Manuscript details

Manuscript number

Title

Article type

Abstract

Keywords

HE_2015_297

Hydrogen as electron donor for copper removal in bioelectrochemical systems

Full length article

Hydrogen gas is an attractive alternative electron donor since it is produced in large quantities as a side product in the metallurgical industry. Aim of this study was to demonstrate that microbial anodic hydrogen oxidation on a non-catalyzed graphite electrode can be coupled with cathodic copper reduction in a BES to simultaneously recover copper and produce power. The strategy was first to grow an anodic biofilm on acetate, then replace the acetate with hydrogen as electron donor, and finally combine hydrogen oxidation with copper reduction in the cathode. The maximum current density was 1.8 A/m2 at -250 mV anode potential vs Ag/AgCl. When coupled with Cu2+ reduction, the maximum power density was 0.25 W/m2 at a current density of 0.48 A/m2. Anode overpotentials were higher compared to acetate oxidation, probably a result of limited hydrogen solubility and transfer.

Hydrogen gas; Copper recovery; Bioelectrochemical systems;

Manuscript category

Fuel Cells & Applications

Corresponding Author	Pau Rodenas Motos	
Order of Authors	Eleftheria Ntagia, Pau Rodenas Motos, Annemiek Ter Heijne, Cees Buisman, Tom Sleutels	
Suggested reviewers	Cesar Torres, Xia Huang, Oskar Modin	

Submission files included in this PDF

File Type	File Name		
Manuscript	manuscript for Journ Hydr c.docx		
Highlights	Highlights c.docx		
Response to reviewers	response to reviewers.docx		
Figure	figure1.TIF		
Figure	figure2.TIF		
Figure	figure3a.TIF		
Figure	figure3b.TIF		
Graphical Abstract	toc.tif		
Cover Letter	cover letter Intern Journ Hydro Ene.docx		

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Hydrogen as electron donor for copper removal in bioelectrochemical systems

3E. Ntagia^{1,*}; P. Rodenas^{1,2,*}; A ter Heijne²; C.J.N. Buisman^{1,2};T.H.J.A.

4Sleutels^{1,#}

51 Wetsus, european centre of excellence for Sustainable Water Technology, Oostergoweg 9, 68900 CC Leeuwarden, The Netherlands

72 Sub-Department of Environmental Technology, Wageningen University, Bornse Weilanden

89, 6708 WG Wageningen, The Netherlands

9* Both authors contributed equally

10# corresponding author tom.sleutels@wetsus.nl

12**Abstract**

13Hydrogen gas is an attractive alternative electron donor since it is produced in large quantities as a 14side product in the metallurgical industry. Aim of this study was to demonstrate that microbial anodic 15hydrogen oxidation on a non-catalyzed graphite electrode can be coupled with cathodic copper 16reduction in a BES to simultaneously recover copper and produce power. The strategy was first to 17grow an anodic biofilm on acetate, then replace the acetate with hydrogen as electron donor, and 18finally combine hydrogen oxidation with copper reduction in the cathode. The maximum current density 19was 1.8 A/m² at -250 mV anode potential vs Ag/AgCl. When coupled with Cu²⁺ reduction, the maximum 20power density was 0.25 W/m² at a current density of 0.48 A/m². Anode overpotentials were higher 21compared to acetate oxidation, probably a result of limited hydrogen solubility and transfer.

23Introduction

24Significant volumes of heavy metal containing wastewaters are produced continuously at metal 25producing or electroplating companies. Heavy metals have been proven highly toxic for human, 26microbial and plant life[1,2]. Because heavy metals, even at low concentrations have negative effects 27on the environment, there is a need to remediate metal containing waste streams. Moreover, metal 28resources are finite, making their recycling crucial, since their production and transportation results in 29high energy consumption and arises unwanted and highly pollutant gas, solid and liquid 30emissions[3,4].

31Conventional heavy metal removal technologies include electrochemical, chemical precipitation and 32ion-exchange. Although these technologies are applied in practice and a large scale their economic 33and environmental impact could still be improved by reduction of the high operational costs due to 34chemicals and energy consumption and the reduction of excessive production of hazardous wastes. 35This improvement could possibly also be achieved by the removal of these heavy metals in 36bioelectrochemical systems (BES).*[3,5]*. In order to improve economic revenue and environmental 37impact of heavy metal treatment, we propose the removal of metals with BES. In BES the costs for 38chemicals and also for energy are minimized, and even power is produced in some cases; the 39biological oxidation of organic substrates provides part of required energy input. Three basic 40components comprises a BES: an anode, where substrate is oxidized and electrons are entering an 41electrical circuit, a cathode where a reduction reaction takes place and electrons are leaving the 42electrical circuit and, in most of the cases, an ion-exchange membrane that keeps the anolyte and 43catholyte separated and prevents substrate/product crossover[6].

44Several metals have been demonstrated as electron acceptors in BES cathodes such as silver [7,8], 45iron [9–11], Nickel [12], zinc [13] and copper [13–19]. A common feature in all these studies is that the 46electron donor in the anodic compartment is an organic substrate. Electrochemically active bacteria 47are efficient oxidizers of organic substrates such as glucose, ethanol, glycerol, cellulose feedstocks, 48sewage sludge and aromatic compounds, but also inorganic such as hydrogen and sulfur 49compounds[20–22]. The use of organic substrates limits application of BESs to certain locations were 50organic wastewaters are available, but in reality organic waste streams are not ubiquitous. At the same

51time, mining and metal industries, being the ones most interested in metal recovery, produce large 52amounts of hydrogen as a side product of their electroplating activities [23,24]. Hydrogen is also 53produced in reduction furnace operations[25] and as a side product of electro-catalytic treatment for 54acidity in mine waters[26,27].

55Hydrogen can be used as electron donor in chemical fuel cells where it reacts with oxygen to produce 56electric current. The drawbacks of fuel cells is that they utilize noble metal catalysts like platinum, 57which are expensive and rare materials, and often operate at extreme conditions [28,29]. 58Microorganisms could serve as an alternative catalyst for the hydrogen oxidation reaction. Production 59of current by hydrogenotrophic anodophilic bacteria in MECs has already been reported by a number 60of researchers [30–32]. Rozendal et al. (2008) [33] used hydrogen as electron donor in order to grow a 61bioanode, which was after start-up changed to a hydrogen producing biocathode by reversing the 62polarity of the electrode. Moreover, Wang et al. (2014) [34], succeeded in perchlorate reduction in a 63bioelectrochemical reactor utilizing autotrophic hydrogen oxidizing bacteria. Both studies did not 64analyze the performance of a hydrogen oxidizing biofilm on the anode.

65The main objective of this study was to explore the feasibility to utilize hydrogen as electron donor in 66combination with electrochemically active microorganisms at the anode for the recovery of copper at 67the cathode of a bioelectrochemical system. The strategy was to first to grow an anodic biofilm on 68acetate, then replace the acetate with hydrogen as electron donor and finally couple the hydrogen 69oxidation to copper reduction in the cathode. The performance of this system was studied by analyzing 70current as a function of anode potential, and, when coupled to copper reduction, power production.

72Materials and methods

73<u>Experimental set up</u>. Two identical cells (biotic and abiotic control) with a surface area of 22 cm² were 74constructed, as described by ter Heijne et al (2008) [35]. Each of them comprised of two graphite 75plates (Müller & Rössner GmbH & Co., Troisdorf, Germany) serving as anode current collector and 76cathode. The anode material was graphite foil (1.0 g/cm³ density, 99% purity; Coidan Graphite 77Products Ltd., York, UK), which was pressed on the anode current collector.

78Two plexiglass plates with a single flow channel as middle compartments contained anolyte and 79catholyte and were separated by a Ralex anion exchange membrane (MEGA a.s.,Stráž pod Ralskem, 80Czech Republic). Two additional plexiglass plates served as temperature control (30 °C) on the 81outside of the cell.

82Temperature and pH were continuously logged (Endress + Hauser, Liquiline data logger) through pH 83electrodes (Endress + Hauser, CPS41 D) that were placed in the recirculation of anolyte and catholyte. 84In the headspace of each of the recirculation bottles, a gas sampling point was placed. The outgoing 85gas flow was measured using a bubble counter (MilliGascounter, Type MGC-1, Ritter, Bochum, 86Germany).

87<u>Electron donor and electrolyte composition</u>. The anode of both cells was first fed with an acetate 88containing solution (20 mM) at a rate of 2 mL/min. This solution furthermore contained the following 89buffer and nutrients: 0.68 g/L KH₂PO₄, 0.87 g/L K₂HPO₄, 0.74 g/L KCl, 0.58 g/L NaCl, 0.28 g/L NH₄Cl, 900.1 g/L MgSO₄.7H₂O, 0.1 g/L CaCl₂·2H₂O and 0.1 mL/L of a trace element mixture [36].

91The anolyte chamber was operated in a continuous mode and the catholyte in a batch mode. The 92anolyte was recirculated at 200 mL/min via two recirculation bottles of 0.5 L each. The catholyte was 93recirculated with the same rate in a 1 L bottle, which was shared by both the biotic and abiotic cell.

94During stage 1, the bioanode tests on acetate and hydrogen, the catholyte consisted of 10 mM of 95phospha During stage 1, the bioanode tests on acetate and hydrogen, the catholyte consisted of 10 96mM of phosphate buffer solution (pH 7). In stage 2, the bioanode coupled to copper reduction, the 97catholyte consisted of 1 g/L Cu²⁺ (prepared from CuCl₂ and deionized water; pH = 4).

98The cathode was kept anaerobic by flushing with nitrogen gas.

99In the second stage both cells were fed with hydrogen gas as electron donor. This hydrogen gas inflow 100was controlled with a mass flow controller (Bronkhorst HICH-TECH BV, Ruurlo, Nederland) at 3, 10 101and 30 mL/min. Hydrogen was sparged in the recirculation bottles and continuously recirculated 102through the headspace of the recirculation bottles with a vacuum pump to achieve saturation of the 103anolyte with hydrogen. Buffer and nutrients remained the same when the hydrogen gas served as 104electron donor.

105<u>Experimental strategy:</u> The biotic cell was inoculated with a mixed microbial culture from an active 106MFC utilizing acetate as electron donor. The abiotic cell was not inoculated and served as control. This 107cell was tested under the same hydrogen flows as for the biotic cell and with the anode potential 108ranging from -400 to -200 mV.

109Table 1 gives an overview of the two experimental stages. First, the cell was started with acetate as 110electron donor and during the experiment the acetate was first partly and later fully replaced with 111hydrogen. For both cells the anode potential was controlled with a potentiostat ranging from -400 mV 112to -200 mV (50 mV steps), in order to see the response of current generation as a function of the 113anode potential. When a stable current was reached, the acetate concentration was decreased 114stepwise to 10 and 5 mM acetate. For these last two concentrations of acetate the anolyte was flushed 115with 3 mL/min H₂ at the same time. Next, only hydrogen was provided to the cells as a sole electron 116donor with a flow of 3, 10 and 30 mL/min were tested.

117In the second experimental stage, hydrogen oxidation at the anode was combined with copper 118reduction at the cathode. The potentiostat was replaced by an external resistor and the resistance was 119stepwise reduced when the current reached a stable value; 1000, 500, 250, 100, 75, 50 Ω . The 120hydrogen flow provided during these experiments was 30 mL/min, while the catholyte was a

121Copper solution replaced regularly to keep copper concentration constant during the experiment. The 122copper concentration was checked twice every day to be sure that concentration was close to 1g/L, 123and never lower than 0,7 g/L.

124. Finally, bicarbonate was added as carbon source in addition to hydrogen as electron donor (30 125mL/min H_2). Again, the anode potential was controlled with a potentiostat between -400 and -200 mV 126in 50 mV steps.

127The biotic cell was inoculated again a few times during the experiment when a sharp drop in current 128was observed. At these points the system was also supplied with additional acetate. The experiment 129was only resumed when the supplied acetate was completely depleted, as confirmed by IC 130measurements.

131Table 1 Overview of the three experimental stages for the biotic cell.

Stage of experiment	Electron donor	Anode potential vs Ag/AgCl	Duration (days)	Cathode reaction
		or		
		value of resistor		
1: bioanode development	20 mM Acetate	-400 mV to -200 mV	10	Hydrogen production
on acetate and switch to	10 mM acetate + 3ml/min H ₂	-350 mV to -250 mV	8	
hydrogen	5 mM acetate + 3ml/min H_2	- 400 mV to -200 mV	8	
	3 ml/min H ₂	- 400 mV to -200 mV	11	
	10 ml/min H ₂	-350 mV and -200 mV	11	
	30 ml/min H ₂	- 400 mV to -200 mV	7	
2. Bioanode on hydrogen	30 ml/min H₂	1000 Ω to 50 Ω	14	Copper reduction
coupled to copper	30 ml/min H ₂ + HCO ₃ ⁻	400 mV to -200 mV	15	
reduction and addition				
carbon source				

134<u>Electrochemical control.</u> The anode potential was controlled with a potentiostat (BANK ELEKTRONIK, 135WENKING POTENTIOSTAT KP 3A5V). Since the anode potential was controlled and the cathode was 136anaerobic, the cell voltage was negative, such that hydrogen gas was produced in the cathode. The 137potentials of the cell compartments were controlled and measured versus Ag/AgCl 3M KCl reference 138electrodes (Qis, QM711X – Reference electrode, Epoxy, Refillable, 4mm) that were placed in the 139electrolyte, for both the anodic and the cathodic compartment. All potentials are reported versus the 140Ag/AgCl reference electrode (+0.201 vs SHE).

141The cell voltage, anode, cathode and membrane potential (defined as the potential difference between 142the two reference electrodes), as well as the current produced were recorded every minute on a data 143logger (Endress + Hauser RSG40). Chronoamperometry tests were conducted in order to check the 144current production of the abiotic cell with different hydrogen flows and anode potential control, using 145Autolab equipment (Metrohm Autolab B.V., PGSTAT 302N).

146<u>Analytical procedures and calculations</u>: The acetate concentration in the cells was measured using ion 147chromatography (Metrohm 761 Compact IC equipped with a conductivity detector and a Metrosep 148Organic Acids 6.1005.200 ion exclusion column) and the bicarbonate concentration was determined 149using a total carbon analyzer (Shimadzu TOC-VCPH). The gas composition in the headspace of the 150cells was also examined with a gas chromatographer (Varian Inc. (Part A) - CP-4900 Micro-GC).

151When the catholyte was changed to the copper solution, the current and power production were 152calculated according to

$$\frac{V_{cell}}{R_{ext} * A_{an}}$$
(2)

$${}_{154}P = \frac{Vcell^2}{R_{ext} * A_{an}}$$
(3)

155where V_{cell} is the cell voltage, R_{ext} is the external resistance (Ω), A_{an} is the projected surface area of the 156anode (0.0022 m²).

158Results and discussion

159**Hydrogen as electron donor for an electroactive biofilm.** The performance of the anodic biofilm 160with acetate and hydrogen as electron donors is summarized in Figure 1.

161Initially an acetate solution (20 mM) was used to achieve fast growth of an electroactive anodic biofilm 162and a stable current. The performance of this bioanode was analyzed at controlled anode potentials in 163steps of 50 mV (every 50 hours) ranging from -400 to -200 mV. The bioanode produced a maximum 164current of 3.98 A/m² at -250 mV. After that, the acetate concentration was stepwise decreased while an 165inflow of hydrogen was introduced to the system. When the acetate concentration decreased to 10 166mM and 3 mL/min H₂ was flushed in the anolyte, the maximum current density was 1.80 A/m² at -250 167mV. When the acetate concentration was decreased further to 5 mM, the maximum current density 168was 0.92 A/m² at an anode potential of -250 mV.

169In the next experimental stage, after depletion of acetate, the anolyte was provided only with hydrogen 170at a flow of 3 mL/min. The maximum current density was 0.67 A/m² at an anode potential of -250 mV. 171This was the highest current obtained during this experimental cycle. Increase of the anode potential 172to -200 mV led to a slight decrease in current density (0.62 A/m²). These results show that also in 173absence of acetate, hydrogen can be used as electron donor for the bioanode.

174Nevertheless, the produced current with hydrogen as electron donor was a factor 6 lower than with 175only acetate. A possible limitation of current production from hydrogen is the availability of hydrogen 176gas for the microorganisms, due to its low solubility. To test if hydrogen was limiting, after 11 days, the 177hydrogen inflow into the anolyte was increased from 3 mL/min to 10 mL/min H₂ gas. For -200 mV (the 178only anode potential measured at this H₂ flow), the bioanode now produced 1.84 A/m². A further 179increase of the H₂ gas flow to 30 mL/min and repeating the experiments at anode potentials in the 180range of -350 to -200 mV did not lead to a further increase in current density. The maximum current 181density at this hydrogen flow was 1.57 A/m² at an anode potential of -200 mV.



183Figure 1- Performance of biotic cell when shifting from acetate to H₂ as electron donor

184**Abiotic control experiments.** Hydrogen can be oxidized in the absence of microorganisms at noble 185metal based catalysts like Pt. Possibly, it can also be oxidized on a plain graphite electrode. To verify if 186the biological catalysis, and not the graphite electrode, was responsible for hydrogen oxidation, the 187current produced in an abiotic cell was compared to the current produced in the biotic cell. In Figure 2 188the current density obtained from the biotic cell is compared with the current produced from the abiotic 189cell, at an anode potential of -200 mV and at different hydrogen inflows. The current density in the 190abiotic cell was much lower than the current produced in the biotic experiment at all flow rates. Some 191negative current was measured for the abiotic cell, meaning that a reduction reaction, instead of an 1920xidation reaction, occurred at -200 mV electrode potential, due to the potentiostatic control of the cell.

193According to these results, it can be concluded that the electroactive biofilm oxidized hydrogen at the 194graphite electrode.



196Figure 2 Abiotic cell vs biotic cell performance at an anode potential of -0.200 V at different hydrogen 197inflow rates.

198**Cathodic copper reduction coupled with anodic hydrogen oxidation.** Finally, experiments were 199conducted where the electrons from hydrogen oxidation at the bioanode were used to reduce copper 200at the cathode. The anolyte was continuously flushed with 30 mL/min H₂, in order to operate at high 201hydrogen concentration, while the catholyte was a copper solution ([Cu²⁺]=1 g/L). Copper 202concentration was replenished regularly so that copper concentration was always >0.7 g/L.

203Figure 3A shows the performance of the system in a polarization and power curve. During the 204experiment the external resistor was stepwise reduced: 1000, 500, 250, 100, 75 and 50 Ω . The initial 205cell voltage and current density obtained with 1 k Ω were 0.61 V and 0.28 A/m², respectively, giving a 206power density of 0.17 W/m². The maximum power density obtained was 0.25 W/m². At that point the 207cell voltage was 0.53 V and the current density was 0.48 A/m². The highest current density was 0.66 208A/m² in combination with a power density of 0.24 W/m².

209The following equations show the potentials for acetate and hydrogen oxidation, copper reduction and 210the overall cell voltages.

211Acetate oxidation

 $212CH_3COO^{-} + 4H_2O \rightarrow 2HCO_3^{-} + 9H^+ + 8e^-$

 $E_{an} = -0.496 V \text{ at pH 7}$ (1)

213Hydrogen oxidation

 $214H_2 \rightarrow 2 H^+ + 2 e^-$

 $E_{an} = -0.554V \text{ at pH}=7$ (2)

215Copper reduction

216Cu ²⁺ + 2 e ⁻ → Cu (s)	$E_{cat} = 0.082 \text{ V at pH}=3$	(3)

217Overall

$218CH_{3}COO^{-} + 4H_{2}O + 4Cu^{2+} \rightarrow 2HCO_{3}^{-} + 9H^{+} + 8e^{-} + 4Cu(s)$	E _{cell} = 0.578 V	(4)
$219H_2 + Cu^{2+} \rightarrow 2H^+ + Cu$ (s)	E _{cell} = 0.636 V	(5)

220Figure 3B shows the anode, cathode and membrane potential and their contribution to the total cell 221voltage. The maximum cell voltage close to the open circuit voltage was 0.608 V; which is very close 222to the theoretical open cell voltage of 0.636 V for copper reduction combined with hydrogen oxidation 223(equation 5). The anode potential close to open circuit was -0.468 V, slightly more positive than the 224theoretical potential (equation 2). The cathode potential was quite stable throughout the range of 225measured current densities varying between 0.097-0.141 V, close to the theoretical potential of copper 226reduction (equation 3). The copper concentration in the catholyte decreased in the course of the 227experiment, indicating that indeed copper was recovered at the cathode. Copper recovery and its 228efficiency was not further analyzed in this study, but has been analyzed by others [37].

229As can be seen from this figure, both the membrane and cathode potential are stable over the whole 230current range. The drop in cell voltage at higher current densities is caused by increase in anode 231potential. This increase in anode potential is most likely caused by limited transport of hydrogen 232towards the biofilm. Even though the gas flow was increased hydrogen supply to the biofilm might be 233limited due to its limited solubility in water at atmospheric pressure.



235Figure 3 (A) Power and polarization curve for hydrogen oxidation coupled to copper recovery and (B) 236contribution of the anode, cathode and membrane potential to the cell voltage.

237

238**Perspectives and long term performance.** The results presented here show the proof of principle for 239the use of hydrogen as electron donor for the recovery of copper in BESs. The current and power 240densities obtained in this study (0.48 A/m² in combination with 0.25 W/m²) are in the same order as 241previous investigations of copper removal in BESs. However, all previous studies used an organic 242electron donor while here for the first time hydrogen was used. Tao et al. produced 0.26 W/m² power 243density at 0.86 A/m² current density [18], using glucose as electron donor. Ter Heijne et al obtained a 244maximum power density for acetate oxidation coupled to copper reduction of 0.43 W/m² at a current 245density of 1.7 A/m² in exactly the same cell configuration as used in this study [16].

246Second, our analysis of overpotentials shows that the anode overpotential for hydrogen oxidation is 247higher than for acetate oxidation. A probable reason for this higher overpotential is limited hydrogen 248availability due to its low solubility, even at higher flow rates. A future configuration should avoid these 249limitations, for example through more efficient gas transport and elevated gas pressure.

250Finally, the long term performance of this system should be investigated Hydrogen acts only as an 251electron donor, while microorganisms require also a carbon source for growth. In the final stage of our 252experiment, we observed a slow decrease in current density, with limitations in carbon source as a 253possible reason. To test the effect of carbon source, we provided the cell with bicarbonate and were 254able to run it under stable conditions (~1.2 A/m²) for over two weeks (data not shown). It should be 255further investigated what the minimum requirement of carbon source is and if an inorganic source is 256sufficient for these microorganisms to survive for long periods of time. For example, Jeremiasse et al 257showed that a hydrogen producing biocathode required two times lower startup time with acetate 258compared to a biocathode starting on bicarbonate [38]. When adding a carbon source, either in the 259form of acetate or bicarbonate, care should be taken not to produce methane instead of electricity, 260since both acetate and bicarbonate are suitable substrates for methanogens. In the present research, 261already small amounts of methane (max 4%) were detected in the headspace.

263 Conclusions

264With a BES, biologically catalyzed hydrogen oxidation at the anode can be coupled with copper 265reduction Results show a maximum current density of 0.67 A/m² combined with a power density of 2660.25 W/m² when hydrogen is used as single electron donor Further research and optimization of the 267system could lead to a novel practical application for BESs for metal recovery.

269Acknowledgement

270This work was performed in the cooperation framework of Wetsus, european centre of excellence for 271sustainable water technology (www.wetsus.nl). Wetsus is co-funded by the Dutch Ministry of 272Economic Affairs and Ministry of Infrastructure and Environment, the European Union Regional 273Development Fund, the Province of Fryslân, and the Northern Netherlands Provinces. The authors 274would like to thank the participants of the research theme "Resource Recovery" for the fruitful 275discussions and their financial support. This work is also part of BioelectoMET project. Title: 276Bioelectrochemical systems for metal recovery. Funded under the Seventh Framework Programme 277(FP7) Research area: ENV.2011.3.1.9-1 (Eco-innovation).

278References

- Babich H, Stotzky G. Heavy metal toxicity to microbe-mediated ecologic processes: A review
 and potential application to regulatory policies. Environ Res 1985;36:111–37.
 doi:http://dv.doi.org/10.1016/0013.0251(95)00011.8
- 281 doi:http://dx.doi.org/10.1016/0013-9351(85)90011-8.
- Islam EU, Yang X, He Z, Mahmood Q. Assessing potential dietary toxicity of heavy metals in
 selected vegetables and food crops. J Zhejiang Univ Sci B 2007;8:1–13.
 doi:10.1631/jzus.2007.B0001.
- 285[3] Barakat M a. New trends in removing heavy metals from industrial wastewater. Arab J Chem 286 2011;4:361–77. doi:10.1016/j.arabjc.2010.07.019.
- Norgate TE, Jahanshahi S, Rankin WJ. Assessing the environmental impact of metal
 production processes. J Clean Prod 2007;15:838–48.
- 289[5] Norgate TE, Rankin WJ. The Role of Metals in Sustainable Development, 2002, p. 49–55.
- Rabaey K, Angenent L, Schröder U, Keller J. Bioelectrochemical systems : from extracellular
 electron transfer to biotechnological application. 2010.
- Lim BS, Lu H, Choi C, Liu ZX. Recovery of silver metal and electric power generation using a microbial fuel cell. Desalin Water Treat 2014:1–7. doi:10.1080/19443994.2014.923191.
- 294[8] Choi C, Cui Y. Recovery of silver from wastewater coupled with power generation using a microbial fuel cell. Bioresour Technol 2012;107:522–5.
- 296[9]Lefebvre O, Neculita CM, Yue X, Ng HY. Bioelectrochemical treatment of acid mine drainage297dominated with iron. J Hazard Mater 2012;241-242:411–7. doi:10.1016/j.jhazmat.2012.09.062.
- 298[10]Ter Heijne A, Hamelers HVM, Buisman CJN. Microbial fuel cell operation with continuous299biological ferrous iron oxidation of the catholyte. Environ Sci Technol 2007;41:4130–4.
- Khosa MK, Jamal MA, Hussain A, Muneer M, Zia KM, Hafeez S. Efficiency of Aluminum and
 Iron Electrodes for the Removal of Heavy Metals [(Ni (II), Pb (II), Cd (II)] by Electrocoagulation
 Method. J Korean Chem Soc 2013;57:316–21. doi:10.5012/jkcs.2013.57.3.316.
- 303[12] Qin B, Luo H, Liu G, Zhang R, Chen S, Hou Y, et al. Nickel ion removal from wastewater using
 the microbial electrolysis cell. Bioresour Technol 2012;121:458–61.
 doi:10.1016/j.biortech.2012.06.068.

- 306[13] Modin O, Wang X, Wu X, Rauch S, Fedje KK. Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute solutions. J Hazard Mater 2012;235-236:291–7.
- 308[14] Zhang LJ, Tao HC, Wei XY, Lei T, Li JB, Wang AJ, et al. Bioelectrochemical recovery of
 ammonia-copper(II) complexes from wastewater using a dual chamber microbial fuel cell.
 Chemosphere 2012;89:1177–82.
- Cheng S-A, Wang B-S, Wang Y-H. Increasing efficiencies of microbial fuel cells for
 collaborative treatment of copper and organic wastewater by designing reactor and selecting
 operating parameters. Bioresour Technol 2013;147:332–7. doi:10.1016/j.biortech.2013.08.040.
- Heijne A Ter, Liu F, Weijden R Van Der, Weijma J, Buisman CJN, Hamelers HVM, et al. Copper
 recovery combined with electricity production in a microbial fuel cell. Environ Sci Technol
 2010;44:4376–81. doi:10.1021/es100526g.
- Wang Z, Lim B, Lu H, Fan J, Choi C. Cathodic reduction of Cu 2+ and electric power
 generation using a microbial fuel cell. Bull Korean Chem Soc 2010;31:2025–30.
- Tao HC, Liang M, Li W, Zhang LJ, Ni JR, Wu WM. Removal of copper from aqueous solution by
 electrodeposition in cathode chamber of microbial fuel cell. J Hazard Mater 2011;189:186–92.
 doi:10.1016/j.jhazmat.2011.02.018.
- Rodenas Motos P, Weijden R van der, ter Heijne A, Saakes M, Buisman CJN, Sleutels THJA.
 High Rate Copper and energy recovery in Microbial Fuel Cells. Front Microbiol 2015;6.
 doi:10.3389/fmicb.2015.00527.
- Pant D, Van Bogaert G, Diels L, Vanbroekhoven K. A review of the substrates used in microbial
 fuel cells (MFCs) for sustainable energy production. Bioresour Technol 2010;101:1533–43.
 doi:10.1016/j.biortech.2009.10.017.
- Lovley DR. Bug juice: harvesting electricity with microorganisms. Nat Rev Microbiol
 2006;4:497–508. doi:10.1038/nrmicro1442.
- Hamelers HVM, Ter Heijne A, Sleutels THJA, Jeremiasse AW, Strik DPBTB, Buisman CJN.
 New applications and performance of bioelectrochemical systems. Appl Microbiol Biotechnol
 2010;85:1673–85. doi:10.1007/s00253-009-2357-1.
- Cobley AJ, Saez V. The use of ultrasound to enable low temperature electroless plating. Circuit
 World 2012;38:12–5. doi:10.1108/03056121211195003.
- 335[24] Speck JA. Mechanical fastening, joining, and assembly. vol. 109. CRC Press; 1997.
- 336[25] Cox R. Waste/By-Product Hydrogen. Fuel Cell Hydrog Energy Assoc 2011.
- 337[26] Bouwer H, Chaney RL. Land treatment of wastewater. Adv Agron 1974;26:133–76.
- 338[27] Treharne R, Wright D. Acid mine water treatment process 1974.
- 339[28]Debe MK. Electrocatalyst approaches and challenges for automotive fuel cells. Nature3402012;486:43–51. doi:10.1038/nature11115.
- MINH N. Solid oxide fuel cell technology?features and applications. Solid State Ionics
 2004;174:271–7. doi:10.1016/j.ssi.2004.07.042.

343[30]Freguia S, Rabaey K, Yuan Z, Keller J. Syntrophic processes drive the conversion of glucose in
microbial fuel cell anodes. Environ Sci Technol 2008;42:7937–43. doi:10.1021/es800482e.

- Lee HS, Torres CI, Parameswaran P, Rittmann BE. Fate of H2 in an upflow single-chamber
 microbial electrolysis cell using a metal-catalyst-free cathode. Environ Sci Technol
 2009;43:7971–6. doi:10.1021/es900204j.
- Lee HS, Rittmann BE. Significance of biological hydrogen oxidation in a continuous singlechamber microbial electrolysis cell. Environ Sci Technol 2010;44:948–54.
 doi:10.1021/es9025358.
- 351[33]Rozendal RA, Jeremiasse AW, Hamelers HVM, Buisman CJN. Hydrogen production with a
microbial biocathode. Environ Sci Technol 2008;42:629–34. doi:10.1021/es071720+.
- Wang Z, Gao M, Zhang Y, She Z, Ren Y, Wang Z, et al. Perchlorate reduction by hydrogen autotrophic bacteria in a bioelectrochemical reactor. J Environ Manage 2014;142:10–6.
 doi:10.1016/j.jenvman.2014.04.003.
- 356[35] Ter Heijne A, Hamelers HVM, Saakes M, Buisman CJN. Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. Electrochim Acta 2008;53:5697–703.
- 358[36] Zehnder AJB, Huser BA, Brock TD, Wuhrmann K. Characterization of an acetatedecarboxylating, non-hydrogen-oxidizing methane bacterium. Arch Microbiol 1980;124:1–11.
 doi:10.1007/BF00407022.
- Heijne AT, Liu F, Weijden RVD, Weijma J, Buisman CJN, Hamelers HVM, et al. Copper
 recovery combined with electricity production in a microbial fuel cell. Environ Sci Technol
 2010;44:4376–81.
- 364[38] Jeremiasse AW, Hamelers H V, Croese E, Buisman CJ. Acetate enhances startup of a H 2producing microbial biocathode. Biotechnol Bioeng 2012;109:657–64.

Highlights:

- Biological hydrogen oxidation was successfully combined with copper reduction.
- Open circuit voltage was close to theoretical voltage of the cell.
- Power density of 0.25 W/m² was achieved at 0.48 A/m².
- Hydrogen solubility was limiting the rate of the process.

Reviewer 1:

Overall I think the paper is clearly written. However, the following information should be added:

-Give information about the chemical composition of the catholyte. This info could be provided in the paragraph on lines 92-95.

The composition of the catholyte has been added to lines 92 to 95:

During stage 1, the bioanode tests on acetate and hydrogen, the catholyte consisted of 10 mM of phosphate buffer solution (pH 7). In stage 2, the bioanode coupled to copper reduction, the catholyte consisted of 1 g/L Cu^{2+} (prepared from CuCl₂ and deionized water; pH = 4).

-Show Cu concentration data. On line 223, it is stated the the Cu concentration in the catholyte decreased. Although the focus of the paper is on the H2-utilizing anode, there is no reason not to show the Cu data as well (for example in a supplementary material). Also, use the Cu concentration measurements to estimate a coulombic efficiency for the cathode.

The copper solution was replaced regularly regularly copper concentration constant during the experiment. Copper concentration was checked twice every day to be sure that concentration was close to 1g/L, never lower than 0,7 g/L.

-Discuss the possible reactions taking place at the anode. On line 256 it is stated that small amounts of CH4 was produced during H2-feed. It is also possible that acetate is produced from H2 and bicarbonate. That acetate could then potentially be used by the bioanode or by methanogens. Was any acetate detected during feed with only H2?

Indeed, alternative electron donors besides H2 could play a role. We did analyze the anolyte for Acetate concentration, and it was below detection limit, on the other hand, methane was detected in the headspace of the cell, likely originating from H2 and CO2 We have added this to the text.

-I think the sentence on lines 252-253 should have the word acetate in it somewhere. Please check.

The word acetate has been added to line ...:

Jeremiasse et al showed that a hydrogen producing biocathode required two times less time for startup with acetate compared to a biocathode starting on bicarbonate

-Clarify what led you to add bicarbonate in the last phase of the study. Was there any evidence that the bioanode was not stable with only H2 feed? Or was it just to check if the current could be increased with a higher concentration of the carbon source?

When H2 was used as energy source for a longer period, current density decreased gradually (data not shown in manuscript) well known that microorganisms need a carbon source, besides an energy source to survive a longer period of time. Therefore, we decided to demonstrate that a combination of bicarbonate as carbon source combined with hydrogram allowed to maintain the cell performance for a longer period of time or even improve it.

_The focus of the article is clear and experiments are well described.

-In the introduction all the treatments look very bad and only BES are the only good. Comments should be soften.

We have changed line to :

Conventional heavy metal removal technologies including electrochemical, chemical precipitation and ion-exchange. Although these technologies are applied in practice and a large scale their economic and environmental impact could still be improved by reduction of the high operational costs due to chemicals and energy consumption and the reduction of excessive production of hazardous wastes. This improvement could possibly alternative achieved by the removal of these heavy metals in bioelectrochemical systems (BES).

<u>-</u>It is not clear excess H2 in the metallurgical industry for use in the anode. In fact it is ususal produce H2 at the cathode in many BES, as evidenced by numerous articles in this journal

We agree with the reviewer that both processes have been studied before. However, they have not been combined in a single study before; and in addition, the performance of a hydrogen oxidizing bioanode (e.g. as function of anode potential) has not been studied before. This study looks for a combined solution of use the hydrogen oxidation process by bacteria and the copper reduction process, as explained in the introduction: There have been a number of reports of heavy metal removal in BESs. These reports all make use of an organic electron donor. These electron donors are generally not available at sites where heavy metals are produced and their transport to these facilities would be expensive. On the other side, an excess of H2 is produced in smelting companies in the reduction furnace or by electrochemical production as secondary reaction when nickel, cobalt or zinc are plated.

_The main objective of this study is to explore the feasibility to utilize hydrogen as electron donor in combination with electrochemically active microorganisms at the anode for the recovery of copper at the cathode. This approach is not very new.

-Oxidation of H2 at the anode is known. The reduction of copper is also known.

Oxidation of H2 at the bioanode is a novel process that has been reported, but its performance has not been studied before. The reduction of copper is not new, but the combination of both processes in a single device is a novel application of this technology, as discussed in the comment above.

_Discussion of results should increase and use less comments in conclusions

Changes already done in the manuscript.











Leeuwarden, July 14th 2015

Editorial Office

International Journal of Hydrogen Energy

Dear editor,

Hereby we would like to submit the enclosed manuscript entitled "Hydrogen as electron donor for copper removal in bioelectrochemical systems" as a research paper for *International Journal of Hydrogen Energy.*

The recovery of heavy metals is an important challenge in the hydrometallurgical industry. Recently, the recovery of copper coupled to oxidation of organic matter in a bioelectrochemical system was proposed as a viable option. However, organic electron donors are not always available on-site. Hydrogen gas is an attractive alternative electron donor since it is produced in large quantities as a side product in the metallurgical industry. The aim of this study was to demonstrate that microbial anodic hydrogen oxidation on a non-catalyzed graphite electrode can be coupled with cathodic copper reduction in a BES to simultaneously recover copper and produce power. We show here, for the first time, that it is possible to combine hydrogen oxidation with copper reduction. We believe the results achieved in this study show great promise for the future recovery of heavy metals in bioelectrochemical systems.

We hope you will consider our manuscript for publication in *International Journal of Hydrogen Energy.*

Sincerely,

Dr Tom Sleutels Oostergoweg 9 P.O. box 1113 8900 CC Leeuwarden, the Netherlands Tom.sleutels@wetsus.nl

T: +31 58 2843000