

A novel biorefinery:

Murray, A. J.; Zhu, J.; Wood, J.; Macaskie, L. E.

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1 **A Novel biorefinery: biorecovery of precious metals from spent automotive**
2 **catalyst leachates into new catalysts effective in metal reduction and in the**
3 **hydrogenation of 2-pentyne**

4 *A.J. Murray¹, J.Zhu², J. Wood² and L.E. Macaskie¹

5
6 Schools of ¹Biosciences and ²Chemical Engineering, University of Birmingham Edgbaston,
7 Birmingham B15 2TT UK

8
9 * Corresponding author L.E.Macaskie: L.E.Macaskie@bham.ac.uk

10
11 With the aim to recover precious metals (PMs) from spent automotive catalyst leachates into
12 new catalysts, cells of *E. coli* first reduced Pd(II) or Pt(IV) physiologically to nanoparticulate cell-
13 bound Pd(0) and Pt(0). Metallised cells were then used as chemical catalysts for the reductive
14 recovery of precious metals from model solutions and from *aqua regia* leachates of crushed
15 spent automotive catalyst. Metal removal, which was slower from real leachate due to
16 interference by other contaminants, was complete after 60 h. Biofabricated PM catalyst from
17 waste reduced 0.5 mM Cr(VI) to a similar extent to commercial 5% Pd catalyst but at ~ half the
18 rate. The hydrogenation of 2-pentyne was examined using commercial Pd on Al₂O₃ catalyst and
19 biofabricated Pd/Pt catalyst, the latter showing more than 3-fold enhanced selectivity towards
20 the desired *cis*-pentene product. Hence, biorefined PMs offer a clean route to waste treatment
21 and effective neo-catalyst biomanufacture.

22 Key words: Precious metals. Biorecovery. Automotive catalysts waste. Neo-catalyst. Cr(VI)
23 reduction. 2-pentyne hydrogenation

24 **1. Introduction**

25 Platinum group metals (PGMs) are scarce high value metals with a wide range of applications
26 from jewellery and commercial catalysis to use within car catalytic converters for atmospheric
27 protection (Xiao and Laplante, 2004; Bernardis *et al.*, 2005; Wiseman and Zereini, 2009). No
28 suitable alternative has yet been found for PGM (particularly Pt) in many applications as they
29 have low substitutability, except with other PGMs (Bernardis *et al.*, 2005; Yang, 2009). PGM
30 catalysts are used in low temperature fuel cells (Anon, 2006). This highlights future tensions
31 between today's transport requirements and tomorrow's energy needs. Supply and price of PGM
32 are critical to both (Anon, 2008). To safeguard future supplies of PGMs it is increasingly important
33 to recover and re-use the metals effectively and sustainably.

34 All new motor vehicles are fitted with a catalytic converter, each containing up to 2.4 g of
35 precious metals which are routinely 'thrifed' by adjusting the catalytic composition according to
36 the PGM market price (Mouza *et al.* 1995; Johnson Matthey, 2001; Xiao and Laplante, 2004).
37 PGM loadings on catalytic converters are unlikely to decrease in future (Bloxham, 2009) and will
38 probably increase slightly in order to meet stringent standards (Yang, 2009).

39 Under load the PGMs on the catalytic surface become abraded from the support and become
40 deposited within road dust (Cinti *et al.*, 2002; Schafer & Puchelt, 1998). The PGM levels found
41 within some urban wastes were shown to be equivalent to that of an ore from a low grade mine
42 (Jackson *et al.* 2007) e.g. a small city the size of Sheffield, UK produces around 8000 tonnes of
43 road dust per year. Consideration of such secondary wastes as 'urban mines' is attractive due to
44 the negligible comminution costs of powdered materials as well as the resource they contain.
45 However upgrading of bulk materials to obtain PGM levels that are economic for extraction

46 remains a challenging area (Murray, 2011).

47 We take automotive catalysts as an example as these are the source material from which
48 environmental PGMs are derived. Yong *et al.* (2003) showed a new approach to recovery of PGMs
49 from acidic spent automotive catalyst leachates using cells of the bacterium *Desulfovibrio*
50 *desulfuricans* which deposits precious metals via their reduction from soluble ionic forms. The
51 ability of *D. desulfuricans* and many other bacteria (Deplanche *et al.*, 2011) to reduce various
52 metals, including PGMs, onto their surface through hydrogenase activity is well documented (e.g.
53 see Lloyd *et al.*, 1998; Deplanche *et al.*, 2010; 2011). The deposited metals form nanoparticles on
54 the cell surface. This ability has been exploited to create “bionanocatalysts” comprising bacterial
55 cells coated with a well distributed layer of metallic nanoparticles (NPs) (see Deplanche *et al.*,
56 2011 for review). Studies have illustrated the use of metals biorecovered from wastes to produce
57 these catalysts (Mabbett *et al.*, 2006; Murray *et al.*, 2007; Macaskie *et al.*, 2011). Some can
58 produce catalysts with higher activity than those made with just one metal (Yong *et al.*, 2010; see
59 Macaskie *et al.*, 2011). However, although for applications in fine chemicals synthesis an
60 undefined ‘dirty’ catalyst may be unattractive, for other applications such as decontamination of
61 pesticides (Mertens *et al.*, 2007) or chlorinated organic compounds in groundwater (Deplanche
62 *et al.*, 2009) a mixed metal ‘dirty’ catalyst may suffice. This approach pioneers a new area of
63 environmental nanotechnology. However the potential hazards of NP migration would need to
64 be minimised. This can be done via the retention of multiple catalytic NPs onto micron-sized
65 ‘carrier’ bacterial cells that are structurally robust and can be immobilised on bacterial biofilm for
66 continuous use (Beauregard *et al.*, 2010; Yong *et al.*, 2015), with negligible catalyst attrition from
67 bacterially-bound nanoparticles (Bennett *et al.*, 2013).

68 A continuous biorecovery system for PGMs from waste was pioneered by Yong *et al.* (2003).
69 These authors used electrochemically-generated hydrogen to supply a film of PGM-reducing
70 bacteria on the outside of a Pd/Ag thimble electrode immersed in PGM solution, with the
71 hydrogen generated at the back-side. When loaded, the bacteria fell from the electrode for
72 harvest (Yong *et al.*, 2003). The bacteria removed more than 80% of the presented Pd and Pt from
73 an industrial processing waste and up to 75% of the presented Rh (Yong *et al.*, 2003).

74 Recovery of metals from very acidic solutions such as waste leachates is difficult. This is due
75 to the strength of acid required to dissolve PGMs (noble metals typically require *aqua regia*). This
76 is incompatible with biochemical activity. Therefore a two step approach was developed whereby
77 bacteria were first allowed to reduce (e.g.) Pd(II) to Pd(0) 'seeds' under physiologically compatible
78 conditions. These pre-metallised cells then functioned as chemical catalysts in the recovery of
79 PGMs from acidic solutions (Creamer *et al.*, 2006; Mabbett *et al.*, 2006).

80 An early study showed that 5% by mass loading of Pd(0) onto *D. desulfuricans* gave a
81 hydrogenation catalyst comparable to commercial 5% Pd on carbon (Creamer *et al.*, 2007) but
82 'thrifting' Pd(0) on cells of *D. fructosovorans* resulted in an inferior catalyst; i.e. cells at 5% and 2%
83 Pd(0) mass loading released, respectively, 0.7 and 0.3 ml H₂/min/mg Pd from hypophosphite,
84 while the respective hydrogenation of 0.4 mM itaconic acid (methylene succinate) to methyl
85 succinate after 1 h was 70% and 50% (Skibar *et al.*, 2005). The discrepancy was even greater in
86 the bio-Pd- catalysed reduction of Cr(VI) (CrO₄²⁻ anion). Here, less than 10% of 0.5 mM Cr(VI) was
87 reduced after 3 h by cells with 2% Pd(0) mass loading whereas 5% loading achieved > 30%
88 reduction (Skibar *et al.*, 2005). Clearly a mass loading of 5 wt% Pd is preferable and a way to
89 reduce this to 2wt% Pd from a primary source while retaining catalytic efficacy would be useful

90 from an economic viewpoint. One option is to 'top up' the cellular Pd(0) by sourcing the metal
91 from a wastes.

92 The dual aims of this study were firstly to use a microbial biorecovery method to convert a
93 waste leachate into catalytically active biomaterial and secondly to show that the biorecovered
94 metal gave catalytic activity over and above that of metallised bacteria bearing only the initial
95 'seeds'.

96 Previous work has focused on Pd (e.g. Creamer *et al.*, 2007). Many PGM wastes and especially
97 catalytic converters and road dusts contain both Pd and Pt (Shelef and McCabe, 2000, Ek *et al.*,
98 2004) as well as Rh. This study focused on Pd and Pt since these are the major PGM components
99 (Murray, 2011). Hence, cells were 'seeded' using both Pd and Pt to various loadings prior to metal
100 removal from, initially, model metal mixtures and then from real automotive catalyst leachate.
101 Initial studies focused on reduction of Cr(VI) but in order to assess the potential for this approach
102 in chemical manufacturing applications ('green chemistry') the bionanocatalysts were also
103 evaluated with respect to their ability to hydrogenate 2-pentyne, focusing on the ability to
104 produce the preferred *cis*-pentene isomer.

105 Many studies have reported the application of microbial processes to the recovery of base
106 metals and precious metals from wastes but relatively few have progressed from model solutions
107 to actual wastes, i.e. that contain also other metallic and non-metallic components. Bio-
108 conversion of a metal recovered from a waste into a neo-catalyst has received little attention;
109 examples include bioconversion of a relatively benign PGM-processing wastewater into a catalyst
110 for reduction of toxic Cr(VI) (Yong *et al.*, 2015) and a fuel cell electrocatalyst (Yong *et al.*, 2010)
111 but showing the potential for neo-catalysts biomanufactured from an aggressive waste leachate

112 is a novel development. The goal of this study is to illustrate this potential.

113

114 **2. Materials and Methods**

115

116 *2.1. Growth of organisms*

117

118 *Escherichia coli* MC4100 cells were cultured in 12 litres of nutrient broth under anaerobic
119 conditions (i.e. with exclusion of air: Deplanche and Macaskie, 2008). Cells were harvested by
120 centrifugation, washed three times in 20mM MOPS-NaOH buffer pH 7.0 and resuspended in a
121 known volume of buffer. The cell density was checked by OD₆₀₀ which was converted to bacterial
122 dry weight by a previously determined calibration, whereby suspended samples of cells at a
123 known OD₆₀₀ and known volume were dried to constant weight after washing with water to
124 remove residual salts. With a dry weight of cells between 20-30 mg/ml the cell suspensions were
125 then split into six aliquots in preparation for pre-metallisation.

126

127 *2.2. Pre-metallisation of cells*

128 Cells were metallised as described by Taylor (2012). Solutions of 2 mM Pd(II) and Pt(IV) were
129 prepared in 1 mM HNO₃ using Na₂PdCl₄ and K₂PtCl₆ salts respectively. The required volume of
130 metal solution was then added to aliquots of cells (known mass: above) to achieve the desired
131 metal loadings of 1%, 2% or 5% by mass as stated. H₂ was bubbled through the suspension (30
132 min) and suspensions were then incubated at 30 °C under H₂ for reduction of metal onto the cells.
133 Complete metal reduction and removal was confirmed in sample supernatants using a SnCl₂ –

134 based assay for residual soluble metal as described previously (Creamer *et al.*, 2008). Following
135 full reduction of metals (within 30 min) the 'seeded' cells were harvested by centrifugation,
136 washed once using distilled water and resuspended in distilled water (30 ml).

137

138 2.3. *Recovery of target metals from model solution and catalyst production*

139 The seeded cells (1%, 2% or 5% of Pd, or Pt as specified; 16 mg of pre-loaded cells) were exposed
140 to a mixed solution of 0.34 mM Pt(IV) and 0.42 mM Pd(II) in HNO₃ (target metal solution: chosen
141 as an approximation to a real catalyst leachate: Taylor, 2012). The volume of solution added was
142 calculated as that required to give a final loading of metals on pre-palladised cells, following target
143 metal reduction of, respectively, 15 wt%, 16 wt% and 20 wt% in a background of 1 mM HNO₃.

144 The reducing agent (H₂) was bubbled into the solution as described in the seeding step with
145 metal reduction monitored in withdrawn samples using SnCl₂ as above. No attempt was made to
146 assess selectivity of metal removal. The results were expressed as percentage target metal
147 reduction against time, using five independent batches for each test to assess the inter-batch
148 variability (standard error of the mean was within 5%). After complete metal reduction (loss of
149 metals by assay of the spent solution) the cells were harvested by centrifugation, washed once in
150 H₂O and once in acetone. They were then dried and ground in an agate mortar to give a black
151 powder which was passed through a 100 micron sieve to obtain a fine powder catalyst.

152

153 2.4. *Catalytic evaluation via reduction of Cr(VI) to Cr(III)*

154 Catalyst prepared as described above (10 mg powder) was added to a 12 ml serum bottle and 5

155 ml 0.5 mM $\text{Na}_2\text{Cr}_2\text{O}_7 \cdot 4\text{H}_2\text{O}$ in 20 mM MOPS-NaOH buffer pH 7.0 was then added. The bottle was
156 sealed with a butyl rubber stopper, degassed under vacuum (via a needle) and sparged with
157 oxygen free nitrogen. It was then placed onto a rotary shaker (180 rpm; 10 min, room
158 temperature) to ensure mixing and distribution of catalyst. Sodium formate (1 ml of a 25 mM
159 solution) was added. The bottle (still under N_2) was returned to the shaker and sampled at 30
160 minute intervals. Sample supernatants were analysed for residual Cr(VI) using diphenylcarbazide
161 (Mabbett *et al.*, 2004).

162

163 2.5. Catalytic evaluation via hydrogenation of 2-pentyne

164

165 2-pentyne hydrogenation experiments were carried out in a three-phase 500 ml stainless steel
166 autoclave reactor (Baskerville, Manchester, U.K.) To this, 150 ml solvent (2-propanol) and a
167 weighed mass (usually 0.056 mmol Pd(Pt) unless stated otherwise) of ground catalyst were
168 added. The comparator was 2wt% mass of Pd on Al_2O_3 ('Pural SB': Condea). The mixture was
169 heated to 40°C, nitrogen was purged in order to remove residual oxygen, and the catalyst was pre-
170 reduced by bubbling a flow of hydrogen (0.5 L/min) through the system for 20 min with gentle
171 stirring (500 rpm). 4 ml of 2-pentyne (98+%, Alfa Aesar UK) was then added. The reactions were
172 stirred (1000 rpm) at 40 °C under a constant 2 bar of hydrogen pressure. Liquid samples were
173 withdrawn periodically. The composition of the reaction mixture was determined by gas
174 chromatographic (GC) analysis using a Varian CP-3380 with a flame ionisation detector (FID) and
175 a 30 m Gamma DEX™ 225 capillary column (Thermo Electron Corporation UK) at 40°C after
176 equilibration for 10 min.

177 The major products from 2-pentyne hydrogenation are partially hydrogenated *cis/trans*-
178 pentene and fully hydrogenated pentane. The performance of the catalyst was assessed in terms
179 of selectivity toward *cis*-pentene; selectivity was calculated as the number of moles of *cis*-
180 pentene divided by the total number of moles of all products detected.

181 Hydrogenation experiments were done twice, independently, with a difference between them
182 of within 10% throughout and pooled data are shown.

183

184 2.6. Metal recovery from spent automotive catalyst by leaching and preparation of catalyst from 185 leachate

186

187 Preparation of catalyst leachate was developed from methods described by Yong *et al.* (2003) as
188 detailed by Murray (2011). A used 'three way' car catalyst (Peugeot 106 aftermarket catalyst
189 provided by Humphries Garage, Bearwood, Birmingham) was stripped of its outer cladding to
190 expose the cordierite and washcoat monolith, the latter containing the PGMs. The monolith was
191 processed by jaw crushing to $d < 3.5$ mm (Sturtevant 150 mm jaw crusher with corrugated jaw
192 plates), ground using a roll crusher (Sturtevant 150 mm Roll Crusher) and then passed through a 1
193 mm screen. Any oversize material was reground in the Roll crusher so that all test material was of
194 diameter $d \leq 1$ mm. The automotive catalyst used for leachate production had 600 channels per
195 square inch, thus each channel was 1.04 mm wide. Any material greater than 1mm was reground in
196 order to avoid over-crushing but to facilitate maximum acid - washcoat interaction.

197 For leaching *aqua regia* (60 ml; 3 parts 37 % HCl to 1 part 70 % HNO₃) was added to 6 g of milled
198 catalyst and allowed to stand in an open vessel (30 min). The vessel was then sealed and placed in a
199 microwave (CEM Microwave Accelerated Reaction System 5) set to ramp (106°C in one min using a

200 power of 600W). That temperature was maintained (15 min) followed by a cooling cycle (5 min). The
201 contents of the vessel were transferred with washings (half the volume of distilled water to *aqua*
202 *regia*; final *aqua regia* concentration 67% vol/vol aq.), centrifuged (4000 rpm; 10 min) and the
203 supernatant was retained for biomass metallisation tests. Commercial analysis of leachate was done
204 by Engelhard Corporation (ICP-MS) with a stated ICP limit of detection of 0.1ppm for PGMs.

205 The procedure for making the catalyst from leachate was as follows. Due to the low level of
206 Pd(II) (see Results and Discussion) the leachate used in this study was 'spiked' to 400 ppm with
207 Pd(II). The leachate was diluted ten-fold in distilled water to reduce the concentration of acids to
208 6.7% (to avoid destruction of the biomass support) and it was brought to pH 2.2 with 6 M NaOH.
209 Pre-palladised ('seeded') cells of *E. coli* (1 ml, 5 wt% initial Pd loading) were added to 77 ml of
210 leachate (and model solution in parallel to a comparable metal loading; see 2.3) and H₂ was
211 bubbled through this mixture (2 h) and then left to stand until the PGMs were removed (by assay
212 of spent solution using SnCl₂). The other components of the catalyst, and their extraction by this
213 method, were not analysed.

214 In order to implicate compound(s) responsible for the slower PGM deposition from the waste
215 (see Results and Discussion) a simple test was carried out. Model leachates (Pd(II) and Pt(IV))
216 were prepared as above using fresh *aqua regia* and aliquots were spiked with Pd(II) (to 400 ppm
217 final concentration), neutralised and diluted as before. The pH was adjusted to 2.0. Aliquots of
218 the model leachates were spiked with silica (SiO₂ (to 173 ppm)) and Al₂O₃ (to 173 ppm) final
219 concentrations) and also a mixture of both. The Pd- 'seeded' bioinorganic catalyst was added in
220 each reactor and PGM removal was followed as before.

221

222 **Results and Discussion**

223

224 *3.1. Analysis of leachates and leaching of PGMs from wastes*

225

226 Commercial analysis of the leachate gave 24ppm Pd, and 4ppm Rh but no detectable Pt (although
227 this method was confirmed to give effective leaching of Pt from solid scraps: (Murray, 2011)).
228 Subsequent analysis of the leach residue solids (by copper collection and XRF of copper button)
229 confirmed >95% Pd extraction during leaching but only 50% Rh extraction. A comparison of the
230 catalyst used in this work against other typical spent automotive catalysts showed that PGM
231 levels were unusually low (probably due to losses onto roads during use), with the Pd content
232 being approximately 10% of the value of another catalyst processed under the same conditions
233 (this catalyst was retained for testing in another study). Hence the leachate of the catalyst used
234 in this study was 'spiked' with additional Pd(II) (see Materials and Methods).

235 Optimal leaching conditions were initially developed as described by Murray (2011) to give the
236 procedure described in Materials and Methods. Two solid samples were treated and analysed in
237 order to determine the initial PGM content of both crushed catalysts (i.e. the catalyst providing
238 material as used in this study and for a parallel catalyst which was used in other tests which will form
239 the basis for a future publication). Since Yong *et al.*, (2003) had reported relatively high PGM
240 recoveries (>80% of maximum of Pd and Pt) using 50% *aqua regia* tests were conducted using both
241 50% and 100% *aqua regia* as shown in Table 1. The results (Fig. 1) show that for each condition
242 approximately 90% of the Pd was recovered but 100% *aqua regia* was required for the highest
243 recovery of Rh (> 80%). For Rh no clear conclusion could be drawn regarding the advantage of using

244 a solid:liquid ratio of 10:1 as compared to 5:1 but use of 100% *aqua regia* gave enhanced extraction
245 over 50% *aqua regia* at both liquid:solid ratios (Fig. 1). Use of a finely ground sample did not improve
246 the extraction efficiency of Rh at a liquid:solid ratio of 5:1 (Fig. 1). The conclusion from this study is
247 that effective metal recovery is only achieved using 100% *aqua regia* but that 50% *aqua regia* is
248 sufficient for Pd recovery. The possibility to develop a selective method to separate Pd and Rh (i.e.
249 concentration of Rh into the unextracted fraction) was not explored, while the occurrence of Rh in
250 the final catalyst sample was not measured, and hence the 'finished' neo-catalyst was probably a
251 mixture of Pd and a small amount of Rh which was not tested. However use of 50% *aqua regia* would
252 represent a distinct advantage with respect to savings in acid costs as well as minimising the potential
253 damage to the biomass support. Hence, subsequent tests used 50% *aqua regia* with microwave
254 processing at 106°C for 15 minutes (see Materials and Methods). The advantage of microwave
255 processing has been described elsewhere (Jafarifar *et al.*, 2005) and the conditions were optimized
256 for these samples previously (Murray 2011).

257

258 3.2. Hydrogenation of 2-pentyne using the model system with cells pre-seeded at 2 wt% Pd

259 A full description of the data with respect to catalytic activity of bio-catalyst made on cells that
260 were 'seeded' to 1 wt%, 2 wt% and 5 wt% Pd was given by Taylor (2012). Cells seeded using 1
261 wt% Pd gave an inferior catalyst and hence in this study a pre-loading of 2 wt% was used in the
262 hydrogenation tests.

263 Bennett *et al.* (2009) showed that bioPd functioned in the hydrogenation of 2-pentyne but a
264 different reactor system and catalyst loading was used in the earlier work as compared to this
265 study so direct comparisons are not possible. Fig. 2 shows that the slowest conversion rate was

266 seen using 2wt% bioPd alone but by supplementing with the additional metals the rates for the
267 commercial and biocatalyst became comparable. Other tests (not shown) revealed that 5wt%
268 pre-palladised cells (i.e. Pd alone) had a similar activity to the commercial catalyst shown in Fig.
269 2 and hence no further enhancement occurred by augmenting with additional Pd/Pt.

270 It is concluded that supplementing the initial Pd 'seeds' with additional Pt and Pd from the
271 model mixture produced a catalyst comparable to a commercial catalyst. Bennett *et al.* (2010)
272 noted that (under their conditions) the bioPd had only ~ 30% of the activity of its commercial
273 counterpart but it showed a higher selectivity to the *cis*-ene product. Hence, the present study
274 also examined the ability of the biomaterial to promote reaction specificity since production of
275 the *cis* alkene over *trans* is highly desirable industrially. The results (Table 2) show that, with
276 respect to the *cis/trans* products, the bio-catalyst gave much lower selectivity to *trans*-pentene
277 (below 20 mol%; i.e. a higher selectivity to the *cis*-product), while commercial 2 wt% and 5 wt%
278 Pd/Al₂O₃ gave above 35 mol% selectivity to undesirable *trans*. Using commercial catalyst the
279 *cis/trans*-pentene ratio was 0.71 for 2 wt% Pd/Al₂O₃ and 0.68 for 5 wt% Pd/Al₂O₃. Hence, using
280 bio-catalyst gave a 3-4- fold higher selectivity (data are averaged for the two loadings). However,
281 Table 2 shows that, overall, there was no advantage (or disadvantage) in using the catalyst made
282 from the mixture as compared to the 'seeded' cells alone. Hence, the advantage of providing
283 additional catalyst from the mixture was that the activity of 2%wt bioPd was enhanced by
284 approximately two-fold to become slightly better than the commercial comparator (Fig. 2)
285 without loss of selectivity (Table 2). However such assessments are subject to a number of
286 variables (e.g., solvent, reactor etc) and a more detailed investigation is warranted.

287

288 3.3. Recovery of PGMs from waste using 'seeded' cells

289

290 Due to the high acidity of the leachate native cells were not used to make catalyst from waste
291 leachate, as the low pH was not physiologically compatible. Instead, recovery of PGMs from the
292 waste leachate used 'seeded' cells (5 wt% Pd) as shown in Fig.3. Note that, whereas PGM
293 recovery from model solutions was complete within five minutes (Taylor, 2012), the reaction took
294 ~ 60 h to proceed to completion in a real waste (Fig. 3). The observed slow PGM reduction from
295 the catalyst leachate by the pre-palladised *E. coli* cells proceeded in three distinct phases (Fig. 3).
296 An initially rapid rate of metal removal (0-12 h) was followed by a ~ halving of the rate between
297 12-35 h. Selectivity of metal removal was not tested. Removal of the final ~20% of the metals was
298 very slow over the final 20h. Full disappearance of PGM species from solution was achieved after
299 ~60 hours of contact.

300 In order to implicate the compound responsible for the inhibition of PGM reduction. Model
301 leachates were spiked with Pd(II) as before, and were also spiked with silica (SiO₂ (to 173 ppm)
302 and Al₂O₃ (to 173 ppm) final concentrations) and also a mixture of both. The addition of Pd-
303 'seeded' bioinorganic catalyst and then addition of either Al or Si inhibited PGM reduction:
304 Pd(II)/Pt(IV) disappearance from the supplemented model solution was observed only after 6
305 and 14 hours of contact with the bioinorganic catalyst with SiO₂ or Al₂O₃ respectively. Complete
306 PGM removal was not observed from the model solution supplemented with both Si and Al even
307 after 48 h. In contrast metal was removed from the unsupplemented control (model leachate +
308 distilled water) within 5 mins (i.e. as seen with the model solutions). These results suggest that

309 the presence of Al and Si inhibit PGM recovery and are responsible for a more than 30-fold
310 increase in reduction time observed with the spent car catalyst leachate.

311 These preliminary tests suggest that, since the actual composition of a waste is likely to vary
312 according to source of the material (and any upstream processing) there is little to be gained by
313 an in depth model study of critical inhibitory concentrations. This is because the potency of the
314 inhibitory agent(s) may be synergistic (or moderated) by other agents present in the waste. Such
315 studies are beyond the scope of this work but the preliminary results we describe suggest that
316 wastes would need to be evaluated on a case by case basis for their amenability to 'biorefining'.
317 Despite this, this combined biochemical and chemical approach shows potential for recovery of
318 PGMs from leachates, albeit with longer contact periods. Although samples in this study were
319 diluted (precluding re-use of the acid in this case) a previous study (Yong *et al.*, 2003) showed
320 metal recovery from 50% *aqua regia* which is suitable for Pd leaching with the application of
321 microwave energy (Fig. 1). Hence, although acid re-use was not tested in subsequent leaching
322 cycles, there is clear scope for a continuous metal recovery process (e.g. as described by Yong *et*
323 *al.*, 2003) with acid recycle, which is an important economic consideration for further
324 development.

325

326 3.4. *Catalytic activity of the PGM recovered from waste leachate*

327
328 Cells pre-palladised with 5 wt% Pd(0) were used in the reduction and removal of Pd and Pt from
329 the model solution and from the catalyst leachates prepared as described above. Both catalysts
330 were active in Cr(VI) reduction (Fig. 4), with similar initial reaction rates. Near-complete Cr(VI)
331 reduction was obtained with the catalyst made from model leachate after 120 min whereas the

332 catalyst obtained from real leachate showed a \sim 2-fold slower rate after 30 min, probably
333 attributable to the presence of non-PGM contaminants (possibly Si and Al) which could partially
334 poison catalytic PGM nanoparticles (above). Nevertheless, more than 90% of the Cr(VI) was
335 reduced after 180 min by the biorecovered material. A similar conclusion was reached by Yong
336 et al (2015) who showed, using immobilised neo-catalyst, that the slower rate was easily
337 compensated by increasing the flow residence time in a continuous flow column system.

338

339 *4.0. Conclusions and future scope*

340

341 This study shows that via use of microwave assisted leaching Pd is recovered with high efficiency
342 from spent car catalyst using 50% *aqua regia*. The biorecovered material reduced Cr(VI) at
343 approximately half the rate as a similar biocatalyst prepared from model solution. Si and Al were
344 shown to reduce the rate of removal of PGMs and were implicated in a reduced catalytic activity
345 of the biorefined material, with the reaction requiring 180 min as compared to 120 min in the
346 model system. Potential application to commercially-relevant industrial reactions is also
347 indicated. Bio-reprocessing of waste PGMs into neo-catalysts is a key development towards
348 realising added value from wastes. Future supplies of PGMs would be safeguarded as well as
349 reducing the environmental burden of PGM primary processing from ores (comminution of ore
350 is highly energy-expensive, e.g. overall, over 14 tonnes of CO₂ are generated per kilo of Pt
351 produced (Anon 2008). On the other hand, recycling processes also carry impacts and
352 consequences. Towards reducing these, waste *E. coli* bacteria left over from other processes have
353 been used to make Pd bio-catalyst for use in hydrogenation (Zhu et al., 2016). However the true

354 impact of the 'double benefit' can only be assessed by a side by side comparison via a full life
355 cycle analysis which is currently in progress incorporating both economic and environmental
356 factors, which is not trivial. This considers 'second life catalyst from waste' against use of primary
357 resources and also loss of catalyst in 'once through' systems as compared to metal recovery and
358 re-use. With respect to the latter the use of immobilised bacteria brings the additional benefit
359 of continuous catalyst use (Yong et al., 2015).

360

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365

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Table 1. Leaching conditions applied to the spent automotive catalyst as used in this study

Leaching Scheme	<i>Aqua Regia</i> Concentration	Liquid to Solid Ratio (ml/g)	Coarse^(a) or Fine^(b) material
A	50%	5:1	Coarse
B	50%	10:1	Coarse
C	100%	5:1	Coarse
D	100%	10:1	Coarse
E	50%	5:1	Fine

Scheme A and E are similar experiments but one uses coarsely ground catalyst (with a particle size range of $1000\mu\text{m} \geq d \leq 45\mu\text{m}$)^(a) and the other uses fine material (ground in a tema mill for 30 seconds so that $d \leq 38\mu\text{m}$)^(b) in order to test the hypothesis that fine grinding does not increase leaching efficiency i.e. that gentle crushing to open the channels is sufficient for complete extraction of the PGM/washcoat layer.

Table 2. Comparison of selectivity between commercial catalyst and bio-catalyst in 2-pentyne hydrogenation

Catalyst	commercial catalyst		bio-catalyst on <i>E.coli</i>			
	Pd/Al ₂ O ₃		pre-palladised bio-Pd		after target metal recovery bio-PdPt	
loading (wt%)	2	5	2	5	16	20
selectivity to <i>trans</i> -pentene (mol%)	37.63	35.1	19.65	19.91	20.65	15.93
<i>cis/trans</i> -pentene ratio	0.71	0.68	2.82	2.58	1.52	3.45
Average	0.7		2.7		2.5	

* Values of selectivity and *cis/trans* ratio obtained after achieving 100% 2-pentyne conversion.

Average is obtained from the 2wt% and 5wt% samples in each case. Each datum is the mean of two experiments with a variation between them of less than 10%.

Highlights

- Bacteria recover precious metals from automotive catalyst leachate
- Metal recovery is slower than from pure solution but is eventually complete
- Neo-catalyst from waste reduces Cr(VI) comparably to purpose-made catalyst

Legends to Figures.

Figure 1. PGM recovery in leachate from spent automotive catalyst used in this study. A: concentrations of Pd and Rh recovered under various leaching conditions as shown in Table 1. B: Pd extraction (%). C: Rh extraction (%). C: coarse sample; F: fine sample. % is *aqua regia* concentration. Ratio is liquid to solid ratio. Error bars are $\pm 3.6\%$ for Pd and $\pm 7.7\%$ for Rh.

Figure 2. Activity of 2 wt% bioPd in the hydrogenation of 2-pentyne and supplemented with additional metals from the model solution. For comparison results using commercial 2 wt%Pd/ Al_2O_3 (\square) are also shown. The biocatalyst samples were as follows: \blacksquare , 2wt% Pd/*E. coli*; \blacktriangle , 16 wt%Pd/Pt/*E. coli* (starting material 2wt% bioPd). The conditions were 4 ml of 2-pentyne in 150 ml of isopropanol; T = 40 °C; pH_2 = 2 bar; Stirring = 1000 rpm. The data are averaged from two experiments with a reproducibility between them of within 10%.

Figure 3. PGM Recovery from leachate using 5% pre-palladised cells. Data are the average from two independent preparations with a reproducibility between them of within 10%.

Figure 4. Catalytic activity of biorecovered catalyst using 5% pre-palladised cells as shown in Fig. 2. Open circles: catalyst made from model mix (see Materials and Methods) Closed circles: catalyst made from real waste leachate (see text). Data are means \pm standard error of the mean from three experiments.

Fig. 1

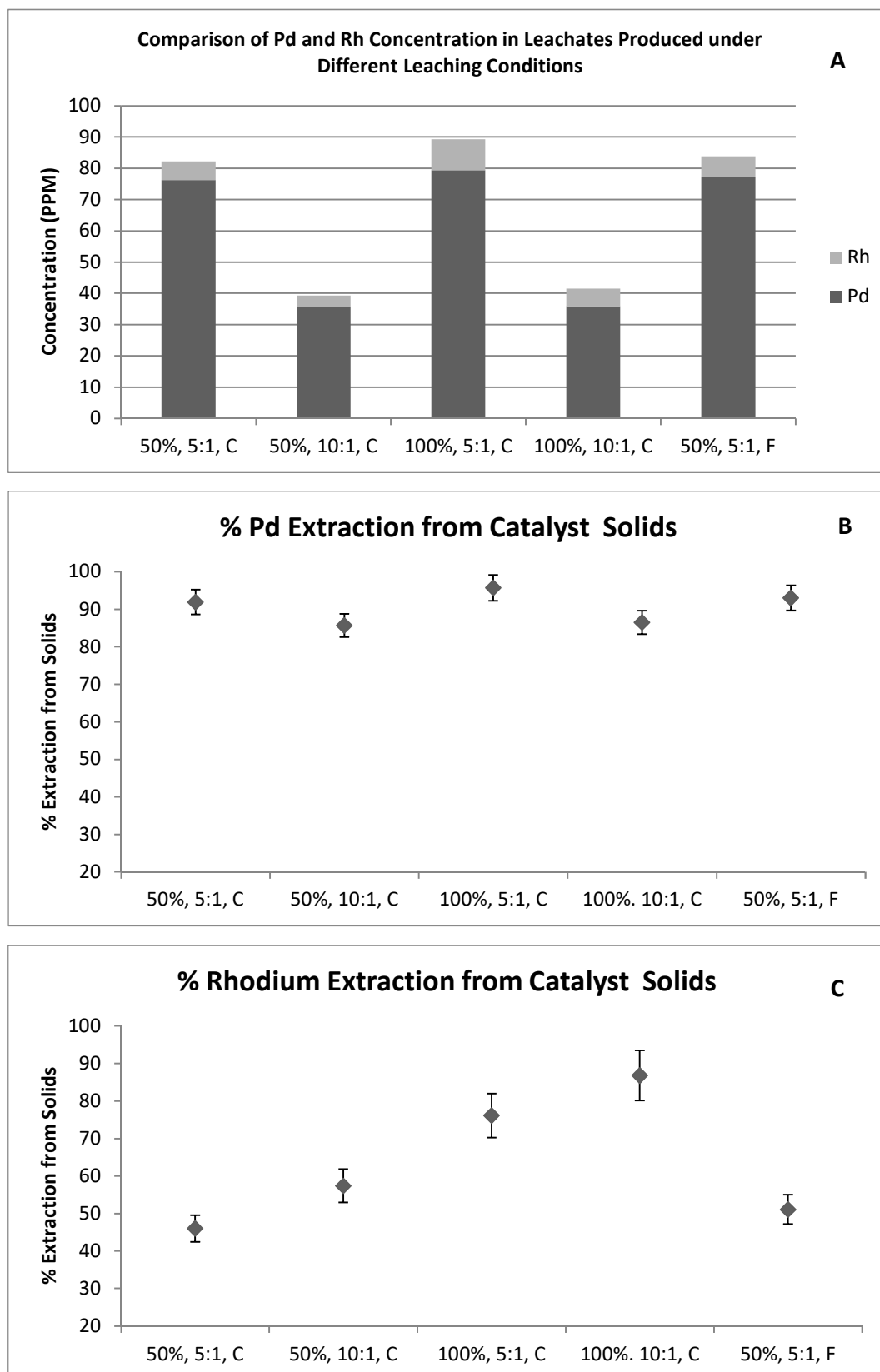


Fig. 2

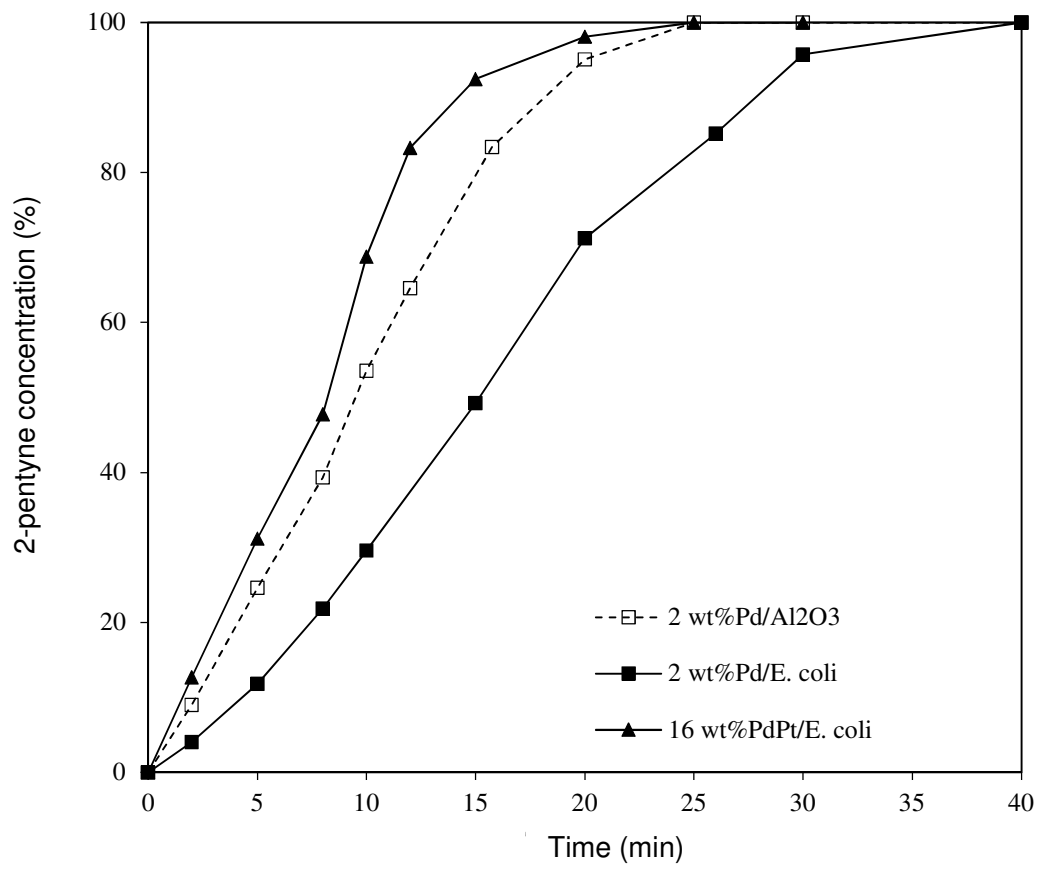


Fig. 3

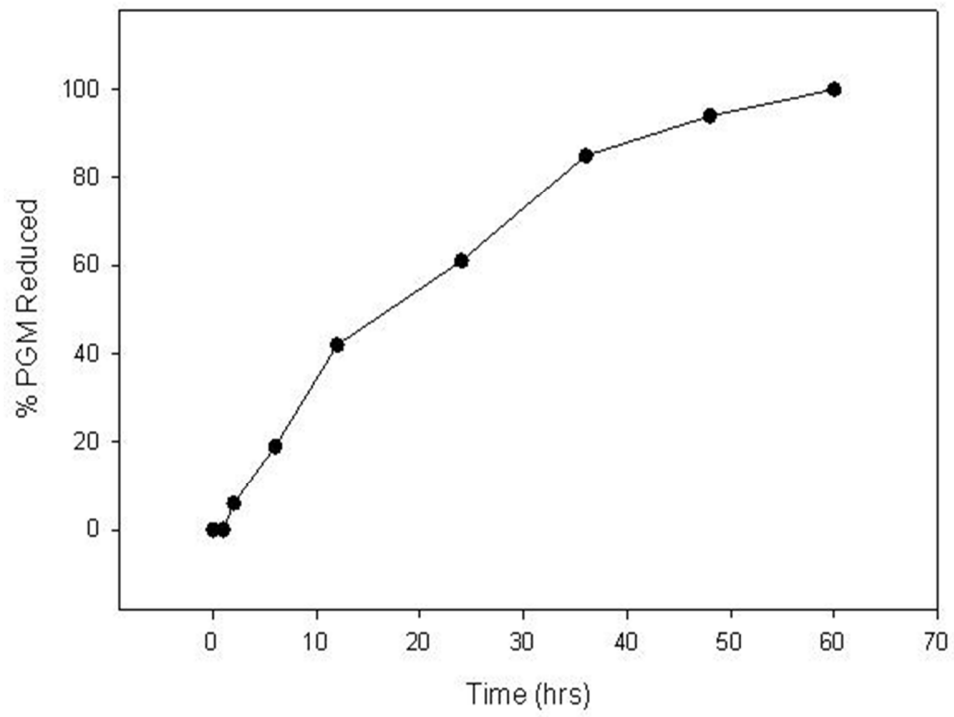


Fig. 4

