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# Forming microbial anodes with acetate addition decreases their capability to treat raw paper mill effluent

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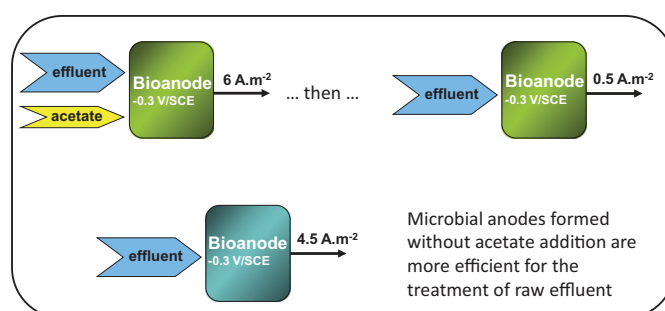
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## HIGHLIGHTS

- Microbial anodes were formed at  $-0.3$  V/ECS from paper mill effluent.
- Currents were increased by the addition of acetate into the raw effluent.
- Bioanodes formed with acetate addition lost their ability to treat the raw effluent.
- Bioanodes formed without acetate addition gave up to  $4.5$  A/m<sup>2</sup> in continuous mode.
- Addition of acetate decreased the diversity of the microbial diversity of bioanodes.

## GRAPHICAL ABSTRACT



## ABSTRACT

Microbial anodes were formed under polarization at  $-0.3$  V/SCE on graphite plates in effluents from a pulp and paper mill. The bioanodes formed with the addition of acetate led to the highest current densities (up to  $6$  A/m<sup>2</sup>) but were then unable to oxidize the raw effluent efficiently ( $0.5$  A/m<sup>2</sup>). In contrast, the bioanodes formed without acetate addition were fully able to oxidize the organic matter contained in the effluent, giving up to  $4.5$  A/m<sup>2</sup> in continuous mode. Bacterial communities showed less bacterial diversity for the acetate-fed bioanodes compared to those formed in raw effluents. *Deltaproteobacteria* were the most abundant taxonomic group, with a high diversity for bioanodes formed without acetate addition but with almost 100% *Desulfuromonas* for the acetate-fed bioanodes. The addition of acetate to form the microbial anodes induced microbial selection, which was detrimental to the treatment of the raw effluent.

## Keywords:

Microbial anode  
Bioanode  
Microbial fuel cell  
Paper industry effluent  
*Desulfuromonas*

## 1. Introduction

Microbial fuel cells (MFCs) and microbial electrolysis cells are proposing alternative processes for wastewater and effluent

treatment (Logan and Rabaey, 2012). Microbial bioanodes constitute the heart of these systems. A huge amount of research has thus logically been aimed at increasing microbial bioanode performance (Wei et al., 2011). In this objective, most works have been carried out in synthetic media, i.e. in buffered solutions containing nutrients and vitamins and using acetate as the substrate. Even when raw media are used, acetate is often added to increase the

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current or to accelerate the formation of EA biofilms (Parot et al., 2008). Acetate is a highly efficient substrate for bioanodes, in particular because it does not allow fermentation, which is an alternative pathway to electroactivity (Pant et al., 2009). Using acetate in synthetic media (He et al., 2011) or raw media (Pocaznoi et al., 2012) has allowed high current densities to be generated: above 30 A/m<sup>2</sup> has thus been reported with carbon fiber bioanodes, 85 A/m<sup>2</sup> with specific inoculum (Rousseau et al., 2013) and up to 390 A/m<sup>2</sup> with multi-layered carbon electrodes (Chen et al., 2012).

In contrast, in the domain of effluent treatment, in raw conditions without any addition of substrate, nutrients or buffer compounds, microbial bioanodes have produced significantly lower currents (Fornero et al., 2010). A chocolate effluent has allowed 3 A/m<sup>2</sup> to be reached (Patil et al., 2009); it probably represents a maximum value, while most bioanodes implemented in raw effluents have provided drastically lower current densities so far (Velasquez-Orta et al., 2011a). Bioanodes operating in real wastewaters or real effluents remain far from the performance reached in synthetic media with acetate as substrate. Lefebvre et al. (2013) have recently illustrated such a marked difference by comparing bioanodes formed in synthetic wastewater and in real domestic wastewater: they obtained coulombic efficiencies as different as 54% and 1%, respectively. Similarly, the comparison of different substrates (acetate, glucose, starch) has shown that the bioanode performance drastically decreased with the complexity of the substrate (Velasquez-Orta et al., 2011b). The complexity of the substrates contained in raw effluents can consequently be a main cause of the drastic diminution of performance compared to acetate in culture media.

If MFCs and other related technologies have to be developed for the treatment of domestic or industrial effluents, it remains essential to investigate any possible track that could raise the performance of microbial anodes in raw effluents. The purpose of the present work was to advance in this direction by checking whether the addition of acetate might boost the formation of bioanodes able to oxidize raw effluents or not. To the best of our knowledge, this was the first attempt to check the suitability of acetate-oxidizing bioanodes for the treatment of raw effluents.

The choice was made to use effluent from the paper industries. This industrial sector produces worldwide large amounts of highly concentrated effluents, the treatment of which implies high energy costs. MFCs may offer a promising alternative solution as shown by Velasquez-Orta et al. (2011a), who compared the suitability of paper, dairy, brewery and bakery wastewaters to feed MFC. They reported that paper wastewaters provided current density (125 ± 2 mA/m<sup>2</sup>) at least five times higher than the other tested effluents. Huang and Logan (2008) managed to produce 144 mW/m<sup>2</sup> (current density 0.6 A/m<sup>2</sup>) in a batch single-compartment MFC fed with paper effluent supernatant and 210 ± 7 mW/m<sup>2</sup> in continuous flow (Huang et al., 2009). By adding 100 mM phosphate buffer to increase the conductivity of the solution a power density of 672 ± 27 mW/m<sup>2</sup> was reached (current density 2.8 A/m<sup>2</sup>). In these studies, current and power densities were related to the cathode surface area, because it was the rate-limiting step of the system. Recent studies have revealed paper mill effluent to be a promising candidate to form, and be treated by, microbial bioanodes. Current densities up to 6 A/m<sup>2</sup> have been reached with a specific procedure for bioanode formation (Ketep et al., 2013a).

The purpose of the present study was to use paper mill effluents to compare microbial anodes formed with and without addition of acetate and to determine whether the initial addition of acetate could help in the designing of bioanodes appropriate for raw effluents or not. All experiments were conducted in 3-electrode set-ups in order to characterize the bioanodes separately from the numerous other interactions that can occur in whole MFCs or electrolysis cells. Actually, in an MFC the bioanode is polarized at very different

potential values during its formation: from potential values close to the open circuit potential of the cathode, at the beginning, to final values that can be considerably inferior depending on the balance between the anode and cathode kinetics, on the ratio of the anode and cathode surface areas, on the internal resistance of the cell, and on other parameters like biofouling of the cathode surface. The characteristics of a bioanode obtained with an MFC set-up greatly depend on the MFC design. In order to avoid most of these biases, the bioanodes were formed and characterized here in 3-electrode set-ups, as commonly done in electroanalytical domain when the objective is to focus the intrinsic kinetics of an electrode.

## 2. Methods

### 2.1. Effluent

At the paper mill from which effluent was collected, the effluent undergoes a first fermentation, the outlet is then decanted and partially converted to biogas in a fluidized bed digester. The effluent was collected at the outlet of the digester, with pH of 7.2 ± 0.1 and conductivity of 3.3 mS/cm and COD ranging from 1090 to 1500 mg/L. Until used, it was stored at 4 °C under nitrogen. It was generally used without any supplementation but, when indicated, nutrients were added in the proportions COD/N/P of 200/5/1, which corresponds to the supplementation in nitrogen (urea) and phosphorus (phosphoric acid) commonly applied in the treatment of paper mill effluents. In this case, highly concentrated solutions of urea (10 g/L) and pure phosphoric acid (85% diluted with deionized water at the ratio 1:100) were used.

### 2.2. Bioanode design

The electrochemical reactors contained 500 mL of effluent and were equipped with a 3-electrode set-up. Working electrodes were flat graphite plates 2 × 5 × 0.5 cm (Goodfellow) electrically connected via a 2-mm-diameter screwed titanium wire (Alfa Aesar). Before use, the graphite electrodes were cleaned by immersion in 1 M HCl for 1 h, rinsing with distilled water for 20 min and immersion in 1 M NaOH for 1 h. Auxiliary electrodes were 90% platinum–10% iridium grids (Heraeus) around 10 cm<sup>2</sup> surface area cleaned by heating in a blue flame. Potential was controlled and expressed versus a saturated calomel electrode (SCE; potential +0.24 V/SHE; Radiometer, Copenhagen) using a multi-channel potentiostat (Bio-Logic SA).

Bioanodes were formed under constant polarization at –0.30 V/SCE. The reactors were kept in batch mode for a week and then switched to continuous mode by turning on the peristaltic pump, which ensured a volumetric flow of 20.83 ml/h to get a hydraulic retention time of 24 h. The solution in the reactors was continuously sparged with nitrogen to maintain anaerobic conditions. The continuous gas flow also removed dihydrogen that was formed on the auxiliary electrode by water reduction. Temperature was maintained at 25 °C by air conditioning of the room and the storage tanks that contained the inlet and outlet solutions were stored in a refrigerator at 4 °C. Control experiments were carried out with the same set-up but without polarization of the graphite plates. Batch experiments were carried in the same set-up but no solution flow.

Samples were collected daily from the reactor inlet and outlet and the chemical oxygen demand (COD) was measured according to the ISO 15705 standard micro-methods using Merck reagents and a WTW Photolab® 6000 spectrophotometer. Volatile fatty acids (VFA) were analyzed by liquid chromatography (DIONEX) with ion exchange resin (Transgenomic IC Sep ICE-COREGEL

87H3) as the stationary phase, sulfuric acid 0.01 N as the eluent (0.6 mL/min) and UV-detection performed at 205 nm.

### 2.3. SSU rRNA gene pyrosequencing and community composition analysis

Biofilms were scraped from the electrodes and bacterial DNA was extracted and stored at  $-80^{\circ}\text{C}$  before analysis as previously described (Erable et al., 2009). SSU rRNA gene amplification was performed with barcoded primers for the V1–V3 regions. The 16S universal Eubacterial primers 27Fmod (5'-AGRGTTTGATCMTGGCT-CAG) and 519R (5'-GTNTTACNGCGGCKGCTG) were used for the amplification of the 500 bp region of the 16S rRNA genes. The 454 Titanium sequencing run was performed on a 70675 GS Pico-TiterPlate by using a Genome Sequencer FLX System (Roche, Nutley, NJ). Each individual sequence was trimmed to a Q25 average and data derived from the sequencing process was processed using a proprietary analysis pipeline ([www.mrdnalab.com](http://www.mrdnalab.com)). Sequences were depleted of barcodes and primers, then sequences that were less than 250 bp long after quality trimming were not considered, and those with ambiguous base calls or homopolymer runs exceeding 6 bp were removed. Sequences were then denoised and chimeras were removed. The resulting sequences were evaluated using the classify.seqs algorithm (Bayesian method) in MOTHUR against a database derived from the Greengenes set using a bootstrap cut-off of 65%. On the basis of sequence identity, each bacterium was identified to its closest relative and taxonomic level. Sequences were clustered into different operational taxonomic units (OTUs) and the resulting clusters were assessed at 3% and 5% dissimilarity to provide the data needed for diversity analysis.

The resulting sequences were processed using the quantitative insights into microbial ecology (QIIME) software package, version 1.5.0. Briefly, QIIME clusters 16S rRNA gene sequences into OTUs using UCLUST at 97% sequence similarity, assigns taxonomic classification using RDP (Wang et al., 2007), generates data summaries and processes diversity between groups using unweighted and weighted Unifrac distances (Lozupone et al., 2006).

### 2.4. Calculations

In a reactor operating in continuous mode, the COD removal ( $\Delta\text{COD}$ , g/L) was the difference between input and output COD:

$$\Delta\text{COD} = \text{COD}_{\text{input}} - \text{COD}_{\text{output}} \quad (1)$$

Generally expressed as the percentage with respect to the inlet COD:

$$\%\Delta\text{COD} = \frac{\Delta\text{COD}}{\text{COD}_{\text{input}}} \cdot 100 \quad (2)$$

The values were averaged over one week from daily COD measurements. Standard deviations were of the order of 6%.

Coulombic efficiency (CE) was the ratio of the charge passed through the circuit ( $Q$ , coulomb), calculated by integrating the current over a period of time  $\Delta t$  (hour), to the charge that would be produced if COD removal was totally due to electrochemical oxidation ( $Q_{\text{max}}$ ):

$$\text{CE} = \frac{Q}{Q_{\text{max}}} \quad (3)$$

with:

$$Q_{\text{max}} = nF 0.0208 \Delta t \Delta\text{COD} / M_{\text{O}_2} \quad (4)$$

where  $n=4$  is the number of electrons exchanged per mole of oxygen,  $F=96485$  C/mol is the Faraday constant,  $M_{\text{O}_2}=32$  g/mol

is the molar mass of oxygen, and 0.0208 is the flow rate (L/h) for a hydraulic retention time of 24 h and a reactor volume of 0.5 L.

## 3. Results and discussion

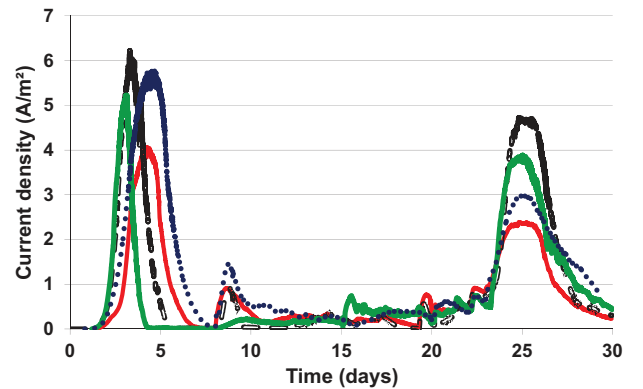
### 3.1. Microbial bioanodes designed with and without acetate addition

Four reactors were run in parallel, each containing 500 mL of effluent and a graphite plate electrode polarized at  $-0.3$  V/SCE. The reactors were initially kept in batch mode for one week, with 5 mM of added acetate (equivalent to 300 mg/L COD). Current densities increased up to 4–6 A/m<sup>2</sup> and then returned to zero because of substrate depletion (Fig. 1). After the initial week, the reactors were switched to continuous mode by feeding them with the raw effluent without acetate addition at a hydraulic retention time (HRT) of 24 h. After a maximum around 1 A/m<sup>2</sup> current densities remained stable below 0.5 A/m<sup>2</sup>, during the 15 days of continuous feeding. From day 23 on, 5 mM acetate was added into the effluent continuously supplied to the reactors, leading to immediate increase of the current densities to around 2.4–4.7 A/m<sup>2</sup>.

The COD of the inlet effluent was monitored throughout the experiment. It varied slightly, between 1700 and 1500 mg/L, and the COD outlet ranged from 1400 to 1000 mg/L. The amount of oxidizable organic matter provided to the reactors was consequently not limiting, but coulombic efficiencies remained very low, around 0.7%. A significant part of this COD was related to the presence of volatile fatty acids (VFAs), mainly composed of acetic acid (200 mg/L, i.e. 3.2 mM), propionic acid (98 mg/L) and butyric acid (36 mg/L). Also found in trace amounts (<10 mg/L) were lactic, formic, isobutyric, valeric and isovaleric acids. The very low current densities and coulombic efficiencies observed with the raw effluent showed that the organic matter it contained was hardly oxidized by the bioanodes formed with acetate addition, while the bioanodes recovered almost full performance when fed again with acetate. It must be concluded that the microbial bioanodes designed in the presence of added acetate were poorly suited to the treatment of real effluents.

### 3.2. Microbial bioanodes designed directly in raw effluent without acetate addition

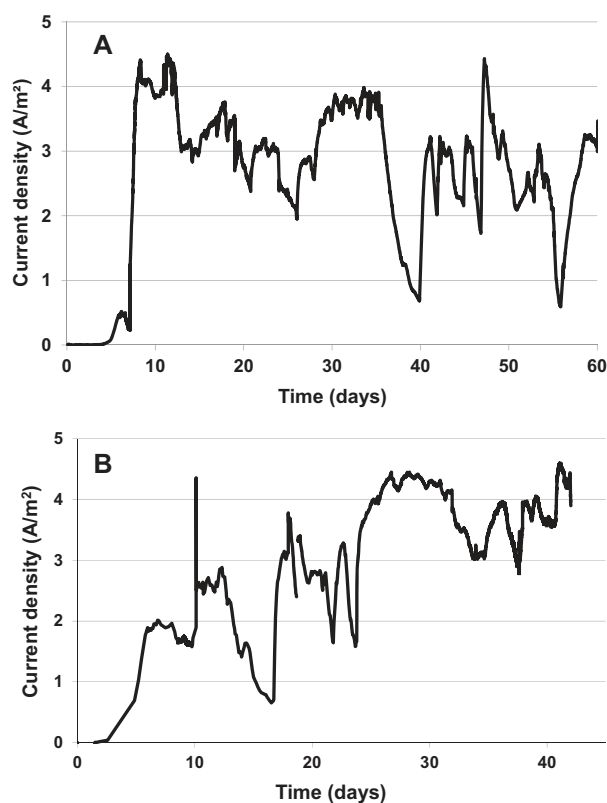
Bioanodes were formed in identical conditions but without any addition of acetate. Reactors were filled with 500 mL of raw effluent, left in batch mode for one week, and then continuously fed



**Fig. 1.** Current density versus time for four bioanodes run in parallel. The first 7 days were run in batch mode with 5 mM acetate, then the reactors were fed in continuous mode (HRT 24 h) with raw effluent. From day 23 onwards, 5 mM acetate was introduced in the inlet flow. Working electrodes were 10 cm<sup>2</sup> graphite plates, polarized at  $-0.3$  V/SCE. Anaerobic conditions were maintained with continuous N<sub>2</sub> sparging.

with the raw effluent. Despite some experimental contingencies due to pipe clogging, which sometimes brought the current down, the current density was mainly between 3 and 4 A/m<sup>2</sup> for around 4 weeks and then between 2 and 3 A/m<sup>2</sup> for additional 3 weeks (Fig. 2A).

These current densities were pretty high compared to values commonly reported in the literature for microbial bioanodes operated in continuous mode with real effluents without any supplementation of substrate, nutrients or buffer solution. To our knowledge, the highest current densities obtained in continuous mode were 525 mA/m<sup>2</sup> with a landfill leachate effluent (Tugtas et al., 2013) and 600 mA/m<sup>2</sup> with paper mill effluent (Huang et al., 2009). Moreover, currents were not enhanced here by a particular morphology of the electrode surface (high roughness, woven fibers, etc.) or use of a 3-dimensional structure (fiber brush, felt, multilayered architecture, etc.), but only flat graphite plates were used. Actually, most previously reported studies were carried out in whole MFCs. Several processes not related to the anode kinetics (low solution ionic conductivity, slow cathode kinetics, cathode fouling, etc.) may interact in MFCs and limit the current generation. The 3-electrode set-ups used here implemented the bioanode in the most favorable electrochemical conditions. The current generated by the bioanodes was controlled by its own kinetics, with no detrimental interactions from the other parts of the system. The high current densities recorded here in electro-analytical conditions confirmed the values of 4 A/m<sup>2</sup> reported in a previous study that treated the same raw effluent in identical continuous 3-electrode set-ups (Ketep et al., 2013b). It suggests that the intrinsic performance of bioanodes may be underestimated when extracted from MFC experiments and that bioanodes formed in raw effluents should be more efficient than suspected so far.



**Fig. 2.** Current density versus time of a bioanode formed in raw paper mill effluent. The first 7 days were run in batch mode, then the reactor was fed continuously with raw effluent (HRT 24 h). The 10 cm<sup>2</sup> graphite plate electrode was polarized at  $-0.3$  V/SCE. Anaerobic conditions were maintained with continuous N<sub>2</sub> sparging. (A) first experiment; (B) duplicate.

A 42-day replicate performed in the same conditions confirmed the same behavior, with weekly average current densities increasing from 1.5 to 3.5 A/m<sup>2</sup> from week 2 to 6 (Fig. 2B, Table 1). In this case COD values were measured in the inlet and outlet fluxes and the total COD removals were calculated (Table 1). For the last two weeks, the reactors were fed with freshly collected effluent, which explained the increase of total COD removals and the mathematically induced decrease of coulombic efficiencies (Eq. (3)). The part of COD removal that was due to the electrochemical process, calculated as:

$$\% \Delta \text{COD}_{\text{electro}} = \% \Delta \text{COD} \cdot \text{CE} \quad (5)$$

never exceeded 7% (Table 1). The part of organic matter consumed by the electrochemical process remained low and was not affected by the freshness of the effluent. In parallel, a control reactor was run in identical conditions, fed with exactly the same effluents but with a non-polarized electrode. The total COD removals were of the same order of magnitude than with the polarized anode, just slightly lower (Table 1), which confirmed that most COD removal was not related to the electrochemical process. It is widely agreed that a substantial fraction of the organic matter contained in complex media is oxidized by alternative aerobic or anaerobic pathways due to the presence of dissolved electron acceptors (oxygen, nitrate, sulfate, etc.) (Min et al., 2005; Lu et al., 2009). Fermentation processes could not be ruled out and biomass growth has also been suggested to account for a part of COD removal (Liu et al., 2004). Most MFC studies implementing real wastewaters have commonly obtained very low CE, between 1% and 5% (Lefebvre et al., 2013; Liu et al., 2004). The Coulombic efficiencies obtained here, ranging from 12% to 30%, are among the highest obtained to date for MFCs using real raw effluents. For example, under continuous flow in an air cathode MFC, Liu et al. (2004), with the effluent from a treatment plant, obtained CE of 12% at HRT of 33 h; Cheng et al. (2006) with domestic wastewater, found maximal CE of 27% at the lower HRT of 3.4 h. The bioanodes formed directly in raw effluents consequently led to very good performance, according to the current state of the art. Nevertheless, it should be recalled that the total COD removals remained low (19–47%, Table 1) and the impact of the electrochemical process less than 7%. These data showed that Coulombic efficiencies are not sufficient to assess the suitability of a microbial anode for the treatment of effluents because the part of COD removal really due to the electrochemical process ( $\% \Delta \text{COD}_{\text{electro}}$ ) should also be systematically calculated.

It should be noted that the 3-electrode set-up used here was designed to characterize the bioanode kinetics but not to improve coulombic efficiencies. These are two opposite objectives in terms of reactor design (Ketep et al., 2013b). Despite this experimental choice detrimental to Coulombic efficiencies, fair values were recorded, which emphasizes the promising capability of bioanodes formed directly in raw effluents.

### 3.3. Analysis of the bacterial communities with and without acetate addition

Six bioanodes were formed in parallel in raw effluent under identical conditions but with different supplementations. Two reactors were run in batch mode with an initial load of 5 mM acetate and 4 successive additions of acetate at the same concentration. Operating in batch mode ensured that the bioanodes were actually exposed to 5 mM acetate, while in continuous mode a significant part of the acetate added into the storage tank or into the inlet flow might be consumed before reaching the reactor. These two reactors gave similar current densities, which increased with the successive additions of 5 mM acetate ( $1.0 \pm 0.5$ ,  $5.0 \pm 0.2$ ,  $7.2 \pm 0.2$ ,  $9.0 \pm 0.1$ ,  $10.3 \pm 0.05$  A/m<sup>2</sup>). Two reactors were continuously fed with raw effluent without any addition of acetate and

**Table 1**

Current densities, COD removals and Coulombic efficiencies obtained in continuous mode. Bioanodes were polarized at  $-0.30$  V/SCE with continuous supply (HRT 24 h) of raw paper mill effluent without any addition of acetate. The control experiment was performed in parallel in identical conditions but without polarization. Experimental data were averaged over every week from daily measurements. The first week (not reported) corresponded to the initial bioanode formation in batch mode in raw effluent.

	Polarization $-0.30$ V/SCE				Control at open circuit			
Week number	2	3	4	5	2	3	4	5
COD input (mg/L)	1090	1200	1505	1275	1090	1200	1505	1275
Current density ( $A/m^2$ )	2.0	2.8	3.0	3.5	0	0	0	0
% $\Delta$ COD	19	21	47	35	17	18	34	29
CE (%)	22	30	15	12	0	0	0	0
% $\Delta$ COD <sub>electro</sub>	4	6	7	4	0	0	0	0

% $\Delta$ COD is the percentage of total COD removal and CE is the Coulombic efficiency, calculated according to Eqs. (2) and (3), respectively; % $\Delta$ COD<sub>electro</sub> is the part of COD removal due to the electrochemical process defined by Eq. (5).

two others were continuously fed with effluent supplemented with the nutrients urea and phosphoric acid. The four continuous reactors generated current densities ranging from 2 to 5  $A/m^2$  for more than 20 days. Chaotic variations of the current were observed as previously (Fig. 2) related to the difficulty to implement raw effluents in pretty small-scale reactors for long periods, such as partial pipe clogging, irregularities of the inlet flow provoked by biofouling, small changes in effluent composition. As illustrated in Fig. 3, the reactors supplied with urea and phosphoric acid and the reactors without any addition gave similar current densities. At day 23, the nutrient addition was stopped for a few days in the reactor that had been supplied with up to this time and the nutrient feed was switched to the inlet of the other reactor. The current decreased when nutrient feeding was stopped, while the current increased immediately in the other reactor when it was supplied with nutrient. Each bioanode was consequently well adapted to the composition of the effluent, in which it was formed. Despite experimental deviations, the four electrodes with and without nutrient addition gave similar average current densities of the order of 3  $A/m^2$ . Each electrode was so adapted to its effluent that it was immediately affected in positive (sudden nutrient supply) or negative (stop of nutrient supply) way when the composition changed.

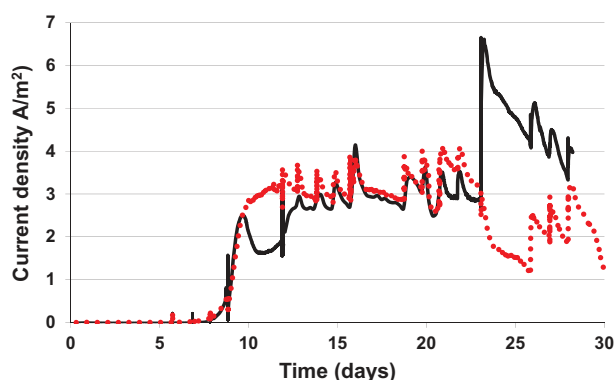
After 1 month of polarization, the microbial communities of the six bioanodes were characterized. On the basis of pyrosequencing data, the bacterial composition of duplicates from the same feeding system was similar. *Proteobacteria* were found to predominate (40–50%) in all bioanodes and, among them, *Deltaproteobacteria* was the most abundant taxonomic group, especially for acetate-fed

bioanodes (Fig. 4). These taxonomic groups have also been found as dominant members in bioanodes formed from complex inocula, for example from soil organic matter (Ringelberg et al., 2011; Dunaj et al., 2012) or wastewater diluted in synthetic medium with acetate as substrate (Yates et al., 2012).

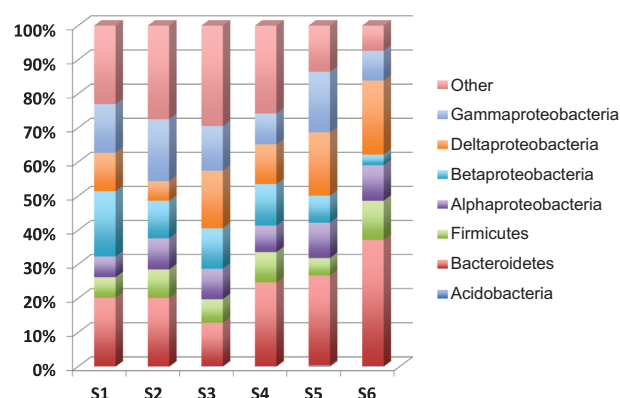
Considering the whole bacterial composition, *Bacteroidetes* was nearly the most abundant taxonomic group. *Bacteroidetes* have already been found in abundance in microbial bioanodes formed from soil (Dunaj et al., 2012), wastewater with acetate (Miceli et al., 2012) or raw wastewaters (Bond et al., 2002; Kiely et al., 2011; Katuri et al., 2012). Nevertheless, the role of this bacterial group in terms of extracellular electron transfer or contribution to biofilm formation has not been elucidated yet.

Considering *Deltaproteobacteria*, a high diversity was observed within this group for the bioanodes that were not supplemented with acetate, with a predominance of *Desulfuromonadaceae*. In contrast, almost 100% of bacteria from this group were closely related to *Desulfuromonas* in the acetate-fed bioanodes (Fig. 5). *Desulfuromonas* sp. have already been identified among dominant members of electroactive biofilms (Bond et al., 2002; Ketep et al., 2013a), which is consistent with the high current observed here. Surprisingly, despite the relatively high diversity of *Desulfuromonadaceae*, bacteria closely related to *Geobacter* were rare or absent. These data suggest that the *Deltaproteobacteria* group may contain efficient electroactive bacteria and that their diversity is greater than generally believed.

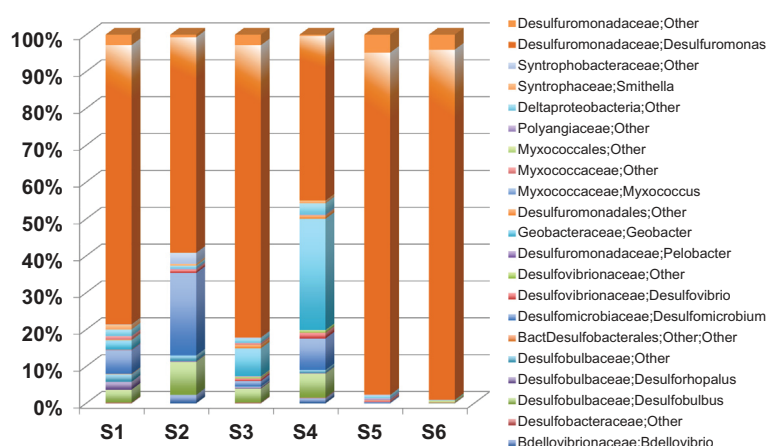
The Shannon and Chao diversity indexes confirmed the lower bacterial diversity in the acetate-fed bioanodes than in the bioanodes formed only with raw effluents. Rarefaction curves using both Mothur and QIIME corroborated these findings (Fig. 6). Supplementing with urea and phosphorous also tended



**Fig. 3.** Current density versus time of bioanodes formed in raw paper mill effluent with (dotted red line) or without (continuous black line) addition of urea and phosphoric acid. The first 7 days were run in batch mode, then the reactor was fed continuously with raw effluent (HRT 24 h). The 10  $cm^2$  graphite plate electrode was polarized at  $-0.3$  V/SCE. Anaerobic conditions were maintained with continuous  $N_2$  sparging. At day 23 urea and phosphoric acid supply was stopped in one reactor and switched to the other. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Relative abundance of major taxonomic groups of bioanodes formed under polarization at  $-0.3$  V/SCE in S1–S2: raw effluent, S3–S4: raw effluent supplemented with urea and phosphoric acid, S5–S6: raw effluent with addition of 5 mM acetate.



**Fig. 5.** Comparison of microbial composition of genus-level OTUs among *Deltaproteobacteria*. S1–S2: raw effluent, S3–S4: raw effluent supplemented with urea and phosphoric acid, S5–S6 raw effluent with addition of 5 mM acetate. S1–S2: raw effluent, S3–S4: raw effluent supplemented with urea and phosphoric acid, S5–S6 raw effluent with addition of 5 mM acetate.

to restrict the biodiversity but to a significantly smaller extent than the addition of acetate.

The addition of the nutrients urea and phosphoric acid, which are often required to boost the conventional paper effluent treatments, induced a slight reduction of the microbial diversity of the bioanodes, which did not affect their capacity to oxidize the raw effluent, but did not increase the current density. This is an interesting result showing that the addition of nutrients was not necessary to form the bioanodes. MFC technology would consequently have the great advantage of avoiding the necessity for nutrient addition.

The effect of medium on the composition of the microbial communities of bioanodes has already been evidenced. For instance, [Yu et al. \(2012\)](#) have shown different microbial compositions for bioanodes obtained in synthetic medium vs. real domestic wastewater. [Chung and Okabe \(2009\)](#) have presented bioanodes dominated by *Gammaproteobacteria* in a glucose-fed MFC, while *Firmicutes* dominated in acetate-fed MFCs. Nevertheless, to our knowledge, the

present work provides the first demonstration that the simple addition of acetate in a raw effluent significantly reduced the diversity of bacteria making up the bioanodes. The presence of acetate induced the selection of species closely related to *Desulfuromonas* among the *Deltaproteobacteria*.

#### 4. Conclusions

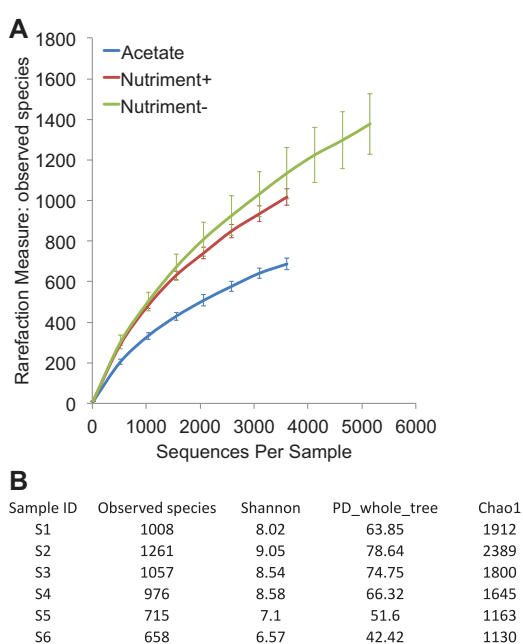
The demonstration was provided here that addition of acetate into a raw effluent drastically decreases the ability of the formed bioanodes to treat the effluent. This loss of capacity was correlated to a significant reduction in the diversity of the bioanode bacterial communities, which gave the bioanode a strong ability to oxidize acetate. In contrast, the bioanodes formed directly in raw effluents gave current density up to 4 A/m<sup>2</sup> in continuous mode. If similar results were enlarged to other effluent types, laboratory and field conditions would have to be considered as different worlds in the future research works.

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**Fig. 6.** Diversity estimates. Rarefaction curves (A) and richness/diversity estimators (B) as calculated by MOTHUR at a level of 3% dissimilarity.

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