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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Renewable sustainable biocatalyzed electricity production in a photosynthetic algal microbial fuel cell (PAMFC)

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Abstract Electricity production via solar energy capturing by living higher plants and microalgae in combination with microbial fuel cells are attractive because these systems promise to generate useful energy in a renewable, sustainable, and efficient manner. This study describes the proof of principle of a photosynthetic algal microbial fuel cell (PAMFC) based on naturally selected algae and electrochemically active microorganisms in an open system and without addition of instable or toxic mediators. The developed solar-powered PAMFC produced continuously over 100 days renewable biocatalyzed electricity. The sustainable performance of the PAMFC resulted in a maximum current density of 539 mA/m^2 projected anode surface area and a maximum power production of 110 mW/m^2 surface area photobioreactor. The energy recovery of the PAMFC can be increased by optimization of the photobioreactor, by reducing the competition from non-electrochemically active microorganisms, by increasing the electrode surface and establishment of a further-enriched biofilm. Since the objective is to produce net renewable energy with algae, future research should also focus on the development of low energy input PAMFCs. This is because current algae production systems have energy inputs similar to the energy present in the outcoming valuable products.

Keywords Alga · Bioenergy · Electricity · Microbial fuel cell · Photobioreator · Renewable

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Introduction

The current climate change threat caused by CO_2 emission from combustion of fossil fuels, in combination with the instabilities in the fossil fuel markets and increasing energy prices, accelerates the urgency for development of alternative renewable reliable energy technologies (IPCC 2007). Solar energy conversion by plants and algae into biomass, also called bioenergy, may encompass considerable shares in future sustainable renewable energy production as heat, hydrogen, methane, electricity, and other biofuels (Ragauskas et al. 2006). A promising biotechnology for producing electricity or hydrogen from bioenergy is the rapidly evolving microbial fuel cell (MFC; Logan et al. 2006). In a MFC, electron donors are oxidized at the anode and concomitant-produced electrons flow through an energy consumer toward the cathode where oxygen is reduced to water (Kim et al. 1999). Hereby, electrochemically active microorganisms act as the biocatalyst in the anode and/or cathode (He and Angenent 2006). When the MFC is coupled to a proton-reducing cathode, by means of a power supply, hydrogen is produced (Rozendal et al. 2006).

Recently proof of principle was delivered of renewable electricity generation via living higher plants in MFCs (Strik et al. 2008a; De Schamphelaire et al. 2008; Kaku et al. 2008). Herein, the living plant is photosynthesizing and releasing a part of the produced organic materials via its roots into the root surroundings. These materials, the so-called rhizodeposits, were then in situ oxidized in the bioanode of the MFC and transformed into electrical power. In the present study, we focus on living microalgae for solar energy capturing and bacteria in the MFC for electricity generation. These concepts of living green biomass and MFCs are attractive, since they can, in potential, generate energy in a renewable, sustainable, and efficient manner

(Rittmann 2008; Strik et al. 2008a, b; Ter Heijne et al. 2006).

The so far investigated algae and microbial fuel cell combinations, also called solar-powered or photosynthetic fuel cells, are promising with regards to efficiency since Yagishita et al. (1997) developed a system that transformed light energy into electricity with a conversion efficiency of 3%. This is similar to conversion efficiencies achieved by organic solar cells (Hoppe and Sariciftci 2004). However, the current algae or cyanobacteria and microbial fuel cell combinations are unsustainable because they make use of expensive catalysts like platinum for in situ hydrogen oxidation (Rosenbaum et al. 2005) or make use of instable and toxic mediators for electron shuttling (Berk and Canfield 1964; Tanaka et al. 1985; Yagishita et al. 1997; Chiao et al. 2006; Cho et al. 2008). Furthermore, current systems make use of pure algae cultures in closed systems. This makes them less robust when those algae would be used in an open system, which is vulnerable to algae and other infections.

Therefore, the objective of this study was to prove the principle of a photosynthetic algal microbial fuel cell (PAMFC) for renewable sustainable electricity production with three novel attributes: (1) naturally selected algae cultures in an open system, (2) electrochemically active bacteria as low-cost self-sustaining catalysts, and (3) cost-effective electrodes made from graphite (Pham et al. 2004). This would mean that platinum or added instable and toxic mediators are not needed.

Hereby, it was our strategy to employ the well-known processes of photosynthesis by algae for the conversion of light energy into chemical energy and electron production by the electrochemically active bacteria at graphite bioanode of the MFC. The intermediate energy transfer from the micro-algae to the bacteria is expected to proceed via processes like alga lyses, excretion of dissolved organic compounds, or hydrogen production (Richmond 2004; Malinsky-Rushansky and Legrand 1996). These outcoming products can most likely be degraded by the electrochemically active bacteria.

For this study, we started an autotrophic operating photobioreactor with a mixture of algae, CO_2 from air as the carbon source, and a nutrient medium. Separately, a MFC was started, which was inoculated with electrochemically active bacteria and fed with acetate for initial start-up. Thereafter, both reactors were linked to form the PAMFC, which was investigated on principles, efficiencies, and perspectives.

Materials and methods

Experimental setup

A schematic overview of the experimental setup is shown in Fig. 1; numbers used in the scheme are also referred in the text between square brackets. The initial start-up algal photobioreactor (AR1) [1] was a bubble column from glass with a height of 40 cm and diameter of 10 cm. Ambient air

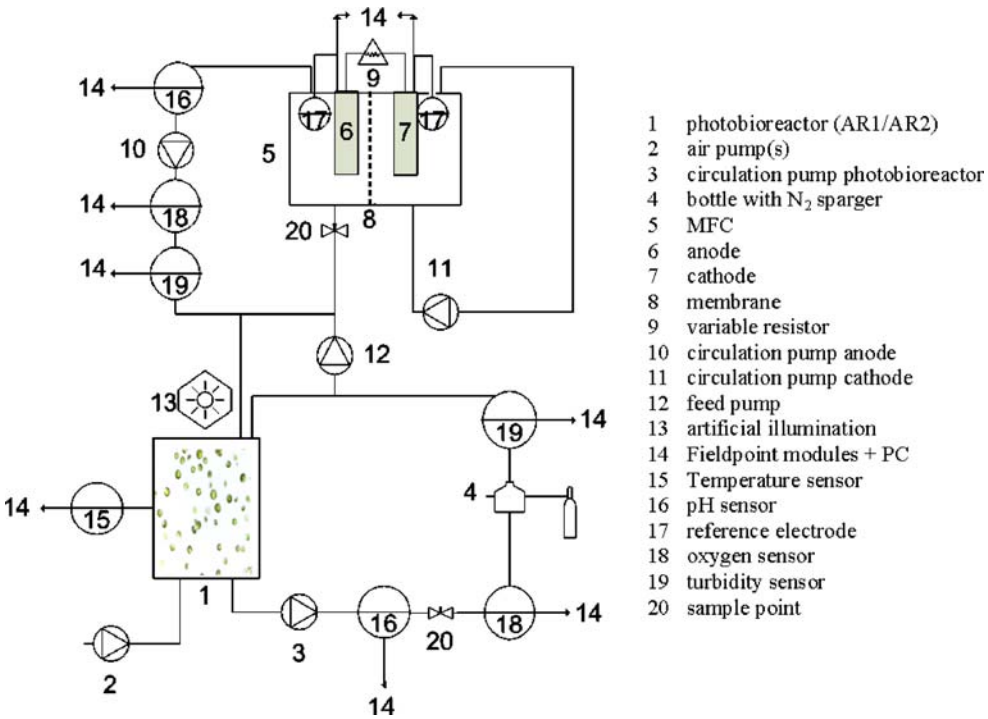

Fig. 1 Schematic overview of the experimental setup

was dosed via a sparger into the reactor by an air pump [2] at a flow rate of 3.2 l h^{-1}. The second algal photobioreactor (AR2) was a flat plate glass (4-mm thick) photobioreactor (height, 31 cm; width, 24 cm; and depth, 3 cm). Ambient air was pumped into the reactor via a wash bottle to moisten the air and two spargers into the reactor by two pumps with a total flow rate of 60 l h^{-1} [2]. The topside of the algal photobioreactor was open to the ambient air and functioned as the gas outlet. The reactor solution was continuously circulated with a peristaltic pump (3.2 l h^{-1}) [3] in a circuit containing a 2-l flask, which was continuously stirred and covered with aluminum foil, glass holders with the online sensors, and a 500-ml bottle with a tunable N_2 gas sparger (maximum, 1 l min^{-1}) [4]. The total solution volume of the flat plate algal reactor was 4.5 l. The ambient temperature near the algal photobioreactor was between 298 and 303 K. The used flat plate MFC [5] with graphite felts (National Electrical Carbon B.V., The Netherlands) at the anode [6] and cathode [7] was, except for the type of membrane, identical to the one used by Ter Heijne et al. (2006). In this study, we used a cation exchange membrane (type FKB, Fumapsep) [8]. The anode and cathode electrodes were connected to a resistor with a range of 0 to 100 Ω [9]. The MFC was operated in a temperature-controlled room at 303 K. The anolyte and catholyte, with both a volume of total 2 l, were continuously circulated by peristaltic pumps [10, 11] through a circuit containing the online sensors with a flow rate of, respectively, 12 and 10.8 l h^{-1}. The PAMFC was formed by connecting the algal photobioreactor via a tube circuit to the anode compartment of the MFC. A time-switch-controlled peristaltic pump [12] was used for mixing the algal photobioreactor and the anolyte. The artificial illumination [13] was emitted to the algal photobioreactor by two 400 W Master SON-T PIA Green Power (Philips) lamps and by two 400 W Master HPI-T Plus (Philips) lamps placed in a cabinet. The average photon flux density of photosynthetic active radiation (PAR; 400–700 nm) at the surface of the flat plate algal photobioreactor was 139 μmol m^{-2} s^{-1} or 28 W m^{-2}. The lights were time switch controlled resulting in a continuous illumination period of 14 h day^{-1}.

Data acquisition, on- and offline measurements, microscopic, electrochemical, and chemical analysis

Online data was acquired using Fieldpoint modules (National Instruments) and a personal computer with Labview Software (National Instruments) [14]. A thermocouple was used for measurement of the ambient temperature near the algal photobioreactor [15]. pH [16] was measured online as well as cell voltage and anode and cathode potential (vs. Ag/AgCl reference electrodes; 3 M KCl, +205 mV vs. standard hydrogen electrode, ProSense Qis [17]. Oxygen was measured online (CellOX325, WTW) [18]. The density of the algae suspension was monitored online according to Hoekema et al. (2002). Hereby, turbidity sensors were used with a red-light-emitting diode peaking at 668 nm [19]. The method validity was checked by optical density (OD or OD_{660}) measurements with a spectrophotometer (Dr. Lange Xion 500) at 660 nm, which resulted in a correlation of >0.99. Scanning electron microscopy (SEM) of the anode graphite felt was performed as described by Rozendal et al. (2008). Samples (0.25 cm^2) of the graphite felt anode of PAMFC 1 and 2 were taken by deconstructing the MFC during the experiment. Light microscopy (Nikon Eclipse E400) was performed using a magnification of 1,000 to analyze algae morphology, species, and vitality. Images were taken digitally by using a mounted Nikon camera connected to a personal computer. The PAR light intensity was measured with a quantum/radio/photometer (Li-Cor, model LI-250). Quantum spectra of the growing lights were provided by Philips (The Netherlands) and used to calculate the average energy content of the photons. The photosynthetic efficiency of the algae was calculated with Planck's Law and the Gibbs free energy expression assuming that by the algae produced chemical oxygen demand (COD) was glucose. This is because glucose is the main building bloc of carbohydrates like polysaccharides, which are common energy storage products in algae (Larkum et al. 2003). The coulombic efficiency (CE) and MFC energy recovery from the removed COD was calculated assuming that all COD consisted of glucose as described by Logan et al. (2006) and Ter Heijne et al. (2006). Current density was expressed in mA per square meter projected anode electrode surface unless otherwise stated. The algae's photosystem II efficiency and the determination of which group the algae in the samples belonged to (green algae, diatoms or cyanobacteria) were determined with Phyto-PAM according to Lürling and Verschoor (2003). The algal biovolume and amount was measured with an electronic particle counter (Coulter Multisizer II) as described (Lürling and Verschoor 2003). The MFC internal resistance was determined by using a variable external resistance as described (Logan et al. 2006) and by electrochemical impedance spectrometry (EIS) as described by Strik et al. (2008b). Cyclic voltammetry was performed with a potentiostat (IviumStat, Ivium, The Netherlands) connected to a personal computer used at a scan rate of 25 mV/s in the potential range from −1,000 to 1,000 mV. The working electrode was an 8-cm^2 graphite rod that was cleaned in ethanol and deionized water prior to use, and the counter electrode was a golden wire. An Ag/AgCl electrode was used as a reference. COD analysis was conducted by using a COD cuvette test (LCK114; Dr Lange, GmbH, Düsseldorf, Germany).

Experimental procedures

The first algal photobioreactor (AR1) was filled with modified Hoagland medium (Taiz and Zeiger 2006) and inoculated with water samples (approximately 50 ml), containing algae and a diversity of other microorganisms, obtained from ditches in the surrounding of Wageningen (The Netherlands) and Berlin (Germany), a fish aquarium, and from a cooling mantle of a laboratory bioreactor. This way, we started cultivation of mixed algae populations in an open reactor. After 89 days, another algal photobioreactor (AR2) was used and filled with Hoagland medium and inoculated with a 100-ml sample of the bubble column (AR1). The algal photobioreactors were operated autotrophically, which means that only CO_2 was provided as carbon source. Evaporated water was replenished with demineralized water, and the volume of the taken samples was replenished with modified Hoagland medium.

The MFC was started with a nutrient solution adapted from Van der Zee et al. (2001), a phosphate buffer (K_2HPO_4 and KH_2PO_4; mixed to a final concentration of 20 mM and pH 7.0), and batch fed with potassium acetate (final concentration, 20 mM). The MFC was inoculated with effluent containing microorganism of another MFC running on acetate (Ter Heijne et al. 2008). The pH of the anolyte was controlled with NaOH to maintain a minimum pH of 8.0 (controller of Endress+Hauser Liquisys S) to provide favorable conditions for algae and electrochemically active bacteria (Richmond 2004; Ter Heijne et al. 2006). The catholyte contained 50 mM potassium hexacyanoferrate and 20 mM phosphate buffer (K_2HPO_4 and KH_2PO_4; mixed to pH 7.0). At a decreasing cathode potential, the catholyte was replenished.

After 35 days of start-up, the algal photobioreactor (AR1) was coupled via tubes to the anode compartment of the MFC to form the PAMFC 1. Before this, the anolyte of the MFC was removed so that no acetate was left and thereafter refilled with the Hoagland medium. The MFC was everyday batchwise-fed with a peristaltic pump, feeding for a period of 3 to 5 h at a flow rate of 4.0 l h^{-1}. It was prevented that oxygen would intrude in the anode compartment of the MFC. Here, the algal photobioreactor and the anolyte of the MFC were mixed after some time that the illumination was turned off. This way, oxygen production by the algae was halted, and in the meantime, oxygen could be consumed by the algae. In addition, the circulation flow of the algal photobioreactor was flushed with N_2 during the first 8 weeks of the experiment to strip oxygen out of the algae suspension. To investigate the effect of the present electrochemically active bacteria in the bioanode, the MFC was replaced by an identical MFC on day 118 to form the non-biocatalyzed PAMFC 2. This MFC contained a clean graphite felt and was filled with 0.8-l medium of the algal photobioreactor. The PAMFC 2 was replenished with the Hoagland medium. After 2 days, the biocatalyzed MFC was placed back to form PAMFC 1 again. Subsequently, PAMFC 1 was operated as before. From day 131, the daily feeding of the MFC stopped by decoupling of the algal photobioreactor from the MFC.

Results

Start phase and long-term operation of PAMFC 1

Right from the first day that the PAMFC 1 was formed, by connecting the algal photobioreactor to the anode compartment of the MFC, current production started (Fig. 2). PAMFC 1 was operated autotrophically during weeks 1 to 17 and resulted in continuous current production.

During the whole experiment, there was a nutrient neutral operation since the nutrients remained in the PAMFC 1. And throughout the period in which the MFC was fed daily, the photobioreactor and the MFC showed, as explained further on, typical daily characteristics.

Performance of the photobioreactor of PAMFC 1

Figure 3 shows the typical daily characteristics of the photobioreactor of PAMFC 1. During the illumination, the optical density at 660 nm (OD_{660}), as a measure of the algae density, increased. Two phases were distinguished during illumination. Throughout the first 40 min, the rate of OD_{660} increase was 0.014 OD_{660} min^{-1}; thereafter, the increases was 0.00045 OD_{660} min^{-1}. During the dark period, the OD_{660} decreased steadily. During the feeding period, the algae suspension was mixed with the low OD_{660} anolyte of the MFC, and therefore, the OD_{660} decreased even more. When illumination was turned on again, the OD_{660} increased again. The oxygen concentration in the algal photobioreactor was, during illumination, maximum of 6 mg l^{-1} and decreased, without illumination, to 0 within 2 to 4 h. During the 14-h illumination period, the PAR light was transformed into chemical energy with 6.3% efficiency. This represented a total 1.2 g COD production within these 14 h. During illumination, the soluble COD concentration in the photobioreactor remained quite constant at 0.32 g l^{-1} (sample interval, 2 h; standard deviation, 0.006 g l^{-1}).

Performance of the MFC of PAMFC 1

Figure 4 shows the typical daily characteristics of the MFC of PAMFC 1. During the feeding period, the OD_{660} increased to the maximum. Thereafter, the OD_{660} gradually decreased linearly until the next feeding period. As a result of the feeding, the cell voltage first increased for 7 h and

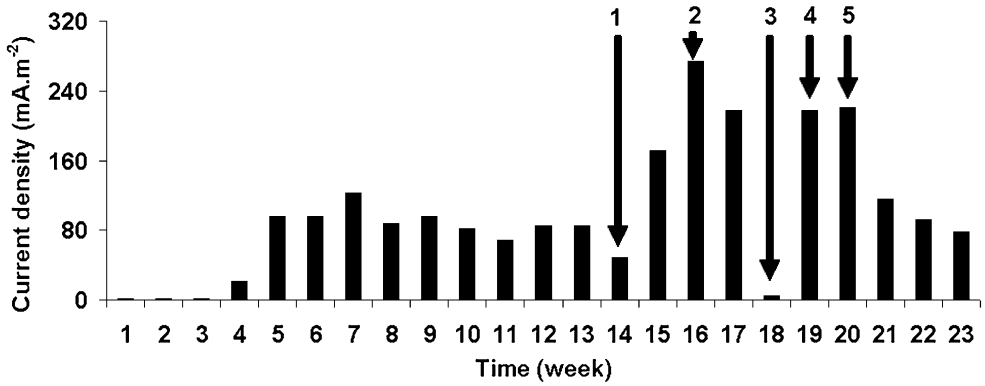

Fig. 2 Long-term operation of the PAMFCs showing the average week current density per square meter projected anode surface. *1* Installment of AR2; this photobioreactor had a higher algae productivity since the maximum OD660 of AR2 was 1.3 times higher than AR1. In week 14, this did not result in a higher current density, since algae productivity was lost due a leakage. In week 15, the current density was 171 mA m^{-2}, so the installment of AR2 resulted in a more current generation compared to AR1. *2* External resistance was set from 100 to 25 Ω, which was near the estimated internal resistance of the MFC (see data further on). This resulted in a higher average current density during the next 2 weeks of 246 mA m^{-2}; *3* formation of PAMCF 2; this resulted in a lower average current production by PAMFC 2 of 4.2 mA m^{-2}. *4* Return of PAMFC 1 that resulted in a current production of 217 mA m^{-2}. *5* Disconnection of AR2 and MFC. Then, the current production proceeded for more than 4 weeks without additional feeding

thereafter gradually decreased toward a stable value. Therefore, the trends of OD_{660} and current were not the same. It was likely that the first current peak was due to fast degradable COD and the remaining current to slower degradable COD. The soluble COD concentration remained quite constant at 0.32 g l^{-1} during the whole day (sample interval of 2 h; standard deviation, 0.008 g l^{-1}). At the moment that the cell voltage started to decrease still, 1.6 g l^{-1} total COD was present. CE was calculated from the produced Coulombs and removed COD. CE was 2.8% for the period on day 125. The MFC energy recovery from the removed COD was 1.0% on day 125. On day 95, the CE of the MFC was investigated by adding the additional electron donor potassium acetate. The CE from the removed acetate was 21%.

Total PAMFC 1 efficiency

The light energy radiation on the photobioreactor of the PAMFCs was 105 kJ day^{-1}. The total produced renewable electricity on day 125 was 92 J. Thus, on the selected day, the overall efficiency of PAMFC 1 transforming PAR light energy into electricity was 0.1%. This was an average power production of 14 mW m^{-2} used surface area of the photobioreactor.

Polarization, power density, and Nyquist plots of PAMFC 1

Because the cell polarization curve did not show a linear relationship for the whole polarization range, it was not possible to estimate the internal resistance from this data

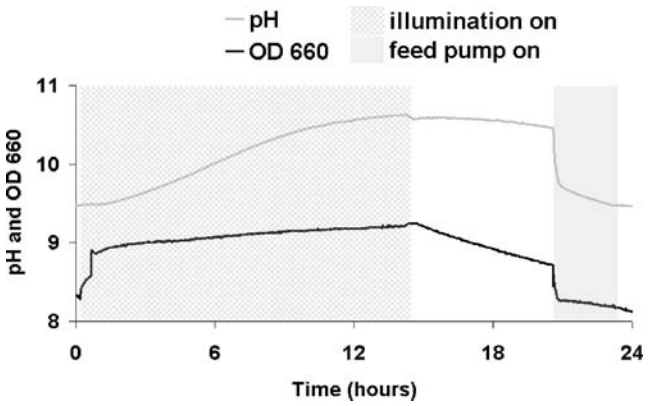

Fig. 3 Typical daily characteristics of the photobioreactor of PAMFC 1 showing the pH and the optical density of the algae suspension calculated from the online turbidity measurements during day 124. The *crosses-pattern* area represents the illumination period and the *gray area* the feeding period

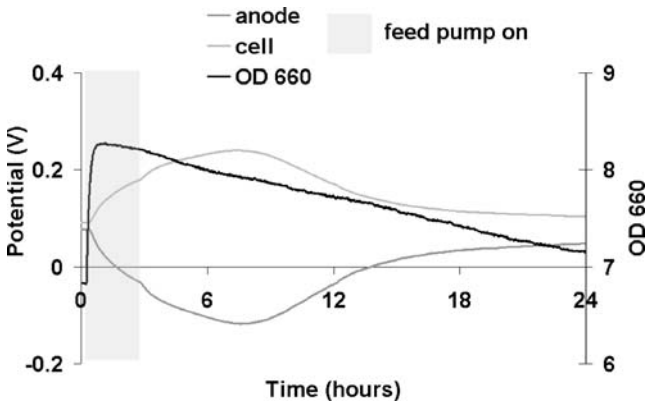

Fig. 4 Typical daily characteristics of the MFC of PAMFC 1 showing the anode potential, cell voltage, and the optical density of the algae suspension calculated from the online turbidity measurements during day 125. The applied external resistance was 25 Ω. The *gray area* represents the feeding period

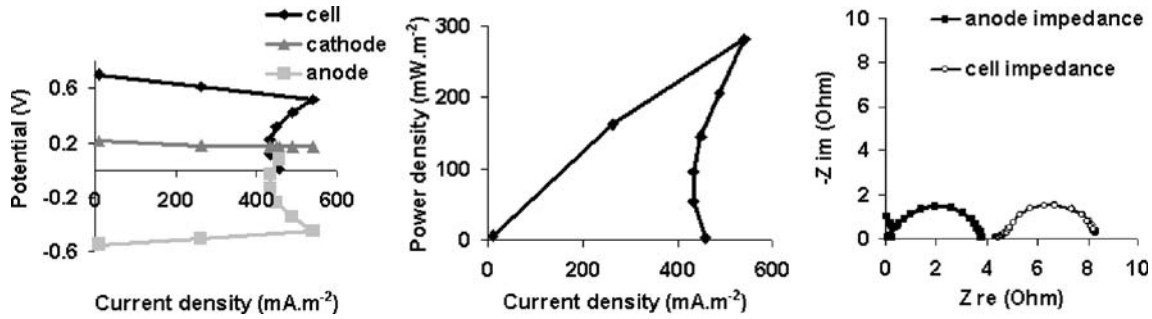

Fig. 5 Polarization, power, and Nyquist plots of the biocatalyzed PAMFC 1. The current density was expressed in mA per square meter projected anode surface

(Fig. 5). Therefore, the maximum power point from the power curve was used to estimate the internal resistance. Total internal resistance of the MFC of PAMFC 1 on day 101 was 33 Ω. During the recording of the polarization curves, the cathode potential remained constant and the anode potential dropped. This means that the cathode was not limited, and thus, the anode processes were limiting the current flow. The open circuit voltage was 0.705 V. The maximal power density was 282 mW m^{-2} projected anode surface at a current density of 539 mA m^{-2} projected anode surface and an anode potential of −0.445 V. The maximum power production per square meter surface area of the photobioreactor was 110 mW m^{-2}. The Nyquist plots of the anode (at −0.503 V) and cell (at 0.550 V) impedance measurements of PAMFC 1 on day 105 revealed more insights. The EIS data met the four criteria necessary for valid EIS results. Because EIS measurements were not performed at the maximum power density, the total internal resistance measured with EIS (8.2 Ω) was lower than estimated from the power curves (33 Ω). The 4.2 Ω of the measured cell impedance internal resistance was due to ohmic resistance. Four ohm was due to resistance of the anode. The remaining resistance due to the cathode over-potential was <1 Ω.

Effect of oxygen in the anode compartment

On day 119, the MFC of PAMFC 1 was fed during daytime, which resulted into oxygen presence in the anolyte medium up to 5 mg l^{-1} (Fig. 6). First, a feed period of 30 min was applied, which resulted in a reduced cell voltage, but no clear increase in oxygen was observed. During the second feed period, oxygen increase was observed and associated with an increase of anode potential and a subsequent decrease of the cell potential from 119 to 50 mV. At the moment, the feed pump was stopped, the oxygen concentration started to drop, and cell potential increased. The estimated CE for the period when oxygen was present in the anode compartment was 0.06%. This was 25% compared to the operation without oxygen presence. After the oxygen disappeared, the cell voltage recovered toward the typical daily values.

Presence of *Chlorella* algae and electrochemically active bacteria

By light microscopic analyses, it was determined that different alga species were present in the inocula of the algal photobioreactor. The Phyto-PAM analyses revealed on day 112 that the present microorganisms belonged to the green algae and that the algae present in the algal photo-bioreactor and anolyte of the MFC were alife. The algae's photosystem II efficiency was high (0.71), which was similar to values of green *Scenedesmus* alga under favorable growing conditions (Lürling and Roessink 2006). It was morphologically determined that only algae belonging to the genus *Chlorella* with a diameter of approximately 3 μm remained in the algal photobioreactor and anolyte of PAMFC 1 (see Fig. 7a). Coulter Multisizer II showed that over 95% of the particles were of size 2.8 to 6 μm, which matched the size of the algae. Therefore, it

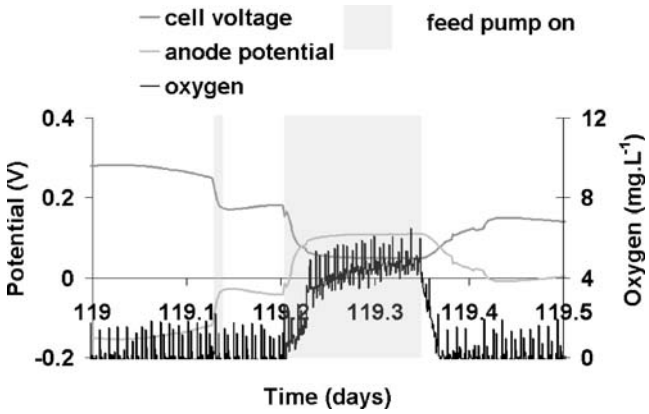

Fig. 6 Characteristics of the MFC of PAMFC 1 showing the effect of oxygen in the anode compartment. First, a feed period of 30 min was applied, thereafter, a feed period of 216 min. The applied external resistance was 25 Ω

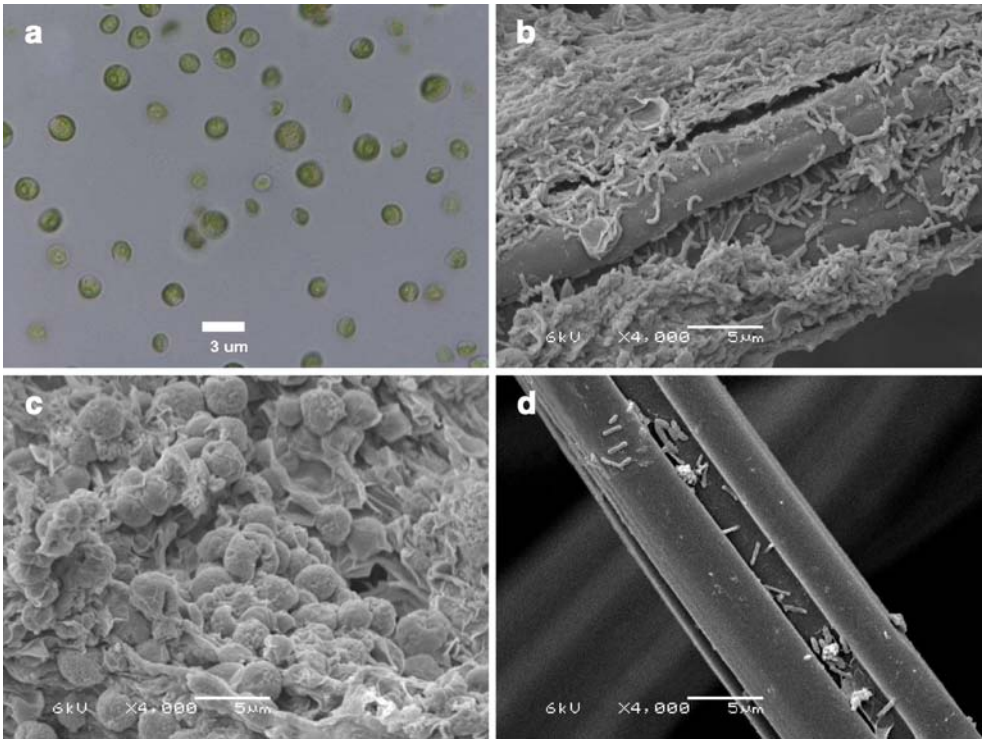

Fig. 7 **a** Light microscopic photo showing algae present in algal photobioreactor (sample day 112). **b**–**d** SEM photos from graphite felt anodes. **b** Anode of the PAMFC 1 after 4 months operation showing bacteria (**b**) and algae (**c**). **d** Anode of PAMFC 2 after 2 days of operation

was determined that *Chlorella* algae were the dominant species. No bacteria were observed in these samples. The findings proved that *Chlorella* algae were responsible for transforming light energy into chemical energy as measured by COD.

It was determined that a biofilm of approximately 1 mm was formed at the graphite anode felt of PAMFC 1. SEM photos from the graphite felt anodes revealed that a biofilm with bacteria (Fig. 7b) and algae (Fig. 7c) was present at the anode of PAMFC 1, and no such biofilm or algae were present on the anode felt of PAMFC 2 (Fig. 7d).

To reveal the role of the present biofilm in PAMFC 1, a MFC without inoculation of electrochemically active bacteria was constructed and replaced PAMFC 1 for 2 days. This setup was named PAMFC 2. Right from the first day that the PAMFC 2 was created, current production started. The open circuit voltage was 0.534 V. The power curve (recorded after 12 h of operation) showed a maximal power density of 3.6 mW m^{-2} projected anode surface at a current density of 11 mA m^{-2} projected anode surface and an anode potential of 0.030 V. The estimated internal resistance was 98 Ω. Thus, current production was possible without the presence of a dense biofilm. The maximum power density was 79 times higher with the presence of a biofilm at the anode. Therefore, the biofilm present in PAMFC 1 was thus acting as a kind of biocatalyst, and therefore, the present bacteria were electrochemically active. From the measurements, we were not able to determine whether *Chlorella* algae could also be responsible for current generation.

Cyclic voltammograms (CV) of the Hoagland medium and PAMFC 1 anolyte were performed to reveal whether redox-mediating compounds were present. The CV of the Hoagland medium and PAMFC 1 anolyte (Fig. 8) showed three oxidation and reduction peaks covering the whole anode potential operation range (−0.6 to 0.2 V vs. Ag/AgCl). Centrifuged algae and resuspended in demineralized water did not show any peaks. This means that

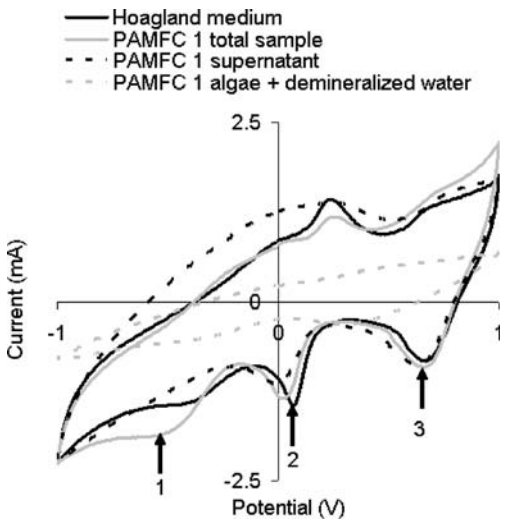

Fig. 8 Cyclic voltammograms of the Hoagland medium and PAMFC 1 anolyte. The *arrows* show the three peaks present in the anolyte sample of PAMFC 1

dissolved molecules were present in the anolyte, which could act as redox mediators.

Discussion

Renewable sustainable biocatalyzed electricity production in PAMFC 1

In the present study, we proved the principle of a novel PAMFC, which produced continuous renewable electricity. This is the first report of a solar-powered MFC, wherein living algae and biocatalyzing bacteria cooperate to produce renewable electricity (PAMFC 1). The enriched out-selected *Chlorella* algae were photosynthesizing and transforming light energy into chemical energy as biomass from which electricity was generated with electrochemically active bacteria at graphite bioanode of the MFC. The present bacteria in the biofilm were electrochemically active bacteria, since the maximal power output of PAMFC 1 was 79 times higher than the not-inoculated PAMFC 2. This study used naturally selected culture, low-cost self-sustaining solar energy capturer, electrochemically active bacteria as the primary biocatalysts and low-cost graphite electrodes. Cyclic voltammetry revealed that compounds were present in the anolyte and Hoagland medium, which could act as redox mediators. However, Hoagland medium does not contain instable or toxic mediators.

The dissolved nutrients were preserved in the system, which makes the PAMFC sustainable regarding nutrient use. The PAMFC was unsustainable regarding cathode process, since we applied the non-sustainable hexacyanoferrate-reducing cathode. However, literature reports many promising biocathode alternatives that make use of cost-effective graphite electrodes (He and Angenent 2006). PAMFC 1 showed a sustained performance, since operation was prolonged over 100 days including day–night intervals without any down time. The temporary oxygen presence in the bulk anolyte of bioanode did not irreversibly reduce the power output. This is in contrast to reports on solar-powered algae MFCs that use platinum catalysts. After operation of several days platinum showed an almost complete disappearance in current output due to poisoning of the platinum by algal byproducts or possible by aging of the algal culture (Cho et al. 2008; Rosenbaum et al. 2005). After the disconnection of the photobioreactor from the MFC, current production continued for over 4 weeks with chemical energy stored in the PAMFC 1. This shows that electricity generation can prolong during periods in which photosynthesis cannot proceed. The phenomenon that oxygen presence in the bulk anolyte did not suppress the present electrochemically active bacteria shows that they are not likely strictly anaerobes. This corresponds with findings by Ringeisen et al. (2007) who operated a MFC with an aerobic bioanode.

Transfer of energy from the algae to the microbial fuel cell was limiting the current output

The bioanode of PAMFC 1 was substrate-limited, since we observed that addition of potassium acetate resulted in a direct increase of current production. This was despite the presence of minimal 1.3 g total COD l^{-1} and 0.3 g soluble COD l^{-1}. Therefore, most of the present COD was apparently not directly available for uptake by the electrochemically active bacteria. The substrate limitation may be improved by forcing the algae to excrete more easily biodegradable dissolved organic carbon compounds and to produce more easily degradable organic acids via dark fermentation (Malinsky-Rushansky and Legrand 1996; Richmond 2004). Cyclic voltammetry revealed that redox-active species were present in the anolyte medium with peaks within the operative anode potential of PAMFC 2. These kinds of peaks indicate the presence of electron shuttles (Rabaey et al. 2004), which can result in current production. Further research will have to be conducted to reveal the mechanisms of chemical energy transfer from the algae to the microbial fuel cell.

A high photosynthetic efficiency was achieved within the photobioreactor

We achieved during illumination a photosynthetic efficiency of 6.3% (PAR-based), which was 42% of the expected maximum (Janssen et al. 2003). This high efficiency was achieved, since the average photon flux density was near the level of 100 μmol m^{-2} s^{-1} at which light energy utilization is still efficient (Janssen et al. 2003). Photosynthetic efficiency and productivity can be improved by optimization of the photobioreactor (Janssen et al. 2003). In this study, an initial fast OD_{660} increase in the algal photobioreactor during illumination was observed. It was unclear whether this was algae growth or a kind of photosynthetic activation period of the algae.

Coulombic efficiency of PAMFC 1 was low compared to other MFCs

The MFC of PAMFC 1 transferred chemical energy into electricity with an energy recovery of 1.0% of the removed COD. This was due to coulombic loss and internal resistances of the MFC. The CE of PAMFC 1 of 2.8% was relative low compared to MFCs running on (artificial) wastewaters that achieve more often efficiencies in the range of 20–60% (Logan and Regan 2006). Three processes were distinguished for being responsible that not all

captured solar energy was available for the electrochemically active bacteria. Firstly, *Chlorella* algae itself metabolized stored chemical energy in the absence of light. Ogbonna and Tanaka (1996) reported for example that *Chlorella* algae respired 16% of their biomass within a 14-h dark period. This indicates that 23% of all the algae biomass may be fermented during the dark period of 20 h in the anode of the PAMFC. Secondly, we observed a biofilm growth on the anode graphite felt, which contains a part of the chemical energy captured by the algae. Over the total length of the experiment, this was on average 24 mg COD day^{-1}, which is less than 1% of the total COD daily fed to the MFC. The third reason for the coulombic loss were the possible presence of microorganisms, which were unable to utilize the anode electrode as electron acceptor. These organisms will use the substrate for fermentation and/or methanogenesis (Logan et al. 2006). The CE from added acetate was 21%, which supported the indication that competing non-electrochemically active microorganisms were present. CE can possibly be improved by reducing the residence time of the algae in the dark anode during illumination periods and by reducing the competition from other microorganisms.

The internal resistance and overpotentials of the microbial fuel cell can be improved

The estimated total internal resistance at the maximum power output of 33 Ω was higher than the 10 Ω Ter Heijne et al. (2006) achieved with the same MFC design. This study achieved a MFC energy recovery of 1% of the removed COD, and Ter Heijne et al. (2006) achieved a recovery of 29%. This shows that the MFC was not operated at its maximum, and improvement is possible. In this study, the prevailed internal resistances of the MFC were due to anode overpotential and ohmic resistance. The ohmic resistance can likely be reduced by using high concentrated electrolytes in combination with the use of marine algae. The anode overpotential can be reduced by, for example, increasing the electrode surface and establishment of a further-enriched biofilm (Logan et al. 2006). The latter also counts for a PAMFC application with a biocathode.

Total efficiency of the PAMFC 1 processes

The overall efficiency of PAMFC 1 on one selected day transforming PAR light energy into electricity was 0.1%. The PAMFC 1 gross average electricity generation was 14 mW m^{-2} used surface area of the photobioreactor. The maximum power production per square meter surface area of the photobioreactor was 110 mW m^{-2}. This was more than the 67 mW m^{-2} Strik et al. (2008a) achieved with *Glyceria maxima* in a MFC. The variety of solar powered MFC designs, materials, operation conditions (e.g., input of PAR light intensity) and reported measurements makes it meaningless to compare total efficiencies or output. We expect that the PAMFC efficiency will improve in the future, since we revealed ways to improve the total efficiency. We expect that the energy recovery from a MFC will increase from 29% (Ter Heijne et al. 2006) to 60%. This, combined with the maximum PAR photosynthetic efficiency of 15% (Janssen et al. 2003), results in a maximum overall PAMFC efficiency of total light energy conversion into electricity of 3.9%. This is similar to conversion efficiencies achieved by organic solar cells (Hoppe and Sariciftci 2004).

Future applications, perspectives, and challenges

One of the challenges of a renewable sustainable energy production system is to make the net energy production (energy output minus input) as high as possible. When we take a look at successful large-scale algae production systems, most often, raceway ponds are applied and seldom closed photobioreactors (Richmond 2004). Hereby, raceway ponds are more often located near the coast to make use of salt water and not to compete with land for food production. However, raceway ponds and closed photobioreactors have considerable energy input. The paddle wheels needed for mixing raceway ponds consume electrical energy in the range of a minimum 0.2 W m^{-2} up to 4.6 W m^{-2} at a flow rate of 15 cm s^{-1} or even 37 W m^{-2} at a flow rate of 30 cm s^{-1} (Wagener 1983). Closed photobioreactors can have energy inputs needed for mixing and oxygen removal of 6 to 10 W m^{-2} (E. Roebroeck, LGem, 2008, The Netherlands, personal communication). When the typical raceway pond algae productivity of 13 g m^{-2} day^{-1} (energy content of 19 MJ kg^{-1}; Wagener 1983; Richmond 2004) can be transformed into electricity with a prospective net 60% MFC efficiency, this results into 1.7 W m^{-2} electricity. Thus, overall, the net energy production by combining algae production with a MFC system may be, depending on the implementation, positive or negative. Therefore, the challenge to design a net renewable electricity production system with algae as the primary solar energy capturers still remains.

To conclude, this study showed a first proof of principle of a promising, novel, potentially cost-effective, renewable, sustainable electricity generation system, which needs further research and development. Additional research on (1) reducing the energy input, (2) advancing the photosynthetic efficiency of the photobioreactor, (3) improving the chemical energy transfer from the algae to the electrochemically active bacteria, and (4) producing electricity in the fuel cell should allow higher yields and a technology closer to practical implementation.

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