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TITLE:

Comparison of scale in a photosynthetic reactor system for algal remediation of wastewater

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KEYWORDS:

Nutrient removal, algae biomass growth, scale-up, ammonia nitrogen removal, wastewater remediation, large-scale

SHORT ABSTRACT:

An experimental methodology is presented to compare the performance of small (100 L) and large (1000 L) scale reactors designed for algae remediation of landfill wastewater. System characteristics, including surface area to volume ratio, retention time, biomass density, and wastewater feed concentrations, can be adjusted based on application.

LONG ABSTRACT:

An experimental methodology is presented to compare 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 (1000 L) reactors designed for algal remediation of landfill wastewater. Contents of the small and large scale reactors were mixed before the beginning of each weekly testing interval to maintain equivalent initial conditions across the two scales. System characteristics, including surface area to volume ratio, retention time, biomass density, and wastewater feed concentrations, can be adjusted to better equalize conditions occurring at both scales. During the short 8-week representative time period, starting ammonia and total nitrogen concentrations ranged from 3.1-14 mg NH₃-N/L, and 8.1-20.1 mg N/L, respectively. The performance of the treatment system was evaluated based on its ability to remove ammonia and total nitrogen and to produce algal biomass. Mean \pm standard deviation of ammonia removal, total nitrogen removal and biomass growth rates were 0.95 \pm 0.3 mg NH₃-N/L/day, 0.89 \pm 0.3 mg N/L/day, and 0.02 \pm 0.03

g biomass/L/day, respectively. All vessels showed a positive relationship between the initial ammonia concentration and ammonia removal rate ($R^2=0.76$). Comparison of process efficiencies and production values measured in reactors of different scale may be useful in determining if lab-scale experimental data is appropriate for prediction of commercial-scale production values.

INTRODUCTION:

Translation of bench-scale data to larger scale applications is a key step in the commercialization of bioprocesses. Production efficiencies in small-scale reactor systems, particularly those focusing on the use of microorganisms, have been shown to consistently over predict efficiencies occurring in commercial-scale systems ¹⁻⁴. Challenges also exist in scaling up photosynthetic cultivation of algae and cyanobacteria from the laboratory scale to larger systems for the purpose of manufacturing high-value products, such as cosmetics and pharmaceuticals, for production of biofuels, and for the treatment of wastewater. The demand for large-scale algal biomass production is growing with the emerging industry for algae in biofuel, pharmaceuticals/nutraceuticals, and livestock feed ⁵. The methodology described in this manuscript aims to evaluate the influence of increasing scale of a photosynthetic reactor system on biomass growth rate and nutrient removal. The system presented here uses algae to remediate landfill leachate wastewater but can be adapted for a variety of applications.

Production efficiencies of large scale systems are often predicted using smaller scale experiments; however, several factors must be considered to determine the accuracy of these predictions, as scale has been shown to affect the performance of bioprocesses. For example, Junker (2004) presented results from a comparison of eight different-sized fermentation reactors, ranging from 30 L to 19,000 L, which showed that actual productivity at pilot- or commercial-scales was almost always lower than the values predicted using small-scale studies ⁴. Inequalities in vessel dimension, mixing power, agitation type, nutrient quality, and gas transfer were predicted to be the major causes for the decreased productivity ⁴. Similarly, it has been shown in algae growth reactors that biomass growth and biomass related products are nearly always reduced when scale is increased ⁶.

Biological, physical, and chemical factors change with the size of a reactor, with many of these factors influencing microbial activity at small scales differently than at larger scales ^{2,7}. Since most full-scale systems for algae, such as raceway ponds, exist outdoors, one biological factor to consider is that microbial species and bacteriophages can be introduced from the surrounding environment, which may alter the microbial species present and thus the microbial function of the system. The activity of the microbial community will also be sensitive to environmental factors, such as light and temperature. Mass transfers of gasses and fluid motion are examples of physical factors that are influenced in the scale up of microbial processes. Achieving ideal mixing in small reactors is easy; however with increasing scale, it becomes a challenge to engineer ideal-mixing conditions. At larger scales, reactors are more likely to have dead zones, non-ideal mixing, and reduced efficiencies in mass transfer ². Since algae are photosynthetic organisms, commercial growth must account for changes in light exposure due to changes in water depth and surface area when increasing volume. High biomass density and/or low mass transfer rates can cause decreased CO₂ concentrations and increased O₂ concentrations, both of which may result in inhibition of biomass growth ⁸. Chemical factors in an algae growth system

are driven by pH dynamics of the aquatic environment ², which is consequently affected by changes in pH buffering compounds such as dissolved CO₂ and carbonate species. These factors are compounded by complex interactions among the biological, physical, and chemical factors, often in unpredictable ways ⁹.

This study presents a paired reactor system designed to regulate and compare growth conditions in vessels of two different scales. The experimental protocol focuses on quantifying leachate treatment and algae growth; however, it could be adapted to monitor other metrics such as changes in the microbial community over time or the CO₂ sequestration potential of algae. The protocol presented here is designed to evaluate the effect of scale on algal growth and nitrogen removal in a leachate treatment system.

PROTOCOL:

1. System Set Up

Note: A 'paired system' refers to one aquarium tank and one raceway pond, run in parallel.

1.1. 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.

1.2. Inoculate all vessels with the same algae culture. Use a high density of the inoculation, resulting in a final density of no less than 0.1 g/L once diluted to the full volume in the tank or pond ¹⁰. It may take a considerable amount of time (weeks to months) to grow enough algae for this step.

1.3. Use untreated landfill leachate as the nutrient source. Use leachate taken from a landfill that accepts mostly domestic waste and has low levels of toxins. Composition analysis for the leachate should be available from the landfill. The amount of leachate used in each tank or pond can vary depending on the strength of the wastewater, but final ammonia concentrations should measure 5-75 mg NH₃-N/L.

1.4. Start the 100 L aquaria tank with a 60 L working volume, and the raceway pond with a 600 L working volume. This study started with approximately 1 L leachate in 59 L of water in the aquaria tank, and 10 L leachate in 590 L of water in the raceway pond. Increase the concentration of leachate used over the course of this study.

2. Weekly Operation and Sampling

2.1. Operate the aquaria tank and raceway pond as semi-batch reactors with hydraulic retention times of three weeks. Each sampling period spans one week.

2.2. Take a 125 mL sample from each vessel. This is the beginning of the week sample. Test samples according to the **Sample Analysis** protocol in sections 3.1-3.3.

2.3. At the end of the week, take 125 mL samples from each vessel for analysis. After the end-of-week samples have been taken, empty the entire volume of the aquarium tank into the

raceway pond.

2.3.1. Once per week, pump the entire volume of the aquarium tank into the raceway pond.

2.4. Remove one-third of the volume (for an average hydraulic retention time of 3 weeks) from the raceway pond. Replace volume removed with water and untreated leachate.

2.5. Transfer approximately 60 L from the raceway pond back into the aquarium tank. This ensures that the aquarium tank and the raceway pond are starting with the same nutrient and biological conditions each week.

2.6. Take 125 mL samples from all vessels for the analysis of the starting conditions for the next week.

3. Sample Analysis

3.1. Test all beginning-of-the-week and end-of-the-week samples for ammonia-N, nitrate-N, nitrite-N, and biomass density.

3.2. Measure biomass by standard total suspended solids (TSS) protocol, ASTM-D5907, using 0.45 μm filters.

3.2.1. First weigh a filter paper and then filter 20-40 mL of sample using a vacuum filtration system. Dry the biomass/filter paper in an oven at 105 °C for one hour, or until the weight of the biomass/filter paper no longer changes.

3.2.2. Weigh biomass/filter paper, and subtract the initial mass of the filter paper. Divide this mass by the volume filtered to calculate the biomass density. Run in duplicate ¹¹.

3.3. Measure ammonia, nitrate, and nitrite spectrophotometrically using a spectrophotometer.

3.3.1. Use 100 μL of sample in the commercial method kit to determine ammonia concentration. Refer to the manufacturer's protocol.

3.3.2. Use 1 mL of sample in the commercial method kit to determine nitrate concentration. Refer to the manufacturer's protocol.

3.3.3. Use 10 mL of sample in the commercial method kit to determine nitrite concentration. Refer to the manufacturer's protocol.

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. Refer to the manufacturer's protocol.

4. Statistical Analysis of Results

4.1. Determine if the data collected is statistically normal. Determine normality of the data set using a Q-Q plot¹².

4.2. Determine correlations among parameters using Pearson's r or Spearman's ρ for normal and non-normal data, respectively¹³. Correlation parameters should include at least the following parameters: initial ammonia concentration, initial total nitrogen concentration, initial biomass density, ammonia removal rate, total nitrogen removal rate, biomass growth rate, and all environmental conditions.

REPRESENTATIVE RESULTS:

The aim of this study is to compare the biomass growth and nutrient removal capabilities of algal cultures grown in small- and large-scale reactors. This study uses two paired systems, referred to as System 1 and System 2, to duplicate its findings. These representative results are from an 8-week period, February through April, 2016. The first raceway pond was inoculated with algae originally sourced from an outdoor pond in Philadelphia, PA¹⁴. This culture was grown to a high density in an aquarium tank. This inoculation resulted in a biomass density of 0.12 g/L in the RWP. After 2.5 weeks the second raceway pond and aquarium tank were inoculated, resulting in starting biomass densities of approximately 0.18 g/L. After a few weeks, all ATs and RWPs were mixed together for a uniform biomass density and microbial population among all vessels; regular operation and monitoring began as described in the above protocol.

Starting and ending parameters were measured on a weekly basis as described in the Sample Analysis section¹⁵. Initial conditions for biomass, ammonia and total nitrogen concentrations in all vessels ranged from 0.2-1.0 g/L, 3.1-14 mg NH₃-N/L, and 8.1-20.1 mg N/L, respectively. The mean and standard deviation of the removal and growth rates recorded from each vessel are presented in Table 1. These conditions yielded biomass growth rates, and ammonia and total nitrogen removal rates ranging from -0.04-0.07 g/L/day, 0.39-1.61 mg N/L/day, and 0.26-1.47 mg N/L/day, respectively from all four vessels. Weekly nitrogen removal rates and biomass growth rates from System 1 and System 2 can be seen in Figure 2.

[Place Figure 2 here]

Statistical correlations were used to compare parameters and identify possible trends. Input parameters were: initial ammonia concentration, initial nitrate concentration, initial nitrite concentration, initial total nitrogen concentration, starting biomass concentration, ammonia removal rate, nitrate removal rate, nitrite removal rate, total nitrogen removal rate, biomass growth rate, water temperature, pH. The data collected was not statistically normal so Spearman's ρ , the nonparametric correlation, was used. The strongest significant correlation was between the initial ammonia concentration and ammonia removal rate ($\rho=0.90$). The trend between initial ammonia concentration and the ammonia removal rate can be seen in Figure 3.

[Place Figure 3 here]

Figure 1. Examples of an aquarium tank and raceway pond.

An example of an aquarium tank (A) and raceway pond (B) are shown.

Figure 2. Summary of productivity over the representative study period.

Ammonia removal rates (A), total nitrogen removal rates (B), and biomass growth rates (C) are

presented in the top, middle and bottom panels, respectively. Results from system 1 are presented on the left, and system 2 on the right. Results from aquaria tanks and raceway ponds are represented on all graphs by X, and Δ, respectively.

Figure 3: Ammonia removal rate as a function of starting ammonia concentration. Data from all vessels over the representative 8 weeks are presented. Trendline $R^2 = 0.76$.

Table 1. Mean \pm standard deviation of productivity rates in individual vessels

DISCUSSION:

System performance:

Over the course of an 8-week study, the productivity of the small- and large-scale vessels in a system were compared. In this study nitrogen and ammonia removal rates and biomass growth rates were used as measures of productivity of the treatment system. The system was operated as a semi-batch reactor, where each week was operated under discrete conditions. 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.

The methodology described above calls for mixing the paired system (small- and large-scale vessels) together every 7 days. Therefore the difference in productivity between the two scales over this time period depends solely on the different conditions in the two sized vessels. For example, if the light exposure in one of the reactors is significantly less than in the other, the biomass growth rates will be significantly different. Dead zones due to incomplete mixing, poor mass transfer, variable CO₂ or pH, along with any other discrepant conditions between the two scales could cause differences in the productivity of the vessels within the paired systems. On the other hand, 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.

The treatment capacity of this system was evaluated based on its ability to remove nitrogen. Statistical correlations among all factors revealed a strong, positive correlation ($\rho=0.90$) between the starting ammonia concentration and the ammonia removal rate. This same positive correlation was seen in a previous study conducted in the aquaria tanks¹⁴. This positive trend between the ammonia removal rate and the starting ammonia concentration can be seen in Figure 3, which includes data collected from all RWPs and ATs. The nitrogen removal rates from the two vessel types can be compared to identify scale-specific trends once more data has been collected.

Variable reactor parameters:

Key reactor variables include the surface area to volume ratio and residence time. Reactors were operated in a semi-batch manner, with a complete mix of small and large vessels and 1/3 total reactor system volume replacement every 7 days. While the mixing period in this study was one week, as stated in section 2.3, this time could be modified depending on the growth and nutrient consumption rates of the photosynthetic cultures, as well as the ultimate application of the full-

scale system. The surface area to volume ratio, which can be modified by changing the volume, will influence the mass transfer rate of gases as well as light exposure for photosynthetic organisms.

The volumes 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 both scales were equal. The length of time between mixing the two vessels can be modified based on the application. Since most algae 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. Too much time between mixing the scales would allow for the microbial communities to diverge significantly, at which time the comparison between scales would no longer be accurate of the reactor conditions. Even when extending the length of time between mixing the two scales it is important to complete several repetitions in order to verify that any difference (or lack of differences) in productivities is significant.

The surface area to volume ratio can be modified by adjusting the working volume. This ratio impacts the mass transfer of gasses in and out of the vessel, as well as the amount of light the algae is exposed to. Depending on the type of vessel, the surface area to volume ratio (SA:V) and the light exposed surface area to volume ratio (LE-SA:V) may be different. In this study the walls of the aquaria tanks are transparent, allowing light in on all sides and through the top, whereas gas transfer will only occur through the water surface, meaning the SA:V and the LE-SA:V are unequal. However, the raceway ponds used in this study have opaque walls, so the SA:V and LE-SA:V are equal.

When focusing on scale up, the light exposed surface area to volume (LE-SA:V) ratio is important^{1,7}. A dense algae culture will result in minimal light penetration beyond the first few centimeters of water. Continuous mixing of a dense culture and a high LE-SA:V ratio will increase overall light exposure and should result in higher production yields. Continuous mixing will also aid in the mass transfer of gasses. To verify that the small-scale vessel accurately predicts large-scale productivity a full comparative study would need to be done.

Reactor constraints:

When setting up and operating this system for the first time there are a few things that may cause difficulties. First, it is very important to have at least 0.1 g/L of algae biomass in any vessel when scaling up. If the density is too low it is highly likely that the inoculated algae will die off rapidly¹⁰. Secondly, this system can handle high concentrations of ammonia, however the input ammonia concentration has to be increased slowly over many weeks^{14,16,17}. In this study the input ammonia concentration was raised at a very conservative rate, an approximate increase of 10 mgN/L every 3 weeks. Lastly, while monitoring all dissolved nitrogen species it is important that the nitrite concentrations are kept low. Nitrite can be toxic to algae and other organisms at high concentrations¹⁸. If nitrite concentrations increase above 150 mg N/L, then additional volume should be removed and replaced with water to dilute the toxic nitrite concentrations.

Potential applications:

This methodology can be applied to verify the accuracy of input data used to simulate full-scale production processes in life cycle assessments (LCAs) and techno-economic analyses (TEAs) of full-scale production systems. Frequently, biomass growth and nutrient consumption rates from small-scale studies overestimate the abilities of a scaled-up system. Despite this, the vast majority of LCAs and TEAs use input values from small-scale studies to predict full-scale production values for their estimation of full-scale technologies¹⁹⁻²³. Before using the results from small-scale studies in this way, it should be verified that these results are a good representation of what can be expected from a full-scale system. Currently, there is no standardized methodology for collecting data for predictive studies of large-scale systems. The methodology presented here could be applied as a verification study.

In this study, nitrogen removal and biomass growth were used as the metrics for determining the effectiveness of treatment. This system could be easily adapted for other applications, including other waste streams (domestic or agricultural wastewater), monitored for other parameters (BOD, heavy metal, pathogen removal), observations on changes in microbial community, or altered from a semi-batch reactor to a continuously mixed reactor system. In any of these applications the protocol described here can be used to evaluate lab-scale and larger-scale systems.

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DISCLOSURE:

The authors have nothing to disclose.

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Figure 1

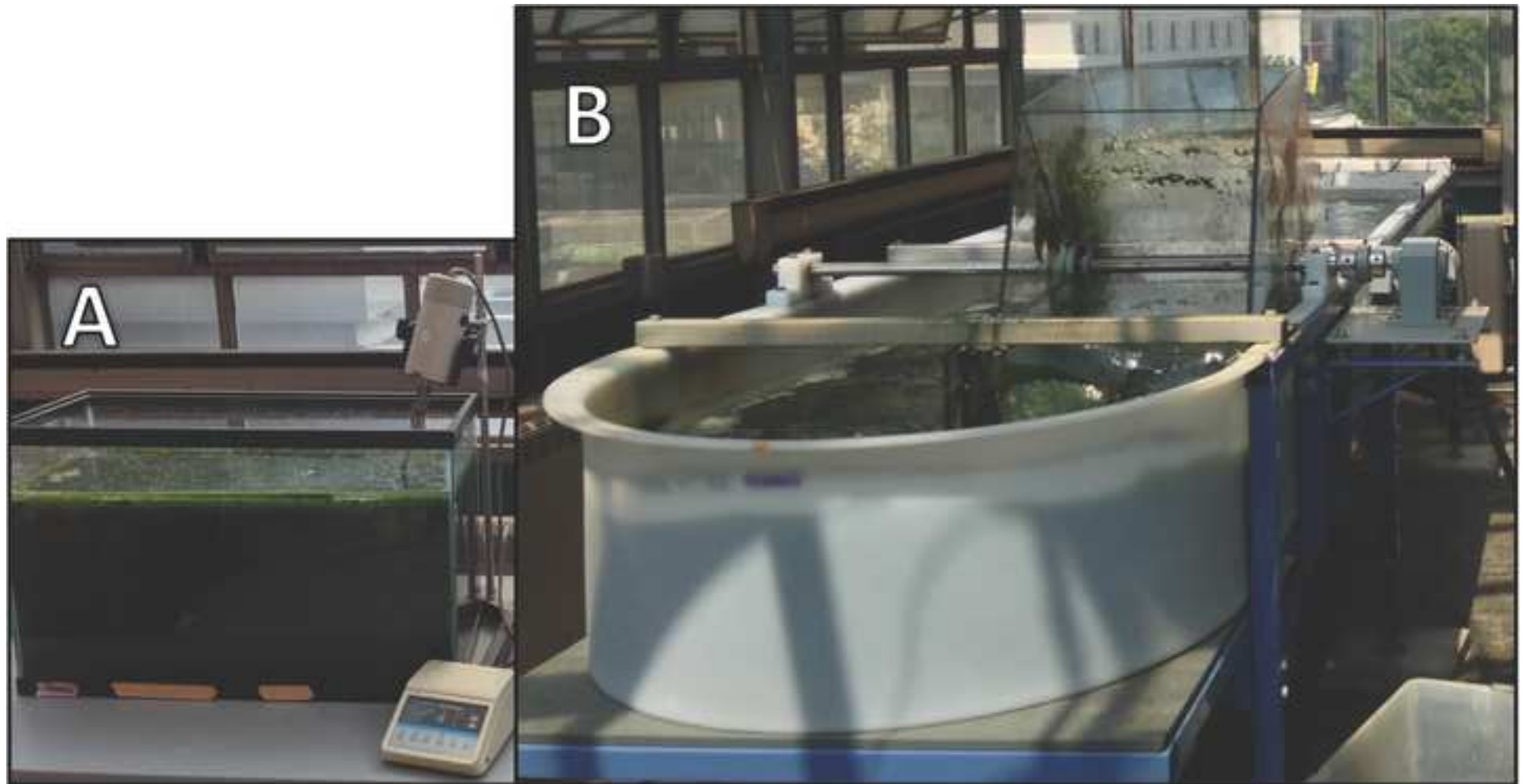


Figure 2

[Click here to download Figure JoVE_Sniffen_Figure2.png](#)

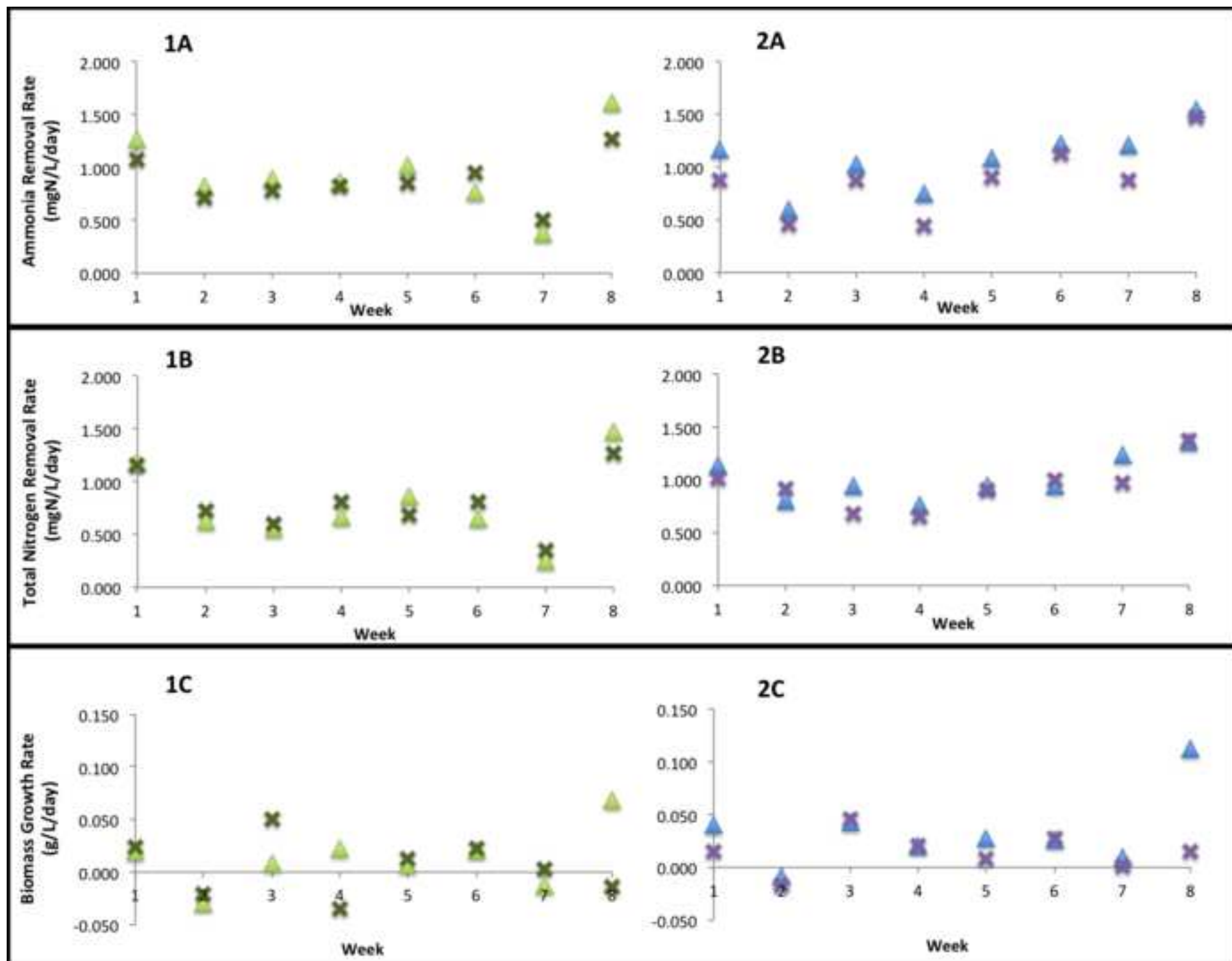
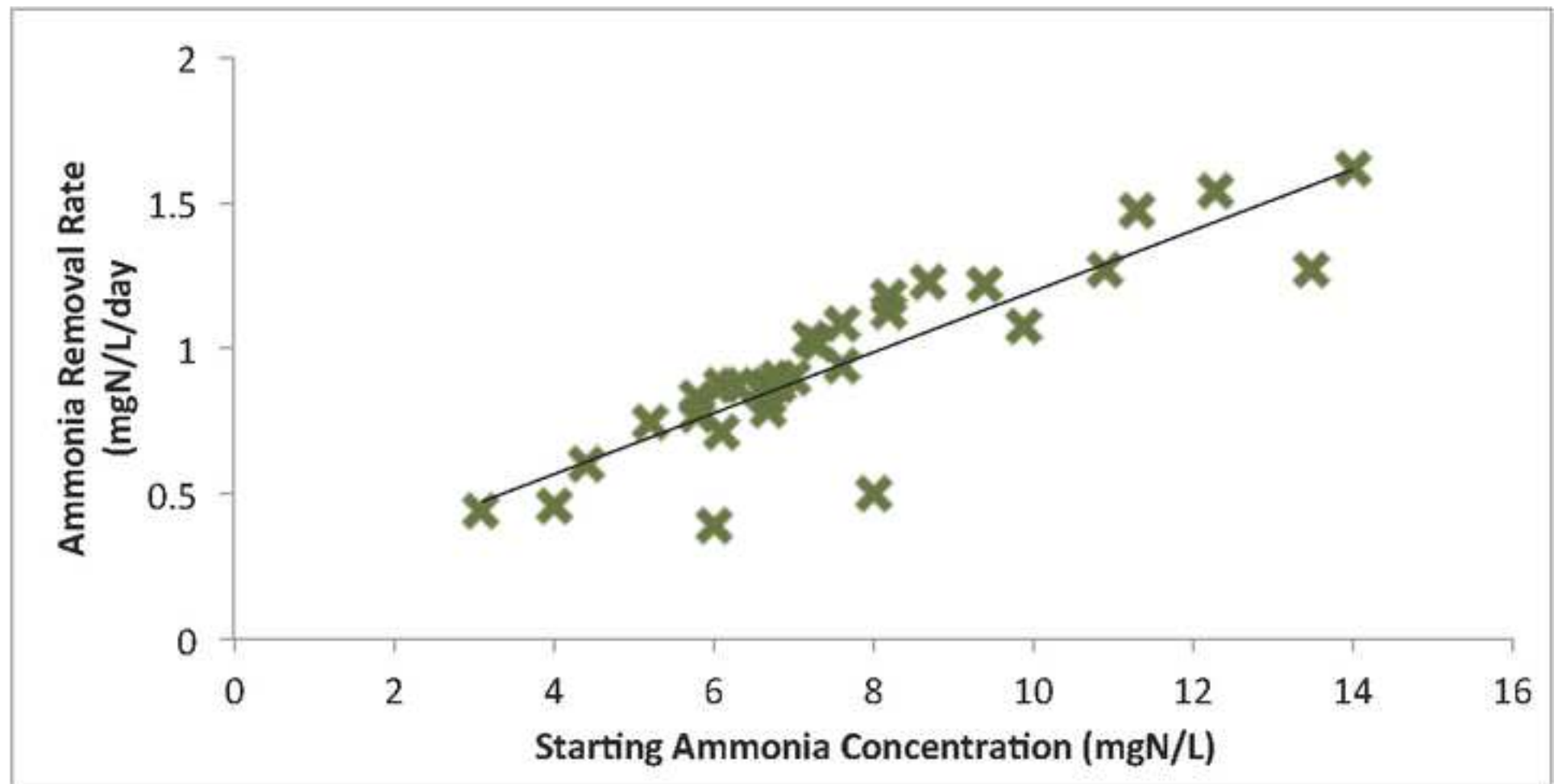


Figure 3



	Ammonia Removal Rate (mgN/L)	Total Nitrogen Removal Rate (mgN/L)	Biomass Growth Rate (g biomass/L)
RWP 1	0.95 ± 0.36	0.79 ± 0.38	0.013 ± 0.029
RWP 2	1.08 ± 0.30	1.01 ± 0.21	0.034 ± 0.036
Aquarium Tank 1	0.87 ± 0.23	0.803 ± 0.30	0.005 ± 0.028
Aquarium Tank 2	0.88 ± 0.33	0.94 ± 0.22	0.015 ± 0.019

<u>Name of Material/ Equipment</u>	<u>Company</u>	<u>Catalog Number</u>
Aquarium Tank		
	MicroBio	
RW 3.5	Engineering	
Eurostar 100 digital	IKA	4238101
Leachate	Sandtown Landfill	
Sampling Bottles	Nalgene	
Transfer Pumps		
AmVer Salicylate Test 'N Tube	Hach	2606945
NitraVer X Nitrogen - Nitrate Reagent Set	Hach	2605345
NitriVer 2 Nitrite Reagent Powder Pillows	Hach	2107569
Hach DR2400 Spectrophotmeter	Hach	
EMD Microbiological Analysis Membrane Filters	Millipore	HAWG047S6

Comments/Description

Any 100+L aquarium tank with optically clear glass can be used

[Raceway Pond](#)

Overhead mixers

Plastic or glass, lab grade, 125-200mL

Garden type pump with drinking water quality hoses will be suitable

High Range Ammonia Tests

High Range Nitrate Tests

High Range Nitrite Tests

The DR2400 was discontinued, but any DR series Hach spectrophotometer can be used in this application.

0.45µm filters



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1.1. 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"

Commented [KS1]: One of the reviewers or editors made a significant change to this portion without tracking the changes; this was part of those changes.

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 small 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 $\rho=0.90$. This is statistically significant and strong correlation, and is presented in the last paragraph of the results section. The R^2 value shows variability since this study was not done in a highly controlled environment. An R^2 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 RWP 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 bioprocesses for any purpose should be operated until steady state conditions were obtained for the 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 of 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

