

Combining biocatalyzed electrolysis with anaerobic digestion

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ABSTRACT

Biocatalyzed electrolysis is a microbial fuel cell based technology for the generation of hydrogen gas and other reduced products out of electron donors. Examples of electron donors are acetate and wastewater. An external power supply can support the process and therefore circumvent thermodynamical constraints that normally render the generation of compounds such as hydrogen unlikely. We have investigated the possibility of biocatalyzed electrolysis for the generation of methane. The cathodically produced hydrogen could be converted into methane at a ratio of 0.41 mole methane mole⁻¹ acetate, at temperatures of 22 ± 2°C. The anodic oxidation of acetate was not hampered by ammonium concentrations up to 5 g NL⁻¹. An overview is given of potential applications for biocatalyzed electrolysis.

Key words | ammonium toxicity, BEAMR, biological cathode, hydrogen, manure, methane, MFC, urine

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INTRODUCTION

The principle of acetate oxidation with concomitant hydrogen production through a biocatalyzed electrolysis cell, also called a bio-electrochemically assisted microbial reactor (BEAMR), was established by Liu *et al.* (2005a) and Rozendal *et al.* (2006a). The anodic oxidation of acetate is the same reaction as the one in the anode of microbial fuel cells that harvests electrical energy through an external resistance (Rabaey *et al.* 2005a). In the case of biocatalyzed electrolysis, hydrogen is produced at the cathode with platinum as a catalyst. An external power source is needed to realize hydrogen production. It has been suggested to use the produced hydrogen as a fuel for chemical fuel cells (Rozendal *et al.* 2006a). Also, mixing of

hydrogen with biogas has been proposed as a strategy to improve the combustion properties of biogas (Aelterman *et al.* 2006a). Dark fermentation of glucose is limited to production of 4 moles hydrogen and 2 mole acetate per mole glucose. Aided by biocatalyzed electrolysis, acetate can also be converted into hydrogen gas. The combination of both technologies results in a theoretical production of 12 moles hydrogen per mole glucose (Liu *et al.* 2005a).

In this research we have tested the ability of using biocatalyzed electrolysis for anodic liquid streams with a high ammonium content and the ability of converting the produced hydrogen into methane at room temperature.

METHODS

Microbial fuel cell setup

The MFCs were constructed of two similar Perspex frames ($7.5 \times 7.5 \times 2 \text{ cm}^3$ for one frame). A robust cation exchange membrane (Ultrex[®]CMI7000, Membranes International Inc., USA) was used between the anodic and cathodic frame of the MFCs. The anode electrode consisted of graphite granules (type 00514, diameter between 1.5 and 5 mm, Le Carbone, Belgium) and a graphite rod (5 mm diameter, Morgan, Belgium) to collect the electrons. Prior to use, the granules were washed five times with distilled water before being submerged overnight in turns in 1 N NaOH and 1 N HCl. Afterwards the granules were washed five times with distilled water. The cathodic electrode consisted of 41.25 cm^2 graphite woven web with 5 g Pt m^{-2} (LT 140EW ELAT[®]GDE, E-TEK, USA) fixed to a graphite rod with 3 cable-ties (3M) for providing electrical contact.

Operational conditions

The anodic compartment of the MFCs was inoculated with effluent originating from highly performing MFCs (Aelterman *et al.* 2006b). The anodic and cathodic medium consisted of $4.4 \text{ g KH}_2\text{PO}_4 \text{ L}^{-1}$, $3.4 \text{ g-K}_2\text{HPO}_4 \text{ L}^{-1}$, $2 \text{ g NaHCO}_3 \text{ L}^{-1}$, $0.5 \text{ g NaCl L}^{-1}$, $0.0146 \text{ g CaCl}_2 \text{ L}^{-1}$ and trace elements as previously described (Rabaey *et al.* 2005b). For the tests where ammonium was present in the anodic medium, $(\text{NH}_4)_2\text{HPO}_4$ and KH_2PO_4 were used as phosphate buffer in the same molar ratio ($[\text{H}_2\text{PO}_4^-]/[\text{HPO}_4^{2-}] = 1.66$) to obtain the desired ammonium concentration. For the test with a high ammonium concentration, both the anodic and cathodic medium were continuously fed with fresh medium at a rate of 0.65 L d^{-1} with a peristaltic pump (Watson Marlow, Belgium). Peristaltic pumps were used to recirculate the anodic liquid out of a recirculation vessel (0.5 L) at 6 L h^{-1} . Acetate was continuously dosed to the anodic recirculation vessel with a syringe pump. For the tests where hydrogen was converted into methane, the anodic system was batch fed with 1 g sodium acetate upon depletion and the anodic liquid was recirculated at 6 L h^{-1} . A 6 cm^2 slice was made at the bottom of the membrane in order to facilitate proton transport to the cathode. Non-woven cloth (Liplisse 3, Libeltex, Belgium)

was placed in this slice to avoid migration of anodic graphite granules. The headspace of the cathodic compartment was bubbled through an external vessel (0.4 L) with a peristaltic pump. This external vessel was inoculated with 10 mL non-granular anaerobic sludge for the conversion of hydrogen gas into methane. Gas bags were used to trap the gas produced in the cathodic systems.

Measurements

The voltage was applied by a DC power supply or by a potentiostat (PAR Bi-Stat Potentiostat, Princeton Applied Research, France). When a DC power supply was used, the current was measured by placing a 1.07Ω resistor in the electrical circuit. The voltage difference over this resistor divided by the 1.07Ω results in the current, according to Ohm's law. The current was expressed per cathode projected surface (CPS). A data acquisition unit (HP 34970A, Agilent, USA) recorded the voltage difference every minute. The hourly averaged values with standard deviation and the value of the external resistance were then used for further calculations. The coulombic efficiency (CE) was calculated as the ratio of current produced and theoretical current production from the substrate dosed. The cells were operated at a room temperature of $22 \pm 2^\circ\text{C}$. Ammonium was measured by a Kjeltac distillation apparatus according to Greenberg *et al.* (1992). Methane and carbon dioxide were analyzed with an Intersmat IGC 120 MB gas chromatograph connected to a Hewlett-Packard 3390A. The qualitative presence of hydrogen gas was determined using a H_2 sensor (OPUS, Zellweger Analytics, U.K.).

RESULTS

Biocatalyzed electrolysis at high ammonium concentrations

Acetate was oxidized in the anode by microorganisms while hydrogen gas was chemically produced in the cathode, similar to the process described by Liu *et al.* (2005a) and Rozendal *et al.* (2006a). A DC power supply was used to obtain an applied voltage of $-0.600 \pm 0.010 \text{ V}$ and both anode and cathode were operated in a continuous mode (HRT = 2.2 h). The acetate loading rate was $2.56 \text{ kg acetate-COD m}^{-3}$ total anodic compartment (TAC) d^{-1} .

Starting with an anodic influent without ammonium, the ammonium concentration was increased to 1 g NL^{-1} and was then further increased with 0.2 g NL^{-1} every two days in the range 1 to 2.5 g NL^{-1} and with 0.5 g NL^{-1} every two days in the range 2.5 to 5.5 g NL^{-1} . The current production was in the order of $2\text{--}3 \text{ A m}^{-2}$ CPS without ammonium and the coulombic efficiency for the acetate conversion (CE) was between 20 and 30%. The current production increased up to 5.3 A m^{-2} CPS at 5 g NL^{-1} and the coulombic efficiency was between 40 and 45%. When the loading rate was lowered to $1.60 \text{ kg COD m}^{-3} \text{ TAC d}^{-1}$ the current production was not affected resulting in a coulombic yield of 75 to 85%. When the ammonium concentration was 5.5 g NL^{-1} , the current production was severely hampered. This effect was not due to degradation of the membrane nor the cathodic electrode, since replacement of both did not enhance the current production when the ammonium concentration was 0.2 g NL^{-1} (data not shown). With the use of a potentiostat, the effect of the applied voltage was verified at different ammonium concentrations (Figure 1). The current production increased proportionally with an increasing applied voltage.

The overall acetate to hydrogen efficiency was $46 \pm 8\%$ or $1.85 \pm 0.32 \text{ mol H}_2 \text{ mol}^{-1}$ acetate. Of the ammonium dosed to the anodic medium $87 \pm 7\%$ was found in the anodic effluent and $14 \pm 3\%$ was found in the cathodic effluent by passage through the cation selective membrane. This corresponds with a maximal ammonium flux of

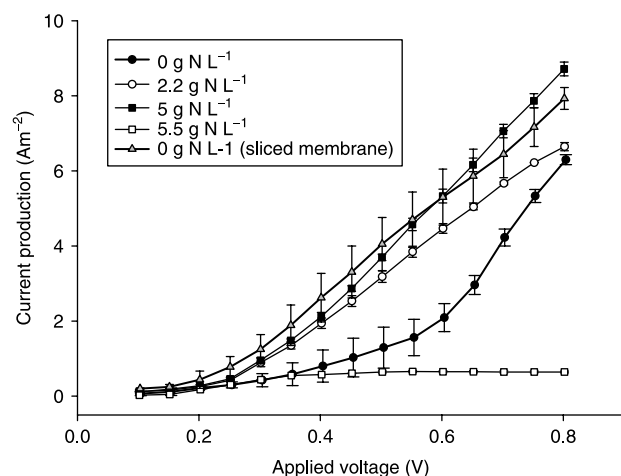


Figure 1 | The current production in function of the applied voltage provided by a potentiostat. Bars indicate minimum and maximum of the duplicate scans.

$0.51 \pm 0.11 \text{ mg N per cm}^2$ membrane per hour. The pH in the anodic effluent and cathodic effluent was 6.7 ± 0.1 and 8.0 ± 0.1 respectively.

Methanogenesis at $22 \pm 2^\circ\text{C}$

Typically 70% of the methane production in natural and engineered environments is due to acetoclastic methanogenesis (Angenent *et al.* 2004), except for systems where *Methanosaetaceae* is absent (Karakashev *et al.* 2006). Hydrogen production is the rate limiting step for hydrogenotrophic methanogenesis, and is mainly determined by the hydrogen partial pressure (Lovley & Goodwin 1988). Since hydrogen can be produced by MFC technology at room temperature in the cathode of a biocatalyzed electrolysis cell, we investigated if the production of hydrogen gas could be combined with methane formation at room temperature. Therefore the gas from the cathodic headspace was recirculated over an external bottle that contained 10 mL not granulated anaerobic sludge in 400 mL of the same medium as used in the cell. The external bottle was covered from the light to avoid phototrophic growth.

Since the pH of the anodic liquid decreased to 4.8 and the pH of the cathodic liquid increased up to 11.8 in a batch system, we modified the system by removing 6 cm^2 of the cation exchange membrane (sliced membrane) for a more efficient ion exchange. Insufficient proton transfer through NAFION membranes has also been reported (Rozendal *et al.* 2006b). A non-woven cloth prevented migration of anodic granular graphite towards the cathode. In this way the pH of the anodic and cathodic liquid remained between 6 and 7. The performance of this cell was evaluated with a potentiostat (Figure 1) and the better performance was likely due to a lower ohmic resistance over the membrane. It took 28 days for the hydrogenotrophic methanogens to adapt and for the overall system to obtain a recovery of $0.41 \text{ mole CH}_4 \text{ mole}^{-1}$ acetate at a current density of 6.0 A m^{-2} CPS ($1.6 \text{ kg COD m}^{-3} \text{ TAC d}^{-1}$; $0.600 \pm 0.010 \text{ V}$ applied to the cell). The gas consisted of 57% CH_4 and 1% CO_2 at that time, the remainder was most likely hydrogen gas. The conversion of acetate to electricity (CE) was typically between 75 and 85%.

DISCUSSION

Hydrogen production from acetate in the presence of high concentrations of ammonium

In this research we addressed the possibility to use biocatalyzed electrolysis for COD conversion into hydrogen gas in the presence of ammonium concentrations up to 5 g NL^{-1} . This is of particular interest for wastestreams that contain high levels of ammonium (eg. animal wastewater, urine, etc.). Urine after urea hydrolysis can contain up to 8.2 g NL^{-1} (the ratio total COD to total ammonia: $0.88 \pm 0.14 \text{ g COD g}^{-1} \text{ N}$) (Udert *et al.* 2003a). The high COD content combined with a high salinity (0.206 M) (Udert *et al.* 2003b) make urine an interesting substrate for anodophilic oxidation if it is diluted to ammonium levels below 5 g NL^{-1} .

Also for UASB reactors, methane production was inhibited above 5 g NL^{-1} (Borja *et al.* 1996). For hydrogen fermentation in continuous flow tests, both hydrogen production rates and yields were adversely affected by ammonia ($\text{NH}_3 + \text{NH}_4^+$) (Salerno *et al.* 2006). In the anodic conditions tested here (5.5 g NL^{-1} , $\text{pH} = 6.7 \pm 0.1$, $22 \pm 2^\circ\text{C}$) the NH_3 concentration in solution was between 12 and 19 mg NL^{-1} . NH_3 toxicity for methanogens is described to start from concentrations higher than 80 mg NL^{-1} (Angelidaki & Ahring 1993). Probably another inhibitory mechanism causes the low current production, as also suggested for hydrogen fermentation in the pH range of 5.2 to 6.2 where the NH_3 concentration was between 1.3 and 13 mg NL^{-1} (Salerno *et al.* 2006).

The hydrogen gas recovery efficiency was slightly lower than achieved by Rozendal *et al.* (2006a) for a recirculating system and it is believed that gas diffusion through the membrane and the tubing causes this low recovery. The current density was higher than the results obtained by Rozendal *et al.* (2006a), but this difference is due to the reactor design and size. Liu *et al.* (2005a) obtained similar current densities for a 28 mL reactor and also described that the reactor configuration had an important influence on the current production. Moreover, a higher ionic strength has been described to enhance the current production by a decrease of the ohmic resistance of the electrolyte (Liu *et al.* 2005b).

Hydrogenotrophic methanogenesis in combination with biocatalyzed electrolysis

In this research we have described that methane formation could account for 60% (v) of the gas produced in a biocatalyzed electrolysis cell. Hence, the hydrogen gas, produced in biocatalyzed electrolysis cells, can easily be converted into methane in the presence of carbonate in a cathodic liquid. Degradation of hydrogen gas to methane for combustion purposes implies an energy loss of approximately 20% (calculated from standard redox potentials). Further research might reveal whether methanogens can grow faster in the presence of a hydrogen producing cathode. In this research typically 0.600 V (or $2 \text{ kWh kg}^{-1} \text{ COD}$) was used to obtain a current production up to 6.8 A m^{-2} (or $1.8 \text{ kg COD m}^{-3} \text{ TAC d}^{-1}$). Further technological improvement in the field of biocatalyzed electrolysis should result in higher conversion rates at lower applied voltages, since aerobic combustion of methane yields approximately $3.5 \text{ kWh kg}^{-1} \text{ COD}$ of which only $1 \text{ kWh kg}^{-1} \text{ COD}$ can be recovered as electricity. Microbial fuel cells have the potential to provide the applied voltage in order to achieve a sustained biocatalyzed electrolysis. In order to obtain a higher recovery of hydrogen to methane, we suggest the use of hollow-fiber membranes as has successfully been used for hydrogen based denitrification and perchlorate reduction (Nerenberg & Rittmann 2004). Also, the loss of gas out of the system needs to be addressed. Further research is also needed to investigate if the hydrogen gas partial pressure can further be lowered to values typical for anaerobic digesters (10^{-5} atm). A lower hydrogen partial pressure will improve the thermodynamics of the cathodic reduction which might result in a decreasing need of additional voltage in order to maintain the same current density.

Recent studies have shown that bacteria can retrieve electrons from a cathodic electrode for nitrate and oxygen reduction without hydrogen gas as an intermediate (Gregory *et al.* 2004; Rhoads *et al.* 2005; Clauwaert *et al.* 2007). Further research is needed to reveal whether cathodic biofilms can be adapted to methane or hydrogen formation without the use of mediators by direct electron transfer from the cathodic electrode to the microorganisms.

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