

## Electrolysed palladium has the potential to increase methane production by a mixed rumen population in vitro

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H<sub>2</sub>-transfer between methanogens and bacteria or fungi from the rumen have been demonstrated (Wolin and Miller, 1988, in: *The Rumen Microbial Ecosystem*, Hobson, ed). Methanogens attach to protozoa in the rumen and it has been hypothesised that this is a commensal relationship where the methanogens use hydrogen (H<sub>2</sub>) generated by the protozoa (Vogels et al, 1980, *Appl Environ Microbiol*, 40, 608-661). Boone and colleagues (1989, *Appl Environ Microbiol*, 55, 1735-1741) argue that H<sub>2</sub> cannot diffuse to the cell surface of methanogens from the rumen liquid at a rate fast enough to account for the amount of methane (CH<sub>4</sub>) produced. When H<sub>2</sub> was bubbled into the rumen the proportion of protozoa with attached methanogens decreased, however no estimate of CH<sub>4</sub> production under these circumstances was made (Stumm et al, 1982, *Br J Nutr*, 47, 95-99). We report here preliminary findings from a study to use electrolysed palladium to provide a source of H<sub>2</sub> for methanogens in a mixed rumen population in vitro.

Palladium (Pd) can be saturated with up to 0.88 moles H<sub>2</sub> per mole of metal by electrolysis (Green and Quickendon, 1994, *J Electroanal Chem*, 368, 121-131). Palladium as the cathode was electrolysed in 1M H<sub>2</sub>SO<sub>4</sub> using 200 mA and a platinum anode. The rate of release of H<sub>2</sub> after electrolysis is determined by the ratio of surface-area to volume of the metal; after electrolysis for 16 h a 10g lump of Pd continued to release H<sub>2</sub> for 2 h, whereas c.a. 300mg of Pd wire (250µm diameter) released H<sub>2</sub> for only 10 min.

Six 120 ml serum vials containing 30 ml ovine rumen contents were filled with H<sub>2</sub>:CO<sub>2</sub> (80%:20%). Pd wire (162 and 177mg, 250 µm diameter) was added to two of the vials, electrolysed Pd wire (283 mg and 286 mg 250 µm diameter) was added to two of the vials, and

the remaining two vials were controls. The vials were re-filled with H<sub>2</sub>:CO<sub>2</sub>, sealed with butyl rubber stoppers and incubated at 39°C with shaking. The volume of gas evolved was measured and duplicate analyses were made of the composition of the gasses in the headspace at the start of incubation and 2 h later. Gas samples were analysed by gas chromatography for CH<sub>4</sub> and H<sub>2</sub> using a carbosphere column, a thermal conductivity detector and argon as the carrier gas.

At the beginning of the incubation there was no detectable CH<sub>4</sub> and the average concentration of H<sub>2</sub> was 45.1 µmoles/ml. After 2h there was no significant difference (*p* < 0.05) in the concentration of CH<sub>4</sub>, H<sub>2</sub> or the ratio of CH<sub>4</sub>:H<sub>2</sub> between the control and non-electrolysed Pd. Replication in the two vials containing electrolysed Pd was poor; the concentrations of hydrogen in the headspace gasses were similar but the concentration of CH<sub>4</sub> in one vial was double that in the other. This suggests that electrolysed Pd provides H<sub>2</sub> that is more easily accessed by the methanogens than the H<sub>2</sub> in the gas headspace. Analysis of the surface of the electrolysed Pd from both vials by scanning electron microscopy revealed a coating of nickel on the surface of the Pd in the vial with the lowest CH<sub>4</sub> concentration. This piece of Pd wire had been electrolysed in an earlier experiment using a stainless steel anode. Since repeated electrolysis reduces the amount of H<sub>2</sub> that can be absorbed by Pd (Green and Quickendon, 1994) this may explain the difference in methane production in the two vials.

These data indicate the potential to use electrolysed Pd as a source of H<sub>2</sub> for methanogens in vitro. The value of electrolysed Pd for an in vivo study of interspecies H<sub>2</sub>-transfer will be the subject of further investigations.

Concentration (µmoles/ml) in the headspace (mean ± SD)

Time (h) from the start of incubation	Methane (2)	Hydrogen (2)	CH <sub>4</sub> :H <sub>2</sub>
Control	5.4 ± 0.02	39.0 ± 0.05	0.14
Palladium	5.9 ± 0.95	55.5 ± 12.8	0.11
Electrolysed Pd (used)	5.3	35.6	0.15
Electrolysed Pd (new)	12.6	28.3	0.44