

# 1 **Hormetic effect of ionic liquid 1-ethyl-3-methylimidazolium acetate on** 2 **bacteria**

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## 12 **Highlights**

- 13 • Among three ILs tested, only [EMIM]Ac exhibited hormesis in Gram -ve and Gram +ve  
14 bacteria
- 15 • Growth of Gram -ve, aerobic bacterium *Pseudomonas putida* was increased by 4-fold in  
16 presence of 0.5 g L<sup>-1</sup> of [EMIM]Ac
- 17 • Growth of Gram +ve anaerobic bacterium *Clostridium* sp. was increased by 0.4-fold in  
18 presence of 0.5 g L<sup>-1</sup> of [EMIM]Ac
- 19 • Hormesis of [EMIM]Ac on bacterial growth was mediated via regulation of medium pH

20

## 21 **Abstract**

22 The biological effect of ionic liquids (ILs) is one of the highly debated topics as they are being  
23 contemplated for various industrial applications. 1-ethyl-3-methylimidazolium acetate  
24 ([EMIM][Ac]) showed remarkable hormesis on anaerobic *Clostridium* sp. and aerobic  
25 *Pseudomonas putida*. Bacterial growth was stimulated at up to 2.5 g L<sup>-1</sup> and inhibited at >2.5 g  
26 L<sup>-1</sup> of [EMIM][Ac]. The growth of *Clostridium* sp. and *P. putida* were higher by 0.4 and 4-fold  
27 respectively, in the presence of 0.5 g L<sup>-1</sup> [EMIM][Ac]. Assessment of the effect of [EMIM][Ac]  
28 under different growth conditions showed that the hormesis of [EMIM][Ac] was mediated via

29 regulation of medium pH. Hormetic effect of [EMIM][Ac] was evident only in medium with  
30 poor buffering capacity and in the presence of a fermentable substrate as the carbon source. The  
31 hormetic effect of [EMIM][Ac] on bacterial growth is most likely associated with the buffering  
32 capacity of acetate anion. These observations have implications in ILs toxicity studies and  
33 ecological risk assessment.

34 Keywords: 1-ethyl-3-methylimidazolium acetate; *Biomass pretreatment*; *Clostridium sp.*;  
35 hormesis; imidazolium ionic liquids; *Pseudomonas putida*.

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## 38 1. Introduction

39 Ionic liquids (ILs) are novel class of organic salts with low melting points ( $< 100\text{ }^{\circ}\text{C}$ ),  
40 increasingly considered as green replacements for volatile organic compounds (Passos et al.,  
41 2014; Patel et al., 2012). Ionic liquids are typically made up of two components, a bulky organic  
42 cation (i.e. N,N'-dialkylimidazolium, N-alkylpyridinium, alkylammonium, alkylphosphonium,  
43 alkylsulphonium and triazolium) and an organic or inorganic anion (i.e. halides,  
44 tetrafluoroborate, hexafluorophosphate, alkylphosphates, acetate) (Bubalo et al., 2014). These  
45 compounds have been in the spotlight of scientific and industrial community as novel green  
46 solvents for replacement of conventional volatile solvents (Bubalo et al., 2014). Ionic liquids are  
47 attractive due to their low vapor pressure, non-flammability, and high thermal stability.  
48 Importantly, ionic liquids offer unprecedented flexibility in designing several classes of  
49 compounds with novel physical and chemical properties by means of tuning cation and anion  
50 structure (Earle and Seddon, 2000). Ionic liquids are extensively studied for applications in  
51 organic synthesis, separation technology, biocatalysis, corrosion inhibitors, biomass pretreatment  
52 and in use as corrosion inhibitors and antimicrobials (Brand et al., 2013; Pham et al., 2010;  
53 Plechkova and Seddon 2008; Nancharaiah et al., 2012).

54 Lignocellulosic materials (i.e. wood, agricultural or forest residues) are most abundant on our  
55 planet and available at a much lower cost than starch and sucrose based materials for production  
56 of biofuels (Brandt et al., 2013). However, major obstacle in using the lignocellulosic materials  
57 is the non availability of cost-effective pretreatment technologies for hydrolysis and  
58 deconstruction to readily fermentable products (Datta et al., 2010). Ionic liquid based  
59 pretreatment methods show promise for cellulose dissolution and biomass deconstruction (Brand

60 et al., 2013; Zavrel et al., 2009). Ionic liquids with chloride, acetate, and phosphate anions  
61 showed good cellulose dissolution capacities (Vitz et al., 2009). The cellulose dissolving abilities  
62 of 1-ethyl-3-methylimidazolium acetate ([EMIM][Ac]), 1-ethyl-3-methylimidazolium  
63 dimethylphosphate ([EMIM][DEP]) and 1-methyl-3-methylimidazolium dimethylphosphate  
64 ([MMIM][DMP]) were reported to be in the range of 8%, 10% and 12 -14% (w/v) respectively  
65 (Vitz et al., 2009). Moreover, the pretreatment methods should neither introduce nor generate  
66 compounds that would negatively impact the downstream processes such as fermentation.  
67 Interestingly, the growth and fermentative metabolism of *Clostridium* sp. was not inhibited by 1-  
68 ethyl-3-methyl imidazolium and 1-methyl-3-methyl imidazolium ionic liquids with anions such  
69 as acetate, dimethylphosphate or diethylphosphate up to 2.5 g L<sup>-1</sup> (Nancharaiah and Francis,  
70 2011).

71 High volume production and wide applications of ionic liquids could lead to pollution of  
72 aquatic environments due to water solubility of ILs (Zhang et al., 2011). Many studies have  
73 shown that ILs are persistent in the environment and exhibit toxicity towards prokaryotic and  
74 eukaryotic organisms (Bubalo et al., 2014; Pham et al., 2010). However, the toxicity of IL is  
75 dependent on cation, alkyl chain length of substituent of cation, and anion. Recently, hormesis  
76 was observed in case of certain ionic liquids, particularly those with short alkyl chains (Ge et al.,  
77 2010; Nancharaiah and Francis 2011; Wang et al., 2011; Zhang et al., 2013). Hormesis was  
78 originally applied to describe the effect of low doses of ionizing radiation, but now it is generally  
79 used to describe biphasic dose-response of biological systems to environmental conditions or  
80 stress (Davies et al., 2006). In toxicology, hormesis is defined as a biphasic dose-response  
81 phenomenon primarily characterized by stimulation of biological response at lower  
82 concentrations while inhibition at higher concentrations. The hormetic response of ionic liquids

83 is a poorly understood phenomenon and the chemical and biochemical mechanisms are  
84 unknown. Among the three ionic liquids (i.e. [EMIM][Ac], [EMIM][DEP], [MMIM][DMP]),  
85 tested for their influence on the growth and fermentative metabolism of *Clostridium* sp. BC1,  
86 only [EMIM][Ac] showed hormetic effect. Consequently, the aim of the present study was to  
87 investigate the mode of action of hormesis by determining the hormetic effect of [EMIM][Ac] on  
88 anaerobic Gram +ve and Gram -ve bacteria under different growth conditions.

## 89 2. Materials and methods

### 90 2.1 Ionic liquids

91 The structures of ILs [EMIM][Ac], [EMIM][DEP] and [MMIM][DMP] used in the present  
92 study are shown in Table 1. All the ionic liquids were obtained from Sigma–Aldrich and used as  
93 received.

### 94 2.2 Ionic liquid stock solutions

95 Stock solutions of ionic liquids were prepared in de-ionized water as described earlier  
96 (Nancharaiah and Francis, 2011). The ionic liquid solutions were sterilized by filtering through  
97 0.22 µm Millex filter. The ionic liquid solutions were transferred to serum bottles, closed with  
98 butyl rubber stoppers, aluminum crimp sealed and deoxygenated by purging with ultra high  
99 purity (UHP) N<sub>2</sub> gas. The ionic liquid stock solutions were stored at room temperature.

### 100 2.3 Bacterial cultures and growth conditions

101 *Clostridium* sp. BC1 (ATCC 53464), gram-positive, anaerobic, fermentative bacterium, is  
102 phylogenetically closely related to *C. pasteurianum*. It was grown in mineral salts medium in  
103 serum bottles as described earlier (Nancharaiah and Francis, 2011). The mineral salts medium  
104 contained the following: glucose, 10.0 g; NH<sub>4</sub>Cl, 0.5 g; glycerol phosphate, 0.3 g; MgSO<sub>4</sub>.7H<sub>2</sub>O,  
105 0.2 g; CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.5 g; FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.005 g; peptone, 0.1 g; yeast extract, 0.1 g; deionized  
106 water, 1 L; pH, 6.8. The medium contained glycerol phosphate as the P source. The medium was  
107 pre-reduced by boiling for 10 min while purging with UHP nitrogen gas. The medium was  
108 dispensed as 40 mL aliquots into 60 mL serum bottles in an anaerobic chamber (Coy Laboratory  
109 products, USA). The serum bottles containing media were fitted with butyl rubber stoppers,  
110 crimp sealed with aluminum caps and autoclaved. The culture was maintained by repeated sub-  
111 culturing in serum bottles by inoculating autoclaved MS medium with 1 mL of log phase culture.  
112 The serum bottles were incubated at 26 °C.

113 *Pseudomonas putida* TUM-PP12 (Nancharaiah et al., 2003, 2008), a gram-negative  
114 bacterium, was maintained in Luria Bertani agar (Difco, USA) plates supplemented with 50 µg  
115 mL<sup>-1</sup> kanamycin under aerobic conditions. For liquid cultures, *P. putida* was routinely grown in  
116 250 mL Erlenmeyer flasks containing 100 mL sterile mineral salts medium by inoculating with  
117 log phase culture. The culture flasks were incubated at 30 °C in an orbital shaker set at 100 rpm.

#### 118 *2.4 Effect of ionic liquids on Clostridium sp. and P. putida*

119 To determine the effect of ILs on *Clostridium* sp. different concentrations (0.5 to 10 g L<sup>-1</sup>  
120 w/v) of ILs were added to serum bottles containing sterile mineral salts medium, Sterile mineral  
121 salts medium without ionic liquids was used as control. The serum bottles with and without

122 ionic liquids in mineral salts medium were inoculated with 1 mL of 24 h-old *Clostridium* sp.  
123 culture (OD 0.4). The serum bottles were incubated at 26 °C. At periodic time intervals, total gas  
124 production was measured. After measuring the total gas production, a 4 mL of the culture sample  
125 was removed with a syringe for monitoring growth and medium pH.

126 To determine the effect of ILs on *P. putida*, Erlenmeyer flasks (250 mL volume) containing  
127 100 mL of sterile mineral salts medium with and without [emim]Ac were inoculated with 1 mL  
128 of 24 h old culture of *P. putida*. The flasks were incubated at 30 °C in an orbital shaker set at 100  
129 rpm. Liquid samples were collected at regular time intervals for measuring growth and pH. The  
130 effect of [EMIM][Ac] on the growth of *P. putida* was also determined in MS medium  
131 supplemented with acetate as sole carbon source and tryptic soy broth (Difco, USA) under the  
132 similar experimental conditions.

### 133 *2.5 Effect of ionic liquids on growth in phosphate-buffered mineral salts medium*

134 In order to understand the hormetic effect of [EMIM][Ac], the growth of *Clostridium* sp. and  
135 *P. putida* was determined in phosphate buffered mineral salts (PMS) medium (Nancharaiah et  
136 al., 2012). The PMS medium consisted of the following: glucose, 10.0 g; NH<sub>4</sub>Cl, 0.5 g;  
137 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 g; KH<sub>2</sub>PO<sub>4</sub>, 5g; K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 6.55g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.005 g; peptone, 0.1 g;  
138 yeast extract, 0.1 g; deionized water to 1 L; pH, 6.8. The serum bottles and flasks were prepared  
139 with PMS medium and autoclaved as mentioned above. [EMIM][Ac] was added to serum bottles  
140 or culture flasks containing PMS medium. PMS medium without ionic liquid was used as the  
141 control. The serum bottles and flasks with and without ionic liquid were inoculated with  
142 *Clostridium* sp. and *P. putida* respectively and incubated as described above. All experiments

143 were setup in triplicate serum bottles or culture flasks. The liquid samples were collected at  
144 regular time intervals for monitoring growth and pH.

## 145 2.6 Analytical methods

146 Bacterial growth was monitored by measuring turbidity at 600 nm using Spectronic 20  
147 spectrophotometer (Thermo Scientific, USA) or UV-3600 UV-Vis spectrophotometer  
148 (Shimadzu, Japan). Total gas produced by *Clostridium* sp. was measured using a pressure gauge  
149 connected to a syringe (Francis and Dodge, 1987). An aliquot of the culture sample was filtered  
150 through 0.45  $\mu\text{m}$  Millex filter and the pH was determined using a Beckman 350 pH meter with a  
151 Beckman 511275-AB combination electrode. Glucose was analyzed by HPLC. The HPLC  
152 system consisted of a SCL-10A controller, a SIL-10A sample autoinjector, and a LC-10AS  
153 liquid chromatograph (Shimadzu, Japan). The HPLC system was fitted with a Bio-Rad HPX-  
154 87H column (Bio-Rad labs, USA) and mobile phase (0.003 N  $\text{H}_2\text{SO}_4$ ) was pumped at flow rate  
155 of 0.7  $\text{ml min}^{-1}$ . Glucose concentration was determined using RID-6A refractive index detector  
156 (Shimadzu, Japan).

## 157 3. Results and discussion

### 158 3.1 Effect of ionic liquids on *Clostridium* sp. growth

159 The effect of water miscible imidazolium ILs with short alkyl chains on the cation (-ethyl  
160 and -methyl) and three different anion groups (acetate, diethylphosphate and dimethylphosphate)  
161 on *Clostridium* sp. growth indicated that the toxicity was dependant with the type of IL and its  
162 concentrationconcentration. The growth curves of *Clostridium* sp. could be characterized with

163 distinct rapid exponential and extended stationary phases. *Clostridium* sp. grew rapidly in the  
164 first 24 h by fermenting the glucose. This rapid exponential growth has caused a significant drop  
165 in the medium pH to ~2.8 (Fig. 1), which has lowered the subsequent growth of *Clostridium* sp.  
166 The growth of *Clostridium* sp. was higher by almost 40% in mineral salts medium in the  
167 presence of lower concentrations of [EMIM][Ac]. Delay and complete inhibition in the growth  
168 of *Clostridium* was observed in the presence of moderate and higher concentrations of  
169 [EMIM][Ac], respectively. The growth of *Clostridium* sp. was not significantly altered in the  
170 presence of [EMIM][DEP] up to 2.5 g L<sup>-1</sup>. However, the final growth was decreased in the range  
171 of 9 - 60% in the presence of higher concentrations of [EMIM][DEP]. The growth was not  
172 significantly altered by [EMIM][DMP] up to 4 g L<sup>-1</sup>. The growth of *Clostridium* sp. was either  
173 retarded or completely inhibited at higher concentrations of [MMIM][DMP]. Overall, ionic  
174 liquids used in the present study did not cause inhibition in the growth and fermentative  
175 metabolism of *Clostridium* sp. up to 2.5 g L<sup>-1</sup> (Fig. 1, Figs. SM-1 to SM-2). The toxicity of ionic  
176 liquids was evident in either delaying the growth or causing complete growth inhibition, which is  
177 seen only at higher concentrations above >2.5 g L<sup>-1</sup>. Interestingly, [EMIM][Ac] exhibited  
178 hormesis, thereby increasing the growth of *Clostridium* sp. at lower concentrations ranging from  
179 0.5 to 2.5 g L<sup>-1</sup> (Fig. 1). At higher concentrations (>2.5 g L<sup>-1</sup>) of [EMIM][Ac], the growth of  
180 *Clostridium* sp. was inhibited significantly. The growth of *Clostridium* sp. was completely  
181 inhibited at 7.5 g L<sup>-1</sup> and higher concentrations of [EMIM][Ac]. Whereas the other two ionic  
182 liquids, [EMIM][DEP] and [MMIM][DMP] caused a concentration dependent inhibition in the  
183 growth (Fig. SM-1) and fermentative metabolism (Figs. SM-2 and SM-3) of *Clostridium* sp.

### 184 3.2 Hormetic effect of [EMIM][Ac]

185 In the presence of [EMIM][Ac], the pH of mineral salts medium was always found to be  
186 higher than that of control (Figs. 1 and 2). The pH of MS medium remained at 4 or higher in the  
187 presence of ionic liquid that facilitated growth. *Clostridium* sp. growth was almost 40% higher in  
188 mineral salts medium supplemented with 0.5 g L<sup>-1</sup> of [EMIM][Ac] as compared to the growth  
189 observed in mineral salts medium without ionic liquid (Fig. 2). Enhanced growth of *Clostridium*  
190 sp. was corroborated with higher glucose utilization (Fig. SM-3) and total gas production (Fig.  
191 SM-2).

192 The hormetic effect of [EMIM][Ac] was even observed on growth of a Gram negative  
193 bacterium *P. putida*. In fact, the enhancement in the growth of *P. putida* was much higher in  
194 mineral salts medium supplemented with [EMIM][Ac] (Fig. 3). The growth of *P. putida* was  
195 almost 400% higher in mineral salts medium supplemented with 0.5 g L<sup>-1</sup> of [EMIM][Ac]. The  
196 pH of the mineral salts medium decreased to ~3.5 within 40 h of inoculation and remained stable  
197 thereafter. In the presence of ionic liquid, pH of the medium was maintained above 6.0 until 40 h  
198 and subsequently decreased to ~3.5. Based on the data, it was hypothesized that the hormetic  
199 effect of [EMIM][Ac] was mediated via medium pH regulation. This was verified by  
200 determining the growth of *Clostridium* sp. and *P. putida* in phosphate buffered mineral salts  
201 medium (PMS) with and without 0.5 g L<sup>-1</sup> [EMIM][Ac]. The growth of *Clostridium* sp. or *P.*  
202 *putida* was much higher in PMS medium compared to the growth observed in MS medium. The  
203 higher growth was possible because the pH of PMS medium maintained at ~6.5 throughout  
204 growth phase. However, the growth of *Clostridium* sp. and *P. putida* were not enhanced by the  
205 addition of [EMIM][Ac] to PMS medium (Figs. 4 and 5). These results were in agreement with  
206 earlier findings that the stimulatory effect of [EMIM][Ac] on *Clostridium* sp. growth and  
207 fermentative metabolism could be simulated by the addition of bicarbonate to MS medium

208 (Nancharaiah and Francis, 2011). Moreover, [EMIM][Ac] did not show hormesis on *P. putida*  
209 grown either in tryptic soy broth or in MS medium supplemented with a non-fermentable carbon  
210 source such as acetate (data not shown).

211 The toxicity of ionic liquids is associated with the type of cation, anion or the alkyl chain  
212 length of cation substituent's (Nancharaiah and Francis, 2011; Ranke et al., 2004; Nancharaiah et  
213 al., 2012; Wang et al., 2011). Certain ionic liquids, particularly those with short alkyl side chains  
214 have shown hormesis on bacteria (Ge et al., 2010; Nancharaiah and Francis 2011; Wang et al.,  
215 2011; Zhang et al., 2013a; Zhang et al., 2013b;), microalgae (Cho et al., 2007; Cho et al., 2008),  
216 IPC-81 leukemia cells (Ranke et al., 2004) and HeLa cells (Stepnowski et al., 2004) . The  
217 luminescence of *Vibrio qinghaiensis* sp. –Q67 was induced remarkably by 1-ethyl-3-  
218 methylimidazolium tetrafluoroborate (Wang et al., 2011). Ge et al. (2010) have predicted  
219 hormetic effects of ionic liquid mixtures on luciferase activity using concentration addition  
220 model. The mechanistic aspects of hormesis exhibited by ionic liquids are largely unknown.  
221 Dipeolu et al., (2009) hypothesized that N, N-dimethylethanolammonium acetate increased the  
222 growth rate of *Clostridium sporogenes* by either increasing the bioavailability of nutrients or by  
223 biodegradation of ionic liquid itself (Dipeolu et al., 2009). Based on the data obtained in this  
224 study, it can be concluded that the hormesis of [EMIM][Ac] on bacterial growth was is  
225 dependent on the medium buffering capacity and the type of fermentable and/or non-fermentable  
226 nature of the substrate. Bacterial growth was enhanced [EMIM][Ac] only in a poorly buffered  
227 medium that contained a fermentable substrate as the carbon source. It is apparent that the  
228 hormetic effect of [EMIM][Ac] on *Clostridium* sp. or *P. putida* in poorly buffered medium  
229 supplemented with glucose as carbon source is mostly associated with buffering action of IL in  
230 particular acetate anion group.

## 231 4. Conclusions

232 Among the three water miscible ionic liquids, 1-ethyl-3-methylimidazolium acetate, 1-ethyl-3-  
233 methylimidazolium diethyl phosphate and 1-methyl-3-methylimidazolium dimethylphosphate,  
234 tested only [EMIM][Ac] showed remarkable hormesis on *Clostridium* sp. and *Pseudomonas*  
235 *putida* growth. The growth was stimulated at up to 2.5 g L<sup>-1</sup> and inhibited at >2.5 g L<sup>-1</sup> of  
236 [EMIM][Ac]. The growth of *Clostridium* sp. and *Pseudomonas putida* increased by 0.4 and 4-  
237 times, respectively in mineral salts medium supplemented with 0.5 g L<sup>-1</sup> of [EMIM][Ac]. The  
238 enhancement in bacterial growth due to [EMIM][Ac] was not evident in tryptic soy broth,  
239 phosphate buffered mineral salts medium, or mineral salts medium that contained a non-  
240 fermentable substrate. It is evident that the bacterial growth was enhanced only in poorly  
241 buffered medium that contained a fermentable substrate as carbon source. Therefore, the  
242 hormesis of [EMIM][Ac] could be alleviated by growing bacterial cultures in a phosphate  
243 buffered mineral salts medium or mineral salts medium supplemented with a non-fermentable  
244 substrate. **Acknowledgments**

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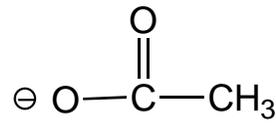
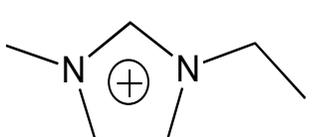
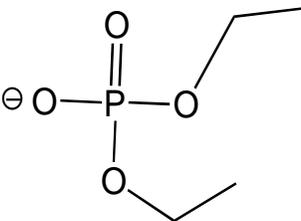
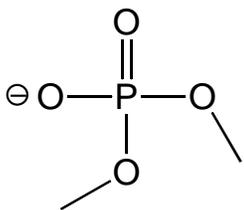
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329 of 1-alkyl-3-methylimidazolium chloride and their mixtures on *Vibrio qinghaiensis* sp.-  
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331

332

333 Table 1. The structure of ionic liquids used in the present study.

334	IL name	Abbreviation	Structure	
335	1-ethyl-3-methylimidazolium acetate	[EMIM][AC]		
336				
337	1-ethyl-3-methylimidazolium diethylphosphate	[EMIM][DEP]		
	1,3-dimethylimidazolium dimethylphosphate	[MMIM][DMP]		

338

### 339 Figure legends

340 Figure 1. Growth (A) and medium pH (B) of *Clostridium* sp. BC1 in mineral salts medium  
341 supplemented with different concentrations of 1-ethyl-3-methylimidazolium acetate  
342 ([EMIM][Ac]). Growth measured at 96 h of inoculation is shown. Symbols and error bars  
343 represent averages and standard deviations from triplicate experiments.

344 Figure 2. *Clostridium* sp. BC1 growth (A) and medium pH (B) in mineral salts (MS) medium  
345 (control), and MS medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols and error bars  
346 represent averages and standard deviations from triplicate experiments.

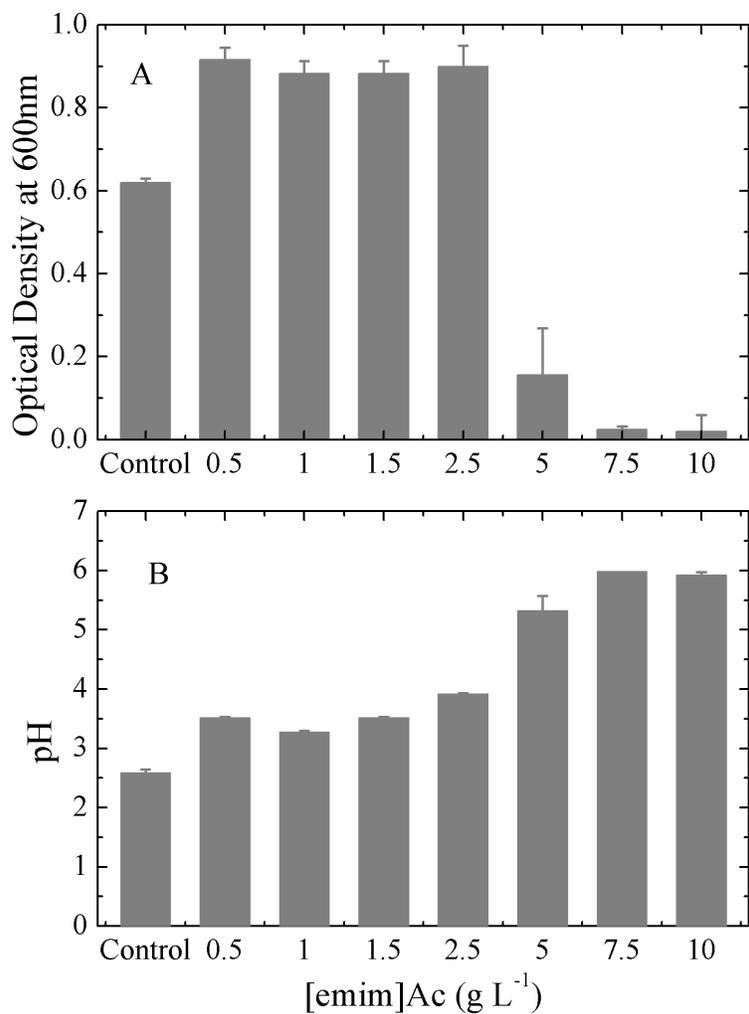
347 Figure 3. *Clostridium* sp. BC1 growth (A) and medium pH (B) in phosphate buffered mineral  
348 salts (PMS) medium (control), and PMS medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols  
349 and error bars represent averages and standard deviations from triplicate experiments.

350 Figure 45. *P. putida* growth (A) and medium pH (B) in mineral salts (MS) medium (control), MS  
351 medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols and error bars represent averages and  
352 standard deviations from triplicate experiments.

353 Figure 5. *P. putida* growth (A) and medium pH (B) in phosphate-buffered mineral salts (PMS)  
354 medium (control), PMS medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols and error bars  
355 represent averages and standard deviations from triplicate experiments.

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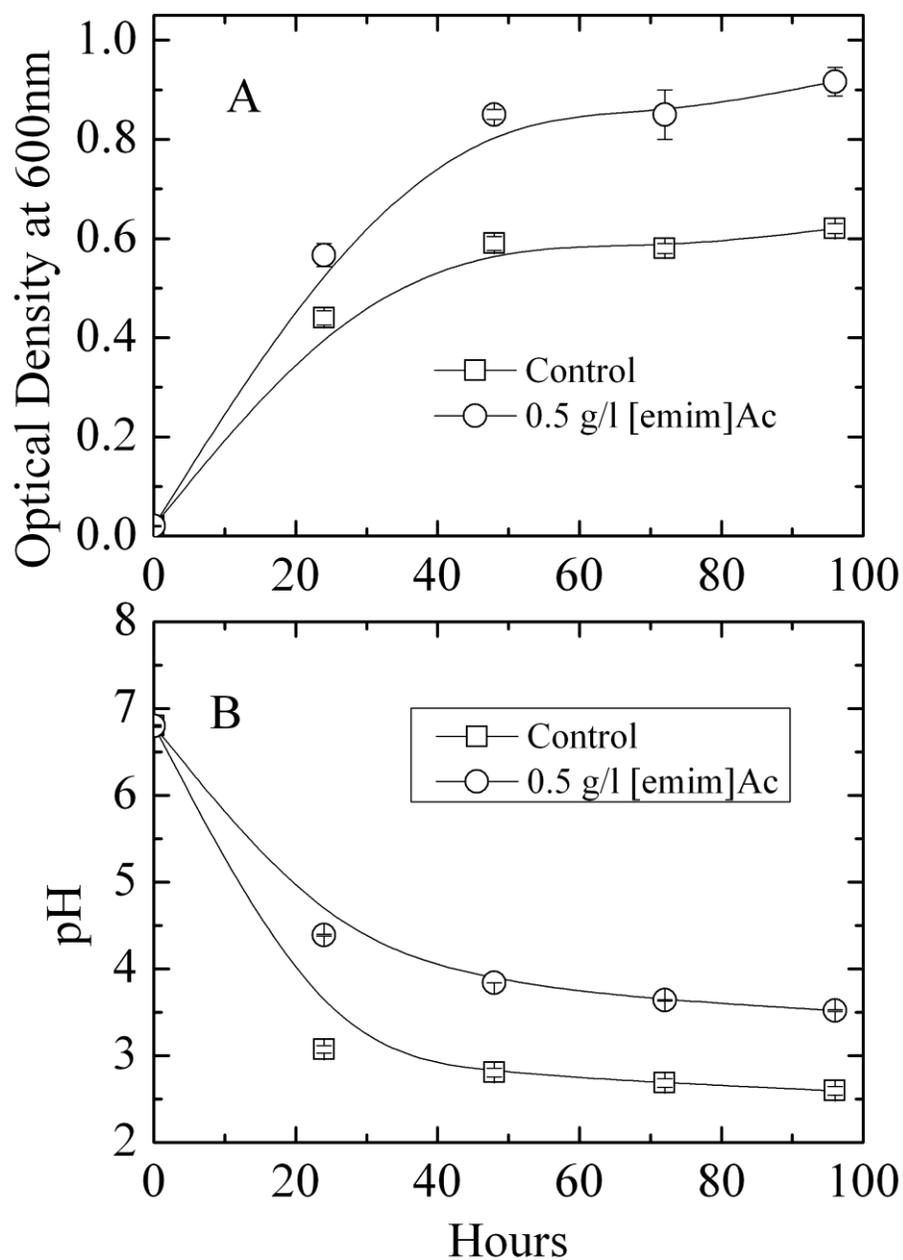
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359 Figure 1. Growth (A) and medium pH (B) of *Clostridium* sp. BC1 in mineral salts medium  
 360 supplemented with different concentrations of 1-ethyl-3-methylimidazolium acetate  
 361 ([EMIM][Ac]). Growth was measured at 96 h of inoculation is shown. Symbols and error bars  
 362 represent averages and standard deviations from triplicate experiments.

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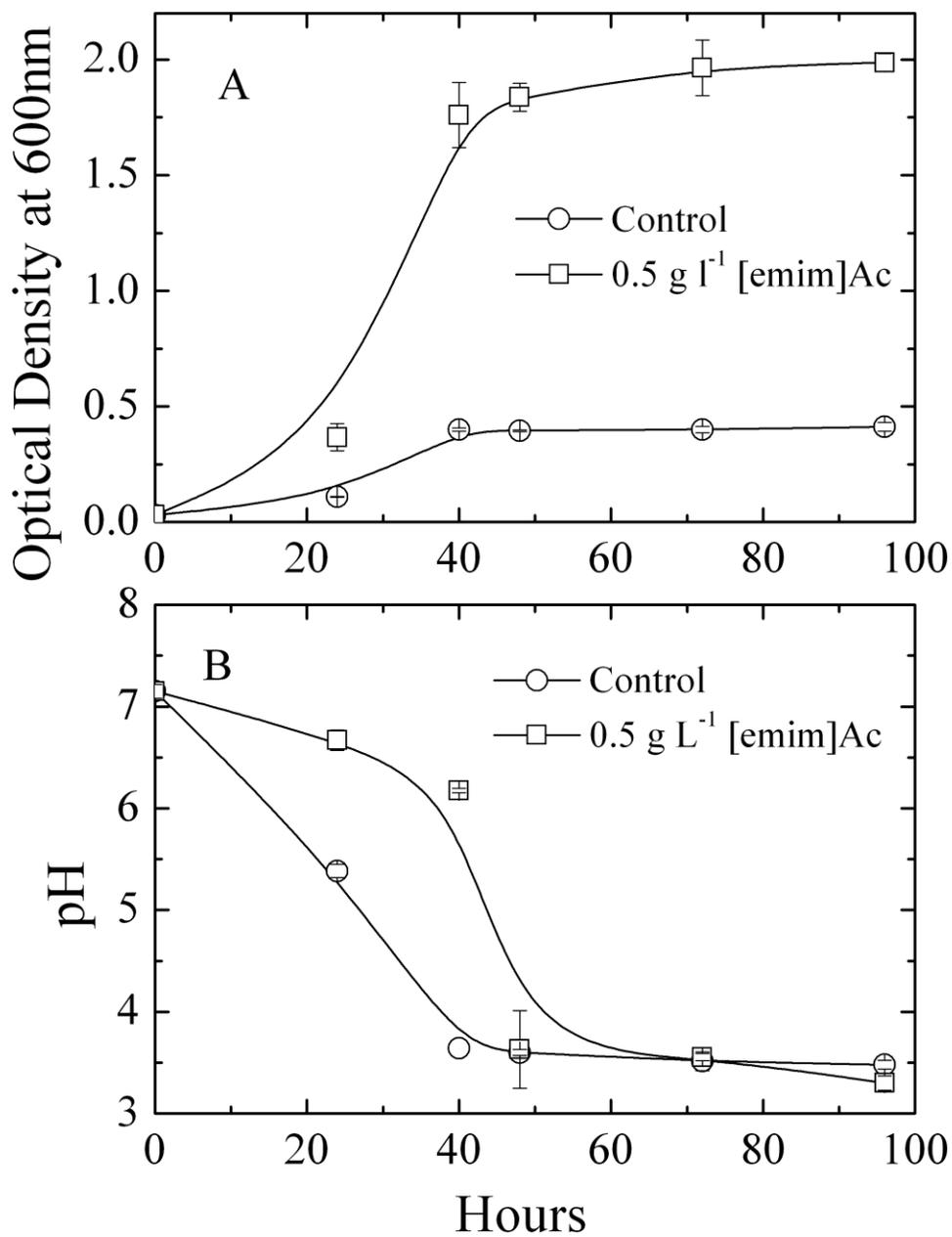
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367 Figure 2. *Clostridium sp.* BC1 growth (A) and medium pH (B) in mineral salts (MS) medium  
368 (control), and MS medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols and error bars  
369 represent averages and standard deviations from triplicate experiments.

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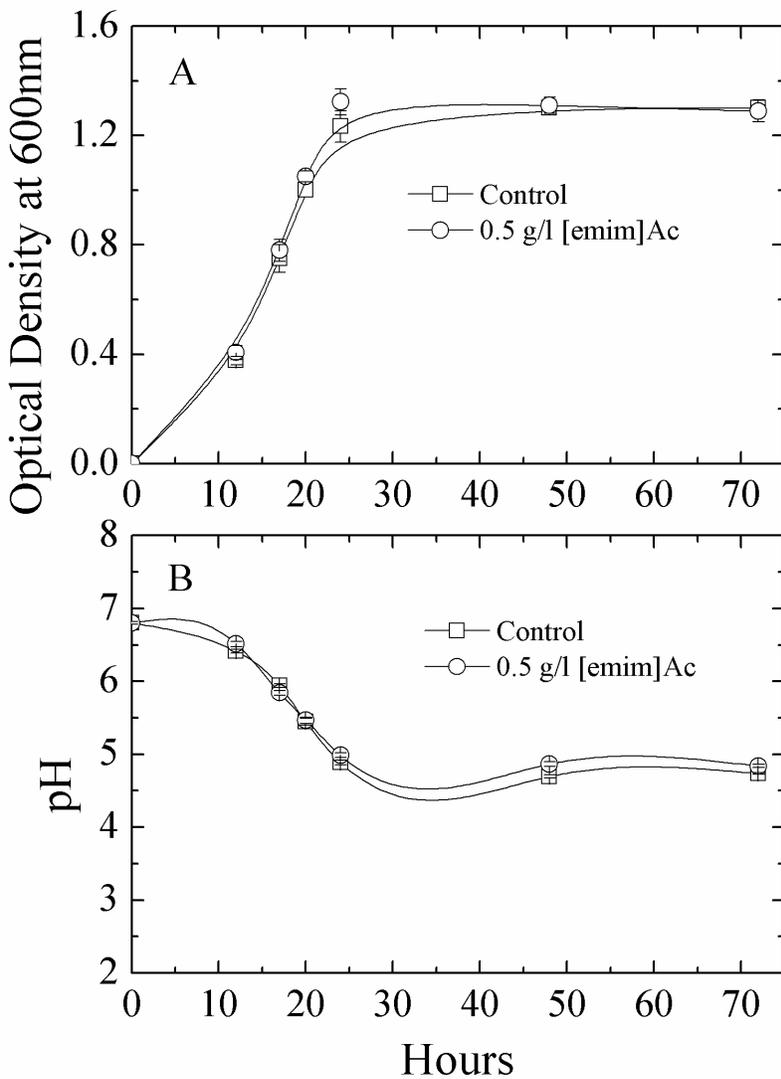


372

373 Figure 3. *Clostridium sp.* BC1 growth (A) and medium pH (B) in phosphate buffered mineral  
 374 salts (PMS) medium (control), and PMS medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols  
 375 and error bars represent averages and standard deviations from triplicate experiments.

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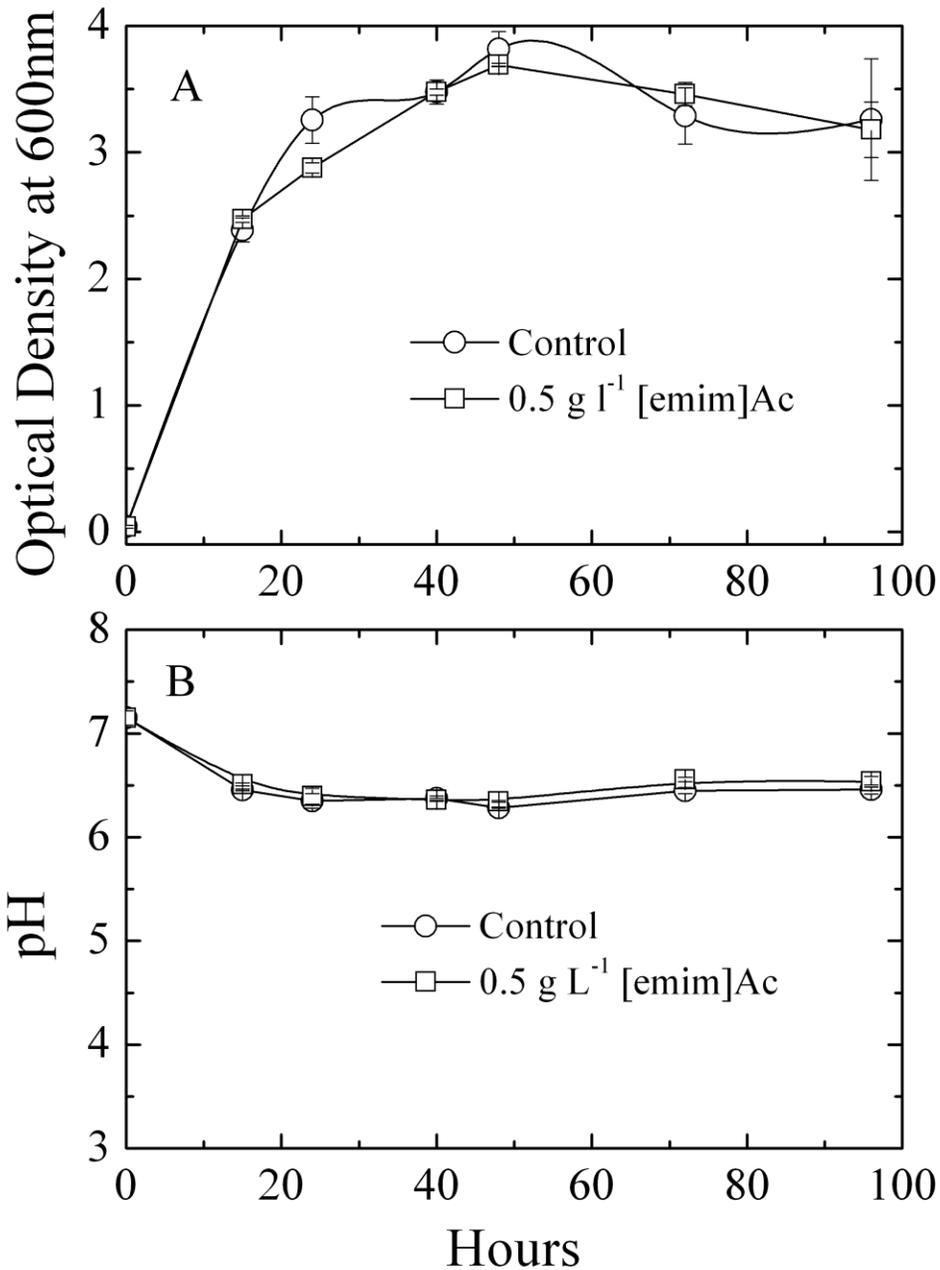
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380 Figure 4. *P. putida* growth (A) and medium pH (B) in mineral salts (MS) medium (control), MS  
 381 medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols and error bars represent averages and  
 382 standard deviations from triplicate experiments.

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386 Figure 5. *P. putida* growth (A) and medium pH (B) in phosphate-buffered mineral salts (PMS)  
 387 medium (control), PMS medium amended with 0.5 g L<sup>-1</sup> [EMIM][Ac]. Symbols and error bars  
 388 represent averages and standard deviations from triplicate experiments.

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## Supplementary Information

392 Hormetic effect of ionic liquid 1-ethyl-3-methylimidazolium acetate on bacteria

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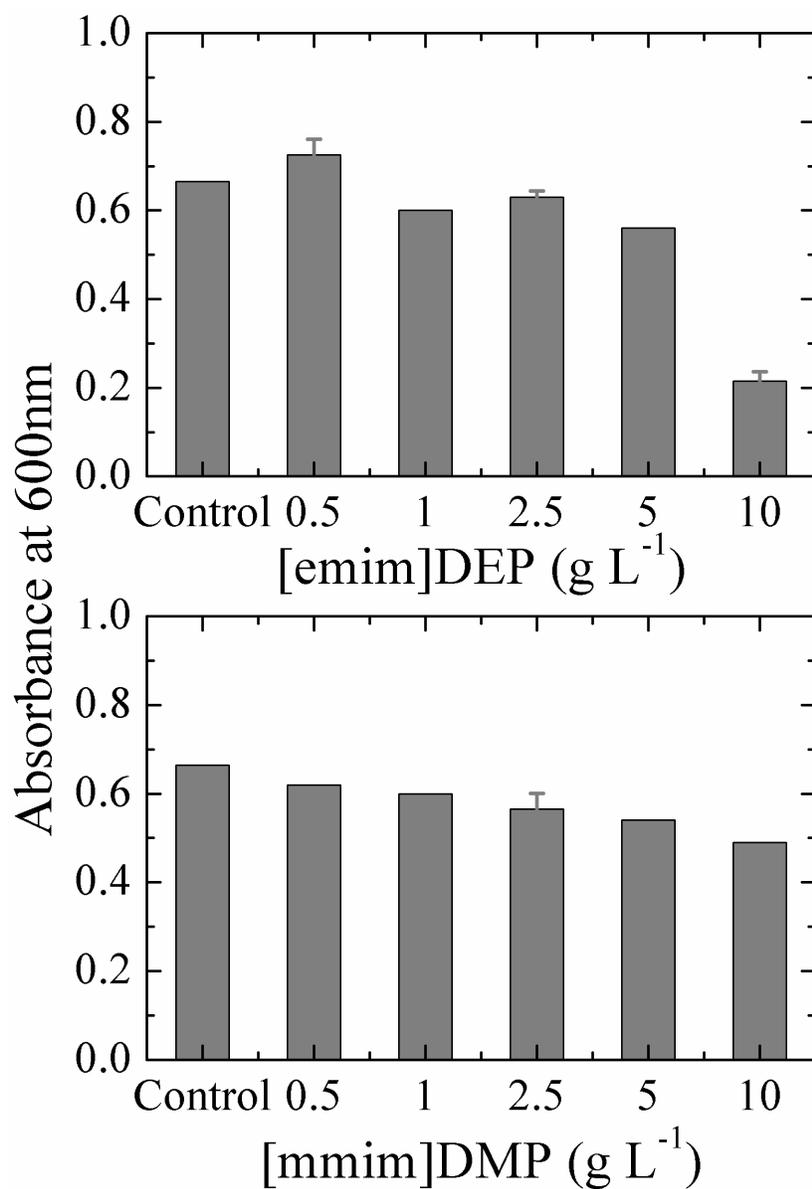
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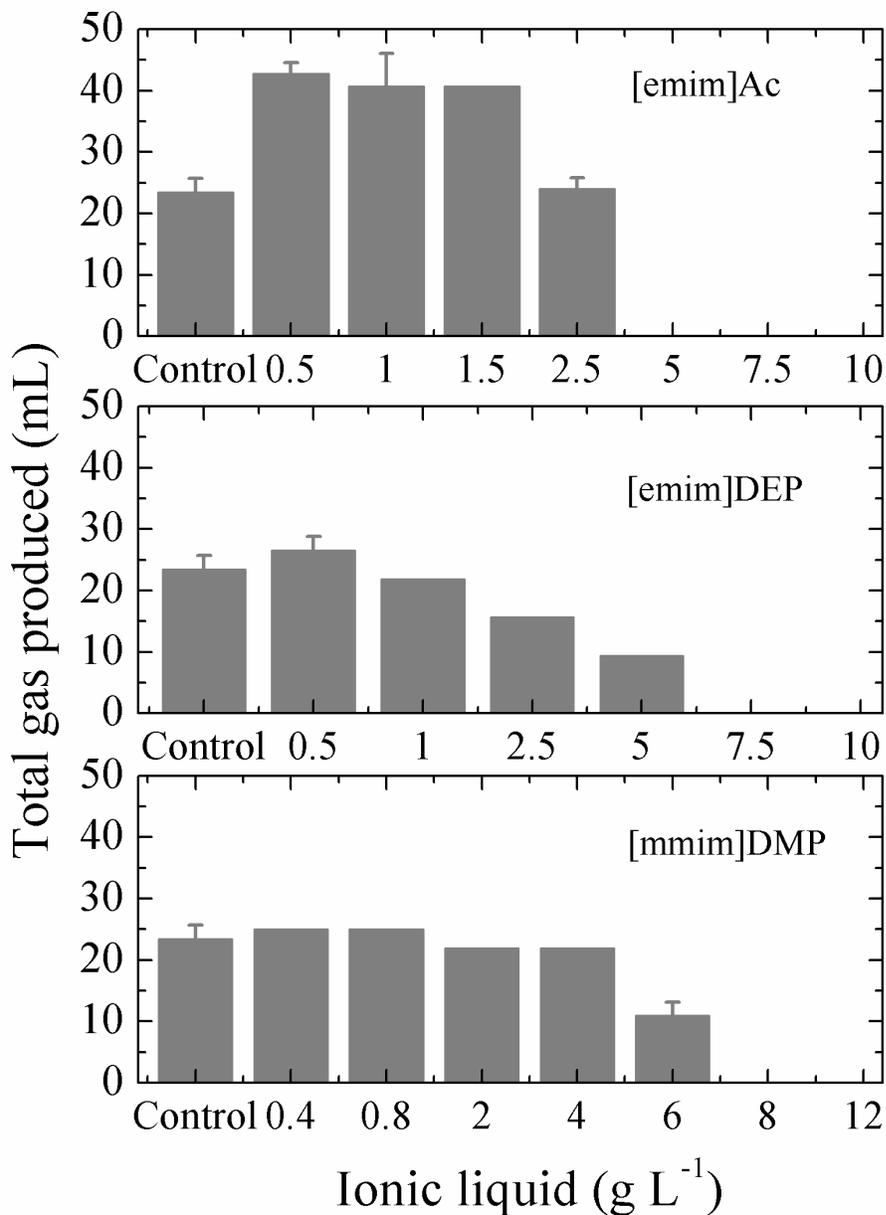
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405 Figure SM-1. Growth of *Clostridium* sp. BC1 in mineral salts medium supplemented with  
 406 different concentrations of ionic liquids, 1-ethyl-3-methylimidazolium diethylphosphate  
 407 ([emim]DEP) and 1-methyl-3-methylimidazolium dimethylphosphate ([mmim]DMP). Growth  
 408 was measured at 96 h of inoculation is shown. Symbols and error bars represent averages and  
 409 standard deviations from triplicate experiments.

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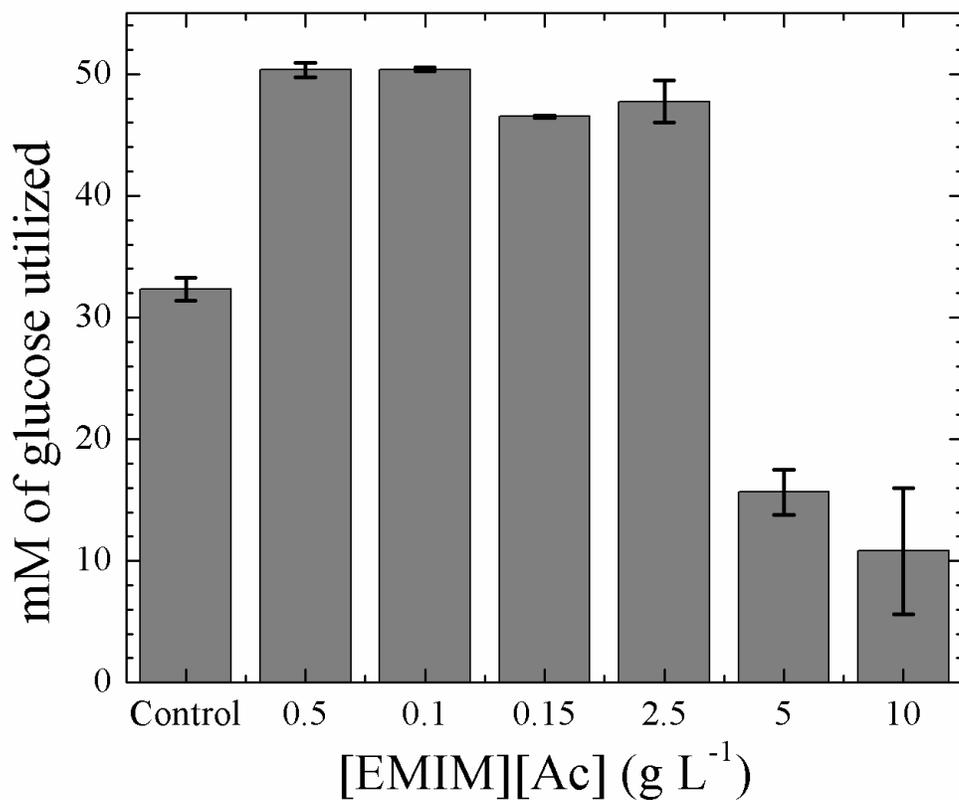
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 413 Figure SM-2. Total gas produced by *Clostridium* sp. BC1 in mineral salts medium supplemented  
 414 with different concentrations of ionic liquids, 1-ethyl-3-methylimidazolium acetate([emim]Ac),  
 415 1-ethyl-3-methylimidazolium diethylphosphate ([emim]DEP) and 1-methyl-3-  
 416 methylimidazolium dimethylphosphate ([mmim]DMP). Total gas was measured at 96 h of  
 417 inoculation. Symbols and error bars represent averages and standard deviations from triplicate  
 418 experiments.

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422 Figure SM-3. Total amount of glucose utilised by *Clostridium* sp. in mineral salts medium  
 423 supplemented with different concentrations of 1-ethyl-3-methylimidazolium acetate  
 424 ([EMIM][Ac]). Total glucose utilized was measured at 96 h of inoculation is shown. Data  
 425 represent average of triplicate experiments and error bars represent standard deviation.

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