1	Pilot-scale bioelectrochemical system (BES) for simultaneous nitrogen and carbon
2	removal in urban wastewater treatment plants.
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10 11	Keywords: Microbial electrolysis cell, scaling up, nitrogen removal, centrate, biocathode.
12	Short title: Pilot BES for nitrogen and carbon removal from WW.
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20 ABSTRACT

21 This study aims to characterize the performance of a 150 L bioelectrochemical system-22 based plant, during the simultaneous carbon and nitrogen removal from several waste 23 streams of wastewater treatment plants. The bioelectrochemical system (BES) contained 24 five electrode pairs (operated hydraulically and electrically in parallel) and was fed with 25 either wastewater, centrate (nutrient-rich liquid stream produced during the dewatering 26 of digested biomass), or a mixture of both over 63 days, with a hydraulic retention time 27 of one day. Total Organic Carbon (TOC) and Total Nitrogen (TN) removal rates 28 averaged 80% and 70%, respectively, with a specific energy consumption of 0.18 kWh \cdot m⁻³ (BES + ancillary equipment). This work also underlines the challenges of 29 30 using BES for nitrogen removal, highlighting the limitations of the current design, and 31 suggesting some strategies for improvement.

32 INTRODUCTION

The presence of nitrogen in wastewater (WW) has for years been a source of health and environmental issues (1)(2). For urban WW (uWW) in particular, nitrogen is usually present as organic nitrogen, ammonium, and nitrates (3), and it is conventionally removed by a two-step process that involves autotrophic aerobic oxidation of ammonia (nitrification), and heterotrophic reduction of nitrate and nitrite to nitrogen gas (denitrification) (4).

39 The uWW entering a WW treatment facility, which represents the main source of 40 nitrogen for the water line, usually contains a low concentration of nitrogen that is 41 typically around 45 mg \cdot L⁻¹ (5). Another important source of nitrogen comes from the 42 sludge centrifugation (after anaerobic digestion) which produces a liquid stream, known 43 as centrate (6), that is usually returned to the head of the water line. Nitrogen content in 44 centrates can exceed 1300 mg·L⁻¹ (total Kjedahl nitrogen) (7) and may account for 15-45 20% of the nitrogen entering the plant (8). Its removal demands extra power input and 46 the implementation of larger treatment tanks in the water line (9), all of which have a 47 significant impact on the economic balance of the plant. Moreover, as pollutants in the 48 centrate get diluted when entering the uWW, their elimination becomes more difficult. 49 Thus, developing new strategies for treating centrates without their being returned to the 50 water line may represent an important source of monetary and energy savings for the 51 plant.

Bioelectrochemical systems (BESs), are an emerging technology with a wide range of
potential applications in the field of WW treatment (10). One of their main benefits,
compared to more conventional technologies, is that they allow the recovery of part of

55 the chemical energy content present in the WW, while removing organic pollutants. 56 BESs can also be used for removing inorganic contaminants such as nitrogen (11), and 57 when designed for simultaneous carbon and nitrogen removal they have the potential to 58 become an effective and efficient WW treatment technology (12)(13). Thus, several 59 strategies for simultaneous carbon and nitrogen removal from different waste streams 60 using BESs have already been put forward, and have been validated at the laboratory 61 scale (14)(15). These experiments have provided valuable information for determining 62 the optimal operating conditions such as pH and temperature ranges, Chemical Oxygen 63 Demand (COD)/N ratio or materials (16). However, larger-scale experiments are vital 64 for evaluating how laboratory designs can be implemented at a practical scale, to 65 identify where the limitations are, and to assess the chances of practical applicability 66 (17).

67 The aim of this study is to obtain a preliminary characterization of the operation of a 68 150 L BES-based pilot plant (Fig. 1) for simultaneous carbon and nitrogen removal 69 from two waste effluents: uWW and centrate. The BES unit consist of five independent 70 modules hydraulically and electrically connected in parallel. The fresh waste effluent is 71 first fed to anode side of each module for organic matter oxidation. Then it is conducted 72 to a packed-bed column reactor to convert ammonia into nitrate. Finally, the effluent of 73 the column is led to the cathode side of each module for denitrification. The plant was 74 operated in continuous mode for 63 days, and this paper presents results for TOC and 75 TN removal rates, as well as energy usage/recovery. It also highlights the main 76 limitations of the current design and advances some strategies for improvement.

The BES was designed within a framework of easy-to-manufacture, easy-to-maintain considerations to improve its scalability and its practical applicability. Although the whole set-up was initially planned to operate with centrate as the feed, the results show that it can also work with urban wastewater, which highlights its flexibility to operate both in the water line or in the sludge line in a WWTP.

82

83 MATERIALS AND METHODS

84 BES and nitrification reactors design

85 The BES reactor consisted of a polypropylene tank (0.7 m x 0.32 m x 1.2 m) with a 86 working liquid volume of 150 L (Fig. 1). It contained five electrode pairs that were 87 mounted on polypropylene frames, and were hydraulically and electrically operated in parallel. The anodes and cathodes were made of 5 mm-thick graphite felt (Sigratherm®, 88 Germany), with a projected area of 0.47 m² per electrode (0.98 m x 0.48 m) and were 89 90 pretreated according to (18). Each anode and cathode were separated by an anion 91 exchange membrane, AMI-7001 CR (Membranes International INC., USA), and the 92 electrodes were connected to the external electrical circuit through stainless steel current 93 collectors. Thus, the reactor was divided into six chambers (three anode chambers and 94 three cathode chambers) with a volume of 30 L each, except for the two outer chambers 95 which have half that volume. Every chamber was fitted with a recirculation loop and a 96 pump to maintain mixing conditions.

97 Nitrification was carried out in an external PVC, tubular, fixed-bed reactor (0.25 m in
98 diameter and 1.7 m in height) which was filled with plastic rings (4 cm in diameter) to

99 provide a high surface area. A peristaltic pump was used to continuously recirculate the100 effluent.

101 Reactor inoculation and start-up

102 During the inoculation and start-up process, the BES reactor was operated as an MEC 103 by applying 1 V between the anode and cathode. The anode was inoculated with WW 104 collected from the aerobic zone of an activated sludge reactor (WWTP, north-west of Spain) and was supplemented with 0.5 g \cdot L⁻¹ of acetate. The cathode was inoculated 105 106 with WW collected from the denitrification zone, and was supplemented with 50 mg N-107 NO₃·L⁻¹. Finally, the inoculum for the nitrification reactor consisted of WW collected 108 from the nitrification zone, and was supplemented with 100 mg N-NH₄·L⁻¹. The anode 109 and cathode chambers were filled simultaneously to avoid differential pressures that

110 may damage the membrane. The start-up process was completed in nine days.

111 **Operating conditions**

The reactor was initially operated in MFC mode by connecting electrical resistances between the anode and the cathode. Voltage across resistances was measured continuously, and current was determined using Ohm's law. The ohmic values of the external resistances were determined according to the results of polarization tests (that were performed daily) to maximize the power output.

When the BES was operated in MEC mode the resistances were removed and a voltageof 1 V was applied between anode and cathode by means of power supplies that were

119 computer controlled using a PCI-6713 analog output board (National Instruments,

120 Texas). The current was measured and recorded every 10 minutes across a 16 Ω fixed 121 resistor, using a data acquisition system.

Raw wastewater was pumped from the feed tank (maintained at 4 °C) to the anode 123 chambers by C1R peristaltic pumps (Dosiper, Spain). Identical pumps were used to 124 drive the water from the column to the cathode chambers. The BES and nitrification 125 reactors were operated at room temperature. Wastewater was recirculated by DC15/5 126 centrifugal pumps (Xylem, UK) in the anode and cathode chambers, and with NLAV 127 peristaltic pumps (Dosiper, Spain) in the external tubular fixed bed (Fig. 2). Hydraulic 128 retention in the BES was fixed at one day.

129 The pilot plant was meant to be operated and evaluated within the facilities of the 130 WWTP. However, due to project constraints, this was not possible, and the pilot plant 131 performance was evaluated within the facilities of the authors' laboratory. Therefore, 132 WW and centrate samples were received regularly from the WWTP, and were stored at 4 °C for less than 10 days. 133

134 Following the start-up period, the BES was operated as an MFC. Since no significant 135 current was produced, the BES was switched to MEC mode for a period of 63 days that 136 was further subdivided into three stages. During the first stage, which lasted for 39 days, 137 the plant was fed with uWW. During the second stage, which lasted for 20 days, the 138 plant was fed with a mixture of uWW and centrate. The proportion of the latter was 139 gradually increased to favor microbial acclimation to the new feeding conditions. 140 During the third stage, the influent consisted only of centrate.

141 The received samples of raw wastewater (collected from the primary treatment) and

142 centrate had Total Organic Carbon (TOC) concentrations around 90 ± 30 and 180 ± 50

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143 mg·L⁻¹, respectively, and Total Nitrogen (TN) concentrations of 40 ± 25 mg·L⁻¹ in the

144 WW, and $1460 \pm 80 \text{ mg} \cdot \text{L}^{-1}$ in the centrate. The pH was 7.8 ± 0.3 , for both WW and

145 centrate, and conductivity was $0.8 \pm 0.1 \text{ mS} \cdot \text{cm}^{-1}$ in the WW and $10 \pm 0.7 \text{ mS} \cdot \text{cm}^{-1}$ in

the centrate.

147 Analytical methods and calculations

148 Liquid samples were taken daily after inoculation. Samples were taken from the influent

and effluent of the anode, cathode, and tubular fixed bed. TOC and TN were measured

150 (duplicate samples) using a TOC multi N/C 3100 (Analytikjena, Germany).

151 Ammonium, nitrites, and nitrates were measured according to the Standard Methods

152 (19) using an ion-selective electrode NH₃ 781 pH/Ion meter (Metrohm, Switzerland)

and a DU640 spectrophotometer (Beckman, USA), respectively. The pH, conductivity,

and dissolved oxygen were measured using a GLP 21 pH meter (Crison Instruments,

155 Spain), a TetraCon 325 conductivity meter (WTW, Germany) and a HQ40d dissolved

156 oxygen meter (Hach Company, USA), respectively. Gas production in the MEC was

157 measured using an MGC-1 PMMA Milligascounter (Ritter, Germany), and the gas

158 composition was determined using a CP 3800 GC gas chromatograph (Varian, USA)

159 where the carrier gas was argon.

160 Coulumbic efficiency was calculated according to eq. 1 as:

161 Coulombic efficiency =
$$\frac{\int_0^t I \, dt}{(COD_{in} - COD_{out})/M \cdot Q \cdot e \cdot F}$$
(1)

Where COD_{in} and COD_{out} are the COD concentration of BES influent and effluent,
respectively, I is the circulating electrical current (A), M is the weight of 1 mol of COD

164 (32 g·mol⁻¹), Q is the influent flow rate $(L \cdot d^{-1})$, e is the number of mol of electrons

165 exchanged per mol of COD equivalent consumed (4 mol·mol⁻¹), and F is the Faraday

166 constant (96,485 C·mol⁻¹). TOC was converted to COD as $COD = 49.2 + 3.00 \cdot TOC$

167 according to Dubber and Gray (20).

168 Cathodic efficiency was calculated according to eq. 2 as:

169
$$Cathodic \ efficiency = \frac{(NO_3^-_{in} - NO_3^-_{out})/M \cdot Q \cdot e \cdot F}{\int_0^t I \ dt}$$
(2)

170

171 Where NO_{3 in} and NO_{3 out} are the nitrate concentration of BES influent and effluent,

172 respectively, M is the weight of 1 mol of nitrate (62 $g \cdot mol^{-1}$) and e is the number of mol

173 of electrons exchanged per mol of nitrate consumed (5 mol \cdot mol $^{-1}$).

Specific energy consumption of a pump (EC_{Pump}, kWh·m⁻³) was calculated according to
eq. 3 as:

176
$$EC_{Pump} = \frac{\nu \pi r^2 \cdot \left(\rho g h + \frac{\rho v^2}{2}\right)}{1000 \cdot \eta} \cdot \frac{1}{Q}$$
(3)

177 where v (m·s⁻¹) is the water velocity, r (m) is the radio of the tube, ρ (kg·m⁻³) is the 178 water density, g (m·s⁻²) is the gravitational acceleration, η (%) is the efficiency of the 179 pump (we assumed 70% for centrifugal pumps and 100% for peristaltic pumps) and Q 180 (m³·h⁻¹) is the water flow rate.

181 RESULTS AND DICUSSION

182 After inoculation, and once the current stabilized in all the five modules, the reactor was 183 operated in MFC mode for 10 days to explore the possibility of electrical energy 184 recovery, while removing nitrogen and carbon. Polarization tests were performed daily, yielding power densities lower than $0.06 \text{ mW} \cdot \text{m}^{-2}$ of the electrode projected surface area 185 186 (Fig. 3), well below the power densities reported in other studies performed at the 187 laboratory scale using similar MFC configurations (21)(22). Moreover, TOC and TN 188 removal efficiencies were below 40%, all of which led to the abandonment of the idea 189 of operating the reactor as an MFC. Thus, taking advantage of the reversibility of 190 bioelectrochemical systems, the reactor was switched to MEC operation mode (by 191 applying a voltage of 1 V to every module) to "force" the circulation of current, and 192 therefore speed up the rates of carbon and nitrogen removal. As a result, current density almost immediately increased from ~0.001 $A \cdot m^{-2}$ in MFC mode to ~0.200 $A \cdot m^{-2}$ in 193 194 MEC mode.

195 The reactor performance was monitored for a period of 63 days that was further 196 subdivided into three stages (each characterized by the effluent fed to the plant) as 197 described in the M&M section.

198 TOC removal

During the first stage, when the plant was fed with primary treatment effluent, TOC removal in the anode chamber achieved almost 100% efficiency for most of the time except for the first 10 days, where it averaged 40% (Fig. 4A). This poorer performance is attributed to the adaptation of microbial communities during the transition from MFC to MEC operation mode. It is also important to note that, despite the high variability of TOC concentrations in the entering WW (between 50 to 150 mg·L⁻¹), the effluent TOC was almost constant, which highlights the flexibility of the plant to absorb changes inthe characteristics of the effluent, at least when dealing with uWW

207 During the second stage, the centrate concentration in the feed was gradually increased 208 (Fig. 4A). It is striking that, despite using a higher strength effluent, the TOC entering 209 the BES did not rise accordingly. This is explained by the variability of the WW and 210 centrate samples received from the plant, which depended greatly on the time of day 211 when they were collected, as well as the weather conditions. Despite the TOC 212 concentration of the feed being similar to that used in the first stage, the TOC of the effluent increased, reaching up to 50 mg \cdot L⁻¹. This translated into a sharp decline in the 213 214 TOC removal efficiency, which occasionally descended below 40%, most probably as a 215 result of the lower biodegradability of the centrate (23).

216 The third stage, in which the plant was fed solely with centrate, lasted for only five days

due to project constraints and difficulties regarding the logistics of the samples. TOC

218 concentration in the feed was ~200 mg·L⁻¹, falling slightly below 100 mg·L⁻¹ in the

219 outlet, which resulted in a 50% removal efficiency. Although it is difficult to draw

220 conclusions from the limited number of results in stage III, it seems that the removal

rate tended to stabilize (Fig. 4A).

Finally, it is important to remark that the aerobic treatment in the column also

223 contributed some organic matter removal, which amounted to less than 10% of TOC

removal for all three stages. Thus total TOC removal in the whole systems (BES +

column) was slightly higher than the values provided in Figure 4. TOC did not suffer

any significant modification during the cathodic treatment (±2 mg TOC variation

throughout the three stages).

228 Nitrogen removal

229 During the first stage, the TN concentration in the feeding water was below 100 mg \cdot L⁻¹, 230 as expected from urban wastewater samples (5). As with the TOC, TN removal was 231 relatively low during the first 10 days of operation (averaging 48%), which was again 232 attributed to the need of the microorganisms to adapt to the new operating conditions. 233 Following this adaptation period, the TN concentration in the effluent dropped sharply, 234 thus improving nitrogen removal efficiency, which averaged 70% (Fig. 4B). However, a 235 high variability was still observed throughout the entire duration of experiment, which 236 contrasted with the much more stable removal rates for TOC (Fig. 4). One possible 237 explanation lies in the fact that nitrogen must go through three different treatments, 238 which makes its removal a more sensitive process (see Fig. 2). In addition, 239 denitrification is a delicate process that involves several transformations (24), all of 240 which amplifies the effect of disturbances that might appear in any of these steps. In 241 contrast, organic matter oxidation in the anodic chamber is a much more robust process 242 that only requires the anodic oxidation step. During stages II and III, as the nitrogen 243 concentration in the feed gradually increased, the nitrogen concentration in the effluent 244 also increased, although the mean nitrogen removal efficiency did not vary significantly 245 (Fig. 4B).

One of the main bottlenecks in this setup was found in the external nitrification reactor, where not all of the ammonia entering the column was converted into nitrate. In fact, the conversion rate averaged only 40% (Fig. 5A), further declining as the nitrogen concentration in the feed increased (stage II and, notably, stage III). It is known that the nitrification process can be affected by the free ammonia concentration, pH, and

251 insufficient O_2 or CO_2 (25). In this plant the CO_2 concentration, as measured using a 252 TOC analyzer, always remained above the limiting thresholds for nitrification (26), and 253 the pH was in the range between 7.5 and 8.5, which is within the growth range for pure 254 cultures of ammonia oxidizing bacteria (5.8 to 8.5) (27). Moreover, free ammonia 255 inhibition has been reported to occur at concentrations beyond 3700 mg N-NH4⁺ L⁻ 256 $^{1}(28)$, which is far from the NH₄⁺ concentration levels entering this nitrification reactor. 257 Therefore, free ammonia, insufficient CO₂, and pH can be rejected as limiting factors 258 for the nitrification process. It is believed that an insufficient aeration rate was the main 259 cause behind the poor performance of the nitrification reactor, as the dissolved oxygen concentration ($6.2\pm0.9 \text{ mg O}_2 \text{ L}^{-1}$) was generally (especially during stages II and III) 260 261 below the stoichiometric concentration required for the oxidation of ammonium to nitrate (NH₄⁺ + 2O₂ \rightarrow NO₃⁻ + 2H⁺ + H₂O). Therefore, improving the performance of 262 263 the overall pilot setup would demand rethinking the external nitrification reactor, either 264 by increasing the column size, or by improving the aeration rates.

265 The poor performance of the nitrification reactor led to further limitations in the 266 denitrification (cathodic) process, as most of the nitrogen entering the cathode was in 267 the form of ammonia (Fig. 5A). This means that the relatively high nitrogen removal 268 efficiencies observed cannot entirely be explained through cathodic (electrogenic) NO₃⁻ 269 to N₂ conversion. This, together with cathodic efficiencies (calculated as the ratio of 270 electrons required for the reduction of nitrate to nitrogen gas, versus the electrons that 271 reach the cathode) above 100% (see Fig. S1A in the supplementary information) led to 272 the consideration of alternative nitrogen elimination pathways. One feasible alternative 273 is NH₃ volatilization due to relatively high cathodic pHs, a phenomenon previously 274 observed in other studies (29) and could explain, at least partially, the ammonium loss

275 detected during the cathodic treatment. In addition, the coexistence of NH₄⁺ and NO₃⁻ in 276 the cathodic medium makes it reasonable to hypothesize the occurrence of anaerobic 277 ammonium oxidation (anammox), a phenomenon previously observed in BESs and 278 reported in other studies (30)(31)(32). Low concentrations of nitrite detected in the 279 catholyte (below 20 mg \cdot L⁻¹) would support this hypothesis. Finally, it is also possible 280 that some nitrate would diffuse back to the anodic chamber through the anion exchange 281 membrane, although nitrate was not detected in the anolyte of our BES. Unfortunately, neither NH3 nor N2 concentrations were measured for in the off-gas, and the data 282 283 collected through the tests is not sufficient to provide a solid estimation of to what 284 extent these routes (NH₃ volatilization, nitrate diffusion, and anammox) would proceed.

285

286 Current, gas production, and energy efficiency

287 The average current densities on each module were 0.26, 0.27, 0.21, 0.21, and 0.17 288 $A \cdot m^{-2}$ based on the projected electrode surface area (see Fig. S1C in the supplementary 289 information), which are within the range of current densities reported by other 290 researchers feeding MECs with real-waste effluents (33), but still far from the threshold proposed to make BES a feasible technology (5-10 $A \cdot m^{-2}$ electrode surface area), at 291 292 least from an economic point of view (34)(35). Coulombic efficiencies were estimated 293 from TOC measurements as described in M&M section. Results showed coulombic 294 efficiencies in the range between 3-10%, which are indeed rather low values, thus 295 revealing the existence of a significant potential COD loss that could be explained by 296 the presence of alternative electron acceptors as reported in previous studies (36).

297 In the current BES design, the anodic and cathodic chambers opened at the top to a 298 single space, where the gas was collected for analysis. Chromatography results showed 299 that the percentages of methane and carbon dioxide in the gas were highly variable (see 300 Fig. S1B in the supplementary information), although the methane concentration tended 301 to increase as the proportion of centrate in the feed increased (probably as a result of 302 increased methanogenic activity). Interestingly, the hydrogen concentration in the off-303 gas kept growing during the first days of the experiment (peaking at 32%), and then 304 stabilized at around 10%. However, during stage II the percentage of hydrogen 305 decreased to zero, and was totally absent when the BES was fed with centrate. Low gas 306 production, along with the low percentage of hydrogen and methane makes this gas 307 barely usable.

308 Energy consumption was calculated based on the amount of energy delivered to the 309 BES by the power source (kWh) and normalized per kilogram of TOC removed (pumping energy was not considered). It averaged 0.55 kWh \cdot kg⁻¹-TOC (kWh per kg of 310 TOC removed), which corresponded to 0.12 kWh \cdot m⁻³ (kWh per m³ of treated WW) 311 312 (Fig. 6). Energy consumption in the ancillary equipment (pumps) amounted to 0.06 kWh·m⁻³ (see Table 1) and thus total energy consumption in the plant (BES + pumps) 313 was estimated to be 0.18 kWh·m⁻³. This represents a significant saving when compared 314 315 to the average energy consumption in aeration reactors in WWTPs, which in Spain is 316 typically 50–60% of the total energy demand in the facilities (0.53 kWh \cdot m⁻³) (37). 317 Energy consumption increased as the proportion of centrate increased, although it 318 tended to decrease as the microorganisms adapted to the new feeding conditions. A 319 similar phenomenon of a sudden rise and subsequent decline of energy consumption 320 was observed when the feed was only centrate. Therefore, it might be expected that

energy consumption decreases as the microbial communities adapt to the new feeding
conditions. Again, due to project constraints, this is a hypothesis that could not be
further evaluated.

324 In conclusion, when the pilot-scale BES was operated as an MFC, TOC and TN 325 removal were almost negligible. However, when switched to MEC (using uWW as a 326 feed), removal rates reached almost 100% several times, although they declined when 327 using centrate. Energy consumption was within the values traditionally attributed to 328 conventional aerobic systems. The main bottleneck was found in the external 329 nitrification reactor, which was unable to convert all the ammonia into nitrate. As a 330 result, the denitrification process in the cathode was limited, and alternative nitrogen 331 removal pathways (ammonia volatilization and annamox) needed to be considered to 332 explain the results observed.

333 ACKNOWLEDEGMENTS

- 334 Funding for this study was provided by the CDTI INTERCONECTA Programm-
- 335 Project 'Treatment and regeneration of centrate through Forward Osmosis and
- 336 Bioelectrochemical Systems'. M.I. San-Martín thanks the Spanish Ministry of Economy
- and Competitiveness the FPU fellowship granted FPU13/04014.
- 338 The authors declare no conflict of interest.

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449 FIGURE LEGENDS

450 Figure 1. Photograph of the reactor in situ in the Natural Resources Institute of León451 (Spain).

452 Figure 2. Schematic diagram of the pilot-scale BES set up. Each anodic and cathodic 453 chamber was provided with a recirculation pump (only two of them are represented for 454 simplicity). On the right are shown 5 electrode pairs that are mounted on polypropylene 455 frames inside of a polypropylene tank.

456 Figure 3. A) Polarization curve and B) power density versus current density for the five
457 different electrode pairs (C1, C2, C3, C4, and C5) during operation of the reactor in MFC
458 mode.

- 459 Figure 4. A) TOC and B) TN concentration $(mg \cdot L^{-1})$ in the MEC influent and effluent,
- 460 and A) TOC and B) TN removal efficiency. Three stages are differentiated for the feed
- 461 (stage I: WW, stage II: WW + centrate, stage III: centrate).
- 462 Figure 5. Ammonium and nitrate percentages (without considering nitrite concentration)
- 463 in A) the nitrification column and in B) the cathode.
- 464 Figure 6. Energy consumption (kWh·kg⁻¹-TOC_{removal}) in the MEC during three stages
- 465 differentiated for the feed (stage I: WW, stage II: WW + centrate, stage III: centrate).

466 TABLE LEGEND

467 Table 1. Energy consumption by the pumps (feeding and recirculation pumps)











