Huntsman Environmental Research Center

# BIENNIAL REPORT 2016-2017

## PROJECTS AND PRODUCTS

Summary. During the years 2016-2017, HERC themes emphasized included biodegradation, recycling, and air quality. Toxic algae blooms have become a severe problem for users of recreational water bodies and for drinking water supplies in the state of Utah. HERC worked with the Utah Department of Environmental Quality to test the destruction of algal toxins using biodegradation within anaerobic digestion systems. HERC collaborated with WesTech-Inc. in recycling metals and other chemicals in mining wastewaters using microalgae. HERC also collaborated with the Logan City Environmental Department to evaluate wastewater treatment by recycling a by-product of cement manufacturing to remove turbidity and suspended solids in an effective and cost-effective process. Also, organic wastes from agricultural processes related to dairy and swine operations were recycled into animal feed by cultivating algae on the waste, and then extracting the proteins from the algae for the Circle Four industry and for the dairy industry.

2017

**Project: Hazardous Algal Blooms (HABs): Cyanobacterial Microcystin Biodegradation through Anaerobic Digestion**

**HERC Theme**: Biodegradation

**Collaborators:** Utah Department of Environmental Quality, the Central Valley Water Reclamation Facility (Salt Lake City), and the Utah Water Research Laboratory

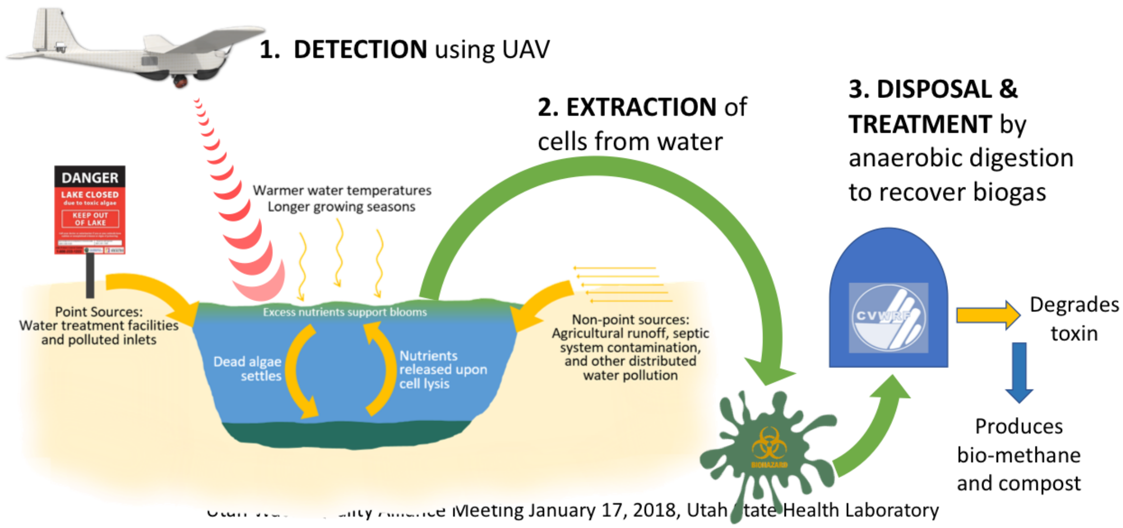
**Students Supported:** Kyle Hillman and the Senior Capstone Design Team (BS)

**Results/Products/Outcomes:** The cyanobacterial toxin, Microcystin-LR is known to be carcinogenic and toxic to humans, domestic animals, fish, and wildlife and is occurring with increasing frequency in Utah lakes and reservoirs, and is a high priority for the Utah Department of Environmental Quality (UDEQ) and the Utah Department of Natural Resources (UDNR). Microcystin-LR withstands high temperatures, changes in pH, photolysis, and hydrolysis. This project tested the biodegradation of Microcystin-LR using anaerobic biodegradation based on literature reports from China that indicated that anaerobic biodegradation was effective at treating cyanobacterial contamination. The ABRAXIS Microcystin Recreational Water Dipstick ELISA Test was used to monitor the presence of Microcystin-LR over time in a control bioreactor containing only distilled water and experimental bioreactors containing anaerobic biomass from the Central Valley Water Reclamation Facility (CGWRF). Results showed a consistent reduction in the presence of Microcystin-LR over a 35-day treatment period that demonstrated the potential for anaerobic biodegradation of Microcystin-LR. Plans for 2018 include working with our collaborators to harvest algae blooms and to test additional cyanotoxins for treatment using anaerobic biodegradation with applications to the HABs occurring in Utah lakes and reservoirs.

**Dissemination of Results**: Presentations at national professional meetings

1. Platform presentation at the National Institute of Biological Engineering (IBE) Conference, Salt Lake City, March 30-April 1, 2017.
2. Poster presentation at the Algae Biomass Organization Summit. Salt Lake City, October 30-November 1, 2017.
3. Presentation to the Utah Water Quality Alliance, Utah State Health Laboratory, January 17, 2018.

# Three-step Response to Utah HABs




Water Quality Alliance Meeting, January 17, 2018, Utah State Health Laboratory

# Anaerobic Digestion

- Studies show MC biodegradation using anaerobic microorganisms
- CVWRF anaerobic digesters are locally available and have suitable volumetric capacity
- HAB disposal through anaerobic digestion responsibly turns an environmental and public health liability into biogas, a value-added product

Central Valley Water Reclamation Facility Anaerobic Digesters

**Project: Treatment of mercury, chloride, and sulfate in wastewater from gold mining in the Intermountain West**

**HERC Theme**: Recycling

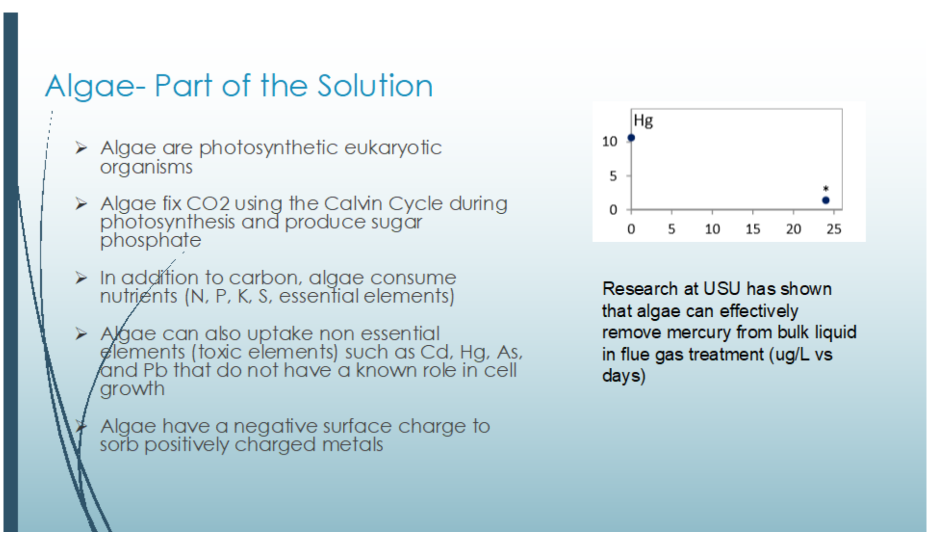
**Collaborators**: WesTech-Inc. Engineering, Salt Lake City

**Students Supported**: Alex Beeston and James Vandermeyden (BS)

**Results/Products/Outcomes:** A gold mine in Nevada has been oxidizing gold ore with sodium hypochlorite, and wastewater has migrated into the underground mine where it is pumped to the surface. The objective of this project was to determine if algae can grow in the wastewater and reduce the concentrations of mercury in the water by recycling the chemicals in the form of microalgae because algae can take up non-essential elements including those of interest in this project. Algae that were harvested from the Logan City Wastewater Treatment Lagoons were inoculated into wastewater from the gold mine at 100% concentration as well as 50% and 25% dilutions to evaluate the ability of the algae to grow and to uptake mercury, sulfate, and chloride. At the 25% dilution concentration, algae could be cultivated on the wastewater and removed 96% of the mercury in the wastewater, however did not significantly remove sulfate and chloride from the wastewater. The mercury taken up by the algae represents a way to recycle the mercury for recovery from the microalgae.

**Dissemination of Results**: Presentations at national professional meetings

1. Platform presentation at Algae Biomass Organization (ABO) Summit. Salt Lake City, October 30-November 1, 2017.
2. Poster presentation at Institute of Biological Engineering Intermountain Conference November 11, 2017. Utah State University.



Presented at the Algae Biomass Organization (ABO) Summit in Salt Lake City, 2017

**Project**: **Expanded shale as treatment for removal of suspended solids from wastewater**.

**HERC Theme**: Biodegradation

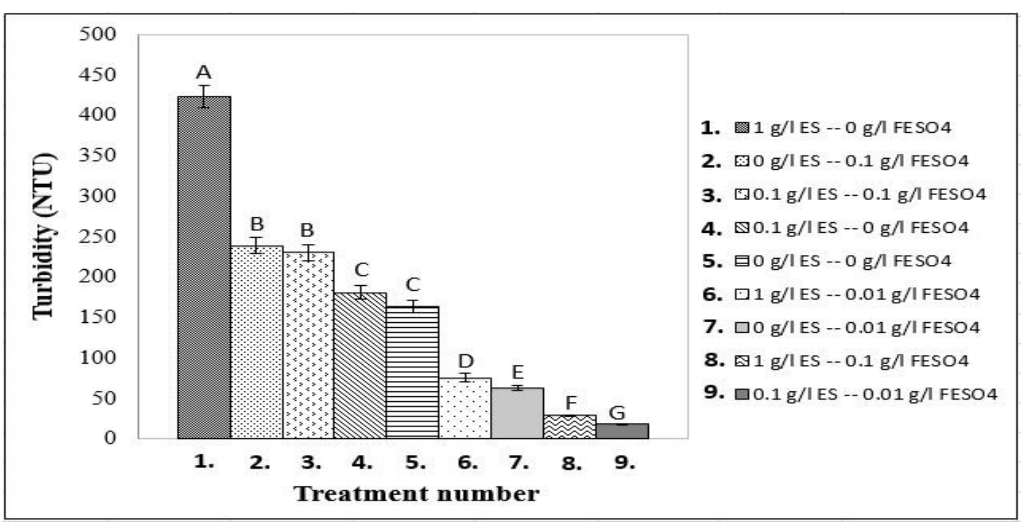
**Collaborator**: Logan City Environmental Department and South Davis Sewer District

**Students Supported**: Alan Hodges (MS), Tyler Ayers (BS), and Kateyln Ellis (BS)

**Results and Research Outcomes:** Municipal wastewater treatment systems in Utah are currently undergoing upgrades in more than a dozen cities and communities, including Logan City, and a significant proportion of this wastewater is treated using coagulation-flocculation systems. Therefore, there is an interest to develop new effective, low cost coagulants to be utilized in these systems that will improve the efficiency of biodegradation of the remaining chemicals. This study demonstrates the feasibility of a novel, low cost coagulant blend of expanded shale, a ceramic derived from the concrete industry, and an industry standard coagulant, ferric sulfate. Triplicate jar tests were operated using synthetic wastewater flocculated with varying concentrations of either ferric sulfate, expanded shale, or a combination of both components. The wastewater was subsequently analyzed for turbidity removal. The optimum concentration for turbidity removal, 0.1 g/L expanded shale and 0.01 g/L ferric sulfate, removed 84.7% and 91% of turbidity and suspended solids, respectively. This project demonstrated that expanded shale is an effective additive to traditional coagulation-flocculation systems.

**Results/Products/Outcomes:** Presentations at national professional meeting

1. Poster presentation at the Institute of Biological Engineering Intermountain Conference November 11, 2017. Utah State University.
2. Expanded shale flue dust as an additive for the coagulation of wastewater. 2017. Hodges, A., I. Hamud, R. C. Sims, and C. Miller. International Journal of Innovative Research in Science, Engineering, & Technology, 6(9).




From reference 2 showing superior performance of shale treatment number 9.

**Project**: **Algae lipid-protein extract characterization for food and feed**

**HERC Theme**: Recycling

**Collaborator:** Caine Dairy Center at USU and Circle Four Farms, Milford, Utah

**Students Supported**: Tyler Marlar (BS), Michael Hansen (BS), Justin Marriott (BS), and Ashik Sathish (MS)

**Results/Products/Outcomes**: The objective of this project was to determine the efficacy of an algal lipid-protein precipitate generated from the lipid-protein extraction procedure as a protein source in lactation dairy diets. Algal biomass derived from dairy waste-water was used throughout this study. Algal lipid-protein precipitate (ALP) was generated using optimized lipid-protein extraction procedure (L-PEP). Efficacy for implementing the algal lipid-protein precipitate in lactation dairy diets was determined by comparing total protein and total fatty acid content to traditional supplements. Additionally, concentrations of essential amino acids and fatty acids were compared to ideal bovine diets. The algal lipid-protein precipitate was determined to be 35% crude protein and 3% total fatty acids by mass. The lysine:methionine ratio was determined to be 2:1. Omega-3 and omega-6 fatty acids accounted for 22% of the extracted lipids.

**Dissemination of Results:** Presentations at national professional meeting

1. Poster presentation at the Algae Biomass Organization (ABO) Summit. Salt Lake City, October 30-November 1, 2017.
2. Poster presentation Institute of Biological Engineering Intermountain Conference November 11, 2017. Utah State University.

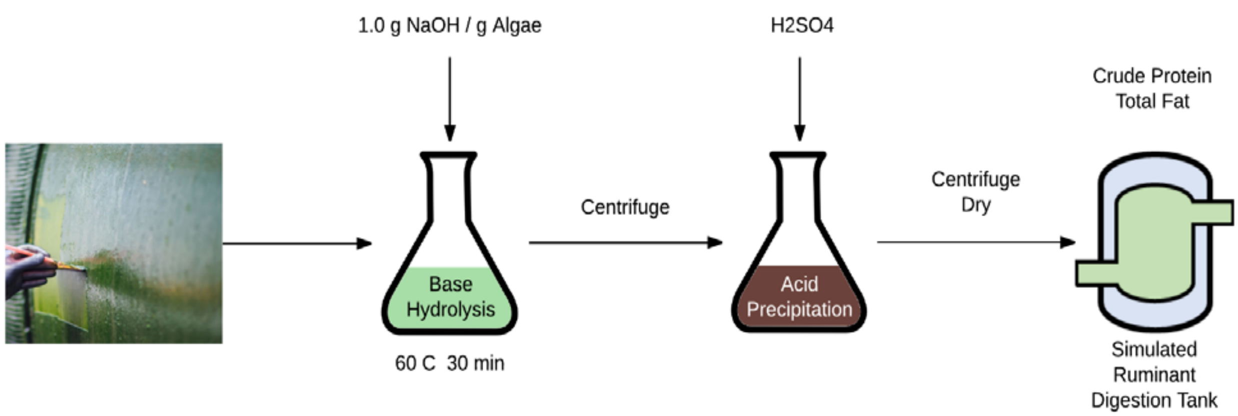



**Figure. Biofilm algae growing on dairy wastewater was harvested from the USU Rotating Algae Biofilm Reactor (RABR). Protein and lipids were extracted from the algae using the procedure developed through the project.**

**Project: Optimization of algae growth on L-PEP aqueous waste media**

**HERC Theme**: Recycling and Air Quality

**Collaborator: Circle Four Farms, Milford, Utah and Caine Dairy Center, USU**

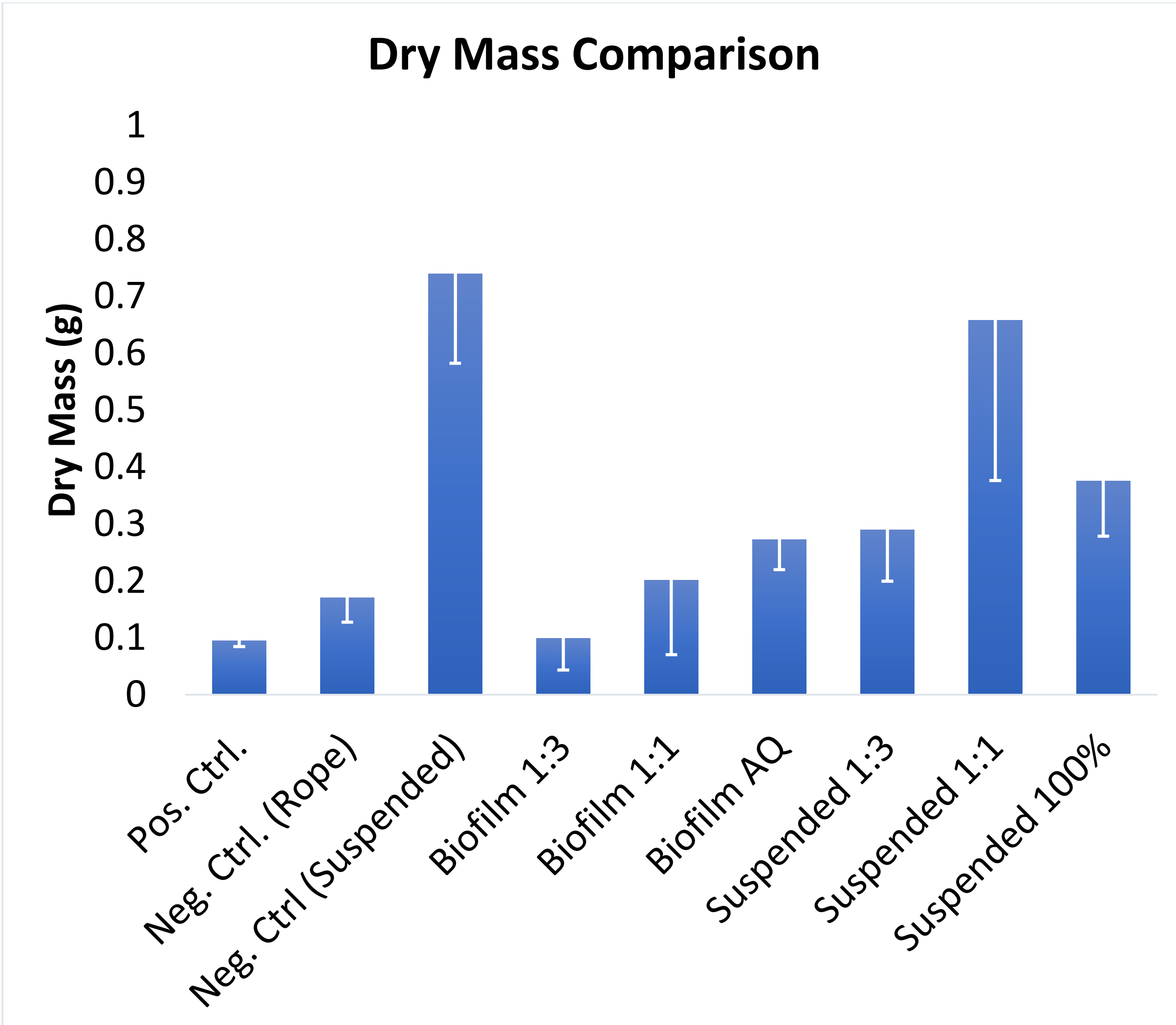
**Students Supported**: Daniel Kade Derrick (BS), Jake Accordino (BS), Tyler Marlar (BS), Justin Marriott (BS), Jason Peterson (MS), and Ben Peterson (MS)

**Results/Products/Outcomes:** The lipid-protein extraction procedure (L-PEP) can be applied as a growth media for microalgae. Algae is grown on wastewater to remove nutrients found at harmful concentration, and can then be used to create bioproducts. The Lipoprotein extraction process (or L-PEP) is an algal bioprocess that can be utilized to generate high-value bioproducts, including bio-oils and biogas. One bioproduct of particular interest is the algal lipid-protein precipitate (ALP) which has been applied as a feed supplement in agricultural systems. Through a series of steps, the protein is isolated from algae lysate, and precipitated from a final aqueous solution. Previously, the aqueous solution was discarded as a waste stream. However, nutrient analyses revealed the aqueous phase to be high in nitrogen, phosphorous, and organic compounds. By recycling the aqueous phase as a nutrient-rich growth media, USU's L-PEP has potential to become a self-sustaining system and to remove carbon dioxide, which is a greenhouse gas (GHG), from the atmosphere through the process of photosynthesis.

Results showed the high nitrogen concentration (992 mg/L) and phosphorus concentration (347 mg/L) of the protein extracted algae aqueous phase. Results presented in the figure below for the amount of algae cultivated show that the aqueous phase was a viable growth medium for algae in both a biofilm and suspended growth setting when diluted. The positive control was a standard algae growth medium. Therefore, the "waste" produced as a result of extracting protein and lipids from algae grown on dairy waste can be recycled to grow more algae because of the high concentration of algae nutrients in the aqueous phase of the protein extraction process.

**Dissemination of Results:** Presentations at national professional meeting

1. Poster presentation at the Utah State University Research Symposium, April 7, 2017.
2. Poster presentation at the Institute of Biological Engineering Intermountain Conference November 11, 2017. Utah State University.



Dry Mass Comparison
Dry Mass (g)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Pos. Ctrl.
Neg. Ctrl. (Rope)
Neg. Ctrl (Suspended)
Biofilm 1:3
Biofilm 1:1
Biofilm AQ
Suspended 1:3
Suspended 1:1
Suspended 100%

**2016**

**Project: Pairing of Anaerobic and Aerobic Treatment of Petroleum Wastewater**

**HERC Theme:** Biodegradation, Recycling, and Air Quality

**Collaborator:** Wes-Tech Engineering, Salt Lake City; Utah Petroleum company

**Students Supported:** Zak Fica (MS), Alan Hodges (MS), Jessica VanDarlin (BS), Jordan Wanlass (BS)

**Results/Products/Outcomes:** Wastewater derived from petroleum refining accounts for 33.6 million barrels per day globally. Currently, few wastewater treatment strategies exist to produce value-added products from petroleum refining wastewater, while successfully reducing nutrients, total suspended solids (TSS), and biological oxygen demand (BOD). The theory behind RABR wastewater treatment is that a 40% submerged rotating drum, with appropriate substratum, allows phototrophic microalgal biofilms to be exposed to two growth stimulating environments: 1) nutrients in the wastewater and 2) light and carbon dioxide in the air. As the biofilm grows, macro and micronutrients from the wastewater can be converted to biomass in the biofilm. The biofilm is then easily removed from the system through mechanical scraping, allowing for removal of these nutrients. The Rotating Algae Biofilm Reactor (RABR) has demonstrated the ability to effectively remove macronutrients and produce various bioproducts including biodiesel, bioplastics, acetone, ethanol, and protein feed for livestock. The goal of this project was to develop a novel treatment strategy for petroleum wastewater that meets the following State of Utah effluent guidelines: 25 mg/L BOD (38 mg/L COD) and 25 mg/L TSS, and also secondary objectives to reduce nitrogen to at or below 10 mg/L, reduce phosphorus to at or below 1 mg/L, and produce value-added products through recycling of nutrients.

The pairing of aerobic and anaerobic treatment was shown to be a possible strategy for remediation of petroleum wastewater. When compared to a negative control of evaporative lagoons, aerobic RABR treatment not only produced biofilm algae that could be used downstream for value added streams, but was shown to be statistically significant for reduction of soluble nitrogen, soluble phosphorus, and total suspended solids. The capture of methane and carbon dioxide, both greenhouse gases (GHG), are beneficial for the environment, and represents a potential sustainable “bridge” from fossil fuels to biofuels.

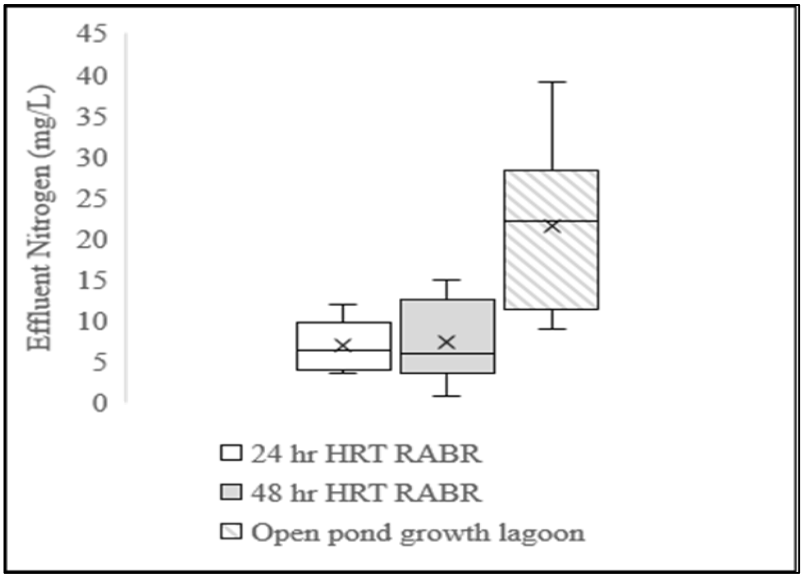
**Dissemination of Results:** Presentations at national professional meeting and publications

1. Nutrient and suspended solids removal from petrochemical wastewater via microalgal biofilm cultivation. 2017. Chemosphere 174, 46-48. Hodges, A., Fica, A., Wanlass, J., VanDarlin, J., and Sims, R.C.
2. Poster presentation at the Institute of Biological Engineering Annual Meeting, Greenville, SC, April 7-9, 2016.

Triplicate Rotating algal bioreactors (RABRs) were fed with petrochemical wastewater.
24-hr and 48-hr hydraulic retention times (HRT)
Open pond lagoon control operated at 36 hr HRT

| Constituent | Objective Effluent | Average Observed Effluent | Method of removal |
| --- | --- | --- | --- |
| **BOD** | 25 | 15.67 | BMP/UASB |
| **TSS** | 25 | 16.85 | RABR |
| **Nitrogen** | 10 | 6.7 | RABR |
| **Phosphorus** | 1 | 0.85 | RABR |

All of the objectives for the project were met as shown in the table above. The method of removal included BMP (BioMethane Potential) and RABR (Rotating Algae Biofilm Reactor).




Influent Nitrogen = 25 mg/L
Nitrogen removal:

24 hr HRT RABR: 18.1 mg $L^{-1}$ (72.4%)
48 hr HRT RABR: 17.7 mg $L^{-1}$ (70.8%)
Open pond growth lagoon: 3.47 mg $L^{-1}$ (13.9%)

The sample median is represented by the horizontal line through the box, the mean of the sample is denoted by an x, the lower and upper bounds of the boxes represent the $25^{th}$ and $75^{th}$ percentile of sample, and whiskers represent maximum and minimum values.
RABR groups performed statistically similarly to each other (p-value 0.41) and significantly outperformed the open pond growth lagoons (p-value <0.001).

**Project: Algae-based Biofilm Productivity and Treatment of Dairy Wastewater: Effects of Temperature and Organic Carbon Concentration**

**HERC Theme:** Biodegradation, Recycling, and Air Quality

**Collaborator:** Caine Dairy Research Center, Circle Four Farms, Milford, Utah

**Students Supported**: Zak Fica (MS), Jay Barlow (MS), Justin Marriott (BS)

**Results/Products/Outcomes:** Biofilm-based microalgal growth was determined as functions of organic chemical loading and water temperature utilizing dairy wastewater from a full-scale dairy farm. The dairy industry is a significant source of wastewater worldwide that could provide an inexpensive and nutrient rich feedstock for the cultivation of algae biomass for use in downstream processing of animal feed and aquaculture applications.

Algal biomass was cultivated using a Rotating Algal Biofilm Reactor (RABR) system. The RABR is a biofilm-based technology that has been designed and used to remediate municipal wastewater, and was applied to treat dairy wastewater, through nutrient uptake, and simultaneously provide biomass for the production of renewable bioproducts.

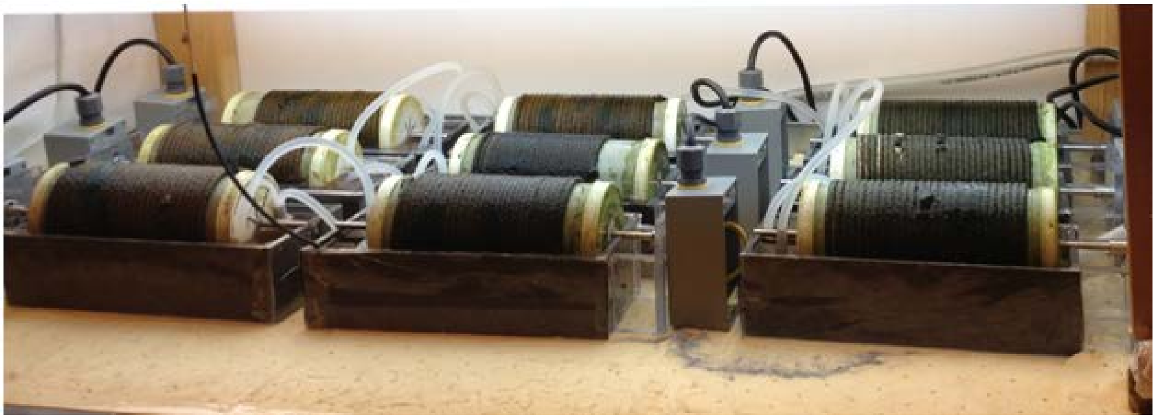
Algal biomass was grown at temperatures ranging from 7-27 °C, and organic carbon concentrations ranging from 300-1200 mg/L of Total Organic Carbon (TOC). Aerial algal biofilm growth rates were calculated, and analysis of Variance (ANOVA) calculations indicated that both the temperature of the wastewater and the level of organic carbon contributed significantly to the rate of biomass growth in the system. However, the interaction of temperature and organic carbon content was not significantly related to the biofilm-based growth rate.

Equations were developed using an Arrhenius linearization and temperature correction coefficient that can be used to evaluate algal biomass productivity and nutrient removal rates in future experiments and designs for dairy wastewater.

In addition to contributing to biodegradation and recycling technologies, this project improves air quality by capturing carbon dioxide from the atmosphere and incorporating it into biomass through the process of photosynthesis using the same process of trees.

**Dissemination of Results:** Presentations at national professional meeting and publications

1. Algae-based biofilm productivity utilizing dairy wastewater. Journal of Biological Engineering 10:18-24. DOI: 10.1186/s13036-016-0039-y. 2016. Fica, Z. and Sims, R.C.
2. Poster presentation at the national Institute of Biological Engineering Annual Conference, Greenville, SC, April 7-9, 2016.



**Green color shows successful growth of algae on dairy wastewater at temperatures from 45 degrees to 81 degrees Fahrenheit. (From Journal of Biological Engineering publication).**

**Project: Anaerobic Digestion of Wastewater: Effects of Inoculants and Nutrient Management on Biomethane Production and Treatment**

**HERC Theme:** Biodegradation, Conservation of trees, and air quality

**Collaborator:** WesTech Engineering, Circle Four Farms, Woods Cross Petroleum Processing plant site

**Student Supported**: Jason Peterson (MS)

**Results/Products/Outcomes:** Anaerobic digestion of wastewater can reduce cost by utilizing methanogens to treat high amounts of organic chemicals in wastewater without the need for oxygenation. Anaerobic digestion accomplishes biodegradation of organic chemicals, provides methane, which is a renewable energy source and alternative to trees, and can decrease methane emissions when controlled and utilized properly.

Wastewaters with high carbon content have been shown to produce higher amounts of the greenhouse gas (GHG) methane when balanced with nitrogen. If this GHG can be generated and utilized under controlled condition, there is the potential for both conservation of trees that are used for energy production and improved quality of air through reduction of GHG. It has been suggested that microalgae be added to increase the nitrogen content to help balance the high carbon to nitrogen ratio of the wastewater. One challenge with the use of algae is the initial biodegradation of microalgae. Using a digester with algalytic microbes, algae can be biodegraded and production of enhanced methane. The augmentation of wastewater by microalgae with algalytic microbes could provide the balance needed for the methanogens to treat wastewater and provide methane.

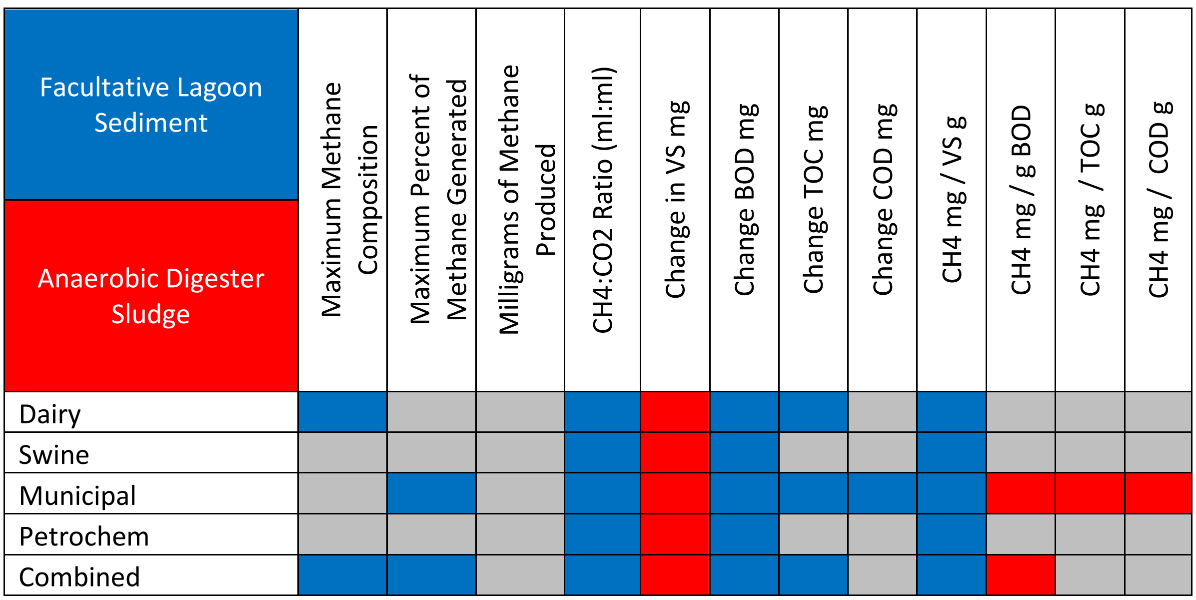
A biomethane potential test was used to compare the ability of two inocula, facultative lagoon sediment and anaerobic digester sludge, to digest algae under anaerobic conditions and produce biomethane. Each inoculant treated, dairy, swine, municipal, and petrochemical wastewaters augmented with algae and sodium acetate to increase and balance the carbon to nitrogen ratio. The ability to degrade augmented wastewaters and produce methane was determined by measuring the volume and composition of biogas produced over time.

Both treatments were successful in the production of enhanced methane. Facultative lagoon sediment showed a higher ratio of methane produced per carbon dioxide than anaerobic digester sludge. Facultative lagoon sediment showed a larger reduction in biological oxygen demand, where anaerobic digester sludge showed a larger decrease in volatile solids. Facultative lagoon sediment showed more methane produced per gram of volatile solids than anaerobic digester sludge.

**Dissemination of Results:** Thesis and professional presentation

1. M.S. Thesis "Anaerobic Digestion of Wastewater: Effects of Inoculants and Nutrient Management on Biomethane Production and Treatment. Jason Peterson. Biological Engineering Department. 2017. All Graduate Theses and Dissertations. 5699.
2. Jason Peterson. Bioenergy and Treatment for four Wastes. Presentation to USTAR Director, Dr. Andrew Sweeney, Utah State University, July 7, 2016.

Results of this project demonstrated that sediment taken from the City of Logan Wastewater Lagoons contained microorganisms that were more effective in the treatment of the four wastes tested than microbes taken from the anaerobic digester of a conventional municipal wastewater treatment plant.



Visual representations of significant Yield and composition parameters found from treatment of dairy, swine, municipal, and petrochemical wastewaters by Facultative Lagoon Sediment (FLS) and Anaerobic Digestive Sludge (ADS). Non-significant findings are marked in gray, FLS significant findings are marked in blue and ADS significant findings are marked in red. Bottom row (Combined) show if significance difference was found between treatments based on all wastewaters treated. [VS = Volatile Solids; BOD = Biochemical Oxygen Demand; TOC = Total Organic Carbon; COD = Chemical Oxygen Demand; mg = milligrams]