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Electrochemically and Bioelectrochemically Induced Ammonium Recovery

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Abstract:	<p>Streams such as urine contain high levels of ammonium nitrogen, 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. The current and consequent restoration of the charge balance across the cell allows 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, as well as the energy input required to drive the extraction. Finally, a comparative analysis considering key aspects such as reliability, electrode cost, and rate is made.</p> <p>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.</p>
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Attn: The Editor of The Journal of Visualized Experiments

Dear editor,

Please find herewith our manuscript titled “**Electrochemically and bioelectrochemically induced ammonia recovery**” which we kindly submit for publication in JoVE. The authors are Sylvia Gildemyn, Amanda K. Luther, Stephen J. Andersen, Joachim Desloover and Korneel Rabaey.

This study presents two approaches for recovery of nutrients, in particular ammonium, from ammonium-rich waste streams such as digestate, urine or wastewater. Both an electrochemical and a bioelectrochemical recovery system are discussed in this work. Here we provide a step-by-step approach to build and operate each reactor system. The modular reactor system we use offers many possibilities for alternative applications. The results presented are thoroughly discussed and calculations are provided. We provide guidelines for the audience to build and optimize their own test set-up.

In the field of bioelectrochemical systems we are creating an open community of scientists. Clear and open communication on experimental methods and results is an important aspect of this. We believe that this video article can contribute towards further development of research in the field of bioelectrochemical systems.

We would like to thank Elizabeth Sheeley for assisting us in the submission process. The author contributions are as follows: Sylvia Gildemyn advised on experimental design, conducted the experiments with the bioanode, processed the data, wrote the paper and submitted the manuscript. Amanda K. Luther advised on experimental design, processed the data, conducted the experiments with the bioanode and reviewed the paper. Stephen J. Andersen advised on experimental design and reviewed the paper. Joachim Desloover designed the experiments, conducted the abiotic experiments and reviewed the paper. Korneel Rabaey coordinated the study and reviewed the paper.

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Thank you for considering this manuscript. We believe this paper and video will be valuable to environmental engineers and scientists both in and outside the field of (bio)electrochemical systems, and are excited to submit the work to The Journal of Visualized Experiments.

Looking forward to a positive outcome,

Prof Dr. Ir. Korneel Rabaey



TITLE:

Electrochemically and bioelectrochemically induced ammonium recovery

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KEYWORDS:

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SHORT ABSTRACT:

We demonstrate the extraction of ammonium from an ammonium-rich stream using an electrochemical and a bioelectrochemical system. The reactor setup, operation and data analysis are discussed.

LONG 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.

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 loop¹. 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 ammonia²³. However, the need for chemical addition is a downside of these processes⁴. 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 temperature⁶. 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 nutrients⁴. 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 WWTP⁷.

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.

1.1) 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.

1.2) 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.

1.3) Prepare a stock of 1 M H_2SO_4 for the absorption column. Increase this concentration as necessary to accommodate higher loads of ammonia.

1.4) 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 minutes. Rinse

the carbon felt with demineralized water⁸. The stable anode for electrochemical experiments does not require a pretreatment.

1.5) 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

1.6) 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

1.7) 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.

1.8) 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.

1.9) 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.

1.10) Add Raschig rings in both the strip and absorption column to fill the columns halfway.

1.11) 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.

1.12) Fill the absorption column with 250 ml of 1 M H₂SO₄, 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.

[Place Figure 1 here]

[Place Figure 2 here]

[Place Figure 3 here]

2. Bioanode driven extraction.

2.1) Preparing the media.

2.1.1) Prepare analyte for the bioreactor as described in Table 1⁹. Increase the ammonium

concentration in the medium to mimic a nitrogen-rich waste stream.

2.1.2) 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.

2.1.3) Flush the medium by purging with nitrogen gas for at least 30 minutes to remove oxygen. To do this, insert a tube or needle into the medium and turn on the nitrogen gas stream.

2.1.4) Prepare a conductive solution as catholyte. In this case, use 0.1 M NaCl to allow caustic production.

[Place Table 1 here]

2.2) 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.

2.2.1) 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.

2.2.2) Connect a gas bag filled with N₂ 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.

2.2.3) Using a syringe, fill the anode and cathode compartment simultaneously with their respective solutions. Connect a gas bag filled with N₂ 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.

2.2.4) When both reactor compartments are filled, turn on the recirculation pump at a recirculation rate of approximately 6 L/h.

2.2.5) Connect the potentiostat cable with the three electrodes, using the anode as working electrode. Position the reference electrode in the anode compartment.

2.2.6) Switch on the potentiostat in chronoamperometry mode using the potentiostat

software. Select a fixed anode potential of -200 mV vs. Ag/AgCl.

2.3) 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).

2.3.1) 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 hours.

2.3.2) 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.

2.3.3) Refresh the medium three times per week. Prepare fresh anolyte and catholyte as described in the steps 2.1.1-2.1.4.

2.3.4) After these steps, attach a gas bag filled with N₂ 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.

2.3.5) 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.

2.3.6) 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.

2.3.7) 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).

2.3.8) 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.

[Place Table 2 here]

[Place Table 3 here]

3. Electrochemical extraction.

3.1) Preparing the media.

3.1.1) Prepare a synthetic wastewater stream as anolyte according to Table 4⁴. Add ammonium sulfate to reach a final concentration of 1, 3, or 5 g N/L.

3.1.2) Prepare a 0.1 M NaCl solution for the catholyte.

[Place Table 4 here]

3.2) Running a continuous reactor for ammonium extraction.

3.2.1) Switch on the feed pump to fill the reactor compartments. To speed up the process temporarily increase the pump rate.

3.2.2) Reduce the pump speed to obtain an HRT of 6 hours 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.

3.2.3) Start the strip and absorption unit. Operation of this unit is the same as for the bioreactor.

3.2.4) 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.

3.2.5) When the system has been polarized for 24 h, 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.

3.2.6) 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.

3.2.7) Depending on the stability of the anode influent, take a new anode influent sample if necessary. This is necessary when real wastewater is used.

3.2.8) 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.

3.2.9) When the pH of the absorption column approaches 4, replace the absorbent with fresh 1 M sulfuric acid solution.

4. **Sample analysis.**

4.1) 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.

4.2) 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 NH₃ loss.

4.3) Measure nitrogen as TAN by the standard steam distillation method or any other reliable method for measuring TAN¹⁰.

4.4) Measure fatty acids as acetate by any reliable method, such as ion chromatography or gas chromatography¹¹.

5. **Data analysis and calculations**

5.1) 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.

5.2) Collect all measured data (pH, ammonium, VFA) in one data file for calculations. The calculations are discussed in the results section.

5.3) Calculate the current production by the bioreactor. This is best represented as current density, which is calculated as follows (Equation 1,¹²):

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

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.

5.4) Calculate the parameters related to ammonium extraction.

5.4.1) 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):

$$J_N = \frac{(C_{AN,in} - C_{AN,out}) \times Q}{A} \text{ Equation 2}$$

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 (m^2) is the membrane surface area (equal to projected anode and cathode surface area).

5.4.2) Present the nitrogen flux as current density (I_N , A/ m^2):

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

where $z_{NH_4^+}$ (-) is the charge of NH_4^+ , F the Faraday constant (96485 C/mol) and M the molecular weight of nitrogen (14 g/mol).

5.4.3) Calculate the current efficiency (CE, %) as:

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

where $I_{Applied}$ (A/ m^2) is the applied (electrochemical extraction) or measured (bioelectrochemical extraction) current density.

5.4.4) Calculate the theoretical nitrogen flux. Calculate the maximum theoretical nitrogen flux ($J_{N,max}$, g N/ m^2 /d) for a given applied current and membrane surface area (Equation 5) as:

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

5.4.5) 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).

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

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

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

where $J_{N,applied}$ ($\text{g N m}^{-2} \text{d}^{-1}$) is the applied current density expressed as a nitrogen flux.

5.5) Calculate gas/liquid ratio as (Equation 8):

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

5.6) 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):

$$\text{H}_2\text{SO}_4 \text{ (mol/L)} = \frac{\frac{J_{Nmax} \times \frac{TAN}{14} \times A \times t}{2}}{V} \quad \text{Equation 9}$$

5.7) Calculate the stripping efficiency SE (%) (Equation 10):

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

5.8) 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 hours, resulted in a continuous increase in current production until a plateau was reached at 3.5 A/m^2 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 production¹³.

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).

[Place Figure 4 here]

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-workers¹³.

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 al⁴.

[Place Table 5 here]

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 for the electrochemical system (Figure 5). Comparison of the data is not straightforward as the nitrogen concentration in the anolyte was not the same for both systems. The last data points on the graph however allow a comparison. The bioreactor was treating an influent at 4.5 g N/L and 5.1 g N/L while producing an average current of 27 A/m². These conditions are similar those of the electrochemical cell where the applied current and influent nitrogen concentration were 30 A/m² and 5 g N/L respectively. This higher flux for the bioreactor can be explained by the difference in anolyte pH between the two systems. The higher pH in the case of the bioreactor anolyte resulted in less competition with protons for transport across the membrane. More ammonium cations were proportionally available to restore the charge balance compared to protons. The well-buffered anolyte of the bioreactor is necessary to maintain stable conditions for electricity production by the electroactive bacteria, and is also an advantage for the ammonium extraction. This explains the higher current efficiency in the bioreactor as compared to the electrochemical system.

[Place Figure 5 here]

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 NH₃ 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 NH₃ to gaseous NH₃. 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 Chatelier¹⁴. 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 ($C_{\text{CAT,out}}$). 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).

[Place Table 6 here]

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.

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.

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 materials table.

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.

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.

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

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

extraction

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).

Table 4: Anolyte composition for electrochemical ammonium extraction⁴

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 where 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 A/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.

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 system 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 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 electrode¹⁵. 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 \$1000 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 attractive¹⁶.

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 h 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 input⁴. 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 Harnisch¹⁸.

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 Ca²⁺ and Mg²⁺, and the high solids content¹⁹. 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 O₂ 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 biofilm²⁰.

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 NH₃ to the anode compartment. Therefore, a minimum gas to liquid ratio of 1000 (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 NH₃ 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.

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DISCLOSURES:

The authors have nothing to disclose.

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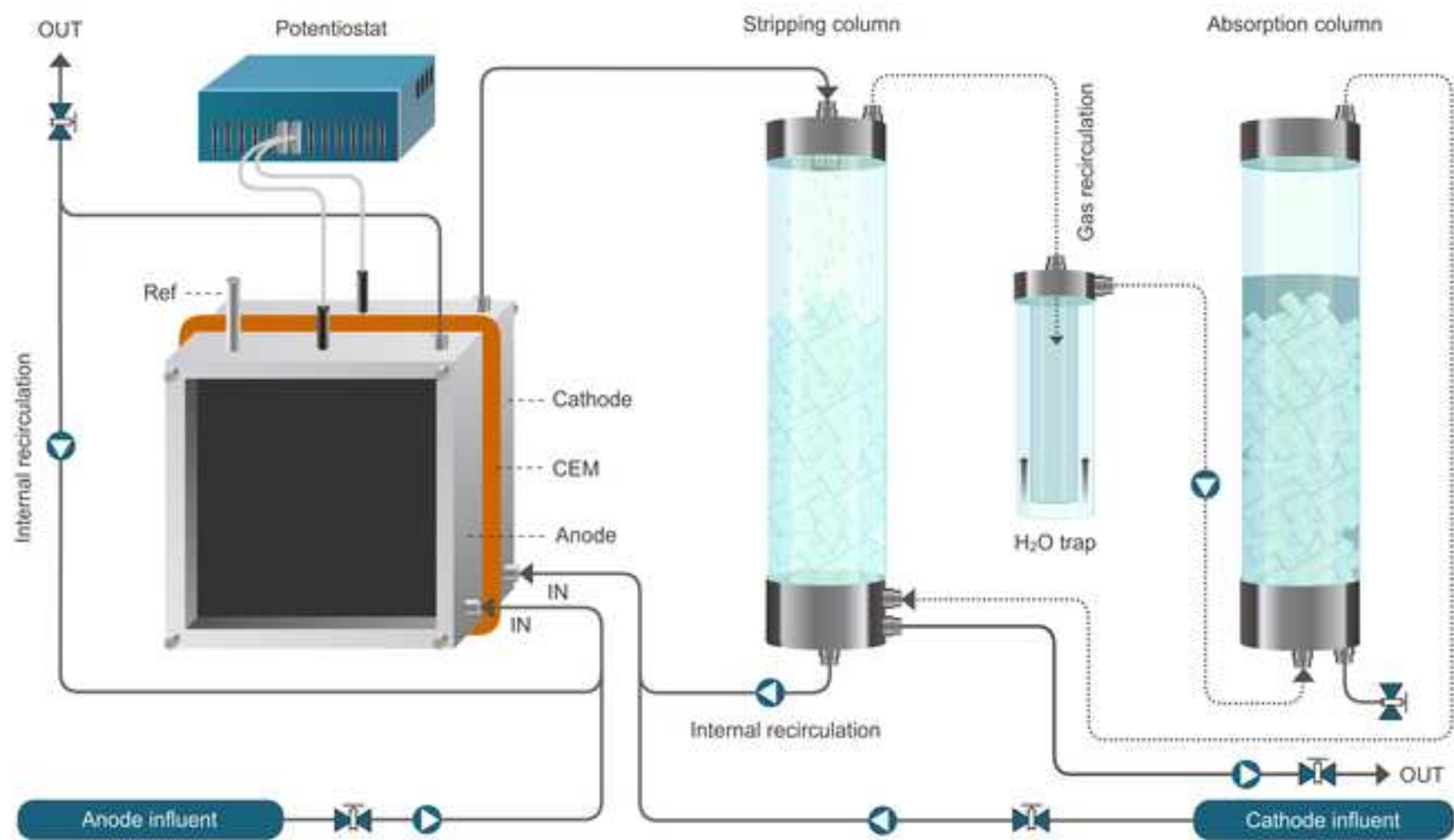


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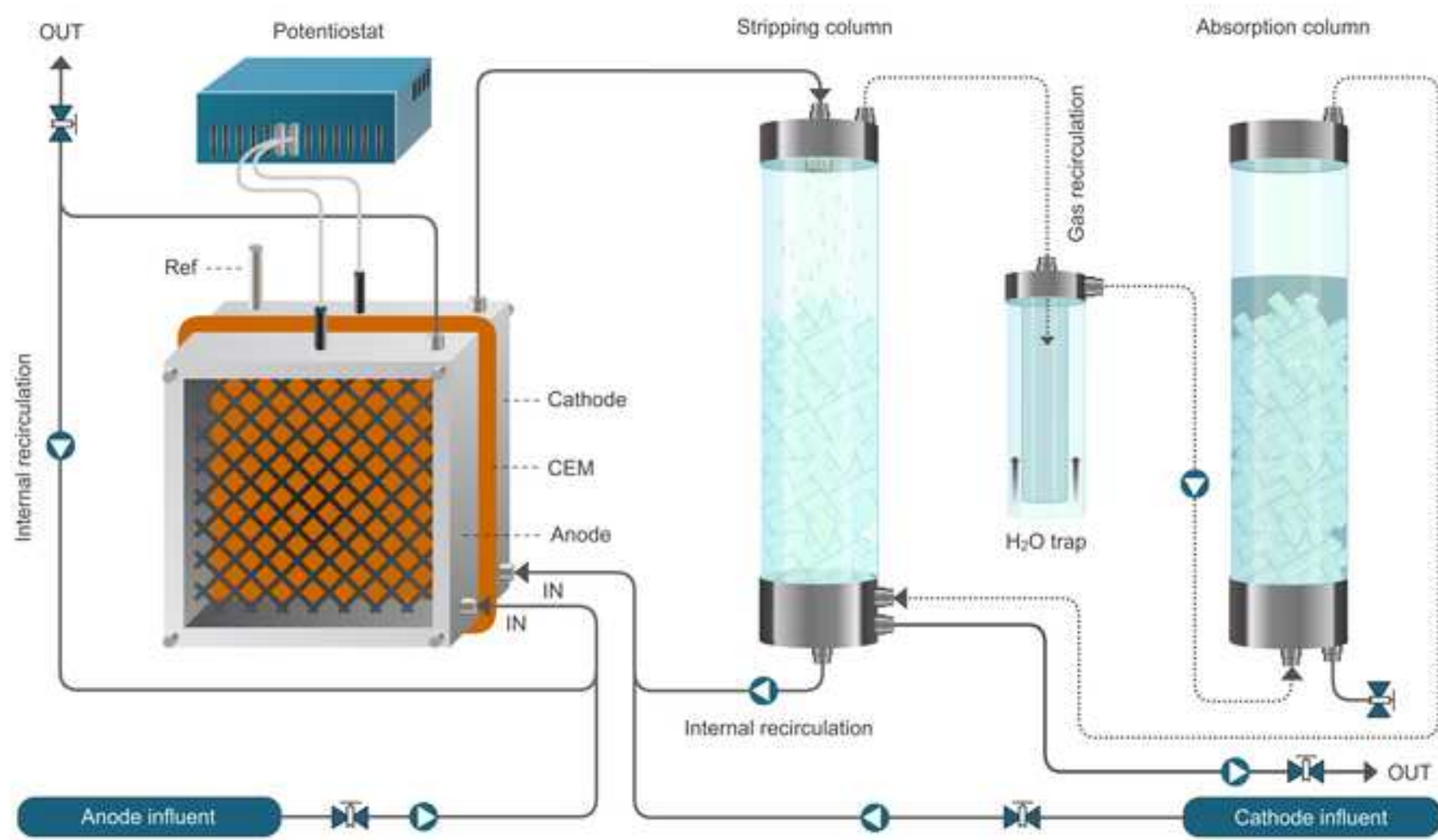
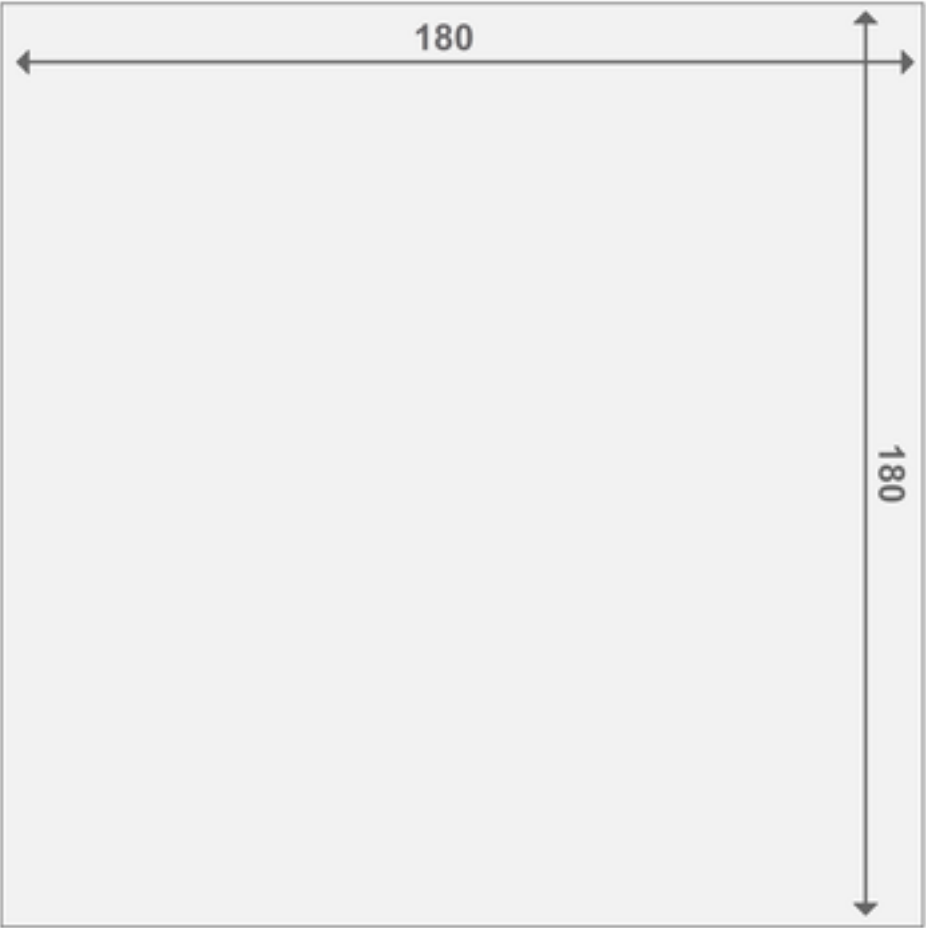
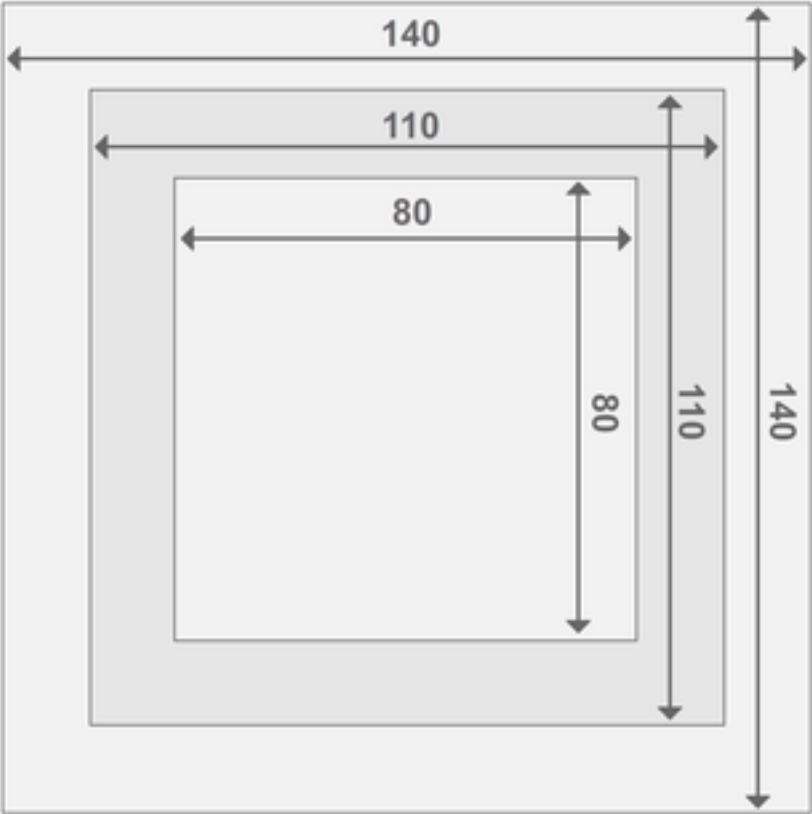


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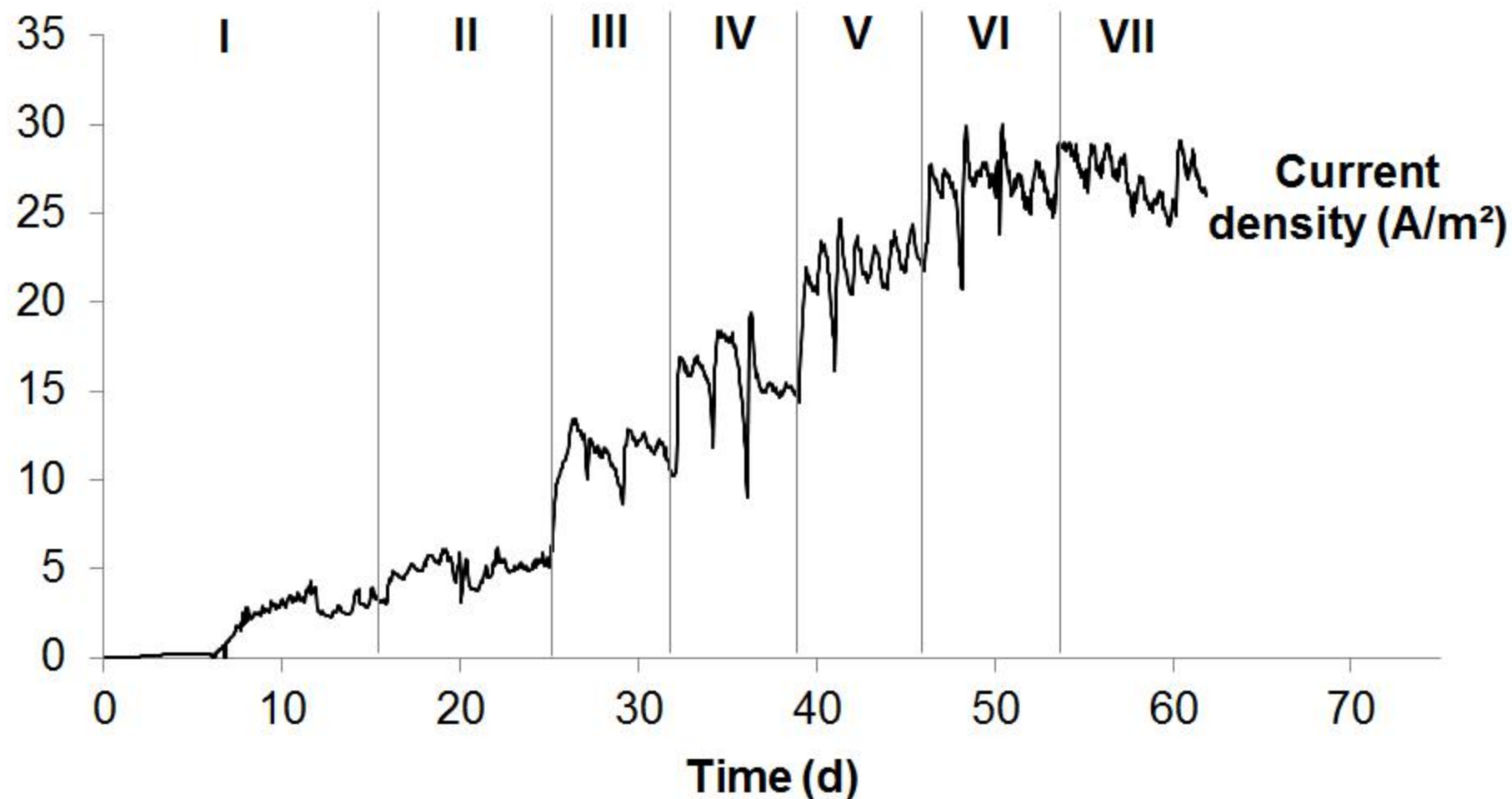


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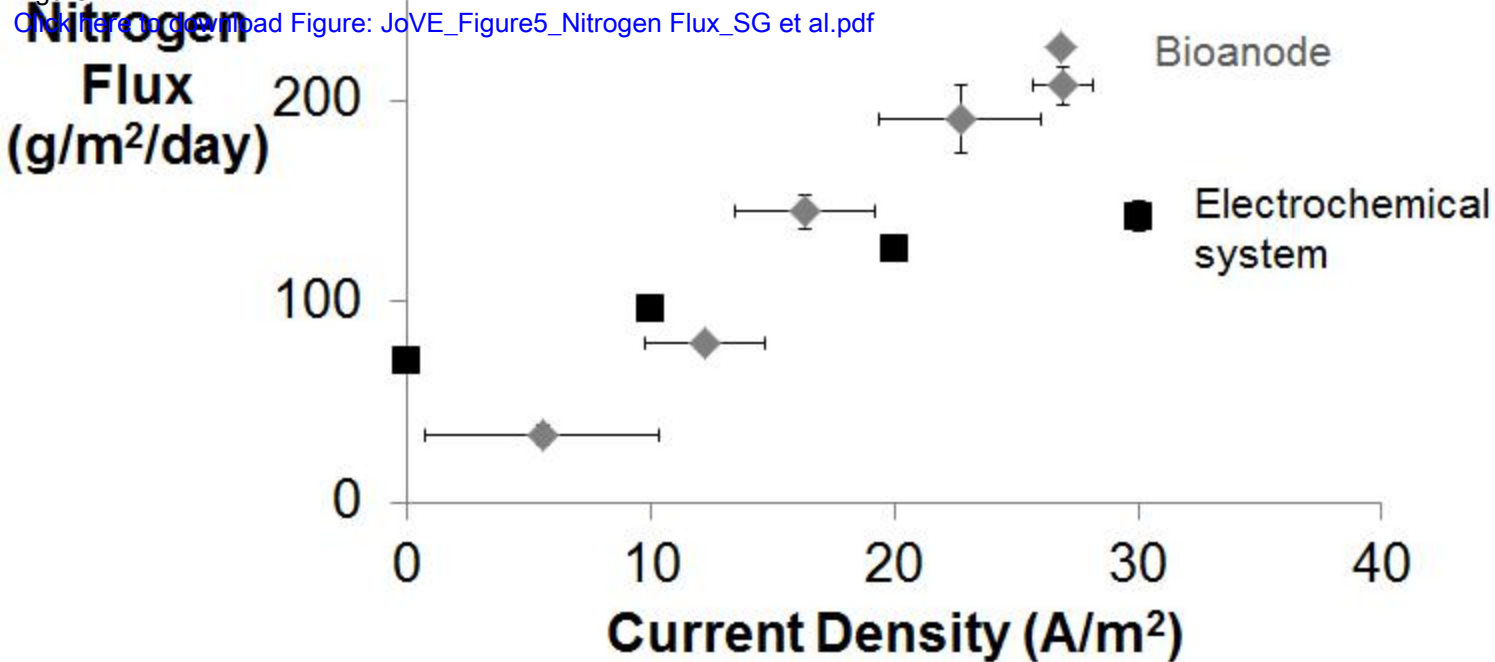


Table 1
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Component	Amount
Na ₂ HPO ₄	6 g/L
KH ₂ PO ₄	3 g/L
NaCl	0.5 g/L
NH ₄ Cl	0.5 g/L
MgSO ₄ ·7H ₂ O	0.1 g/L
CaCl ₂ ·2H ₂ O solution (14.6g/L)	1ml
Sodium Acetate	2 g/L (for
Trace Elements	1ml
Vitamin solution	1mL

Trace Elements (1000x)	g/L	Vitamins (1000x)	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 hydrocholride	0.01
		sodium ascorbate	0.01

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

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 4
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Component	Amount
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	1.03 g/L
KH_2PO_4	0.58 g/L
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 g/L
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.02 g/L
$(\text{NH}_4)_2\text{SO}_4$	depending on the experiment, to obtain 1/3/5 g N/L final concentration

Current density	Bioanode (V)	Electrochemical system (V)
0 A/m ²	N/A	N/A
10 A/m ²	1.69 ± 0.05	2.73 ± 0.06
20 A/m ²	2.20 ± 0.11	2.99 ± 0.08
30 A/m ²	2.32 ± 0.14	3.35 ± 0.21

Parameter	Bioanode	Electrochemical system
Current efficiency (%)	67.1 ± 0.28	38 ± 0.6
Removal efficiency (%)	51 ± 0.5	41 ± 2
Nitrogen flux (g N/m ² /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

Name of Material/ Equipment	Company	Catalog Number
Carbon Felt 3.18 mm Thick	Alfa Aesar	ALFA43199
Ti electrode coated with Ir MMO	Magneto Special Anodes (The Netherlands)	RVS 554/64: material AISI 316L, mesh width: 564 micron, wire thickness: 140 micron, mesh number: 36,6
Stainless steel mesh	Solana (Belgium)	inox 304 sheet, thickness: 0,5mm
Stainless steel plate	Solana (Belgium)	A-012167 RE-1B
Ag/AgCl Reference Electrode	Bio-Logic (France)	
Potentiostat (VSP	Bio-Logic (France)	
Multipotentiostat)	Bio-Logic (France)	
EC Lab	Membranes International (USA)	Ultrex CMI-7000
Cation Exchange Membrane	ElectroCell Europe A/S (Tarm, Denmark)	EPC20432-PP-2
Turbulence Promotor mesh	Serto	1,281,161,120
Connectors		
Strip and absorption column	Masterflex	HV-06404-16
Tubing	Keika Ventures	
Gas bag		
Rashig Rings	Glasatelier Saillart (Belgium)	Raschig rings 4 x 4 mm
Rubber sheet		
Perspex reactor frames	Vlaeminck, Beernem	

Comments/Description

Used as bioanode, 110 mm x 110 mm

Used as stable anode for electrochemical tests

Used as cathode, 110 mm x 110 mm

Used as current collector for the bioanode

software for performing electrochemistry measurements

Pretreated according to the manufacturers' instructions

spacer material, 110 mm x 110 mm

Other sizes possible, dependant on tubing type and size of holes in frames

In house design

Kynar gas bag with Roberts valve

Put inside the strip and absorption column to improve the air/liquid contact. Available with many suppliers

Cut to fit on the perspex frames

In-house design, see tab "reactor frames" in this file



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ELECTROCHEMICALLY AND BIOELECTROCHEMICALLY INDUCED AMMONIUM RECOVERY

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13th June 2014

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Answers to Reviewers of Manuscript JoVE52405R1 entitled 'Electrochemically and Bioelectrochemically Induced Ammonium Recovery'**The comment made by the reviewer is written in bold.**

The answers by the authors are provided under the comment, as italic text. All changes to the manuscript have been made using the "track-changes" mode in Microsoft Word.

Text from the manuscript appears as plain text. The line numbers that are referred to are the line numbers for the final document, with "show markup" off.

Editorial comments:

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (52405_R1_062514.docx) is located in your Editorial Manager account under "File Inventory." Please download the .docx file and use this updated version for any future revisions.

We have accepted all changes and started from that document to answer the comments written below. We used the "track changes" function when text was modified.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

We have carefully checked the manuscript for spelling and grammar mistakes. All changes are included as tracked changes.

2. In the trouble shooting in the Discussion, please amend the following: "(ii) too great a _____ between the electrodes,"

The text has been adapted as:

Line 673:

(ii) too great a space between the electrodes

3. Step 1.1 should reference a table or materials list.

A reference to the list of materials included in the paper has been added.

Line 120:

1.1) Collect all necessary material to build the reactor: electrodes, frames and rubbers (See List of Materials).

4. The Representative Results section is not written according to JoVE format. It includes what should be protocol steps that have not been previously discussed, such as how to calculate things like efficiency and flow rate. It also contains several items which belong in the Discussion.

We have adapted the Representative Results section to correspond to the JoVE format. All calculations have been moved to the Protocol section. We have moved discussion on the effect of temperature to the discussion section and the discussion on

membrane fouling to the troubleshooting part.

5. Length Warning: The highlighted length is at our upper limit, so care should be taken if material is added after peer review.

The protocol length was checked to not exceed the upper limit of 2.75 pages of highlighted protocol.

Reviewers' comments:

Reviewer #1:

The topic is well introduced. The manuscript provides the details required to build an ammonium recovery cathode. The results obtained were previously published in other high-impact journal. This demonstrates the quality of the research and the interest of this protocol.

The authors thank Reviewer #1 for these positive comments

Reviewer #2:

Manuscript Summary:

This manuscript presents a useful description of protocols for the construction, operation, analysis of data, and presentation of data for electrochemical experiments to remove ammonium from wastewater streams. This information will help to standardize the way information is presented and make it easier to compare results by different researchers.

We thank Reviewer #2 for these comments. We indeed believe that this article can contribute to standardization in our field of research.

Major Concerns:

1. The authors need to rewrite the abstract, introduction, and discussion to more clearly present the differences between urine, anaerobic digestate, and other ammonia-rich waste streams of interest, and the relevance of their actual experimental work to these different applications. The abstract mentions only urine, but the synthetic wastewater used by the authors is not representative of urine (acetate as C source) - it seems more like a simulant of digestate but the authors should state what they are trying to simulate. The introduction mentions only ammonia rich waste streams and anaerobic digestion of manure. The implications of the electrochemical vs bioelectrochemical approach should be discussed for different types of waste streams.

The aim of the manuscript and video are to provide guidelines for the operation of a bioelectrochemical or electrochemical cell for ammonium recovery. The experimental work and representative results form the core of the paper and therefore we did not elaborate on all possible waste streams that could potentially be treated by the technique, as well as the outcomes of all these particular cases. We have now elaborated on the possible applications in the introduction of the paper to clarify the aim.

Line 103:

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. 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

2. How does the authors bioelectrochemical cell compare with that used by Kuntke et al (2012)? This paper should be cited and the approach and results compared with the current work.

Kuntke, P., Śmiech, K. M., Bruning, H., Zeeman, G., Saakes, M., Sleutels, T. H. J. A., et al. (2012). Ammonium recovery and energy production from urine by a microbial fuel cell. *Water Research*, 46(8), 2627-2636. doi:10.1016/j.watres.2012.02.025

We agree that the work by Kuntke and co-workers is similar to the bioanode results presented here. The main difference is the use of an MFC in the case of the work by Kuntke and an MEC in the case of the work presented here. The current density produced by the bioanode in the MEC was much higher (27 A m^{-2}) compared to the work of Kuntke et al. (0.5 A m^{-2}). The resulting flux of ammonium was thus much higher in the MEC case ($200 \text{ g N m}^{-2} \text{ d}^{-1}$ for the MEC compared to a maximum of $3.29 \text{ g N m}^{-2} \text{ d}^{-1}$ for the MFC). The small energy investment for the MEC results in much higher recovery rates.

We have included a discussion on these results in our manuscript.

Line 604:

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.

3. Line 191-193. Please expand on the discussion of inoculum. What is the rationale for using a mixture? Which inocula did the authors use in the reported research?

We chose to use a mixture to increase the likelihood of quickly enriching for an electroactive community, then used what was available in the lab. Step 2.2.1 states what we used in this reactor.

Minor Concerns:

4. Line 642 - missing word?

There was indeed a word missing, this has been adapted.

Line 673:

(ii) too great a space between the electrodes

Reviewer #3:

Manuscript Summary:

The manuscript details how an electrochemical and bioelectrochemical cell can be set up with the objective to recover ammonium from waste streams.

Major Concerns:

In general the manuscript is clear and well set up. There are some issues lacking or requiring more detail/explanation that I will outline below.

What is unclear to me is what, in the authors' opinion, is the main objective of this treatment. In the abstract, urine is mentioned as a potential source, in the introduction the focus seems on decreasing ammonia toxicity (line 102-104). The objective could be phrased more clearly, as it may influence the results of the experiment.

We agree that the objective of the treatment could be emphasized more in the text. We have adapted the text and included the two processes that, to our opinion, provide the best applications of this technology: decreasing ammonium toxicity in anaerobic digestion of nitrogen-rich waste streams (as discussed in the original manuscript by Desloover and co-workers) and the treatment of urine. The text is now as follows:

Line 103:

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 nutrients⁴. 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.

Minor Concerns:

ABSTRACT

line 53: a word is missing: a higher voltage to produce?

A word is indeed missing, this has been adjusted.

Line 52:

... electroactive bacteria catalyze the anodic reaction, whereas in the electrochemical cell the potentiostat applies a higher voltage to produce a current.

line 59-60: the phrasing is unclear, what is included in the energy input required to drive the extraction? This makes the reader believe that e.g. energy for stripping is included, which is not the case.

The extraction which is mentioned in this paragraph only concerns the membrane extraction. The energy input required for the extraction of ammonium through the cation exchange membrane can be compared for the electrochemical and bioelectrochemical system. We have clarified this in the text:

Line 59:

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.

INTRODUCTION

please clarify the aim

The last paragraph of the introduction has been adapted to clarify the aim.

Line 109:

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. I don't find a description for installing the stripper. Also no Raschig rings are mentioned

A protocol step has been added to explain the use of Raschig rings in the strip and absorption unit. The connection of the unit is detailed on Figure 1 and will be visualized in the video. The protocol text now includes:

Line 158:

1.10) Add Raschig rings in both the strip and absorption column to fill the columns halfway.

2. Why is a potential of -200 mV vs Ag/AgCl chosen? This needs some explanation or discussion in the discussion section. Why is potential controlled operation favored compared to current controlled operation?

We agree with this comment and have included this in our discussion section. We refer to the following paper to explain the choice of the anode potential:

Aelterman, P., Freguia, S., Keller, J., Verstraete, W. & Rabaey, K. The anode potential regulates bacterial activity in microbial fuel cells. Applied Microbiology and Biotechnology. 78 (3), 409-418, doi:10.1007/s00253-007-1327-8 (2008).

Line 585:

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 at the same time to regulate 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

REPRESENTATIVE RESULTS

Is the bioelectrochemical cell operated in MEC mode? Perhaps the authors can provide typical cathode potentials (or cell voltages) to be found to verify correct operation

Yes, the bioelectrochemical system is operated as MEC. We have clarified this in the introduction. We have also provided the cathode potentials obtained in our test, but it is important to note that the potentials are highly dependent on the material used. The text was adapted as follows:

Introduction section (line 111):

The main aim of the protocol focuses is to provide a means for the comparison of electrochemical nutrient ammonium recovery and bio-electrochemical nutrient ammonium recovery using an electrolysis cell.

Representative results section (line 459):

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.

Also for the electrochemical system, I would suggest to include a graph/data which show the performance of the EC, in terms of I vs V.

The cell voltage is the most important variable in the case of the electrochemical system, as it will determine the necessary energy input for electrochemical ammonium extraction. This variable is shown in table 5. It is our choice not to provide details on specific anode and cathode potentials to avoid confusion by the reader. We have however added a reference to the original paper by Desloover et. al. (2012) in which the potentials can be found for the conditions tested.

Line 465:

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.

Line 388: It is not clear how "the cell potential of the biological system started to increase". Is this related to anode potential (decrease)? Please rephrase.

The increased cell potential was probably due to scaling or diffusional limitations, as explained in the same paragraph. We have clarified that the anode potential remained fixed at -200 mV vs Ag/AgCl.

Line 452:

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.

Line 404: Do the authors have a suggestion of how to deal with scaling?

For laboratory tests, in which the membrane can easily and frequently be changed, scaling does not form a major problem. In other cases, intermittent polarity reversal could resolve this problem, as well removal of Ca^{2+} and Mg^{2+} prior to operation, e.g. via struvite precipitation. We have chosen not to detail this in the manuscript, as it represents a highly different line of research and development.

Line 411-471: Equations to calculate the energy required/produced per kg N are missing, while this aspect is discussed later. Including it would improve the analysis.

We agree with this comment and have included the equation in the protocol section.

The conditions for which the calculation are made are also described in table 6: 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.

Line 405:

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

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

With ΔV the measured potential difference between anode and cathode.

Line 475: Stripping efficiency is also dependent on liquid distribution, as it largely affects the gas liquid transfer. Perhaps this can be included (here or in the troubleshooting part)

We agree with this comment. We have added this to the troubleshooting part:

Line 701:

Therefore, a minimum gas to liquid ratio of 1000 (G/L) is advised. The use of Raschig rings is imperative to favor the liquid/gas transfer during stripping.

Line 616: I agree that the bioelectrochemical system is even more sensitive to disturbances. Therefore a troubleshooting chapter that focuses on the bioanode would be useful. What if the current production is hampered unexpectedly?

As written in our article, we suggest to watch the JoVE video article by Gimkeiwicz and Harnisch regarding the operation of a bioanode. We believe that this video article combined with the protocol presented here offer a good basis for operation of bioanode reactors.

Gimkiewicz, C. & Harnisch, F. Waste Water Derived Electroactive Microbial Biofilms: Growth, Maintenance, and Basic Characterization. JoVE (82), e50800, doi:doi:10.3791/50800 (2013).

DISCUSSION

Line 580/581: Numbers are mentioned for energy input, but it is not clear where these numbers come from.

The formula to calculate the energy input has been added to the protocol section (5.8). The conditions for which the calculation are made are also described in table 6: 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.

Line 405:

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

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

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 periode, for the electrochemical reactor the average for the entire run is taken.

Line 642: word is missing (there is only a line)

There was indeed a word missing, this has been adapted.

Line 673:

(ii) too great a space between the electrodes

REFERENCES

Why is there no reference to the work of Jung-rae Kim and Philipp Kuntke?

We agree that the studies by Kuntke and co-workers are an interesting basis for comparison. We have included references to their work in our JoVE article. The work of Jung-rae Kim and co-workers discussed possible routes for ammonia loss. As we make use of the same principles for our work, we have added a reference to this publication.

The following references were added:

Line 90:

1. Kim, J. R., Zuo, Y., Regan, J. M. & Logan, B. E. Analysis of ammonia loss mechanisms in microbial fuel cells treating animal wastewater. *Biotechnology and bioengineering*. **99** (5), 1120-1127, doi:10.1002/bit.21687 (2008).

Line 107:

2. Kuntke, P., Sleutels, T. H. J. A., Saakes, M. & Buisman, C. J. N. Hydrogen production and ammonium recovery from urine by a Microbial Electrolysis Cell. *International Journal of Hydrogen Energy*. **39** (10), 4771-4778, doi:http://dx.doi.org/10.1016/j.ijhydene.2013.10.089 (2014).

Line 610:

3. Kuntke, P. *et al.* Ammonium recovery and energy production from urine by a microbial fuel cell. *Water Research*. **46** (8), 2627-2636, doi:10.1016/j.watres.2012.02.025 (2012).

Additional Comments to Authors:

Nice idea to submit your work to this journal.