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Production of Electricity and Butanol from Microalgal Biomass in Microbial Fuel Cells

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Abstract *Chlorella vulgaris* (a freshwater microalga) and *Dunaliella tertiolecta* (a marine microalga) were grown for bulk harvest, and their biomass was tested as feedstock for electricity production in cubic two-chamber microbial fuel cells (MFCs) at 37°C. The anode inoculum was anaerobic consortium from a municipal sewage sludge digester, enriched separately for the two microalgal biomass feedstocks. After repeated subculturing of the two anaerobic enrichments, the maximum power density obtained in MFCs was higher from *C. vulgaris* (15.0 vs. 5.3 mW m^{-2}) while power generation was more sustained from *D. tertiolecta* (13 vs. 9.8 J g^{-1} volatile solids). Anolytes of algal biomass-fed MFCs also contained substantial levels of butanol (8.7–16 mM with *C. vulgaris* and 2.5–7.0 mM with *D. tertiolecta*), which represents an additional form of utilizable energy. Carryover of salts from the marine *D. tertiolecta* biomass slurry resulted in gradual precipitation of Ca and Mg phosphates on the cathode side of the MFC. Polymerase chain reaction-denaturing gradient gel electrophoresis profiling and sequencing of bacterial communities demonstrated the presence of *Wolinella succinogenes* and *Bacteroides* and *Synergistes* spp. as well as numerous unknown bacteria in both enrichments. The *D. tertiolecta* enriched consortium contained also *Geovibrio thiophilus* and *Desulfovibrio* spp. Thus, the results indicate potential for combining fermentation and anaerobic respiration for bioenergy production from photosynthetic biomass.

Keywords Butanol · Electricity · *Chlorella vulgaris* · *Dunaliella tertiolecta* · Microalgal biomass · Microbial fuel cell

Introduction

Production of biomass-based fuels and energy carriers has been widely studied due to finite petroleum supplies and concerns for environmental effects caused by fossil fuel utilization. Microalgae may prove an alternative to terrestrial crops as they have higher photosynthetic efficiencies, higher yields and growth rates, and lower cultivation area requirements. They may also be cultivated in brackish and saline waters and in ponds, channels or photobioreactors constructed in arid or agriculturally uncultivable land areas [1, 2]. Biochemical conversion of algal biomass to energy carriers via anaerobic microbial metabolism does not require cost-intensive dewatering of the biomass [3]. In addition, algal biomass has high content of lipids, starch, and proteins and does not contain recalcitrant lignin, thus making it amenable to anaerobic digestion processes [1, 2, 4].

Microbial fuel cells (MFCs) convert chemically bound energy into electricity via anaerobic microbial respiration that couples with anode as the final electron acceptor. Many MFC studies have been conducted using model substrates such as acetate, butyrate, and glucose [5, 6]. Some studies have reported the use of complex substrates such as cellulose [7], domestic wastewater [8], paper recycling wastewater [9], and solid animal manure [10]. Power production

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varies greatly due to differences in MFC configurations and electrogenic microbial consortia [11]. MFCs have been operated with pure cultures or mixed cultures on the anode. Pure cultures have relatively predictable metabolic capabilities but require aseptic process conditions, are prone to process disturbances, have relatively low power outputs, and are usually unable to degrade complex substrates such as plant or algal biomass [12]. Microalgal biomass is potential feedstock for electrogenic bacteria on the anode as electricity production has been reported from suspensions of powderized macro- and microalgal biomass [13], natural marine plankton suspensions [14, 15], and effluent of an anaerobic microalgal biomass digester [16].

The purpose of this work was to utilize the biomass of two microalgal species, freshwater *Chlorella vulgaris* and marine *Dunaliella tertiolecta*, as feedstocks in cubic two-chamber MFCs. These were inoculated with microbial consortia derived from a municipal sewage sludge digester and enriched separately for several passages with the two microalgal biomass slurries as the feedstock. The anodic microbial communities were characterized using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) profiling. Previous MFC studies with algal biomass have focused on power output without much regard to value-added co-products. The present study is to our knowledge the first to report on concurrent electricity and butanol production from untreated microalgal biomass in MFCs. It is inevitable that MFC systems using plant- or algae-based feedstocks will not accomplish complete mineralization of biomass residues. Thus, it is worthwhile to explore the possibility that metabolic pathways in MFC systems be manipulated to generate metabolites that have fuel value or other useful industrial properties. This study was initiated to address this proof-of-concept, an important aspect in the development of sustainable biorefinery approaches.

Experimental

Algal Biomass and Inoculum Enrichment

C. vulgaris (UK strain 211/11B, Culture Collection of Algae and Protozoa, SAMS Research Services Ltd., Oban, Argyll, UK) and *Dunaliella tertiolecta* (strain SAG 13.86, Sammlung von Algenkulturen Göttingen, Germany) were grown photoautotrophically and harvested with chitosan and NaOH flocculation, respectively [17]. *C. vulgaris* biomass contained 36, 13, and 8% proteins, lipids and sugars on dry weight basis, respectively. The corresponding biomass composition of *D. tertiolecta* was 15, 11, and 4% [17]. These values fall within the generally broad range reported for microalgal biomass composition [17].

Anaerobic cultures were enriched from a sample of an anaerobic digester operating at 35°C and treating primary and secondary sludge from a municipal activated sludge process in the Viinikanlahti wastewater treatment plant (City of Tampere, Finland). Cultures were first enriched separately with the two algal feedstocks for CH_4 production as a series of batch incubations at 37°C with 5 g volatile solids (VS) L^{-1} algal biomass [17]. The cultures were further enriched in cubic two-chamber MFCs (37°C, 120 rpm) by transferring the culture suspension from the anode compartment to the next enrichment step. Enrichments were batch incubations of 15 days in step 1 and 28 days in step 2 with 5 g VS L^{-1} algal biomass. Then the enrichment was continued in the MFCs in fed-batch mode with an initial algal biomass loading of 5 g VS L^{-1} and addition of 2.5 g VS L^{-1} during each feed cycle in 2- to 4-day intervals in enrichment steps 3 to 6. These fed-batch enrichment steps were incubated for 16, 12, 16, and 13 days, respectively. Thus, the enrichment cultures went through six passages in MFCs with microalgal biomass as the feedstock. These consortia were developed separately for the two algal biomass types. The *C. vulgaris*-fed consortium is hereafter referred to as U-C and *D. tertiolecta*-fed consortium as U-D. The enrichment procedure is described in detail in Table 1.

MFC Configuration and Experimental Conditions

The cubic two-chamber MFCs consisted of two polycarbonate or poly(methyl methacrylate) halves separated by an Ultrex proton-exchange membrane (CMI-7000, Membranes International, Ringwood, NJ) (Fig. S1 in the Electronic Supplementary Material (ESM)). The working volumes of both anode and cathode were 75 mL. Graphite plate electrodes (4.6×2.7×0.6 or 5×2.9×0.6 cm, McMaster-Carr, Aurora, OH) were used in both chambers and they were pretreated as described by Bond and Lovley [18] before and between each MFC run. Modified Zehnder medium was used as the anolyte [19, 20]. One liter of medium contained 0.4 g KH_2PO_4, 0.4 g Na_2HPO_4, 0.3 g NH_4Cl, 0.3 g NaCl, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g $MgCl_2{\cdot}6H_2O$, 4 g $NaHCO_3$, 2 mg $FeCl_2{\cdot}4H_2O$, 50 μg H_3BO_3, 50 μg $ZnCl_2$, 38 μg $CuCl_2{\cdot}2H_2O$, 41 μg $MnCl_2{\cdot}2H_2O$, 50 μg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 50 μg $AlCl_3$, 50 μg $CoCl_2{\cdot}6H_2O$, 50 μg $NiCl_2{\cdot}6H_2O$, 0.5 mg EDTA, 0.24 g $Na_2S{\cdot}9H_2O$, 26.3 μg $Na_2SeO_3{\cdot}5H_2O$, 32.9 μg $NaWO_4{\cdot}5H_2O$, 0.5 g cysteine-HCl, and 0.2 g yeast extract and vitamin solution according to Karlsson et al. [19]. Thus, cysteine was both the oxygen scavenger and assimilatory sulfur source. K-ferricyanide (50 mM $K_3Fe(CN)_6$) in phosphate buffer (100 mM Na_2HPO_4, pH 7.0) was used as the catholyte. Ferricyanide was used in this study as it is more soluble in water and thus a more efficient electron acceptor at the cathode than dissolved O_2. The anode compartment was

Table 1 Steps in the enrichment of electrochemically active microbial community

Experimental step	Operation mode	Substrate (initial loading; addition during each feeding)	Inoculum source
Enrichment step 1	Batch	*Chlorella vulgaris* (5 g VS L^{-1})	Methanogenic sludge fed with *C. vulgaris*
		Dunaliella tertiolecta (5 g VS L^{-1})	Methanogenic sludge fed with *D. tertiolecta*
Enrichment step 2	Batch	*C. vulgaris* (5 g VS L^{-1})	*C. vulgaris*-fed anolyte from step 1
		D. tertiolecta (5 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 1
Enrichment step 3	Fed-batch	*C. vulgaris* (5 g VS L^{-1}; 2.5 g VS L^{-1})	*C. vulgaris*-fed anolyte from step 2
		D. tertiolecta (5 g VS L^{-1}; 2.5 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 2
Enrichment step 4	Fed-batch	*C. vulgaris* (5 g VS L^{-1}; 2.5 g VS L^{-1})	*C. vulgaris*-fed anolyte and *D. tertiolecta*-fed anolyte from step 3 (1:1)
		D. tertiolecta (5 g VS L^{-1}; 2.5 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 3
Enrichment step 5	Fed-batch	*C. vulgaris* (5 g VS L^{-1}; 2.5 g VS L^{-1})	*C. vulgaris*-fed anolyte from step 4
		D. tertiolecta (5 g VS L^{-1}; 2.5 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 4
Enrichment step 6	Fed-batch	*C. vulgaris* (5 g VS L^{-1}; 2.5 g VS L^{-1})	*C. vulgaris*-fed anolyte from step 5
		D. tertiolecta (5 g VS L^{-1}; 2.5 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 5
Production potential experiment	Fed-batch	*C. vulgaris* (5 g VS L^{-1}; 2.5 g VS L^{-1})	*C. vulgaris*-fed anolyte from step 6
		D. tertiolecta (5 g VS L^{-1}; 2.5 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 6
		Pre-digested *C. vulgaris* (1.2 g VS L^{-1}; 0.6 g VS L^{-1})	*C. vulgaris*-fed anolyte from step 6
		Pre-digested *D. tertiolecta* (2.1 g VS L^{-1}; 1.05 g VS L^{-1})	*D. tertiolecta*-fed anolyte from step 6
		Glucose (5 gL^{-1}; 2.5 gL^{-1})	*C. vulgaris*-fed anolyte from step 6
		Glucose (5 gL^{-1}; 2.5 gL^{-1})	*D. tertiolecta*-fed anolyte from step 6

The methanogenic sludges for the enrichment step 1 originated from a previous study [17]

flushed with nitrogen prior to the experiments. The MFCs were inoculated (10%, *v*/*v*) with the enrichment culture and operated at 37°C and 120 rpm shaking in fed-batch mode with feed cycle every 2 days. During each feeding, catholyte was replaced by fresh K-ferricyanide, sample was taken from the anolyte and the sample volume replaced by fresh medium and feed. If the anode pH was below 7.0, it was adjusted to 7.0± 0.2 with degassed 1 MNaOH.

A fixed external resistance (R) of 100 Ω was connected between the electrodes and the closed circuit potentials of the MFCs were recorded every 2 min with an Agilent 34970A data logger. The power density was calculated according to the equation, $P = I \times V/A$, where V is voltage (V), I ($I=V/R$) the current (amp), and A the surface area of the anode (m^2). Polarization characteristics of the MFCs were determined by varying the resistance (between 1 MΩ and 5 Ω) using a variable resistor box from the open circuit voltage stepwise in 5-min intervals. The internal resistance (R_i) of the fuel cells was estimated according to the equation $R_i = (V_o - V_r)/I$, where V_o is the open-circuit potential and V_r the potential across the external resistance. Coulombic efficiency was calculated by comparing the actual coulombs produced (by integrating the current over time) to the theoretical production of coulombs based on removal of total chemical oxygen demand (COD_{tot}), using a conversion factor of 8 g COD per mole of electrons [21, 22]. The conversion factor is derived from the stoichiometric ratio of 4 mol e^-/mol O_2 reduced (4e^-/32 g O_2=1e^-/8 g O_2). The energy content of the produced electricity was calculated by integrating the power over time for the highest electricity producing batch cycle.

In addition to non-pretreated *C. vulgaris* or *D. tertiolecta* biomass slurry, electricity production was tested with glucose, pre-digested *C. vulgaris* and pre-digested *D. tertiolecta* (biomass after 4–6 weeks of anaerobic digestion). Algal biomass was pre-digested in anaerobic serum bottles with sewage sludge as the inoculum as previously described by Lakaniemi et al. [17]. Aliquots of *C. vulgaris* and *D. tertiolecta* biomass were added to the MFCs initially at 5 and subsequently 2.5 g VS L^{-1} during each feed batch cycle, and the corresponding additions of glucose were 5 and 2.5 g L^{-1}, respectively. For the pre-digested algal biomass, the initial feedstock concentration was 1.2 and 2.1 g VS L^{-1} and subsequently 0.6 and 1.05 g VS L^{-1} of pre-digested *C. vulgaris* and pre-digested *D. tertiolecta*, respectively. Controls without substrate and without inoculum were also included in the experiments.

Analytical Methods

The pH of anode solution was measured with a WTW pH 3301 pH meter and WTW pH SenTix 41 electrode. The formation of volatile fatty acids (VFAs; including

acetate, propionate, butyrate, isobutyrate, valerate, and caproate) and alcohols (ethanol and butanol) was analyzed with a Perkin Elmer Clarus 500 GC system equipped with an HP-5MS column and a flame ionization detector. The temperatures of injector and detector were 250 and 280°C, respectively. Oven temperature was held at 50°C for 3 min, increased from 50 to 100°C at the rate of 20°C min^{-1}, from 100 to 150°C at 10°C min^{-1} and finally held at 150°C for 5 min. Chemical oxygen demand (COD) was analyzed with dichromate method according to standard SFS 5504 [23]. COD_{tot} was based on unfiltered samples that included all soluble metabolites as well as feedstock biomass, bacteria, cellular debris and digestion products. Gas formation on the anode was measured by connecting tedlar bags (Zefon International, Ocala, FL) to the anode chamber. The gas volume in the gas bags was determined with water displacement method. The gas composition in the gas bags (H_2, CH_4 and CO_2) was measured using Shimadzu gas chromatograph GC-2014 equipped with Porapak N column (80/100 mesh) and a thermal conductivity detector. The oven, injector and detector temperatures were 80, 110 and 110°C, respectively. Nitrogen was used as carrier gas at a flow rate of 20 mL min^{-1}. Conductivity of the anode solution was measured with a WTW LF95 conductivity meter. Samples of precipitates formed on cathode electrode and cathode side of the membrane in MFCs fed with *D. tertiolecta* biomass were air dried, mounted on Al-stubs, and carbon coated for examination with Philips XL-30 scanning electron microscope (SEM) equipped with ion microprobe for elemental analysis. Concentration of chloride ions was analyzed with Dionex DX-120 ion chromatograph equipped with AS40 auto sampler and IonPac AS23 (4×250 mm) anion exchange column. Concentration of sodium ions was analyzed with inductively coupled plasma emission-mass spectrometry according to industry standard DIN EN ISO 17294.

Microbial Community Analyses

Duplicate samples of suspended cultures (1.5 mL) were taken from the anode chamber at the end of the MFC experiment and stored at −20°C. Prior to DNA extraction samples were pelleted by centrifugation (10,000×*g*, 5 min) and the supernatant was removed. DNA was extracted from the pellets with PowerSoil™ DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). The extracted DNA sample was used as a template for PCR. Partial bacterial 16S rRNA genes of the community DNA were amplified by using primer pair GC-BacV3f [24] and 907r [25] as described by Lakaniemi et al. [17]. DGGE was performed with INGENYphorU2×2-system (Ingeny International BV, Goes, The Netherlands) using 8% polyacrylamide gels with denaturing gradient from 30 to 70 % as described by Lakaniemi et al. [17] (100% denaturing solution contains 7 M of urea and 40% formamide). The dominant bands were excised from the gels, eluted in 20 μL of sterile water at +4°C overnight, stored at -20°C and reamplified for sequencing. Sequencing was conducted at Macrogen Inc. (Seoul, Korea). Sequence analyses were performed with BioEdit-software and online BLAST software tool.

Results

Enrichment of Electrochemically Active Microbial Community

In first two batch enrichment steps, current generation remained below 0.09 mA with both microalgal feedstocks. Thereafter, fed-batch operation mode was started, and the current increased up to 0.40 mA with *D. tertiolecta* biomass and U-D, whilst the current still remained very low (0.04 mA) with *C. vulgaris* and U-C. In the beginning of enrichment step 4, the *C. vulgaris* MFC was inoculated with 1:1 ratio of anode solution from *C. vulgaris* and U-C MFC and anode solution from *D. tertiolecta* and U-D MFC from the previous enrichment step to enhance the current generation from *C. vulgaris*. This increased the current generation up to 0.28 mA from *C. vulgaris*. During enrichment steps 5 and 6, current generation remained at similar levels as in previous steps, being at maximum 0.32 and 0.42 mA from *C. vulgaris* and *D. tertiolecta*, respectively.

The sum of VFAs and alcohols at the end of each MFC enrichment step increased up to step 5, being 26.7 and 11.7 mM at highest with *C. vulgaris* and U-C, and *D. tertiolecta* and U-D, respectively (Fig. S2A in the ESM). Butanol and propionate were the main metabolites detected under these conditions.

Electricity Production

In the electricity production assay, electricity was produced from *C. vulgaris* with U-C, *D. tertiolecta* with U-D, glucose with U-C, glucose with U-D as well as from pre-digested *D. tertiolecta* with U-D (Fig. 1). In contrast, no current was produced from pre-digested *C. vulgaris* with U-C.

Repeated cycles of substrate addition resulted in a rapid current increase (<5 min) in MFCs fed with glucose and a slower current increase (<11 h) in MFCs fed with *C. vulgaris*, *D. tertiolecta*, or pre-digested *D. tertiolecta* (Fig. 1). In glucose-fed MFCs the current dropped steeply after the maximum, whereas in MFCs fed with algal biomass or pre-digested *D. tertiolecta* the decrease in the current was slower. The current was at the minimum prior to the next feed batch cycle.

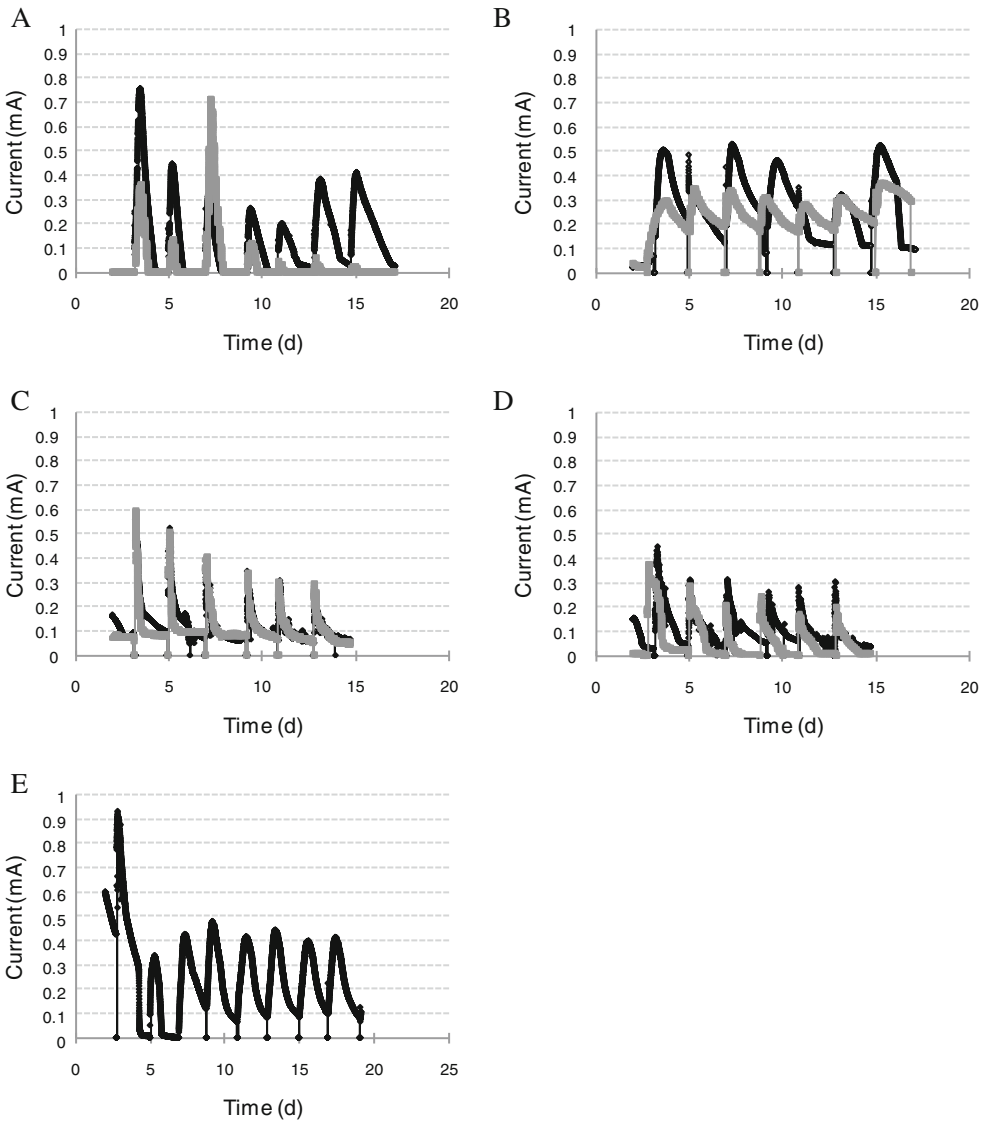

Fig. 1 Current over time in fed-batch MFCs with *C. vulgaris* and U-C (**A**), *D. tertiolecta* and U-D (**B**), glucose and U-C (**C**), glucose and U-D (**D**), and pre-digested *D. tertiolecta* and U-D (**E**). U-C = *C. vulgaris*-fed enrichment culture, U-D = *D. tertiolecta*-fed enrichment culture. *Black graph* represents MFC A, and *grey graph* MFC B of the duplicate treatments. Only one replicate was included with the pre-digested *D. tertiolecta* and U-D

The maximum power density was higher from *C. vulgaris* with U-C than from *D. tertiolecta* with U-D (Table 2). Similarly, the maximum power density was higher from glucose with U-C than from glucose with U-D (Table 2). The maximum power density was highest from the pre-digested *D. tertiolecta* with U-D, but this power was attained only once and all the other current peaks were much lower (Table 2; Fig. 1E). Current peaks after each feeding were wider from *D. tertiolecta* or glucose with U-D than from *C. vulgaris* or glucose with U-C (Fig. 1). With *C. vulgaris* and U-C the voltage decreased to near zero between the feed cycles whereas with D. *tertiolecta* and U-D the current remained above 0.07 mA at all times. Thus, although higher maximum power densities were obtained with enrichment culture U-C, electricity production was more sustained with enrichment culture U-D. When the energy content of the produced electricity was calculated by integrating the power over time during the highest electricity producing batch cycle, the produced energy was clearly higher from *D. tertiolecta* than from *C. vulgaris* (Table 2). Electricity produced from pre-digested *D. tertiolecta* and from glucose with either enrichment culture was lower than that from the algal biomasses.

COD Removal and Coulombic Efficiency

The relative removal of COD_{tot} with enrichment culture U-C was 18.4 and 17.6% from *C. vulgaris* biomass and glucose, respectively. The corresponding values for *D. tertiolecta* and glucose with enrichment culture U-D were 16.8 and 7.7%. Although the COD_{tot} removal was generally higher with U-C than U-D, the electrons were more efficiently utilized in electricity generation with U-D based on the coulombic efficiencies (Table 2). Relatively low removals of COD_{tot} were obtained with glucose as the electron donor. Anaerobic glucose oxidation, involving some fermentation, was fast and

Table 2 Electricity generation from the various substrates

Parameter	*C. vulgaris* and U-C	*D. tertiolecta* and U-D	Glucose and U-C	Glucose and U-D	Pre-digested *D. tertiolecta* and U-D
Maximum voltage (mV)	73.5±3.2	44.8±11.1	57.7±2.5	41.3±4.7	93.0
Maximum current (mA)	0.74±0.03	0.45±0.11	0.58±0.03	0.41±0.05	0.93
Maximum power density (mW m^{-2}/mW m^{-3})	15.0±0.1/722±62	5.3±2.6/277±133	9.3±1.7/444±39	4.5±1.0/229±51	25.7/1,150
Coulombic efficiency of the highest voltage peak (%)	1.7	8.1	1.4	8.0	7.2
Energy content of the highest voltage peak (J/g VS biomass and J/g glucose)	9.8	12.9	2.5	2.6	8.3

Maximum voltage, current and power density results are given as averages±standard error from the duplicate MFCs. However, only one replicate was included with the pre-digested *D. tertiolecta* and U-D. U-C = *C. vulgaris*-fed enrichment culture, U-D = *D. tertiolecta*-fed enrichment culture

resulted in accumulation of VFAs at relatively high rates and decrease in pH, which inhibited further metabolism of these intermediate products seen also as low overall COD removal.

Metabolic Products

Methane and hydrogen were not detected in headspace samples of the MFCs. The overall concentrations of VFAs and alcohols increased in the MFCs fed with algal biomass or glucose at the beginning of the incubation (Fig. S2B in the ESM). Metabolite concentrations stabilized to 23.6–28.9 and 11.5–14.2 mM in *C. vulgaris*- and *D. tertiolecta*-fed MFCs by day 9 but continued to increase in glucose-fed MFCs (Fig. S2B in the ESM). In algal biomass-fed MFCs, the main metabolites (in a descending order of concentration) were butanol (Fig. 2A)>propionate>butyrate, while in glucose-fed MFCs they were butanol (Fig. 2B)>butyrate> propionate. In *C. vulgaris*-fed MFCs, the concentration of butanol was 16.1 mM at maximum and 12.7 mM in the end of the experiment, whereas in *D. tertiolecta*-fed MFCs the corresponding values were 7.0 and 2.5 mM (Fig. 2A). In the MFC fed with pre-digested *D. tertiolecta*, only low levels (<0.1 mM) of ethanol and propionate were produced. Acetate was not detected under any experimental conditions.

Other MFC Parameters

Anode solution conductivities were on average 9.8, 15.4, 13.9, and 6.6 mS cm^{-1} in the MFCs fed with *C. vulgaris*, *D. tertiolecta*, pre-digested *D. tertiolecta*, and glucose, respectively. Internal resistances of the MFCs were calculated from the region of constant voltage drop in the center of the polarization curves representing ohmic losses (data not shown). Internal resistances (±standard error) were 136±2, 310±100, 131±0, 2,780±890, and 2,130±33 Ω for MFCs with *C. vulgaris* and U-C, *D. tertiolecta* and U-D, pre-digested *D. tertiolecta* and U-D, glucose and U-C, and glucose and U-D, respectively.

Initially, the pH of anode solution decreased in all MFCs from days 0 to 3. Subsequently, the pH remained relatively constant (near 7.0) with *C. vulgaris* and *D. tertiolecta* (Fig. 3A). The pH of anode solution varied more in the beginning with pre-digested *D. tertiolecta*, but after day 9 the pH stabilized to near 7.5. Anode pH changed more with glucose (Fig. 3B). During each feeding the pH was adjusted to 7.0±0.2 with NaOH, but it dropped to as low as pH 5.7 during incubation indicating VFA production.

The average Cl^- concentration in the anode solutions was 1.2, 8.1, and 4.7 gL^{-1} and Na^+ concentration was 1.6, 4.1,

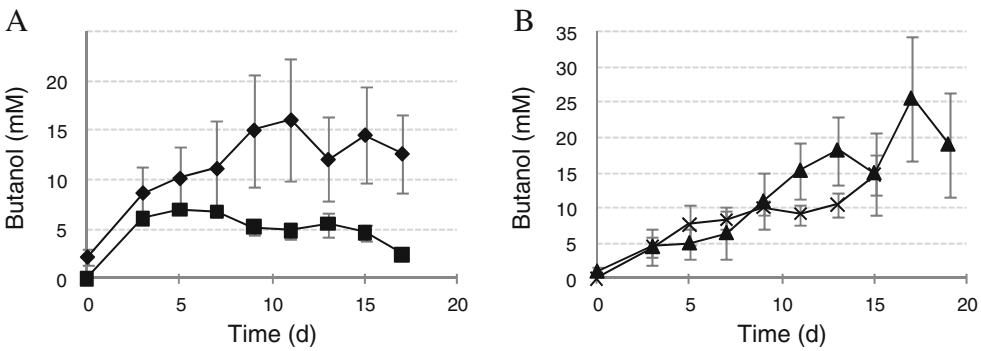

Fig. 2 Changes in butanol concentration over time in the anolyte of MFCs with *C. vulgaris* and U-C (*filled diamonds*), *D. tertiolecta* and U-D (*filled squares*) (**A**), glucose and U-C (*filled triangles*), and glucose and U-D (*error marks*) (**B**)

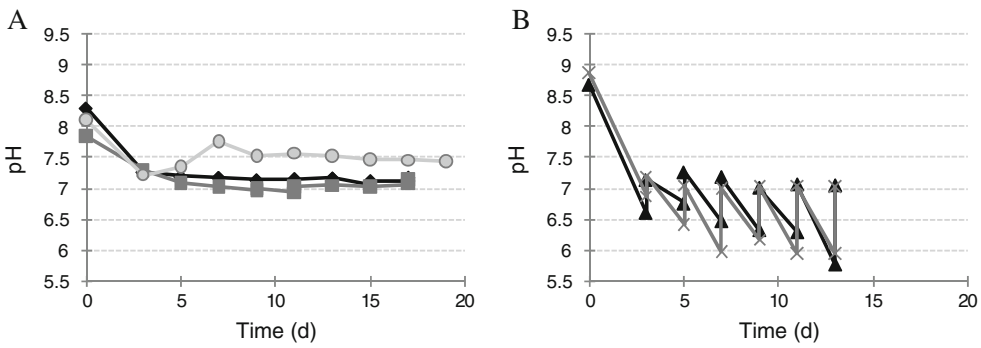

Fig. 3 Changes in anode solution pH over time in MFCs with *C. vulgaris* and U-C (*filled diamonds*), *D. tertiolecta* and U-D (*filled squares*), pre-digested *D. tertiolecta* and U-D (*filled circles*) (**A**), glucose and U-C (*filled triangles*), and glucose and U-D (*error marks*) (**B**)

and 2.6 gL^{-1} in MFCs fed with *C. vulgaris*, *D. tertiolecta* and pre-digested *D. tertiolecta*, respectively.

Microbial Community Composition

Suspended cultures were repeatedly transferred to the anode compartment during enrichment. Their bacterial compositions were profiled using PCR-DGGE. Both anaerobic consortia had been originally enriched separately with *C. vulgaris* and *D. tertiolecta* biomasses for CH_4 production [17]. The cultures were further enriched for electricity production, again separately for the two algal biomass types. The DGGE profiles of U-C and U-D were dissimilar prior to further enrichment for electricity production and developed increasingly differently in the MFCs (Fig. 4).

Some bacterial 16S rDNA sequences amplified from the MFC enrichments matched uncultured bacteria with no genus or species-level information (Fig. 4; Table 3). The matches with species level information in the U-C enrichment were a *Bacteroides* sp. (band 1), *Wolinella succinogenes* (band 3), an uncultured *Synergistes* sp. (band 4) and *C. vulgaris* (band 5). In the U-D enrichment they were a *Bacteroides* sp. (bands 1 and 8), *W. succinogenes* (band 3), an uncultured *Synergistes* sp. (band 4), *Geovibrio thiophilus* (band 10), a *Roseobacter* sp. (band 11), and a *Desulfomicrobium* sp. (band 14).

Precipitate Formation in the MFCs

Two types of precipitates were gradually formed on the cathode electrode and the cathode side of the membrane in the MFCs that were fed with *D. tertiolecta* biomass and pre-digested *D. tertiolecta* (Fig. 5A). Microprobe analysis with SEM revealed that many precipitates contained Mg, P, and O (P1 in Fig. 5B) and some contained Ca, P, and O (P2 in Fig. 5B).

Discussion

This study demonstrated electricity production in MFCs from untreated *C. vulgaris* and *D. tertiolecta* biomass. Electricity was also produced from anaerobically pre-digested *D. tertiolecta*, but not from pre-digested *C. vulgaris*. No optimization of the MFC design was undertaken in this study, but the anaerobic inoculum was extensively enriched for these experiments. The sequential enrichment increased current generation from 0.02 to 0.76 mA with *C. vulgaris* and from 0.03 to 0.53 mA with *D. tertiolecta*. Differences in electricity production were likely related to different chemical compositions and cell wall structures in the two microalgal biomass stocks. *C. vulgaris* had a higher content of proteins, lipids and carbohydrates than *D.*

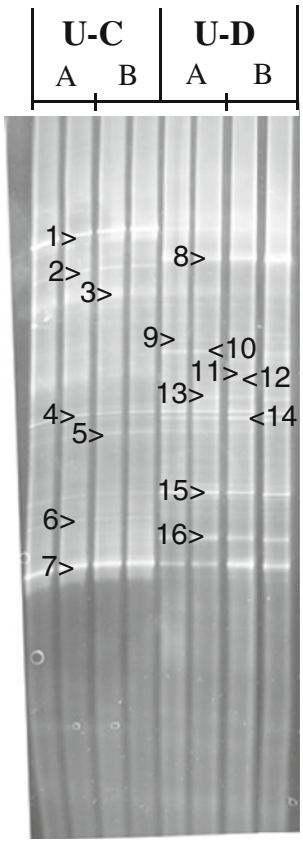

Fig. 4 Bacterial community profiles of the enrichment cultures U-C and U-D. Samples were taken from MFCs fed with *C. vulgaris* and *D. tertiolecta*, respectively. MFCs were run in duplicate (**A**, **B**) and duplicate samples for microbial community analyses were taken from each MFC. See Table 3 for the labeled bands

Table 3 Matches of selected band identities of PCR-DGGE samples from the MFCs

Band label[a]	SL[b]	Sim (%)[c]	Affiliation (acc)[d]	Phylum/Family	Origin of the sample with the closest match
1	456	100	*Bacteroides* sp. (AY554420)	Bacteroidetes/Bacteroidaceae	A landfill leachate bioreactor
2	469	81.7	Uncultured bacterium (FN563291)	Unknown/unknown	A mesophilic and fuzzy logic controlled two phase biogas reactor
3	455	100	*Wolinella succinogenes* (NR_025942)	Proteobacteria/Helicobacteraceae	American Type Culture Collection
4	438	99.3	Uncultured *Synergistes* sp. (EU721766)	Synergistetes/Synergistaceae	Production water from an Alaskan mesothermic petroleum reservoir
5	252	90.4	*Chlorella vulgaris* chloroplast (AB001684)	Chlorophyta/Chlorellaceae	*C. vulgaris* grown in M-4NA medium
6	428	92.8	Uncultured spirochete (AY648566)	Spirochaetes/unknown	Anaerobic bioreactors processing sulfate-rich waste streams
7	490	99.2	Uncultured rumen bacterium (HQ616113)	Unknown/unknown	Rumen bacteria in cattle
8	481	99.4	*Bacteroides* sp. (AY695842)	Bacteroidetes/Bacteroidaceae	*Bacteroides* sp. SA-11
9	343	72.0	Uncultured beta proteobacterium (FJ184029)	Proteobacteria/unknown	Artificial biofilms
10	457	96.3	*Geovibrio thiophilus* (NR_028005)	Deferribacteres/Deferribacteraceae	Isolate from methanogenic mixed culture degrading acetone
11	426	98.6	*Roseobacter* sp. (FJ984834)	Proteobacteria/Rhodobacteraceae	Unknown
12	436	97.7	Uncultured bacterium (EU592330)	Unknown/unknown	The Salton Sea
13	436	95.7	Uncultured Firmicutes bacterium (CU927841)	Firmicutes/unknown	Full-scale mesophilic anaerobic digester
14	437	99.8	*Desulfomicrobium* sp. (AM419442)	Proteobacteria/Desulfomicrobiaceae	Sediments of the Adour Estuary
15	452	100	Uncultured bacterium (FR669200)	Unknown/unknown	Hydrogen-producing biocathode in a microbial electrolysis cell
16	444	99.8	Uncultured bacterium (CT574327)	Unknown/unknown	A municipal anaerobic sludge digester

[a] Band lable in Fig. 4

[b] Sequence length

[c] Similarity (%)

[d] Closest species in GenBank database and its accession number

tertiolecta, but lower conductivity due to carry-over of salts with the *D. tertiolecta*-biomass. *C. vulgaris* has a rigid cell wall which is relatively recalcitrant unlike the fragile *D. tertiolecta*, which is prone to lyse. Thus, current peaks were higher with *C. vulgaris* but current generation was more sustained with *D. tertiolecta*. Inhibition of electricity production by salt water ions was not apparent.

During pre-digestion, methanogenic conversion of *D. tertiolecta* was incomplete, whilst *C. vulgaris* was more efficiently converted to CH_4 [17]. Conversely, pre-digested *C. vulgaris* was less amenable to electricity production than pre-digested *D. tertiolecta*. Thus most of the chemical energy content of *C. vulgaris* was already converted to CH_4 during pre-digestion before the MFC experiments.

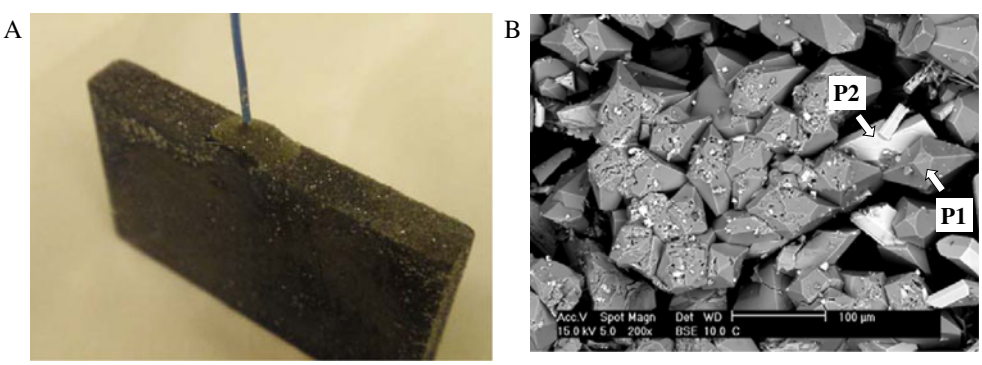

Fig. 5 A photograph (**A**) and SEM micrograph (**B**) of precipitates formed on cathode electrode and cathode side of the membrane in the MFCs fed with *D. tertiolecta* biomass and pre-digested *D. tertiolecta*. The darker crystals (*P1*) consisted of Mg, P, and O, and the lighter crystals (*P2*) of Ca, P, and O

In this study glucose was used as a positive control to establish that the electrogenic culture in the MFCs was metabolically active, but no effort was made to define the corresponding kinetics or maximum power yields with glucose. The conditions in the MFCs were designed for electricity production from algal biomass based feedstock rather than glucose. Glucose was metabolized fast by the mixed population in the anodic chamber as shown by the rapid current increase in glucose-fed MFCs. This resulted in accumulation of VFAs faster than they were further utilized by the consortium. VFA accumulation lowered the anodic pH and thus inhibited the electrogenic activity. Similar phenomenon was observed in a previous methanogenic study [26], where the pH of positive controls with both acid-hydrolyzed reed canary grass (5.76 gL^{-1} soluble sugars) and glucose (5 gL^{-1}) decreased to as low as pH 3.9–4.3 due to excessive accumulation of VFAs. Consequently, methane production remained low despite subsequent pH adjustment to above 6.0 [26]. In glucose-fed MFCs, the conductivity of anode solution was also lower than in MFCs fed with algal biomass. As a consequence, the internal resistance in the glucose-fed MFCs was significantly higher (approximately 2,100–2,800 Ω) than in MFCs fed with *C. vulgaris*, *D. tertiolecta*, or pre-digested *D. tertiolecta* (130–400 Ω), resulting in a lower maximum power density. Internal resistance is generally lower with soluble substrates than with particulate substrates [10] while suboptimal pH and low conductivity have been reported to increase internal resistance and thus reduce power generation of an MFC [9, 11].

Dominant anaerobic metabolic pathways of microalgal biomass degradation were different in the MFCs as compared with hydrogenic and methanogenic incubations described in a previous study with the same original inoculum [17]. In previous experiments, main soluble metabolites in methanogenic enrichment cultures were acetate and propionate [17], whereas in the MFC cultivations of this study they were butanol, propionate and butyrate. The lack of acetate may be due to the absence of acetogens or presence of electrogenic organisms rapidly oxidizing acetate.

Neither hydrogen nor methane was detected in the anode headspace. The reason for the lack of methanogenesis in anode chambers is not clear, especially since the inocula for MFCs were developed with methanogenic consortia. It is plausible that the biodegradation of algal biomass feedstock did not proceed to H_2 and acetate production in amounts that would support detectable CH_4 formation. In glucose-fed MFCs methanogenesis was likely limited by the low pH. At near neutral pH values, in the microalgal biomass-fed MFCs, the absence of methanogenesis may also have been due to low levels of oxygen, which may have diffused to the anode chamber from non-gas tight cathode [8, 27]. Some fermentative and electrogenic bacteria are facultative anaerobes and thus less susceptible to oxygen inhibition than methanogens, which are strictly anaerobic organisms [27, 28]. Oxygen diffusion to the anode may also have been an additional reason for the low coulombic efficiencies attained in these systems [27].

The precipitation of Ca and Mg phosphates on the cathode and the cathode side of the cation exchange membrane in MFCs resulted from carryover of Ca^{2+} and Mg^{2+} ions in the *D. tertiolecta* slurry. Salt concentrated biomass and new catholyte with phosphate buffer added to the system during each feed cycle lead to gradual precipitation as Ca^{2+} and Mg^{2+} ions were translocated to the cathode chamber through the cation exchange membrane. Ca and Mg phosphate precipitation would also be possible in salt water algae-fed MFCs with biocathodes, if the catholyte contains phosphate buffer. For example, Jeremiasse et al. [29] reported formation of Ca-phosphate precipitates on graphite felt biocathode of a two-chamber microbial electrolysis cell and postulated that the precipitate gradually decreased the current density. Although ferricyanide cathodes were used successfully in this proof-of-concept study, they would not be amenable to commercial MFC applications due to unsustainable nature of ferricyanide as it requires chemical regeneration from ferrocyanide back to ferricyanide [21].

Rabaey et al. [30] have reported that both suspended and attached bacteria perform efficient electron transfer. In this study, suspended (planktonic) bacteria were characterized by analysis of 16S rDNA sequence. The U-C consortium contained *Bacteroides* spp., *W. succinogenes* and *Synergistes* spp. None of these were detected in the preceding methanogenic enrichment cultures that were the original inocula of *C. vulgaris*-fed MFC cultures [17]. *Bacteroides* spp., *W. succinogenes*, and *Roseobacter* spp. in the U-D consortium were already identified in the original inocula for *D. tertiolecta*-fed MFCs. *Synergistes* spp., *G. thiophilus*, and *Desulfomicrobium* spp. were not detected in the original inoculum [17]. *Bacteroides* spp. and *W. succinogenes* in the electrogenic U-C enrichment culture may also have their origin in the U-D enrichment as it was used to boost electricity production in *C. vulgaris*-fed MFCs. *W. succinogenes* [31], *Synergistes* spp. [32], and *Geovibrio* spp. [7] have been previously reported in bioelectrochemical systems, but their specific roles in electricity generation are not clear. *Bacteroides* spp. transfer electrons to Fe(III) [33], and *Desulfomicrobium* spp. are sulfate-reducers [34] that contain cytochromes with the ability to link electron transfer to the anode electrode [35]. *Roseobacter* spp. are strictly aerobic bacteria, which are generally found in marine environments and likely originated from the *D. tertiolecta* slurry [36], but the sequence here may represent a related species capable of growing under anaerobic conditions.

With consortium U-C, the COD removal was generally higher but the coulombic efficiency significantly lower than with consortium U-D. The coulombic efficiencies estimated

in this study, 1.4–8.1%, were low and in the 1.3–5.2% range previously reported by Zheng and Nirmalakhandan [10] for MFCs fed with solid animal manure. The maximum power densities obtained in this study were also low compared with other complex substrates. For example, the maximum power densities of 55 mW m^{-2} from cellulose with a two-chamber MFC with ferricyanide cathode [7], 67 mW m^{-2} from solid animal manure with a single-chamber MFC with air-cathode and brush type anode [10], and 980 mW m^{-2} from dried *C. vulgaris* powder with a single chamber MFC with air-cathode and brush type anode [13] have been reported. The recalcitrant nature of untreated *C. vulgaris* biomass due to its rigid cell wall and the high salinity of *D. tertiolecta* biomass slurry may limit the anaerobic conversion of the algal biomass stocks [17, 37]. Energy yields as electricity (9.8 and 12.9 J g-VS^{-1}) were orders of magnitude lower than the yields of 10 and 0.86 kJ g-VS^{-1} recovered as CH_4 from *C. vulgaris* and *D. tertiolecta*, respectively [17]. It is conceivable that MFC design modification and pretreatment of biomass will lead to improved coulombic efficiency and power density.

In addition to electricity, substantial levels of butanol were detected in the anolytes of MFCs fed with *C. vulgaris*, *D. tertiolecta*, or glucose. Finch et al. [38] also reported high butanol concentration in MFC anolytes fed with glucose and inoculated with *Clostridium acetobutylicum*. Accumulation of high butanol levels retains electrons that would otherwise be shunted in the closed circuit. However, butanol is a prospective candidate as a biofuel and solvent. Based on the butanol concentration in the end of the incubation, the number of feeding cycles and lower heating value of butanol (33.07 kJ g^{-1}) [39], the energy converted to butanol was 1.4 kJ g-VS^{-1} in *C. vulgaris*-fed MFCs and 270 J g-VS^{-1} in *D. tertiolecta*-fed MFCs. Thus, the energy content of butanol greatly increased the overall energy production of the MFCs and made their energetic yields more comparable with H_2 and CH_4 production.

Conclusions

This work demonstrated, without optimization of the MFC design, simultaneous electricity and butanol production from untreated biomass of the fresh water microalga *C. vulgaris* and the marine microalga *D. tertiolecta*. The maximum power density was higher from *C. vulgaris* (15 mW m^{-2}), but the power generation was more sustained from *D. tertiolecta* (13 J g-VS^{-1}). Butanol was produced in the anodes of the MFCs, contributing to the overall energy output of the systems and increasing the estimated energy yield to 1.4 kJ g-VS^{-1} in *C. vulgaris*-fed MFCs and 270 J g-VS^{-1} in *D. tertiolecta*-fed MFCs. Carry-over of salts with the *D. tertiolecta* biomass increased solution conductivity but also caused gradual precipitation of Ca and Mg phosphates on the cathode side, which may hinder electricity generation during long-term operation. PCR-DGGE profiling provided matches to bacteria previously described in bioelectrochemical systems.

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