* 1. **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 (55256\_R1\_081516.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.  
       
     1. Please include an Acknowledgements and Disclosure section.  
      These were added.   
       
     2. Please abbreviate all journal titles.

A copy of the Reference section with abbreviated journal titles was uploaded as a supplemental document and labeled “ Corrected JoVE References”.  
  
3. Formatting: Please fill out the acknowledgement and disclosure sections.  
 These were added and filled out.

4. Grammar:  
-3.2.1 – “weight” should be “weigh”

This was changed.

-3.3.3 – “commercialferrous’  
 Changed to “commercial method kit”

5. Additional detail is required:  
-Section 2 – Please clarify system 1 vs system 2. Both involve raceway ponds, so how does this involve a comparison between small and large scale?

This was clarified by editing Section 1.1 to:

“For one paired system, use one 100 L aquaria tanks (AT), with an overhead mixer for the small-scale vessel and one 1000 L raceway pond (RWP), with a paddle wheel mixer for the large-scale vessel. Vessels used in this system are pictured in Figure 1.”

As described in 1.1, the AT are small scale vessels, and the RWPs are large scale vessels. The results from the small scale ATs and the large scale RWPs are compared. I also added a line in the results section to help further clarify the difference between system 1 and 2:

“This study uses two paired systems, referred to as System 1 and System 2, to duplicate its findings.”

-3.4 – How are these conditions monitored?  
The following text was changed to:

3.4 Monitor environmental conditions (air temperature, solar radiation, wind speed) using a commercial weather station as well as tank/pond conditions (water temperature, pH, dissolved oxygen) using commercial probes and data logger.

6. Results: The data in Figure 2 and Figure 3 should be discussed in the Results section, not the  
  
7. Discussion. Please move the figures to this section, and describe what they show in the Results section.  
 Figure 2 and 3 were moved to the results section.

**Reviewers' comments:**  
  
Editor’s Note:  We do not require in depth or novel results for publication in JoVE, only representative results that demonstrate the efficacy of the protocol. However, please ensure that all claims made throughout the manuscript are supported by either results or references to published works.  
  
**Reviewer #1:**  
*Manuscript Summary:*  
N/A  
  
*Major Concerns:*  
Since this article objective is to compare performance and condition of small and large scale reactor for algal remediation of leachate, the retention time used in this study is too short and experiment set up does not represent the real situation. For wastewater treatment especially high concentration like leachate, longer experiment cycle time or longer continuous experiment time should be tested to see how variation of condition will happen after long period of treatment time then analyze the variation and propose some design equation or factor for better translation of mall to larger scale. So the protocol presented in this article is not really useful for designing full scale or commercial scale from experimental scale.  
 We do not disagree that 8 weeks is too short for a full study. The 8 weeks of data are presented as “representative results” as described in the Editors notes and JoVE’s scope. The last sentence of the first paragraph of the discussion acknowledges exactly this:

“Representative results account for the first 8 weeks of system operation, however a full study could extend for much longer periods to account for seasonal variability in environmental conditions.”

A longer retention time is also suggested in the second paragraph of the Variable reactor parameters section:

“The length of time between mixing the two vessels can be modified based on the application. Since most alga are relatively slow-growing microorganisms, one week is recommended as the shortest amount of time that should be used. A longer period of time between mixing may reveal some variation in the productivity caused by small differences in environmental conditions between the two scales.”

The protocol presented in this article is stated in the last paragraph of the introduction as follows:

“The protocol presented here is designed to evaluate the effect of scale on algal growth and nitrogen removal in a leachate treatment system.”

*Minor Concerns:*  
The discussion of the system performance does not give clear explanation to the factor that effect the performance of the bioprocess. For example, the author explain that light exposure, mixing, CO2 concentration and pH could effect performance of the process but the author do not explain why the small and large scale in this experiment give same result while lab scale has different SA:V and LE-SA:V comparing with the large scale. More explanation with references are required in the discussion.  
 In the second paragraph under the System performance section in the Discussion we discuss potential reasons why these two scales yield similar results:

“…if the productivity values of the small- and large-scale vessels in each system are equal, then it is likely that the small-scale vessel creates similar growth conditions as the large-scale vessel or any differences between the two different scaled reactors affect productivity negligibly. In this situation, the values from the small-scale system would likely be representative predictors of productivity in a full-scale system.”

*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
Author reported experimental results on comparison of the performance of two different sized reactors designed for wastewater treatment. In this study, ammonia removal, nitrogen removal and algal growth are compared over an 8-week period in paired sets of small (100 L) and large 37 (1000 L) reactors designed for algal remediation of landfill wastewater. The content of the manuscript has technical value and it is publishable. I have following comments:

1. How did authors choose 100 L is small and 1000 L is large reactors? For example as author mentioned, Junker (2004) presented results from a comparison of eight different-sized fermentation reactors, ranging from 30 L to 19,000 L.

100L and 1000L reactors were chosen based cost and facility size limitations.

2. What is difference between total nitrogen concentrations and total dissolved nitrogen in the abstract? Need to be consistent all over the manuscript.

These are the same; the abstract was changed in order to keep terminology consistent throughout the manuscript.

3. R2 mentioned in the abstract is low that may need to explain

The statistical correlation between these two factors (initial ammonia concentration and ammonia removal rate) has a ρ=0.90. This is statistically significant and strong correlation, and is presented in the last paragraph of the results section. The R2 value shows variability since this study was not done in a highly controlled environment. An R2 value of 0.76 is still strong.

4. In Fig1A and 1B, why data going down (7week) then up (8week)? Author may need to plot controls too.

I think this is referring to Figure 2, not Figure 1. The ammonia and total nitrogen removal rates of system 1 are much higher in week 8, than in week 7. This may be due to the fact that the water temperatures rose by 7 degrees F during that week (data not shown), however, due to the small sample size of this data set there is not enough evidence to present the variability in this one data point.

5. Was there any byproducts of total nitrogen and how was the mass balance?  
Nitrate, nitrite, and ammonia were all measured throughout this study. Ammonia was the most abundant species, with nitrite never reaching about 2mgN/L. Nitrate was present, data not shown.

*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #3:**  
*Manuscript Summary:*  
The manuscript investigates the comparison of nitrogen removal and algae productivity in semi-batch operated small and large scale reactors from leachate. The bioprocesses used in the study are suspended growth aquarium tank and raceway pond. They were operated sequentially. Ammonia removal rate, total nitrogen removal rate and biomass growth rate in the reactors were evaluated. The main parameter studied is the initial ammonia concentration.  
  
*Major Concerns:*  
-The main parameter studied is the initial ammonia concentration. Treatment of leachate by algae is an original contribution to the literature. The author states that it is a scale comparison study. But the reactors used are aquarium tank and raceway with different sizes. The operation principles of these two bioprocesses are not the same. Therefore, I do not see the main purpose of this comparison. In addition, the reactors were operated sequentially. Therefore, the process should be considered as single bioprocess as far as I am concerned.

The aquarium tank and raceway ponds are an order of magnitude different in volume. These two vessels were operated in parallel, not sequentially, as described in the protocol sections 1-2. This has been clarified in the note following the System set up and throughout the protocol:

“**Note:** A ‘paired system’ refers to one aquarium tank and one raceway pond, run in parallel.“

A line was also added to the first paragraph of the discussion section:

“The system was operated as a semi-batch reactor, where each week was operated under discrete conditions.”

The ATs and RWPs were different sizes on purpose, so that the bioprocesses occurring within these vessels could be compared. Comparing the growth rates and removal rates occurring at potentially different rates is the point of this manuscript.

-A bioproceses for any purpose should be operated until steady state conditions were obtained for he target response parameter or parameters such as ammonia, dissolved nitrogen as they were measured in this study. Lines:201-206 states that the "the volume of the each tanks were mixed every week". What I understand from this sentence is that the water from race way were mixed with the aquarium tank. It means that the biomass concentration and nutrient concentrations in each tank were somehow equalized. As far as I am concerned it shouldn't have been done. Because, the biomass production and nutrient removal capacities of tanks would be different. In other words, the concentrations of nutrients in the each tank were different and they were made the same by mixing. I understand that the microbial population could diverge. In fact this is what should have been observed. The rate of removal could change by this mixing as well. Another problem with this mixing is to reach the steady state conditions in the reactors. If the conditions are changed during operation, steady state conditions can not be reached.  
 The volumes of the tanks were mixed in between sampling, (after the “end of the week sample” was taken, but before the “beginning of the week sample” was taken). This is described in Section 2.2-2.6. The lines referenced in the comment above are misquoted. They actually say:

“The volume from the raceway pond and aquarium tank of each system were mixed at the beginning of each week to ensure that the starting conditions, specifically the inoculum culture, in the two scales were equal. These volumes were mixed at the beginning of each weekly cycle to reduce the likelihood that the microbial community of the cultures would diverge over the course of the study, which would have led to an inaccurate comparison of reactor conditions.“

The AT and RWP were not mixed continuously throughout the week. The wording of 2.3.1 was adjusted to clarify this

“Once per week, pump the entire volume of the aquarium tank into the raceway pond”

The wording throughout the protocol section was adjusted to help further clarify this point.

The RWP and AT are mixed once before the start of the week to ensure that the microbial population in the two vessels are equal. In the discussion section it is stated that:  
 “The length of time between mixing the two vessels can be modified based on the application. Since most alga are relatively slow-growing microorganisms, one week is recommended as the shortest amount of time that should be used. A longer period of time between mixing may reveal some variation in the productivity caused by small differences in environmental conditions between the two scales.”

The results presented in this manuscript are a small representative of what a full study would include. This is included according to the JoVE Editor’s note, and scope of this journal. It is also stated in the last sentence of the first paragraph of the discussion section:

“Representative results account for the first 8 weeks of system operation, however a full study would extend for much longer periods to account for seasonal variability in environmental conditions.”

*Minor Concerns:*  
-Lines 108-109: "A 'paired system' refers to one aquarium tank and one raceway pond. This study uses two paired systems to duplicate its findings." As far as I understood, aquarium tanks and raceway systems were operated sequentially (lines 142- 143) and two sequential systems were run in parallel. System 1 and System 2 states the each or these parallel operations. Please define system 1 and system 2 in Lines 108 and 109 (for example) if they are parallel operations.

The note under system set up was changed to include that these systems were operated in parallel. “**Note:** A ‘paired system’ refers to one aquarium tank and one raceway pond, run in parallel.“

-Lines 142-143: "Pump the tank of system 1 into the raceway pond of system 1. Pump the tank of system 2". It means that the run was sequential. Raceway was operated with the effluent of tank. The initial nutrient and biomass concentrations were the conditions in the effluent of the tank. The questions is that Was the rate calculations done by considering this fact? Because, the rates given in Figure 2 are different than that of Table 1.

These systems were not run sequentially. The protocol was reworded slightly to help clarify this. A line was also added to the discussion to help clarify this further: “The system was operated as a semi-batch reactor, where each week was operated under discrete conditions.”

-System was partially loaded with fresh leachate every 3 weeks. It means that the system was loaded 2.5 times for 8 weeks operations. Form Figure 2, It looks that system performance substantially changed on 8th week especially for the system 1. Can this be explained?

The system was partially loaded with leachate every week. Leachate was added every week (2.4), for a total of 8 times over the 8-week representative time period. One third of the volume was removed each week (section 2.4), which results in a 3-week hydraulic retention time.

-Representative results: Only second paragraph gives some results. But first paragraph repeats the protocol. It can be removed.

The first paragraph was cut down so that the protocol description was removed but important information about starting conditions remains.

-Discussion section: This section gives general knowledge about the effect of variables on algae photobioreactors.  
  
*Additional Comments to Authors:*  
N/A