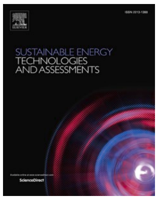
Sustainable Energy Technologies and Assessments 47 (2021) 101423

Contents lists available at ScienceDirect

Sustainable Energy Technologies and Assessments

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



Bio-electrochemical degradability of prospective wastewaters to determine their ammonium recovery potential

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ARTICLE INFO

Keywords:
Bio-electrochemical systems
Wastewater
Ammonium recovery
Recovery potential
Biodegradability

ABSTRACT

Bio-electrochemical ammonium recovery (BEAR) can close the cycle between anthropogenic emission of reactive nitrogen and energy intensive nitrogen fixation in the Haber-Bosch process. BEAR is currently limited by the bio-electrogenic degradability of the treated wastewater. Here, we investigated the degradability of blackwater, hydrolyzed human urine, cow manure and pig manure as prospective wastewaters for BEAR in a standardized experimental design.

We found that bio-electrochemical conversion efficiencies ranged from 63% (blackwater) to 42% (cow manure) and 41% (urine) to 26% (pig manure) after 5 days. These values correspond well with the relative VFA content of soluble COD for blackwater and cow manure, while additional compounds must have been converted for urine and pig manure.

The degradability of blackwater and cow manure was sufficiently high to theoretically be able to remove all TAN already after < 0.5 d. The actual recovery potential (consisting of conversion efficiency and COD/TAN ratio) of pig manure was just high enough to remove all TAN. Human urine would require additional electron donor to remove all TAN in BEAR. Therefore, combining the maximum recovery potential with the relative VFA content of soluble COD can give a good estimate of the actual recovery potential of a wastewater.

Introduction

An increasing world population will require higher agricultural output and therefore more fertilizer to sustain human food production [1]. At the same time, high concentrations of nutrients in wastewater need to be removed to protect waterbodies from eutrophication. Therefore, nutrient recovery from wastewaters is important to create a more sustainable future. For example, recovering ammonia from wastewaters via gas stripping in sulfuric acid can provide ammonium sulfate as a nitrogen fertilizer [2].

Bio-electrochemical Systems (BESs) are sustainable technologies to recover ammonium (NH_4^+) as part of total ammonia nitrogen (TAN) at low energy cost [3,4]. In BESs, bio-degradable organic compounds in wastewaters are converted into electricity, thereby driving ammonium across a cation exchange membrane (CEM) and separating it from the wastewater. This separated TAN can then be recovered from the catholyte, i.e. by using a membrane distillation process [5,6]. While bio-electrochemical ammonium recovery (BEAR) provides an elegant solution to both pollutant removal and nutrient recovery, its key challenge lies in the production of sufficient electrical current to separate the TAN from the wastewater [4]. Ideally, one electron as electrical current can transport one positive charge of cations across the membrane. Therefore, the ratio of charge of electrons as electricity over charge of ammonium loaded can be expressed as load ratio and give a good estimate for expected TAN removal efficiency and threshold for optimal system operation [7].

In BES, this current depends on the availability of oxidizable matter (measured in chemical oxygen demand, COD) in the wastewater, meaning the available amount, and how much of it can be converted into electricity by bacteria, the electrogenic biodegradability. Furthermore, not all biodegraded COD is converted into electricity due to competing processes and electron acceptors (metal ions, sulfate, nitrate, methane, aerobic respiration, microbial growth and secondary metabolites) and toxicity, as from free ammonia nitrogen or heavy metals,

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https://doi.org/10.1016/j.seta.2021.101423
Received 30 September 2020; Received in revised form 25 April 2021; Accepted 17 June 2021

might hamper the resulting current production and block degradation pathways [8,9].

Several wastewater streams have been investigated experimentally for ammonium recovery using BESs and potential wastewaters have been identified from theoretical considerations [4,10–13]. Yet, these theoretical examinations cannot account for all aspects of suitability of potential wastewaters for BEAR and experimental investigations lack comparability. This lack in comparability is due to the fact that these experimental studies vary in system parameters such as initial pH, pH buffering, temperature, liquid and gas retention times, reactor design, electrode materials, as well as ratios of microbial available electrode surface area to electrolyte volume [14,15]. Furthermore, comparison of bio-anodes from different studies is difficult due to differences in bio-anode performances and biofilm acclimation and adaption strategies [16] as well as the use of non-comparable inocula [17].

All these reasons make a comparable experimental investigation of wastewaters for BEAR suitability necessary. Other bio-degradation tests, such as biochemical oxygen demand (BOD) and biochemical methane potential (BMP), cannot be used to estimate bio-electrogenic degradability since the involved microbial consortia differ from those on bio-anodes. Hence, a standardized method for bio-electrochemical degradation is still needed. Making bio-electrochemical tests comparable would require a standardized and optimized BES that should control pH, organic loading, and avoid conversions to other electron acceptors, such as methane and sulfate, that compete with bio-electrogenesis. The anode potential serves as controlled thermodynamic driving force for the conversion at the anode [8], while using a comparable, adapted and acclimatized microbial community in the bio-anode would serve comparability and reproducibility [18].

This study analyzes the suitability of wastewaters for BEAR in a comparable manner, based on wastewater characteristics and bio-electrogenic degradation tests combined with calculated recovery potentials. The degradability of potential wastewaters for BEAR was tested in simple, reproducible BES. Resulting bio-electrogenic substrate conversion efficiencies were selected for comparability based on reproducibility criteria. The same mixed inoculum was used for the degradation of each wastewater, and the microbial community was allowed to acclimate and adapt to the respective wastewater.

The results from the degradation experiments were used to assess the recovery potential in BEAR for each wastewater.

Materials and methods

BES design

The experiments were performed in H-type cells (see Supplementary material S1) [19]. Each H-type cell consisted of two 325 mL cylindrical glass compartments (Laboratory Glass Specialists B.V., the Netherlands). Each compartment had a short tubular opening on its lower side to connect both compartments with a flange connection. A bipolar membrane (BPM; 7 cm^2, Ralex membrane, MEGA, Czech Republic) was clamped in between both flange connectors and sealed off with rubber O-rings. The bipolar membrane was oriented with the anion exchange side towards the bio-anode to neutralize the produced protons during bio-electrogenic conversion. Opposite to the flange opening in each compartment were three smaller, vertically aligned flange openings, which were sealed off with rubber stoppers and metal caps. These were used to insert a Luggin capillary (Laboratory Glass Specialists B.V., the Netherlands) including the 3 M Ag/AgCl reference electrode (QM711X, QIS, the Netherlands) together with the 3-way liquid sampling port (Servoprax, Germany) and the connecting wire of the bioanode, from bottom to middle opening, respectively. The top of each compartment had a GL45 opening that was closed with a rubber stopper and screwing lids with silicon inlay.

The anode consisted of a 4 cm × 10 cm carbon felt (SGL Carbon, Germany) of approximately 0.3 cm thickness. The cathode was made of a 4 cm × 10 cm Pt coated Ti mesh (0.5 mg/cm^2, Dexmet, Ct, USA). Both electrodes were bent on their longest edge to form half circles with an opening towards the BPM. Both anode and cathode compartments were stirred with magnetic stirring bars at 200 rpm.

All potentials in this work are reported versus 3 M KCl type Ag/AgCl reference electrodes (+205 mV vs NHE).

Media

Four different wastewater were investigated: Hydrolyzed human urine, black water, cow manure, and pig manure.

Hydrolyzed human urine from source separated male urinals from the Wetsus institute was drawn from a collection tank of 600 L with an HRT of 20 days at 0.5 L/min mixing. Source separated black water (toilet water) samples were collected from a neighborhood equipped with vacuum toilets in Sneek (The Netherlands). The liquid fraction of dairy manure was obtained after passing a screw press at a dairy farm in the area of Groningen (The Netherlands). The pig manure was taken directly from the pit at a farm in Friesland (The Netherlands). The dairy and pig manure were sieved (200 μm) to remove coarse solids. All wastewaters were locally stored in closed containers at 3 °C prior to use.

All wastewaters were diluted to 0.5 g_{COD}/L in 0.15 L to serve as anolyte and provide the same standardized amount of substrate to all bio-anodes. Phosphate buffer (PB) of 50 mM was added to the anolyte to achieve a stable and buffered pH between 7.5 and 8. The PB composed of K_2HPO_4 and KH_2PO_4 varied for each wastewater due to differing buffer capacities (see Supplementary material S2). A substrate concentration of 0.5 g_{COD}/L was chosen not only to have the same initial concentration, but also to avoid competing conversions [8,19,20]. The catholyte consisted of 50 mM PB at pH 7.1.

Black water, cow manure and pig manure were also paper filtered (Whatman black ribbon ashless filter paper, grade 598: 8–10 μm) prior to dilution in BESs. This lowered retention of mostly undegradable particulate COD, as well as possible clogging due to solids accumulation, membrane fouling and bio-anode passivation due to formation of a layer of solids on the bio-anode.

Inoculum

The mixed inoculum consisted of equal volumes of biomass samples from an anaerobic digester treating black water [21], bio-electrochemical systems fed with hydrolyzed human urine and artificial urine, containing acetate as carbon and energy source [22], syntrophic propionate oxidizer cultures [23] as well as unfiltered samples of each wastewater.

Experimental strategy

Each wastewater batch was investigated in reactor duplicates. BESs were first injected with 1% v/v of inoculum mixture to each of the differently diluted wastewaters (prepared as described in the media section). The first inoculation batch with wastewater was considered bio-anode preculturing. Each diluted and phosphate buffered wastewater batch was replaced with the same fresh solution after 7 days to allow the formation of a microbial community specialized in bio-electrogenesis from the respective wastewater in the anodic biofilm. During replacement, anodes were gently rinsed with deionized water to dilute remaining suspended microorganisms not partaking in current generation. The first batch was considered as start-up and not included in the results.

Before the start of each batch, anode potentials were controlled at −0.2 or −0.1 V vs. 3 M KCl type Ag/AgCl by a potentiostat (Ivium-N-stat, Ivium Technologies, the Netherlands) and the anode headspace was purged with N_2 for 30 min. These two potentials were chosen to test whether this driving force for current generation influenced substrate degradability. The cathode headspace was constantly flushed with N_2 to remove cathodically produced H_2 and avoid H_2 diffusion to and conversion at the bio-anode.

Reproducibility criteria applied

Since biological systems performed differently due to changes in wastewater composition and changes in the microbiome, we applied the following reproducibility criteria:

1. Values were defined as comparable when fulfilling the following equation of observed bio-electrogenic conversion efficiencies (hereafter abbreviated as conversion):

$$|\text{conversion}_{\text{batchA}} - \text{conversion}_{\text{batchB}}| < 0.1*(\text{conversion}_{\text{batchA}} + \text{conversion}_{\text{batchB}}) \tag{1}$$

   with conversions given in %. For example, if 50% COD was converted bio-electrogenically in batch A and 60% in batch B, then the results of the equation is 10% < 11%, which is a true statement, making both conversions comparable. On the contrary, if 20% is converted in batch A and 25% in batch B, then the equation results in a false statement of 5% < 4.5%, signifying that the conversions are not comparable.
2. Highest conversion efficiencies needed to be comparable for both reactor duplicates of the same wastewater batch and for at least two different batches of the same wastewater.
3. In the batches chosen by the previous criteria, the current density over time profiles generated must be similar in peak height and peak duration. Similar peak areas were found by loading the same amount of COD in each batch and applying the first two criteria.

Sampling and analysis

Sampling and analysis were conducted as described in Supplementary material S3.

Calculations

In this work we analyzed the current generation by the EAMs in order to predict the possible maximum NH_4^+ recovery (recovery potential). However, all our calculation assume two main points of a BES system to recover ammonia from wastewater:

1. The dominant transport mechanisms through the (in practice applied) CEM is migration of cations.
2. There is no build up of ammonia/ammonium in the cathode/concentrate compartment.

Hence, TAN removed from the anode/feed compartment through the CEM will be efficiently recovered from the cathode/concentrate compartment using an additional process (i.e., membrane stripping or gas stripping). This integration was shown in several publications (reviewed in [4]).

The recoverable TAN in BEAR systems is dependent on the electrical current generated from converting COD from the fed wastewater. In theory, 1 mol of electrons (e^-) can transport 1 mol of NH_4^+. The ratio of current over ammonium loading is defined in the load ratio concept [7]:

$$L_N = \frac{i_{bio}}{Q_{TAN}*z_{t_{NH_4^+}}*F} \tag{2}$$

where i_{bio} is the electrical current generated by the bio-anode in ampere (A = C/s), Q_{TAN} is the influx of TAN into the BES in $mol_{NH_4^+}/s$, $z_{t_{NH_4^+}}$ as 1 mol NH_4^+ being transported per 1 mol electrons generated as electricity, and F the Faraday number of 96,485C/mol electrons.

The bio-electrical current can be described as a conversion of the COD flux into electricity:

$$i_{bio} = \frac{[COD_f]*z_{O_2}}{M_{O_2}}*Q*F*\eta_{COD_{rem}}*CE \tag{3}$$

With $[COD_f]$ being the COD concentration after solids removal in g_{COD}/L, z_{O2} the amount of electrons (4) transferred per amount of COD in mol_{e}^{-}/mol_{O2}, M_{O2} the molar mass of oxygen at 32 g_{O2}/mol_{O2}, Q the flux of medium in L/s, $\eta_{COD_{rem}}$ the COD removal efficiency and CE the Coulombic efficiency.

The influx of TAN can be calculated as

$$Q_{TAN} = \frac{[NH_4^+]}{M_{NH_4^+}}*Q \tag{4}$$

With $[NH_4^+]$ being the TAN concentration in $g_{NH_4^+}/L$ that can be removed as NH_4^+ and $M_{NH_4^+}$ as molar mass of ammonium with 18 g_{NH4+}/mol_{NH4+}.

The TAN transport efficiency η_{TAN} is defined as the ratio of removed TAN flux across the membrane over the achieved current:

$$\eta_{TAN} = \frac{Q_{TAN,removed}}{i_{bio}} \tag{5}$$

With $Q_{TAN,removed}$ being the difference of flux between anode feed and anode effluent in mol_{TAN}/s.

The load ratio L_N in equation (2) can be rewritten as biological load ratio $L_{N,bio}$:

$$L_{N,bio} = \frac{[COD_{wastewater}]*z_{O_2}*M_{NH_4^+}}{[NH_4^+]*z_{t_{NH_4^+}}*M_{O_2}}*\eta_{COD_{rem}}*CE*\eta_{TAN} \tag{6}$$

Assuming an ideal maximum 100% for η_{CODrem}, CE and η_{TAN}, this $L_{N,bio}$ can be expressed as maximum achievable bio–electrochemical TAN recovery potential (RP_{max}, adapted from [4]) and simplified to

$$RP_{max} = 2.25*\frac{[COD_f]}{[NH_4^+]} \tag{7}$$

When η_{CODrem} and CE are not 100%, the conversion of substrate to electricity can be defined as bio-anode current achieved from the provided substrate:

$$\text{conversion} = \eta_{COD_{rem}}*CE = \frac{[COD_{rem}]}{[COD_{start}]}*\frac{\int_0^t idt*M_{O_2}}{[COD_{rem}]*z_{O_2}*V*F} \tag{8}$$

$$\therefore \text{conversion} = \frac{\int_0^t idt*M_{O_2}}{[COD_{start}]*z_{O_2}*V*F} \tag{9}$$

the electric charge in Coulomb is obtained from the integral of current over time, $[COD_{start}]$ is the starting concentration of COD of around 0.5 g_{COD}/L and V is the batch liquid volume of 0.15 L diluted wastewater. Therefore, the conversion is the combination of COD removal efficiency and Coulombic efficiency.

Combining equation (7) and (9) then gives the actual recovery potential (RP_{act}) achieved after time t with 100% assumed N-transport efficiency (η_{TAN}):

$$RP_{act} = RP_{max}*\text{conversion} \tag{10}$$

Assuming 60% N-transport efficiency as an average value suggested in literature [4] lowers the resulting RP_{max} by a factor of 0.6 and therefore would require 1.67 times the RP_{act} to be able remove all NH_4^+. This transport number is also influenced by the wastewater ion composition, among many other factors, which we did not investigate further in this study.

When assuming that only volatile fatty acids (VFAs) are converted into electricity and that at 100% COD removal efficiency, CE, and TAN transport efficiency, the relative VFA content of COD after filtration (COD_f) and the RP_{max} can indicate an expected recovery potential (RP_{exp}) of a wastewater:

$$RP_{exp} = RP_{max} * \frac{[VFA]}{[COD_f]} \qquad (11)$$

With [VFA] as the total concentration of COD present as VFAs in g_{COD} (calculated according to Supplementary material S4).

Results and discussion

Wastewater compositions and implications

Several wastewaters were investigated because of their high COD and TAN concentrations, which makes them attractive for BEAR. These wastewaters were: source separated human black water, hydrolyzed human urine, cow manure and pig manure. They were characterized in terms of solids, pH, predominant buffer components (TAN and inorganic carbon), total COD (COD_t) and COD after paper filtration (COD_f), volatile fatty acids (VFAs) and acetate concentrations (Table 1).

All wastewaters contained more than 1 g/L of NH_4^+. Furthermore, all wastewaters contained more than 3 g/L of COD that may be converted into electricity. In addition, black water, cow and pig manure contain high amounts of solids that may make solids removal by filtering necessary. Also, the pH of most wastewaters is sufficiently close to the physiological optimum pH of 7 for electro-active microorganisms (EAMs). This is the case for all wastewaters except hydrolyzed urine, where the pH is above 9 and highly buffered by carbonate and TAN. This buffering at high pH, together with an increased NH_3 toxicity at that pH, may limit EAM activity [24].

The compositions of the different wastewaters reported here are mostly comparable to those found in literature [25–29]. Slight differences in inorganic carbon (IC), NH_4^+, COD and acetate could be the result of seasonal effects on the wastewater as well as the filtration method due to volatilization.

Assuming that acetate is the main COD component that EAMs can readily convert into electricity, around 28% of cow manure, 26% of black water, 15% of hydrolyzed urine, and only 8% of pig manure COD_f is readily convertible (1.08 $g_{COD}/g_{acetate}$, see Supplementary material S4). However, while acetate content of COD_f can be an indicator for bio-degradability, it is not the only readily bio-degradable component [30,31]. Therefore, the ratio of COD from volatile fatty acids (VFAs, such as acetate, propionate and butyrate) over COD_f is a more comprehensive parameter to describe bio-electrochemical degradability. This ratio of VFA over COD_f is 63% for black water, 40% for cow manure, 15% for hydrolyzed urine, and only 8% for pig manure.

Apart from wastewater characteristics, also COD removal and Coulombic efficiency (CE), which multiplied give the bio-electrogenic conversion efficiency (equation (8) and (9)), differ strongly between studies. This is often the case due to differences in system design and operational strategies [18] (for examples, see [5,18,32,33]). Therefore, we investigated the bio-electrogenic conversion efficiency of each wastewater in specifically designed, comparable bio-electrochemical degradation tests at 0.5 g_{COD}/L organic loading for all following experiments.

Bio-electrogenic conversion efficiencies of wastewaters in BES are reproducible

Applying the described reproducibility selection criteria, four batches with the highest reproducible bio–electrogenic conversion efficiencies (see equation (9)), hereafter abbreviated as "conversion") were identified for each wastewater and comparable current density profiles over time are displayed below (Fig. 1).

The current density over time profiles of black water, cow manure and pig manure show one relatively sharp peak around 0.5 days, while the peak for urine occurs later and is more distributed over time. These peaks accounts for 42% conversion for black water, 36% for cow manure, 31% for urine, and 16% for pig manure (Table 2). For cow manure and black water, the conversion within their peaks can likely be attributed to the conversion of readily convertible substrates such as acetate, while the conversion after the peak until day 5 may be attributed to other VFAs.

For hydrolyzed urine and pig manure, conversions were higher than expected based on their VFA content (see Table 2). This indicates that more complex organic compounds were converted into electricity. For black water and cow manure, degradability may be limited by less biodegradable organic compounds included in the COD, such as lignins shown to be present therein [37]. This conversion of complex organic compounds contributed to an additional conversion into electricity, approximately doubling the conversion after the peak. In relative terms, the conversion after the peak up to 5 days contributed an additional 50% for black water, 44% for pig manure, 32% for urine and 17% for cow manure. Interestingly, conversion efficiencies for black water (63%) and cow manure (42%) until day 5 corresponded well with relative amounts of VFAs present (63% of COD for black water and 40% for cow manure). This may indicate that mainly VFAs were converted into electricity.

The bio-electrogenic conversion efficiencies found here are comparable to literature methanation efficiencies (Table 2, expressed as temperature corrected BMP/BMP_{max}, with BMP_{max} of 0.35 L_{CH4}/g_{COD} at 0 °C and 1 atm) for black water (63 vs. 60%) and slightly lower for cow manure (42 vs. 59%). For hydrolyzed urine, only the lower methanation efficiency range is comparable to the conversion found here (41 vs. 46%). The high methanation efficiency of pig manure (23 vs. 55%) and the higher range of methanation of urine (41 vs. 86%) indicate that

Table 1

Wastewater compositions in parameters relevant for bio-electrochemical systems and in particular, bio-electrochemical ammonium recovery. All parameters are represented for paper-filtered samples in quadruple replicates for this study, apart from TS, TSS and total COD (COD_t). RP_{max} calculated with COD_f (COD after paper filtration) and NH_4^+. * meaning single replicate, "-" meaning no data; [1] de Graaff et al., 2010, [2] Zamora et al. 2017, [3] Maurer et al. 2006, [4] Timmerman et al. 2015, [5] Hernandez et al. 2011 [25–29].

	Black water		Hydrolyzed urine			Cow manure		Pig manure		
	this study	[1]	this study	[2]	[3]	this study	[4]	this study	[4]	[5]
TS[g/kg]	–	–	<0.1 *	–	–	58 ± 2	84 ± 5	66 ± 8	78 ± 6	35–56
TSS[g/kg]	5 ± 4	–	<0.1 *	–	–	39 ± 2	–	49 ± 4	–	–
pH	8.2 ± 0.2	8.6–8.8	9.3 ± 0.1	9.4 ± 0.1	9–9.1	7.8 ± 0.1	7.5 ± 0.3	8.3 ± 0.2	7.5 ± 0.3	9
IC[g_C/L]	0.4 ± 0.1	0.7–1.2	2 *	–	–	2 ± 0.5	–	5.8 ± 0.8	–	–
NH_4^+[g_{NH4+}/L]	1 ± 0.1	1.1–1.8	4.9 *	5.1 ± 0.6	5.5–10.4	3.1 ± 0.4	2.2 ± 0	6.7 ± 1.1	5.3 ± 1	4–6.7
COD_t[g_{COD}/L]	11 ± 6	7.7–9.8	3.3 ± 0.4	4.5 ± 0.6	6–10	69 ± 4	102 ± 3	58 ± 6	82 ± 5	30–69
COD_f[g_{COD}/L]	4.3 ± 1.6	2.8–4.7	–	–	–	40 ± 5	–	19 ± 3	–	–
VFA[g_{COD}/L]	2.7 ± 0.9	1.2–1.5	–	–	–	16 ± 2	10 ± 3	1.6 ± 1.1	3.3 ± 2	3.7–5.3
acetate [g_{COD}/L]	1.1 ± 0.5	–	0.5 *	–	–	11 ± 1	–	1.5 ± 1.1	–	2.5–3.7
RP_{max}(COD_f)	10	5.7–5.8	1.5	2	2.2–3.1	29	–	6	–	–

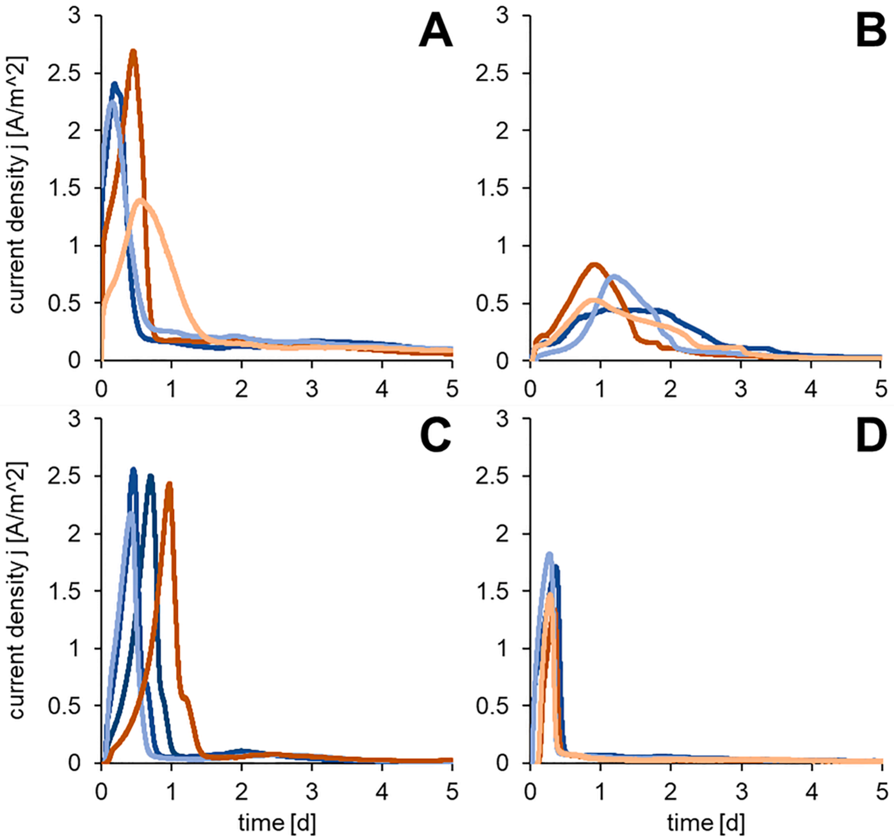

Fig. 1. Bio-electrochemical conversion of filtered black water (A), hydrolyzed urine (B), filtered cow manure (C) and filtered pig manure (D) displayed as current density generated over time. Shades of blue indicate separate replicates of anode potentials of –0.2 V, shades of red −0.1 V vs. 3 M KCl type Ag/AgCl. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary of reproducible bio-electrochemical wastewater conversions to electricity from four replicates. BMP/BMP$_{max}$ recalculated from maximum values from [1] de Graaff et al., 2010, [2] Barbosa et al., 2019, [3] Yao et al., 2017, [4] Shin et al., 2019 [26,34–36].

	Black water	Urine	Cow manure	Pig manure
Peak current density j [A/m^2]	2.2 ± 0.6	0.6 ± 0.2	2.4 ± 0.1	1.6 ± 0.2
Conversion efficiency after peak [%]	42 ± 8	31 ± 2	36 ± 5	16 ± 2
Conversion efficiency after 5 d [%]	63 ± 4	41 ± 5	42 ± 4	23 ± 2
Acetate/COD$_f$ [g$_{CODf,Ac}$/g$_{CODf}$] [%]	26	15	28	8
VFAs/COD$_f$ [g$_{CODf,VFAs}$/g$_{CODf}$] [%]	63	15	40	8
BMP/BMP$_{max}$ [%]	60 [1]	46–86 [2]	59 [3]	55 [4]

higher conversions for both substrates may also be achievable. We hypothesize that some methanations are higher than conversions because the experiment durations differ strongly between BMP tests (several weeks) and bio-electrogenic conversion tests (1 week). This provides more time for fermentation of complex organic molecules and thereby increases the available substrate for methanation.

Conversions found for urine (31–41%) and pig manure (16–23%) were higher than expected based on the VFAs present (15% urine, 8% pig manure; both mostly as acetate). This means that additional electron donors were converted. EAMs have been found to directly oxidize substrates other than acetate, such as hydrogen gas and ethanol [19,38,39]. Yet, at least for ethanol, this oxidation may not occur as quickly as for acetate [19].

As mentioned earlier, urine peak conversion was more spread over time compared to the other wastewaters. This spread of the current profile might be caused by free ammonia nitrogen (FAN) toxicity inhibiting the microbial metabolism [40]. FAN toxicity might be caused by the relatively higher ammonium to COD ratio and the relatively low initial COD concentration compared to the other wastewaters. Both these facts have led to less dilution to the target 0.5 g$_{COD}$/L starting concentration and therefore less dilution of ammonium and carbonate in the urine. Thereby, high concentrations of ammonium and carbonate can overcome the phosphate buffer capacity and increased the pH above 8, which would have led to more FAN and could have inhibited the microbial metabolism [24,41].

While for back water, urine, and pig manure, all reproducibility criteria could be satisfied, cow manure could only be reproducibly converted in one reactor because of operational problems, such as loss of electrode contact and liquid leakages. Therefore, reproducibility for cow manure is only shown for one reactor, but for four different batches.

Electron transfer from COD to electron acceptors other than the anode (nitrate/nitrite, sulfate, metal ions) and methanogenesis were not inhibited, but their influence on the conversions found here was limited. Contrary to bio-electrogenic microorganisms in the anodic biofilm, suspended microorganisms were washed out regularly with each batch's medium replacement [19]. Furthermore, concentrations of other electron acceptors in the wastewaters were much lower than COD concentrations and therefore their contributions as alternative electron donors limited. For example, the COD conversion efficiency to methane was sporadically measured to be below 1% (data not available) and confirms previous findings [19].

For a well-acclimated and -adapted microbial community, VFAs already present in the wastewater would become less important. We hypothesize that an acclimated and adapted biofilm would be less rate limited in how fast it can hydrolyze and subsequently acetify complex organic matter, as indicated by previous findings [19]. Faster acetification provides additional substrate to EAMs, which could then oxidize more COD overall. That would reduce the influence of the initial concentration of readily bio-electrochemically degradable compounds, such as acetate, in the wastewater on the actual recovery potential (RP_{act}, converted COD/TAN) and increase RP_{act} depending on how fast the substrate can be acetified.

Fig. 1 also includes a change in applied anode cultivation potential from −0.2 V (shades of blue) to −0.1 V (shades of red). We tested this to study whether a change in anode potential would affect the conversion efficiency. Most biochemical standard reaction potentials (pH 7, 1 atm, 298 K, 1 mol/L reactants concentrations) of bio-electrochemically convertible organic compounds are around or more negative than −0.3 V vs. 3 M KCl type Ag/AgCl [42]. This would leave 0.1–0.2 V as energy gain for EAMs. However, we found no difference in bio-anode conversion of organics between −0.2 and −0.1 V anode potential, neither in maximum current density, maximum conversion nor current density profiles over time (Fig. 1). This indicates that the conversion of complex substrates to electricity was not limited by the applied anode potential.

Maximum recovery potential can be linked to hydraulic retention time

Considering equation (7), a maximum achievable bio-–electrochemical TAN recovery potential (RP_{max}) higher than 1 means that theoretically all of the TAN can be recovered bio-electrochemically using the COD present in the wastewater. The RP_{max} was very high for cow manure with 29, black water with 10, and pig manure with 6 (Table 1). A RP_{max} of 1.5 for hydrolyzed urine shows that an additional electron donor may be necessary to recover all TAN. However, RP_{max} assumes 100% COD degradability and CE and therefore can only indicate the bio-electrochemical TAN recovery potential of a wastewater.

As described in equation (10), conversion can be linked with the RP_{max} to give the actual recovery potential ($RP_{act,}$ meaning converted COD/TAN) of a wastewater. For black water, the actual recovery potential RP_{act} is higher than 1 before 0.5 d, which indicates that most NH_4^+ could be removed in a relatively short time (Fig. 2 A). However, for a common transport efficiency for NH_4^+ of 60% [4], a RP_{act} of 1.7 would be necessary to remove close to 100% NH_4^+ (see explanation of equation (10)). This RP_{act} of 1.7 is achieved around 0.5 d. This short batch time could be translated into a short HRT for a system in continuous feed mode. Therefore, it is likely that all TAN from black water can be recovered in BEAR at a short HRT. For urine, the RP_{act} is below 0.6 even after 5 d (Fig. 2 B). The conversion found here of around 40% is higher than the previously found 13% for batch operation [33] and similar to previously found 37 – 46% for continuous feeding [5]. However, the similar conversions also confirm the desired high efficiency of the system design chosen for these experiments. Still, an RP_{act} of 0.6 (meaning 60% TAN removal at 100% assumed N-transport efficiency) even at prolonged operation time means that TAN removal efficiency close to 100% from urine is not feasible with the COD present in urine. Yet, high TAN concentrations in urine make it an attractive wastewater to recover TAN, especially when the aim is not to remove/recover all TAN and a certain TAN concentration in the effluent is acceptable.

Pig manure follows a similar, yet more ambivalent reasoning with RP_{act} values of around 1 at 0.5 d (Fig. 2 D). Considering an average TAN transport efficiency of 60%, it is not likely that all TAN can be removed at short HRT. However, after 5 d the RP_{act} increases up to 1.5, meaning that at higher HRT almost all TAN could be removed. Considering the very high TAN concentrations in pig manure, operating a BEAR system

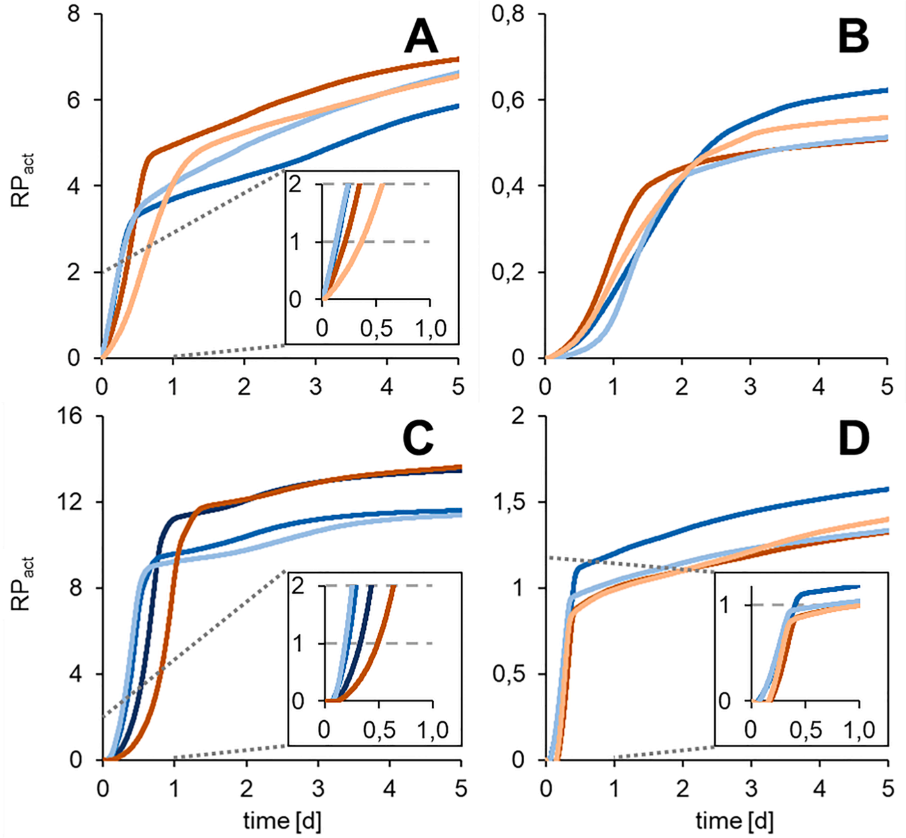

Fig. 2. Maximum bio-electrochemical ammonium recovery potential for average filtered COD concentrations achieved per hydraulic retention time for filtered black water (A), hydrolyzed urine (B), filtered cow manure (C) and filtered pig manure (D). Shades of blue indicate separate replicates of anode potentials of –0.2 V, shades of red −0.1 V vs. 3 M Ag/AgCl. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

on pig manure would require high conversion and TAN transport efficiencies to remove all TAN. Yet, the RP_{act} may be increased by improving the pre-treatment, either by removing less VFAs during filtration or by preceding fermentation of complex organics to VFAs.

The most promising RP_{act} of around 10 after 1 d was observed for cow manure (Fig. 2 C). This is due to the high concentration of available COD and the high ratio of acetate to COD, which indicates a high concentration of readily convertible substrate. This makes high TAN removal efficiency and rate possible for cow manure and makes cow manure a very attractive wastewater for BEAR.

When sieving (200 μm) and paper-filtration (8–10 μm) is applied as pre-treatment, as we did in our experiments, the relative VFA content of filtered COD may give a good estimate of bio-electrochemical degradability. This ratio is 63% for black water, 40% for cow manure, 15% for hydrolyzed urine and 8% for pig manure (Table 2). Combining the relative VFA content of filtered COD with the RP_{max} for filtered COD (see equation (11)), an expected recovery potential (RP_{exp}) of 6 for black water, 12 for cow manure and 0.5 for pig manure can be estimated. The RP_{exp} for unfiltered urine samples would be 0.2. The actual RP_{act} found here after 1 d (cow manure: 10; black water: 4; pig manure: 1; urine: 0.2) therefore correspond very well with the expected RP_{exp} estimated from RP_{max} and relative VFA content of COD.

However, the recovery potentials found in batch here can only indicate how efficient ammonia recovery in continuous operation could be like since neither ammonium removal nor recovery were determined here. Furthermore, the varying composition of COD in real wastewaters would affect continuous BEAR. Competing electron acceptors, such as nitrate and sulfate, as well as methanogenesis, would reduce the recovery potential in practice. Also, the transport efficiency may vary depending on the reactor design and transport of ions competing with NH_4^+ would lower the ammonia removal efficiency. A preceding fermentation of complex organic matter to VFA step might help stabilize the wastewater COD composition predominantly as VFAs, increase fast COD conversion to electricity and thereby the recovery potential. Finally, real wastewaters pose different challenges in batch compared to continuous systems and not all applications require all TAN to be removed since discharge limits differ between wastewaters and treatments. Therefore, while a continuous system recovering ammonia from cow manure or black water appears very promising for BEAR, its testing is still pending. The bio-electrochemical degradability test we suggest here would allow simple and fast wastewater testing to assess anaerobic bio-electrogenic degradability in a comparable manner.

Conclusion

The NH_4^+ recovery potential of wastewaters in bio-electrochemical systems is largely predetermined by their composition, in particular the concentrations of NH_4^+ and COD. The ratio of these two parameters gives the maximum recovery potential RP_{max} (based on the COD/TAN ratio found in the respective wastewater). Black water as well as cow and pig manure have RP_{max} values high enough to make TAN removal efficiencies close to 100% possible. For hydrolyzed human urine, high TAN removal efficiencies are not possible based on its RP_{max} value and would require additional electron donor to remove all TAN.

In addition, the relative amount of COD that can be readily converted into electricity can be indicated by the relative VFA content of soluble COD. The expected recovery potential RP_{exp} for our experiments based on RP_{max} and relative VFA content of COD accurately predicted the actual recovery potential RP_{act} found in our experiments.

Experiments in comparable batches confirm that TAN removal (based on RP_{act}) close to 100% is most feasible with black water and cow manure, and close to 90% is possible with pig manure at longer HRT. In contrast, BEAR systems treating hydrolyzed human urine should focus on high feed and TAN removal rates instead of high removal efficiencies, or additional electron donors, such as acetate or hydrogen gas, need to be supplied to achieve high removal efficiencies.

While possible (ammonia) toxicity remains an issue for human urine, one issue that diminishes the recovery potential of pig and cow manure as well as black water is the high solid content that would require extensive pre-treatment or cause fouling inside the BES. Apart from that, while pig manure could benefit from fermentation of complex organics to VFAs preceding BEAR, the high recovery potentials of black water and (in particular) cow manure indicate high potential as attractive future waste streams for BEAR.

Using the standardized experimental design we present here, the degradability of a wastewater being considered for a certain BES application can be estimated. Furthermore, determining the recovery potentials described here can give early indicators of how bio-electrogenically degradable a specific wastewater is with BES. When taking confounding factors, such as transport efficiency, into consideration as well, this can help estimate the performance of BES systems based simply on the composition of the wastewater. In principle, this approach is transferrable to any BES applications that rely on substrate degradation (resource recovery, desalination, energy or hydrogen production) and therefore can serve as a benchmark for what is achievable with BES.

Data availability

https://doi.org/10.4121/uuid:7abd3fdc-c71e-4923-8ca0-a7ba72f71934.

CRediT authorship contribution statement

S. Georg: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization, Supervision. **C. Schott:** Investigation. **J.R. Courela Capitao:** Validation, Investigation. **T. Sleutels:** Conceptualization, Methodology, Writing - original draft, Supervision. **P. Kuntke:** Conceptualization, Methodology, Writing - original draft, Supervision. **A. ter Heijne:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration. **C.J.N. Buisman:** Conceptualization, Methodology, Writing - original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

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

Acknowledgements

This work was performed in the cooperation framework of Wetsus, European Centre of Excellence for Sustainable Water Technology (www.wetsus.eu). Wetsus is co-funded by the Dutch Ministry of Economic Affairs and Ministry of Infrastructure and Environment, the European Union Regional Development Fund, the province of Fryslân, and the Northern Netherlands Provinces. This research has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 665874. The authors like to thank the participants of the research theme "Resource Recovery" for the fruitful discussions and their financial support. Furthermore, the authors would like to thank Hardy Temmink for the fruitful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seta.2021.101423.

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