

## Shortcut Biological Nitrogen Removal (SBNR) in Microbial Fuel Cells (MFCs)

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Microbial Fuel Cells (MFCs) represent nowadays a promising technology for the treatment of industrial wastewater. In this work the Shortcut Nitrification/Denitrification process in H-type MFC was investigated. The cell was fed by sodium acetate and fumaric acid, as organic carbon source, and ammonium sulphate, sodium nitrite and sodium nitrate as nitrogen source. Anaerobic digestion supernatant (digestate) was used as bacterial source. Batch tests were performed at a TOC/N ratio of 0.35, and Total Organic Carbon (TOC), pH and Open Circuit Voltage (OCV) were daily monitored. High organic carbon removal (up to 85%) in short time (within 6 days) were achieved. The nitrification proved to be independent of organic carbon amount and composition: an ammonium content reduction of about 45% was observed. Regarding the denitrification step, an almost quantitative removal of nitrite and nitrate was observed when fumaric acid was used as a carbon source.

### 1. Introduction

Nitrogen is a pollutant present in domestic, agricultural and industrial wastewater in form of ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ). The eutrophication is the big consequence due to their releasing in the environment. Generally, nitrogen removal from wastewater is performed using a biological process consisting of two steps: nitrification and denitrification. During nitrification, ammonium content is converted in nitrite by ammonia oxidizing bacteria and then nitrite in nitrate using nitrite oxidizing bacteria. Both reactions are performed in aerobic condition (Ruiz et al., 2003) according to Eq(1) and Eq(2).



Denitrification is characterized by a reductive reaction pathway shown in Eq(3) and Eq(4) (De Filippis et al., 2013), performed in anaerobic condition (Pan et al., 2012).



These processes are commonly performed in two different reactors (Khin and Annachatre, 2004), but a lot of studies proposed the possibility to obtain simultaneous nitrification and denitrification (SND) by heterotrophic bacteria able to convert nitrate into nitrite and then nitrogen in the same reactor used for nitrification step by ammonia oxidizing bacteria (Chiu et al., 2007). The Shortcut Biological Nitrogen Removal (SBNR) is an optimization of biological nitrogen cycle characterized by the accumulation of nitrite in controlled dissolved oxygen condition (Peng et al., 2006). The shortcut pathway, displayed in Eq(5), consists of a partial

ammonium oxidation to nitrite (nitritation) directly converted into nitrogen (denitritation) thus overcoming nitrate formation (Ciudad et al., 2005; Ruiz et al., 2006).



Wastewaters can be identified by Total Organic Carbon (TOC) and Nitrogen (N) concentration ratio (TOC/N). Lower value of this ratio can compromise biological treatment such as anaerobic digestion (Yenigun and Demirel, 2013). The Microbial Fuel Cell (MFC) is a promising technology that allows simultaneous removal of organic carbon and nitrogen (Virdis et al., 2010). MFC is a bioelectrochemical system (BES) that converts chemical energy to electrical energy using heterotrophic electrogenic biomass (Du et al., 2007; Logan et al., 2006). The electrons provided from organic electron donors are transferred from the anodic compartment to the cathodic chamber through an external circuit, while the protons cross a proton exchange membrane (Rozendal et al., 2006). The objective of this work was to evaluate the possibility to achieve nitritation and denitritation in an H-type MFC using a complex synthetic wastewater characterized by a TOC/N ratio equal to 0.35.

## 2. Materials and method

### 2.1 Microbial Fuel Cell

A classical H-Type MFC was used to perform all tests. Both chambers, 300-mL pyrex glass bottles, were provided by carbon paper electrode (Goodfellow Cambridge Limited, LS366112 SJP Carbon Foil) connected by an external titanium wire closed with a 203.4  $\Omega$  resistor. The two compartments of the cell were separated by a Cationic Exchange Membrane (Ultrax CMI-7000, Membranes International, USA,  $\phi$ = 53 mm). The anodic chamber was provided with a reference electrode (Ag/AgCl Crison 5240). In cathodic chamber an air diffuser was used.

### 2.2 Synthetic solutions

The anodic chamber was filled with synthetic wastewaters containing ammonium sulphate, sodium nitrite and sodium nitrate as nitrogen source and different organic substrates (sodium acetate and fumaric acid). The composition of the solutions used in the different tests is reported in Table 1. All the reagents were dissolved in a buffer phosphate solution (50 mM) to ensure pH neutral condition. Digestate was used as a bacterial inoculum (Bavasso et al., 2016). All reagents were purchased from Sigma Aldrich. The cathodic compartment was filled only with buffer phosphate (50 mM). All tests were performed at room temperature.

Table 1: Summary of runs performed and characterization of synthetic solutions used. Composition is expressed in term of TOC % and N%

Run	Wastewater characteristic		TOC load composition		N load composition			Operating condition
	N [mg L <sup>-1</sup> ]	TOC [mg L <sup>-1</sup> ]	Sodium Acetate [%]	Fumaric Acid [%]	Ammonium [%]	Nitrite [%]	Nitrate [%]	Time [day]
1	480	168	100	-	100	-	-	7
2	480	168	50	50	100	-	-	7
3	270	94.5	100	-	-	100	-	7
4	270	94.5	50	50	-	100	-	7
5	100	35	50	50	33	33	33	7

### 2.3 Analytical measurement

Chemical properties were daily monitored: TOC (Total Organic Carbon analyzer TOC-L Shimadzu), NH<sub>4</sub><sup>+</sup> (UDK 139- Velp), NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> (Dionex ICS 1100 Thermo Scientific), pH (GLP21 Crison). At the end of each test the differences between the starting and the final values of organic carbon and nitrogen contents were evaluated and expressed as  $\Delta$ TOC,  $\Delta$ NH<sub>4</sub><sup>+</sup>,  $\Delta$ NO<sub>2</sub><sup>-</sup> and  $\Delta$ NO<sub>3</sub><sup>-</sup>. Open circuit voltage (OCV) was measured (VSP Potentiostat BioLogic) and evaluated referred to a saturated Ag/AgCl electrode (5240 Crison).

### 3. Results and discussion

The aim of this work was to evaluate the possibility to achieve organic carbon and nitrogen removal in an H-type MFC system. The effect of different organic carbon source in the shortcut nitrogen cycle was studied.

#### 3.1 Nitritation

In a previous work nitrite accumulation in the anodic chamber during ammonium reduction was confirmed and a positive effects of lower value of TOC/N ratio was found (Bavasso et al., 2006). In this study, the ammonium reduction degree in tests conducted using different organic carbon source was analyzed. As can be observed in Figure 1, ammonium reduction was not affected by TOC composition. In both tests a conversion of ammonium nitrogen around 55% ( $\pm 1\%$ ) was achieved after a 7-days treatment.

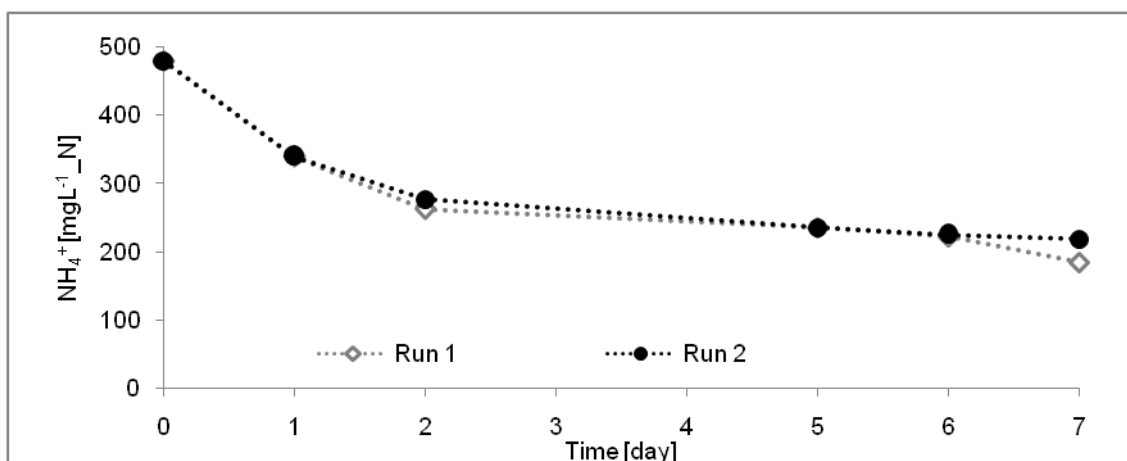


Figure 1: Daily ammonium concentration during nitritation step.

The composition of wastewaters at the end of the test was described in Table 2. Nitrite accumulation corresponding to the ammonium oxidation without or with negligible nitrate formation was observed in both runs. This behaviour can be attributed to the microaerobic condition in the anodic chamber granted by the lower oxygen permeability of the membrane. Moreover the reaction between oxygen and organic carbon occurred rather than oxidation reaction leading to total conversion of ammonium to nitrate. In Run 1, when only sodium acetate was used as organic load, higher values of TOC removal ( $85.72\% \pm 5.5\%$ ) and  $OCV_{max}$  were observed due to sodium acetate high biodegradability (Baker J.A. et al., 2000) and its electron donor capacity. During each tests pH was monitored and neutral values were recorded.

Table 2: Synthetic wastewater composition after 7 days of nitritation step.

Run	$\Delta TOC$ [mg L <sup>-1</sup> ]	$\Delta NH_4^+$ [mg L <sup>-1</sup> N]	$NO_2^-$ [mg L <sup>-1</sup> N]	$NO_3^-$ [mg L <sup>-1</sup> N]	$OCV_{max}$ [mV]
1	155.22	219.46	232.31	-	120.03
2	123.38	214.08	248.74	-	98.00

#### 3.2 Denitritation

With the aim of simulating a continuous process, the synthetic wastewaters prepared to perform denitritation step was characterized by a nitrite concentration similar to that obtained at the end of the nitritation. In this case air supply in cathodic chamber was stopped to avoid oxygen losses through the membrane and to ensure anaerobic condition in anodic chamber. In Run 3 and Run 4 the effect of organic carbon composition on nitrite removal was analyzed. The obtained results showed that denitritation process was positively affected when a mix of organic carbon source was used. It can be concluded that it is possible to perform denitritation step whatever organic carbon composition used, unless they are toxic and/or not biocompatible compounds.

After 7 days, the process was stopped and the results recorded are collected in Table 3. Nitrite reduction was 23.48% ( $\pm 3.6\%$ ) and 46.56% ( $\pm 1.2\%$ ) in Run 3 and Run 4 respectively. Formation of nitrate was not observed, thus confirming that stopping the air supply in the cathodic chamber was sufficient to ensure anaerobic condition in the anodic compartment. Organic carbon removal was 41.29% ( $\pm 4\%$ ) in Run 3 and 54.72% ( $\pm 4.8\%$ )

in Run 4. Different  $OCV_{max}$  values were achieved depending on the electron donor (sodium acetate) concentration. Comparing this results with  $OCV_{max}$  measured in nitrification step, the lower values observed in the denitrification can be explained considering that in this case the organic carbon source is progressively consumed in the reaction for nitrite removal. Also in these tests neutral pH values were measured.

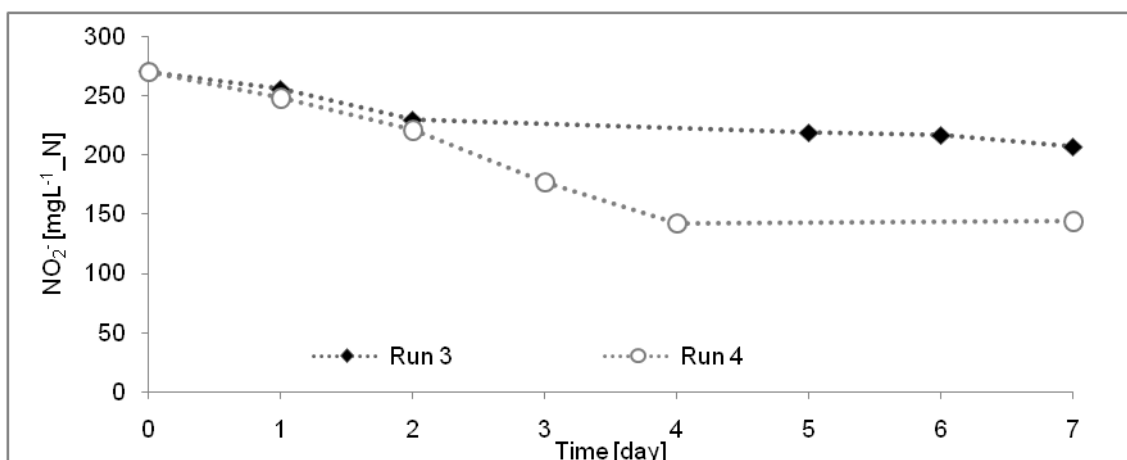


Figure 2: Daily nitrite concentration during denitrification step.

Table 3: Synthetic wastewater composition after 7 days of denitrification step.

Run	$\Delta TOC$ [mg l <sup>-1</sup> ]	$\Delta NO_2^-$ [mg l <sup>-1</sup> _N]	$NO_3^-$ [mg l <sup>-1</sup> _N]	$OCV_{max}$ [mV]
3	39.02	63.42	-	102.00
4	51.71	125.73	-	65.00

### 3.3 SBNR in MFCs

Further tests were conducted to verify the feasibility of a continuous shortcut process in the same compartment using an H-type MFC system. In particular, in Run 5 a complex solution characterized by the copresence of sodium acetate, fumaric acid, ammonium, nitrite and nitrate was fed in the anodic chamber in order to simulate a real wastewater. During the first 6 days nitrification step was performed, then, in order to allow the occurrence of the denitrification step, air feeding at the cathode was stopped and organic carbon was added at the anode to restore the TOC/N starting value. The experiment was monitored for further 9 days. Regarding of the first part of the shortcut process, results showed in Figure 3 confirmed the oxidation of ammonium and the increase of nitrite concentration mainly in the first three days; nitrate remained almost constant thus revealing that nitrification step was achieved. In the second part, when anaerobic condition was obtained and organic carbon source was added, the complete removal of nitrite and nitrate was reached.

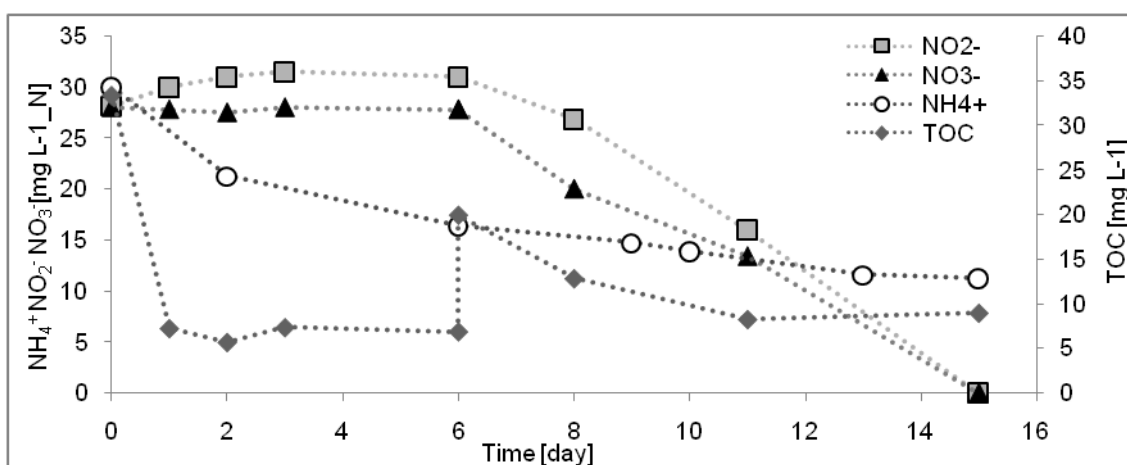


Figure 3: Shortcut process in anodic compartment of H-type MFC. Daily concentration of all compounds.

The removal of organic and nitrogen content achieved during nitritation and denitritation steps in the shortcut process is shown in table 4. Because of the presence of dissolved oxygen concentration in the anodic chamber during the nitritation,  $\Delta\text{TOC}$  measured was higher than that observed in the denitritation step. A decrease of ammonium concentration was found in both steps of the shortcut process: this suggests that during denitritation, when anaerobic condition does not make possible the oxidation of ammonium according Eq(1), the action of specific bacteria (Anammox) can not be excluded (Di Domenico et al., 2015; Di Palma et al., 2015).

Table 4: Synthetic wastewater composition after each step of shortcut process.

Run 5	Time [day]	$\Delta\text{TOC}$ [mg L <sup>-1</sup> ]	$\Delta\text{NH}_4^+$ [mg L <sup>-1</sup> _N]	$\Delta\text{NO}_2^-$ [mg L <sup>-1</sup> _N]	$\Delta\text{NO}_3^-$ [mg L <sup>-1</sup> _N]	$\text{OCV}_{\text{max}}$ [mV]
Nitritation	6	16.11	13.57	-	-	60.00
Denitritation	9	11.50	5.21	31.50	27.80	53.00

In Figure 4  $\text{OCV}_{\text{max}}$  and pH values monitored during the test were collected. As reported in a previous paper (Bavasso et al., 2016), at the operative condition adopted, the  $\text{OCV}_{\text{max}}$  value, determined by the initial TOC concentration, was reached after a short period (2 days) probably because of the persistence of a biofilm on the surface of the anode used in this test. pH trend during shortcut preliminary test showed a circumneutral constant value.

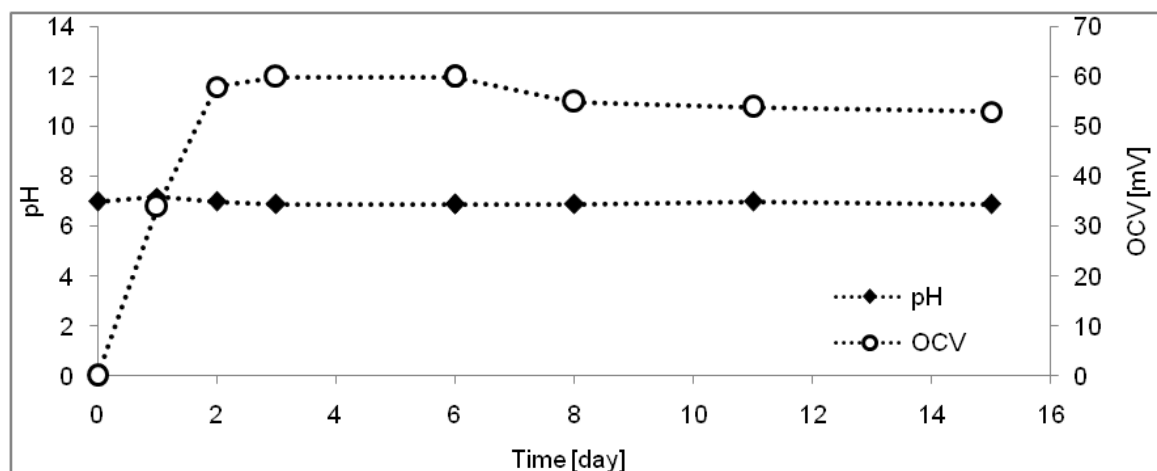


Figure 4: Open Circuit Voltage [mV] and pH behaviour during shortcut process.

#### 4. Conclusions

The aim of this work was to evaluate the feasibility of a shortcut biological nitrogen removal using an H-type Microbial Fuel Cell. The advantage of using a double chamber MFC for shortcut implementation consists in the flexibility in changing microaerobic/anaerobic conditions. Synthetic wastewaters using different organic carbon and nitrogen sources were prepared. The effect of organic carbon source during nitritation and denitritation steps was assessed. During nitritation TOC and  $\text{NH}_4^+$  reduction was achieved with  $\text{NO}_2^-$  accumulation in the anodic compartment and  $\text{NO}_3^-$  formation was not observed. Tests conducted with different organic carbon sources led to almost the same ammonium removal (45%) thus indicating that nitritation is not affected by the organic composition of the wastewater. In the denitritation step, nitrite reduction was obtained: a  $\text{NO}_2^-$  reduction around 46.56%, using sodium acetate and fumaric acid, was observed. A preliminary shortcut process was performed. After 6 days of nitritation and 9 days of denitritation, a total removal of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  was attained. In all tests  $\text{OCV}_{\text{max}}$  was reached after 2/3 days with values positively affected by increasing initial TOC concentration.

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