Video Article

Electrochemically and Bioelectrochemically Induced Ammonium Recovery

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Abstract

Streams such as urine and manure can contain high levels of ammonium, which could be recovered for reuse in agriculture or chemistry. The extraction of ammonium from an ammonium-rich stream is demonstrated using an electrochemical and a bioelectrochemical system. Both systems are controlled by a potentiostat to either fix the current (for the electrochemical cell) or fix the potential of the working electrode (for the bioelectrochemical cell). In the bioelectrochemical cell, electroactive bacteria catalyze the anodic reaction, whereas in the electrochemical cell the potentiostat applies a higher voltage to produce a current. The current and consequent restoration of the charge balance across the cell allow the transport of cations, such as ammonium, across a cation exchange membrane from the anolyte to the catholyte. The high pH of the catholyte leads to formation of ammonia, which can be stripped from the medium and captured in an acid solution, thus enabling the recovery of a valuable nutrient. The flux of ammonium across the membrane is characterized at different anolyte ammonium concentrations and currents for both the abiotic and biotic reactor systems. Both systems are compared based on current and removal efficiencies for ammonium, as well as the energy input required to drive ammonium transfer across the cation exchange membrane. Finally, a comparative analysis considering key aspects such as reliability, electrode cost, and rate is made.

This video article and protocol provide the necessary information to conduct electrochemical and bioelectrochemical ammonia recovery experiments. The reactor setup for the two cases is explained, as well as the reactor operation. We elaborate on data analysis for both reactor types and on the advantages and disadvantages of bioelectrochemical and electrochemical systems.

Video Link

The video component of this article can be found at https://www.jove.com/video/52405/

Introduction

Recovery of valuable products from wastewater gains importance as valuable resources become scarce and treatment without recovery represents only a cost. Wastewater contains both energy and nutrients that can be recovered, and nutrient recovery can help to close the production loop[1]. Recovery of energy through anaerobic digestion is a well-established process, while recovery of nutrients is less common. Recovery of nutrients from liquid waste streams such as urine and manure has been widely investigated, *e.g.*, through the production of struvite and direct stripping of ammonia[2,3]. However, the need for chemical addition is a downside of these processes[4]. Here we present a technique for the recovery of cationic nutrients from waste streams, including both potassium and ammonium. The cationic form of these nutrients allows recovery using an ion selective membrane in an electrochemical system. In this case, the electrochemical system consists of an anode chamber (where oxidation takes place), a cathode chamber (where reduction takes place) and an ion selective membrane to separate the compartments. A voltage is applied across the cell to produce a current flow from anode to cathode. This voltage can be generated by an external power source to drive water oxidation and reduction reactions. Alternatively the anodic oxidation, *e.g.*, of organics, can be catalyzed by electroactive bacteria, requiring less power. To close the circuit and maintain the charge balance, a charged species must migrate between the electrodes for each electron generated. Ammonium transport from the anode chamber to the cathode chamber across a cation exchange membrane (CEM) can thus compensate the flux of electrons [4,5].

The technique presented here not only removes ammonium from waste streams, but also enables its recovery. Total ammonia nitrogen (TAN) exists in equilibrium of both ammonium (NH_4^+) and ammonia (NH_3), and is dependent on pH and temperature[6]. NH_4^+ is abundantly available due to high TAN concentration and near neutral pH in the anode chamber and this positively charged species can therefore be driven by the current across the CEM into the cathode chamber. The current drives the reduction of water at the cathode, leading to the production of hydroxide ions and hydrogen gas. The TAN equilibrium shifts to nearly 100% NH_3 due to the high pH in the cathode chamber (> 10.0). NH_3 is a gas that can be easily transferred via air circulation from the stripping unit to the absorption column where it is trapped and concentrated in an acid solution.

This technology has the potential to decrease ammonium toxicity during anaerobic digestion of N-rich streams like manure, thus increasing the energy recovery from these waste streams, while simultaneously recovering nutrients[4]. Electrochemical and bioelectrochemical extraction of

ammonium can also be applied as nutrient recovery technique on waste streams with a high TAN content such as urine thereby avoiding costs for nutrient removal at a WWTP[7].

The protocol presented here can serve as a basis for many different electrochemical and bioelectrochemical experiments, as we use a modular reactor. Different electrode types, membranes and frame thicknesses can be combined as explained in the protocol below. The main aim of the protocol is to provide a means for the comparison of electrochemical ammonium recovery and bio-electrochemical ammonium recovery using an electrolysis cell. The systems are evaluated in terms of extraction efficiency, power input and reproducibility.

Protocol

1. Assembling the Reactor and Connecting the Stripping and Absorption Units

1. Collect all necessary material to build the reactor: electrodes, frames and rubbers (See List of Materials). Carefully cut all parts to the same dimensions to avoid leaks while assembling the reactor.
2. Drill holes in the reactor compartments to fit a male to male connector. Drill one additional hole in the middle of the side of one of the reactor compartments to fit the reference electrode.
3. Prepare a stock of 1 M H_2SO_4 for the absorption column. Increase this concentration as necessary to accommodate higher loads of ammonia.
4. Ensure that the membrane is pretreated according to the manufacturer's instructions. Pretreat the carbon felt electrode by soaking it in 2 mM CTAB (detergent) for 3 min. Rinse the carbon felt with demineralized water[8]. The stable anode for electrochemical experiments does not require a pretreatment.
5. Stack the different reactor parts in order according to the reactor type. For the bioreactor: Perspex endplate, rubber, stainless steel current collector, pretreated graphite felt, Perspex reactor compartment, rubber, cation exchange membrane, rubber, spacer material, stainless steel mesh electrode, rubber, Perspex reactor compartment, rubber, Perspex endplate
6. Stack the reactor parts for the electrochemical cell as follows: Perspex endplate, rubber, IrOx anode through the endplate, Perspex reactor compartment, rubber, spacer, rubber, cation exchange membrane, rubber, spacer material, stainless steel mesh electrode, rubber, Perspex reactor compartment, rubber, Perspex endplate.
7. Use Teflon to seal the connection ports of the reactor. Place the reference electrode in the same compartment as the working electrode: the anode in the case of a bioelectrochemical cell, the cathode or anode in the case of an electrochemical cell.
8. Use nuts and bolts to close the reactor. Tighten bolts on opposite sides to equalize the pressure. Do not use tools to close the reactor as finger-tight is enough to ensure a completely sealed reactor.
9. Fill the reactor with water to test if the reactor is leak-free. If leaks appear, check if the bolts are tightened enough or if one of the reactor parts moved while assembling the reactor. If no leaks are detected, empty the water from the reactor.
10. Add Raschig rings in both the strip and absorption column to fill the columns halfway.
11. Calibrate the flow rate of all the pumps. Connect the feed and recirculation pumps to the reactor and the air pump to the stripping and absorption units (**Figure 1**). Minimize the length of the tubing as much as possible.
12. Fill the absorption column with 250 ml of 1 M H_2SO_4, it should cover the Raschig rings. Ensure that the air stream mixes the acid well when the pump is switched on. Increase or decrease the volume of acid based on the stripping column design and air pump capacity.
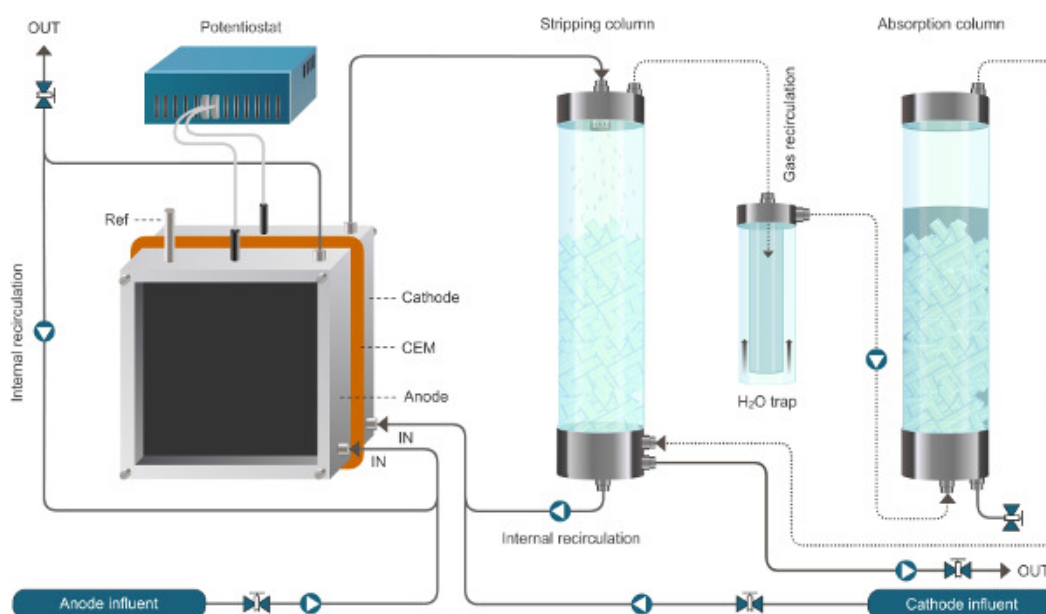

Figure 1. Reactor setup for the bioelectrochemical system enabling ammonium extraction. The system presented here operates in continuous mode. Solid lines represent liquid flow, dotted lines represent gas flow. Please click here to view a larger version of this figure.
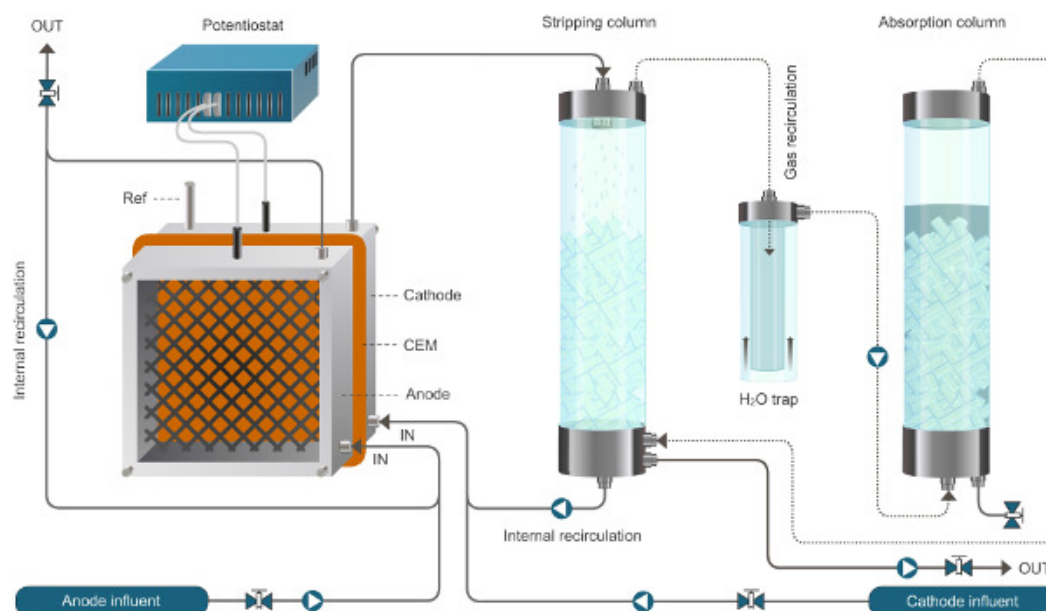
Figure 2. Reactor setup for the bioelectrochemical system enabling ammonium extraction. The system presented here operates in continuous mode. Solid lines represent liquid flow, dotted lines represent gas flow. Please click here to view a larger version of this figure.
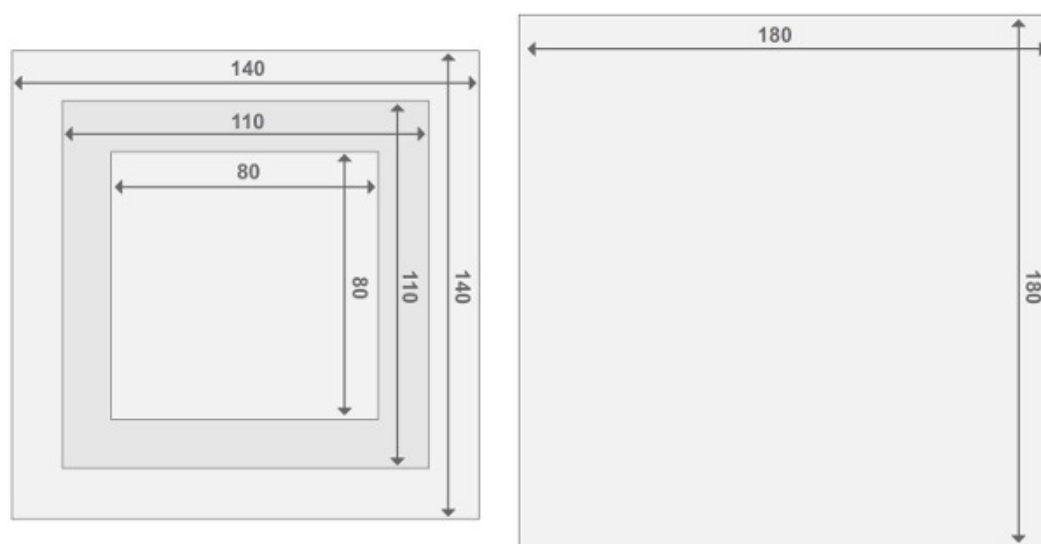

Figure 3. Design of the Perspex reactor frames. Each reactor is comprised of two endplate reactors and 2 reactor compartments. All parts have a thickness of 2 cm. Details concerning the size of other materials can be found in the List of Materials. Please click here to view a larger version of this figure.

2. Bioanode Driven Extraction

1. Preparing the media.
    1. Prepare anolyte for the bioreactor as described in **Table 1**[9]. Increase the ammonium concentration in the medium to mimic a nitrogen-rich waste stream.
    2. To store the medium prior to use, autoclave the medium to ensure the carbon source is not depleted through contamination. Prepare vitamins and trace-elements according to **Table 1** and add after autoclaving and cooling the medium.
    3. Flush the medium by purging with nitrogen gas for at least 30 min to remove oxygen. To do this, insert a tube or needle into the medium and turn on the nitrogen gas stream.
    4. Prepare a conductive solution as catholyte. In this case, use 0.1 M NaCl to allow caustic production.

Component	Amount		
Na_2HPO_4	6 g/L		
KH_2PO_4	3 g/L		

NaCl	0.5 g/L		
NH$_4$Cl	0.5 g/L		
MgSO$_4$·7H$_2$O	0.1 g/L		
CaCl$_2$·2H$_2$O solution (14.6 g/L)	1 ml		
Sodium Acetate	2 g/L (for start-up)		
Trace Elements	1 ml		
Vitamin solution	1 ml		
Trace Elements (1,000x)	**g/L**	**Vitamins (1,000x)**	**g/L**
CoCl$_2$	0.1	biotin	0.004
Na$_2$MoO$_4$.2H$_2$O	0.01	folic acid	0.004
H$_3$BO$_3$	0.01	pyridoxine hydrochloride	0.02
Mg$_2$Cl$_2$.6H$_2$O	3	riboflavin	0.01
ZnCl$_2$	0.1	thiamine hydrochloride	0.01
CaCl$_2$.2H$_2$O	0.1	nicotinic acid	0.01
NaCl	1	DL-calcium pantothenate	0.01
nitrilotriacetic acid	1.5	Vit B12	0.0002
AlCl$_3$.6H$_2$O	0.01	*p*-aminobenzoic acid	0.01
CuCl$_2$	0.01	lipoic(thioctic) acid	0.01
FeCl$_2$	0.1	*myo*-inositol	0.01
MnCl$_2$.2H$_2$O	0.5	choline chloride	0.01
Adjust to pH 6.5 using KOH		niacinamide	0.01
		pyridoxal hydrochloride	0.01
		sodium ascorbate	0.01

Table 1. Anolyte composition for bio-anode driven ammonium extraction.

2. Inoculation of the bioreactor
   NOTE: Working in sterile conditions is not necessary for this bioreactor, as a mixed culture inoculum is used and reactor conditions will select for the specific electroactive organisms.
   1. Prepare the inoculum. For this bioreactor, prepare a 30 ml mixture of effluents from active anaerobic bioreactors including a fermenter, a bioanode, an anaerobic digester and/or raw wastewater. Collect the mixture in a syringe.
   2. Connect a gas bag filled with N$_2$ to the anolyte bottle in order to keep the pressure stable while not allowing oxygen to enter. Mix the inoculum source with a volume of anolyte (here, 100 ml of anolyte for 30 ml of inoculum source) by emptying the syringe with inoculum into the medium bottle. Be sure to obtain the volume necessary to fill the anode compartment.
   3. Using a syringe, fill the anode and cathode compartment simultaneously with their respective solutions. Connect a gas bag filled with N$_2$ to the anolyte bottle so that the anolyte solution can be removed through a sampling port without introducing oxygen. Close the sample port with a tap between transfers.
   NOTE: Perform this step together with a colleague to ensure that both reactor compartments are filled simultaneously.
   4. When both reactor compartments are filled, turn on the recirculation pump at a recirculation rate of approximately 6 L/hr.
   5. Connect the potentiostat cable with the three electrodes, using the anode as working electrode. Position the reference electrode in the anode compartment.
   6. Switch on the potentiostat in chronoamperometry mode using the potentiostat software. Select a fixed anode potential of -200 mV vs. Ag/AgCl.

3. Running a continuous reactor for ammonium extraction
   NOTE: As the biofilm develops, current will be produced with the consumption of acetate. As a consequence of acetate depletion, the current will drop (see Results section, **Figure 3**).
   1. To change to continuous feeding, switch on the feed pump for the anode and cathode. The pump speed will determine the hydraulic residence time (HRT). Here, operate the reactor at a HRT of 6 hr.
   2. Switch on the air pump of the strip and absorption unit. Recirculate the air in a closed loop, or circulate in an open loop using the ambient air. Air flow configuration can affect absorption efficiencies.
   3. Refresh the medium three times per week. Prepare fresh anolyte and catholyte as described in the steps 2.1.1-2.1.4.
   4. After these steps, attach a gas bag filled with N$_2$ to the closed feed bottle, stop the feed pump, put a clamp on the influent line, switch the old and new bottles and finally remove the clamps and restart the pump.
   5. Each time the feed is refreshed, take 5 ml liquid samples of the effluent and influent of the anolyte and catholyte for measurement of conductivity, pH, acetate content and ammonium concentration.

6. When changing the feed, also take a 3 ml sample of the absorption column to monitor the pH and for TAN analysis. When the pH approaches 4, replace the absorbent with fresh 1 M sulfuric acid solution to ensure high absorption efficiency.
7. As the current will first increase and then reach a plateau, measure the acetate content in the anolyte influent and effluent to ensure this is not caused by carbon limitation: acetate concentrations in the anolyte effluent below 100 mg/L indicate carbon limitation. Increase the acetate concentration in the feed in that case (**Table 2**).
8. If the current stabilization is not caused by acetate limitations, gradually increase the ammonium concentration in the feed, and wait for stabilization of the current in order to assess extraction efficiencies (**Table 3**).
   NOTE: As the ammonium concentration is increased, ammonia toxicity and high conductivity will challenge the biofilm and the current will eventually drop as a consequence.

Time	Amount of sodium acetate added to the anode feed (g/L)
Day 0 – Day 35	2
Day 35 – Day 37	3
Day 37 – Day 51	4
Day 51 – Day 61	5

Table 2. Concentration of sodium acetate in the anolyte for the bioanode driven ammonium extraction.

Time	Amount of NH_4HCO_3 added to the anode feed (g/L)	Phase
Day 0 – Day 16	2.26	I
Day 16 – Day 26	4.5	II
Day 26 – Day 33	9	III
Day 33 – Day 40	14.1	IV
Day 40 – Day 47	20	V
Day 47 – Day 54	25.4	VI
Day 54 – Day 63	31	VII

Table 3. Concentration of ammonium in the anolyte for the bioanode driven ammonium extraction. The phases are indicated on the current density graph (**Figure 2**).

3. Electrochemical Extraction

1. Preparing the media
   1. Prepare a synthetic wastewater stream as anolyte according to **Table 4**[4]. Add ammonium sulfate to reach a final concentration of 1, 3, or 5 g N/L.
   2. Prepare a 0.1 M NaCl solution for the catholyte.

Component	Amount
$Na_2HPO_4.2H_2O$	1.03 g/L
KH_2PO_4	0.58 g/L
$MgSO_4 \cdot 7H_2O$	0.1 g/L
$CaCl_2.2H_2O$	0.02 g/L
$(NH_4)_2SO_4$	depending on the experiment, to obtain 1/3/5 g N/L final concentration

Table 4. Anolyte composition for electrochemical ammonium extraction[4].

2. Running a continuous reactor for ammonium extraction
   1. Switch on the feed pump to fill the reactor compartments. To speed up the process temporarily increase the pump rate.
   2. Reduce the pump speed to obtain an HRT of 6 hr once the reactor is filled. Switch on the recirculation pump at a rate of 6 L/hr. Take a sample of the influent (5 ml).
      NOTE: Measure the flow rate periodically throughout the experiment to ensure it does not vary.
   3. Start the strip and absorption unit. Operation of this unit is the same as for the bioreactor.
   4. Switch on the potentiostat in chronopotentiometry mode using the potentiostat software. First apply a low current density of about 0.5 A/m² to polarize the membrane and to determine nitrogen flux due to diffusion alone.
   5. When the system has been polarized for 24 hr, apply the current density necessary for the experiment. Test different current densities, usually ranging from 10 A/m² to 50 A/m². Take samples of the anode and cathode effluents, and the absorption column before increasing the current density.
      NOTE: After 3 HRT cycles, the reactor should approach steady state.

6. Once the reactor has reached steady state, take at least 3 samples over a time course. Take samples from the anode and cathode effluents, and the absorption column (5 ml each). Write down the sampling volume, date and time.
7. Depending on the stability of the anode influent, take a new anode influent sample if necessary. This is necessary when real wastewater is used.
8. Change the test conditions, such as applied current density and TAN concentration. After each change, let the reactor stabilize for at least 3 HRTs before taking samples.
9. When the pH of the absorption column approaches 4, replace the absorbent with fresh 1 M sulfuric acid solution.

4. Sample Analysis

1. Measure the pH and the conductivity of the samples the same day as sampling to reduce inaccuracies due to loss of volatile ammonia. Measure pH and conductivity using adequately calibrated pH and conductivity probes.
2. If the sample are not measured immediately, store samples for TAN analysis (both reactors) and fatty acid analysis (bioreactor) at 4 °C. Filter samples from the bioreactor anode effluent and influent through 0.45 μm filters to remove biomass and help preserve fatty acids. Fill all sample tubes to the rim in order to minimize NH_3 loss.
3. Measure nitrogen as TAN by the standard steam distillation method or any other reliable method for measuring TAN[10].
4. Measure fatty acids as acetate by any reliable method, such as ion chromatography or gas chromatography[11].

5. Data Analysis and Calculations

1. Export the potentiostat data file from the software and import it to a spreadsheet program. Calculate averages per hour for the electrochemical variables to decrease the number of data points and smooth the curves when plotting them.
2. Collect all measured data (pH, ammonium, VFA) in one data file for calculations. The calculations are discussed in the results section.
3. Calculate the current production by the bioreactor. This is best represented as current density, which is calculated as follows (Equation 1,[12]):

$$ j \left(\frac{A}{m^2} \right) = \frac{I\ (A)}{A\ (m^2)} \qquad \text{Equation 1} $$

with j as the current density, I the absolute current, and A the projected surface area of the electrode. In certain software it is possible to have this calculated automatically by entering the anode surface area before the start of the experiment.
4. Calculate the parameters related to ammonium extraction
    1. Calculate the nitrogen flux. Normalize nitrogen flux (g N/m²/d) to the membrane surface area then expressed as a current density (I_N). Use this value to calculate the CE (Equation 2, 3, and 4):

$$ J_N = \frac{(C_{An,in} - C_{An,out})\ x\ Q}{A} \qquad \text{Equation 2} $$

where $C_{An,in}$ (g N/L) and $C_{An,out}$ (g N/L) are the measured ammonium concentrations coming in and out the anode compartment, respectively. Q (L/d) is the anode flow rate and A (m²) is the membrane surface area (equal to projected anode and cathode surface area).
    2. Present the nitrogen flux as current density (I_N, A/m²):

$$ I_N = \frac{J_N\ x\ z_{NH_4^+}\ x\ F}{M\ x\ 86400\ s\ d^{-1}} \qquad \text{Equation 3} $$

where z_{NH4+} (-) is the charge of NH_4^+, F the Faraday constant (96,485 C/mol) and M the molecular weight of nitrogen (14 g/mol).
    3. Calculate the current efficiency (CE, %) as:

$$ CE = \frac{I_N}{I_{Applied}}\ x\ 100 \qquad \text{Equation 4} $$

where $I_{Applied}$ (A/m²) is the applied (electrochemical extraction) or measured (bioelectrochemical extraction) current density.
    4. Calculate the theoretical nitrogen flux. Calculate the maximum theoretical nitrogen flux ($J_{N,Max}$, g N/m²/d) for a given applied current and membrane surface area (Equation 5) as:

$$ J_{N,max} = \frac{I_{Applied}\ x\ z_{NH_4^+}\ x\ M\ x\ 86400\ s\ d^{-1}}{F\ x\ A} \qquad \text{Equation 5} $$

    5. Calculate the nitrogen removal efficiency (RE, %). Refer to the percentage of ammonium that is removed from the anolyte as the removal efficiency. Calculate from the anode influent and effluent TAN concentrations (Equation 6).

$$ RE = \frac{C_{AN,in} - C_{AN,out}}{C_{AN,in}}\ x\ 100 \qquad \text{Equation 6} $$

    6. Calculate the maximum theoretical nitrogen removal efficiency (RE_{max}, %) for a given influent TAN load and applied current (Equation 7):

$$ RE_{max} = \frac{J_{N,applied}\ x\ A}{C_{An,in}\ x\ Q}\ x\ 100 \qquad \text{Equation 7} $$

where $J_{N,applied}$ (g N m^{-2} d^{-1}) is the applied current density expressed as a nitrogen flux.
5. Calculate gas/liquid ratio as (Equation 8):

$$\left(\frac{G}{L}\right) = \frac{\text{Gas flow rate } (\frac{m^3}{h})}{\text{Liquid flow rate } (\frac{m^3}{h})} \quad \text{Equation 8}$$

6. Calculate the maximal capacity of the absorption column. Calculate the maximum theoretical N load to the absorption column from the maximum theoretical nitrogen flux J_{Nmax}, the TAN concentration in the influent (mol/L), the time of operation t, the membrane surface area A, and the volume of absorbent V (Equation 9):

$$H_2SO_4 \text{ (mol/L)} = \frac{\frac{J_{Nmax} \times \frac{TAN}{14} \times A \times t}{2}}{V} \quad \text{Equation 9}$$

7. Calculate the stripping efficiency SE (%) (Equation 10):

$$SE = \frac{C_{AN,in} - C_{AN,out} - C_{CAT,out}}{C_{AN,in}} \times 100 \quad \text{Equation 10}$$

8. Calculate the energy input for ammonium extraction through the cation exchange membrane (E_N, expressed as kWh/kg N) (Equation 11):

$$E_N = \frac{j * A * \Delta V * 24 / 1000}{(C_{An,in} - C_{An,out}) \times Q} \quad \text{Equation 11}$$

With ΔV the measured potential difference between anode and cathode. In the case of the bioreactor, ΔV was calculated as the average for the sampling period, for the electrochemical reactor the average for the entire run is taken.

Representative Results

Chronoamperometry results from the bioreactor

The chronoamperometry results, calculated according to Equation 1, show a typical graph for a continuous reactor (**Figure 4**). At the start of the experiment, the anode and cathode were operated in recirculation mode. This allows a biofilm to develop and the onset of the current production. After 5 days of operation, the current density reached a maximum, followed by a decrease in current production. This is an indication that the biofilm lacks a carbon/electron source (*e.g.*, acetate) to produce current. The change to continuous operation on day 6, using an HRT of 6 hr, resulted in a continuous increase in current production until a plateau was reached at 3.5 A/m² between day 12 and 16. A plateau was necessary to obtain sufficient data on ammonium extraction for a certain current density.

The ammonium concentration in the feed was increased in several steps (**Table 2**). Each step resulted in an increase of the current density that ultimately reached an average current of 27 A/m². This current increase was linked to an increased conductivity of the anode feed, in which the addition of ammonium bicarbonate increased the concentration of ions and thus the conductivity. A higher conductivity decreases ohmic resistance and thus favors current production[13]**Chronoamperometry results from the bioreactor**

Acetate measurements showed the complete removal of the carbon source by the anodic biofilm from day 27 to 37. During this period, the current density produced by the biofilm decreased prior to medium change. As the medium was not kept in sterile conditions, the acetate concentration in the feed dropped over time due to consumption by non-electroactive microorganisms in the feed bottle. The current density increased again as soon as the medium was replenished. This indicated that the current production by the biofilm was limited by the carbon source concentration in the feed. Several increases in acetate concentration were necessary to prevent carbon limitation for the second half of the test (**Table 2**).
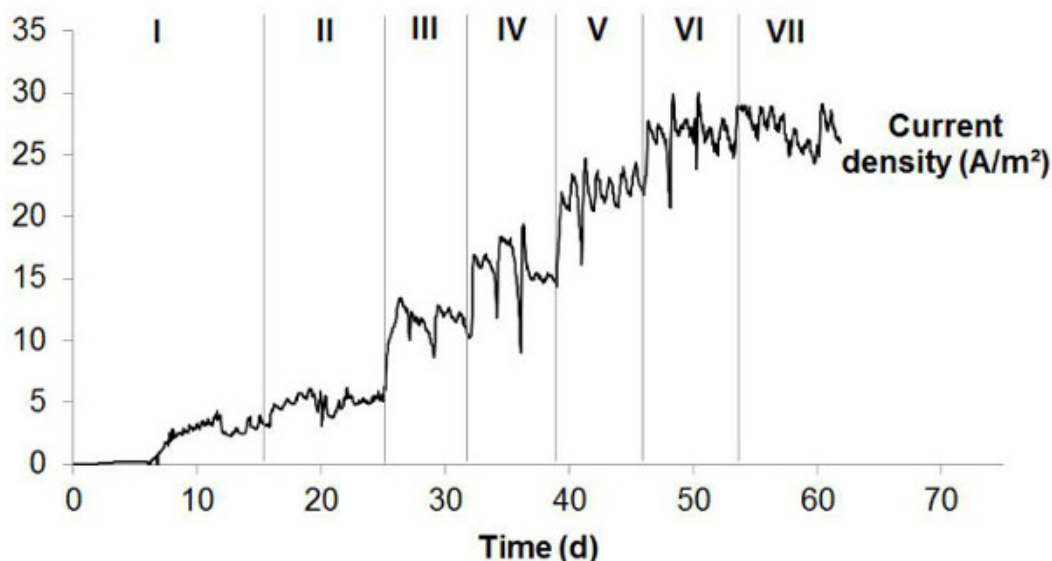
Figure 4. The current density over time for the bioelectrochemical system. After the change to continuous mode on day 6, an increase in current can be observed. Each phase (II – VII) indicates an increase in the ammonium feed concentration, which resulted in an increase in current.

Cell potential

The cell potential is calculated based on the difference between the anode and cathode potential, the overpotentials at the electrodes and the Ohmic resistance. The cell potential relates to the total power necessary to drive the electrochemical cell. For equations and elaboration on this topic, we refer to the review paper by Clauwaert and co-workers[13].

In the case of the biological ammonium extraction, the anode potential was fixed at -200 mV vs. Ag/AgCl and the biofilm produced the current. As a consequence the cathode potential varied in order to sustain the current produced by the biofilm. In this case, the resistance across the cell affected the cathode potential. On day 16 the cell potential of the biological system started to increase though no increase in current was observed and the anode potential remained fixed at -200 mV vs. Ag/AgCl. This was a consequence of an increased resistance in the system, which may be a result of membrane resistance (*e.g.*, scaling on the membrane) or diffusional limitations caused by poor mixing between the anode and the membrane. The reactor was carefully emptied and opened, and the membrane was replaced. The anode was placed further away from the membrane to improve mixing. The anode compartment was filled again with the anolyte that had been previously removed. This operation restored the cell potential to the same level as at the start of the continuous experiment (0.5 V), with the cathode potential stable around -700 mV vs. Ag/AgCl.

In the abiotic electrochemical extraction experiments, the cell potential is calculated similarly as for the bioelectrochemical extraction, including overpotentials and ohmic resistance. Both the anode and cathode potential were subject to variations. The cell voltage for the electrochemical system is higher than for the bioreactor (**Table 5**). This is mainly due to the higher anode potential required for electrochemical oxidation of water to oxygen. Specific anode and cathode potentials for the conditions tested are described by Desloover *et al.*[4].

Current density	Bioanode (V)	Electrochemical system (V)
0 A/m²	N/A	N/A
10 A/m²	1.69 ± 0.05	2.73 ± 0.06
20 A/m²	2.20 ± 0.11	2.99 ± 0.08
30 A/m²	2.32 ± 0.14	3.35 ± 0.21

Table 5. Comparison of the cell potentials (V) for the bioreactor and electrochemical system at different current densities. The results for the bioreactor are calculated from steady state periods were the current density value reached between the indicated current density value ± 2 A/m². For the biosystem the anolyte feed concentration increased from 1.62 g N/L (10 A/m²) to 5.1 g N/L (30/m²) across this range of currents. All values for the electrochemical system were calculated for a system operating at 5 g N/L in the anolyte feed.

Ammonium extraction and stripping

The electrochemical parameters presented in the two previous sections are the factors that determine the efficiency of ammonium extraction through the cation exchange membrane. The following parameters are calculated in order to compare the performances of the biotic and abiotic systems in terms of ammonium extraction.

Nitrogen flux (J_N) and Current efficiency (CE) of extraction

Ammonium ions cross the cation exchange membrane to restore the charge balance over the cell. For each electron being released at the anode, one positive charge must be displaced from the anode to the cathode compartment. If ammonium restored 100% of the charge balance, one would obtain a current efficiency of 100%.

The nitrogen flux for the bioreactor is higher than the electrochemical system (**Figure 5**). This can be explained by the lower alkalinity of the feed of the electrochemical system, resulting in a lower anolyte pH. This resulted in a higher competition between ammonium and protons to restore the charge balance over the membrane.
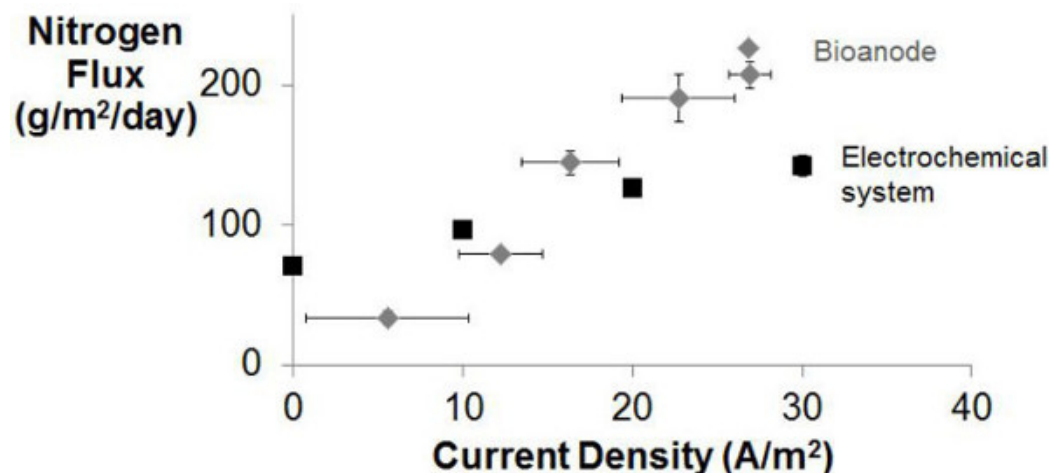

Figure 5. The nitrogen flux for the bioreactor compared to the nitrogen flux for the electrochemical system for different current densities. The flux for the bioreactor is calculated for a range of TAN concentration in the anode influent; for the electrochemical system the flux is given only for a concentration of 5 g N/L. The error bars for the electrochemical system are smaller than the symbols.

Stripping efficiency

The liquid recirculation rate and the air pump performance can be adjusted in order to obtain higher stripping efficiency. The choice of an open or closed air circulation loop will also have an effect on the stripping efficiency. An open air stream is favorable when the absorption efficiency is high and all the NH_3 gas is trapped during its passage through the acid. The open air system ensures that the air going through the stripping column is free of ammonia, resulting in a higher driving force for the conversion of dissolved NH_3 to gaseous NH_3. In case of a low absorption efficiency the closed system will prevent ammonia losses. The ammonia gas captured in the gas flow must be absorbed into an acid solution to make the stripping process thermodynamically favorable, as expressed by the principle of Le Chatelier[14]. When the pH of the absorbent starts to rise it must be replaced, as this indicates that there are no longer protons available to protonate the ammonia. The absorption capacity can be estimated beforehand. For every mole of H_2SO_4, 2 moles of N from NH_3 can be captured.

Stripping efficiency (SE, %) is calculated based on the ammonia nitrogen removed from the anode, and the cathode effluent concentration ($C_{CAT,out}$). This method is more accurate than methods using the measured TAN inform the absorption column as these are subject to evaporation/precipitation. It is important to note that Equation 10 is only valid for equal flow rates of the anolyte and catholyte.

Overall comparison of the biotic and abiotic systems

The bioreactor and the electrochemical system are compared for the most similar conditions of the test: a concentration of 5.1 g N/L for the bioreactor anolyte, which resulted in a current density of 27 A/m² and a concentration of 5 g N/L combined with an applied current density of 30 A/m² in the case of the electrochemical system (**Table 6**).

Parameter	Bioanode	Electrochemical system
Current efficiency (%)	67.1 ± 0.28	38 ± 0.6
Removal efficiency (%)	51 ± 0.5	41 ± 2
Nitrogen flux (g N/m²/d)	226 ± 1	143 ± 7
Cell voltage (V)	2.12 ± 0.09	3.35 ± 0.21
Energy input (kWh/kg N removed)	6.04 ± 1.78	16.8 ± 1.4
Anolyte pH	7.39 ± 0.13	1.56 ± 0.14
Catholyte pH	12.53 ± 0.07	12.92 ± 0.08

Table 6. Overall comparison of the bioreactor and electrochemical system. The bioreactor was operating at steady state at 5.1 g N/L feed concentration, resulting in an average current density of 27 A/m². The electrochemical system was run at 30 A/m² for a nitrogen feed concentration of 5 g/L.

Discussion

This manuscript provides the necessary tools to set up a bioelectrochemical and an electrochemical cell for ammonium recovery. The calculations presented in the results section provide the parameters for evaluation of the system performance. The biological and electrochemical systems are similar in setup and function. The main difference between the two systems is the choice of a fixed current for the electrochemical cell versus a fixed anode potential for the bioelectrochemical setup. The fixed current for the abiotic setup is necessary to drive the electrode reactions and allows also allows for regulation of the processes in the bulk phase, thus leading to steady state conditions. For the bioelectrochemical system on the other hand, a fixed anode potential of -200 mV vs. Ag/AgCl was chosen to enable electron transfer to the electrode[15]. The two-compartment electrochemical cell allows the extraction of ammonium over a membrane, driven by an electrical current. Each system presents certain advantages over the other. Some of the possible problems with the systems are described.

The bioelectrochemical system offers several advantages with regards to the cost of the system. The cost of the graphite felt anode is much lower than the cost for the stable anode used in the electrochemical system. For a 1 m² electrode surface, the capital costs of the anode is decreased by a factor of 10, from \$1,000 to \$100 per m². The operational cost of the bioelectrochemical system is also lower. In a bioanode reactor, the current is produced at a much lower anode potential by the biofilm compared to the electrochemical reactor, hence the required cell voltage is much lower in a bioelectrochemical setup. In the electrochemical cell the extraction requires an energy input of 16.8 kWh/kg N extracted, while for the bioanode operating under the same conditions the energy input is more than halved to 6.04 kWh/kg N extracted. The electroactive bacteria catalyze the anodic reaction at a lower potential as opposed to the electrochemical oxidation of water, which substantially reduces the operational cost of the bioreactor. Other operational costs such as power for pumps and stripping and absorption are not included, but are anticipated to be similar for both systems. An even lower energy input is obtained when using a microbial fuel cell (MFC) instead of a microbial electrolysis cell. The low extraction rates obtained with an MFC make the investment of electrical energy in the case of the MEC attractive[16].

While cost favors the bioelectrochemical system, operational stability and reproducibility is an advantage of the electrochemical cell. As a biological system, the electroactive biofilm is sensitive to the environment and can be easily be disrupted. The biofilm is sensitive to changes in pH, concentration of toxic compounds and changes in temperature. The influent should be well buffered to maintain the pH around the neutral value during the oxidation reaction. The anode reaction will enforce a pH decrease if the anolyte is not sufficiently buffered, as was the case for the electrochemical system. This is a critical point to address when using the biological system for the treatment of real wastewater. The effect of the temperature was clearly visible in the bioelectrochemical test presented here. It is best to place the reactor in a temperature-controlled environment to exclude the influence of temperature on bacterial kinetics but this was not the case in the bioelectrochemical test presented here, where temperature fluctuation can be observed to impact the chronoamperometry. Daily variations between night (cold, low current) and day (warm, high current) can be seen in the graph (**Figure 4**), in particular between day 42 and 46, when no other factors such as low availability of carbon source were inhibiting the bacterial activity[13,17].

Another disadvantage is that the biological system requires a longer startup time. The biofilm develops over a few days on the electrode, but changes to the feed characteristics such as the TAN concentration must be applied gradually in order to reduce stress to the microbial biofilm. In our system, the electrochemical system only requires 24 hr of polarization and 3 HRTs to reach stable operating conditions.

An electrochemical system allows a greater degree of control over operational parameters. For example, the current density can be controlled to obtain an optimal ratio between product recovery and power input[4]. Current densities higher than those presented here (over 30 A/m²) can be used, while for a bioelectrochemical system the current production cannot be controlled in the present state-of-the-art. Limiting the carbon source, or providing excess carbon can alter the current output of the biological system, but as discussed in the results section more factors affect current production by the biofilm, thus making it difficult to optimize process parameters.

The elements described above provide a basis for evaluation of a reactor for a given influent, and can help with determining whether a bioelectrochemical or electrochemical system should be chosen. We hope that this instructional video provides the necessary tools to operate a simple electrochemical or bioelectrochemical system for ammonium extraction.

Troubleshooting during experimental operation

Many factors affect the performance of an electrochemical cell. The bioelectrochemical system is even more sensitive to disturbance. The most common problems in reactor operation are discussed here, but other problems might occur. Reactor operation is most easily learned hands-on and confrontation with problems will allow you to operate more easily in the next run. Other aspects regarding bioelectrochemical systems are dealt with in the JoVE video article by Gimkeiwicz and Harnisch[18].

Sizes of the Materials

Other reactor sizes are possible for ammonium extraction. For example, the reactor compartment can be rectangular instead of square, with inner dimensions of 5 x 20 cm². The most important aspect is that all elements should fit properly. The rubbers should always cover the outer side of the reactor compartment frame. The membrane should be cut larger than the exchange surface area. For the 8 x 8 cm² reactor 13 x 13 cm² is a suitable size. The same accounts for the graphite felt. The stainless steel current collector for the bioanode has outer dimensions of 13 cm x 13 cm and inner dimensions of 11 cm x 11 cm in order not to be in direct contact with the anolyte.

Potentiostat

Assure proper functioning of the potentiostat by executing a dummy-cell test prior to the start of the reactor experiment.

Ohmic resistance

Keep a close eye on the ohmic resistance of the system, which will negatively affect the cell potential at higher values. A sudden increase of the ohmic resistance of the system might indicate a variety of problems: (i) malfunctioning of the ion exchange membrane, (ii) too great a space between the electrodes, (iii) poor electrode connections, (iv) low electrolyte conductivity, or (v) insufficient mixing. A steep increase of the ohmic resistance can be detected very quickly by checking the required compliance voltage that has to be delivered by the potentiostat. If this becomes too high (> 10 V), the potentiostat software program will interrupt the experiment, though this is dependent on the equipment.

Membrane fouling and scaling can be expected over time especially when real wastewater is used as anolyte due to the presence of bivalent cations such as Ca^{2+} and Mg^{2+}, and the high solids content[19]. This will lead to an increased ohmic resistance and a higher cell voltage, rendering the system less efficient.

Reference electrode

The reference electrode should be checked weekly relative to a stable reference electrode (*e.g.,* Calomel electrode) to assure that the system is operated at the correct fixed potential. Place the reference electrode in the system in such a way that gas bubbles cannot be trapped near the reference electrode (connect to the side of the reactor, not to the top).

Oxygen intrusion

As the biofilm is oxygen-sensitive, oxygen intrusion should be avoided at all times. The influent vessel and anode compartment should be flushed with nitrogen gas during start-up of the reactor. Whilst the experiment is running, a low current density might indicate the use of O_2 as electron acceptor instead of the anode electrode. Check all connections and tubing (especially pump tubing) to detect air leaks. Oxygen intrusion can be detected by using resazurin, however this compound might interfere with the electrode-active biofilm[20].

Stripping and absorption efficiency

High stripping efficiency should be maintained to avoid ammonia loss from the cathode effluent as well as to avoid back-diffusion of dissolved NH_3 to the anode compartment. Therefore, a minimum gas to liquid ratio of 1,000 (G/L) is advised. The use of Raschig rings is imperative to favor the liquid/gas transfer during stripping. The absorption efficiency should be high to maintain a low concentration of NH_3 in the stripping gas. The pH of the absorption column should be kept below 4.

Insufficient gas recirculation

The power of the gas recirculation pump (membrane vacuum pump, VWR) and hence the gas flow rate may decrease over time due to the influence of moisture and scaling. Install a water trap prior to the inlet of the vacuum pump and clean the membrane head of the pump regularly to prevent and remove scaling.

Disclosures

The authors have nothing to disclose.

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