

1 **Electricity generation and struvite recovery from human urine using microbial fuel cells**

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1 **ABSTRACT**

2

3 BACKGROUND: Urine is an abundant waste product which requires energy intensive treatment
4 processes in modern wastewater treatment plants. However urine can be utilised as fertiliser in the
5 form of struvite. Microbial fuel cells (MFCs) are a promising technology for treating waste whilst
6 producing electricity. Combining these two approaches, a 3-stage MFC/struvite extraction process
7 system was developed and its feasibility tested in order to maximise urine utilisation in terms of
8 electricity generation and struvite recovery.

9 RESULTS: At the first stage, whilst generating electrical energy, MFCs accelerated urea hydrolysis,
10 which was beneficial for the struvite precipitation process in the following stage. After collecting
11 struvite by adding magnesium into the initial effluent, the supernatant was used at the final stage for
12 additional power and more efficient COD reduction. In total, 82 % of $\text{PO}_4^{3-}\text{-P}$ and 20 % of COD of
13 undiluted human urine were removed by the 3-stage system. Also 14.32 W/m^3 (absolute power: 358
14 μW) and 11.76 W/m^3 (absolute power: $294 \mu\text{W}$) of power was produced from the 1st and 3rd stages of
15 the system, respectively, during operation.

16 CONCLUSION: This work shows how MFCs and struvite precipitation could be integrated for both
17 energy generation and resource recovery from urine, leading to a truly sustainable energy future.

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19 Keywords - microbial fuel cells, struvite, source-separated urine, resource recovery

20 INTRODUCTION

21

22 The average individual human being can produce approximately 2.5 litres of urine per day, and
23 taking into account that cattle can produce up to three times as much, gives an estimated global annual
24 urine production of ~6.4 trillion litres ¹. Urine is normally just flushed down the toilet to be treated in
25 wastewater treatment plants (WWTPs) before returning to natural water bodies. Although urine
26 consists of less than 1 % of municipal wastewater in volume, it contains about 50 % of total
27 phosphorus (P) and 75 % of total nitrogen (N) in municipal wastewater ². Phosphorus and nitrogen are
28 two main elements that require removal from the wastewater since an accumulation of these can lead
29 to eutrophication. The annual social and ecological damage cost of freshwater eutrophication was
30 estimated as £75 – 114M in England and Wales only ³. Hence most modern WWTPs adapt energy
31 intensive treatment processes for treating both phosphorus and nitrogen, which are also very costly to
32 build and operate.

33 In the 1990s, various European groups started working on the concept of source-separated urine
34 for improving the sustainability of wastewater management ⁴. Source-separated urine can reduce the
35 operation cost of WWTPs and also contribute to better effluent quality of WWTPs by changing
36 wastewater composition ². In addition, nitrogen and phosphorus can be recovered and utilised from
37 source points since urine has a high concentration of these elements, and phosphorus recovery through
38 struvite precipitation has received increased attention. Struvite (magnesium ammonium phosphate,
39 $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) is usually formed in stale urine through the following chemical reaction and
40 recovering struvite from urine has two big attractive advantages.



42 First it contains both nitrogen and phosphorus, which renders the simultaneous removal of the
43 two compounds from the main stream of wastewater achievable. In addition to the aforementioned
44 reduction in operation cost and improvement in effluent quality, this could also reduce pipe blockage
45 occurrences, which are caused by struvite formation at WWTPs. Once struvite has formed and
46 blocked water pipes, these need to be cleaned or even replaced, and in many cases other elements of
47 WWTPs such as pumps, valves, centrifuges and aerators are also liable to fouling by struvite deposits

48 ^{5,6}. These ‘undesirable’ struvite deposits increase maintenance costs, and reduce the piping system
49 capacity of WWTPs. Second, struvite can be used as a slow-release fertiliser ⁷⁻⁹, which is not a
50 completely new concept since many communities have used or once used human excretion including
51 urine for growing crops before commercial fertilisers appeared.

52 Further to this well-known approach of utilising urine, recently it has been presented that
53 electrical energy can also be generated from urine with the use of the microbial fuel cell (MFC)
54 technology ^{1,10}. MFCs are transducers that convert chemical energy of feedstock into electricity
55 through the metabolic activity of microorganisms. Whilst producing electricity, MFCs can also treat
56 the feedstock by consuming nutrients through microbial metabolism. For this reason, a wide range of
57 organic compounds have been tested for MFC power production and urine is one of the attractive
58 options due to its abundance and natural properties such as neutral pH and high conductivity.

59 Only recently recovery of resources whilst treating waste using bioelectrochemical systems
60 (BES) including MFCs and microbial electrolysis cells (MECs) has begun to gain attention. Recent
61 progress and findings can be found in a comprehensive review¹¹. In the case of resource recovery
62 from urine, nitrogen recovery in the form of ammonium (both NH_3 and NH_4^+) from the cathode have
63 been attempted using MFCs and MECs^{12,13}. Zang et al.¹⁴ reported that phosphorus and nitrogen
64 recovery in the form of struvite from stale urine is compatible with MFC operation. However the
65 current study’s thesis is that more effort should be made for better understanding the processes and
66 implementing the systems especially when dealing with fresh urine such as a MFC system directly
67 connected to urinals.

68 In order to maximise urine utilisation in this work, a 3-stage MFC/struvite extraction process
69 system that generates electricity whilst collecting phosphorus and nitrogen in the form of struvite was
70 proposed and its feasibility was tested. With this system, electricity generation is maximised, thus
71 increasing the consumption of organic matter, and high concentrations of nitrogen and phosphorus are
72 recovered through the struvite precipitation process. Furthermore this can be easily integrated with
73 existing source-separated urinals. Therefore, the aim of this study was to combine the MFC electricity
74 production with struvite recovery and investigate whether the two processes can complement each

75 other. This work could contribute to a sustainable urine treatment process through recovery of
76 resources and generation of energy as well as treating urine from the point of source.

77

78 **EXPERIMENTAL**

79

80 **MFC design**

81 The MFCs consisted of 6.25 mL anode chambers and open-to-air cathodes as previously
82 described¹⁵. The anode compartments had inlets and outlets, which allowed the anolyte to fill up from
83 the bottom and then overflow from the top; the overflow anolyte fed into the downstream MFC. Plain
84 carbon fibre veil electrodes (PRF Composite Materials, UK) with 12 layers of 4.18 cm² (width: 2.2
85 cm, length: 1.9 cm) were used for the anodes. A cation exchange membrane (CMI-7000, Membrane
86 International, USA), 25 mm diameter, was sandwiched between the anode and cathode frames. The
87 cathode electrodes, which were identical for all MFCs, were made of hot-pressed activated carbon
88 onto carbon fibre veil and had a total macro surface area of 4.9 cm². Nickel-chromium (0.45 mm
89 thickness) wire was used for connection and current collection.

90

91 **Urine and inoculum**

92 Neat (untreated without dilution) human urine was used for this work since the final
93 MFC/struvite extraction process system is aimed to fit directly into urinals. Urine was donated from
94 male and female healthy individuals, on a normal diet and without any medical conditions, and pooled
95 together prior to use. Unless otherwise stated, urine was used on the day of donation.

96 The anodes were inoculated with activated sewage sludge supplied from the Wessex Water
97 Scientific Laboratory (Saltford, UK). Sludge was mixed with 0.5 % yeast extract and 1 % tryptone
98 (both Sigma-Aldrich) as an initial feedstock. Following the inoculation of the MFCs and the maturing
99 of the biofilm communities on the anodes for a week, neat human urine was provide as the sole
100 energy source (fuel).

101

102 **Proposed system operation**

103 For a more efficient use of urine with MFCs in terms of power production and nutrient recovery,
104 a system of MFCs which could be fitted into urinals was developed. This proposed system consisted
105 of two MFC groups and each group had four MFC units. In each group, four MFCs were cascaded
106 and had a single flow of substrate, which was provided continuously, using a 16-channel peristaltic
107 pump (205U, Watson Marlow, UK). The four MFCs were connected in a series/parallel configuration
108 (MFCs 1 & 4, and MFCs 2 & 3 were connected in parallel, and the two pairs were connected in series)
109 in order to produce sufficient power for demonstration of a practical application. An external load of
110 1K Ω was applied to both groups.

111 In the first stage, untreated urine was supplied to Stage 1 (see Fig.1) at a flow rate of 96 mL/hr,
112 which resulted in 16 minutes of hydraulic retention time (HRT) for all 4 MFCs. Once the effluent was
113 collected, magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, Fisher Scientific) was added in Stage 2 and
114 mixed using a magnetic stirrer at 100 rpm for 5 min. Following this, the mixing was stopped and the
115 solution was allowed to settle for 45 min before the supernatant was fed into the last stage (Stage 3) of
116 the treatment. The amount of magnesium addition was 1.2 times the phosphate presented in the initial
117 effluent in molar ratio ¹⁶. In the final stage, the MFCs treated the struvite-deprived effluent (2nd stage
118 effluent) at a flow rate of 42 mL/hr (HRT: 38 min). The final effluent was collected for analysis.

119 Figure 1 shows the whole system operation. All experiments were carried out in a temperature
120 controlled laboratory, with 22 ± 2 °C and repeated at least 3 times.

121

122 **Chemical & physical analysis**

123 The pH, redox potential (ORP) and conductivity of the suspension were measured using a
124 pH/ORP meter (pH 209, Hanna Instruments, UK) and conductivity meter (470 Cond Meter, Jenway,
125 UK). For measuring soluble elements such as COD, NH_4^+ -N and PO_4^{3-} -P, urine samples were filtered
126 through 0.45 μm filters (Millex, USA) and then analysed according to the standard methods ¹⁷.

127 Characterisation of the dried crystals from the struvite precipitation stage was performed by X-
128 ray diffraction (XRD) (D8 Advance Diffractometer, Bruker, UK) and the results were analysed using
129 the EVA software package (Bruker). The precipitates were filtered through 0.45 μm filters, and then

130 dried at 40 °C prior to analysis. Microscopic images of the precipitates were also taken by a digital
131 microscope (KH-7700, Hirox, Japan).

132

133 **Electrical measurement and power output calculations**

134 Power output of the MFCs was monitored in real time in volts (V) against time using a HP
135 Agilent multiplex logging system (34907A, HP, USA). Polarisation experiments were performed
136 periodically by connecting a decade variable resistor box (Centrad Boite A Decades De Resistances
137 DR07, ELC, France) between the anode and cathode electrodes and varying the external resistance
138 from 30 k Ω to 10 Ω in time intervals of 5 minutes after the MFCs had established a steady-state open
139 circuit voltage. The current (I) in amperes (A) was determined using Ohm's law. Power density (P_D)
140 and current density (I_D) were calculated according to the anodic chamber volume; $P_D = P/v$, $I_D = I/\alpha$,
141 where α is the anode chamber volume in cubic metres (m³).

142

143 **RESULTS AND DISCUSSION**

144

145 **Effect of struvite collection on the MFC performance**

146 Before operating the 3-stage MFC/struvite extraction process system, the effect of struvite
147 collection on the MFC performance was investigated in two steps. First, in order to observe the effect
148 of naturally occurring struvite on MFC performance, untreated neat urine as a fuel was provided to
149 both MFC groups continuously at a flow rate of 21.2 mL/h (HRT: 18 min for individual MFCs) for 5
150 days. During this period, the MFCs were operated individually without inter-connection and the urine
151 was stored at room temperature in an open container. Power performance and struvite precipitation
152 were monitored and shown in Fig. 2a. In these 5 days, urine pH rose naturally due to urea hydrolysis,
153 which resulted in increased precipitation. Furthermore, soluble phosphate concentration decreased
154 accordingly since part of it went into the precipitate. Power output from individual MFCs remained
155 relatively stable suggesting that it was not significantly affected by the change of pH or phosphorus
156 concentration in the feedstock. Hence naturally occurring struvite precipitation did not seem to have
157 an effect on MFC performance as long as the same untreated urine was provided.

158 For the second step of this work, rapid struvite precipitation from fresh urine was pursued by
159 manually changing the pH and adding $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. Sodium hydroxide (NaOH) pellets were used for
160 increasing the pH to 11 and 0.5 M H_2SO_4 solution was used for bringing the pH back to its initial
161 value after removing the precipitate. The two MFC groups were fed both with untreated freshly
162 collected urine as well as with struvite-deprived urine at a flow rate of 21.2 mL/h. Using this method,
163 20 % of $\text{NH}_4^+ \text{-N}$ (from 363 mg/L to 290 mg/L) and 82 % of $\text{PO}_4^{3-} \text{-P}$ (from 202 mg/L to 36 mg/L)
164 were recovered in the form of precipitate (Table 1). ORP and conductivity increased whilst the pH
165 decreased slightly. In both cases of neat- and struvite-removed urine, the change in pH, ORP and
166 conductivity between feedstocks and effluents, demonstrated a similar pattern. The degree of change
167 was proportional to the initial value of the influent. It was also observed that the power output was
168 higher from the struvite-removed urine than from the neat urine. This is thought to be the result of
169 conductivity increase of the anolyte after adding magnesium - $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (molar ratio; $\text{Mg}^{2+} : \text{PO}_4^{3-}$
170 $\text{-P} = 1.2 : 1$).

171 Thus from the two tests, it was suggested that a decrease of phosphorus concentration in urine
172 through struvite precipitation did not significantly affect the MFC power performance. This finding
173 can be useful when designing a MFC system that can be connected to urinals. For MFC operation,
174 reducing any insoluble matter in the feedstock solution is preferred for minimising blockages of the
175 anode or membrane. Since the amount of precipitate in urine increases with time, precipitate removal
176 or fresh urine (in both cases of undiluted and diluted urine) is required before adding into MFCs.
177 Relatively high ammonium concentration of urine have been reported not to hinder the MFC power
178 performance^{12,14}. However no published information was found on the inter-relationship between
179 MFC operation and high phosphorus concentration of urine. These findings demonstrate that it is
180 possible to remove the precipitate before the MFC system without negatively affecting the power
181 output.

182 Struvite removal with Mg addition has the added advantage of higher phosphorus recovery
183 rates in shorter periods of time. Approximately 38 % of $\text{PO}_4^{3-} \text{-P}$ was removed in 5 days of storage
184 (Fig. 2a) whereas 82 % of $\text{PO}_4^{3-} \text{-P}$ was recovered through the Mg addition process, which required a
185 period of less than 1 hour. In terms of struvite recovery, struvite precipitation using additional

186 magnesium is a very well established and efficient way to remove phosphorus from urine. A previous
187 study¹⁸ reporting struvite recovery from the cathode electrode observed deterioration of electricity
188 generating performance due to struvite deposition on the cathode and membrane which impedes the
189 mass transfer of ions and oxygen. Therefore in the case of utilising urine which has high concentration
190 of phosphorus, phosphorus recovery through struvite precipitation process seems more convenient
191 and efficient way to pursue.

192

193 **Urea hydrolysis acceleration by microbial activity of MFCs**

194 Figure 2b shows the general pattern of pH change occurring in undiluted urine that was stored
195 on a laboratory bench in ambient temperature. The initial pH of 6.4 did not significantly change for
196 approximately the first 10 hours (less than 0.2 pH units), but then increased relatively rapidly for the
197 next 20 hours. After 30 hours of storage, the pH stayed at approximately 9.2, which is consistent with
198 previous reports¹⁹.

199 As shown in Table 1, the pH of untreated urine however, rose to the same level after running
200 through a group of 4 MFCs in only 72 min (HRT of the whole group). This clearly showed that MFCs
201 accelerated urea hydrolysis, which was not attributed solely to the electricity generating activity of
202 microorganisms in the MFCs, since a similar degree of pH increase was also recorded for the same
203 MFCs without an external load (open circuit condition). Therefore the acceleration effect could be the
204 result of urine being exposed to a higher population of microorganisms for a given time.

205 The pH of urine naturally increases as a result of urea hydrolysis by ubiquitous microorganisms
206²⁰. It is very likely that electricity generating microorganisms existing in MFCs could also hydrolyse
207 urea. Further investigation is needed to confirm if they are the same species to the urease-positive
208 bacteria that hydrolyse urea in urine and if their activity is affected. Nevertheless it seems that
209 introducing MFCs to fresh urine shortens the time for urea hydrolysis thus increases the pH of urine
210 rapidly.

211 Previous studies^{8,21,22} proposed an optimum pH range of 8 - 9 for struvite precipitation. Since
212 the effluent pH is in this range, no additional NaOH is necessary for pH adjustment. This could be an
213 added advantage when struvite collection is expected from both cases of diluted or undiluted urine.

214 Most studies on struvite recovery from urine used either stored urine for allowing the pH of urine to
215 increase naturally or fresh urine but increasing the pH by adding alkalis like NaOH. In both cases,
216 large storage capacity or high cost for pH increase is required, and it seems that both operational costs
217 can be significantly reduced, by accelerating the pH increase through MFC systems.

218

219 **Urine treatment and nutrient recovery**

220 Based on the above findings, a 3-stage MFC/struvite extraction process system was designed.
221 The first stage, running the 1st group of MFCs, was for generating power and increasing the pH for
222 struvite removal. The second stage centred on the struvite precipitation process by adding Mg. In the
223 third and final stage, the 2nd group of MFCs was fed with the supernatant from Stage 2 at a slower
224 flow rate in order to further reduce COD and generate electricity. Change of pH, conductivity, NH_4^+ -
225 N, PO_4^{3-} -P, COD and power output from each stage is shown in Figure 3.

226 After the first stage of treatment, the pH of urine increased to 9.37 and conductivity rose by
227 11.8 mS/cm, which implied a considerable amount of urea hydrolysed within 15.6 minutes of HRT.
228 At the same time, NH_4^+ -N concentration increased almost 7 times whilst PO_4^{3-} -P concentration
229 decreased 26 %. Again the increase in NH_4^+ -N concentration was a result of urea hydrolysis
230 accelerated by microbial activity in MFCs and it led to pH increase. The PO_4^{3-} -P concentration
231 reduction could probably be explained by solubility decline of phosphate due to pH increase, and
232 bacterial uptake for growth. Struvite solubility depending on pH value is well described in the
233 literature ²³; generally solubility decreases with increasing pH and this results in struvite precipitation
234 within the system. In addition, phosphorus is one of the essential elements for microbial growth thus it
235 could be absorbed by microorganisms in MFCs. However this phosphate behaviour was not consistent
236 in repeated tests as also has been the case in previous reports ^{24,25}. The biological phosphorus removal
237 process adapted in modern WWTPs uses specific microbial species, so called, polyphosphate
238 accumulating organisms (PAOs) under certain environmental conditions, switched from anaerobic to
239 aerobic ^{26,27}.

240 Once neat urine was treated in the first stage, magnesium was added without any pH adjustment.
241 This addition brought about a decrease in pH, NH_4^+ -N, PO_4^{3-} -P and COD but an increase in

242 conductivity. Since the PO_4^{3-} -P concentration was much lower than NH_4^+ -N concentration in the 1st
243 stage effluent (thus a limiting factor for precipitation), the recovery rate of PO_4^{3-} -P was far higher
244 than the recovery rate of NH_4^+ -N. Approximately 7 % of NH_4^+ -N and 78 % of PO_4^{3-} -P were
245 recovered by collecting the precipitate.

246 X-ray diffraction (XRD) analysis showed this precipitate had a similar pattern to struvite (Fig.
247 4). Also the microscopic image showed that the precipitate consisted of mainly transparent rod-like
248 crystals, which is a typical orthorhombic structure of struvite²¹. However the purity of struvite needs
249 to be investigated further. In a process like this, besides struvite, other minerals such as
250 montgomeryite ($\text{Ca}_4\text{Al}_5(\text{PO}_4)_6(\text{OH})_5 \cdot 11\text{H}_2\text{O}$, $\text{Ca}_4\text{MgAl}_4(\text{PO}_4)_6(\text{OH})_4 \cdot 12\text{H}_2\text{O}$), epsomite
251 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and brucite ($\text{Mg}(\text{OH})_2$) may also be formed depending on the amounts of other
252 divalent or trivalent metal cations available²⁸.

253 During the final stage of treatment, pH and conductivity slightly increased again due to the
254 hydrolysis of urea that remained from the previous treatment. Consequently NH_4^+ -N concentration
255 also increased which was in contrast to previous studies reporting NH_4^+ -N reduction in the anodic
256 chamber of MFCs^{12,14}. This is thought to be due to different conditions of substrate and MFC
257 operation (fresh neat urine without dilution and continuous feeding mode in this study). Also PO_4^{3-} -P
258 concentration increased slightly this time. However, the phosphate behaviour at the final stage was -
259 as in the first stage - inconsistent. The inconsistent phosphorus behaviour might be attributed to the
260 dynamic physical-chemical reactions and equilibrium conditions of the system as well as bacterial
261 activity. In the case of COD, the highest removal of 15.6 % was achieved at this stage. Longer HRT
262 of the stage was thought to be one of the reasons for the higher COD removal. After the 3-stage
263 system treatment, COD was still relatively high (above 5000 mg/L), which could make further
264 utilisation of urine as a MFC substrate possible.

265 In total, 82 % of PO_4^{3-} -P and 20 % of COD were removed by the 3-stage system.

266

267 **Electricity generation**

268 During the first treatment, 358 μW of power ($P_D = 14.32 \text{ W/m}^3$) was generated by the 1st MFC
269 group. The 2nd MFC group produced 294 μW ($P_D = 11.76 \text{ W/m}^3$) from the 2nd stage effluent. However

270 it cannot be concluded that untreated urine always gives higher power than the 2nd stage effluent. In
271 order to evaluate urine as a fuel source at different treatment stages, several factors need to be
272 considered. Firstly, any differences in the microbial community developed at each stage need to be
273 taken into account. In each group, microorganisms had different environmental conditions such as pH,
274 conductivity and salts concentration of the feedstock thus there could have been changes in the
275 abundance and diversity of the complex microbial community in the system. This may have caused
276 differences in the performance between the two MFC groups. In this case, the 1st group was better
277 performing than the 2nd group as shown in Figure 5. Therefore the 1st group was expected to produce
278 higher power than the 2nd group if the same urine was supplied. When the same untreated urine was
279 provided to the 2nd group, the output was 291 μW ($P_D = 11.64 \text{ W/m}^3$) which was almost identical to
280 the output generated from the same MFC group fed with the 2nd stage effluent. In repeated tests, the
281 power output from the same group fed with untreated urine was similar or only slightly higher than
282 the 2nd stage effluent. Moreover, when the position of the two groups was swapped, higher power was
283 produced by the 1st MFC group at the 3rd stage.

284 An important aspect to consider is the flow rate of urine supply resulting in different HRT, and
285 in this study different flow rates were set for the MFC processes. The higher flow rate (96m L/hr) for
286 the first stage was in order to prevent precipitation inside the system but still result in a good level of
287 power. For operating MFC systems in continuous feeding mode, the flow rate of feedstock needs to
288 be optimised since too low or high flow rate can cause performance decline²⁹. Different flow rates
289 were tested in order to find the optimum flow rate for the system used in this work and with the flow
290 rate of 42 mL/hr the system showed the maximum power output. For this reason, this was the flow
291 rate used during the final stage. Therefore more power could be expected from the 3rd stage, if all
292 other conditions were identical. It is also important to give consideration to the concentration change
293 of readily available organic matter. In most cases, the second MFCs in each group produced higher
294 output than the first units when they were monitored individually. It is likely that the amount of
295 readily available organic matter increased after the first MFC units. In a similar fashion, effluent from
296 the 1st stage might have had more easily utilisable organic matter than untreated urine. A similar
297 pattern has been witnessed in previous studies using complex feedstocks where the downstream

298 MFCs outperformed the upstream^{30,31}. Therefore the optimum flow rate, HRT, group positioning and
299 amount of magnesium addition need to be chosen accordingly when designing a MFC system for
300 maximising power output and nutrient recovery.

301

302 **Practical application**

303 For demonstration purposes, it was attempted to operate a commercially available electronic air
304 freshener with the 2 groups of 4 MFCs (8 in total) used in this study. The air freshener originally
305 required two D sized batteries to operate. The original circuit board of the automatic air freshener was
306 modified with a 240 mF super-capacitor (Cellergy, Israel) which would allow a maximum voltage of
307 up to 4.2 V. When the charged voltage of the capacitor reached 2.8 V, the air freshener operated the
308 integrated motor, which actuated to press the nozzle of an inserted compressed air spray can. After the
309 firing motion, the voltage of the capacitor decreased to 2.1 V, the system stopped and the capacitor
310 began to charge again. The 4 MFCs within each group were connected as already described above,
311 and the two groups were then connected in series.

312 Figure 6 shows the temporal profiles of the MFC stack whilst operating the automatic air
313 freshener. Each trough and peak represent one charge/discharge cycle where the MFC stack voltage
314 increased as the capacitor was charged. When the capacitor discharged at 2.8 V, the voltage of the
315 stack dropped to 2.1 V then quickly started charging up again. This charge/discharge cycle repeated
316 every 15-25 minutes for 4 weeks continuously. This exemplar practical application demonstrated
317 successfully the capability of the MFC stack, with only 8 MFCs of 6.25 mL anodic volume each.

318

319 **CONCLUSIONS**

320 In this work, a 3-stage MFC/struvite extraction process system demonstrated how the MFC
321 technology and struvite precipitation could be integrated and beneficial to each other in this
322 integration for both energy generation and resource recovery from urine. Three major conclusions
323 were drawn in this study.

- 324 (1) Placing MFCs before the struvite precipitation process helps struvite collection by
325 accelerating urea hydrolysis, and removing struvite from urine before the MFCs helps by
326 minimising the element of system blockage without hindering the MFC performance.
- 327 (2) With the 3-stage system proposed in this work, 82 % of $\text{PO}_4^{3-}\text{-P}$, 20 % of COD of undiluted
328 human urine were removed and 14.32 W/m³ (absolute power: 358 μW), 11.76 W/m³
329 (absolute power: 294 μW) of power was produced, which was put to practical use.
- 330 (3) Besides the potential benefits of the proposed system concept, several design factors such
331 as flow rate and amount of magnesium addition are suggested for further consideration,
332 which can be the first steps to a truly sustainable energy future.

333

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339

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413 **Table legend**

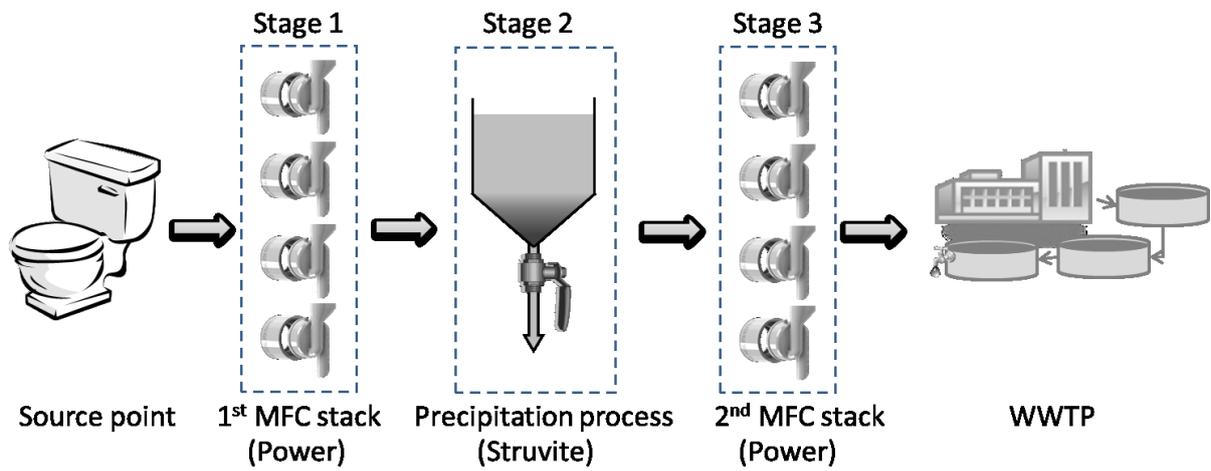
414 Table 1. Comparison of neat urine and struvite removed urine as a feedstock in terms of pH, ORP,
 415 conductivity, NH_4^+ -N, PO_4^{3-} -P and power output. Data presented as the mean and error (n=2 for
 416 NH_4^+ -N and PO_4^{3-} -P, n=4 for power output).

	untreated urine (neat)	treated urine from group 1	treated urine from group 2	untreated urine (struvite removed)	treated urine from group 1	treated urine from group 2
pH	6.57	9.28	9.24	6.49	9.25	9.23
ORP (mV)	-14	-171	-166	-2	-165	-163
Conductivity (mS/cm)	12.3	23.0	22.5	13.5	24.4	24.2
NH_4^+ -N (mg/L)	363 ± 8	3268 ± 10	3246 ± 15	290 ± 6	3114 ± 14	3106 ± 12
PO_4^{3-} -P (mg/L)	202 ± 6	254 ± 11	244 ± 6	36 ± 2	36 ± 1	36 ± 2
Power (μW)		101 ± 7	82 ± 3		104 ± 6	86 ± 4

417

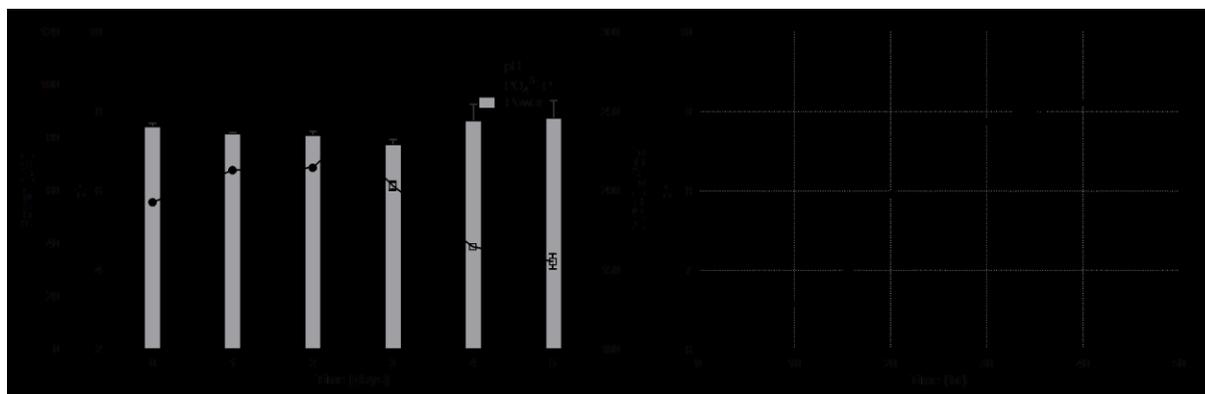
418 **Figure Captions**

419 Figure 1. Schematic diagram of the 3-stage MFC/struvite extraction process system. The diagram also
420 illustrates how this can be implemented in real life.



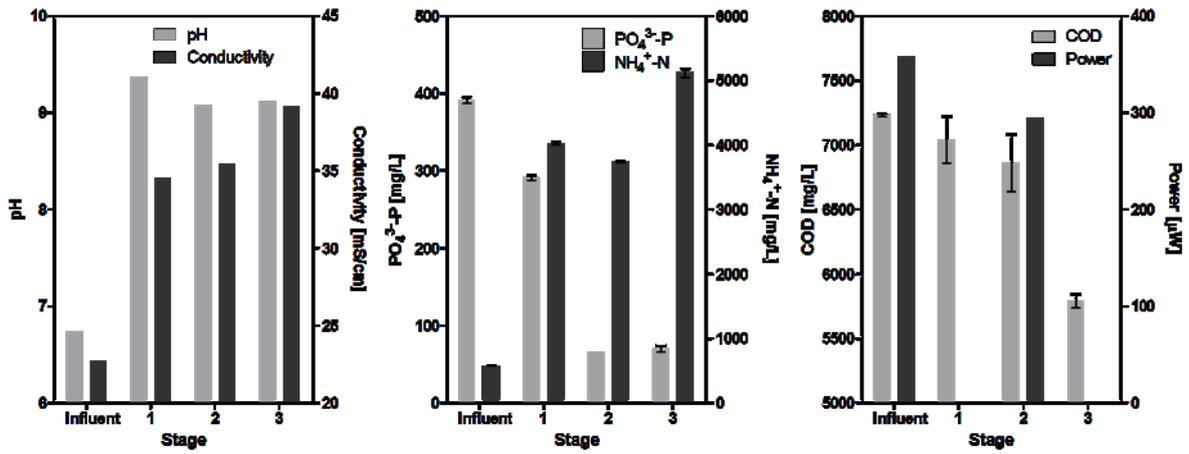
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422 Figure 2. Urine pH change with time and its use as a substrate for MFCs (a) profile of influent pH,
423 soluble phosphate and MFC power output. Data are based on mean values (n=2 for $\text{PO}_4^{3-}\text{-P}$, n=4 for
424 power output). (b) typical pH behaviour of urine when stored in a bottle at room temperature; pH was
425 measured *in situ* every 30 minutes for 50 hours.



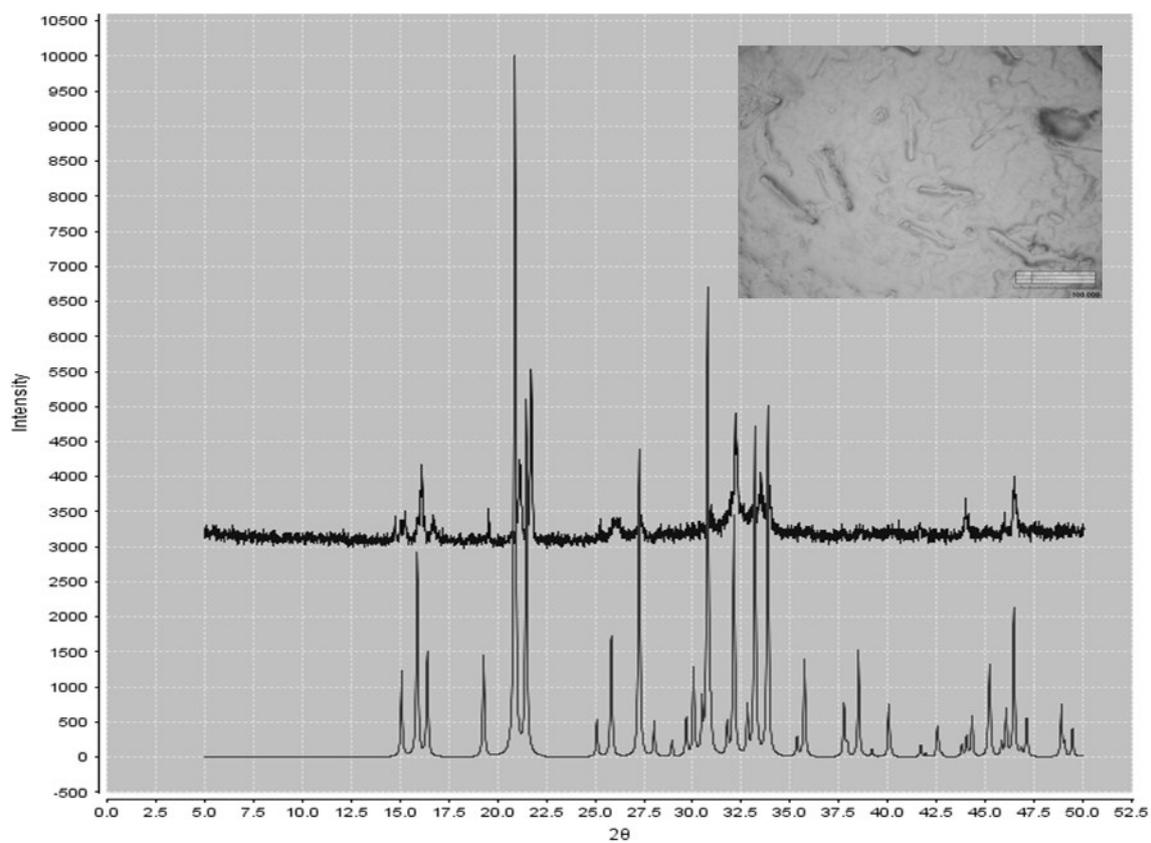
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427 Figure 3. Profile of pH, conductivity, $\text{PO}_4^{3-}\text{-P}$, $\text{NH}_4^+\text{-N}$, COD and power output of each stage. Data
 428 presented as the mean and range (n=3).



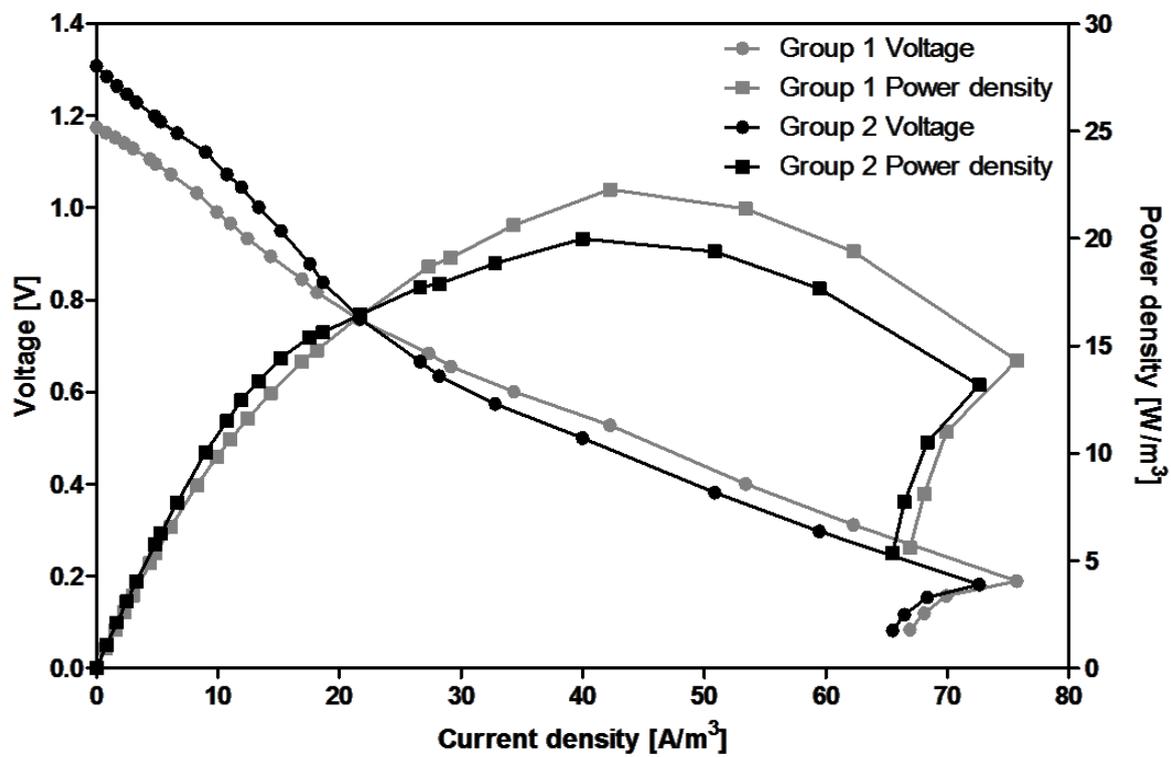
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430 Figure 4. XRD analysis of the struvite precipitate (grey line: standard struvite, black line: precipitate
431 sample from the 2nd stage). The inset shows the microscopic image of the struvite.



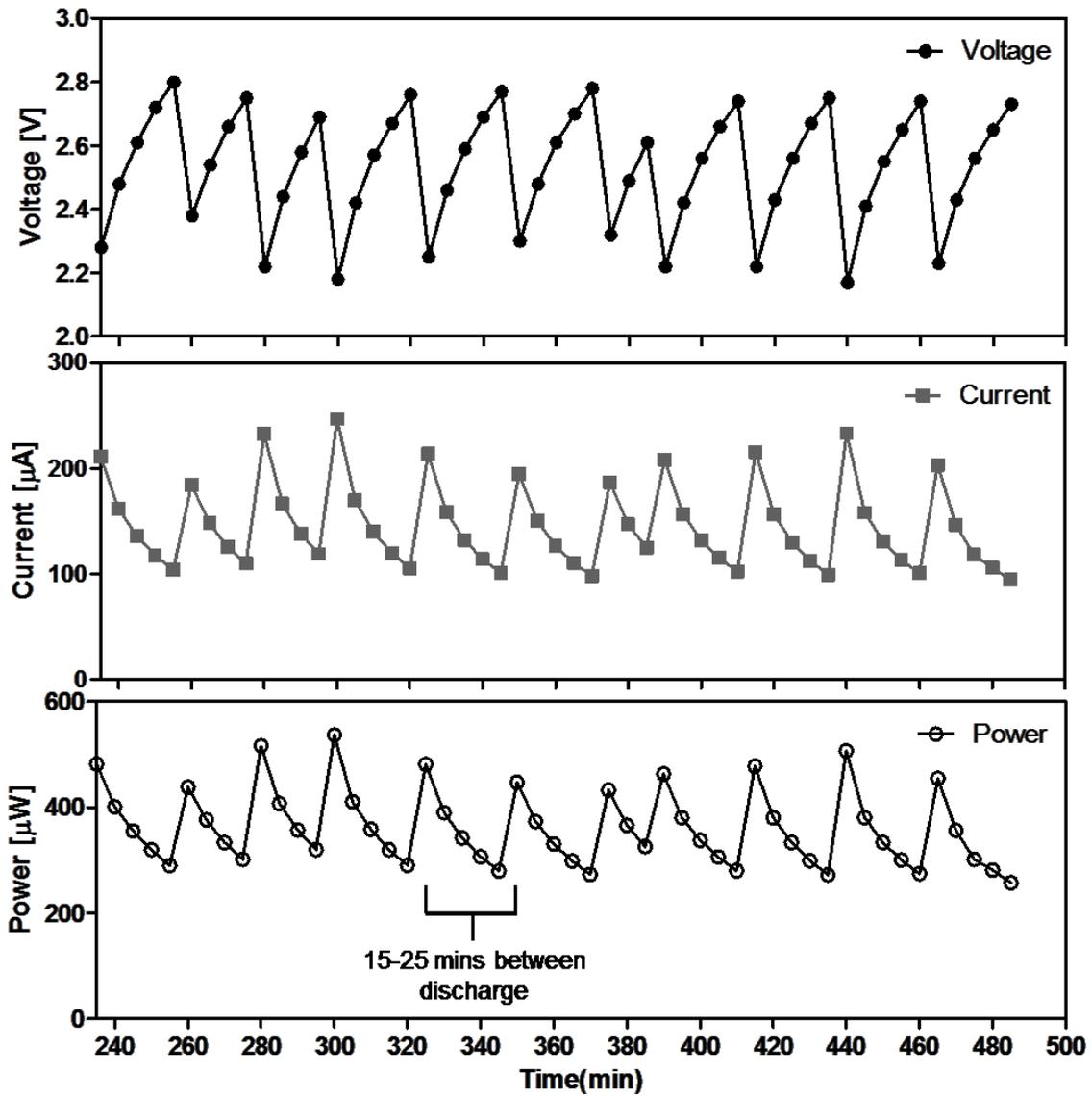
432

433 Figure 5. Polarisation curves of MFC group 1 and MFC group 2; each group consisted of 4 MFC units
434 in cascade.



435

436 Figure 6. Temporal profiles of the MFC stack when connected to a commercial electronic air
437 freshener; charge/discharge cycle in voltage, current and power (from the top).



438