Experimental Assessment of a Process Including Microbial Fuel Cell for Nitrogen Removal from Digestate of Anaerobic Treatment of Livestock Manure and Agricultural Wastes

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The main objective of the research was to verify the effectiveness of a MFC (Microbial Fuel Cell) based reactor for the removal of nitrogen from digested sludge (from anaerobic treatment of livestock manure and agricultural wastes).
Preliminary tests were performed to study the development of biofilm at the electrodes, by running small H-type MFC under different conditions. Results of the preliminary lab scale tests showed an increase of more than 60 % of the potential (of both, E_{OCV} and E_{CCV}), in the case of biofilm enrichment.
Moreover, scale-up tests were run in continuous, using a 17 L reactor designed and assembled to the scope, constituted by an anaerobic anodic chamber, followed by an aerobic cathodic stage and an anoxic stage.
Results of five months operation showed that anodic potential quickly achieved a stable negative potential, in the typical range of the MFC, also leading to a volatile solids removal of about 60 %, thus confirming the good efficiency of the double anaerobic/aerobic system in carbon degradation. However, current generation and power produced was rather low, in comparison to previous results obtained in small batches. In the aerobic chamber, a good nitrogen removal was observed (up to 60 %), as a consequence of both, carbon degrading biomass uptake and nitrification. Mass balances performed, considering the nitrogen content in the waste sludge (about 3 % with respect to TS content), allowed to exclude a significant ammonia nitrogen volatilization.

1. Introduction

The most common technology for the treatment of agricultural waste and food industry wastes is anaerobic digestion for biogas production. Due to the high organic content of those residues, high biogas production rate is expected and, correspondingly, high removal effectiveness towards organics is achieved.
The final product (the so-called digestate) from biogas power plant is a nutrient-rich sludge commonly used as a fertiliser. However, according to the EU Directive 91/676/CE, treatment of digestate may be mandatory, prior its sparging over soil, in order to reduce the impact of eutrophication.
A growing interest is therefore devoted to finding alternative technology pathways, aiming at reducing the nitrogen content in digestates (Ahn, 2006). The conventional biological nitrogen removal (i.e. nitrification and denitrification) is typically performed on wastewater containing low nitrogen concentration, such as municipal wastewater (De Filippis et al., 2013). In any case, several novel and cost-effective biological nitrogen removal

processes have been developed in the last few years, including partial nitritation, denitrification (Aytimur et al., 2008), anaerobic ammonium oxidation, and its combined system (Jetten et al., 2002).

Among the biotechnologies available to treat organic and nitrogen rich wastewaters, microbial fuel cells (MFCs) appear to be a sustainable promising technology (Virdis et al., 2008).

Microbial fuel cells are electrochemical devices that generate electric current by direct or mediated electron transfer to electrodes, through the metabolic activity of microorganisms (Schröder, 2007). Organic matter is oxidized by microbial metabolism in the anodic cell, where electrons are transferred to the anode. In the cathodic chamber, oxygen or oxidized compounds are reduced either via an abiotic process or by microbially mediated reduction (He and Angenent, 2006). To date, the mechanisms of nitrogen removal in a microbial fuel cell remain unsolved and are still under investigation, representing a challenge for the future, since treating nitrogen rich wastewater coupled to electrical energy direct production could enhance MFC implementation in municipal wastewaters treatment plants, or organic-based industrial effluents and wastes.

The aim of this study was to develop and optimize a process for nitrogen and organic matter removal from digestate, through bioelectrochemical processes. Particular attention was dedicated to the selection of a microbial community (biofilm) with enhanced bioelectrochemical ability.

Preliminary MFC batch experiments were performed to evaluate electroactive biofilm formation from digestate. Subsequently, a lab scale continuous membrane-less reactor was operated over 4 months: carbon and nitrogen conversion was investigated, and mass balances were performed to assess the effectiveness of the combined process.

2. Materials and methods

2.1 H-type experiments

H-type MFC experiments were run mainly to evaluate the effect of the development of a suitable biofilm on the bioelectrochemical system. A first set of experiments was performed, in order to better understand the biological contribution to nitrogen removal and organic matter degradation, leaving the circuit of the cell open (thus excluding the electrochemical part), and using digestate as substrate and inoculum (without the enrichment with activated sludge). This was considered as our blank. The digestate was diluted 1:1 (v/v) with synthetic growth medium, as reported by Zhan et al. (2012) and inoculated in both chambers. In order to facilitate start up and initial biomass growth, 10 g/L glucose were also added. Anodic chamber was flushed for 5 minutes with argon in order to favour the development of an anaerobic community, while the cathode chamber was constantly flushed with O_2. A typical H-type MFC, consisting of two bottles (separated by a Nafion membrane) with 250 mL working volume each, was used. The electrodes were made of carbon paper (2x3 cm^2) and the electrochemical performance of the cells was tested by a AUTOLAB potentiostat, by means of current potential curves (IV curves).

2.2 Continuous experiments

On the basis of the above-mentioned tests, a process was proposed to reduce nitrogen and carbon content of digestates, involving an MFC section and a post denitrification treatment.

The first two stages of the reactor, one anaerobic (A) and the other aerobic (C1), were equipped with graphite electrodes connected through an external resistance thus realizing a membrane-less microbial fuel cell. The following anoxic stage (C2) ensured the denitrification of the residual nitrates or formed during nitrification. A final settling tank (C3) was added to separate the liquid from the solid phase. The total volume of the reactor was 17 L. The sludge mean retention time in the system was 10 days.

The digestate was periodically collected at an anaerobic digestion plant where agricultural wastes and cow manure are treated (Azienda Agrozootecnica Bruni Sutri - VT). At the beginning of the study, the collected digestate was characterized by specific analyses as described in the following section. The digestate was first subjected to homogeneization for 5 minutes in a mixer at 200 rpm. The obtained suspension was then stored in plastic container at -20°C and then used to feed the reactor after the desired dilution. After a 10 days acclimation in batch conditions, the reactor was operated for about 150 days at a digestate/water equal to 1.

The voltage (V) across an external resistor (Rext, equal to 180 Ω except in selected electrochemical tests as described below) in the MFC section of the reactor was monitored at 30 min intervals using a multichannel potentiostat/galvanostat VSP (Biologic Sas). Current (I) was measured using the same instrument in a chronoamperometric mode. Power was calculated as $P=VI=RI^2$, the power density was normalized by the projected surface area of one side of the cathode, and the volumetric power density was normalized by the volume of the liquid media. Cyclic voltammetry was carried out in order to characterize the substrate oxidation reaction at the biofilm anode surface: scan rate of 1 mV/s was used, ranging from -1 V to 1 V (Di Domenico et al., 2015).

A conventional three-electrode system was employed (Logan, 2008), with the anode as the working electrode, an Ag/AgCl reference electrode, and a platinum wire as the counter electrode (Marsili et al., 2008).

Polarization curves were obtained by measuring the stable voltage generated at various external resistances and then used to evaluate the maximum power density (Venkata Mohan et al., 2010).

The chemical and physical parameters controlling process efficiency were measured daily in each stage of the reactor. pH was measured using a GPL 42 instrument (Crison); the dissolved oxygen was measured with a 913 OXY oxymeter (Mettler-Toledo). Total and volatile suspended solids by gravimetric procedure, and Chemical Oxygen Demand (COD) by acid digestion and dichromate titration, were determined according to standard methods (APHA, AWWA, WEF, 2005). Ammonia nitrogen and Total Kjeldahl Nitrogen (TKN) were determined by spectrophotometry after distillation of liquid samples and Nessler reagent addition, using a T80+ UV Vis spectrometer (PG Instruments, Ltd). Nitrites and nitrates were determined by ionic chromatography, using a DX 120 instrument (Dionex).

3. Results and discussion

The main characteristics of the collected digestate are reported in Table 1. The digestate main parameters were in the typical range of digestate obtained in the co-digestion of manure wastes and agricultural residues.

Table 1: Selected characteristics of the raw digestate

Parameter	Value
pH	7.98
VS (g/kg)	31.7
TKN (g NH_4^+/kg)	2.66
NH_4^+ (g/kg)	1.85
NO_3^- (g/kg)	n.d.

3.1 H-type experiments

A first set of experiments (MFC1) was performed, in order to evaluate the biological capacity of the system.

The results obtained with the open circuit suggested that there was an important biological contribution in the organic matter degradation and ammonium conversion into nitrate. Around 47% of ammonium (corresponding to 420 mg/L NH_4^+-N) was converted in the cathodic chamber, with a concomitant increase of nitrate (from 57 to 550 NO_3^--N), while no nitrite was observed. This was in good agreement with Virdis and colleagues (2010), under open circuit conditions. Maximum voltage obtained during a period of 2 weeks experimentation reached a value of 0.391 V. Seemingly, nitrifying bacteria (aerobic bacteria belonging to the family Nitrobacteraceae) caused the ammonium conversion in the oxygenated cathode chamber. These microorganisms are important in the nitrogen cycle, converting ammonia to nitrates (Koops and Pommerening-Roeser, 2009).

It is worth noting that, even though no appreciable ammonium oxidation was observed in the anodic chamber (no increase in nitrite or nitrate), however there was a decrease of NH_4^+-N. Similar results were also reported by Virdis and colleagues (2010). Several authors reported ammonium diffusion through the cation exchange membrane, but this was probably not the case in our study, which used a proton exchange membrane. It might be possible that nitrogen removal process might have partially proceeded through anaerobic ammonium oxidation (anammox) by utilizing NH_4^+-N as electron donors and nitrite as acceptors (Van Hulle et al, 2010). Recently, new technologies have been under investigation, such as simultaneous partial nitrification, anammox and denitrification (SNAD) (Kumar and Lin, 2010). Even though the presence of organic carbon is commonly not suitable for anammox processes, the presence of mixed cultures from the digestate (adding numerous fermentation pathways) can easily cause significant decrease of organic matter and, thus, C/N ratio. Furthermore, it has been found that anammox bacteria can be competitive with heterotrophic denitrifiers for the utilization of organic matter fermentation products, such as propionate or formate (Kumar and Lin, 2010) and in the presence of glucose. The authors also reported that excess dissolved oxygen in the system could be consumed by ammonia oxidizers in the outer layers of the biofilm, and simultaneously, the anammox bacteria could develop in the anoxic layers. A similar mechanism, depending on the three-dimensional structure and mutualistic interaction of the biofilm, might also be active to decrease the C/N ratio.

Thus, the mechanism occurring in the anodic chamber still needs to be understood and will have to be further investigated. Organic matter was degraded in both chambers (due to fermentation in the anodic chamber and to aerobic degradation in the cathodic one), as observed in other studies (i.e. Virdis et al., 2010). A second set of experiment (MFC2) was performed in the same conditions of MFC1, but with a closed circuit, for the evaluation of the bio-electrochemical contribution to the cell. In order to obtain an enhanced development of the biofilm, 10 g/L glucose were also added (as in MFC1). As shown in Figure 1a, the voltage measured with

open circuit (E$_{OCV}$) increased from initial values close to zero, up to 0.47 V, while the potential measured with the closed circuit (E$_{CCV}$) showed an increase from initial 0.05 to 0.23 V, within approximately 2 weeks of experimentation. Moreover, the performance of the MFC2 showed a clear enhancement over time, as can be seen from the comparison of Table 2 (measurements after 10 days operation) and Figure 1 (measured after 15 days).
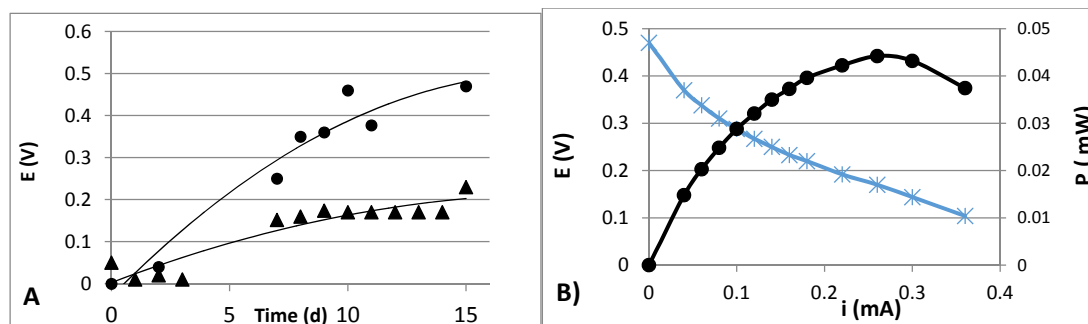

Figure 1: Voltage generation in MFC2: trend of the open circuit voltage, OCV (circles) and closed circuit voltage CCV (triangles) (A); Galvanostatic curve. Trend of the potential is shown by the black circles (referring to right axis P), while the IV curve (current potential) is represented by the stars (B)

Table 2: MFC2 potentiometric measurements obtained after 10 days operation

i (mA)	E (V)	P (mW)
0.000	0.480	0.000
0.020	0.406	0.008
0.040	0.338	0.013
0.060	0.294	0.018
0.080	0.247	0.020
0.100	0.210	0.021
0.120	0.186	0.022
0.140	0.154	0.021

This was in good agreement also with the comparison between the IV curves, obtained after 10 and 15 days operation, clearly showing the enhanced performance of the MFC2 over time (Figure 2), which was also confirmed by the faster recovery of the cell, observed between one cycle (anodic cyclic voltammetry, ranging from -0.5 to 0.5 V, with rate 50mV/sec) and the other (at the same applied current). Around 82% of ammonium was converted in the cathodic chamber, with nitrate increasing from 57 – 1645 mg/L NO$_3$$^-$N. The chemical oxygen demand (COD) measured after 2 weeks experimentation showed a degradation of about 47% (from the centrifuged fraction) in the anodic chamber, while only half was obtained in the cathode chamber. The higher organic matter degradation of the anodic chamber might depend on the possibility to discharge electrons through the anode to the external circuit, during the oxidation of organic matter.

A third set of experiments (MFC3) was performed with the aim to evaluate the effect of biofilm enrichment on the bioelectrochemical system, by means of activated sludge, in order to select a higher number of bioelectrogenic bacteria. The underlying hypothesis was that the activated sludge would represent a better inoculum compared to the autochtonous one contained in the digestate alone. In this case, we decided not to use any additional carbon source, besides digestate, in order to evaluate the efficiency of the enriched inoculum on the digestate alone (thus without the contribution of any glucose). The results showed a better performance of the MFC3, already from the first days of operation.

The potential at the anode (E$_{anode}$) reached a value of – 0.280 V vs. SHE in only few days, while the potential at the cathode (E$_{cathode}$) was + 0.463 V vs. SHE. As can be observed in Figure 3, the voltage measured with open circuit (E$_{OCV}$) reached a maximum value of 0.75 V in about 16 days, while E$_{CCV}$ increased from initial 0.02 up to 0.35 V within approximately 2 weeks. The E$_{CCV}$ corresponded to almost half the value obtained with the open circuit, thus confirming the development of an effective biofilm. An additional prove of the development of an efficient biofilm come from the increase of both E$_{OCV}$ and E$_{CCV}$ compared to MFC2 experimentation: in fact the voltage increased by more than 60% in both cases, compared to the experiments with non-enriched inoculum (despite the use of additional 10 g/L of glucose in MFC2). This further suggests that the development of a suitable biofilm does not require the use of an additional carbon source.
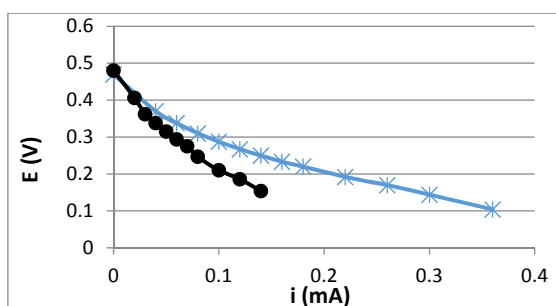
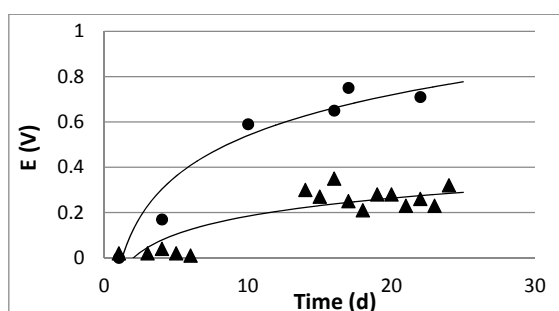Figure 2: Comparison of IV curves obtained after 10 days operation (black circles) and 15 days (stars)

Figure 3: Voltage measurements at open circuit E_{OCV} (circles) and closed circuit E_{CCV} (triangles), obtained during 3 weeks operation with the MFC3

3.2 Continuous experiments

Figure 4 a,b shows the time trends of the main physical and electrical parameter, and carbon cycle along time.
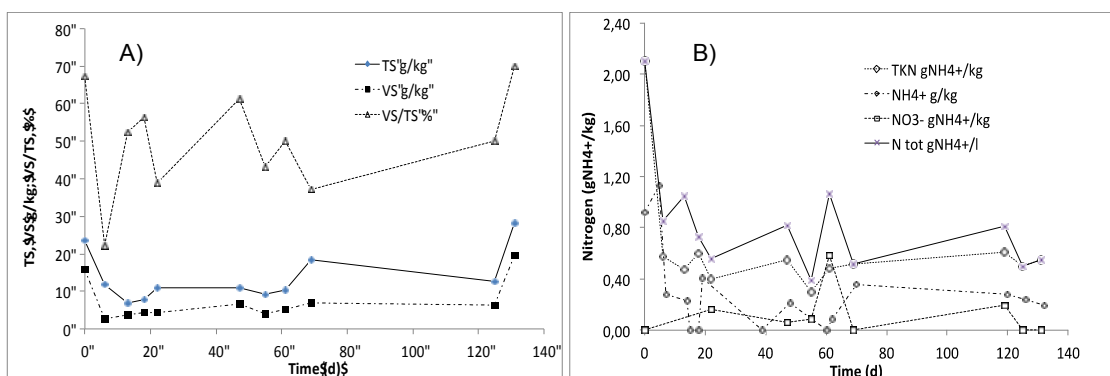

Figure 4: Solids concentration and nitrogen species in the aerobic chamber (C2) along time

Results show that the anodic potential quickly achieved a stable negative potential, in the typical range of the MFC. However fully aerobic conditions were not achieved in the cathodic chamber (C1): due to air diffusers fouling. A cathodic potential oscillating from slight negative to about 50 mV was generally observed. Only few measurements were in the range between 100 and 150 mV. As a consequence, the OCV measured in the MFC section of the reactor was mostly in the range between 100 and 300 mV, far from the optimal conditions for current generation. Volatile solids removal of about 60% was achieved, thus confirming the good efficiency of the double anaerobic/aerobic system in carbon degradation. Despite a periodic sludge withdrawal from the chamber C2, an additional periodical sludge withdrawal from chamber C1 was necessary, due to the partial settling of suspended matter in that stage.

Nitrogen removal in the anaerobic stage was consistent with the carbon degrading biomass uptake; in the aerobic chamber C1, a huge nitrogen removal was observed, as a consequence of both carbon degrading biomass uptake and nitrification. The nitrification process determined the production of nitrates, fully removed

in the following anoxic stages. Mass balances performed also considering the nitrogen content in the waste sludge (about 3% with respect to TS content) allowed to exclude a significant ammonia nitrogen volatilization. Finally, cyclic voltammetries show that only a negligible activity was observed after about 150 days of experiments, thus confirming a low electroactivity in the system (data not shown).

4. Conclusions

A process able to remove nitrogen and carbon content of digestates was proposed. Preliminary batch tests in H-type cells demonstrated that biofilm enrichment by means of activated sludge represented a better inoculum, compared to the digestate alone. An increase of more than 60% in E_{OCV} and E_{CCV} was detected, with respect to the experiments with non-enriched inoculum (despite the addition of 10 g/L glucose in the latter one). Such results suggest that the development of a suitable biofilm does not require the use of additional carbon sources. The process was then tested in a three-stage reactor, involving a MFC section in the first two stages (anaerobic and aerobic reactors) and a post-denitrification treatment in the third stage (anoxic reactor). Several tests have been performed under different operating conditions, both in small batches and up-scaled reactors. During the five-month continuous operation of the upscaled 17 L reactor, a 60% nitrogen removal was observed, due to both, carbon degrading biomass uptake and nitrification in the aerobic reactor. Nitrates produced by the nitrification process were fully removed in the anoxic part of the reactor. In addition, volatile solids removal of about 60% was achieved, showing a good carbon degradation efficiency.

The good results obtained encourage further studies and experimental tests in order to optimize the proposed upscaled process, in view of its implementation in the treatment of digestate.

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