthetic wastewater was used as feed to both SBRs. The reactors were seeded with
91 aerobic activated sludge from a local municipal wastewater plant (Bailonggang
92 Wastewater Treatment Plant, Shanghai, China), and operated under the same conditions
93 for 60 days. The aeration intensity was set at 0.75 m³/h and the hydraulic retention time

94 (HRT) was 8 h with extra 1 h setting time. No sludge waste occurred throughout 60
95 days from both reactors, except samples taken to measure the suspended solids. The
96 experiment temperature was conducted at 28-32 °C.

97 *2.2 Raw wastewater and physiochemical analysis*

98 Raw wastewater was prepared every day referring to Liu et al. (2005). Chemical
99 oxygen demand (COD), ammonia-nitrogen ($\text{NH}_4^+\text{-N}$) and mixed volatile suspended
100 solids (MLSS) were measured according to Chinese NEPA standard methods (2002).
101 The influent COD were 290.1 ± 7.4 mg/L and 287 ± 4.57 mg/L for the C-SBR and
102 MAS-SBR, respectively. The influent $\text{NH}_4^+\text{-N}$ were 65.7 ± 2.7 mg/L and 64.6 ± 2.15
103 mg/L for the C-SBR and the MAS-SBR, respectively. The particle sizes of magnetic
104 powder and sludge samples were measured using a static light scattering particle size
105 analyzer (Malvern 2000, Britain). The measurement of sludge volume after 30 min of
106 settling (SVI_{30}) was used for describing sedimentation performance of sludge. The
107 sludge activity was measured by pH value method whose principle is the concentration
108 of CO_2 accumulated by cellular respiration (Li et al., 2007)

109 *2.3 Microbial community and diversity analysis*

110 *2.3.1 DNA extraction and PCR amplification*

111 To comprehensively analyze the microbial communities in the SBRs, 2 mixed liquor
112 samples (one sample for each) were collected from the C-SBR and the MAS-SBR at the
113 end of the experiments for Illumina MiSeq sequence analysis. Microbial DNA was
114 extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.)

115 according to manufacturer's protocols.

116 *2.3.2 454 sequencing and phylogenetic classification*

117 Amplicons were extracted, purified and quantified according to the manufacturer's
118 instructions. Purified amplicons were pooled in equimolar and paired-end sequenced (2
119 × 250) on an Illumina MiSeq platform. The raw reads were deposited into the NCBI
120 Sequence Read Archive (SRA) database (Accession Number: SRP101681). Operational
121 Units (OTUs) were clustered with 97% similarity using UPARSE, and chimeric
122 sequences were identified and removed using UCHIME. The taxonomy of each 16S
123 rRNA gene sequence was analyzed by RDP Classifier against the silva (SSU123)16S
124 rRNA database using the confidence threshold of 70%. According to the rarefaction
125 curves, Shannon and Chao indices were calculated to compare the microbial diversity
126 and richness between the C-SBR and the MAS-SBR samples.

127 **3. Results and Discussion**

128 *3.1 The performance of the SBRs*

129 After nearly one month acclimatization, both SBRs successfully reached steady state.
130 The COD and NH_4^+ -N removals are provided in Figure 1. It can be seen from Figure 1
131 that adding magnetic powder in the SBR had no negative impact on the removals of
132 COD and NH_4^+ -N. The average COD and NH_4^+ -N removal efficiencies increased from
133 $83.60 \pm 11.02\%$ to $88.36 \pm 7.43\%$, $78.54 \pm 18.59\%$ to $86.3 \pm 13.13\%$, respectively. Thus,
134 adding magnetic powder in the SBR successfully enhance the removal of the pollutants.

135 *3.2 Activated sludge properties*

136 At the beginning of the experiments, the MLSS concentrations of both SBRs were
137 3.146 ± 0.055 g/L. By the end, the MLSS concentrations of the C-SBR and the
138 MAS-SBR reached up to 5.376 ± 0.067 g/L and 5.160 ± 0.061 g/L respectively. The
139 amount of sludge increased by 70.88% in the C-SBR and 64.02% in the MAS-SBR,
140 suggesting that 6.86% sludge reduction was obtained by adding magnetic powder in the
141 SBR. At the same time the SVI₃₀ tests demonstrated that adding magnetic powder could
142 improve the sedimentation property of sludge. In other words, the possibility of sludge
143 bulking can be partly decreased by adding magnetic powder. Meanwhile, according to
144 the pH value method testing, the sludge activity measured as $\Delta[\text{CO}_2]$ increased from
145 0.088 $\mu\text{mol/g}\cdot\text{min}$ of the C-SBR to 0.119 $\mu\text{mol/g}\cdot\text{min}$ of the MAS-SBR, indicating that
146 adding magnetic powder in the SBR had a positive effect on sludge activity. The
147 average particle size distribution size was 40.821 μm for the C-SBR and 37.765 μm for
148 the MAS-SBR. Thus, adding magnetic powder had little bioflocculation effect on
149 activated sludge. It can be inferred that magnetic bio-effect may affect the microbial
150 communities in the SBRs, and further improved the reactor performance. Thus it is
151 necessary to further analyze the diversity, composition and dominant species of
152 microbial communities in the SBRs.

153 *3.3 Microbial community and diversity analysis*

154 *3.3.1 Overall analysis of amplicon sequencing*

155 The microbial community and composition of activated sludge samples in the SBRs
156 were investigated through high-throughput sequencing. After necessary quality filtering,

157 the valid sequences of activated sludge samples in the C-SBR and the MAS-SBR were
158 32454 and 37494, respectively. Alignment and clustering of the sequences showed that
159 the OTUs based on a 97% sequence identity of the C-SBR and the MAS-SBR were 492
160 and 493, respectively. In summary, the microbial communities in the SBRs consist of
161 30 phyla, 58 classes, 95 orders, 156 families, 223 genera, 344 species (relative
162 abundance $\geq 0.1\%$). The rarefaction curves approached the plateau phase at 400 reads for
163 both samples, suggesting that the sequencing depth was sufficient to capture most of the
164 microbial diversity. Also, the Good's coverage of the sludge samples in both SBRs were
165 more than 0.998, indicating that the sequence library obtained from each sludge sample
166 could cover the microbial diversity of the SBR. Moreover, Shannon indices showed that
167 the MAS-SBR (4.8) contained higher level of community diversity than the C-SBR
168 (4.59) whereas Chao indices confirmed the opposite result in species richness.

169 3.3.2 Taxonomic classification of the microbial communities

170 To better understand the microbial communities of the C-SBR and the MAS-SBR
171 systems, the sequence reads were taxonomically classified and the relative abundance
172 was calculated at the phylum and genus level for each sample. The results are visualized
173 in Figure 2 and Table 1. At the phylum level, the top 10 dominant phyla present as $\geq 1\%$
174 of the sequence reads in the C-SBR and the MAS-SBR were similar, including
175 *Proteobacteria* (33.9% and 28.1%), *Bacteroidetes* (32.7% and 26.0%), *Chloroflexi*
176 (11.9% and 18.6%), *Saccharibacteria* (5.0% and 5.1%), *Chlorobi* (4.2% and 5.4%),
177 *Firmicutes* (4.2% and 4.5%), *Actinobacteria* (2.95% and 3.85%), *Acidobacteria* (2.05%

178 and 3.58%), *Planctomycetes* (1.02% and 1.43%) and *unclassified_Bacteria* (1.11% and
179 1.45%), respectively. It can be seen that the relative abundance of *Proteobacteria* and
180 *Bacteroidetes* simultaneously declined from 33.9% and 32.7% to 28.1% and 26.0%,
181 respectively, whereas the other phyla increased by 6.7%, 0.1%, 1.2%, 0.3%, 0.9%, 0.8%,
182 0.41% and 0.34%, respectively. It is reported that *Bacteroidetes* play a critical role in
183 degradation of polysaccharides and starch which are considered to be COD in
184 wastewater (Shu et al., 2015). Although the relative abundance of *Bacteroidetes*
185 declined by 6.7% with added magnetic powder, there is no negative effect on COD
186 removal efficiency, suggesting functional-sosurplus *Bacteroidetes* existed in both SBRs.
187 *Chloroflexi* have been identified as the major participants in the biological treatment in
188 digesting long chain fermenting sugars into short chain fatty acids precursors (Yamada
189 and Sekiguchi, 2009). Herein, the increased abundance of *Chloroflexi* in the MAS-SBR
190 may provide evidence for improved COD removal. At the genus level, the relative
191 abundance of *Nitrosomonas* fell from 0.017% in the C-SBR to 0.004% in the
192 MAS-MBR, whereas the relative abundance of *Nitrosomonadaceae*, *Nitrospira* and
193 *Candidatus_Nitrotoga* increased from 0.665%, 0.065% and 0.017% in the C-SBR to
194 0.743%, 0.128% and 0.021% in the MAS-MBR, respectively. *Nitrosomonas* and
195 *Nitrosomonadaceae* are related to the process of ammonia oxidization to nitrite, and
196 *Nitrospira* and *Candidatus_Nitrotoga* are closely associated with the process of nitrite
197 oxidation to nitrate (Ma et al., 2017). Thus, the genera variation consequently affected
198 the processes of ammonia oxidation and nitrite oxidation in the SBR, which provided

199 evidence of 7.76% higher NH_4^+ -N removal efficiency by adding magnetic powder in the
200 SBR. Similarly, the relative abundance of *Clostridium_sensu_stricto_13* increased from
201 3.28% in the C-SBR to 3.94% in the MAS-SBR. It has been reported that the genera
202 *Clostridia* had the ability to degrade biomass and fermentation of the wastewater (Lynd
203 et al., 2002). Maybe it can be used to explain the 6.68% sludge reduction by the
204 addition of magnetic powder in the SBR. Compared to the C-SBR, the relative
205 abundances of some genera (e.g. *Simplicispira*, *Commonadaceae*, *Thauera*,
206 *Chryseobacterium* and *Saprospiaceae*) decreased or increased in the MAS-SBR. Thus,
207 adding magnetic powder in the SBR significantly affected the microbial diversity and
208 richness of activated sludge, and consequently affected the reactor performance.

209 4. Conclusion

210 Adding magnetic powder in the SBR has definite potential in removal pollutants and
211 reducing sludge production. The differences between the C-SBR and the MAS-SBR
212 were clearly observed, such as COD and NH_4^+ -N removals, sludge properties and
213 dominant bacteria in activated sludge. High-throughput sequencing helped to
214 understand the effect of adding magnetic powder on the reactor performance and
215 microbial community in activated sludge.

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217 More detailed information such as PCR amplification, compositions of synthetic
218 wastewater, the SV_{30} results during the operation and SEM images of C-SBR and
219 MAS-SBR can be found in Supplementary Material.

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289 ironporphyrin modified Fe₃O₄ nanoparticles as an efficient biomimic catalyst. *Chem.*
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295 **Figure Captions**

296 Figure 1: Effect of adding magnetic powder in the SBR on COD and NH_4^+ -N removals

297 Figure 2: Taxonomic classification of the microbial communities at phylum level in

298 both SBRs, present as $\geq 1\%$ of the sequence reads in at least one sample.

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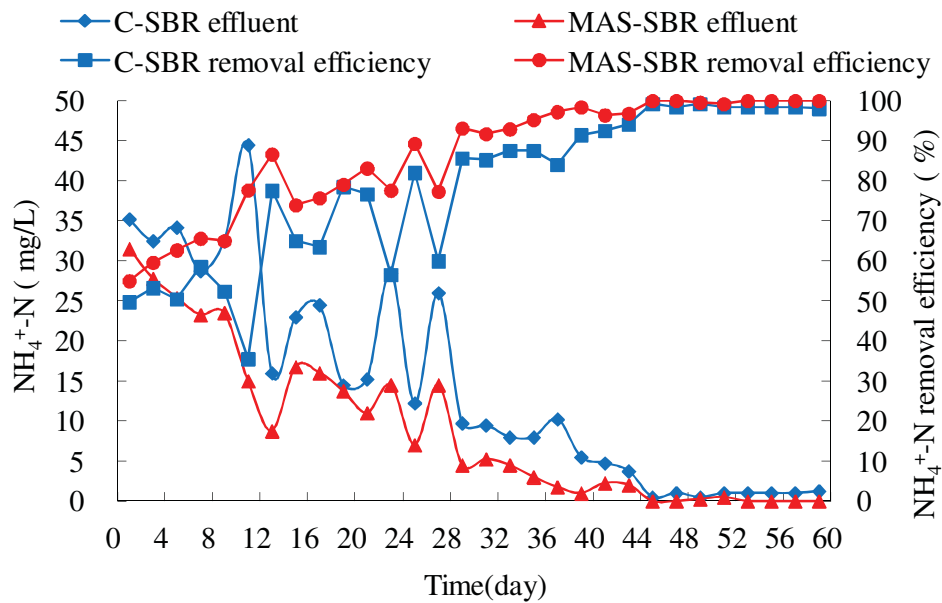
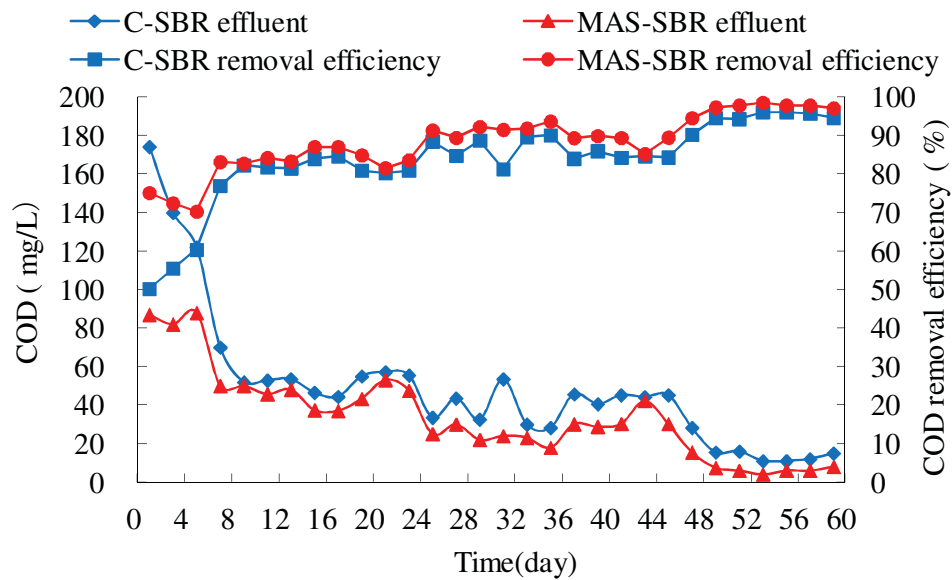
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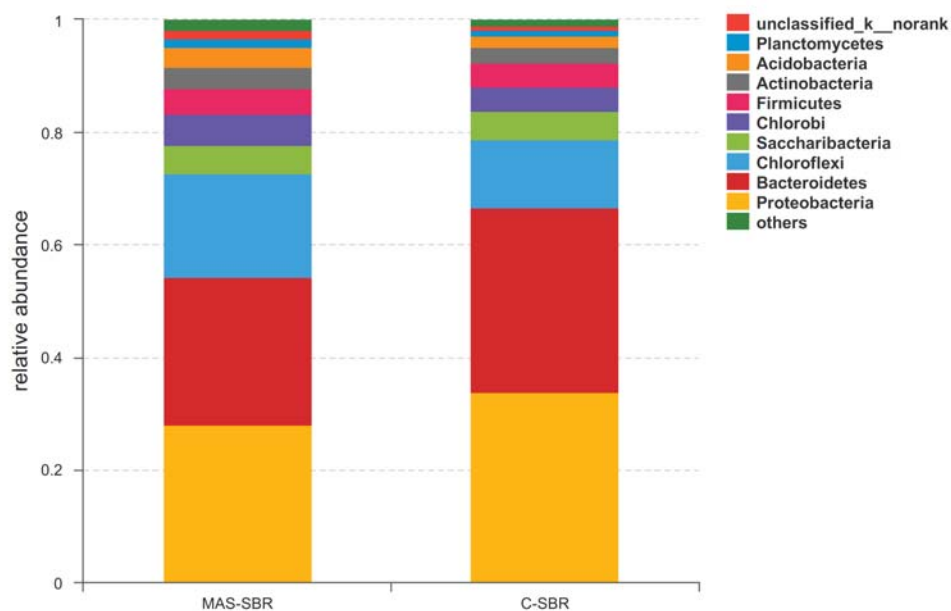
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337 **Fig. 1.**

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339 **Fig. 2.**

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352 **Table 1**

353 The comparison of the main genera in the C-SBR and the MAS-SBR

Genera	C-SBR (%)	MAS-SBR (%)
Nitrosomonas	0.017	0.004
Nitrosomonadaceae	0.665	0.743
Nitrospira	0.065	0.128
Candidatus_Nitrotoga	0.017	0.021
Clostridium_sensu_stricto_13	3.28	3.94
Simplicispira	4.08	2.78
Commonadaceae	6.67	6.47
Thauera	3.30	1.66
Chryseobacterium	12.25	7.16
Saprospiaceae	8.86	8.14
Anaerolineaceae	5.24	9.02
Ardenticatenia	2.53	3.33
Saccharibacteria	5.16	5.01
PHOS-HE36	2.07	2.82

354

355 **Highlights**

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357 • The effect of magnetic powder addition on the performance of MAS-SBR was
358 studied

359 • Adding magnetic powder could enhance the removal of pollutants and sludge
360 reduction

361 • Magnetic powder addition significantly enriched the microbial diversity