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# Generation of electricity in microbial fuel cells at sub-ambient temperatures

Tunc Catal [a], Paul Kavanagh [a], Vincent O'Flaherty [b], Dónal Leech [a,*]

[a] School of Chemistry, National University of Ireland, Galway, Ireland
[b] Department of Microbiology, National University of Ireland, Galway, Ireland

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ABSTRACT

Direct generation of electricity from a mixture of carbon sources was examined using single chamber mediator-less air cathode microbial fuel cells (MFCs) at sub-ambient temperatures. Electricity was directly generated from a carbon source mixture of D-glucose, D-galactose, D-xylose, D-glucuronic acid and sodium acetate at 30 °C and <20 °C (down to 4 °C). Anodic biofilms enriched at different temperatures using carbon source mixtures were examined using epi-fluorescent, scanning electron microscopy, and cyclic voltammetry for electrochemical evaluation. The maximum power density obtained at different temperatures ranged from $486 \pm 68\ mW\ m^{-2}$ to $602 \pm 38\ mW\ m^{-2}$ at current density range of $0.31\ mA\ cm^{-2}$ to $0.41\ mA\ cm^{-2}$ (14 °C and 30 °C, respectively). Coulombic efficiency increased with decreasing temperature, and ranged from $24 \pm 3$ to $38 \pm 1\%$ (20 °C and 4 °C, respectively). Chemical oxygen demand (COD) removal was over 68% for all carbon sources tested. Our results demonstrate adaptation, by gradual increase of cold-stress, to electricity production in MFCs at sub-ambient temperatures.

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## 1. Introduction

Recent research has focused on alternative strategies for energy production due to increased demand and the prospect of diminishing fossil fuel resources, and the application of microbial fuel cells (MFCs) for energy production is suggested as an alternative promising technology [1–3]. Microorganisms catalyze the conversion of carbon sources into electricity, attaching onto anode surfaces to form a biofilm which is a common structure of microbial communities in MFCs [4–6]. The property of anodic biofilms and their features are determined by the microbial community, which is affected by substrate availability [7]. MFCs, based on anodic biofilms, can utilize inexpensive organic substrates sourced from natural resources, including lignocellulosic substrates and wastewaters, to generate electricity whilst effecting wastewater treatment under mild operational reaction conditions [8–12]. As an operational parameter, the effect of temperature on electricity generation by microorganisms remains important, especially for MFC applications in cold climate environments, since microorganisms can be significantly affected by temperature. For example, the effect of sub-ambient temperatures on microbial dynamics using anaerobic granular sludge systems has been previously reported [13], and MFC performance at 20 °C has been analyzed [14]. However, knowledge of the operating, microbial, morphological and electrochemical characteristics of MFCs based on anodic biofilms operating at different temperatures is still very limited, and acclimation of electricity generation starting at psychrophilic conditions in MFCs and voltage generation at sub-ambient temperature, such as 4 °C, has not been reported, yet.

In this study, we report on power generation in air-cathode single chamber MFCs, using a carbon source mixture of D-glucose, D-galactose, D-xylose, D-glucuronic acid and sodium acetate, under mesophilic (30 °C) and sub-ambient temperatures (<14 °C). The features of anode biofilms enriched at 30 °C and 14 °C were also examined morphologically and electrochemically.

## 2. Materials and methods

### 2.1. MFC construction

Eight mediator-less single chamber MFCs were constructed of plexiglass, volume of $8\ cm^3$ [9,14]. Briefly, the anode and cathode were placed in parallel on the opposite sides of the chamber separated by a distance of 1.4 cm. Non-wet proofed carbon cloth (type B, BASF Fuel Cell Inc., Somerset, NJ, USA) was used as the anode ($5.7\ cm^2$) without any treatment. The cathode ($5.7\ cm^2$) carbon cloth was coated with carbon/poly(tetrafluoroethylene) (PTFE) layers on the air-facing side and platinum ($0.5\ mg\ cm^{-2}$ cathode area) with Nafion as binder on the water-facing side, according to the published procedures [15].

* Corresponding author. Tel.: +353 91493563; fax: +353 91525700.
*E-mail address:* Donal.leech@nuigalway.ie (D. Leech).

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### 2.2. Enrichment of bacteria and operation of an MFC under psychrophilic condition

MFCs were inoculated with a mixed bacterial culture that was originally enriched from domestic wastewater (Mutton Island Wastewater Treatment Plant, Galway, Ireland). D-Glucose ($1.2\,g\,L^{-1}$) was initially used as carbon source during enrichment of microbial consortium in a medium solution (100 mM phosphate buffer; pH 7.0) containing: $NH_4Cl$ ($0.31\,g\,L^{-1}$); $NaH_2PO_4{\cdot}H_2O$ ($5.84\,g\,L^{-1}$); $Na_2HPO_4{\cdot}7H_2O$ ($15.47\,g\,L^{-1}$); KCl ($0.13\,g\,L^{-1}$), a mineral solution (12.5 mL) and a vitamin solution (12.5 mL) was used, as reported previously [16].

When a stable voltage output across a 1000 Ω resistor was obtained over several batches at temperatures of 30 °C and 20 °C initially, using glucose ($1.2\,g\,L^{-1}$), the carbon source was replaced with one of the following carbon sources, and continuously changed after each operation ($n = 2$ for each temperature): sodium acetate ($1.2\,g\,L^{-1}$) (Sigma Chemical Co., St. Louis, MO, USA), D-galactose ($1.2\,g\,L^{-1}$) (BH 15 ITD, England), D-glucuronic acid ($1.2\,g\,L^{-1}$), D-xylose ($1.2\,g\,L^{-1}$) (Fluka Chemie GmbH, Buchs, Switzerland).

In another experimental set, two MFCs were started-up at 15 °C temperature using glucose ($1.2\,g\,L^{-1}$) ($n = 2$). The operational temperature was then increased to 20 °C, since voltage generation was not significant at the initial 15 °C temperature. After reaching a repeatable voltage at 20 °C over several batches, the carbon source was replaced with a carbon source mixture consisting of D-glucose, sodium acetate, D-galactose, D-glucuronic acid and D-xylose ($250\,mg\,L^{-1}$ each). After reaching repeatable voltage, the MFC operating temperature was gradually decreased from 20 °C down to 4 °C, with batches repeated at each temperature level. In another experimental set-up, MFCs were started up as described above, but the temperature decrease was stopped at 14 °C in order to prepare power density curves, obtained using series of external resistors ranging from 1 kΩ to 80 Ω to measure the voltage drop across the MFC. MFCs were allowed run for at least two batches to ensure repeatable power output can be achieved for each resistance. All experiments were performed in a temperature-controlled incubator, and the accuracy of temperature was verified using a digital thermometer.

### 2.3. Calculations and analyses

Voltage was measured using a multimeter with a data acquisition system (Pico Technology, Data logger, UK). Power density ($mW\,m^{-2}$) was calculated according to $P = IV/A$, where $I$ is the current, $V$ is the voltage, and $A$ is the projected area of the anode. The Coulombic efficiency (CE), is an important parameter in evaluating MFC performance and is described as the percentage of electrons recovered from the organic matter versus the theoretical maximum whereby all electrons are used for electricity production. CE was calculated as $CE(\%) = C_P/C_{Ti} \times 100$, where $C_P$ is the total coulombs calculated by integrating the current over time, $C_{Ti}$ is the theoretical amount of coulombs based on the added substrate.

A sample from each MFC at the end of the batch experiment for each temperature was filtered through a sterile syringe filter (0.2 μm), and was used for the determination of COD according to a standard method [17]. Comparison of the planktonic bacterial concentrations in the MFC solutions was made by measuring optical density (OD) at 450 nm using a spectrophotometer (Agilent 8453 UV–visible spectrophotometer).

All electrochemical experiments were performed using a potentiostat (Model CHI600A electrochemical analyzer, CH Instruments, Austin, TX, USA) in single-chamber MFCS containing an Ag/AgCl reference electrode (Bioanalytical Systems), a platinum counter electrode (Goodfellow Cambridge Ltd., Huntingdon, UK) and working electrodes (anode) in a 100 mM phosphate buffered saline at room temperature.

Biofilm-covered anodes ($n = 2$) were gently washed in saline phosphate buffer (25 mM, pH 7.0) to remove planktonic cells and then stained with SYBR gold nucleic acid gel stain (20× diluted) (Invitrogen, Eugene, OR, USA). Anodic biofilms were examined using an epi-fluorescence microscope (Olympus BX40F4, Japan). Ten randomly selected fields of vision were observed at a magnification of 30× and 100×. For SEM analysis, anode samples ($n = 2$) containing biofilm were treated with 1% osmium tetroxide (Sigma Chemical Co., St. Louis, MO, USA) overnight, and dehydrated in a graded series of ethanol. Finally, samples were mounted on aluminum stubs, sputtered with gold and examined using SEM (Model S-4700 Hitachi, Japan) [3]. All chemicals were of analytical grade and obtained from commercial sources.

## 3. Results and discussion

### 3.1. MFC voltage generation and adaptation to carbon sources and temperature

Glucose was used as the carbon source for all eight MFCs during the start-up period. When a stable voltage (>0.5 V) was generated, the culture medium was replaced with a fresh solution containing alternate carbon sources. The following carbon sources were tested after reaching stable voltage output using glucose, sodium acetate, D-galactose, D-glucuronic acid, and D-xylose, in that order. All carbon sources generated the expected voltage profiles for each batch addition without adding new bacterial inoculum at 30 °C and 20 °C across a 1 kΩ external resistance (Fig. 1A and B). However, slight differences in the voltage profile for batch addition of carbon source are observed between the two operating temperatures and when comparing xylose to the other carbon sources. For example, a lower voltage maximum for each carbon source was observed for the MFC operating at the lower temperature, with a maximum of ~0.5 V achieved at 30 °C compared to ~0.3 V at 20 °C for glucose as a carbon source. The tested carbon sources used here have all been individually examined for electricity generation in MFCs. However there is little information on the effect altering a carbon source in a single MFC has on the voltage output, as reported here. Previously, adaptation times of microorganisms were reported when the carbon source was changed from glucose to a separate carbon source in similarly configured MFCs as used here, with adaptation times of 12–18 h for D-galactose, D-glucuronic acid, and D-xylose [9]. In the present MFC configuration and under the conditions used, adaptation times were much shorter, with less than 1 h required for adaptation to acetate, D-galactose and D-glucuronic acid. It may be that the lower MFC volume, with reduced electrode spacing, as well as the difference in inoculum may play a role in reducing adaptation times to new substrates. However, adaptation to xylose as a carbon source at both 30 °C and 20 °C (Fig. 1A and B) seems longer, with a lower voltage maximum over the batch period, compared to other carbon sources in our MFCs. It should be noted that alteration of carbon source from xylose back to glucose restores the voltage profile to that previously observed prior to use of xylose. It is possible that biofilms containing microorganisms enriched with glucose may require less adaptation time to six carbon sugars compared to five carbon sugars [9]. Nevertheless, our results show very flexible utilization of carbon sources to generate electricity in the MFC configuration and conditions, including different temperatures, operating here.

Voltage generation in an MFC across a 1 kΩ external resistance over repetitive batch addition of a mixture of the carbon sources (Fig. 1C and Table 1) at 30 °C is not significantly different to that observed when glucose is used as a single carbon source (Fig. 1A),

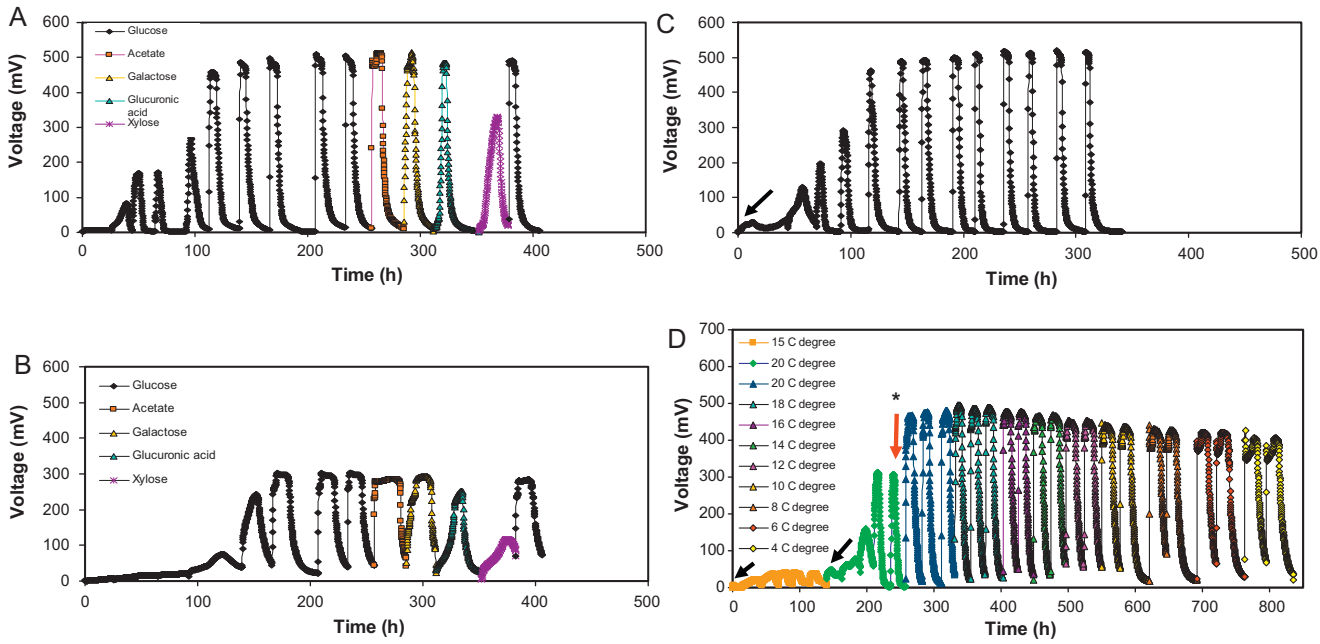


**Fig. 1.** Voltage generation across a 1 kΩ resistance in single-chamber MFCs by various individual substrates continuously changed at 30 °C and 20 °C temperatures (A and B, respectively), and by a carbon source mixture at 30 °C (C). Acclimation of electricity generation at sub-ambient temperatures with gradually increased cold stress using glucose (black arrow) and the carbon source mixture (red arrow, D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

with several batches required to produce a repeatable voltage profile. In a separate MFC trial, initial attempts to produce significant voltage, across the 1 kΩ external resistance, using glucose as a carbon source under psychrophilic conditions (15 °C) proved unsuccessful, with voltage outputs of less than 50 mV observed for repeated additions of carbon source (Fig. 1D). Increasing the temperature to 20 °C resulted in generation of repeatable (over 2 batch additions) voltage profiles, with a voltage maximum of ~0.3 V, similar to that observed in the MFC operated at 20 °C previously (Fig. 1B). However, voltage generation increased significantly, by about 70% from ~0.30 V to ~0.50 V, when the carbon source was changed from glucose to the carbon mixture at this temperature. Subsequently cold stress, with at least two batch additions for each temperature change, was gradually increased by altering the operating MFC temperature from 20 °C down to 4 °C, in steps of 2 °C (Fig. 1D). Voltage generation remained relatively constant over this series of temperature changes, with only a slight drop in maximum voltage observed, from ~0.5 V at 20 °C to ~0.4 V at 15 °C. Mixed bacterial cultures of various electricity-generating bacteria can utilize a wide range of substrates [9] whilst pure cultures may require specific substrates for surviving and addition of electron transfer mediating compounds for electricity generation [18]. Results from this study appear to suggest that using a carbon source mixture has an advantageous effect when a mixed bacterial culture is subject to electricity production in MFCs under environmental stress conditions, such as cold stress. Since wastewaters and lignocellulosic biomass hydrolysates contain a wide range of carbon sources [9–19], practical use of MFCs might envisage wastewater treatment using mixed bacterial culture. However, the treatment process conditions in cold climate regions and operation under these conditions should be considered. Recently, the application of low-temperature anaerobic biotreatment of wastewaters for long-term bioprocessing has been reported [20]. The initial results in our MFC trials suggest that microbial communities in anodic biofilms in these MFCs can be adapted to generate electricity under psychrophilic conditions, under a 1 kΩ external resistance load utilizing a model mixture as a carbon source. This provides a route to offering an alternate, or indeed a complementary process, to the anaerobic digestion of fuels and wastes for energy generation under these conditions.

**Table 1**
MFC performance at different temperatures using the carbon source mixture.

| Temperature (°C) | Voltage (V)[a] | CE (%)[a] | COD removal (%) | $OD_{450\,nm}$ |
|---|---|---|---|---|
| 30 | 0.56 ± 1 | 28 ± 1 | 78 ± 1 | 0.417 |
| 20 | 0.48 ± 1 | 24 ± 3 | 77 ± 3 | 0.325 |
| 18 | 0.48 ± 1 | 25 ± 0 | 75 ± 1 | 0.226 |
| 16 | 0.45 ± 2 | 30 ± 0 | 74 ± 0 | 0.189 |
| 14 | 0.44 ± 2 | 31 ± 1 | 73 ± 4 | 0.188 |
| 12 | 0.44 ± 1 | 34 ± 1 | 68 ± 0 | 0.188 |
| 10 | 0.42 ± 2 | 36 ± 1 | 70 ± 0 | 0.189 |
| 8 | 0.42 ± 3 | 33 ± 1 | 72 ± 1 | 0.128 |
| 6 | 0.41 ± 2 | 38 ± 2 | 69 ± 1 | 0.189 |
| 4 | 0.40 ± 3 | 38 ± 1 | 71 ± 2 | 0.155 |

Median value ($n = 2$) ± range.
[a] Results were obtained using 1 kΩ resistance.

### 3.2. Comparison of MFC performance

Maximum power density (based on 5.4 $cm^2$ cathode surface area) of 486 ± 68 mW $m^{-2}$ was obtained at 14 °C with a current density of 0.31 mA $cm^{-2}$, following the adaptation of MFC operation to these psychrophilic conditions. In comparison, a maximum of 602 ± 38 mW $m^{-2}$ power density was achieved at a current density of 0.41 mA $cm^{-2}$ under 30 °C condition (Fig. 2A), about 20% higher than that at 14 °C using this carbon source mixture. Previously, maximum power densities (based on 7 $cm^2$ cathode surface area) were reported using individual monosaccharides; glucose (619 mW $m^{-2}$), xylose (684 mW $m^{-2}$) galactose (600 mW $m^{-2}$), glucuronic acid (785 mW $m^{-2}$) using a similar MFC configuration, with 1.7 cm electrode spacing and 12 $cm^3$ volume [9]. Liu et al. reported that decreasing the electrode spacing reduces the internal resistance [14]. They also reported that the maximum power density was reduced only 9% from 720 mW $m^{-2}$ to 660 mW $m^{-2}$ (current density of 0.22 mA $cm^{-2}$, 200 Ω, based on 7 $cm^2$ cathode

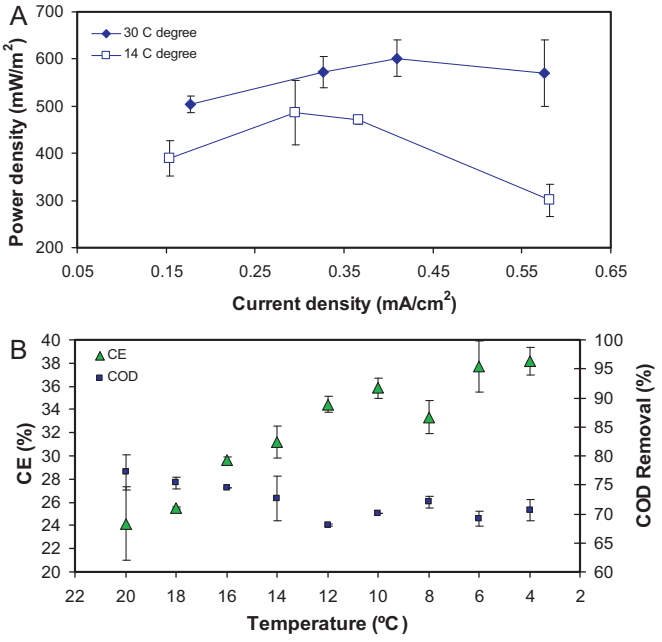


**Fig. 2.** Power density (A) and CE (B) observed at 30 °C and 14 °C for the single-chamber MFCs operated using a carbon source mixture.

surface area with reactor volume of 28 $cm^3$) when the operation temperature was reduced from 32 °C to 20 °C [14].

About 68% of COD was removed for all carbon source mixtures tested at different temperatures at the end of the each batch addition when the voltage was lower than 0.05 V. COD removal decreased with decreasing temperature (77 ± 3% vs. 60 ± 1% at 20 °C and 4 °C, respectively) (Fig. 2B). However, the CE that was calculated based on the total substrate concentration added was only in the range of 24 ± 3–38 ± 1% under a 1 kΩ external resistance load for temperatures of 20 °C and 4 °C, respectively, possibly indicating both incomplete oxidation and incomplete capture of electrons by the electrode (Fig. 2B). Optical density (OD) of the solution, corresponding to the presence of planktonic microorganisms, decreased with decreasing temperature (Table 1). The increased CE, coupled to a decrease in OD indicative of lower levels of planktonic bacteria, at the lower temperatures may therefore be partially correlated to a reduced efficiency of substrate turnover by planktonic microorganisms. It may be that whilst the presence of planktonic microorganisms in MFCs is related to high voltage generation, they consume substrate for growth, which affects CE during MFC operation. Obviously, other factors could contribute to low CE in the air-cathode MFCs, including electron transfer from substrate to other electron acceptors in solution, such as oxygen, and methanogenesis [9,11]. An increased CE as MFC temperature is lowered has been previously reported suggesting that prevention of methanogenesis might contribute to increased CE [21]. Chae et al. have shown methanogenic activity at different temperatures, and reported that a series of temperature reductions from 28 °C to 15 °C, had a negligible effect on both the methanogenic activity and current generation in MFCs [22]. Although we did not measure methanogenic activity at different temperatures, it is debatable whether increased CE resulted from decreased methanogenic activity, or not, a topic that therefore merits further investigation.

### 3.3. Voltammetric analysis of biofilms

Cyclic voltammetry is increasingly used to examine the redox properties of species in MFCs, and to confirm the capability of

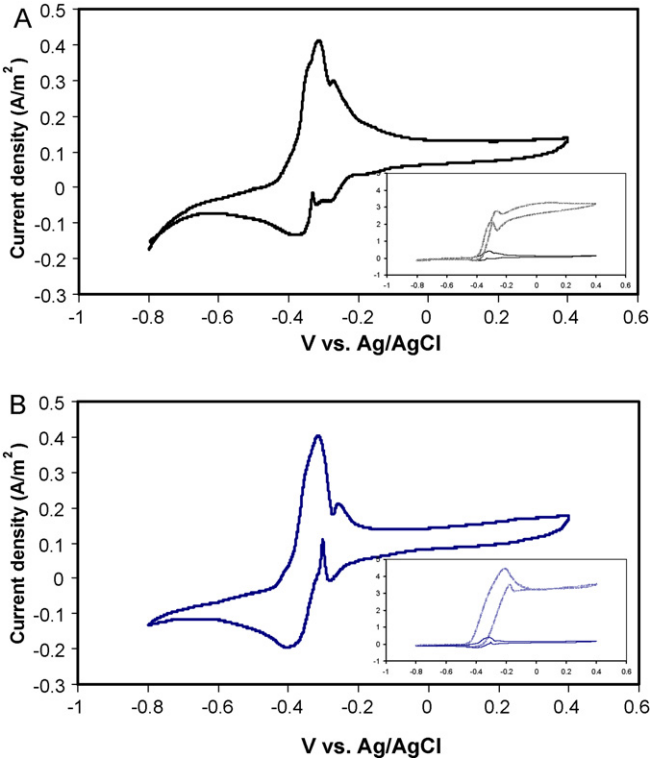


**Fig. 3.** Cyclic voltammograms, recorded at room temperature in substrate-limiting culture medium, of anode biofilm enriched at 30 °C (A) and 14 °C (B) at 1 mV $s^{-1}$ of scan rate. Inset figures show comparison of 1 mV $s^{-1}$ scan rate CVs recorded before (solid line) and 1 h after (dotted line) addition of the carbon source.

MFC anodic biofilms to electrocatalyze substrate oxidation. Fig. 3 shows typical cyclic voltammograms (CVs) obtained for the anodic biofilms, enriched at 30 °C (Fig. 3A) and 14 °C (Fig. 3B) ($n$ = 2 for each). The CVs of the anodic biofilms generated on carbon cloth electrodes, recorded in the culture medium at room temperature, at the end of the batch operation when substrate is depleted, exhibit very similar CVs, with oxidation and reduction peaks indicative of the presence of several redox active species. The voltammograms are comparable to those reported previously for biofilms generated from inoculation with a single culture of *G. sulfurreducens* [18,23,24], and for those generated from inoculation with a mixed culture [3]. These CVs display a complex shape, most likely due to the presence of multiple redox transitions from the multiple heme-based cytochromes implicated in electron transfer between electroactive bacteria and electrodes [25,26]. At least two redox transitions are observed in the CVs recorded in the substrate-depleted culture, centred around redox potentials of −0.35 V (I) and −0.25 V (II) vs the Ag/AgCl reference electrode. By alteration of the CV switching potential (not shown) it can be observed that the oxidation peak at −0.3 V is coupled to the reduction peak observed at −0.4 V (transition I), whilst that observed at −0.24 V is coupled to the reduction peak at −0.26 V (transition II). The fact that the anodic peak current for transition I scales linearly with scan rate, at scan rates <10 mV $s^{-1}$, indicates finite diffusion, and thus that the redox transition is between the electrode and a surface-confined species, as observed for biofilms of electroactive bacteria [3,23–26]. When slow-scan rate CVs are recorded in the presence of the mixed carbon source substrate, insets in Fig. 3, the CV waveshape is drastically altered, with a plateau current observed for the anodic scan direction, indicative of bioelectrocatalytic oxidation of the substrate, with transfer of electrons to the electrode, as described recently for *G. sulfurreducens*-based biofilms on electrodes [24,26]. It is difficult to determine, given the uncertainty in the electrode areas, if

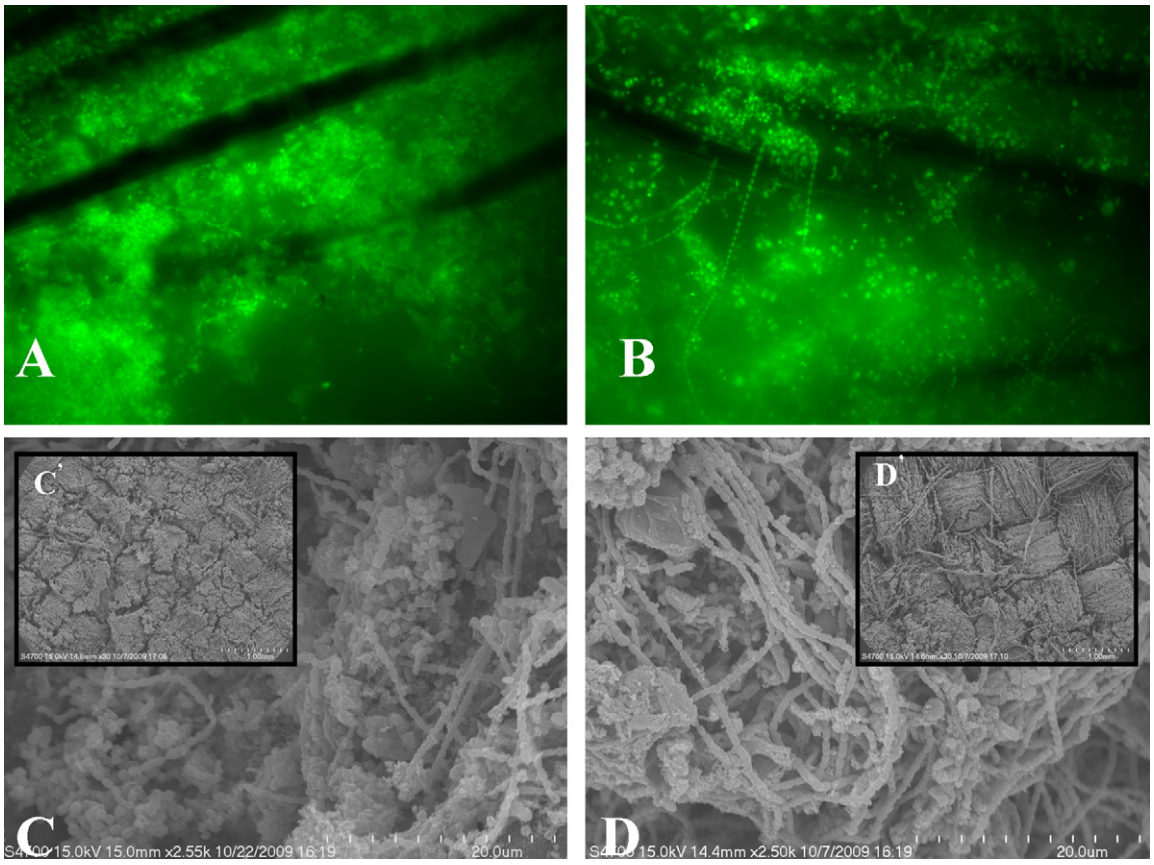


**Fig. 4.** Epi-fluorescent (A, B) and SEM (C, D) images of biofilm enriched at 30 °C (A, C) and 14 °C (B, D) using carbon mixture after more than three months of batch operation. Epi-fluorescent images were taken at the magnification of 100×.

the biofilms enriched at the different temperatures differ in their voltammetric characteristics.

The morphologies of the biofilms samples enriched at 30 °C and 14 °C do differ slightly. Epi-fluorescent images show that biofilm enriched at 30 °C (Fig. 4A) is more dense when compared to the biofilm sample of 14 °C (Fig. 4B), and bacterial colonies are clearly observed around carbon fibrils. SEM images seem to indicate thicker biofilm formation in the sample enriched at 30 °C (Fig. 4C) when compared to that for the 14 °C sample (Fig. 4D).

## 4. Conclusions

Electricity was successfully generated using a carbon source mixture of D-glucose, D-galactose, D-xylose, D-glucuronic acid and sodium acetate with a mixed bacterial culture in single-chamber air cathode mediator-less MFCs at 30 °C and sub-ambient temperatures (<20 °C, down to 4 °C). Anodic biofilms enriched at different temperatures using carbon source mixtures were examined using epi-fluorescent, SEM and CV for electrochemical evaluation. The maximum power density obtained at different temperatures ranged from $486 \pm 68\ mW\ m^{-2}$ to $602 \pm 38\ mW\ m^{-2}$ at current density range of $0.31\ mA\ cm^{-2}$ to $0.41\ mA\ cm^{-2}$ (14 °C and 30 °C, respectively). Coulombic efficiency increased with decreasing temperature, and ranged from $24 \pm 3$ to $38 \pm 1\%$ (20 °C and 4 °C, respectively). Chemical oxygen demand (COD) removal was over 68% for all carbon sources tested. Anodic biofilms showed different morphological, though similar voltammetric, features depending on the carbon source and temperature. Our results demonstrate that using carbon source mixtures might be advantageous for electricity production, and electricity could be successfully generated using lignocellulosic biomass at sub-ambient temperatures in MFCs.

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