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Abstract: The aim of this work were to study terracotta-based porous air-water separators (4 mm thickness) in microbial recycling cells (MRCs) fed with cow manure (CM), swine manure (SM) and dairy wastewater (DW). Over 125 days, besides the removal of 60-90% of soluble-COD, considerable fractions of the main macronutrients (C, N, P, K, Fe, Mn, Ca, Mg) were removed from the wastewater and deposited on the terracotta separators as both inorganic salts and biomass deposits. Water evaporation at air-water interface as well as the high cathodic pH (10-12), induced by oxygen reduction to OH-, were the predominant factors leading to precipitation. The separators were saturated of up to 10 g per kg of terracotta of the main macronutrients, with negligible concentrations of the main inorganic contaminants. These materials could be directly reused as nutrients-enriched solid conditioners for agricultural soils.

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Milan, 07th Jan, 2019

Sub: Revision of Orginal Manuscript, submitted to Bioresource technology special issue BIORESTEC VSI. Following up the oral presentation [O3.2A]

Dear Editor and scientific committee of Bioresource technology,

Kindly find enclosed the revised version of manuscript entitled "**Microbial recycling cells (MRCs): first steps into a new type of bioelectrochemical systems, aimed at recovering nutrients from wastewater**", after a careful revision, according to the reviewers and the editor's requests.

The interest in research on microbial electrochemical technologies needs no introduction and in recent years, the numbers of papers on this topic have grown exponentially. We recently published a new concept of METs called **microbial recycling cells (MRCs)**, definitively beyond energy-harvesting and with the specific goal of **recovering carbon and nutrients from organic-rich wastewater streams from agro-food production chains** and fabricating **bio-based renewable fertilizers**.

In the paper '**Microbial recycling cells (MRCs): A new platform of microbial electrochemical technologies based on biocompatible materials, aimed at cycling carbon and nutrients in agro-food systems'**, **Science of The Total Environment Volume 649, 1 February 2019, Pages 1349-1361**, we first explained this concept. Initially focused on electricity generation from wastewaters through Microbial Fuel Cells (MFCs), the focus of research community has shifted to recovering organic matter and nutrients and producing **renewable fertilizers and soil conditioners**. Low-cost and low-grade materials such as **terracotta** or biomass-based materials (see our previous publication **Marzorati et al., Journal of Cleaner Production 170 (2018) 1167e1176: Ligno-cellulosic materials as air-water separators in low-tech microbial fuel cells for nutrients recovery**) could be used to fabricate this new generation of METs.

Here, we present an **exploratory experiment** where we analyze the possibility of using **terracotta** as air-water separator, to be saturated by nutrients and fully re-used in agriculture.
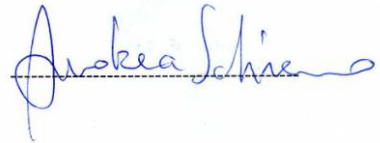
We also certify that:

1.   It is the original work of the authors and all authors have made substantial contributions to the following aspects: the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be submitted.
2.   All the authors (Andrea Goglio, Stefania Marzorati, Laura Rago, Deepak Pant, Pierangela Cristiani, Andrea Schievano) have mutually agreed that it should be submitted to Bioresource Technology.
3.   This research has not been published, nor is being considered for publication elsewhere, either in whole or substantial part. This manuscript was never submitted previously to Bioresource Technology.

We very much hope that the manuscript will fit the scope of **Bioresource Technology (classification 20.060 : Biological nutrients removal)**

Best regards,

(Andrea Schievano)

REVIEWERS' COMMENTS

1) Abstract: delete - Inorganic salts deposition and biofouling have been hindering long-term operation of air- exposed biocathodes applied to wastewater treatment. Recently, microbial recycling cells (MRCs) were proposed to turn these phenomena into an advantage. MRCs are fabricated using low cost, biocompatible and fully-recyclable materials which, after getting saturated, could be directly recycled as nutrients-enriched fertilizers/conditioners in agricultural soil. In this exploratory laboratory trial - add a new sentence as "The aim of this work was to study ....", then give main results.

Thank you for the suggestions, we changed the structure of the abstract.

2) Language needs improvement; use past tense to describe the word done and results.

Ok. We checked for errors and corrected accordingly.

3) Conclusions need to be re-written; mention here only inferences drawn from results.
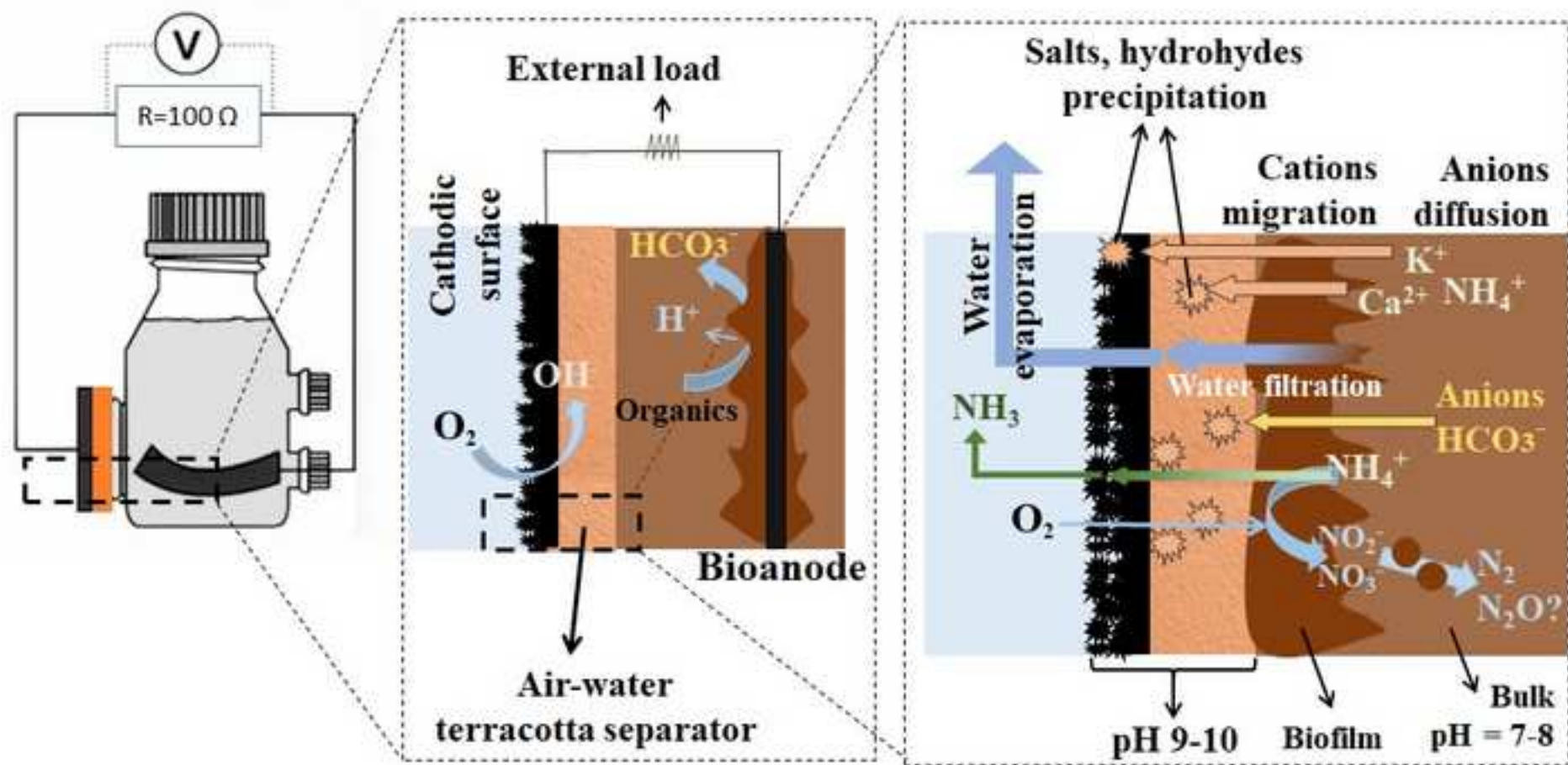
Thank you for the suggestions, we changed the structure of conclusions.

4) Refs can be maximum 50. Delete older ones.

Thanks, we corrected

5) Page length can be maximum 35.

Thanks, we corrected

Graphical Abstract (for review)

- Microbial Recycling Cells were recently proposed as new concept in METs
- Fully-recyclable materials are saturated with nutrients from wastewater
- This is an exploratory experiment studying terracotta-based separators
- Inorganic and organic forms of nutrients are deposited on separators
- Main causes are current-derived cathodic pH increase and water evaporation

Microbial recycling cells: first steps into a new type of microbial electrochemical technologies, aimed at recovering nutrients from wastewater

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Abstract

The aim of this work were to study terracotta-based porous air-water separators (4 mm thickness) in microbial recycling cells (MRCs) fed with cow manure (CM), swine manure (SM) and dairy wastewater (DW). Over 125 days, besides the removal of 60-90% of soluble-COD, considerable fractions of the main macronutrients (C, N, P, K, Fe, Mn, Ca, Mg) were removed from the wastewater and deposited on the terracotta separators as both inorganic salts and biomass deposits. Water evaporation at air-water interface as well as the high cathodic pH (10-12), induced by oxygen reduction to OH^-, were the predominant factors leading to precipitation. The separators were saturated of up to 10 g per kg of terracotta of the main macronutrients, with negligible concentrations of the main inorganic contaminants. These materials could be directly reused as nutrients-enriched solid conditioners for agricultural soils.

Keywords

Nutrients recovery; wastewater treatment; microbial electrochemical technologies; microbial recycling cells; terracotta.

1. Introduction

It is estimated that demand for food will continue to increase, as a result of population growth, but at the same time, food production will increasingly face huge constraints, such as water scarcity, soil desertification and the increase of fertilizers prices (Gustavsson et al., 2011). The recovery of organic/inorganic carbon and mineral nutrients from wastewater derived from food production chains is widely recognized as a priority to minimize environmental contamination, while treating

water (Verstraete et al., 2009). Nutrients removal from water solution and recovery as renewed fertilizers is of particular interest, because of the potential threat of natural water bodies over-fertilization, the limited mining resource and high cost associated with nutrient production (Rittmann et al., 2011).

Research in the field of microbial electrochemical technologies (METs) has been tremendously expanding over the last decades. In microbial fuel cells (MFCs), microporous layers (MPL) electrodes have been used to separate the liquid in the anodic chamber from the air-exposed cathodic surface, while increasing cathodic surface area (Ghadge et al., 2015) and guaranteeing low ionic resistance   (Cristiani et al., 2013). Usually, MPL are based on blends of hydrophobic polymeric binders (PTFE, Nafion® etc. and conductive powders used as catalysts (carbon black, metals etc.), spread on carbon fibres or other conductive materials (Santoro et al., 2015).

Several mechanisms have been limiting the development of applicable MFC technologies. Over relatively short-term operations (50-100 days), clogging and biofouling due to organic materials as well as inorganic salts deposition on electrodes surfaces, strongly invalidate cell performances (Santini et al., 2017). When the system works properly, pH tends to increase in close proximity of the cathode (pH > 9), leading to inorganic salts (carbonates, phosphates, hydroxides, etc.) precipitation and accumulation as a layer on cathode's surface, thus impeding charge transfer processes (Santini et al., 2015). In addition, water evaporation from MPLs or separators at air-water interface contributes to salts precipitation (Santini et al., 2017). Combined biofouling and salts depositions tend to clog the MPL, increasing the internal resistance and deactivating the system (Mathuriya and Pant, 2018).

In a recent review, we proposed a new type of METs, called Microbial Recycling Cells (MRCs), dedicated to recover carbon and nutrients from wastewater, to fabricate bio-based renewable fertilizers (Goglio et al., 2019). In MRCs, biofouling and salts deposition are viewed as a key-advantages: air-exposed cathodes and MPLs could be used as 'sponges' for recalcitrant organic matter, inorganic carbon and nutrients. Once saturated (typically after 40-80 days) (Santini et al., 2017), MRC modules could be substituted with new ones and fully recycled as organic-mineral fertilizers for agricultural soil application. This concept works only if alternative air-water separators and electrodes were fabricated using low-cost, largely available, biogenic/biocompatible and fully-recyclable materials (e.g. clay, biomass, etc.). The whole material of MRCs (enriched by mineral/organic forms of nutrients) can return to soil, and thereby return to the original status, i.e. be 'recycled'.

Air-water interface porous separators based on polymeric binders (used in MPLs) and membranes would not be applicable for these purposes, because of their high cost and their incompatibility with

the environment. Alternatively, terracotta (earthenware) was introduced in recent studies as low-cost and biocompatible air-water separator (Pasternak et al., 2015). Terracotta cylinders were used to build air-cathode MFCs and to separate anode and cathode (Winfield et al., 2016). Current generation in cylindrical terracotta-MFCs contributed to produce an electro-osmotic drag of water molecules, cations migration and catholyte formation in proportion to MFC power performance (Gajda et al., 2014). Also, increased pH and water evaporation from the air-water interface contributed to salts precipitation and heavy metals recovery (Gajda et al., 2015). Moreover, the possibility of recovering macronutrients (e.g. N, P, K, Ca, Mg, etc.) was shown. Ammonium ions are dragged by electro-osmotic forces to the cathodic surface, where pH is typically higher than pH=9; under such conditions ammonia stripping (Santoro et al., 2013) and struvite precipitation (Merino-Jimenez et al., 2017) can be favoured to recover nutrients as insoluble salts. Ammonium was also reported to undergo anaerobic or microaerophilic oxidations to nitrites and nitrates, respectively at the anode and at the air-water interface. Successive denitrification was also reported, in presence of bioavailable organic carbon (Virdis et al., 2010). Finally, suspended organic matter can be recovered by biofouling. Organic matter in the form of microbial cells, extracellular polymeric substances, complex and recalcitrant organic molecules usually contribute to create layers on the electrode or separator surfaces (Noori et al., 2016).

In this work, the behaviour of terracotta air-water separators was studied in lab-scale MRCs, proposing them as mean of organic matter and nutrients recovery from organic-rich wastewater streams coming from agro-food chains, such as farming and cheese production.

2. Materials and Methods

In this experiment, terracotta separators were studied as air-water interface in air-cathode MRCs, to recover organic matter, inorganic carbon and nutrients, from 3 different types of wastewater: cow manure (CM), swine manure (SM) and dairy-industry wastewater (DW) (sampled from a cheese factory). Six MRCs (built with glassy lab-scale reactors) were studied over a relatively long period (125 days): 2 trials for each wastewater were run using terracotta as air-water separator. Hereafter, each couple of reactors were called, according to the wastewater fed, CM, SM and DW.

The electrochemical performances of the MRCs and the water losses by evaporation from the air-water interface were measured over time. The fate of soluble fractions of organic matter and nutrients was monitored in the liquid phase along the observed period. Particular attention was focused on nitrogen and its main forms. Microbial analyses were performed to describe the system. At the end of the experiment, terracotta separators and cathodes were characterized, to evaluate the enrichment in organic matter and nutrients achieved along the test.

2.1 MRCs configuration

Figure S1 shows the MRC reactors configurations with the terracotta separator. The MFCs were built with simple Pyrex® bottles of 120 mL volume. The anode was fabricated using 30 cm^2 of plain carbon cloth (SAATI C1, Appiano Gentile, Italy) and it was rolled on itself to increase the chance of bacterial growth and placed at the bottom of the cell. Cathodes (25 cm^2 of geometric area, 1 g of dry weight) were made of carbon cloth, modified by a MPL made of activated carbon/PTFE mixture (10:7 on dry weight basis) (Santoro et al., 2015). Cathodes were positioned at the air-exposed side of the terracotta separator. Anode and cathode were electrically connected through an external load of 100 Ω and positioned at a distance of 2 cm. Connections were insulated with non-conductive epoxy resin. The terracotta separator was characterised by 4 mm of thickness, pore size in the range 60-500 nm, 10 g of dry weight and 25 cm^2 of geometric area, between the anolyte and the cathode.

2.2 BET analysis

The Brunauer-Emmett-Teller (BET) specific surface area was obtained from N_2 adsorption/desorption isotherms at 77 K using a Micromeritics Tristar II apparatus (Tristar II 3020). Specific surface area was determined by the instrumental software. Porosity distribution was evaluated for each sample by using BJH method. Before measurements, sample powders were heat-treated at 150 °C for 4 h under a N_2 flow to remove adsorbed foreign species.

2.3 Inoculation and experimental set-up

All MRCs were inoculated with raw wastewaters and then always fed in parallel with identical timing. The chemical characteristics of the raw wastewaters and their content of organic matter and nutrients are reported in Table 1.

The first batch cycle was considered as an acclimation. When the current dropped over time, the reactors were emptied and fed again with fresh wastewater. After this, the reactors were periodically refilled with additional wastewater, to compensate the volume decrease due to water evaporation through air-water separators. At day 56, sodium acetate (3 g L^{-1}) was added as standard substrate, to compare MRCs parameters for clarity, the experimental operations are resumed in Table 2.

2.4 Electrochemical analyses

Throughout the duration of the experiment, several electrochemical measurements were carried out: current density trends, open circuit potential (OCP) and power curves. For each MRC, the potential difference across an external load of 100 Ω was recorded every 20 minutes using a multichannel Data Logger (Graphtech midi Logger GL820). The anodic OCP were periodically measured versus an Ag/AgCl reference electrode after 30 min equilibration time.

Power curves were periodically recorded with a two-electrode configuration. Before each electrochemical measurement, 1 h of equilibration time was found necessary to allow the system, disconnected from the data logger, to reach OCP. The anode was set as working electrode and the cathode as reference electrode. A linear sweep polarization (scan rate v=0.010 V min^{-1}) was recorded from the cell OCP to 10 mV. The power was calculated by $P = I \cdot V$, normalized by the cathodic area and plotted vs current density.

2.5 Physico-chemical characterization of the process

Water evaporation from the air-water interfaces were monitored over time by weight measurements of the reactors. pH profiles near the air-water interface were measured, using a potentiometric microelectrodes and the measurements were performed under open circuit conditions and the positions of microelectrode were determined by a computer controlled micromechanical movement system (NSC-A1 Stepper Motor Controller, Newmark Systems Inc.) (Guerrini et al., 2013).

The chemical composition of the wastewaters along the cycles was monitored by several parameters: Chemical oxygen demand (COD), Total Kjeldahl Nitrogen (TKN) and total content of the main macronutrients (P, Ca, K, Mg, Fe, Mn) analysed by inductively coupled plasma mass spectrometry (ICP-MS). In addition, the enrichment in these elements on the terracotta separators was determined at the end of the experiment.

2.6 Evaluation of nitrogen removal mechanisms

A control experiment was run in double with SM, to evaluate the different mechanisms that play a role in Nitrogen removal. NH_3 evaporation from the air-water interface was evaluated as shown in **Figure S1**). A counter-chamber was used on the cathode side, to capture and quantify NH_3 emissions due to stripping at the cathode. A trap of H_2SO_4 was used to entrap ammonia and quantify it at the end of the experiment. $N-NH_4^+$, $N-NO_2^-$ and $N-NO_3^-$ were monitored in the liquid phase along a batch cycle (days 34-56). Sodium acetate (3 g L^{-1}) was spiked in the solution, to evaluate denitrification mechanisms in presence of a readily-available carbon source as electron donor.

2.7 Details of analytical procedures

The soluble fractions of COD (sCOD) in the anolyte were determined using spectrophotometric method after specific reactions using test kits (Hach Company, Loveland, CO, USA). The samples were filtered (0.2 µm Nylon filters) before COD analysis. TKN was determined by titration of ammonium ions, after digestion in concentrated H_2SO_4, as indicated by standard methods (APHA). ICP-MS was used to measure total and soluble contents of single elements in the anolyte, membrane and electrodes. For total content, weighted amounts of materials were digested by a microwave digestion system (Anton Paar MULTIWAVE-ECO) in Teflon tubes filled with 10 mL

of 65% HNO_3 by applying a one-step temperature ramp (210 °C reached in 10 min and maintained for further 10 min). After 20 min of cooling time, the mineralized samples were transferred in polypropylene test tubes. Both solutions of mineralized samples and extracted soluble fractions were diluted 1:100 with 0.3 M HNO_3 in MILLI-Q water and the concentration of elements was measured by ICP-MS (BRUKER Aurora-M90 ICP-MS).

2.8 DNA extraction

DNA samples were obtained from the air-cathode MRCs at the end of the experiment. Small pieces of anodic carbon cloth were cut and combined for DNA extraction. The terracotta biofilm sample obtained scraping the cathodic biofilm from the internal side of the terracotta with a sterile spatula. Total DNA was extracted from approximately 0.25 g of samples using a PowerBiofilm DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Quantity and quality of the DNA were measured by spectrophotometer (BioPhotometer, Eppendorf,). DNA was visualized under UV light in a 1% gel electrophoresis with TBE 0.5× (Tris-Borate 50 mM; EDTA 0.1 mM; pH 7.5–8).

2.9 Illumina MiSeq sequencing

Genomic DNA was PCR amplified using a two-stage "targeted amplicon sequencing (TAS)" protocol (Bybee et al., 2011; Green et al., 2015). The sequencing was performed as described previously (Rago et al., 2018). The primers contained 5' common sequence tags (known as common sequence 1 and 2, CS1 and CS2) as described previously (Moonsamy et al., 2013). Two primer sets were used for this study, including CS1_341F/CS2_806R (Bacteria), CS1_ARC344F/CS2_ARC806R (Archaea) (Rago et al., 2017).

Library preparation and pooling was performed at the DNA Services (DNAS) facility, Research Resources Center (RRC), University of Illinois at Chicago (UIC). Sequencing was performed at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign (UIUC).

Forward and reverse reads were merged using PEAR (Zhang et al., 2014). Ambiguous nucleotides and primer sequences were trimmed (quality threshold p = 0.01). After trimming, reads containing internal ambiguous nucleotides, lacking either primer and/or shorter than 300 bp were discarded. Chimeric sequences were identified with the USEARCH algorithm (Edgar, 2010) and removed. Further analyses were performed with the QIIME tools (J Gregory Caporaso et al., 2010). Sequences with a similarity higher than 97% were grouped in Operational Taxonomic Units (OTUs) and representative sequences for each OTU were aligned to the SILVA SSU Ref dataset (Quast et al., 2013) using the PyNAST method (J. G. Caporaso et al., 2010). In Figure 5 are

3. Results and Discussion

3.1 Current generation trends

The current density trends during the operational period of 125 days are plotted in Figures 1 - A, B, and C. CM reactors produced peak current densities of around 500 mA m^{-2} (referred to cathodic geometric area), along 125 operation days. After 8 days of lag phase during acclimation, the system started producing current. After acclimation, the current density slightly increased and remained almost constant for 15 days. During the cycle fed with sodium acetate, the current production of CMs reached around 300 mA m^{-2}, lasting less than previous cycles. SM systems produced an oscillating current signal along 125 days, with peak current density of around 800 mA m^{-2}, the highest among the tested wastewater types. This is likely due to the fact that SM was richer in readily bioavailable sCOD, as compared to CM. DWs showed trends current densities around 150 mA m^{-2}. A significant current production began at day 4, yielding the maximum current density. Then, for this system, throughout its operational period, current density was lower, consistent with the lower concentrations of sCOD (844 mg$_{COD}$ L^{-1}, Table 1).

After day 75, all MRCs in parallel showed decreasing trends of current density. This might be attributable to deposition phenomena of inorganic salts as well as of organic matter. Santini et al. (Santini et al., 2017) recently observed a biocathode inactivation over time due to carbonate scale deposition in air-cathode MFCs, already after around 40-50 days. Previous works by the same group (Santini et al., 2015) documented the presence of a thick layer of carbonate formed as a consequence of the alkalinity induced by the ORR on cathodes operated for long time in single chamber MFCs. In particular, this phenomenon was found to significantly hinder MFC performances after around 60 days of operation.

3.2 Electrochemical characterizations

Figure 1-D shows the power density curves measured on all reactors, during average current production (avoiding peaks of electrochemical performances). The power density curves evidenced a consistent difference among the different wastewaters confirming the data obtained during the monitoring of the current density trend. In Table 3 some electrochemical parameters are summarized, to characterize the MRCs. The terracotta separator was likely impeding proper electrolytic contact of the cathode with the solution, resulting in relatively low power densities. In this experiment, the plain terracotta surface was particularly exposed to air contact, and consistent

water evaporation (**Figure 2**, Section 3.3) probably contributed to high electrolytic resistance. However, the goal of MRCs systems is not to produce high amount of electricity and energy harvesting, while to recover nutrients and treat wastewater. The observed power densities might be sufficient to induce cations migration and other mechanisms to drive salts sequestration from the anolyte. In addition, other configurations of terracotta MRCs (e.g. cylindrical, with the cathode placed in the internal surface) were reported with much lower internal resistance (Santoro et al., 2015). Therefore, there is quite sufficient space for optimizing these systems and maximizing the electrochemical performances.

The configuration also affected the CE, that achieved relatively low percentages on the bioavailable sCOD (**Table 3**). The consistent water evaporation occurred and the high electrolytic resistance likely affected the CE. Also, the direct contact of wastewater with air through the porosity of terracotta, probably favoured carbon removal through aerobic respiration, both at the terracotta-air interface and at the liquid surface. Other carbon-consuming processes, such as denitrification at the terracotta interface might have counted in lowering CE (Rago et al., 2018). An optimized configuration would in this case improve the contribution of bioelectrochemical paths towards COD removal. However, it is important to keep in mind that in MRCs, unlike in MFCs, achieving high CE is not the goal and might not correspond to high nutrients recovery. The electrochemical process is one of the mechanisms that can drive to organics mineralization and nutrients removal from the liquid phase. These aspects are further discussed in Section 3.3 and 3.4.

3.3 - Effects of the MRCs on the wastewater liquid phase

Cumulative water evaporation trends are reported in **Figure 2-A** for all MRCs along the observed period. Water evaporation resulted in all cases considerable and it was favored with more diluted wastewaters (such as DW), while higher organic matter concentrations (CM and SM) likely limited water evaporation throught the porous separator. Indeed, water evaporation rates were comparable for CM and SM, while being relatively higher in DW (**Table 3**).

Evaporation was mainly favored by the relatively big pores of the terracotta layer. **Figure 3** reports the distribution of pores volume and surface area on pores diameters. The total specific surface area was around 0.868 m^2/g with pore size in the range. Thus, the total surface area available to air-water exposure in each MRC reactor (each terracotta separator weighted around 10 g) was around 8.7 m^2. Water capillary diffusion, as well as electro-osmotic forces induced by the electric field, as previously found in other experiments (Gajda et al., 2015), contributed to water evaporation in contact with this relatively abundant surface area.

Figure 2-B shows that the pH in the anodic compartment remained almost constant in CMs and slightly higher for both in SMs and DWs. Depending on the buffer capacity of the liquid medium,

slight increases in pH is typical in anaerobic environments, as result of volatile fatty acids consumption (De Bere, 2000). Regardless to the type of wastewater, the pH never exceeded pH 8.5 in the bulk liquid phase.

Contrarily, in the vicinity of the air-water interface, the pH profile measured by microelectrodes was found always over pH 9 (**Figure 2-C**). In the vicinity of the terracotta separators on the anodic side, pH was nearly 10 in all MRC systems. Although the presence of terracotta separators induced a considerable internal resistance to the electrochemical cells (**Table 3**), the current densities obtained by all MRCs were sufficient to induce consistent pH increases (around pH 10) at the air-water interface. The pH profile within the pores of the terracotta separators and at the cathode were indirectly measured at the end of the experiment, after shredding the material. In all cases, the pH in the separator as well as at the cathode was in the range 9.5 – 10 (**Table 3**). This phenomenon was already observed in previous experiments and linked to the surplus of hydroxyl free radicals liberated by incomplete the cathodic oxygen reduction reaction (Winfield et al., 2016). Measurements on the catholyte formed by electroosmotic water transport through terracorra separators was found up to pH 13 (Gajda et al., 2015). This is indeed a key point to favor inorganic salt depositions (e.g. struvite, Ca-carbonates, Mg-Carbonates, organophosphates, oxydes and other salts etc.) within the internal porosity of the terracotta separators (Santini et al., 2015;). This finding encourages the use of MRCs, made of low-cost air-water porous separators, to improve nutrients recovery as precipitated salts within the terracotta separators and at the cathode.

Figure 4 shows that in all MRCs a considerable amount of the soluble fractions (after filtration at 2 μm) of organic carbon (measured as COD), N, P, Ca, K, Mg, Fe and Mn and were removed from all the wastewaters. Although the configuration and architecture of this lab-scale system was not optimized, organic carbon (as soluble-COD) was removed with relatively high efficiency.

Soluble nitrogen forms were removed from the liquid phase with particular efficiency. In the case of DW, N was undetectable at the end of the experiment, while SM showed over 80% removal and CM around 50% (**Figure 4**). A discussion on the possible fate of N forms is reported more in detail in the following Section 3.4. The soluble forms of the other elements (P, Ca, K, Mg, Fe, Mn) could be removed from the bulk liquid in principle by: a) precipitation of inorganic salts on the air-water interface separators, thanks to high pH and locally increased concentrations due to water evaporation; b) deposition of organic fractions on the terracotta inner surface; c) fouling, precipitation or settling in the anodic chamber. Moreover, are also investigated the amount of the heavy metals in the **Table 5** to confirm the possibility to use these kinds of wastewater as a substrate for a treatment to recovery nutrients.

3.4 – Nitrogen removal and analysis of microbial communities

Total nitrogen was efficiently removed from the liquid phase (**Figure 4**). To give more details to this aspect, ammonium ion removal was measured along one batch cycle (days 34-54) of the SM-systems. **Figure 5** reports the trends of replicate experiments, together with the trends of sCOD, N-NH_4^+, N-NO_2^- and N-NO_3^- concentrations. Ammonium removal followed a parallel trend to sCOD. The ammonium removal rate observed in **Figure 5** could be ascribable to a range of different phenomena: a) anaerobic ammonium oxidation (Anammox) (Qiao et al., 2018); b) exoelectrogenic NH_4^+ oxidation at the anode (Yang et al., 2017); c) nitrification at the air-water interface, thanks to the possible presence of microaerophilic conditions in the biofilm at the water-side surface of the terracotta separator; d) gaseous ammonia (NH_3) stripping at the terracotta/cathode, due to high pH and water evaporation (**Figure 2**) and e) nitrogen deposition as part of precipitated salts (e.g. struvite) either on the terracotta or within the anodic chamber.

After around 20 days (from day 34 to day 56), N-NH_4^+ reached the minimum value (around 300 mg L^{-1}) while nitrites and nitrates concentrations increased to their maximum values (up to 180 mg L^{-1} and 65 mg L^{-1} respectively). After the acetate addiction of 3 g L^{-1}, the accumulation of nitrites and nitrates showed a fast inversion of tendency, and they decreased till the lowest values after five days (day 61) in both cases. In this period, it was present also a fast consumption of sCOD (from around 4.6 to 1.3 g L^{-1}). These results indicate that the nitrifying metabolism was associated to the presence of sCOD and so probably it was carried out by heterotrophic microorganisms. The amount of N-NH_3 captured by the H_2SO_4 trap was modest. It corresponded to around 4% of the initial amount of N-NH_4^+ in the wastewater. Instead, nitrification and denitrification processes showed that the highest contribution to N removal is given by nitrification/denitrification processes in the reactor. The ability of air-exposed MFC systems (with a range of different architectures) to remove mineral nitrogen was often reported (Sotres et al., 2016; Virdis et al., 2010; Yan et al., 2012; Zhao et al., 2016).

Here, anodic and terracotta biofilms were analysed to investigate the microbial community. The cathodic microbiology was not analysed because the pore dimension of terracotta substantially impeded microorganism to reach the external surface of the separator and the cathode, as already explained in a previous study (Rago et al., 2018). In fact, pores diameters were below 100 nm by 99.9% of their total volume. Around 50% of pores volume was associated to pores diameters <2 nm (micropores), while the other half was in the range 2 – 50 nm (mesopores) (**Figure 3**).

Both anodic and terracotta biofilm communities showed a similar composition, as shown by the phylum representation (**Figure 6A**). *Bacteriodetes* phylum was the main present (28-31% in anodic and terracotta biofilms) and it is often reported in the MFC biofilms (Montpart et al., 2018).

Proteobacteria (around 25% in both samples) and *Firmicutes* (18-14% in anodic and terracotta biofilms) phyla are commonly found in bioelectrochemical systems playing important roles in bioelectroactive biofilms (Logan, 2009; Parameswaran et al., 2010; Patil et al., 2009; Rago et al., 2016). *Euryachaeota* phylum was more present in anodic (4%) than in terracotta community (1%) indicating that the anodic condition was more anoxic (Rago et al., 2017).

The genus representation (**Figure 6B**) showed that fermentative bacteria mainly colonized both communities. The anodic exoelectrogenic community was more present on the anode (conductive material) than on terracotta (inert material), as already observed in a previous study (Rago et al., 2018). The main difference in composition between the two communities was the high presence of well-known electroactive genera only in the anodic community and the presence of microaerophilic and facultative microorganisms in terracotta biofilm. This exoelectrogenic community was mainly represented by *Clostridium sensu stricto I* (4.3%), *Desulfuromonas* (3.5%) *Desulfomicrobium* (1.1%) genera (Rago et al., 2017, 2018). On the other side, several members of Leptonema sp., *Taibaiella* sp., *Brumimicrobium* sp., *Acholeplasma* sp., that were present only in terracotta biofilm, were previously described as microaerophilic, or strictly or facultative aerobic Bacteria (Bowman, 2015; Brown et al., 2015; Huntemann et al., 2013; Zhang et al., 2013). These microbes might have played an important role at the air-water interface, to justify the observed efficient nitrification (**Figure 5**). Some facultative anaerobes or anaerobic genera retrieved in both anodic and terracotta communities (*Sterolibacterium* sp., *Vulcanobacillus* sp. and *Thiopseudomonas* sp.) were associated to nitrate reduction (or denitrification) process (Cai et al., 2015; Chiang et al., 2007; Tan et al., 2015), in presence of available organic carbon. This was evident after the addition of sodium acetate at day 56 (**Figure 5**).

Instead, the 16S rRNA gene sequencing in both the anodic and terracotta communities (with a presence higher than the 1% of the total OTUs) did not show the presence of well-known anammox or electroactive-nitrifying microorganisms (**Figure 6**). However, we cannot exclude that such processes might have contributed to ammonium removal. Some of the observed genera, that were not previously associated to these metabolisms, likely contributed to the observed nitrification process. For a deeper insight into these mechanisms, future experiments should aim at looking at metabolic pathways

3.5 – Deposition of nutrients on air-water separators

In all cases, MRCs were efficient in removing most nutrients from the wastewater and one of the reasons was the deposition of salts and organic molecules on the terracotta separator. A complete balance of nutrients was not possible to be addressed in this experiment. The systems were not tight

enough and, possible precipitation/settling in the anodic chamber was not taken into account. Also, the systems were not optimized to maximize nutrients deposition on the separator. However, it is interesting to consider the amount of nutrients stored on the terracotta separators along the observed period (**Table 4**). These amounts were calculated as difference of total elements measured at the end of the experiment and on the raw terracotta, before the experiment. Overall, all nutrients were recovered in comparable amounts in all the MRC systems. The terracotta separators increased their content in elements by over 10-fold, as compared to their initial amount. Ammonium and metals cations were likely to be dragged by electro-osmotic forces to the cathode, through the terracotta pores, where different mechanisms favoured their immobilization. Typically, terracotta has intrinsically high cation exchange capacity and the flux of cations to the cathode could be 'intercepted' by sorption on the terracotta. Also, the high pH in the vicinity of the cathode likely induced insoluble salts precipitation (e.g. carbonates, hydroxides) within the porous structure of terracotta.

The amount of TOC found on the terracotta was relatively low, as compared to the other elements. Especially for P, Ca, Fe, Mg and Mn, the TOC/element ratios measured on the terracotta at the end of the experiment (**Table 4**) were particularly low, as compared to the raw wastewater (**Table 1**). Thus, inorganic salts and hydroxides deposition/precipitation were probably prevalent phenomena, over organic matter fouling/deposition on the terracotta. These data evidence the selective deposition of inorganic forms of most nutrients, over organic molecules.

P is typically present in manures and wastewaters either in organic forms and/or as inorganic anions (Ekpo et al., 2016). Organic P forms could deposit on the terracotta separator or the cathode only by fouling mechanisms. Most of organic P was expected to be retained and/or settled in the anodic chamber. Inorganic forms of P, as they are found mainly as anions, were expected to be attracted to the anode, by electroosmotic forces. Possible precipitation of P as struvite at the cathode, reported in several articles to be related to high pH (Almatouq and Babatunde, 2016), probably worked only for a small fractions of total P. Phosphorous was already found to precipitate in microbial electrochemical systems (Kim et al., 2018). Struvite minerals are typically insoluble at pH>9 (Tansel et al., 2018).

Deposition of Ca and Mg is likely to happen as insoluble carbonates at pH over 9. This was already observed in several cases, at cathodic air-water interfaces in METs (Marzorati et al., 2018; Santini et al., 2017).

3.6 – Perspectives

After a terracotta separator gets saturated with organic and inorganic forms of various nutrients, it could be ready to be re-utilized as soil conditioner. Soil conditioners and amendments have the

characteristic of improving physical and chemical characteristics of soil, such as its structure, making slight fertilization, facilitate water retention, cation exchange capacity and plant roots growth (Wu et al., 2016). Terracotta has the composition of clay, which is known to have high cation exchange capacity and be a good soil conditioner (Rossini-Oliva et al., 2017). Soil amendments with terracotta and other residual ceramic materials have been suggested to alleviate the soil compaction, to increase water retention, the hydraulic conductivity, the cation-exchange capacity and to improve other soil physical properties (Wu et al., 2017).

The enrichment in nutrients after the experiment in MRCs likely happens due to particularly high pH conditions and ionic concentrations during the process. The reuse of the terracotta separators, enriched of nutrients (**Table 4**), might bring the additional advantage of releasing macro- and micro- nutrients, once applied to agricultural soils. Once distributed as soil amendment, these nutrients would return in soluble forms, available for plant roots uptake, having a partial fertilization effect. More detailed chemical characterization and study could help in elucidate the forms and the availability of the nutrients, measured here as total content. Future study should also aim at applying these materials to soil and study their effects on plant growth.

Another important point should be assessed, as part of the MRCs concept. Any wastewater, even when produced at farm level or in food-transformation industries, might contain relevant concentrations of both organic (antibiotics, herbicides, pesticides, etc.) and inorganic contaminants (e.g. heavy metals). Organic contaminants could undergo biodegradation and bioanodic oxidation, according to their recalcitrance (Domínguez-Garay et al., 2016). Specific assays should assess the fate of specific contaminants in MRCs systems, to avoid possible accumulation on the solid material, by adsorption.

Regarding inorganic contaminants, their possible occurrence at significant concentrations in wastewater might hinder the possibility to directly re-use the materials after MRCs' life cycle, as soil conditioners. Here, the main heavy-metals (Ni, Cu, Zn, As, Cd and Pb) were measured in the wastewaters at the end of the experiment and their possible accumulation verified on the terracotta separators (**Table 5**). According to their provenience (farming and food-transformation industry), substantially low concentrations of heavy metals initially were present in the tested wastewaters (**Table 1**). In the case of CM, most heavy-metals were likely bounded to the organic suspended fractions and tended to accumulate in the liquid phase (negative removal efficiency). Contrarily, MRCs treating SM and DW were relatively efficient in removing the large part of heavy metals. In all cases, a net increase in content was measured on the terracotta separator (**Table 5**). When compared to a relatively restrictive voluntary standard-limit (EU ECO Label) for Soil improvers and growing media (**Table 5**), the retrieved concentrations of all heavy-metals on the ceramic

materials confirmed their possible direct reuse as soil conditioners. However, depending on the type of wastewater treated, these values might change and accurate characterization should be done. Eventually, post treatments to recover heavy metals from the ceramic materials might be proposed, before agricultural re-use.

4. Conclusions

The electrochemical forces generated by anodic oxidations, the local increase of cathodic pH driven by incomplete oxygen reduction reaction and local water evaporation were the main drivers towards cation migration to the cathode and to inorganic salts or organic matter deposition on the separator. After an operational period of around 125 days, terracotta-based porous air-water separators were saturated with relatively high amounts of the main macronutrients. Terracotta is a relatively low-cost and biocompatible material, which could be directly recycled as base for organic-mineral fertilizers and soil conditioners, in agricultural applications. Further studies should elucidate in more detail a mass balance of nutrients and carbon, to better understand their organic/inorganic forms and improve deposition mechanisms.

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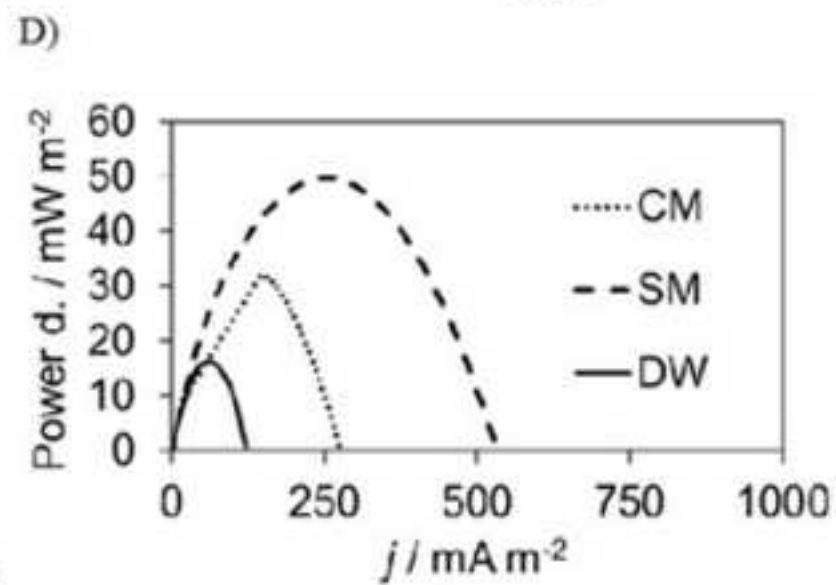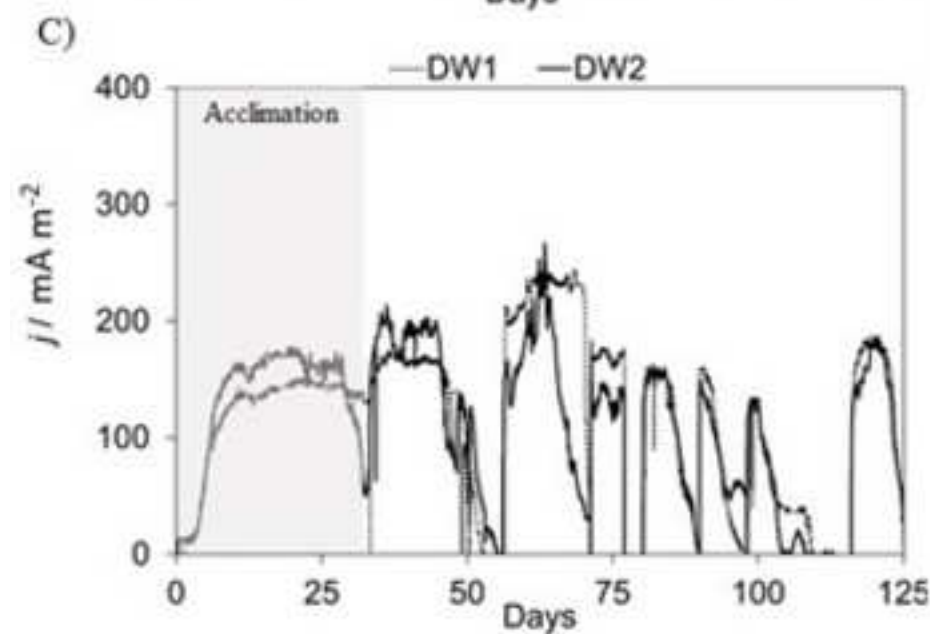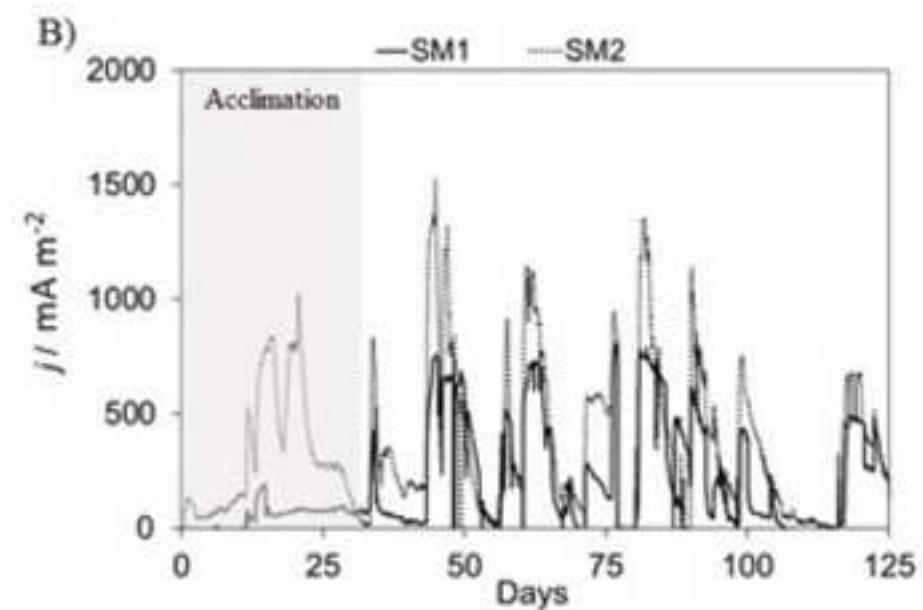
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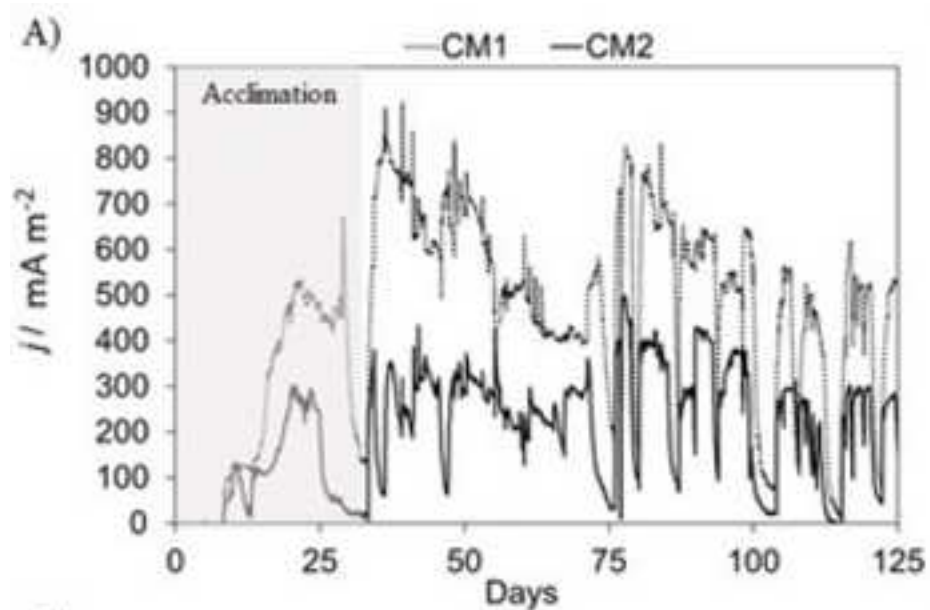**Click here to download high resolution image**
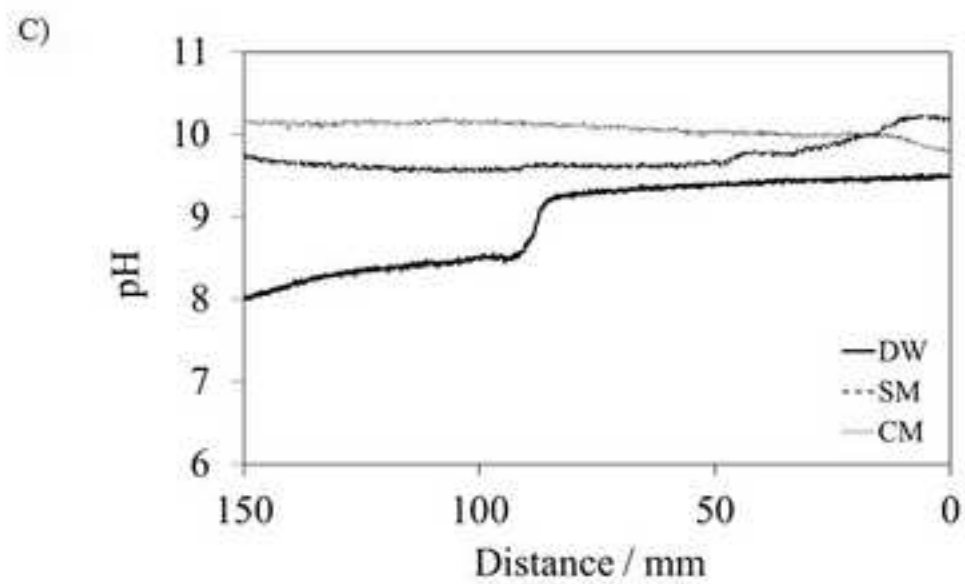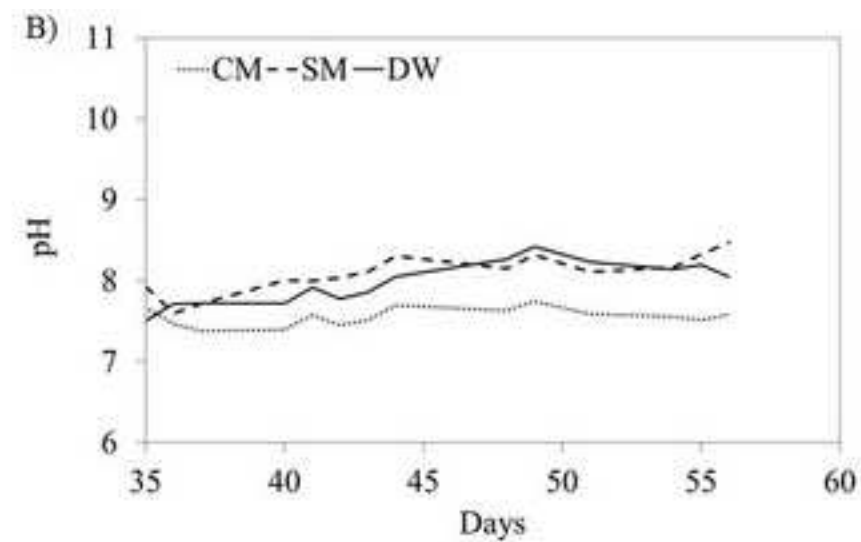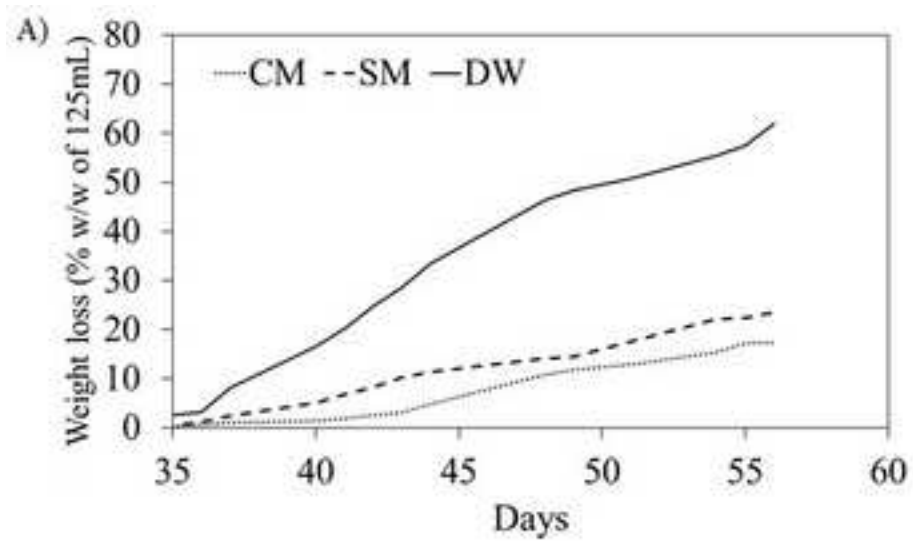
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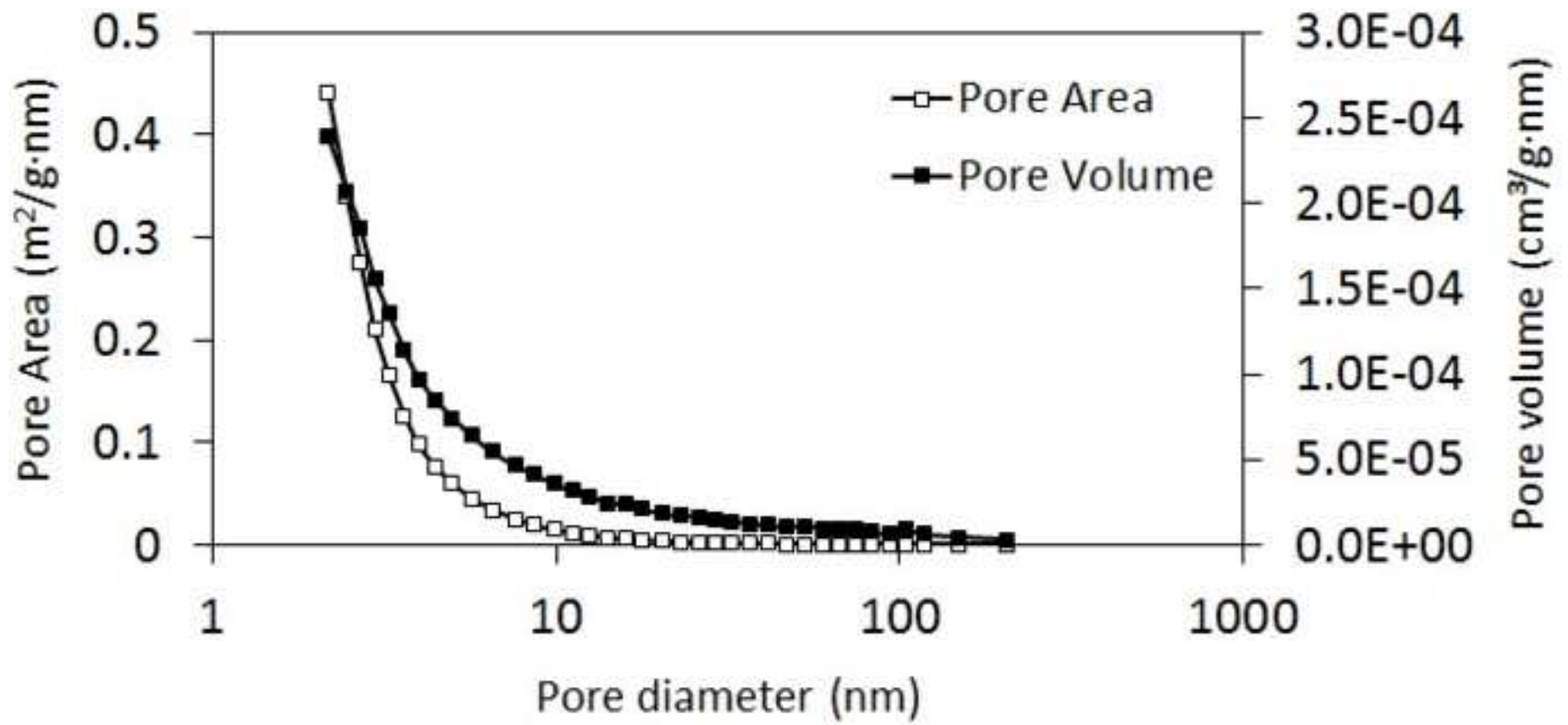
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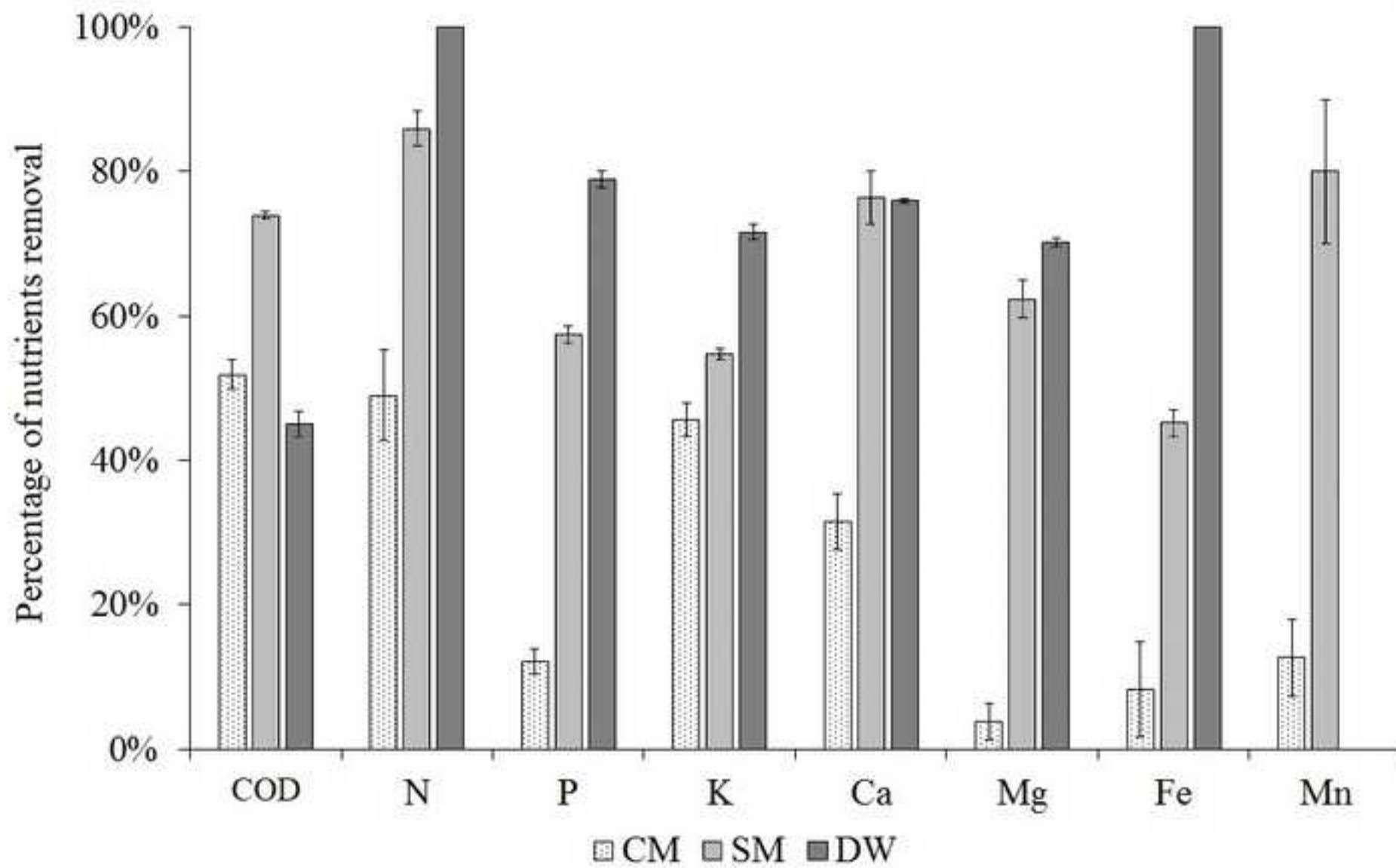
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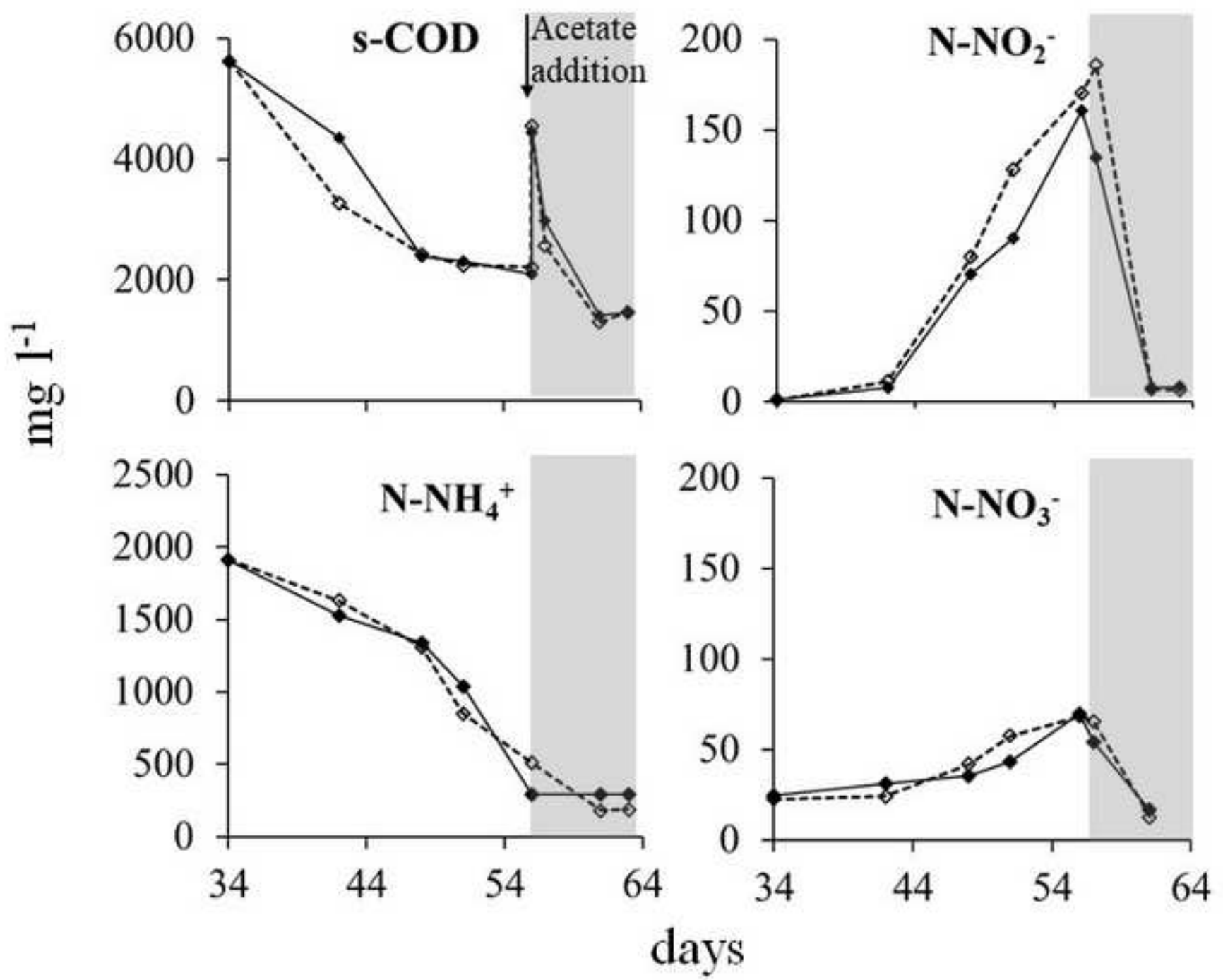
Figure6
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A)

SM anode | SM terracotta

Legend:
- <1%
- Unassigned
- Deinococcus-Thermus
- Tenericutes
- Synergistetes
- Verrucomicrobia
- Actinobacteria
- Spirochaetae
- Atribacteria
- Chloroflexi
- Cloacimonetes
- Firmicutes
- Proteobacteria
- Bacteroidetes
- Euryarchaeota

B)

SM anode legend:
- <1%
- Unassigned
- Atribacteria uncult
- Anaerolineaceae uncult
- Alkaliflexus
- Candidatus Cloacamonas
- Clostridium sensu stricto 1
- Thiopseudomonas
- Desulfomicrobium
- Desulfuromonas
- Saprospiraceae uncult
- Fastidiosipila
- Proteiniphilum
- Truepera
- Limnobacter
- Mariniphaga
- BD1-7 clade
- W27 uncult
- vadinHA17 uncult
- vadinBC27 ww-sludge gr.
- VC2.1 Bac22 uncult
- Methanosaeta

SM terracotta legend:
- <1%
- Unassigned
- Saccharibacteria uncult
- Synergistaceae uncult
- Leptonema
- Taibaiella
- Brumimicrobium
- Sterolibacterium
- Erysipelotrichaceae UCG-004
- Vulcanibacillus
- Acholeplasma
- Desulfobacterium
- Saprospiraceae uncult
- Fastidiosipila
- Proteiniphilum
- Truepera
- Limnobacter
- Mariniphaga
- BD1-7 clade
- W27 uncult
- vadinHA17 uncult
- vadinBC27 ww-sludge gr.
- VC2.1 Bac22 uncult

Figures' captions

Figure 1 – Current density trends during 125 days observation of the MRCs fed with cow-manure (**A**), swine manure (**B**), dairy wastewater (**C**). Power curves (**D**) representing average electrochemical performances of the tested MRCs systems, along the observed period.

Figure 2 – Weight loss due to water evaporation (**A**), pH trends in the bulk liquid of anodic chambers (**B**), along a representative period (days 35 – 55) and pH profiles in the vicinity (15 mm, measured by microelectrodes) of the anodic side of the terracotta separator of MRCs fed by cow manure, swine manure and dairy wastewater (**C**).

Figure 3 – BET analysis of the terracotta separator: pore area and volume distribution over pore diameters.

Figure 4 – Removal efficiency of the soluble fraction of the main nutrients from the bulk liquid phase after 125 days, reported as percentage of the initial concentration in the wastewater. MRCs were fed with cow manure (**A**), swine manure (**B**) and dairy wastewater (**C**). Error bars stand for standard deviations of duplicate experiments, under identical conditions.

Figure 5 – Removal trends for sCOD, $N-NH_4^+$, $N-NO_2^-$ and $N-NO_3^-$ from the liquid phase of MRCs fed with swine manure, in duplicate experiments under identical conditions. One representative batch cycle (days 34 – 56) followed by a spike of sodium acetate (3 g L^{-1}) are shown.

Figure 6 – Phylum (**A**) and genus (**B**) representation of Illumina 16S rRNA gene amplicon sequencing, resulted from biofilms sampled on the anode and on the terracotta-water interface, in MRCs fed with swine manure.

Table 1 – Characterization of the wastewater streams. All values for elements refer to soluble fractions (after filtration with 0.45 µm mesh). Values in brackets report the ratios TOC/element.

Parameter	Unit	CM	SM	DW
pH		7.46	8.35	8.16
El. Conductivity	mS cm^{-2}	9.88	8.54	1.48
COD	g L^{-1}	12.5	5.66	0.84
TOC	mg L^{-1}	4.22	1.87	0.28
Macronutrients				
TKN	g L^{-1} (TOC/N)	1.55 (2.7)	1.92 (1)	0.1 (2.8)
P	mg L^{-1} (TOC/P)	262 (16.1)	114 (16.4)	12 (23.3)
K	g L^{-1} (TOC/K)	2.92 (1.4)	1.72 (1.1)	0.51 (0.5)
Ca	mg L^{-1} (TOC/Ca)	853 (4.9)	151 (12.4)	91 (3.1)
Mg	mg L^{-1} (TOC/Mg)	265 (15.9)	39 (47.9)	16 (17.5)
Fe	mg L^{-1} (TOC/Fe)	44 (95.9)	5 (347)	1 (280)
Mn	mg L^{-1} (TOC/Mn)	8 (527.5)	2 (935)	<1 (-)
Micronutrients / heavy metals				
Ni	mg L^{-1}	0.02	0.008	<0.001
Cu	mg L^{-1}	0.2	0.11	0.011
Zn	mg L^{-1}	2.4	1.6	0.018
As	mg L^{-1}	0.003	0.003	<0.001
Cd	mg L^{-1}	0.004	<0.001	<0.001
Pb	mg L^{-1}	<0.001	<0.001	<0.001

Table 2 – Experimental set-up and wastewater feeding conditions (refilled volumes after water evaporation)

Operation days	Feeding	CM	SM	DW
Day 0	Raw sewage (mL)	120	120	120
Day 34	Raw sewage (mL)	120	120	120
Day 56	Sodium acetate (g $_{COD}$ L^{-1})	2.34	2.34	2.34
Day 72	Raw sewage (mL)	15	24	68
Day 85	Raw sewage (mL)	13	34	57
Day 100	Raw sewage (mL)	10	24	59
Day 117	Raw sewage (mL)	1	6	33

Table 3 – Electrochemical characteristics, average water evaporation rates and pH within the

terracotta and cathodic materials, along the observation period

Reactors		CM	SM	DW
Coulombic Efficiency	% on initial sCOD	16	16	28
Average power density	mW m^{-2}	33	67	17
Anodic OCP	mV *vs* Ag/AgCl	-492	-498	-523
Water evaporation rates	mL cm^{-2} day^{-1} of terracotta	0.25	0.31	0.94
pH at the terracotta separator		9.66±0.11	9.69±0.04	9.75±0.01
pH at the cathode		9.37±0.04	9.64±0.20	10.02±0.02

Table 4 – Total amounts of nutrients (measured as total element) recovered per kg of terracotta separators along 125 days of operation for MRCs and TOC/element ratios.

	CM	SM	DW
Mg ($g\ kg^{-1}$)	1.86	2.33	0.23
K ($g\ kg^{-1}$)	0.62	2.35	-
Ca ($g\ kg^{-1}$)	3.75	5.45	-
Mn ($mg\ kg^{-1}$)	51	164	-
Fe ($g\ kg^{-1}$)	2.89	9.14	2.41
P ($g\ kg^{-1}$)	0.20	1.05	0.38
TKN ($g\ kg^{-1}$)[a]	0.32	0.30	0.10
TOC ($g\ kg^{-1}$)[b]	1.32	1.93	0.74
TOC/Mg	0.71	0.83	3.27
TOC/K	2.12	0.82	**-**
TOC/Ca	0.35	0.35	**-**
TOC/Mn	0.03	0.01	**-**
TOC/Fe	0.46	0.21	0.31
TOC/P	6.60	1.84	1.93
TOC/TKN	4.13	6.43	7.40

[a] TKN = Total Kjieldahl nitrogen. [b] TOC = Total Organic Carbon

Table 5 – Concentrations of potential inorganic contaminants recovered on the terracotta separators after their working period (125 days).

	Ni	Cu	Zn	As	Cd	Pb
Concentration on terracotta separator (mg kg^{-1})						
CM	<0.2	36.1	51.4	<0.2	<0.2	<0.2
SM	30.6	6.1	33.8	<0.2	<0.2	<0.2
DW	24.4	<0.2	7.7	1.6	<0.2	<0.2
Standards/limits for soil conditioners						
European union[1]	25	70	300	10	1	100
USA[2]	420	1500	2800	41	39	300

[1] Voluntary standard EU ECO Label for Soil improvers and growing media

[2] EPA CFR40/503 Sludge Rule

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