Supplementary material

A role for microbial–palladium nanoparticles in extracellular electron transfer

Xuee Wu[1], Feng Zhao[1,2*], Nelli Rahunen[3], John R. Varcoe[1], Claudio Avignone–Rossa[3], Alfred E. Thumser[4], Robert C.T. Slade[1]

[1]Chemical Sciences, [3]Microbial Sciences, [4]Biological Sciences,

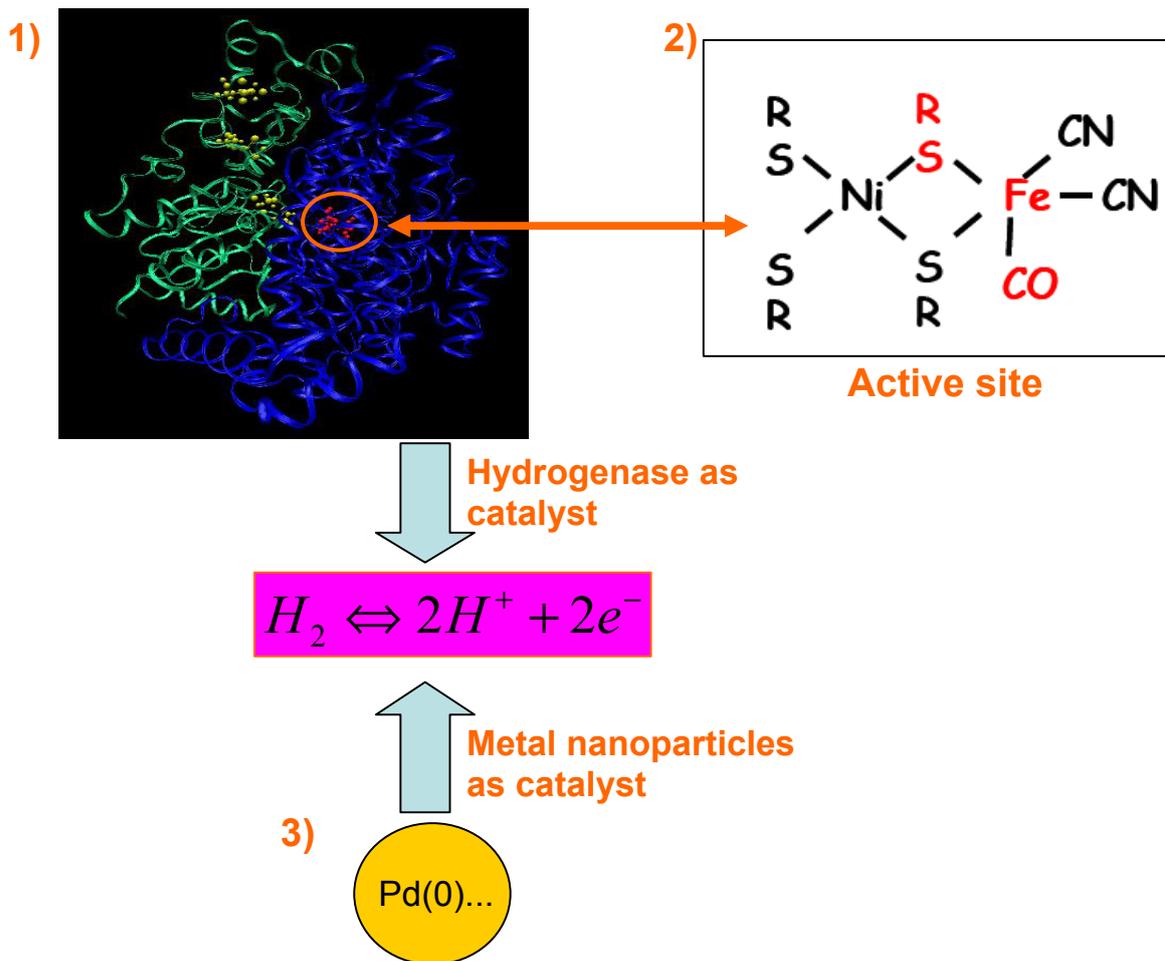*University of Surrey, Guildford, GU2 7XH, United Kingdom*

[2]Institute of Urban Environment, Chinese Academy of Sciences

Xiamen 361021, P. R. China

Email:; fzhao@iue.ac.cn; f.zhao@surrey.ac.uk

Supplementary Fig. 1. Summary of previously reported elemental transformation into lower valence species via microbial reduction reactions[1–7]. Products of elemental metals (valence = 0), elemental non–metals (valence = 0), metallic oxides/ions (valence ≠ 0) and metalloid (valence = 0) indicated by red, yellow, green and brown respectively, and the methylation of Hg, As, Se, Sn and Te etc are not considered in this diagram. The elements (i.e. O, S, Se and Te), which are in the same group, can be reduced to the products of valence = 0. However, no bio-produced Fe, Ni or Cu (valence = 0) particles, which elements are in the same group with the noble metals, were reported. Through microbial evolution of billions of years, there is potentially a wide diversity of electron transport pathways/mechanisms that are yet to be fully elucidated and understood e.g. what features make the microbial nano–pili electronically conductive?[1] Are there any effects of the membrane bounded metallic-nanoparticles of high electronic conductivity on microbial growth or metabolism?

1)

2)

R S\
S Ni
S
R

R S CN
S Fe — CN
S CO
R

Active site

Hydrogenase as catalyst

$$H_2 \Leftrightarrow 2H^+ + 2e^-$$

Metal nanoparticles as catalyst

3)

Pd(0)...

Supplementary Scheme 1 hydrogenase and metal nanoparticles as catalysts for hydrogen oxidation and production. **1)** [NiFe]-hydrogenase; **2)** the structure of active site; **3)** membrane bound bio-nanoparticles. The following mechanism of the nanoparticle production is proposed: Pd(II) is absorbed into the cell and then reduced by hydrogenases and/or cytochrome to elemental Pd(0); periplasmic [FeFe]–hydrogenases serve as the nucleation sites for the initial formation of the metallic nanoparticles, which are then secreted through the cell membrane and undergo size growth. The bio-nanoparticles (e.g. Pd, Pt, Rh and Au) have outstanding catalytic properties in various redox reactions. Fe or Ni (valence = 0) particles generally have little catalytic activity, but the [Fe-Ni] unit (i.e. active site) are key part of hydrogenase, which has been known as catalyst for hydrogen reactions of energy chain in membrane.
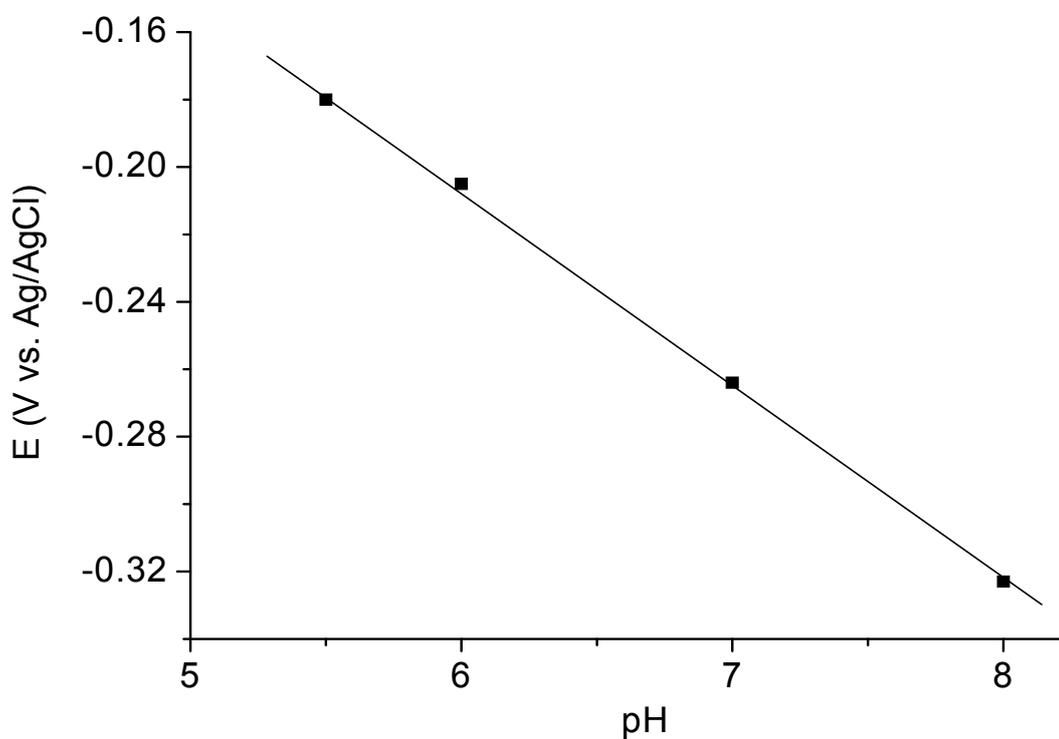
Supplementary Fig. 2. Effect of solution pH on the reduction potential for the direct electron transfer reaction of a *D. desulfuricans* modified GC electrode. An increase in pH causes a negative shift in the reduction peak with a slope of -60 mV pH^{-1} (the oxidation peaks show a similar profile – data not shown). The results indicate that proton transport inside the biofilm limits current generation in bioelectrochemical systems (e.g. microbial fuel cells[8]).
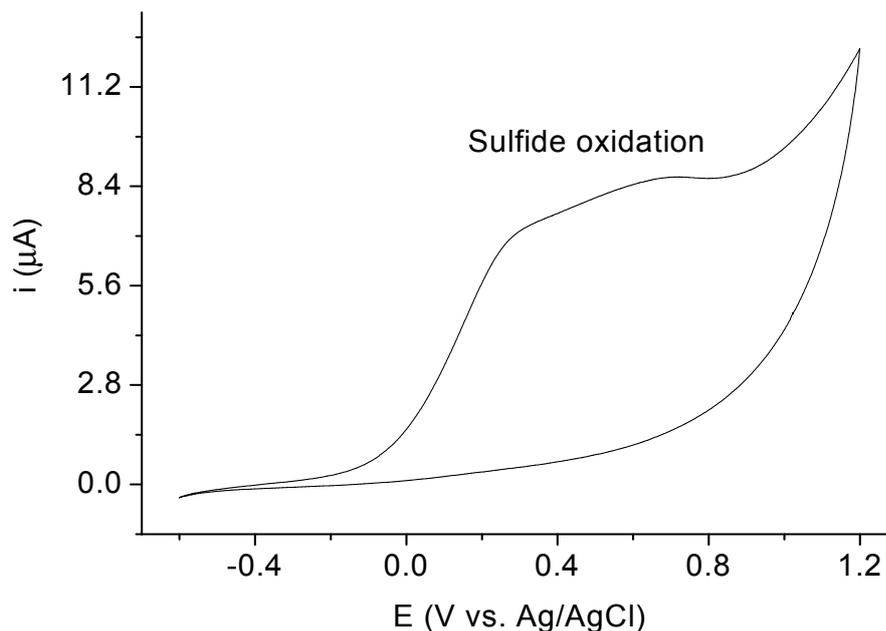
Supplementary Fig. 3. Cyclic voltammogram of a pristine glassy carbon electrode in 50 mM phosphate buffer (bacteria-free) in presence of sulfide with a scan rate was 10 mV s^{-1}. Sulfide, in principle, can be simultaneously oxidized to elemental sulphur and polysulphide species in neutral aqueous solutions because equilibrium potentials for these processes are similar; further oxidation, in parallel or consecutively, can occur depending on the prevailing experimental conditions (*e.g.* sulfate can be generated on increased potential). The broad oxidation peak suggests a mixture of products of sulfide oxidation (*i.e.* elemental sulfur, sulfite, thioshlfate and sulfate etc.)[9]. For detailed investigations of microbial electron transfer mechanisms, the use of careful background/control experiments is required to exclude interferences from the indirect electron transfer via electrochemical oxidation of microbially produced sulfide. In the present case, the cell suspensions were centrifuged and washed to remove the electroactive metabolites and secreted products before coating on the glassy carbon electrode; where immediate production of current was observed in Figure 2.
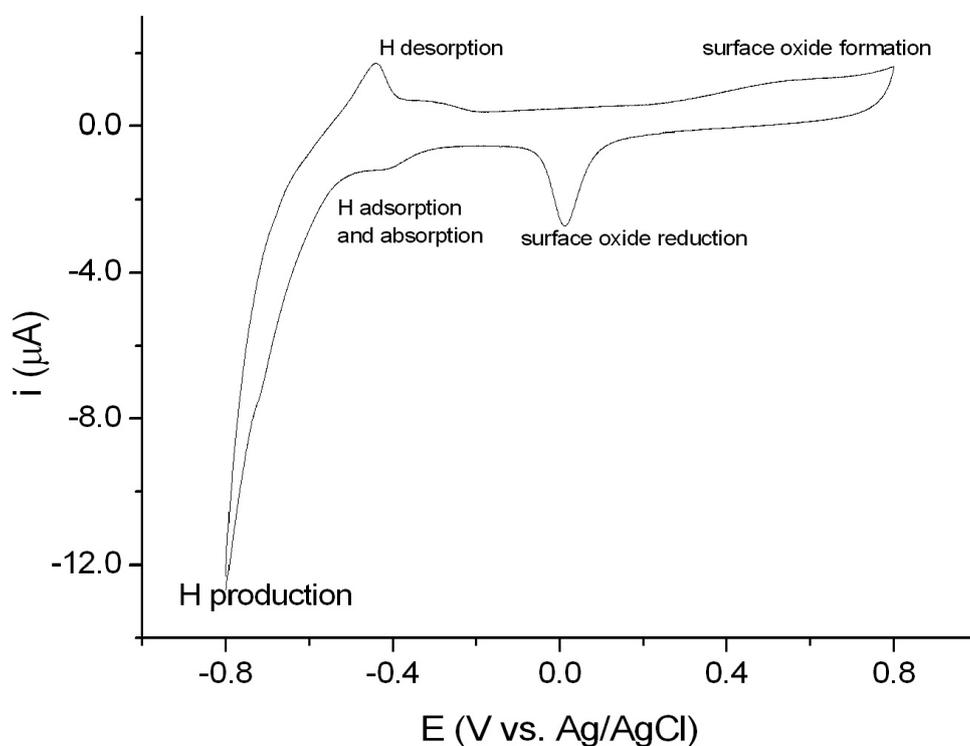
Supplementary Fig. 4. Cyclic voltammogram of a Pd–only modified GC electrode (bacteria–free) in 50 mM pH=7 phosphate buffer purged with nitrogen at a scan rate of 10 mV s^{-1}. The redox waves at high positive potentials are caused by the formation and reduction of surface PdO and PdO_2 compounds. The peaks at –0.4 V are considered to be the results of hydrogen desorption and adsorption on the surface of α– or β–phases of Pd. Hydrogen evolution on Pd occurs at more negative potentials[10–12].
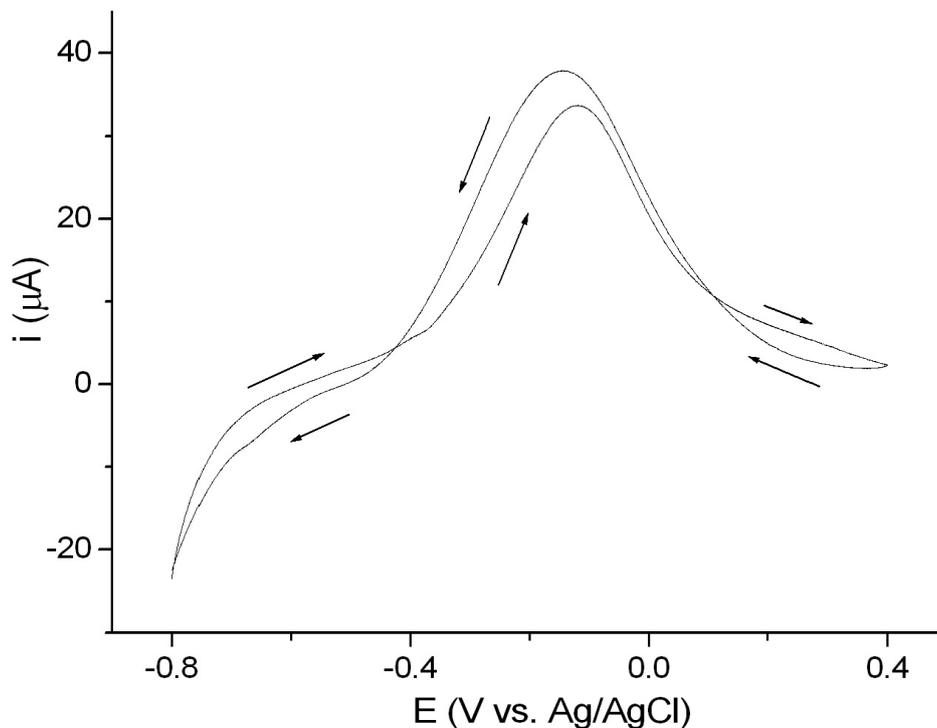
Supplementary Fig. 5. Cyclic voltammogram of Pd–only modified GC electrode (bacteria–free) in 50 mM pH=7 phosphate buffer in the presence of formate (concentration) at a scan rate of 50 mV s^{-1}. Pd and other platinum–group metals exhibit high catalytic activity towards abiotic electrochemical processes such as hydrogen evolution and absorption and the reduction/oxidation of organic compounds. The voltammogram is in good agreement with previous reports[13,14]. Formate oxidation to CO_2 on a Pd surface mainly occurs via dehydrogenation; the CO intermediate (identifiable by infrared reflectance spectroscopy) is a poisoning species when adsorbed and is formed on the Pd surface at higher positive potentials. The high oxidation current obtained on the reverse potential sweep is due to the regeneration of the active Pd surface sites.
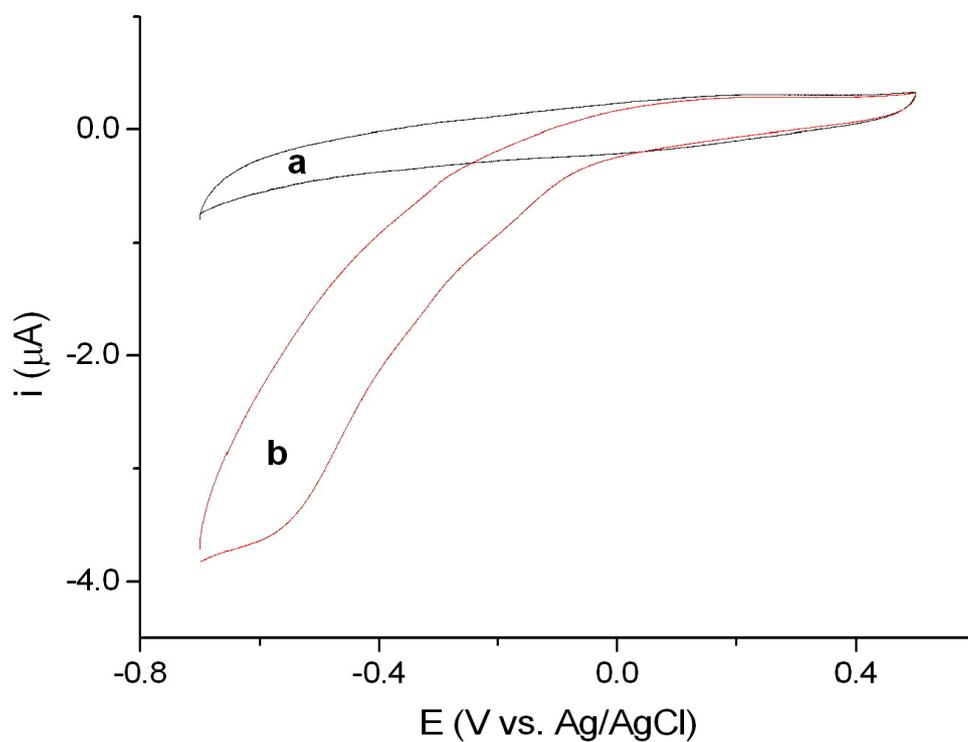
Supplementary Fig. 6. Cyclic voltammograms of bio–Pd–modified *D. desulfuricans* coated onto a GC electrode in 50 mM phosphate buffer in the presence of nitrogen (a) and air (b) at a scan rate of 10 mV s^{-1}. The results show that bio–produced Pd is an excellent oxygen reduction catalyst due to the small crystal sizes and high kinetic activity; this raises the possibility of utilization in the cathodes of bioelectrochemical systems (*e.g.* microbial fuel cells or microbial electrolysis cells)[15, 16] and abiotic electrochemical systems (*e.g.* traditional chemical fuel cells).

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