Video Article

Comparison of Scale in a Photosynthetic Reactor System for Algal Remediation of Wastewater

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URL: https://www.jove.com/video/55256
DOI: doi:10.3791/55256

Keywords: Environmental Sciences, Issue 121, nutrient removal, algae biomass growth, scale-up, ammonia nitrogen removal, wastewater remediation, large-scale

Date Published: 3/6/2017

Citation: Sniffen, K.D., Sales, C.M., Olson, M.S. Comparison of Scale in a Photosynthetic Reactor System for Algal Remediation of Wastewater. *J. Vis. Exp.* (121), e55256, doi:10.3791/55256 (2017).

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 (1,000 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 NH_3-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 ± standard deviation of ammonia removal, total nitrogen removal and biomass growth rates were 0.95±0.3 mg NH_3-N/L/day, 0.89±0.3 mg N/L/day, and 0.02±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.

Video Link

The video component of this article can be found at https://www.jove.com/video/55256/

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 [1,2,3,4]. 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 CO_2

concentrations and increased O_2 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 CO_2 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 CO_2 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 Setup

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

1. For one paired system, use one 100 L aquaria tanks (AT), with an overhead mixer for the small-scale vessel, and one 1,000 L raceway pond (RWP), with a paddle wheel mixer for the large-scale vessel. Vessels used in this system are pictured in **Figure 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.
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 NH_3-N/L.
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.

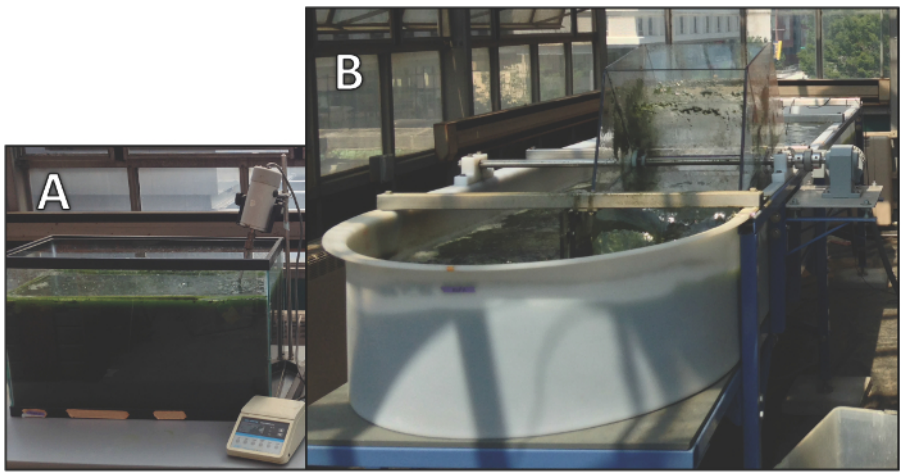

Figure 1. Examples of an aquarium tank and raceway pond. An example of an aquarium tank (**A**) and raceway pond (**B**) are shown. Please click here to view a larger version of this figure.

2. Weekly Operation and Sampling

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. 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.
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.
    1. Once per week, pump the entire volume of the aquarium tank into the raceway pond.
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.
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.
6. Take 125 mL samples from all vessels for the analysis of the starting conditions for the next week.

3. Sample Analysis

1. Test all beginning-of-the-week and end-of-the-week samples for ammonia-N, nitrate-N, nitrite-N, and biomass density.
2. Measure biomass by standard total suspended solids (TSS) protocol, ASTM-D5907, using 0.45 µm filters.
    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.
    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. Measure ammonia, nitrate, and nitrite spectrophotometrically using a spectrophotometer.
    1. Use 100 µL of sample in the commercial method kit to determine ammonia concentration. Refer to the manufacturer's protocol.
    2. Use 1 mL of sample in the commercial method kit to determine nitrate concentration. Refer to the manufacturer's protocol.
    3. Use 10 mL of sample in the commercial method kit to determine nitrite concentration. Refer to the manufacturer's protocol.
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

1. Determine if the data collected is statistically normal. Determine normality of the data set using a Q-Q plot[12].
2. Determine correlations among parameters using Pearson's r or Spearman's p for normal and non-normal data, respectively[13]. 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, PA [14]. 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 section [15]. Initial conditions for biomass, ammonia and total nitrogen concentrations in all vessels ranged from 0.2-1.0 g/L, 3.1-14 mg NH_3-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**.

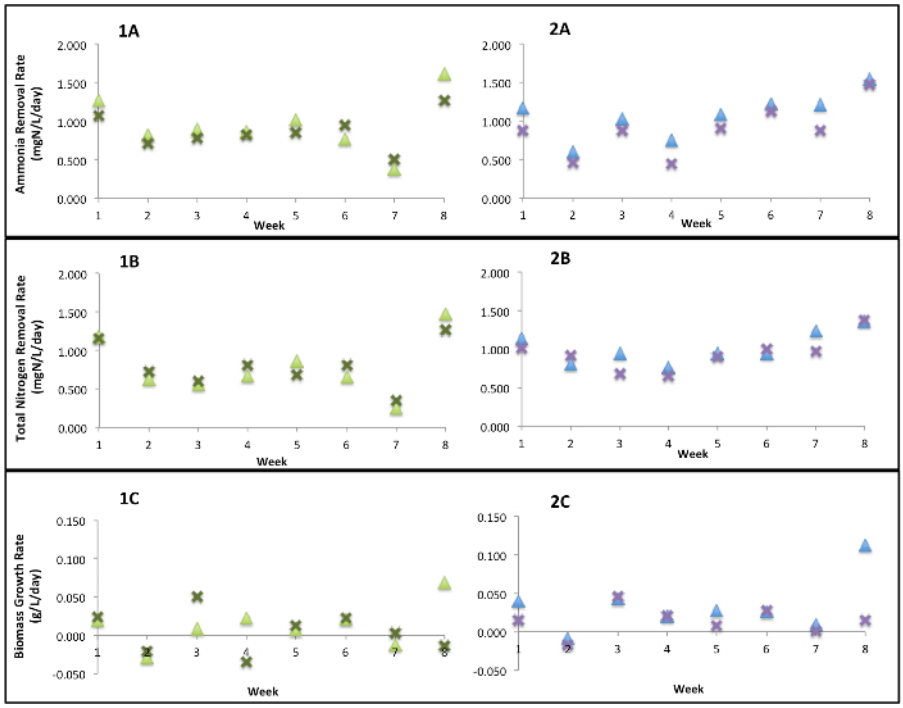

Figure 2. Summary of productivity over the representative study period. Ammonia removal rates (**A**), total nitrogen removal rates (**B**), and biomass growth rates (**C**) are presented in the top, middle and bottom panels, respectively. Results from system 1 are presented on the left, and system 2 on the right. Results from aquaria tanks and raceway ponds are represented on all graphs by **X**, and **Δ,** respectively. Please click here to view a larger version of this figure.

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 rho, the nonparametric correlation, was used. The strongest significant correlation was between the initial ammonia concentration and ammonia removal rate (ρ=0.90). The trend between initial ammonia concentration and the ammonia removal rate can be seen in **Figure 3**.

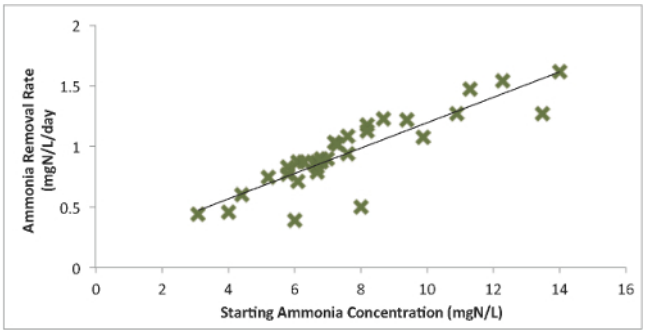

Figure 3: Ammonia remova fo:keep-together.within-page="1"l rate as a function of starting ammonia concentration. Data from all vessels over the representative 8 weeks are presented. Trend line R^2 = 0.76. Please click here to view a larger version of this figure.

	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

Table 1. Mean ± 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 CO_2 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 (ρ=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 tanks [14]. 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 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. 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 aide 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 rapidly [10]. 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 concentrations [18]. 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 technologies [19,20,21,22,23]. 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.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors would like to thank the Sandtown Landfill in Felton, DE for sharing their knowledge and leachate.

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