Manuscript Details

Manuscript number	HE_2016_1199
Title	Signal trends of microbial fuel cells fed with different food-industry residues
Article type	Full length article

Abstract

A microbial fuel cell (MFC) is an anaerobic bioreactor where soluble metabolites liberated by hydrolysis and fermentation of macromolecules are simultaneously available for anode respiring bacteria (ARB). ARB can be influenced by chemical imbalances in the liquid phase of the bioreactor. The objective of the work was to explore the trend of electric signals generated by MFCs, in relation to anaerobic biodegradation of four different solid food-industry residual substrates. Four sets of membraneless single-chamber MFCs were operated in batch mode, with solid waste substrates characterized by a different base component: i) mixed kitchen waste (fibers), ii) whey from dairy industries (sugar), iii) fisheries residues previously processed to recover oils (proteins), iv) pulp waste from citrus juice production (acidic). All the tested MFCs were able to produce an electric output with different trends, depending on the principal component of the solid substrate. MFC potential varied as function of the COD and the feeding cycle, as well as of the substrate. The pH variability during the fermentative process significantly affected the electric output. Citrus (acidic) pulp fed MFCs started to operate only when the pH raised up 6.5. MFCs fed with mix food wastes had a relatively stable electric signal; fish based waste caused spiking in the MFC signal and an averaging in the COD degradation trend. This phenomenon was attributed to a pH instability induced by proteins degradation forming ammonia. The fermentation process was strongly predominant with respect the electrochemical process in MFCs and the coulombic efficiency (CE) was low, ranging between 2 to 10 %. This result call for a deeper exploration of harvesting power from solid wastes and pointed also to the possibility of using a MFC to monitor important parameters of fermentation processes in biotech production plants.

Keywords	microbial fuel cells, solid food waste, citrus pulp, fish wastes, diary whey
Manuscript category	Fuel Cells & Applications
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Milan, 11 August 2016

Dear Scientific Committee of Journal of Hydrogen Energy,

It is my pleasure to submit to Your attention the revised version of the manuscript that was previously entitled: " Different solid-phase fuels in microbial fuel cells" and now entitled "***Signal trends of microbial fuel cells fed with different food-industry residues***" which I am submitting for exclusive consideration of publication as an original research article for the Special Issue of the conference EFC2016, held in Napoli (Italy) in December 2015.

We acknowledged all the suggestions and the requests of the Reviewer. We thank them, as they give us the opportunity of strongly improve the manuscript, indeed. At the end of this letter there is the punctual answer to each request and, in following, the

We also confirm, what declared in the previous cover letter:
The paper present, for the first time, the result of an experimentation with sets of membraneless single-chamber Microbial Fuel Cells (MFCs) operated with solid waste dried matrices, characterized by different principal components. All the tested MFCs were able to produce an electric output. Power varied as function of the substrate principal content (protein, sugar, acids, mix), the COD and the feeding cycle. The pH variability during the fermentative process significantly affected the electric output.
The result pointed to the possibility of using a MFC to monitor fermentation processes, such as the biogas production.

The publication is approved by all the authors and by the responsible authorities where the work was carried out.

I can confirm with the other authors that each of us have made substantial contributions to the work and that there are no conflict of interest, including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work.

Hoping in a positive response, and thanking for Your consideration,
I look forward to the challenge of publishing with the journal.

Truly
Pierangela Cristiani

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ANSWERS TO REVIEWERS

We thank the reviewers for the comments that help us to strongly improve the manuscript and we apologies ourselves for the refuses and mistakes in the English of the original version.

We answered to all the requests of the Reviewers and we provided to strongly improve the manuscript, enlarging substantially the discussion, and strongly revising the English syntaxes.

We included the final version of the revised manuscript and a version of the revised manuscript with underlined in red all changed and insert words and phrases and in ~~red~~ all deleted worlds.

Comments from the editors and reviewers:

-Reviewer 1

- The manuscript describes MFC applications for solid-phase fuels. The manuscript must solve the following issues before the publications.

1. First of all, the authors make sure if the work is for power generation or just treatment of solid-phase matters. If the former one is the target for this work, the manuscript must include the detailed relationship between COD and coulombic efficiencies (CE). Also, thorough analysis of the CE data must be made since the CE determines the amount of COD that can be captured as electrical current by the end of each fed-batch cycle.

We thank the reviewer to give us the opportunity of better explain the objective of the work. Our objective was to explore and analyze microbial fuel cells output in function of different organics components of wastes that are usually digested in anaerobic bioreactors. The results indicated the possibility of exploiting the signal achieved, at least, for monitor the biodegradation process. This is a novelty and the most interesting aspect in our opinion.

Under this point of view, the low CE is not an issue, because the lowest is CE, the best for monitoring purpose. In fact, in this condition the MFCs don't influence the biodegradation process much, but it is influenced by it.

The original text was not sufficiently clear about this point, because we expressed this concept just at the end of the discussion and in the conclusion. Now we deeply modified the text, strongly improving the discussion part, and better address the objective in the Abstract and Conclusion.

We also improve the English syntaxes and the lay-out of the figure in order to make more visible all the details.

The abstract was modified adding the following sentence at the beginning:

"A microbial fuel cell (MFC) is an anaerobic bioreactor where soluble metabolites liberated by hydrolysis and fermentation of macromolecules are simultaneously available for anode respiring bacteria (ARB). ARB can be influenced by chemical imbalances in the liquid phase of the bioreactor. The objective of the work was to explore the trend of electric signals generated by MFCs, in relation to anaerobic biodegradation of four different solid food-industry residual substrates"

The introduction was also strongly improved with the following sentences (l. 59-82):

"Anaerobic bioconversion processes such as anaerobic digestion and dark fermentation rely on sequential microbial hydrolytic and fermentative processes that solubilize different substances in the liquid phase. Macromolecules are degraded to soluble molecules that become available to

secondary fermentations or anaerobic respirations, by other microbes forming part of complex consortia [3]. In many cases, the liberation of soluble metabolites might significantly change the chemical equilibria of the liquid and gaseous phases where microbes live [4]. This is often the cause of inhibition of more sensible microbial species and possible imbalances of the overall biodegradation process [5].

In anaerobic biodegradation, secondary metabolisms (e.g. acetogenesis, acetoclastic methanogenesis, hydrogenophilic methanogenesis, denitrification, sulphate reduction, etc.) rely on the availability of short-chain organic molecules, inorganic ions and soluble gasses, such as volatile fatty acids, di-hydrogen, hydrogen sulphide, ammonium, nitrate, carbon dioxide, bicarbonates, etc [6,7].

Similarly, in microbial electrochemical systems like microbial fuel cells (MFC), electroactive microbial species like anode respiring bacteria (ARB) rely on the same substances to transfer electrons and produce current [8]. Macromolecules, in regular MFCs, should be at least hydrolyzed and pre-fermented by fermentative microbial species before being available to ARB, which preferentially oxidize low-carbon carboxylates, as indicated in various literature contributions [8–11].

For this reason, MFCs can be used as a mirror-process for secondary biodegradation metabolisms. In anaerobic environments, the electrical signal produced by ARB activity can give real-time hints on the trend of the ongoing biodegradation mechanisms and biochemical conditions, such as availability of soluble low-carbon organics, availability of mineral nutrients, favorable chemical equilibria in the liquid medium like pH, electrical conductivity, etc. A widely recognized issue is the competition of methanogenic populations and ARB for the same organics [12,13]. This corroborates the assumption that ARB activity, measured as voltage generation, could be used as monitor of the interactions between fermentative and methanogenic microbial populations in anaerobic biodegradation environments."

The discussion was improved in the Result and Discussion chapter, adding a sub-paragraphs for each different substrates used, in this way:

3.1 Electrical signal trend in CW-fed MFCs

where, other than a better revision of the language and English, the follow sentence has been add at the end:

This effect point to the possibility of monitoring on-line possible accumulations of that soluble metabolites inhibiting biodegradation processes. This would be particularly useful in high-solids anaerobic digestion plants [4], or other biodegradation processes at high organic loading rates [20].

3.2 Electrical signal trend in KW-fed MFCs

Where KW is "Kitchen Waste" that substitutes the name of waste previously named "mix from municipal wastes", because more simple and adherent to the substrate used, as the preparation of the mix were made, in this case, starting from the single components specified in the Table 1, and not from a real wastes).

In the paragraph 3.2, this part has been included (l. 220-235):

"Interestingly, the maximum potential decreased cycle by cycle, in parallel with the peak of COD at the beginning of each cycle. The peak value of COD was over 3000 mg_{O2} L^{-1} in the first cycle, and decreased progressively to less than 2000 mg_{O2} L^{-1} in cycle 5.

This condition might be due to the accumulation of mineralized substances in the liquid phase, such as ammonium ions, and other nutrients, creating an inhibitory environment for microbial population and for ARB. The MFC signal followed the general trend of the primary phases of biodegradation (hydrolysis and fermentation). Progressively less organic matter was hydrolyzed from the solid phase (visible as decreasing peaks of COD). Additionally, each cycle lasted for longer time as compared to the first one. Biodegradation rate was visibly decreasing. All these effects were reflected in progressively decreasing peaks of the electrical signals.

The MFCs potential trends were, again, a mirror of an increase of limiting (or toxic) conditions for the overall microbial population in the bioreactor. In this case the electrical signals were indicating a progressive increase of inhibition conditions. This application of MFCs in bioreactors would be useful for monitoring the accumulation of potentially toxic metabolites of biodegradation (ammonia, H_2S, Na^+, etc.) on long-term operations of bioreactors [5, 21].

3.3 Electrical signal trend in FW-fed MFCs

Here, the following sentence was mainly add (lines 261-267):

The peak values of COD were very similar at each cycle. Differently from CW and KW, peak of COD didn't correspond to peaks of cell potential, especially at cycles 2 and 3. This is likely to be due to the recalcitrants hydrolyzed from complex proteins of FW (Table 1). Contrarily, cell potentials lasted for longer time at relatively high values, as compared to COD values.

In this case, MFCs can be thought as monitors of the presence of long-term biodegradable fractions of the organic matter.

3.4 Electrical signal trend in CP-fed MFCs

Here, the following sentences was added, Line 282-289:

"In the first two cycles, acidic conditions (pH 4 – 5) were established in the bioreactor and the pH raised over 6.5 only after 40 days. Alkalinity was evidently insufficient to buffer the acidity of CP (rich in citric acid and other organic acids). COD decreased from 3400 mg_{O2}/l to 200 mg_{O2} L^{-1} along over 45 days of operation of cycle 1 and nearly 50 days in cycle 2.

At cycle 3, a buffer medium (potassium bicarbonate, 5 g L^{-1}) was added to the bioreactor to equilibrate the pH, which remained stable in the range 7 – 7.8 along cycle 3. COD peaked to more than double (over 8000 mg_{O2} L^{-1}), due to more efficient microbial hydrolysis and fermentation of the solids accumulated in the bioreactor."

And at the end (l. 320-324):

When stable pH was guaranteed by equilibrating CP acidity, cell potential trends followed exactly the trend of COD consumption in the liquid phase. These results highlighted another aspect

of the chemical equilibria in anaerobic biodegradation environments, which can be efficiently correlated to the trend of the electrical signal produced by ARB, living in the same environment. pH-related inhibition of microbial activity reflects very promptly in drops of MFCs cell potentials.

The following lines was deleted, because discussing about a phenomenon which data were not reported:

~~Additional CP-fed SMFCs were operated with 0.2 g $COD_{substrate}$/$gVS_{inoculum}$. An increase in potential was observed after 2 days of operation as already obtained with the other substrates (**results not shown here**). As already known from biogas and anaerobic digestion field [11], the results from CP-SMFCs highlighted that the substrate/inoculum ratio is another key limiting factor when organic solid wastes are used to fed microbial metabolism.~~

Also the discussion in chapter 3.5 Electrode polarization curves was improved at the end, from line 341:

This indicates, first of all, that the cathode was predominantly characterized by microbially-catalyzed reduction reactions. As reported in previous works for single-chamber MFCs, bio-cathodic instead of abiotic mechanisms drive oxygen reduction reactions [25]. This is an important aspect to consider, in the case that cell potential has to be used as indicator of microbial consortia activity in a bioreactor. To maximize the MFC system response to inhibitory effects, due to chemical imbalances in the liquid medium of biodegradation environments, single chamber MFCs might be the ideal solution. A double chamber architecture with abiotic cathode would be less sensible to inhibitory conditions for microbes.

FW biodegradation started showing instability at cycle 3. Error bars reported for SCOD measured in two MFCs indicate a condition of partial inhibition of the system. In biodegradation of protein-rich organic materials (see Table 1 for FW composition), it might typically be related to accumulations of ammonia, hydrogen sulfide or other toxic metabolites [26].

The considerable decrease of polarization currents from both bioanodes and biocathodes, already registered during cycle 2 (days 62 – 64, Figure 5), can be considered as an early-warning for inhibiting conditions for the whole bioreactor environment. Future experiments should more deeply focus on this aspect. Polarization of electrodes, as mirror of both anodic and cathodic microbial communities, could be studied as early-warning sensors for inhibiting conditions in anaerobic biodegradation environments.

3.6. Just monitoring or influencing the biodegradation process of COD removal?

This sub-chapter concludes the discussion (l. 372-380):

This indicates that biodegradation were negligibly influenced by the MFC process. In future applications of MFCs as sensors for monitoring biodegradation process, electrode surface/reactor volume ratio might be even scaled down, to monitor specific environments.

On the contrary, enlarging the electrodes and improving their surface/volume ratio using a different geometry, will allow to reach and enhance the performance of the MFC process in degrading solid phase organics with respect the current literature [14].

Finally, the optimization of the electrode surface finishing [29] and the use of different materials such as stainless steel [28] would address other possible needs for an useful application in both cases: monitoring or influencing the biodegradation process.

Finally, the Table 2 was simplified as below, reporting just three cycles, as the others are not performed for all the substrates:

Table 2 Coulombic efficiency and COD removal during fed-batch MFC operation.

	Sodium acetate (control)		**Cheese whey**		**Kitchen waste**		**Fish waste**		**Citrus pulp**	
	CE (%)	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**
Cycle 1	22.4	98.1	0.76	95.1	3.87	84.37	7.77	88.69	3.13	93.52
Cycle 2	21.3	95.9	2.02	96.9	9.91	64.25	4.37	85.94	2.74	89.70
Cycle 3	23.2	97.4	1.87	95.8	9.40	61.40	3.53	89.20	-	-

2. Although the authors claim that the low CE was due to other non-optimized conditions in MFCs, the CE the authors provide in table 2 is significantly low compared to other studies and does not support that the COD removal is due to electron transfer reactions. Rather, the COD removal might be other anaerobic or aerobic processes. Then the treatment of the solid-phase matters might be made through simple microbial reactions not in the MFCs. The authors clearly mention about this.

Please see the answer above, valid also for this point.

3. The reviewer wonders if we can see this work as an actual solid-phase matters' treatment method. This is because all the matters were completely grinded and mixed with liquid phase solution. The actual raw wastewater does not look like this.

The referee is right and we thank you for underlying this important issue.

Although some other authors [14] already referred to "solid MFCs in similar cases, the definition of 'solid' MFCs was consequently removed from the text, and used just MFCs.

Grinded materials in suspension in a bulk sludge at around 20 g per liter of volatile solids is a typical concentration for optimized anaerobic digestion tests. In this test we worked at standard conditions for anaerobic biodegradation. To clarify this, the M&M section was deeply improved.

4. There are so many typos/errors in the manuscript and non-technical writing phrases/words. The authors need to thoroughly check the manuscript again.

We apologies us again for the mistakes, due to a quite hurry in submitting the manuscript within the deadline. We improved the manuscript with a deep revision of all the parts.

5. The figure 1, 2, & 3 must be more readable and clear so that the readers can understand. The labels are needed. Or some enlarged portion will be needed.

The figure 1, 2, 3 and 4 were completely revised and improved, to make them readable. Figure 6 (old version) should clarify a single cycle, now better visible, so it was removed in the new version of manuscript. The trends are now readable in the graphs of figures 1,2,3,4, split into two parts of 60 days each. Cycles were also indicated.

The new Figures are:

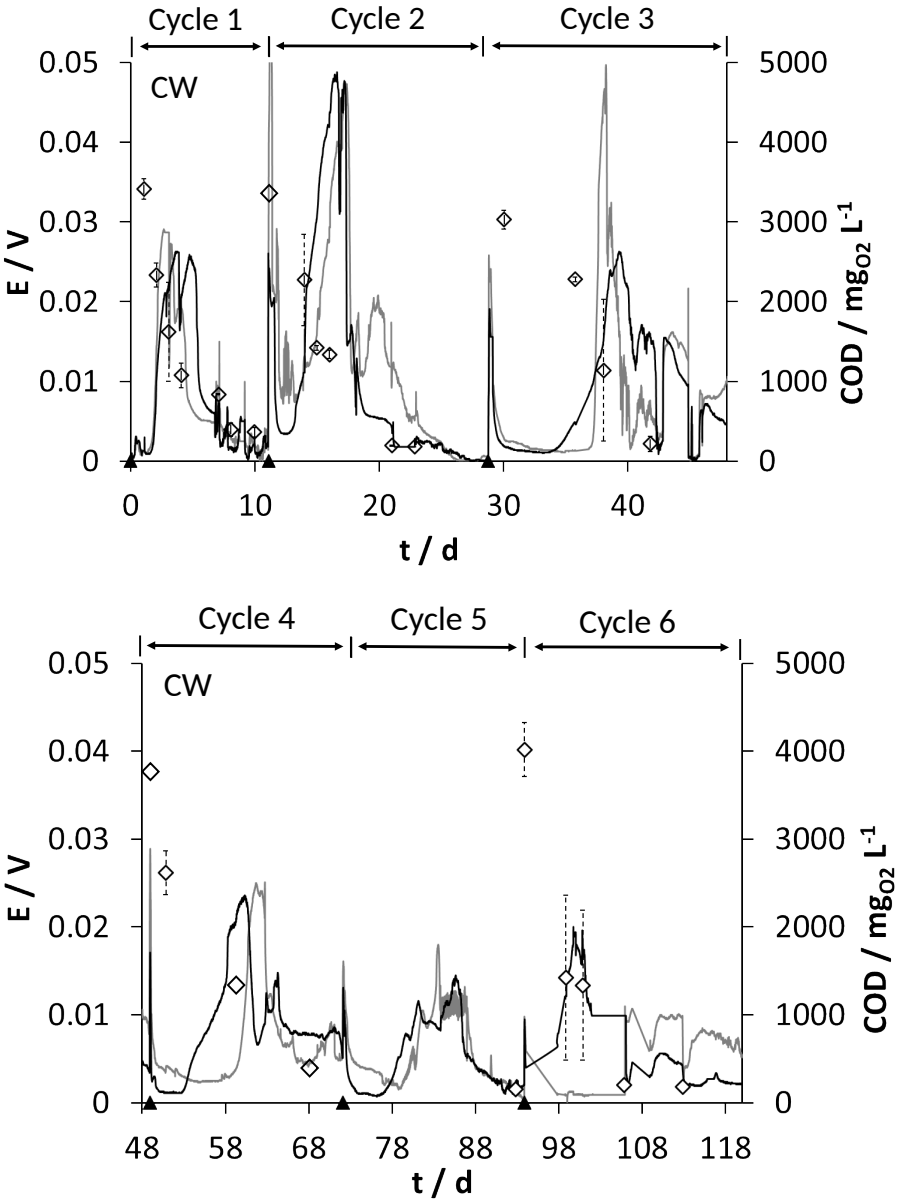

Fig. 1 Graphics of potential trends of two CW-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and six batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

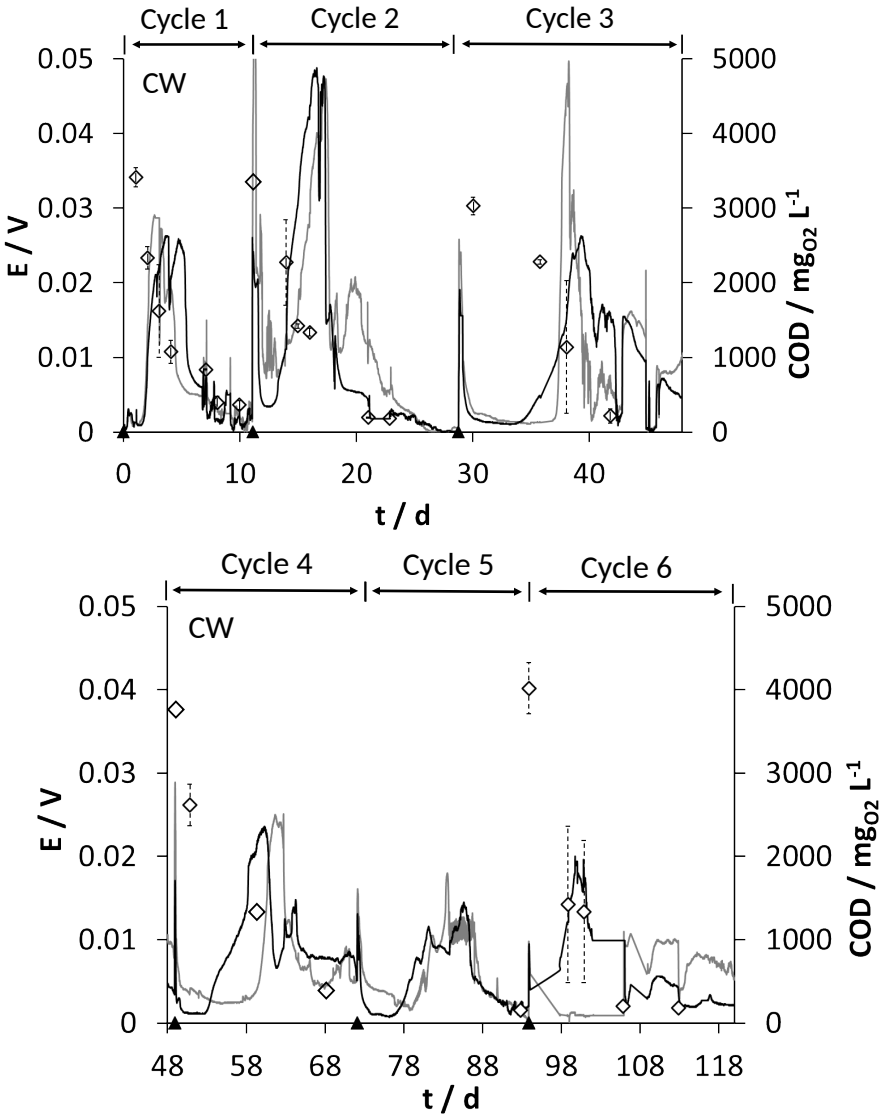

Fig. 1 Graphics of potential trends of two KW-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and six batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

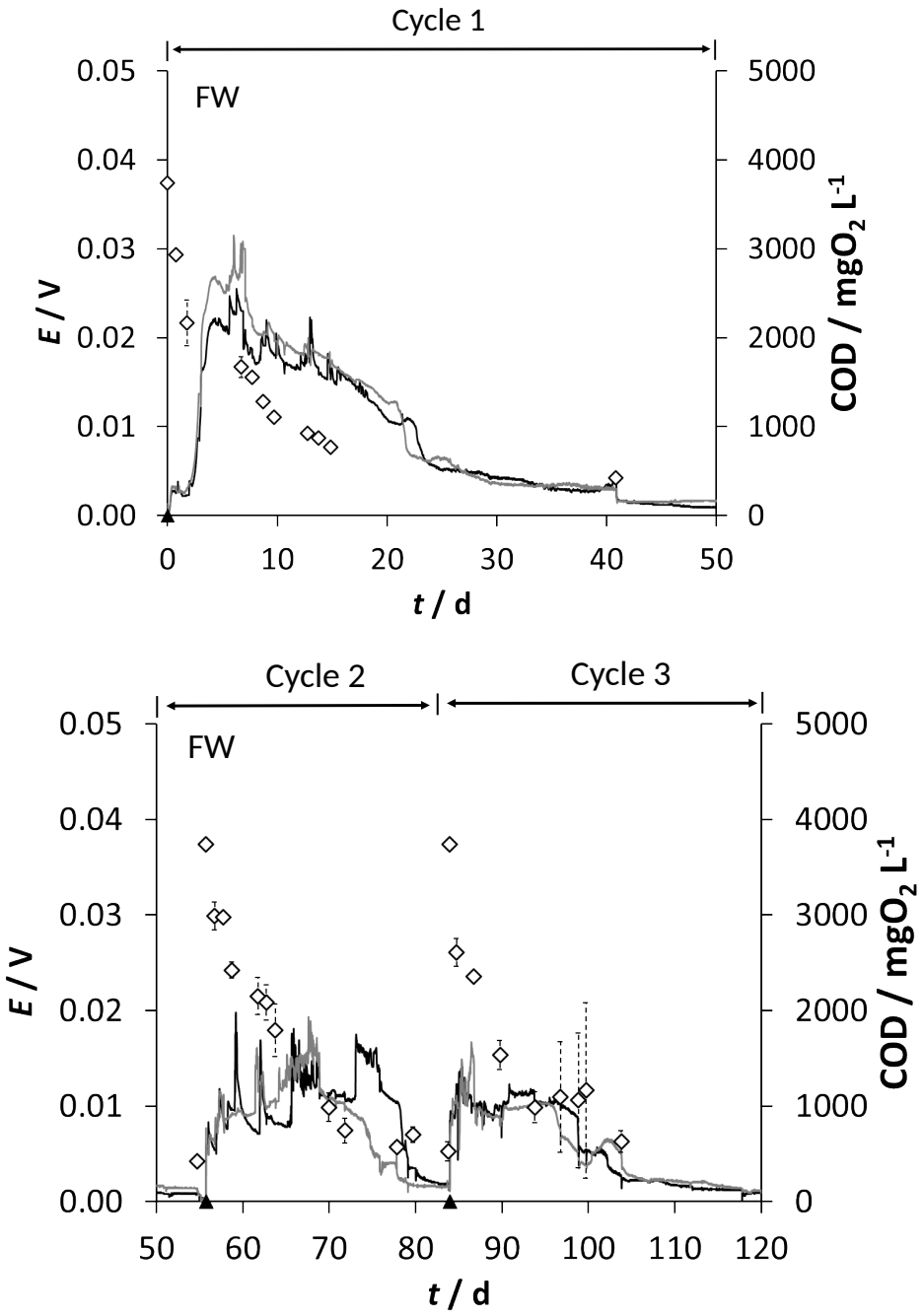

Fig. 2 Graphics of potential trends of two FW-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and three batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

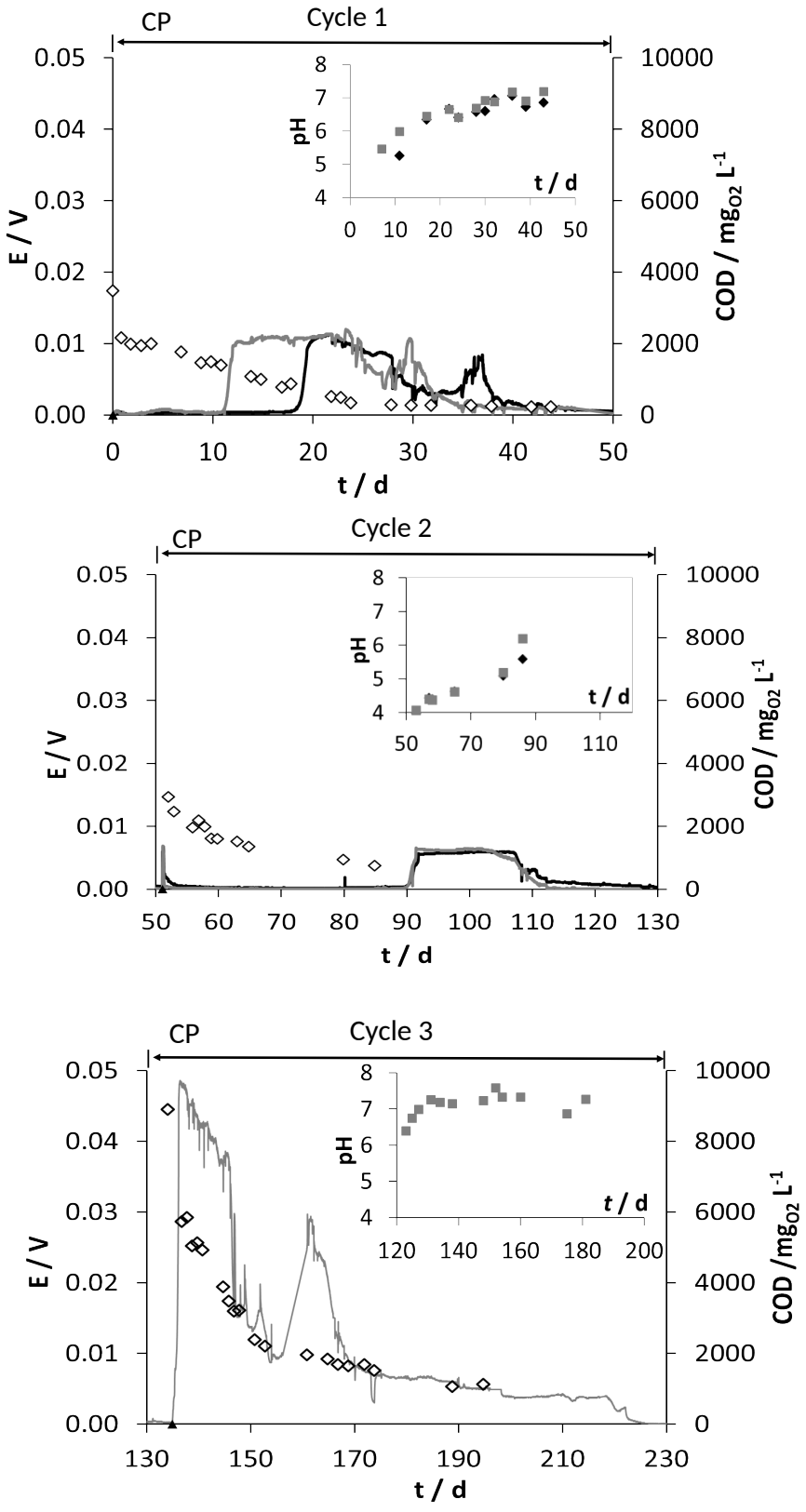

Fig. 4 Graphics of potential trends of two CP-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 230 days of operation and three batch cycles. Standard deviation of COD values is reported with vertical bars. Insets report the trend of pH over time.

-Reviewer 2

In this manuscript, the authors investigated the power output of solid-phase microbial fuel cells with four solid organic substrates. The coulombic efficiency and COD removal were also obtained. In

my opinion, major revision is needed for publication in this journal based on the following comments:

(1) The title 'Different solid-phase fuels in microbial fuel cells' means the key point is the fuel, obviously, it can not accurately summarize the content of this manuscript.

The referee is completely right. We deeply modified the title and the abstract, accordingly. The results and discussion chapter was also revised, according to the main meaning of the work that was better underlined.

Please see the previous answers and the revised version with the track-changes underlined in red.

(2) In Fig. 1, there is no sCOD value in the 4th and 5th cycle, the same for the 4th cycle in Fig. 2. Why the test is discontinuous?

Some cycles were not monitored in deep. However, they are a minor part and the overall trend of the bioreactors are clear with the available data. We improved by analysing at least the beginning and the end of the cycle, where there were missing samples along the cycle.

(3) In Fig. 5, the insert figure can not show the pH value after day 90, the authors should present enough pH results. In addition, there is delay in producing power in the 1st and 2nd feeding cycle, however, why no delay exists in the 3rd cycle?

Thank you for the note about pH. The request was approached. Please see the revised figure 4. In addition, the range of pH in all other experiments was reported along the text. See lines 164, 183 and 210.

The other point of the referee was deeply explained in the discussion, in the section *3.4 Electrical signal trend in CP-fed MFCs*

(4) There are two 'Fig. 6' in the manuscript. In the first 'Fig. 6', no legend description for a, b, c and d. And there is no discussion in the text for the second 'Fig. 6'.

This was deeply improved. The ''first figure 6'' was removed, as it is included in Figures 1,2,3,4 with an improved view of the trends of Cell potentials.

Please see the new versions of all figures, also according to Referee 1.

(5) Some words in the manuscript are not the common expressions in microbial fuel cells, such as 'power intensity' and 'potential output'. Generally speaking, the cell performance is evaluated by 'power density'. In Fig.1-3 and Fig.5, I guess the left y-axis is the voltage of the fuel cell, not the potential output.

All these mistakes were due to hurry in the deadline for submission. We deeply improved the text and the terminology throughout the manuscript.

(6) The manuscript needs careful editing paying attention to 'space' and spelling such as:
in lines 125, 126, 130 and 144 'SMFC s',
in line 218 'SMFCat',
in line 249 'SMFCoperation',
in line 257 'byporducts'.

All these mistakes were due to hurry in the deadline for submission. We deeply improved the text and the terminology throughout the manuscript.

Here THE FULL revised MANUSCRIPT

~~Different solid-phase fuels in microbial fuel cells~~

Signal trends of microbial fuel cells fed with different food-industry residues

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ABSTRACT

A microbial fuel cell (MFC) is an anaerobic bioreactor where soluble metabolites liberated by hydrolysis and fermentation of macromolecules are simultaneously available for anode respiring bacteria (ARB). ARB can be influenced by chemical imbalances in the liquid phase of the bioreactor. The objective of the work was to explore the trend of electric signals generated by MFCs, in relation to anaerobic biodegradation of four different solid food-industry residual substrates. ~~Solid-phase microbial fuel cells (SMFCs) treat solid organic waste mixed in liquid phase as fuel to harvest power.~~ Four sets of membraneless single-chamber ~~S~~MFCs were operated in batch mode, with solid waste substrates characterized by a different base component: : i) mixed kitchen waste (fibers), ii) whey from dairy industries (sugar), iii) fisheries residues previously processed to recover oils (proteins), iv) pulp waste from citrus juice production (acidic).

All the tested ~~S~~MFCs were able to produce an electric output with different trends, depending on the principal component of the solid substrate. ~~Power intensity~~ MFC potential varied as function of the COD and the feeding cycle, as well as of the substrate. ~~The space occupied by the anode was less than 0.1 of the anode-chamber volume, consequently.~~

The pH variability during the fermentative process significantly affected the electric output. Citrus (acidic) pulp fed SMFCs started to operate only when the pH raised up 6.5. ~~S~~MFCs fed with mix food wastes had a relatively stable electric signal; fish based waste caused spiking in the ~~S~~MFC signal and an averaging in the COD degradation trend. This phenomenon was attributed to a pH instability induced by proteins degradation forming ammonia.

The fermentation process was strongly predominant with respect the electrochemical process in MFCs and the coulombic efficiency (CE) was low, ranging between 2 to 10 %. This result call for a deeper exploration of harvesting power from solid wastes and pointed also to the possibility of using a ~~S~~MFC to monitor important parameters of fermentation processes in biotech production plants.

Highlights

- Four different component of solid organic wastes were investigated ~~as fuel~~ in membranelessMFCs
- Cell potential trends varied in function of different waste components in the bioreactors
- ~~All the SMFCs fed with different fuels were able to produce an electric output~~
- Cell potential trends varied as function of the substrate, the COD and the feeding cycle
- The pH in the anodic chamber significantly affected the electric output
- Results call for exploration of MFCs as sensors for fermentation/biodegradation processes

Index Terms – microbial fuel cells, solid food waste, citrus pulp, fish wastes, diary whey

1. Introduction

The new paradigm of circular economy claims new technological approach for energy and resource recovery. Agro-food industry produces massive amounts of organic materials as secondary streams and waste [1]. Microbes naturally evolve enzymes and pathways that can convert solid biomass-derived carbon sources into valuable fuels and products, such as biomethane, biohydrogen, biodegradable polymers, carboxylates [2]. Biological conversions ~~might~~ play a fundamental role in waste refinery chains and especially of agricultural and food-industry residues. In this context, microbial electrochemical technologies (METs) offer potential innovative approaches in wastes treatment.

Anaerobic bioconversion processes such as anaerobic digestion and dark fermentation rely on sequential microbial hydrolytic and fermentative processes that solubilize different substances in the liquid phase. Macromolecules are degraded to soluble molecules that become available to secondary fermentations or anaerobic respirations, by other microbes forming part of complex consortia [3]. In many cases, the liberation of soluble metabolites might significantly change the chemical equilibria of the liquid and gaseous phases where microbes live [4]. This is often the cause of inhibition of more sensible microbial species and possible imbalances of the overall biodegradation process [5].

In anaerobic biodegradation, secondary metabolisms (e.g. acetogenesis, acetoclastic methanogenesis, hydrogenophilic methanogenesis, denitrification, sulphate reduction, etc.) rely on the availability of short-chain organic molecules, inorganic ions and soluble gasses, such as volatile fatty acids, di-hydrogen, hydrogen sulphide, ammonium, nitrate, carbon dioxide, bicarbonates, etc [6,7].

Similarly, in microbial electrochemical systems like microbial fuel cells (MFC), electroactive microbial species like anode respiring bacteria (ARB) rely on the same substances to transfer electrons and produce current [8]. Macromolecules, in regular MFCs, should be at least hydrolyzed and pre-fermented by fermentative microbial species before being available to ARB, which preferentially oxidize low-carbon carboxylates, as indicated in various literature contributions [8–11].

For this reason, MFCs can be used as a mirror-process for secondary biodegradation metabolisms. In anaerobic environments, the electrical signal produced by ARB activity can give real-time hints on the trend of the ongoing biodegradation mechanisms and biochemical conditions, such as availability of soluble low-carbon organics, availability of mineral nutrients, favorable chemical equilibria in the liquid medium like pH, electrical conductivity, etc. A widely recognized issue is the competition of methanogenic populations and ARB for the same organics [12,13]. This corroborates the assumption that ARB activity, measured as voltage generation, could be used as monitor of the interactions between fermentative and methanogenic microbial populations in anaerobic biodegradation environments.

~~SMFCs fed with solid organic waste are generally indicated as solid-phase SMFCs (SMFCs~~

Electricity harvesting with complex biomass ~~in solid-phase MFCs~~ was recently achieved by Mohan et al. [14], who fed open-air cathode, single-chamber ~~S~~MFCs with different types of canteen-based food waste. The best performing configuration, where a proton exchange membrane separated the cathode from the anodic chamber, achieved a peak power density of 170 mW m^{-2} with open circuit potential (OCP) of 463 mV. Similar results with similar substrates were obtained by Goud et al[15], who tested increasing organic loading rates (OLR) in bio-electrochemical reactors fed continuously. ~~Above~~ OLR of 1.39 kg COD/m^3-day both power density and OCP started decreasing, due to inhibiting concentrations of volatile fatty acids (>800 mg/L) and acidic pH conditions (pH=6).

Pretreatment of wastes from agro-food is achieved in several ways (e.g. to extract essential oils and proteins from specific wastes, such as citrus pulp, residual fish) and MET can be though as downstream processing for energy harvesting in Microbial Fuel Cells [14] or for further bio-processing (e.g. electrofermentation [12]), however, very little is reported in literature about biodegradation pathways of complex organic matrices in the bulk medium. In particular, the relationships between the electric signal produced by ARB and the biodegradation process as-a-whole (hydrolysis of macromolecules, fermentative metabolisms, etc.).

Here, we studied the electrical signals produced by MFCs during anaerobic biodegradation of four different types of agro-industrial residual materials of interest in Mediterranean agro-food sectors: citrus pulp, fishery waste, cheese whey and ~~organic fraction of municipal solid~~ kitchen waste. Voltage trends were monitored on long-term, ~~operation of SMFCs was monitored~~ ,over 100 days, with successive batch cycles, to evaluate the response of the electrochemical system to the anaerobic biodegradation of the solid matrices.

2. Materials and methods

2.1 ~~S~~MFC configuration and setup

Four sets of membraneless single-chamber ~~S~~MFCs were operated in duplicate and in parallel over more than 100 days. The total volume of each MFC was 125 ml and the design was previously reported [16]. Anodes were made of 3×5 cm rolled carbon cloth sheet (Saati C1, Legnano, Italy), electrically connected to a copper wire. Three layers of non-conductive high-viscosity epoxy resin (Mapei Epojet) were applied to ensure insulation at the connection between copper and carbon cloth. Cathodes were made of 5×5cm carbon cloth sheets modified by the addition of a Gas Diffusion Layer (GDL) on the air side. The GDL composition has

been described in [17] and the PTFE content is 80%$_{w/w}$ with respect to carbon powder. The geometric cathodic surface area exposed to the solution was 3 cm^2. Anode and cathode were then connected through an external circuit with a resistance of 100 Ω.

All SMFCs were operated at mesophilic temperature of 35±1 °C in batch mode without pH adjustment. SMFCs were inoculated with 90 ml of anaerobic mesophilic sludge obtained from a municipal wastewater treatment plant (Cremona, Italy). The volatile solids (VS) content in the sludge was 15 g $_{VS}$kg^{-1}. This concentration is typically used in standard batch-like anaerobic digestion tests [18]. The sludge was not subjected to any pretreatment. A concentrated solution of nutrients was added at the beginning of the experimentation. The stock solution of nutrients contained (in g/L): KH_2PO_4 (0.27), $Na_2HPO_4 \cdot 12H_2O$ (1.12), NH_4Cl (0.53), $CaCl_2 \cdot 2H_2O$ (0.075), $MgCl_2 \cdot 6H_2O$ (0.10), $FeCl_2 \cdot 4H_2O$ (0.02). Analytical grade reagents and double distilled water were used.

Two SMFCs were fed with each organic substrate in the form of dried powder (1 mm particle size): i) cheese whey powder (CW); ii) ~~dried organic fraction municipal solid~~ kitchen waste (KW); iii) ~~dried~~ fish waste (FW); iv) ~~dried~~ citrus pulp (CP). The macromolecular composition of the four ~~solid waste~~ substrates was reported in Table 1. CW was a commercial by-product from dairy industries (Cremona, Italy) used as animal feed. ~~OFMSW~~KW was a mixture of animal and vegetal food waste prepared in lab according to the following recipe: 30 g egg shells; 30 g dried bread, 50 g corn flour, 100g grated cheese, 75 g cracker, 10 g coffee grounds, 130g apple peel, 300g green salad, 145 g orange peel, 85 g zucchini peel, 68 g banana peel, 56 g carrots, 30 pumpkin skin, 20 g kiwi peel, 30 g fennels, 16 g potato peel. Food mixture was grinded with a kitchen blender, homogenized and finally dried at 105 °C. FW was obtained from fish after an enzymatic pretreatment to remove oils (no alcohols used). CP was obtained from citrus juice production plant (Catania, Italy).

Table 1 Main macromolecular constituents of the four solid wastes

Substrate	Carbohydrates (% of DM)	Fibers (% of DM)	Fats (% of DM)	Proteins (% of DM)	Ashes (% of DM)
CW	70 (lactose)	-	-	12	8.5
KW	53.4	19.2	9.6	14.3	3.5
FW	0.3	-	3.8	51.2	20.1
CP	8.5	43.1 (33.2 cellulose, 9.9 hemicellulose)	3.1	26.9 (19.7 pectin)	18.4

The amounts of inoculum and organic substrates introduced in each SMFCs were determined on the basis of preliminary analytics determination (volatile solids and total solids). The organic substrate to inoculum ratio was 0.35 g sCOD$_{substrate}$/g VS$_{inoculum}$. A new dose of feed was added when negligible potential values were obtained and soluble Chemical Oxygen Demand (COD) fell down to a constant value.

2.2 Tests

2.2.1 Data acquisition, electrochemical experiments and calculations

The potential difference across the 100 Ω resistance (*R*) was acquired every 10 minutes, via a multichannel Data Logger (Graphtech midi Logger GL820). The generated current (*I*) was calculated by the equation $I = V/R$, where *I* is the current flowing through the external resistance. The total charge flowed into the electrical circuit at the end of each batch cycle was calculated by integrating the current over time. Coulombic efficiency (CE) was then evaluated on the basis of degraded soluble COD.

Quasi-steady stationary polarization curves were recorded *in situ* on anodes and cathodes. Experiments were performed with a classical three-electrode configuration, using a Compactstat IVIUM potentiostat connected to a personal computer. Anodes and cathodes were used as working electrode, a platinum wire as counter electrode and an Ag/AgCl (3M) electrode as reference. All the potentials throughout the text are referred to the Ag/AgCl (3M) electrode. For polarizations on the cathode, a Luggin capillary was adopted to minimize the ohmic drop into the solution. Before each experiment, ~~S~~MFC s were allowed to equilibrate at the open circuit potential (o.c.p.) for at least 30 minutes. Potential was then moved at a scan rate of 10 mV/min from the o.c.p. to 0.1 mV for polarization on anodes, and from the o.c.p. to -0.5 for polarization on cathodes.

2.2.2 Chemical ~~oxygen demand analysis~~ characterizations

The soluble Chemical Oxygen Demand was periodically measured by a spectrophotometric method. A portion of solution sampled from each ~~S~~MFC was centrifuged for 15 minutes at 6000 rpm, carefully added to HT-COD cuvette test (Hach Lange Gmbh), and digested at 175°C for 15 min (Lange HT 200 S). Upon cooling, the COD value was read by an UV- spectrophotometer (Lange DR 3900).

3. Results and discussion

3.1 Electrical signal trend in CW-fed MFCs

Error! Reference source not found. reports the evolution of potential trend for two ~~S~~MFC s fed by CW along with the degradation of the soluble COD. pH was stably in the range 6.5 – 7.5, in all cycles. The acquisition of cell potential over all the time and the measurements of sCOD provided an indication of the productivity of each ~~S~~MFC and of the rate of organic substrate degradation.

The ~~S~~MFC produced power within 2 days, along with the establishment of anaerobic conditions ~~inside~~in the ~~SMFC s~~anodic chamber and the colonization of anode and cathode by biofilms. ~~S~~MFC produced a peak of potential at days 4-5 and then potential rapidly decreased down to a negligible value. COD continuously decreased from the first day. ~~The first cycle~~Cycle 1 of CW degradation ~~got~~was completed within 11 days, reaching 95% of COD removal. When a new dose of CW was added at day 11, ~~S~~MFC immediately produced a ~~sudden~~ spike of cell potential followed by dramatic drop and a new broader peak with a maximum of 50 mV at day 16. After the ~~decaying~~decay of cell potential to a negligible values and the decrease of COD, a third dose of CW was added. Six cycles of feeding were operated over 125 days. pH varied in the range 6.7 – 7.5.

The same trend in cell potential ~~output~~ was observed for all cycles, with the generation of a spike just after the addition of a dose of feed followed by the development of a broader peak after a lag time. The duration

of the ~~cycle~~cycles became longer from the third cycle on (about 21 days) and the maximum ~~generated~~cell potential decreased from the fourth cycle on. The rates of soluble COD removal, however, seem quite similar in each cycle. These two aspects pointed to a progressive deactivation of the ~~whole S~~MFCs, over long-term operation, due to electrode scaling, as documented for wastewater operated with the same ~~S~~MFC in previous works [19] as consequence of the alkalinity generated at cathode [20].

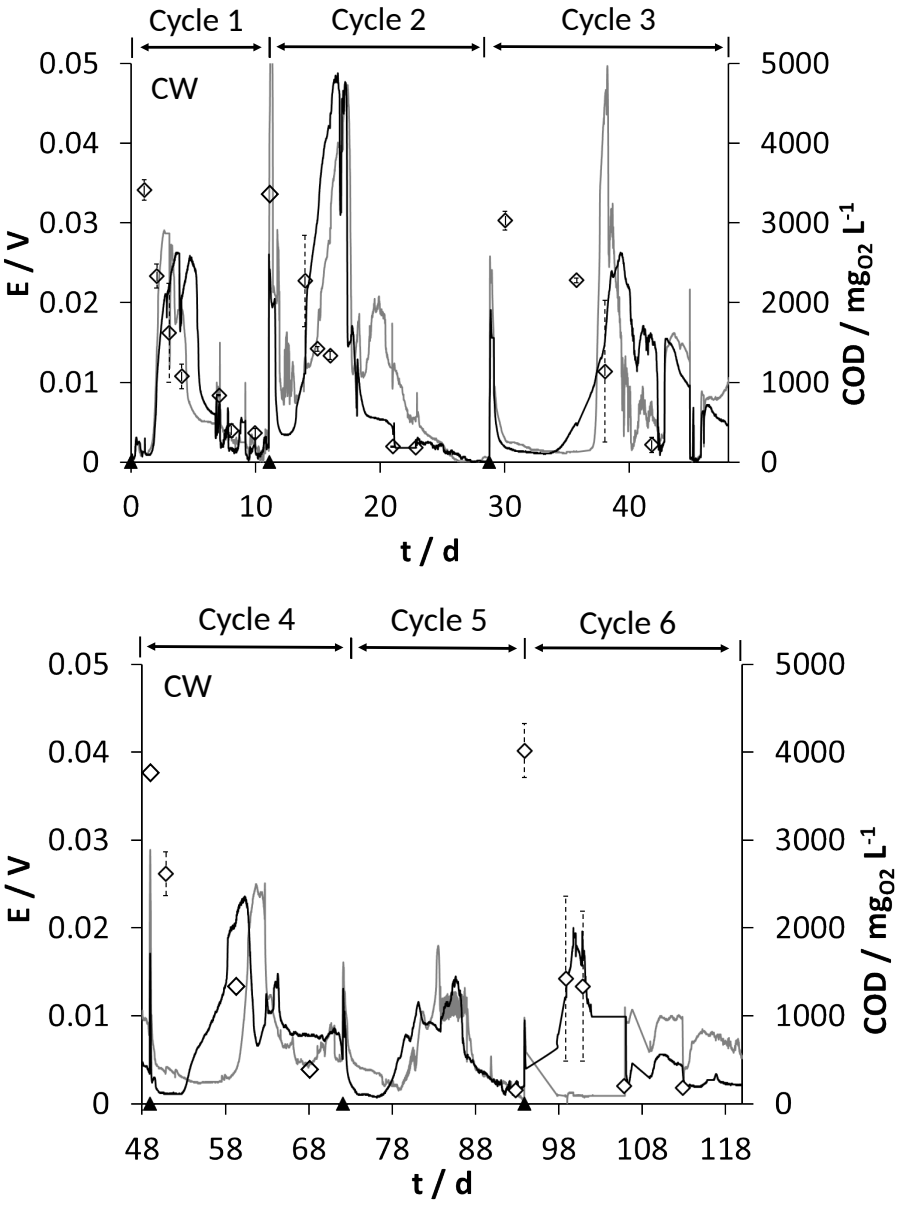


Fig. 1 Graphics of potential trends of two CW-fed SMFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and six batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

The initial spike of cell potential, found at each cycle, can be attributed to a rapid increase of easily degradable molecules in the liquid phase, as a consequence of acidogenic fermentation of lactose. Lactose is easily hydrolyzed to glucose and galactose; sugars fermentation to short chain fatty acids in the bulk anaerobic medium happens at high rates [21]. The sudden drop of cell potential is likely due to a temporary

inhibitory effect of soluble metabolites (e.g. volatile fatty acids) on ARB activity, with a detrimental effect on the electrochemical signal of ~~S~~MFCs.

This effect point to the possibility of monitoring on-line possible accumulations of that soluble metabolites inhibiting biodegradation processes. This would be particularly useful in high-solids anaerobic digestion plants [4], or other biodegradation processes at high organic loading rates [22].

3.2 Electrical signal trend in KW-fed MFCs

Fig. 3Fig. 3 reports the evolution of cell potential over time for two ~~S~~MFCs fed by ~~OFMSW~~KW along with the ~~degradation of~~ the soluble COD degradation. pH was stably in the range 6.5 – 7.5, in all cycles.

Just after the first day of operation, an increase in ~~the~~cell potential ~~output~~ was observed. The potential reached a maximum of 40 mV at day 2 and then stepwise decreased. COD values continuously decayed from the first day ~~to a constant value of about~~of each cycle to around 500 mg_{O2}~~/l,~~ L^{-1}. Further degradation beyond ~~that it was not further degraded. The~~this value was never achieved. At the first cycle, 84% of ~~removal~~ initial COD could be ~~reached~~ removed within 11 days. The second cycle was operated from day 11, when a new dose of ~~OFSMW~~KW was added into the ~~S~~MFCs. An initial spike of potential was observed, immediately followed by two broader shoulders. The duration of the second cycle was 17 days.

Five cycles of feeding were operated over ~~125~~120 days. The two MFCs gave similar cell potential ~~output~~signals during all ~~the~~ cycles, even though the duration of the cycles became longer and the resolution between the two shoulders of potentials trends became less defined.

The evolution of potential over time is significantly different for KW than CW, even if the duration of COD removal is quite similar. ~~In the case of CW, the produced potential is characterized by sudden increases and rapid drops. On the other hand, when SMFCs are fed by OFMSW~~ In this case, the potential remained higher and more stable for longer time. This aspect highlights that the accumulation of VFA is not the main issue in the degradation process, since the composition of KW is more complex than CW.

It is possible to deduce that the macromolecular degradation, producing still complex organics other than volatile acids, might prevent the inhibition of the bacterial activity and had an effect on the stabilization of the electrochemical signal of the whole MFCs.

Interestingly, the maximum potential decreased cycle by cycle, in parallel with the peak of COD at the beginning of each cycle. The peak value of COD was over 3000 mg_{O2} L^{-1} in the first cycle, and decreased progressively to less than 2000 mg_{O2} L^{-1} in cycle 5.

This condition might be due to the accumulation of mineralized substances in the liquid phase, such as ammonium ions, and other nutrients, creating an inhibitory environment for microbial population and for ARB. The MFC signal followed the general trend of the primary phases of biodegradation (hydrolysis and fermentation). Progressively less organic matter was hydrolyzed from the solid phase (visible as decreasing peaks of COD). Additionally, each cycle lasted for longer time as compared to the first one. Biodegradation rate was visibly decreasing. All these effects were reflected in progressively decreasing peaks of the electrical signals.

The MFCs potential trends were. Again, a mirror of an increase of limiting or toxic conditions the overall microbial population in the bioreactor. The MFCs potential trends were, again, a mirror of an increase of limiting (or toxic) conditions for the overall microbial population in the bioreactor. In this case the electrical signals were indicating a progressive increase of inhibition conditions. This application of MFCs in bioreactors would be useful for monitoring the accumulation of potentially toxic metabolites of biodegradation (ammonia, H2S, Na+, etc.) on long-term operations of bioreactors [5, 23].

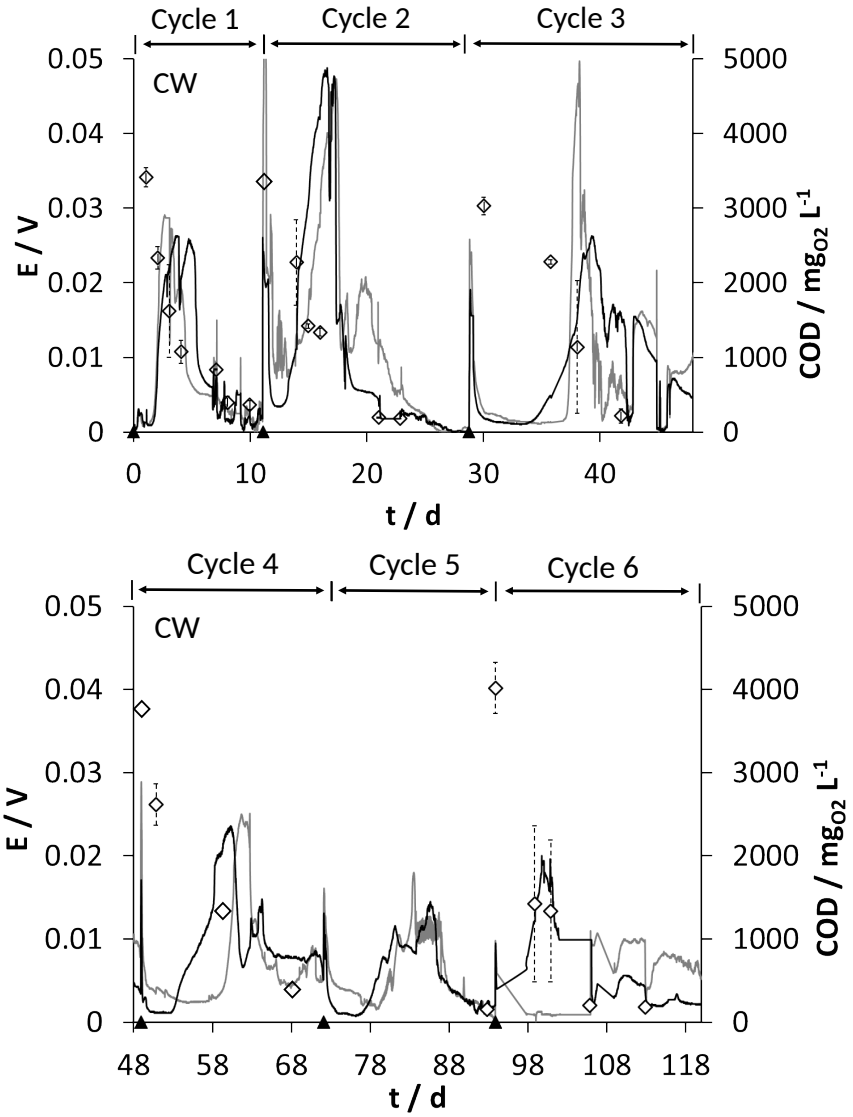
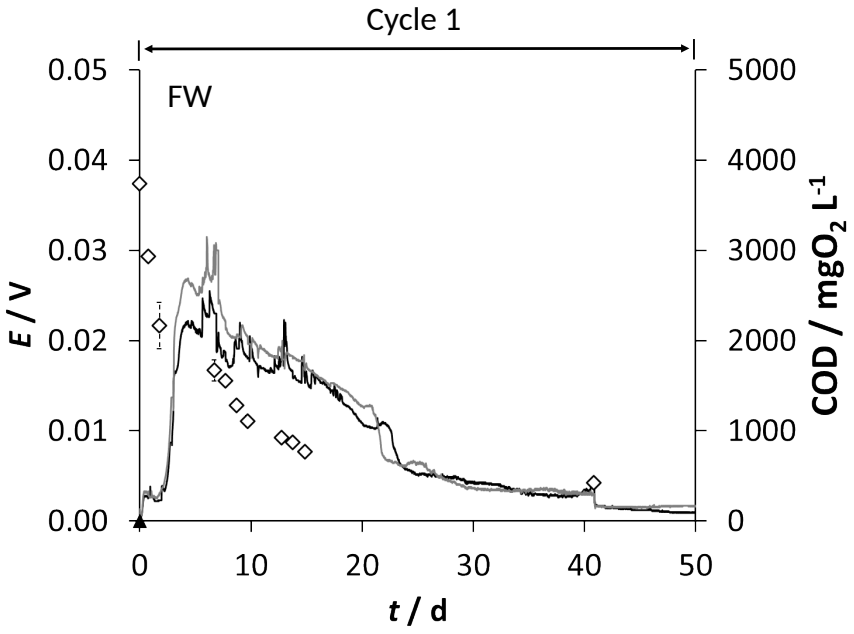


Fig. 4 Graphics of potential trends of two KW-fed ~~S~~MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and six batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

3.3 Electrical signal trend in FW-fed MFCs

Figure 3Fig. 5 reports the evolution of cell potential over time for two ~~S~~MFCs fed by FW along with the ~~degradation of~~ the soluble COD degradation. pH was stably in the range 7.5 – 7.9 in all cycles. Both MFCs started to generate electric signal from day 2, with ~~the~~cell potential that rapidly increased up to a maximum of 30 mV and then slowly decreased from day 7 to day 25. ~~In~~At the same time, COD spiked to nearly 3700 mg_{O2} L^{-1} and continuously decreased ~~from 3700~~ to 400 ~~mg(O_2)/l and no further decreased beyond this~~

~~value.~~mg_{O2} L^{-1}. During the first cycle, ~~the~~ 88% of COD removal was achieved~~.~~ – in around 40 days. Electric signal and COD varied with a really similar trend along the first cycle.



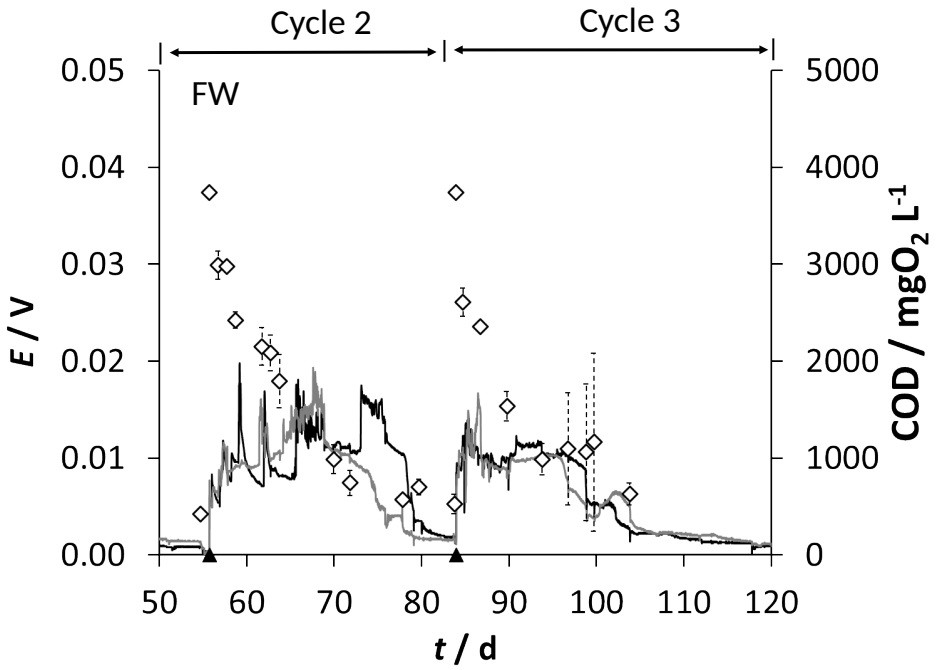


Fig. 5 Graphics of potential trends of two FW-fed SMFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and three batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

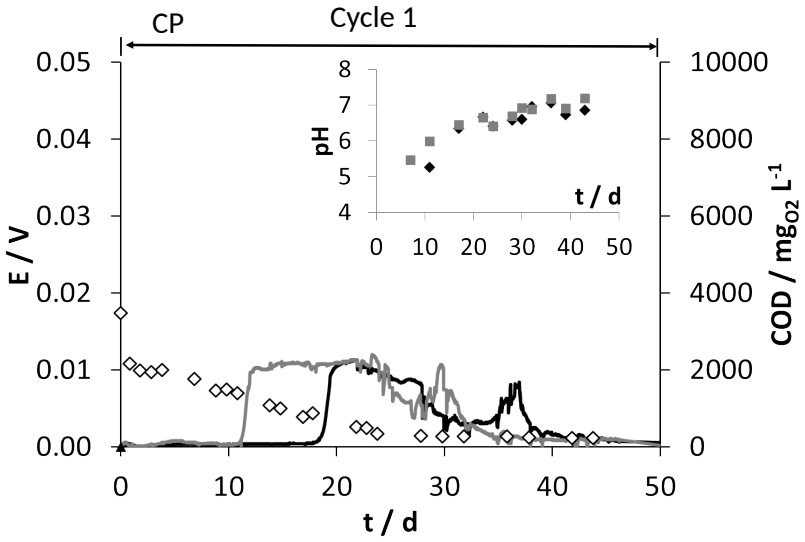
At day 55, a new dose of FW was added. Upon addition, the potential firstly rapidly increased up to 10 mV, then maintained in the range between 10 mV and 20 mV for at least 25 days. The first cycle of operation was the more efficient in term of electrochemical performance and COD degradation, and different behavior was observed in the following cycles. At day 84, a new dose of FW was added and evolution of potential and

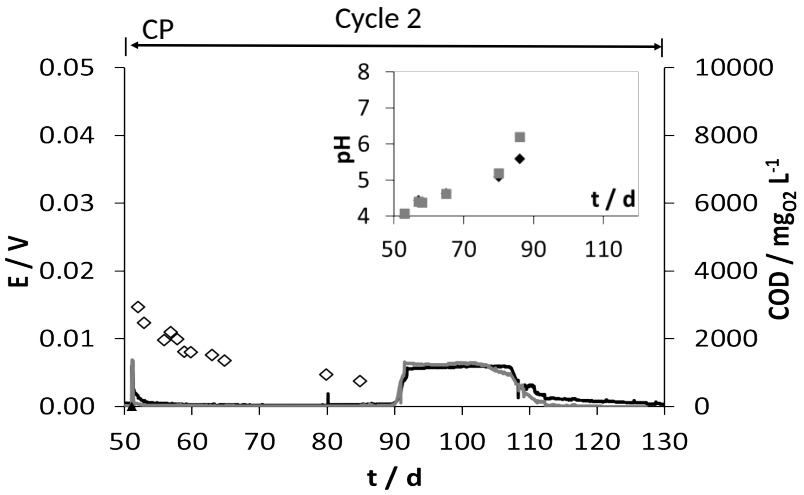
COD similar than in the second cycle were achieved. ~~In the first days after the feed addition, the COD was removed very fast for each cycle, while in the last days of each cycle the~~ In cycle 2 and 3, COD removal happened with nearly a double rate, ~~of degradation experienced a slowing down,~~, in parallel with ~~an increasing residual COD concentration cycle by cycle.~~ cell potentials. The peak values of COD were very similar at each cycle. Differently from CW and KW, peak of COD didn't correspond to peaks of cell potential, especially at cycles 2 and 3. This is likely to be due to the recalcitrants hydrolyzed from complex proteins of FW (Table 1). Contrarily, cell potentials lasted for longer time at relatively high values, as compared to COD values.

In this case, MFCs can be thought as monitors of the presence of long-term biodegradable fractions of the organic matter.

The production of uric acids and ammonia, could affected the pH stability on the electrodes, contrasting the effect of acidic fermentation. It was recently proved that urea is quickly oxidized at the anode, inducing an increase of the electric output in single chamber MFCs and pH increase over 9 due to ammonia [24]. A variability in the concentration trend of ammonia, in contrast with acidic components, including volatile fatty acids, could cause the signal instability in the ~~S~~MFCs fed with fish waste. Furthermore, pH variability and the accumulation of less degradable byproducts in time could stressed the microbial communities on both the electrodes (bioanode and biocathode), globally lowering the ~~S~~MFC performances and making also instable the COD degradation (see error bar in Fig. 3). In fact, the pH of those ~~S~~MFCs, measured periodically close to the anode in the bulk solution, was in the range of 7-8, and never decrease below 7 since the first days, while in the case of CW and ~~OFMSK~~W, with a lower protein content (Table 1) the pH was around 6.7 ± 0.2 during the whole experimentation time.

3.4 Electrical signal trend in CP-fed MFCs





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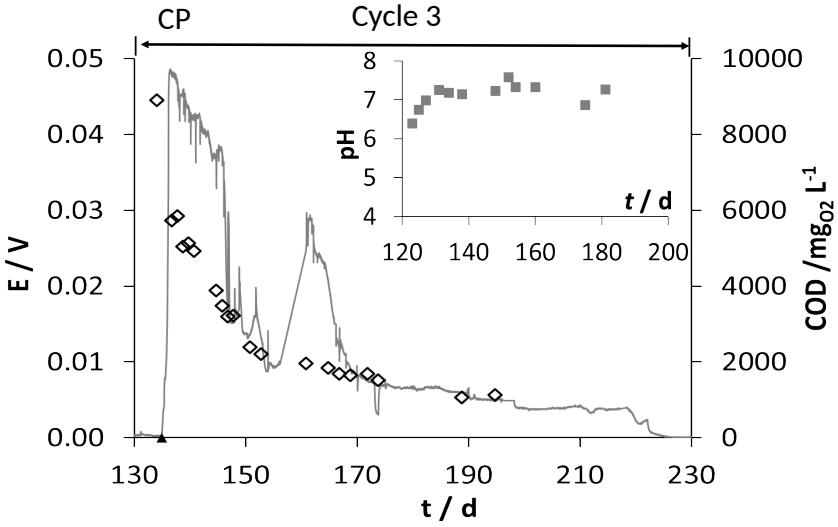

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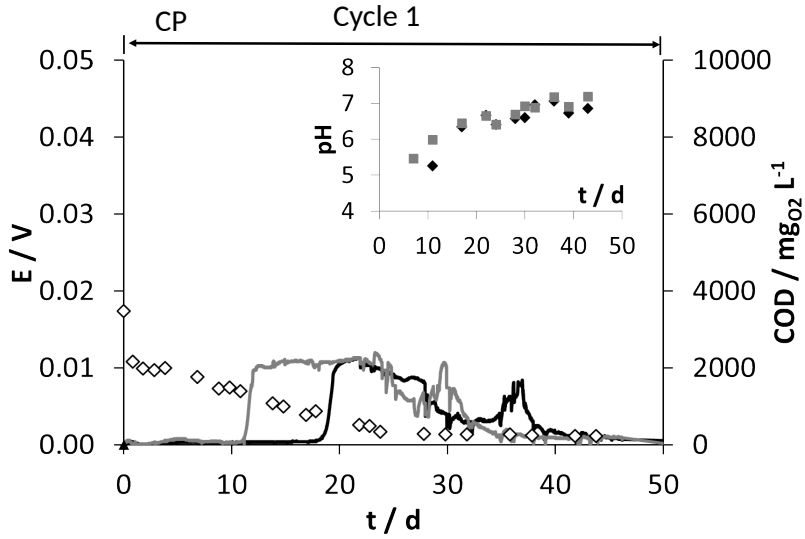
291 **Fig. Figure 4 reports the evolution of potential over time for two ~~S~~MFCs fed by CP along with the degradation of**
292 **the soluble COD. In the inset of Figure 4**



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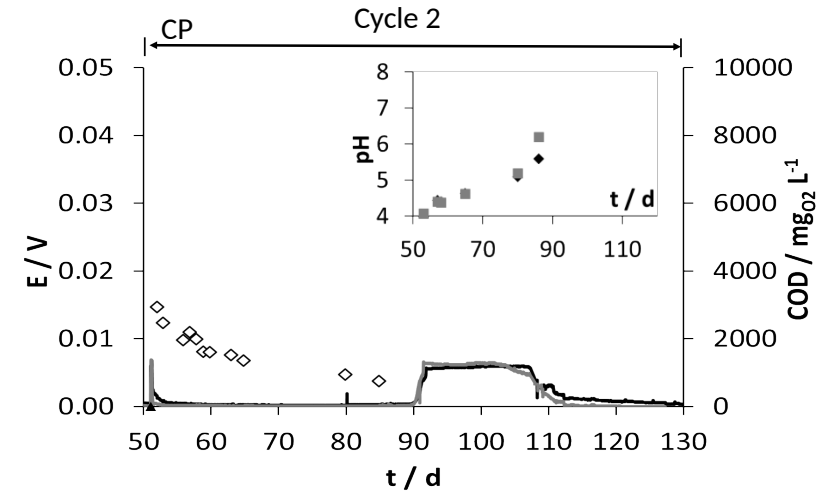

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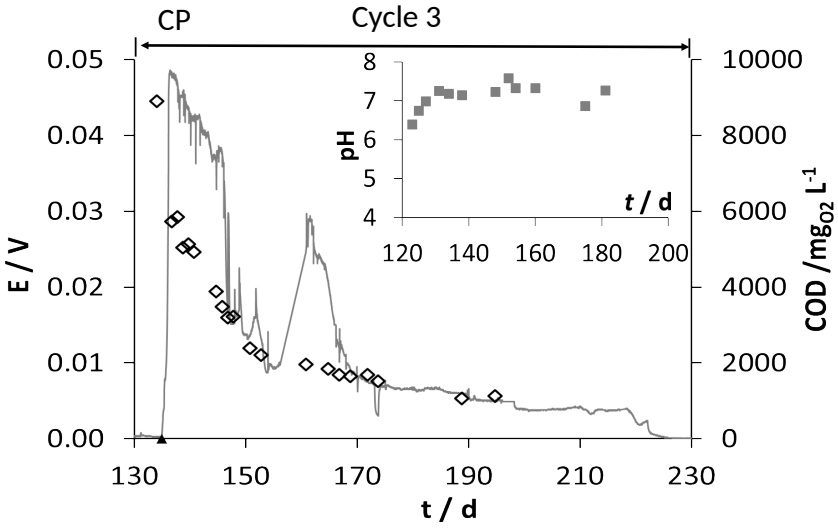

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296 Fig. the variation of pH in the ~~bulk~~ liquid phase of the MFCs bioreactor is shown. In the first two cycles,
297 acidic conditions (pH 4 – 5) were established in the bioreactor and the pH raised over 6.5 only after 40 days.
298 Alkalinity was evidently insufficient to buffer the acidity of CP (rich in citric acid and other organic acids).
299 COD decreased from 3400 mg_{O2}/l to 200 mg_{O2} L^{-1} along over 45 days of operation of cycle 1 and nearly 50
300 days in cycle 2.
301 At cycle 3, a buffer medium (potassium bicarbonate, 5 g L^{-1}) was added to the bioreactor to equilibrate the
302 pH, which remained stable in the range 7 – 7.8 along cycle 3. COD peaked to more than double (over 8000
303 mg_{O2} L^{-1}), due to more efficient microbial hydrolysis and fermentation of the solids accumulated in the
304 bioreactor.
305 The ~~S~~MFCs did not show any current generation until day 11 and 18. One of the ~~S~~MFCs started to produce
306 power at day 11 and the other ~~S~~MFCs at day 18. The rapid increase of cell potential was associated with the
307 ~~establishment~~ increase of ~~a bulk~~ pH ~~of at least~~ in the liquid phase over 6.5. After the rapid increase up to 10
308 mV, the potential remained stable for about 10 days then started to slowly decay~~. Another peak of potential~~
309 ~~was observed~~, along with COD. This happened identically during ~~the decaying. The delay in producing~~
310 ~~power can be certainly attributed to the low value of pH that~~ cycle 2. pH acidic conditions inhibited in
311 perfect parallel the overall biodegradation rate and the activity of exoelectrogenic bacteria. The absence of

electric signal from ARB was accomplished by an evidently slow-rate biodegradation, due to inhibited microbial activity.

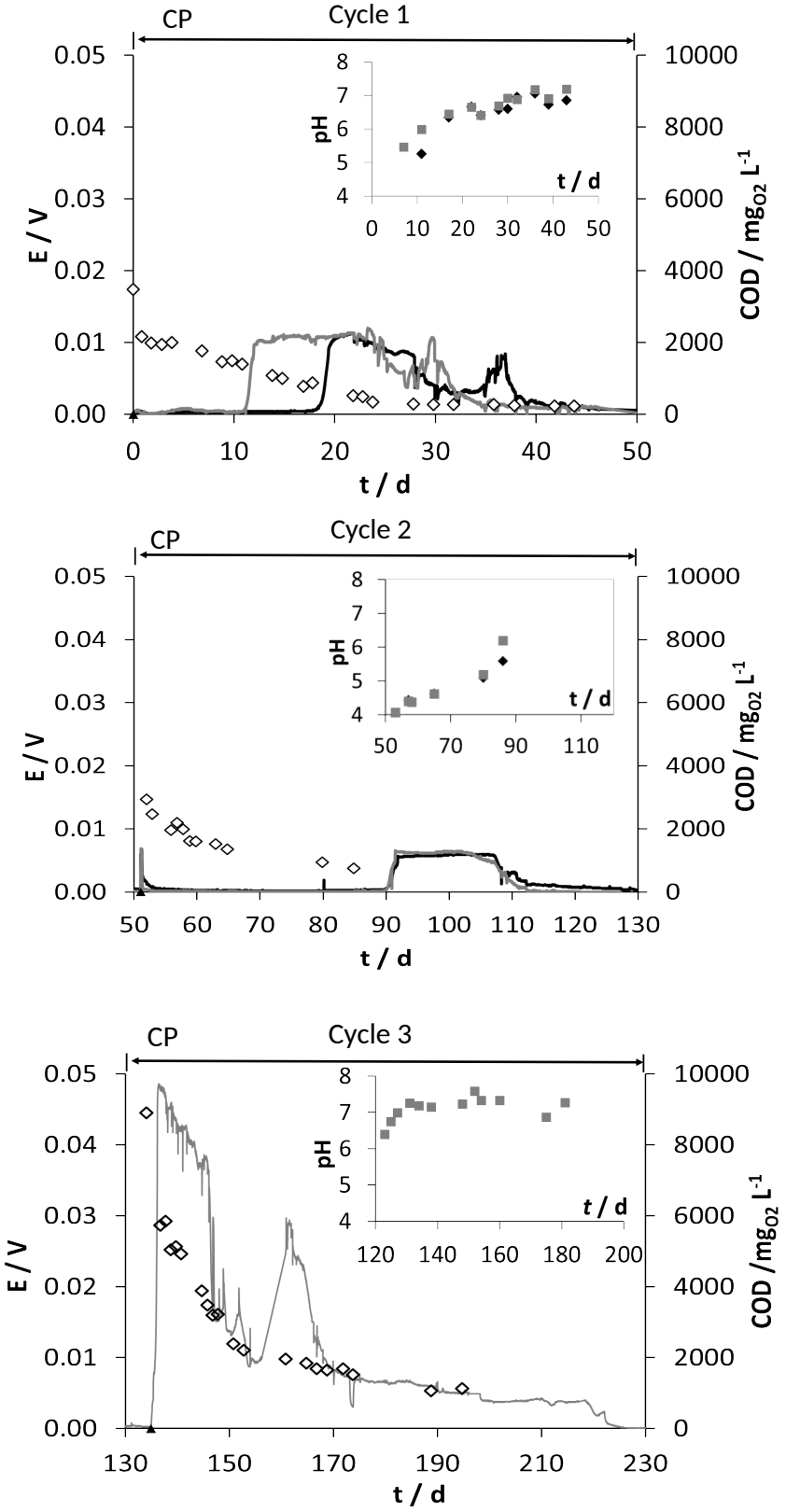

Fig. 4 Graphics of potential trends of two CP-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 230 days of operation and three batch cycles. Standard deviation of COD values is reported with vertical bars. Insets report the trend of pH over time.

However, the absence of electric signal was not accomplished by absence of microbial activity inside the MFC. As a matter of fact, COD decreased from 3400 mg_{O2}/l to 200 mg_{O2}/l during all the 45 days of operation of the first cycle. When a new dose of CP was added at day 51, the bulk pH lowered below 4.5 As long as the pH stayed lower than 6.5, apart from an initial spike, no electrical output was obtained from the SMFCs. After day 90, the potential rapid increased up to 7 mV and fixed there for 20 days then dropped to negligible values. Fermentative anaerobic degradation of the substrate took place, but electricity generation was initially inhibited.

Since the electric signal is severely affected by the pH, the increase of the potential is a clear indication that optimal condition of pH has been achieved inside the MFC. The kinetics of degradation is slightly lower in the second cycle and this is likely due variation of not-buffered pH.

~~Additional CP-fed SMFCs were operated with 0.2 g $COD_{substrate}$/$gVS_{inoculum}$. An increase in potential was observed after 2 days of operation as already obtained with the other substrates (results not shown here). As already known from biogas and anaerobic digestion field [11], the results from CP-SMFCs highlighted that the substrate/inoculum ratio is another key limiting factor when organic solid wastes are used to fed microbial metabolism.~~

When stable pH was guaranteed by equilibrating CP acidity, cell potential trends followed exactly the trend of COD consumption in the liquid phase. These results highlighted another aspect of the chemical equilibria in anaerobic biodegradation environments, which can be efficiently correlated to the trend of the electrical signal produced by ARB, living in the same environment. pH-related inhibition of microbial activity reflects very promptly in drops of MFCs cell potentials.

3.5 Electrode polarization curves

Polarization curves recorded on anodes and cathodes of a FW-fed MFC at different days of operation are reported in Fig. . Polarizations recorded at day 7 and 9 refer to cycle 1, polarization at day 62 and 64 refers to cycle 2. Anodes exhibited a peak around -0.35 V, which can be related to exogenous redox mediator and which position did not change significantly with the time. This potential range can be typically related to the oxidation of short-chain carboxylates, like volatile fatty acids [25]. The current delivered by the anode is about 0.5 mA lower in cycle 2. Similarly, polarization of cathodes exhibited considerably higher currents in cycle 1 than in ~~the second one~~ cycle 2.

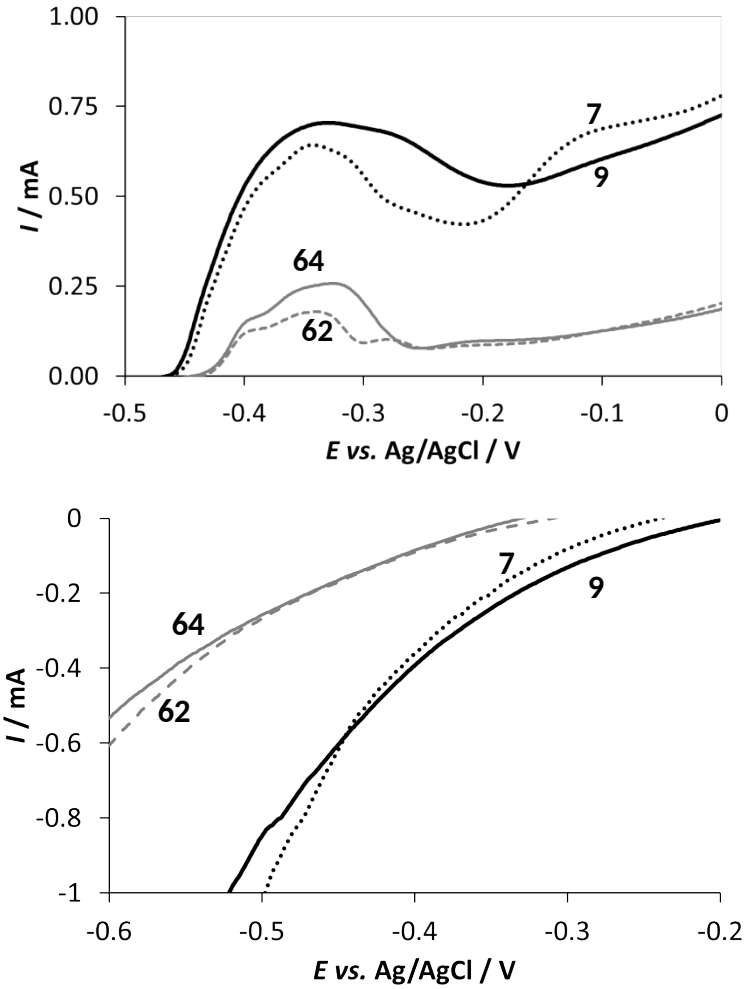

Fig. 5 Polarization curves of anodes (a) and cathodes (b) recorded at different days in a FW-fed MFC. Numbers near the curves indicate the day of experiments.

This indicates, first of all, that the cathode was predominantly characterized by microbially-catalyzed reduction reactions. As reported in previous works for single-chamber MFCs, bio-cathodic instead of abiotic mechanisms drive oxygen reduction reactions [16, 26]. This is an important aspect to consider, in the case that cell potential has to be used as indicator of microbial consortia activity in a bioreactor. To maximize the MFC system response to inhibitory effects, due to chemical imbalances in the liquid medium of biodegradation environments, single chamber MFCs might be the ideal solution. A double chamber architecture with abiotic cathode would be less sensible to inhibitory conditions for microbes.

FW biodegradation started showing instability at cycle 3. Error bars reported for SCOD measured in two MFCs indicate a condition of partial inhibition of the system. In biodegradation of protein-rich organic materials (see Table 1 for FW composition), it might typically be related to accumulations of ammonia, hydrogen sulfide or other toxic metabolites [24, 27].

The considerable decrease of polarization currents from both bioanodes and biocathodes, already registered during cycle 2 (days 62 – 64, Figure 5), can be considered as an early-warning for inhibiting conditions for the whole bioreactor environment. Future experiments should more deeply focus on this aspect. Polarization

of electrodes, as mirror of both anodic and cathodic microbial communities, could be studied as early-warning sensors for inhibiting conditions in anaerobic biodegradation environments.

3.6. Just monitoring or influencing the biodegradation process of COD removal*?*

~~The~~ kinetics of the various cycles of each ~~waste~~ organic substrates is reported in Table 2 -**Error! Reference source not found.**. Overall there was a more rapid kinetic of the first days and the first cycle. This points to a component of degradation due to an aerobic metabolism which preceded the formation of anaerobic biofilm on the anode and on the cathode.. The trend of kinetics is slower in subsequent cycles for all matrices, while coming practically going to 100% in the case of the milk and near 90% of COD in all the other cases. As expected, the ~~most quick~~quickest degradable substrate was ~~milk~~CW and the less one was food wastes (the most complex) ~~, that~~KW. For KW, COD removal was never ~~overcame~~higher than 85% due to the significant presence of ~~oils~~ recalcitrant fractions (e.g. fats and fibers). ~~lignocellulose compounds, which byporducts accumulated cycle by cycle.~~

For sake of comparison, in Table 2 the COD removal and CE are listed for the four different substrates.

The results showed that the COD removal ranged from 61.4% to 98.77% and CE ranged from 0.76 to 9.9% respectively. The so low CE achieved is consequence of the un-optimized anode electrode surface/volume ratio, as the anode occupied a marginal part of the cell volume. The maximum COD removal (98.77) was obtained for the CW substrate (cycle 6) and the maximum CE (9.91) was determined for the KW substrate (cycle 2).

This indicates that biodegradation were negligibly influenced by the MFC process. In future applications of MFCs as sensors for monitoring biodegradation process, electrode surface/reactor volume ratio might be even scaled down, to monitor specific environments.

On the contrary, enlarging the electrodes and improving their surface/volume ratio using a different geometry, will allow to reach and enhance the performance of the MFC process in degrading solid phase organics with respect the current literature [14].

Finally, the optimization of the electrode surface finishing [29] and the use of different materials such as stainless steel [28] would address other possible needs for an useful application in both cases: monitoring or influencing the biodegradation process.

Table 2 Coulombic efficiency and COD removal during fed-batch MFC operation.

	Sodium acetate (control)		**Cheese whey**		**Kitchen waste**		**Fish waste**		**Citrus pulp**	
	CE (%)	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**
Cycle 1	22.4	98.1	0.76	95.1	3.87	84.37	7.77	88.69	3.13	93.52
Cycle 2	21.3	95.9	2.02	96.9	9.91	64.25	4.37	85.94	2.74	89.70
Cycle 3	23.2	97.4	1.87	95.8	9.40	61.40	3.53	89.20	-	-

4. Conclusions

Four sets of membraneless single-chamber Microbial Fuel Cells were operated in duplicate and in parallel over more than 100 days, inoculated with anaerobic sludge of a biogas production plant and cyclically fed with the following different organic substrates: i) organic fraction of Kitchen waste (KW), ii) Cheese whey from dairy industries (CW), iii) residues of fish previously processed to recover oils (FW), iv) pulp waste from citrus juice production (CP).

~~All the tested SMFCs were able to produce an electric signal that varied in intensity as function of the substrate principal component, the COD concentration and the feeding cycle. Nevertheless, the pH mostly seems affect the electric signal.~~

~~This suggest the possibility of using SMFC output as sensor to control the pH and other parameters in several industrial anaerobic fermentation processes, such as biogas plants~~.

All MFCs were able to produce electric signals that varied in intensity as function of the chemical equilibria in the liquid phase of the bioreactor and the biodegradation rates achieved.

Sudden upcoming inhibiting conditions for microbial community in the MFC bioreactor corresponded to sudden drops in cell potentials. Progressive decrease of biodegradation efficiency in successive batch cycles corresponded to diminishing peaks of cell potentials. pH drops below 6.5 inhibited both biodegradation and anodic exoelectrogenic activity. The presence of recalcitrant fractions gave a delay between soluble COD degradation trend and the electrical signal over time. Both anodic and cathodic polarization curves, gave lower currents corresponding to incoming inhibiting conditions in the bioreactors.

Biotic mechanisms driving cathodic reduction reactions can help in having more sensible responses from MFCs coupled to bioreactors. A deeper study should follow these preliminary indications in considering MFCs as sensor to monitor and control anaerobic biodegradation processes.

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Signal trends of microbial fuel cells fed with different food-industry residues

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ABSTRACT

A microbial fuel cell (MFC) is an anaerobic bioreactor where soluble metabolites liberated by hydrolysis and fermentation of macromolecules are simultaneously available for anode respiring bacteria (ARB). ARB can be influenced by chemical imbalances in the liquid phase of the bioreactor. The objective of the work was to explore the trend of electric signals generated by MFCs, in relation to anaerobic biodegradation of four different solid food-industry residual substrates. Four sets of membraneless single-chamber MFCs were operated in batch mode, with solid waste substrates characterized by a different base component: i) mixed kitchen waste (fibers), ii) whey from dairy industries (sugar), iii) fisheries residues previously processed to recover oils (proteins), iv) pulp waste from citrus juice production (acidic).

All the tested MFCs were able to produce an electric output with different trends, depending on the principal component of the solid substrate. MFC potential varied as function of the COD and the feeding cycle, as well as of the substrate.

The pH variability during the fermentative process significantly affected the electric output. Citrus (acidic) pulp fed MFCs started to operate only when the pH raised up 6.5. MFCs fed with mix food wastes had a relatively stable electric signal; fish based waste caused spiking in the MFC signal and an averaging in the COD degradation trend. This phenomenon was attributed to a pH instability induced by proteins degradation forming ammonia.

The fermentation process was strongly predominant with respect the electrochemical process in MFCs and the coulombic efficiency (CE) was low, ranging between 2 to 10 %. This result call for a deeper exploration of harvesting power from solid wastes and pointed also to the possibility of using a MFC to monitor important parameters of fermentation processes in biotech production plants.

1. Introduction

The new paradigm of circular economy claims new technological approach for energy and resource recovery. Agro-food industry produces massive amounts of organic materials as secondary streams and waste [1]. Microbes naturally evolve enzymes and pathways that can convert solid biomass-derived carbon sources into valuable fuels and products, such as biomethane, biohydrogen, biodegradable polymers, carboxylates [2]. Biological conversions play a fundamental role in waste refinery chains and especially of agricultural and food-industry residues. In this context, microbial electrochemical technologies (METs) offer potential innovative approaches in wastes treatment.

Anaerobic bioconversion processes such as anaerobic digestion and dark fermentation rely on sequential microbial hydrolytic and fermentative processes that solubilize different substances in the liquid phase. Macromolecules are degraded to soluble molecules that become available to secondary fermentations or anaerobic respirations, by other microbes forming part of complex consortia [3]. In many cases, the liberation of soluble metabolites might significantly change the chemical equilibria of the liquid and gaseous phases where microbes live [4]. This is often the cause of inhibition of more sensible microbial species and possible imbalances of the overall biodegradation process [5].

In anaerobic biodegradation, secondary metabolisms (e.g. acetogenesis, acetoclastic methanogenesis, hydrogenophilic methanogenesis, denitrification, sulphate reduction, etc.) rely on the availability of short-chain organic molecules, inorganic ions and soluble gasses, such as volatile fatty acids, di-hydrogen, hydrogen sulphide, ammonium, nitrate, carbon dioxide, bicarbonates, etc [6,7].

Similarly, in microbial electrochemical systems like microbial fuel cells (MFC), electroactive microbial species like anode respiring bacteria (ARB) rely on the same substances to transfer electrons and produce current [8]. Macromolecules, in regular MFCs, should be at least hydrolyzed and pre-fermented by fermentative microbial species before being available to ARB, which preferentially oxidize low-carbon carboxylates, as indicated in various literature contributions [8–11].

For this reason, MFCs can be used as a mirror-process for secondary biodegradation metabolisms. In anaerobic environments, the electrical signal produced by ARB activity can give real-time hints on the trend of the ongoing biodegradation mechanisms and biochemical conditions, such as availability of soluble low-carbon organics, availability of mineral nutrients, favorable chemical equilibria in the liquid medium like pH, electrical conductivity, etc. A widely recognized issue is the competition of methanogenic populations and ARB for the same organics [12,13]. This corroborates the assumption that ARB activity, measured as voltage generation, could be used as monitor of the interactions between fermentative and methanogenic microbial populations in anaerobic biodegradation environments.

Electricity harvesting with complex biomass was recently achieved by Mohan et al. [14], who fed open-air cathode, single-chamber MFCs with different types of canteen-based food waste. The best performing configuration, where a proton exchange membrane separated the cathode from the anodic chamber, achieved a peak power density of 170 mW m^{-2} with open circuit potential (OCP) of 463 mV. Similar results with similar substrates were obtained by Goud et al[15], who tested increasing organic loading rates (OLR) in bio-

electrochemical reactors fed continuously. OLR of 1.39 kg COD/m^3-day both power density and OCP started decreasing, due to inhibiting concentrations of volatile fatty acids (>800 mg/L) and acidic pH conditions (pH=6).

Pretreatment of wastes from agro-food is achieved in several ways (e.g. to extract essential oils and proteins from specific wastes, such as citrus pulp, residual fish) and MET can be though as downstream processing for energy harvesting in Microbial Fuel Cells [14] or for further bio-processing (e.g. electrofermentation [12]), however, very little is reported in literature about biodegradation pathways of complex organic matrices in the bulk medium. In particular, the relationships between the electric signal produced by ARB and the biodegradation process as-a-whole (hydrolysis of macromolecules, fermentative metabolisms, etc.).

Here, we studied the electrical signals produced by MFCs during anaerobic biodegradation of four different types of agro-industrial residual materials of interest in Mediterranean agro-food sectors: citrus pulp, fishery waste, cheese whey and kitchen waste. Voltage trends were monitored on long-term, over 100 days, with successive batch cycles, to evaluate the response of the electrochemical system to the anaerobic biodegradation of the solid matrices.

2. Materials and methods

2.1 MFC configuration and setup

Four sets of membraneless single-chamber MFCs were operated in duplicate and in parallel over more than 100 days. The total volume of each MFC was 125 ml and the design was previously reported [16]. Anodes were made of 3×5 cm rolled carbon cloth sheet (Saati C1, Legnano, Italy), electrically connected to a copper wire. Three layers of non-conductive high-viscosity epoxy resin (Mapei Epojet) were applied to ensure insulation at the connection between copper and carbon cloth. Cathodes were made of 5×5cm carbon cloth sheets modified by the addition of a Gas Diffusion Layer (GDL) on the air side. The GDL composition has been described in [17] and the PTFE content is 80%$_{w/w}$ with respect to carbon powder. The geometric cathodic surface area exposed to the solution was 3 cm^2. Anode and cathode were then connected through an external circuit with a resistance of 100 Ω.

All MFCs were operated at mesophilic temperature of 35±1 °C in batch mode without pH adjustment. MFCs were inoculated with 90 ml of anaerobic mesophilic sludge obtained from a municipal wastewater treatment plant (Cremona, Italy). The volatile solids (VS) content in the sludge was 15 g $_{VS}kg^{-1}$. This concentration is typically used in standard batch-like anaerobic digestion tests [18]. The sludge was not subjected to any pretreatment. A concentrated solution of nutrients was added at the beginning of the experimentation. The stock solution of nutrients contained (in g/L): KH_2PO_4 (0.27), $Na_2HPO_4 \cdot 12H_2O$ (1.12), NH_4Cl (0.53), $CaCl_2 \cdot 2H_2O$ (0.075), $MgCl_2 \cdot 6H_2O$ (0.10), $FeCl_2 \cdot 4H_2O$ (0.02). Analytical grade reagents and double distilled water were used.

Two MFCs were fed with each organic substrate in the form of dried powder (1 mm particle size): i) cheese whey powder (CW); ii) kitchen waste (KW); iii) fish waste (FW); iv) citrus pulp (CP). The macromolecular composition of the four substrates was reported in Table 1. CW was a commercial by-product from dairy

industries (Cremona, Italy) used as animal feed. KW was a mixture of animal and vegetal food waste prepared in lab according to the following recipe: 30 g egg shells; 30 g dried bread, 50 g corn flour, 100g grated cheese, 75 g cracker, 10 g coffee grounds, 130g apple peel, 300g green salad, 145 g orange peel, 85 g zucchini peel, 68 g banana peel, 56 g carrots, 30 pumpkin skin, 20 g kiwi peel, 30 g fennels, 16 g potato peel. Food mixture was grinded with a kitchen blender, homogenized and finally dried at 105 °C. FW was obtained from fish after an enzymatic pretreatment to remove oils (no alcohols used). CP was obtained from citrus juice production plant (Catania, Italy).

Table 1 Main macromolecular constituents of the four solid wastes

Substrate	Carbohydrates (% of DM)	Fibers (% of DM)	Fats (% of DM)	Proteins (% of DM)	Ashes (% of DM)
CW	70 (lactose)	-	-	12	8.5
KW	53.4	19.2	9.6	14.3	3.5
FW	0.3	-	3.8	51.2	20.1
CP	8.5	43.1 (33.2 cellulose, 9.9 hemicellulose)	3.1	26.9 (19.7 pectin)	18.4

The amounts of inoculum and organic substrates introduced in each MFCs were determined on the basis of preliminary analytics determination (volatile solids and total solids). The organic substrate to inoculum ratio was 0.35 g $sCOD_{substrate}$/g $VS_{inoculum}$. A new dose of feed was added when negligible potential values were obtained and soluble Chemical Oxygen Demand (COD) fell down to a constant value.

2.2 Tests

2.2.1 Data acquisition, electrochemical experiments and calculations

The potential difference across the 100 Ω resistance (R) was acquired every 10 minutes, via a multichannel Data Logger (Graphtech midi Logger GL820). The generated current (I) was calculated by the equation $I = V/R$, where I is the current flowing through the external resistance. The total charge flowed into the electrical circuit at the end of each batch cycle was calculated by integrating the current over time. Coulombic efficiency (CE) was then evaluated on the basis of degraded soluble COD.

Quasi-steady stationary polarization curves were recorded *in situ* on anodes and cathodes. Experiments were performed with a classical three-electrode configuration, using a Compactstat IVIUM potentiostat connected to a personal computer. Anodes and cathodes were used as working electrode, a platinum wire as counter electrode and an Ag/AgCl (3M) electrode as reference. All the potentials throughout the text are referred to the Ag/AgCl (3M) electrode. For polarizations on the cathode, a Luggin capillary was adopted to minimize the ohmic drop into the solution. Before each experiment, MFC s were allowed to equilibrate at the open circuit potential (o.c.p.) for at least 30 minutes. Potential was then moved at a scan rate of 10 mV/min from the o.c.p. to 0.1 mV for polarization on anodes, and from the o.c.p. to -0.5 for polarization on cathodes.

2.2.2 Chemical characterizations

The soluble Chemical Oxygen Demand was periodically measured by a spectrophotometric method. A portion of solution sampled from each MFC was centrifuged for 15 minutes at 6000 rpm, carefully added to HT-COD cuvette test (Hach Lange Gmbh), and digested at 175°C for 15 min (Lange HT 200 S). Upon cooling, the COD value was read by an UV- spectrophotometer (Lange DR 3900).

3. Results and discussion

3.1 Electrical signal trend in CW-fed MFCs

Error! Reference source not found. reports the evolution of potential trend for two MFC s fed by CW along with the degradation of the soluble COD. pH was stably in the range 6.5 – 7.5, in all cycles. The acquisition of cell potential over all the time and the measurements of sCOD provided an indication of the productivity of each MFC and of the rate of organic substrate degradation.

The MFC produced power within 2 days, along with the establishment of anaerobic conditions in the anodic chamber and the colonization of anode and cathode by biofilms. MFC produced a peak of potential at days 4-5 and then potential rapidly decreased down to a negligible value. COD continuously decreased from the first day. Cycle 1 of CW degradation was completed within 11 days, reaching 95% of COD removal. When a new dose of CW was added at day 11, MFC immediately produced a spike of cell potential followed by dramatic drop and a new broader peak with a maximum of 50 mV at day 16. After the decay of cell potential to a negligible values and the decrease of COD, a third dose of CW was added. Six cycles of feeding were operated over 125 days. pH varied in the range 6.7 – 7.5.

The same trend in cell potential was observed for all cycles, with the generation of a spike just after the addition of a dose of feed followed by the development of a broader peak after a lag time. The duration of the cycles became longer from the third cycle on (about 21 days) and the maximum cell potential decreased from the fourth cycle on. The rates of soluble COD removal, however, seem quite similar in each cycle. These two aspects pointed to a progressive deactivation of the MFCs, over long-term operation, due to electrode scaling, as documented for wastewater operated with the same MFC in previous works [19] as consequence of the alkalinity generated at cathode [20].

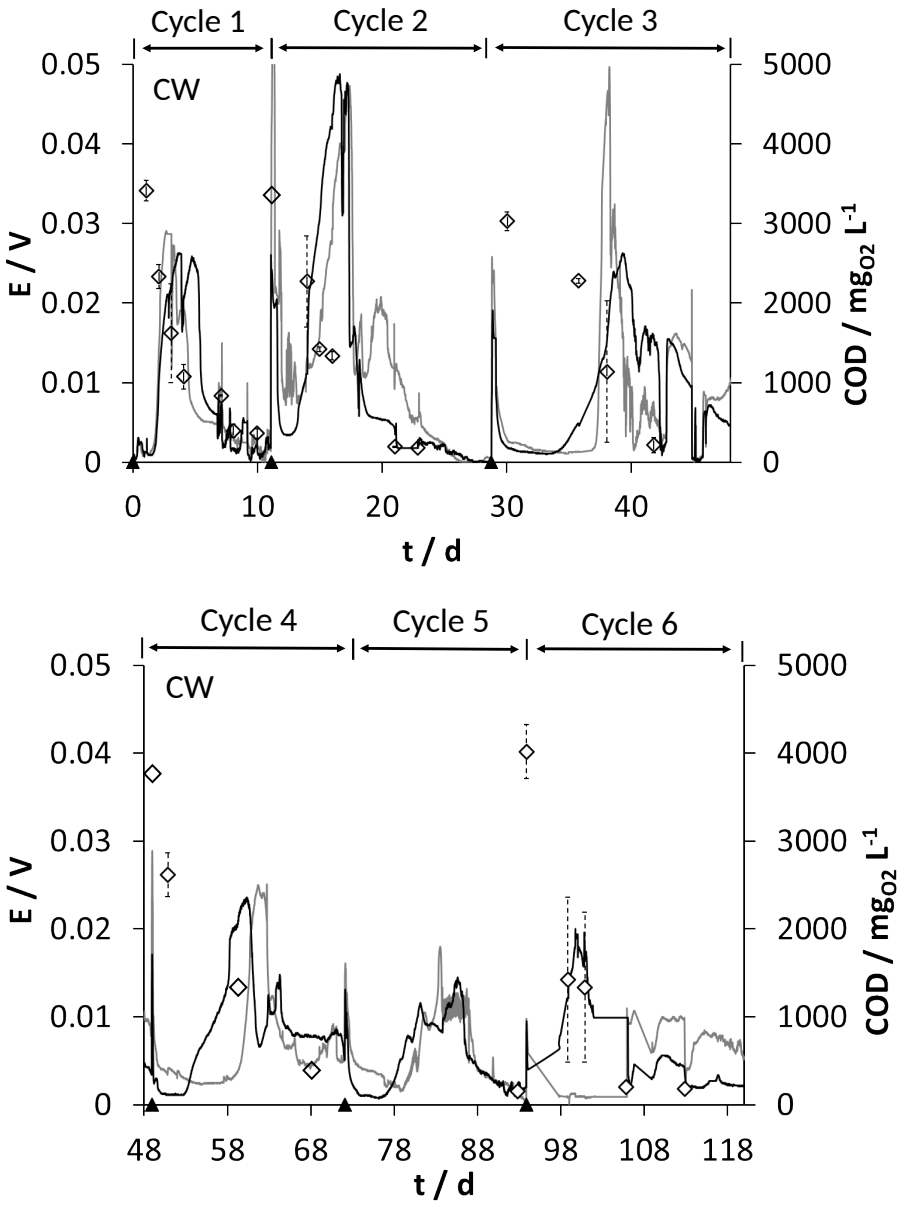


Fig. 1 Graphics of potential trends of two CW-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and six batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

The initial spike of cell potential, found at each cycle, can be attributed to a rapid increase of easily degradable molecules in the liquid phase, as a consequence of acidogenic fermentation of lactose. Lactose is easily hydrolyzed to glucose and galactose; sugars fermentation to short chain fatty acids in the bulk anaerobic medium happens at high rates [21]. The sudden drop of cell potential is likely due to a temporary inhibitory effect of soluble metabolites (e.g. volatile fatty acids) on ARB activity, with a detrimental effect on the electrochemical signal of MFCs.

This effect point to the possibility of monitoring on-line possible accumulations of that soluble metabolites inhibiting biodegradation processes. This would be particularly useful in high-solids anaerobic digestion plants [4], or other biodegradation processes at high organic loading rates [22].

3.2 Electrical signal trend in KW-fed MFCs

Fig. 1Fig. 1 reports the evolution of cell potential over time for two MFCs fed by KW along with the soluble COD degradation. pH was stably in the range 6.5 – 7.5, in all cycles.

Just after the first day of operation, an increase in cell potential was observed. The potential reached a maximum of 40 mV at day 2 and then stepwise decreased. COD values continuously decayed from the first day of each cycle to around 500 mg_{O2} L^{-1}. Further degradation beyond this value was never achieved. At the first cycle, 84% of initial COD could be removed within 11 days. The second cycle was operated from day 11, when a new dose of KW was added into the MFCs. An initial spike of potential was observed, immediately followed by two broader shoulders. The duration of the second cycle was 17 days.

Five cycles of feeding were operated over 120 days. The two MFCs gave similar cell potential signals during all cycles, even though the duration of the cycles became longer and the resolution between the two shoulders of potentials trends became less defined. The evolution of potential over time is significantly different for KW than CW, even if the duration of COD removal is quite similar. In this case, the potential remained higher and more stable for longer time. This aspect highlights that the accumulation of VFA is not the main issue in the degradation process, since the composition of KW is more complex than CW.

It is possible to deduce that the macromolecular degradation, producing still complex organics other than volatile acids, might prevent the inhibition of the bacterial activity and had an effect on the stabilization of the electrochemical signal of the whole MFCs.

Interestingly, the maximum potential decreased cycle by cycle, in parallel with the peak of COD at the beginning of each cycle. The peak value of COD was over 3000 mg_{O2} L^{-1} in the first cycle, and decreased progressively to less than 2000 mg_{O2} L^{-1} in cycle 5.

This condition might be due to the accumulation of mineralized substances in the liquid phase, such as ammonium ions, and other nutrients, creating an inhibitory environment for microbial population and for ARB. The MFC signal followed the general trend of the primary phases of biodegradation (hydrolysis and fermentation). Progressively less organic matter was hydrolyzed from the solid phase (visible as decreasing peaks of COD). Additionally, each cycle lasted for longer time as compared to the first one. Biodegradation rate was visibly decreasing. All these effects were reflected in progressively decreasing peaks of the electrical signals.

The MFCs potential trends were. Again, a mirror of an increase of limiting or toxic conditions the overall microbial population in the bioreactor. The MFCs potential trends were, again, a mirror of an increase of limiting (or toxic) conditions for the overall microbial population in the bioreactor. In this case the electrical signals were indicating a progressive increase of inhibition conditions. This application of MFCs in bioreactors would be useful for monitoring the accumulation of potentially toxic metabolites of biodegradation (ammonia, H2S, Na+, etc.) on long-term operations of bioreactors [5, 23].

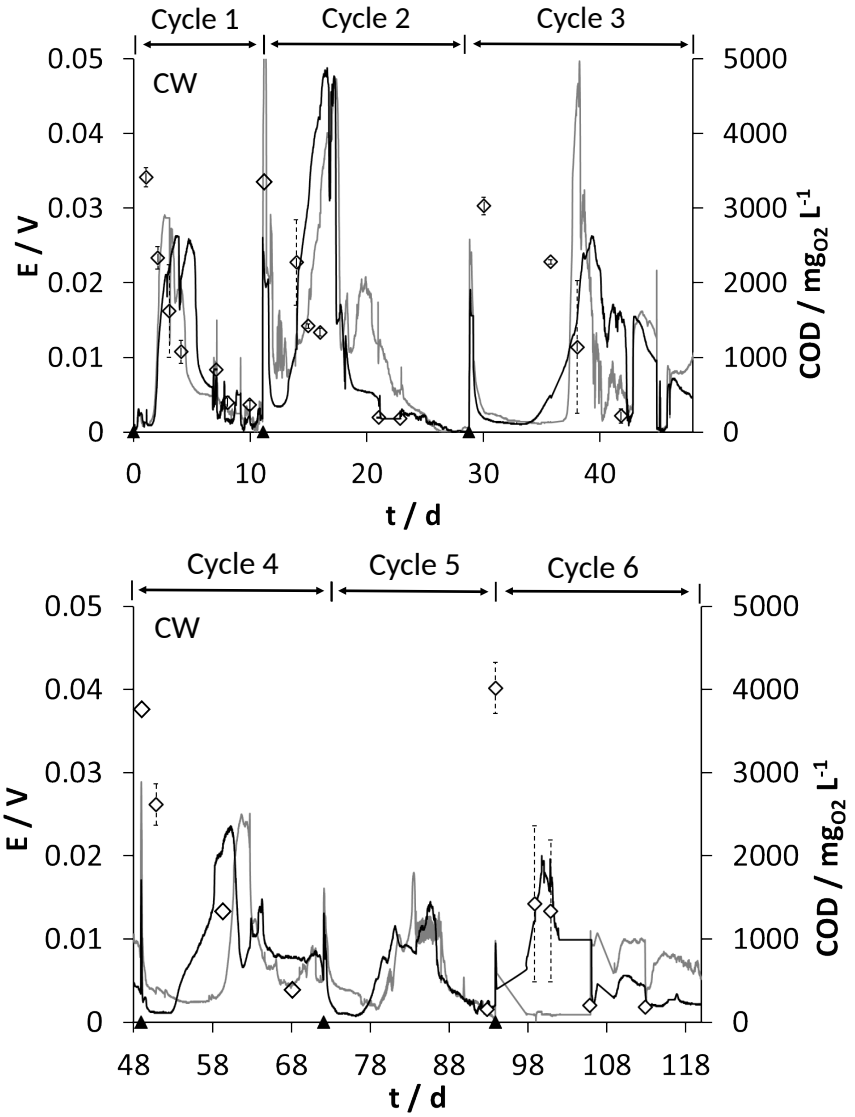


Fig. 2 Graphics of potential trends of two KW-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and six batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

3.3 Electrical signal trend in FW-fed MFCs

Figure 3Fig. 3 reports the evolution of cell potential over time for two MFCs fed by FW along with the soluble COD degradation. pH was stably in the range 7.5 – 7.9 in all cycles. Both MFCs started to generate electric signal from day 2, with cell potential that rapidly increased up to a maximum of 30 mV and then slowly decreased from day 7 to day 25. At the same time, COD spiked to nearly 3700 mg_{O2} L^{-1} and continuously decreased to 400 mg_{O2} L^{-1}. During the first cycle, 88% of COD removal was achieved in around 40 days. Electric signal and COD varied with a really similar trend along the first cycle.

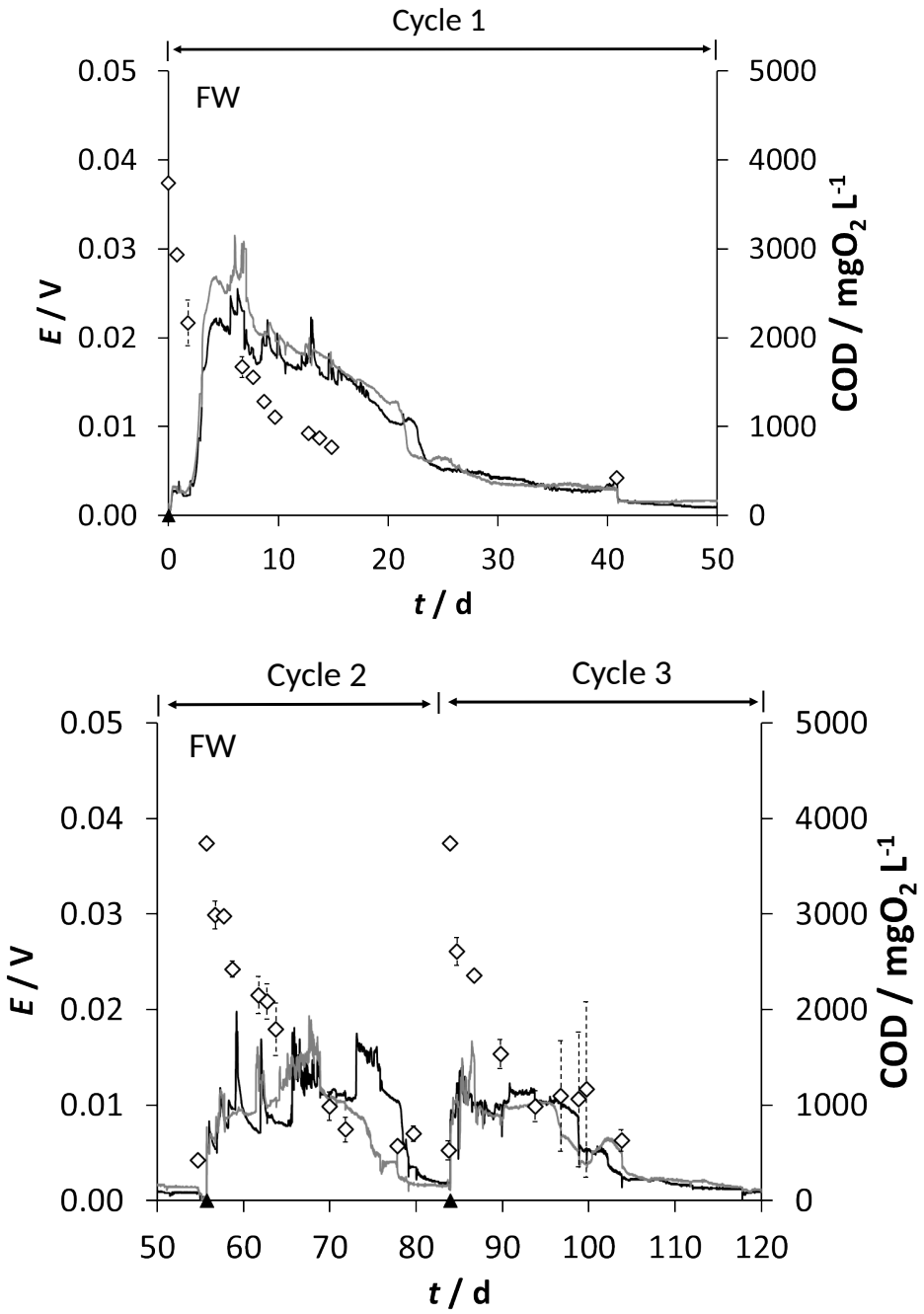


Fig. 3 Graphics of potential trends of two FW-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 125 days of operation and three batch cycles. Standard deviation of COD values is reported with vertical bars. Triangles on the x-axis indicate the day of feed addition.

At day 55, a new dose of FW was added. Upon addition, the potential firstly rapidly increased up to 10 mV, then maintained in the range between 10 mV and 20 mV for at least 25 days. The first cycle of operation was the more efficient in term of electrochemical performance and COD degradation, and different behavior was observed in the following cycles. At day 84, a new dose of FW was added and evolution of potential and COD similar than in the second cycle were achieved.

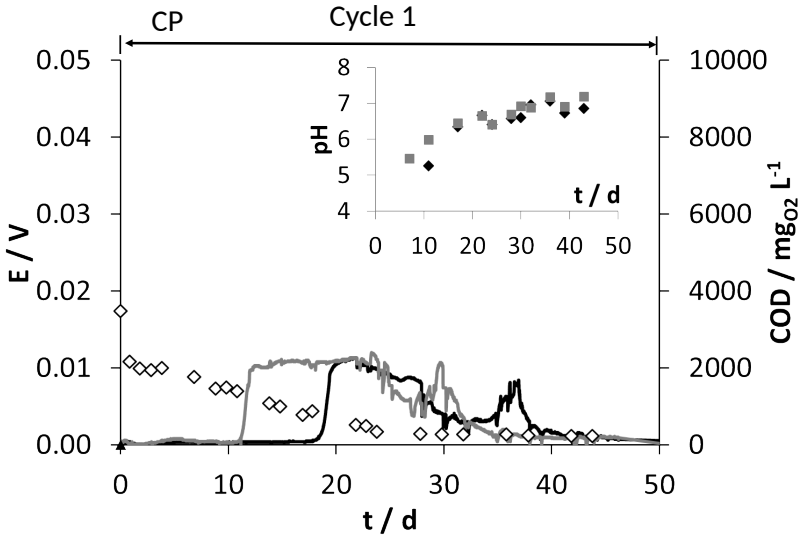
In cycle 2 and 3, COD removal happened with nearly a double rate,, in parallel with cell potentials. The peak values of COD were very similar at each cycle. Differently from CW and KW, peak of COD didn't

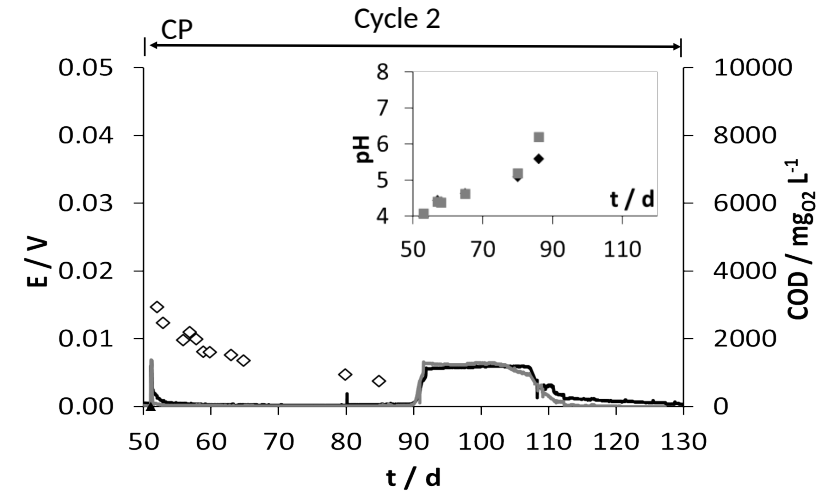
correspond to peaks of cell potential, especially at cycles 2 and 3. This is likely to be due to the recalcitrants hydrolyzed from complex proteins of FW (Table 1). Contrarily, cell potentials lasted for longer time at relatively high values, as compared to COD values.

In this case, MFCs can be thought as monitors of the presence of long-term biodegradable fractions of the organic matter.

The production of uric acids and ammonia, could affected the pH stability on the electrodes, contrasting the effect of acidic fermentation. It was recently proved that urea is quickly oxidized at the anode, inducing an increase of the electric output in single chamber MFCs and pH increase over 9 due to ammonia [24]. A variability in the concentration trend of ammonia, in contrast with acidic components, including volatile fatty acids, could cause the signal instability in the MFCs fed with fish waste. Furthermore, pH variability and the accumulation of less degradable byproducts in time could stressed the microbial communities on both the electrodes (bioanode and biocathode), globally lowering the MFC performances and making also instable the COD degradation (see error bar in Fig. 3). In fact, the pH of those MFCs, measured periodically close to the anode in the bulk solution, was in the range of 7-8, and never decrease below 7 since the first days, while in the case of CW and KW, with a lower protein content (Table 1) the pH was around 6.7 ± 0.2 during the whole experimentation time.

3.4 Electrical signal trend in CP-fed MFCs





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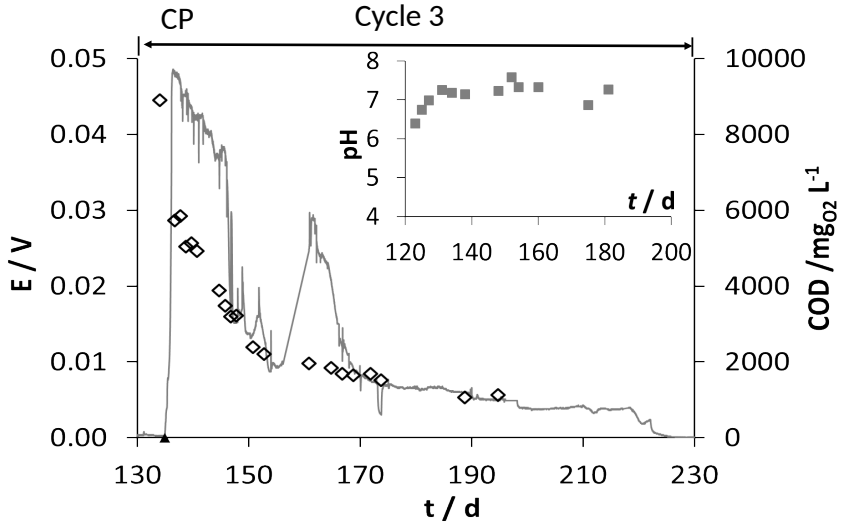

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278 **Fig. Figure 4 reports the evolution of potential over time for two MFCs fed by CP along with the degradation of**
279 **the soluble COD. In the inset of Figure 4**
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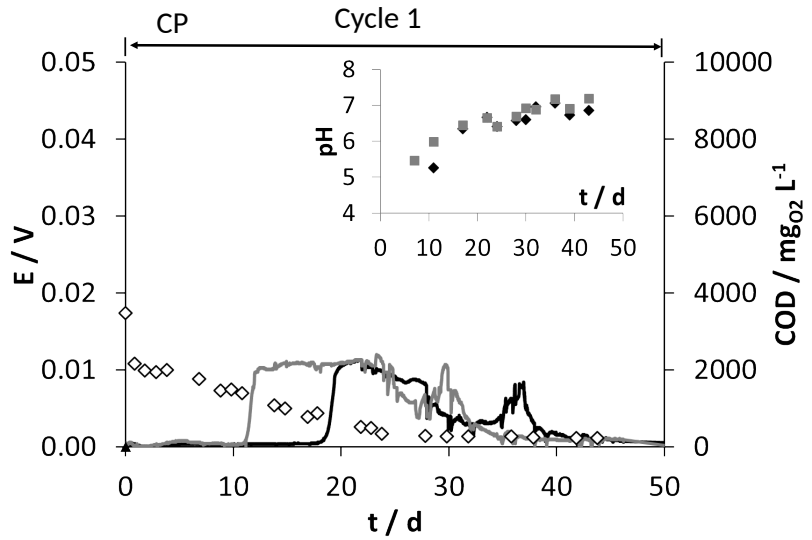

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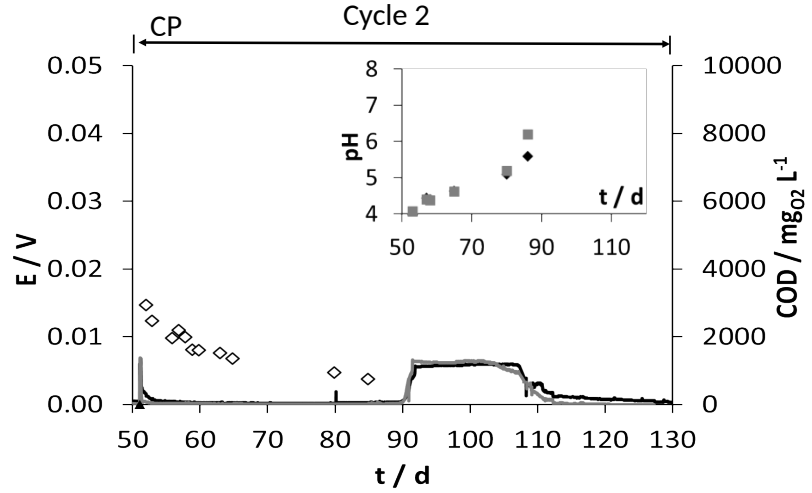

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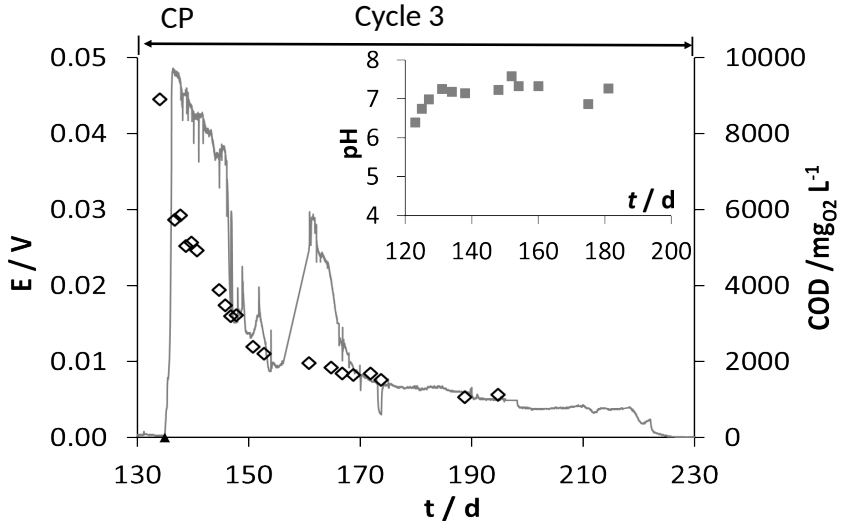

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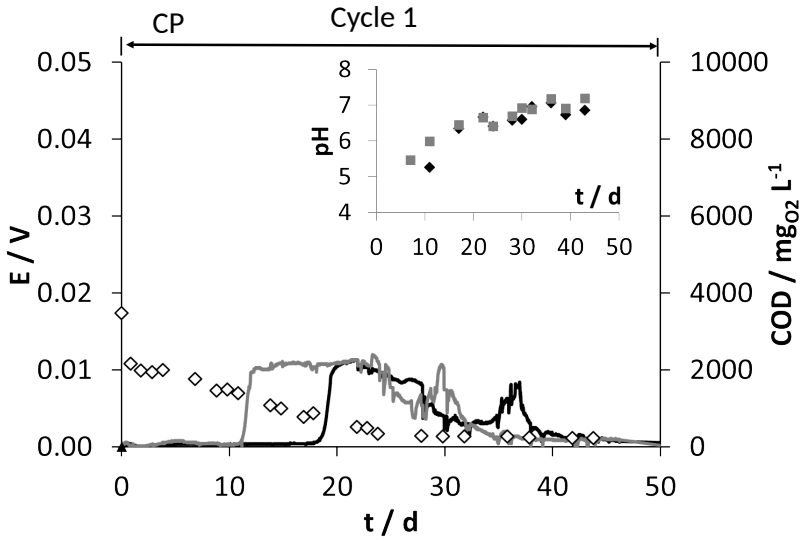
290 Fig. the variation of pH in the liquid phase of the MFCs bioreactor is shown. In the first two cycles, acidic
291 conditions (pH 4 – 5) were established in the bioreactor and the pH raised over 6.5 only after 40 days.
292 Alkalinity was evidently insufficient to buffer the acidity of CP (rich in citric acid and other organic acids).

COD decreased from 3400 mg_{O2}/l to 200 mg_{O2} L^{-1} along over 45 days of operation of cycle 1 and nearly 50 days in cycle 2.

At cycle 3, a buffer medium (potassium bicarbonate, 5 g L^{-1}) was added to the bioreactor to equilibrate the pH, which remained stable in the range 7 – 7.8 along cycle 3. COD peaked to more than double (over 8000 mg_{O2} L^{-1}), due to more efficient microbial hydrolysis and fermentation of the solids accumulated in the bioreactor.

The MFCs did not show any current generation until day 11 and 18. One of the MFCs started to produce power at day 11 and the other MFCs at day 18. The rapid increase of cell potential was associated with the increase of pH in the liquid phase over 6.5. After the rapid increase up to 10 mV, the potential remained stable for about 10 days then started to slowly decay, along with COD. This happened identically during cycle 2. pH acidic conditions inhibited in perfect parallel the overall biodegradation rate and the activity of exoelectrogenic bacteria. The absence of electric signal from ARB was accomplished by an evidently slow-rate biodegradation, due to inhibited microbial activity.



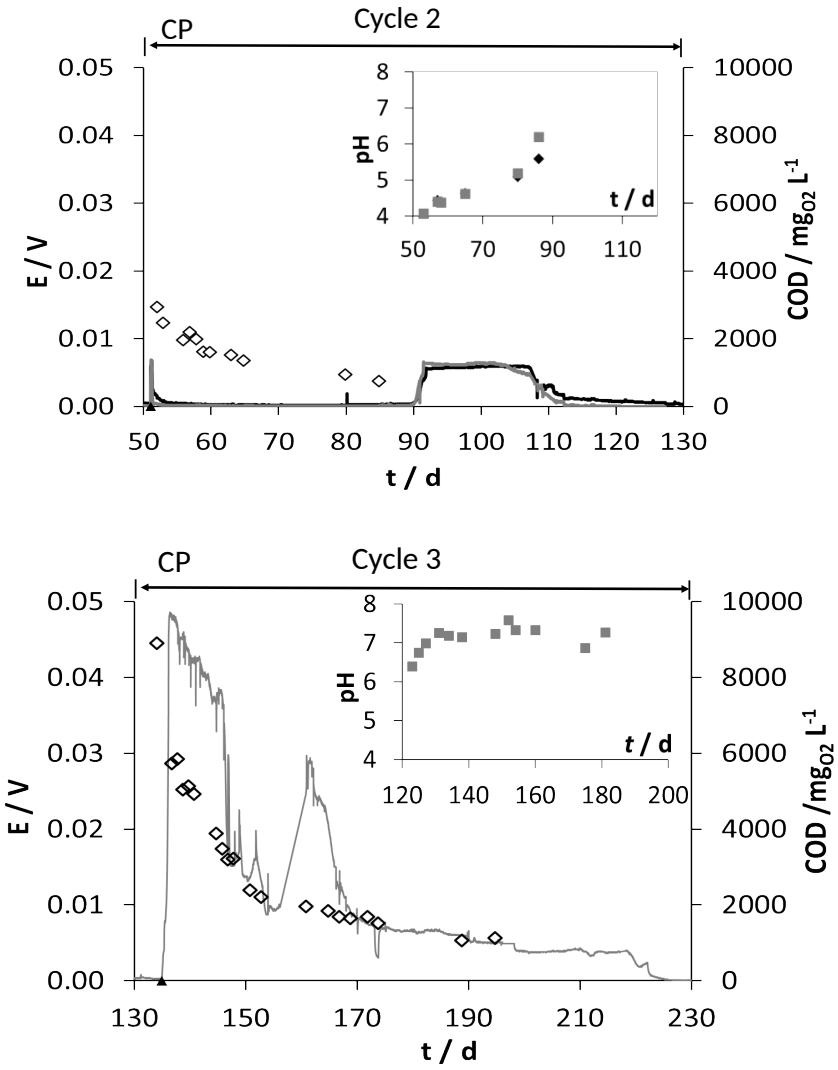


Fig. 4 Graphics of potential trends of two CP-fed MFCs (solid lines, left y-axis) and COD evolution (◊, right y-axis) over 230 days of operation and three batch cycles. Standard deviation of COD values is reported with vertical bars. Insets report the trend of pH over time.

However, the absence of electric signal was not accomplished by absence of microbial activity inside the MFC. As a matter of fact, COD decreased from 3400 mg_{O2}/l to 200 mg_{O2}/l during all the 45 days of operation of the first cycle. When a new dose of CP was added at day 51, the bulk pH lowered below 4.5 As long as the pH stayed lower than 6.5, apart from an initial spike, no electrical output was obtained from the MFCs. After day 90, the potential rapid increased up to 7 mV and fixed there for 20 days then dropped to negligible values. Fermentative anaerobic degradation of the substrate took place, but electricity generation was initially inhibited.

Since the electric signal is severely affected by the pH, the increase of the potential is a clear indication that optimal condition of pH has been achieved inside the MFC. The kinetics of degradation is slightly lower in the second cycle and this is likely due variation of not-buffered pH.

When stable pH was guaranteed by equilibrating CP acidity, cell potential trends followed exactly the trend of COD consumption in the liquid phase. These results highlighted another aspect of the chemical equilibria in anaerobic biodegradation environments, which can be efficiently correlated to the trend of the electrical

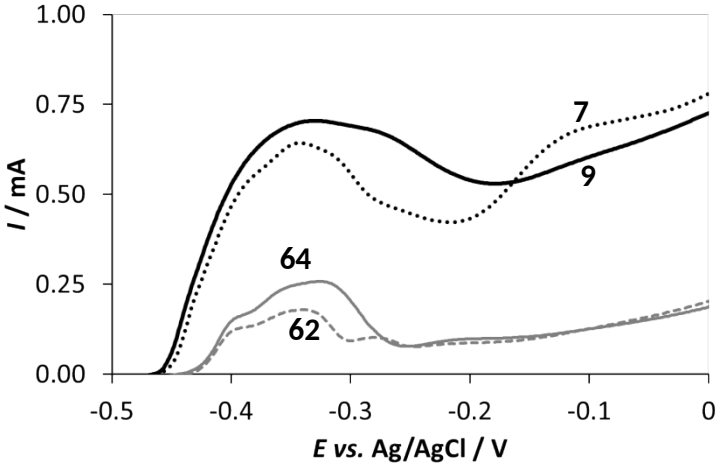
signal produced by ARB, living in the same environment. pH-related inhibition of microbial activity reflects very promptly in drops of MFCs cell potentials.

3.5 Electrode polarization curves

Polarization curves recorded on anodes and cathodes of a FW-fed MFC at different days of operation are reported in Fig. . Polarizations recorded at day 7 and 9 refer to cycle 1, polarization at day 62 and 64 refers to cycle 2. Anodes exhibited a peak around -0.35 V, which can be related to exogenous redox mediator and which position did not change significantly with the time. This potential range can be typically related to the oxidation of short-chain carboxylates, like volatile fatty acids [25]. The current delivered by the anode is about 0.5 mA lower in cycle 2. Similarly, polarization of cathodes exhibited considerably higher currents in cycle 1 than in cycle 2.

This indicates, first of all, that the cathode was predominantly characterized by microbially-catalyzed reduction reactions. As reported in previous works for single-chamber MFCs, bio-cathodic instead of abiotic mechanisms drive oxygen reduction reactions [16, 26]. This is an important aspect to consider, in the case that cell potential has to be used as indicator of microbial consortia activity in a bioreactor. To maximize the MFC system response to inhibitory effects, due to chemical imbalances in the liquid medium of biodegradation environments, single chamber MFCs might be the ideal solution. A double chamber architecture with abiotic cathode would be less sensible to inhibitory conditions for microbes.

FW biodegradation started showing instability at cycle 3. Error bars reported for SCOD measured in two MFCs indicate a condition of partial inhibition of the system. In biodegradation of protein-rich organic materials (see Table 1 for FW composition), it might typically be related to accumulations of ammonia, hydrogen sulfide or other toxic metabolites [24, 27].



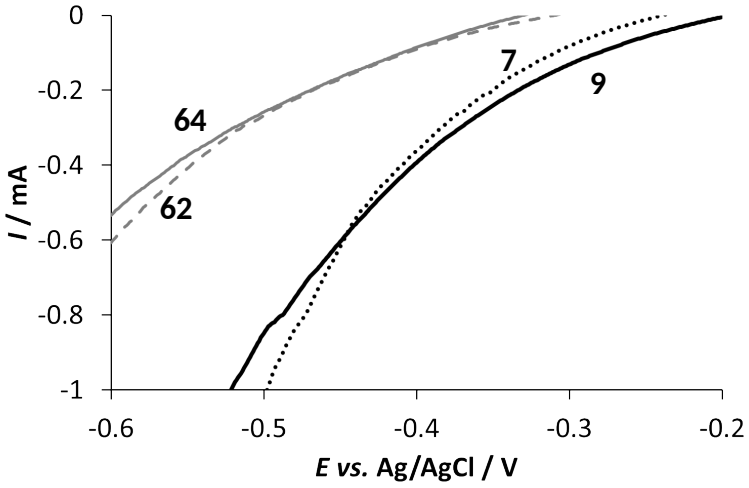


Fig. 5 Polarization curves of anodes (a) and cathodes (b) recorded at different days in a FW-fed MFC. Numbers near the curves indicate the day of experiments.

The considerable decrease of polarization currents from both bioanodes and biocathodes, already registered during cycle 2 (days 62 – 64, Figure 5), can be considered as an early-warning for inhibiting conditions for the whole bioreactor environment. Future experiments should more deeply focus on this aspect. Polarization of electrodes, as mirror of both anodic and cathodic microbial communities, could be studied as early-warning sensors for inhibiting conditions in anaerobic biodegradation environments.

3.6. Just monitoring or influencing the biodegradation process of COD removal*?*

kinetics of the various cycles of each organic substrates is reported in Table 2 **Error! Reference source not found.**. Overall there was a more rapid kinetic of the first days and the first cycle. This points to a component of degradation due to an aerobic metabolism which preceded the formation of anaerobic biofilm on the anode and on the cathode.. The trend of kinetics is slower in subsequent cycles for all matrices, while coming practically going to 100% in the case of the milk and near 90% of COD in all the other cases. As expected, the quickest degradable substrate was CW and the less one was food wastes (the most complex) KW. For KW, COD removal was never higher than 85% due to the significant presence of recalcitrant fractions (e.g. fats and fibers).

For sake of comparison, in Table 2 the COD removal and CE are listed for the four different substrates.

The results showed that the COD removal ranged from 61.4% to 98.77% and CE ranged from 0.76 to 9.9% respectively. The so low CE achieved is consequence of the un-optimized anode electrode surface/volume ratio, as the anode occupied a marginal part of the cell volume. The maximum COD removal (98.77) was obtained for the CW substrate (cycle 6) and the maximum CE (9.91) was determined for the KW substrate (cycle 2).

This indicates that biodegradation were negligibly influenced by the MFC process. In future applications of MFCs as sensors for monitoring biodegradation process, electrode surface/reactor volume ratio might be even scaled down, to monitor specific environments.

On the contrary, enlarging the electrodes and improving their surface/volume ratio using a different geometry, will allow to reach and enhance the performance of the MFC process in degrading solid phase organics with respect the current literature [14].

Finally, the optimization of the electrode surface finishing [29] and the use of different materials such as stainless steel [28] would address other possible needs for an useful application in both cases: monitoring or influencing the biodegradation process.

Table 2 Coulombic efficiency and COD removal during fed-batch MFC operation.

	Sodium acetate (control)		**Cheese whey**		**Kitchen waste**		**Fish waste**		**Citrus pulp**	
	CE (%)	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**	**CE (%)**	**sCOD removal (%)**
Cycle 1	22.4	98.1	0.76	95.1	3.87	84.37	7.77	88.69	3.13	93.52
Cycle 2	21.3	95.9	2.02	96.9	9.91	64.25	4.37	85.94	2.74	89.70
Cycle 3	23.2	97.4	1.87	95.8	9.40	61.40	3.53	89.20	-	-

4. Conclusions

Four sets of membraneless single-chamber Microbial Fuel Cells were operated in duplicate and in parallel over more than 100 days, inoculated with anaerobic sludge of a biogas production plant and cyclically fed with the following different organic substrates: i) organic fraction of Kitchen waste (KW), ii) Cheese whey from dairy industries (CW), iii) residues of fish previously processed to recover oils (FW), iv) pulp waste from citrus juice production (CP).

All MFCs were able to produce electric signals that varied in intensity as function of the chemical equilibria in the liquid phase of the bioreactor and the biodegradation rates achieved.

Sudden upcoming inhibiting conditions for microbial community in the MFC bioreactor corresponded to sudden drops in cell potentials. Progressive decrease of biodegradation efficiency in successive batch cycles corresponded to diminishing peaks of cell potentials. pH drops below 6.5 inhibited both biodegradation and anodic exoelectrogenic activity. The presence of recalcitrant fractions gave a delay between soluble COD degradation trend and the electrical signal over time. Both anodic and cathodic polarization curves, gave lower currents corresponding to incoming inhibiting conditions in the bioreactors.

Biotic mechanisms driving cathodic reduction reactions can help in having more sensible responses from MFCs coupled to bioreactors. A deeper study should follow these preliminary indications in considering MFCs as sensor to monitor and control anaerobic biodegradation processes.

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Highlights

- Four different component of solid organic wastes were investigated in membranelessMFCs
- Cell potential trends varied in function of different waste components in the bioreactors
- Cell potential trends varied as function of the substrate, the COD and the feeding cycle
- The pH in the anodic chamber significantly affected the electric output
- Results call for exploration of MFCs as sensors for fermentation/biodegradation processes