



Research paper

Self-sustainable electricity production from algae grown in a microbial fuel cell system

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ABSTRACT

This paper describes the potential for algal biomass production in conjunction with wastewater treatment and power generation within a fully biotic Microbial Fuel Cell (MFC). The anaerobic biofilm in the anodic half-cell is generating current, whereas the phototrophic biofilm on the cathode is providing the oxygen for the Oxygen Reduction Reaction (ORR) and forming biomass. The MFC is producing electricity with simultaneous biomass regeneration in the cathodic half-cell, which is dependent on the nutrient value of the anodic feedstock. Growth of algal biomass in the cathode was monitored, assessed and compared against the MFC power production (charge transfer), during this process. MFC generation of electricity activated the cation crossover for the formation of biomass, which has been harvested and reused as energy source in a closed loop system. It can be concluded that the nutrient reclamation and assimilation into new biomass increases the energy efficiency. This work is presenting a simple and self-sustainable MFC operation with minimal dependency on chemicals and an energy generation system utilising waste products and maximising energy turnover through an additional biomass recovery.

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1. Introduction

To limit the environmental impact of greenhouse gasses, future energy sources should be renewable and carbon neutral. This would include biomass products for energy generation such as growth plants and crops, algae and organic wastes. From the food security point of view, both micro and macro algae have attracted considerable interest as a potential feedstock for a bio-based economy [1]. Microalgae, as very efficient converters of solar power, have been used in mass culture for both biomass as well as high value product production. However, the cost of algal growth and biomass harvesting is limiting the technology at large scale. It has been more apparent that High Rate Algal Ponds (HRAP) and microalgal biofilms [2] are allowing recovery of nutrients such as nitrate and phosphate from municipal wastewater [3] as well as toxic waste removal [4]. In this process, microalgae use the end products of bacterial metabolism (for example CO₂ and ammonia) and in turn, supply aerobic bacteria with the oxygen required for

the degradation of organic compounds. This process can increase resource efficiency turning eutrophication into an opportunity for biomass production. Wastewater treatment HRAPs could provide cost-effective and efficient tertiary-level wastewater treatment with the co-benefit of algal biomass production for biofuel use [5]. The high biomass productivity of wastewater-grown microalgae suggests that this cultivation method offers real potential as a viable means for sustainable energy [6] being beneficial to the food chain in the local ecosystem. The suitability of this biomass may not be recognised as being appropriate for the food industry however it would be suitable for energy conversion technologies including Anaerobic Digestion or Microbial Fuel Cell. The Microbial Fuel Cell (MFC) is an attractive renewable energy technology, in which photosynthetic organisms can be incorporated for direct electricity generation [7,8]. Microbial Fuel Cells employ the anaerobic respiration of microorganisms to convert organic waste (fuel) directly into useful electricity [9], which can be used to energise practical applications [10]. The MFC performance however, is still limited and one way to improve the technology's longevity would be to facilitate the Oxygen Reduction Reaction (ORR) for the cathodic half-cell, without employing expensive catalysts. The development of bio-cathodes is a viable alternative to chemical and noble metal cathode catalysts, improving sustainability and cost effectiveness [11]. Incorporation of photosynthesis into the MFC system can be

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done through various configurations [12]. Its operation has an important additional advantage of using both chambers for simultaneous treatment under different conditions, which helps with the removal of pollutants [13]. For example, incorporating photosynthetic organisms can provide active electron acceptors for the cathode, as well as dissolved oxygen for the ORR [7,14–16]. It has been previously shown that the cathodic half-cell can be part of a carbon capture system via an open to air configuration [17] where carbon capture can be achieved by (i) the mineralisation of CO₂ into trona and (ii) the growth of phototrophic organisms in double-chamber MFCs [18,19]. The use of phototrophs as biocatalysts in the cathode half-cell aims to: (i) meet the oxygen level requirements for the ORR [14] and (ii) produce biomass that can be subsequently used directly as the fuel for the MFC anode. Algal powder has been previously shown to be a suitable feedstock for MFCs [20,21]. Photo-cathodes have been recognised as the most promising option for incorporation of photosynthesis into MFC systems [22] and enabling energy production with the added value of carbon fixation [23]. The key innovation on a large scale would lay in low cost biomass regeneration and nutrient recovery (such as nitrogen and phosphorus) for use as fertilisers. However, from the practical point of view, most microalgal MFC studies contain specific, controlled media and growth conditions, and often CO₂ addition to support algal growth [15,24]. Here, it is proposed that a microalgal culture can be maintained in the cathode chamber simply by the MFC operation, addition of water and exposure to light. Moreover, the algal culture is of natural origin, may be spontaneous and is not controlled or limited. During the MFC operation, cations other than protons are being transported from the anode to the cathode through a PEM [25], which is a mechanism that may support algal micronutrient requirements to sustain and enhance algal growth. This experimental work is presenting the further development of previously published work on photosynthetic cathodes [16] and the lagooning photosynthetic ponds [26]. The advantages of algal cathodes include eliminating the need for a mechanical air supply at the cathode therefore lowering the running costs and reducing the overall CO₂ emissions from the anodic bacterial respiration. Dual-chamber MFCs were evaluated under batch-fed mode using sewage sludge and sodium acetate as the carbon-energy source, with mixed anaerobic bacteria as the anode biocatalyst. The cathode compartment contained mixed photosynthetic consortia. The cathode chamber was connected to photo-reactors, which acted as oxygen reservoirs/photosynthetic ponds. Algal biomass produced in the cathodic photoreactors has been harvested and used directly as fuel in the anode, thereby closing the loop and demonstrating self-sustainability. Unlike previous work, the algal biomass was fed neat, i.e. it has not been processed or dried. Furthermore, the system has not used any CO₂ fertilisation, pH control, growth media, catalysts, exotic metals, compressed air mixing or externally forced air-flow to optimise and simplify MFC conditions in order to present its suitability for future practical applications.

This work is aiming to: i) demonstrate the operation of a fully biological microbial fuel cell with an anaerobic anode and a photosynthetic cathode colonised by the mixed culture of photosynthetic organisms; ii) investigate the relationship between the development of the cathodic biofilm and MFC power generation and iii) utilise the harvested biomass directly as a feedstock for the MFC anodes.

2. Materials and methods

2.1. MFC design

MFC reactors comprised 25 mL anode chambers and 25 mL cathode chambers, separated by a cation exchange membrane

(VWR International) as previously described [16]. The electrode material was carbon fibre veil with a total area of 270 cm² (20 g/m²) (PRF Composite Materials, Poole, UK) used in both the anode and cathode chambers. Carbon veil sheets were folded down into rectangular cuboids and connected with a nickel-chrome wire (thickness-0.45 mm) to the external circuit. The cathode electrodes were modified in 5 different experimental groups (Table 1) and included two control conditions, i.e. one control group with no electrode modification and an abiotic control (algae water) and 4 experimental groups with: a) non modified cathode electrode (algae); b) cotton string (thickness-2 mm) wrapped around the electrode (algae string); c) cellulose layer (thickness-1 mm) coating around the electrode (algae cellulose); d) stainless steel wire (type 316, thickness 0.45). The modifications were employed to support algal biofilm development on the cathode electrode and for current collection. Each experimental condition was tested in triplicate resulting in a total of 15 MFCs. No growth media, pH control or chemical pre-treatment were used.

2.2. MFC inoculation and operation

For the cathode inoculum, fresh pond water (Frenchay, Bristol) was cultivated in a well-illuminated laboratory environment for 2 months prior to the start of experiments, to allow algal growth and development. When the sample became visibly green, it was used as the inoculum for the cathode half-cell and operated in batch mode to ensure biofilm establishment and electrode colonisation. Sterilised deionised water was used as the catholyte in the control MFCs. Anodes were inoculated with activated anaerobic sludge provided by the Wessex Water Scientific Laboratory (Saltford, UK), mixed with 0.1 M sodium acetate prior to use (pH 7.2) and employed thereafter for periodic feeding. After 40 days, each of the cathode chambers was connected to the 0.5 L Schott photoreactor bottles in which, again algae were re-suspended in fresh pond water. The photoreactor bottles were connected via a 16-channel peristaltic pump (205U, Watson Marlow, UK) to the MFCs in a closed loop recirculation manner, at a flow rate of 123 mL/h, as shown in Fig. 1. The MFCs and photoreactor bottles were placed in a temperature- and light-controlled incubator (LMS Ltd., Kent, UK), fitted with two Cool White Daylight Tubes (3500 lux), and controlled by a programmable timer under a 14 h light/10 h dark regime at 22 °C.

MFCs were operated under 8.2 kΩ external resistive loads for 8 months while the cathode half cells as well as the photoreactors showed visibly established green communities. Microscopic observation showed a dominant community of green algae and other species such as cyanobacteria, heterotrophic bacteria and protozoa. During the long-term MFC operation, the control (abiotic) cathodes became serendipitously biotic, showing growth of photosynthetic organisms visible to the naked eye in the chambers as reported earlier [16]. All MFCs have shown a well-developed photosynthetic biofilm in the cathode side half-cell. This is when all the photoreactors were filled with fresh deionised water and the growth of photosynthetic organisms was observed and assessed as shown in Fig. 1.

2.3. Data capture

Polarisation experiments were performed using a resistorstat tool [27] in the range of 30 kΩ to 10Ω and the time constant for each resistance value was 3 min. Data were logged using an ADC-24 16-Channel Data Logger (Pico Technology LTD., Cambridgeshire, UK). The data were processed using the Microsoft Excel and GraphPad Prism software packages. Current and power were calculated as previously described [28].

Table 1
Electrode modifications in the cathode biotic and abiotic environment.

Cathode name	Cathode type	Catholyte
Algae water	Carbon veil (control)	(Initially) abiotic – (subsequently) turned biotic
Algae	Carbon veil	Biotic
Algae string	Carbon veil wrapped with cotton string	Biotic
Algae cellulose	Carbon veil wrapped with cellulose coating	Biotic
Algae wire	Carbon veil wrapped with stainless steel wire	Biotic

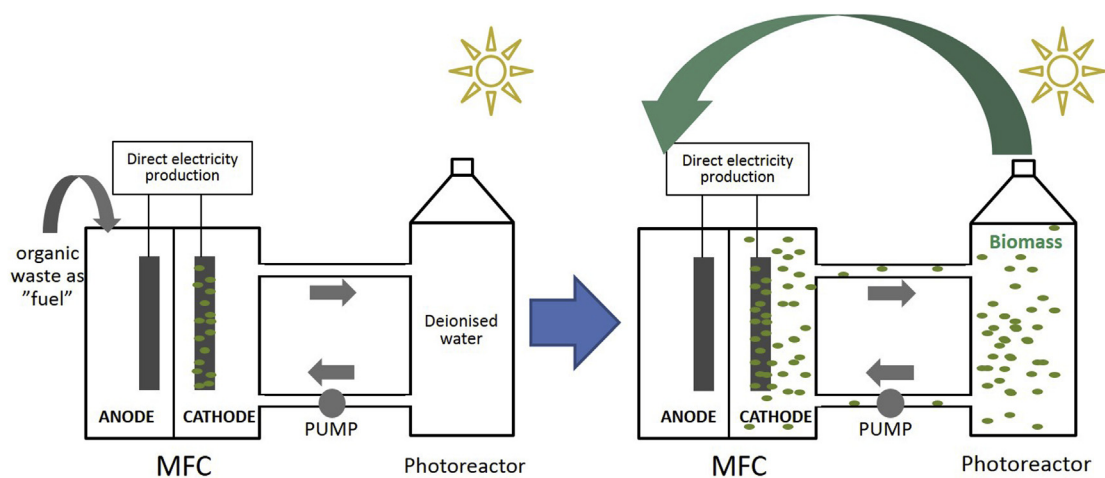


Fig. 1. Microbial Fuel Cell (MFC) with a biomass harvesting photoreactor. The source of fuel was organic waste (sludge+20 mM sodium acetate) or biomass harvested from the cathodic photoreactor.

2.4. Biomass assessment

A direct microscopic count was performed on the harvested samples of microalgal suspension using a Neubauer bright line haemocytometer (Marienfeld, Germany) and a transmitted light microscope (AxioStar Plus, Carl Zeiss) 4 weeks after the start of the experiment. Optical density was measured using a 6300 spectrophotometer (Jenway, UK) at 678 nm (*Chl a* absorption peak). Microalgal dry weight (mg/L) was assessed using a vacuum filtration unit (Millipore, UK) and 47 mm (0.2 μm pore size) sterile membrane filters (Whatman, VWR, UK). Dry weight was determined by the analytical balance (HR120, Metler Toledo) after obtaining constant weight from drying filter papers for 24 h under room temperature and 1 h under 100 W lamp. Calibration was performed using dilutions in the range of dilution factor (DF) of 1–0.1.

2.5. Cathode surface analysis

Surface morphology image of cathode electrodes with the attached photosynthetic biofilms, was observed by scanning electron microscopy (SEM). Dry samples were mounted on aluminium using contact adhesive. Images were observed and captured using a Philips XL30 scanning electron microscope (SEM). Samples were further prepared for microscopy by sputter coating in gold using an Emscope SC500.

3. Results & discussion

3.1. MFC power performance

The maximum absolute power generation is shown in Fig. 2, where the best performing MFC was the algal based cathode giving 128 μW , algae water 81 μW , algae string 74 μW , algae wire 67 μW

and algae cellulose 61 μW maximum power in comparison with the abiotic MFC (control) with the lowest performance of 46 μW . The results are consistent with previously reported data, where the algae were shown to improve the system power performance and longevity [16]. The biomass accumulation in the connected to the MFC photoreactors, shows dependence on the charge transfer of the MFCs and can be directly linked with the produced power. Therefore it is suspected that the more electricity the MFC generates, the more biomass may be obtained from its cathode photoreactor. The harvested biomass showed that the cell density for the algal cathode was up to $31 \times 10^7/\text{L}$. A platinum based cathode was previously shown to maintain a monoculture of *Chlorella vulgaris* [18] for CO_2 sequestration and oxygen generation. Here not only the same process is supported but it is further hypothesised that the cationic flux of ions such as NH_4^+ through the exchange membrane, which is dependent on the MFC performance, can influence biomass growth [29] in a carbon based MFC system. This can significantly lower the cost of MFC technology making it feasible for practical applications.

3.2. Algal growth within the reactor

The relationship between optical density, cell density and dry weight was established by linear regression, as shown in Fig. 3 (left). As the samples were taken from the photoreactor bottles, it was observed that algae, algae wire and algae water showed uniform cell densities, whereas more aggregation was observed in the string and cellulose units. The aggregation in the MFC with string and cellulose was limiting the optical density measurement as a reliable tool of biomass assessment. Therefore, the dry weight was chosen to correlate with the maximum power performance (Fig. 3, right). Microalgal biomass growth at the cathode was assessed to correlate with the power output. It shows that the most promising configuration for the algal cathode is the non-coated carbon veil

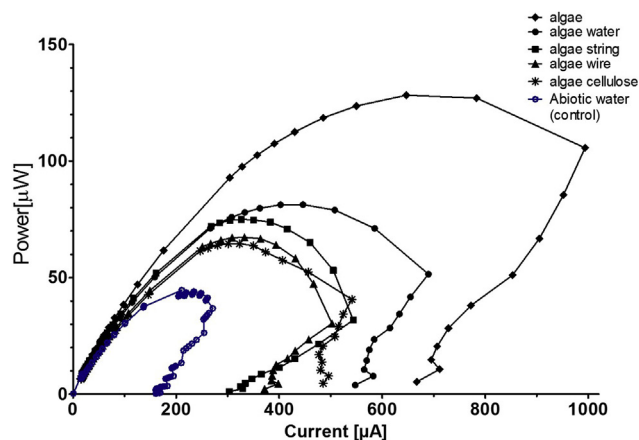


Fig. 2. Power performance derived from MFC polarisation experiments.

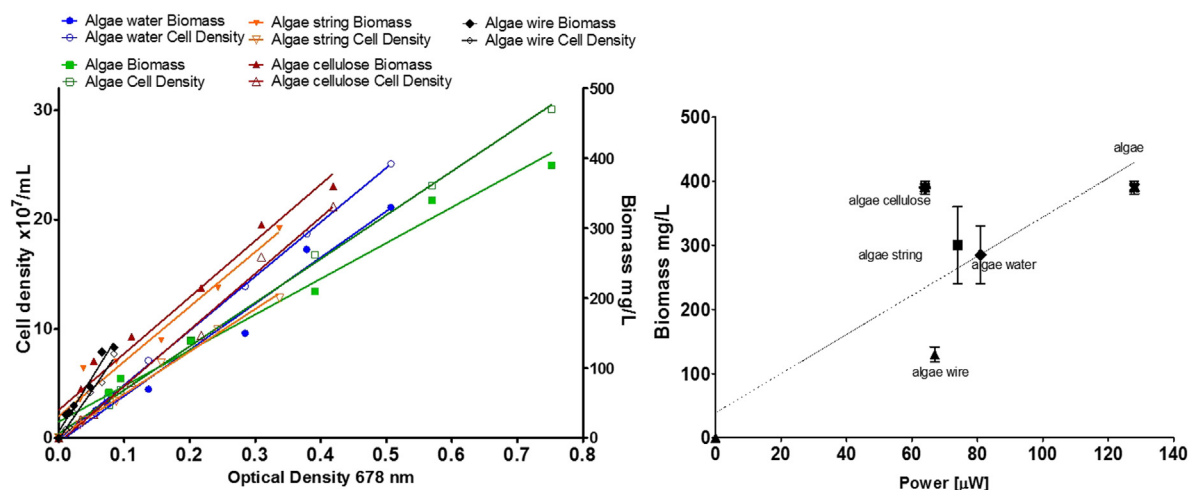


Fig. 3. Calibration curves of optical density at A678, cell density and biomass (left). Relationship between the harvested biomass from the photoreactors and MFC power performance (right).

matrix to allow better diffusion of dissolved oxygen to the electrode.

The high cost of CO₂ as a feedstock for algal growth is a major obstacle, which is why there is interest in CO₂ regeneration techniques [30] and algal growth has already been correlated with power performance in a wastewater supplemented cathode [31]. In the present study, it is suggested that the cathode acts more like a polishing chamber, since no other nutrients were supplied, other than naturally occurring pond water (inoculum) and deionised water.

3.3. Photosynthetic biofilm

The SEM images (Fig. 4) show algal biofilm formed on all tested cathode electrodes in comparison with the abiotic control. String and cellulose coating showed a layer consisting of microorganisms embedded in a microbial extracellular polymeric substance (EPS) matrix formed on the electrode surface. The thicker biofilm formed in these two conditions possibly limited the oxygen diffusion and MFC performance as indicated before [32], which suggests that the power generation as well as biomass production is favoured up to a certain thickness of photosynthetic biofilm on the cathode.

Oxygenic biofilm has already been shown to enhance the MFC

current production [33]. The charge-balancing cation transport from the anode to the cathode compartment [34] may be responsible for the increased growth in better performing MFCs, which in turn may influence the increased power generation. Previous research on cathodic biomass growth was studying light intensity [35], showing that intermittent illumination provides more efficient and prolonged operation of MFCs [36].

In addition, it has previously been shown that the amount of recovered salt from the cathode was related to power generation [17]. Therefore it may be assumed that the level of charge transfer connected to the proton/cation transfer is influencing the biomass growth within the given reactor. The more power the MFC generates, the more protons/cations are being transported from the anode to the cathode and thus made accessible for photosynthetic organisms. MFC-activated crossover of minerals extracted from the anode plays a vital part in nutrient recovery when ceramic was used as the separator [37] therefore replacing CEM with ceramic

could be one very cost-effective option for future scale-up. The co-existence of the attached biofilm, suspended biomass in solution and aggregate forming, made it difficult to accurately quantify the total biomass with optical density or haemocytometer. Analyses were performed on the least aggregated samples however the highest aggregation was observed in the cellulose and string based cathodes, which might be due to the dense electrode colonisation as shown in Fig. 4.

Surface texture is one of the factors that influence microalgal attachment to different substrata. In general, rough or porous surfaces have higher surface area and better cell attachment, and are thus preferred as natural substrata for algae harvesting [38]. It has been observed that the biomass growth was the lowest in the algae wire MFCs, and may be related to stainless steel bio-corrosion affecting the output and limiting algal growth. Microscopic observation had shown a predominant colonisation by unicellular as well as mixed algae and cyanobacteria, bacterial species and protozoa suggesting it is a dynamic and balanced close-to-natural ecosystem. Natural biofilm communities include a number of microbes such as fungi, algae, protozoa and bacteria showing symbiotic interactions [39]. The development of a natural and low maintenance biocathode for active biomass fixing will help to make MFCs a carbon-neutral technology with enhanced efficiency and self-sustainability.

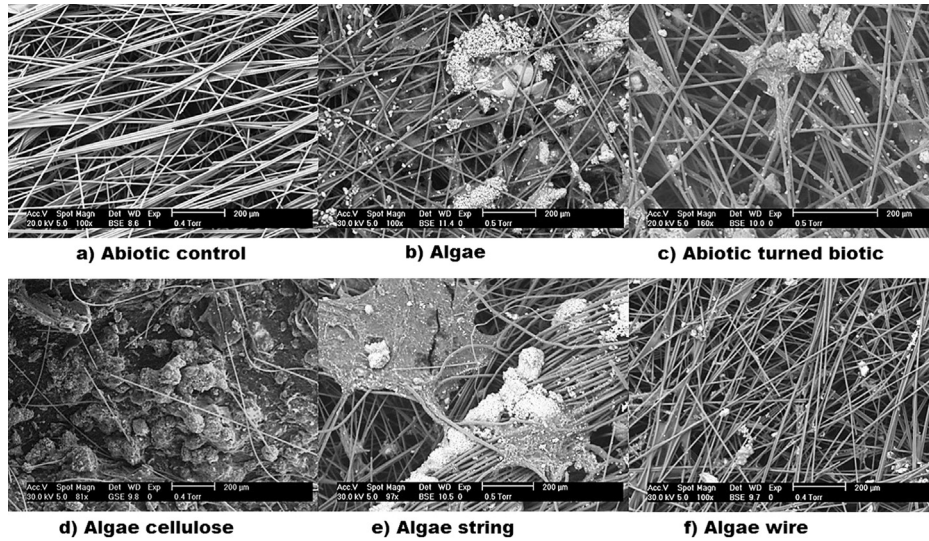


Fig. 4. SEM images of cathode electrodes with developed biofilm (b, d, e, f) and abiotic control electrode (a) which spontaneously turned biotic (c).

3.4. Algae used as feedstock

The catholyte collected from the photoreactors was harvested and separated into 4 feedstock solutions dependent on the type and the amount of biomass: non aggregated feedstock whose biomass content was: a) 0.25 g/L, b) 0.39 g/L and aggregated feedstock: c) dry mass 0.56 g/L d) dry mass 0.72 g/L. The biomass was used as feedstock directly without any pre-treatment. The graph in Fig. 5 shows the average power of all five types of MFC when fed with sludge+0.1 M acetate in comparison with algal feedstock used as indicated. It shows that the algal feedstock produced by the MFC cathode may be successfully utilised as the anodic substrate. The performance increased with the amount of biomass fed to the anode half-cell. The slow-release nature of this feedstock (Fig. 6) in

comparison to acetate, is indicating that algal biomass seems to be a more complex substrate due to its mineral composition.

A similar closed loop system was already presented [40] where an Anaerobic Digester had been supplied with algal feedstock in a pre-treatment stage. The proof of concept has been presented in strictly controlled conditions and supplemented with growth media and CO₂ fertilisation. Here these control mechanisms were avoided to represent the sustainable MFC utilising natural processes. In this set up the control environment was minimised to show the possibility of implementation in real world applications. Algae are produced in abundance in high-rate algal oxidation ponds from the tertiary phases of the sewage treatment process [41]. Algae have previously been used as feedstock for MFCs as powders [20,21], or pre-treated microalgae [42] and macroalgae [43].

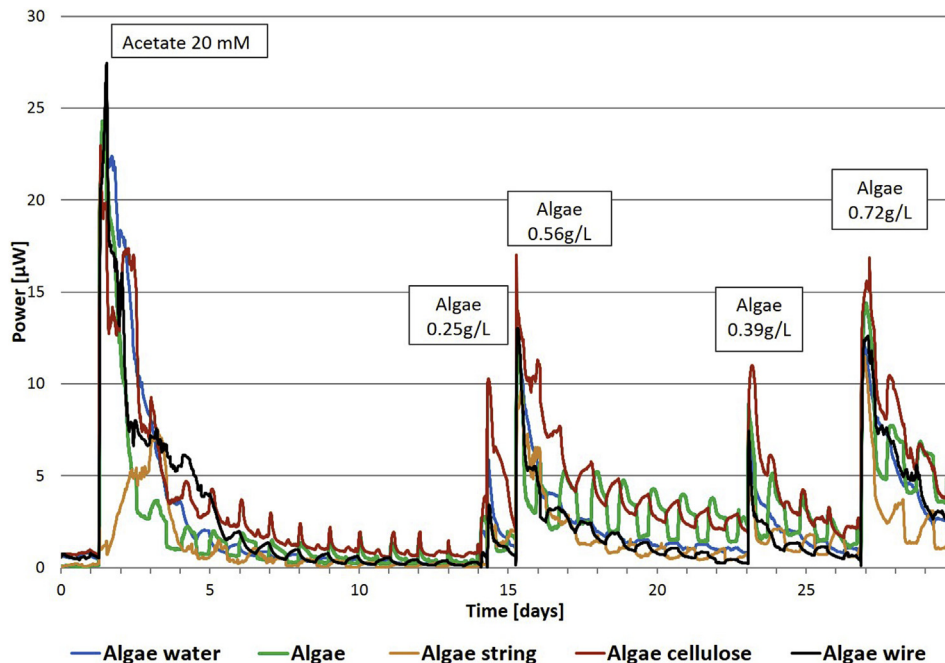


Fig. 5. Algal biomass used as feedstock in comparison to acetate used at the beginning of the test.

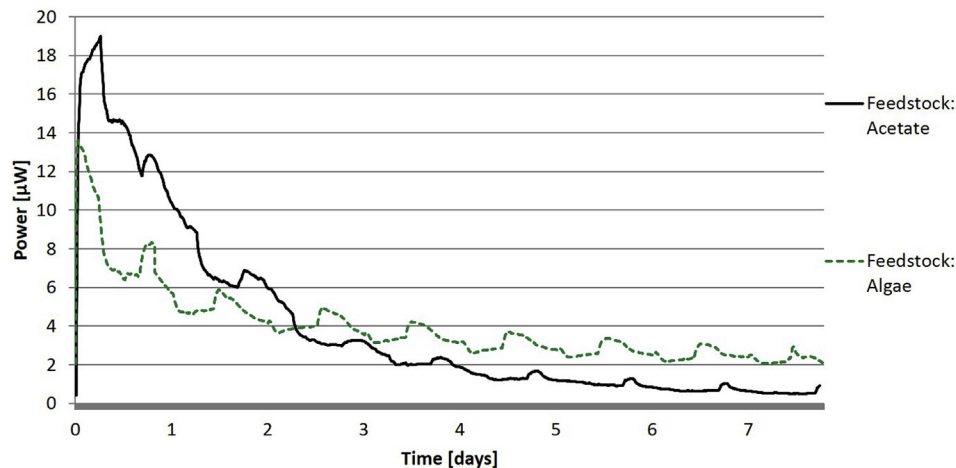


Fig. 6. Overview of feedstock comparison.

Phototrophic biofilms grow in response to light, carbon dioxide and inorganic nutrients where the availability of nutrients influences the type of biofilm formed [44]. Third generation biofuels from algal cells grown on non-arable land provide a solution in the food-fuel debate [45]. Wastewater seems to be the best option for reducing the environmental burden from the cultivation of algal biomass, therefore the current work aims to contribute to the development of algal biofuels through self-sustainable MFC systems. In the development of renewable energy sources and carbon sequestration technologies, one of the most popular methods of CO₂ reduction is the use of photosynthetic organisms such as algae and cyanobacteria that convert CO₂ into biomass. Incorporating such photo-assisted cathodes for Microbial Fuel Cells (MFCs) provides active oxygenation for the oxygen reduction reaction with simultaneous carbon capture.

4. Conclusions

This study presents a fully biotic system that is able to continuously generate electricity with simultaneous biomass production in cathodic photoreactors. It shows the sustainable recovery of biomass enhancing carbon capture and its reuse for electric output in the same system. Whilst the algal activity supports the oxygen reduction reaction *in situ*, the MFC operation provides cations for microalgal growth in the MFC cathode. There is a large potential for the development of algal based cathode MFC systems for wastewater treatment. At present, the infrastructure of wastewater treatment systems provides an opportunity to evaluate large scale operation of algal based biofilm technologies, integrating waste remediation and biomass production. The capture of the energy locked within the organic contaminants of wastewater to produce electric energy and the improvement in nutrient recovery, could serve as a sustainable option increasing the energy recovery balance. Enclosed biofilm MFCs and photo-bioreactors therefore offer a potentially more economical alternative to conventional tertiary treatment processes for nutrient removal [46]. It has been proposed that the separation of wastewater treatment in the anode and biomass production in the cathode can only be truly linked and combined when electricity is generated, therefore the three valuable functions can be integrated.

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