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Principle and perspectives of hydrogen production through biocatalyzed electrolysis

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Abstract

Biocatalyzed electrolysis is a novel biological hydrogen production process with the potential to efficiently convert a wide range of dissolved organic materials in wastewaters. Even substrates formerly regarded to be unsuitable for hydrogen production due to the endothermic nature of the involved conversion reactions can be converted with this technology. Biocatalyzed electrolysis achieves this by utilizing electrochemically active micro-organisms that are capable of generating electrical current from the oxidation of organic matter. When this biological anode is coupled to a proton reducing cathode by means of a power supply, hydrogen is generated. In the biocatalyzed electrolysis experiments presented in this article acetate is used as a model compound. In theory, biocatalyzed electrolysis of acetate requires applied voltages that can be as low as 0.14 V, while hydrogen production by means of conventional water electrolysis, in practice, requires applied voltages well above 1.6 V. At an applied voltage of 0.5 V the biocatalyzed electrolysis setup used in this study, produces approximately 0.02 $m^3 H_2/m^3$ reactor liquid volume/day from acetate at an overall efficiency of $53 \pm 3.5\%$. This performance was mainly limited by the current design of the system, diffusional loss of hydrogen from the cathode to the anode chamber and high overpotentials associated with the cathode reaction. In this article we show that optimization of the process will allow future volumetric hydrogen production rates above 10 $m^3 H_2/m^3$ reactor liquid volume/day at overall efficiencies exceeding 90% and applied voltages as low as 0.3–0.4 V. In the future, this will make biocatalyzed electrolysis an attractive technology for hydrogen production from a wide variety of wastewaters.

Keywords: Hydrogen production; Biocatalyzed electrolysis; Electrochemically active micro-organisms

1. Introduction

Stimulated by the depletion of fossil fuels and the threat of global warming, society is widely considering renewably produced hydrogen as an alternative clean fuel for transportation [1]. To deal with future hydrogen demands independent of fossil fuels, it will be necessary to consider all available renewable resources for hydrogen production [2]. In theory, large amounts of renewable hydrogen can be produced from dissolved organic materials in wastewaters using fermentation technology. However, the efficiency of dark fermentation of carbohydrate-rich wastewater, the most promising of the currently known technologies, is generally less than 15% [3]. Besides methanogenic consumption of hydrogen [4–6], thermodynamical limitations are an important reason for this low yield. Due to these

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thermodynamical limitations, the majority of the substrate is converted to byproducts (e.g. acetate, butyrate) instead of hydrogen. According to Benemann [7], economic feasibility can only be reached if hydrogen yields on dissolved organic material can approach 60–80%. In order to achieve this, the produced byproducts need to be converted to hydrogen as well. Dark fermentation is not capable of doing this, because the conversions involve endothermic reactions. Therefore, much research has been directed over the years towards photofermentations [8–10], which use sunlight to overcome this thermodynamical barrier. However, the diffuse nature of solar radiation and the limited conversion efficiencies severely limit the economic feasibility of these processes due to the enormous reactor surface areas that are required [11].

Alternatively, biocatalyzed electrolysis [12,13], a recently discovered technology that is related to the microbial fuel cell [14–22], overcomes this thermodynamical barrier by means of a small input of electrical energy. This makes the process independent of the reactor surface area, which benefits the economic feasibility. Biocatalyzed electrolysis achieves this by utilizing electrochemically active micro-organisms, which convert dissolved organic material to bicarbonate, protons and electrons. Either by direct contact with an electrode surface [23,24] or aided by (excreted) redox mediators [21], these micro-organisms release the produced electrons to an electrode surface in order to generate a current. By coupling this biological anode to a proton reducing cathode by means of a power supply, a direct conversion of dissolved organic material to hydrogen is accomplished. The complete process takes place in an electrochemical cell in which oxidation of dissolved organic material and proton reduction are separated in two chambers (Fig. 1). The separation between these chambers is established by means of a cation exchange membrane (e.g. Nafion®). Externally, the anode and the cathode are connected to the power supply using an electrical circuit. While the power supply drives the released electrons from the anode to the cathode, an equal number of protons permeates through the membrane. At the cathode, protons and electrons combine to form pure hydrogen gas.

Acetate is used as a model compound for the biocatalyzed electrolysis experiments presented in this study, as acetate cannot be directly converted to hydrogen through dark fermentation

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2. \quad (1)$$

Based on Gibbs free energy calculations, hydrogen production from acetate requires an energy input of

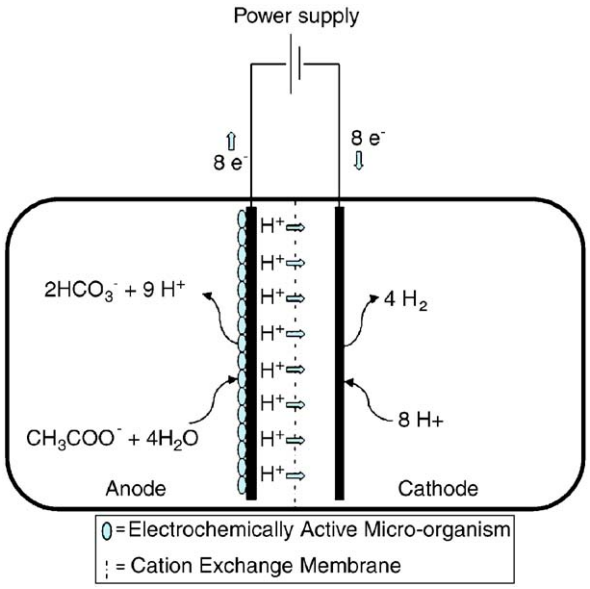

Fig. 1. Schematic representation of hydrogen production through biocatalyzed electrolysis of acetate.

104.6 kJ/mol under standard conditions [25]. Accordingly, in theory, an applied voltage of only 0.14 V is required for hydrogen production through biocatalyzed electrolysis of acetate. At pH 7, this corresponds to equilibrium potentials for the oxidation of acetate (1 mol/l) and proton reduction of −0.28 and −0.42 V (NHE), respectively. In practice, however, it can be expected that more than 0.14 V will be required for this reaction to proceed. Firstly, this is caused by the fact that electrochemically active micro-organisms consume part of the available energy themselves for growth and maintenance purposes. Consequently, the micro-organisms release the electrons at a higher potential than the equilibrium potential. Secondly, the required applied voltage is also expected to be affected by other losses in the cell as a consequence of the ohmic resistance of the electrochemical system and the occurrence of overpotentials. Nevertheless, at pH 7 anode potentials are found in literature that are typically around −0.2 V (NHE) or lower under operating conditions [17,18] and, therefore, it can be expected hydrogen production through biocatalyzed electrolysis will already be possible at applied voltages around 0.22 V. In comparison, hydrogen production through water electrolysis, in practice, operates at applied voltages that are well above 1.6 V [26–28].

Essentially, dissolved organic material is *electrolyzed* into carbon dioxide and hydrogen gas during the process with the electrochemically active micro-organisms acting as the catalyst. Therefore, we call this technology

"biocatalyzed electrolysis" [12]. Independent of the work done by our laboratories, the researchers Liu et al. [13] recently published their preliminary findings on the same technology and refer to it as "electrochemically assisted microbial production of hydrogen". In line with their work, this article aims to further elucidate the working principle, to pinpoint the possible bottlenecks of biocatalyzed electrolysis and to evaluate what this technology implies for the future of hydrogen production from wastewaters.

2. Methods

2.1. Electrochemical cell

Biocatalyzed electrolysis was studied in a two-chambered electrochemical cell made of poly(methyl methacrylate). The cylindrical chambers (290 mm inside diameter; length: 50 mm; wall thickness: 5 mm) had a total volume of 3.3 l each and were separated by a Nafion® 117 cation selective membrane (surface area: 256 cm^2). Both the anode and the cathode chamber were equipped with an Ag/AgCl reference electrode (+0.2 V vs. NHE [29]) to be able to measure the separate electrode potentials. The anode consisted of a disc-shaped piece of graphite felt (diameter: 240 mm; thickness: 3 mm—FMI Composites Ltd., Galashiels, Scotland); the cathode of a disc-shaped titanium mesh electrode with a 50 g/m^2 platinum coating (diameter: 240 mm; thickness: 1 mm; specific surface area: 1.7 m^2/m^2—Magneto special anodes BV, Schiedam, The Netherlands). To minimize ohmic resistance, both electrodes were placed in direct contact with the membrane. During the experiments 400 cm^2 of the surface area of both electrodes was submerged in the respective media and regarded electrochemically active. The electrodes were connected to a potentiostat (μAutolabIII, Eco Chemie B.V., Utrecht, The Netherlands) using an isolated electrical wire through a gas tight connection in the chamber wall. The potentiostat was used to control the applied voltage on the total system (as the power supply) or to control the anode potential during the growth of the micro-organisms after inoculation. Prior to the experiments, the electrochemical cell was sterilized (1 h) with 6% hydrogen peroxide and rinsed with autoclaved Milli-Q water.

2.2. Medium preparation

The anode chamber was filled with 3 l of autoclaved anode medium (pH 7) containing (in Milli-Q): 2.22 g/l KCl, 0.61 g/l KH_2PO_4, 0.96 g/l K_2HPO_4, 0.28 g/l NH_4Cl, 0.1 g/l yeast extract, 100 mg/l $MgSO_4 \cdot 7H_2O$, 10 mg/l $CaCl_2 \cdot 2H_2O$ and 1 ml/l of a trace element mixture [30]. The cathode chamber was filled with 3 l of autoclaved cathode medium (pH 7) containing (in Milli-Q): 2.22 g/l KCl, 0.61 g/l KH_2PO_4 and 0.96 g/l K_2HPO_4. The described amounts of acetate were added to the anode medium as an autoclaved concentrated (100 g/l) solution of $NaCH_3COO \cdot 3H_2O$ in Milli-Q.

2.3. Electrochemically active micro-organisms

The inoculation (3%) of the anode chamber was done with 90 ml of effluent from an identical electrochemical cell that had been running biocatalyzed electrolysis of acetate for over 5 months. That cell had previously been started up with sludge from a fullscale UASB reactor treating sulfate-rich papermill wastewater [31] (Industriewater Eerbeek, Eerbeek, The Netherlands). The 5 month operation period had resulted in the natural selection of a consortium of electrochemically active micro-organisms from this sludge.

Immediately after the inoculation, an applied voltage scan was recorded. After this, the microbial community was grown in the anode chamber by controlling the anode potential with the potentiostat. The anode potential was set to −0.1 V (vs. Ag/AgCl) for 3 days and then switched to −0.4 V (vs. Ag/AgCl) for 2 days. In this period, the current generation increased from ∼ 1 to ∼ 16 mA. Two applied voltage scans were then recorded. To check whether the microbial community had stabilized, the anode potential was set to −0.4 V (vs. Ag/AgCl) for 3 more days. During this period, the current remained stable. After this second growth period, two applied voltage scans were recorded again. During both growth periods, the pH was regularly checked and corrected to 7 if necessary.

2.4. Experimental procedures

During the applied voltage scans both chambers were flushed continuously with nitrogen gas. The applied voltage was gradually increased from 0 to 0.75 V at a rate of 0.1 V/h. This relatively low rate was chosen to reduce non-Faradaic currents to a negligible level. After every scan an equilibration time of 4 h at an applied voltage of 0 V was taken into account, preventing non-Faradaic currents from the potential switch back to 0 V to influence the next scan. All scans were taken in duplicate, except for the scan taken just after

inoculation of electrochemically active micro-organisms (single scan).

During the constant applied voltage experiments (0.5 V, in duplicate) only the anode chamber was continuously flushed with nitrogen to enhance mixing; the cathode chamber was only flushed intensively prior to the experiment. The cathode chamber was kept closed during the experiment, thereby capturing all produced hydrogen in the headspace. To create equilibrium between the headspace volume and the bulk liquid, the headspace gas was continuously recycled through the cathode chamber at a recycle rate of 280 ml/ min. Total cathode headspace volume was 275 ml.

During all experiments the temperature of the electrochemical cell was kept at 303 K. Current and applied voltage recordings were performed by the potentiostat. All reported potentials are against the NHEreference. An ion chromatograph (Metrohm 761 Compact IC) equipped with a conductivity detector and an ion exclusion column (Metrosep Organic Acids 6.1005.200) was used to measure the acetate concentrations. Gas samples were analyzed with a gas chromatograph (Shimadzu GC-2010) equipped with a thermal conductivity detector and a molesieve column (Varian molsieve 5A). Total hydrogen production was determined by measuring the hydrogen in the headspace and calculating the dissolved hydrogen from Henry's law [32]. All reported hydrogen volumes refer to hydrogen at 303 K and 1 bar.

2.5. Electrochemical calculations

The theoretically required applied voltage was calculated from the Gibbs energy according to $\Delta G = -nF\Delta E$ [29]. This calculation excludes all potential losses in a practical biocatalyzed electrolysis system caused by microbial energy consumption, ohmic resistances in the system and overpotentials suffered at the electrodes. Including these potential losses will always lead to a higher required applied voltage. Consequently, the theoretically required applied voltage is also the minimally required applied voltage. The equilibrium cathode potential (proton reduction) at pH 7 was calculated by converting the potential of the NHE (by definition: 0 V) to its value at pH 7 by means of the Nernst equation [29]. The Nernst equation was also used for calculating other concentration effects. The equilibrium anode potential (acetate oxidation) at pH 7 was calculated from the difference between the theoretically required applied voltage and the equilibrium cathode potential at pH 7.

3. Results and discussion

The start-up of the biocatalyzed electrolysis process (10 mM acetate) was followed by evaluating the system performance by means of applied voltage scans (Fig. 2). Without biocatalysis (open circles) and directly after inoculation of electrochemically active micro-organisms (closed circles) no significant currents were generated as a result of the applied voltage. However, enhanced current generation was observed in the scans recorded after a 5-day (open squares) and after a subsequent 3-day growth period (closed squares) at controlled anode potential (see Methods). The similarity of both of these scans indicates that the microbial community was stable. From the scans it becomes clear that the presence of a developed culture of electrochemically active micro-organisms is essential for the process; without biocatalysis no current is generated. The scans recorded after both growth periods also show that to enhance current generation an increasing voltage needs to be applied. This increased voltage represents the additional energy that needs to be supplied at higher current densities to compensate for the increasing energy losses associated with the electrode reactions (i.e. overpotential/polarization) and the ohmic resistance in the system. The fact that current generation occurred below the theoretically required applied voltage of 0.16 V (at 10 mM acetate) can be explained from the continuous nitrogen purging of the cathode compartment during the scans. This constantly removed the generated hydrogen from the cathode, favoring new hydrogen formation.

To confirm that the current generation in the applied voltage scans indeed originated from acetate oxidation, the system was operated for 4 h at a constant applied voltage of 0.5 V without acetate in the anode medium. In this case, current generation was negligible ($< 15\,mA/m^2$ anode surface) and no hydrogen was detected in the cathode headspace. When the same procedure was repeated with acetate in the anode medium (2 mM), current generation and hydrogen production were significant. The average measured current density during this period was $470 \pm 74.3\,mA/m^2$ anode surface (18.8 ± 3.0 mA). After 4 h 0.38 ± 0.034 mmol of acetate was consumed and 0.80 ± 0.13 mmol (20 ± 3.2 ml) of hydrogen was produced. This corresponded to a volumetric hydrogen production rate of approximately $0.02\,m^3H_2/m^3$ reactor liquid volume/day.

Further examination of these results, gave a clear indication of the current performance of the system and especially also pinpointed the bottlenecks that limit this performance. Table 1 shows the efficiencies that were calculated from these measurements. The Coulombic

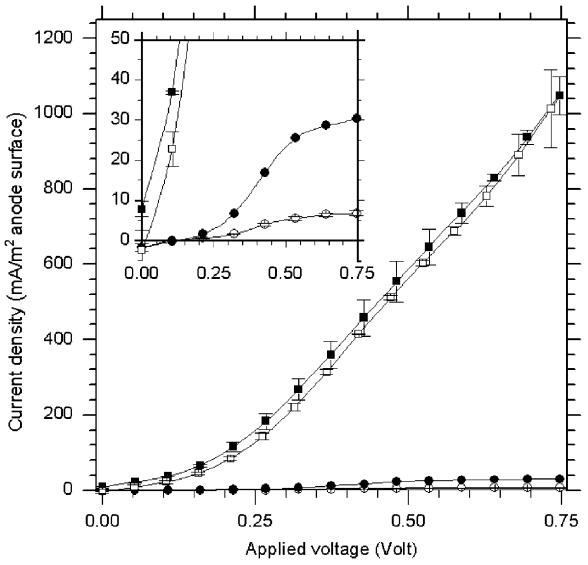

Fig. 2. Applied voltage scans from 0 to 0.75 V for biocatalyzed electrolysis of acetate (10 mM). Current generation was measured in the absence of electrochemically active micro-organisms (duplicate scans—open circles) and in the presence of electrochemically active micro-organisms just after 3% inoculation (single scan—closed circles), after a 5-day growth period at controlled anode potential (duplicate scans—open squares) and after a subsequent 3-day growth period at controlled anode potential (duplicate scans—closed squares). See Methods for details on the growth periods. The inset shows the same measurements on an adapted current density scale. Bars indicate minimum and maximum of the duplicate scans.

Table 1
Calculated efficiencies of hydrogen production through biocatalyzed electrolysis of acetate

% Coulombic efficiency (acetate to e^-)	92 ± 6.3
% Cathodic hydrogen efficiency (e^- to H_2)	57 ± 0.1
% Overall hydrogen efficiency (acetate to H_2)	53 ± 3.5

These yields were calculated from duplicate experiments that lasted for 4 h at a constant applied voltage of 0.5 V. The experiment started with an acetate concentration of 2 mM. Hunderd percent efficiency corresponds to: 1 acetate $\rightarrow 8e^- \rightarrow 4H_2$.

efficiency ($92 \pm 6.3\%$), i.e. the conversion of acetate to electrons, compares well with that found for biological anodes that have been applied in microbial fuel cell systems [14,15,20,21] and even seem to be slightly higher. This is probably due to the fact that no coulombic efficiency losses are suffered by aerobic microbial conversion of substrate, as is the case in microbial fuel cells, [18]. In microbial fuel cells, oxygen can diffuse from the aerobic cathode chamber to the anaerobic anode chamber through the cation exchange membrane. In biocatalyzed electrolysis this problem is circumvented as the cathode chamber is anaerobic as well. The small coulombic efficiency loss that is found for the biocatalyzed electrolysis system is partly caused by the presence of methane-producing bacteria in the anodic chamber, as confirmed by the presence of trace amounts of methane in the nitrogen purge flow. Furthermore, as in every microbial process, a few percent of the organic substrate is expected to be consumed for growth of the microbial consortium and thus lost for current generation. Nevertheless, all these losses are negligible compared to the loss of cathodic hydrogen efficiency, i.e. the conversion of electrons to hydrogen, suffered during the experiment. The cathodic hydrogen efficiency was found to be only $57 \pm 0.1\%$. Based on the generated current, 100% cathodic hydrogen efficiency would have yielded 35 ± 5.6 ml of hydrogen gas. However, only 20 ± 3.2 ml was measured. Consequently, approximately 15 ml of hydrogen was lost during the experiment. This loss is likely caused by diffusion of hydrogen from the cathode into the anode chamber through the Nafion® 117 membrane, as is the case with oxygen in microbial fuel cells. Hydrogen that enters the anode chamber is consumed by micro-organisms or removed from the reactor with the nitrogen purge. Analysis indeed confirmed the presence of trace amounts of hydrogen in the nitrogen purge flow leaving the anode compartment. Assuming hydrogen saturation in the Nafion® membrane, and using the hydrogen diffusion data as determined by other researchers [33], it was estimated [18] that during the experiment a maximum amount of hydrogen of between 19 and 26 ml could have diffused to the anode chamber. Although the hydrogen concentration in the headspace at the end of the experiment was only $6.1 \pm 0.9\%$, the hydrogen concentration at the membrane can still be close to saturation due the direct physical contact between the cathode and the membrane. During the experiment hydrogen bubble formation was clearly observed on the cathode surface, thus indicating saturated conditions in proximity of these bubbles. Mainly due to the loss in cathodic hydrogen efficiency, the resulting overall hydrogen efficiency of biocatalyzed electrolysis system tested in this study was found not to be higher than $53 \pm 3.5\%$.

By analyzing the electrode potentials (Fig. 3a) and comparing them with the equilibrium potentials, it was evaluated how the applied voltage of 0.5 V was consumed in the electrochemical cell during the 4 h experiment (Fig. 3b). In this way, another bottleneck in the system was revealed. At an acetate concentration of 2 mM, the theoretically required applied voltage necessary to start hydrogen production is 0.16 V. The remaining 0.34 V was thus lost to irreversibilities in the

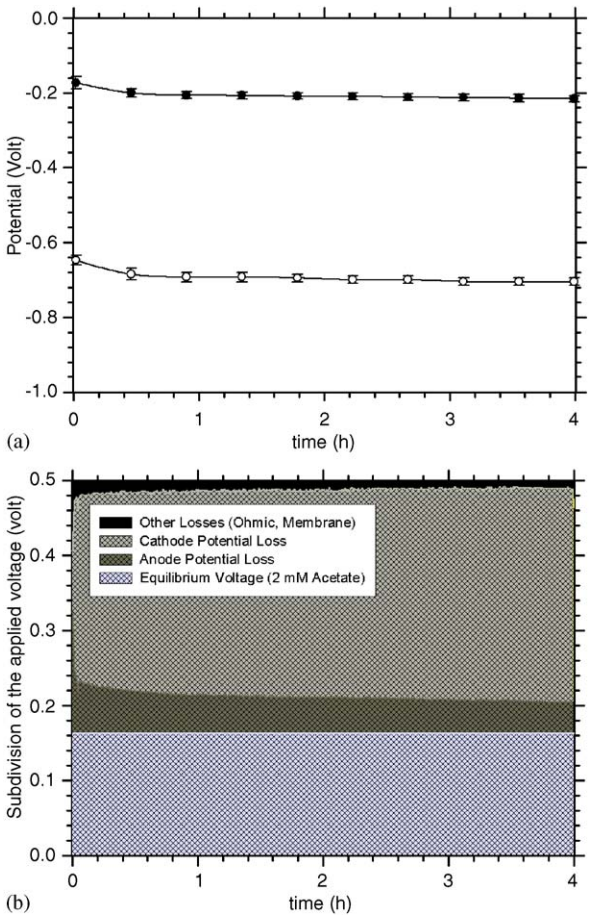

Fig. 3. (a) Measured potential of the anode (closed circles) and cathode (open circles) during biocatalyzed electrolysis of acetate (2 mM) at an applied voltage of 0.5 V (duplicate experiments). The average measured current density during this period was $470 \pm 74.3\,\mathrm{mA/m^2}$ anode surface. Bars indicate minimum and maximum of the duplicate experiments. (b) Subdivision of the applied voltage (0.5 V) during biocatalyzed electrolysis of acetate (2 mM).

system. From Fig. 3 it can be clearly seen that in the current experimental setup, most of this overvoltage is consumed by the cathode. At the end of the experiment ($t = 4$ h), for example, more than 0.28 V was lost by the cathode reaction (cathode potential: −0.71 V). At the same time, only 0.04 V was lost in the anode reaction (anode potential: −0.22 V) and 0.01 V to the other parts of the electrochemical cell (e.g. membrane and ohmic loss). So, despite the fact that platinum catalysis ($50\,\mathrm{g/m^2}$) is applied on the cathode, this part of the system still suffers from the largest potential losses. This is in contradiction to what is commonly accepted for conventional polymer electrolyte water electrolysis, where the proton reduction step is known to be associated with low overvoltages. Even at a current density exceeding $10\,000\,\mathrm{A/m^2}$ the overvoltage for the hydrogen evolution reaction can be as low as 0.025 V [27]. Therefore, much lower potential losses should be possible at the relatively low current densities ($1–10\,\mathrm{A/m^2}$) as encountered in bio-electrochemical processes. One explanation might be the relatively mild conditions (pH 7, 303 K) under which the experiments have been conducted in this study. From the literature not much is known about hydrogen evolution under those conditions [34].

4. Perspectives

The presented analysis of the experimental results has clearly indicated two important issues in the current experimental setup for hydrogen production by means of biocatalyzed electrolysis, i.e. the cathodic hydrogen efficiency loss and the cathode potential loss. Together with the relatively low volumetric hydrogen production rate of approximately $0.02\,\mathrm{m^3\,H_2/m^3}$ reactor liquid volume/day at an applied voltage of 0.5 V, it is clear that improvements are necessary to come to a mature hydrogen production technology. However, since this is one of the first articles describing biocatalyzed electrolysis, there is much room for improvements.

First of all, optimizations can be made with respect to the design of the electrochemical cell. The currently used system has a large liquid volume in relation to the anode surface area, while most of the generated current from acetate is believed to originate from the microorganisms that are directly attached to the anode [17]. Assuming it is possible to reduce the anode liquid volume to a small layer around the anode (total compartment length: ~ 5 mm) without influencing the current density and replacing the complete cathode chamber by a gas diffusion electrode as commonly applied in water electrolysis [35], the total reactor volume could be reduced to about 3% of its current size. This would already increase the volumetric hydrogen production rate to over $0.66\,\mathrm{m^3\,H_2/m^3}$ reactor liquid volume/day.

Furthermore, according to literature data on the performance of biological anodes in microbial fuel cell studies [21], current densities can be improved by at least one order of magnitude. However, in order to achieve this, much attention first needs to be spent towards lowering the above-mentioned cathode potential loss. When the cathode potential loss can be decreased, more of the applied voltage becomes available for other parts of the electrochemical cell, thus allowing higher current densities. At these higher current densities the cathodic hydrogen efficiency also is expected to increase

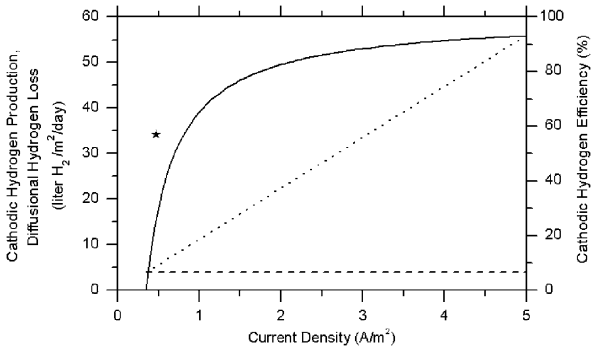

Fig. 4. Estimation of the dependency of the cathodic hydrogen efficiency (solid line) on current density calculated for the experimental setup presented in this study. In the calculations it is assumed 100% H_2-saturated conditions apply in the cathode at all current densities. Therefore, a constant diffusional hydrogen loss is predicted of $4\,l/m^2/day$ (dashed line—prediction based on [18,33]). The dotted line shows the hydrogen production at 100% cathodic hydrogen efficiency. The star indicates the cathodic hydrogen efficiency of the experiment presented in this study. The fact that the star is not on the line, is caused by the fact that the experiment was not conducted under 100% H_2-saturated conditions.

significantly, because the hydrogen diffusion through the membrane is only dependent on the hydrogen concentration gradient and not on the hydrogen production rates. This is schematically presented in Fig. 4 for the experimental setup that was used in this study. At higher current densities, the absolute amount of hydrogen lost through the Nafion® membrane remains the same. However, in relative sense this loss becomes less significant, because more hydrogen is produced at the cathode at higher current densities. Furthermore, other cation conducting membranes that are less hydrogen permeable might be available.

As can be seen from Fig. 4, the current system was running the biocatalyzed electrolysis process in the lower part of the current density scale, explaining the relatively low cathodic hydrogen efficiency. If, on the other hand, a current density of $5\,A/m^2$ anode surface area is realized in an optimized system, the cathodic hydrogen efficiency and hence the overall hydrogen efficiency can reach values above 90%. This exceeds the mark for economic feasibility as mentioned by Benemann [7] by far. However, current densities of this magnitude can only be reached if the cathode potential loss can be reduced significantly (to $< 0.1\,V$ at $5\,A/m^2$). In combination with an optimized reactor design, volumetric hydrogen production rates will then become possible that exceed $10\,m^3\,H_2/m^3$ reactor liquid volume/day at applied voltage of 0.3–0.4 V. The volumetric organic matter conversion rates associated with this increased performance are comparable to that of commercial anaerobic wastewater treatment plants. Therefore, next to being an efficient hydrogen generating process, the process then also functions as an efficient wastewater treatment process. Furthermore, with an expected required applied voltage of 0.3–0.4 V, biocatalyzed electrolysis achieves extremely low energy requirements for hydrogen production. Water electrolysis in practice operates at applied voltages well above 1.6 V [26–28], biocatalyzed electrolysis requires at least four times less. The price of hydrogen produced through water electrolysis is strongly dependent on the electricity price [2]. As this can be expected for biocatalyzed electrolysis as well, the reduced consumption of electrical energy per unit of hydrogen is a strong advantage of biocatalyzed electrolysis.

Besides acetate, a compound normally considered to be a byproduct of dark fermentation of glucose [3], many other substrates qualify for hydrogen production through biocatalyzed electrolysis. Recently, biological anodes have been studied in microbial fuel cells under various conditions. These conditions ranged from working with pure microbial cultures, e.g. with *Shewanella putrefaciens* [16], *Geobacter sulfurreducens* [14] and *Rhodoferax ferrireducens* [15], on synthetic wastewater to mixed microbial cultures [17,21,22] on real wastewaters [18–20]. These studies showed that electrochemically active micro-organisms are able to generate current from many different substrates, varying from organic substrates like sugars [15,21,22] and fatty acids [14,17,36] to inorganic substrates, like elemental sulfur [24]. By coupling comparable biological anodes to a proton reducing cathode many different kinds of substrates become available for hydrogen production. This diversity makes biocatalyzed electrolysis a robust and versatile hydrogen production process suitable for many kinds of wastewaters. Even more so, because this can be achieved by using a mixed culture of micro-organisms. This obviously benefits the economics of the process, as no effort needs to be spent towards keeping the culture pure.

Another advantage of hydrogen produced through biocatalyzed electrolysis is its potential purity. Other hydrogen production processes from organic matter (e.g. dark fermentation, biomass gasification) produce a mixture of hydrogen, carbon dioxide and traces of other gases (e.g. H_2S, CO) and therefore require expensive gas treatment processes. Biocatalyzed electrolysis produces the hydrogen separately in the cathode chamber. However, also with biocatalyzed electrolysis some contamination of the hydrogen might occur by the diffusion of gases produced in the anode chamber (e.g. CO_2)

through the cation exchange membrane. As the biocatalyzed electrolysis experiments presented in this article were not optimized for the purity of the produced hydrogen, no definitive conclusion can yet be drawn about this. Hydrogen purity therefore needs to be subject for future research, as the product purity will eventually determine the application of this technology. PEM fuel cells, for example, which are considered to be most suitable for transportation purposes, require pure hydrogen as a fuel [37] and are irreversibly damaged by traces of H_2S and CO.

As a hydrogen production process, biocatalyzed electrolysis exhibits an enormous hydrogen production potential worldwide. In the case of the Netherlands, for example, all collected urban wastewater [38] already contains enough biodegradable material for the generation of 1.1 billion m^3 of hydrogen gas per year. Assuming a fuel efficiency of 0.5–1 kg hydrogen per 100 km for a fuel cell powered vehicle [39], this is already enough hydrogen for driving 9.4–19% of the total car km in the Netherlands [38]. In its optimized configuration, biocatalyzed electrolysis will thus certainly proof its right of existence in the future hydrogen economy. However, as with any hydrogen-producing technology, the exploitation of this potential only becomes fully possible when a complete hydrogen infrastructure is established. Applying this technology as a centralized wastewater treatment, connection to a hydrogen transport network is necessary to distribute the produced hydrogen from the wastewater treatment to the consumer. Alternatively, one can think of the development of a decentralized system, allowing people to produce hydrogen gas from their own wastewater at home. Until that time niche applications can be investigated in hydrogen-consuming industries that have to treat effluents rich in organics (e.g. food-processing, petrochemical industry).

Acknowledgments

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