

COMPARING ATTACHED GROWTH AND  
SUSPENDED CULTURES FOR THE ALGAL  
REMEDICATION OF ARSENIC

BY CHASE STEARNES

NM WRRI  
STUDENT WATER RESEARCH GRANT  
FINAL REPORT

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## 1. Researchers

**Student Researcher:** Chase Stearnes

**Faculty Advisor:** Andrew J Schuler

## 2. Project title:

### **Comparing Attached Growth and Suspended Cultures for the Algal Remediation of Arsenic**

## 3. Research Problem and Objectives.

Arsenic (As) contamination of water is one of the greatest challenges to clean water and health internationally. Millions in countries such as Bangladesh have widespread As groundwater contamination. As commonly enters into water sources through natural (volcanic activity, As bearing minerals, etc.) and anthropogenic (mining, smelting, agriculture, etc.) activities (Bahar, Megharaj, & Naidu, 2012; Wang, et al., 2015). The microalgae *Scenedesmus* sp. has been seen to accumulate by adsorption and uptake a majority of total As in 8 days in suspension, depending on initial concentrations and As species present (Bahar, Megharaj, & Naidu, 2012). Biomass production of algae is greatly increased when given a surface for attachment (Hoh, Watson, & Kan, 2015; Lee, et al., 2014; Kunitz, Kumar, Gross, & Wen, 2016; Johnson & Wen, 2009).

The objectives of this research are to determine: the potential of algal biofilms for remediation of arsenic from contaminated waters, the best application for algal remediation (mining, drinking water, etc.), and the efficiency of As removal by algal biofilm.

## 4. Methodology

### 4.1 Algal polyculture:

The algae polyculture was obtained from existing bioreactors that are maintained at the Biofuels Center of Excellence at the Santa Fe Community College (SFCC). This polyculture is dominated by *Scenedesmus*, but is in the open air outside and is therefore exposed to many local species. Samples were taken for Illumina genetic sequencing at introduction of algae to the reactors, again prior to the introduction of arsenic to the synthetic feed, and once more at the end of the experiment to monitor the predominant species existing within each reactor.

### 4.2 Bioreactors:

Bioreactors were constructed of ¼ inch thick acrylic. The dimensions of these reactors were 6 in. by 4 in. by 48 in., with a total volume of 18.9L. Media was maintained at a depth such that the total media volume was approximately 13L. At the start of Day 17, the biofilm reactors were screened with aluminum foil to limit light entering the media and discourage suspended growth. Each reactor had a water pump that provided constant mixing.

## 4.2a Reactor media

Algae were grown in reactors using Bold's Basal Medium (Bold, 1949). The reactors were run with a 6-day HRT, in which half of each reactor volume was replaced with feed. This ensured that nutrients were being replaced every 3 days and that the cultures were never in starvation.

## 4.2b Suspended growth

To maintain an aerobic environment, air stones supplying fine bubble air were placed at two locations within the reactor and a propeller supplied consistent mixing.

## 4.2c Attached growth

One 3 in. diameter by 48 in. length PVC pipe was rotated at 7rpms, partially submerged in the reactor. The PVC pipe was wrapped in fabric to provide more area for attachment. One reactor had cotton fabric and the other had nylon. The pipes were rotated using a Dayton 115V, AC, permanent split capacitor gear motor, with V-belt pulleys as shown in Figure 1.



Figure 1. Full rotating algal biofilm reactor setup



### 4.3 Arsenic removal test

After 35 days of system equilibration an aliquot of arsenic was added to the feed at a concentration of 240 µg/L bringing the initial concentration in each reactor to 120 µg/L. This 120 µg/L concentration was maintained in the synthetic feed which replaced half of each reactor volume every 3 days.

#### 4.4a Media sampling and analysis

3 mL samples of the media were taken each day and analyzed by Hach Nitraver X, to monitor nitrate concentrations as a surrogate for biological activity. At the time of arsenic introduction, 15 mL samples were also taken daily for analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to monitor arsenic concentrations over the duration of the experiment.

#### 4.4b Biomass sampling for genetic sequencing

Samples for genetic sequencing were collected and frozen at Days 0, 8, 25, and 49. At day 0, the inoculated media was filtered on a .45-micron glass fiber filter, and collected off of the filter. At Day 8, 100mL of media from each reactor were filtered on a .45-micron glass fiber filter, and collected off of the filter. At day 25, prior to the addition of arsenic, algae were collected from the biofilm growth on the cotton-wrapped biofilm reactor and frozen. At the end of the arsenic uptake experiment, samples were taken, once again, from the biofilms that had formed in each of the three reactors. These 10 samples were sent to Molecular Research Laboratory for genetic sequencing using Illumina.

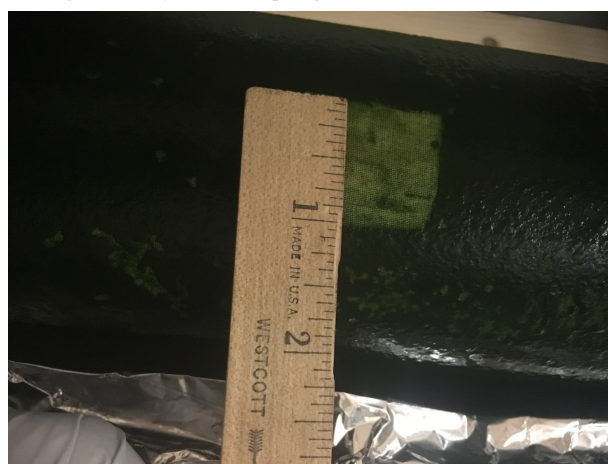
#### 4.4c Biomass sampling for SEM

Initially, Scanning Electron Microscopy was performed using wet, untreated samples. These 1 cm<sup>2</sup> samples were cut from the reactor while maintaining the integrity of the attachment surface and biofilm. Later, samples were freeze-dried and carbon-coated. The freeze-drying process included placing fresh 1 cm<sup>2</sup> samples into a -70°C freezer, and then vacuum drying the frozen samples using a lyophilizer.

#### 4.4d Algal biomass quantification

For suspended biomass, between 50-200 mL was filtered using a .45-micron glass fiber filter and dried for 1 hour at 100°C to obtain a dry mass, in this report referenced as total suspended solids or TSS. Biofilm biomass was quantified by scraping a 1 in<sup>2</sup> patch of biofilm (see Figure 2) and drying for 1 hour at 100°C. Initially, a biofilm sample was taken at one location along the bioreactor surface; later, the sample was taken at 3 locations along the surface and the dry weights of all 3 samples were averaged for a more uniformly representative result. The result

Figure 2. Dry mass sampling



was assumed representative of the whole surface area and the total biofilm dry mass was divided by reactor volume to obtain a mg/L dry mass.

## 5. Results and Conclusions

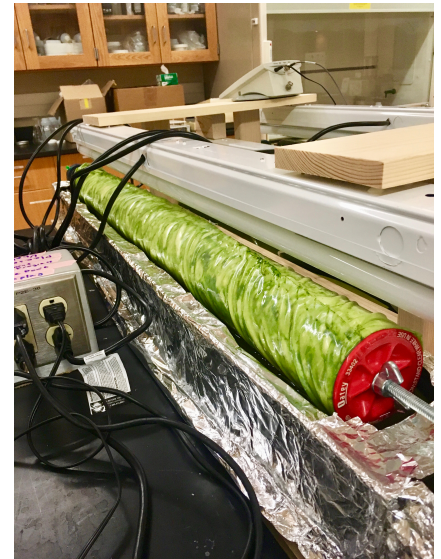
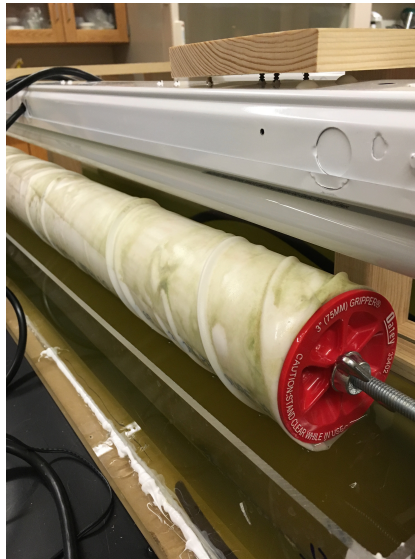
### 5.1 Results

#### 5.1.1 Microalgae culture preference in reactor type and attachment substrate

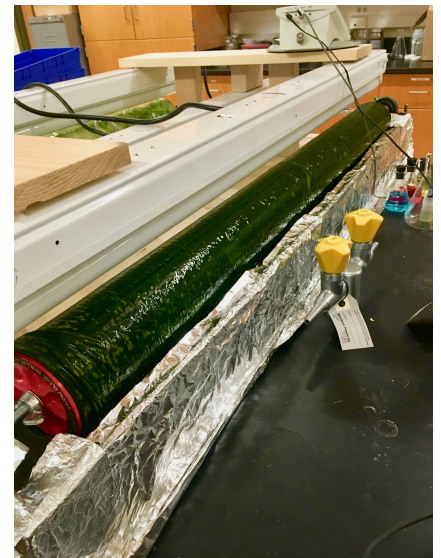
It was shown that the microalgal culture used in this experiment has a definite preference to grow on cotton rather than nylon or in suspension. (Figures 3 & 4) A significant visual difference between the nylon and cotton reactors was evident from day 1. It can be seen that the cotton wrapped RABR had significantly more growth initially and throughout the experiment. (Figures 3 & 4)

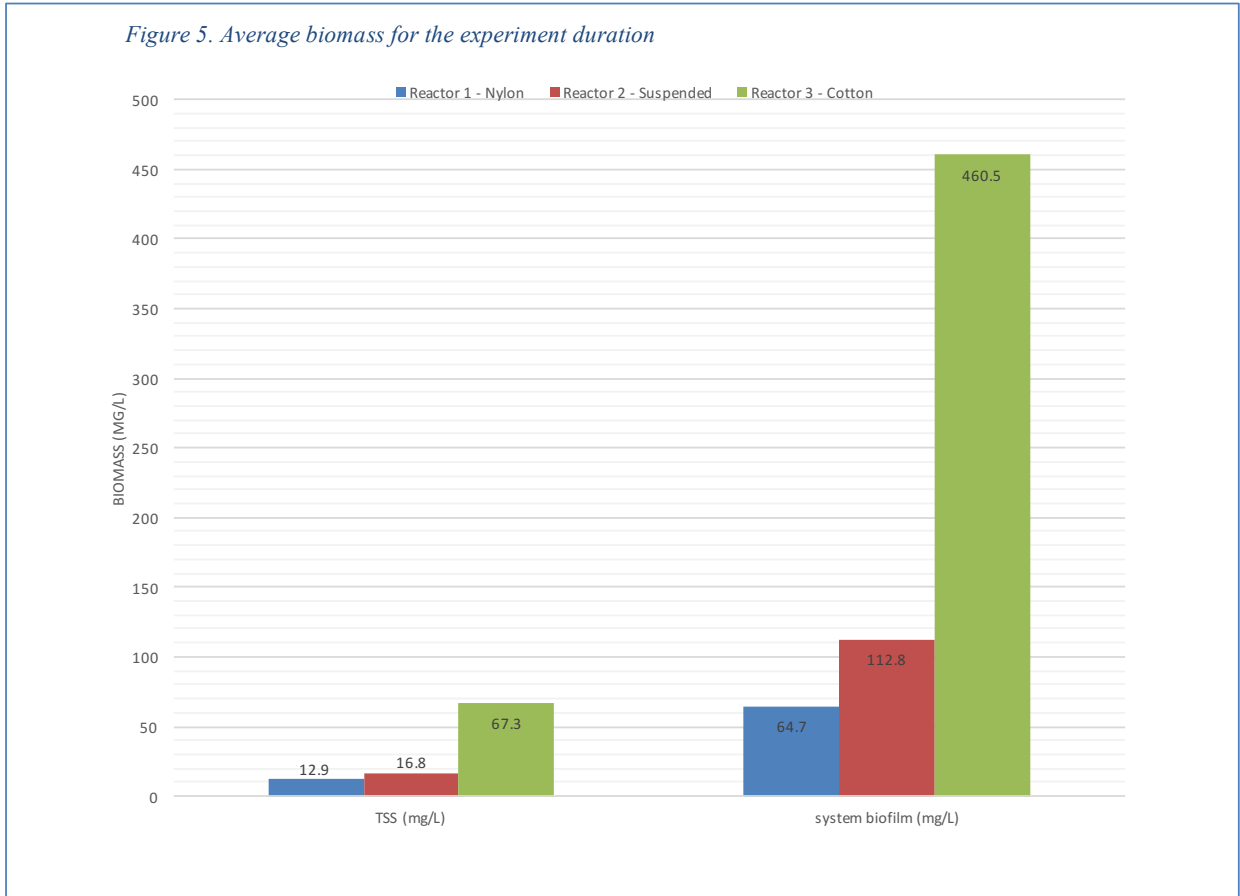
Average biomass was significantly increased in the cotton reactor when compared to the nylon and suspended growth reactors (Figure 5). The cotton reactor averaged more than 4 times greater suspended solids – which were assumed to be principally algal biomass - than the suspended growth reactor. The nylon reactor averaged lowest in suspended solids. Average attached biomass ranked similarly, with average attached biomass being the greatest in the cotton reactor, by more than 400%, and the nylon reactor having the least biofilm growth of the three reactors.

*Figure 3. Nylon reactor at days 2 & 29*



*Figure 4. Cotton reactor at days 2 & 29*





### 5.1.2 Arsenic uptake varies between reactor and attachment types

After an initial spike of uptake in the first day, there was a 5-day lull in uptake before a moderately linear continuation of arsenic uptake began (Figure 6). This increasing uptake continued for the remainder of the experiment. Comparing the 3 reactors, the cotton RABR accumulated the most arsenic out of solution, followed by the nylon RABR and the suspended growth reactor.

Looking at the period of linear cumulative uptake, the rate of uptake had a slight variation (Figure 7). The cotton RABR had the greatest uptake rate at  $4.2 \text{ mgL}^{-1}\text{d}^{-1}$ . The suspended growth reactor followed with a rate of  $4.0 \text{ mgL}^{-1}\text{d}^{-1}$  and the nylon RABR had the lowest rate,  $3.8 \text{ mgL}^{-1}\text{d}^{-1}$ .

Figure 6. Cumulative arsenic accumulation

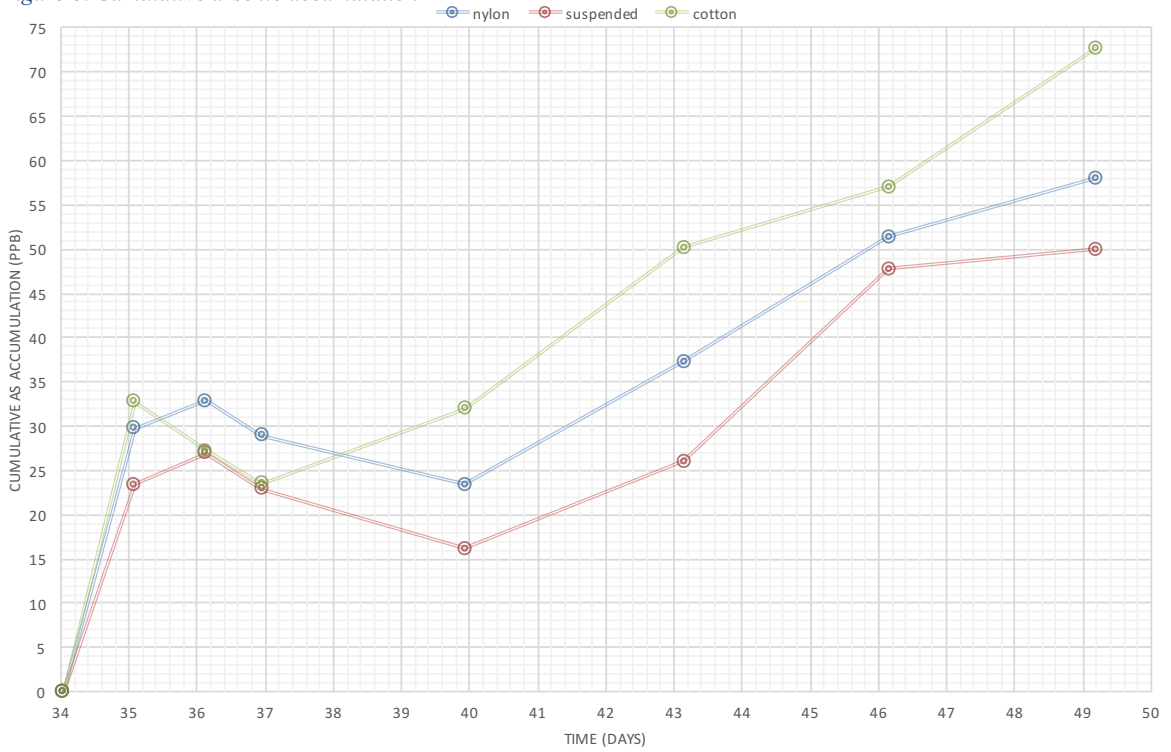
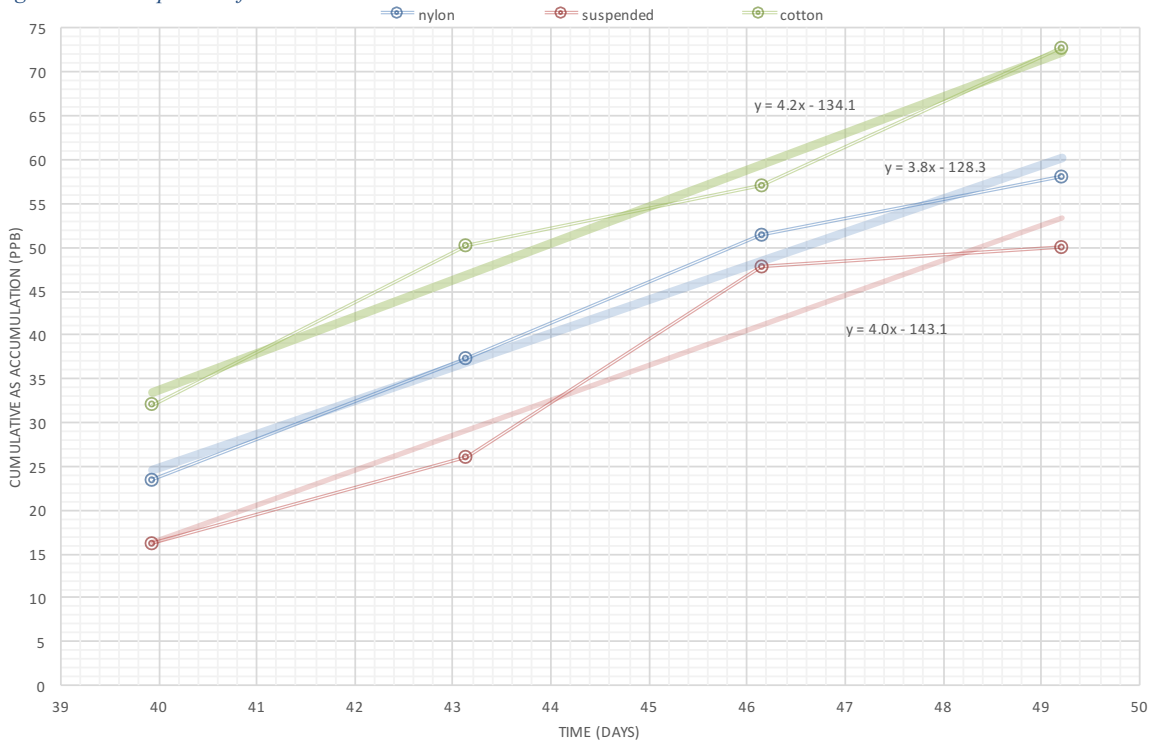


Figure 7. Linear period of cumulative arsenic accumulation





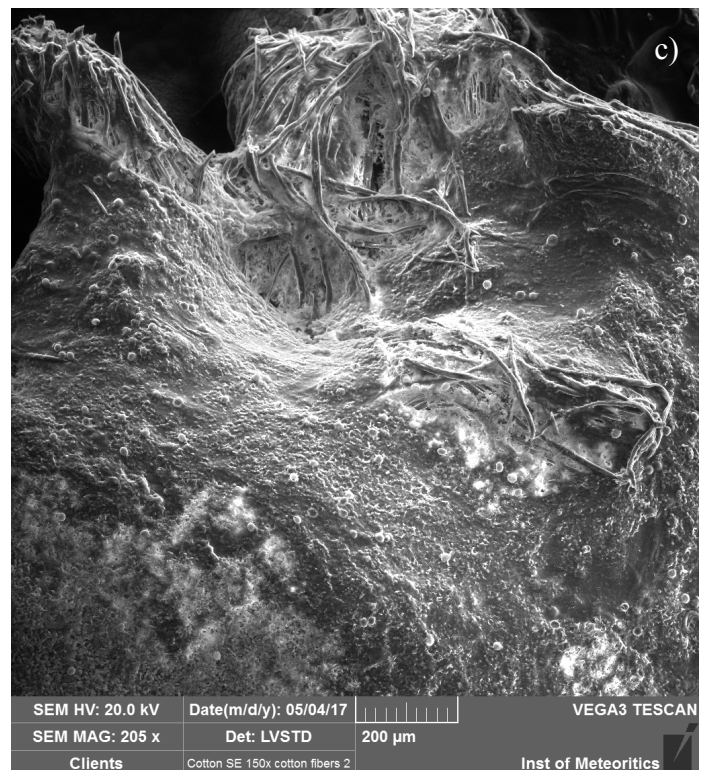
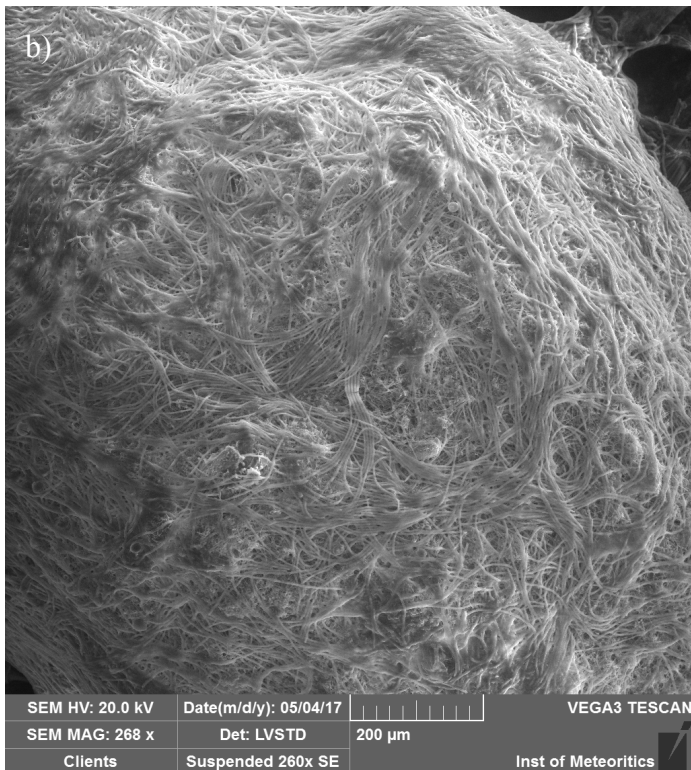
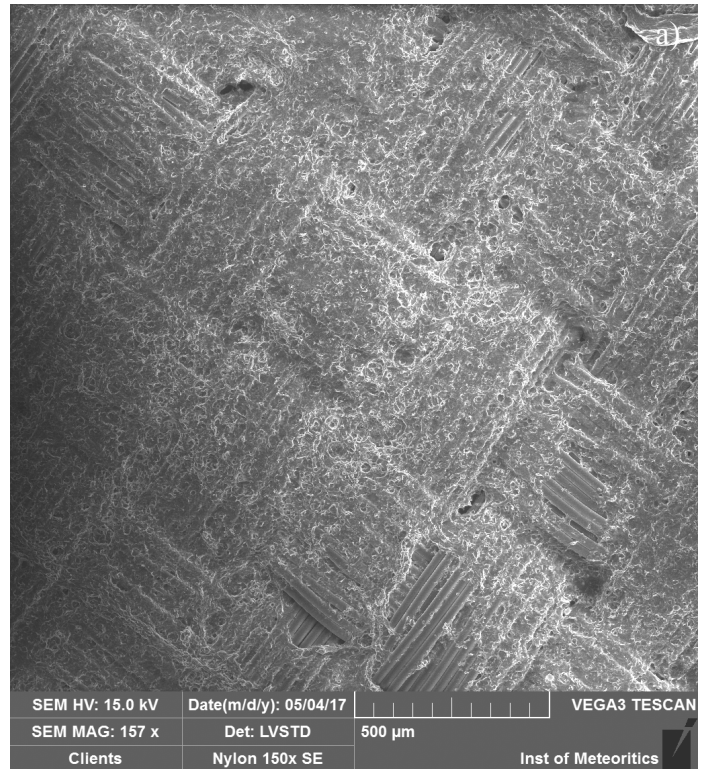
### 5.1.3 Microalgal community composition using genetic sequencing

Results from genetic sequencing will be available mid-July.

### 5.1.4 Scanning Electron Microscope Imaging

Preliminary imaging using SEM without freeze drying provided a distinct view of the differences between biofilms growing in each reactor (Figure 8). Varying biomass density is evident as well as distinctive growth structure in each community. Further imaging is pending for samples that were freeze-dried and coated. These sample will provide a higher resolution perspective of the inner workings of these communities. Having further processed the biomass samples will also allow individual cells to stay intact while samples that have not been coated often have imploded cells from the exceedingly high vacuum pressure used in SEM imaging.

Figure 8. Preliminary SEM images from biofilm in each reactor; a) nylon, b) suspended, c) cotton



## 5.2 Conclusions – A preference and efficiency in the cotton RABR

The higher biomass accumulations in the cotton RABR undeniably indicate a greater ability for microalgal communities to grow on cotton. These results were similar to those of others (Christenson & Sims, 2012) who found that in comparing use of cotton rope and fabric to polyester substrate, the provision of cotton products significantly increased dry algal mass.

A definite difference was also observed in cumulative arsenic uptake rates with the cotton RABR having the highest. Future experiments should investigate the use of different growth conditions while solely growing algae on cotton RABRs.

Current research does not yet wholly understand why cotton makes such a great growth substrate for microalgae. Therefore, perhaps some of the most important task for future work is in examining the use of various forms of cotton substrate and attempting to uncover what makes cotton such an exceptional substrate.

## 6. Target Beneficiaries

The beneficiaries of the results are primarily people living in areas of high arsenic concentrations and minimal developments in water treatment technology. These people live in rural and low-income areas around the world. If the HRT of these reactors could be minimized, it is possible that algal biofilm technologies could be scaled up for municipal utilities, such as Albuquerque’s, to reduce arsenic in distributed drinking water.

## 7. Budget

<b>Expense</b>	<b>Budget</b>	<b>Actual</b>	<b>Remaining</b>
<b>Lab Supplies</b>	\$1244	\$1914.39	-\$670.39
<b>Travel</b>	\$1540	\$671.28	\$868.72
<b>Technical Services</b>	\$1600	\$2,063.55	-\$463.55
<b>Student Salary</b>	\$1600	\$1,349.95	\$250.05
<b>University Charges</b>	\$16	\$0.83	\$15.17
<b>Total</b>	\$6000	\$6000	0

## 8. Presentations

October 7, 2016 – Poster Presentation at the NMWRRRI Annual Water Conference,  
Silver City, NM

April 20-21, 2017 – Poster Presentation at the New Mexico Water Workshop,  
Albuquerque, NM

April 27, 2017 – Poster Presentation at the NM EPSCoR All Hands Meeting,  
Albuquerque, NM



## 9. Publications

In addition to the final report required by NMWRRRI, this research is intended to inform a research publication on the use of algal biofilms for arsenic remediation in the later part of this year.

## 10. Contributors

Phil Roveto – UNM Center for Water and the Environment Graduate Student, Blade Allen – Student and Outreach Coordinator for the Center for Water and the Environment at UNM, Chason Simpson – SFCC Student, Luke Spangenburg – Director at SFCC Biofuels Center of Excellence, Stephen Gomez - Chair of SFCC Sustainable Trades and Technologies Department), Abdul-Mehdi S. Ali – Analytical Chemistry Lab Manager UNM.

## 11. Recognition

N/A

## 12. Student Career Outlook

Currently, Chase Stearnes is working towards completion of his Bachelor of Science in Civil Engineering with an expected graduation date of December 2017. Chase has been accepted into the Shared Credit program and is taking graduate level courses to be applied to both his BS and a Master of Science in Civil Engineering at UNM. He is planning on continuing research in biological water treatment systems as a graduate student, with particular interest in the bioremediation of heavy metals. Chase is currently working as an intern at Daniel B Stephens & Associates in Albuquerque, where he hopes to stay and learn as much as possible about water infrastructure, engineering, and hydrology. With this knowledge, he hopes eventually to assist rural and developing communities around the world to obtain clean and healthy water.

## Works Cited

- Bahar, M., Megharaj, M., & Naidu, R. (2012). Toxicity, transformation and accumulation of inorganic arsenic species in a microalga *Scenedesmus* sp. isolated from soil. *Journal of Applied Phycology*.
- Bold, H. C. (1949, March). The morphology of *Chlamydomonas chlamydogama*, sp. nov. *Bulletin of the Torrey Botanical Club*, 76(2), 101-108.
- Christenson, L. B., & Sims, R. C. (2012). Rotating Algal Biofilm Reactor and Spool Harvester for Wastewater Treatment with Biofuels By-Products. *Biotechnology and Bioengineering*, 1674-1684.
- Hoh, D., Watson, S., & Kan, E. (2015). Algal biofilm reactors for integrated wastewater treatment and biofuel production: A review. *Chemical Engineering Journal*.
- Johnson, M. B., & Wen, Z. (2009, July 28). Development of an attached microalgal growth system for biofuel production. *Applied Microbiology and Biotechnology*.
- Kunetz, T., Kumar, K., Gross, M. A., & Wen, Z. (2016). Evaluation of the Performance of a Revolving Algae Biofilm System for recovering Nitrogen and Phosphorous from Municipal Wastewater. *WEFTEC 2016*.
- Lee, S.-H., Oh, H.-M., Jo, B.-H., Lee, S.-A., Shin, S.-Y., Kim, H.-S., . . . Ahn, C.-Y. (2014). Higher Biomass Productivity of Microalgae in an Attached Growth System, Using Wastewater. *Journal of Microbiology and Biotechnology*.
- Wang, Y., Xu, P., Liu, M., Wang, Y., Wang, C., Zhang, C., & Ge, Y. (2015). Review of arsenic speciation, toxicity and metabolism in microalgae. *Reviews in Environmental Science and Bio-Technology*.