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1 **Removal characteristics of organics and nitrogen in a novel**
2 **four-stage biofilm integrated system for enhanced treatment of**
3 **coking wastewater under different HRTs**
4

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11

12

13 **Abstract**

14 Coking wastewater contains substantial organics and nitrogen, posing a great threat on water
15 environment. In this work, organics and nitrogen removal characteristics within each single
16 reactor of a pilot-scale four-stage biofilm anaerobic/anoxic/oxic/oxic (FB-A²/O²) coking
17 wastewater treatment system were specifically investigated at various hydraulic retention
18 times (HRTs). The long-term experiment showed chemical oxygen demand (COD) was
19 greatly degraded in Reactors A₂ and O₁, while ammonia-nitrogen (NH₄⁺-N) was mostly
20 removed in Reactor O₂. 116 h was considered to be optimum for treating coking
21 wastewater, achieving the total COD and NH₄⁺-N removal efficiencies of 92.3 % and
22 97.8 %, respectively. Experimental data presented good linear correlations between
23 volumetric loading and removed loading rates among 0.15~0.65 kgCOD/m³·d and

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24 0.03~0.07 kgNH₄⁺-N /m³·d, much lower than treating other kinds of wastewater due to its
25 complex composition and high toxicity. HRT also strongly influenced removal
26 characteristics and process performance of each biofilm bioreactor. Vertical spatial
27 distributions in DO, COD, NH₄⁺-N and NO₃⁻-N concentration profiles along the reactor
28 height were obviously observed in the upflow biofilm bioreactor filled with granular media,
29 facilitating the enhancements of organics removal, nitrification and denitrification. The
30 FB-A²/O² system integrating with hydrolysis-acidification, denitrification, carbonization
31 and nitrification identified by dominant bacterial populations in each single reactor was
32 proved to be feasible and efficient to treat poor-degraded and high-toxic coking
33 wastewater.

34

35 **Keywords:** coking wastewater; four-stage biofilm anaerobic/anoxic/oxic/oxic
36 (FB-A²/O²); hydraulic retention time (HRT); removal characteristics; bacterial
37 composition

38

39 **Introduction**

40 Coking wastewater, one of the most toxic and complex industrial effluent from iron and
41 steel production facilities, originates from the process of destructive distillation of coal at
42 high temperatures (900-1100 °C) in the absence of air.¹ Its composition varies depending on
43 the types of raw coal, process modifications and operating conditions in the coke ovens.^{2,3}
44 In China, for example, typical influent coking wastewater generally contains 200-300mg/L
45 biochemical oxygen demand (BOD₅), 1000-2000 mg/L chemical oxygen demand (COD),
46 200-400 mg/L suspended solids (SS), 200-400 mg/L ammonia-nitrogen (NH₄⁺-N), 250-350

47 mg/L phenols and 5-20 mg/L cyanide as well as large amounts of highly toxic substances
48 involving mono- and polycyclic aromatics hydrocarbons (PAHs) and heterocyclic aromatic
49 hydrocarbons containing nitrogen, oxygen and sulfur.⁴⁻⁶ Therefore, proper disposal of coking
50 wastewater has become a highly severe issue to be solved urgently for coking industries not
51 only in China but also other countries.

52 Compared with physico-chemical processes, biological treatments as high-efficient,
53 cost-effective and environment-friendly technologies have been widely applied to treat
54 various domestic and industrial wastewaters. Since the 1970s, quite a wide range of
55 bio-systems have been developed for treating coking wastewater involving conventional
56 activated sludge (CAS),^{1,7} fixed biofilm,⁸⁻¹⁰ biological nitrogen removal (BNR) process,^{1,}
57 ^{3, 6, 11-13} sequencing batch reactor (SBR),^{2, 14, 15} fluidized-bed reactor (FBR)¹⁶⁻¹⁸ and
58 membrane bioreactor (MBR).^{19, 20} Unfortunately, most of biological processes were
59 insufficient to successfully remove organic matters and ammonia-nitrogen, leading to the
60 biologically treated effluent greatly exceeding the first-grade discharge standard for coking
61 wastewater in China ($\text{COD} \leq 100$ mg/L and $\text{NH}_4^+ \text{-N} \leq 15$ mg/L), despite quite high removal
62 efficiencies of BOD_5 , phenols and cyanide were achieved.

63 Actually, there are strongly inhibitory effects on both heterotrophic and autotrophic
64 bacteria in aerobic CAS processes for treating coking wastewater due to its complicated
65 composition and high toxicity,^{3, 5} resulting in some undesirable problems such as low
66 process efficiency,^{1, 2, 7} poor sludge settle-ability^{21, 22} and unstable system performance.^{23, 24}
67 During recent several decades, attached biofilm systems have been demonstrated as one of
68 the most effective and competitive alternatives for the treatment of high-strength hazardous
69 coking wastewater^{5, 11, 13, 25} based on their high volumetric loading rate, high microbial

70 biomass and long mean cell retention time (MCRT) for effective nitrification efficiency and
71 stable effluent quality. It has been also proved that anaerobic process as the pre-treatment
72 can effectively promote the biodegradability and reduce the toxicity of refractory organics
73 in coking wastewater.²⁵⁻²⁸ The utilization of anoxic reactor is capable of not only removing
74 total nitrogen, but also enhancing the degradation of refractory organic matters through
75 denitrification at the presence of oxidized nitrogen.²⁹ In terms of NH_4^+ -N removal, enough
76 autotrophic nitrifying bacteria can be easily enriched in two-step aerobic biofilm reactors
77 with very long MCRT, which organic matters are removed in the first aerobic reactor and
78 nitrification is significantly performed in the second aerobic reactor under lower organic
79 loading and toxicity.³⁰

80 Based on the above, a novel four-stage biofilm anaerobic/anoxic/oxic/oxic (FB-A²/O²)
81 system, within which hydrolysis-acidification, denitrification, carbon oxidation and
82 nitrification were integrated, was developed to enhance simultaneous carbon and nitrogen
83 removal from high-strength coking wastewater to meet increasingly more stringent
84 discharge standard in China.³¹ In the present study, specific removal characteristics of each
85 biofilm bioreactor of the FB-A²/O² system were investigated at various hydraulic retention
86 times (HRTs). Additionally, bacterial compositions linked with bioreactor performance at
87 optimum HRT were also identified by pure culture and microbial analysis.

88

89 **Materials and methods**

90 **Experimental setup**

91 Fig. 1 depicts a pilot-scale integrated coking wastewater treatment system operated for over
92 two years consisting of four up-flow fixed biofilm bioreactors: anaerobic (A₁)-anoxic

93 (A₂)-oxic (O₁) and oxic (O₂) located at a coking plant of Tongshida Co.Ltd in Linfen, Shanxi
94 Province. The working volumes of four reactors were respectively 3.0 m³, 4.8 m³, 4.8 m³
95 and 4.8 m³. Sampling ports were evenly installed at different heights of the packing layer in
96 Reactors A₁, A₂ and O₂. Reactors A₁, A₂ and O₂ were packed with ceramsite to maintain high
97 biomass and develop vertical microbial distribution and fine filtration along the reactor
98 height, while Reactor O₁ was filled with hollow plastic balls to enhance mass transfer
99 efficiency and capable of largely removing COD at high organic loading via filamentous
100 bacteria. The specification of the used bio-packings is listed in Table 1.

101 **Start-up of the system**

102 The seed sludges for anaerobic/anoxic reactors and two-stage aerobic reactors of the
103 whole system were, respectively, obtained from the anoxic tank and aerobic aeration tank
104 of a coke-plant wastewater treatment facility of Tongshida Co.Ltd in Linfen. The raw
105 coking wastewater via pre-treatments (ammonia stripping, oil isolating and air floatation)
106 was collected in the wastewater tank. The characteristics of the influent wastewater are
107 given in Table 2. In the beginning, to relieve high toxicity of coking wastewater, about 0.04
108 m³/d raw wastewater diluted with 200 % tap water continuously flowed into the system
109 operated at 28-30°C. Dissolved oxygen (DO) concentration was kept above 4 mg/L in the
110 aerobic reactors using a lower gas-liquid ratio to avoid the washing out of young biofilm
111 during biofilm growth. K₂HPO₄ (C: N: P=100: 5: 1) was added into the influent tank each
112 day to provide sufficient nutrient elements for the normal growth of microorganisms. After
113 about 2-week period acclimation operated in continuous-flow, with increased influent
114 loading based on continuous a small increase of the flow rate, 0.2 m³/d of coking wastewater
115 with 50 % tap water was flowed into the system for the stability establishment of the biofilm

116 reactors during biofilm growth. After 32 d, the removal efficiencies for COD and $\text{NH}_4^+\text{-N}$ of
117 the system without dilution were achieved above 70 % and 65 %, respectively, indicating the
118 microorganisms were successfully acclimated.

119 **Operational conditions**

120 Allowing for HRT considered as one of the most import process parameters to be optimized
121 during coking wastewater treatment,^{12, 32} the system continuously operated for 267 days
122 after its successful start-up was investigated under long-term steady-state operational stages
123 (Runs 1-5) shown in Table 3. Throughout the experimental period, the temperature was in
124 the range of 25–35 °C, pH and alkalinity were controlled through the addition of NaHCO_3
125 solution into the second aerobic reactor so as to compensate the loss of alkalinity due to
126 nitrification. The final effluent pH was maintained above 7.0 and the alkalinity was not be
127 less than 80 mg/L (as CaCO_3). DO concentrations in the aerobic reactors supplied by the
128 compressor were kept around between 3.5–5 mg/L and nitrifying recirculation ratio from
129 Reactor O_2 to Reactor A_2 was controlled at 3.0 based on the optimization of internal nitrate
130 recycling in the previous lab-scale study. Only twice back washings of biological filters
131 (Reactors A_2 and O_2) were conducted to wash out intercepted solids substances and aging
132 biofilms for avoiding media clogging during the experiment.

133 **Microbial analysis**

134 *Sample collection and handling*

135 A certain amount of bio-packings (ceramic particles from Reactors A_1 , A_2 and O_2 and
136 polypropylene polyhedral hollow ball from Reactor O_1) were collected and mixed in the
137 laboratory glass bottles at 4 °C and then immediately eluted with 50 mL of 0.85 % normal
138 saline. And the eluent was diluted with 300 mL of sterile water.

139 ***Culture medium***

140 Culture mediums for facultative anaerobic, aerobic heterotrophic and nitrifying bacteria
141 were specifically listed in Table 4.

142 ***Isolation and purification***

143 The qualitative analysis for dominant microbial populations within four biofilm reactors at
144 the optimum operational run was undertaken by bacterial isolation and pure culture. 10 mL
145 eluents at different diluted multiples were inoculated into a 500 mL triangle bottle
146 containing 100 mL liquid medium and incubated for 2 d (facultative anaerobic and aerobic
147 bacteria) and 35 d (aerobic nitrifying bacteria) in a rotatory shaker at 30°C, 150 r/min. After
148 that, 0.1 mL culture solution was coated on the plate at 30°C incubator for 2 d (facultative
149 anaerobic and aerobic bacteria) and 6 d (aerobic nitrifying bacteria) to develop the colonies.
150 The more colonies and faster growing strains were scribed and purified on the plate and then
151 continuously operated for 3-5 times, respectively.

152 ***Identification of strains***

153 The morphological, physiological, biochemical characteristics of the strains were identified
154 according to the Bergey's Manual of Determinative Systematic Bacteriology.³³

155 ***Analytical methods***

156 Temperature, pH, DO and alkalinity were measured daily. COD, NH₄⁺-N, NO₃⁻-N were
157 analyzed weekly. Temperature and pH were measured with a pH meter (WTW Multi340i).
158 The DO was measured using a portable DO meter (YSI-500). COD, NH₄⁺-N, NO₃⁻-N and
159 alkalinity both in the influent and effluent of each single bioreactor were analyzed according
160 to Standard Methods.³⁴

161

162 **Results and discussion**

163 **Overall removal efficiency**

164 Figure 2 depicts the average COD and NH_4^+ -N concentrations from the influent to effluent
165 and corresponding removal efficiencies at different HRTs. It was clearly demonstrated that
166 COD gradually dropped along the four-stage biofilm system where anoxic and subsequent
167 aerobic reactors were the major contributor of the overall organic removal, yet anaerobic
168 process only played a minor role in COD removal. Organic matters removal efficiencies
169 during the experimental periods were steadily maintained almost over 75 % with the influent
170 COD between 1000-1200 mg/L, accordingly, final effluent COD concentrations were less
171 than 300mg/L, even at rather short HRT of 45h, indicating the FB-A²/O² system possessing
172 a strong adaptive ability to the organic loading shock. Nevertheless, NH_4^+ -N removals were
173 almost entirely occurred at aerobic stages, especially in the second aerobic bioreactor and
174 NH_4^+ -N seemed to some extent increased in the anaerobic/anoxic reactors. Ammonia
175 nitrogen removal efficiencies drastically varied between 23.1 % and 99.4 % among different
176 experimental runs greatly influenced by HRT.^{1,2,7}

177 In Run 1, average COD and NH_4^+ -N in the final effluent dropped to 123 mg/L and 0.7
178 mg/L, respectively. In this case, 88.1 % COD and 99.4 % NH_4^+ -N were removed at HRT of
179 136 h, concluding that COD and NH_4^+ -N were thoroughly degraded at such an extremely
180 long HRT. In Run 2, COD removal further increased to 92.3 % along with a slight decrease
181 in NH_4^+ -N removal (97.8%), correspondingly, effluent average COD and NH_4^+ -N
182 concentrations were 97.8 mg/L and 1.7 mg/L, respectively, which met the demand of the
183 first level of the coking wastewater discharge standards (GB8978–1996). An obvious
184 increase in organic removal was likely explained by the acceleration of biofilm renewals and

185 weakening of media clogging effects at faster rising filtration velocity due to properly
186 increased hydraulic loading. In Runs 3-5, however, effluent COD and $\text{NH}_4^+\text{-N}$ considerably
187 raise with further decrease of HRT, indicating that deteriorated treatment capacity perhaps
188 attributed by breaking the biomass for media and lowering microbial activity due to stronger
189 scour and shear at excessively shortened HRT.³⁵ Especially, it was found that $\text{NH}_4^+\text{-N}$
190 removal efficiency was sharply reduced at shorter HRTs from 86 h to 46.5 h owing to
191 detrimental effects of high organic loading on nitrification. Based on above results, the
192 optimal total HRT of the integrated system seemed 116 h in this study.

193 The Figure 3 reveals the relationship between influent volumetric loadings and
194 removed loading rates. In accord with other correlation studies,^{36, 37} experimental data
195 showed good linear correlations for both COD and $\text{NH}_4^+\text{-N}$ between volumetric loading and
196 removed loading at high correlation coefficient (R^2 above 0.9) with above 75% COD
197 removal efficiency among 0.15~0.65 $\text{kgCOD/m}^3\cdot\text{d}$ and above 60% $\text{NH}_4^+\text{-N}$ removal
198 efficiency at 0.03~0.07 $\text{kg NH}_4^+\text{-N/m}^3\cdot\text{d}$. Compared with treating medium and low-strength
199 wastewater, such relatively low volumetric loading rates were required for the treatment of
200 high-strength coking wastewater containing considerable complex compounds and toxic or
201 harmful substances.^{1, 38}

202 **Bioreactor performance and microbial characteristics in Reactor A₁**

203 In Reactor A₁, anaerobic fermentation mainly acted as the pre-treatment for complex and
204 poorly degradable organic matters in the coking wastewater. Its efficiency depended on the
205 increasing rate of BOD_5/COD (B/C ratio) in the anaerobic effluents,^{4, 9} because some
206 biodegradable organic compounds such as volatile fatty acids (VFA) and low molecular
207 organics were produced at the bottom of Reactor A₁ based on partial scission of heterocyclic

208 or polycyclic rings though transformation of macromolecular structure rather than excessive
209 degradation of organics at shorter HRTs during hydrolysis-acidification, leading to enhanced
210 B/C increasing ratio and indistinctive removal of COD. As shown in Fig.4, it was clearly
211 observed that apparent differences in increasing rate of B/C ratio and COD removal in
212 anaerobic reactor at different HRTs. It was interesting that B/C ratios distinctly increased
213 but COD was not largely removed at Runs 2 and 4 occurring favorable
214 hydrolysis-acidification performance, while slight rises in B/C ratios but notable COD
215 removals instead at three other runs, concluding that pretreatment efficiency was strongly
216 influenced by anaerobic HRT. In this study, anaerobic HRT of 20 h seemed optimum for
217 hydrolysis-acidification with 175 % increasing rate of B/C ratio and only 14.6 % COD
218 removal at Run 2. It was concluded that anaerobic HRT optimization played an important
219 role in enhancing hydrolysis-acidification efficiency to improve the biodegradability of
220 coking wastewater and provide enough readily biodegradable organics for subsequent
221 anoxic and aerobic treatment processes,²⁶ while increasing-efficiency in B/C ratio was
222 greatly low at short HRT due to incomplete transformation of refractory compounds (Run 1)
223 or at too long HRT owing to excessive mineralization of biodegradable organics (Runs 4-5).
224 The performance of the anaerobic biofilm reactor was also confirmed by its microbial
225 characteristics, because a mass of facultative anaerobic bacteria commonly found in
226 anaerobic wastewater treatment systems such as *Bacillus*, *Aeromonas*, *Flavobacterium* and
227 *Paracoccus* (in Fig.5 a-d) which were capable of performing hydrolysis-acidification effect
228 were identified.

229 **Bioreactor performance and microbial characteristics in Reactor A₂**

230 Figure 6 depicts DO, COD, NH₄⁺-N and NO₃⁻-N profiles along the height of Reactors A₂

231 at different HRTs. It was clearly that the submerged fixed bed with granular filter media
232 had uneven spatial distribution characteristics in oxygen, carbon and nitrogen, unlike
233 complete-mixing reactors. Sudden drops in DO and COD were evidently observed at the
234 initial of the packing layer height (at 0.2 m) due to abundant organic substrates rapidly
235 utilized by higher biomass at the bottom of the reactor.^{31, 39} Similarly with DO and COD,
236 concurrent decreasing trends of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ implied that organic removal,
237 nitrification and denitrification occurred simultaneously at the bottom, closely linked with
238 diverse microbial populations. And then COD and $\text{NO}_3^-\text{-N}$ at the upper part of the reactor
239 were concurrently removed by denitrification along the height of above 0.2 m at gradual
240 decreased DO, partly preventing adverse impacts of high oxygen concentration from
241 internal recycling liquid on anoxic denitrification based on continuous plug-flow
242 characteristics of the granular fixed bed reactor.^{39, 40} But $\text{NH}_4^+\text{-N}$ converted by organic
243 nitrogen and cyanide compounds (CN^-) increased above 0.2 m due to ammonification.⁴¹
244 Furthermore, DO profiles among different runs were closely related with HRT, longer HRT
245 led to higher DO due to low loading rates. Carbon and nitrates removal rates at short HRT
246 (Runs 2 and 4) were higher than at very long HRT (Run 1), because excessive nitrates were
247 accumulated, causing insufficient denitrification due to low C/N ratio and higher nitrate
248 loading. On the other hand, organics and nitrogen were poorly removed at too short HRT
249 because of low degrading speeds at high volume loading rates. Thus, appropriate control of
250 HRT could improve simultaneous carbon and nitrogen removals, especially enhance anoxic
251 degradation of refractory organic compounds under the denitrifying condition at the
252 presence of $\text{NO}_3^-\text{-N}$.^{29, 42, 43} In this study, about 134 mg/L COD and 35 mg/L $\text{NO}_3^-\text{-N}$ were
253 simultaneously removed via almost complete denitrification at the optimal anoxic HRT of

254 32 h at Run 2. Compared with conventional biological processes,^{1, 10, 44} COD/NO₃⁻-N ratio
255 was just only about 3.8 for thorough denitrification, demonstrating that internal carbon
256 source was greatly economized in the FB-A²/O² system to treat coking wastewater with
257 low B/C and C/N ratios. A large number of observed organisms in the anoxic reactor at
258 Run 2 were identified as *Alkaligenes*, *Pseudomonas stutzeri*, *Flavobacterium* and other
259 bacilliform facultative bacteria in Fig.7 a-c, some of which were heterotrophic nitrifying
260 and anoxic/microaerophilic denitrifying bacteria^{45, 46} coexisting in the single anoxic reactor
261 under abundant organic substrates, resulting in significantly removing nitrogen.

262 **Bioreactor performance and microbial characteristics in Reactors O₁ and O₂**

263 The Figure 2 showed that COD was mostly removed in Reactor O₁ due to aerobic
264 oxidization by heterotrophic bacteria and NH₄⁺-N was slightly removed because
265 autotrophic nitrifiers such as AOB and NOB tended to fail to compete with other
266 heterotrophic bacteria at higher COD loading and toxicity, while nitrifying bacteria could
267 easily occupy predominance to significantly remove NH₄⁺-N at lower organic loading in
268 Reactor O₂.

269 In Fig. 8, similarly in the Reactor A₂, obvious variations were presented in oxygen,
270 organic and nitrogen concentrations profiles along the Reactor O₂ height at different
271 HRTs due to identical reactor configuration and operational pattern. DO levels strongly
272 affected by HRT almost linearly dropped along the reactor height due to vertical
273 distribution characteristics created in the up-flow fixed bed reactor filled with granular
274 filter media.^{39, 40} COD concentrations swiftly decreased at 0.6 m at the bottom of the
275 reactor due to quick degradation of biodegradable organic matters via higher biomass,
276 while NH₄⁺-N concentrations reduced drastically along with continuous nitrates

277 accumulation at the upper part of the reactor, especially above 1.2 m, implying significant
278 nitrification performed by enough autotrophic nitrifying bacteria, which were dominant
279 bacterial groups due to lack of available biodegradable organics at the upper and top
280 height. As Fig.8 shown, remarkable NH_4^+ -N removal were achieved at Runs 1 and 2 due
281 to long HRT and low organic loading, while ammonia were poorly removed at Run 4
282 owing to limited nitrification by high organic loading. Consequently, it was concluded
283 that suitable selection of HRT was essential for efficiently removing nitrogen from coking
284 wastewater.

285 In terms of microbial characteristics in aerobic reactors, aerobic heterotrophic
286 microorganisms including *Bacillus*, *Flavobacterium*, *Zoogloea* and *Nocardia* (Fig.9 a-d)
287 existed in Reactor O₁, while autotrophic nitrifying bacteria such as *Nitrobacter*,
288 *Nitrococcus*, *Nitrosomonas* and *Nitrosococcus* (Fig.10 a-d) outgrown by heterotrophs
289 under lower organic loading were dominant bacteria in Reactor O₂, consistent with
290 operational characteristics and process efficiencies of two-step aerobic treatment system.

291

292 **Conclusions**

293 Bioreactor performance and microbial characteristics in a novel pilot-scale four-stage
294 biofilm anaerobic/anoxic/oxic/oxic (FB-A²/O²) system to steadily enhance the treatment of
295 coking wastewater treatment were specifically investigated at various HRTs. Through
296 optimization, the best coking effluent quality was obtained at 116 h achieving the total
297 92.3 % COD removal and 97.8 % NH_4^+ -N removal efficiencies at rather low volumetric
298 loading rates due to complex composition and high toxicity of the wastewater. Some
299 dominant bacterial populations related with bioreactor performance were also identified by
300 pure culture and microbial analysis, implying hydrolysis-acidification, denitrification,

301 carbonization and nitrification were integrated within a system to efficiently treat poorly
302 degraded and highly toxic coking wastewater.

303

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436 List of Figure Legends

437 Fig.1 Schematic diagram of a pilot-scale A^2/O^2 biofilm system (a) process chart; (b) actual system; (c)
438 packing media.

- 439 Fig.2 Average influent and effluent concentration and corresponding removal efficiency during
440 operational periods (a) COD; (b) NH_4^+ -N.
- 441 Fig.3 Relationship between influent volumetric loadings and removed loading rates (a) COD; (b)
442 NH_4^+ -N.
- 443 Fig.4 Increasing rates of B/C ratio and COD removals in Reactor A₁.
- 444 Fig.5 Morphology of dominant bacteria in Reactor A₁ (a: *Bacillus*; b: *Aeromonas*; c: *Flavobacterium*;
445 d: *Paracoccus*).
- 446 Fig.6 Concentration profiles along the height of Reactors A₂ (a) DO; (b) COD; (c) NH_4^+ -N and (d)
447 NO_3^- -N.
- 448 Fig.7 Morphology of dominant bacteria in Reactor A₂ (a: *Alkaligenes*; b: *Pseudomonas stutzeri*;
449 c: *Flavobacterium*).
- 450 Fig.8 Concentration profiles along the height of Reactors O₂ (a) DO; (b) COD; (c) NH_4^+ -N and (d)
451 NO_3^- -N.
- 452 Fig.9 Morphology of dominant bacteria in Reactor O₁ (a: *Bacillus*; b: *Flavobacterium*; c: *Zoogloea*; d:
453 *Nocardia*).
- 454 Fig.10 Morphology of dominant bacteria in Reactor O₂ (a: *Nitrobacter*; b: *Nitrococcus*; c:
455 *Nitrosomonas*; d: *Nitrosococcus*).

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457 List of Table Titles

458 Table 1 Specification of the used bio-packings

459 Table 2 Characteristics of the influent wastewater

460 Table 3 Operational conditions under different experimental stages

461 Table 4 Culture medium

462

463

464

Table 1. Specification of the used bio-packings

Specification	Hollow plastic balls	Ceramic particles
Type	Ball	Granular
Specific surface area (m ² /m ³)	236	3900
Porosity (%)	90%	≥55%
Hydrochloric acid soluble rate (%)	≤0.22	≤0.22
Sodium hydroxide soluble rate (%)	≤15.0	≤15.0
Diameter (mm)	50	3-7

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Table 2. Characteristics of influent coking wastewater

Parameter	Unit	Average value	SD,standard deviation	N,sampling number
pH	—	8.3	0.3	32
BOD ₅	mg/L	303	61	24
COD	mg/L	1195	297	30
BOD ₅ /COD	—	0.23	0.07	18
NH ₄ ⁺ -N	mg/L	228.2	55.5	32
Phenol	mg/L	255	117	5
Cyanide	mg/L	8	2	5

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Table 3 Operational conditions under different experimental stages

Run	COD (mg/L)	NH ₄ ⁺ -N (mg/L)	HRT(h)	Operation periods (days)
1	1036±42	233.9±9.7	174	49
2	1230±231	278.6±51	116	63
3	1046±73	221.8±32.5	87	49
4	1212±95	147.5±26.6	69.6	42
5	1079±79	227.2±23.2	43.5	64

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Table 4 Culture medium

Microorganisms	Culture medium containing (per liter)
facultative anaerobic bacteria	glucose 10.0 g, beef extract 10.0 g, peptone 10.0 g, NaCl 10.0 g, AGAR powder 20.0 g with distilled water 1000 mL and pH 7.0.
aerobic heterotrophic bacteria	beef extract 10.0 g, peptone 10.0 g, NaCl 10.0 g, AGAR powder 20.0 g with distilled water 1000 mL and pH 7.0.
nitrite bacteria	NaCl 0.3 g, MgSO ₄ ·7H ₂ O 0.14 g, FeSO ₄ ·7H ₂ O 0.3 g, KH ₂ PO ₄ 0.14 g, (NH ₄) ₂ SO ₄ 0.66g, CaCO ₃ powder 6.0 g, AGAR powder 20 g, trace elements solution ^a 0.4 mL with distilled water 1000 mL and pH 7.2.
nitrate bacteria	NaCl 0.3 g, MgSO ₄ ·7H ₂ O 0.14 g, FeSO ₄ ·7H ₂ O 0.3 g, KH ₂ PO ₄ 0.14 g, (NH ₄) ₂ SO ₄ 0.66g, NaNO ₂ 0.5g, CaCO ₃ powder 6.0 g, AGAR powder 20 g, trace elements solution ^a 0.4 mL with distilled water 1000 mL and pH 7.2.

557 ^atrace elements solution that consists of the following components (per liter): ZnSO₄·7H₂O 0.003g, MnSO₄·7H₂O 0.003g,
558 CoSO₄·7H₂O 0.001g and CuSO₄·5H₂O. 0.003g.

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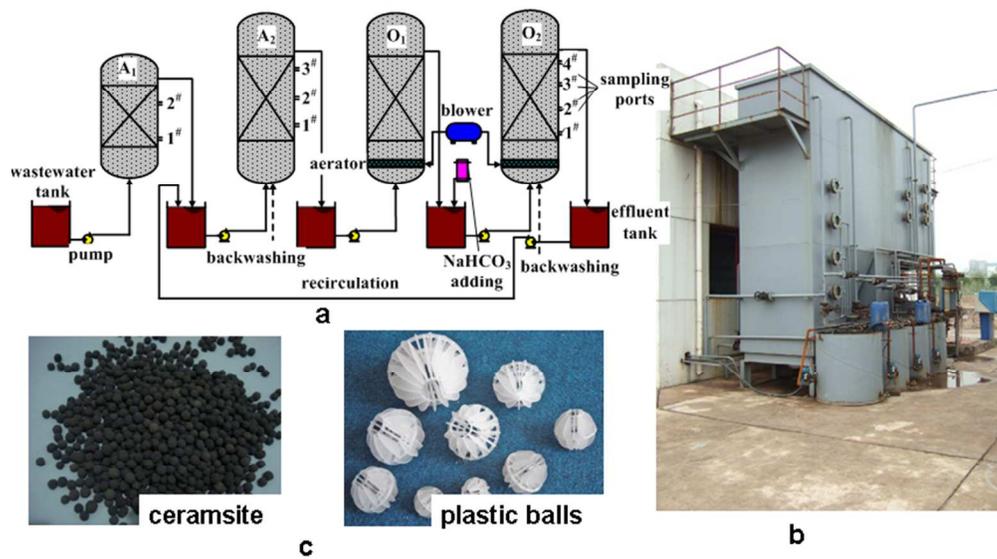


Fig.1 Schematic diagram of a pilot-scale A2/O2 biofilm system (a) process chart; (b) actual system; (c) packing media.
226x128mm (96 x 96 DPI)

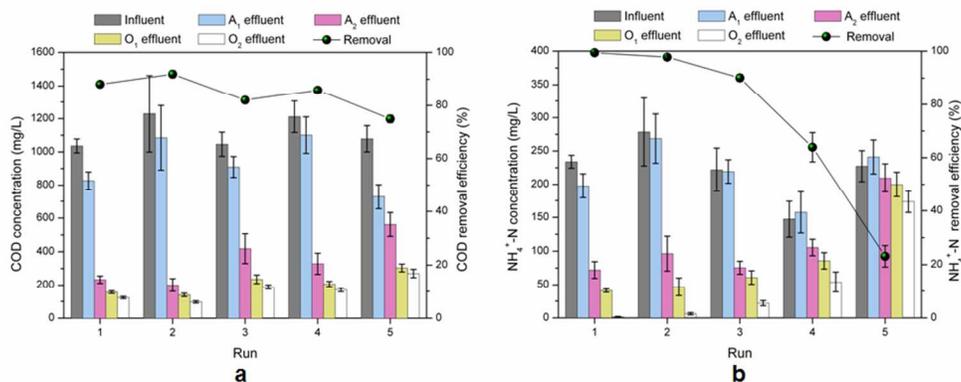


Fig.2 Average influent and effluent concentration and corresponding removal efficiency during operational periods (a) COD; (b) NH₄⁺-N.
270x105mm (96 x 96 DPI)

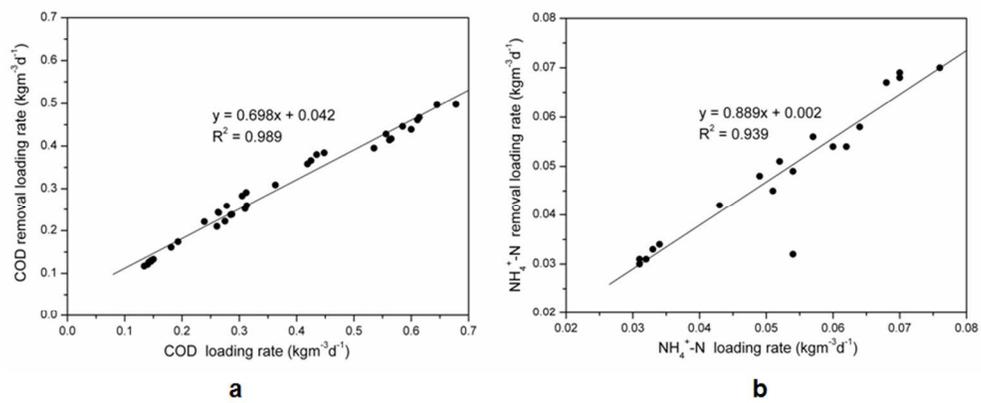


Fig.3 Relationship between influent volumetric loadings and removed loading rates (a) COD; (b) $\text{NH}_4^+\text{-N}$.
250x105mm (96 x 96 DPI)

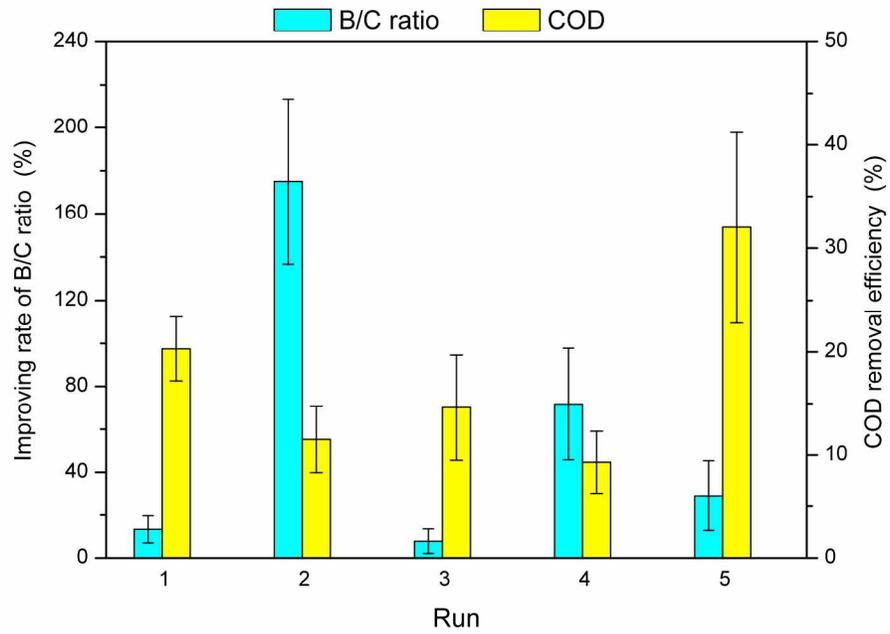


Fig.4 Increasing rates of B/C ratio and COD removals in Reactor A1.
208x156mm (300 x 300 DPI)

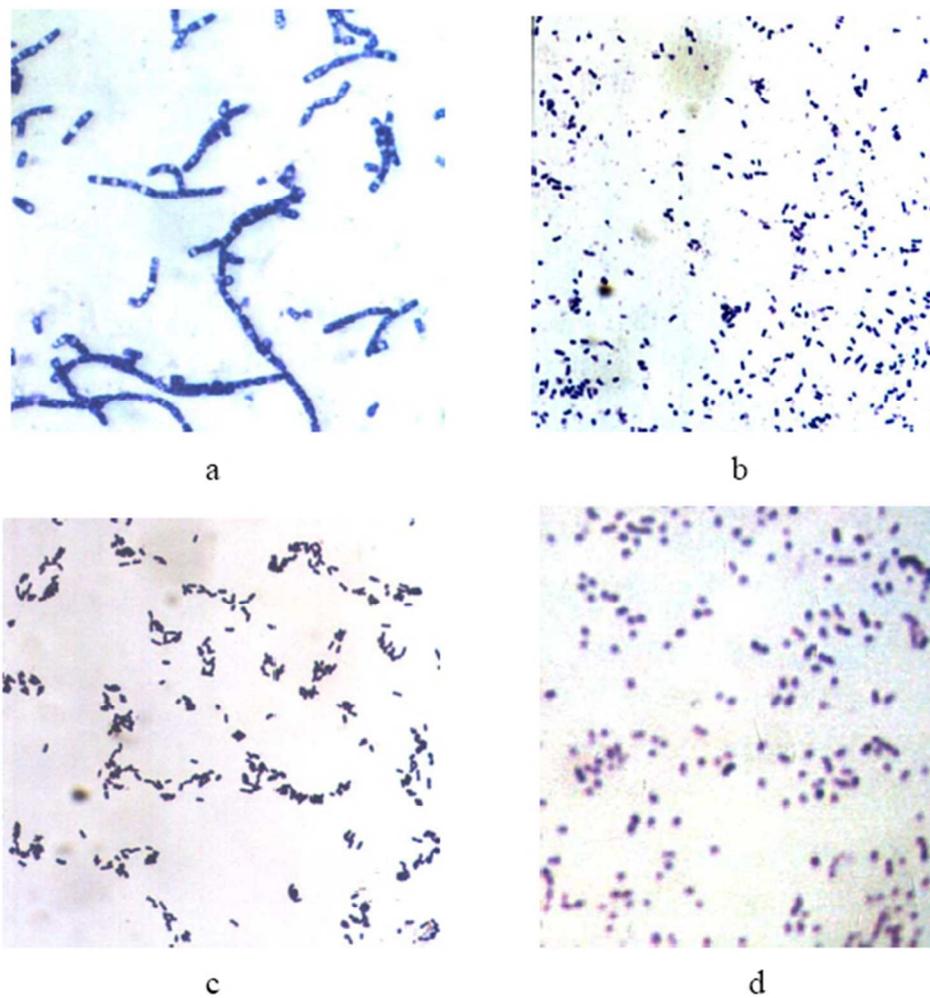


Fig.5 Morphology of dominant bacteria in Reactor A1 (a: Bacillus; b: Aeromonas; c: Flavobacterium; d: Paracoccus).

156x158mm (96 x 96 DPI)

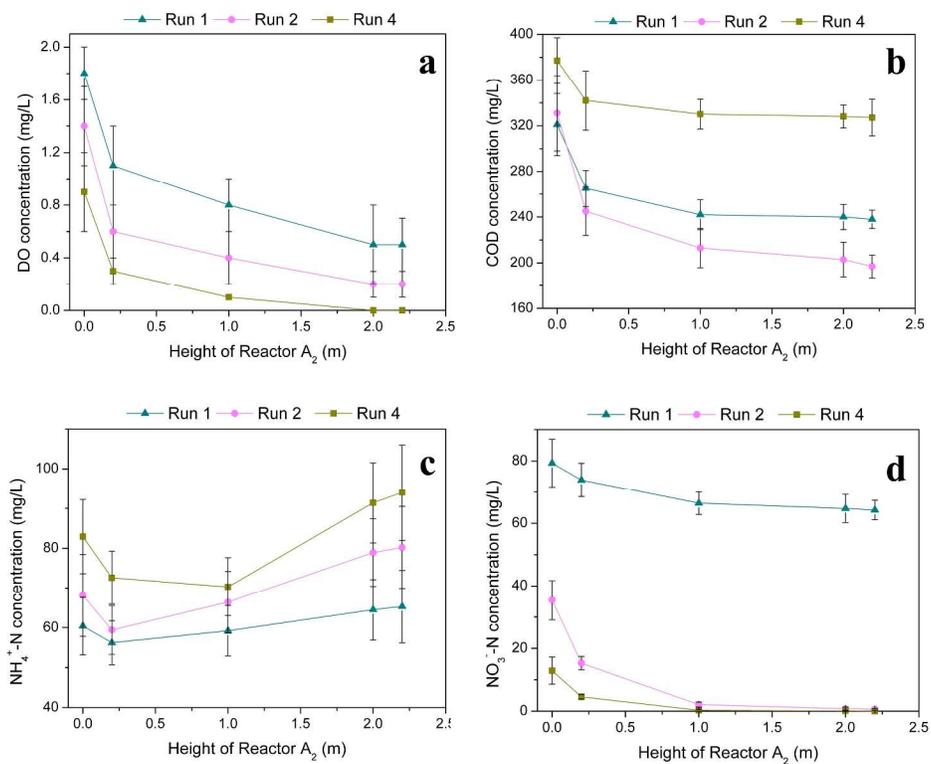


Fig.6 Concentration profiles along the height of Reactors A2 (a) DO; (b) COD; (c) NH₄⁺-N and (d) NO₃⁻-N.

331x273mm (300 x 300 DPI)

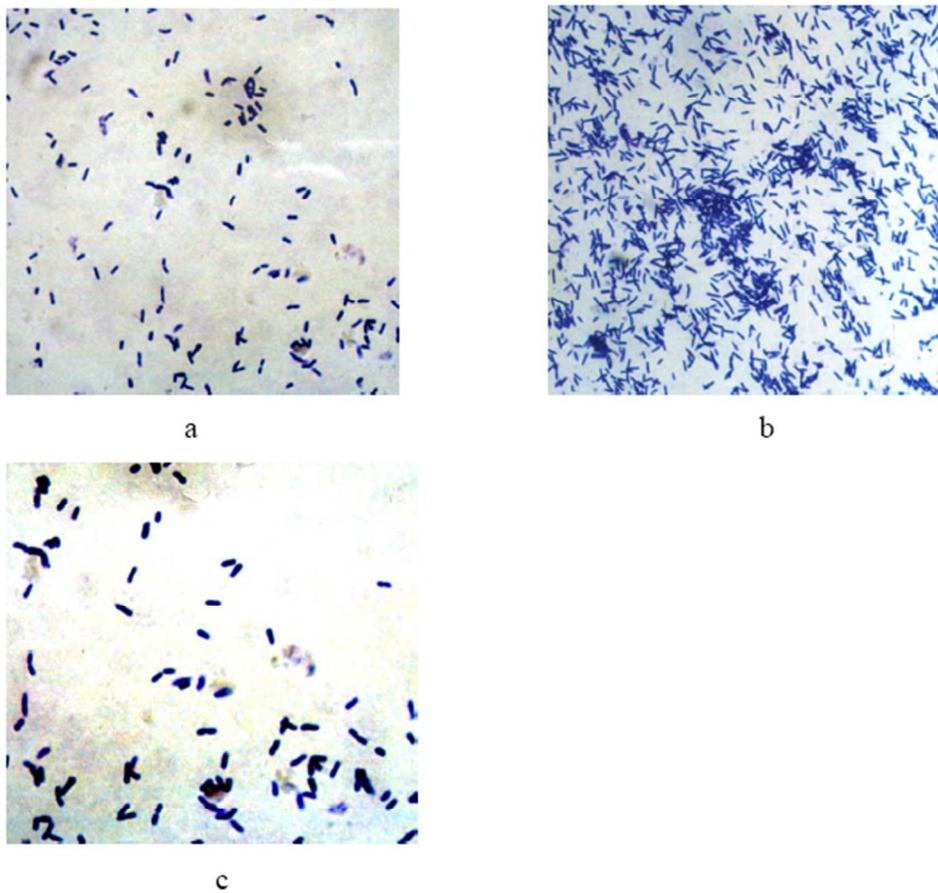


Fig.7 Morphology of dominant bacteria in Reactor A2 (a: *Alkaligenes*; b: *Pseudomonas stutzeri*; c: *Flavobacterium*).

170x156mm (96 x 96 DPI)

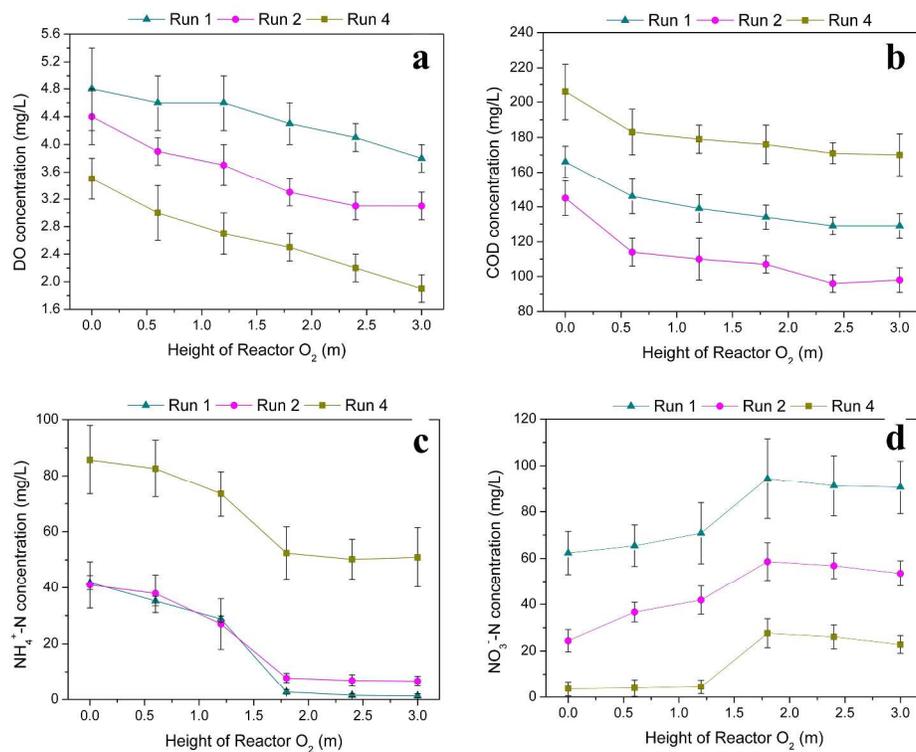


Fig.8 Concentration profiles along the height of Reactors O₂ (a) DO; (b) COD; (c) NH₄⁺-N and (d) NO₃⁻-N.

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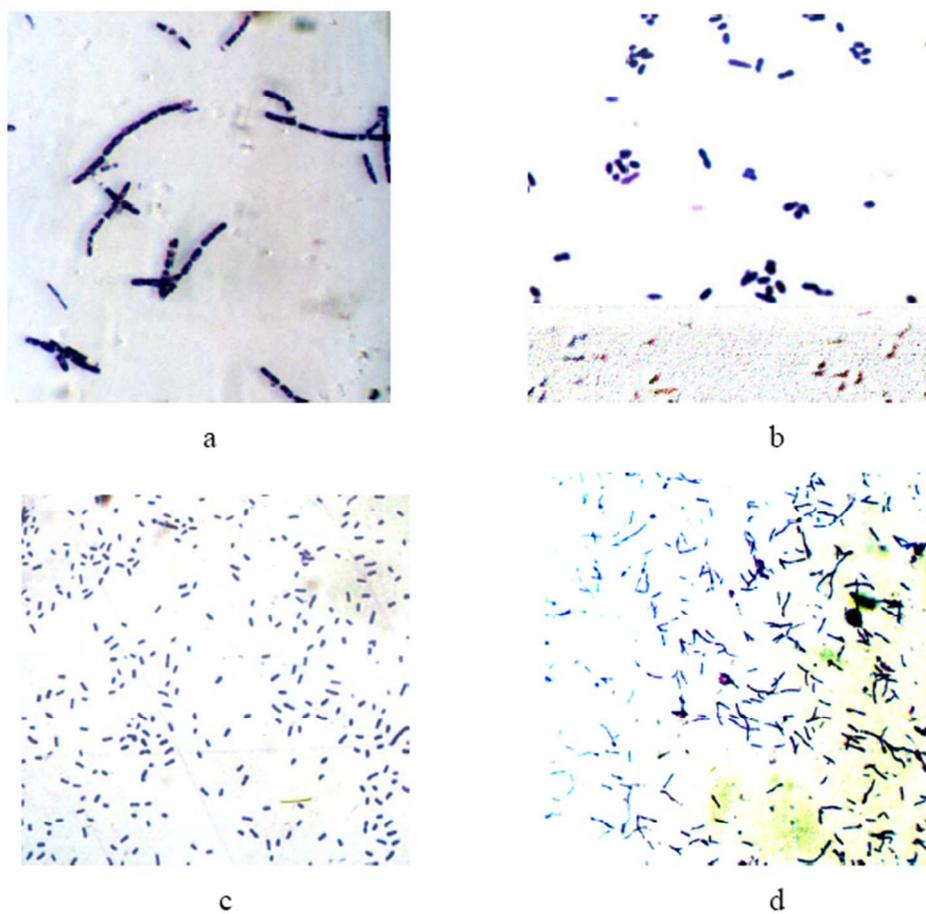


Fig.9 Morphology of dominant bacteria in Reactor O1 (a: Bacillus; b: Flavobacterium; c: Zoogloea; d: Nocardia).
167x156mm (96 x 96 DPI)

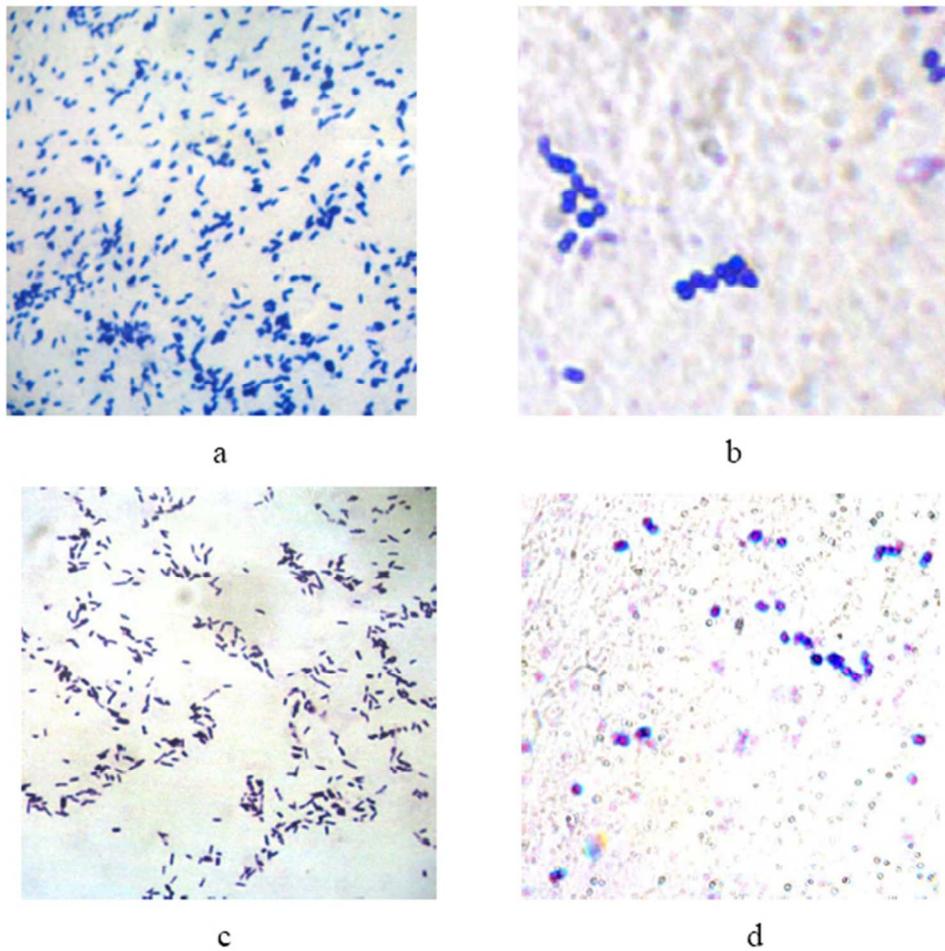
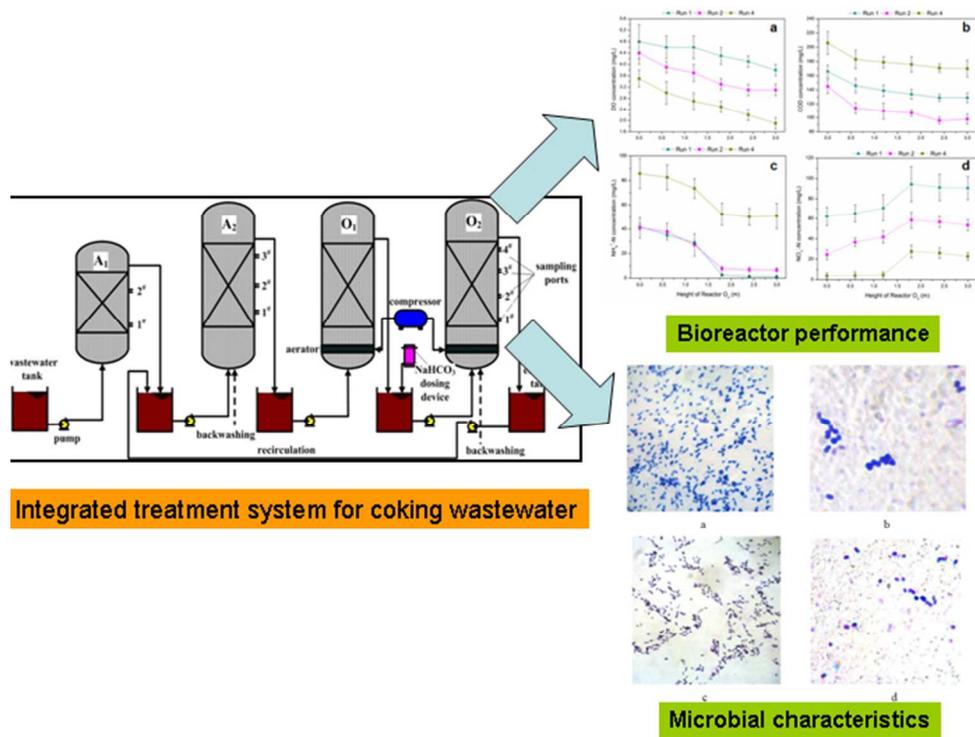


Fig.10 Morphology of dominant bacteria in Reactor O2 (a: Nitrobacter; b: Nitrococcus; c: Nitrosomonas; d: Nitrosococcus).
158x153mm (96 x 96 DPI)



Graphical Abstract
188x138mm (96 x 96 DPI)