*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 g N m-2 d-1 for the MEC compared to a maximum of 3.29 g N m-2 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 innocula 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. Add Raschig rings in both the strip and absorption column to fill the columns halfway.

1. **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 Ca2+ and Mg2+ 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 (EN, expressed as kWh/kg N) (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 (EN, expressed as kWh/kg N) (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 Jungrae 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:*

1. 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:*

1. 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.**