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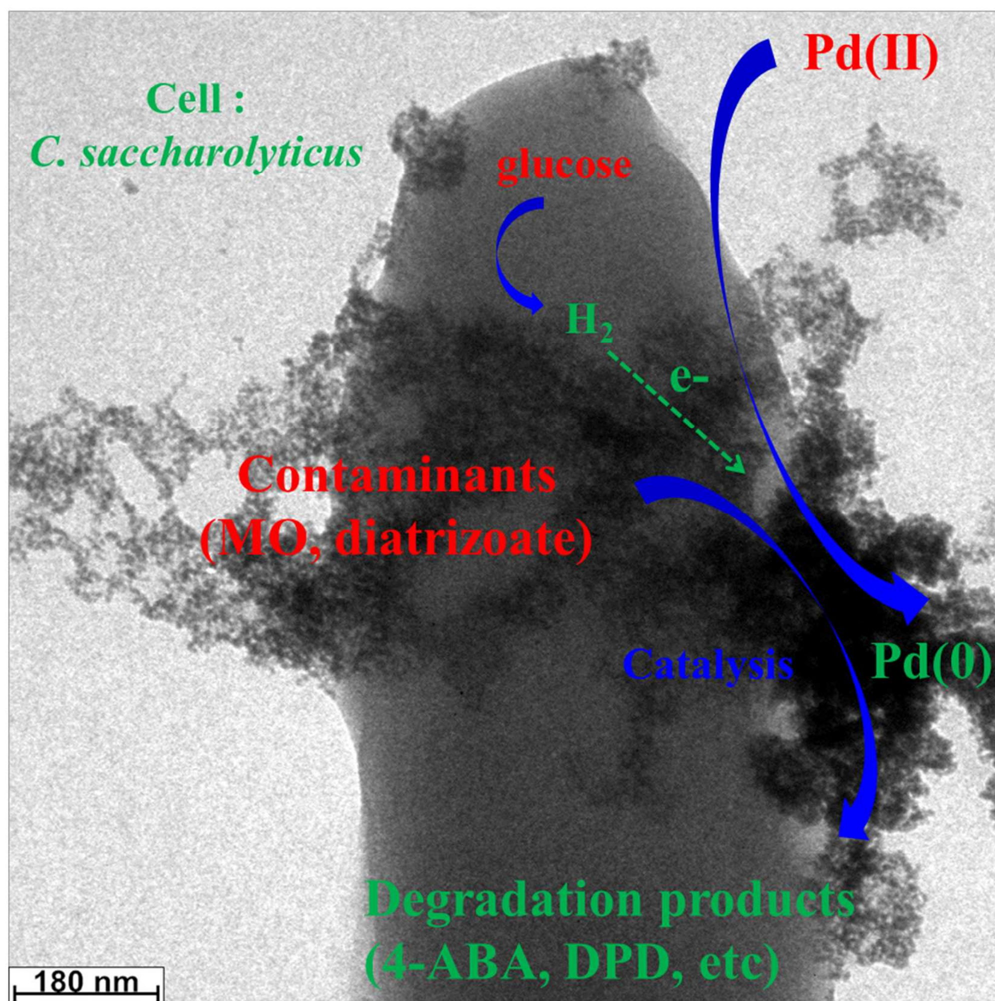
Graphical Abstract



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1 **Palladium nanoparticles produced and dispersed by *Caldicellulosiruptor***

2 ***saccharolyticus* enhance the degradation of contaminants in water**

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14 **Abstract**

15 This study focused on examining the general applicability of coupling bio-palladium
16 (Pd) nanoparticle generation and bio-H_2 produced by *Caldicellulosiruptor*
17 *saccharolyticus* for wastewater treatment under extreme thermophilic conditions.
18 Na_2PdCl_4 was added to cell cultures to achieve a final Pd concentration of 50 mg/L.
19 Methyl orange (MO) and diatrizoate were chosen as the contaminants in water. In the
20 cultures with, and without, Pd added, MO (100 mg/L) was degraded within 30 min
21 and in over 6 h, respectively. Diatrizoate (20 mg/L) was degraded within 10 min in
22 Pd-added cultures. However, no diatrizoate degradation happened without Pd addition.
23 The degradation rates were correlated positively with dissolved hydrogen generated
24 by *C. saccharolyticus*. Furthermore, the catalytic actions of Pd(0) nanoparticles and
25 cells were distinguished during the degradation process. MO was degraded under the
26 combined action of Pd(0) and hydrogenase. However, Pd(0) was the essential catalyst,
27 and hydrogenase had no effect on the deiodination of diatrizoate within 20 min. Pd(0)
28 particles were dispersed well by the cells of *C. saccharolyticus* and showed a better
29 catalytic activity than Pd(0) formed without cells. Dissolved hydrogen produced by *C.*
30 *saccharolyticus* should be the perfect reduction equivalent for Pd formation and for
31 reducing degradation. Therefore, Pd should be added to *C. saccharolyticus* cultures to
32 enhance the degradation of contaminants in water.
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34 **1. Introduction**

35 Nanoparticles (NPs) of Pd(0) have a high catalytic activity in a standard
36 hydrogenation reaction [1]. Conventional production methods of these NPs require the
37 use of a series of toxic and expensive chemical agents, such as $NaBH_4$ and H_2. Both
38 stabilizers and carrier materials are needed to prevent the particles from aggregating in
39 a solvent. These materials would be released and pollute the environment, resulting in
40 an overall increase in cost. However, without these materials, Pd(0) could be formed in
41 bulk instead of the nanoscale. It is known that bulk Pd(0) has a lower catalytic activity
42 compared to NPs [2]. Nanopalladium catalysts can also be synthesized by the
43 precipitation of Pd on the surface of bacteria, leading to the production of biogenic Pd
44 nanoparticles [3]. This synthesis process is considered a more 'green' or
45 environment-friendly, low-cost technique. Hence, there is growing interest in
46 synthesizing metal NPs by biological methods.

47 Bio-Pd(0) formation and hydrogenation reactions need the addition of an
48 external electron donor, such as hydrogen, in the biosystem. Hydrogen is consistently
49 identified as one of the most reactive electron donors. To avoid the safety concerns
50 with the use of hydrogen and to reduce the cost of synthesis, many investigators
51 prefer to use hydrogen produced by bacteria *in situ*. Thus, several strains of
52 hydrogen-producing bacteria were investigated in the reduction of Pd(II) or other
53 metals [4-6]. Hennebel et al. studied how bacteria could be used to produce Pd(0) under
54 fermentative conditions. *In situ* hydrogen produced by bacteria was coupled to the
55 formation of Pd(0). Then Pd(0) was tested for the ability to dehalogenate the

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56 recalcitrant aqueous pollutants, diatrizoate and trichloroethylene [6]. Those studies were
57 all under mesophilic conditions. Whether extreme thermophilic bacteria have the
58 same or better ability to reduce metals using *in situ* hydrogen is not known. How Pd(0)
59 particles are formed and act under the extreme thermophilic conditions has also not
60 been reported. Other studies have reported that the biochemical reaction rate of
61 anaerobic digestion is higher at high temperatures [7, 8]. Thus, in this study, the
62 degradation rate of contaminants and the catalytic activities of Pd(0) particles under
63 extreme thermophilic conditions and mesophilic conditions were compared.

64 *Caldicellulosiruptor saccharolyticus*, isolated from thermal springs, is an
65 extreme thermophilic fermentation bacterium with an optimum growth temperature of
66 70 °C. *C. saccharolyticus* can use saccharides or polysaccharides for growth, and has
67 high hydrogen yields (up to 3.5 mol H_2/mol glucose) [9]. More importantly, it contains
68 Ni-Fe hydrogenase bound to the cytoplasmic membrane [10]. Ni-Fe hydrogenase is
69 involved in both the uptake and release of H_2 [11]. When dissolved hydrogen is reduced
70 by Ni-Fe hydrogenase, the generated electrons could be transferred to other electron
71 acceptors, such as heavy metals. For example, the reduction of Pd by *Escherichia coli*
72 is catalyzed by three hydrogenases [12]. Others have reported the extracellular iron
73 reduction of neutral red by *E. coli*, mediated by hydrogen and with the aid of a
74 hydrogenase [13]. With these properties (hydrogen production and hydrogenase), *C.*
75 *saccharolyticus* is expected to produce Pd(0) from Pd(II).

76 In this study, the *in situ* application of hydrogen produced by *C. saccharolyticus*
77 was investigated under extreme thermophilic conditions. Methyl orange (MO) and

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78 diatrizoate were chosen as the contaminants in water. This study aimed at getting Pd(0)
79 out of Pd(II), and studying the catalysis in decolorization and deiodination. First, it
80 was verified that Pd(0) was formed under the action of *in situ* hydrogen produced by
81 *C. saccharolyticus*. Second, the effects of Pd(0) on enhancing decolorization and
82 deiodination were studied. Third, the catalysis of Pd(0) and hydrogenase in the
83 processes of decolorization and deiodination was distinguished. Furthermore, the role
84 of cells in dispersion was confirmed.

85

86 **2. Materials and methods**

87 **2.1. Microorganism and growth medium**

88 Pure culture of *C. saccharolyticus* (DSM 8903) was purchased from Deutsche
89 Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and
90 was cultivated in the DSM640-medium as described previously without supply of
91 cysteine, trypticase and $FeCl_3$ [14]. Glucose was supplied as the carbon source.

92 **2.2. Experimental setup**

93 2.2.1. Batch experiment setup: with glucose

94 The experiments were conducted in 165-mL serum bottles with 60 mL DSM640
95 medium. After being purged with N_2 gas to ensure anaerobic conditions, the serum
96 bottles were sealed with butyl rubber stoppers and aluminum caps, sterilized by
97 autoclaving under 105 °C for 20 min and then incubated at 70 °C. After reaching
98 70 °C, each bottle was inoculated with the microbial culture, which was in its
99 exponential growth phase, to a final optical density (OD_{620}) of 0.03. Two groups of

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100 experiments, one with Pd(II) added and the other without Pd(II), were conducted at
101 the same time. The experiments were carried out in triplicate. Normally, a Na_2PdCl_4
102 stock solution (500 mg Pd/L) was used to achieve a final Pd(II) concentration of 50
103 mg/L. Then 100 mg/L of MO or 20 mg/L of diatrizoate were added into the two
104 groups, respectively. When Pd(II) was reduced to Pd(0) through the action of
105 hydrogen produced by *C. saccharolyticus*, the effect of Pd(0) was investigated.
106 2.2.2. Batch experiment setup: without glucose
107 To confirm the reducing action of hydrogen produced by *C. saccharolyticus,* and
108 distinguish the catalytic action of Pd(0) and cells, glucose was removed from the
109 medium. Hydrogen or nitrogen was bubbled into the DSM640 medium without
110 glucose. Thus, four groups of experiments were conducted at the same time: (1) 60%
111 H_2 and Pd(II); (2) 60% H_2, cells and Pd(II); (3) N_2 and Pd(II); (4) N_2, cells and Pd(II).
112 After autoclaving, 50 mg/L of Pd(II) was added into the serum bottles. Thirty minutes
113 later, cells in the logarithmic growth phase were centrifuged (6000×g for 8 min) and
114 collected, and then re-suspended into the medium.
115 The difference in the catalytic activity between Pd(0) particles produced with and
116 without cells was investigated in three groups: (1) H_2 and Pd(0); (2) H_2 and
117 inactivated cells; (3) H_2, activated cells and Pd(0). The headspace contained 60% H_2
118 and 40% N_2. Harvested cells were washed with medium and added into the anaerobic
119 serum bottles until the OD_{620} value was 1.0.
120 **2.3. Pd reduction and nanopalladium observation**
121 The concentration of Pd(II) was determined by atomic absorption spectrometry

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122 (AAS, Shimadzu AA-360, Kyoto, Japan). Palladium particles produced by hydrogen
123 without cells were observed directly by scanning electron microscopy (SEM,
124 JSM-6700F, JEOL Co., Japan). The palladium particles produced in the presence of
125 cells were fixed in 5% glutaraldehyde for 12 h at 4 °C, and then dehydrated in
126 increasing concentrations of ethanol (30%, 50%, 70%, 80%, 95% and 100%) for 15
127 min in each, and freeze-dried. After the samples were prepared, they were observed
128 by SEM. Transmission electron microscopy combined with energy dispersive
129 spectrometry (TEM-EDS) analysis was also performed to observe the morphology of
130 the cells and particles, and their distribution.

131 **2.4. Chemical analysis**

132 Concentrations of residual MO and the intermediates of MO decolorization were
133 determined using a HPLC system (LC-1100, Agilent Inc., USA) equipped with a UV
134 detector and a Hypersil ODS C18 column. Methanol (solution A) and water with 0.1%
135 (v/v) acetic acid and 0.1% (w/v) ammonium acetate (solution B) were used as the
136 isocratic mobile phase. The gradient elution program was as follows: 5% (v/v)
137 solution A and 95% (v/v) solution B at 0 min; 20% solution A and 80% solution B at
138 15 min; 100% solution A at 30 min; 5% solution A and 95% solution B at 38 min. The
139 flow rate was 0.8 mL/min, the wavelength of the UV detector was set at 450 nm and
140 254 nm, and 20 μL of sample were injected for analysis.

141 The concentration of diatrizoate was monitored by HPLC (LC-1100, Agilent Inc.,
142 USA) equipped with a UV detector and a Hypersil ODS C18 column. Elution was
143 performed isocratically at 25 °C and at a flow rate of 1 mL/min with 5% solvent A

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144 (100% acetonitrile) and 95% solvent B (0.1% formic acid). The injection volume was
145 50 μL, and the elution program ran within 15 min.

146 Hydrogen was sampled by a gas-tight syringe (SGE Analytical Science, Australia)
147 and the hydrogen concentration was determined by a GC (Lunan model SP7890, CN)
148 equipped with a thermal conductivity detector and a 1.5-m stainless steel column
149 packed with a 5-Å molecular sieve. The temperatures of the injector, detector, and
150 column were kept at 80, 100, and 50 °C, respectively. N_2 was used as the carrier gas.

151

152 **3. Results and discussion**

153 **3.1. Pd nanoparticle formation**

154 The reduction of Pd(II) and Pd(0) nanoparticle formation could be confirmed by
155 the results of AAS, TEM images and EDS (Fig. 1). The results of AAS showed that
156 Pd(II) disappeared completely from the solution (data not shown). The color change
157 of the medium, from pale yellow to black, indicated that Pd(0) nanoparticles formed
158 in the system. EDS analysis of the point marked in the TEM image indicated that Pd(0)
159 was indeed formed in this system. This result also illustrated that hydrogen, as a
160 reducing power produced by *C. saccharolyticus*, could reduce Pd(II) to Pd(0) via the
161 action of hydrogenase, as reported by others [6, 12, 15]. Furthermore, the TEM image
162 illustrated that Pd(0) particles were formed and that the diameter of the Pd(0) particles
163 was around 10–20 nm. Most of the particles were distributed around the cells.

164 Initially, the Pd(II) ions spread over the cell culture including the extracellular
165 polymeric substances (EPS) of *C. saccharolyticus*. EPS are high-molecular-weight

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166 polymers that are secreted by microorganisms into the surrounding environment [16].
167 Many particles could hide in the EPS. Hydrogen produced by *C. saccharolyticus*
168 escaped from the cytomembrane, and then reached the EPS and the bulk solution.
169 Then Pd(0) formed with the aid of hydrogen. It is possible that the EPS facilitated the
170 dispersion of the Pd(0) particles. Thus the Pd(0) particles could disperse well without
171 the addition of a chemical dispersant. The EPS prevented the aggregation of the
172 Pd(0) particles. Conventional production methods of Pd(0) particles required the use
173 of a series of toxic or expensive chemical agents, such as stabilizers and reductant [6, 17].
174 Thus, our study has shown that EPS can act as an environmental bio-dispersant, and
175 that hydrogen is produced *in situ* by *C. saccharolyticus*.

176 **3.2. Enhanced decolorization and dehalogenation**

177 The *in situ* application of the Pd(0) particles and hydrogen produced by *C.*
178 *saccharolyticus* was investigated in cases of decolorization and dehalogenation. MO
179 (100 mg/L) was added into all the batches with and without Pd(0) particles. The rates
180 of decolorization with Pd(0) particles were higher than that without Pd(0) particles
181 (Fig. 2). MO was completely removed within 30 min in the group with Pd(0) particles.
182 However, more than 6 h were required to completely remove 100 mg/L of MO in the
183 group without Pd(0) particles. To eliminate the possible effect of adsorption by Pd(0)
184 particles, the intermediates of MO decolorization were determined.
185 4-aminobenzenesulfonic acid (4-ABA) and N',N-dimethyl-p-phenylenediamine (DPD)
186 were produced as the intermediates during the decolorization of MO. The
187 concentrations of both 4-ABA and DPD increased with the decrease of MO (Fig. 2a,

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188 b). As shown in Fig. 2b, 100 mg/L of MO (0.30 mM) disappeared completely within
189 30 min, which corresponded to the stoichiometric appearance of 50 mg/L 4-ABA
190 (0.29 mM) and 40 mg/L DPD (0.29 mM). This result demonstrated that MO was
191 reduced rather than adsorbed by cells and Pd(0) particles.

192 The complete decolorization in the group without Pd(0) particles was realized
193 with the combined action of hydrogen and hydrogenase. This phenomenon was
194 confirmed in our previous study [18]. In the group with Pd(0) particles, the complete
195 decolorization was the combined effects of hydrogen, hydrogenase and Pd(0) particles.
196 The nanopalladium enhanced the decolorization of MO. MO decolorization driven by
197 cells alone needed longer time as other researchers reported. For example, MO (100
198 mg/L) was completely removed within 7 h by *S. oneidensis* MR-1 [19]. A 95%
199 decolorization by *P. luteola* was observed at 6 h and 9 h for 100 and 350 mg/L MO,
200 respectively [20].

201 Pd(0) particles have been reported frequently as the catalyst of the reduction of
202 halides, such as trichloroethylene (TCE), chlorophenols and diatrizoate [21-24]. This
203 study chose diatrizoate as the halide to test the catalytic activity of Pd(0) particles in
204 extreme thermophilic condition. As shown in Fig. 3, 20 mg/L of diatrizoate
205 disappeared within 10 min in the two groups with Pd(0) particles during different time
206 slot. However, in the group without Pd(0) particles diatrizoate was not reduced within
207 20 min. This result indicates that the catalytic activity of Pd(0) particles was essential
208 in the deiodination, whereas cells had no catalytic effect. Furthermore, the rate of
209 deiodination was slightly higher when the reaction of deiodination started at 20 h

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210 compared to 10 h. The time span, i.e. 10 and 20 h, was calculated from the inoculation
211 time. The hydrogen content in the headspace was 16 mL at 20 h and 10 mL at 10 h.
212 From the Michaelis-Menten model, it can be seen that the enzymatic catalytic reaction
213 rate is positively related to the concentrations of catalyst and substrate. In this study,
214 the catalyst was Pd(0) particles and the substrate was hydrogen. Thus, higher
215 concentrations of hydrogen accelerated the rate of deiodination.

216 The rate of deiodination observed in this present study was higher than that
217 reported in a study of *Citrobacter braakii* [6], which was also able to reduce Pd(II) to
218 Pd(0) through hydrogen produced *in situ* under mesophilic conditions. The same
219 amount of Pd(II) (50 mg/L) was added into both systems. More hydrogen (1.31 mmol
220 vs. 0.6 mmol in our study) was produced under mesophilic conditions when the
221 reaction of deiodination started. However, it took over 30 min to remove 20 mg/L of
222 diatrizoate in the study of *C. braakii* [6], whereas in the present study it took only 10
223 min. It is likely that temperature played an important role in enhancing the
224 deiodination process in our experiments. Thus, extreme thermophilic conditions
225 should be beneficial to deiodination. It is also possible that the extreme thermophilic
226 fermentation bacterium *C. saccharolyticus* performed better in coupling bio-Pd
227 nanoparticle generation and bio-H_2 for the deiodination process than many mesophilic
228 bacteria reported previously.

229 **3.3. Catalysis of hydrogenase and/or Pd(0)**

230 In our previous study, we found that hydrogen could decolorize MO with the
231 action of hydrogenase [18]. Pd(0) enhanced the degradation of MO and also had the

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232 catalytic activity of hydrogenation, as in the deiodination of diatrizoate. What role did
233 hydrogenase and Pd(0) play in, respectively, decolorization and deiodination? The
234 role of hydrogenase and Pd(0) in decolorization and deiodination was determined in
235 this study. Glucose was removed from the medium, and four groups of experiments
236 were conducted at the same time. As shown in Fig. 4a, MO (50 mg/L) was removed
237 completely within 30 min in the group with H_2, cells and Pd(0), as the concentration
238 of MO was measured only at 30 min. The disappeared MO (50 mg/L) corresponded to
239 the appearance of 29 mg/L 4-ABA and 23 mg/L DPD, indicating the completed
240 degradation of MO, rather than adsorption by Pd(0). However, only 25 mg/L of MO
241 was removed in the group with H_2 and cells within 30 min. In the group with N_2, cells
242 and Pd(II), only 10 mg/L of MO was removed, which was a result of adsorption. In
243 the group with N_2 and Pd(II), there was almost no reduction of MO. Pd(II) could not
244 be reduced to Pd(0) in all the batches without hydrogen in our test. Thus, there was no
245 catalytic activity of Pd(0) in both groups without hydrogen. It was speculated that
246 about 20% MO was adsorbed by cells and 30% was catalyzed by hydrogenase, which
247 was comparable to the catalysis of Pd(0) and hydrogenase within 30 min. Thus, Pd(0)
248 greatly enhanced the decolorization of MO, and the catalytic activity of hydrogenase
249 and the adsorption of cells could be instead by the result of the catalytic effect of Pd(0)
250 particles, because the catalytic activity of Pd(0) particles was sufficiently powerful to
251 completely remove 50 mg/L of MO within 30 min.

252 Fig. 4b shows that 20 mg/L of diatrizoate was removed within 5 min in the group
253 with H_2, cells and Pd(0). Diatrizoate was not degraded in the groups without Pd(0)

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254 formation. About 3 mg/L of diatrizoate was removed in the group with N_2, cells and
255 Pd(II), resulted from adsorption to cells within 20 min. This phenomenon indicated
256 that Pd(0) was the essential catalyst, and that hydrogenase had no effect in the
257 deiodination process within 20 min. In other studies, diatrizoate could be effectively
258 degraded solely by Pd(0). For example, the removal of 20 mg/L diatrizoate by a 10
259 mg/L Pd suspension and 100% hydrogen was completed after 4 h [21]. However, in our
260 study, 50 mg/L Pd and 60% hydrogen were added. The degradation rate was different,
261 as the degradation rate of diatrizoate was related positively to the dosage of Pd(0) and
262 hydrogen. Furthermore, the temperature were different in two studies. Compared with
263 the catalytic effect in the batches with glucose in the previous section (Fig. 3), it can
264 be seen that the catalytic effect was better in the batches without glucose in this
265 section, because the content of hydrogen in the headspace in the batches with glucose
266 was between 0.45 and 0.71 mM, and was around 2.68 mM in the batches without
267 glucose. Hydrogen was filled artificially in this section, whereas hydrogen was
268 produced by cells in the batches with glucose in the previous section. This could also
269 be explained by the Michaelis-Menten model [18].

270 **3.4 Dispersive action of cells on Pd(0) particles**

271 The difference in size and catalytic activity between Pd(0) particles produced
272 with and without cells was also investigated in this study. Cells were sterilized after
273 Pd reduction to eliminate the effect of hydrogenase. This design aimed to confirm the
274 dispersive action of cells. The Pd(0) particles aggregated together and formed a bulk
275 in the system without dispersant (Fig. 5a, b). The diameter of the Pd(0) particles

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276 formed under this condition (chemical-Pd(0)) was between 1 and 2 μm. By contrast,
277 in the system with the cells as a 'green' dispersant, the Pd(0) particles could be finely
278 dispersed (Fig. 5c, d). The diameter of most Pd(0) particles formed in the presence of
279 cells was under 100 nm. These Pd(0) particles were polyporous and homogeneous.
280 Previous research on EPS offered a sufficient theoretical foundation for this study [25-28];
281 that is, the EPS of *C. saccharolyticus* could adsorb the Pd(0) particles and prevent
282 them from aggregating together. The Pd(0) particles in the system with cells had a
283 smaller size and a larger specific surface area compared with the Pd(0) particles in the
284 system without cells. Thus the Pd(0) particles in the system with cells should
285 theoretically have a higher catalytic activity. The following experiments with these
286 systems, i.e. with and without cells, provided an answer.

287 MO was chosen as the contaminant to verify the catalytic activity of the Pd(0)
288 particles in three groups. As shown in Fig. 6a, only 25 mg/L of MO was removed
289 within 15 min in the group with H_2 and Pd(0) (Fig. 6a), and the tendency of the
290 intermediates is shown clearly in Fig. 6b, indicating the degradation of MO. Fig. 6c
291 shows that there were no intermediates detected in the group with H_2 and cells. This
292 result indicates that reduced MO was not degraded but adsorbed by the cells. Not
293 more than 20 mg/L of MO was adsorbed by cells within 30 min, which indicates that
294 the adsorption by the cells played only a small role. However, 100 mg/L of MO was
295 removed completely within 15 min in the group with H_2, cells and Pd(0), and the MO
296 was degraded to 4-ABA and DPD (Fig. 6d). These results confirmed that Pd(0)
297 particles formed in the group with cells had a higher catalytic activity in the

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298 decoloration of MO. The cells of *C. saccharolyticus* were ‘green’ dispersants in this
299 system.
300

301 **4. Conclusions**

302 Pd(0) particles were produced from Pd(II) with *in situ* hydrogen generated by *C.*
303 *saccharolyticus* during glucose fermentation. The degradation of MO and diatrizoate
304 were both enhanced by Pd addition. The removal of MO was the result of the
305 combined action of hydrogen, hydrogenase and Pd(0) particles. However, Pd(0)
306 particles played an essential role in the removal of diatrizoate. Furthermore, the Pd(0)
307 particles were well dispersed by cells of *C. saccharolyticus* and showed a better
308 catalytic activity than chemical Pd(0) without dispersant. Generally speaking, the
309 addition of Pd would enhance the degradation of contaminants in water.
310

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319 **References**

320 1. L. E. Macaskie, V. S. Baxter-Plant, N. J. Creamer, A. C. Humphries, I. P.

RSC Advances Accepted Manuscript

321 Mikheenko, P. M. Mikheenko, D. W. Penfold and P. Yong, *Biochem Soc T*,
322 2005, 33, 76-79.
323 2. V. L. Nguyen, D. C. Nguyen, H. Hirata, M. Ohtaki, T. Hayakawa and M.
324 Nogami, *Advances in Natural Sciences: Nanoscience and Nanotechnology*,
325 2010, 1, 035012.
326 3. T. Hennebel, B. De Gusseme, N. Boon and W. Verstraete, *Trends Biotechnol*,
327 2009, 27, 90-98.
328 4. K. Deplanche and L. E. Macaskie, *Biotechnol Bioeng*, 2008, 99, 1055-1064.
329 5. O. A. Zadvornyy, M. Allen, S. K. Brumfield, Z. Varpness, E. S. Boyd, N. A.
330 Zorin, L. Serebriakova, T. Douglas and J. W. Peters, *Environ Sci Technol*, 2010,
331 44, 834-840.
332 6. T. Hennebel, S. Van Nevel, S. Verschuere, S. De Corte, B. De Gusseme, C.
333 Cuvelier, J. P. Fitts, D. Van der Lelie, N. Boon and W. Verstraete, *Appl*
334 *Microbiol Biot*, 2011, 91, 1435-1445.
335 7. Y. Xia, T. Zhang and H. H. P. Fang, *Procedia Environ Sci*, 2012, 12, 3-8.
336 8. P. Intanoo, P. Rangsanvigit, P. Malakul and S. Chavadej, *Bioresource Technol*,
337 2014, 173, 256-265.
338 9. K. Willquist and E. W. J. van Niel, *Int J Hydrogen Energ*, 2012, 37,
339 4925-4929.
340 10. H. J. G. van de Werken, M. R. A. Verhaart, A. L. VanFossen, K. Willquist, D.
341 L. Lewis, J. D. Nichols, H. P. Goorissen, E. F. Mongodin, K. E. Nelson, E. W.
342 J. van Niel, A. J. M. Stams, D. E. Ward, W. M. de Vos, J. van der Oost, R. M.

RSC Advances Accepted Manuscript

343 Kelly and S. W. M. Kengen, *Appl Environ Microb*, 2008, 74, 6720-6729.

344 11. G. Meshulam-Simon, S. Behrens, A. D. Choo and A. M. Spormann, *Appl*
345 *Environ Microb*, 2007, 73, 1153-1165.

346 12. K. Deplanche, I. Caldelari, I. P. Mikheenko, F. Sargent and L. E. Macaskie,
347 *Microbiol-Sgm*, 2010, 156, 2630-2640.

348 13. J. B. McKinlay and J. G. Zeikus, *Appl Environ Microb*, 2004, 70, 3467-3474.

349 14. K. Willquist and E. W. J. van Niel, *Metab Eng*, 2010, 12, 282-290.

350 15. T. Hennebel, H. Simoen, W. De Windt, M. Verloo, N. Boon and W. Verstraete,
351 *Biotechnol Bioeng*, 2009, 102, 995-1002.

352 16. H. L. Zhang, W. Fang, Y. P. Wang, G. P. Sheng, C. W. Xia, R. J. Zeng and H.
353 Q. Yu, *Bioresource Technol*, 2013, 142, 714-718.

354 17. S. D. Corte, *Microb Biotechnol*, 2012.

355 18. N. Shen, Y. C. Huo, J. J. Chen, F. Zhang, H. Zheng and R. J. Zeng, *Chem Eng*
356 *J*, 2015, 262, 847-853.

357 19. P. J. Cai, X. Xiao, Y. R. He, W. W. Li, J. Chu, C. Wu, M. X. He, Z. Zhang, G. P.
358 Sheng and M. H. W. Lam, *Appl Microbiol Biot*, 2012, 93, 1769-1776.

359 20. C. C. Hsueh and B. Y. Chen, *J Hazard Mater*, 2007, 141, 842-849.

360 21. T. Hennebel, S. De Corte, L. Vanhaecke, K. Vanherck, I. Forrez, B. De
361 Gusseme, P. Verhagen, K. Verbeken, B. Van der Bruggen, I. Vankelecom, N.
362 Boon and W. Verstraete, *Water Res*, 2010, 44, 1498-1506.

363 22. B. De Gusseme, T. Hennebel, L. Vanhaecke, M. Soetaert, J. Desloover, K.
364 Wille, K. Verbeken, W. Verstraete and N. Boon, *Environ Sci Technol*, 2011, 45,

RSC Advances Accepted Manuscript

365 5737-5745.

366 23. T. Hennebel, J. Benner, P. Clauwaert, L. Vanhaecke, P. Aelterman, R.
367 Callebaut, N. Boon and W. Verstraete, *Biotechnol Lett*, 2011, 33, 89-95.

368 24. T. Hennebel, H. Simoen, P. Verhagen, W. De Windt, J. Dick, C. Weise, F.
369 Pietschner, N. Boon and W. Verstraete, *Environ Chem Lett*, 2011, 9, 417-422.

370 25. T. Urashima, H. Ariga, T. Saito, T. Nakamura, S. Tanaka and I. Arai,
371 *Milchwissenschaft*, 1999, 54, 190-193.

372 26. P. d'Abzac, F. Bordas, E. Joussein, E. D. van Hullebusch, P. N. L. Lens and G.
373 Guibaud, *Environ Sci Pollut R*, 2013, 20, 4509-4519.

374 27. F. X. Kang, P. J. Alvarez and D. Q. Zhu, *Environ Sci Technol*, 2014, 48,
375 316-322.

376 28. J. Xu, G. P. Sheng, Y. Ma, L. F. Wang and H. Q. Yu, *Water Res*, 2013, 47,
377 5298-5306.

378

379

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380 **A list of figures**

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394 formed with cells and hydrogen supplied).

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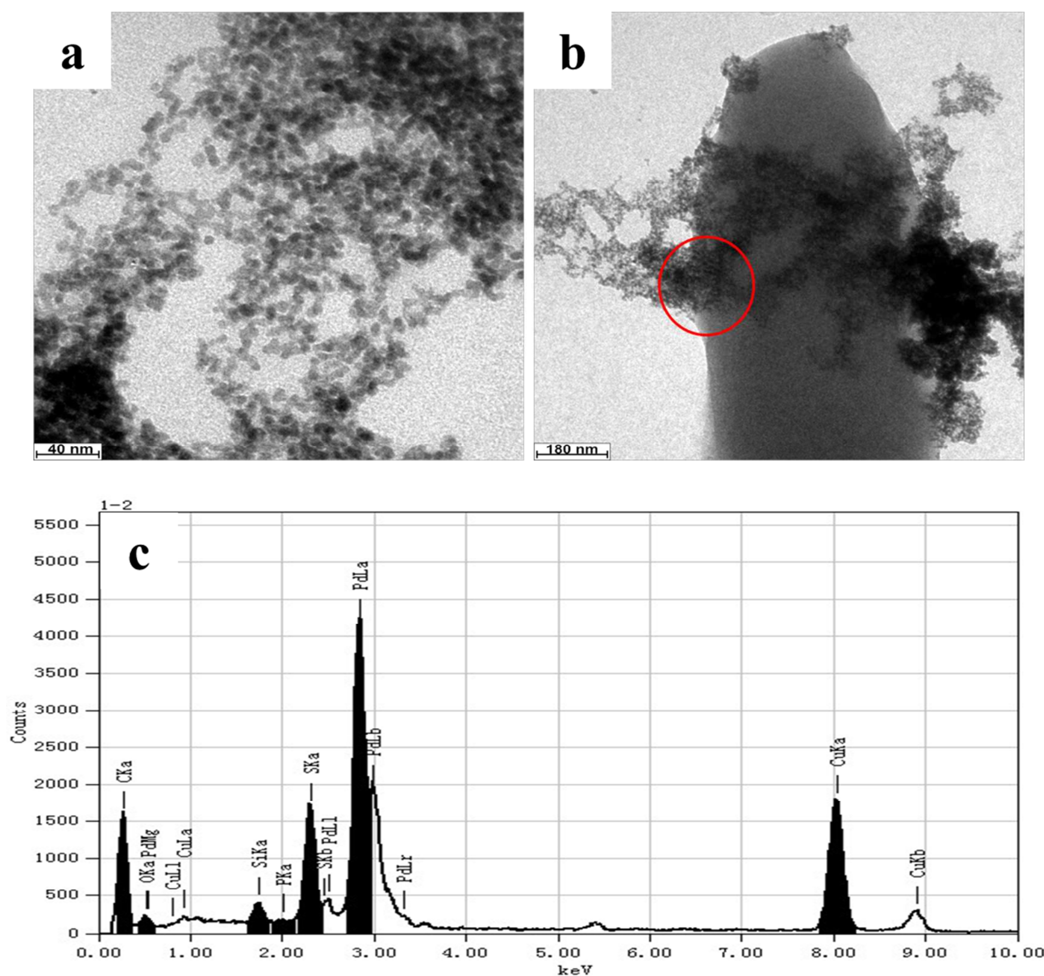

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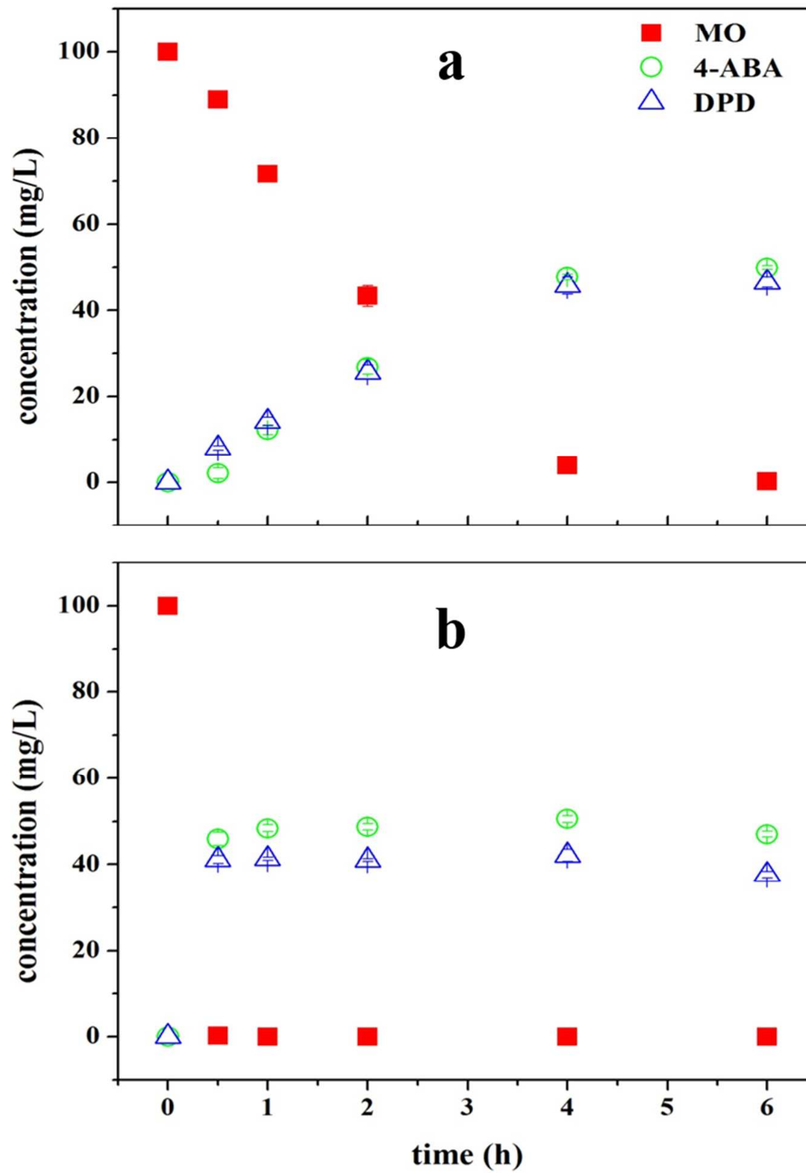

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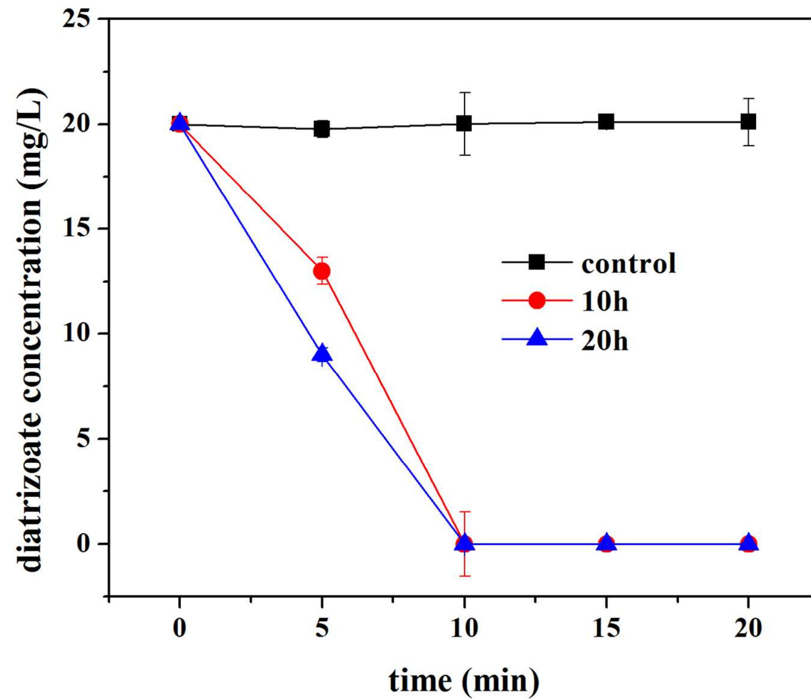

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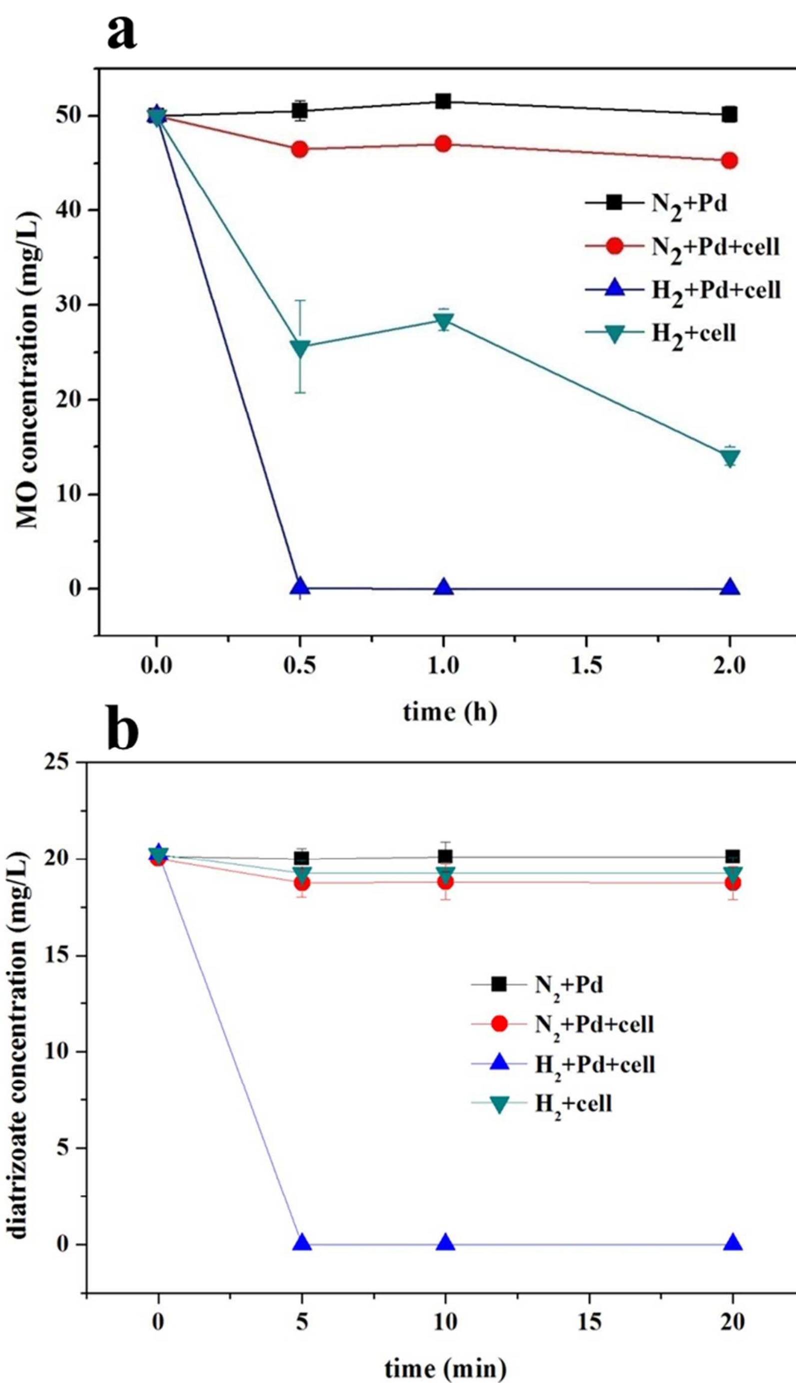

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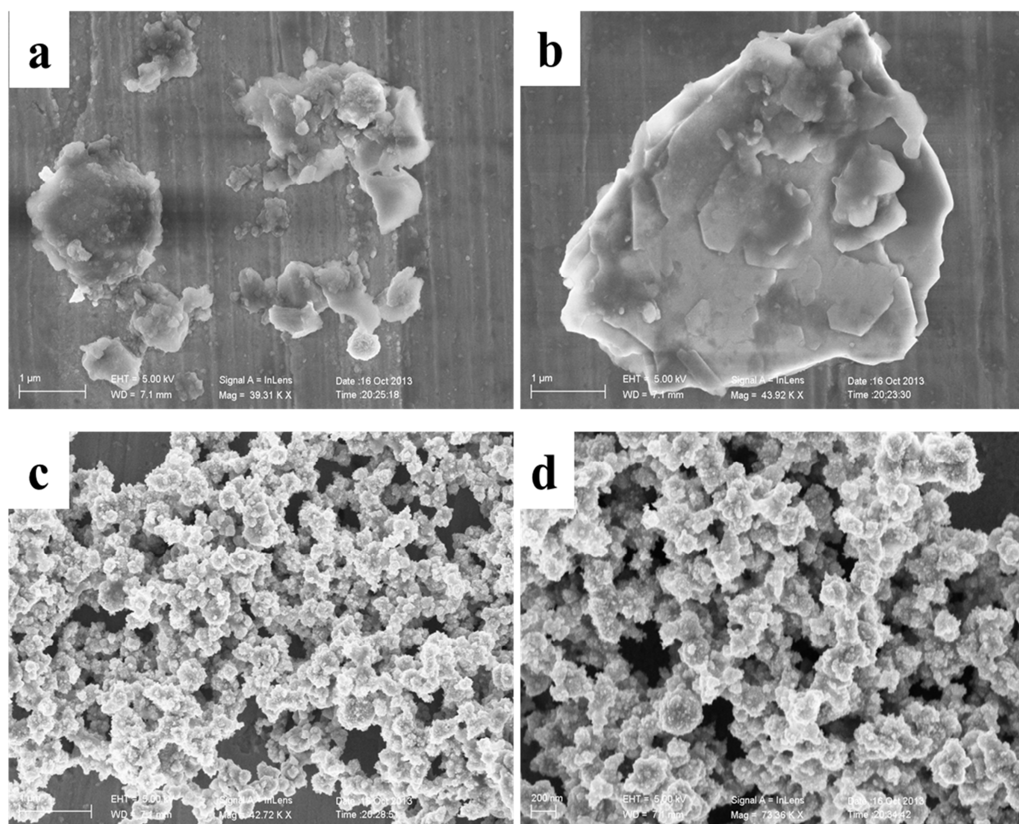

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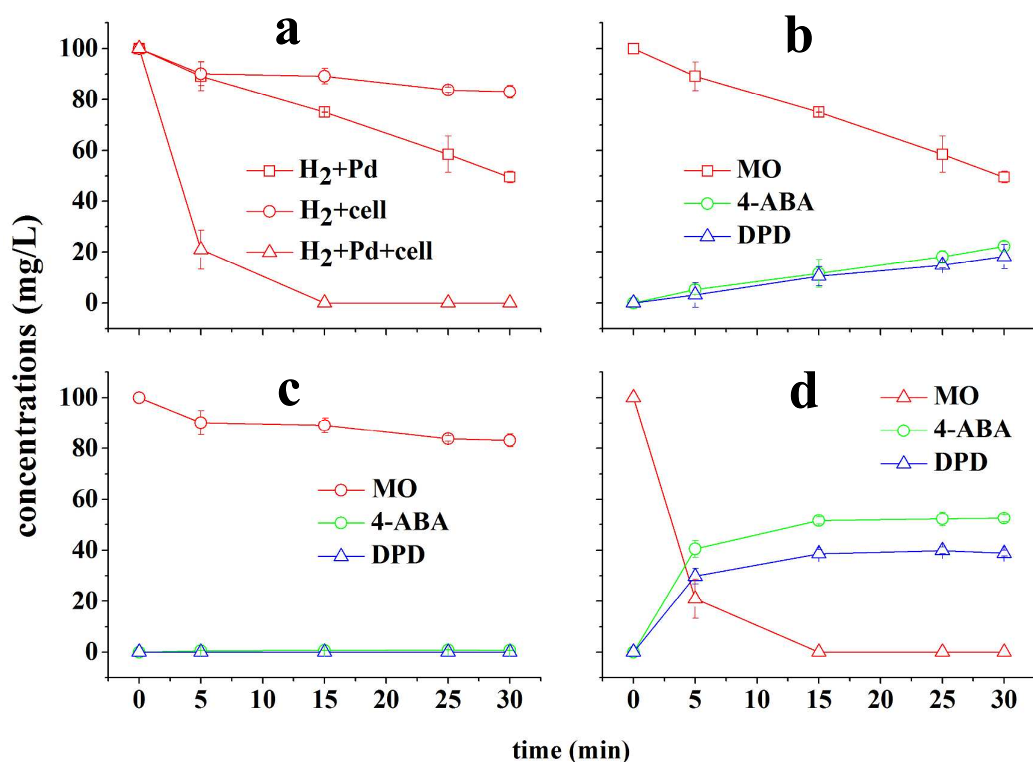

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