**JoVE5515R3 Author’s Responses (John Lisle, USGS)**

**Editorial comments:**

1. Line 34 “Because the groundwater source strictly anaerobic, extremely reduced (< -365 mV) and approximately 400 m below land surface…” – fragmented sentence please revise.

Author response: Completed

2. Line 70 “…land surface where day time temperatures can reach 50°C” – daytime

Author response: Completed

3. Line 77 “For this protocol, use a the diffusion chamber is an alternative design…” fragmented sentence, please revise

Author response: Completed

4. Line 81 “This type of diffusion chambers is used…”

Author response: Completed

5. Note Line 81-84 please break-up or edit for sentence flow.

Author response: Completed

6. Line 89 “choose an appropriate sized membrane” appropriately

Author response: Completed

7. Line 130 “Obtain the inner walls of the outer container that is commercially available polypropylene tank”.

Author response: Need clarification on the specific issue with this sentence.  
  
8. Results: Figure 3: please provide a photographic image showing the diffusion chambers (some or all of them) in place in the inner container.

Author response: Completed  
  
9. References: Please use abbreviated journal titles and provide DOI for references where possible.

Author response: Journal abbreviations inserted and DOI information entered for all of the references except #6, 7, 10, 14, 16 and 17. DOI numbers are not available for those publications.

**Reviewer #1:**  
  
1. What is the expected "life-time" of these chambers? Is it possible to estimate the turnover time for their use? What materials are most likely to fail and/or need replacing?

Author response: I did not perform any type of tolerance experiments for any of the materials described in this protocol, so providing an estimation of the “failure” rate for these materials is not possible. I think it’s apparent in the way the protocol was written that the researcher should select materials for the fabrication of the diffusion chambers and the inner and outer containers that’s resistant to the geochemical conditions of the water which will be in contact with those materials for the expected exposure time.

2. Although the materials appear to be from accessible sources (scientific supply companies and retail stores found in the United States for example), sourcing the construction materials may be somewhat prohibitive to groups from developing countries. But, this in no way invalidates the novelty and utility of the instrument developed and presented.

Author response: Agreed.

**Reviewer #2:**  
  
1. The experimental protocol language is confusing and long. It can be made shorter.

Author response: I disagree.

2. The experimental protocol should be reported in past tense

Author response: This comment is the opposite of previous editorial comments made by the JoVE staff.

3. At some places in the manuscript the sentence making is improper and it does not make any sense.

Author response: Please provide specific examples.

4. The Sampling for the colony forming units (CFU) was it done in sets? Was the standard deviation and Standard Error accounted for in the samples of CFU?

Author response: The data and interpretations are included as an example of how the diffusion chambers and above ground mesocosm can be used to answer a specific research question. Accordingly, only a single sample was collected for each time point and no experimental repetition was attempted.

5. Why was there an initial rapid inactivation rate followed by a much slower rate? How long was the sampling done? Explanation can be added in the manuscript

Author response: The manuscript is supposed to focus on, in this case, the fabrication of diffusion chambers and an above ground mesocosm, not on describing or defending trends in the representative data. I disagree that an explanation of what might be causing the data to follow a biphasic inactivation model would add substantially to the length of the paper and, in my opinion, be outside the original objective of the manuscript submission.

6. The name of Agent/Equipment and catalog number can be added as supplementary material in the manuscript.

Author response: Perhaps I’m misinterpreting the comment, but per the JoVE guidelines the used of “…agent/equipment and catalog number(s)…” is not permitted.

7. The field application of this research should be elaborated in the discussion.

Author response: I think the field applications for the diffusion chambers and above ground mesocosm are adequately described for the JoVE format.

8. Were other variables like dissolved oxygen,reduction potential etc. monitored for the whole experimental period? If yes then it can be reported as a table.

Author response: Please see the author’s response to Comment #5 from Reviewer #2.

9. The authors can add some literature review in the introduction of what previous studies have been attempted

Author response: When considering the objective of the manuscript, I think the “literature review” of previous studies is adequate. I do not agree that increasing the introduction or discussion of previous studies on the inactivation of *E. coli* in groundwater will increase the value of the manuscript.  
  
10. Line 77. For this protocol,use "of a" diffusion chamber

Author response: Completed

11. Line 78. 'Membranes used were....

Author response: Completed

12.Line 149 'Drill a hole of same diameter'

Author response: Completed

13.Line 180. 'Width of the gasket should be equal to the thickness'

Author response: Completed

14.Line 324. As in step 4.1.12 pr 4.1.2?

Author response: Corrected

15. Line 326 needs to be reframed

Author response: Please provide more detail on what needs to be “reframed”.

16. Line 360 'Research questions being asked'

Author response: Corrected

**Reviewer #3:**

1. Introduction has no references to previous studies? Or other approaches that have been adopted to measure microbial decay rates?

Authors response: The focus of the manuscript is the construction and application of diffusion chambers and an above ground mesocosm, not microbial decay rates. The data presented in the Representative Results section was included just as an example. The diffusion chambers and mesocosm can be used to study a variety of microbial responses to almost any environmental stimulus.

2. Suggest include a literature summary of previous decay rates and methods, thereby outlining the gap that this article tries to fill.

Authors response: See author’s response to Comment #1 for Reviewer #3.

3. Also why is it important to measure the decay rates? E.g. for drinking water and water recycling via aquifer risk assessment, such as: \*Toze, S., Bekele, E., Page, D, Sidhu, J., Shackelton, M. (2010) Use of static Quantitative Microbial Risk Assessment to determine pathogen risks in an unconfined carbonate aquifer used for Managed Aquifer Recharge, Water Research, 44, 1038-1049. \*Page, D., Dillon, P., Toze, S., Bixio, D., Genthe, B., Jiménez Cisneros, B., E. and Wintgens, T. (2010) Valuing the subsurface pathogen treatment barrier in water recycling via aquifers for drinking supplies, Water Research, 44, 1841-1852. \*Page, D., Vanderzalm, J., Barry, K., Levett, K., Sidhu, J., Toze, S., Kremer, S. and Dillon, P. (2010) Managed Aquifer Recharge (MAR) staged risk assessment framework applied to the Parafield Aquifer Storage Transfer Recovery system to assess human and environmental risks, Journal of Environmental Quality, 39, 6, 2029-2039.

Author response: This is a very relevant collection of publications from a very productive research group, all of which do describe bacterial inactivation rates in a variety of aquatic systems. However, as previously stated, the selection of inactivation data was just to serve as a representative data set which could be collected from the diffusion chambers and mesocosm. Inactivation rates were not the focus of the manuscript and dedicating text to this topic would detract from that describing the diffusion chambers and mesocosm.

4. The protocol seems very involved. It could be better describv3ed by referencing a similar design e.g. Sidhu et al (2015) - already cited and outlining differences.

Author response: I admit the protocol is involved and painstakingly broken down into as many simple steps as possible. This format follows that outlined by the JoVE editorial staff.

5. The biphasic model has also been referred to as the broken stick model by other researchers.

Author response: I agree but would prefer to us a more technical description.

6. Line 542: Sentences such as "treated waters must be free of E. coli prior to recharging and during recovery" is simply untrue. Water is commonly recharged with E. coli present and the aquifer can have a treatment effect on the water quality, e.g. \*Page, D.W., Vanderzalm, J.L., Barry, K.E., Torkzaban, S., Gonzalez, D. and Dillon, P. J. (2015) E. coli and turbidity attenuation during urban stormwater recycling via Aquifer Storage and Recovery in a brackish limestone aquifer, Ecological Engineering 84, 427-434

Author response: I disagree but recognize that the differences in opinion may be due to differences in regulatory statutes between the reviewer’s and author’s state and possibly country. The state of Florida regulations that govern the microbiological quality of water injected into groundwater systems like those described in this manuscript clearly state the concentration of E. coli should be zero.

7. Line 547 use SI units.

Author response: Corrected

8. Line 548 and elsewhere, need to quantify these statements such as "extremely reduced geochemical conditions"

Author response: There are five references within the current version of the manuscript that define the reduced conditions for the example aquifer as < -300 mV. I don’t agree that this value has to be inserted after each reference to the reduced conditions within the example aquifer as this is the only water source described in the manuscript.

9. Line 543, why is this important. How is this an improvement over current down hole methods? Given that groundwater chemistry should be quite stable, why would you need to take so many measurements?

Author response: First, I think it’s obvious that the time required to open up and extract a set of diffusion chambers in the described above ground mesocosm would be significantly less than the time required to retrieve the same diffusion chambers from 1000’s of feet below surface (i.e., few minutes vs. hours) using some type of cable retrieval system. Secondly, there are several scenarios where the geochemistry of the groundwater would change (e.g., contamination plume, recharged surface water with relatively elevated concentrations of dissolved oxygen and nutrients) and the several collection events for samples and diffusion chambers would be necessary to characterize changes in the geochemistry.

10. Line 551 No discuyssion of why the decay is biphasic.

Author response: See author’s response to Comment #5 from Reviewer #2.

11. How do these results compare to other E. coli decay rates? A comparison table would be useful here.

Author response: See author’s response to Comment #5 from Reviewer #2.