

1                                   **Combining electro-bioremediation of nitrate in saline**  
2                                   **groundwater with concomitant chlorine production**

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17   **Highlights:**

- 18           -       3-compartment BES configuration for nitrate and salinity removal from  
19                   groundwater.
- 20           -       Electro-bioremediation of saline groundwater reached drinking water  
21                   standards.
- 22           -       Water recovery and sustainable chlorine production from contaminated  
23                   groundwater.
- 24           -       Stable active chlorine production suitable for *in-situ* water disinfection.
- 25           -       Successful groundwater remediation within a circular economy-based  
26                   approach.

1 **Abstract:**

2 Groundwater pollution and salinization have increased steadily over the years. As the  
3 balance between water demand and availability has reached a critical level in many  
4 regions of the world, a sustainable approach for water resources and salinity  
5 management has become essential. A 3-compartment cell configuration was tested for  
6 the simultaneous denitrification and desalination of nitrate contaminated saline  
7 groundwater. The cells were initially operated in potentiostatic mode to promote  
8 autotrophic denitrification at the bio-cathode and then switched to galvanostatic mode to  
9 improve the desalination of groundwater in the central compartment. The average  
10 nitrate removal rate achieved was  $39 \pm 1 \text{ mgNO}_3^- \text{-N L}^{-1} \text{ d}^{-1}$  and no intermediates (i.e.,  
11 nitrite and nitrous oxide) were observed in the effluent. The salinity of groundwater was  
12 considerably reduced ( $63 \pm 5\%$  of chloride removal on average). Within a circular  
13 economy approach, part of the removed chloride was recovered in the anode  
14 compartment and converted into chlorine, which reached a concentration of  $26.8 \pm 3.4$   
15  $\text{mgCl}_2 \text{ L}^{-1}$ . The accumulated chlorine represents a value-added product, which could  
16 also be dosed for disinfection in water treatment plants. With this cell configuration,  
17 WHO and European legislation drinking water threshold limits for nitrate ( $11.3 \text{ mgNO}_3^-$   
18  $\text{-N L}^{-1}$ ) and salinity ( $2.5 \text{ mS cm}^{-1}$ ) were met, with low specific power consumptions  
19 ( $0.13 \pm 0.01 \text{ kWh g}^{-1} \text{NO}_3^- \text{-N}_{\text{removed}}$ ). These results are promising, and pave the ground for  
20 the development of a sustainable technology that could successfully tackle an urgent  
21 environmental issue.

22 **Keywords:** circular economy; denitrification; microbial electrochemical technology;  
23 saline groundwater; value-added products; water recovery.

24 **List of abbreviations**

25 AEM Anion-Exchange Membrane  
26 BER Bioelectrochemical Reactor

1	BES	Bioelectrochemical System
2	CE	Current Efficiency
3	CEM	Cation-Exchange Membrane
4	CL <sup>-</sup> -RE	Chloride Removal Efficiency
5	CL <sup>-</sup> -RR	Chloride Removal Rate
6	EC-RE	Electrical Conductivity Removal Efficiency
7	ECT	Electric Charge Transferred
8	ER	Electroreduction
9	HRT	Hydraulic Retention Time
10	IEM	Ion-Exchange Membranes
11	MDC	Microbial Desalination Cells
12	N-RE	Nitrate Removal Efficiency
13	N-RR	Nitrate Removal Rate
14	OCV	Open Circuit Voltage
15	SEC	Specific Energy Consumption
16	SHE	Standard Hydrogen Electrode
17	Ti-MMO	Titanium coated with mixed metals oxide
18	TSS	Total Suspended Solids
19	WHO	World Health Organization

20

21

## 22 **1. INTRODUCTION**

23 Groundwater represents one of the main sources of drinking water in many countries of  
 24 the world (Zhang et al., 2017). However, this crucial water resource is threatened by

1 multiple polluting sources, both natural and anthropogenic (Burri et al., 2019), which  
2 limit its possible exploitation for human consumption.

3 Nitrate is one of the most widespread pollutants and it can accumulate in groundwater  
4 mainly due to agricultural-related activities such as the spread of inorganic fertilizers  
5 and animal manure on crops (Menció et al., 2016). The consumption of nitrate can  
6 cause severe health risks (Carrey et al., 2021; Ward et al., 2018; Coss, 2004). Besides  
7 nitrates, groundwater salinity is a matter of concern since it limits the potential use of  
8 water for drinking purposes. Saline water consumption has been associated with high  
9 blood pressure (Naser et al., 2017). Groundwater salinity is variable and depends on  
10 both the aquifer geology and anthropogenic impacts. Over-exploitation of groundwater  
11 in coastal areas leads to a significant drop in groundwater levels, causing an alteration  
12 of the hydrodynamic balance between seawater and freshwater, with the consequent  
13 seawater intrusion and salinization of the aquifer (Liu et al., 2020).

14 Nitrate and salinity content simultaneously affect groundwater quality in many  
15 countries around the world, especially in coastal areas of the Mediterranean Basin, East  
16 Africa, and China (Troudi et al., 2020; Alfarrach et al., 2018; Gounari et al., 2014; Hu et  
17 al., 2005). For this reason, the World Health Organization (WHO) and the European  
18 Council (Council Directive 98/83/EC) established strict threshold limits for nitrates  
19 ( $11.3 \text{ mg NO}_3^- \text{-N L}^{-1}$  or  $50 \text{ mg NO}_3^- \text{ L}^{-1}$ ) and salinity ( $2.5 \text{ mS cm}^{-1}$ ) in water for human  
20 consumption.

21 Conventional technologies for groundwater treatment used to remove both nitrate and  
22 salinity, such as reverse osmosis, nanofiltration, ion exchange, and electrodialysis are  
23 mainly based on separation processes (Della Rocca et al., 2007). Besides being  
24 effective, these technologies are characterized by: i) high costs for energy and chemicals  
25 consumptions, ii) the production of wastes/brines that are difficult to be disposed of, iii)

1 the need for regular rejuvenation of materials (ion exchange) and iv) the loss of  
2 efficiency due to scaling and fouling (electrodialysis, nanofiltration, reverse osmosis)  
3 (Aliaskari et al., 2021; Epsztein et al., 2015; Koter et al., 2015; Twomey et al., 2010;  
4 Bamforth et al., 2005). It must be considered also that separation-based processes  
5 remove all the ions present in water so they cannot selectively remove nitrate (Rezvani  
6 et al., 2019).

7 Among biological treatment processes, autotrophic denitrification represents the key  
8 metabolism for nitrate contaminated groundwater bioremediation, since groundwater is  
9 usually characterized by low organic carbon concentration (Regan et al., 2017).

10 Bio-electrochemical systems (BES) proved to be a promising sustainable and efficient  
11 alternative for nitrate removal from groundwater (Li et al., 2019; Pous et al., 2018). In  
12 such systems, the electrochemical redox processes are enhanced by electro-active  
13 bacteria, which can use a solid electrode as electron donor or acceptor (Rabaey et al.,  
14 2009). Previous studies have demonstrated the possibility to achieve complete nitrate  
15 conversion into dinitrogen gas in BES via autotrophic denitrification at the bio-cathode,  
16 with no nitrite nor nitrous oxide production (Ceballos-Escalera et al. 2021; Puig et al.  
17 2011; Desloover et al., 2011).

18 Several studies were also carried out with bioelectrochemical technologies applied to  
19 desalination, i.e., Microbial Desalination Cells (MDC), which exploit the oxidation of  
20 organic matter in wastewater as a source of energy for desalination. The electric  
21 potential gradient created by the exoelectrogenic bacteria desalinates water by driving  
22 ion transport through a series of ion-exchange membranes (IEM) (Ramírez-Moreno et  
23 al., 2019; Sevda et al., 2015; Kim et al., 2013).

1 However, to our knowledge, there is only one study concerning the simultaneous  
2 removal of nitrate and salinity from groundwater using BES. Zhang et al. (2013) tested  
3 a submerged 2-compartment desalination-denitrification cell for the treatment of  
4 synthetic groundwater affected by high salinity and nitrate concentrations, using  
5 simulated municipal wastewater as the source of electrons. A higher nitrate removal  
6 (99%) was achieved at high ionic strength compared to low ionic strength conditions  
7 (91%), even though salinity removal was lower (60% versus 95%). In this regard, it  
8 must be considered that groundwater is usually characterized by low conductivity (<1  
9 mS cm<sup>-1</sup>), which would lead to more ohmic and transport losses and higher pH gradients  
10 (Logan et al., 2006), thus hindering BES treatment performances. In this sense, high  
11 salinity groundwater could be more suitable for BES treatment, since nitrate removal  
12 efficiency should not be limited by low conductivity.

13 Within this framework, a proof-of-concept based on a 3-compartment bio-  
14 electrochemical cell configuration was designed and tested treating saline groundwater  
15 contaminated by nitrates. The main objective of the study was to investigate the  
16 feasibility of coupling bioelectrochemical nitrate removal with salinity reduction in a  
17 continuously fed BES. The 3-compartments cell was operated in both potentiostatic and  
18 galvanostatic mode, and different operating conditions were tested. Moreover, the  
19 possibility to sustainably produce value-added chemicals while treating groundwater  
20 was assessed, within a circular economy-based approach. Specifically, the conversion of  
21 chlorides into free chlorine, which is a strong disinfecting agent widely used for water  
22 disinfection in water treatment plants, was investigated.

## 1 2. MATERIALS AND METHODS

### 2 2.1 Reactors set-up

3 Two identical 3-compartment bioelectrochemical cells made of transparent Plexiglas  
4 were used in this study. Each cell consisted of a bio-cathode compartment ( $8 \times 8 \times 2 \text{ cm}^3$ ,  
5 net volume 110 mL), an anode compartment ( $8 \times 8 \times 2 \text{ cm}^3$ , net volume 130 mL), and a  
6 central “desalination” compartment ( $8 \times 8 \times 0.5 \text{ cm}^3$ , net volume 30 mL).

7 The cathode and the central compartments were separated by a cation-exchange  
8 membrane (CEM 7000-S, Membrane International Inc., USA) with a surface of  $64 \text{ cm}^2$ .

9 Carbon felt (thickness 1.12 cm, degree of purity 99.9%, AlfaAesar, Germany) with a  
10 surface of  $64 \text{ cm}^2$  was used as the bio-cathode (working electrode), and connected to a  
11 stainless steel mesh which worked as the current collector. A reference electrode

12 (Ag/AgCl, +0.197 V vs SHE, mod. MF2052, BioAnalytical Systems, USA) was also  
13 placed in this compartment. The anode and the central compartments were separated by

14 an anion-exchange membrane (AEM 7001-CR, Membranes International Inc., USA)  
15 with a surface of  $64 \text{ cm}^2$ . Titanium coated with mixed metals oxide (Ti-MMO,  $15 \text{ cm}^2$ ,

16 NMT-Electrodes, South Africa) was used as anode (counter electrode), and connected  
17 to a titanium wire (thickness 0.75 mm, degree of purity 99.98%, AlfaAesar, Germany)

18 which worked as the current collector. Cathode, anode, and reference electrodes were  
19 connected to a multichannel potentiostat (Ivium technologies, IviumNstat, NL). A

20 schematic representation of the setup is shown in Figure 1.

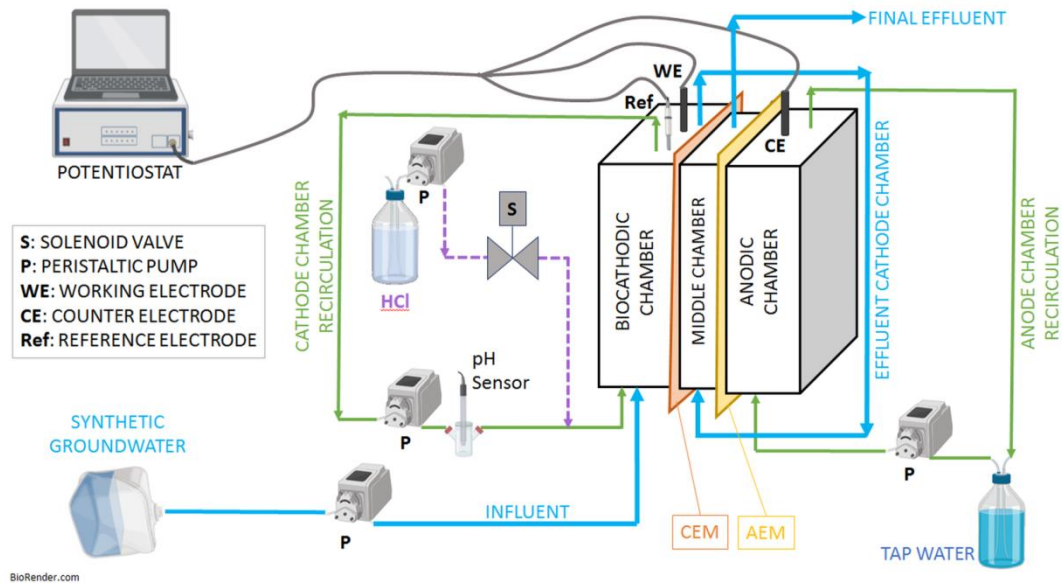


Figure 1: Schematic process flow diagram.

## 2.2 Synthetic groundwater characteristics

A synthetic medium mimicking nitrate concentration and salinity of groundwater from the nitrate vulnerable zone of Arborea (Sardinia, Italy) was fed to the bio-cathode compartment (Medium A):  $216.6 \text{ mg L}^{-1} \text{ KNO}_3$  (corresponding to  $30.0 \text{ mgNO}_3^{-}\text{-N L}^{-1}$ );  $10 \text{ mg L}^{-1} \text{ NH}_4\text{Cl}$  (corresponding to  $2.6 \text{ mgNH}_4^{+}\text{-N L}^{-1}$ ),  $4.64 \text{ mg L}^{-1} \text{ KH}_2\text{PO}_4$ ;  $11.52 \text{ mg L}^{-1} \text{ K}_2\text{HPO}_4$ ;  $350 \text{ mg L}^{-1} \text{ NaHCO}_3$ ;  $2000 \text{ mg L}^{-1} \text{ NaCl}$  and  $100 \mu\text{L L}^{-1}$  of trace elements solution (Patil et al., 2010). The resulting electric conductivity and pH were  $3.3 \pm 0.3 \text{ mS cm}^{-1}$  and  $8.2 \pm 0.2$ , respectively. Medium B (same composition as Medium A, but without  $\text{KNO}_3$  and  $\text{NH}_4\text{Cl}$ ) was used to fill the anode and central compartments during batch mode operation, in order to avoid conductivity and pH gradients during biofilm development and enrichment. All media were prepared using distilled water, and Medium A was pre-flushed with  $\text{N}_2$  gas for 15 minutes to avoid any presence of oxygen.

## 2.3 Experimental procedure

Table 1 summarizes the experimental procedure followed in this study. Both cells were started up in batch mode (Phase 1). The supernatant of activated sludge liquor drawn from the municipal wastewater treatment plant of Cagliari (Italy) and the effluent from a



1 parent electro-denitrifying system were mixed in a 60:40 ratio (v:v) and used as  
2 inoculum. The bio-cathode compartment was initially filled with synthetic groundwater  
3 (Medium A) and inoculum ( $<100 \text{ mgTSS L}^{-1}$ ) in a 50:50 ratio (v:v). A proper amount of  
4  $\text{KNO}_3$  solution (0.2 M) was periodically added when nitrate concentration measured  
5 inside the bio-cathode compartment dropped below  $3.5 \text{ mgNO}_3^- \text{-N L}^{-1}$ , in order to bring  
6 nitrate concentration up to  $30 \text{ mgNO}_3^- \text{-N L}^{-1}$ . The anode and central compartments were  
7 filled with Medium B, which was periodically replaced when pH dropped below 3 in  
8 the anode compartment, or salinity was below  $2 \text{ mS cm}^{-1}$  in the desalination  
9 compartment, respectively. Three peristaltic pumps were used to recirculate the  
10 solutions in each compartment with a flow rate of  $50 \text{ mL min}^{-1}$ , thus providing thorough  
11 mixing of the media. The working, reference, and counter electrodes were connected to  
12 a potentiostat set in potentiostatic mode (Thasar, Ivium-N-Stat, NL). Bio-cathode was  
13 poised at  $-0.500 \text{ V vs Ag/AgCl}$  ( $-0.303 \text{ V vs SHE}$ ), a potential suitable for nitrate  
14 removal (Pous et al., 2015). During Phase 2, the bio-cathode compartment was  
15 continuously fed with Medium A, and the effluent was sent into the central  
16 compartment to achieve desalination. Tap water was batch-fed and recirculated in the  
17 anode compartment. The potentiostat was kept in potentiostatic mode, and the electrical  
18 parameters remained the same as in Phase 1.

19 During Phase 3, the potentiostat was switched to galvanostatic mode and three different  
20 currents were applied, namely 2, 5, and 10 mA.

21 In the last experimental phase (Phase 4), pH control was introduced to keep the pH at  
22 values  $<7.5$  by dosing HCl (1 M) in the cathode recirculation line. The sensor for  
23 continuous pH measurement was connected to a transmitter (Mettler Toledo, mod.  
24 M300, USA), which recorded data every 10 minutes. During Phase 4, the cells were  
25 operated in galvanostatic mode with a fixed current of 10 mA.

26

1 Table 1: Experimental procedure.

Phases	Days of experimentation [d]	Hydraulic operation	Hydraulic retention time [h]	Electrical operation	Controlled parameter	pH control
1	30	Batch mode (inoculation)	-	Potentiostatic	Cathode potential: -0.500V vs Ag/AgCl	NO
2	40	Continuous mode	18	Potentiostatic	Cathode potential: -0.500V vs Ag/AgCl	NO
3a	7	Continuous mode	24	Galvanostatic	Applied current: 2 mA	NO
3b	5	Continuous mode	24	Galvanostatic	Applied current: 5 mA	NO
3c	5	Continuous mode	24	Galvanostatic	Applied current: 10 mA	NO
4	30	Continuous mode	18	Galvanostatic	Applied current: 10 mA	YES

2

### 3 2.4 Control tests

4 Abiotic tests were performed in duplicate to evaluate the different contributions to  
5 nitrate removal during operation in galvanostatic mode.

6 The abiotic tests were carried out in a cell identical to those used for the main  
7 experiments, in open circuit and galvanostatic mode, with an applied current of 10 mA.  
8 Synthetic groundwater was continuously fed to the cathode compartment, then  
9 transferred into the central compartment before being discharged. Tap water was batch-  
10 fed and recirculated into the anode compartment. The different contributions were  
11 obtained by monitoring the nitrate concentration in each compartment of the cell. All  
12 tests lasted 24 hours.

## 1 **2.5 Analytical methods**

2 Samples were periodically taken from influent (once per week), effluent (three times per  
3 week), cathode and anode compartments (three times per week) in order to evaluate  
4 overall cells performances. Liquid samples were analyzed for quantification of anions,  
5 i.e., chloride ( $\text{Cl}^-$ ), nitrite ( $\text{NO}_2^-$ -N), nitrate ( $\text{NO}_3^-$ -N), phosphate ( $\text{PO}_4^{3-}$ ), and sulfate  
6 ( $\text{SO}_4^{2-}$ ), using an ion chromatograph (ICS-90, Dionex-ThermoFisher, USA) equipped  
7 with an AS14A Ion-PAC 5  $\mu\text{m}$  column. Samples were filtered (acetate membrane filter,  
8 0.45  $\mu\text{m}$  porosity) and properly diluted with distilled water. The concentrations of the  
9 main cations, i.e., potassium ( $\text{K}^+$ ) and sodium ( $\text{Na}^+$ ), were determined using an  
10 ICP/OES (Optima 7000, PerkinElmer, USA): samples were filtered (acetate membrane  
11 filter, 0.45  $\mu\text{m}$  porosity), acidified (1% v:v of nitric acid) and diluted with grade 1  
12 water.

13 Electrical conductivity and pH were measured using a benchtop meter (HI5522, Hanna  
14 Instruments, Italy).

15 The concentration of free chlorine was analyzed using spectrophotometric techniques  
16 (DR1900, Hach Lange, Germany) and the DPD free chlorine method (DPD free  
17 chlorine reagent powder pillows Cat. 2105569, Hach Lange, Germany).

18 Nitrous oxide ( $\text{N}_2\text{O}$ ) was measured using an  $\text{N}_2\text{O}$  liquid-phase microsensor (Unisense,  
19 Denmark) located in the effluent line of the reactors, thanks to a dedicated glass  
20 measuring cell.

21 The resulting currents and potentials were recorded every five minutes during Phases 1-  
22 2 and Phases 3-4, respectively, through potentiostat. Cell potential was periodically  
23 checked using a multimeter (K2M, mod. KDM-600C, Italy).

24 SEM images of ion-selective membranes were captured using a FEI Quanta 200 SEM  
25 microscope. The membranes did not undergo any kind of preparation, they were simply  
26 fixed on the stub using a double-sided graphite adhesive. The analyses were performed

1 in low vacuum mode (i.e., residual pressure in the experimental chamber in the range of  
 2 0.3-0.9 Torr), to minimize electrostatic charge effects, or high vacuum (pressure below  
 3  $10^{-4}$  Torr). Images were collected in either secondary electrons or backscattered  
 4 electrons.

## 5 **2.6 Calculations**

6 Nitrate Removal Efficiency (N-RE) and Nitrate Removal Rate (N-RR) were calculated  
 7 according to equations 1 and 2, respectively:

$$8 \quad N - RE [\%] = \frac{C_{NO_3^- - N(\text{inf})} - C_{NO_3^- - N(\text{eff})}}{C_{NO_3^- - N(\text{inf})}} \quad (1)$$

$$9 \quad N - RR [mg\ N\ L^{-1}\ d^{-1}] = \frac{C_{NO_3^- - N(\text{inf})} - C_{NO_3^- - N(\text{eff})}}{HRT} \quad (2)$$

10 Where  $C_{NO_3^- - N(\text{inf})}$  and  $C_{NO_3^- - N(\text{eff})}$  [ $mg\ L^{-1}$ ] are nitrate concentrations in the influent and  
 11 the effluent, respectively, while HRT [d] is the hydraulic retention time considering the  
 12 cathode and central compartments volumes.

13 The desalination performance was evaluated by calculating the electrical conductivity  
 14 removal efficiency (EC-RE, equation 3), the chloride removal efficiency (Cl<sup>-</sup>-RE,  
 15 equation 4), and the chloride removal rate (Cl<sup>-</sup>-RR, equation 5).

$$16 \quad EC - RE [\%] = \frac{EC_{(\text{inf})} - EC_{(\text{eff})}}{EC_{(\text{inf})}} \% \quad (3)$$

$$17 \quad Cl^- - RE [\%] = \frac{C_{Cl^- (\text{inf})} - C_{Cl^- (\text{eff})}}{C_{Cl^- (\text{inf})}} \% \quad (4)$$

$$18 \quad Cl^- - RR [mg\ L^{-1}\ d^{-1}] = \frac{C_{Cl^- (\text{inf})} - C_{Cl^- (\text{eff})}}{HRT} \quad (5)$$

19 where  $EC_{(\text{eff})}$  [ $mS\ cm^{-1}$ ] and  $C_{Cl^- (\text{eff})}$  [ $mg\ L^{-1}$ ] represent the effluent electric conductivity  
 20 and chloride concentration, respectively. The  $EC_{(\text{inf})}$  [ $mS\ cm^{-1}$ ] and  $C_{Cl^- (\text{inf})}$  [ $mg\ L^{-1}$ ]  
 21 represent the influent electric conductivity and chloride concentration for Phases 1-3,  
 22 respectively. Instead, during Phase 4, the  $EC_{(\text{inf})}$  and  $C_{Cl^- (\text{inf})}$  corresponded to the electric  
 23 conductivity and chloride concentration of the solution in the bio-cathode compartment  
 24 (i.e., the influent to the central compartment), respectively, in order to consider the  
 25 chloride input due to the acid dosage in the cathode chamber. The HRT [d] is the  
 26 hydraulic retention time of the central compartment.

1 The coulombic efficiency for nitrate and nitrite reduction ( $\varepsilon NO_x$ ) was calculated  
 2 according to equation 6 (Virdis et al., 2008):

$$3 \quad \varepsilon NO_x [\%] = \frac{I}{n \Delta C_{NO_x} Q_{in} F} \quad (6)$$

4 where I is the current [A], n is the number of electrons that can be accepted by 1 mol of  
 5 oxidized nitrogen compound present in the bio-cathode compartment assuming N<sub>2</sub> is the  
 6 final product;  $\Delta C_{NO_x}$  is the difference between the nitrate concentration in the cathodic  
 7 influent and effluent [molNO<sub>3</sub><sup>-</sup>-N L<sup>-1</sup>]; Q<sub>in</sub> is the influent flow rate [L s<sup>-1</sup>] and F is  
 8 Faraday's constant [96485 C e<sup>-</sup>mol<sup>-1</sup>].

9 The current efficiency (CE) was expressed as the percentage of the charge associated  
 10 with the chloride removed from the central compartment to the amount of electric  
 11 charge transferred (ECT) across the membranes (Ramírez-Moreno et al., 2019). CE [%]  
 12 and ECT [C m<sup>-3</sup>] were calculated using equations 7 and 8, respectively:

$$13 \quad CE [\%] = \frac{\nu z F (C_{Cl^-}(\text{inf}) - C_{Cl^-}(\text{eff}))}{ECT} \quad (7)$$

$$14 \quad ECT [C m^{-3}] = \frac{\int I dt}{V} \quad (8)$$

15 where,  $\nu$  and  $z$  represent the stoichiometric coefficient and the valence of the chloride  
 16 ion, respectively, V [m<sup>-3</sup>] is the volume of water treated and dt the time [s].

17 The specific energy consumption (SEC) was calculated according to equation 9 for  
 18 potentiostatic mode (Ben Sik Ali et al., 2010), and according to equation 10 for  
 19 galvanostatic mode (Djouadi Belkada et al., 2018):

$$20 \quad SEC_{\text{pot.}} [\text{kWh m}^{-3}] = \frac{E \int I dt}{V} \quad (9)$$

$$21 \quad SEC_{\text{gal.}} [\text{kWh m}^{-3}] = \frac{I \int E dt}{V} \quad (10)$$

22 where E is the cell potential [V].

23 Energy losses were calculated as reported by Sleutels et al. (2009). Specifically, the  
 24 cathode overpotential ( $\eta_{\text{cat}}$ ) was calculated using the calculated cathode potential

1 ( $E_{\text{NO}_3^-/\text{N}_2}$ ) and the measured cathode potential, while the anode overpotential ( $\eta_{\text{an}}$ ) was  
2 calculated using the calculated anode potential ( $E_{\text{O}_2/\text{H}_2\text{O}}$ ) and the measured anode  
3 potential. pH gradient losses ( $E_{\Delta\text{pH}}$ ) were determined using the Nernst equation,  
4 rendering a potential loss of  $-0.059$  V per pH unit. Ionic losses ( $E_{\text{ionic}}$ ) were calculated  
5 at each side of the membranes, considering the distance between the anode and the  
6 AEM for anode compartment (1 cm), the AEM and the CEM for the central  
7 compartment (0.5 cm) and the cathode and the CEM for the bio-cathode compartment  
8 (1 cm).

### 9 **3. RESULTS AND DISCUSSION**

#### 10 **3.1 Cells performances in potentiostatic mode**

11 The two cells worked as duplicates during the whole experiment, which started with the  
12 inoculation period in batch mode (Phase 1). Denitrification took place in the bio-  
13 cathode compartment, while the electromigration of ions through the membranes and  
14 therefore the desalination, occurred in the central compartment. During Phase 1, an  
15 average nitrate removal rate of  $6.8 \pm 0.4 \text{ mgNO}_3^- \text{-N L}^{-1} \text{ d}^{-1}$  was achieved and a significant  
16 reduction in electric conductivity was also observed in the central compartment, from  
17  $4.11 \pm 0.2$  to  $0.17 \pm 0.2 \text{ mS cm}^{-1}$ .

18 Once stable conditions were achieved, the reactors were switched to continuous mode  
19 (Phase 2), with an HRT of 18 h. This new operation mode resulted in increased nitrate  
20 removal compared to Phase 1. The average nitrate removal rate and removal efficiency  
21 were  $10 \pm 5 \text{ mgNO}_3^- \text{-N L}^{-1} \text{ d}^{-1}$  and  $23 \pm 11\%$ , respectively. Although no nitrite and nitrous  
22 oxide were detected in the effluent, the highest value of coulombic efficiency obtained  
23 during this period was about 50%, which could indicate the occurrence of side reactions  
24 (e.g., oxygen oxidation).

1 The current density was close to  $0.03 \text{ A m}^{-2}_{\text{membrane}}$ , which suggests a limited demand  
2 for electrons at the bio-cathode, likely due to the high internal resistance of the system.  
3 As reported by Cao et al. (2009) for MDCs, the lower the conductivity of the central  
4 compartment, the higher the resistance of the ion exchange membranes. In our cell  
5 configuration, the presence of the two membranes between the compartments hindered  
6 the transfer of protons from the anode to the bio-cathode compartment. An efficient  
7 transfer is indeed necessary for the successful denitrification reaction as the four steps  
8 of nitrate reduction require the presence of protons (Nguyen et al., 2015). In addition, an  
9 increase in the pH gradient between the anode and bio-cathode compartment also causes  
10 an increase in the internal resistance of the system (Puig et al., 2012). However,  
11 according to calculations reported by Sleutels et al. (2009), energy losses due to pH  
12 gradient between the compartments were only 1.6% of the total energy loss  
13 (corresponding to -0.1 V), while the most important energy losses are attributable to  
14 cathode overpotential, ionic and transport losses, amounting respectively to 14.9% (-  
15 0.96 V), 11.8% (-0.76 V), and 59.5% (-3.86 V).

16 Even though nitrate removal was observed in potentiostatic mode, low chloride removal  
17 efficiency was achieved ( $4 \pm 3\%$ ). The low measured current density ( $0.03 \text{ A m}^{-2}_{\text{membrane}}$ )  
18 was not sufficient to promote electromigration of ions: in fact, at such current density,  
19 the theoretical maximum chloride removal would be about  $220 \text{ mg L}^{-1} \text{ d}^{-1}$ ,  
20 corresponding to a removal efficiency of 2%. Coherently, no significant  
21 electromigration of ions across the membranes with consequent reduction of  
22 conductivity in the effluent was observed. Electromigration is directly related to the  
23 applied (or generated) current, together with the 2 perm-selectivities imposed by the  
24 membranes (Dykstra et al., 2021). Kim et al. (2013) reported that the maximum  
25 current densities for microbial desalination cells range from 0.7 to more than 8.4 A

1  $\text{m}^{-2}_{\text{membrane}}$ . Based on the results achieved during Phase 2, the operating conditions were  
 2 thoroughly modified in order to work at higher current conditions and maximise both  
 3 nitrate and salinity removal.

### 4 **3.2 Cells performances in galvanostatic mode**

#### 5 3.2.1 BES operation without pH control

6 During Phase 3, the reactors operation was switched to galvanostatic mode and three  
 7 different currents were tested (Table 2).

8 Table 2: Operating conditions and main results obtained during tests in galvanostatic  
 9 mode with different applied currents.

Phase	Applied current [mA]	Nitrate removal rate [ $\text{mgNO}_3\text{-N L}^{-1}\text{d}^{-1}$ ]	Nitrate removal efficiency [%]	Effluent nitrate concentration [ $\text{mgNO}_3\text{-N L}^{-1}$ ]	Chloride removal efficiency [%]	Effluent conductivity [ $\text{mS cm}^{-1}$ ]
3a	2	6.5±1.7	30±7	26.5±2.6	0	5.4±0.4
3b	5	12.1±4.9	65±37	11.7±6.9	68±37	3.6±4.0
3c	10	19.6±1.1	89±3	3.4±0.1	97±2	0.2±0.1

10

11 The best results in terms of nitrate and salinity removal were obtained during Phase 3c,  
 12 when 10 mA was applied (current density of  $1.6 \text{ A m}^{-2}_{\text{membrane}}$ ): average nitrate removal  
 13 efficiency was  $89\pm3\%$  (corresponding to an effluent nitrate concentration of  $3.4\pm0.1$   
 14  $\text{mgNO}_3\text{-N L}^{-1}$ ), and desalination efficiency was  $97\pm2\%$  (corresponding to an effluent  
 15 conductivity of  $0.2\pm0.1 \text{ mS cm}^{-1}$ ).

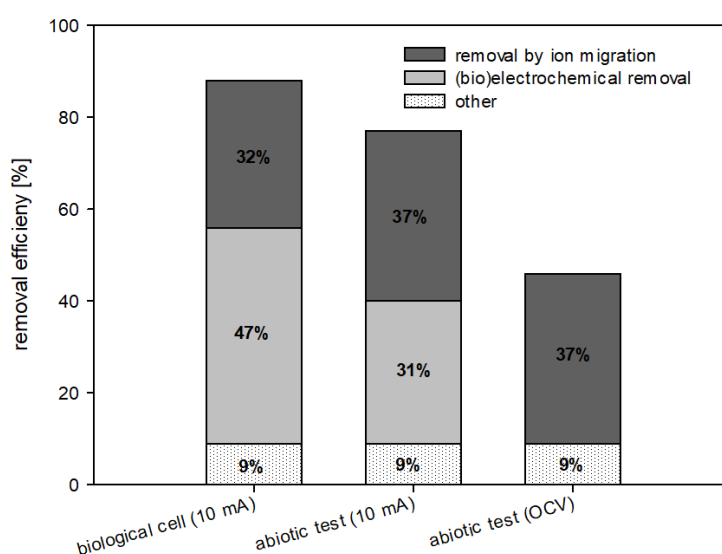
16 The short duration of Phases 3a, 3b, and 3c (Table 1) due to rapid membranes  
 17 deterioration (as it will be explained below), did not allow to observe the migration of



1 the major cations ( $K^+$  and  $Na^+$ ) through the CEM, and their possible accumulation in the  
2 bio-cathode compartment.

3 The coulombic efficiency ( $\epsilon_{NO_x}$ ) related to nitrate reduction was always greater than  
4 100%, with increasing values as the applied current increased. This was because the  
5 applied current was higher than that required only for nitrate removal (i.e., about 3 mA).  
6 The current efficiency (CE) related to the removal of chlorides from the central chamber  
7 was zero (desalination was negligible),  $83\pm 73\%$ , and  $28\pm 1\%$  during Phases 3a, b, and c,  
8 respectively. Although the highest CE was achieved during Phase 3b, it must be noticed  
9 that the process was highly unstable.

10 Abiotic tests were carried out to determine different contributions (i.e.,  
11 bioelectrochemical, electrochemical, and migration across AEM) to nitrate removal.  
12 The denitrifying performance of the biological cell in galvanostatic operation (10 mA)  
13 was compared with that of the abiotic cell in galvanostatic (10 mA) and open circuit  
14 (OCV) operation (Figure 2).



15  
16  
17  
18

Figure 2: Different contributions to nitrate removal (i.e., bioelectrochemical, electrochemical, and migration of nitrate ions through the AEM) determined with biotic and abiotic tests.

1 Recent studies have shown that electrochemical technologies, including  
2 electroreduction (ER), are effective in removing nitrates in wastewater due to their high  
3 reactivity (Xu et al., 2018). The reaction mechanism depends strongly on the type of  
4 cathode material, cathode potential, and solution pH. To the authors' knowledge, there  
5 are no specific studies on electroreduction applied with carbon felt cathodes, but the  
6 conditions established in the cathode chamber in galvanostatic mode may be favorable  
7 for nitrate electroreduction, as clearly indicated by the abiotic test. However, the results  
8 proved that the bioelectrochemical contribution significantly improved nitrate removal,  
9 which was 16% higher than that obtained electrochemically in the abiotic cell.

10 Although significant nitrate and salinity removal was achieved with galvanostatic  
11 operation, the high reaction rate caused an increase in pH (>10) in the bio-cathode  
12 compartment, resulting in membranes damage and a subsequent decline in overall  
13 process performance, including denitrification capacity. Even though the optimal pH  
14 working range of both AEM and CEM is between 0 and 10 pH, the worst deterioration  
15 was observed in the anion-exchange membrane (Figure S1), which resulted to be  
16 particularly sensitive to high pH values.

### 17 3.2.2 Effect of pH control on BES performance

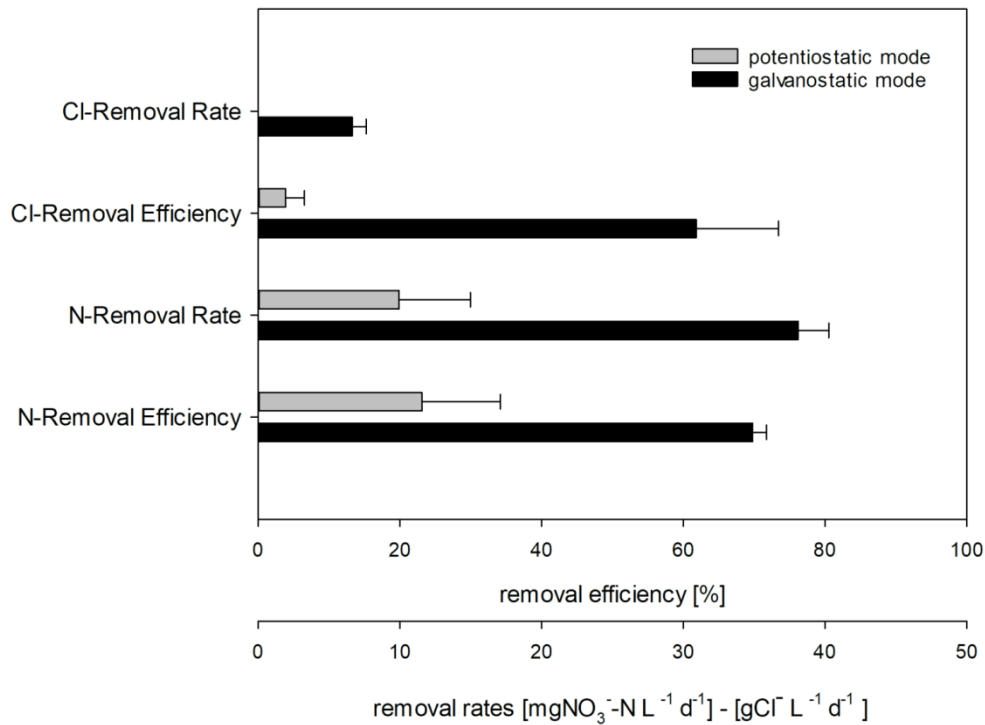
18 In order to improve the stability of the process and the lifetime of the membranes,  
19 several tests were carried out, which included the on/off operation of the potentiostat  
20 and the periodic washing of the bio-cathode compartment. However, no improvements  
21 were observed in terms of performance and process stability (data not shown).  
22 Implementing pH control (<7.5), based on acid dosage in the recirculation line of the  
23 bio-cathode compartment, significantly improved process stability (Phase 4). Average  
24 nitrate removal rate was  $39 \pm 1 \text{ mgNO}_3^- \text{-N L}^{-1} \text{ d}^{-1}$  (corresponding to a nitrate removal  
25 efficiency of  $69 \pm 2\%$ ), while the chloride removal rate was  $13 \pm 2 \text{ gCl}^- \text{ L}^{-1} \text{ d}^{-1}$

1 (corresponding to a chloride removal efficiency of  $63\pm 5\%$ ). A decrease in conductivity  
2 between the inflow and outflow of the central compartment was observed, from about 6  
3 to  $2 \text{ mS cm}^{-1}$  respectively, corresponding to an EC removal efficiency of  $59\pm 13\%$ . The  
4 current efficiency related to chloride migration was  $104\pm 16\%$ , indicating that all the  
5 applied current was used for chloride migration.

6 During Phase 4, a significant accumulation of major cations ( $\text{K}^+$  and  $\text{Na}^+$ ) was observed  
7 in the bio-cathode compartment (Figure S2). This result was consistent with the trend of  
8 anions, in particular chlorides, and confirmed that in such conditions it was possible to  
9 promote the electromigration of ions from the central compartment to the anode and  
10 bio-cathode compartments. Besides, the concentrations of cations in the effluent showed  
11 a constant increasing trend over time and they equalled the influent concentrations after  
12 25 days of experiments (Figure S3). Despite such increase in cations concentration, no  
13 precipitates or deposits on the electrode were observed at the end of Phase 4.

14 Figure 3 shows a comparison of the performances, in terms of denitrification and  
15 desalination, obtained during the potentiostatic (Phase 2) and galvanostatic (Phase 4)  
16 operation modes of the reactors. The galvanostatic mode with pH control, significantly  
17 enhanced desalination as expected, but a significant improvement in denitrification rates  
18 was also observed. Energy losses in Phase 4 were significantly higher than those in  
19 Phase 2, and mainly associated with ionic and transport losses, corresponding to 33%  
20 and 54% of the total energy losses, respectively.

21 During galvanostatic operation with pH control, nitrate concentration and conductivity  
22 in the effluent ( $11.4\pm 0.5 \text{ mgNO}_3^- \text{-N L}^{-1}$  and  $2.2\pm 0.3 \text{ mS cm}^{-1}$ , respectively) were close  
23 to threshold limits for drinking water, corresponding to  $11.3 \text{ mgNO}_3^- \text{-N L}^{-1}$  (91/767/EU)  
24 and  $2.5 \text{ mS cm}^{-1}$  (98/83/CE), respectively.



1  
2 Figure 3: Comparison of overall performance observed during potentiostatic  
3 (Phase 2) and galvanostatic operation (Phase 4) of the reactors.

4 During Phase 4 (galvanostatic mode with pH control), the specific energy consumption  
5 (SEC) was  $0.13 \pm 0.01 \text{ kWh g}^{-1} \text{NO}_3^- \cdot \text{N}_{\text{removed}}$ , comparable with those previously reported  
6 in the literature concerning bioelectrochemical reactors operated with similar  
7 conditions. Zhou et al. (2009) reported a SEC of  $0.07 \text{ kWh g}^{-1} \text{NO}_3^- \cdot \text{N}_{\text{removed}}$  in a  
8 bioelectrochemical reactor (BER) fed with real groundwater and operated in  
9 galvanostatic mode. Although Pous et al. (2015) achieved lower SEC in  
10 bioelectrochemical systems operated in potentiostatic mode ( $0.7 \cdot 10^{-2} \text{ kWh g}^{-1} \text{NO}_3^- \cdot$   
11  $\text{N}_{\text{removed}}$ ), it should be noticed that the energy provided in our system was used not only  
12 for nitrate removal but also to promote ions electromigration and achieve a reduction in  
13 salinity of the treated water. In this sense, a direct comparison of SEC may be  
14 misleading. The average SEC (per unit volume of treated water) was  $3.48 \pm 0.13 \text{ kWh}$   
15  $\text{m}^{-3} \text{water treated}$ , which is comparable or lower than the consumption reported for well-  
16 established desalination technologies, such as membrane processes (1-12 and 2-12 kWh

1 m<sup>3</sup> for electrodialysis and reverse osmosis, respectively) or thermal processes (14-25  
2 and 7-25 kWh m<sup>-3</sup> for multi-stage flash desalination and multi-effect  
3 evaporation/distillation, respectively) (Al-Amshawee et al., 2020). Furthermore, it is  
4 important to consider that these processes exploit established technologies that operate  
5 on a pilot or full scale, while the system in this study, although already showing  
6 competitive results, represents a proof of concept with significant scope for  
7 improvement in terms of process performance (i.e., removal and desalination rates) and  
8 energy consumption.

### 9 **3.3 Chloride recovery and synthesis of disinfectants**

10 During Phase 4, a progressive accumulation of chloride ions in the anolyte solution (i.e.,  
11 tap water) was observed. The chloride recovered was partially converted into chlorine  
12 (Cl<sub>2</sub>) thanks to the anodic potential (+1.49±0.06 V vs SHE), which was close to the  
13 minimum required for chlorine production (i.e., +1.4 V vs SHE). After about 15 days of  
14 operation, the concentration of chlorides in the anode compartment reached a value of  
15 2300 mgCl<sup>-</sup> L<sup>-1</sup>, while chlorine concentration stabilized at a value of 26.8±3.4 mgCl<sub>2</sub> L<sup>-1</sup>  
16 from day 6th of the experiment. This concentration is higher than the typical dosage  
17 required for disinfection purposes in water treatment plants (0.5–2.0 mg Cl<sub>2</sub> L<sup>-1</sup>). Thus,  
18 in the perspective of an on-site application of this technology, the chlorine produced  
19 could be slightly dosed for disinfection of the treated water (Ragazzo et al., 2020).

20 Moreover, the oxidation of chloride to chlorine ( $\Delta G^{\circ}=2.72$  eV) appears to be  
21 particularly convenient also from an energy point of view, since it is 45% less energy-  
22 consuming than water electrolysis ( $\Delta G^{\circ}=4.92$  eV), which is the reaction mainly used at  
23 the anode in BES systems for denitrification (Batlle-Vilanova et al., 2019).

24 Therefore, the production of chlorine compounds in the anode compartment, which  
25 could be used for water disinfection in water treatment plants, shows both economic

1 value and application potential. Currently, chlorine-based disinfectant products are  
2 priced at 2.20 € kg<sup>-1</sup> (averaged from different providers).

3 The production of chlorine in the anodic chamber of the BES has considerable  
4 advantages over conventional technologies (i.e., the chlor-alkali process) from a  
5 sanitary and environmental point of view. In fact, BES do not require the use of toxic  
6 chemicals and do not produce highly concentrated brines. It also offers advantages from  
7 a management point of view, considerably reducing energy costs, which are high in the  
8 chlor-alkali process, as well as disposal costs of the brine.

9 Based on these considerations, the possibility of producing chlorine spontaneously from  
10 a groundwater treatment process, which does not involve the production of  
11 intermediates or waste products and with reduced energy consumption, is of particular  
12 interest for the development of increasingly sustainable processes.

### 13 **3.4 Comparison with state of the art and perspectives**

14 To the authors' best knowledge, this is the first study addressing simultaneous nitrate  
15 removal, desalination, and chlorine synthesis in a bioelectrochemical system. The  
16 results achieved in this study were compared with those reported in the literature  
17 concerning groundwater denitrification and desalination by BES and other technologies  
18 (Table 3).

19 Previous studies showed that high nitrate removal rates can be achieved, working under  
20 both potentiostatic (Ceballos-Escalera et al., 2021; Liu et al., 2019; Pous et al., 2015)  
21 and galvanostatic conditions (Zhou et al., 2007). In particular, Pous et al. (2015) showed  
22 that denitrification rates increased as the energy input increased. It must be considered  
23 that denitrification rates were achieved under very different operating conditions, and  
24 direct comparisons may be difficult. For example, Ceballos-Escalera et al. (2021)

1 reported removal rates of  $519 \pm 53 \text{ mgNO}_3^- \text{-N L}^{-1} \text{ d}^{-1}$ , much higher than those reported in  
2 our study ( $39 \pm 1 \text{ mgNO}_3^- \text{-N L}^{-1} \text{ d}^{-1}$ ). However, if nitrate removal rates are calculated per  
3 cathode electrode surface area available for biomass growth, an average of  $600 \text{ mgNO}_3^-$   
4  $\text{-N m}^{-2} \text{ d}^{-1}$  was obtained in our study, about twice the value achieved by Ceballos-  
5 Escalera et al. (2021) (i.e.,  $300 \text{ mgNO}_3^- \text{-N m}^{-2} \text{ d}^{-1}$ ). This means that by increasing the  
6 electrode surface area available, it will be possible to develop a greater amount of  
7 biomass, thus optimizing denitrification performance.

8 The simultaneous denitrification and desalination of groundwater was investigated only  
9 by Zhang et al. (2013), using a submerged microbial desalination denitrification cell.  
10 The oxidation of organic matter at the anode was used to generate the electrons required  
11 to drive electromigration (i.e., desalination), and much lower nitrate removal rates were  
12 achieved compared to other studies (Table 3).

13 Concerning groundwater desalination, previous studies that have focused particularly on  
14 nitrate removal used technologies such as electrodialysis and reverse osmosis. Some of  
15 these technologies require the addition of chemicals, and none of them is oriented  
16 towards the recovery of value-added products (Table 3). Although the addition of  
17 hydrochloric acid was necessary for the present study during Phase 4 for active pH  
18 control, a significant part of chloride was recovered as free chlorine, which is a value-  
19 added chemical commonly used in water and wastewater disinfection and may  
20 contribute to reducing management costs.

21 As for energy requirements, the proof-of-concept 3-compartment BES investigated in  
22 our study already showed SEC comparable with those reported in the literature  
23 (Pirsaheb et al., 2015; Bi et al., 2011). In this sense, there is still considerable room for  
24 improvement in terms of SEC reduction, since the process can be further optimized in

- 1 terms of operating conditions (e.g., by lowering the HRT), geometrical configuration
- 2 (e.g., the distances between electrodes and membranes), and materials.

3



1 Table 3: Comparison of operating conditions and main results with previous studies.

Reference	Type of reactor or process	Influent type	Fixed parameter	Nitrate removal efficiency [%]	Nitrate removal rates [mgNO <sub>3</sub> -N <sup>-</sup> L <sup>-1</sup> d <sup>-1</sup> ]	Desalination efficiency [%]	Energy consumption [kWh m <sup>-3</sup> ]	Addition of chemicals	Recovery/ Production of value-added substances
This study (Phase 4)	3-chamber BES (bioelectrochemical system)	synthetic groundwater	current	69±2	39±1	63± 5	3.48 ± 0.13	Yes, hydrochloric acid	Yes, Cl <sub>2</sub>
Ceballos-Escalera, 2021	Tubular BES (bioelectrochemical system)	synthetic groundwater	potential	90 ±6	519±53	-	-	No	No
Pous et al., 2015	2-chamber BES (bioelectrochemical system)	real groundwater	potential	96±2	98.2	-	0.20	No	No
Zhou et al., 2007	3D BER (Biofilm Electrode Reactor)	real groundwater	current	97	n.m.	-	0.44	Yes, ethanol and sulphuric acid	No
Zhang et al., 2013	SMDDC (Submerged Microbial Desalination Denitrification Cell)	synthetic groundwater	-	91	17	94	n.m.	Yes, sodium acetate	No
Liu et al., 2019	a combined SMFC (Sediment Microbial Fuel Cell)	real groundwater	potential	n.m	93	-	n.m.	No	No
El Midaoui et al., 2002	Electrodialysis	real groundwater	potential	93	n.m.	77	0.08	No	No
Bi et al., 2011	Electrodialysis	synthetic groundwater	potential	99	n.m.	n.m.	1.7	No	No
Pirsaheb et al., 2015	Electrodialysis	real groundwater	current	47	n.m.	72	2	Yes, hydrochloric acid	No
Pirsaheb et al., 2015	Reverse osmosis	real groundwater	current	91	n.m.	73	1.2	Yes, hydrochloric acid and antiscalant	No

## 1 **4. CONCLUSIONS**

2 A proof-of-concept based on a 3-compartment bio-electrochemical cell configuration  
3 was designed and tested in this study for the treatment of saline groundwater  
4 contaminated by nitrates. The proposed system successfully combined simultaneous  
5 nitrate reduction, desalination, and production of a value-added chemical in a single  
6 reactor, within a circular economy-based approach. Several operating conditions were  
7 tested, and the galvanostatic mode (applied current: 10 mA) with active pH control in  
8 the bio-cathode compartment allowed to achieve high nitrogen and salinity removal, and  
9 significant recovery of free chlorine (i.e., a disinfectant commonly used in the water  
10 treatment sector), with much improved process stability and low power consumption.  
11 The contribution of bioelectrochemical and electrochemical denitrification, as well as of  
12 ion migration across membranes to nitrate removal was assessed. Standard quality  
13 requirements for drinking water in terms of nitrate concentration (91/767/EU) and  
14 conductivity (98/83/CE) were successfully met with this cell configuration, paving the  
15 ground for the development of a sustainable technology to tackle such an urgent  
16 environmental issue.

## 17 **DECLARATION OF COMPETING INTEREST**

18 The authors declare that they have no known competing financial interests or personal  
19 relationships that could have appeared to influence the work reported in this paper.

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2

3

4 **List of Figures**

5 Figure 1: Schematic process flow diagram.

6 Figure 2: Different contributions to nitrate removal (i.e., bioelectrochemical,  
7 electrochemical, and migration of nitrate ions through the AEM) determined with biotic  
8 and abiotic tests.

9 Figure 3: Comparison of overall performance observed during potentiostatic (Phase 2)  
10 and galvanostatic operation (Phase 4) of the reactors.

11

12

13 **List of Tables**

14 Table 1: Experimental procedure.

15 Table 2: Operating conditions and main results obtained during tests in galvanostatic  
16 mode with different applied currents.

17 Table 3: Comparison of operating conditions and main results with previous studies