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MICROBIAL ELECTROCHEMICAL TECHNOLOGIES FOR BIOFUELS AND BIOENERGY PRODUCTION

A thesis submitted for the degree of Doctor of Philosophy in

DESIGN, MODELING AND SIMULATION IN ENGINEERING

and

CIÈNCIA I TECNOLOGIA DE L'AIGUA

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Preface

The work reported in this Ph.D. thesis, entitled "Microbial Electrochemical Technologies for Biofuels and Bioenergy Production", was conducted mainly at LabTA$_2$ (Laboratorio di Trattamenti Avanzati per l'Ambiente), Department of Civil Engineering and Architecture (DICAr), University of Pavia from October 2017 to September 2020. A joint doctorate agreement was signed in October 2019 with University of Girona, following a one year long abroad research period at LEQUiA (Laboratori d'Enginyeria Quimica i Ambiental). Professor Andrea G. Capodaglio (University of Pavia) and Professor Sebastià Puig Broch (University of Girona) jointly supervised the present thesis.

The thesis contains both published and unpublished material; the publications herein reported were included in the Ph.D. thesis.

Bolognesi, S., Cecconet, D., Callegari, A., Capodaglio, A.G., 2020. Bioelectrochemical treatment of municipal solid waste landfill mature leachate and dairy wastewater as co-substrates. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-10167-7

Bolognesi, S., Cecconet, D., Callegari, A., Capodaglio, A.G., 2021. Combined microalgal photobioreactor/microbial fuel cell system: performance analysis under different process conditions. Environ. Res. 192, 110263. https://doi.org/10.1016/j.envres.2020.110263

Bolognesi, S., Bernardi, G., Calegari, A., Dondi, D., Capodaglio, A.G., 2019. Biochar production from sewage sludge and microalgae mixtures: properties, sustainability and possible role in circular economy. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-019-00572-5

The following experimental and research work was conducted in the period of the present Ph.D. study, but not included in the Ph.D. thesis.

Bolognesi, S., Cecconet, D., Capodaglio, A.G., 2020. Agro-industrial wastewater treatment in microbial fuel cells, in: Abbassi, R., Yadav, A.K., Khan, F., Garaniya, V. (Eds.), Integrated Microbial Fuel Cells for Wastewater Treatment. Butterworth-Heinemann, pp. 93–133.

Callegari, A., Bolognesi, S., Cecconet, D., 2019. Operation of a 2-Stage Bioelectrochemical System for Groundwater Denitrification. Water 11, 1–13. https://doi.org/10.3390/w11050959

Callegari, A., Bolognesi, S., Cecconet, D., Capodaglio, A.G., 2020. Production technologies, current role, and future prospects of biofuels feedstocks: a state-of-the-art review. Crit. Rev. Environ. Sci. Technol. 50, 384–436. https://doi.org/10.1080/10643389.2019.1629801

Capodaglio, A. G., Bolognesi, S., 2019. 2 - Ecofuel feedstocks and their prospects, in: Azad, K.B.T.-A. in E.-F. for a S.E. (Ed.), Woodhead Publishing Series in Energy. Woodhead Publishing, 15–51. https://doi.org/https://doi.org/10.1016/B978-0-08-102728-8.00002-4

Capodaglio, A.G., Bolognesi, S., 2020. 14 - Microbial Fuel Cells: Treatment Efficiency and Comparative Bioelectricity Production from Various Wastewaters, in Tiquia-Arashiro, S. (Ed.), Pant, D. (Ed.). Microbial Electrochemical Technologies. Boca Raton: CRC Press, https://doi.org/10.1201/9780429487118

Cecconet, D., Bolognesi, S., Callegari, A., Capodaglio, A.G., 2019. Simulation tests of in situ groundwater denitrification with aquifer-buried biocathodes. Heliyon 5, e02117. https://doi.org/10.1016/j.heliyon.2019.e02117

Cecconet, D., Bolognesi, S., Callegari, A., Capodaglio, A. G. 2019. Controlled sequential biocathodic denitrification for contaminated groundwater bioremediation. Science of the Total Environment, 651, 3107–3116. https://doi.org/10.1016/j.scitotenv.2018.10.196

Cecconet, D., Bolognesi, S., Daneshgar, S., Callegari, A., Capodaglio, A.G., 2018. Improved process understanding and optimization by multivariate statistical analysis of Microbial Fuel Cells operation. Int. J. Hydrogen Energy 43, 16719–16727. https://doi.org/10.1016/j.ijhydene.2018.07.056

Cecconet, D., Bolognesi, S., Molognoni, D., Callegari, A., Capodaglio, A.G., 2018. Influence of reactor's hydrodynamics on the performance of microbial fuel cells. J. Water Process Eng. 26, 281–288. https://doi.org/10.1016/j.jwpe.2018.10.019

Abstract

Wastewater treatment and energy production are key points to grant the next generations health, economic, and environmental sustainability. The water–energy nexus represents the synergy between water, energy and the environment, leading to complex relationships between stakeholders, authorities, resources and environmental management. Nowadays, research is focused on developing efficient, green technologies based on water responsible use, sustainable energy management and environmental preservation. On the other hand, green energy production processes and resource recovery from waste streams are essential factors in environmental sustainability.

Microbial electrochemistry is a branch of bioelectrochemistry based on the study and application of the interactions between living microbial cells and a solid-state electrode, serving well the purpose of the water-energy nexus. In the last two decades many biologist and microbiologists, chemists, biotechnologists, engineers have focused their interest on the different applications and interaction mechanisms of this new research field. Microbial Electrochemical Technologies (METs) present attractive applications, such as: (i) wastewater treatment, (ii) groundwater pollutants removal, (iii) water desalination and (iv) synthesis of added value carbon chemicals.

In this context, two applications are studied and operated in the present Ph.D. thesis, to investigate potential energy recovery options from waste streams: Microbial Fuel Cells (MFCs) and Microbial Electrosynthesis (MES). MFCs rely on direct conversion of the chemical energy of a substrate into electrical energy, while MES technology focus on electron (energy) utilization for chemical commodities production (for example, biofuels) using a liquid or gaseous waste stream as feedstock.

The implementation of METs in scaled-up applications however depends upon the optimization of microbial, technological, and economic issues: 1) use of genetically engineered bacteria, 2) biological or chemical catalysts, 3) materials, reactors and electrode design. Other strategies may rely on integration of METs with different technologies. The present thesis also explores the possibility of combining microalgae and METs, and the possible products that can be recovered from waste streams in the attempt to improve the water-energy nexus balance.

Sommario

Il trattamento delle acque reflue e la produzione di energia sono punti chiave per garantire alle generazioni future sostenibilità sanitaria, economica e ambientale. Il *water-energy nexus* rappresenta la sinergia tra acqua, energia e ambiente, che a sua volta implica complesse relazioni tra stakeholder, autorità, risorse e gestione ambientale. La ricerca oggigiorno si sta concentrando sullo sviluppo di efficienti tecnologie ecologiche basate sull'uso responsabile dell'acqua, sulla gestione sostenibile dell'energia e sulla conservazione dell'ambiente; d'altro canto, i processi di produzione di energia rinnovabile e il recupero delle risorse da materiali di scarto sono fattori essenziali per la sostenibilità ambientale.

L'elettrochimica microbica è una branca della bioelettrochimica basata sullo studio e l'applicazione delle interazioni tra microorganismi e un elettrodo a stato solido, che si integra bene allo scopo del *water-energy nexus*. Negli ultimi due decenni molti biologi e microbiologi, chimici, biotecnologi, ingegneri hanno concentrato il loro interesse sulle diverse applicazioni e sui meccanismi di interazione di questo nuovo campo di ricerca. Le Tecnologie Elettrochimiche Microbiche (METs) hanno una grande varietà di applicazioni (i) nel trattamento delle acque reflue, (ii) nella rimozione degli inquinanti delle acque sotterranee, (iii) nella desalinizzazione dell'acqua e (iv) nella sintesi di sostanze chimiche carboniose a valore aggiunto.

In questo contesto, due applicazioni sono quindi studiate e operate nella presente tesi, per indagare le potenziali opzioni di recupero di energia da materiali di scarto: le celle a combustibile microbiche (MFCs) e l'elettrosintesi microbica (MES). Le MFC si basano sulla conversione diretta dell'energia chimica di un substrato in energia elettrica, mentre la tecnologia MES si concentra sull'utilizzo di elettroni (energia) per la produzione di prodotti chimici (ad esempio, biocarburanti) utilizzando un flusso di rifiuti liquidi o gassosi come materia prima.

Tuttavia, l'implementazione delle MET in applicazioni su larga scala dipende anche dall'ottimizzazione di parametri microbici, tecnologici ed economici: 1) uso di batteri geneticamente modificati, 2) catalizzatori biologici o chimici, 3) materiali, reattori e progettazione di elettrodi. Altre strategie si basano sull'integrazione dei MET con diverse tecnologie. La presente tesi esplora anche la possibilità di combinare microalghe e MET, e i possibili prodotti che possono essere recuperati dai flussi di materiali di scarto.

Resumen

El tratamiento de aguas residuales y la producción de energía son puntos clave para garantizar la sostenibilidad sanitaria, económica y medioambiental de las próximas generaciones. El *water-energy nexus* representa una sinergia entre el agua, la energía y el medio ambiente, lo que genera relaciones complejas entre las partes interesadas, las autoridades, los recursos y la gestión ambiental. La investigación actual se centra en el desarrollo de tecnologías ecológicas eficientes basadas en el uso responsable del agua, la gestión sostenible de la energía y la preservación del medio ambiente. Por otro lado, los procesos de producción de energía verde y la recuperación de recursos de las corrientes de residuos son factores esenciales en la sostenibilidad ambiental.

La electroquímica microbiana es una rama de la bioelectroquímica basada en el estudio y la aplicación de las interacciones entre los microorganismos y un electrodo, que premite la sinergia entre agua y energía. En las últimas dos décadas múltiples disciplinas se han centrado en la aplicación y los mecanismos de éste pionero campo de investigación. Las Tecnologías Electroquímicas Microbianas (METs) tienen gran variedad de aplicaciones: i) en el tratamiento de aguas residuales; ii) en la eliminación de contaminantes de aguas subterráneas; iii) en la desalinización de agua y iv) en la síntesis de productos químicos de carbono de valor añadido.

En la presente tesis se estudian y operan dos tecnologias basadas en la recuperación de energía de los flujos de residuos: las Celdas de combustible microbianas (MFC) y la electrosíntesis microbiana (MES). En las MFC existe una conversión directa de la energía química de un sustrato a energía eléctrica. En cambio, las MES se centran en el uso de electrones (energía) para la producción de productos químicos (por ejemplo, biocombustibles) utilizando una corriente de desechos líquidos o gaseosos como materia prima.

Sin embargo, la implementación de METs en aplicaciones reales depende de la optimización de los parámetros microbianos, tecnológicos y económicos: 1) uso de microorganismos modificados genéticamente, 2) catalizadores biológicos o químicos, 3) materiales, reactores y diseño de electrodos. Otras estrategias se basan en la integración de METs con diferentes tecnologías. La presente tesis también explora la posibilidad de combinarlos con microalgas para investigar la recuperació de productos de los materiales de desecho.

Resum

El tractament d'aigües residuals i la producció d'energia són punts claus per garantir la sostenibilitat sanitària, econòmica i ambiental de les properes generacions. El *water-energy nexus* representa una sinergia entre aigua, energia i medi ambient, que condueix a relacions complexes entre els grups d'interès, les autoritats, els recursos i la gestió ambiental. Actualment, la investigació es centra en el desenvolupament de tecnologies ecològiques i eficients basades en un ús responsable de l'aigua, la gestió sostenible de l'energia i la preservació del medi ambient. D'altra banda, els processos de producció d'energia verda i la recuperació de recursos a partir de corrents de residus són factors essencials per a la sostenibilitat ambiental.

L'electroquímica microbiana és una branca de la bioelectroquímica basada en l'estudi i l'aplicació de les interaccions entre microorganismes i un elèctrode, que permet la sinèrgia entre aigua i energia. En les últimes dos dècades, múltiples disciplines s'han centrat en l'aplicació i els mecanismes d'aquest camp d'investigació pioner. Les Tecnologies Electroquímiques Microbianes (METs) tenen gran varietat d'aplicacions: (i) en el tractament d'aigües residuals, (ii) en l'eliminació de contaminants subterranis, (iii) en la dessalinització d'aigües i (iv) en la síntesi de productes químics de carboni de valor afegit. En la present tesis s'estudien i utilitzen dos sistemes basats en la recuperació d'energia a partir de corrents residuals: les Cel·les de combustible microbianes (MFC) i l'electrosíntesi microbiana (MES). En les MFC existeix una conversió directa de l'energia química d'un substrat a energia elèctrica. En canvi, lesMES es centren en l'ús d'electrons (energia) per a la producció de productes químics (per exemple, biocombustibles) utilitzant un flux de residus líquids o gasosos com a matèria prima.

Tanmateix, la implementació de METs en aplicacions reals depèn de l'optimització dels paràmetres microbians, tecnològics i econòmics: 1) l'ús de microorganismes modificats genèticament, 2) catalitzadors biològics o químics, 3) materials, reactors i disseny d'elèctrodes. Altres estratègies es basen en la integració dels METs amb tecnologies diferents. La tesi en qüestió també explora la possibilitat de combinar-los amb microalgues per tal d'investigar la recuperació de productes dels residus de materials.

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for me to face this journey. This thesis is dedicated to you. To my grandparents, I always feel you by my side, watching my back and leading my way.

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1. Introduction

The depletion of global fossil fuel reserves leads researchers to investigate and develop alternative technologies for energy production. Renewable bioenergy from wastes, presenting a neutral or negative carbon footprint, is one of the possible alternatives to alleviate the current fossil fuels crisis and might also mitigate global warming effects. Conventional wastewater treatment plants require large amounts of electrical energy: 0.5 – 2 kWh m^{-3} are generally necessary for carbon and partial nitrogen removal, depending on process applied and wastewater composition (Capodaglio and Olsson, 2020). Current wastewater treatment processes are energy and chemical intensive and require large investments with low to no energy recovery. Greenhouse gases (GHGs) such as carbon dioxide (CO_2) and nitrous oxide (N_2O) are produced throughout the process and released to the atmosphere: it was reported that for every 1000 tons of wastewater treatment, 1500 tons of greenhouse gases are released (Wang et al., 2010). Furthermore, conventional treatment processes (i.e., activated sludge process) produce excess sludge which needs further disposal, requiring high capital and operation costs (Li et al., 2014).

In this context, the development of Microbial Electrochemical Technologies (METs) has been pursued by many scientists for the last two decades. METs represents an ensemble of technologies for energy and resources recovery from waste streams, and includes Microbial Fuel Cells (MFCs), Microbial Electrolysis Cells (MECs) and Microbial electrosynthesis (MES). The latter are two different technologies requiring an external power supply, designed to use small amounts of electricity to be stored in energy carriers (such as hydrogen, H_2) or to convert it into valuable chemicals production.

1.1. Energy and fossil fuels crisis: a critical era for our future

The growing pressure that massive demographic increase exerts on society, in combination with challenges determined by the energy needs and the disposal of wastes, imposed a marked turnaround in the definition of national and international energy goals. To face the increasing environmental issues, a balance should be reached between the development of clean and renewable energy sources, and the waste reduction/recovery of raw materials. Fossil fuels combustion and CO_2 emissions from anthropic activities also contribute to ongoing climate change effects, with the first decade of the new millennium registered as the warmest ever (Arndt et al., 2010). Researchers in the last decades have intensified the study and development of alternative technologies, aimed at producing renewable fuels and bioenergy to meet future energy demand and satisfy present and future sustainability. Estimates predicting that some fossil fuels could deplete within the next 50 years (BP, 2016). This, together with the concretization of environmental damages associated to various phenomena caused by greenhouse gases (GHG) emissions (i.e. climate warming, urban smog and acid rain), have prompted governments and international

organizations to target reductions of carbon emissions into the atmosphere (Arens et al., 2017; L. B. Cui et al., 2014; Kanemoto et al., 2014). Industry, transportation and energy sector are accountable for GHG release in the atmosphere. Carbon dioxide, a byproduct of fossil fuel combustion, is the principal greenhouse gas contributing to global warming, accounting for about 76% of total GHG emissions. Other greenhouse gases including methane (16%), nitrous oxide (6%), and a few industrial-process gases also are important contributors to climate change (IPCC, 2014). Thus, the European Union set a target of GHG emissions 20% below 1990 levels by 2020, 40% by 2030 and a fully sustainable zero carbon economy by 2050. In EU-28, the objective for 2020 was reached in 2017 with a decrease of 8.8 tonCO_2 equivalent per capita, but next objectives will be more and more ambitious and challenging (European Environment Agency, 2019).

A shift towards renewable energy sources considered less environmentally harmful, such as solar, wind, biofuels, etc., has also been strongly encouraged (Dincer, 2000; Jacobsson and Lauber, 2006). The term biofuel includes products derived from biomasses, their residuals, microalgae or bacteria: (bio)gas, (bio)diesel, (bio)ethanol, (bio)methanol, (bio)ethers, (bio)fuels, (bio)hydrogen, and vegetable oils (Lin et al., 2014). Considering the current levels of production, markets seem primarily focused at the moment on biogas, biodiesel and bioethanol (Raboni et al., 2015). However, EU economy is still largely dependent on fossil fuels, accounting for 65% of the energy supply (Dessì et al., 2021).

At this time, renewable energy sources such as photovoltaic and aeolic energy are widely developed. However, they are susceptible to one major drawback, i.e., transportability. Transport of large quantities of electricity without powerlines, or with relatively inefficient batteries (as far as current technology allows) is challenging. Liquid biofuels, on the contrary, are easily stored, have a reasonable energy density, and may be used in existing engine technology with only minor modifications (Agarwal, 2007; Lapuerta et al., 2008).

At the same time, water systems, including wastewater treatment facilities, have been indicated among major energy consumers at municipal level worldwide (Rosso and Stenstrom, 2008). It was estimated that they alone may require 1-3% of the total electrical energy output of a country (US DOE, 2014). Current wastewater treatment state-of-the-art technology requires energy consumption between 20 and 45 kWh/PE-year (population equivalent). Consequently, it is not only highly energy intensive, but also a significant source of GHG emissions (Sabba et al., 2018), whose reduction has been recently mandated by European Union and other countries' policies. Energy savings and wastewater valorization by exploitation of its residual resources content, may provide significant contribution to Circular Economy and GHGs reduction (Capodaglio and Olsson, 2020). Based on current knowledge, novel concepts of biorefinery could be developed to satisfy the need of more sustainable environmental protection technology and, at the same time, recover necessary energy and resources (Cherubini, 2010).

In this context, new technologies and materials are studied and tested to find suitable solutions to mitigate the problems emerging today to be ready to face present and future challenges. The reasons for the enthusiasm that METs have aroused in the scientific community lie in the extreme urgency of developing solutions to face some of the great emergencies of today's society: the constant availability of clean and safe water, the need for energy and the minimization of the carbon footprint of waste streams. As an example, MFCs can potentially provide "green" electricity by exploiting as fuel the organic substance contained in domestic and industrial wastewater, obtaining simultaneous removal of

contaminants, although it has not been proven in real world applications yet (Gajda et al., 2018). MES platform instead can convert carbon based wastes (liquid or gas) into value added chemicals and biofuels, granting a nearly zero carbon footprint (Jiang et al., 2019).

1.2. Microbial Electrochemical Technologies (METs)

METs or bioelectrochemical systems (BES), when referring to the device used, can be defined as "an electrochemical system in which electrochemically active microorganisms catalyze the anode and/or the cathode reaction" (Schröder et al., 2015). The main characteristics of BES are the presence of two electrodes, concurring to a redox reaction, anode and cathode respectively, and the role of microorganisms in the catalysis of reactions happening at one or both electrodes. A microbial electrochemical technology is thus a hybrid approach that uses microorganisms to catalyze bioelectrochemical reactions to convert waste carbon materials into bioenergy and bioproducts.

Each reaction can occur when applying its own redox potential; the difference between the redox potential of each reaction at the anode and at the cathode defines the spontaneity of a reaction, also determining the classification of the type of BES operated (Figure 1 and Figure 2).

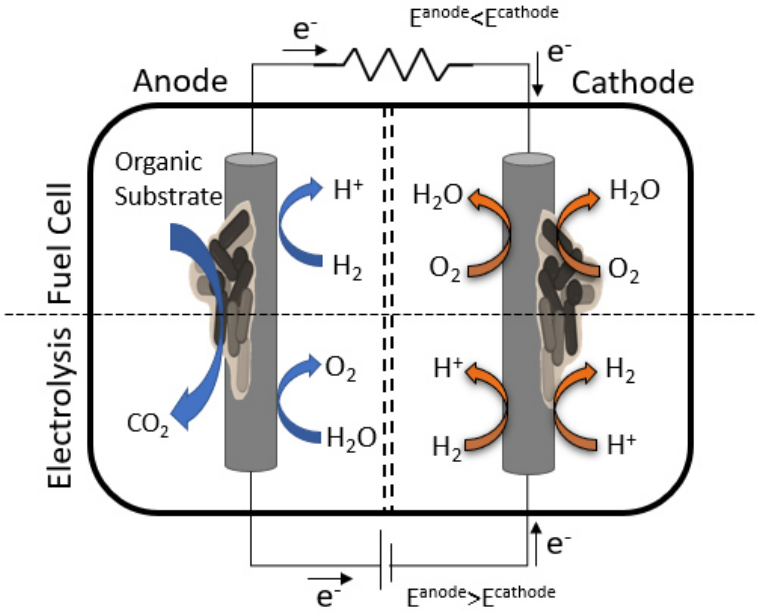

Figure 1. Schematic overview of the possible combinations of microbial and chemical oxidation (anode) and reduction (cathode) reactions in BESs. Microbial fuel cells (MFCs) are characterized by a spontaneous reaction (energy producing), while microbial electrolysis cells (MECs) require an external energy supply (unspontaneous reaction). Figure modified from Clauwaert et al. 2008.

The theoretical cell voltage of a BES, or electromotive force (*emf*) determines the spontaneity of the reaction and if an additional power supplement is needed. The *emf* is

also known as open circuit voltage (OCV), which is the maximum voltage measured without current flow in the circuit. The *emf* can be calculated from the Gibbs free energy of the overall cell reaction:

$$emf = -\frac{\Delta G}{nF} \quad (1)$$

where *emf* is the electromotive force (V), n is the amount of electrons involved in the reaction (mol), ΔG is the Gibbs free energy of the reaction (J mol^{-1}) and F is the Faraday constant (96485.3 C mol^{-1}). Gibbs free energy is a thermodynamic potential that can be used to calculate the maximum of reversible work that may be performed by a thermodynamic system at a constant temperature and pressure. Alternatively, the *emf* can be easily calculated as the difference between the cathode potential and the anode potential, assuming that both potentials are calculated from Gibbs free energy:

$$emf = \Delta E^0 = E^0_{cat} - E^0_{an} \quad (2)$$

where E^0_{cat} and E^0_{an} are the standard electrode potential at pH 7 (V vs SHE; Standard Hydrogen Electrode) at the cathode and at the anode, respectively.

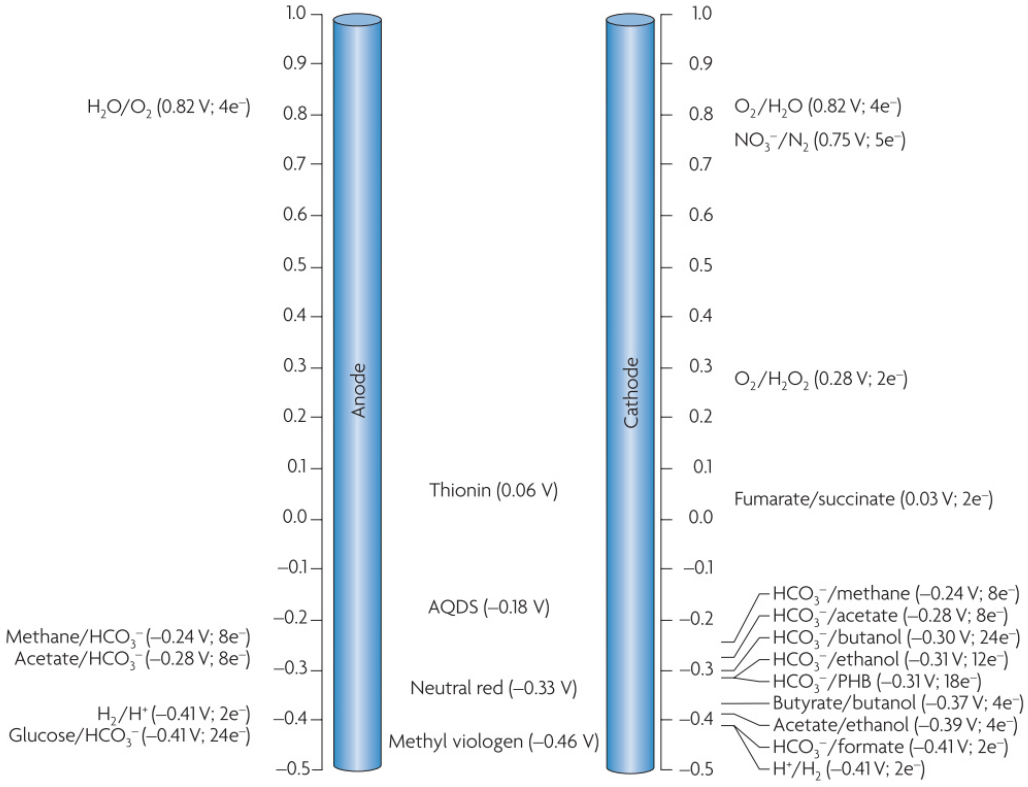

Figure 2. Standard potential for different redox couples. The potential are all referred to the standard hydrogen electrode (SHE), and taken with permission from Rabaey and Rozendal (2010).

However, theoretical OCV is affected by a series of losses that may limit the energy production in the case of MFC and increase the energy demand in the case of MEC and MES, requiring extra energy supply. These losses are called overpotentials. The losses that may occur in a bioelectrochemical system are the result of the resistances of different parts

of the cell: the pH gradient over the membrane ($E_{\Delta pH}$), anode overpotential (η_{an}), cathode overpotential (η_{cat}), ionic losses (E_{ionic}) and transport losses (E_T) all concur to increase energy losses of the system. The real cell voltage can be calculated as follows (Sleutels et al., 2009):

$$\Delta E = OCV - E_{\Delta pH} - \eta_{an} - \eta_{cat} - E_{ionic} - E_T \quad (3)$$

The anode and cathode overpotential (V) were calculated as:

$$\eta_{an} = E^{an,measured} - E^{an};\ \eta_{cat} = E^{cat} - E^{cat,measured} \quad (4)$$

where $E^{an,measured}$ and $E^{cat,measured}$ are the measured anode and cathode potential, respectively, and E^{an}, E^{cat} are the theoretical anode and cathode potentials calculated according to the Nernst equation.
Ionic losses depend on electrolyte resistance of anolyte and catholyte, and can be estimated as:

$$E_{ionic} = I_{ions}\left(\frac{1}{2}R_{an} + \frac{1}{2}R_{cat}\right) = I_{ions}\left(\frac{d_{an}}{2A_{\sigma an}} + \frac{d_{cat}}{2A_{\sigma cat}}\right) \quad (5)$$

where I_{ions} is the measured current density (A m^{-2}), d_{an} and d_{cat} are respectively the distances between the anode and cathode and the membrane (m), A is the surface area (m^2), σ_{an} is the anode conductivity (S m^{-1}) and σ_{cat} is the cathode conductivity (S m^{-1}).
The voltage ohmic losses other than the ionic losses of the system are usually included in the anode and cathode overpotentials, and they are mostly imputed to electron transfer processes to the electrode, affected by the resistivity of the conductor materials (and electrolytes) operated in the system. Activation and concentration overpotentials also need to be taken into account (Clauwaert et al., 2008).

Finally, the transport loss E_T is the potential loss caused by transport of ions through the membrane. It can be calculated using all other components from eq. (3), or alternatively by considering the potential difference between the two reference electrodes (anodic and cathodic chamber), corrected for the ionic resistance and pH gradient of the electrolyte between the two faces of the membrane. The ionic exchange membrane, if present in the system, separates hydraulically the two chambers, allowing an ionic flow between the two compartments. According to the polarity of ions allowed to pass through the membrane, most used membranes in BES are proton exchange membrane (PEM), cation exchange membrane (CEM), anion exchange membrane (AEM), or bipolar exchange membrane. In the case of a PEM, only protons can pass through the membrane. CEM allows the movement of other cations other than protons, while AEM allows the passage of the sole anions. Bipolar membranes are two-sided and joint an AEM and a CEM, allowing one-way only ion flow from each side of the membrane.

1.2.1. Microbial Fuel Cells (MFC)

Microbial fuel cells (MFCs) are a specific type of bioelectrochemical system (BES) that rely on the catalytic action of electrochemically active bacteria (EAB) to oxidize an organic substrate in the anodic chamber, releasing carbon dioxide, electrons and protons. Electrons travel through an external electric circuit from the anode to the cathode electrode, while protons (or other charge-balancing ions) pass through an ionic selective membrane (if present) to reach the cathode. There, both electrons and protons are combined with the

terminal electron acceptor (TEA), usually oxygen (Logan and Rabaey, 2012). MFCs can also be equipped with a biocathode, where an electrotrophic biomass acts as catalyst of the oxygen reduction reaction (ORR), improving sustainability of the cell in comparison to the use of expensive metallic catalysts (He and Angenent, 2006; Nikhil et al., 2017). The additional benefit of MFCs is that, besides successful organic matter removal, electric energy is generated, and can be harvested by low-power management systems (Dallago et al., 2016). Two chamber MFC is the most common architecture for this technology, but also air-cathode MFCs with cathode exposed to air or single-chamber MFCs were extensively studied. Extracellular electron transfer (EET) operated by EABs can be achieved in three different ways, as shown in Figure 3.

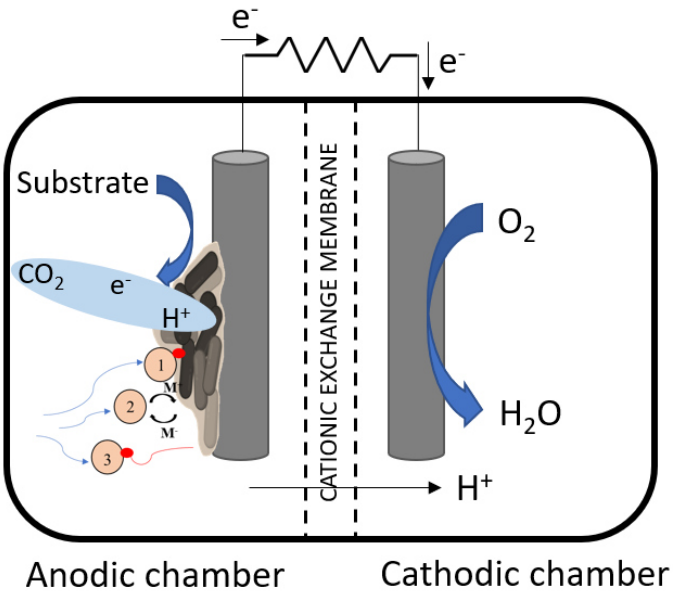

Figure 3: Simplified view of a two-chamber MFC; possible electron transfer mechanisms are shown: (1) direct electron transfer; (2) electron transfer through mediators; (3) electron transfer through nanowires.

The electrochemical reactions in an MFC are exergonic, the overall reaction possesses a negative Gibbs' free energy, which means the reaction proceeds spontaneously with (electrical) energy release.
Typical oxidation and reduction reactions and their respective theoretical maximum *emf* are reported in equations 6-8. Acetate was considered as anodic substrate (electron donor), and oxygen as TEA at the cathode in standard conditions (pH 7, 298.15 K, pO_2=0.2 bar) (Rozendal et al., 2008). Different electron donors and TEAs (i.e., NO_3^-) lead to different bioelectrochemical reactions, with different yields in terms of bioelectricity production.
Anodic and cathodic reactions:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \ (E^0 = -0.289\ V\ vs.SHE) \quad (6)$$

$$2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O \ \ (E^0 = 0.805\ V\ vs\ SHE) \quad (7)$$

Overall reaction:

$$CH_3COO^- + 2O_2 \rightarrow 2HCO_3^- + H^+ \quad (\Delta E^0 = 1.094\,V) \qquad (8)$$

where E^0 is anode/cathode potential, ΔE^0 is the electromotive force. The Gibbs free energy of the reaction is -847.60 kJ mol^{-1}.

The use of MFCs for organic matter removal has shown promising results: in some cases, an average COD (Chemical Oxygen Demand) removal efficiency up to 90% has been achieved. Although the studies reported in the literature confirm the promising nature of this technology as a renewable energy source, MFCs require further improvements that make them economically attractive. Previous experimental works on laboratory scale MFCs report an organic matter removal rate of up to 7 $kgCOD_{REMOVED}$ m^{-3} d^{-1}, in line with that of conventional treatment systems (a range of 0.5-2 $kgCOD_{REMOVED}$ m^{-3} d^{-1} is reported for activated sludge and 8-20 $kgCOD_{REMOVED}$ m^{-3} d^{-1} for anaerobic processes, Logan et al., 2006). Practical MFCs application in WWTP design, however, has been long delayed by the instability of the engineered systems, and the low power density and output voltages obtained so far are not sufficient to cover the energy requirements of the system itself (pumps, aeration). Anodic side reactions such as methanogenesis, aerobic or anoxic respiration by competitive microorganisms represent drawbacks of the process, even though can be partly limited by appropriate operational strategies (Rozendal et al., 2008).
Various kinds of substrate can be operated in MFCs for bioelectricity production, for example pure compounds and complex mixtures of organic matter, such as those occurring in wastewater. Choosing a suitable substrate is a critical factor affecting the systems' performance. Among all the wastewater tested, agro-industrial wastewater rich in organic content proved to be an ideal substrate for MFCs (Bolognesi et al., 2020). MFCs also found application in the bioremediation of specific pollutants and nutrients in wastewaters recovery of heavy metals, decolorization of dyes, are other applications of MFCs (Mathuriya and Yakhmi, 2016; Venkata Mohan et al., 2014). MFC performance is also influenced by many variables such as operating conditions, surface area, type of electrodes and inoculum type; MFC architecture is also a major discriminant in bioelectricity production (Capodaglio et al., 2015).

1.2.2. Microbial Electrolysis Cells (MEC)

Microbial electrolysis cell (MEC) is a technology for hydrogen production from organic matter, including wastewater and other renewable resources (Kadier et al., 2016b). The application of a voltage between anode and cathode is necessary in the case of MEC since the reaction is not spontaneous ($E^0_{cat}<E^0_{an}$). In an MEC, the anodic reaction is the same as MFCs, where EAB oxidize organic matter generating CO_2, electrons and protons. The electrons then travel through an electric circuit to a cathode and combine with the free protons in solution. The reduction may happen directly on the cathode surface or be also mediated by electroactive bacteria (Kitching et al., 2017).
However, in order to produce hydrogen at the cathode from the combination of these protons and electrons, MEC reactors require an externally supplied voltage (0.2 – 0.8 V), relatively low if compared to typical water electrolysis values (1.23-1.8 V) (Zhang and Angelidaki, 2014). The required energy can be supplied through a power supply, a potentiostat to directly fix the cathode potential, or an alternative renewable energy source, such as solar power or even the electrical output of one or more MFCs.

Reactions occurring at the anode (9), cathode (10) and overall (11) in an MEC are the following:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (9)$$

$$8H^+ + 8e^- \rightarrow 4H_2 \quad (E^{cat} = -0.421\ V\ vs\ SHE) \quad (10)$$

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2 \quad (11)$$

Gibbs free energy of the reaction is positive (ΔG=93.14 kJ mol^{-1}), consequently, a negative *emf* is detected; Gibbs free energy is the minimum amount of energy that has to be supplied to achieve cathodic hydrogen production. The presence of system overpotentials may increase the energy needed to produce hydrogen.

Regarding technology architecture, the most common setup is the two-chamber (dual chamber) setup, shown Figure 4; materials used for electrodes and membranes are similar to the ones operated in MFCs. An ion exchange membrane is used to separate the system into two different liquid compartments, allowing the ion flux between chambers. Another option is the single-chamber MEC to decrease the ohmic losses generated by the presence of a membrane, reducing also costs and complexity of architecture. This setup is possible due to the fact that both anode and cathode require an anaerobic environment, differently from the MFC process; however, methanogens can be competitive and partially use the hydrogen produced (Kadier et al., 2016a).

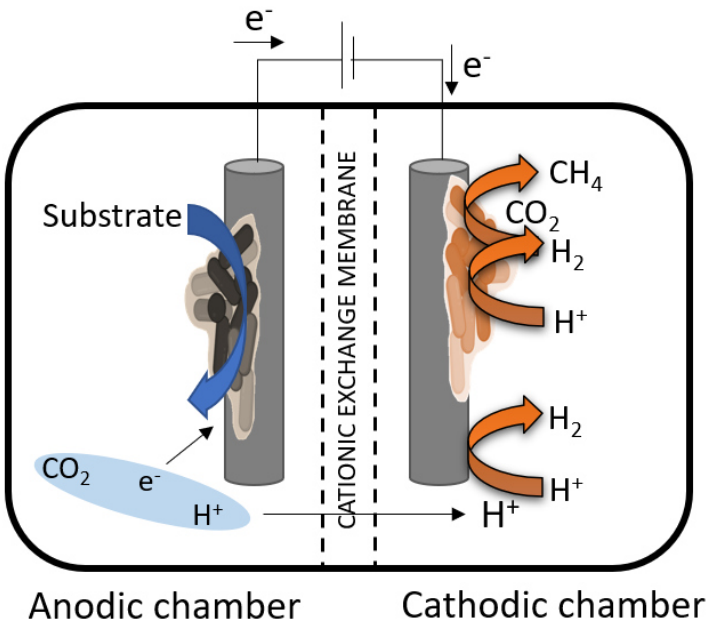

Figure 4. Scheme of a typical microbial electrolysis cell.

MEC has also been operated in other applications to mitigate other environmental problems, such as recovery of metals (Cu, Pb, Cd, and Zn) at the cathode (Modin et al., 2012), recalcitrant contaminants removal such as nitrobenzene (Wang et al., 2012) and 4-chlorophenol (Wen et al., 2013); biosensor, simpler and more reliable than MFC biosensors (Zhang and Angelidaki, 2014).

1.2.3. Microbial Desalination cells (MDCs)

An MDC is a bioelectrochemical system developed from a microbial fuel cell, in which anode and cathode compartments are further divided by ionic selective membranes (anion and cation exchange membrane) (Gude et al., 2013; Santoro et al., 2017). The purpose of this technology is obtain desalination in the middle chamber, in addition to wastewater treatment and bioenergy production (Saeed et al., 2015). The most diffused configuration is reported in Figure 5: the organic substrate is oxidized at the bioanode, the ORR closes the redox reaction at the cathode, while in the additional chamber in the middle, called desalination chamber, operates desalination.

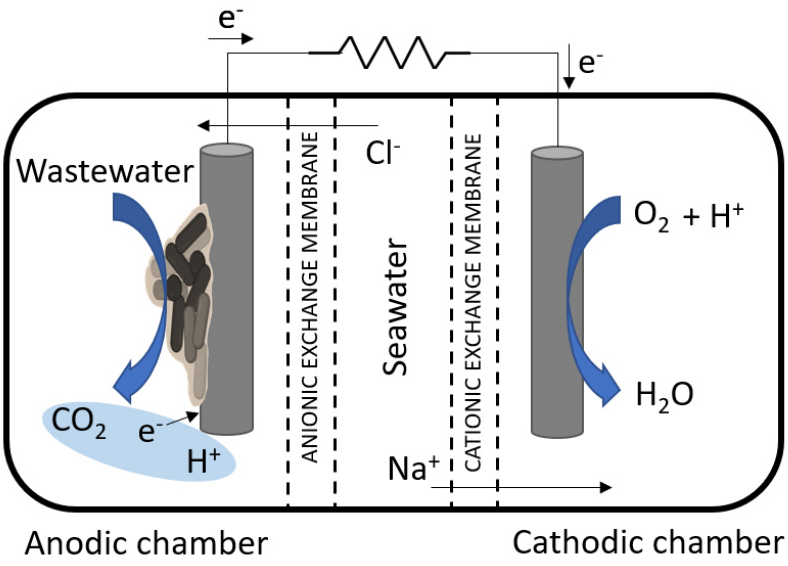

Figure 5. Typical scheme of microbial desalination cell.

The principle of operation of the middle chamber is very simple: seawater (or brackish water) is fed to the desalination chamber, and the ions are attracted by the electrode with the opposite polarity, allowing the removal of salts by the separation of ions that compose them: anions will move towards the anode chamber through the AEM while cations will migrate in the direction of the cathode passing through the CEM. The process is driven by the organic matter oxidation at the anode, and the cathode closes the electrical circuit. Forrestal et al. (2012) reported a positive energy balance for an MDC, meaning that the system can generate more energy than the amount needed for the operation. The open circuit voltage (OCV) is similar to the voltage in MFCs but total power and current generated are lower due to increased losses associated with the increased complexity of the system (E_T, E_{ionic}). In case of saltwater characterized by high salinity, MDCs achieve higher current densities, because the high conductivity in the desalination chamber would lower the ionic losses (Saeed et al., 2015); for this reason, salinity in the middle desalination chamber ought to be higher than that of the electrolytes, to avoid reverse concentration gradients between saltwater and electrolytes (Yuan et al., 2012). Several configurations for MDCs have been developed: biocathode MDC in which bacteria acts as catalyst in the cathodic reactions (Wen et al., 2012); stack-structured MDC, where multiple pairs of ion exchange membranes are inserted to allow creating multiple concentrating and desalting

chambers (Kim and Logan, 2013); air-cathode MDC (Gude et al., 2013); microalgae biocathode MDC, where the TEA, oxygen, is provided by microalgae (Kokabian and Gude, 2015); microbial electrolysis desalination cell (MEDC), created by the combination of a MEC and a MDC, where a voltage is applied between anode and cathode to allow the production of H_2 at the cathode (Chen et al., 2012); osmotic MDC (Zhang and He, 2013), with the integration of forward osmosis in the MDC setup and the supercapacitive MDC (Santoro et al., 2017).

1.2.4. Microbial Electrosynthesis (MES)

In the last decade, with increased interest on resource recovery and circular economy, a new category of BESs called microbial electrosynthesis systems (MES) has emerged. Microbial electrosynthesis technology is developed on the MEC platform, using the cathode for the synthesis of more complex chemicals rather than hydrogen. In MESs, microbial metabolism is driven by electric current for the production of valuable compounds (Rabaey and Rozendal, 2010). A large variety of substrates can be used for bioelectrosynthesis of value-added products. In addition to the acetate and glucose, the choice of substrates for the bioelectrochemical conversion of wastes into value-added chemicals has gradually been expanded to various wastewaters, solid wastes, and waste gas, as summarized in Figure 6 (Kong et al., 2020).

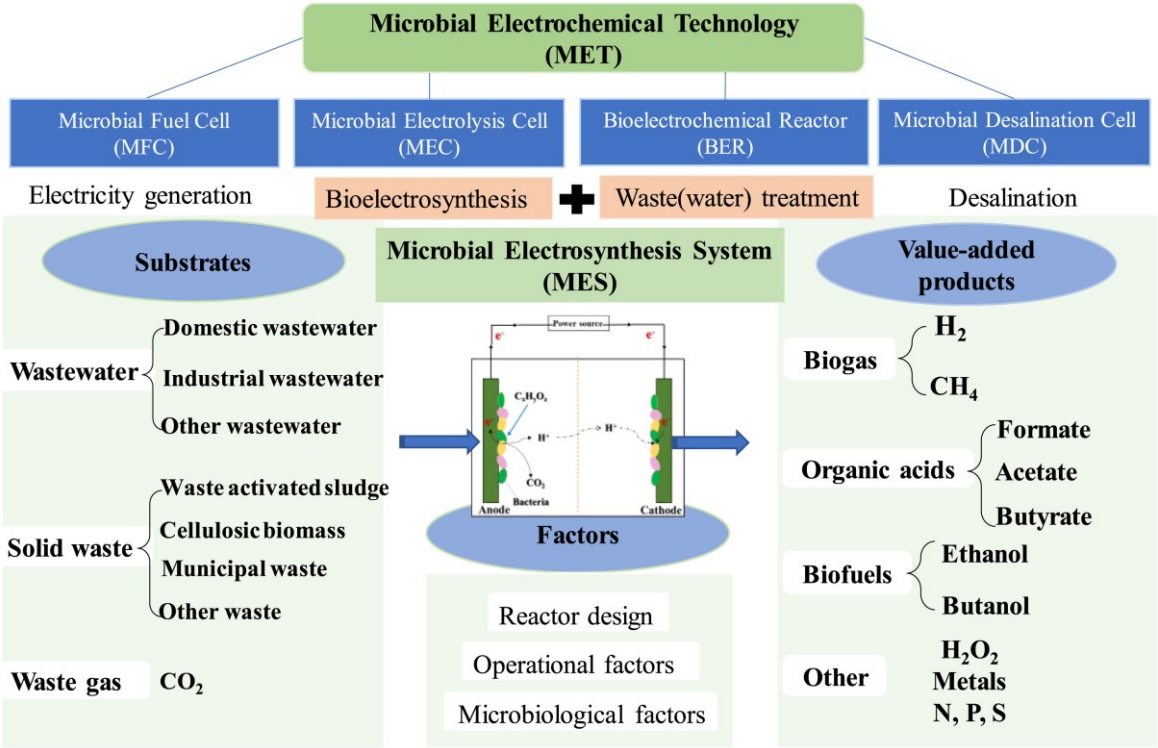

Figure 6. Overview of microbial electrochemical technology and its use for bioelectrosynthesis of value-added products. Taken with permission from Kong et al. (2020).

A wide variety of products can be synthetized, including hydrogen, methane, volatile fatty acids, alcohols, hydrogen peroxide and metals.
The utilization of CO_2 as a renewable carbon feedstock for chemicals production not only takes advantage of a low cost and abundant carbon resource, but also actively mitigates carbon emissions (Jiang et al., 2019). That is why interest for direct biocathodic CO_2 electro conversion has increased in the latest years in comparison to other liquid and solid waste substrates. A schematic of the process is shown in Figure 7.

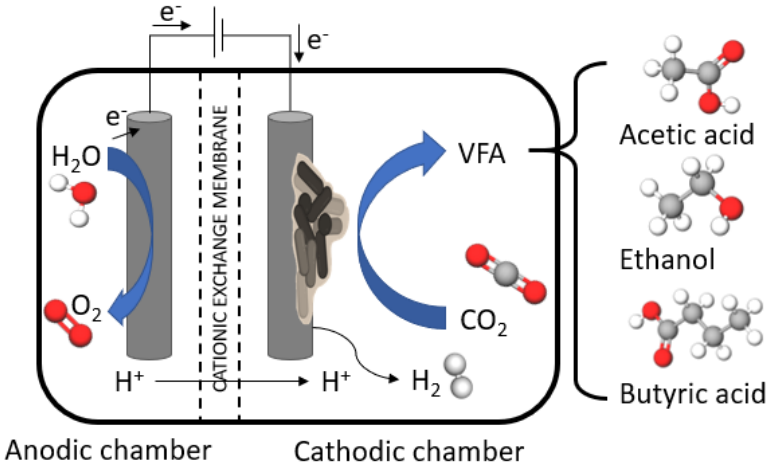

Figure 7. Schematic overview of chemical production in a typical MES for CO_2 bioelectrorecycling.

Methane is the intend product of power-to-gas (P2G) process for renewable electricity conversion and storage, and methanogens can readily use H_2, formate or acetate to produce CH_4. In MESs, synthesis of methane is no longer a side-effect as in MFCs and MECs, but the actual target of the process (Cheng et al., 2009; Villano et al., 2010).
Methane can be produced following two pathways: direct production at the cathode (Eq. 12) or indirect production mediated by H_2 production (Eq 10 and 13):

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (E^{cat} = -0.244\,V\ vs\ SHE) \qquad (12)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (E^{cat} = 0.170\,V\ vs\ SHE) \qquad (13)$$

Electromethanogenesis reactors have shown long term stability and have been already operated at pilot scale, but the production rates are still lower than traditional anaerobic digestion. However, integration of MES in anaerobic digestion (AD) has shown to improve sludge hydrolysis and acidification, increase the conversion of volatile fatty acids (VFAs) by microbial electrolysis, their removal and subsequent methane production, opening the way for a new combined technology for simultaneous energy recovery and wastewater treatment (Kong et al., 2020).

Short chain organic acids (or VFAs) and relative alcohols are other major products of CO_2 reduction in MESs. Among all, acetic acid is the most abundant compound produced in the carboxylate platform (Agler et al., 2011; May et al., 2016). The bioelectrosynthesis of acetate by CO_2 reduction using the cathode as electron donor was demonstrated by Nevin

et al. (2011), at a set cathode potential of -0.4 V. Acetic acid is central in the production of chemicals beyond CH_4 and H_2. Acetic acid production has been observed in reactors containing pure or mixed cultures, and it can be theoretically produced at a potential of -0.28 V vs SHE. However, a much lower potential may be required (-0.6 to -1.1 V vs SHE) due to potential losses associated with reactor design and electrolytes (Batlle-Vilanova et al., 2017; Mohanakrishna et al., 2016; Nevin et al., 2011).
The reaction for acetic acid production starting from CO_2 (or bicarbonate) is the following:

$$2CO_2 + 8H^+ + 8e^- \rightarrow CH_3\text{COOH} + 2H_2O \quad (E^{cat} = -0.28\,V\ vs\ SHE) \qquad (14)$$

Butyric acid can be synthesized both from chain elongation reaction from acetate and ethanol and bioelectrochemically through the Wood-Ljungdahl pathway coupled with acetyl-CoA reduction (Agler et al., 2011). Biolectrochemically produced butyric acid can be formed from acetic acid, protons and electrons, or alternatively with acetate and hydrogen at a cathode potential applied of -0.8 V vs SHE (Ganigué et al., 2015).

$$4CO_2 + 20H^+ + 20e^- \rightarrow CH_3CH_2CH_2\text{COOH} + 6H_2O$$
$$(E^{cat} = -0.8\,V\ vs\ SHE) \qquad (15)$$

Another key product from CO_2 bioelectroconversion is ethanol, and its availability is very important in the chain elongation process. Ethanol has been obtained by the reduction of acetate using methyl viologen as mediator and an electrode as electron donor by Steinbusch et al. (2010) at a cathode potential of -0.55 V vs SHE: the production of ethanol was obtained only in presence of the mediator, so the source of electrons was not clear. However, by controlling the operational conditions (pH, hydrogen partial pressure pH2, carbon content), Blasco-Gómez et al. (2019) found out the conditions to stimulate the Wood-Ljungdahl + Acetyl-CoA metabolic pathway for ethanol production, switching the metabolic route from acetogenesis to solventogenesis.

$$2CH_3\text{COOH} + 4H^+ + 4e^- \rightarrow CH_3CH_2\text{OH} + H_2O \qquad (16)$$

Various other organic compounds have been produced in MESs from CO_2 reduction, including formic acid (Nevin et al., 2011), propionic acid (Modestra and Mohan, 2017), butyric acid (Bajracharya et al., 2016; Ganigué et al., 2015), isopropanol (Arends et al., 2017), isobutanol, isobutyric acid, hexanol and caproic acid (Vassilev et al., 2018).

Other value-added chemicals have been reported being produced by MES: hydrogen peroxide (Rozendal et al., 2009; Zhang et al., 2015), methanol (Sharma et al., 2013), and polyhydroxyalkanoates (PHA) (Srikanth et al., 2012). However, one of the main drawbacks of the technology is the difficulty of achieving selective production at high production rates and titres (Prévoteau et al., 2020).

Chain elongation has been considered as strategy to convert short chain fatty acids (such as acetate and butyrate) into medium chain carboxylates (caproate C6 and n-caprylate C8) in the presence of organic electron donors (Raes et al., 2017). Fermentation and electrofermentation following MES are the most commonly applied processes to achieve an effective chain elongation.

1.2.5. METs as environmental strategy for renewable energy production and GHG containment

MFC technology has captured researchers' interest because of its dual role as both wastewater treatment and energy generation device, with low impact in terms of GHG emissions. However, technical viability for real-world operational dimensions is still under discussion. MFCs have been built lab-to-pilot scale, and transition to industrial scale is challenging (Ge and He, 2016). First, an efficient industrial design for MFCs is challenging: larger volume units have shown low power densities, so the solution proposed is to use a stack of MFCs to avoid big volume/electrode surface ratios (Ieropoulos et al., 2010; Zhuang et al., 2012). For example, large-scale reactors can include modular MFCs reaching up to 1000 L, achieving up to 90% COD removal and power density up to 60 W m^{-3} when operated on municipal wastewater (Liang et al., 2018). It must be considered that organic removal rates granted from conventional treatment technologies should also be guaranteed by MFCs, and not all experiences collected so far could grant a sufficient effluent quality, even at lab-scale (Gude, 2016). Last, but not least, power densities are still quite low to grant a positive net energy balance, especially when considering costs connected to pumping and mixing, and maintenance costs; but part of this factor may be overcome with scaling-up and cost-effective design (Tommasi and Lombardelli, 2017). Most of the material components can be expensive, even at prototype level, so the major issue that researchers have to face is to identify alternatives that would perform equally well and for prolonged periods but, most of all, be inexpensive and widely available (Gajda et al., 2018). Future real-scale applications for MFC reactors can be applied to *in-situ* treatment, to degrade pollutants and generate electricity simultaneously, potentially decreasing cost, energy consumption and treatment cycle (Walter et al., 2018).

On the other hand, MES platform may have a significant role in CO_2 emissions mitigation. As previously stated, CO_2 accounts for 76% of total GHG emissions, and it is mainly produced from fossil fuels and industrial facilities. MES can contribute to CO_2 capture of exhausted industrial gases and their subsequent conversion into valuable carbon-based compounds and may contribute to achieve the ambitious target of a climate neutral economy. MES rely on cheap and self-regenerating biocatalysts that can achieve over 80% electricity-to-product conversion at mesophilic conditions (Bajracharya et al., 2017). MES platform could be potentially applied for bioelectrochemical decarbonization of spent off-gases from the energy and carbon-intensive industry sectors such as steel mills (20-30%), ceramic (15-20%), glass (10-15%), refineries (10-20%), cement industries (25-30%), and power plants (10-15%), or anaerobic digestion process (Dessì et al., 2021). Industrial application of MES requires CO_2 capture and transportation to the treatment facility with an estimated cost of up to 180 €/ton CO_2 (Leeson et al., 2017). Hence, integrated on site plants seem to be a more viable solution. Gas cooling up to 30°C (using heat exchangers to recover thermal energy to be reused), and industrial effluent pre-treatment may be necessary to remove detrimental compounds for the microbial community (e.g., NO_X, SO_X, and O_2): on-line monitoring of the flue gas is thus required to prevent system breakdown (Dessì et al., 2021).

Electric energy is necessary for non-spontaneous (bio)electrochemical processes, and if MES were powered with fossil fuel-based energy sources, they could contribute to carbon emissions instead of their mitigation (Christodoulou et al., 2017). The use of renewable and low-cost energy is a key factor to make MES biorefineries both economically and

environmentally sustainable. Renewable energy sources can be integrated into MES either indirectly, by using renewable electric energy sources, or directly, by solar-to-product conversion using photoactive electrodes (photo-MES) producing the required power density (Dessì et al., 2021).

Amongst carbon-based products in the carboxylate platform, ethanol production has the most positive effect on GHG emissions, with a negative global warming potential of –753 tons CO_{2eq}, while acetic acid production requires more energy with subsequent superior carbon emissions generation if a non-neutral carbon source is used for its production (154,747 GJ, generating 6164 tons CO_{2eq}), with rectification energy cost as most impacting (~92% of the total energy spent in the process). Comparing MES reactors instead, ethanol (16,707 GJ) and methanol (9310 GJ) bioelectrosynthesis used the most energy (Christodoulou et al., 2017). In terms of global warming potential, according to Christodoulou and co-workers MES-synthetized methanol (-2.2), ethanol (-1.8) and formic acid (-1.1) production presented a negative carbon footprint, while, conventional methods for their production yielded positive carbon emissions. Propionic (0.7) and acetic (0.04) production instead yielded a positive global warming potential, as a consequence of the energy required for their purification. This leads to two different strategies to develop cost-effective MES reactors for CO_2 recycling: (i) maximize production rate of low-value products with high market request (i.e., acetic acid or methane gas), possibly with direct reuse to avoid rectification costs or (ii) steer production towards more valuable products (i.e., ethanol or medium chain fatty acids), though at lower production rates.

H_2 electrolyzers relying on water splitting or photoelectrochemical water splitting process have been developed and are already consolidated at a commercial scale (producing up to 4% of overall hydrogen produced worldwide; IRENA, 2018); electrochemical carbon dioxide reduction process has improved in recent years. Production of simpler C_1 products (i.e., CO and formic acid) has become possible by using simple catalysts and electrodes materials, while more expensive catalyst, electrolytes, and cell engineering are necessary to grant selectivity for C_{2+} products because of the difficulty of C-C coupling, but encouraging values in terms of current density (100 mA m^{-2}) and faradaic efficiency (up to 60%) have already been reported. For example, a conversion efficiency of 0.95 kg CO_2 per kg ethanol, and a global warming potential (connected mainly to energy costs) of 1.79 kgCO_2/kg product were assessed (De Luna et al., 2019).

To make MES competitive with other CO_2 conversion technologies, many factors must be considered. According to Prévoteau et al., researchers must pay attention to three different key points: i) high internal resistances associated with bioelectrochemical systems as a major drawback for MES, in particular associated with distance of electrodes, unbalance of electrolytes between adjacent compartments, too low electric conductivity; ii) cathode material should be biocompatible and highly conductive (avoiding the use of expensive metal-based catalysts), stable on the long-term, a good compromise among specific surface availability for biomass adhesion and mass (electron/CO_2) transfer efficiency; iii) careful selection of the microbial community to be used as biocatalyst, and operational conditions control. On this note, genetic engineering of microorganisms may rely on increased production performance or variety of products, increased tolerance to toxicity or stress factors (Mukhopadhyay, 2015), increase metabolic rates or electrons uptake (Jensen et al., 2010). A combination of electrochemical/bioreactors should also be considered to enhance chain elongation and target chemicals production.

1.3. Microalgae: a potential resource for biofuels production and resource recovery

Biofuels industry can be broadly divided into four different generations, according to feedstock used for their production: first generation biofuels, produced from food and feed crops; second generation biofuels, which used starch-based residues from agro-food industry as feedstock; third generation biofuels refer to algae-derived fuels; fourth generation biofuels rely on genetic modification/metabolic engineering of bacteria and microalgae to enhance their properties and composition to better fit the target biofuel qualities (Abdullah et al., 2019; Dutta et al., 2014). Biorefineries of first and second generation were competitive against land use for primary human needs and uses, while microalgae can achieve extensive cultivation, as an interesting alternative to land-based plants and biomasses. In recent years, microalgae have been studied for biofuels and bioenergy production, due to various characteristics such as faster growth rates, simplicity of growth operation and management, higher biomass yield with CO_2 capture abilities and their possible integration in high-value product chains (Sevda et al., 2019). Algae and microalgae, similarly to land crops, can produce energy directly by extraction of lipid, or indirectly as feedstock for fermentation processes (Dragone et al., 2010; Maity et al., 2014). Microalgae are (mainly) autotrophic organisms, growing by photosynthesis, with strong mitigation potential against CO_2 emissions, and providing valuable substrate for producing diverse fuels, due to two specific characteristics. First, microalgae contain oil that can be easily refined into diesel or even gasoline components; second, special fermentative bacteria can degrade the biomass for biobutanol production and, more importantly, can be genetically manipulated to directly produce a number of "biological" fuels, from biodiesel, to biobutanol, biomethane, bioethanol, vegetable oil, and even jet fuel (Lakaniemi et al., 2013; Milledge and Heaven, 2014).

Algae can use a wide array of carbon sources: algae-growing facilities could, for example, be tied to carbon-emitting point sources (power plants, industries, etc.) to convert gaseous emissions into fuel directly, without CO_2 release into the atmosphere (this would be insufflated in the cultivation basins); alternatively, to wastewater treatment facilities, where they would take up dissolved nitrogen and phosphorus, either way reducing total emissions. Heterotrophic (dark) cultivation with synthetic substrate (acetate or glucose) and oxygen or wastewater as a carbon source was also assessed (Heredia-Arroyo et al., 2010; Leu and Boussiba, 2014).

Photosynthesis is a complex metabolic process, composed by light and dark reactions as summarized in Figure 8.

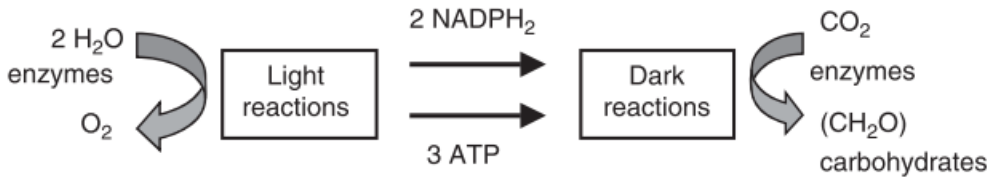

Figure 8. Major products of the light and dark reactions of photosynthesis. The process of oxygenic photosynthesis is divided into two stages, the so-called light reactions and dark reactions. The light reactions include light absorption, transfer of excitons and electron and proton translocation resulting in the production of $NADPH_2$, ATP and O_2. The other phase, the dark reactions, which occur in the stroma, represent the reduction of carbon dioxide and the synthesis of carbohydrates using the $NADPH_2$ and ATP produced in the light reactions (Richmond, 2004).

The light reaction is called photophosphorylation and can be expressed as:

$$2\,NADP + 3\,H_2O + 2\,\mathrm{ADP} + 2\,P_i \xrightarrow{\substack{light\ energy \\ chlorophyll}} 2\,NADPH_2 + 3\,\mathrm{ATP} + O_2 \qquad (17)$$

The fixation of carbon dioxide happens in the dark reaction using the $NADPH_2$ and ATP produced in the light reaction of photosynthesis. The reaction can be expressed as:

$$CO_2 + 4H^+ + 4e^- \xrightarrow{\substack{enzymes \\ 2\,NADPH_2, 3\,ATP}} carbohydrates + H_2O \qquad (18)$$

Carbohydrates and water are finally formed from the dark reaction.

In case of heterotrophic/mixotrophic microalgae, synthesis reactions for heterotrophically cultivated *Chlorella sorokiniana* (Baroukh et al., 2017) using acetate or butyrate as carbon source are the following:

$$3.5\,H^+ + 2\,C_2H_3O_2^- + 2\,O_2 \rightarrow C_4H_6O_4 + 1.5\,H_2O \qquad (19)$$

$$7\,H^+ + 1\,C_4H_7O_2^- + 1.5\,O_2 \rightarrow C_4H_6O_4 + 5\,H_2O \qquad (20)$$

Fatty acids (including acetate and butyrate) are degraded to Acetyl-CoA, which is then transformed to succinate (SUC, $C_4H_6O_4$) thanks to the glyoxylate cycle. SUC is primary precursor to produce metabolites and energy via the tricarboxylic acid (TCA) cycle for protein, DNA, RNA, carbohydrate and lipid synthesis. Heterotrophic biomass synthesis reaction is expressed as follows:

$$7.30\,H^+ + 4.61\,O_2 + 4.15\,C_4H_6O_4 + 0.98\,NH_4 + 0.12\,P_i + 0.02\,SO_4 + 0.01\,Mg_2 \rightarrow 1\,Biomass + 7.04\,H_2O + 8.06\,CO_2 \qquad (21)$$

General photosynthesis reaction for *Chlorella sorokiniana* can be summarized as follows:

$$24\,light + 3\,CO_2 + 2H_2O + 1\,P_i \rightarrow 1\,C_3H_7O_6P + 3\,O_2 \qquad (22)$$

During photosynthesis, inorganic carbon (CO_2) is assimilated using light energy to produce a 3-carbon sugar glyceraldehyde 3-phosphate (GAP, $C_3H_7O_6P$), where GAP is used as

primary precursors to produce precursor metabolites and energy via the TCA cycle for protein, DNA, RNA, carbohydrate and lipid synthesis. Photosynthetic biomass synthesis reaction is expressed as follows:

$$4.15\ C_3H_7O_6\mathrm{P} + 2.53\ O_2 + 0.98\ NH_4 + 0.02\ SO_4 + 0.01\ Mg_2 \rightarrow 0.99\ H^+ + 1\ Biomass + 2.89\ H_2O + 3.91\ CO_2 + 4.02\ P_i \quad (23)$$

Microalgae can be cultivated anywhere if temperatures are sufficiently warm and present a variety of cultivation possibilities: open ponds, in which algae is grown within a pond in the open air, simple and low-cost, but less efficient than other and more at risk of external contamination (Borowitzka and Moheimani, 2013); closed-loop systems, similar to open ponds, but not exposed to the atmosphere and tapping a CO_2 source before it is even released in the atmosphere (Klemenčič and Griessler-Bulc, 2010); or photobioreactors, the most advanced and most complex systems to implement, with high capital costs but with maximum advantages in terms of process yield and control (Ugwu et al., 2008).

Algae have only a major downside, in that they require large amounts of water and nutrients (N & P) to grow (Yang et al., 2011). Yang ad co-workers (2011) calculated that to generate 1 kg of biodiesel from microalgae, 0.33 kg N, 0.71 kg P and 3726 kg of water are necessary: the use of wastewater as growth environment would reduce by 90% the need for water and completely fulfill the nutrient demand of microalgae.

Microalgae, compared to other first and second generation feedstocks, were reported to have the potential to reduce biodiesel production costs by 60-90% (Kiss, 2010). Third generation biodiesel from high oil-content microalgae may grant sustainability for production of this type of fuel (Dragone et al., 2010). The main advantage of algal biomass is its high photosynthetic efficiency and productivity; but unfortunately the main difficulty still lies in the extraction of oil, corresponding to the main limiting factor in large scale biodiesel production (Scott et al., 2010; Vasudevan and Fu, 2010). The extracted oil is then subjected to a transesterification process, further increasing biodiesel production cost (Singh et al., 2013). Some microalgae species, among all *Auxenochlorella protothecoides*, appear to be particularly adapt to produce biodiesel compatible microalgal oil, since their cells are capable to accumulate large amounts of lipids (up to 50% of their total weight, when cultivated heterotrophically). Heterotrophic and mixotrophic cultivation conditions enhance lipid accumulation, since medium-long term dark metabolism stresses algae, increasing lipid "reserves" production (Espinosa-Gonzalez et al., 2014; Gao et al., 2014; Richmond, 2004).

Microalgae are generally characterized by a high content of carbohydrates (> 40%), that can be converted into simple sugars for fuel production, making them the ideal feedstock also for biobutanol production via fermentation (Ho et al., 2013). Harvested algal biomass is pretreated to release monosaccharides (microalgal sugars) and then fermented. Using microalgal feedstock for biobutanol production, *Clostridium acetobutylicum* can convert the residual solid matter in the liquor to butanol, and thus the butanol yield is higher when unfiltered hydrolysate is processed (containing 21.96 mg/g residues vs 10.03 mg/g of the filtered one) (Cheng et al., 2015). Notwithstanding these promising characteristics, the main obstacle for an efficient production of biobutanol from microalgae is the high capital and operating cost of their cultivation (400€/ton biomass) (Wang et al., 2017). Currently, only a few reports were published concerning biobutanol production from

wastewater-cultivation of microalgal feedstock (Ellis et al., 2012; Jernigan et al., 2013; Y. Wang et al., 2017). While growth of microalgal biomass from polluted water could lower overall process costs, the quality of monocultural species is more consistent and may yield higher carbohydrate content, making them better suited to industrial applications.

It has also to be taken into account that high-value co-products have been produced by extraction from algae to improve the overall economics of microalgae biorefineries: examples of these high-value products are pigments, proteins, lipids, carbohydrates, vitamins and anti-oxidants, with applications in cosmetics, nutritional and pharmaceuticals industries (Chew et al., 2017; Michalak and Chojnacka, 2015; Steinman et al., 2017; Wang et al., 2015). Microalgal biochar is also an interesting recovery material; biochar is a solid residue of pyrolysis, according to its properties can be used as a solid biofuel, or it can find recovery in different areas: as a fertilizer, with slow release of the nutrients contained in the char, or even as electrode, due to its high carbon content and conductive properties (Goglio et al., 2019; Yu et al., 2018, 2017).

To promote sustainability of such processes, innovative microalgae biorefinery schemes are being implemented. The feasibility of multi-product cogeneration has already led to more efficient production pathways and enhanced recovery of materials and energy (Chew et al., 2017). Significant improvement must however still be achieved on the economics of algal biofuel production to make them competitive in the market with fossil fuels. Some of the conversion processes still in use are complex and expensive, but they could turn commercially viable by optimization of all by-products proper exploitation (Adeniyi et al., 2018). In conclusion, biofuels from algae are technically feasible due to their high sustainability, and this puts them in the best position to potentially displace fuels obtained from crude oil. The future of algal biofuels is based on developing cost-effective approaches for the most operationally efficient technologies (Adeniyi et al., 2018).

1.4. Integrating microalgae in METs: A biorefinery approach

MFCs are a promising technology for wastewater treatment (see section 1.2), characterized by electrical energy recovery coupled with low GHG emissions and reduced sludge production (Capodaglio et al., 2013; Logan et al., 2006). In case of abiotic cathode reaction, metallic materials can catalyze the reduction of oxygen to form water. However, in such processes, the use of expensive catalysts such as platinum makes the process economically unfavorable, and development of biocathodes (with biomass directly catalyzing the ORR) appeared to be a possible solution to mitigate costs (Xia et al., 2013). Recently, the use of microalgae for co-treatment of wastewater was proposed, being an effective process for both resources recovery and CO_2 sequestration (Gabriel et al., 2018; Wang et al., 2010). Microalgae are well known as potential candidates as feedstock in biorefineries (third generation feedstocks) for biofuels and biomaterials production. Substantial research is being conducted to explore the potential of microalgae in different BES systems, especially in MFCs. Algae in MFCs may be used as efficient electron acceptors during the photosynthetic reactions at the cathodic end or as electron donors when applied at the anode, and their ability in removing organic substrates (Commault et al., 2017; Gude et al., 2013). In the cathode chamber and in the presence of sunlight, algae carry out photosynthesis and convert CO_2 to generate organic matter, oxygen and biomass; on the contrary, in the dark stage, they consume oxygen to produce energy by direct

oxidation of organic materials (Ndayisenga et al., 2018; Richmond, 2004; Saba et al., 2017a). Many factors can alter overall performance: light/dark cycles influence O_2 production, growth rate and algal stress of these processes; consequently, they may influence both bioelectricity production and possible recovery products from the effluent, affecting the global energy and economic balance of the system (León-Vaz et al., 2019). Several designs and setup have been studied so far in the context of MFCs and MDCs.

Several authors studied developing mechanisms in single chamber MFCs with bacterio-algal consortia. Yuan et al. (2011) demonstrated that blue-green algae could be effective feed for bioelectricity generation in a single-chamber tubular MFC, achieving a maximum power density of 114mW m^{-2} was yielded at a blue-green algae concentration of 1113 mgCOD L^{-1} with contextual good removal efficiencies of COD, total nitrogen and ammonium. Fu et al. (2010) operated an MFC containing *Spirulina platensis* under different conditions of light, spacing of the electrodes, pH, temperature and connection methods, achieving better performance in the dark configuration in terms of energy production.

To avoid problems linked to the production of oxygen, two chamber MFCs has been the most studied setup. Different microalgae strains such as *Chlorella vulgaris* (Khazraee Zamanpour et al., 2017; Wang et al., 2010; Zhang et al., 2011), mixed algal cultures (He et al., 2017; Jiang et al., 2013) and *Pseudokircheneriella subcapitata* (Xiao et al., 2012) have been operated in double chamber MFCs. Wang et al. (2010) operated an algae-based microbial carbon capture cell (MCC) to mitigate CO_2 emissions, reporting significant CO_2 conversion and good performance in terms of power production. Bazdar et al. (2018) investigated the effect of light intensities and illumination regimes on simultaneous production of bioelectricity, biomass and wastewater treatment in a who chamber photosynthetic microalgae microbial fuel cell (PMMFC), demonstrating that light dark regimes influence both MFCs performance and microalgae's lifetime. Some authors explored the possibility of enhancing nutrients removal by using algal biocathodes (Nguyen and Min, 2020).

Besides MFC, other studies have investigated the use of algae in microbial desalination cells (MDCs). Saba et al. (2017b) with *Nannochloropsis salina* as catholyte and Zamanpour et al. (2017) with *Chlorella vulgaris* demonstrated good performances and excellent salinity removal when using a microalgal biocathode MDCs. However, researchers' interest did not go too far from MFC setup when considering microalgae, even though integrated microalgae METs is still an engaging topic among researchers (Cui et al., 2014; Do et al., 2018; Gouveia et al., 2014; Sevda et al., 2019). Despite most studies focused on bioelectricity production and nutrients removal, among the advantages of integrating microalgae there is the possibility to recover the algal effluent and convert it into algal biorefinery valuable products. This thesis aims at contributing to the already present literature on the topic, attempting to integrate microalgae in different METs. Other than more conventional microalgae integration in MFCs, their integration with microbial electrosynthesis is also explored, by exploiting the dark metabolism of heterotrophic microalgae species such as *A. protothecoides* to take advantage of the short chain VFAs produced as carbon source. Microalgae grown on biocathode effluent then can be further processed to recover, for example, biodiesel or other biofuels, products form thermal conversion (biochar) and other valuable chemical commodities (proteins, pigments).

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2. Objectives and structure of the thesis

Based on the concepts introduced in Chapter 1, it is possible to determine the objectives and the structure of this Ph.D. thesis. The main objective is to study Microbial Electrochemical Technologies applications through the design, construction, and operation of different METs, to produce electrical energy and biofuels. Microalgae integration in METs led to consider other possible materials recovery options from waste streams through microalgae biorefinery. Secondary objectives are the optimization of the examined processes to maximize resource recovery while minimizing the external energy input to the systems.

Two specific MET technologies (MFC and MES) were studied and operated to evaluate different strategies to store energy from a liquid or gaseous waste stream. Experimental studies conducted during this Ph.D. thesis are reported starting from Chapter 3. In Chapter 3, two MFCs were built and operated with a mixture of an ideal substrate, dairy wastewater, and landfill leachate. Energy losses and performance were evaluated while increasing the percentage of landfill leachate in the mixture. Other strategies to minimize energy-related costs in MFCs were evaluated in Chapter 4, where a microalgal biocathode was integrated in the same MFC design. In this case, a synthetic substrate (acetate) was operated to better assess the response of the system to the conditions tested. Microalgae provided the TEA, i.e., oxygen; algal stress was then evaluated under different light/dark and aeration conditions, and correlated to electrical production. Chapter 5 marks the change of focus by switching the main topic of study to a different technology, Microbial Electrosynthesis (MES). Building on the knowledge and expertise on microalgae previously acquired, and exploring possible additional outcomes of microalgal biorefinery, Chapter 5 is an attempt to (unconventionally) integrate microalgae in METs by microalgae integration in microbial electrosynthesis. Chapter 6 investigated novel strategies for direct integration of microalgae in BESs. Finally, Chapter 7 discussed possible materials and energy recovery options from microalgae and sewage sludge mixture for biochar production.
Hereafter, a short summary of subsequent chapters is presented.

Chapter 3: Bioelectrochemical treatment of municipal solid waste landfill mature leachate and dairy wastewater as co-substrates.
In this chapter a strategy for the treatment of mature landfill leachate in MFCs is discussed. Mature leachate is a difficult substrate, characterized by low biodegradability. Leachate was combined with dairy wastewater, a highly biodegradable substrate to enhance treatment by MFCs. Start-up and operation of two dual-chamber MFCs fed with an influent solution at increasing percentage of landfill leachate in the mixture is described. The effect of operational conditions, reactor architecture and materials on their performances are analyzed and discussed.

Chapter 3 has been published as: Bolognesi, S., Cecconet, D., Callegari, A., Capodaglio, A.G., 2020. Bioelectrochemical treatment of municipal solid waste landfill mature leachate and dairy wastewater as co-substrates. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-10167-7

Chapter 4: Combined microalgal photobioreactor/microbial fuel cell system: performance analysis under different process conditions.

Chapter 4 investigates the performance of a combination of microbial fuel cell (MFC) technology with microalgal-based process in MFC-PBR (photobioreactor) and its possible advantages in terms of energy and material recovery. Microalgae has potential to reduce GHG emissions from wastewater treatment facilities by capturing the CO_2 produced at the anode, and converting it into oxygen. Two MFC-PBR system configurations were tested and compared to a control MFC, under different light/dark ratios, air/CO_2 provision methods, and using both synthetic and agro-industrial wastewater as anolytes. The system equipped with microalgal biocathode proved to be equally capable to efficiently treat real wastewater when compared to the control experiment, varying results according to applied experimental conditions.

Chapter 4 has been published as: Bolognesi, S., Cecconet, D., Callegari, A., Capodaglio, A.G., 2021. Combined microalgal photobioreactor/microbial fuel cell system: performance analysis under different process conditions. Environ. Res. 192, 110263. https://doi.org/10.1016/j.envres.2020.110263

Chapter 5: Power-to-algae: carbon dioxide conversion using electricity as feed for microalgal biorefinery.

In Chapter 5 an experimental work involving microbial electrosynthesis as a novel integrated technology is introduced. Bioelectrochemical conversion of CO_2 into acetic acid, ethanol and butyric acid is already a well-established process, but further chain elongation is hard to reach in single-step MES processes. Thus, new strategies have to be explored to increase variety and economic value of bioelectrochemically synthetized commodities.

Two BES H-type reactors were built and operated in batch mode at a cathodic potential of -0.8 V vs SHE for 120 days. As a secondary treatment, the effluent from the biocathode was transferred in a batch reactor containing heterotrophic microalgae *Auxenochlorella protothecoides* to evaluate oil production yield, producing a biodiesel compatible bio-oil, and possible recovery options for the solid fraction.

Results described in Chapter 5 are currently being elaborated into a manuscript that will be submitted for publication at a later date.

Bolognesi, S., Bañeras, L., Perona-Vico, E., Capodaglio, A. G., Balaguer, M. D., Puig. S., 2021. Power-to-algae: carbon dioxide conversion using electricity as feed for microalgal biorefinery.

Chapter 6: Power-to-algae: a prototype three-chamber reactor for efficient bioelectroCO_2conversion.

In this chapter a novel three chamber setup for microbial electrosynthesis is described, which was built and operated for three months. The limited extension of the system's

experimentation was a forced, unfortunate consequence of the Covid-19 pandemic-related generalized shutdown. The additional middle chamber in the system was designed to perform direct conversion of biocathodic produced acetate. Two BES replicates were built and operated in batch mode, but the system was designed to be easily converted into continuous mode operation.

Chapter 7: Biochar production from sewage sludge and microalgae mixtures: properties, sustainability and possible role in circular economy.
In this chapter, a possible option for microalgae biomass conversion is explored. Cultivated microalgae *Chlorella*, sewage sludge, and a mixture of sewage sludge and microalgae from a phytoremediation process were characterized by thermogravimetric analysis and then pyrolyzed through slow pyrolysis process, in order to maximize biochar production. The solid residue was then characterized and difference between the samples' composition and characteristics were highlighted. Possible opportunities for reuse of the material recovered are analyzed in the latter part of the chapter.
Chapter 7 has been published as: Bolognesi, S., Bernardi, G., Callegari, A., Dondi D., Capodaglio, A.G., 2019. Biochar production from sewage sludge and microalgae mixtures: properties, sustainability and possible role in circular economy. Biomass Conv. Bioref. https://doi.org/10.1007/s13399-019-00572-5.

Chapter 8: Conclusions.
This last chapter sums up results obtained in the present thesis, also giving an insight on future development of the research topics presented.

3. Bioelectrochemical treatment of landfill leachate and dairy wastewater as co-substrates

This chapter addresses experiments with MFC bioelectrochemical treatment of liquid wastes. Despite the recent reduction of municipal solid waste (MSW) landfill disposal due to recent European legislation, landfill leachate disposal remains a significant problem, and will be for many years in the future, since its production may persist for years after a site's closure. Among process technologies proposed for its treatment, Microbial Fuel Cells (MFCs) can be effective, achieving both contaminant removal and simultaneous energy recovery. Start-up and operation of two dual-chamber MFCs fed with a mature landfill leachate is reported in this study. The influent solution (a mix of dairy wastewater and landfill leachate) was fed to the anodic chambers of the units under different conditions, at increasing leachate concentration. The maximum COD removal efficiency achieved was 84.9% at low leachate/dairy mix (5% leachate), and 66.3% with 7.6% coulombic efficiency (CE) at a leachate/dairy ratio of 20%. Operational issues, the effects of cells' architecture and materials operated on their performance are analyzed and discussed. The Chapter contains material published in: *Bolognesi, S., Cecconet, D., Callegari, A., Capodaglio, A.G., 2020. Bioelectrochemical treatment of municipal solid waste landfill mature leachate and dairy wastewater as co-substrates. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-10167-7.*

3.1. Introduction

Municipal solid waste (MSW) disposal is a problem with no easy or unique solution. In 2015, 242.3 Mt of MSW were produced in the European Union, 62 Mt of which discarded in landfills. Italy, in this context, produced about 29.5 Mt MSW in 2015, of which 7.8 landfilled (ISPRA 2017). Despite the gradual reduction, or outright ban, of MSW landfill disposal due to recent European legislation (EU, 2018a; EU, 2018b), leachate generated from decomposition of MSW in landfills is still a significant problem nowadays, and will be for many years in the future, since its production may persist for years after a site's closure. The risk of groundwater pollution by leachate spills from damaged landfill containment is significant, and specific monitoring is normally required in these situations due to the possible spread of harmful pollutants (Capodaglio et al., 2016a).

Leachate characteristics are quite variable, affected by landfill construction and age, local meteorology, waste type and composition; it is normally high in COD and ammonia content (Kulikowska and Klimiuk, 2008; Youcai, 2018). Typically, its BOD/COD ratio decreases from around 0.7 to 0.04 with landfill ageing (Sonawane et al. 2017). Leachate contains organics constituents that may be degraded by bacteria already within the landfill, but it also contains ammonia at high concentrations (Kjeldsen et al. 2002), toxic metals and

other refractory organic and inorganic compounds that may accumulate, inducing microbial toxicity and microbial inhibition (Renou et al. 2008).

Leachate is typically hauled to off-site treatment facilities, where it may interfere with biological processes due to toxic metals content, high ammonia concentration or the presence of other xenobiotic pollutants (PAHs, organic halogens, PCBs) that may be refractory, inhibitory, or otherwise affect such processes (Callegari and Capodaglio, 2017). Leachate may also present unbalanced C/N ratio content (especially in leachate from end of operation or closed landfills), making them poorly biodegradable, and affect other processes due to its characteristics, e.g., reduce ultraviolet disinfection effectiveness by quenching UV light. All these factors may represent a major ordeal for many conventional facilities, often requiring special pre-treatment onsite, or at destination. A specific pre-treatment for leachate could be designed to be performed directly onsite, or even full treatment for subsequent discharge to a municipal sewer or a water body; however, it may often be not cost-effective. The most common processes for leachate treatment are biological (aerobic or anaerobic) and/or physicochemical, according to characteristics and differential removal capacities of the pollutants contained, and advanced technologies may also be appropriate (Wiszniowski et al., 2006). These include treatment or pre-treatment by chemical oxidation (Kim and Hu, 2009), adsorption (Foo and Hameed, 2009), ammonia removal by biodegradation (Capodaglio et al., 2016b) or stripping (Cheung et al., 1997), evaporation, filtration and reverse osmosis (Di Palma et al., 2002), sonication (Nazinudheen et al., 2018) and others, depending on leachate characteristics, and discharge or site-specific constraints.

Microbial Fuel Cells (MFCs) have been pointed out as a promising bioelectrochemical technology for various types of liquid waste streams, including domestic (Logan et al. 2006, Ahn and Logan, 2010) and industrial wastewaters (Molognoni et al., 2018). They were also indicated as appropriate for landfill leachate treatment (Puig et al., 2011). MFCs have been used to treat easily biodegradable industrial wastewater (Callegari et al., 2018) and difficult-to-treat substrates (Abbasi et al., 2016; Shrikant et al., 2016) in laboratory scale. In the latter case, like in any other biologically-mediated processes, biomass acclimation to the specific pollutants is a key element for success (Capodaglio et al., 2010).

Landfill leachate as a substrate for MFCs has been investigated under different circumstances (Hu et al., 2017; Huang et al., 2018; Li and Chen, 2018; Zhang et al., 2015a; Zhang et al., 2015b) either alone or in combination with other processes (Mahmoud et al., 2014; Velasquez-Larios et al., 2014).

The addition of a co-substrate is a common strategy in wastewater treatment to treat biologically substrates normally not suitable for biological treatment and increase overall process efficiency (Luo et al. 2009). Simultaneous treatment of landfill leachate and wastewater with MFCs was already explored in literature. Hernandez-Flores et al. (2017) reported the combined treatment of leachate and municipal wastewater by adding 30, 50 and 70% of highly biodegradable leachate in the mixture. In this case, the presence of an increased biodegradable organic matter (leachate) enhanced electricity production.

However, only few studies dealt with leachates characterized by a low biodegradability so far. In this study, mature leachate from a closed landfill, together with agro-industrial (dairy) wastewater as a co-substrate, was fed to a dual-chamber MFC at increasing rates to evaluate system performance and overcome limitations connected to the use of poorly biodegradable leachate as a substrate for bioenergy production.

3.2. Materials and methods

3.2.1. System setup and operation

Two dual-chamber MFCs, consisting of an anodic and a cathodic chamber on the opposite sides of a methacrylate rectangular frame, separated by a cationic exchange membrane (CEM, CMI-7000, Membranes International Inc., USA), were operated and closely monitored during the study. The two identical cells (from now on, indicated as MFC1 and MFC2) differed only for the chosen electrode material. MFC1 was built with graphite-coated stainless steel (GCSS) mesh (a single 200x200 mm sheet) electrodes in both chambers, while MFC2 anodic and cathodic chambers were filled with granular graphite (model 00514, diameter 1.5–5 mm, EnViro-Cell, Germany) serving as electrode material. The final free volume of each chamber was 800 mL (net anodic chamber, NAC, and net cathodic chamber, NCC) in MFC1 and 450 mL (NAC and NCC) in MFC2, respectively. In order to allow external circuit connection, graphite rod electrodes (250 × 4 mm) were inserted in both chambers. A 33 Ω resistance was connected to MFC's external circuit: this value was determined to be as close as possible to the static internal resistance of the MFCs. An Ag/AgCl reference electrode was placed in the anodic chamber (+197 mV vs SHE, Xi'an Yima Opto-Electrical Technology Co., China). Oxygen was the TEA, provided directly into the cathodic chambers by a porous diffuser connected to a fish tank air pump. The scheme of the experimental system is shown in Figure 9.

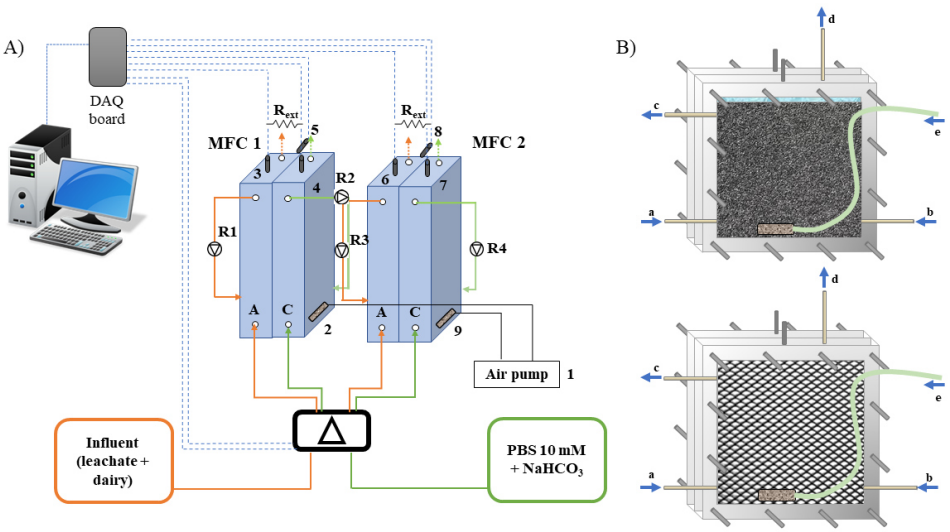

Figure 9. A) Hydraulic and electrical connections. Continuous lines: hydraulic connections (anode orange lines, cathode green lines), R1, R2, R3, R4 recirculation pumps; dashed lines: electrical connections to DAQ board; dotted lines (green and orange): effluents discharge. Legend: (1) air pump; (2, 9) porous diffuser; (3, 6) anode electrode; (4, 7) cathode electrode; (5, 8) reference electrode. B) Cathode chamber setup: MFC2 filled with granular graphite, MFC1 with stainless steel graphite coated mesh. a) inlet; b) recirculation inlet; c) recirculation outlet; d) outlet; e) air inlet.

Influent dosage and recirculation were performed using computer-controlled peristaltic pumps (BT100N, Baoding Shenzhen Precision Pump Co., China). Close-circuit recirculation (100 L d^{-1}) was operated continuously to accomplish well-mixed conditions within anodic chambers, influent flow rate was set at 1 L d^{-1} in step-feeding mode (20 minutes each hour). The two MFC were inoculated with a mixture of activated sludge and effluent of a parent MFC treating only dairy wastewater (DW).

A mixture of DW and screened leachate from a nearby landfill was fed to the anodic chambers during the study. Landfill leachate (LL) and DW characteristics are reported in Table 1, the former were constant throughout the study (resulting from a single sample collection), while DW quality varied slightly during the study due to the different process cycles operated at the cheese factory. Fresh DW was collected once per week due to its quickly biodegradable characteristics. Both landfill leachate and dairy wastewater were stored at 4°C after collection and until use. Phosphate buffer solution (PBS, 10 mM, pH=7) was used as pH-control medium for the cathodic chamber, with the following composition: 507 mg L^{-1} NaH_2PO_4, 819 mg L^{-1} Na_2HPO_4, 1000 mg L^{-1} $NaHCO_3$, 130 mg L^{-1} KCl, 310 mg L^{-1} NH_4Cl (Xia et al. 2013).

Table 1: Main characteristics of leachate and dairy influent

Parameter	Units	Leachate	Dairy (range during study)
pH		8.28	5.5-8.9
Electric conductivity (20°C)	mS/cm	22.1	9-16
COD	mg/kg	2420	1150-2670
BOD_5	mg/kg	215	710-1230
NH_4^+	mg/kg	2595	8-23
$N\text{-}NO_2^-$	mg/kg	<1	2-9
$N\text{-}NO_3^-$	mg/kg	16.8	7-21
P_{tot}	mg/kg	158	30-84
Total suspended solids	mg/kg	41	42-170
Toxic metals	mg/kg	Traces	Not tested

3.2.2. Data collection and evaluation

Anodic potentials were monitored with Ag/AgCl reference electrode, and continuously acquired at 1-min intervals by an automated data acquisition system (NI USB-6008, National Instruments Italy) connected to a computer, and MFCs' voltages were simultaneously recorded. Power (P) and current (I) were determined from continuous voltage measurement (V) in the MFCs. Current (dI) and power (dP) densities were calculated dividing the respective value of I and P by the NAC volume of each cell. Anodic coulombic efficiency (CE) was computed using daily average data of flow-rate and current intensity.

Determination of effluent COD (one daily composite sample per MFC) and influent wastewater COD (one sample for every feed batch) was performed according to "Standard Methods" (APHA, 2017). Anodic Organic Loading Rate (OLR) was calculated as the daily organic matter concentration (in terms of COD) divided by the anode's hydraulic retention

time (HRT). Organic matter removal efficiency (ηCOD, percent) was determined as described in Molognoni et al. (2014). Conductivity and pH were measured at least once every 5 days for both anode and cathode influents and effluents (IntelliCAL™ probes + HQd™ Digital Meter, Hach Lange).

The normalized energy recovery (NER) of the MFCs, a parameter that expresses the amount of energy recovered per removed mass of COD (NER_S, kWh $kgCOD_{removed}^{-1}$) and per volume of treated wastewater (NER_V, kWh $m^{-3}_{treated}$) was calculated for each period and for the total experiment with the following equations, as proposed by Ge et al. (2014):

$$NER_V = \frac{P \cdot t}{V_{treated}} \tag{24}$$

$$NER_S = \frac{P \cdot t}{kg_{CODremoved}} \tag{25}$$

Polarization and power curves were performed to verify the internal resistance of the system and identify differences between the two setups by using a potentiostat (NEV 4, Nanoelectra, Spain). Energy losses were calculated as in Sleutels et al. (2009).

3.2.3. Experimental procedure

The experimental study was divided into 11 successive phases, each operated for five days, to give the biomass the time necessary to adapt to the new conditions. Cells inoculation was initiated with the effluent of a parent MFC and sewage sludge, both from dairy industry origin, run until establishment of a suitable biomass response (in terms of electricity production) was observed. In phase 0, both MFCs were fed with dairy wastewater only, during phases 1-10 the feed consisted of an LL and DW mix at increasing ratios, at 5% step increases at each subsequent phase. The main characteristics of the influent feed during the study are reported in Table 2.

Table 2: Characteristics of anodic influent throughout the study.

Study Phase	Leachate %	OLR [kgCOD m^{-3} d^{-1}]	$pH_{,IN}$	$Conductivity_{,IN}$ [mS/cm]
Phase 0	0	1.49	7.85	1.99
Phase 1	5	1.16	7.42	4.16
Phase 2	10	0.87	8.72	2.74
Phase 3	15	2.39	7.15	4.64
Phase 4	20	2.14	8.17	5.23
Phase 5	25	1.15	7.94	4.64
Phase 6	30	0.71	7.99	5.02
Phase 7	35	1.20	8.38	5.03
Phase 8	40	1.19	7.33	5.61
Phase 9	45	1.34	8.24	6.20
Phase 10	50	1.34	8.00	6.75

3.3. Results and discussion

3.3.1. Electric production

MFCs rely on biological oxidation of wastewater, which mainly depends on the nature of the substrate. LL used in the present experimentation is a poorly biodegradable substrate, to enhance its suitability for biological treatment DW, a highly biodegradable substrate, was used as co-substrate.

Observed energy production did not reflect a specific trend correlated to the LL fraction in the feed, however, upon results examination it can be assessed that the most favorable operating condition was observed in phase 4 (15% leachate), where maximum output power peaks were recorded for both MFCs. It must be stressed out that the characteristics of leachate remained constant during the study, while DW parameters changed slightly, as previously shown in Table 1, although previous studies on the same source showed consistent excellent degradability (up to 90% COD removal) and energy production when fed to similar MFCs (Callegari et al., 2018). Maximum voltage achieved for MFC1 and MFC2 were 151.1 mV and 509.3 mV, respectively, corresponding to current densities of 4.6 and 15.4 A m^{-3}. Power density monitored throughout the experimentation is represented in Figure 10. MFC1 showed much lower electrical production than MFC2 throughout the whole study, highlighting how important setup design and materials affect the performance. MFC1 maintained a fair power generation throughout phases 3 and 4, dropping considerably during phase 5 (voltage measured between electrodes stabilized at around 10 mV). MFC2 maintained, instead, higher and stable values of electrical production up to phase 7, after which measured voltage dropped to below 170 mV (corresponding to current density of 5 A m^{-3}) under all subsequent operating condition tested.

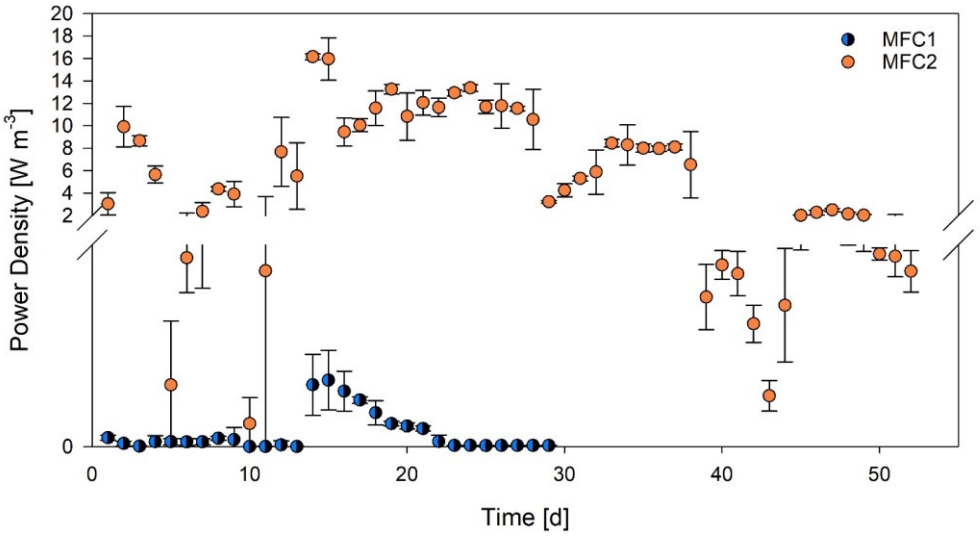

Figure 10. Power density monitored throughout the experimentation. Error bars report the power range monitored each day.

In both systems, after the shift from DW-only feed to the 5% LL-DW mix, an instantaneous drop in energy production was observed, which could be attributed to ongoing acclimation of the MFCs' anodic biomass to the new substrate composition. This acclimation is confirmed by the rapid recovery observed in the following days, with rapid exoelectrogenic biomass activity recovery, which maintained and improved high current production throughout phases 2 and 3 for MFC1, and up to phase 7 for MFC2, even at increasing leachate ratios in the feed. At this point it seems evident that MFC2 architecture proved to be more efficient for energy recovery than MFC1's as, both being operated under the same conditions, the latter showed a consistently lower power generation.

3.3.2. Electric and organic matter removal efficiency

Organic matter removal efficiency (ηCOD) throughout the study was measured for each condition tested, and coulombic efficiency (CE) was calculated. In the first phases of the study, CE was very low for both systems, probably due to a slow adjustment of the exoelectrogenic population. Concerning MFC1, CE showed a linear incremental trend (Figure 11), with values ranging from 1 to 6% in the last condition tested, while MFC2 showed more variability, with sudden increase under phases 5 and 6, where the maximum efficiency (26%) was observed, decreased down to around 10% afterwards.
COD removal efficiency started at 82.9% for MFC2 and 58.1% for MFC1 in phase 0. It increased in phase 1, achieving the best values for both MFCs, 84.9% and 69.1% for MFC2 and MFC1, respectively, decreasing gradually with the increase of leachate ratio in the feed. During phase 5, COD removal dropped drastically in MFC1, at 7.6%. The unit was then operated until the end of phase 6, with no increase in voltage generation and even lower ηCOD, at 5.7%, therefore it was decided to stop operation of this unit. MFC2 maintained high COD removal efficiency (generally at or above 66%, save for a low of about 55% during phase 3) until phase 5. At 25% LL ratio in the feed conditions became critical: from the previous ηCOD of 66.3%, removal dropped by almost half to 36.5%. This content level of landfill leachate in the influent affected both systems, and thus can be considered their operational limit in the studied conditions. MFC2 maintained, however, removal efficiency greater than 30% until phase 10 (LL/DW = 50%), when ηCOD dropped to a low of 8.6%.

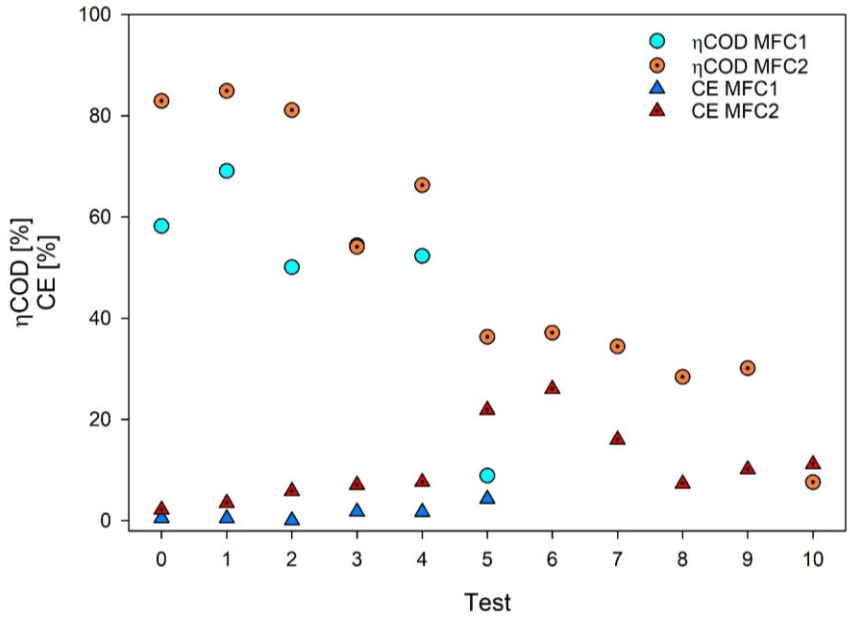

Figure 11. CE and ηCOD in MFC1 and MFC2.

3.3.3. Polarization curves

A final analysis concerned the systems' polarization curves: in addition to representing the electrical behavior of the cells, they allow to establish the real internal resistance value; it was already reported that, to maximize energy production in MFCs, external resistance should be equal to the internal resistance (Molognoni et al., 2016). Polarization curves (Figure 12) were determined for each experimental condition: early examination of the observed power curves of the MFCs, showed that MFC2's internal resistance was 21±10 Ω, quite close to the external resistance actually applied (33 Ω), while MFC1's internal resistance resulted in a staggering 170±18 Ω, five times higher. This explains both the initial lower power generation and CE of this unit. After phase 2, the external resistance of MFC1 was modified to 150 Ω, showing a detectable increase in power density, but no benefit in COD removal efficiency during subsequent tests. This modification did not prevent the system from substantially stop being efficient in terms of COD removal and energy recovery between phase 5 and 6.

The internal resistance detected for MFC2, instead, was similar to the external resistance applied, therefore further analysis of energy losses in the unit was performed. It was found that the largest part (E_t = 55%) of these could be attributed to membrane losses, while the second largest factor affecting energy production was cathode efficiency (η_{cat} = 32%). Anode efficiency and pH gradient only accounted for 7% and 5% loss respectively,

while ionic exchange between anode and cathode could be considered negligible (<1% loss).

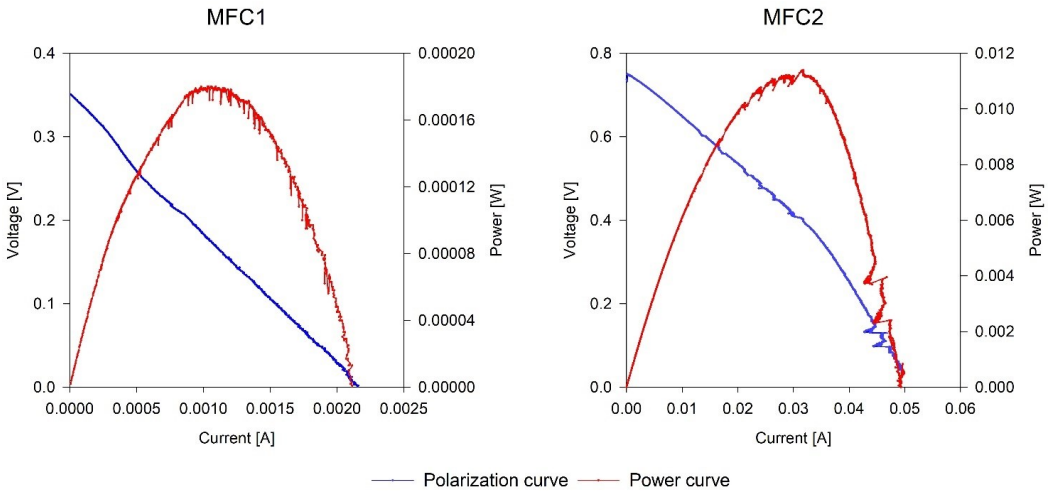

Figure 12. Polarization curves performed during phase 2.

3.3.4. Comparative analysis

Net energy recovery (NER) throughout the study was evaluated for both units, in volumetric (NER_V, net energy recovery per m^3 influent treated) and massive (NER_S, net energy recovery per kg COD removed) specific terms. Results are summarized in Figure 13: it can be noticed that it was not possible to establish a consistent trend of this parameter in relationship with observed COD removal and CE. MFC1 (Figure 13, upper) recovered almost no energy during the first tests, due to suboptimal electric design conditions. When sufficient energy production started (phases 3 and 4), values up to 0.022 kWh $m^{-3}_{treated}$ were observed. As already confirmed by the previously shown data, MFC2 showed better performance, reaching values of NER_V of 0.149 kWh $m^{-3}_{treated}$ during phase 6 (30% leachate). In terms of specific net energy recovery, best rates were also obtained in phase 6, where a NER_S of 0.019 kWh $kgCOD^{-1}$.

To compare the results of the present study to others reported in literature, phase 4 was taken as reference for both units. Reported studies taken for comparison are summarized in Table 3. When considering landfill leachate as a substrate, the type of landfill, age and wastes collected strongly influence performance of a bioelectrochemical system, and must be taken into account. Also, pretreatment increase the bioavailability of organic matter in leachate, for example by performing fermentation, enhancing electricity production and substrate conversion (Mahmoud et al. 2014). Along with COD removal, in many studies nutrients' removal, such as ammonia and phosphorus, were evaluated. However, not being the main focus in the present work, these were not taken into account for the comparison. Young landfill leachate can have relatively high BOD_5/COD ratio (0.4–0.6) indicating good biodegradability (Özkaya et al. 2014). This ratio generally decreases with the age of

the landfill: the present study operated on leachate from a closed landfill, characterized by a low BOD_5/COD ratio of 0.1.

Puig et al. (2011) operated an air cathode MFC with both diluted and raw landfill leachate characterized by low BOD_5/COD ratios (0.02 – 0.2) and high salinity, comparable with that used in the present study. During operation with diluted leachate (507 mgCOD L^{-1}, OLR=1.48 kgCOD m^{-3}), an air cathode MFC achieved 32% COD removal, and average power density of 6.1±4.2 mW m^{-3}. With raw leachate fed to the system, OLR increased up to 24.42 kgCOD m^{-3}, achieving up to 37% COD removal and power density of 344 mW m^{-3}. Observed coulombic efficiency, however, remained below 2%, indicating that substrate degradation was not carried out primarily by exolectrogenic bacteria, but possibly by methanogens, a commonly found competing species (Molognoni et al., 2016).

Most MFC studies in literature concern the use of young landfill leachate: this is, in fact, easily biodegradable, leading to an easier and more effective biological treatment, but not necessarily to higher energy recovery efficiency. Ozkaya et al. (2014) operated with such substrate as MFC feed, characterized by COD up to 50 g L^{-1}, with BOD_5/COD = 0.65, starting from COD concentration of 1 g L^{-1}, and reducing gradually the applied OLR up to 50 g L^{-1} d^{-1}. Higher OLRs led to lower coulombic efficiency (<1%, against 35% at lower ORLs). The authors stated that, despite the overall increase in voltage output, decrease in CE may be due to uptake of organics by non-exoelectrogenic processes, such as methogenesis. Zhang et al. (2015a, b) operated dual chamber BESs for young landfill leachate treatment, characterized by BOD_5/COD=0.48, achieving 2.16 Wm^{-3} maximum energy recovery and 95.1% COD removal at OLR of 1.2 kgCOD $m^{-3}d^{-1}$. These are the best performance values reported so far in literature. Vázquez-Larios et al. (2014) operated with young landfill leachate with excellent biodegradability (BOD_5/COD = 0.86) in a two chambered MFC in batch mode. COD removal of 72% was achieved, with maximum power density of 1.83 W m^{-3}.

The present study shows that both units (MFC1 for part of the study only), even though using diluted old landfill leachate with low biodegradability, achieved satisfactory degradation values and energy recovery parameters in line with those reported in literature for any type of leachate. It should be also noted that not all cited published studies clearly specify the period during which the observed performances were consistently maintained.

Table 3: Net energy recovery from landfill leachate bioelectrochemical system applications. (NER_V and NER_S calculated according to Iskander et al. (2016)).

System configuration	Leachate COD [mg L^{-1}]	Operational mode	COD removal [%]	CE [%]	NER_V [kWh m^{-3}_{tr}]	NER_S [kWh kg_{CODrem}^{-1}]	Reference
Membrane-less Anoxic/Oxic	19200	Continuous	95.1	-	–	0.04866	(Zhang et al. 2015a)
Dual chamber	50000	Continuous	43	<1.0	0.05400	0.00251	(Özkaya et al. 2014)
Single chamber	12300	Batch	72	6.7	–	0.01986	(Vázquez-Larios et al. 2014)
Single chamber (air cathode)	507 (diluted)	Continuous	32	<2.0	0.0000506	0.00031	(Puig et al. 2011)
Membrane-less Anoxic/Oxic	20100	Continuous	86	-	0.06648	0.00383	(Zhang et al. 2015b)
Dual chamber	11400	Continuous	87	0.6	–	0.00190	(Zhang and He 2013)
Dual chamber	300 (diluted 15%)	Continuous	26	-	-	-	(Nguyen and Min 2020)
Dual chamber	4000 (synthetic)	Batch	65.1	-	-	-	(Huang et al. 2018)
Dual chamber	2216	Step-feed	53.6	6.9	0.0068	0.0058	Present study (MFC1)
Dual chamber	2216	Step-feed	56.2	13.5	0.074	0.00714	Present study (MFC2)

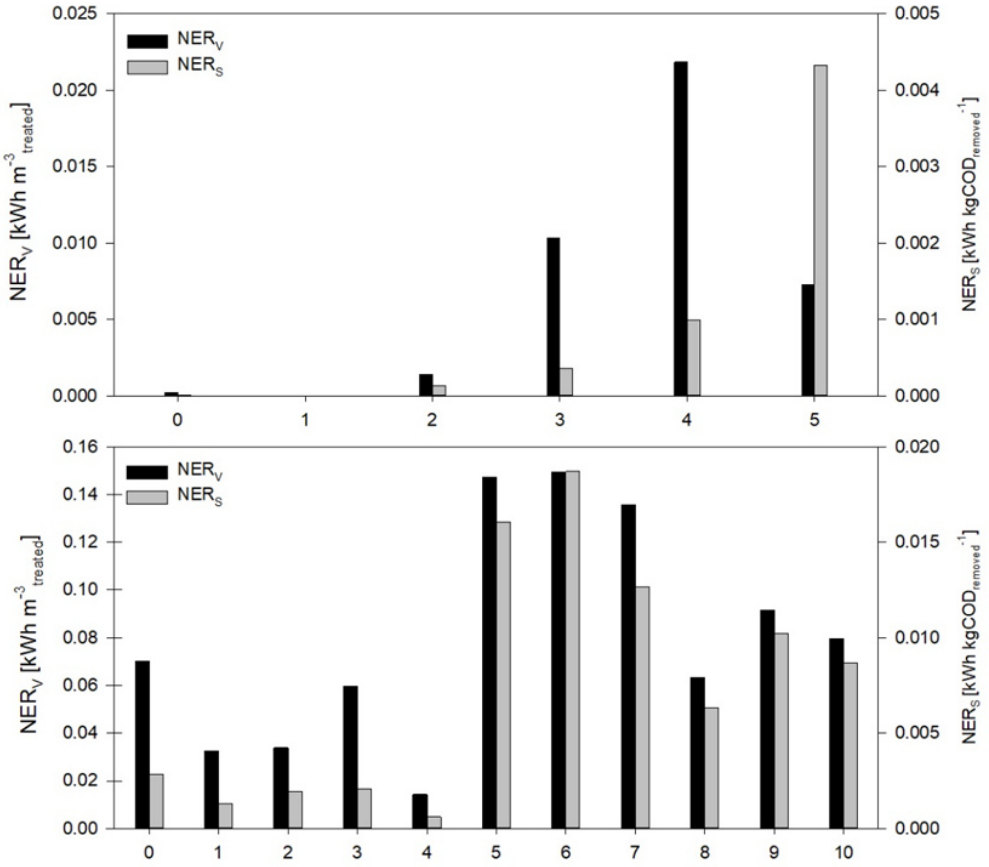

Figure 13. NER_V and NER_S obtained throughout the study: MFC1 (upper) and MFC2 (lower).

3.3.5. End of operation analysis

To better understand the limitations of landfill leachate treatment, and the causes that led to failure of the process when LL/DW=1 (50% ratio) in the feed was reached, an "autoptic" analysis was performed on the cells. After conclusion of the tests, both MFCs were disassembled to analyze the effects of the continuous operation with landfill leachate mix feed on the constituent materials. Figure 14 shows actual photographs of the anodic chamber of MFC2, indicating solid particles obstructing the spaces between the electrode's graphite granules, limiting contact possibility between substrate and electrode surface. Notwithstanding a preliminary screening of the leachate upon collection, the constant flow of raw landfill leachate, in which colloidal and small solid, non-biodegradable particles may have remained, may have caused their gradual accumulation in the anodic chamber, reducing its net free volume, and consequently its hydraulic retention time, affecting overall performance. The effect of internal hydrodynamic conditions and flow distribution on cell performance had already been highlighted in literature (Cecconet et al., 2018, Vilà-Rovira et al., 2015), and this additional evidence confirms previous findings. In addition, non-pretreated landfill leachate could also have caused partial fouling of the cation exchange membrane (CEM), affecting ion transfer efficiency between chambers, and decreasing

overall performance of the unit (Xu et al. 2012). Finally, the presence of trace metals and ammonia may also have affected MFC performance with a potential biomass inhibiting effect (Hang et al., 2020).

Figure 14. Solid residues observed between the electrode's graphite granules in the open anodic chamber of MFC2 after the study. The black sheet material indicated by the arrow in the right panel is the cell's CEM (shown in new original condition in the picture rightmost insert)-

3.4. Discussion

Results of the study showed that one of the MFCs tested for combined leachate and industrial wastewater treatment obtained initially good results both in terms of COD removal and power generation. The use of DW as co-substrate is providing additional nutrients to the system and resulted in improved bioelectrochemical degradation of organics than with LL only. The unit achieving better performance (MFC2) was built with granular graphite electrodes, while the one (MFC1) with GCSS mesh electrodes showed poor results since startup. As pointed out by several studies, the performance of MFCs in terms of power output and durability strongly depends on the key components of these systems, the electrodes, which are one of the limiting factors for a generalized applicability of these systems (Gnanakumar et al., 2013). Anode and cathode materials research is among the most active sector in bioelectrochemical systems, together with units scalability issues (Abdallah et al., 2019). Premature failure of MFC1 could be ascribed to the poor performance of the GCSS mesh electrode material in these conditions. The performance of the granular graphite unit was satisfactory, comparable with that of most similar literature reported studies, until process deterioration, mostly due to physical clogging within the anodic compartment, occurred.

Some of the clogging problems detected during this study could be solved by adequate pretreatment of landfill leachate: a more solid-selective influent screening should be carried

out, possibly in combination with improved cell electrode design allowing efficient free circulation of residual particulate material within the cell. Pretreatment could also be considered in order to enhance leachate biodegradability. Ultrasonication, for example, was shown to increase the content of soluble COD and modify leachate composition in terms of NH_3-N and acetate concentrations (Nazimudheen et al., 2018). High ammonia levels may stripped by air and calcium hydroxide, removing up to 70% of the leachate content (Cheung et al. 1997). Fermentation processes prior to bioelectrochemical treatment was also reported to enhance MFC power recovery, and organics removal by up to 15 times (Mahmoud et al. 2014).

3.5. Conclusions

Two MFCs were operated for treatment of combined poorly biodegradable (BOD/COD = 0.1) landfill leachate and dairy wastewater as a co-substrate at various mixing ratios. The units, with similar architecture but different electrode materials and net cell volumes, were operated under continuous feed. MFC1 was operated for 6 cycle phases, up to 25% leachate percentage in the feed, while MFC2 maintained residual efficiency until reaching a feed composition of 50% leachate, prior to process failure. Both systems achieved their best performance treating a mixture of 20% leachate and 80% dairy wastewater. Premature failure was ascribed in the first cell to poorly electrically performing anodic material, in the second cell, after an examination of the unit at the end of the experimentation, to excessive interference of accumulated feed-contained solids, which determined clogging of the anode cell free volume, favored by suboptimal internal hydrodynamic conditions. Pretreatment of leachate may be the key to operate at higher percentages in the influent solution, lowering the presence of residual non-biodegradable solids or inhibiting waste components. Despite the ultimate process failure, during the first stages of the study, MFC2 performance was quite similar to that reported by other studies.

Bioelectrochemical systems have shown consistent, sustained, long term treatment performance of different substrates and good short-term treatment performance of problem substrates such as landfill leachate, especially when fed “fresh” leachate. Further attempts in this direction should consider adequate substrate pretreatment to overcome the issues experienced in this study, in particular when aged, poorly biodegradable leachate is fed to the system.

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4. Combined microalgal photobioreactor/microbial fuel cell system: performance analysis under different process conditions

This Chapter presents an experimental work on integrate MFC-microalgae systems. Increasing energy demands and greenhouse gases emission from wastewater treatment processes prompted the investigation of alternatives capable to achieve effective treatment, energy and materials recovery, and reduce environmental footprint. Combination of microbial fuel cell (MFC) technology with microalgal-based process in MFC-PBR (photobioreactor) systems could reduce GHG emissions from wastewater treatment facilities, capturing CO_2 emitted from industrial facilities or directly from the atmosphere. Microalgae production could enhance recovery of wastewater-embedded resources. Two system MFC-PBR configurations were tested and compared with a control MFC, under different operating conditions, using both synthetic and agro-industrial wastewater as anolytes. COD removal efficiency (ηCOD) and energy production were monitored during every condition tested, reaching ηCOD values up to 99%. Energy recovery efficiency and energy losses were also evaluated. The system equipped with microalgal biocathode proved to be capable to efficiently treat real wastewater, surpassing the effectiveness of the control unit under specific conditions. Oxygen provided by the algae improves the overall energy balance of this system, which could be further enhanced by many possible resources recovery opportunities presented by post-processing of the cathodic effluent. The Chapter contains material published in: *Bolognesi, S., Cecconet, D., Callegari, A., Capodaglio, A.G., 2021. Combined microalgal photobioreactor/microbial fuel cell system: performance analysis under different process conditions. Environ. Res. 192, 110263. https://doi.org/10.1016/j.envres.2020.110263*

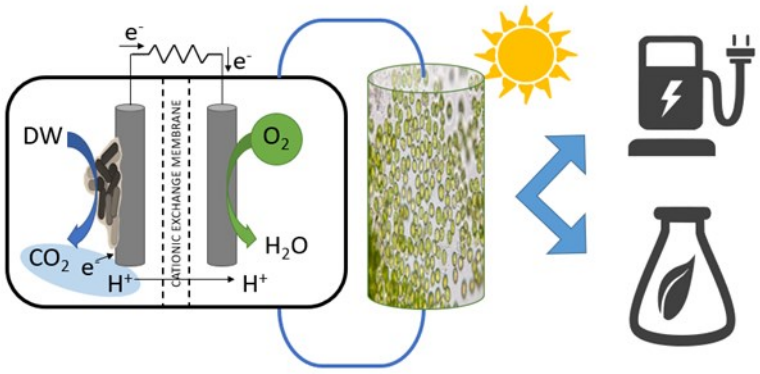

Figure 15. Combined microalgal photobioreactor/microbial fuel cell system. Graphical abstract.

4.1. Introduction

Fossil fuels combustion and CO_2 emissions from anthropic activities contribute to ongoing climate change effects, with the first decade of the new millennium registered as the warmest ever (Arndt et al., 2010). At the same time, water systems, including wastewater treatment facilities, have been indicated among major energy consumers at municipal level worldwide (Rosso and Stenstrom, 2008). Based on current knowledge, novel concepts of biorefinery could be developed to satisfy the need of more sustainable environmental protection technology and, at the same time, recover necessary energy and resources (Cherubini, 2010).

MFCs are a promising technology for wastewater treatment, characterized by electrical energy recovery coupled with low greenhouse gases (GHG) emissions and reduced sludge production (Capodaglio et al., 2013). Recently, the use of microalgae for co-treatment of wastewater was proposed, being an effective process for both resources recovery and CO_2 sequestration (Gabriel et al., 2018; Wang et al., 2010).

Combining MFC technology with algal metabolism, e.g., by coupling a PBR to a MFC cathode, could be an advantageous process improvement: (i) achieve sustainable wastewater treatment (carbon and nutrients removal) by an emerging green technology, MFCs, with low gaseous emission and low solids production, and consequently reduced sludge production (Logan and Rabaey, 2013); (ii) energy recovery by direct conversion of chemical energy in electrical energy (Capodaglio et al., 2016); (iii) CO_2 capture by microalgae with conversion into process-required TEAs and oxygen (Jiang et al., 2013); (iv) production of biofuels or valuable recovered materials from algal process residuals (Brennan and Owende, 2010; Goglio et al., 2019). It is important to point out that in such scheme, microalgae could equally capture anode-produced CO_2, or alternatively utilize gaseous effluents originated from an industrial facility, or atmospheric CO_2, converting it into oxygen (Wang et al., 2019). Some authors explored the possibility of enhancing nutrients removal by using algal biocathodes (Nguyen and Min, 2020). Based on these premises, interest on MFC-PBR systems has increased in the latter years amongst the research and professional communities (Cui et al., 2014; Do et al., 2018; Gouveia et al., 2014; Zamanpour et al., 2017). Light/dark cycles influence O_2 production, growth rate and algal stress of these processes, and consequently may affect both bioelectricity production and possible recovery products from the effluent (León-Vaz et al., 2019). This study evaluates the influence of lighting conditions and electron acceptor supply in an MFC-PBR unit operated both on synthetic substrate (acetate) and real agro-industrial wastewater as anodic feed, in long-term operation (4 months). Energy losses were evaluated under two different aeration conditions in the second part of the study, to highlight how TEA availability affects system performance.

4.2. Materials and methods

4.2.1. Experimental setup and operation

Two identical double-chamber MFCs (MFC1 and MFC2, respectively) were built and operated as described in Chapter 3, but with different configurations for O_2 provision in the cathodic recirculation line (a buffer tank connected to a fishtank aerator, or a

photobioreactor, PBR). The study herein reported was divided into two phases, according to variations of system configuration. In the first phase, two different anolytes were tested: acetate solution (1 g L^{-1}) during period I, then dairy wastewater (DW, collected periodically from a nearby cheese factory) in period II. These were fed continuously, at flow rate of 1 L d^{-1}. In the second phase, only DW was fed to the units. Characteristics of DW varied throughout the study following the production schedule at the factory. DW was stored at 4°C until use to limit bacteria activity, and then fed to the system using collapsible 10 L jerry cans to limit contact with the atmosphere. Table 4 summarizes anolyte characteristics during the study.

Table 4: Summary of the characteristics of the influents used throughout the study.

			Anodic influent			Cathodic influent	
	Substrate	Test	pH	Conductivity [mS cm^{-1}]	COD_{IN} [mgCOD L^{-1}]	pH	Conductivity [mS cm^{-1}]
First phase	Acetate	1	7.80	1.02	553	7.99	3.07
		2	7.47	0.99	529	7.67	3.01
		3	7.54	1.16	544	7.76	2.96
		4	8.10	1.03	528	8.05	2.70
		5	7.78	1.18	568	8.19	3.25
		6	7.71	1.01	527	8.02	3.09
	DW	7	7.90	0.86	426	7.97	4.65
		8	8.07	1.12	946	7.97	3.47
		9	7.34	0.78	707	7.83	3.56
		10	7.62	1.07	1032	7.82	2.08
		11	8.03	3.34	918	7.81	3.38
		12	9.25	3.15	1174	8.14	2.84
Second phase	DW	1	7.10	0.66	1241	7.97	3.02
		2	7.25	0.86	1195	8.12	2.95
		3	7.12	0.78	1142	7.85	2.88
		4	7.85	0.76	742	8.13	2.79
		5	8.76	0.92	652	8.17	3.03
		6	9.39	0.77	374	7.99	3.15
		7	8.45	1.44	952	7.84	2.18
		8	7.48	2.05	1261	7.94	2.25
		9	7.73	1.53	390	7.90	1.63
		10	7.83	2.64	1163	8.02	3.10
		11	7.66	1.67	606	8.24	2.65
		12	6.31	1.57	1195	7.54	1.58

A similar feeding mode was adopted for the cathodic chambers, fed with a phosphate buffer solution (PBS, 10 mM, pH 7) containing macroelements and an inorganic source of

carbon (507 mg L^{-1} NaH_2PO_4, 819 mg L^{-1} Na_2HPO_4, 1000 mg L^{-1} $NaHCO_3$, 130 mg L^{-1} KCl, 310 mg L^{-1} NH_4Cl, modified from Xia et al., (2013)). In MFC2 recirculation of the effluent from the PBR was also returned to the cathode, as explained in Section 4.2.2.

4.2.2. First phase

During the first phase of the study, both MFCs were operated for 60 days, 32 using acetate as substrate, 28 using undiluted raw DW; MFC2 was coupled with the PBR, while MFC1 was individually operated as control. This phase was sub-divided in two separate periods, each corresponding to a different substrate used as anolyte for the MFCs: synthetic wastewater (acetate) in period I, DW in period II. Oxygen (from air) was selected as electron acceptor, introduced according to two different methods in the two MFCs. In the MFC2 setup, a PBR unit consisting of two methacrylate tubular reactors (d=0.03 m, h=0.3 m) and containing a mixed culture of microalgae (*Chlorella*) was operated in the cathode recirculation line. Microalgae Chlorella converted CO_2 (captured from the atmosphere or from the gaseous effluent produced by the anode) into oxygen during daytime. Two different configurations for MFC2's TEA supply were tested during the study: PBR-aerated (PBR-air) configuration, with a fish tank air pump connected to the PBR via an aeration buffer unit to introduce ambient air (CO_2 for conversion, and O_2); and CO_2-capture configuration, in which the PBR was attached to a gas phase separator receiving both liquid and gaseous effluent from the anodic chamber, exploiting the anodic bacterial produced biogas containing CO_2. Gas phase was pushed to the cathodic chamber by the increasing volume of liquid effluent in the methacrylate tube. PBR light source consisted of a conventional led bulb (40 W). Cathode effluent was collected from an overflow device in the topmost section of the PBR. MFC1, acting as experimental control, was equipped with an aeration buffer in in the cathodic recirculation loop, to obtain an oxygen-saturated catholyte. The exhausted catholyte was expelled from the system via an overflow in the same buffer. In either case, the ensemble of cathode plus aeration buffer/PBR will be referred to, from now on, as "cathode system". *Chlorella* was cultivated into an external reactor, and changed in the PBR every 9-10 days (two feeding cycles). The complete experimental setup is illustrated in Figure 16.

PBR performance in MFC2 was evaluated under six different conditions for each period, by varying lighting conditions (light/dark ratio 16/8, 12/12, 24/0) and CO_2 supply conditions (PBR-air and CO_2-capture), while MFC1 was operated as a control throughout the experiment with the same substrate. Each test lasted 4-5 days, and all tests were executed in succession. A summary of the operational conditions operated during the first phase is reported in Table 5.

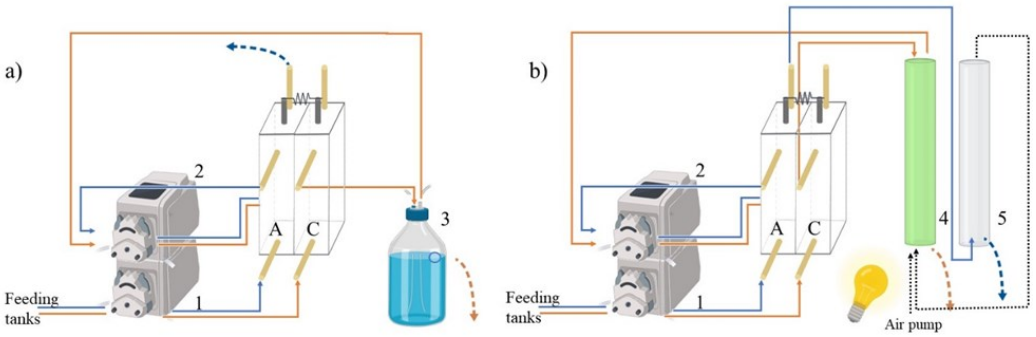

Figure 16. Experimental setup configuration in the first phase. (a) MFC1 with aeration buffer. (b): MFC2 setup with PBR. A: Anode. C: Cathode. R_{ext}: External resistor. (1) Feeding pump; (2) recirculation pumps; (3) aeration buffer; (4) photobioreactor (PBR); (5) CO_2 separator. Orange lines: anodic chamber feeding and recirculation line. Blue lines: cathodic chamber feeding and recirculation line. Black dotted lines: air supply. Dashed lines: effluent discharge.

Table 5: Operational conditions throughout the first phase of the experimentation for MFC2. DW: dairy wastewater.

Test	Substrate	CO_2 source	Dark/light ratio
1	Acetate	PBR-Air	16/8
2		CO_2-Capture	16/8
3		PBR-Air	12/12
4		CO_2-Capture	12/12
5		PBR-Air	24/0
6		CO_2-Capture	24/0
7	DW	PBR-Air	16/8
8		CO_2-Capture	16/8
9		PBR-Air	12/12
10		CO_2-Capture	12/12
11		PBR-Air	24/0
12		CO_2-Capture	24/0

4.2.3. Second phase

During the second phase of the study the two systems were configured as shown in Figure 17: raw DW was fed as anolyte, as in the first phase of the study; both systems cathodes were coupled to a PBR, containing microalgae, applying the best dark/light condition (16/8) determined in the first phase, but with different TEA supply conditions. MFC1 was operated under PBR-air mode, while MFC2 was equipped with the CO_2-capture system. Each test cycle lasted 5 days (except for two cycles lasting only 4 days), for a total duration of 58 days (12 cycles). The aim of the second phase was to highlight the different

performance of the two systems under the same conditions and characteristics, except for the TEA-supply method. During this phase, energy losses of the two MFC-PBR systems were evaluated to determine advantages and drawbacks of each configuration.

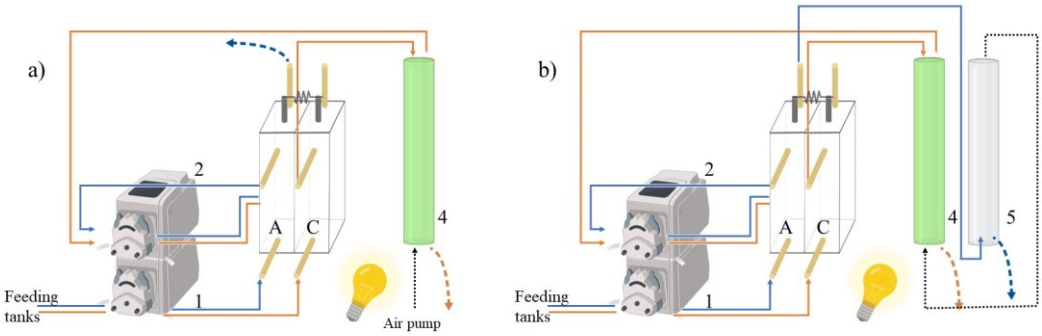

Figure 17. Experimental setup configuration in the second phase. (a) MFC1 under PBR-air configuration. (b): MFC2 under CO_2-capture configuration. A: Anode. C: Cathode. R_{ext}: External resistor. (1) Feeding pump; (2) recirculation pumps; (3) aeration buffer; (4) photobioreactor (PBR); (5) CO_2 separator. Orange lines: anodic chamber feeding and recirculation line. Blue lines: cathodic chamber feeding and recirculation line. Black dotted lines: air supply. Dashed lines: effluent discharge.

4.2.4. Data analysis and evaluation

Anodic potentials were monitored with an Ag/AgCl reference electrode (+197 mV vs Standard Hydrogen Electrode, Xi'an Yima Opto-electrical Technology Co., China) and recorded at 1-min intervals by an automatic data acquisition system (NI USB-6008, National Instruments Italy, Milan) connected to a PC. Overall MFC potentials were recorded with the same time interval, power (P) was determined from continuous current (I) and voltage measurement (V). Current (dI) and power (dP) densities were then calculated dividing the respective value of I and P by the NAC volume of each compartment. Anodic coulombic efficiency (CE) was computed as described in Cecconet et al. (2018). Determination of effluent COD (one sample per MFC per test) and acetate/wastewater influent COD (one common sample for every feed bag refill) was performed using a spectrophotometer (HI83224 Wastewater Treatment Photometer, Hanna Instruments, Italy). Organic matter removal efficiency (ηCOD - %) was determined as described in Molognoni et al. (2014). Conductivity and pH were measured at least once during every test for both anode and cathode influents and effluents (IntelliCAL™ probes + HQd™ Digital Meter, Hach Lange, Italy).

The normalized energy recovery (NER) of the MFCs, a parameter that expresses the amount of energy recovered per removed mass of COD (NER_S, kWh $kgCOD_{rem}^{-1}$) and per volume of treated wastewater (NER_V, kWh $m^{-3}_{treated}$) was calculated for each period and for the total experiment using the equations (24) and (25), as proposed in Ge et al. (2014) and reported in Chapter 3 of the present manuscript.

Energy loss factors were calculated, corresponding to each available polarization curve, using the energy balance equation with the methodology reported by Molognoni et al. (2014). In particular, anode and cathode overpotentials (η_{An} and η_{Cat}), ionic (E_{ionic}), pH gradient ($E_{\Delta pH}$) and membrane transport losses (E_t) were evaluated. Ohmic losses other than ionic were not directly measured, but included in the terms η_{An} and η_{Cat} (Sleutels and Hamelers, 2009).

4.3. Results and discussion

Results for the first and second phases are presented separately, since each one focused on a different specific aspect. The aim of the first phase was to evaluate the system's energy recovery performance and substrate conversion efficiency, by using both synthetic and real wastewater, under different conditions. During the second phase, where MFCs were fed only with DW, evaluation of PBR CO_2 conversion efficiency was the main focus.

4.3.1. Electrical production

The first operational period was characterized by the use of a synthetic substrate as anodic feed. Organic loading rate (OLR) was nearly constant during this phase (1.25 ± 0.06 kgCOD m^{-3} d^{-1}), lasting 32 days. Figure 18a shows voltage generated by the two MFCs during tests 1-6. MFC1 showed constant electricity production throughout this phase, due to the simple characteristics of the treated substrate, achieving an average voltage of 409.71 $\pm$ 46.10 mV (corresponding to a current density of 28.28 ± 2.57 A m^{-3}). MFC2 performance overall was less stable, and more susceptible to variability in different feeding periods due to changes of cathodic conditions. It can be noted that alternation of light and darkness influenced electric production, due to varying availability of oxygen as cathodic TEA. Generally, direct atmospheric O_2 supply led to better performances (as shown in tests 1, 3, 5) than supply of captured anode-produced CO_2 and subsequent conversion into O_2 by algae: in the former case, difference between day/night conditions were detectable, but not inducing large variations in electricity production, with electrical performance presenting an overall increasing trend. During test 1 and 5, electricity production of MFC2 overtook MFC1, achieving the highest voltage of the whole experimentation (573.92 mV). Test under CO_2-capture conditions (2, 4, 6) instead, were more likely influenced by the activity of algae at the biocathode, and presented high voltage drops during night-time, and an overall lower energy production. Light/dark alternation periods seems to influence both availability of TEA and algal stress, resulting in optimal oxygen production with the 16/8 sequence in the atmospheric-aerated test. As for the CO_2-capture configuration, the best electric production was achieved with the 24/0 sequence, although increased algal stress by constant lighting caused a big voltage drop in day 31. Stress conditions for algae entail metabolic changes, affecting metabolic rates. In this case, stress limited photosynthetic activity efficiency in the long run.

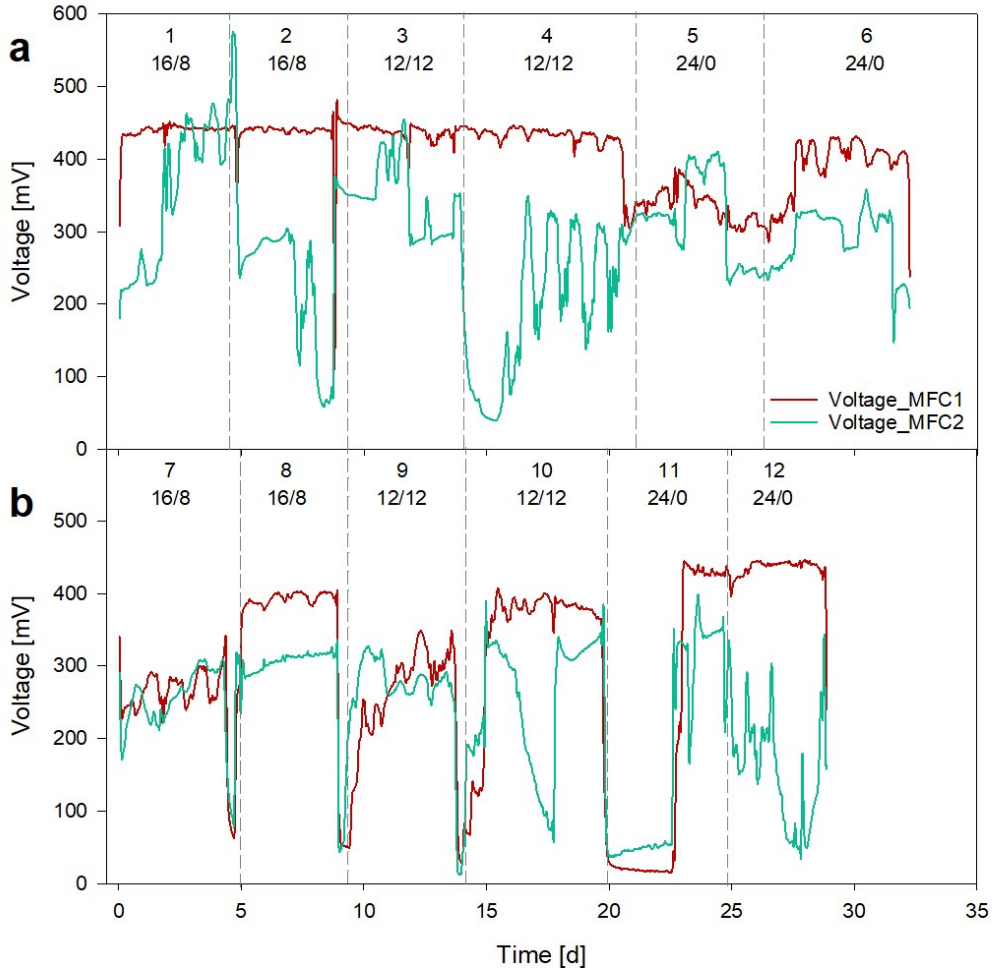

Figure 18. Voltage from MFC1 and MFC2 in the first phase of the experimentation a) with acetate feed; b) with dairy wastewater. Light/dark ratios for MFC2 are reported in the graph. Odd numbers: PBR-air configuration; even numbers: CO_2-capture configuration.

As for period II, during which the MFCs were fed with DW, results are more difficult to interpret, due to the variability of the influent itself, and hydrodynamic issues related to the nature of DW, frequently causing obstructions in the feeding line, which required extra maintenance (Cecconet et al., 2018). Figure 18b shows the voltage profile observed in period II. Although the absolute value of current produced was lower, the voltage gap observed between MFC2 and control MFC1 decreased; in tests 7, 9 and 11 (PBR-air configuration) the two profiles are very close. In CO_2-capture configuration tests (8, 10, 12) the gap is still high, especially in test 12, with algal stress causing voltage drop earlier than in the corresponding test with acetate. Comparing average voltage measured during periods I and II in the two MFCs, a voltage drop due to the change from synthetic to real wastewater in the control experiment is obvious: MFC1 accounted for 387.60 ± 85.65 mV

in period I, against 290.24 ± 130.46 mV, when using DW as anolyte, with difference of about 100 mV. A different behavior is observed for MFC2, with average voltage of 286.77 ± 102.53 mV measured in period I, and of 236.77 ± 97.53 mV in period II. Comparing performance in terms of generated voltage for the two systems in each period, it is evident that MFC1 energy production in period I was significantly higher, while the difference with DW as anolyte between the two systems is not that relevant. When using an easily biodegradable substrate, such as acetate, electron transfer efficiency is limited by cathode TEA availability only. This is obviously lower in MFC2 since it depends on light availability, and algae respiration during night-time. It is encouraging, however, the gap reduction when using real wastewater as substrate: substrate complexity in fact slows down the anodic reactions, limiting the amount of electrons released by substrate degradation, and consequently reducing the limiting influence of microalgal metabolism on cathodic activity.

In the second phase, microalgae were applied at both cathode systems, under a 16/8 light/dark sequence and DW feed. Under PBR-air configuration, the performance of MFC1 showed higher variability in generated voltage and, overall, lower current productions were observed in both MFCs. Average MFC1 voltage throughout the whole phase was 299.34 ± 133.91 mV (corresponding to an average current density of 20.61±9.27 A m^{-3}). The difference with MFC2 (in CO_2-capture configuration) was lower, because the main factor that affected electricity production was the nature of the substrate. MFC2 achieved an average voltage of 231.42 ± 98.70 mV, corresponding to a current density of 15.91 ± 6.83 A m^{-3}. Voltage monitored in this phase of the Study is reported in Figure S1 (supplementary information).

4.3.2. Organic matter removal efficiency and energy efficiency

Organic matter removal (ηCOD) was evaluated throughout the study. MFC1 and MFC2 showed similar behavior in terms of organic matter removal efficiency, with slightly better performance by MFC1, achieving COD removal of 91 ± 8% against 85 ± 14% of MFC2 (Figure 19). With acetate as influent, COD removal efficiency of MFC1 overcame the one obtained by MFC2 (Table 6), while the opposite happened with DW as a feed, where MFC2 achieved the best results, except for test 11, in which the lowest organic matter removal efficiency (56 %) was observed. CE varied throughout the study, depending on the influent feed, and on the TEA supply method, with slightly better results for MFC2. CE of both systems was higher when using acetate as a substrate rather than in the case of DW anolyte: its highest values (17.2–17.7% for MFC1, 23.2–23.8% for MFC2) were obtained with this substrate. The best results in MFC2 operation were achieved in tests under PBR-air mode, due to greater TEA availability at the cathode (Test III and V). The same trend was seen also in test with DW as influent, where PBR-aeration tests overcame CO_2-capture tests in terms of CE values. In PBR-air configuration MFC2's CE was even higher than MFC1's. The lowest CEs (5.3% for MFC1 in Test 11, 3.2% for MFC2 in Test 12) were observed with DW as feed, for both MFCs. For MFC2, this value confirmed the low efficiency of continue lighting. While OLR in acetate-feed tests was constant, in DW tests OLR variability was dependent on variable substrate characteristics. Tests under DW feed were generally characterized by higher OLR (average: 2.09 kgCOD d^{-1}); observed results confirmed reports from previous studies: in presence of high OLR, MFCs tend to develop methanogenic biomass, competitive to EABs, which leads to higher COD removal

efficiency, while decreasing MFC's electric efficiency (Molognoni et al., 2016). Results concerning the second phase have been reported extensively in SI, figure S3. Methanogens also consume organic substrate, increasing the overall COD removal of the system. The present study achieved comparable results in terms of CE and better results in terms of ηCOD (up to 10% more) than previous experiences of the group on similar substrate, operating in the same configuration as MFC1 in the first phase, meaning that the addition of microalgae improved systems' efficiency (Cecconet et al., 2018).

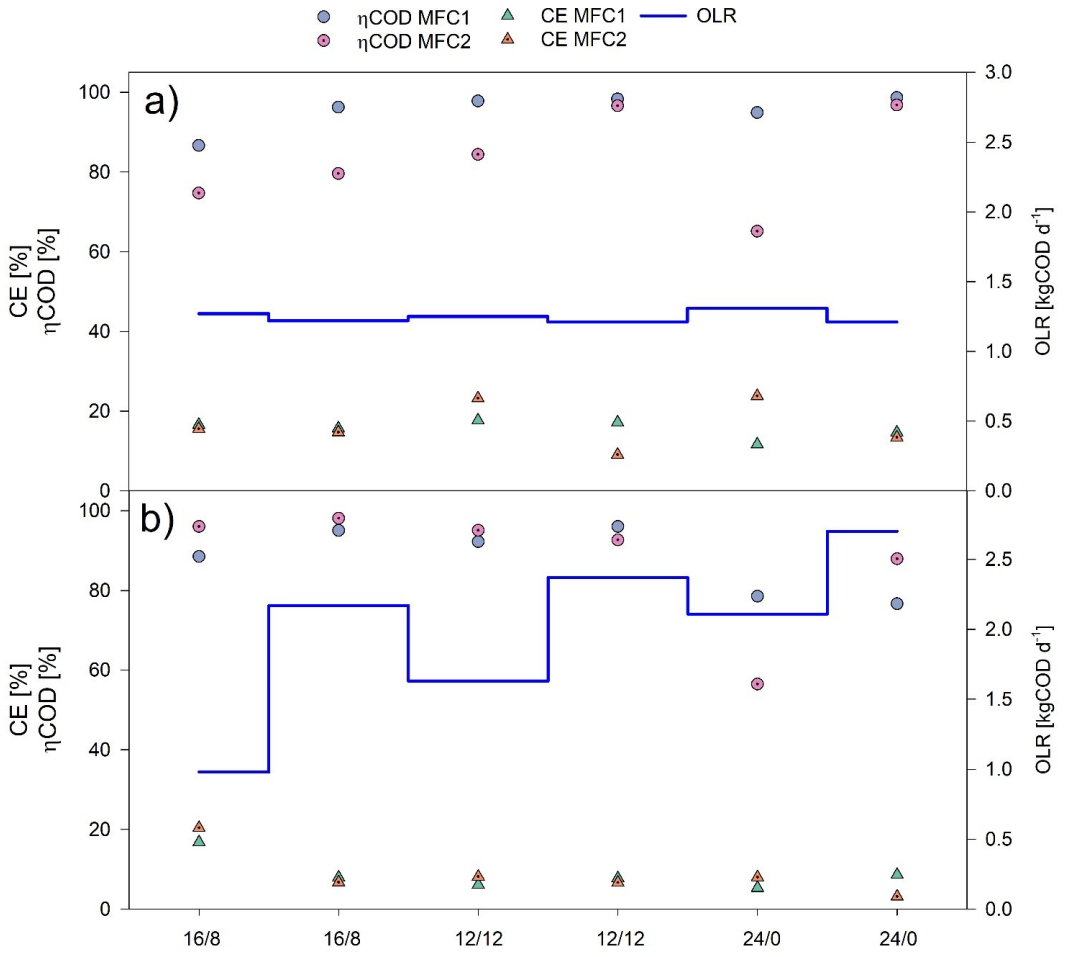

Figure 19. OLR, COD removal and CE for MFC1 and MFC2 throughout the first phase of the study: a) acetate; b) DW. MFC acted as a control with no microalgae in the system.

Table 6: Values of ηCOD, CE, NER_V and NER_S under different substrate and TEA supply conditions in period I.

		MFC1				MFC2			
Substrate	Mode	ηCOD (%)	CE (%)	NER_V (kWh m^{-3})	NER_S (kWh $kgCOD_{rem}^{-1}$)	ηCOD (%)	CE (%)	NER_V (kWh m^{-3})	NER_S (kWh $kgCOD_{rem}^{-1}$)
Acetate	Air	93.10 ± 5.79	15.32 ± 3.21	0.110	0.214	74.73 ± 9.62	20.86 ± 4.62	0.101	0.242
	Capture	97.73 ± 1.32	15.86 ± 1.26	0.111	0.215	90.98 ± 9.87	12.40 ± 2.95	0.052	0.109
DW	Air	86.42 ± 7.07	9.43 ± 6.40	0.102	0.110	82.49 ± 22.58	12.19 ± 7.09	0.054	0.110
	Capture	89.24 ± 10.91	8.12 ± 0.46	0.047	0.091	92.88 ± 5.10	5.53 ± 2.02	0.049	0.052

Net energy recovery (NER) was evaluated for both systems, which achieved comparable NER_V and NER_S values (Figure 20). The best performance in terms of NER in the MFC-PBR system was achieved in Test 3 (0.131 kWh m^{-3} and 0.285 kWh $kgCOD^{-1}$ removed, respectively), while the lowest performance was obtained in the 24/0 light sequence, CO_2-capture configuration. While NER_V does not highlight any coherent pattern in the data, an analysis of NERS data shows that, in tests under PBR-air configuration, MFC2 overcame MFC1 (except for the very first test). Comparing NERS plot with ηCOD's in the first phase with synthetic wastewater, both COD removal efficiency and power production were higher for MFC1, explaining why this specific indicator value is lower. In the second phase with DW as anolyte, ηCOD is higher for MFC2 under two out of three conditions tested (16/8 and 12/12 light sequence), meaning that under these conditions energy recovery is more efficient in the microalgal cathodic configuration. This information is further confirmed by the volumetric normalized indicator, higher than that reported for MFC1, proving that a PBR-biocathode could be beneficial for energy production when using raw DW wastewater as anolyte.

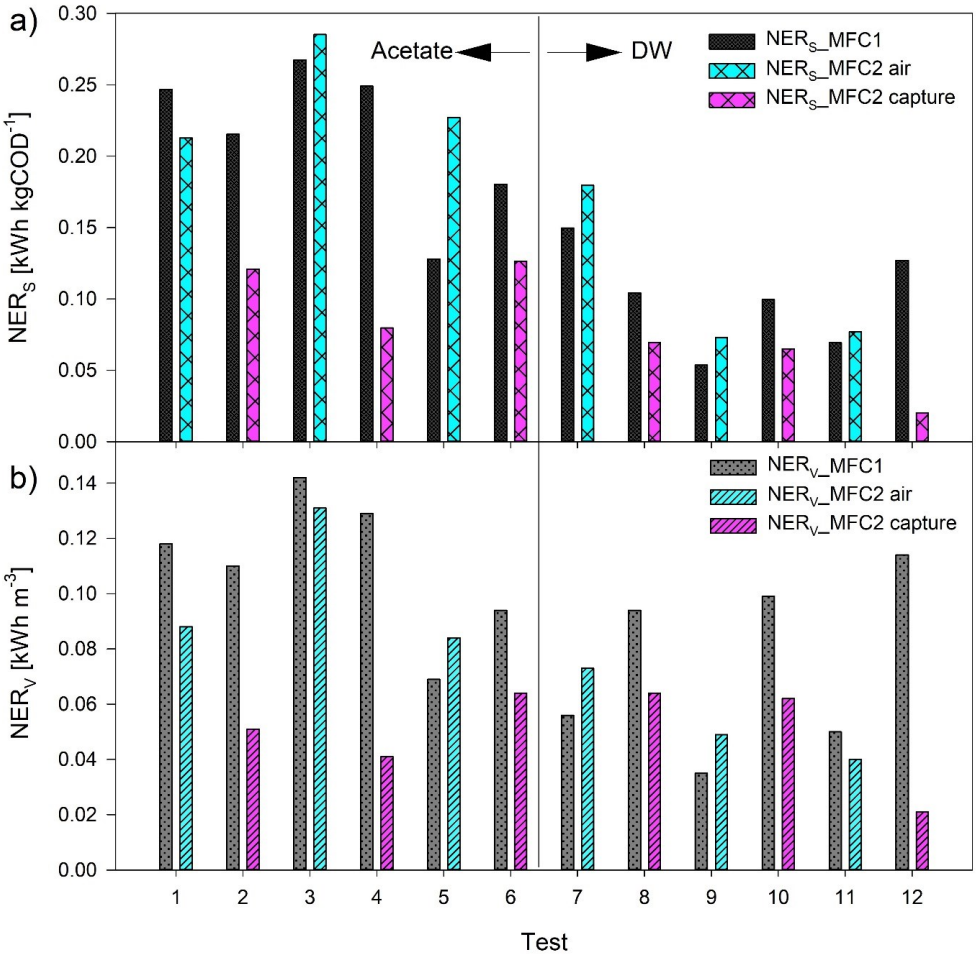

Figure 20. NERS (a) and NERV (b) throughout the first phase of the study.

In the second phase, with DW as feed, energy recovery decreased significantly, achieving values lower than in the second period of phase one (figure S2, supplementary info). The maximum NER_V value reached in CO_2-capture configuration (MFC2) was 0.041 kWh m^{-3}, while in PBR-air configuration (MFC1) was 0.061 kWh m^{-3}. As for NER_S, MFC2 maximum value reached 0.092 kWh $kgCOD^{-1}$, while MFC1's was 0.086 kWh $kgCOD^{-1}$.

4.3.3. Light/dark ratio and CO_2 availability influence on MFC performance

Light/dark sequence affects electricity production, as shown in Figure 21. PBR-air configurations show more stable current productions, even at night-time when algae activity is limited to respiration, consuming oxygen produced during the day. It can be noticed that the 16/8 PBR-aerated operation (Figure 21.a) is the best in terms of current density production (maximum density 39.18 Am^{-3}), with an overall growing trend and low reduction in dark conditions. Under CO2-capture configuration and 12/12 light/dark sequence (Figure 21.d) more stable current output conditions are reached, with current production up to 24.13 Am^{-3}, decreasing in dark conditions. Under 24/0 sequence (Figure

21.e, f), current production is quite stable under light, due to consistent availability of TEA; however, after four days of operation the CO_2-capture configuration (Figure 21.f) shows decreasing energy production, due to excessive algal stress, causing inhibition of algal activity.

Unfortunately, in tests with DW these differences were less consistently detectable due to variable nature of the substrate, leading to some unpredictability in results (voltage drops were sometimes linked to obstructions in feeding/recirculation lines, in addition to the varying quality of the substrate). Day/night behavior with DW is represented in Figure S4 [SI].

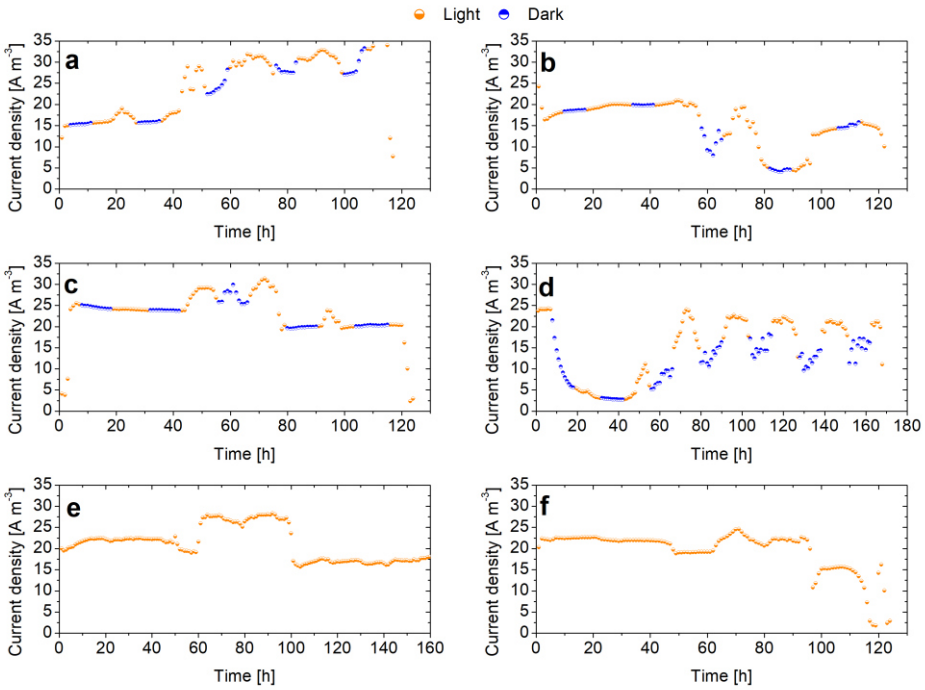

Figure 21. MFC2 performance under different light/dark sequence with acetate as feeding substrate: a) 16/8 with PBR-air; b) 16/8 with CO_2-capture; c) 12/12 with PBR-air; d) 12/12 with CO_2-capture; e) 24/0 with PBR-air; f) 24/0 with CO_2-capture.

4.3.4. Energy losses: differences in PBR-air and CO_2-capture setups

Energy losses represent the difference between MFC electromotive force (i.e. theoretical maximum voltage) and measured voltage at the electrodes. Losses depend on several factors: anode and cathode overpotentials, membrane overpotentials, pH and conductivity (ionic) gradients are easily detectable by performing polarization and power curves. Drawing a polarization curve is an important diagnostic method through which MFC performance efficiency can be assessed, determining also the best external resistance (R_{ext}) value to achieve a MFC's maximum performance, for example applying the maximum power point tracking (MPPT) technique (Molognoni et al., 2014). Different

strategies can be used to overcome or mitigate the problem of energy losses, maximizing energy recovery.

An example of polarization curve performed during the experimentation is shown in Figure 22.

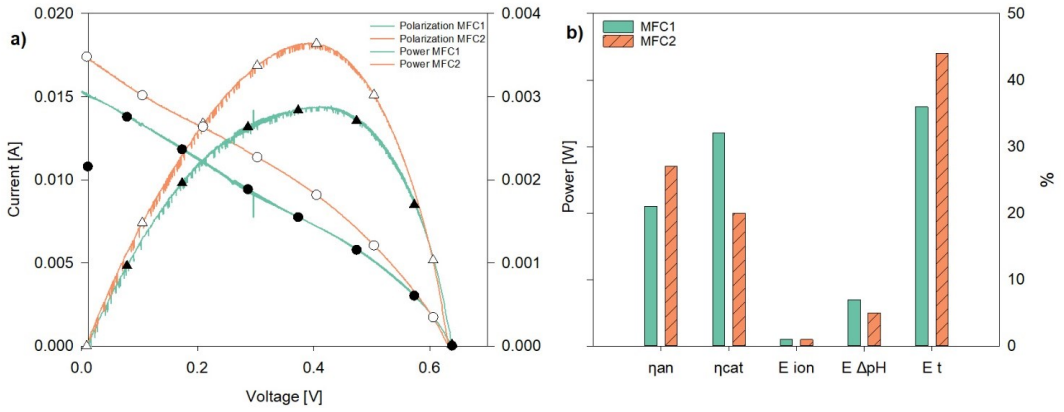

Figure 22. a) Example of polarization and power curve (day 28). Orange: MFC1, green: MFC2. Triangles highlight power curves, dots polarization curves. b) Distribution of energy losses at day 28.

In the present study, it was determined that cathode overpotentials accounted on average for 45% of MFC1's losses, 44% of MFC2's, while membrane overpotentials for 22% in the PBR-air configuration, and 31% in the CO_2-capture configuration. Anodic overpotential and pH gradient only moderately affected energy losses balance. Low pH gradients (between anode and cathode chambers) of maximum one pH unit granted lower losses (less than 10%) than in previous experiences, where significantly higher losses (23%, 2 pH-units) were detected (Molognoni et al., 2018). Anode overpotential accounted on average for 15% of total losses in both MFCs, while electrolyte overpotentials (E_{ionic}) could be considered negligible, representing less than 1% of overall losses, due to low difference in conductivity between anode and cathode media (1.3 ± 0.4 mS cm^{-1} for anolyte, 2.6 ± 0.5 mS cm^{-1} for catholyte). Anodic overpotential may be caused by increased methanogenic community activity. Comparing the first and the second phases' anodic influents, it can be noticed that pH values increased in the latter, reaching pH up to 8, a value suitable for development of a methanogenic biomass, although no microbial analysis were performed to confirm this hypothesis. Feeding an influent with lower pH, pH-gradient related losses would increase; these could be reduced by modifying the system's hydraulic retention time, or by varying its design. Data collected in this phase for MFC1 and MFC2 are reported in Figure 23.

As reported in literature, cathode overpotentials may be reduced by: (i) introducing new, more efficient electrode and catalyzer materials; (ii) improving oxygen transfer kinetics at the cathode; (iii) developing a biocathode. Algal biocathodes, as shown from experimental data of this study, seem to reduce electron transfer efficiency, due to increase in membrane and electrode fouling. However, no significant difference in cathode overpotential was detected between the unit purged with air and the one relying only on

anodic CO_2 conversion. Membrane overpotentials could be reduced by introducing different materials characterized by lower internal resistance, or less subject to biofouling.

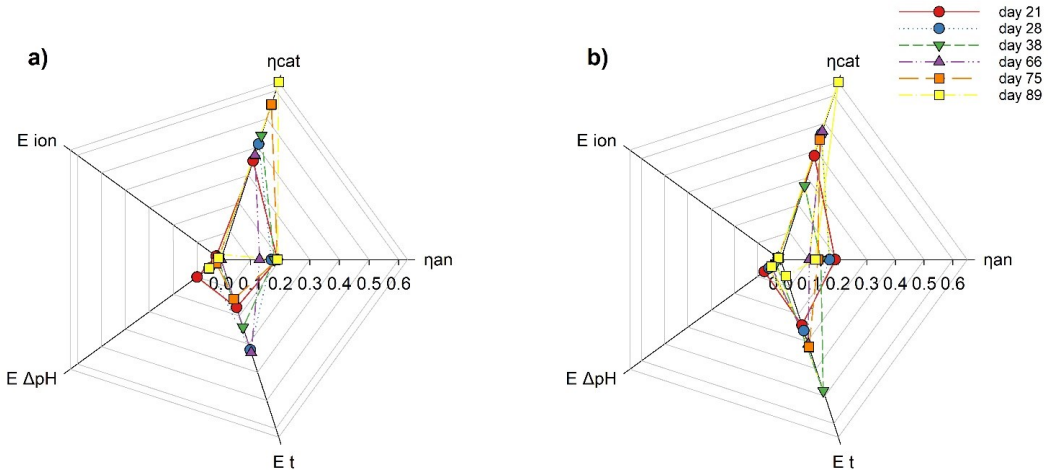

Figure 23. Energy losses in MFC1 (a) and MFC2 (b), respectively.

4.3.5. Energy and circular economy considerations

Few authors explored the possibility of coupling MFC and microalgae. Table 7 reports a summary of studies found in literature, allowing a comparison between the present work and other experiences. It is possible to notice that the system configuration used in this study overcame other architectures' power productions.

Table 7: Reported studies of MFC with microalgae.

MFC type	Influent type	Power production	CE [%]	ηCOD [%]	Microalgal species	Ref.
Two chambers	LL + MW	0.517 W m^{-3} 0.050 W m^{-2}	-	96.8 (A) 0÷56.8 (C)	Not specified	(Nguyen et al., 2017)
Tubular, external PBR	MW (diluted)	0.006 W m^{-2}	-	80.8	*Chlorella*	(Kakarla and Min, 2019)
Two chambers	SUW	0.031 W m^{-2}	<1	40.0÷90.0	*C. vulgaris*	(Gonzalez et al., 2015)
Tubular	MW	0.124 W m^{-3}	57÷78	4.1÷5.5	*C. vulgaris*	(Bazdar et al., 2018)
Two chambers + PBR	AC	2.8 ± 0.9 W m^{-3}	16 ± 5	65.3÷97.2	*Chlorella*	Present study, first phase (AC)
Two chambers + PBR	DW	1.9 ± 0.5 W m^{-3}	9 ± 4	56.1÷98.1	*Chlorella*	Present study, first phase (DW)
Two chambers + PBR	DW	2.5 ± 0.4 W m^{-3}	7 ± 3	85.5 ÷ 99.9	*Chlorella*	Present study, second phase

AC: acetate; DW: dairy wastewater; LL: landfill leachate; MW: municipal wastewater; SUW: synthetic urban wastewater.

Using microalgae as oxygen providers in a MFC system can improve its overall energy balance by decreasing the cost of aeration for TEA supply. The presence of microalgae can also improve the overall energy and economic balance of waste substrate treatment, by exploiting different materials and biofuels precursors potentially recoverable from conversion of algal biomass. Liquid biofuels, e.g., biodiesel, bioethanol, biobutanol and jet fuels, are the most likely outcomes of algal biorefining (Dasan et al., 2019; Liang et al., 2015). Biodiesel may be obtained from oil extraction and following transesterification, with properties complying with EU specifications, bioethanol and biobutanol may be derived from algae fermentation processes (Callegari et al., 2020), while biochar may be obtained by thermal treatment (Yu et al., 2017). Chapter 7 of the present thesis shows an example of this pathway, once the microalgae are harvested and dried. One of the major challenges with microalgae is to achieve efficient and inexpensive oil extraction (Chiew and Shimada, 2013). International regulations and shrinking of fossil fuels reserves will expand the renewable energy market in the next decades. Algal biomass has been indicated as a major component of the future eco-fuel panorama (Callegari et al., 2020), even though, considering current market prices of liquid biofuels, they are still not an economically appealing solution per se, with production costs higher than traditional fossil fuels. Lundquist et al., in fact estimated the cost of large scale production of algae-derived oil from wastewater at 332 $ per barrel when focusing on oil production alone; however, when considering wastewater treatment as the main focus, with algal biomass recovered as a by-product precursor of oil, the calculated cost of algae-derived oil would drop to 28 $ per barrel (lower than the average cost of crude oil) (Lundquist et al., 2010).

Microalgae can also be considered a feedstock for chemicals and materials recovery, such as slow-release fertilizers, since they are capable of accumulating surplus quantities of nutrients, recoverable as dried microalgal biomass or biochar from pyrolysis (Bolognesi et al., 2019). Biofertilizers and biostimulants appear to be one of the most economically appealing fields in algal technology, with market prices in the range of 9-23 € kg^{-1} for biostimulants, and 0.2-0.5 € kg^{-1} for biofertilizers (Voort et al., 2015). Anticipated climatic changes and increasing costs of fertilizers due to reserve shortages (Daneshgar et al., 2018) will open the agronomy field to new green biostimulants development.

Finally, the nutritional value of microalgae could open the possibility for their use in the food and feed (aquaculture or livestock) market, however, food, feed and pharmaceutical reuse of algae grown in wastewater treatment processes still present issues of social acceptance; so far, the most favorable market outlets for microalgae recovery consist of biofuels production, biofertilizers and soil amendment products.

4.4. Conclusions

This study aimed at evaluating the performance of an MFC-PBR system treating synthetic (acetate) and real (dairy wastewater) substrates with energy biorecovery under different operational conditions, and to establish optimal process configuration. Two systems of identical base configuration were operated continuously for up to 60 days at a time, using the same substrate as feed, but using different TEA supply methods. Both systems proved to be effective for wastewater treatment (COD removal), and showed higher power density generation than similar systems described in literature studies. However, concerning bioelectricity production, a traditional system proved to be more

stable and better performing than the MFC-PBR under almost every condition tested, when using synthetic substrate. Systems' performance gap reduced when passing from synthetic substrate to real wastewater feed, showing increasing performance of the MFC-PBR unit, as confirmed by the relative increase of NER_S and NER_V, compared to the same parameters in the conventional unit. This fact was attributed to greater substrate complexity slowing down the anodic reactions in the better performing system, reducing the limiting influence of microalgal metabolism on cathodic activity. This indicates that MFC-PBR combination systems with microalgae may become a feasible option for sustainable wastewater treatment, when the key limitations of MFC will be solved.

Despite many efforts to increase these systems efficiency, in fact, the major issue in MFC technology is linked to internal energy losses, impairing net energy production and recovery, which unfortunately was not sufficiently improved by the introduction of algae as oxygen (TEA) providers. Several existing and envisioned possibilities of recovery and valorization of algal effluent, however, could help improve the overall economic and energy balance of these system, at the same time reducing their atmospheric CO_2 impact.

Supplementary information

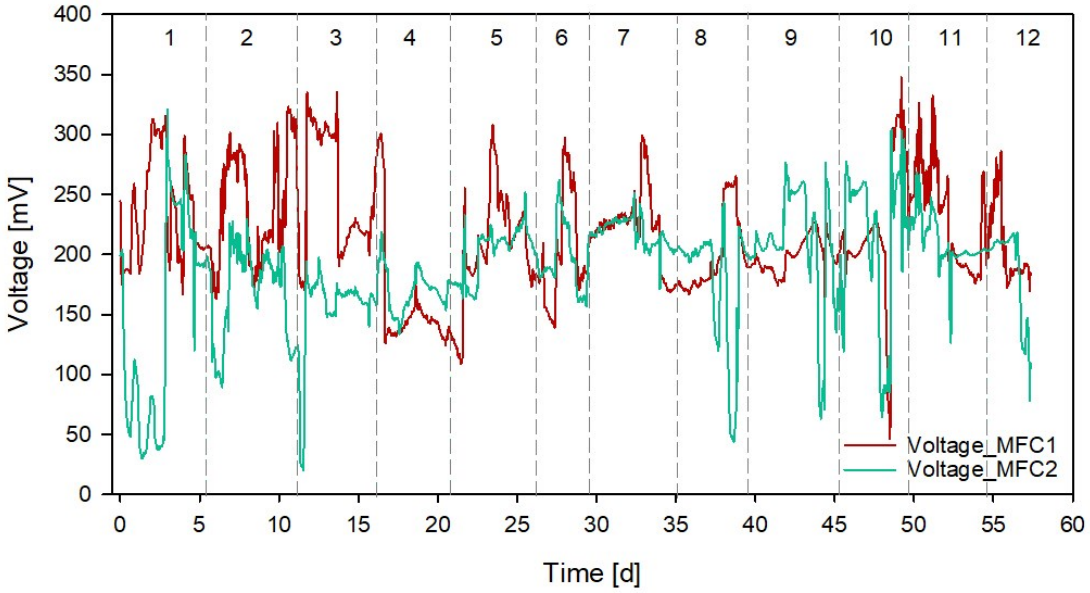

Figure S1. Voltage MFC1 and MFC2 fed with dairy wastewater (second phase).

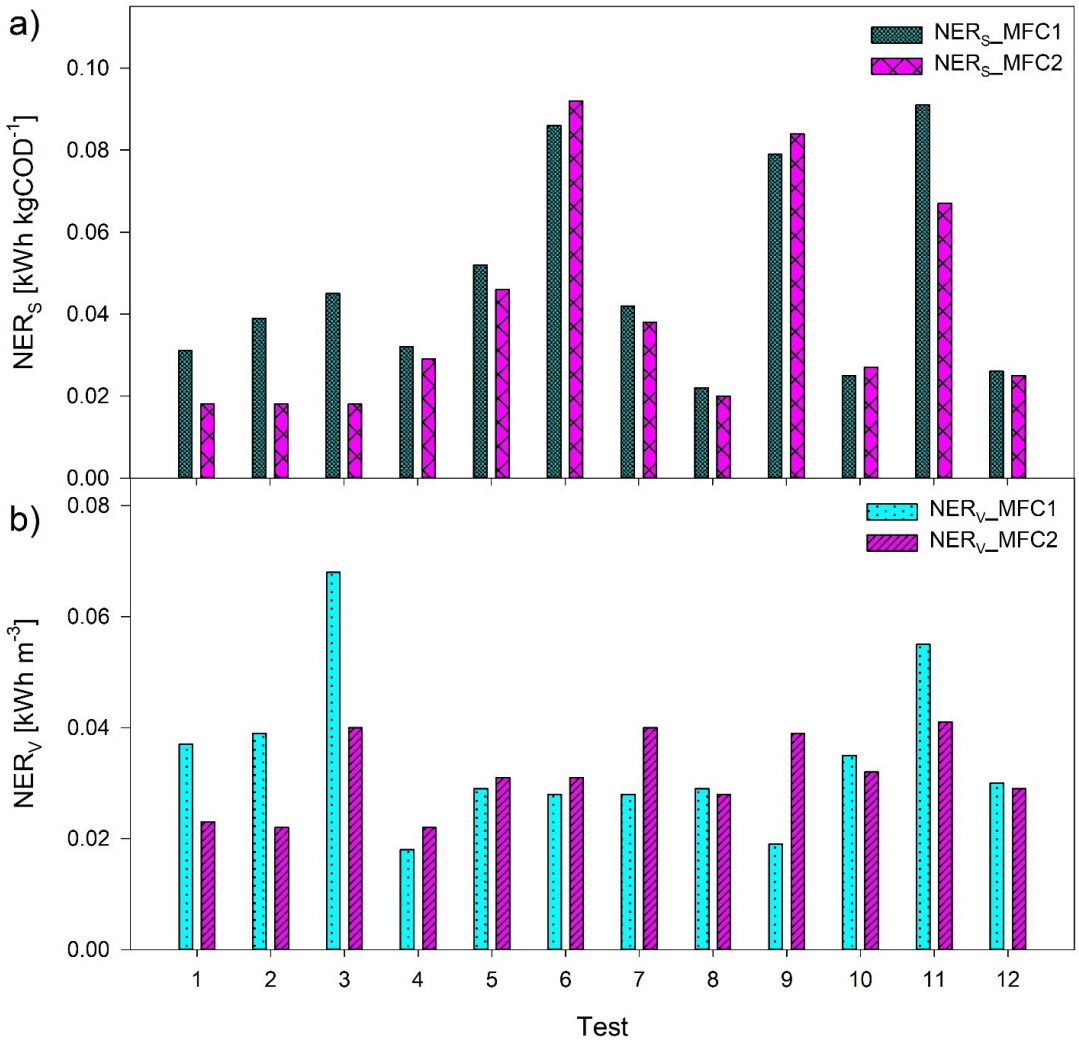

Figure S2. NER_S (a) and NER_V (b) throughout the second phase of the experimentation.

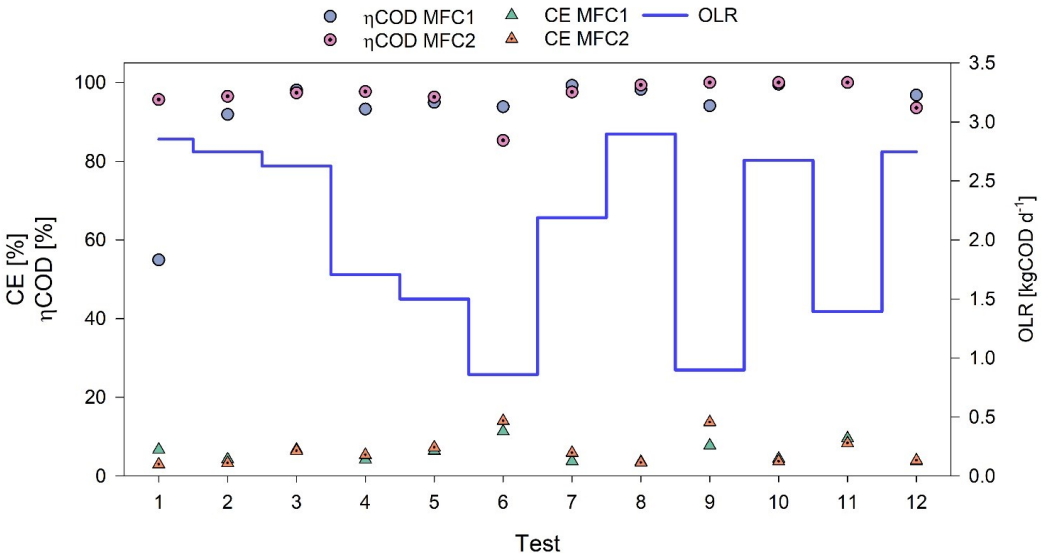

Figure S3. OLR, COD removal and CE for MFC1 and MFC2 throughout the second phase of the study.

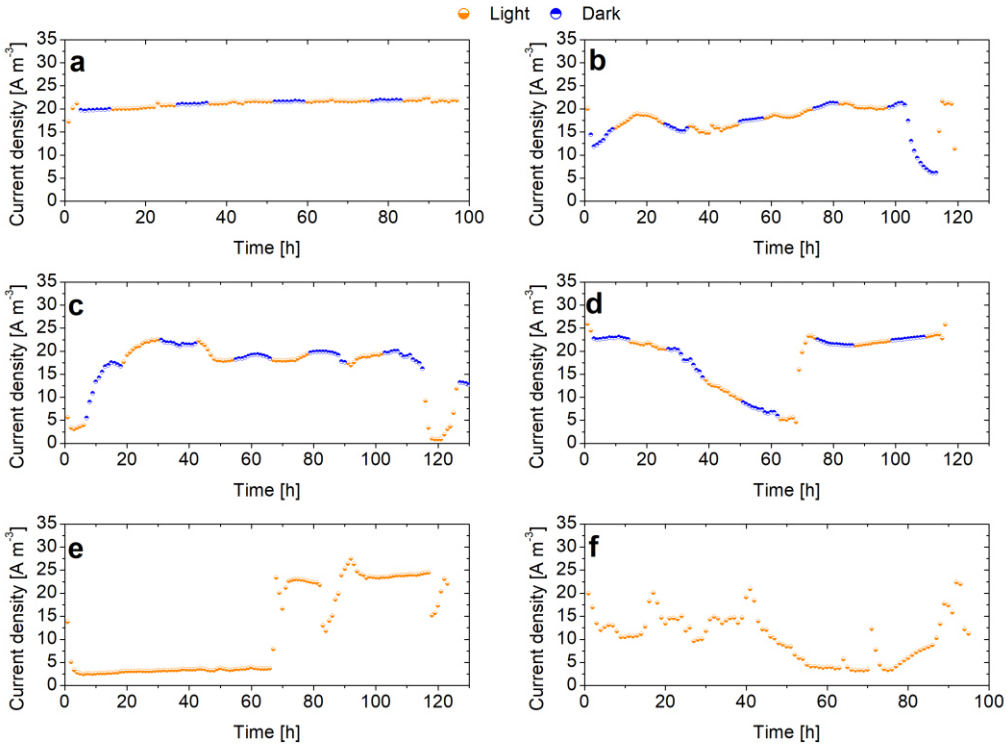

Figure S4. MFC2 performances under different light/dark ratio with dairy wastewater as feeding substrate: a) 16/8 with air; b) 16/8 with CO_2 capture; c) 12/12 with air; d) 12/12 with CO_2 capture; e) 24/0 with air; f) 24/0 with CO_2 capture.

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5. Power-to-algae: carbon dioxide conversion using electricity as feed for microalgal biorefinery

This study aims at establishing the proof of concept of bioelectroCO_2 recycling to oils. Two technologies were coupled for the microbial electrosynthesis of acetate from CO_2 and the subsequent, acetate transformation to oil in a heterotrophic microalgae reactor.
Two BES reactors were built and operated in batch mode at a cathodic potential of -0.8 V vs SHE for 120 days, achieving a high acetic acid concentration (up to 13g L^{-1}), and a maximum production rate of 0.29 g L^{-1} d^{-1}. As a secondary technology, the effluent from the biocathode was transferred in a batch reactor containing heterotrophic microalgae *Auxenochlorella protothecoides* to evaluate the oil production yield, achieving up to 25% w/w oil content, and possible direct recovery options from microalgae. According to the results of this experimentation, approximately 1.11 kg dry algae per kg acetate can be grown, from which 0.03 kg bio-oil per kgCO_2 captured can be recovered. The oil obtained can be further processed to produce an EU biodiesel requirement compatible bio-oil, and possible recovery options can be evaluated for the solid fraction. Combining microbial electrosynthesis and microalgal biorefinery could lead to a fruitful valorisation of biocathodic effluent.

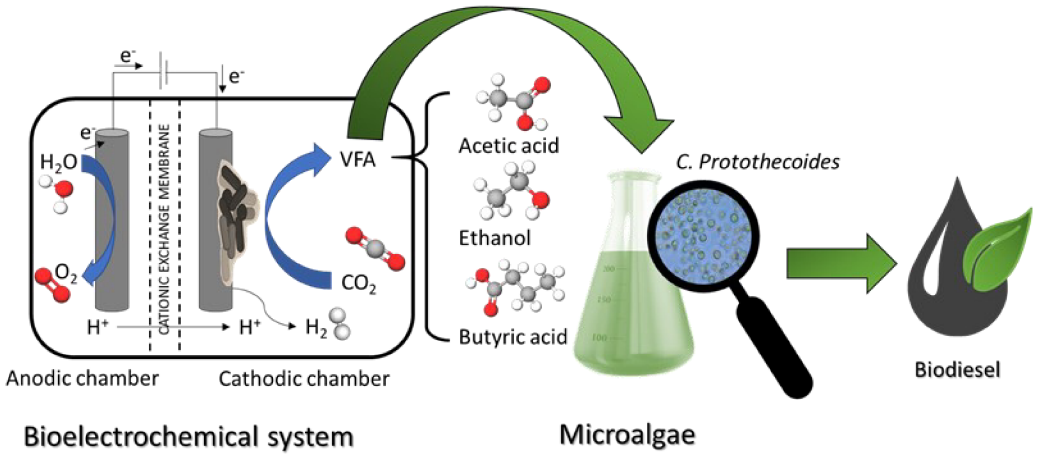

Figure 24. Power-to-algae carbon dioxide conversion using electricity as feed for microalgal biorefinery. Graphical abstract.

5.1. Introduction

Increasing exploitation of fossil-based fuels has led to severe stresses in the energy sector and is the cause of massive release of greenhouse gases in the environment, among all carbon dioxide (CO_2; Bilgili et al., 2017). Key to the process is the capture and sequestration of CO_2, a process that classifies fourth generation biorefineries as carbon neutral fuel sources (Batlle-Vilanova et al., 2019). Amongst many biological methods for CO_2 sequestration and conversion, microbial electrosynthesis (MES) emerges as a promising technology in microbial electrochemistry (ElMekawy et al., 2016; Nevin et al., 2010; Rabaey and Rozendal, 2010). MES relies on bioelectroCO_2 recycling into multi-carbon molecules, such as added value chemicals and biofuels (Dessì et al., 2021). In MESs, the reducing-power is granted by applying a current or fixing the potential at the cathode of a bioelectrochemical system (BES) to provide the desired reducing equivalents to the bacteria (biocatalysts) and perform the bioconversion of CO_2 into short-chain fatty acids (Vassilev et al., 2018). Despite productions of acetic acid plus ethanol (C2 compounds) or butyric acid (C4 compound) have been already assessed, further chain elongation is hard to reach in single-step processes; consequently, the most abundant product is acetic acid, of scarce economic value and appeal, impaired by the high energy-related production cost. Assuming a CE of 90% and an operational voltage of 3 V, Prévoteau and co-workers estimated that about 12 kWh_{el} are necessary to produce 1 kg of acetic acid (Prévoteau et al., 2020).

Many strategies have been evaluated to obtain efficient chain elongation; among all, control of the operational parameters and addition of a subsequent fermentation stage (Blasco-Gómez et al., 2019).

Molitor et al. (2019) proposed a two-stage bioprocessing system as part of a power-to-protein approach to fix CO_2 in a first stage by anaerobic acetogenic bacteria, and use the biocathode effluent to grow yeasts or fungi in a second stage under aerobic conditions. The group obtained a carbon yield of 25% as yeast biomass in continuous mode, with a protein mass-fraction of 40–50% during a proof-of-concept experiment, reaching a protein production rate of up to 0.07 g L^{-1} h^{-1}.

Microalgae are considered as a resource for GHG emissions mitigation; in particular, autotrophic microalgae can capture CO_2 to synthetize new biomass: 1 kg dry microalgae *Chlorella vulgaris* may capture 1.83 kg CO_2 with a fixation rate of 0.73 to 2.22 g L^{-1} day^{-1} (Chisti, 2008; Pires et al., 2012), but when cultivated in raceway ponds part of the CO_2 insufflated is lost (~60%). Furthermore, only the first 10 cm receive sufficient light to perform photosynthesis, and biomass concentration may vary, typically around 0.1 to 8 g L^{-1} in dry weight (Valdovinos-García et al., 2020). Heterotrophic cultures, instead, can generate biomass in large cultivation volumes (many experiences reported over 100000 L cultivation units) which can yield hundreds of kilograms of biomass. These large volumes and high productivity of cultures make the heterotrophic strategy far less expensive than the autotrophic approach (Perez-Garcia et al., 2011).

Heterotrophic microalgae, such as *Auxenochlorella protothecoides*, can use acetate and other short chain volatile fatty acid to grow in presence of oxygen, and require no direct light exposure (Fei et al., 2015). From microalgal biorefinery several valuable products can be obtained (Cheng et al., 2015; Chew et al., 2017; Ho et al., 2013; Liang et al., 2015). Some species of microalgae, among these *A. protothecoides*, can accumulate large amounts

of lipids when cultivated heterotrophically (Gao et al., 2014). Biodiesel can be obtained from microalgal oil extraction and processing; this process is convenient for high mass cultivation units where oil is extracted and then converted through conventional transesterification processes (Gao et al., 2014; Vitova et al., 2014). Biodiesel produced using microalgal oil presents heating value (41 MJ kg^{-1}) and H/C ratio (1.81) fully compatible with ASTM biodiesel standards (Miao and Wu, 2006). The pellet remaining from the extraction process (solid fraction) can be pyrolyzed and recovered as biochar, as presented in Chapter 7 of this thesis (Bolognesi et al., 2019). In the present study, it is proposed a two-stage process based on coupling bioelectrochemical system (BES) and heterotrophic microalgae to convert carbon dioxide and electric power into a biodiesel compatible oil.

5.2. Materials and methods

5.2.1. Experimental setup and inoculation

Two H-type BES were built using two modified 0.25 L bottles (Pyrex V-65231 Scharlab, Spain) and operated, respectively indicated as HT1 and HT2. Cathodic and anodic chambers were separated by a cationic exchange membrane (2 cm^2, CMI-1875T, Membranes International, USA). Carbon cloth (working surface 30 cm^2, thickness 490 μm; NuVant's ELAT, LT2400 W, FuelCellsEtc., USA) connected to a stainless-steel wire was used as cathode (working electrode) while a graphite rod was used as anode electrode (counter electrode, diameter 4 mm, EnViroCell, Germany). An Ag/AgCl reference electrode (+0.197 V vs. SHE, model SE11-S, Sensotechnik Meinsberg, Germany) was placed in the cathodic chamber. Both BES were operated in a three-electrode configuration with a potentiostat (NEV 3.2, Nanoelectra, Spain) controlling the cathode potential at -0.8 V vs SHE. The net liquid volumes for both anode and cathodic chambers were 220 mL. Complete mixing of the cathodic chamber was granted by magnetic stirring. The experiment was conducted at 25±3 °C. Both cathodes were inoculated with 20 mL (10% v/v) of fermentative electroactive inoculum, from a parent thermophilic (50ºC) BES (Rovira-Alsina et al., 2020).

5.2.2. BES operation

The two BESs were operated in batch mode. In the first 30 days of operation, both anodic and cathodic chambers were filled with a low-buffered inorganic medium (modified ATCC1754 PETC medium adjusted to pH 6, as reported in Blasco-Gómez et al. (2019). Starting from day 25, ATCC1754 medium in the anodic chamber was substituted by BG-11 medium ($NaNO_3$ 1.5 g L^{-1}, MgSO4·7H2O 0.075 g L^{-1}, K_2HPO_4 0.040 g L^{-1}, $CaCl_2 \cdot 2H_2O$ 0.036 g L^{-1}, Na_2CO_3 0.020 g L^{-1}, Citric acid 0.006 g L^{-1}, Ferric ammonium citrate 0.006 g L^{-1}, H_3BO_3 $2.86 \cdot 10^{-3}$ g L^{-1}, $MnCl_2 \cdot 4H_2O$ $1.81 \cdot 10^{-3}$ g L^{-1}, EDTA (disodium salt) $1 \cdot 10^{-3}$ g L^{-1}, $NaMoO_4 \cdot 2H_2O$ $0.39 \cdot 10^{-3}$ g L^{-1}, $ZnSO_4 \cdot 7H_2O$ $0.222 \cdot 10^{-3}$ g L^{-1}, $CuSO_4 \cdot 5H_2O$ $0.079 \cdot 10^{-3}$ g L^{-1}, $Co(NO_3)_2 \cdot 6H_2O$ $0.049 \cdot 10^{-3}$ g L^{-1}), typical growth medium for blue green microalgae, to simulate microalgae renewal in an integrated system. CO_2 (99.9%, Praxair, Spain) was the only carbon source, and it was supplied to the systems every 3 days for 5 minutes, saturating the cathodic chamber. pH was monitored throughout the experimentation and

eventually corrected when below 4.70 to avoid the risk of acid crash. Anodic pH dropped up to highly acidic values (up to 2), and medium was replenished or substitute when necessary. Before feeding, gas and liquid samples were collected and analysed to monitor the gas composition and the production of volatile fatty acids (VFAs) and alcohols.

5.2.3. Microalgae cultivation

Two algal strains, *Chlorella vulgaris* 211-11b and *Auxenochlorella protothecoides* 211-7a (SAG, Culture Collection of Algae, Goettingen, Germany) were cultivated in autotrophic conditions throughout the experimentation. *A. protothecoides* was extensively proven in literature to produce good amounts of lipids (Fei et al., 2015; Miao and Wu, 2006; Wei et al., 2009) and thus tested throughout the experimentation, while *C. vulgaris* was only operated in preliminary tests and as a comparison for microalgae growth. Two methacrylate tubular photobioreactors (d= 0.04 m, H= 1 m, 1 L each) were built to preserve the cultures from external contamination. Air was provided through a diffuser from the bottom of the photobioreactor to prevent sedimentation of the microalgae and keep the culture in suspension. BG-11 medium (pH 7) was added periodically for growing.

5.2.4. Heterotrophic tests

Heterotrophic batch tests were performed to evaluate the possibility of growing heterotrophic microalgae using VFAs produced in a biocathode. Starting parameters were assessed according to preliminary tests and values found in literature (Fei et al., 2015). Erlenmeyer flasks (120 mL) were filled with microalgae and cathode effluent at different ratios (Table 8). They were continuously mixed by using a magnetic stirrer, and kept in the dark. Each test was carried out for one week in replicates. 3 mL samples were taken every day from each flask to monitor variations in pH, optical density (OD_{540}), conductivity and chlorophyll a content. VFAs consumption was monitored through GC analysis. At the end of each test, the remaining mixture was then stored to perform oil extraction.

Table 8: Summary of heterotrophic batch tests with microalgae.

Test	Algae:medium/ effluent ratio	Volume	Samples	Microalgae species
PB1	1:1	80 mL	1*	*C. vulgaris*
PB2	1:1	80 mL	1*	*A. protothecoides*
A	1:5	60 mL	2	*C. vulgaris*
B	1:2	60 mL	2	*C. vulgaris*
C	1:1	60 mL	2	*C. vulgaris*
D	3:4	70 mL	2	*A. protothecoides*
E	3:4	70 mL	2	*A. protothecoides*

*Preliminary batch tests were performed with synthetic medium were performed with both inorganic and BG-11 medium, in heterotrophic (2 g L^{-1} acetate) and autotrophic conditions, and at different pH starting values (4, 5.5, 7, 9). Results of preliminary batch tests are reported in supplemental information (Figure S5÷S8).

Chlorophyll a analysis were performed at the beginning of each test and at least every second day. 2 mL of sample was taken and put in a clean tube and centrifuged (10,000 rpm,

10 min) to pellet cells. Supernatant was discarded, and 2 mL of acetone was added to the pellet and mixed. Tubes were left overnight in the freezer (-20 °C) to facilitate complete extraction.

Samples were centrifuged again (10,000 rpm, 10 min), and the supernatant OD was measured at different wavelengths. Chlorophyll a content was calculated as reported in Eq. 26 (modified from Lorenzen (1967)).

$$Chl\ a = (1.56 * (OD_{665} - OD_{830}) - 2.0 * (OD_{645} - OD_{830}) + - 0.8 * (OD_{636} - OD_{830}) * {}^{V_{ac}}/_{V_{sam}} \quad (26)$$

Where V_{ac} is the volume of acetone added to the sample, and V_{sam} is sample volume.

Oil extraction was performed at the end of every batch test. Samples were put in falcon tubes and centrifuged to separate the pellet from water. Algal cells were resuspended in distilled water (DW) to remove impurities and centrifuged again (6,000 rpm, 10 min). 10 mL n-hexane (purity ≥99%, Sigma Aldrich) per gram of wet algae were added, and cell disruption was operated mechanically at first by friction and then using an ultrasonic bath (40kHz, 10 min). Tubes were centrifuged again to separate the different phases (pellet, residual water and n-Hexane with lipids dissolved in it). Supernatant was then separated, and oil was extracted using a rotary evaporator (bath temperature 35°C, 20 rpm).

5.2.5. Analyses and calculations

VFAs and alcohols in the liquid phase were analysed with a gas chromatograph (GC) (Agilent 7890A, Agilent Technologies, USA) equipped with a DB-FFAP column and a flame ionization detector (FID). Unless otherwise stated, the concentration of organic compounds in the liquid phase is expressed throughout the manuscript in mg L^{-1}. Conductivity (EC), pH and optical density (OD_{600}) were also measured on the liquid sample. Gas pressure in the headspace of the reactor was measured before sampling and after feeding with a differential manometer (Model-Testo-512; Testo, Germany). Gas samples were taken using a glass syringe before taking liquid samples, and analysed using a Micro-GC (Agilent 490 Micro GC system, Agilent Technologies, US) equipped with two columns: a CP-molesive 5A for methane (CH_4), carbon monoxide (CO), hydrogen (H_2), oxygen (O_2) and nitrogen (N_2) analysis, and a CP-Poraplot U for carbon dioxide (CO_2) analysis. Both columns were connected to a thermal conductivity detector (TCD). The partial pressure of hydrogen (pH_2) was calculated from the total pressure before taking the gas samples and the composition of the gas detected in the headspace of the biocathode. The concentration of dissolved H_2 and CO_2 were calculated according to Henry's law at 25°C. Both BES after the sampling phase were flushed and then saturated with CO_2, assuming conditions of CO_2 saturation and absence of H_2 in the liquid phase after each feeding. Coulombic efficiency (CE) was calculated according to Rovira-Alsina et al. (2020).

5.2.6. Extraction of DNA and microbial community structure determination

Samples of carbon cloth and bulk liquid for each reactor were taken to assess the microbial community composition at the end of the experimental. Before DNA extraction,

bulk liquid cells were pelleted by centrifugation, whereas carbon cloth samples were used directly. DNA was extracted using the FastDNA® SPIN kit for Soils (MP Biomedicals, USA) following the manufacturer's instructions. The extracts were distributed in aliquots and stored at -20 ºC, and DNA concentration was measured using a NanodropTM 1000 spectrophotometer (Thermo Fisher Scientific, USA). Quality of DNA extracts for downstream molecular applications was checked after PCR detection of 16S rRNA using the universal bacterial primers 515F and 806R.

The hypervariable V4 region of the 16S rRNA gene for all the samples was amplified using the primers 515F and 806R following the method described by Kozich and Schloss, which was adapted to produce dual-indexed Illumina compatible libraries in a single PCR step (Kozich et al., 2013). First, PCR was performed using fusion primers with target-specific portions (Stoeck et al., 2010), and Fluidigm CS oligos at their 5' ends. Second, PCR targeting the CS oligos was used to add sequences necessary for Illumina sequencing and unique indexes. PCR products were normalized using Invitrogen SequalPrep DNA normalization plates and the pooled samples were sequenced using an Illumina MiSeq flow cell (v2) in a 500-cycle reagent kit (2x250bp paired-end reads). Finally, sequencing was done at the RTSF Core facilities at the Michigan State University USA (https://rtsf.natsci.msu.edu/).

Raw sequences from the MiSeq platform were analyzed and treated through DADA2 software from the open-source Bioconductor project as mentioned in (Callahan et al., 2016). Output demultiplex sequences obtained in fastq format were sorted to ensure reads were in the same order, quality-filter with a maximum expected error of 2 to filter out low-quality sequencing reads and trimmed to a consistent length. After trimming, average sequencing reads length were 220 and 200 nt for forward and reverse reads, respectively. Sequences were then dereplicated by combining the identical sequences into unique sequences to remove redundancy, denoised to remove sequences errors and identify the biological sequence in the reads. A sequence table was obtained with the inferred amplicon sequence variant (ASVs), distributed according to length. ASVs with a length between 220-265 nt were kept and checked for chimera removal by comparing each inferred sequence to the others in the table and removing sequences that can be reproduced by stitching two more abundant sequences. Taxonomy was assigned to each ASV sequence using the SILVA 132 database (https://www.arb-silva.de/) as reference to obtain an ASVs relative abundance table. Phylogenetic tree was constructed using *DECIPHER* and *phangorn* R packages to inform downstream analyses, especially for making comparisons between microbial communities and determine phylogenetic diversities. A phyloseq object was created with the data for its further analysis using the *phyloseq* R package. Sequences identified as chloroplasts and mitochondria were removed. Diversity analysis were calculated by different indicators such as species richness (observed number of ASVs) and α-diversity (Shannon Index). To compare community structure between sample groups principal coordinate analysis (PCoA) plots employing weighted UniFrac distance matrices were performed (Lozupone and Knight, 2005). All indices were determined using the R package *phyloseq* (McMurdie and Holmes, 2013) and plotted using ggplot (https://www.r-project.org/).

5.3. Results and discussion

5.3.1. Biocathodes performances

The two biocathodes were inoculated with 20 mL of an AD mixed culture previously operated in a parent BES operated and stored at thermophilic conditions (50°C; Rovira-Alsina et al., 2020). The adaptation to the mesophilic conditions (25°C) lasted 25 days. No significant VFAs production was detected. Figure 25 presents VFAs production over time after acclimatization period for HT1 (a) and HT2 (b).

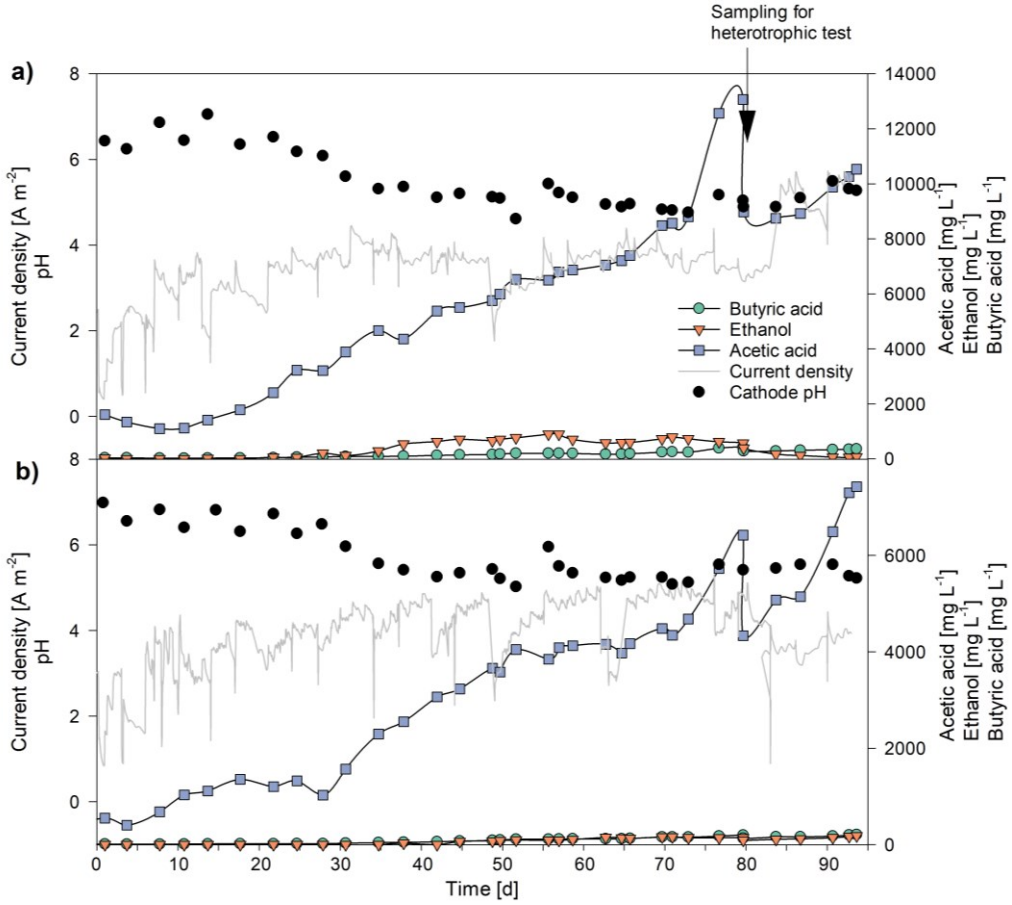

Figure 25. VFAs production throughout the experimentation: a) HT1, b) HT2.

During the first 5 days of operation no significant production was detected in both HT1 and HT2. At day 10th, HT1 started producing acetic (126.33 ± 170.27 mg $L^{-1}d^{-1}$) and butyric acid (5.21 ± 7.35 mg $L^{-1}d^{-1}$), maintaining the production trend constant up to the end of the experimentation. On day 30, pH decreased from 6.3 to 5.6, resulting in ethanol production (15.61 ± 16.73 mg $L^{-1}d^{-1}$). Up to day 60 pH was maintained in the range 5.1÷5.6, leading to an increased ethanol production as expected according to Blasco-Gómez et al. (2019). Later on, pH dropped below 5 linked to in an increment of acetogenic bacteria activity. At day 80, the maximum acetic acid production rate (289.41 mg L^{-1} d^{-1}) and highest concentration of acetic acid in HT1 (13.06 g L^{-1}; Table 9) of the whole experimentation

were obtained at such pH conditions. Comparable values in terms of concentration have been reported only few times in literature. Jiang et al. achieved their maximum concentration of acetate of 13.4 g L^{-1} using an integrated system composed by a slurry electrode and powdered activated carbon (5 g L^{-1}), almost three times the concentration obtained with stainless steel brush only (Jiang et al., 2020). Mohanakrishna et al., (2020) achieved a maximum production of 0.260 g L^{-1} d^{-1} by adding a supplementary inorganic carbon source (15 g HCO_3^- L^{-1}), comparable with peak results obtained in the present study with CO_2 as only carbon source.

On day 80th, one third (60 mL) of the cathodic effluent was extracted to perform microalgae heterotrophic tests, and then, replaced with fresh medium. Production rates for acetic acid and butyric acid were not affected by the liquid extraction. HT2 followed similar trends in production rates and product spectrum.

At the end of the experimentation, on day 95th, both reactors were stopped and microbiology samples from bulk solution and biofilm were collected to perform DNA analysis.

Table 9: Average and peak production rates for acetate, butyrate and ethanol in HT1 and HT2.

	HT1			HT2		
	Acetate	Butyrate	Ethanol	Acetate	Butyrate	Ethanol
mg L^{-1} d^{-1} (Average)	126.33	5.21	15.61	71.83	1.93	4.43
g prod m^{-2}electrode d^{-1}	9.26	0.28	1.19	4.97	0.14	0.20
mg L^{-1} d^{-1} (Peak)	289.41	60.48	59.58	220.60	15.39	56.41
g prod m^{-2}electrode d^{-1}	21.22	4.43	4.37	16.18	1.13	4.14

In terms of CE, both reactors behaviours were different. Since almost no H_2 was found in the headspace of HT1 at every gas sampling point, it was assumed that all the hydrogen produced was consumed in acetic acid production process, while HT2 consumed less H_2 and it was always found in the headspace gas composition, resulting in a lower VFAs production. When comparing coulombs consumed from each reactor as shown in Figure 26, it was evident that the two systems were not equally efficient. HT1 consumed less coulombs and approximately the half of them were devolved to acetate production, while HT2 required way more energy but only 1/3 of the coulombs consumed were devolved to hydrogen or acetic acid production. This could be imputed to fugitive emissions of hydrogen, or the use of electrons to produce undesired compounds. Increased internal resistance due to membrane/electric circuit connections deterioration could explain the difficulty in maintaining the cathode potential fixed at -0.997 V vs Ag/AgCl in HT2 in the latter phase of the experimentation. Maximum CE for acetate production obtained was close to 100 % and 92 % for HT1 and HT2, respectively, for both reactors between days 72-74, while the average CE accounted to 41.5% for HT1 and 20.8% for HT2.

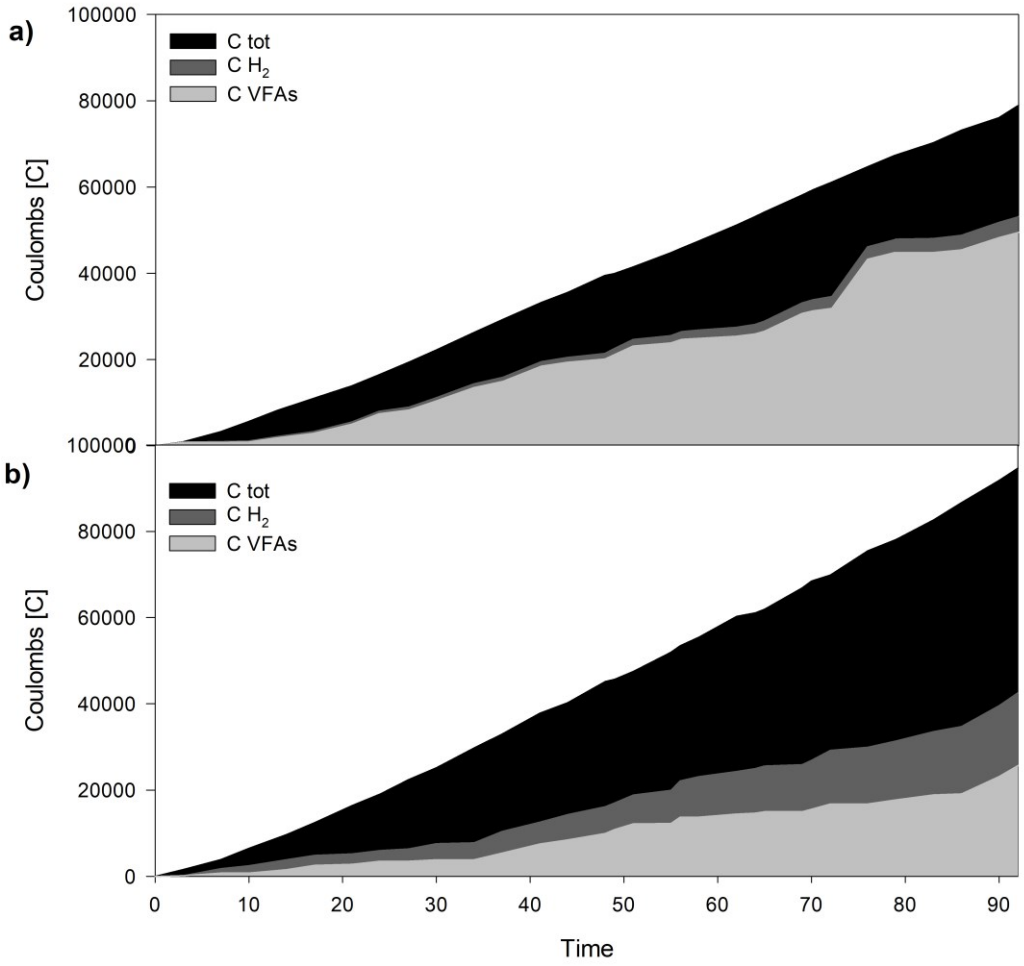

Figure 26. Distribution of the accumulated coulombs in products compared to total coulombs consumed over time (since inoculation) in each reactor in the two H-type BES – a) HT1; b) HT2.

5.3.2. Bacterial community composition

Microbial community structure was analysed by barcoded amplicon sequencing of the 16S rRNA gene. Microbial communities for both BES on day 95th were dominated by *Firmicutes* bacteria, accounting for 93-99% in biofilm and 60-72% in bulk. *Proteobacteria* was also present, being more abundant in liquid samples (26-39%) than in biofilm (1-6%). At the order level, the microbial community was composed by *Clostridiales*, *Betaproteobacteriales* and *Selenomonadales* being the former the most abundant (Figure 27). At the genus level, both reactors were clearly dominated by *Clostridium* spp. Further analyses performed by BLASTn searches revealed the presence of different *Clostridium* species in reactors HT1 and HT2.

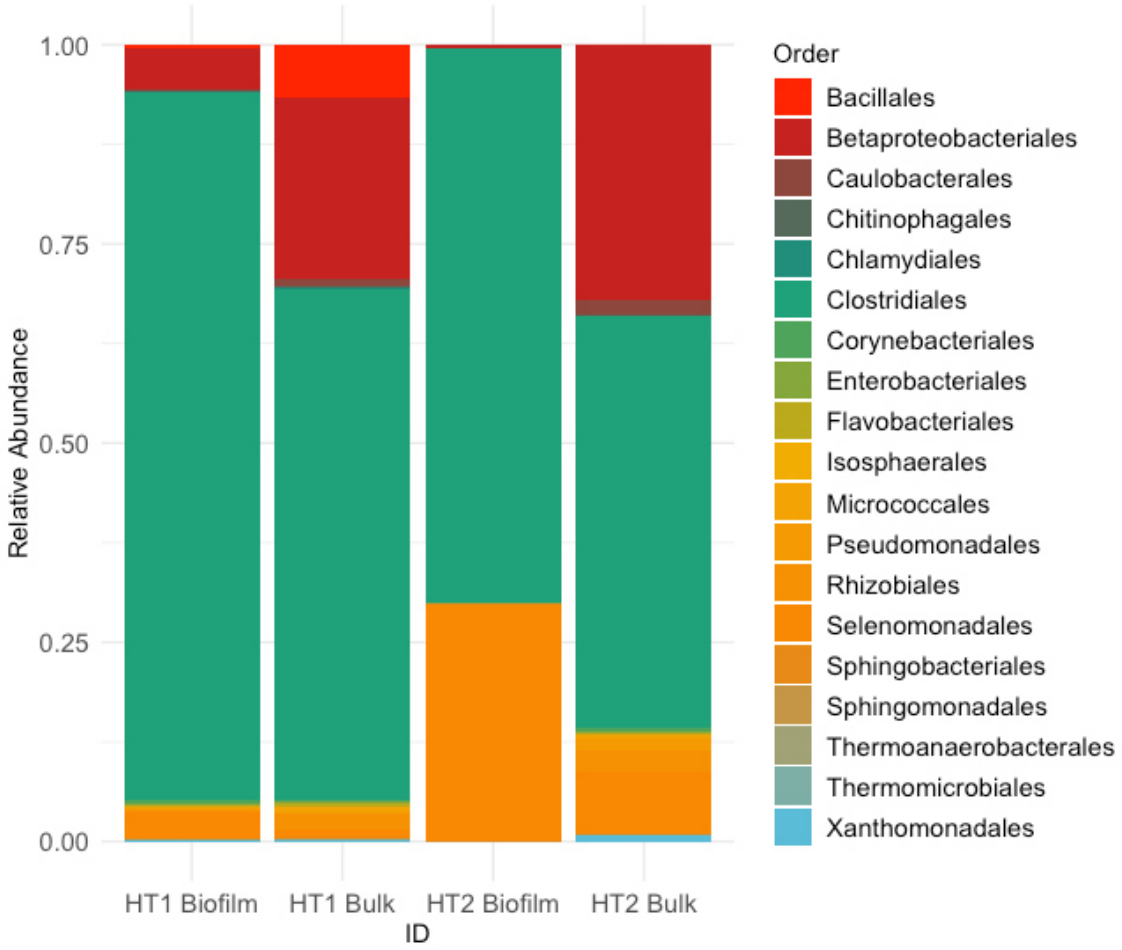

Figure 27. Microbial community composition in biofilm and bulk samples. Bar chart shows relative abundances of main bacterial orders.

Table 10 reports the identification and relative abundance of the most present *Clostridium* spp. in HT1 and HT2. HT1 was characterized by *Clostridium autoethanogenum* and *Clostridium ljungdahlii*. Both species are model homoacetogens, and have been consistently suspected to be able to accept electrons from a cathode (Batlle-Vilanova et al., 2017; Köpke et al., 2010). *Clostridium autoethanogenum* also has a role in autotrophic ethanol production from industrial waste gases (Liew et al., 2017; Liu et al., 2018).

Sequences retrieved from HT2 showed a higher similarity to *Clostridium aciditolerans* and *Clostridium nitrophenolicum* (96 % identity), so far still not reported as typical species in microbial electrosynthesis processes. However, *Clostridium aciditolerans* was proved to survive the acid crash phenomenon, and plays an indispensable role in biohydrogen production and accumulation (Lin et al., 2014). Although additional tests should be performed to confirm the species identity in HT2, the absence of common homoacetogenic clostridia should contribute to the lower acetate production on this reactor.

Table 10: Identification (BLAST, refeseq rna database) and relative abundance of the most present *Clostridium* spp.

ASV	Most probable identification	Similarity (%)	Relative number of sequences (%)			
			HT 1		HT 2	
			Biofilm	Bulk	Biofilm	Bulk
1	*Clostridium autoethanogenum* DSM 10061 NR_121758.1	100	66.2	53.4	< 0.01	<0.01
	Clostridium ljungdahlii DSM 13528 NR_074161.1	100				
2	*Clostridium aciditolerans* strain JW/YJL-B3	96.4	7.5	1.8	70.3	32.2
	Clostridium nitrophenolicum strain 1D	96.4				

5.3.3. Heterotrophic *A. protothecoides* growth tests on biocathode effluent

Heterotrophic tests with cathode effluents were performed. Bothe microalgae *C. vulgaris* and *A. protothecoides* were able to use the acetate-enriched effluent of the biocathodes to grow and replicate. Several one weeklong test were performed with different volumes of microalgae/effluent ratios (Table 8). The results of the preliminary tests with synthetic effluent at different pH were reported in supplementary information (figure S5-S8).

Figure 28 reports results from test A, B and C with *C. vulgaris*. Different initial concentration of VFAs (due to the different microalgae:effluent ratio) were provided to the microalgae. Figure 29 instead shows two replicates obtained from one of the tests performed with *A. protothecoides*. Both tests proved both strains to switch rapidly from autotrophic to heterotrophic metabolism, and to be able to use biocathode produced VFAs for their own growth. In test D and E, previously autotrophically cultivated *A. protothecoides* was diluted up to a concentration of OD_{540} of 1.3 (corresponding to an algal dry weight 0.80 g L^{-1}), by addition of the inorganic medium operated in the BES systems to the concentrated microalgae solution. When cathodic effluent was added, the amount of effluent to be added was calibrated to properly reach a concentration of initial VFAs between 1.5 ÷ 2.0 g L^{-1} in each Erlenmeyer flask. Acetic acid was fully consumed after two days, while more complex molecules (i.e., isobutyric acid) took longer to be assimilated by the microalgae. Chlorophyll a content decreased over time as expected, confirming the fast development of a heterotrophic regime. Microalgae concentration increased by 35%±3% in two days in both flasks. The microalgae concentration decreased again once the substrate was depleted. In a future optimization of the system, feeding cycles for the microalgae can be optimized to last three days, granting the maintenance of the highest growth peak.

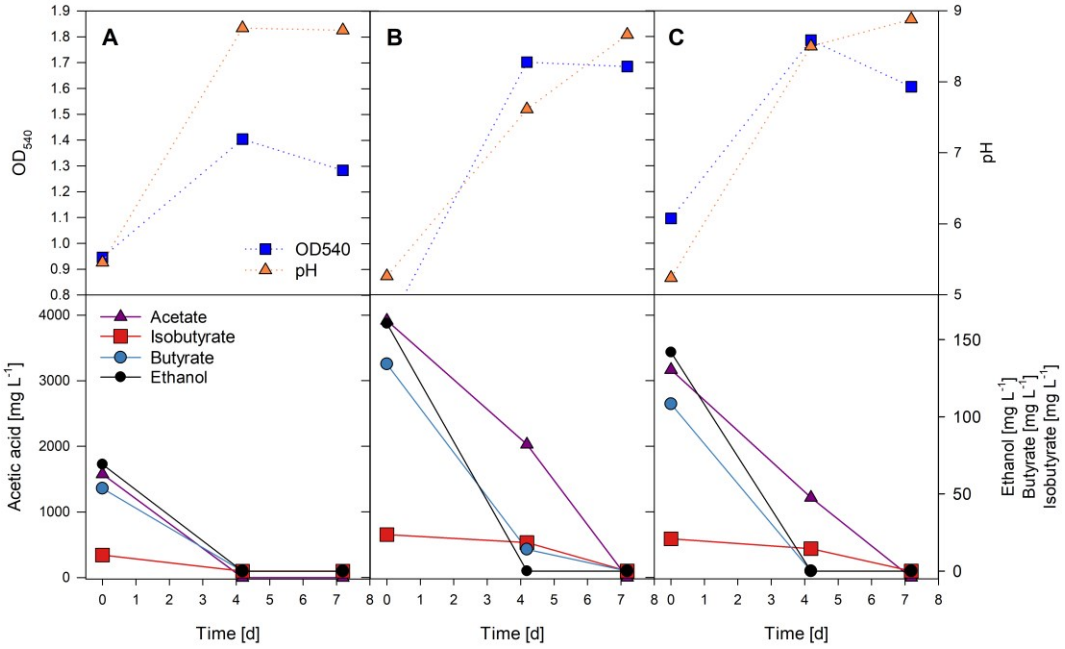

Figure 28. Heterotrophic tests (A, B, C) with cathode effluent at different dilution percentages (*C. vulgaris*).

A quite low quantity of microalgae was collected from each test, since for an 80 mL Erlenmeyer flask only 1-2 g of wet algae were collected. This led to difficult microalgal oil separation and subsequent characterization. However, average oil content obtained from the collected samples in tests D and E accounted to 20±2% w/w, in line with results of previous experiences about batch cultivated *A. protothecoides* with VFAs (Fei et al., 2015). Once optimized the extraction process, biodiesel may consequently be obtained from subsequent transesterification, with product properties complying with EU fuels specifications (Callegari et al., 2020).

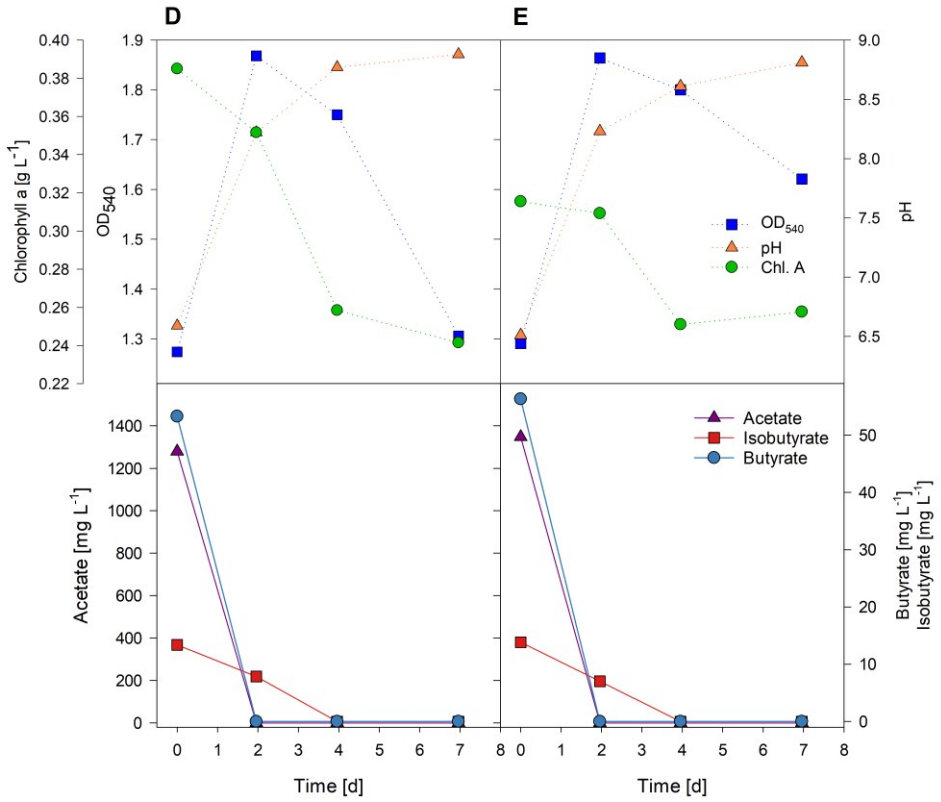

Figure 29. Heterotrophic tests D and E with cathode effluent using *A. protothecoides*.

5.4. Implications

5.4.1. Economic balance

A preliminary economic balance can be assessed with the data collected so far, and literature information concerning microalgal end products. Fei et al. (2015) estimated a cost for biodiesel production at 0.52 € L^{-1} (2.31 \$ gal^{-1}). In this calculation were included 0.29 \$ kg^{-1} for VFAs production cost and nutrients. When using biocathode produced VFAs, considering the performance and consumption of HT1, 12.9 kWh are required to produce 1 kg of acetate (in this estimate electrons spent for ethanol and butyric acid produced were not taken into account), corresponding to a production cost for acetate of 0.83 € kg^{-1}, in line with the values estimated by Prévoteau et al. (2020). Cost of electricity was estimated at 0.07 € kWh^{-1} considering the average non-household price in EU (Eurostat, 2020). Global price of acetic acid has ranged from \$0.35 to \$0.95 per kg between 2010 and 2015 (Wakatsuki, 2015).

Direct reuse of acetic acid produced in MESs avoids the costs for its extraction and purification, lowering the overall economic balance of the process (LaBelle and May, 2017; Xu et al., 2015). In heterotrophic tests D and E conducted in the present study, concentration up to 1.5 g L^{-1} (dry weight) algae were reached in one week-long tests. Lipid yield according to the oil samples collected accounted for ~20% of the cell dry weight,

confirming previous results from Fei et al. (0.19 g/g) for *A. protothecoides* grown on a 2 g L^{-1} VFAs solution.

Considering the values reported in this work, 7.59 $kgCO_2$ and electricity are necessary to produce 1 kg of acetate; starting from that, and without extraction and purification, heterotrophic microalgae can be grown directly on biocathode effluent. Approximately 1.11 kg dry algae per kg acetate can be grown, from which 0.207 kg of lipids could be extracted and further processed for biodiesel production. Costs for microalgae separation and oil extraction however negatively affect the economic balance. Processing costs can be further optimized by producing other commodity compounds from residual pellet (biochar, chemicals) and prior protein extraction. However, it has to be noted that results obtained are based on preliminary data, and only operational costs (excluding the capital costs for reactors construction and maintenance) have been taken into account.

5.4.2. Future developments

H-type BES configuration is useful for proof-of-concept studies; however, one of the major limitations of the present experiment was the low availability of biocathode effluent to perform heterotrophic tests with microalgae. Despite this study proved that heterotrophic microalgae can be efficiently grown on BES produced VFAs, direct conversion possibilities of the algal effluent could not be tested as desired because of this limitation. The first step to improve the value of this study is the design of a setup compatible with continuous mode operation, since H-Type BES only grant ideal conditions for batch operation (high pH_2 in a controlled environment, but difficulty in continuous pH and CO_2 monitoring in the system).

Continuous BES feeding for CO_2 bioelectrorecycling has been tested only a few times in literature so far. Different intermediate strategies, such as continuous CO_2 recirculation, have been operated to enhance acetate production (Mateos et al., 2019). Bajracharya et al. (2017) tested long term operation of a MES reactor in batch and continuous mode. Continuous mode operation was tested at an HRT of 100 h, where influent medium was continuously insufflated with CO_2:N_2 (80:20). An acetate production rate 100 mg L^{-1} d^{-1} was achieved, lower than the maximum amount detected in batch conditions (159 mg L^{-1} d^{-1}). However, long term operation in continuous mode was challenged by the washout effect on suspended micro-organisms that decreased system performance.
Jourdin et al. (2019) showed that it is possible to increase selectivity towards C2, C4 and C6 molecules. Low CO_2 feeding (8.6 L d^{-1}) enhance acetate production (up to 9.8 g L^{-1} d^{-1}); while higher CO_2 loading rate (173 L d^{-1}) and long HRT (14 days) triggers bioelectrochemical chain elongation to longer chain VFAs.

As for microalgae, further characterization of the extracted lipids can be performed via GC-MS or NMR analysis, to fully identify the long chain fatty acid profile (Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2), Linolenic acid (C18:3)) and evaluate its compatibility with biodiesel EU requirements (Diehl and Randel, 2007; Meher et al., 2006). Other recovery options (proteins, commodity chemicals) can also be analysed to increase the economic balance of the process (Liang et al., 2015).

5.5. Conclusions

The two BESs in this study proved to be effective in VFAs production, especially in CO_2 conversion into acetic acid, reaching high concentration (up to 13 g L^{-1} in HT1) and a maximum production rate of 0.29 g L^{-1} d^{-1}. This proof-of-concept study aimed at testing a direct reuse of acetic acid produced in biocathodes as a feed for heterotrophic microalgae aimed at bio-oils accumulation and extraction. Microalgae can use biocathode effluent for their growth. Direct short-chain VFAs reuse by microalgae could optimize the process efficiency, avoiding the costs for acetate extraction and purification. An oil production yield up to 25% w/w oil content was assessed. According to the results of this experimentation, 0.03 kg bio-oil per kgCO_2 captured can be recovered and converted into biodiesel following transesterification process. Future studies will focus on process optimization to further enhance bio-oil production, and to evaluate combined microalgal biorefinery production.

Supplementary Information

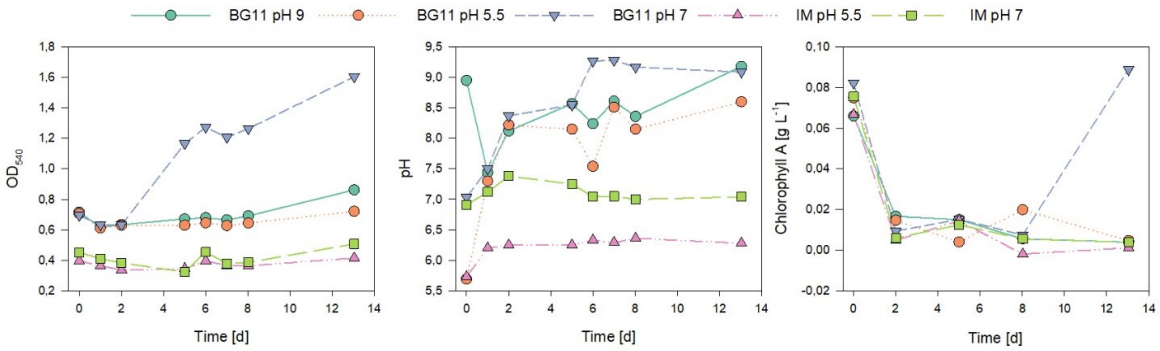

Figure S5. Preliminary *Auxenochlorella protothecoides* autotrophic tests under different medium and starting pH conditions.

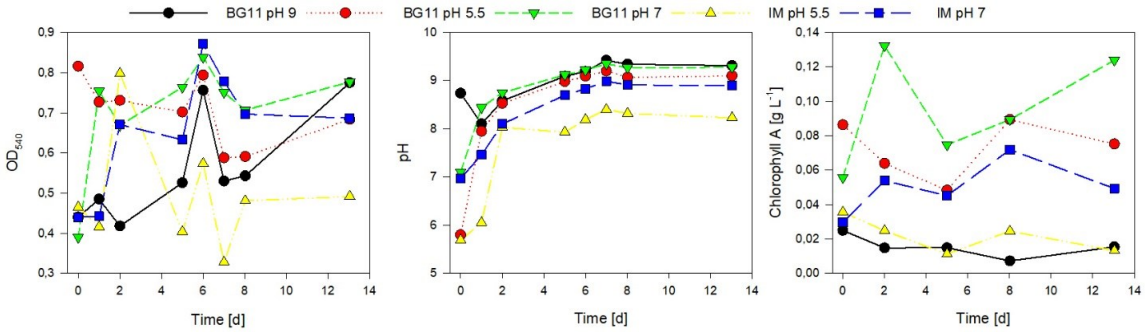

Figure S6. Preliminary *Auxenochlorella protothecoides* heterotrophic tests with synthetic effluent (acetate 2 g L^{-1}) under different medium and starting pH conditions.

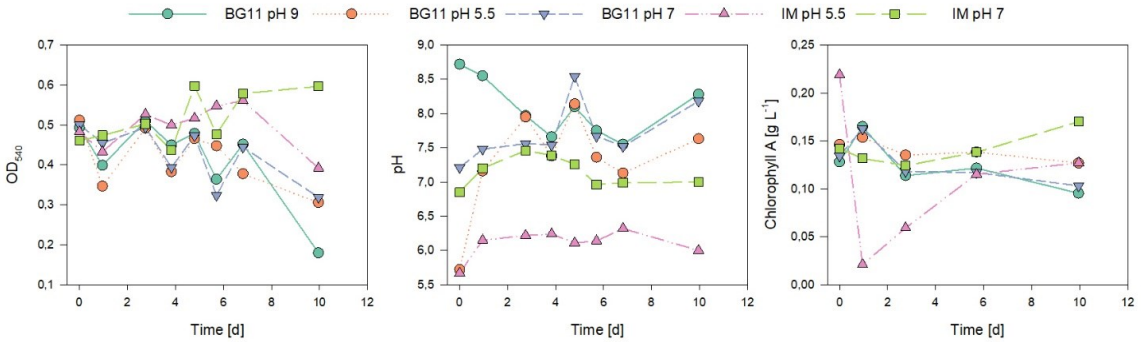

Figure S7. Preliminary *Chlorella vulgaris* autotrophic tests under different medium and starting pH conditions.

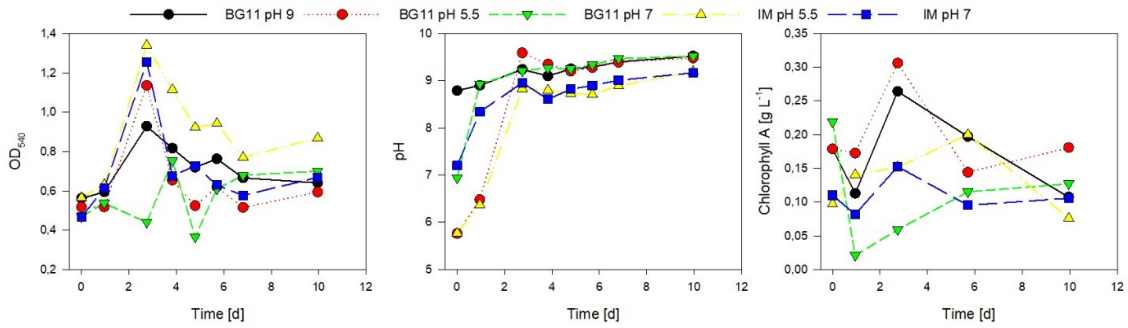

Figure S8. Preliminary *Chlorella vulgaris* heterotrophic tests with synthetic effluent (acetate 2 g L^{-1}) under different medium and starting pH conditions**.**

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6. Power-to-algae: a prototype three-chamber reactor for efficient bioelectroCO_2 conversion

Finding new strategies for mitigation of carbon dioxide (CO_2) emissions and governments sensibilization on environmental problems are at the center of researchers interests nowadays. New global ambitious goals for greenhouse gases (GHG) control have to be set: increase the energy efficiency of productive processes, promote the use of non-fossil fuels, enhance the capture and storage of CO_2 as well as its re-valorization. Microbial electrosynthesis (MES) for bioelectroCO_2 recycling is an interesting, sustainable opportunity to exploit the spent off gases from industrial facilities and convert them into valuable products. The most abundant product from 4th generation biorefineries is acetic acid (C2), of scarce economic value, and high costs for its separation and purification. Hence, a novel design for direct conversion of acetate is herein presented. Two three chamber BES were built and operated in batch mode. Acetic acid production was assessed, and a maximum concentration of acetic acid of 2.2 mg L^{-1} was obtained after two months of operation. The three chambers design allowed the direct reuse of the products in a compact system. The purpose of this study was direct insertion of microalgae in the middle chamber, which were proven to be able to use biocathode effluent for their growth in preliminary batch tests.

6.1. Introduction

In just over a century, human activity has contributed significantly to the constantly increasing greenhouse gas (GHG) emissions worldwide, thus contributing to the global warming phenomenon (Melillo et al., 2009). In recent years, researchers interest switched to find new strategies for mitigation of CO_2 emissions, and sensitized governments to set new global ambitious goals for its control: increase the energy efficiency of productive processes, promote the use of non-fossil fuels, enhance the capture and storage of CO_2 as well as its re-valorization.

CO_2 is a very abundant resource, and it can be obtained both from the atmosphere or directly from industries, moving towards a circular economy and contributing to the decarbonized economy (Rosso and Stenstrom, 2008). One interesting possibility for CO_2 conversion is through microbial electrochemical technologies (MET) (Bian et al., 2020; Nevin et al., 2010; Rabaey and Rozendal, 2010). METs are a versatile platform that can provide multiple solutions to several environmental problems: direct electrical energy recovery from wastewater (Bolognesi et al., 2020; ElMekawy et al., 2015; Kim et al., 2015); groundwater bioremediation (Cecconet et al., 2018; Pous et al., 2018); hydrogen production (Batlle-Vilanova et al., 2014; Kadier et al., 2016a, 2016b); sensing and water quality control (Jia et al., 2018; Wang et al., 2018); microbial electrosynthesis. Microbial electrosynthesis comprehends many microbial biorefinery processes, aiming at converting

electrical energy and a waste substrate (gaseous, liquid or solid) into value added compounds: CO_2 conversion into volatile fatty acids (VFAs) and alcohols (Modestra and Mohan, 2017; Rovira-Alsina et al., 2020); production of chemical compounds of industrial interest (Vassilev et al., 2018), generation of bioplastics (Pepè Sciarria et al., 2018).
In the case of bioelectroCO_2 conversion, process efficiency of conversion of electric energy into soluble products relies on the performance of chemical or biological catalysts. Microbial bioelectrocatalysis based on CO_2 capture and fixation has several advantages: (i) the production of CO_2-neutral commodities; (ii) versatility of operation; (iii) minimization of competitive land use with food production (Rovira-Alsina et al., 2020).
Unfortunately, microbial electrosynthesis has still many challenges to overcome to be considered in industrial applications (Dessì et al., 2021). Efficiency of the process of microbial CO_2 reduction into multi-carbon chemicals is still low and autotrophic growth of electroactive biomass is an energy consuming process (Prévoteau et al., 2020). Costs of separation and purification of chemicals produced in 4th generation biorefineries is still high, and affects the global economic balance of the process, especially in the case of short chain fatty acids (LaBelle and May, 2017).

New strategies for process optimization have to be studied, to ease filling of the technological and economical gap separating this technology from scaling-up (Bian et al., 2020). Nowadays, the coupling of BES systems with microalgae systems is an interesting option to enhance resource recovery by applying a biorefinery approach (Bolognesi et al., 2021; Jiang et al., 2013; Sevda et al., 2019). So far, direct microalgae integration in MES has not been explored. Hence, a three-chamber setup designed to directly recover biocathode produced VFAs is herein presented, aimed to directly integrate microalgae in the system. The use of a three chamber system can lead to several advantages; depending on membranes configuration, different effects can be exploited, for example desalination (Saeed et al., 2015) or concentration of the compounds produced for further extraction and reuse (Gildemyn et al., 2015). When integrating heterotrophic microalgae in the middle chamber, the additional effect of oxygen scavenging could be added to the advantages of using a three chambered device.

6.2. Materials and methods

6.2.1. Experimental setup

Two replicates of three-chambers methacrylate BES were built and operated, named respectively R1 and R2 (Figure 30). Each reactor was composed of three squared frames of 17x17x2 cm (internal hole 9x9x2 cm), one for each chamber. The two external frames were closed by a methacrylate plate of 13x13x1 cm. Each chamber could be opened and inspected individually. Cathodic and middle chamber were separated by a cationic exchange membrane ($2 \cdot 10^{-4}$ m^2, CMI-1875T, Membranes International, USA), while an anionic exchange membrane chamber ($2 \cdot 10^{-4}$ m^2, AMI-7001S Membranes International, USA) separated the anodic and the middle chamber. Carbon cloth (working electrode, surface 0.01445 m^2, thickness 490 μm; NuVant's ELAT, LT2400 W, FuelCellsEtc., USA) connected to a stainless-steel wire was used as cathode while a graphite rod was used as anode electrode (counter electrode). An Ag/AgCl reference electrode (+0.197 V vs. SHE, model SE11, Sensotechnik Meinsberg, Germany) was placed in the cathodic chamber. BES were operated in a three-electrode configuration with a potentiostat (NEV 3.2, Nanoelectra,

Spain) controlling the cathode potential at -0.8 V vs SHE. 3D-printed spacers were added in each chamber to minimize volume variation in the three chambers. The net liquid volumes for anodic, middle, and cathodic chambers were 140 mL, but each chamber was connected to a 250 mL buffer tank, increasing the net liquid volume of each chamber to 250, 250 and 300 mL, respectively. Complete mixing of the cathodic chamber was granted by using a multichannel recirculation pump (40 L d^{-1}, WM 205U, 12 channels, Watson Marlow, USA). The experiment was conducted at 25±3 °C. Both cathodes were inoculated with 100 mL of inoculum from a parent BES (operated in Chapter 5 of the present Ph.D. thesis).

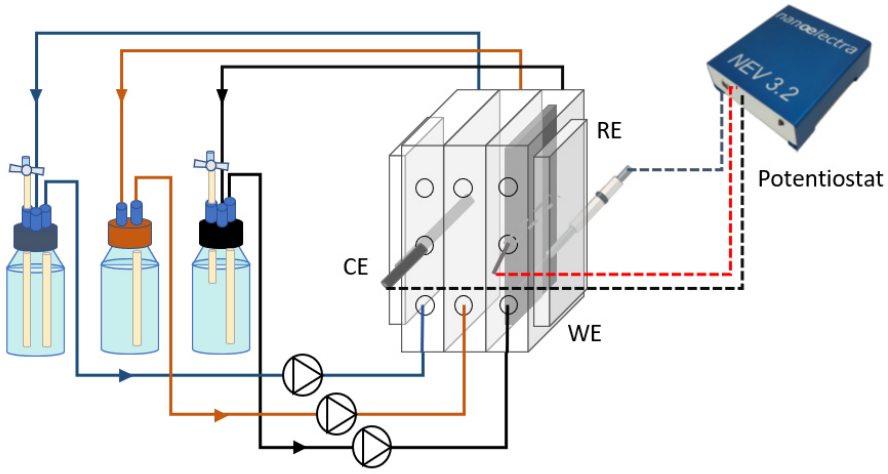

Figure 30. Schematic of the three-chamber BES setup.

6.2.2. BES operation

The two BES were operated in recirculated batch mode. Both anodic and cathodic chambers were filled with a low-buffered inorganic medium (modified ATCC1754 PETC medium adjusted at pH 6, as reported in Blasco-Gómez et al. (2019) (Table 11). CO_2 (99.9%, Praxair, Spain) was the only carbon source, and it was supplied to the systems every 3 days for 1 minute, saturating the cathodic chamber. Conductivity and pH were monitored throughout the experimentation. Before feeding, gas and liquid samples were collected and analysed to monitor the gas composition and the production of volatile fatty acids (VFAs) and alcohols. The experimentation was carried out for 70 days before it had to be stopped due to COVID-19 lockdown.

Table 11: Modified ATCC1754 PETC medium recipe.

	Concentration (g L^{-1})
NH_4Cl	1.0
KCl	0.1
$MgCl_2 \cdot 6H_2O$	0.2
NaCl	0.8
KH_2PO_4	0.1
$CaCl_2 \cdot 2H_2O$	0.02
MES buffer	1.95
L-cysteine HCL	0.4
Vitamins *	1 mL L^{-1}
Trace elements ^	1 mL L^{-1}

* For 1 L distilled water: 2.0 mg Biotin; 2.0 mg Folic acid; 10.0 mg Pyridoxine hydrochloride; 5.0 mg Thiamine . HCl; 5.0 mg Riboflavin; 5.0 mg Nicotinic acid; 5.0 mg Calcium D-(+)-pantothenate; 0.1 mg Vitamin B12; 5.0 mg p-Aminobenzoic acid; 5.0 mg Thioctic acid.

^ For 1 L distilled water: 2.0 g Nitrilotriacetic acid; 1.0g $MnSO_4 \cdot H_2O$; 0.8g $Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$; 0.2g $CoCl_2 \cdot 6H_2O$; 0.2 mg $ZnSO_4 \cdot 7H_2O$; 20.0 mg $CuCl_2 \cdot 2H_2O$; 20.0 mg $NiCl_2 \cdot 6H_2O$; 20.0 mg $Na_2MoO_4 \cdot 2H_2O$; 20.0 mg Na_2SeO_4; 20.0 mg Na_2WO_4.

6.2.3. Analyses and calculations

VFAs and alcohols in the liquid phase were analysed with a gas chromatograph (GC) (Agilent 7890A, Agilent Technologies, USA) equipped with a DB-FFAP column and a flame ionization detector (FID). Unless otherwise stated, the concentration of organic compounds in the liquid phase is expressed throughout the manuscript in mg L^{-1}. Conductivity (EC), pH and optical density (OD_{600}) were also measured on the liquid sample. Gas pressure in the headspace of the reactor was measured before sampling and after feeding with a differential manometer (Model-Testo-512; Testo, Germany). Gas samples were taken using a glass syringe before taking liquid samples, and analysed using a Micro-GC (Agilent 490 Micro GC system, Agilent Technologies, US) equipped with two columns: a CP-molesive 5A for methane (CH_4), carbon monoxide (CO), hydrogen (H_2), oxygen (O_2) and nitrogen (N_2) analysis, and a CP-Poraplot U for carbon dioxide (CO_2) analysis. Both columns were connected to a thermal conductivity detector (TCD). The partial pressure of hydrogen (pH_2) was calculated from the total pressure before taking the gas samples and the composition of the gas detected in the headspace of the biocathode. The concentration of dissolved H_2 and CO_2 were calculated according to Henry's law at 25°C. Both BES after the sampling phase were flushed and then saturated with pure CO_2, assuming conditions of CO_2 saturation and absence of H_2 in the liquid phase after each feeding. Coulombic efficiency (CE) was calculated according to Rovira-Alsina et al. (2020).

6.2.4. Microalgae cultivation and analysis

Two algal strains, *Chlorella vulgaris* 211-11b and *Auxenochlorella protothecoides* 211-7a (SAG, Culture Collection of Algae, Goettingen, Germany) were cultivated in autotrophic conditions throughout the experimentation. Two methacrylate tubular

photobioreactors (d =0.04 m, h = 1 m, 1 L each) were built to preserve the cultures from external contamination. Air was provided through a diffuser from the bottom of the photobioreactor to prevent sedimentation of the microalgae and keep the culture in suspension. BG-11 medium containing $NaNO_3$ 1.5 g L^{-1}, MgSO4·7H2O 0.075 g L^{-1}, K_2HPO_4 0.040 g L^{-1}, $CaCl_2 \cdot 2H_2O$ 0.036 g L^{-1}, Na_2CO_3 0.020 g L^{-1}, Citric acid 0.006 g L^{-1}, Ferric ammonium citrate 0.006 g L^{-1}, H_3BO_3 2.86 $\cdot 10^{-3}$ g L^{-1}, $MnCl_2 \cdot 4H_2O$ 1.81 $\cdot 10^{-3}$ g L^{-1}, EDTA (disodium salt) $1 \cdot 10^{-3}$ g L^{-1}, $NaMoO_4 \cdot 2H_2O$ 0.39 $\cdot 10^{-3}$ g L^{-1}, $ZnSO_4 \cdot 7H_2O$ 0.222*10^{-3} g L^{-1}, $CuSO_4 \cdot 5H_2O$ 0.079 $\cdot 10^{-3}$ g L^{-1}, $Co(NO_3)_2 \cdot 6H_2O$ 0.049$\cdot 10^{-3}$ g L^{-1} and adjusted at pH 7 was added periodically to grow the strains.

Chlorophyll A analysis was performed at the beginning of each test and at least every second day. 2 mL of sample was taken and put in a clean tube and centrifuged (10000 rpm, 10 min) to pellet cells. Supernatant was then discarded, and 2 mL of acetone was added to the pellet and mixed. Tubes were left overnight in the freezer (-20 °C) to facilitate complete extraction. Samples were centrifuged again (10000 rpm, 10 min), and the supernatant OD was then measured at different wavelengths.

Oil extraction was performed at the end of every batch test. Samples were put in falcon tubes and then centrifuged to separate the pellet from water. Algal cells were then resuspended in distilled water to remove impurities and centrifuged again (6000 rpm, 10 min). 10 mL n-hexane (purity ≥99%, Sigma Aldrich) per gram of wet algae were then added, and cell disruption was operated mechanically at first by friction and then using an ultrasonic bath (40 kHz, 10 min). Tubes were centrifuged again to separate the different phases (pellet, residual water and n-Hexane with lipids dissolved in it). Supernatant was then separated, and oil was extracted using a rotary evaporator (R-215 Rotavapor, BUCHI, Switzerland).

6.3. Results and discussion

6.3.1. VFAs production in a three chamber MES reactor

Figure 31 and 32 show the evolution in the activity of the enriched acetogenic culture in the two replicates of the operated bioelectrochemical system. Both reactors were operated under similar conditions (25 ºC, fed with CO_2 every three days and at a fixed potential of -0.8 V vs SHE). The first days of operation correspond to the period of acclimatization of the microbial community to the new configuration. The two reactors responded differently in terms of acclimatization time after the inoculation. In R1, this period was 25 days, while in R2 it was extended to 40 days.

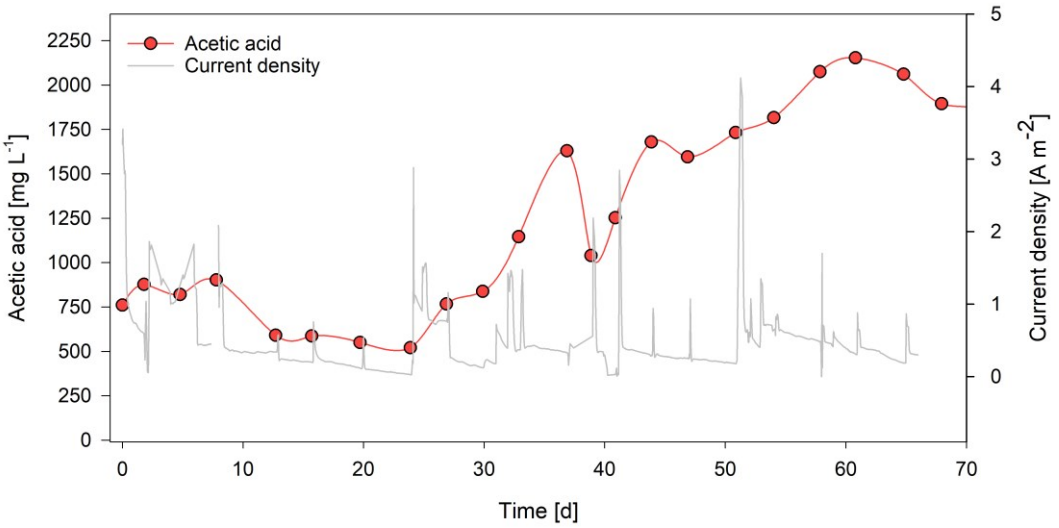

Figure 31. VFAs production profile in R1.

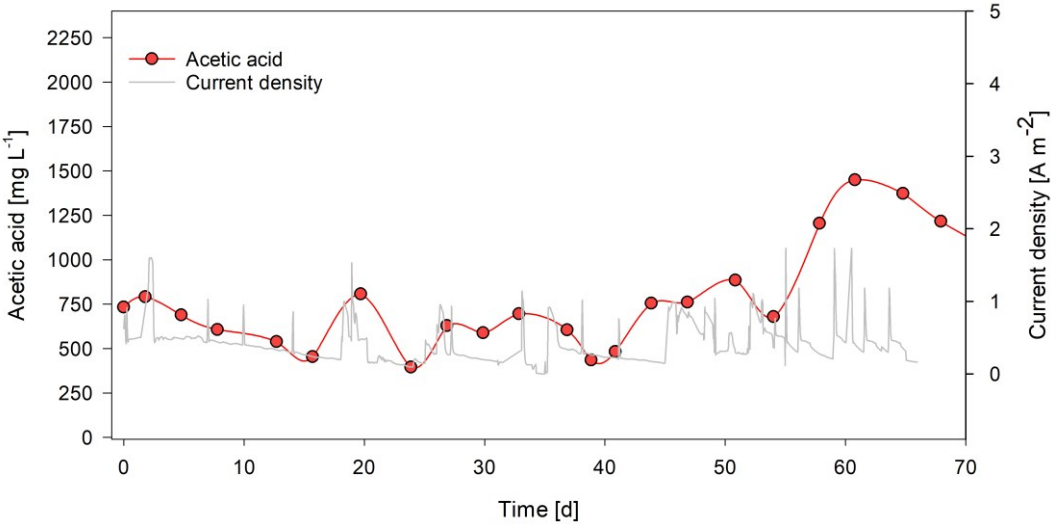

Figure 32. VFAs production profile in R2.

Both reactors had a similar evolution in the electrical behavior. Average current density were 0.449 A m^{-2} and 0.378 A m^{-2} for R1 and R2. The cathode potential of the two cathodic chamber was also oscillating according to the supply of the system with CO_2. Each time CO_2 was fed to the system, the electro-active microorganisms increased their activity and consequently the demand for electrons, as they had more substrate for the production of compounds, explaining the presence of many current density peaks.

No ethanol production was detected in either reactor, traces of butyric acid were instead found. Butyric acid profile evolves differently in the two reactors. In R1, the concentration of this compound increased progressively from day 25 to its maximum; on the other hand, butyric acid present at the beginning in R2 disappeared completely until day 50, when it was detected again. However, in both systems the peak of butyric acid occurs in the last

days of operation, reaching concentrations of 42.6 and 31.9 mg L^{-1} at R1 and R2, respectively. This value however can be considered negligible if compared to acetic acid.

Throughout the experimentation pH in the biocathode had the tendency to increase (7÷8.5), promoting the activity of methanogenic bacteria (Batlle-Vilanova et al., 2017). Following the detection of a small amount of methane (<20%) in the gas phase and traces of methanol in the liquid phase (<50 mg L^{-1}), a methanogen inhibitor (2-Bromoethanesulfonate, BES, 0.5 mM) was added to the biocathode to prevent an increase in this gas effluent. the presence of methanol in the liquid phase gradually decreased in the following samples. However, it was difficult to verify improvements in the gas phase, because gas sampling was often difficult due to low overpressure and variations in headspace volume. The presence of the recirculation pump caused pressure leaks, resulting in very low to no overpressure in biocathode headspace.

The acclimatization period was considered to end when the concentration of acetic acid began to increase, as this was the main product derived from acetogenesis. In R1, the period of acclimatization of microorganisms ended on day 25, when there was a marked increase in the concentration of acetic acid, while in R2 this period was extended to the 40th. The maximum amount of acetic acid was reached on day 60, with concentrations of 2.153 and 1.448 mg L^{-1} in R1 and R2, respectively.

Table 12 compares the average production of the system with that observed during the most productive periods for acetic acid and butyric acid. Acclimatization period was excluded from these calculations.

Gildemyn et al. (2015) achieved way higher production in their three-chamber reactor with simultaneous acetate extraction from the middle chamber, reporting a production rate ten times higher (22.3 g m^{-2} electrode d^{-1}) than the one obtained in the present experimentation. It should be taken into account that in the experiment herein presented, only CO_2 was used as inorganic carbon source, while Gildemyn et al. used a bicarbonate 30 mM buffer solution as catholyte. However, concentrating middle chamber showed a more efficient carbon conversion, probably due to a more efficient electrolyte distribution along the three chambers with lower ion transport resistance related losses.

Finally, the columbic efficiency (CE%) for acetic acid production were calculated, both for the total operation time and for the most productive period. Average CE for R1 accounted to 35 %; while for R2, average CE obtained was 25%. In contrast, assuming only the most productive periods of this compound, CE for acetate was 82% in R1 and 97% in R2.

Table 12: CE, average and peak production rates for acetate and butyrate produced in R1 and R2 biocathodes.

	R1		R2	
	Acetate	Butyric acid	Acetate	Butyric acid
mg L^{-1} d^{-1} (Average)	37.93	0.51	21.01	0.33
g prod m^{-2}electrode d^{-1}	0.79	0.01	0.44	0.01
mg L^{-1} d^{-1} (Peak)	110.89	0.65	98.33	2.92
g prod m^{-2}electrode d^{-1}	2.30	0.02	1.43	0.07
CE [%]	34.88	-	24.71	-

6.3.2. Electrolytes migration

The three-chamber MES reactor was designed with the intention of integrating a culture of microalgae of the *Chlorella protothecoides* species into the middle chamber for bioelectroCO_2 recycling. Once the system reached the steady state, continuous operation would be followed by a recirculation of the cathodic chamber to the middle chamber to provide a carbon source to the microalgae. Before the insertion of the microalgae, the electrolytes migration under the chosen operational conditions in the three-chamber reactor had to be checked. Therefore, the electrolytes flow was monitored by checking various parameters (OD_{600}, pH and EC) in the three chambers (cathodic, middle and anodic). At the start-up, the same inorganic medium was introduced into all three chambers, and the cathode was inoculated with enriched acetogenic culture. Consequently, at first the conditions in the three chambers were similar, while beginning from the second sampling point particular patterns began to be found in each chamber. Figure 33 shows the results of the monitoring of the three chambers during the 70 days of operation.

OD_{600} revealed an initial phase of biocathodic microbial growth in the first 30 days, where it stabilized around 0.18 at R1 and 0.2 at R2, confirming the same acclimatation time assumed from the VFAs production. Optical density increased more rapidly in the cathode chamber than in the other chambers, which can be attributed to the enrichment of the microbial community in the biocathode. From this fact it can be deduced that some of the microorganisms did not fully adhere to the electrode biofilm and remained floating in the bulk solution. The anodic chamber maintained low absorbance values (0-0.05) in both reactors and with a tendency to increase gradually, which could be attributed to the deterioration of the graphite rod (sacrificial anode). Contrary to the similar trend of OD evolution over time in the cathode and anode chambers in the two replicates, in the middle chamber the values recorded did not lead back to any specific pattern. The OD_{600} remained low on the R1 until day 60, when it suffered a sharp rise and began to vary. In contrast, in R2 the OD_{600} oscillated from the first moment between values of 0.02 and 0.1 (Figure 33.A).

In the cathode chamber of both reactors, pH variations between values of 7 and 8 were observed. The peak production periods coincided with a decrease in pH (6.5 ÷ 7) at the cathode due to the use of protons for product generation. Below these high pH values, acetogenesis (acetate formation) is the preferred metabolic pathway and solventogenesis (ethanol formation) is disadvantaged (Mohanakrishna et al., 2016).

In both replicates the pH difference between chambers was important. In the anodic chambers the pH remained very acidic throughout all the experimentation (around 1), due to the production of protons from the electrolysis of water. Theoretically, the protons produced at the anode had to migrate across the membranes and reach the cathode chamber to compensate for the negative charge caused by the transfer of electrons from the anode, but the AEM blocked their passage provoking an accumulation of protons in the anodic chamber. Furthermore, the values recorded at the cathode were between 7 and 8. Such a fact could also be attributed to difficulties protons flow across membranes and relative accumulation of anions in the cathodic chamber. To ensure the good performance of the biomass, pH was corrected to value avoid excessively basic pH in the cathodic chamber by dosing HCl 10M. Finally, in the middle chamber pH values ranged from 2 to 5 increasing when fresh medium was added, and decreasing with the accumulation of H^+ migrating from the CEM (Figure 33.B).

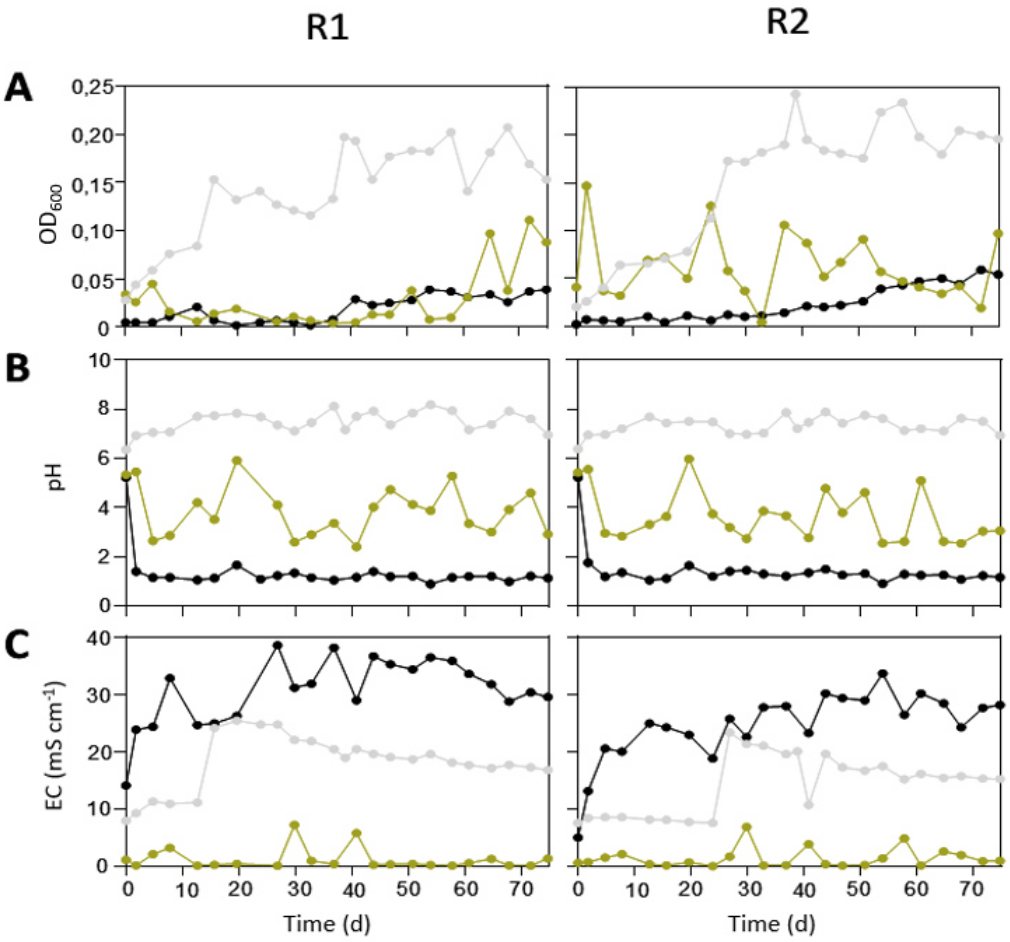

Figure 33. Results in terms of OD_{600} (A), pH (B) and conductivity (C) of the electrochemical behavior of three chambers MES reactor: cathodic chamber (grey), middle chamber (green) and anodic chamber (black). R1 (left) and R2 (right) results over time are reported.

With this configuration of membranes, the middle chamber acted as a desalination chamber (Forrestal et al., 2012; Luo et al., 2011). Due to the presence of semipermeable membranes, negatively charged compounds migrate to the anodic chamber through the AEM, while anions migrate to the cathodic chamber by passing through the CEM. For this reason, the conductivity in the central chamber remained at all times close to 0 mS cm^{-1}. High conductivity values, 20-40 mS cm^{-1}, were recorded in the anodic chamber as a result of accumulation of salts from the central chamber. In terms of conductivity in the cathodic chamber, a decreasing trend was generally observed over time. However, sudden increases were seen on days 12 (R1) and 24 (R2), which coincided with an increase of OD_{600} at the cathode. These variations can be attributed to the rupture of the reference electrode and subsequent release of electrolytes in the cathodic medium (Figure 33.C).

6.3.3. Carbon balance

During the operation of the system the volume of the headspace - gas phase – was slightly variable because of membrane deformation that caused fluctuation in each chamber volume. Complete saturation of medium and 100% CO_2 after feeding was assumed, while before every liquid sampling point a gas sample was collected and analyzed. It must be noted that most times the systems were very close to atmospheric pressure. It was assumed that the microorganisms had consumed all the introduced CO_2 and all the H_2 generated was used for the synthesis of products or lost due to leaks caused by the recirculation pump. Table 13 shows the carbon balance of the system during the 70 days of operation considering acetic acid as end product.

The CO_2 inputs are similar to both reactors; however, carbon balance is slightly more favorable for R1 because of the higher acetate production.

As for acetic acid production, also carbon balance is lower than other works reported in literature.

Rovira-Alsina et al. (2020) assessed an associated carbon ratio from CO_2 to acetic acid of 0.31 ± 0.86 kg of product per kg of CO_2 consumed, way higher than the 0.074 ± 0.022 kg of product per kg of CO_2 consumed of the present experimentation. Furthermore, also results for parent H-Type reactors reported in Chapter 5 in terms of carbon conversion efficiency are better performing (0.089 ± 0.056 kg of product per kg of CO_2).

Table 13: Carbon balance (considering input and outputs) for R1 and R2 biocathodes. Only acetate was considered in the calculations.

	R1	R2
%C conversion	7.7	5.4
molCO_2:molC	12.93	18.44
kgCH_3COOH kgCO_2^{-1}	0.105	0.074

6.4. Implications

Two-chamber reactors have widely been used and studied in microbial electrosynthesis (Marshall et al., 2013; Modestra and Mohan, 2017; Vassilev et al., 2018). In the present study, a three-chamber reactor was designed with the purpose to integrate microalgae into the CO_2 bioelectrorecycling system. However, some limitations of this configuration have occurred during implementation.

As already mentioned, a three-chamber configuration is commonly used as MDC (Microbial Desalination Cell) (Kokabian et al., 2018; Saeed et al., 2015; Santoro et al., 2017). However, literature concerning three chamber MES is limited; it was reported an experimental work from Gildemyn et al. (2015) in which a three-chamber MES batch reactor for production and extraction of acetic acid from CO_2 was operated. The middle chamber was used to accumulate acetate and easily extract it, avoiding excessive stress for the biocathodic biomass and lowering the risk of acid crash (Ibrahim et al., 2018). In their study, Gildemyn et al. (2015) obtained a central chamber maximum concentration of 13.5 g L^{-1} of acetic acid.

Vassilev et al. (2019) proposed a three chambered double (bio)cathode MES reactor for simultaneous acetogenesis, solventogenesis and carbon chain elongation, by application of optimal pH conditions enabling the production of valuable carboxylates and higher

alcohols from CO_2 and electricity. Maximum acetate production in a biotic test reached 9.50 g L^{-1} in cathode compartment 2 (CC2, pH 6.9, optimal acetogenesis conditions), plus the amount migrated through the AEM to cathode compartment 1 (CC1, pH=4.9, abiotic) of 12.4 g L^{-1}, corresponding to 55% of the total production. With both biocathodes equally inoculated, a larger product diversification was observed according to favorable pH and pH_2 conditions, CC1 enhanced solventogenesis. Isobutyrate (1.33 g L^{-1}), butyrate (1.49 g L^{-1}), and caproate (0.27 g L^{-1}) were detected in both CC2 and CC1 (via electromigration), while other C4-C6 alcohols, isobutanol (0.33 g L^{-1}), butanol (0.82 g L^{-1}), and hexanol (0.11 g L^{-1}), were found only in CC1.

So far, despite the increased complexity of the system, three chambered BES reactors led to several advantages in microbial electrosynthesis process improvement; thus, the configuration herein presented with microalgae integration represents a step forward for direct reuse of biocathode produced VFAs.

6.4.1. Possible benefits from microalgae integration

Microalgae integration could lead to several advantages, amongst all direct reuse of acetic acid produced in MESs, avoiding this way the costs for its extraction and purification, and consequently lowering the overall cost of the process (LaBelle and May, 2017; Xu et al., 2015). The presence of microalgae can improve the overall energy and economic balance of waste substrate treatment, by combining the exploitation of different materials and biofuels precursors potentially recoverable from conversion of algal biomass. Liquid biofuels, e.g. biodiesel, bioethanol, biobutanol and jet fuels, are the most likely outcomes of algal biorefining (Dasan et al., 2019; Liang et al., 2015). When comparing biodiesel production between microalgae and vegetable crops, the results are very promising in terms of compatibility with diesel engines (Chisti, 2008). It should be noted that, apart from oils, algal biomass also contains significant amounts of proteins, carbohydrates and other nutrients (Morales-Sánchez et al., 2015). Therefore, once the oil is obtained, the residual pellet from oil extraction could be easily converted into biochar by thermal treatment, increasing the overall economic balance of the systems (Yu et al., 2017).

Currently costs for biodiesel production from microalgae are very high, with (mainly artificial) light (in autotrophic cultivation) and carbon source (CO_2/organic carbon compounds) having the most impact. While considering possible variables to increase the lipid content of biomass, Fei et al. (2015) managed to significantly reduce the costs of biodiesel production, from 5.1 \$ L^{-1} (autotrophic cultivation, 30% lipids) to 2.3 \$ L^{-1} (in heterotrophic cultures with VFAs, up to 50% lipids).

Alternative product outcomes for heterotrophically grown microalgae, such as chemicals and recovery of different materials, can be considered. Examples are slow-release fertilizers, since microalgae accumulate a variety of essential nutrients, which are recoverable in form of dried biomass or biochar from pyrolysis, depending on process operational parameters during thermal treatment (Bolognesi et al., 2019). The nutritional value of microalgae could lead to a possible use in the food, feed and pharmaceutical market, however, algae grown in wastewater treatment processes might still present issues in terms social acceptance; so far, the most favorable market outlets for microalgae recovery consist of biofuels production, biofertilizers and soil amendment products (Mathimani and Pugazhendhi, 2019). Furthermore, the looming fossil fuels crisis encourages an expansion of the renewable energy sector in the next decades. Algal biomass

has been designated a central role in the future eco-fuel panorama (Callegari et al., 2020), even though its production cost is still not competitive with conventional fossil fuels' (Lundquist et al., 2010).
Ruiz et al. (2016) estimated costs and revenues potentially achieved from a 100 ha microalgal biorefinery plant. Production of bulk commodities as fuels, chemicals or food results in a biorefinery costs about 1 € kg^{-1}, with main costs connected to energy required for cell disruption biomass drying, lipid extraction and solvent recovery. It has to be noted that, the lowest revenue per unit of biomass comes from biofuel (0.3 € kg^{-1}), way lower than its production cost; however, using lipids for biofuels and the remaining components for chemicals, could increase biomass market price up to 1.85 € kg^{-1}. A full-circle biorefinery (including biofuels, proteins and other chemical commodities) could result in a net profit potential of 7.6 € kg^{-1} (Ruiz et al., 2016).

6.4.2. Future developments

Some modifications to the herein presented systems must be introduced to improve their efficiency and overcome the drawbacks emerged in the first 70 days of operation. First, by reversing the position of the membranes, problems due to accumulation of protons (anodic chamber) and anions (cathodic chamber), and subsequent pH issues, could be easily solved. Furthermore, concentration of VFAs would occur directly in the middle chamber instead, simplifying the system in terms of recirculation between the two chambers (Gildemyn et al., 2015).

This setup modification step (Figure 34b) may open the way to a continuous operation of the biocathode at 14 days hydraulic retention time (HRT) with fresh medium saturated of CO_2 as a feed, and, eventually, intermittent or continuous gas CO_2 feeding, in order to increase the acetic acid availability for subsequent production. Continuous operation of the system would also partially solve the gas pressure retention problems caused by the recirculation pump, since CO_2 will be dissolved directly in the freshly renewed medium and constantly renovated.

Once reached a stable, consistent production rate, microalgae could be inserted in the middle chamber recirculation line and cultivated heterotrophically by using biocathodic produced VFAs as a carbon source, as previously demonstrated in Chapter 5 of this thesis; the insertion of a microfiltration unit between the buffer tank and the middle chamber can prevent microalgae from overrunning the reactor and causing membrane fouling.

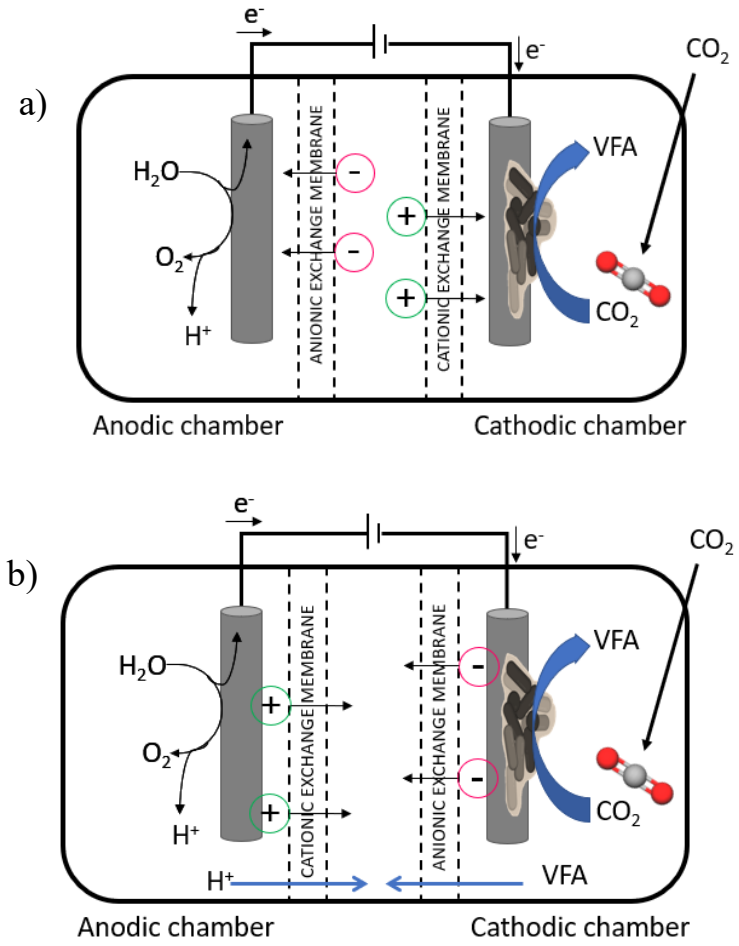

Figure 34. Current setup (a) and proposed modification (b) for direct VFAs migration and exploitation by the microalgae in the middle chamber.

6.5. Conclusions

Microbial electrosynthesis (MES) for CO_2 bioelectrorecycling is an interesting, sustainable opportunity to exploit the spent off gases from industrial facilities and convert them into valuable products. A novel design for direct acetate conversion has been presented. Two three chamber MES reactors were built and operated for 70 days, reaching a concentration of acetic acid of 2.1 g L^{-1}. Results of this preliminary study reported that a

three chamber MES can produce acetic acid in a relatively short time, and despite production rates are lower than the ones reported in other studies, it must be taken into account that this experiment was interrupted prematurely and did not completely fulfilled the objectives set at the moment of conceptualization. This study addresses to future setup improvements towards continuous operation. Direct exploitation of bioelectrochemically produced acetate as substrate for microalgae, which were proven to be able to use biocathode effluent for their growth, is herein proposed and encouraged. Further studies will test this configuration in continuous mode at the cathodic chamber at first, then also for heterotrophic microalgae cultivation and exploitation.

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7. Biochar production from sewage sludge and microalgae mixtures: properties, sustainability and possible role in circular economy

So far, only bioelectrochemical technologies have been considered in this thesis, but the aim of this work was to show that microalgae integration in METs has several advantages, among all, resource recovery from microalgal biorefinery. All previous chapters focused on bioelectrochemically relevant aspects, in terms of electricity production for MFCs and CO_2 bioelectroconversion into acetic acid. In this Chapter a recovery option for microalgae is presented, that potentially can be applied to microalgae grown and collected from the systems presented in the previous chapters. Biochar is considered one of the most interesting final products in wastewater-based circular economy, as proven by the multitude of its possible uses tested so far in different areas, and its production starting from microalgae and sewage sludge mixtures is an interesting opportunity, taking advantage of the characteristics of both matrixes. In this study, a disposal solution was investigated, consisting of pyrolyzation of a mixed sludge/bioalgae matrix under different conditions: in such way, not only landfilled residuals are practically eliminated, but a material with multiple possible beneficial end uses is generated. Initial materials (algae, sludge and combinations thereof) and end-products (biochar and bio-oil) were physically and chemically characterized after pyrolysis under different conditions. Algae alone were also subject to preliminary solvent oil extraction to assess whether increased biochar production would result from this process modification (which did, increasing biochar production by 25-33%). A comprehensive discussion on properties of end products as function of process design, possible applications in a circular economy cycle, and advantages of co-pyrolysis follows. This Chapter has been published as: *Bolognesi, S., Bernardi, G., Callegari, A., Dondi, D., Capodaglio, A.G., 2019. Biochar production from sewage sludge and microalgae mixtures: properties, sustainability and possible role in circular economy. Biomass Conv. Bioref. (2019). https://doi.org/10.1007/s13399-019-00572-5.*

7.1. Introduction

Increasing industrialization, demographic expansion and expansion of the transportation and mobility sector worldwide, and especially in developing countries, are the cause of excessive conventional fossil fuels exploitation, leading not only to repeated energy shortages worldwide, but also to increasing global levels of greenhouse gases emissions (Shuba and Kifle, 2018). Renewable feedstocks and energy sources are thus being investigated to face the demand for cleaner energy alternatives, in order to fulfil growing energy demands. Moreover, increasing carbon dioxide and greenhouse gases

emissions into the atmosphere have prompted the ethical obligation to investigate more sustainable and environmentally neutral energy sources (Raboni et al., 2015; Solomon et al., 2009). A current area of intense investigation is the exploitation of biomasses for energy production (Bilgili et al., 2017; Capodaglio et al., 2016). Amongst them sewage sludge, the final residue of wastewater treatment in the integrated water cycle, is getting increasing attention as not only it normally requires additional expensive treatment and disposal costs by generating utilities, but it is also targeted for sustainable recovery of materials and energy, in compliance with increasingly ambitious EU objectives of generating circular economy cycles from waste streams (Neczaj and Grosser, 2018) in accordance to new paradigms in urban water management. Cost of sludge disposal has been estimated at around 50% of the total cost of wastewater treatment (Capodaglio et al., 2016) while, at the same time, disposal alternatives under current strategies are getting increasingly limited, since accumulation of heavy metals, organic pollutants and pathogenic organisms in the sludge narrow its continued use in commonly adopted practices, such as direct land disposal and composting (Mantovi et al., 2005). Among possible alternatives, incineration would significantly reduce the quantity of waste to be disposed of, allowing energy cogeneration at the same time (Herbert and Krishnan, 2016). However, this involves high costs for effluent gas treatment, which may contain metals, acidic components and dioxins, in addition it generates residual ashes considered hazardous waste. and may be poorly accepted, or outright opposed by public opinion. Therefore, researchers' interest has switched to non-combustion, more environmentally sustainable technologies such as gasification and pyrolysis. Pyrolysis is the thermal degradation of biomass in the absence of oxygen, resulting in the production of liquid (bio-oil) and solid (biochar) residues, and gaseous products (py-gas), effectively transforming wastes into valuable products (Callegari and Capodaglio, 2018; Yang et al., 2019). These show different possible applications, in particular biochar has proven multiple uses as solid fuel, soil conditioner for agricultural land, and industrial applications in flue gas cleaning, as building material, or aid in contaminated sites remediation (Weber and Quicker, 2018). Also, high process temperatures, favour increased stabilization of metals, that concentrate in sewage sludge, into the carbonaceous char matrix, considerably reducing the possibility of their release into soil, and ultimately into the food chain (Callegari and Capodaglio, 2018). Depending on heating velocity and residence time of the process, pyrolysis can be broadly classified as slow (conventional), or fast. Slow pyrolysis maximises solid fraction (biochar) production, and occurs at long residence times and slow heating rates, while liquid and gaseous energy-rich products (bio-oil or py-gas) fractions are increased during fast pyrolysis (Paz-Ferreiro et al., 2018). An increase of pyrolysis temperature generally maximizes the gaseous fraction, minimizing the solid yield (Inguanzo et al., 2002). Properties of the solid residue (biochar) also vary in terms of carbon content and composition. Concerning energetic aspects, bio-oil and biochar could be used as fuels, meeting increasing needs for energy from non-fossil fuels sources (Lakaniemi et al., 2013). However, biochar derived from sewage sludge generally presents high ash content and lower heating value, diminishing its energetic worth (Yang et al., 2019).

For this reason, an interesting opportunity could consist in the application of co-pyrolysis of sludge with microalgae, which have been recently investigated both as a wastewater treatment process and potential energy feedstock (Capodaglio and Bolognesi, 2018). Microalgae are unicellular photosynthetic microorganisms capable of fixing carbon dioxide by photosynthesis, with several characteristics that make them suitable for energy

recovery (Ahmad et al., 2011). These include: (i) absence of competition with food supply, (ii) high productivity with reduced cultivation areas (oil yield of about 70% by weight of dried biomass, with area requirement of just 0.1 m^2/year per kg extracted), (iii) growth possibility on areas not suitable for other crops, without subtraction of soil from food crops cultivation, (iv) production in most types of water (fresh, brackish and waste water), with minimal or positive impact on water resources use (Callegari et al., 2020). Microalgae present positive impact also on carbon dioxide emissions, in fact they contain about 50% C over dry weight derived mainly from atmospheric CO_2, therefore, production of 100 tons of microalgae allows fixation of about 183 tons of carbon dioxide (Sánchez et al., 2003). High growth rate, cultivation ease, high lipid and low ash contents makes microalgae highly appealing, compared to other biomasses, with high yields in terms of both bio-oil and biochar (Yu et al., 2017), as determined with satisfactory results by numerous studies (Chaiwong et al., 2013; Chisti, 2008; Reen et al., 2018). Growth and productivity of microalgae are strongly influenced by environmental and physiological factors, such as temperature, pH, light intensity and nutrient availability (Kumar et al., 2018). Microalgal biochar has lower carbon content than biochar from other feedstocks, lower surface area, and lower cation exchange capacity, while pH, ash and nitrogen contents and extractable inorganic nutrients are high. These properties make it a useful additive to enhance soils characteristics and improve crop productivity, particularly for acidic soils (Yang et al., 2019).

Recently, combined activated sludge (AS)-microalgae wastewater treatment systems have been proposed to remove simultaneously both carbon and nutrients from liquid streams, as a more energy sustainable and economic alternative to conventional technologies (e.g., AS with nitrification and denitrification). The cultivation of microalgae in wastewater allows direct removal of nitrogen and phosphorus contained within, producing up to 1 kg of dry biomass per m^3 of wastewater (Ficara et al., 2014). In this alternative to conventional AS processes, bacteria oxidize the organic substance in wastewater to inorganic compounds consuming oxygen, while microalgae use sunlight to absorb inorganic nutrients released by bacteria, including CO_2, producing oxygen subsequently used by bacteria for oxidation. Although efficient for liquid streams treatment, such systems generate a residue that is more difficult to handle, as algae normally respond poorly to traditional sludge mechanical separation and drying processes. In fact, algal cells are small (2-20 μm), with density similar to that of water, and rather low (0.5-0.3 g L^{-1}) concentration in wastewater (Gabriel et al., 2018).
Purpose of this paper is to evaluate biochar and bio-oil production through thermal pyrolysis processes starting from these initial residues (microalgae and AS waste sludge) and their combination, and to determine which conditions are more favourable to optimal recovery of valuable by-products.

7.2. Materials and methods

Three different materials were tested, characterized and pyrolyzed at two different temperatures throughout the following experiments. Both initial materials and final products were characterised using thermogravimetric analysis (TGA) and infrared spectroscopy (IR). HHV (higher heating value) in recovered biochar samples was also evaluated.

7.2.1. Samples preparation

A mixed culture of microalgae Chlorella was cultivated in four lab-scale open reactors (0.35·0.20·0.10 cm, constant water depth 3 cm) in BG-11 medium (Table 14).

Table 14: Chemical composition of the BG-11 medium.

Compound	Concentration (mg L^{-1})
$NaNO_3$	1500
$MgSO_4 \cdot 7H_2O$	75
K_2HPO_4	40
$CaCl_2 \cdot 2H_2O$	36
Na_2CO_3	20
Citric acid	6
Ferric ammonium citrate	6
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
EDTA (disodium salt)	1
$NaMoO_4 \cdot 2H_2O$	0.39
$ZnSO_4 \cdot 7H_2O$	0.222
$CuSO_4 \cdot 5H_2O$	0.079
Co(NO3)2·6H2O	0.049

A domestic aquarium aerator provided air bubble agitation to keep microalgae in suspension, light was provided by a conventional warm light LED bulb (40 W) under a 16:8 light:dark sequence. Once the culture reached stable growth, microalgae were harvested, dried on nylon filters (ø = 0.25 μm) for 12 h, and pulverized to uniform size in a mortar.

Sewage sludge (mixture of primary and secondary sludge) was collected from a nearby wastewater treatment plant and dried at 100°C for 12 hours (reaching humidity content below 10%).

The third material tested was a mixture of sludge and microalgae with high humidity content, collected from a phytoremediation plant in Spain (kindly supplied by FCC Aqualia S.A.). Fresh material was distributed in 2 cm layers in a crystallizer, and then dried at 100°C for 12 hours to reduce humidity below 10%. Subsequently, dried material was shredded, to obtain a resulting grain size as uniform as possible.

7.2.2. Oil extraction from microalgae

Previous studies assessed that preliminary oil extraction from dried microalgae samples lead to enhanced bio-oil and biochar recovery yields from a subsequent thermal processing. Combination of a two-step lipid extraction and slow pyrolysis processing regime may in fact yield an oil product high in valuable fatty acids, with no variation on its quality, compared to the one-step process, with overall increased yields of liquid and solid fractions over the gaseous one (Grierson et al., 2013, 2012). Therefore, preliminary microalgae solvent oil extraction was performed using a chloroform-methanol ratio 2:1, as described in Kumar et al. (2018). From a fraction of the two algae-containing materials described in

the previous section, 1 g of dried sample was immersed in 20 mL of solvent solution in a flat-bottomed pyrex glass flask, stirred for 25 minutes, then centrifuged for 20 mins at 4000 rpm. The liquid fractions were then filtered and evaporated in a rotary evaporator (Rotovapor, Buchi) to remove solvent and determine the weight of the extracted oil.

7.2.3. Thermogravimetric analysis (TGA) and infrared spectroscopy (IR)

Aliquots (20 g each) of the initial and processed materials (sludge, algae and sludge mix, powdered algae) were subject to thermogravimetric analysis (TGA, 25÷800 °C, heating speed 20 °C min^{-1}, with TGA1 Star System, Mettler Toledo). TGA analysis weights any changes in samples as function of increasing temperatures. Thermal degradation of samples occurs in multiple stages between initial and final temperature settings. TGA was first conducted under nitrogen (nitrogen-TGA) atmosphere (0.4 L min^{-1}) to identify the temperature at which pyrolysis process began, later under air (air-TGA), to determine samples' ash and inorganic material content. Both nitrogen- and air-TGAs were subsequently carried out also on solid residue samples deriving from pyrolysis, to assess the characteristics of processed materials, and compare their ash content. Subsequently, a nitrogen-TGA analysis was carried out on residues of microalgae subject to solvent oil extraction. IR was also used to characterize initial materials, liquid and solid residues from pyrolysis, and to detect any presence of water in liquid samples.

7.2.4. Pyrolysis process and products recovery

Initial substrates were pyrolyzed in a thermostatic sand bath S-70 (FALC instruments) during the experiments. Process equipment is schematized in Figure 35. A flat-bottomed pyrex glass flask containing 20 g of sample was immersed within the heating sand medium in contact with its bottom. The absence of oxygen was ensured by continuous flow of nitrogen blown directly inside the reactor. A three-way glass fitting was connected by silicone tubing to a solvent trap containing acetone, and immersed in crushed ice, for recovery of the oily fraction. Py-gas thus flowed through the tubing, entering the trap where it condensed. The non-condensable py-gas was not further characterized and eliminated from the system. Experiments were conducted at 500°C and 350°C temperatures for each sample. In tests at 500 °C the oven was kept operating at maximum temperature, monitoring the temperature curve with a thermocouple inserted in the sand bath. Once the desired set-point was reached, temperature was kept constant for 30 minutes before switching off the heating device. As for the remaining tests, temperature was monitored with the thermocouple until reaching 350°C, manually maintaining this value for about 30 minutes by acting on the oven's thermoregulator. After cooling, the process' solid and liquid products were recovered. All tests were conducted in triplicate. Table 15 summarizes samples analyses throughout the experiment.

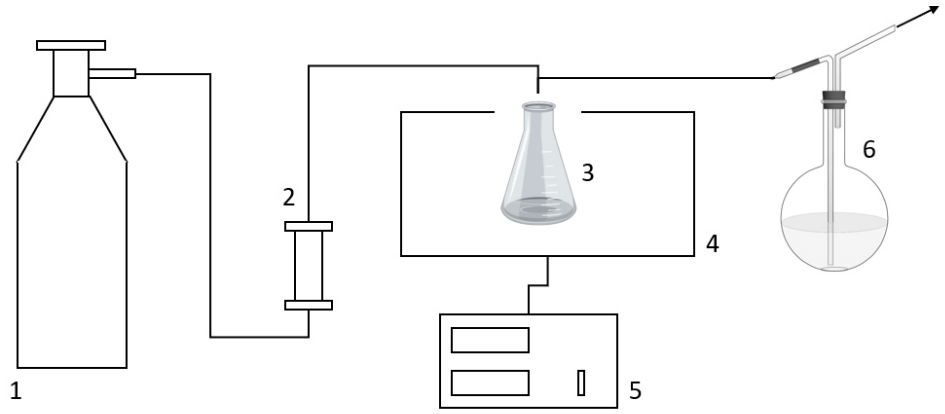

Figure 35. Schematic of the pyrolysis equipment. 1) Carrier gas tank (N_2); 2) flowmeter; 3) Erlenmeyer flask containing sample; 4) sand bath; 5) thermocouple; 6) scrubber with solvent trap.

Table 15: Tests summary.

Sample ID	Substrate	Temperature
1	Microalgae *Chlorella*	500 °C
2	Microalgae *Chlorella*	350 °C
3	Sludge from WWTP	500 °C
4	Sludge from WWTP	350 °C
5	Mix Algae +Sludge	500 °C
6	Mix Algae+Sludge	350 °C

Solid (biochar) and liquid (bio-oil) product fractions were recovered from each test, while the uncondensed gas fraction was considered irrelevant for purposes of this work, and only estimated through mass balance. After completion of each pyrolysis test, all glassware and tubing were washed with acetone to remove all residual solid and oil particles still contained therein. This resulted in a mixture of biochar, bio-oil, acetone and water, subjected to further treatment for components separation. For the solid fraction, filtration with Buchner funnel, with weigh determination before and after filtration to quantify the separated fraction was performed. The liquid fraction (a mixture of acetone and oil) was transferred into a balloon flask, and vacuum evaporated using Rotavapor R-100 (BUCHI) to remove the solvent, weighting the flask before and after the process. In case water were detected in the sample during IR analysis, anhydrous Na_2SO_4 was added to the solution, that was then filtrated and evaporated.
Yields of biochar and bio-oil recovered were calculated as follows (Eq. (27) and (28), respectively):

$$y_{char} = \frac{W_{biochar}}{W_i - W_{H_2O}} \cdot 100 \qquad (27)$$

where $W_{biochar}$ is the weight of biochar recovered, W_i is the initial sample weight (20 g) and W_{H_2O} is the water weight in the initial sample, as determined from TGA analysis, and

$$y_{oil} = \frac{W_{bio-oil}}{W_i - W_{H_2O}} \cdot 100 \quad (28)$$

where $W_{bio\text{-}oil}$ is the weight of bio-oil recovered, W_i is the initial sample weight (20 g) and W_{H_2O} is the water weight in the initial sample, as before.

7.2.5. Biochar thermal properties

The calorific value (HHV - higher heating value) of recovered biochar samples was measured with adiabatic calorimeter IKA C6000 Global Standard, in accordance with UNI EN 14918:2010.

7.3. Results and discussion

7.3.1. Initial materials characterization

TGA was carried out on each initial sample to determine its thermal degradation behaviour. Each material was characterized by both air-TGA (oxidative environment, reproducing a combustion process) and nitrogen-TGA (inert environment), between temperatures of 25 - 800°C. An oxidative environment allows the ash content of the tested material to be evaluated. TGA in inert atmosphere was also needed to determine the temperature range suitable to pyrolyzation of the samples being tested. The thermochemical process in absence of oxygen leads to degradation of volatile substances in the sample, leaving char as residue. Results of the TGA in both air and nitrogen are summarized in Table 16. According to derivative thermogravimetry (DGT) analyses, thermal degradation of microalgae takes place in one single stage, as reported in previous studies (Gong et al., 2014), while that of mixed sludge and algal samples occurs in two distinct phases. It should be highlighted that the temperature range 200-500°C includes the highest degradation peaks for all samples (Figure 36). These are generally associated with carbohydrate and protein de-volatilization (Rizzo et al., 2013). In mixed samples a second peak between 600-700°C also appears, corresponding to degradation of lipids and solid residues (Sotoudehniakarani et al., 2019).

Table 16: Amount of ashes (%) in the three samples based on TGA analyses.

Substrate	% ashes (800 °C)	% residues (char+ashes, 800°C)
Microalgae Chlorella	13.7 ± 2.6	25.1 ± 1.4
Sludge WWTP	30.2 ± 1.8	36.2 ± 2.1
Mix A+S	24.4 ± 3.1	38.7 ± 1.9

Based on ash fractions obtained from TGA analyses, composition of mixed microalgae and sludge from the phytoremediation plant sample was confirmed as 15% and 85% of each, respectively. Ash content in WWTP sludge sample was higher (30.2 ± 1.8%) than in those containing microalgae, meaning that adding even a small amount (15%) of microalgae to the mix positively contributes to the reduction of the ash quantity in residues, improving their energy quality. As for nitrogen-TGA results, it is relevant to see that the

quantity of solid residues from the sludge-microalgae mix, is higher than that produced by the single-sludge matrix, leading to increased yield in solid material recovery.

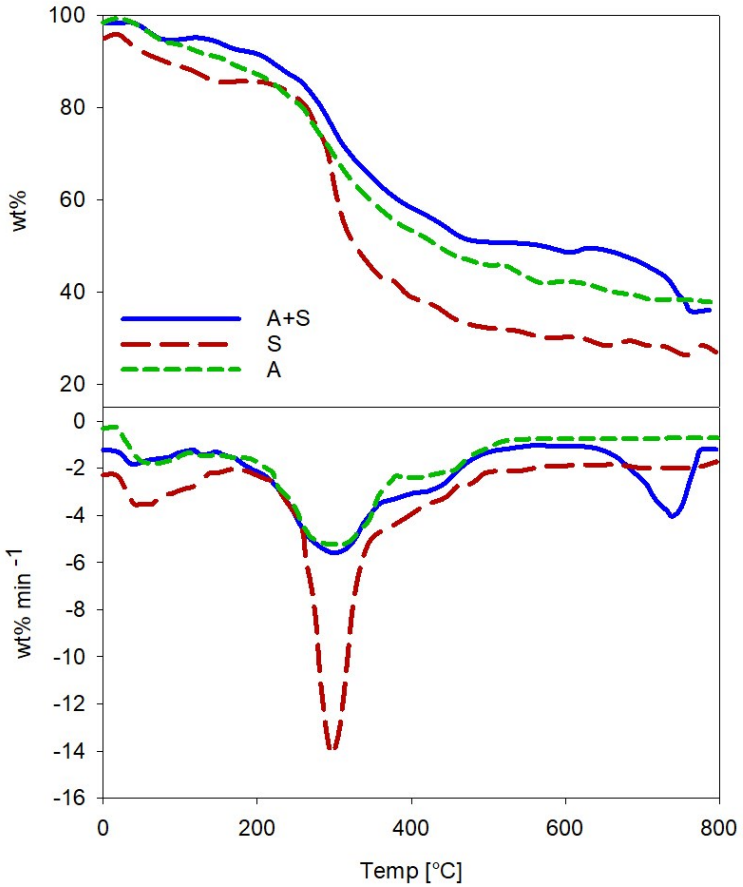

Figure 36. TG and DTG thermograms of the initial materials (microalgae (A), sludge (S), and mix microalgae and sludge (A+S)).

7.3.2. Biochar production and characterization

Resulting pyrolysis products from tests at 350°C and 500°C were solid (biochar) and liquid (bio-oil) residues. After recovering and separating solid and liquid particles remained in the testing equipment, biochar was weighed directly.

Figure 37 represents the product fractions obtained from tests. For all matrixes examined, pyrolysis at 350°C produced the greatest amount of solid residue (biochar), while higher temperatures (500 °C) generally yielded higher production of bio-oil. Considering only the production yield of biochar, WWTP sludge processed at 350°C gave higher values (82.0 ± 4.4 %) along with the mixed sample at the same temperature (82.7 ± 2.1%). As for liquid residues (bio-oil) yields, higher temperatures usually originate higher fractions than those obtained in the present work (Atabani et al., 2013), nevertheless, all samples processed at 500°C produced 13±3% of bio-oil, a fraction higher than at lower temperature.

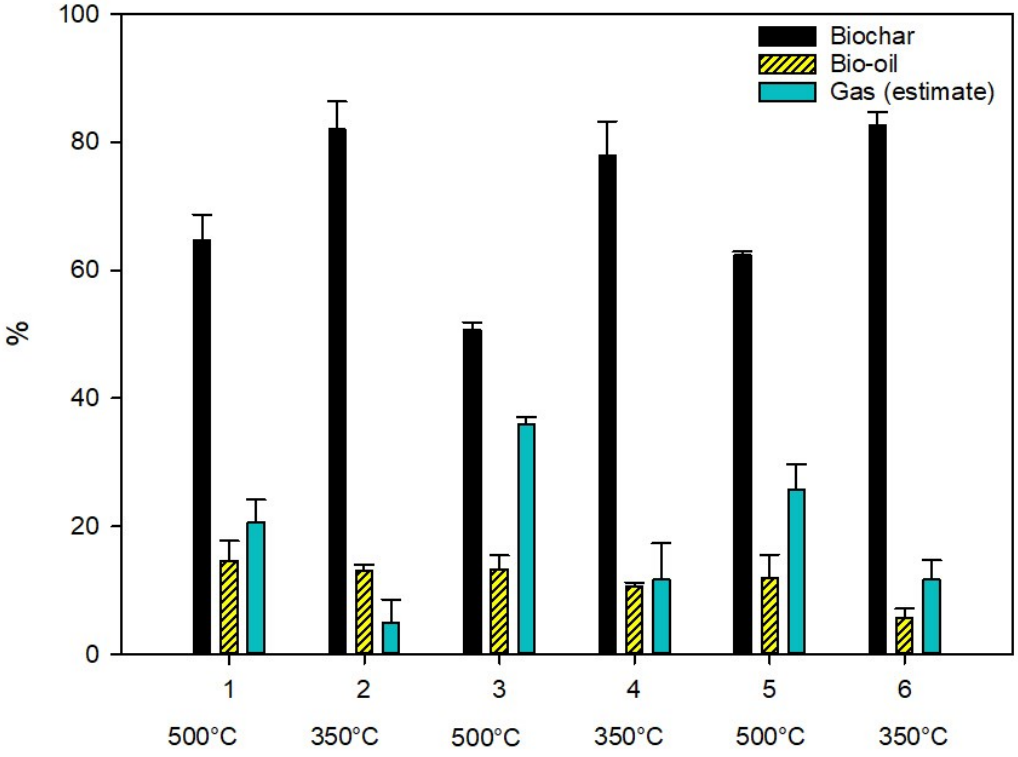

Figure 37. Pyrolysis products: biochar (black), bio-oil (yellow) and gas (light blue, estimated). Error bars represent variability of results between triplicates**.**

Due to the focus of the present work, only the solid residue was fully characterized. Biochar samples from pyrolysis tests were subject to TGA, IR analysis and HHV (High Heating Value, UNI EN 14918:2010). Under visual analysis, all samples appeared different from each other, with appearance changing according to process temperature and initial material. Samples 2 and 4 from tests at 350°C (Figure 39 e, f) presented fairer color (brownish), compared to all others (black or blackish). Among microalgae-derived biochar samples 1 and 2 (Figure 39 a, d, respectively) no colour differences were detected, but they significantly differed in consistence: sample 2 (Figure 39 d) had a dusty structure, while sample 1 was mostly solid (Figure 39 a). Air-TGA analyses were performed to evaluate ash content of the biochar samples, while nitrogen-TGA was used to evaluate the efficiency of the pyrolysis process (Figure 38), by assessing their supplemental weight loss.

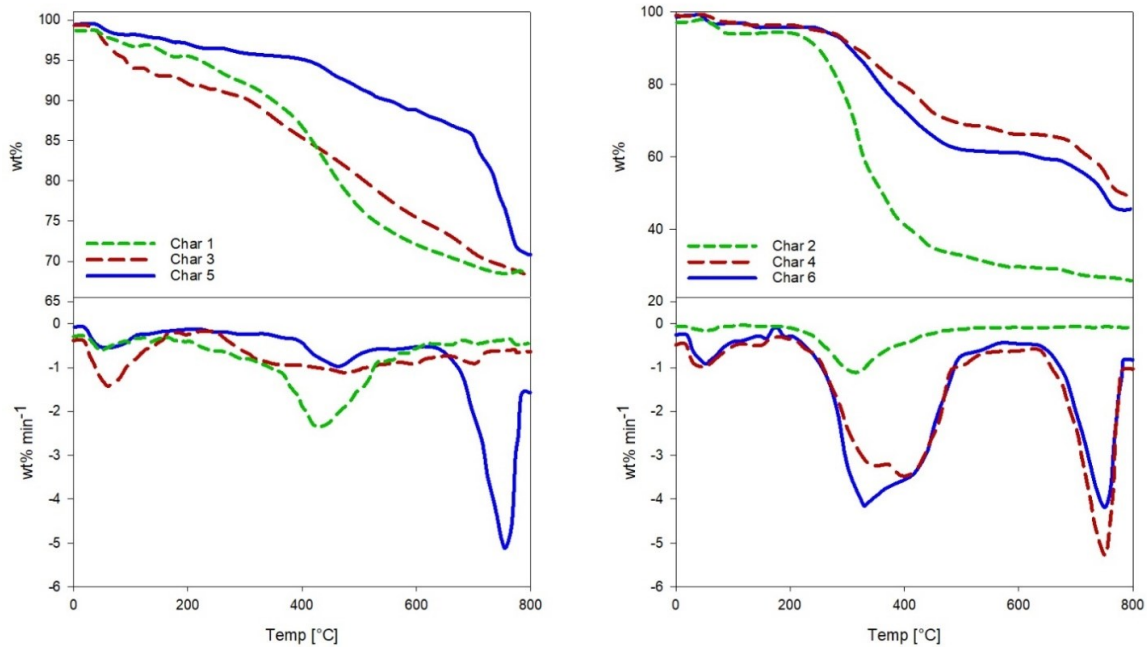

Figure 38. TGA and DTG thermograms of biochars from pyrolysis at different temperatures. Left: 500°C; right: 350°C. Heating speed constant for all processes (20°C min^{-1}).

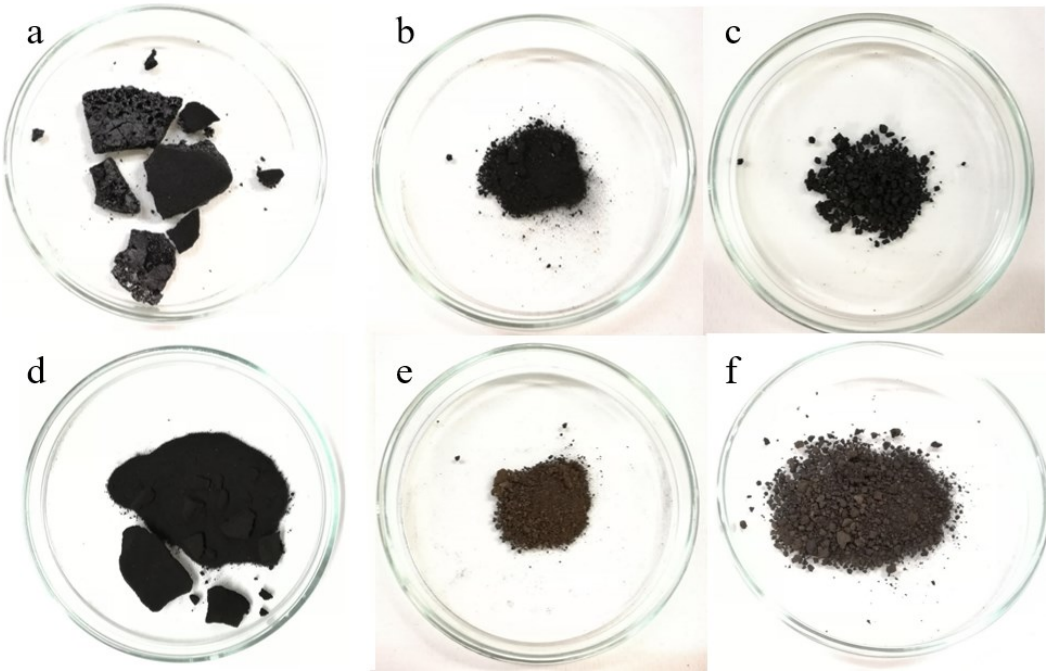

Figure 39. Upper: samples from pyrolysis at 500°C a) microalgae Chlorella; b) sludge from WWTP; c) Mix M+S. Lower: samples from pyrolysis at 350°C: d) microalgae Chlorella; e) sludge from WWTP; f) Mix M+S.

IR analysis was performed before and after pyrolysis to evaluate variation of internal material bonds induced by the process (Figure 40), by determining functional groups and bonds within samples. The most significant information in the graphs are contained in the wavelengths representing water and carboxyl groups (between 3600-2500 cm^{-1}), C-C and C-H bonds (3300 cm^{-1}); esters and fatty acids (1700 cm^{-1}), and Si-O bonds in inorganic material (1100 cm^{-1}). By comparing the different spectra, all samples before pyrolysis appear very similar to each other, although some relationships between components vary. Pyrolyzed samples (only one sample is reported in the figure) show removal of water and organic acids during the process, and reduction of many of the functional groups present. This corresponds to formation of compounds with high carbon content, even if some C-C and C-H bonds are still present. Obviously, Si-O bonds are preserved, as not involved in pyrolysis reactions. Further increasing time of pyrolysis process and temperature would lead to formation of a graphitic carbon, with absence of IR bands detected.

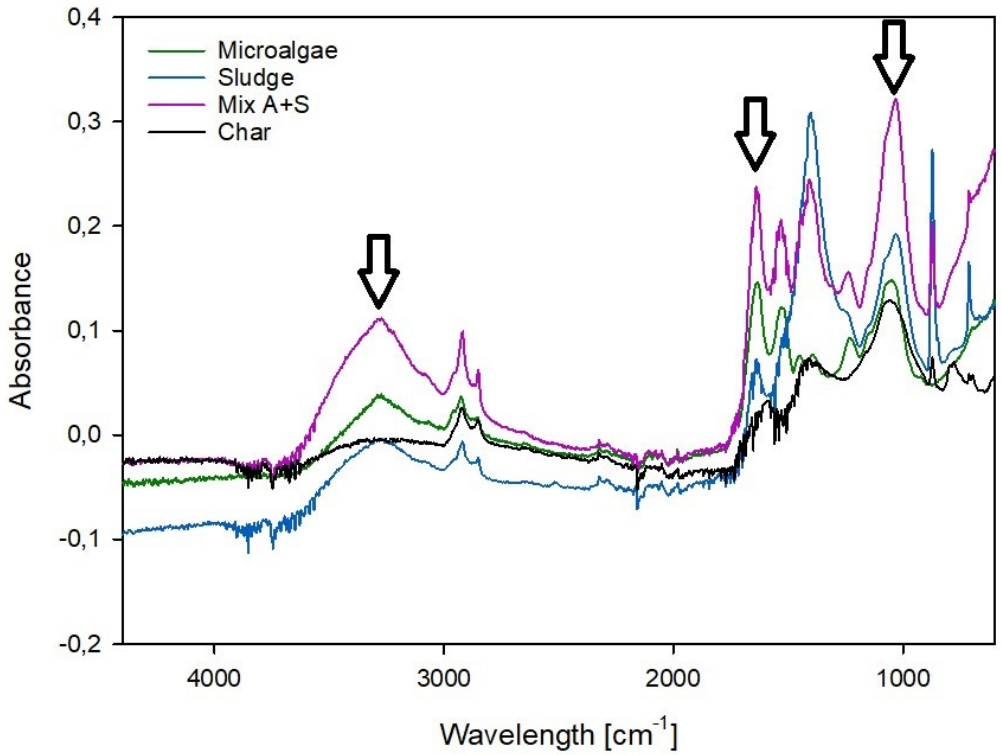

Figure 40. IR analyses results. Absorbance curves for initial materials and for biochar from Mix A+S at 350°C are shown. Arrows show the relevant sections.

HHV analysis shows that microalgae-derived biochar has higher heating value (samples 1 and 2) than others, decreasing with decreasing process temperature. HHVs of remaining samples, are lower, suggesting that thermal uses might not be indicated as the main final application of these biochars. Significant results are summarized in Table 17.

Table 17: Amount of ashes detected by air-TGA, weight loss (incomplete pyrolysis) from nitrogen-TGA analysis, and HHV value of biochars obtained in tests (1-6).

Sample	Pyrolysis temperature [°C]	Ashes [%]	Weight loss [%]	HHV [kJ kg^{-1}]
1	500	41.6 ± 2.3	16.8	29091
2	3\50	31.5 ± 1.7	67.5	26951
3	500	50.1 ± 2.2	23.9	16629
4	350	37.0 ± 1.9	46.2	15648
5	500	44.3 ± 2.7	26.8	16245
6	350	34.5 ± 3.0	49.3	16671

To assess the effect of solvent oil pre-extraction from microalgae on biochar production yield, as suggested in previous studies (Grierson et al., 2013, 2012), solid residues after this pretreatment were subject to nitrogen-TGA, comparing the results with those on raw materials. These samples showed significantly better results, than initial ones: biochar production yield after pre-extraction increased from 25% to 33% in microalgae-only samples. However, no benefits were detected from such preliminary oil extraction in the mixed samples (microalgae and sludge), with 38% biochar yield in both cases.

7.4. Discussion

This work aimed to assess potential advantages in terms of biochar production and characteristics of the combination of sewage sludge and microalgae as feedstock in pyrolysis processes, with a view to improve the final use value of recovered resources. Product analysis was not limited at observing mass weight obtainable from each matrix, but was extended to determine ash fractions in the final products, and their quality. Alternatives for coupling the two matrixes in one feedstock are feasible: one option could consist of separate microalgae production with direct addition to sludge at the time of pyrolysis. However, this strategy would be of small benefit compared to the direct use of a microalgae-sludge mix originated by a wastewater treatment facility of new conception. This novel type of process, in fact, in addition to allowing simultaneous nutrients removal from wastewater by microalgae, without costly bio-denitrification processes, produces a mixed biomass (sludge and microalgae), originating a solid residue with excellent characteristics after thermal treatment, as reported.

7.4.1. Comparative analysis

Observed product yields were compared with those obtained by other authors, to validate present results (Table 18). Sewage sludge biochar was obtained by slow pyrolysis in helium atmosphere using a quartz tubular reactor containing 30 g sludge samples by Sanchez et al. In this study the original matrix had ash content of 3.4% by weight. Tests were conducted at 350, 450, 550 and 950°C, with the largest amount of biochar (52%) produced at the lower temperature of 350 °C, in accordance with the present study.
Microalgae-derived biochar was obtained by Gong et al. using a quartz, fixed-bed reactor under inert (N_2) gas flow, testing 1 g samples at temperatures between 300 and 700°C, with heating rate of 10°C min^{-1}. The study showed that bio-oil fraction increased with

temperature from 30.9% (at 300°C) to a maximum of 60.7% (at 500°C), decreasing afterwards to 48.1% (at 700°C). As for biochar yield, the highest amount was obtained at 300°C (57%), decreasing with temperature to a minimum of 25.5%. HHV of the char also decreased with temperature (from 22.3 to 16.4 MJ kg^{-1}), while gas yield increased along with temperature (from 0.4 to 15.5%). Compared to the results of this study, char production yield from the Chlorella culture was higher, as well as the product HHV.

Results from microalgae and sewage sludge mixtures in this study could not be compared due to the lack of similar literature data concerning these feedstocks co-pyrolysis. The highest productions of biochar were detected in pyrolysis of microalgae alone, while intermediate results were obtained from co-pyrolysis of sewage sludge and microalgae, as expected by preliminary information reported in other studies on co-pyrolysis of microalgae with other, non-sludge matrixes (Yang et al., 2019).

Table 18: Product yields of microalgae and sewage sludge pyrolysis.

Feedstock [Reference]	Pyrolysis type	Temperature [°C]	% biochar	% bio-oil	% gas	HHV [MJ kg^{-1}]
Sewage sludge (Sánchez et al., 2009)	Slow	350	52	10	20	-
Sewage sludge (this study)	Slow	350	74.3	11.5	14.2	15.6
C. vulgaris (Gong et al., 2014)	Slow	500	31.5	49.2	4.6	19.3
C. vulgaris (Sotoudehniakarani et al., 2019)	Fast	500	34.0	41.5	24.5	-
C. vulgaris residues (Wang et al., 2013)	Fast	500	31.0	53.0	11.0	19.4
Chlorella (this study)	Slow	350	78.9	6.0	15.1*	26.9
Chlorella (this study)	Slow	500	58.8	10.2	31.0*	29.1

* gas value estimated

7.4.2. Possible beneficial applications of biochar

Pyrolysis operating conditions are paramount to determine optimal final uses of derived biochar, since these factors directly contribute to the development of different intrinsic characteristics of the product (Hossain et al., 2011). It is therefore important to analyse feedstock materials before thermal processing, in order to establish *a priori* the best application for the biochar that will be obtained under given operating conditions. Results obtained in this study from HHV analysis on obtained biochars, compared with HHV of hard coal (around 30 MJ / kg), prove that biochar from microalgae could in fact be used as fuel (HHVs of 29.1 and 26.9 MJ/kg, not dissimilar from coal's value). As biochar is the product of renewable feedstock, this would substitute the caloric equivalent amount of fossil fuels, offsetting related GHG emissions. However, other alternative uses of this product are known, a more interesting one being its use in agriculture as soil enhancement or in wastewater or contaminated site remediation as pollutants adsorbent, either a use with greater added value compared to outright combustion. Agricultural use will effectively

work as long-term carbon sequestration (also valuable under current policies), uses as adsorbent will substitute other commercial products, after which the spent biochar could be sent to controlled combustion, that would simultaneously serve as final contaminants destruction and exploitation of the char energy content.

The most interesting outcomes for these products, regardless of original feedstock, are in fact considered to be those related to the possibilities of their re-use and valorisation, from an urban (wastewater) based circular economy perspective. An appealing use of biochar is that of soil improver in agriculture, that has shown to allow increase in crop productivity, but also to reduce soil pollution by adsorbing metals and other solute contaminants in groundwater (Arthur et al., 2015). Biochar in fact has excellent adsorbent capacities for both organic and inorganic pollutants, and by virtue of its C content, also acts as a long-term carbon sink. For proper agricultural use, biochar carbon content must be greater than 50% (dry mass), N and P content should be between 1 and 45%, pH should not exceed 10, and particles' specific surface should be greater than 150 m^2g^{-1} (Santos and Pires, 2018; Schmidt et al., 2016). Biochars derived from bagasse and vegetal biomass feedstocks generally fit these specifications, and some studies confirmed that also microalgal biochar presented compatible characteristics (Ding et al., 2017; Yu et al., 2018). Effects of biochar on physical-chemical improvement of soils also depend strongly on the original soil characteristics and on feedstock used for its production (Obia et al., 2016).

A recent study from Oliveira and co-workers (Oliveira et al., 2017) showed that low pyrolysis temperatures (<500°C) favour partial carbonization, producing biochar with smaller pores and reduced surface area, while increasing the presence of oxygen-containing functional groups, making it ideally suitable for removal of inorganic pollutants. On the contrary, biochar produced at high temperatures (> 500°C) could be applied for adsorption of organics, due to higher specific surface area, making it highly suitable for environmental bioremediation of organic pollution and for wastewater treatment applications, specifically for removal of toxic compounds, instead of activated carbon (AC) (Ahmad et al., 2014). In that respect, Alhashimi and Aktas (2017) performed a LCA (life cycle assessment) analysis evaluating the relative economic and environmental performance of biochar as adsorbent, compared to AC. Environmental impact was evaluated in terms of energy demand and GWP (Global Warming Potential) for their production. GWP of biochar generation is usually negative (-0.9 $kgCO_{2eq}$ kg^{-1}), against an average 6.6 $kgCO_{2eq}$ kg^{-1} of commonly used AC. Energy demand for biochar production is lower than that required for AC by one order of magnitude (1.1 ÷ 16 MJ kg^{-1} for biochar, 44 ÷ 170 MJ kg^{-1} for activated carbon). However, it has to be considered that spent AC is usually not discarded immediately, but regenerated for reuse, while spent biochar is usually destroyed after its first use. As for economic aspects, biochar and AC industrial production costs are comparable, estimated as \$5 kg^{-1} and \$5.6 kg^{-1}, but this does not factor in the missed high costs for the original sewage sludge disposal that would otherwise be needed. Some drawbacks of biochar as adsorbent must also be considered, such as less controllable quality and fluctuating efficiency, longer time needed for absorption of certain contaminants, differences in performance of products from different feedstocks. However, environmental advantages are obvious and with adequate optimization biochar may be considered suitable for most adsorption applications. Finally, due to its high carbon content, biochar has found other applications, for example for use as electrode material in bioelectrochemical systems (BES) in lieu of granular graphite or AC, and many others.

7.4.3. Implications for a biochar-based Circular Economy

All the above mentioned and other additional applications of biochar will gradually be investigated and validated as circular economy becomes an effective part of new economic paradigms. In order to encourage the development of a biochar-based circular economy, attempts at certification of biochar are being carried out. Biochar at the moment is defined according to guidelines from the International Biochar Initiative (IBI Biochar Standards) and the European Biochar Foundation. The former concerns the use of biochar uses in soil, the latter consists of guidelines for sustainable production of biochar. Both these guidelines define biochar as material produced by pyrolysis of biomass under low oxygen conditions, without limitations to the origin of the biomass, therefore both sewage sludge and algae fall under this definition. Both guidelines include specifications about maximum toxicants assessment, and their maximum allowed thresholds. Product certification, although at present existing only for a restricted range of applications, is an important step for setting up reliable and lasting circular economy circuits. While circular economy strategies centered on wastewater treatment by-products in the EU generally postulate their direct re-use in energy production, and alternative could be based on their transformation into new products, not necessarily limited to agricultural use. In order to fulfil future certification and regulation requirements for these products, the next challenge for research and development of biochar-based products lies in achieving a greater understanding and control of pyrolysis processes staring with feedstock pretreatment, additives addition, effect of process operating parameters, process yield in terms of specific product properties, such as heavy metal immobilization, specific surface area, elemental analysis, phosphorus and micropollutants contents.

Decentralized biochar production units would constitute the most efficient way to meet local by-products demand with specific characteristics by using homogeneous site-produced feedstock under purpose-designed process conditions, avoiding the economic and environmental impacts of long-range transportation, promoting local business and employment and locally improving resource efficiency and synergistic opportunities for various local sectors in the transition to circular economy paradigms.

7.5. Conclusions

This study aimed at assessing the effects of adopting mixtures of sewage sludge and microalgae as feedstock in terms of these residuals' pyrolysis disposal processes, and specifically of possible improvements of biochar production quantity and quality. Final product analysis was not limited at the determination of relative mass produced from each matrix, but also went further to determine the ash fractions, carbon and energy properties of each final product. Results showed that slow pyrolysis of mixed feedstock (85 and 15% sludge and algae respectively) at temperature of 350°C, yielded 80% of the initial sample by weight as biochar, of which only 24% as ash. Comparing this result to the data from pyrolysis of WWTP sludge at the same temperature, biochar extracted was 74% of the initial sample weight, but with 30% ash content. Therefore, co-pyrolysis of sewage sludge and microalgae yielded a more valuable product, with multiple possible applications. This solution could contribute to the reduction of problems deriving from expensive and/or inappropriate disposal of wastewater treatment residuals.

Various possibilities in terms of implementation of productive biochar reuse have been described. Within a wastewater-based circular economy cycle, biochar is a very valuable material, with multiple possible interesting outlets that need further careful evaluation beyond currently known applications. Standardization and certification of final products characteristics are the keys to a successful circular economy implementation. Some attempts in this sense have been already developed for specific biochar applications. Decentralization of biochar production from local feedstock sources would be the most logical and effective way to implement efficient biochar-based circular economy. Such systems would benefit from more homogeneous feedstock characteristics and the possibility to custom-define and design the required final products characteristics according to local applications, and minimize additional environmental impact.

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8. Conclusions

8.1. Main conclusions

In this Ph.D. thesis, the design and operation of different METs for bioenergy and biofuels production has been explored, analyzed and discussed. Considering the experimental results achieved, these technologies proved to fit in the objectives, and **microalgae integration in METs has led to encouraging results**. Thus, it is possible to draw some further conclusions.

The operation of two MFCs setups with landfill leachate and dairy wastewater as co-substrates for liquid waste conversion into bioelectricity were studied in Chapter 3. Poorly biodegradable (BOD/COD = 0.1) landfill leachate, an unideal substrate, was added to dairy wastewater at various mixing ratios, granting system continuous operation up to 50% leachate in the feed composition in one out of two setups tested. Both systems achieved their best performances treating a lower percentage of leachate (20%) in the mixture. Graphite coated stainless steel mesh proved not to be as good as granular graphite as electrode (anode) material, causing premature failure of one out of the two cells. The main problem affecting both cells after an end of operation analysis was the excess of accumulated solids in the anodic chamber, which in the long run determined clogging of the anode cell free volume and affected hydrodynamic behavior of the system. Use of leachate characterized by higher biodegradability, and/or its pretreatment, may be the keys to operate at higher percentages in the influent solution or to aim at long-term operation, lowering the presence of residual non-biodegradable solids or inhibiting waste components.

The study presented in Chapter 4 aimed at evaluating the performance of an integrated MFC-PBR system containing microalgae *Chlorella,* treating synthetic (acetate) and real (dairy wastewater) substrates. Bioelectricity recovery was evaluated under six different operational conditions to establish optimal process configuration. Two MFCs were operated for 60 days, where one out of two acted as a control with no PBR applied and no microalgae at the biocathode, to evaluate influence of the oxygen provision method. Both systems proved to be effective for wastewater treatment (COD removal up to 99%), and microalgae containing MFC showed generally higher power density generation than similar systems described in literature studies. In a second phase of the experimentation, both MFCs were equipped with PBRs but applying different oxygen provision methods: one was relying on anodic-produced CO_2 capture only, while the other was granted an extra air supply. In this second phase the focus was set on energy losses of the system through performance of polarization curves, resulting in higher transfer losses (31% vs. 22%) for the CO_2 capture configuration. As for cathode overpotentials, in both configurations their percentage increased over time, probably due to electrode fouling and subsequent increased difficulty in electron transfer. However, it has to be pointed out that when considering bioelectricity production with a synthetic substrate, a traditional MFC proved to be more

stable and better performing than the MFC-PBR under almost every condition tested. Systems' performance gap was reduced when dealing with a real substrate of increased complexity, slowing down the anodic reactions in both MFCs, thus reducing the limiting factor of relying in microalgal metabolism for oxygen production. This indicates that MFC-PBR integrated systems with microalgae may become a feasible option for sustainable wastewater treatment. It has to be taken into account that microalgae produced oxygen decrease the cost and the plant complexity for oxygen provision, and CO_2 capture by microalgae positively contributes to reduction of GHG emissions in wastewater treatment plants. Not least, biocathode microalgae effluent can be recovered and converted into valuable microalgal biorefinery products, as exemplified in Chapter 7 of the present thesis, improving the technology global economic balance.

Microalgae integration in METs was the main strategy operated from this point on. In Chapter 5, two H-Type microbial electrosynthesis BESs were built and operated in batch mode. The two reactors proved to be effective in VFAs production, especially in CO_2 conversion into acetic acid, reaching high concentration (up to 13 g L^{-1} in HT1) and a maximum production rate of 0.29 g L^{-1} d^{-1}. Biocathode effluent was then used as feedstock for heterotrophic microalgae *Auxenochlorella prototheocoides*, a species of microalgae capable of accumulating high percentages of lipids in its cellular membrane when cultivated heterotrophically, and it has been extensively reported in literature for microalgal biodiesel production. In this proof-of-concept study it was assessed that microalgae can use directly biocathode effluent for their growth, optimizing the process and avoiding the costs for VFAs extraction and purification. An oil production yield up to 25% w/w oil content was obtained, consequently recovering 0.03 kg bio-oil per kgCO_2 captured. Future studies should focus on process optimization to further enhance bio-oil production and extraction; for these reasons two reactors compatible with continuous mode operation were designed and operated in Chapter 6.

Chapter 6 presents a novel BES design for direct conversion of acetate conversion. Two three chamber MES reactors were built, inoculated, and operated for 70 days, including an acclimatization period of 25 days, and reached a concentration of acetic acid of 2.1 g L^{-1}. The experimentation was prematurely interrupted and did not completely fulfilled the objectives set at the moment of conceptualization, but this preliminary study reported that a three chamber MES design could start producing acetic acid in a relatively short time. Ideally, continuous biocathode operation once the production rate is stabilized, can lead to direct exploitation of bioelectrochemically produced acetate in the middle chamber as substrate for microalgae, which were already proven capable of using biocathode effluent for their own growth. Further studies will test an improved configuration in continuous mode, taking advantage of heterotrophic microalgae cultivation and exploitation.

Finally, Chapter 7 explored one possible recovery option of microalgal effluent. In this study a sewage sludge and microalgae (both in a mixture and separated) were subjected to slow pyrolysis to evaluate biochar production, both in terms of quantity and quality. Mass, ash fraction, carbon and energy properties of char were determined. Results showed that slow pyrolysis of mixed feedstock (85 and 15% sludge and algae respectively) at temperature of 350°C, yielded 80% of the initial sample by weight as biochar, of which only 24% as non-recoverable ash. Biochar extracted from sewage sludge was 74% of the initial sample weight, but with a higher ash content (30%). In both cases, oil extraction prior to pyrolysis did not affect much the composition of the pyrolyzed residue. As for

microalgae sample, biochar percentage obtained was around 80% (17% ashes), but it has to be noted that the residue presented the highest HHV (~30 kJ kg^{-1}). Addition of microalgae to sewage sludge could contribute to the reduction of problems deriving from more and more difficult disposal of wastewater treatment solid residuals, yielding a more valuable product, with multiple possible applications. Biochar production from microalgae (and microalgae mixtures) can be considered as an efficient strategy for a complementary exploitation of side resources generated from bioelectrochemical systems. Since oil extraction prior to pyrolysis did not affect the product quality, and in the case of microalgae as a feedstock increased the percentage of biochar recovered, pellet pyrolysis could be perfectly included after microalgal oil extraction in a microalgal biorefinery approach, as expressed in Chapter 5.

8.2. Process limitations and issues

Considering the results obtained in the present Ph.D. thesis, it is possible to make a point on the state of art of METs for bioenergy and biofuels production. Both MFCs and MES were considered in the experimental works herein reported, and some general considerations can be made on issues common to the whole METs category.

One of the major drawbacks is that only a few experiments at large (or larger than laboratory) scale have been tested so far (Dong et al., 2015; Hiegemann et al., 2016) and mainly related to the production of bioenergy using MFCs or hydrogen production in MECs.

For both MFCs and MES, two major issues have to be overcome in order to reach full scale implementation: the high capital cost and low productivity (He and Angenent, 2006; Prévoteau et al., 2020). In terms of capital costs, the costs associated to construction materials (in particular, electrodes and membranes) and eventual use of chemical/metal catalysts are the voices mostly affecting the economic balance. Anode/cathode material should offer high conductivity to drive electrons from the electrode surface to the biomass (and vice versa) and to grant excellent (electro)chemical stability, coupling both high specific surface area and high porosity to enhance biomass adhesion, favor mass transfer and restrain high overpotentials (Jourdin et al., 2016). An interesting option to decrease costs for MFCs is represented by the application of natural or recovery materials as membrane or electrodes. However, a long way to go is still needed to reach performances comparable to engineered or conventional materials (Goglio et al., 2019).

Electricity production in MFCs is still too low (with power density values below 20 W m^{-3} in most cases) to develop energetically self-sustainable wastewater treatment plants, even though some positive experiences of on-site treatment have been reported (Liang et al., 2018; Velasquez-Orta et al., 2017; Walter et al., 2018; Yu et al., 2021). In the last ten years, researchers' interest has gradually shifted from energy-producing MFCs to hydrogen production (MECs; Kadier et al., 2016; Zhang and Angelidaki, 2014), biogas upgrading or microbial electrosynthesis of chemical commodities (MES) from waste streams (Bian et al., 2020; Cheng and Kaksonen, 2017; Jiang et al., 2019), or selective removal/recovery of chemical compounds, mainly in groundwater (Cecconet et al., 2018; Pous et al., 2018). This change marked a switch in investigation focus from the enhancement of bioanode performance for bioelectricity production to the opportunities and tasks that can be fulfilled by biocathodes. With this in mind, the attention was set to increase value of cathode

performance also in MFCs: the integration of microalgae in biocathodes, for example, helped to partially close the gap with other METs, even though still not sufficient for scaling-up, and opened the door to a variety of new biorefinery applications and recovery opportunities (Zamanpour et al., 2017; Sevda et al., 2019).

When dealing with MES, the key to optimize the overall production process is the enhancement of interactions between bacteria and electrode (Vassilev et al., 2018). Researchers are focusing on exploring novel and composite cathode materials to improve the surface area and electrochemical properties, besides electrocatalytic activity and material biocompatibility, to ensure supply of reducing power to the biocatalysts. Low-cost cathode engineered materials should be targeted for scaling up, avoiding noble metal catalysts, but granting sufficiently high performance for H_2 evolution and long-term stability (Prévoteau et al., 2020). Research interest in MES has been focused towards biocathodes, whereas two different approaches have been applied regarding anodic reactions: i) not focusing at all on anode reaction, avoid the use of pricey mixed-metal oxide anodes (Prévoteau et al., 2020); ii) valorisation of the counter reaction, in the attempt to maximize products recovery and oxygen evolution reaction (OER; Bian et al., 2020). Bian et al. reported some efficient strategies to enhance counter reaction and complement biocathodic products to increase the economic sustainability of MES: deposition of small amounts of stable noble catalyst on non-noble electrode to enhance OER (Bajracharya et al., 2017); photo-assisted MES (photo-anodes; Fu et al., 2018); the use of anodic reactions other than OER for electron generation, such as chlorine generation (Batlle-Vilanova et al., 2019); the use of bioanodes for organic or sulfide oxidation (Xiang et al., 2017). However, the main critical factor is still the cathodic reaction, in particular the study of the microbial extracellular electron transfer (EET) mechanisms and, consequently, production selectivity.

About this last point, although a production of C2-C4 carboxylic acids and relative alcohols was assessed, a delicate equilibrium has to be maintained in managing and controlling the operational conditions to produce, mainly and not exclusively, the target compound (Vassilev et al., 2019); in addition to that, setup "compromise" choices have to be made between reaching high titers while risking biomass inhibition by accumulation of organic acids or alcohols in batch mode operation (e.g., acid crash), or operating in continuous mode to avoid any toxicity effect but achieving lower titers (Prévoteau et al., 2020). Production rates are still not completely fulfilling researchers expectations; when considering the most abundant product, acetic acid, its production rate has increased reaching values up to 685 g m^{-2} d^{-1} (Jourdin et al., 2015); but this value is still too low to be considered for an industrial production, not to mention production of more complex chemicals'. Finally, energy conversion efficiency is still low due to high ohmic resistances in MES reactors (higher than 100 Ω cm^2 ; Prévoteau et al., 2020).

Furthermore, once the target compound is produced, it must be separated from the effluent, so steering the bioelectroconversion to a selected product is essential. Strategies to enhance in-line extraction of microbial electrosynthesis products have been explored, for example the three chamber reactor from Gildemyn et al. (2015); however the costs connected to the extraction of the different VFAs and alcohols produced make the commodity chemicals recovered still not competitive with the ones of industrial production (LaBelle and May, 2017).

However, it has to be taken into account that governments are providing environmental incentives and imposing carbon taxes for CO_2 emission, pushing industries towards the use of carbon conversion technologies to reach the ambitious goal of a zero-carbon economy by 2050 (Leeson et al., 2017). Conventional processes for synthesis and purification of chemical compounds have a negative carbon footprint, even when they are coupled with renewable energy sources. Most of the chemical industry processes require many chemical catalysts and reactors designed to endure reactions at high temperature and pressure conditions (Das et al., 2020). Also electrochemical CO_2 conversion, relying only on chemical catalysts and electricity, presents a negative carbon footprint for most of the compounds (De Luna et al., 2019). MES, instead, is a flexible technology potentially applicable to decarbonize industrial CO_2-containing flue gas, while producing fuels and chemicals, that could replace the existing chemical production facilities with a positive carbon footprint when coupled with renewable energies (Dessì et al., 2021). MES only relies on non-toxic, self-sustainable biocatalysts that operate at room temperature. The highest decarbonization potential at the moment is granted by acetic acid, produced with high selectivity (over 90%) and high production rates (Jiang et al., 2019), which unfortunately is not sustainable from the economic point of view (Christodoulou et al., 2017).

8.3. Future developments

To further enhance MES platform production rates and chemical commodities, several strategies should be evaluated.

First of all, the selection of the microorganism (or the microbial community) plays a fundamental role in process optimization and desired target product. Genetic engineering of homoacetogens is currently being studied by several microbiologists, expanding the portfolio of products that could be produced by MES from CO_2, steering target chemical bioelectrosynthesis, reaching higher concentrations and production rates, to increase their tolerance to stress or toxicity conditions, or to attempt new fermentation pathways (CO rather than $CO_2 + H_2$, for example) (Prévoteau et al., 2020).

Purple phototrophic bacteria (PPB) are a group of anaerobic facultative microorganisms, which can use infrared light as the main energy source. Interest in purple bacteria photo-bioelectrochemistry is growing, due to the capability of purple bacteria to cope and adapt to different environmental stresses (Grattieri, 2020). PPB can be used for the extraction of high value-added products from waste sources, such as biofuels like bio-hydrogen, bioplastics as PHA and single-cell proteins (Vasiliadou et al., 2018).

Alloul et al. (2019) investigated the use of purple non-sulfur bacteria (PNSB) for VFA conversion into proteins and map the effect of acetate, propionate, butyrate and a VFA mixture on growth and biomass yield on different cultures (*Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodospirillum rubrum*, *Rhodobacter capsulatus*). PNSB have a near-perfect substrate-to-biomass COD conversion when grown on VFA, and a biomass productivity of 1.7 g DW L^{-1} d^{-1} was achieved.

The use of electricity to enhance the PPBs metabolic activity aiming to produce high value-added bioproducts is currently under investigation, representing a field with high growth potential in the short-term (Vasiliadou et al., 2018). Perona-Vico et al. (2020) studied the biological production of hydrogen in biocathodes operated at − 1.0 V vs. Ag/AgCl of ten

hydrogenotrophic bacteria species from genera *Rhodobacter*, *Rhodopseudomonas, Rhodocyclus*, *Desulfovibrio* and *Sporomusa*. Eight over ten bacteria strains showed electroactivity and H_2 production rates increased significantly for *Desulfovibrio paquesii* and *Desulfovibrio desulfuricans*. The use of hydrogenotrophic bacteria is fundamental to establish resilient co-cultures, ideally composed of a H_2 producing strain and a homoacetogen, to grant an electron donor for CO_2 reduction in microbial electrosynthesis processes and electro-fermentation by providing a consistent biohydrogen production (Saheb-Alam et al., 2019).

Another option is coupling MES biorefinery with a secondary step to increase the value of the VFAs produced. For example, coupling a BES reactor to a fermenter, as a secondary step aiming at chain elongation, to convert short-chain carboxylates (C2-C4) into medium-chain carboxylates (C6-C8) (Blasco-Gómez et al., 2019; Vassilev et al., 2018). Pepè Sciarria et al. (2018) instead suggested conversion of C2-C4 VFAs produced through MES into bioplastics (PHA). Molitor et al. (2019) proposed a two-stage bioprocessing system as part of a power-to-protein approach to fix CO_2 in a first stage BES inoculated with anaerobic acetogenic bacteria *C. ljungdahlii*, and further use of biocathode effluent to grow yeasts (*Saccharomyces cerevisiae*) or fungi in a second stage under aerobic conditions. The group obtained a carbon yield of 25% as yeast biomass in continuous mode, with a protein mass-fraction of 40–50% during a proof-of-concept experiment, reaching a protein production rate of up to 0.07 g L^{-1} h^{-1}, still far from industrially relevant production rate values of 1 g L^{-1} h^{-1}. Instead of a two-step process, the anodic process can be potentially applied to upgrade MES products to longer chain carboxylic acids using Kolbe electrolysis (Stang and Harnisch, 2016). Dual cathode MES setup with different fixed cathode potential and operational conditions to promote the production of C2–C4 carboxylic acids and alcohols from carbon dioxide by simultaneous acetogenesis, chain elongation and solventogenesis (Vassilev et al., 2019).

If all these strategies are not sufficient to improve enough MES platform production rates, the possibility of decoupling a fast abiotic electrochemical production and a subsequent biological stage should also be taken into account; for example, electrochemically produced H_2 can be fermented with CO_2 in an anaerobic reactor by an homoacetogenic species; abiotic reduction to ethanol has already been assessed, and ethanol can be further processed in a bioreactor with acetic acid to perform chain elongation (Prévoteau et al., 2020).

In Chapters 5 and 6 of this thesis, the secondary step proposed was a heterotrophic microalgae bioreactor, external or directly integrated in the BES reactor, aimed at biofuels and other chemical commodities recovery starting from CO_2 and electricity, producing approximately 1.11 kg dry algae per kg acetate (7.59 kgCO_2 captured and 12.9 kWh are necessary for its production). A final balance in terms of oil produced and extracted from the process aimed at biodiesel production of 0.03 kg bio-oil per kgCO_2 was achieved. These results are expected to be improved in larger-scale, continuous flow experiments, where mass cultivation of microalgae can be easily achieved. Combining METs and microalgae may be fruitful for the variety of recovery options, resilience and flexibility of the microalgal biomass, with possible outcomes varying from food and feed market, to proteins, to chemical compounds and biofuels.

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Figure 9. A) Hydraulic and electrical connections. Continuous lines: hydraulic connections (anode orange lines, cathode green lines), R1, R2, R3, R4 recirculation pumps; dashed lines: electrical connections to DAQ board; dotted lines (green and orange): effluents discharge. Legend: (1) air pump; (2, 9) porous diffuser; (3, 6) anode electrode; (4, 7) cathode electrode; (5, 8) reference electrode. B) Cathode chamber setup: MFC2 filled with granular graphite, MFC1 with stainless steel graphite coated mesh. a) inlet; b) recirculation inlet; c) recirculation outlet; d) outlet; e) air inlet.

Figure 10. Power density monitored throughout the experimentation. Error bars report the power range monitored each day.

Figure 11. CE and ηCOD in MFC1 and MFC2.

Figure 12. Polarization curves performed during phase 2.

Figure 13. NER_V and NER_S obtained throughout the study: MFC1 (upper) and MFC2 (lower).

Figure 14. Solid residues observed between the electrode's graphite granules in the open anodic chamber of MFC2 after the study. The black sheet material indicated by the arrow in the right panel is the cell's CEM (shown in new original condition in the picture rightmost insert).

Figure 15. Combined microalgal photobioreactor/microbial fuel cell system. Graphical abstract.

Figure 16. Experimental setup configuration in the first phase. (a) MFC1 with aeration buffer. (b): MFC2 setup with PBR. A: Anode. C: Cathode. Rext: External resistor. (1) Feeding pump; (2) recirculation pumps; (3) aeration buffer; (4) photobioreactor (PBR); (5) CO_2 separator. Orange lines: anodic chamber feeding and recirculation line. Blue lines: cathodic chamber feeding and recirculation line. Black dotted lines: air supply. Dashed lines: effluent discharge.

Figure 17. Experimental setup configuration in the second phase. (a) MFC1 under PBR-air configuration. (b): MFC2 under CO_2-capture configuration. A: Anode. C: Cathode. R_{ext}: External resistor. (1) Feeding pump; (2) recirculation pumps; (3) aeration buffer; (4) photobioreactor (PBR); (5) CO_2 separator. Orange lines: anodic chamber feeding and recirculation line. Blue lines: cathodic chamber feeding and recirculation line. Black dotted lines: air supply. Dashed lines: effluent discharge.

Figure 18. Voltage from MFC1 and MFC2 in the first phase of the experimentation a) with acetate feed; b) with dairy wastewater. Light/dark ratios for MFC2 are reported in the graph. Odd numbers: PBR-air configuration; even numbers: CO_2-capture configuration.

Figure 19. OLR, COD removal and CE for MFC1 and MFC2 throughout the first phase of the study: a) acetate; b) DW. MFC acted as a control with no microalgae in the system.

Figure S8. Preliminary *Chlorella vulgaris* heterotrophic tests with synthetic effluent (acetate 2 g L^{-1}) under different medium and starting pH conditions**.**

Figure 30. Schematic of the three-chamber BES setup.

Figure 31. VFAs production profile in R1.

Figure 32. VFAs production profile in R2.

Figure 33. Results in terms of OD_{600} (A), pH (B) and conductivity (C) of the electrochemical behavior of three chambers MES reactor: cathodic chamber (grey), middle chamber (green) and anodic chamber (black). R1 (left) and R2 (right) results over time are reported.

Figure 34. Current setup (a) and proposed modification (b) for direct VFAs migration and exploitation by the microalgae in the middle chamber.

Figure 35. Schematic of the pyrolysis equipment. 1) Carrier gas tank (N_2); 2) flowmeter; 3) Erlenmeyer flask containing sample; 4) sand bath; 5) thermocouple; 6) scrubber with solvent trap.

Figure 36. TG and DTG thermograms of the initial materials (microalgae (A), sludge (S), and mix microalgae and sludge (A+S)).

Figure 37. Pyrolysis products: biochar (black), bio-oil (yellow) and gas (light blue, estimated). Error bars represent variability of results between triplicates**.**

Figure 38. TGA and DTG thermograms of biochars from pyrolysis at different temperatures. Left: 500°C; right: 350°C. Heating speed constant for all processes (20°C min^{-1}).

Figure 39. Upper: samples from pyrolysis at 500°C a) microalgae Chlorella; b) sludge from WWTP; c) Mix M+S. Lower: samples from pyrolysis at 350°C: d) microalgae Chlorella; e) sludge from WWTP; f) Mix M+S.

Figure 40. IR analyses results. Absorbance curves for initial materials and for biochar from Mix A+S at 350°C are shown. Arrows show the relevant sections.

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List of Abbreviations

A	anode
AC	acetate
AD	anaerobic digestion
ADP	adenosine diphosphate
AEM	anion-exchange membrane
AS	activated sludge
ATP	adenosine triphosphate
BES	bioelectrochemical system
BOD	biological oxygen demand
C	cathode
CE	coulombic efficiency
CEM	cation exchange membrane
CoA	coenzyme A
COD	chemical oxygen demand
DW	dairy wastewater
EAB	electrochemically active bacteria
EC	electrical conductivity
EET	extracellular electron transfer
emf	electromotive force

FID	flame ionization detector
GAP	glyceraldehyde 3-phosphate
GC	gas chromatograph
GHG	greenhouse gases
HHV	higher heating value
HRT	hydraulic retention time
IEM	ion exchange membrane
IR	infrared spectroscopy
LL	landfill leachate
MCC	microbial capture cell
MDC	microbial desalination cell
MEC	microbial electrolysis cell
MES	microbial electrosynthesis
MET	microbial electrochemical technology
MFC	microbial fuel cell
MSW	municipal solid waste
MW	municipal wastewater
NAC	net anodic chamber
$NADPH_2$	nicotinamide adenine dinucleotide phosphate
NCC	net cathodic chamber
NER	net energy recovery
OCV	open circuit voltage
OD	optical density
OER	oxygen evolution reaction
OLR	organic loading rate
ORR	oxygen reduction reaction
OTU	operational taxonomic units
PAHs	polycyclic aromatic hydrocarbons
PBR	photobioreactor

PBS	phosphate buffer solution
PCBs	Polychlorinated Biphenyls
PCR	polymerase chain reaction
PEM	proton exchange membrane
PHA	polyhydroxyalkanoates
pH_2	hydrogen partial pressure
PMMFC	photosynthetic microalgae microbial fuel cell
pO_2	oxygen partial pressure
PPB	Purple phototrophic bacteria
SHE	standard hydrogen electrode
SUC	succinate
SUW	synthetic urban wastewater
TCA	tricarboxylic acid
TCD	thermal conductivity detector
TEA	terminal electron acceptor
TGA	thermogravimetric analysis
VFA	volatile fatty acid
WWTP	wastewater treatment plant