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Towards an integrated system for bio-energy: Hydrogen production by *Escherichia coli* and use of palladium-coated waste cells for electricity generation in a fuel cell

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Abstract
Escherichia coli strains MC4100 (parent) and a mutant strain derived from this (IC007) were evaluated for their ability to produce hydrogen and organic acids (OAs) via fermentation. Following growth, each strain was coated with Pd(0) via bioreduction of Pd(II). Dried, sintered Pd-biomaterials ('Bio-Pd') were tested as anodes in a proton exchange membrane (PEM) fuel cell for their ability to generate electricity from hydrogen. Both strains produced hydrogen and OAs but 'palladized' cells of strain IC007 (Bio-Pd_{IC007}) produced ~ 3-fold more power as compared to Bio-PdMC4100 (56 and 18 mW respectively). The power output using, for comparison, commercial Pd(0) powder and Bio-Pd made from *Desulfovibrio desulfuricans,* was ~100 mW. The implications of these findings for an integrated energy generating process are discussed.

Key words: biohydrogen, Escherichia coli, fuel cell, palladium

Introduction

Hydrogen and fuel cell technologies offer a feasible alternative to fossil fuels that is consistent with environmental demands for low carbon energy and zero emissions. Hydrogen is currently produced mainly as a by-product from the petrochemical industry. This is unsustainable and, furthermore, commercial H_2 contains traces of CO, a fuel cell catalyst poison. Currently the life time of a proton exchange membrane fuel cell (PEMFC) is less than 4,000 h (~ 167 days) whereas that required for a commercial market launch is 40,000 h (5 years; Aki et al. 2005). Photovoltaically-driven water electrolysis produces clean H_2 but the requirement for solar energy, the large light capture area, and efficiency of photovoltaic materials are currently limiting factors (MacKay 2008). Use of H_2 as a storable energy vector can overcome the problems of intermittency of renewable power systems.

Clean H_2 can also be made via microbial fermentation of organic wastes (Davila-Vazquez et al. 2008; Redwood et al. 2009). Biomass (sugary, starchy, and ligno-cellulosic wastes) is an abundant renewable resource and is, therefore, potentially capable of supporting a future sustainable H_2 economy. The proportion of the energy demand which could be satisfied in this way is difficult to estimate, but it was reported that diverting landfill-destined cellulosic waste into bioH_2 production in the Netherlands would produce enough H_2 to meet 9% of the domestic energy demand (de Vrije and Claassen 2003). Furthermore it has been predicted that bioconversion of organic waste to biogas could provide up to 7.5% of the UK's 2020 renewable energy commitment (Anon 2009).

Escherichia coli, an example of a facultative anaerobe, converts sugar via the mixed acid fermentation to gaseous products (H_2 and CO_2) and soluble products including the organic acids (OAs) acetate, lactate, formate and small amounts of succinate, and also ethanol (Clark 1989). H_2 production

in *E. coli* is mediated by the formate hydrogenlyase (FHL) complex which is located on the inner cell membrane. FHL catalyses the release of H_2 and CO_2 from formate in response to an increase in intracellular formate, which signals the reduction in extracellular pH caused by OA formation (Penfold etal. 2003; Redwood 2007; Redwood et al. 2009). The bioH_2 is sufficiently clean (after CO_2 removal) to be used directly in a PEMFC without further purification (Macaskie et al. 2005).

Formate is the sole precursor of H_2 in *E. coli* with a maximum yield of 2 mol of H_2 per mol of glucose (Clark, 1989). Although other organisms such as *Enterobacter* and *Clostridium* can produce, maximally, 4 mol H_2/mol glucose via redox carrier-linked reversible hydrogenases these systems are inhibited even at a very low partial pressure of H_2, whereas *E. coli* is unaffected due to the use of an irreversible formate-linked hydrogenase system (FHL). The H_2 yield was improved further by utilising the produced OAs in a downstream photobioreactor to make a second H_2 stream via nitrogenase activity; effectively here the *E. coli* fermentation has a dual function in both H_2 and OA production (Redwood 2007; Redwood et al. 2009).

Bio-hydrogen has additional potential due to its compatibility with fuel cells (Macaskie et al. 2005). These utilise precious metal catalyst to split the H_2 for electricity production; hence the second target for process improvement is the fuel cell itself since robustness is a major limitation for commercial development (Aki et al. 2005; above). Previous work showed that spent yeast biomass, coated with platinum nanoparticles can function as the anode material in a proton exchange membrane fuel Cell (PEMFC) (Dimitriadis et al. 2007) while a parallel study showed that *D. desulfuricans* could behave similarly when coated with either Pd(0) or Pt(0) nanoparticles fabricated via the hydrogenase-mediated reduction of Pd(II) or Pt(IV) (Yong et al. 2007). The PEMFC power output was comparable to commercial Pt(0) fuel cell catalyst under similar conditions (Yong et al. 2007). Cells of *E. coli* can fulfil a similar role but the power output using Bio-$Pd_{E.\ coli}$ was substantially lower than that obtained by using Bio-$Pd_{D.\ desulfuricans}$ (Yong et al. 2008). For economy and sustainability via re-use of the H_2-producing biomass to make Bio-Pd/Pt and hence electricity 'in process', the power output from a PEMFC made with Bio-$Pd_{E.\ coli}$ must be increased, but without detriment to the production of either bioH_2 in the 'dark' fermentation or the organic acid co-products which provide the feedstock for the secondary-stage photofermentation to generate the second H_2 stream (Redwood 2008; Redwood et al. 2009). Hence, the dual objectives of this work were to compare hydrogen production and the 'catalytic quality' of Bio-Pd made from cells of *E. coli* strain MC4100 and its mutant IC007 developed within this study. The downstream photofermentation described elsewhere (Redwood et al. 2009) utilises *Rhodobacter sphaeroides* to make additional H_2 via rapid separation and utilisation of the organic acid products of the *E. coli* fermentation. Therefore, the opportunity was also taken to evaluate the potential to use Bio-$Pd_{R.\ sphaeroides}$ in a PEMFC.

Materials and Methods

Bacterial strains and hydrogen production.

Desulfovibrio desulfuricans was as described previously (Yong et al. 2007). *R. sphaeroides* OU001 was grown as described by Redwood (2007). For comparison of *E. coli* strains, *E. coli* HD701 (hydrogen overproducer; derived from the parental strain *E. coli* MC4100 via upregulation of formate hydrogenlyase: Penfold et al. 2003) was also used. *E. coli* strain IC007 (□*focA*, Δ*focB*, □*nirC*, □*hycA*, Δ*ldhA*, Δ*tatABC*::$Apra^R$) was also derived from the parental strain MC4100. Construction of IC007 took advantage of the PCR-targeting approach of Datsenko and Wanner (2000) and the 'Keio Collection' of single, kanamycin resistance-marked *E. coli* deletion strains (Baba et al. 2006). First, for deletion of *focA* and *focB*, the apramycin resistance cassette and flanking FLP recombinase recognition sequences encoded on plasmid pIJ773 (Gust et al. 2003) were amplified using primers based on the

FRT recognition sequences together with the *focA* or *focB* flanking sequences. In each case the PCR-amplified apramycin resistance cassette was transformed into electro-competent *E. coli* strain BW25113 harbouring plasmid pKD20 and double cross-overs were selected by plating the transformants onto LB plates containing apramycin. Disruption of the targeted gene was confirmed by PCR, and the marked deletions were subsequently transduced using P1 phage into MC4100 using the method of Miller (1992) The apramycin resistance cassette was subsequently 'flipped out' of the marked deletions using the method described by Datsenko and Wanner (2000) to give strains IC00A and IC00B. Loss of the apramycin-resistance cassette was confirmed by PCR and apramycin sensitivity. Using identical methods, the *focA* and *focB* mutant alleles were then combined to give a double mutant strain IC001 (as MC4100, Δ*focA*, Δ*focB*), which was used as a base to build additional mutations upon. The *nirC* gene was deleted first, using identical methods, to yield IC002 (as MC4100, Δ*focA*, Δ*focB*, Δ*nirC*). Next, the Δ*hycA*::Kan^R allele from *E. coli* strain JW2695 was moved by P1 transduction into IC002 and flipped out using pCP20 (Amp^R) to yield strain IC003 (as MC4100, Δ*focA*, Δ*focB*, Δ*nirC*, Δ*hycA*). Next, a Δ*ldhA*::Cam^R allele, which was prepared by PCR, was introduced into IC003 and flipped out resulting in IC005 (as MC4100, Δ*focA*, Δ*focB*, Δ*nirC*, Δ*hycA*, Δ*ldhA*). Finally, a Δ*tatABC*::Apra^R cassette was prepared by PCR and introduced into IC005 to yield IC007 (as MC4100, Δ*focA*, Δ*focB*, Δ*nirC*, Δ*hycA*, Δ*ldhA*, Δ*tatABC*::Apra^R). The genotypic identity of strain IC007 is summarised in Table 1.

Stocks of *E. coli* strains were maintained at -80 °C in 75% glycerol, plated on nutrient agar (Oxoid) and incubated overnight at 30 ºC. For experiments, colonies were picked for pre-culture into nutrient broth (Oxoid, 5 mL), with sodium formate (0.5% w/v) pH 7, and shaken (6h, 30 °C). Cells were inoculated (10 µL) in two 2 L Erlenmeyer flasks containing 1 L of the same medium and shaken for 16 h (30 °C). Cell pellets, obtained by centrifugation, were washed twice in 100 mL phosphate buffered saline (PBS: 1.43 g Na_2HPO_4 0.2 g KH_2PO_4, 0.8 g NaCl, 0.2 g KCl /L, pH 7.0) and resuspended in 20 mL PBS to produce a concentrated inoculum for fermentation containing ~ 40—46 g dry weight/L (OD_{600nm} of 1 corresponded to a concentration of 0.48 g dry weight/L (a previously determined conversion factor).

Fermentations (two conditions, each in duplicate) were performed consisting of a 5 L fermenter (Fermac 200-series, Electrolab UK) with pH, temperature and agitation control, a "scrubber" column containing 2 M NaOH, a H_2 collector cylinder full of water and a N_2 gas supply. At least 99.5% of CO_2 was trapped in the scrubber solution as verified by GC (Redwood 2007) and H_2 was collected and quantified by water displacement in a graduated collector cylinder. All tubes and connectors were previously sterilized with 70% ethanol.

The fermentation vessels contained 3 L of fermentation medium (de-ionized water; in each 3 litre batch: 42.60 g Na_2SO_4, 10.46 g K_2HPO_4, 0.20 g KH_2PO_4, 0.20 g $(NH_4)_2SO_4$, pH 5.8). All solutions were prepared using de-ionized water and analytical grade reagents. The following supplements were aseptically added to the fermenter just before inoculation: 1 M $MgSO_4.7H_2O$ (6 mL), 2 M glucose (30 mL; 20 mM final concentration), trace elements solution (9 mL) and polyethylene glycol antifoam (PEG; 0.5 mL). Trace elements solution comprised (quantities in g/L): $CaCl_2.H_2O$ (0.74); $ZnSO_4.7H_2O$ (0.18); $MnSO_4.H_2O$ (0.10); disodium-EDTA (20.1); $FeCl_3.6H_2O$ (16.7); $CuSO_4.5H_2O$ (0.10); $CoSO_4.7H_2O$ (0.21).

The system was purged with oxygen free nitrogen (OFN), (through a 0.2 µm filter) for at least 30 min before inoculation. pH was maintained throughout the fermentation at pH 5.5-5.7 by dosing 2M NaOH and 2M H_2SO_4 via an Electrolab Fermac260 pH controller, at 30 °C with stirring (300 rpm). To start each experiment the OFN was stopped, nitrogen tubes were depressurized and valves to the gas collector cylinder were opened to allow the system to equilibrate for at least 45 min.

Sampling and analysis

For glucose, organic acids and ethanol analysis, samples were periodically withdrawn from the culture, filtered (0.2 µm supor membrane syringe filter) and stored at -20ºC before analysis. Glucose determination was by the colorimetric dinitrosalicylic acid assay (Chaplin 1986). Organic acids were measured by an anion HPLC (Dionex 600-series) as described previously (Redwood & Macaskie 2006). Ethanol was analysed using a Cecil Adept HPLC system equipped with Resex-RCM column (Phenomenex); RI detector; temperature 75 °C; eluate H_2O; flow rate 0.5 ml/min; 30 min experiment time. Bacterial growth was monitored via the OD_{600} of withdrawn samples (OD_{600}:1 ≡ 0.482 g DW/L; Ultrospec 3300 pro). H_2 production was monitored and measured by the displacement of water from graduated cylinders with time-lapse photography for continuous monitoring. CO_2 was removed into 2M NaOH scrubber solution with universal indicator pH 9-13. Hydrogen in collectors was confirmed using a hydrogen sensor detector. Previous work (Sauter et al. 1992 and DW Penfold, unpublished) showed that CO_2 and H_2 are the only gases evolved under these conditions. The H_2 yield was calculated in relation to a theoretical maximum of 2 mol H_2/mol glucose (Clark 1989); 90% of this yield was achieved previously under similar conditions (Redwood 2007).

Preparation of fuel cell electrode materials.

For experiments to test the utility of 'palladised' cells in a fuel cell *D. desulfuricans* (reference organism: Yong et al. 2007) and *E. coli* strains (this study) were grown as described by Yong et al. (2002) and Penfold et al. (2006), respectively. Cells were palladised as described previously (5% Pd by mass; Yong et al, 2007), washed in acetone dried and ground, and then transferred into 10 mL alumina ceramic crucibles for sintering (i.e. carbonising) in a programmable furnace. The temperature was increased from room temperature to 700 ^{o}C within 4 h and held at 700 ^{o}C for a further 4 h before cooling to room temperature in the furnace.

Commercial submicron Pd powder (C-Pd) (Sigma-Aldrich, Germany), and 'Bio-Pds' (20 mg of each, as metal) were mixed separately with pure activated carbon powder (80 mg; BDH Chemicals Ltd, UK). Nafion® perfluorinated ion-exchange resin (0.2 mL; 10 wt % in water, Sigma-Aldrich) and water (1.0 mL) were added to each sample containing 20% of Pd and 80% of C. The sample was mixed well, manually applied evenly onto 16 cm^2 teflon-treated carbon paper (Fuel Cell Scientific, USA), and dried at room temperature. The lab-made electrodes were tested for electricity production versus the commercial Pd powder in a small proton exchange membrane (PEM) fuel cell as described previously (Yong et al. 2007) and the maximum power output (mW) was recorded.

Results and Discussion

Hydrogen and organic acid production by E. coli strains MC4100 and IC007

H_2 production (mL), biomass (g), glucose consumption (mmol), organic acid (OA) and ethanol production (mmol) were measured and quantified; reproducibility was usually within 10% between separate experiments. Hydrogen overproduction by *E. coli* strain HD701 (via derepression of the H_2-producing formate hydrogen lyase complex (Penfold et al. 2003)) and by strains deficient in uptake hydrogenases (Penfold et al. 2006; Redwood et al. 2008) were described previously and production of OA by the latter was also quantified (Redwood & Macaskie 2006). A combination of the *hycA* and *tat* mutations did not increase hydrogen production over that obtained with one mutation alone (Penfold et al. 2006). Hence, in this work, further metabolic engineering of *E. coli* was performed. A new strain (IC007) was prepared that combined mutations additional to those in the *hycA* and *tatABC* genes previously studied (Table 1). Strain IC007 was rendered devoid its formate transporters (FocA and

FocB), and the FocA/B homolog NirC, in an attempt to impair formate secretion during fermentation and utilise early and sustained formate hydrogenlyase (FHL) activity. In addition, strain IC007 lacks an active *ldhA* gene, which encodes an NADH-dependent lactate dehydrogenase that is normally active in the later stages of fermentative growth. The rationale in removing LdhA was to prevent the metabolism of pyruvate to lactate and, instead, shunt additional pyruvate towards formate and hence boost H_2 production.

The fermentation kinetics of strains MC4100 and IC007 are compared in Figure 1 and summarised in the fermentation balances shown in Table 2. During a 24h fermentation, both strains grew very slightly, limited by the low concentration of added nitrogen source; note that prior to the incubation the cells had been grown aerobically to high density, with formate as a FHL inducer (Redwood et al. 2008). The parent strain MC4100 consumed all of the initial added glucose within 24h, whereas the mutant strain IC007 consumed only 70% of the added glucose over 24h. Correspondingly, the MC4100 parent strain evolved H_2 more rapidly than strain IC007 (Fig. 1 a,b), the rate peaking at ~ 4.2 mmol H_2/h during the initial 10h and gradually decreasing within 24h. The overall yields of H_2 produced were 41% and 37% for strains MC4100 and IC007, respectively, and not significantly different on the basis of the two experiments reported (Table 2).

Analysis of OA production (Fig 1 c,d and Table 2) showed that lactate was the main organic product of the parent strain MC4100 but the lactate yield was negligible in strain IC007 due to the *ldhA* mutation. However, the result of this was not an increase in H_2 production but in non-lactate by-products, especially pyruvate, succinate and ethanol. Despite the loss of lactate production, the overall yield of H_2 in strain IC007 was not significantly different from the parent strain MC4100, which can be attributed to the accumulation of alternative byproducts (succinate) and H_2 precursors (pyruvate and formate). The inability of strain IC007 to reassimilate all of the formate initially secreted into the reaction medium may be attributed to the lack of dedicated formate transport machinery in this strain and partially accounts for the reduced H_2 yield observed. Clearly, lactate dehydrogenase is a major route for pyruvate dissimilation and recycling of NADH during fermentation. The loss of *ldhA* leads to the accumulation and ultimate secretion of pyruvate; the shortfall in NADH recycling via removal of lactate production is compensated by an increased production of ethanol and succinate.

Similar observations regarding different *ldh* mutants were made by Mat-Jan et al. (1989) and Zelic et al. (2004) in *ldh* mutants. However, we also observed that when strain IC700 was allowed to ferment for a further 24 h, the pyruvate was reassimilated, which is in accordance with the observations of Sode et al. (1999) where, likewise, the reaction was followed to completion.

We conclude that eliminating lactate production did not benefit the H_2 yield in this study as the additional intermediate either accumulated or gave enhanced production of alternative products, particularly succinate and ethanol. Eliminating succinate production would be difficult due to the key role of succinate-producing enzymes in central metabolism. Further, succinate is potentially useful in the downstream photobioreactor and hence could be re-used 'in process'. Jointly upregulating the activity of pyruvate formate lyase and FHL could hypothetically channel more pyruvate intermediate towards H_2 production, an approach to be addressed in future work. Work is currently in progress to reduce the production of ethanol since this product cannot be transferred easily into the downstream photobioreactor but this metabolic engineering will have implications in the ability of the cells to recycle NADH and such mutants may be highly impaired or non-viable.

Electricity production by palladised cells of E. coli in a fuel cell.

Cells of the *E. coli* strains MC4100 and IC007 (this study), together with strain HD701 (hydrogen production was as described previously: Penfold et al. 2003), *R. sphaeroides* (Redwood 2007) and *D. desulfuricans* (positive control) were coated with palladium nanoparticles as described previously

(Yong et al. 2007) and tested for their ability to produce electricity as anodes in a PEM fuel cell. Sintering of palladised biomass was essential to obtain catalytic activity (P. Yong, unpublished) since biomass is predominantly water and sintering carbonises the biomass to make it electrically conductive. As shown in Fig. 2 and Table 3, Bio-$Pd_{D.\ desulfuricans}$ produced power comparably to commercial finely-ground Pd(0) while Bio-Pd_{MC4100} and Bio-Pd_{HD701} produced little power (~18-28 mW). However Bio-Pd_{IC007} produced ~ 56 mW, which was ~ three-fold higher than the parent strain (~18 mW) or Bio-$Pd_{R.\ sphaeroides}$. (~ 20 mW). Representative data are shown, since the power output is critically dependent on the exact preparation of the electrode but in 3 replicate experiments the Bio-Pd(0) of the mutant outperformed that of the parent strain consistently as shown.

From these results it can be concluded that the catalytic activity of the palladium in a fuel cell relates to the enzymatic composition and localisation in the strain which produced it. A similar conclusion with respect to chemical catalysis was drawn (Rousset et al. 2006) using a mutant of *D. fructosovorans* which lacked its periplasmic hydrogenases; here, the Pd(0) relocated to the site of the remaining cytoplasmic hydrogenase (Mikheenko et al. 2008) and was catalytically more active in the reduction of Cr(VI) (Rousset et al. 2006). In the present case the key difference between strains HD701 and IC007 is that the Tat transport system is missing, and therefore there are no functional periplasm-facing hydrogenases. Therefore, strain IC007, like the *D. fructosovorans* mutant (above) may be location-modified with respect to making Pd-nanoparticles. However no obvious differences were observed in initial comparisons of the Bio-Pd produced by strains MC4100 and IC007 (Yong et al. 2010) and a high resolution TEM study is clearly warranted. Taken together, the two studies using *D. fructosovorans* and *E. coli* indicate that 'relocating' the Pd-nanoparticles may be beneficial to the 'quality' of the catalyst produced. Potential pleiotropic effects of the mutations introduced into strain IC007 were not investigated but warrant further investigation. The extent to which the 'biochemical' component of the Bio-Pd moderates the catalytic activity of its Pd(0) 'partner' after sintering of the material is a subject of current investigation.

Conclusion

In terms of H_2 production strain IC007 offers little benefit over a 24 hour period as compared to its parent strain MC4100, although its ability to produce more succinate and pyruvate could be beneficial as secondary products. However, in terms of an integrated energy process, where biomass is re-used 'in process' the mutant strain offers more potential to make a better fuel cell although this still falls short (by ~ 50%) of the use of *D. desulfuricans* as a support for 'Bio-Pd' for this purpose. The extent to which further molecular engineering could improve the rates and yields of hydrogen production without compromising fuel cell catalyst performance is the subject of ongoing studies.

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Table 1 Genotypic identity of strain *E. coli* IC007

Deletion	Affected gene product (*resultant phenotype*)	Reference
Δ*focA*	Formate transporter (*impaired ability to secrete and take up formate*)	Suppmann et al. 1994
Δ*focB*	Putative formate transporter (*impaired ability to secrete and take up formate*)	Saier et al. 1999
Δ*hycA*	Formate hydrogen lyase (FHL) regulatory protein (*upregulated FHL; H_2 overproduction*)	Sauter et al. 1992; Penfold et al. 2003
Δ*ldhA*	NADH-linked fermentative lactate dehdrogenase (*lactate not produced*)	Bunch et al. 1997
Δ*tatABC*	The twin arginine translocation system: transports folded proteins over membranes (*tat inactivation prevents uptake hydrogenase transport; increases H_2 production*)	Berks et al. 2003; Miller 2005 Penfold et al. 2006
Δ*nirC*	Nitrite transporter, paralogous with FocA/B (*impaired ability to secrete and take up formate*)	Clegg et al. 2002

Strain IC007 was made from its parent MC4100 as described in Materials and Methods. It combines the ability to overproduce hydrogen via deletion of the FHL regulator FhlA and the Tat system with impaired ability to transport formate. It does not make lactate, potentially increasing the metabolic flux into the other fermentation products.

Table 2. Fermentation balance of *E. coli* strains MC4100 and IC007

Strain	Repeat	Time	Product yields (mol/mol glucose)*								Growth (g/mol glucose)	Carbon balance ***
			Lac	Pyr	Form	Succ	Ac	EtOH	H_2	CO_2 **		
MC4100	Expt 1	22.75	0.892	0.000	0.048	0.200	0.354	0.405	0.788	0.51	3.99	99%
	Expt 2	22.75	0.789	0.000	0.024	0.196	0.326	0.438	0.849	0.54	4.49	93%
IC007	Expt 1	23.5	0.008	0.518	0.079	0.492	0.227	0.704	0.735	0.36	4.41	102%
	Expt 2	23.5	0.007	0.527	0.069	0.479	0.222	0.685	0.760	0.36	2.64	99%

* Propionate and butyrate were not detected. Lac: lactate; Pyr: pyruvate; Form: formate; Succ: succinate; Ac: acetate; EtOH: ethanol

** The yield of CO_2 was estimated as described by Redwood et al (2007), equation 2.

*** Carbon balance was calculated as Redwood et al (2007), but including pyruvate (3 carbon equivalents).

Table 3. Maximum power output (P_{max}, mW) from a PEM fuel cell using anodes made from commercial precious metals and Bio-Pd..

Preparation	P_{max} (mW)	Reference
Commercial Pt(0)	170	Yong et al. 2007
Bio-Pt$_{D.\ desulfuricans}$	170	Yong et al. 2007
Commercial Pd(0)	100	This study
Bio-Pd$_{D.\ desulfuricans}$	108	This study
Bio-Pd$_{E.\ coli}$ HD701	28	This study
Bio-Pd$_{E.\ coli}$ MC4100	18	This study
Bio-Pd$_{E.\ coli}$ IC007	56	This study
Bio-Pd$_{R.\ sphaeroides}$ OU001*	19.5	Redwood 2007

Native metallised biomass was inactive in a PEMFC. Samples (all 5% Pd w/w) were, therefore, sintered before use to carbonise the biomass, as described in Materials and Methods.

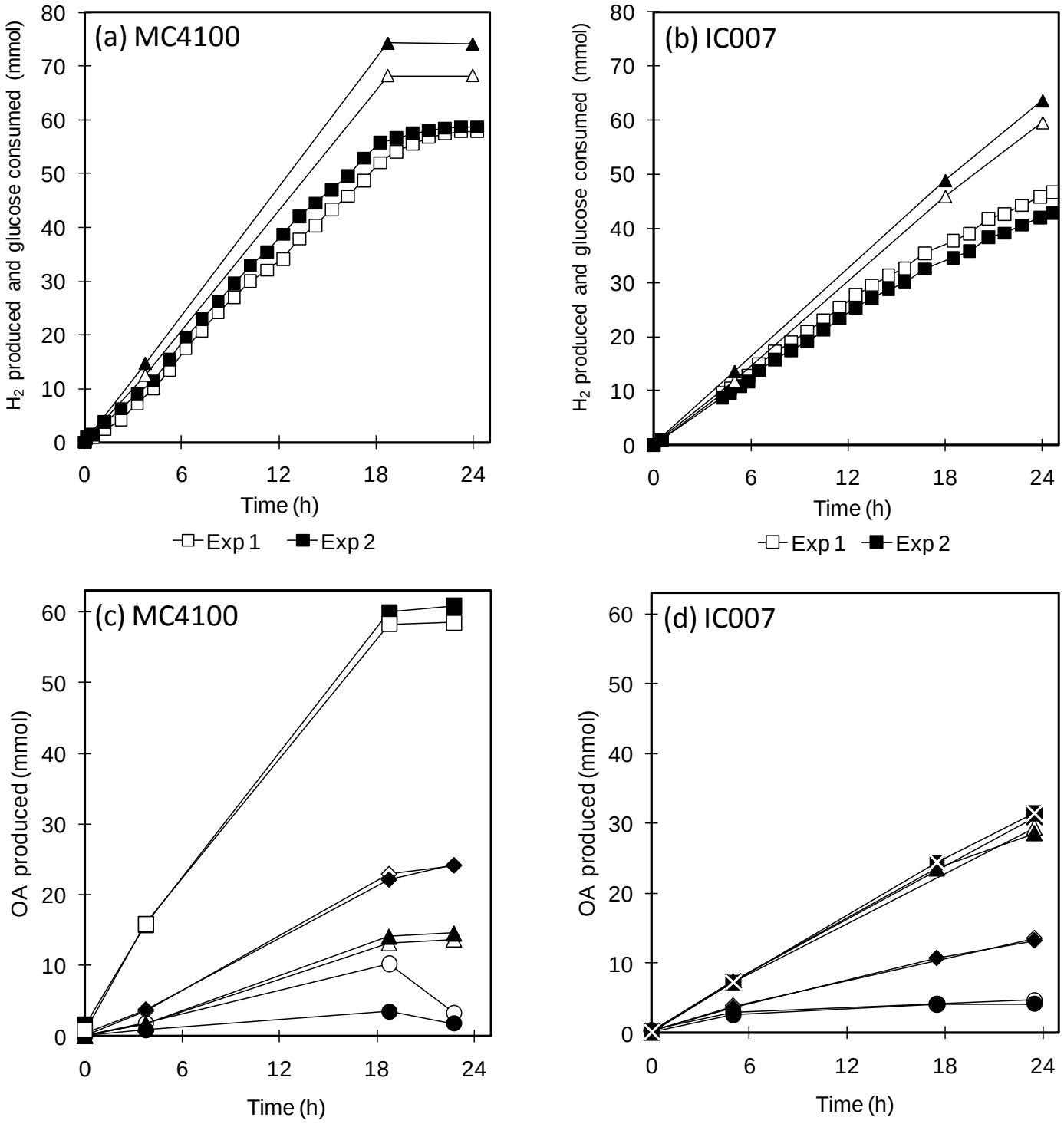

Figure 1. Hydrogen production (a,b) and organic acid production (c,d) from *E. coli* MC4100 (a,c) and IC007 (b,d). Open and filled symbols represent two independent experiments. Hydrogen production and glucose utilization (a,b) are shown as total mmoles to aid comparison. Masses are shown in mmol: (△,▲) Glucose utilization; (□,■) Hydrogen production in a and b. Fermentation products in c and d are: (□,■) lactate; (◇,◆) acetate; (○,●) formate; (△,▲) succinate; (×,⊠) pyruvate.

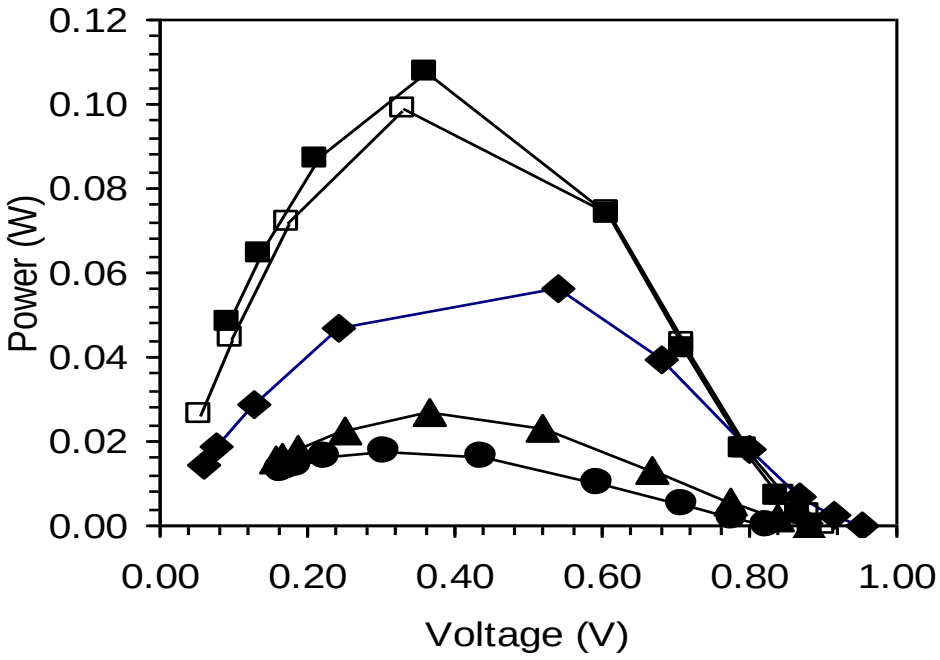

Figure. 2. Power production in a PEM fuel cell using alternative anode catalysts: commercial Pd(0) (□) and palladised, sintered cells (Bio-Pd) made from *D. desulfuricans* (■) and *E. coli* strains MC4100 (●); HD701 (▲) and IC007 (◆).