Renewable Energy 139 (2019) 936–943

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Anaerobic biological fermentation of urine as a strategy to enhance the performance of a microbial electrolysis cell (MEC)

Sónia G. Barbosa [a], Telma Rodrigues [a], Luciana Peixoto [a], Philipp Kuntke [b], Maria Madalena Alves [a], Maria Alcina Pereira [a], Annemiek Ter Heijne [c, *]

[a] CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal
[b] Wetsus, European Centre of Excellence for Sustainable Water Technology, Leeuwarden, the Netherlands
[c] Sub-department of Environmental Technology, Wageningen University, Wageningen, the Netherlands

ARTICLE INFO

Article history:
Received 6 July 2018
Received in revised form
20 February 2019
Accepted 21 February 2019
Available online 25 February 2019

Keywords:
Raw human urine
Fermented human urine
Microbial electrolysis cell
Anaerobic digestion
Two-stage bioconversion process
^{1}H NMR

ABSTRACT

This study investigated the effect of pre-fermented urine on anode performance of a two-chambered microbial electrolysis cells (MECs) compared to raw urine. Pre-fermentation of urine was performed by anaerobic digestion. The effect of this pre-fermentation on anode performance of a MEC was assessed by measuring the removal of chemical oxygen demand (COD), current density and Coulombic efficiency (CE). The MEC using fermented urine achieved a higher average current density (218 ± 6 mA m^{-2}) and a higher CE (17%). Although no significant differences were observed in the COD removal efficiency between both urines, the MEC using fermented urine displayed the highest COD removal rate (0.14 ± 0.02 g L^{-1} d^{-1}). The organic compounds initially found in both urines, as well as the metabolic products associated to the biodegradation of the organic matter were analyzed by proton nuclear magnetic resonance (^{1}H NMR). The main compounds initially identified in the raw urine were urea, creatinine and acetate. In the fermented urine, the main compounds identified were methylamine, acetate and propionic acid demonstrating the effectiveness of the anaerobic fermentation step.

1. Introduction

Urine is a high concentrated wastewater stream, which contributes with around 75% of the total amount of nitrogen (N) and 50% of the total amount of phosphorus (P) present in domestic wastewater [1]. At the same time, urine accounts for only 1% of the total volume of wastewater. Undiluted fresh urine, collected by separation toilets and water-free urinals, has a chemical oxygen demand (COD) concentration of 10 g L^{-1} and a total nitrogen concentration of 8.1 g L^{-1} [2]. In fresh urine most of the N is mainly found as urea [3]. During storage, urea is hydrolyzed due to microbial urease activity into ammonia (NH_3)/ammonium (NH_4^+) and carbonate (CO_3^{2-}) [4]. Urine stored during 6 days, contains a COD concentration of 9.3 g L^{-1} and approximately 1.9 g L^{-1} of ammonium nitrogen (NH_4^+-N) [5]. Thus, urine can be considered as a valuable wastewater stream for energy production and N recovery in bioelectrochemical systems (BESs) [6].

BES can be operated in a microbial fuel cell (MFC) mode if electrical energy is produced or in a microbial electrolysis cell (MEC) mode if energy is supplied for hydrogen (H_2) production [7]. MECs have several advantages over MFCs: (1) oxygen supply to the cathode is not required in an MEC and (2) H_2 gas has a higher economic value than electricity [8,9]. BES for N recovery are commonly composed of two compartments, an anode and a cathode chambers separated by a cation exchange membrane. At the anode, electrochemically active bacteria (EAB) are used to oxidize organic substrate producing protons and electrons [10,11]. Electrons flow to the cathode chamber through an external circuit generating electricity. Electricity will drive the migration of protons and cations such as NH_4^+ from the anode to the cathode through the membrane, where it is converted to NH_3 and easily stripped and recovered [8]. In general, MECs can reach higher current densities and therefore higher N removal rates than MFCs [12]. Kuntke and co-workers demonstrated N-recovery and H_2 production in an MEC as a viable treatment option for diluted urine [8]. However, in their work only 34% of the NH_4^+-N was removed suggesting that further improvements are necessary to increase N-recovery.

MECs fed with fermentable substrates are, in general,

* Corresponding author.
E-mail address: annemiek.terheijne@wur.nl (A. Ter Heijne).

https://doi.org/10.1016/j.renene.2019.02.120

Abbreviations

TSBP	two-stage bioconversion process
SAA	specific acetoclastic activity
SHMA	specific hydrogenotrophic methanogenic activity
C-Tex	Cellulose-based carbon

outperformed (in terms of the Coulombic efficiency (CE)) by MECs using non-fermentable substrates [9]. Several studies have shown the efficient conversion of volatile fatty acids (VFAs) such as acetate and butyrate [13] and propionate [14] directly into electricity. Chae and co-workers investigated the effect of four different substrates (acetate, butyrate, propionate and glucose) on MFC performance [15]. Glucose-fed-MFC showed the lowest CE due to glucose consumption by diverse non EAB via several metabolic pathways including fermentation and methanogenesis [15]. Among several hydrolysable composites such as lipids, proteins and carbohydrates, urine contains fermentable compounds including glucose and lactate [16,17].

The presence of fermentable compounds in urine will promote the growth of non-EAB. Non-EAB will be the responsible of diversion of electrons due to the substrate consumption via other metabolic pathways such as methanogenesis, nitrate and/or sulphate reduction, thus reducing the Coulombic efficiency of the system. In a two-stage bioconversion process (TSBP), the complex biodegradable compounds that are not directly converted by the EAB to electricity are firstly converted in a preliminary anaerobic fermentation step to simple compounds namely VFAs, which can be directly converted by the EAB to electricity in BES [18]. Cerrillo and co-workers investigated the integration of the anaerobic digestion process with a BES in order to improve the COD removal and N recovery from pig slurry [19]. In this study, pig slurry was firstly submitted to a process of anaerobic digestion in a lab-scale continuous stirred tank reactor. The effluent of the anaerobic digestion was then used as feed for a subsequent MEC. MEC operated with digested pig slurry showed higher COD removal and higher CE compared with MEC fed with raw pig slurry [19]. Several works have been highlighting the potential of using BES as a downstream step to recover energy from anaerobic digestion effluent [20–22].

Since urine contains fermentable compounds that can be used by diverse non-EAB via several metabolic pathways, a TSBP may be a suitable option to improve anodic performance of a MEC with urine. In the present work, the MEC operation in combination with a previous anaerobic fermentation step of urine was investigated to improve the COD removal and the current production. This current will drive the migration of NH_4^+ from the anode to cathode. Ammonia can be recovered from the cathode [23]. At first, a preliminary anaerobic biodegradability assay was performed to assess the anaerobic biodegradability of urine. Afterwards, anaerobic urine fermentation over 15 days was performed to convert complex organic compounds present in urine into simpler compounds. The fermented urine was then used as feed for a MEC. Finally, the organic compounds found in urine before and after fermentation as well as the metabolic products associated to urine degradation were analyzed by proton nuclear magnetic resonance (^{1}H NMR).

2. Materials and methods

2.1. Urine source

Human urine was collected and mixed from five male and five female individuals (~ 2.5 L). After storage over 6 days at room temperature, a magnesium oxide (MgO) was added to promote P recovery by struvite precipitation according to Barbosa and co-workers [5]. Following the precipitation process, the urine was submitted to two distinct downstream processes. One portion of the urine (herein referred as raw urine) was used as feed for a first MEC (MEC A). The remaining urine was submitted to an anaerobic digestion process to promote the fermentation of the complex organic compounds present in urine (herein referred as fermented urine). This urine was then used as feed for a second MEC (MEC B). Both urines were stored at −80 °C before use.

2.2. Anaerobic biodegradability of urine

Anaerobic biodegradability batch assays were used to evaluate the anaerobic biodegradability of urine as well as to define the time course of urine fermentation step. Biodegradability assays were prepared by adding the substrate (urine), 30% (v/v) of inoculum (anaerobic granular sludge) and basal medium to a final volume of 50 mL. Batch tests were executed in triplicate and incubated at 37 °C, according to Angelidaki and co-workers [24]. Different concentrations of total COD, 0 g L^{-1}; 0.5 g L^{-1}; 1 g L^{-1}; 2 g L^{-1}; 5 g L^{-1} were tested. The different COD concentrations were obtained by dilution of urine with basal medium. The procedure of diluting the initial COD was adopted to investigate the possible effect of initial COD concentration on methane production. Although during the dilution procedure all the characteristics of the urine change, namely the concentration of ammonia, the initial ammonia concentration was not the critical point since the concentration of ammonia increases during the batch assays due to urea hydrolysis. A blank assay without the addition of urine was also performed to discount the amount of methane (CH_4) produced from residual substrate initially present in the inoculum.

Anaerobic granular sludge (volatile solids (VS) = 0.06 ± 0.01 g g^{-1}) from a brewery industry plant (Central de Cervejas, Portugal) was used as inoculum. The specific acetoclastic activity (SAA) of the anaerobic sludge was 214 ± 17 mL (CH_4) g^{-1} (VS) d^{-1} and the specific hydrogenotrophic methanogenic activity (SHMA) was 389 ± 16 mL (CH_4) g^{-1} (VS) d^{-1}. The SAA and SHMA analysis were performed according to Costa and co-workers [25].

During the anaerobic biodegradability assays, the CH_4 production was monitored by gas chromatography. The biochemical methane production (BMP) and the CH_4 production rate (Rm) were determined. BMP was expressed in L of CH_4 per kg of COD added to each vial and determined according to equation (1). COD_{CH4} (kg) is the amount of COD converted into CH_4, also called cumulative CH_4 production, determined by the theoretical CH_4 production (350 L of CH_4 per 1 kg of COD) and the COD_{added} (kg) is the amount of COD added to each vial [26].

$$BMP = \frac{COD_{CH_4} \times 350}{COD_{added}} \quad (1)$$

The cumulative CH_4 production during batch assays was described by the modified Gompertz according to equation (2) where M(t) is the CH_4 cumulative production (mg), P is the maximum CH_4 production (mg), R_m is the maximum CH_4 production rate (mg d^{-1}), e equals to 2.7182818, λ is the lag-phase (d) and t is the time (d) [26].

$$M(t) = P\, exp\left[- exp\left[\frac{R_m^e}{p}(\lambda - t) + 1\right]\right] \quad (2)$$

Methanation percentage (MP) (%) was defined as the amount of CH_4 produced during the batch assays in relation to the theoretical BMP (*i.e.* 350 L kg^{-1} of COD) and was determined according to

equation (3), where COD_{CH4} (mg) is the amount of COD converted into CH_4 and COD_{added} (mg) is the amount of COD added to each vial [26].

$$MP = \frac{COD_{CH_4}}{COD_{added}} \times 100 \quad (3)$$

2.3. Anaerobic fermentation of urine

Urine fermentation was performed by anaerobic digestion to promote the anaerobic microbial conversion of complex compounds present in urine such as sugars, to VFAs (*e.g.* acetate and propionate). Similar to the previous assays, anaerobic digestion was performed in batch at 37 °C, by adding the substrate (urine, 346 mL) and 30% (v/v) of inoculum (anaerobic granular sludge) to a final volume of 500 mL. In this case, no basal medium was added to avoid urine dilution. The batch assays were conducted until reaching a steady state in terms of soluble chemical oxygen demand (sCOD) concentration, which corresponds to the end of the fermentation process. A preliminary assay, performed to evaluate the time course of the COD solubilization, showed that after approximately 15 days the sCOD concentration stabilizes. Then, the granular sludge was separated from the fermented urine by centrifugation at 4192*g* for 30 min. Afterwards the fermented urine was carefully collected and was submitted to a second centrifugation step (4192 *g* for 30 min) to ensure a sludge free medium. The fermented urine was frozen at −80 °C until use in the MEC to avoid the occurrence of methanogenic microorganisms and minimize loss of organic substrate.

2.4. MEC experimental setup and operation

The experimental setup was composed of two identical dual-chamber MECs to study the effect of urine fermentation by anaerobic digestion on the anode performance of a MEC. MEC A was operated with raw urine (no fermentation) and MEC B was operated with fermented urine. The MECs design used was similar to previously studied MECs [27]. Cellulose-based carbon (C-Tex) (MAST Carbon International Limited, United Kingdom) was used as anode and a graphite insert as a cathode. Flat graphite plates (Müller & Rössner GmbH & Co., Germany) were used as current collectors. A cation exchange membrane (CMH-PP, Ralex, Mega, Czech Republic) was placed directly between the two-chambers. The MECs were operated at room temperature (25 ± 3 °C) at an anode potential of −300 mV vs. Ag/AgCl using a potentiostat (VSP, Biologic, France). Potassium hexacyanoferrate (0.050 mol L^{-1}) in phosphate buffer (0.100 mol L^{-1}) at pH 7 was used as catholyte.

For each type of urine (raw and fermented), three sequential batches were performed until a stable current density was obtained. The urine fed in each cycle was pre-flushed with N_2 gas for 10 min to avoid oxygen in the anodic chamber. The anode chambers were inoculated for a final volume of 5% (v/v) with an anaerobic community previously enriched in "urine-degrading" electroactive microorganisms obtained after an acclimation process in a MFC [28]. The microbial community present in the inoculum was previously investigated by high throughput sequencing in order to identify the dominant bacterial and archaeal communities [28].

2.5. Chemical analysis and measurements

CH_4 produced during the anaerobic digestion assays was analyzed in a gas chromatograph (Chrompack 9000) equipped with a FID detector and a Chromosorb 101 (80e120 mesh, 2 m x 1/8") column, using N_2 as carrier gas (30 mL min^{-1}). The column temperature was 35 °C, the injector temperature was 110 °C and the detector temperature was 220 °C.

VFAs were determined by high-performance liquid chromatography (HPLC) (Jasco, Japan) equipped with a UV detector (210 nm) and a Chrompack column (6.5 × 30 mm^2) at 60 °C and using sulfuric acid (0.5 mol L^{-1}) as mobile phase at a flow rate of 0.6 mL min^{-1}.

The MECs performance was assessed by monitoring sCOD, NH_4^+-N, nitrate (NO_3^-) and sulphate (SO_4^{2-}) concentrations using Hach-Lange cuvette tests (Hach Lange, Düsseldorf, Germany) and a spectrophotometer DR 2800 (Hach Lange, Düsseldorf, Germany). All samples were previously centrifuged at 10 000 rpm for 5 min. For each batch performed, the sCOD removal efficiency (%) was calculated from the difference in measured COD of the influent and effluent of the anode chamber. The sCOD removal rate (g L^{-1} d^{-1}) was determined from the difference in the measured COD of the influent and effluent of the anode chamber normalized by the hydraulic retention time (d). The TAN removal efficiency (%) was calculated from the difference of the measured TAN concentration in the influent and effluent of the anode chamber. The TAN removal rate (g L^{-1} d^{-1}) was determined from the difference of the measured TAN in the influent and effluent of the anode chamber normalized by the hydraulic retention time (d). Ammonia nitrogen (NH_3-N) was calculated based on NH_4^+-N concentration and pH according to equation (4), where NH_3-N and NH_4^+-N were expressed in mg L^{-1} [29].

$$NH_3 - N = \frac{NH_4^+ - N \times 10^{pH}}{exp(\frac{6344}{273+37}) + 10^{pH}} \quad (4)$$

Current density was determined based on projected surface area of the anode (22 cm^2). CE was determined from the measured current and the removed sCOD as described by Logan and co-workers [30].

^{1}H NMR spectroscopy was used to study the course of the biodegradation of the organic matter during MECs operation. Furthermore, the NMR analysis allowed to compare the initial composition of raw and fermented urines. Samples from both MECs were taken at different times (0, 3 and 42 days) and analyzed by NMR using a Bruker Avance II 400 MHz spectrometer. The main functional groups were identified by liquid phase ^{1}H NMR spectroscopy. Deuterated water (D_2O) was used as the solvent.

Statistical significance analysis by using a single factor analysis of variance (ANOVA) was used to compare the results obtained for the different urines (raw and fermented). Statistical significance was established at the $p < 0.05$ level.

3. Results and discussion

3.1. Anaerobic biodegradability of urine

Anaerobic biodegradability batch tests were used to assess the BMP potential of human urine. Urine used in anaerobic biodegradability assays had an initial COD concentration of 10.43 ± 0.06 g L^{-1} and an initial sCOD concentration of 8.74 ± 0.56 g L^{-1}. The initial NH_4^+-N and NH_3-N concentrations were 3.32 ± 0.08 g L^{-1} and 1.88 ± 0.05 g L^{-1}, respectively, which represents a total ammonia nitrogen (TAN, *i.e.* sum of NH_3-N and NH_4^+-N) concentration of 5.20 g L^{-1}. Initial pH of urine was 9.01 ± 0.03. These results are in the same range of values reported in previous works using human urine [1,2,31]. The profile of cumulative CH_4 production is shown in Fig. 1 whereas Table 1 reports the specific CH_4 production and MP obtained during these experiments. The best scenario in terms of specific CH_4 production was observed for a COD concentration of 0.5 g L^{-1} with a specific CH_4 production of

299 ± 43 L kg^{-1} (COD) which corresponds to a MP of 86 ± 12%.

At a COD concentration of 1 g L^{-1}, a BMP of 160 ± 51 L kg^{-1} (COD) corresponding to a MP of 46 ± 14% was obtained. Similar MP of 47 ± 9% was obtained with a COD concentration of 2 g L^{-1}, corresponding to a BMP of 165 ± 33 L kg^{-1}. These results showed a decrease of almost 50% in the CH_4 production for assays with 1 g L^{-1} of COD or more. These assays exhibited a final TAN concentration higher than 1 g L^{-1}. Total inhibition of CH_4 production was observed for assays with 5 g L^{-1} of COD (lower than the exhibited by the blank due to degradation of residual substrate present in the inoculum) which showed a final TAN concentration of 4.3 g L^{-1}. Colón and co-workers, reported a specific CH_4 production of 437 L kg^{-1} (COD) at a TAN concentration of 3 g L^{-1} using undiluted simulant human excreta [31]. The same authors described a strong inhibition of CH_4 production with the increase of TAN concentration. They reported a decrease of 66% at 5 g L^{-1}, a decrease of 86% at 8 g L^{-1} and a decrease of 90% at 10 g L^{-1}. According to Yenigün and co-workers, TAN concentrations between 1.7 g L^{-1} and 1.8 g L^{-1} can cause the failure of the anaerobic digestion [32].

For all concentrations of COD tested, an increase of the TAN concentration at the end of the batch assay was observed. This increase was mainly due to the urea hydrolysis by an enzyme (urease) into carbon dioxide (CO_2) and NH_3/NH_4^+. Furthermore, NH_3-N concentration detectable at the end of the batch assays reached inhibitory levels. Around 1 g L^{-1} of NH_3–N was obtained for the assays with 5 g L^{-1} of COD. According to Hansen and co-workers, NH_3-N concentrations between 0.1 g L^{-1} and 1.1 g L^{-1} have been reported for initial inhibition of unadapted microorganisms [33]. Simultaneously, it is possible to observe that the pH value increased during the assay at 5 g L^{-1} of COD from 7 (initial pH) to 8.5 (Table 1).

With decreasing in dilution of the urine, which corresponds to increase in COD and TAN loading, an increase of inhibition effects was observed. This inhibition was not only seen by the decreasing values of BMP and MP but also by the VFA accumulation. In our experiments, acetic acid was identified as the main VFA reaching a concentration of 2.55 g L^{-1} for batch assay with 5 g L^{-1} of COD (Table 1). Franke-Whittle and co-workers demonstrated that acetic acid concentrations above 0.78 g L^{-1} could indicate process instability [34]. Furthermore, sCOD accumulation was also observed during batch operation with 5 g L^{-1} of COD.

Although CH_4 production was inhibited at high concentrations of TAN and COD, the VFA accumulation, mainly acetate, suggested that urine was fermented during the anaerobic digestion. Thus, anaerobic batch digestion was used as a pre-treatment step to ferment urine and enrich it with VFAs.

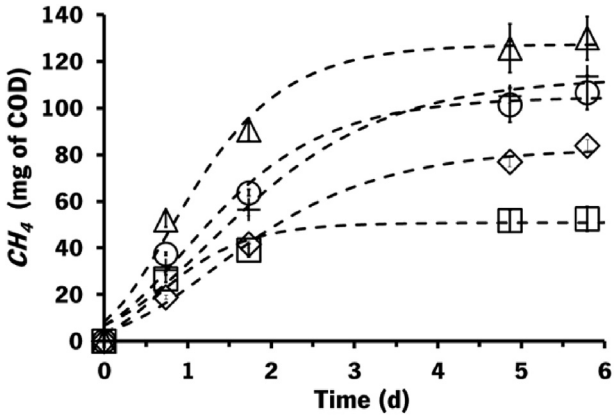

Fig. 1. Cumulative methane production during anaerobic biodegradability assays of urine at COD concentrations of: (◊) 0 g L^{-1} (Blank); (+) 0.5 g L^{-1}; (O) 1 g L^{-1}; (Δ) 2 g L^{-1}; (□) 5 g L^{-1}. The symbols represent the experimental data with respective standard deviation (n = 3 points). The lines represent the predicted data obtained by the modified Gompertz equation.

Table 1
Methane production parameters predicted by the modified Gompertz equation and experimental results obtained at the end of anaerobic biodegradability batch assays.

tCOD	g L^{-1}	0.5	1	2	5
BMP	L kg^{-1}	299 ± 43	160 ± 51	165 ± 33	–
MP	%	86 ± 12	46 ± 14	47 ± 9	–
R_m	mg d^{-1}	34.7	40.7	61.2	31
R^2	–	0.99	0.98	0.99	0.97
pH	–	7.55	7.70	8.00	8.45
sCOD	g L^{-1}	0.81	0.87	0.98	7.83
NH_4^+-N	g L^{-1}	0.50 ± 0.12	0.98 ± 0.06	1.85 ± 0.12	3.39 ± 0.30
NH_3-N	g L^{-1}	0.02 ± 0.04	0.06 ± 0.02	0.21 ± 0.04	0.91 ± 0.01
VFA	g L^{-1}(COD)	n.d.	0.53 ± 0.11	0.45 ± 0.05	2.67 ± 0.55
Acetic acid	g L^{-1} (COD)	n.d.	0.53 ± 0.11	0.43 ± 0.02	2.55 ± 0.55

n.d. not determined.

3.2. MECs performance using raw versus fermented urine

The anodic chamber of each MEC was fed with raw urine (non-fermented) and fermented urine. Table 2 shows the chemical composition of the fed urines. The results showed that fermented urine presents higher sCOD and TAN concentrations compared with raw urine. Concentration of TAN increase in the fermented urine possibly due to urea hydrolysis during the anaerobic digestion pre-treatment process into NH_3/NH_4^+ and carbamate followed by the subsequent carbamate decomposition into NH_3/NH_4^+ and bicarbonate (HCO_3^-) [35]. As a result of the decomposition of urea, the pH of the urine increased from 7.8 to 9.0 (Table 2). Simultaneously, an increase on sCOD concentration was observed in fermented urine. Furthermore, compared to raw urine, fermented urine showed higher concentration of VFAs, namely acetic acid due to the conversion of complex organic compounds into acetate.

3.3. Current generation

The current density generated in the MECs during three sequential batches using raw and fermented urine are shown in Fig. 2.

The shape of the current density profiles was very similar for both MECs. An initial current density peak was observed, with a visible difference between maximum current density reached in both MECs. A peak current density of 836 ± 286 mA m^{-2} was observed for the MEC fed with raw urine, and a peak current density of 1303 ± 717 mA m^{-2} was observed for MEC fed fermented urine. These current density peaks occurred immediately after the urine replacement, suggesting that the electron transfer is independent from the planktonic bacteria and the bacteria attached to

Table 2
Characterization of raw and fermented human urine used as feedings in the microbial electrolysis cells (MECs) (n = 3).

		Raw urine	Fermented urine
sCOD	g L^{-1}	5.17 ± 0.71	8.74 ± 0.56
NH_4^+-N	g L^{-1}	0.44 ± 0.11	5.35 ± 0.64
NH_3-N	g L^{-1}	0.03 ± 0.11	2.73 ± 0.64
SO_4^{2-}	g L^{-1}	0.87 ± 0.06	0.26 ± 0.01
NO_3^--N	mg L^{-1}	7.78 ± 0.11	11.15 ± 1.20
pH	–	7.76 ± 0.05	8.90 ± 0.07
VFA	g L^{-1} (COD)	3.15 ± 0.44	5.72 ± 0.75
Acetic acid	g L^{-1} (COD)	2.78 ± 0.27	4.70 0.75

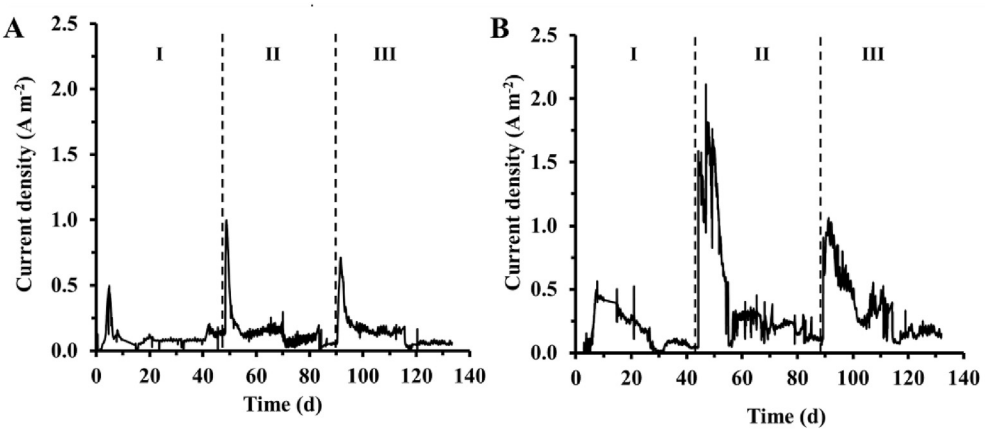

Fig. 2. Current density during experiment on microbial electrolysis cells fed with A) raw urine and B) fermented urine for three sequential batches (I, II and III).

the electrode is possibly the main responsible for current generation [36]. VFA analysis suggest that the initial current peaks were related to the consumption of easily biodegradable compounds such as acetate. Until 13 ± 2 days of operation, an acetate removal rate of $0.15 \pm 0.02\ g\ L^{-1}\ d^{-1}$ was observed for both MECs which corresponds to an acetate removal of 34% for MEC with raw urine ($2.78 \pm 0.27\ g\ L^{-1}$ to $1.84 \pm 0.06\ g\ L^{-1}$) and 40% for MEC with fermented urine ($4.71 \pm 0.75\ g\ L^{-1}$ to $2.83 \pm 0.04\ g\ L^{-1}$). The remaining 53% of acetate removed on MEC with raw urine ($1.84 \pm 0.06\ g\ L^{-1}$ to $0.37 \pm 0.19\ g\ L^{-1}$) and 26% on MEC with fermented urine ($2.83 \pm 0.04\ g\ L^{-1}$ to $1.60 \pm 0.14\ g\ L^{-1}$) occurred over the subsequent 25 ± 5 days of operation. After the initial current peaks, a plateau in current density was observed. Significantly higher average current density was generated from fermented urine ($218 \pm 6\ mA\ m^{-2}$) (Fig. 2B) compared with average current density produced from raw urine ($107 \pm 1\ mA\ m^{-2}$) (Fig. 2A) ($p < 0.05$).

3.4. MECs efficiency

Fig. 3A and B shows the removal efficiencies obtained during the MECs operation. No significant differences on COD removals between both MECs were observed ($p < 0.05$). COD removals were $65 \pm 10\%$ in MEC using raw urine and $54 \pm 3\%$ in MEC using fermented urine. However, MEC using fermented urine displayed a significantly higher COD removal rate ($0.14 \pm 0.02\ g\ L^{-1}\ d^{-1}$) compared with the MEC using raw urine ($0.08 \pm 0.02\ g\ L^{-1}\ d^{-1}$) ($p < 0.05$). This may be explained by the greater sCOD concentration present in the fermented urine.

Although, no significant differences were observed for sCOD removal between both MECs, the higher current density produced on MEC using fermented urine was a result of the higher CE obtained and the higher sCOD at the start of each batch. CE was determined during the operation at the stable current density generation. CE of $14 \pm 2\%$ was obtained for the MEC using raw urine and a CE of $17 \pm 1\%$ was obtained for the MEC using fermented urine. For both MECs the CEs obtained were low, although are comparable to other studies dealing with complex wastewaters with fermentable substrates. Cerrillo and workers reported a CE of 18% using digested pig slurry in a MEC at an anode potential of −100 mV vs. SHE whereas Kuntke and co-workers describes a CE of 10% using raw urine in a MFC [6,19]. Fig. 3 shows the changes in sCOD, TAN, and pH during operation of the MECs.

The MEC using raw urine demonstrated a negative TAN removal efficiency and an effluent TAN concentration of $1985 \pm 420\ mg\ L^{-1}$ (Fig. 3A). The negative TAN removal efficiency can be explained by the urea hydrolysis, which increase the NH_4^+-N concentration in the anode chamber. Urea is hydrolyzed by an enzyme, namely urease, into CO_2 and ammonia increasing the urine pH from 7.7 to 9 during the first 7 days of operation (Fig. 3A). On day 4, a maximum TAN concentration of $8376 \pm 170\ mg\ L^{-1}$ and an increase in the anolyte pH were observed for MEC A (Fig. 3A), which also suggests that urea was hydrolyzed inside the MEC during the first 4 days of operation. Using the TAN concentration after 4 days as the starting concentration, a TAN removal efficiency of 76% was determined in MEC A, which corresponds to a TAN removal rate of $0.15\ g\ L^{-1}\ d^{-1}$. The TAN removal efficiency for the MEC using fermented urine was 90%, which corresponds to a TAN removal rate of $0.17\ g\ L^{-1}\ d^{-1}$ and to an effluent TAN concentration of $791 \pm 127\ mg\ L^{-1}$ (Fig. 3B). In this

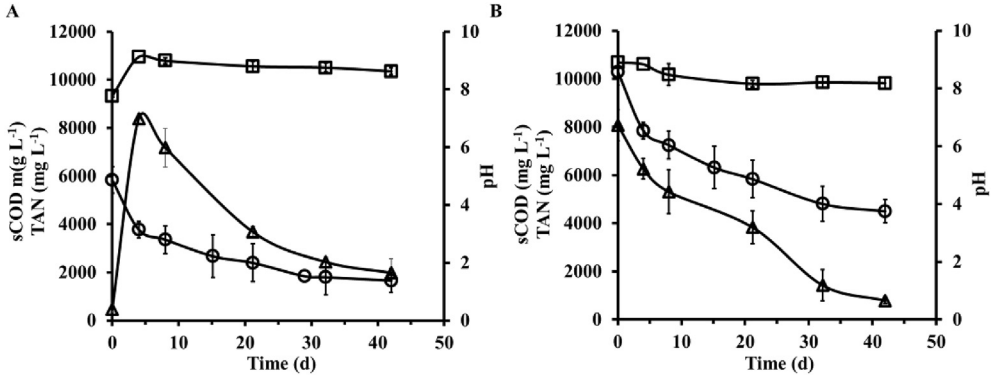

Fig. 3. Averages over the three batches of sCOD (○), TAN (Δ) and pH (□) during experiment on microbial electrolysis cells fed with A) raw urine and B) fermented urine.

study, the NH_4^+-N removal efficiency of 87% (final concentration of 678 ± 127 mg L^{-1}) was higher than the NH_4^+-N removal reported by Kuntke and co-workers who reported an ammonium removal efficiency of $34 \pm 2\%$ using 5x diluted raw urine at an applied cell voltage of 1.0 V vs Ag/AgCl [8]. Statistical analysis confirmed that the TAN removal was significantly different when comparing MEC A and MEC B ($p < 0.05$).

Further, the concentration of NO_3^--N (Table 2) decrease over time, with a final concentration of 4.66 ± 0.14 mg L^{-1} and 5.90 ± 0.76 mg L^{-1}, which corresponds to a NO_3^--N removal of 40% in MEC A (raw urine) and 46% MEC B (fermented urine). Nitrate is an alternative electron acceptor that might be reduced by denitrifying bacteria, thus reducing the CE of the system. The presence of other alternative electron acceptors such as SO_4^{2-} (Table 2) also contribute to the low efficiency obtained. The low CE obtained for both MECs demonstrated that further improvements are needed to improve the conversion of the chemical energy into electrical energy efficiency.

3.5. ^1H NMR analysis

The degradation of organic compounds during the second MEC-batch was studied. Samples from both MECs working with raw and fermented urine were taken at different times (0, 3 and 42 days) and ^1H NMR analyses were performed to study the course of the sCOD biodegradation from the urine. The NMR spectra of the samples taken from both MECs are shown in Fig. 4. D_2O (used as the solvent) peak region (4.8 ppm) was excluded from all the spectra.

The spectra of initial raw urine, which corresponds to day 0 was dominated by signals in the aromatic region (6.5–8 ppm) and in the aliphatic region ascribed to protons attached to O or N (2.5–6.5 ppm). Furthermore, some signals were detected in the aliphatic region (0–3 ppm). The main signals initially detected were at 5.80, 4.08, 3.07 and 1.94 ppm corresponding to the aliphatic region (Fig. 4A/4B). According to Bouatra and co-workers urea and creatinine are two of the principal compounds present in urine [17]. In the ^1H NMR analysis both compounds were identified (urea at 5.08 ppm and creatinine at 4.08 ppm and 3.07 ppm). The signal attributed to urea disappear after the third day of operation supporting the hypothesis that TAN concentration increase in the anode chamber due to urea hydrolysis. Also the signals attributed to creatinine decrease during the first three days of operation. The reduction in the urea and creatinine peaks were possibly due to the presence of microorganisms assigned to *Corynebacterium* and *Tissierella* genus identified in the sludge used to inoculate the MECs which was firstly acclimated in a MFC [28]. Some *Corynebacterium* species have been described as urease positive that catalyze the hydrolysis of urea into CO_2 and ammonia whereas some species of *Tissierella* genus are responsible for metabolize creatinine producing acetate, methylamine, ammonia and CO_2 [37,38]. Simultaneously with the reduction of creatinine peak, an increase in the acetate peak was observed (1.94 ppm) on day 3 due to the conversion of complex organic compounds with a subsequent decrease during the operation (day 42) due to its consumption.

The spectra of initial fermented urine, which corresponds to day 0, was dominated by signals in the aliphatic region (0–3 ppm), namely at 2.60, 1.92 and 1.06 ppm (Fig. 4C/4D). The presence of numerous peaks in the aliphatic region indicates a high biodegradability of the organic compounds found in fermented urine. Urea and creatinine that are two of the main compounds present in urine were not identified in fermented urine, suggesting that during the anaerobic digestion these compounds were hydrolyzed. This observation is in accordance with the increase of TAN concentration during anaerobic digestion. Furthermore, methylamine

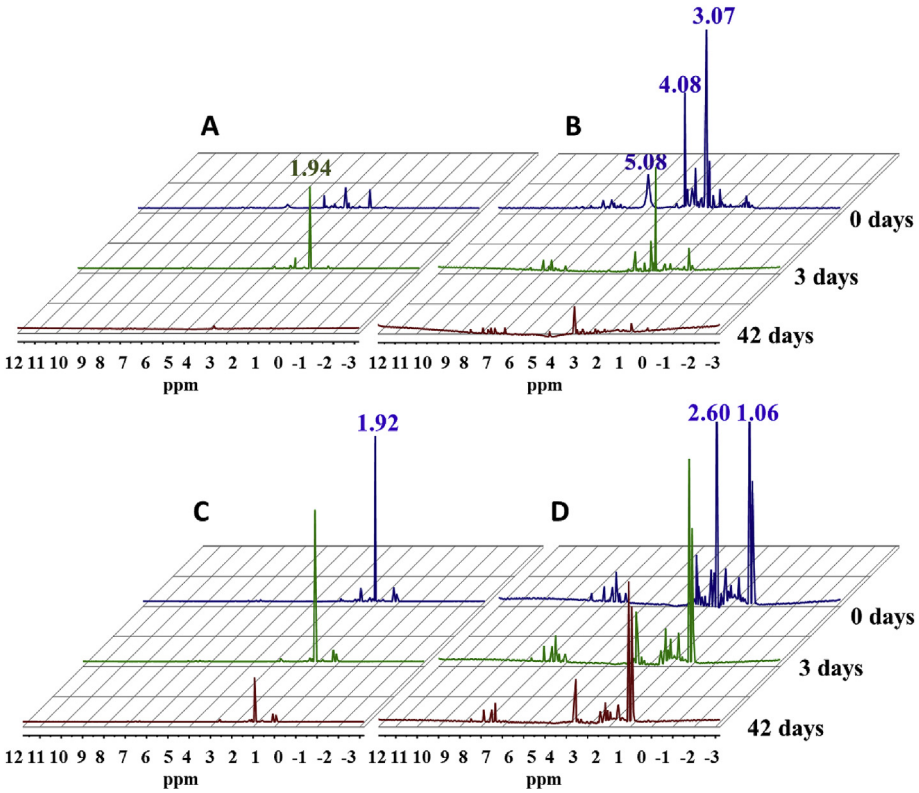

Fig. 4. Proton nuclear magnetic resonance (^1H NMR) spectral data of A) raw urine; B) raw urine after suppression of acetate peak (1.94 ppm); C) fermented urine and D) fermented urine after suppression of acetate peak (1.92 ppm).

(2.60 ppm) identified in fermented urine is a result of creatinine hydrolysis. According to the NMR spectra, its peak almost disappear on day 3 of operation. The peak corresponding to acetate (1.92 ppm) is higher than the one detected in the raw urine demonstrating that complex organic compounds where converted to acetate during the fermentation process. Similar to the spectra of samples taken from the MEC using raw urine, the acetate peak decrease over time evidencing its consumption. Furthermore, and contrary to the raw urine, propionate (1.06 ppm) was identified in the fermented urine demonstrating the success of the fermentation process. According to the NMR spectra, also the propionate peak decrease demonstrating its consumption during the operation. Electricity production from propionate oxidation in a MFC was previously reported by Jang and co-workers [14].

4. Conclusions

Anaerobic digestion was implemented to convert fermentable compounds commonly present in urine into simple compounds, namely VFAs that are directly converted by the EAB to electricity. Therefore, the anodic performance of a MEC was effectively improved through the application of fermented urine, obtained after anaerobic digestion, in comparison to the use of raw urine. The efficiency of the anaerobic digestion process was evaluated through ^{1}H NMR analysis, which showed acetate, propionate and methylamine as the major compounds fermented urine. In opposition, raw urine exhibited as main compounds: urea, creatinine but also acetate. MEC fed with fermented urine produced higher current density and demonstrated higher CE and higher COD removal rate. Moreover, higher current on MEC using fermented urine allowed higher NH_4^+-N removal. Consequently, it can be concluded that the integration of anaerobic digestion of urine with MEC in a two-stage operation is an effective option for the treatment of urine using BES. This provides an insight into the methodology to effectively treat effluents loaded with complex substrates to obtain a polished effluent and enhance the power production of BES.

Acknowledgments

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469 unit and COMPETE 2020 (grant number POCI-01-0145-FEDER-006684) and BioTecNorte operation (grant number NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

This research has received funding from the European Union's Seventh Programme for research, technological development and demonstration (grant number 308535). The authors also would like to acknowledge the support of Wetsus, European Centre of Excellence for Sustainable Water Technology.

References

[1] T.A. Larsen, W. Gujer, Separate management of anthropogenic nutrient solutions (human urine), Water Sci. Technol. 34 (1996) 87–94.
[2] M. Maurer, W. Pronk, T.A. Larsen, Treatment processes for source-separated urine, Water Res. 40 (2006) 3151–3166.
[3] J. Wilsenach, M. van Loosdrecht, Impact of separate urine collection on wastewater treatment systems, Water Sci. Technol. 48 (2003) 103–110.
[4] E. Desmidt, K. Ghyselbrecht, A. Monballiu, K. Rabaey, W. Verstraete, B.D. Meesschaert, Factors influencing urease driven struvite precipitation, Separ. Purif. Technol. 110 (2013) 150–157.
[5] S.G. Barbosa, L. Peixoto, B. Meulman, M.M. Alves, M.A. Pereira, A design of experiments to assess phosphorous removal and crystal properties in struvite precipitation of source separated urine using different Mg sources, Chem. Eng. J. 298 (2016) 146–153.
[6] P. Kuntke, K.M. Smiech, H. Bruning, G. Zeeman, M. Saakes, T.H.J.A. Sleutels, H.V.M. Hamelers, C.J.N. Buisman, Ammonium recovery and energy production from urine by a microbial fuel cell, Water Res. 46 (2012) 2627–2636.
[7] H.V.M. Hamelers, A. Ter Heijne, T.H.J.A. Sleutels, A.W. Jeremiasse, D.P.B.T.B. Strik, C.J.N. Buisman, New applications and performance of bioelectrochemical systems, Appl. Microbiol. Biotechnol. 85 (2010) 1673–1685.
[8] P. Kuntke, T.H.J.A. Sleutels, M. Saakes, C.J.N. Buisman, Hydrogen production and ammonium recovery from urine by a Microbial Electrolysis Cell, Int. J. Hydrogen Energy 39 (2014) 4771–4778.
[9] A. Escapa, M.I. San-Martín, A. Morán, Potential use of microbial electrolysis cells in domestic wastewater treatment plants for energy recovery, Front. Energy Res. 2 (2014) 1–10.
[10] M. Rahimnejad, A. Adhami, S. Darvari, A. Zirepour, S.-E. Oh, Microbial fuel cell as new technology for bioelectricity generation: a review, Alexandria Eng. J. 54 (2015) 745–756.
[11] P.T. Kelly, Z. He, Nutrients removal and recovery in bioelectrochemical systems: a review, Bioresour. Technol. 153 (2014) 351–360.
[12] M. Rodríguez Arredondo, P. Kuntke, A.W. Jeremiasse, T.H.J.A. Sleutels, C.J.N. Buisman, A. Ter Heijne, Bioelectrochemical systems for nitrogen removal and recovery from wastewater, Environ. Sci. Water Res. Technol. 1 (2015) 22–33.
[13] H. Liu, S. Cheng, B.E. Logan, Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell, Environ. Sci. Technol. 39 (2005) 658–662.
[14] J.K. Jang, I.S. Chang, H.Y. Hwang, Y.F. Choo, J. Lee, K.S. Cho, B.H. Kim, K.H. Nealson, Electricity generation coupled to oxidation of propionate in a microbial fuel cell, Biotechnol. Lett. 32 (2010) 79–85.
[15] K.-J. Chae, M.-J. Choi, J.-W. Lee, K.-Y. Kim, I.S. Kim, Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells, Bioresour. Technol. 100 (2009) 3518–3525.
[16] D. Putnam, Composition and Concentrative Properties of Human Urine, 1971. Washington, D.C.
[17] S. Bouatra, F. Aziat, R. Mandal, A.C. Guo, M.R. Wilson, C. Knox, T.C. Bjorndahl, R. Krishnamurthy, F. Saleem, P. Liu, Z.T. Dame, J. Poelzer, J. Huynh, F.S. Yallou, N. Psychogios, E. Dong, R. Bogumil, C. Roehring, D.S. Wishart, The human urine metabolome, PLoS One 8 (2013), e73076.
[18] W.W. Li, H.Q. Yu, From wastewater to bioenergy and biochemicals via two-stage bioconversion processes: a future paradigm, Biotechnol. Adv. 29 (2011) 972–982.
[19] M. Cerrillo, J. Oliveras, M. Viñas, A. Bonmatí, Comparative assessment of raw and digested pig slurry treatment in bioelectrochemical systems, Bioelectrochemistry 110 (2016) 69–78.
[20] A.E. Inglesby, A.C. Fisher, Downstream application of a microbial fuel cell for energy recovery from an Arthrospira maxima fed anaerobic digester effluent, RSC Adv. 3 (2013) 17387–17394.
[21] K.R. Fradler, J.R. Kim, G. Shipley, J. Massanet-Nicolau, R.M. Dinsdale, A.J. Guwy, G.C. Premier, Operation of a bioelectrochemical system as a polishing stage for the effluent from a two-stage biohydrogen and biomethane production process, Biochem. Eng. J. 85 (2014) 125–131.
[22] Z. Ge, F. Zhang, J. Grimaud, J. Hurst, Z. He, Long-term investigation of microbial fuel cells treating primary sludge or digested sludge, Bioresour. Technol. 136 (2013) 509–514.
[23] P. Kuntke, T.H.J.A. Sleutels, M. Rodríguez Arredondo, S. Georg, S.G. Barbosa, A. Ter Heijne, H.V.M. Hamelers, C.J.N. Buisman, (Bio)electrochemical ammonia recovery: progress and perspectives, Appl. Microbiol. Biotechnol. 2 (2018) 3865–3878.
[24] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, J.B. Van Lier, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays, Water Sci. Technol. 59 (2009) 927–934.
[25] J.C. Costa, P.R. Gonçalves, A. Nobre, M.M. Alves, Biomethanation potential of macroalgae Ulva spp. and Gracilaria spP. and in co-digestion with waste activated sludge, Bioresour. Technol. 114 (2012) 320–326.
[26] J.C. Costa, S.G. Barbosa, M.M. Alves, D.Z. Sousa, Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter, Bioresour. Technol. 111 (2012) 141–147.
[27] S.G. Barbosa, L. Peixoto, O.S.G.P. Soares, M.F.R. Pereira, A. Ter Heijne, P. Kuntke, M.M. Alves, M.A. Pereira, Influence of carbon anodes properties on performance and microbiome of microbial electrolysis cells operated on urine 267 (2018) 122–132.
[28] S.G. Barbosa, L. Peixoto, A. Ter Heijne, P. Kuntke, M.M. Alves, M.A. Pereira, Investigating bacterial community changes and organic substrate degradation in microbial fuel cells operating on real human urine, Environ. Sci. Water Res. Technol. 3 (2017) 897–904.
[29] J.V. Oliveira, M.M. Alves, J.C. Costa, Optimization of biogas production from Sargassum sp. using a design of experiments to assess the co-digestion with glycerol and waste frying oil, Bioresour. Technol. 175 (2015) 480–485.
[30] B.E. Logan, D. Call, S. Cheng, H.V.M. Hamelers, T.H.J.A. Sleutels, A.W. Jeremiasse, R.A. Rozendal, Microbial electrolysis cells for high yield hydrogen gas production from organic matter, Environ. Sci. Technol. 42 (2008) 8630–8640.
[31] J. Colón, A.A. Forbis-Stokes, M.A. Deshusses, Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries, Energy Sustain. Dev. 29 (2015) 57–64.
[32] O. Yenigün, B. Demirel, Ammonia inhibition in anaerobic digestion: a review, Process Biochem. 48 (2013) 901–911.

[33] K.H. Hansen, I. Angelidaki, B.K. Ahring, Anaerobic digestion of swine manure: inhibition by ammonia, Water Res. 32 (1998) 5–12.
[34] I.H. Franke-Whittle, A. Walter, C. Ebner, H. Insam, Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities, Waste Manag. 34 (2014) 2080–2089.
[35] H.L.T. Mobley, R.P. Hausinger, Microbial ureases: significance, regulation, and molecular characterization, Microbiol. Rev. 53 (1989) 85–108.
[36] D.R. Bond, D.R. Lovley, Evidence for involvement of an electron shuttle in electricity generation by Geothrix fermentans, Appl. Environ. Microbiol. 71 (2005) 2186–2189.
[37] L. Nolden, G. Beckers, B. Möckel, W. Pfefferle, K.M. Nampoothiri, R. Krämer, A. Burkovski, Urease of Corynebacterium glutamicum: organization of corresponding genes and investigation of activity, FEMS Microbiol. Lett. 189 (2000) 305–310.
[38] C. Harrns, A. Schleicher, M.D. Collins, J.R. Andreesen, Tissierella creatinophila sp. nov., a Gram-positive, anaerobic, non-spore-forming, creatinine-fermenting organism, Int. J. Syst. Bacteriol. 48 (1998) 983–993.