

BIENNIAL REPORT 2014-2015

PROJECTS AND PRODUCTS

Summary. During the years 2014-2015, HERC themes included recycling, air quality (reducing greenhouse gases, GHG), conservation of trees, and biodegradation. HERC collaborated with Carollo Engineers, the Utah Science Technology and Research Initiative, the Utah Water Research Laboratory, and the Algae Biomass Organization to utilize biodegradation to recycle nutrients and to transform wastes into bioproducts including bioplastic materials, phycocyanin, and bioenergy. The Utah On-site Wastewater Treatment Training Program provided workshops for certification to over 200 people throughout the state of Utah and developed revised course manuals that incorporated legislative mandates as well as guidance from the Utah Department of Environmental Quality. The Engineering Education Department developed new curriculum materials for the course Computer Engineering Drafting that address stormwater basins and contaminant mapping for students in the Biological Engineering department and the Civil and Environmental Engineering Department.

2015

Project: Biological Phosphorus and Nitrogen Removal from Lagoon Wastewater using Rotating Algae Biofilm Reactors (RABR)

HERC Theme: Recycling (nutrients), Air Quality

Collaborators: Logan City Environmental Department, Carollo Engineers (Salt Lake City), WesTech Inc. Engineering

Students Supported: Anna Doloman (PhD), Alan Hodges (MS), Sahand Shayan (MS), Celeste Hancock (BS), and Tyler Marlar (BS)

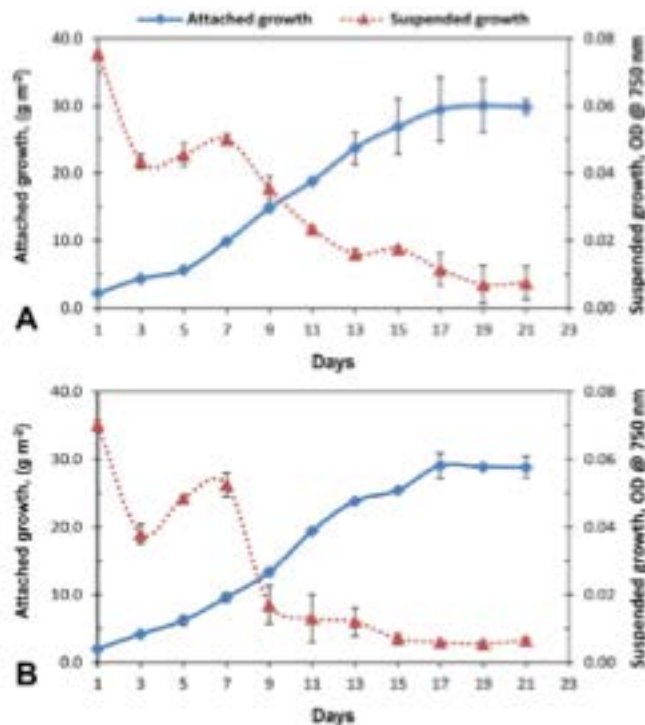
Results/Products/Outcomes: Logan City is currently required to remove phosphorus to low levels (1 mg/L) and was recently required to remove nitrogen from wastewater in order to meet water quality criteria established by the State of Utah in 2015. The biological-based technology, the Rotating Algae Biofilm Reactor (RABR), was tested for phosphorus removal at the City Lagoons Wastewater Treatment Plant. The RABR technology treats wastewater to higher quality (nitrogen removal to less than 5 mg/L) and utilizes the biosolids produced to make valuable bioproducts.

The RABR method for treating wastewater captures carbon dioxide, a greenhouse gas (GHG), in the form of microalgae, thereby providing “CO2 credits” as part of a life cycle analysis of wastewater treatment impacts on the environment. The method also provides a way to avoid landfill disposal of biosolids generated in wastewater treatment by fractionating the algae into several streams that can be used as a feedstock to make valuable bioproducts. The method developed is applicable to other wastewater systems in Utah that depend on lagoons or open ponds for treatment and also to mechanical plants that treat lower volumes of highly concentrated nutrient streams. Implementing RABR microalgae wastewater treatment technology can boost economic growth through creation of new engineering jobs and services in Utah.

We successfully removed nutrients, including phosphorus and nitrogen, from Logan Lagoon wastewater using the RABR technology (Publication 1); developed and tested an improved method for processing the wet harvested algae (Publication 2); and treated Logan Lagoon microalgae in an Upflow Anaerobic Sludge Blanket Reactor (UASB) to produce bioenergy at 90% methane (Publication 3). These results demonstrated the success of mixed culture microalgae for treating municipal wastewater and producing valuable bioenergy and products utilizing all fractions of the algae biomass. As an added benefit, this technology keeps the biomass from wastewater treatment out of the landfill.

Dissemination of Results: Presentations at professional meetings and publications

1. Hydraulic Retention Time Effects on Wastewater Nutrient Removal and Bioproduct Production via Rotating Algae Biofilm Reactor. 2016. *Bioresource Technology* 211:527-533. Sahand Iman Shayan, Foster A. Agblevor, Lorenzo Bertin, and Ronald C. Sims.
2. Optimization of a Wet Microalgal Lipid Extraction Procedure for Improved Lipid Recovery for Biofuel and Bioproduct Production. 2015. *Bioresource Technology* **193**, 15–24. Sathish, A., Marlar, T. & Sims, R. C.
3. Upflow Anaerobic Sludge Blanket Reactor Co-Digestion of Algae and Acetate to Produce Methane. 2016. *Water Environment Research*. DOI: <http://dx.doi.org/10.2175/106143016X14504669767490> Yousef M. Soboh, Darwin L. Sorensen, and Ronald C. Sims.



Algal biomass growth profile and uptake of nutrients (nitrogen and phosphorus) in attached biofilm and suspended medium for 2- day Hydraulic Retention Time (A) and 6-day Hydraulic Retention Time (B) in cases of attached growth (solid lines) and suspended growth (dotted lines). Error bars represent standard deviation ($n = 2$)

Project: Biomass and Phycocyanin Production from Cyanobacteria Dominated Biofilm

Reactors Cultured Using Oilfield and Natural Gas Extraction Produced Water

HERC Theme: Recycling (nutrients), Air Quality (reduce Greenhouse Gas)

Collaborators: Utah Science Technology and Research Initiative, Logan City Environmental Department, State of Utah Water Research Laboratory, LaPoint Produced Water Management (Utah), Integrated Waste Management (Utah)

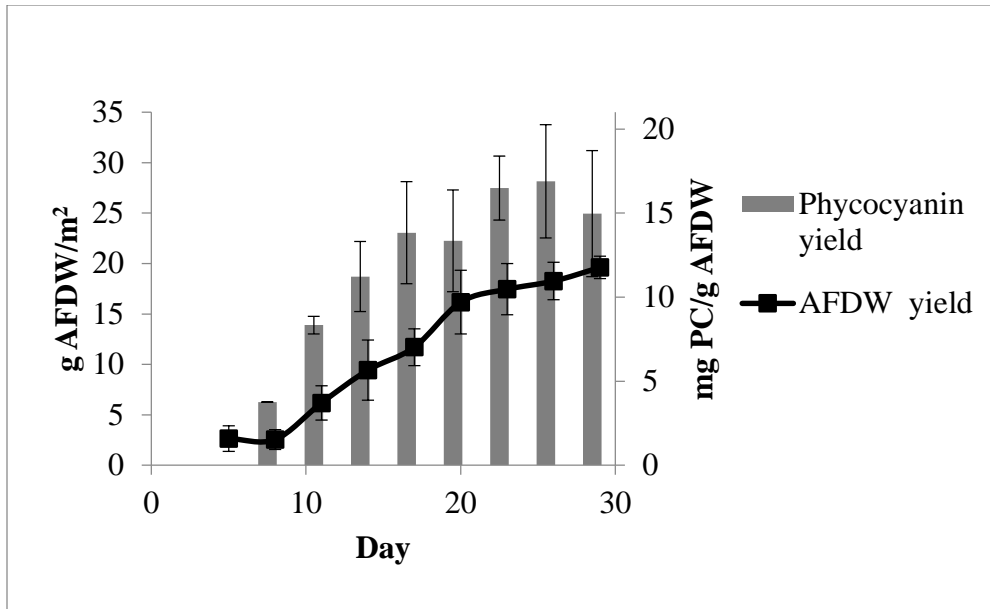
Student Supported: Jonathan Wood (MS)

Results/Products/Outcomes: The production of cyanobacterial biofilm biomass and phycocyanin from Rotating Algal Biofilm Reactors utilizing undiluted produced water from oil and natural gas extraction as a medium was demonstrated in this study. Oil and natural gas extraction produced water is the largest waste stream generated by these industries and may provide an abundant source of non-potable water for the culture of cyanobacteria and phycocyanin. In the present study, a unialgal cyanobacteria isolate from the Logan City, Utah Wastewater Treatment Facility was shown to exhibit an areal ash free dry weight biomass productivity of $4.8 \pm 0.7 \text{ g/m}^2\text{-day}$ when cultured in produced water medium. The cyanobacterial biofilms yielded an areal phycocyanin productivity of $84.6 \pm 9.3 \text{ mg/m}^2\text{-day}$ with a maximum crude extract purity of 0.23 ± 0.03 . The utilization of produced water for the production of cyanobacterial biofilm biomass and associated high value products could provide significant economic and bioremedial advantages to current produced water disposal technologies.

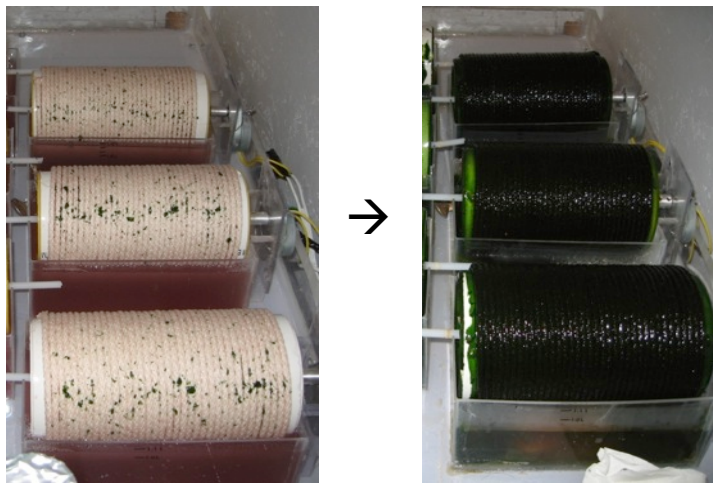
Dissemination of Results:

Biomass and Phycocyanin production from cyanobacteria dominated biofilm reactors cultured using oilfield and natural gas extraction produced water. 2015. *Algal Research*, 11: 165-168. Jonathan L. Wood, Charles D. Miller, Ronald C. Sims, Jon Y. Takemoto.

Applications of Algae Biofilms for Wastewater Treatment and Bioproduct Production. Ed. Singh, B. *Algae and Environmental Sustainability*. Book Chapter 3. Springer Publishing. Pp. 23-31. 2015. Maureen Kesaano, Jonathan L. Wood, Terence Smith, and Ronald C. Sims



Microalgae grows well and produces phycocyanin, a high value bioproduct, using the Rotating Algae Biofilm Reactor (RABR). AFDW = Ash Free Dry Weight of biomass and phycocyanin continues to increase over time on oil field and natural gas extraction wastewater (produced water).



Microalgae isolated from the Logan City Lagoon Wastewater Treatment Plant and inoculated onto the Rotating Algae Biofilm Reactor (RABR) shown immediately after inoculation (left panel) and after 21 days (right panel). The growth of microalgae and increase in phycocyanin concentration is shown in the figure above.

Project: Bioplastic Production Wastewater, Microalgae, and Genetically Engineered Bacteria

HERC Theme: Recycling, Biodegradation, Air Quality

Collaborators: NASA, Algae Biomass Organization (ABO)

Students Supported: Asif Rahman (PhD), Ryan Putman (BS), Ashik Sathish (MS), Terence Smith (MS), Chad Nielsen (MS)

Results/Products/Outcomes: Bioproduct production from wastewater [microalgae](#) has the potential to contribute to societal needs with value added chemicals. Microalgae can remediate wastewater and recycle chemicals to remove nitrogen, phosphorus, and heavy metals and can be processed to produce biofuels and bioproducts. It was previously demonstrated that [recombinant *Escherichia coli*](#) could produce polyhydroxybutyrates (PHBs) when cultured on a wastewater microalgae wet lipid extracted media. In this present project, microalgae were harvested from the effluent of a wastewater treatment facility via centrifugation and hydrolyzed to create a liquid medium for genetically engineered *E. coli* growth and bioplastic production. Standard *E. coli* growth media was supplemented with hydrolyzed algal extract to produce a maximum of 31% bioplastic (polyhydroxybutyrate or PHB) of the *E. coli* dry cell weight.

Dissemination of Results:

Polyhydroxybutyrate Production Using a Wastewater Microalgae Based Media. 2015. Algae Research. Volume 8, 95-98, 2015. Asif Rahman, Ryan Putman, Kadriye Inan, Ashik Slathish, Terence Smith, Chad Nielsen, Ronald C. Sims, and Charles D. Miller.

Production of bioplastic from naturally occurring microalgae and genetically engineered bacteria (*E. coli*).

Microalgae media	Bioplastic (%) of Biomass
1%	31
2%	28
3%	11
10%	5
Control (1.5% glucose)	47

Bioplastic production looks promising using microalgae as a growth medium for the genetically engineered bacteria. However, there was some inhibition of production of bioplastic in the algae media that needs to be determined. (Reference: A. Rahman, et. Al, Algae Research, 2015)).

2014

Project: Algae Biofilm Technology for Wastewater Treatment

HERC Theme: Biodegradation, Recycling (nutrients), Conservation of Trees

Collaborators: Utah Department of Environmental Quality, Utah Water Research Laboratory, Algae Biomass Organization (ABO)

Students Supported: Maureen Kesaano (PhD)

Results/Products/Outcomes: Applications of algal biofilm-based systems in wastewater treatment have been limited despite the potential benefits of a low-cost nutrient removal option and a source of biomass for bioproduct production that can replace products from the destruction of trees. The processes involved with algal biofilm-based systems in wastewater treatment are not adequately addressed in the available literature, which hinders design and scale up of effective systems for applications to municipal, industrial, and agricultural waste streams. A critical review was developed, which examines nutrient removal trends, biomass productivity, growth requirements, and challenges for algal biofilm-based biotechnology as applied to wastewater treatment and recycling both at bench scale and at pilot scale operations. This critical review identified key areas that need to be addressed for designing, building, and testing algal biofilm-based technologies that integrate both nutrient removal and recycling from wastewater and enhanced biomass production to improve the performance of engineered systems. The review identifies the need for research on factors that affect algal growth, mass transport, species selection, algal–bacterial interactions, and validation of laboratory research in field scale tests for the development of an algal biofilm based technology platform for integrating wastewater treatment and biomass production for a variety of industrial and municipal wastewater types.

Dissemination of Results:

Algal Biofilm Based Technology for Wastewater Treatment. 2014. Algal Research Volume 5, 231-240 (July). Maureen Kesaano and Ronald C. Sims

Organizations involved in large scale algal cultivation using attached growth systems

Organization	Bioreactor	Treatment	Products
BioProcess Algae	Grower Harvester	Recycled Water	Animal feed, biofuels
Hydromentia	Algal Turf Scrubber	Nutrients	Compost, animal feed
European Commission	Alga-disc	Carbon Capture	Biomass
Utah State Univ.	Rotating Algae Biofilm Reactor	Nutrient recycling Biodegradation Conservation of trees	Biofuels
Iowa State Univ.	Rotating Algae Biofilm	Nutrients	Biomass

Project: Production of Bioplastic Material in Bacteria using Different Carbon Sources from Wastes

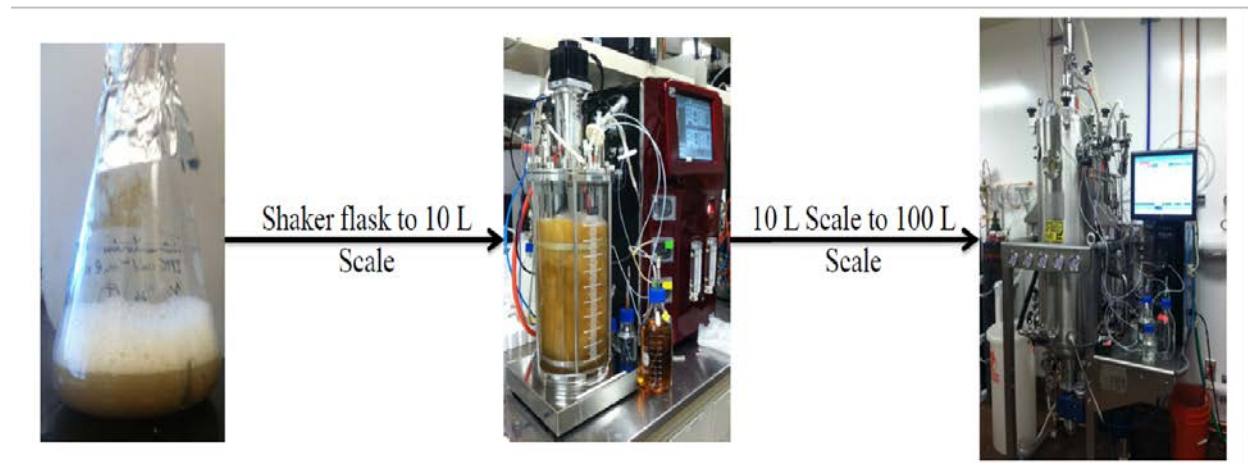
HERC Theme: Recycling, Biodegradation

Collaborators: Logan City Environmental Department, NASA

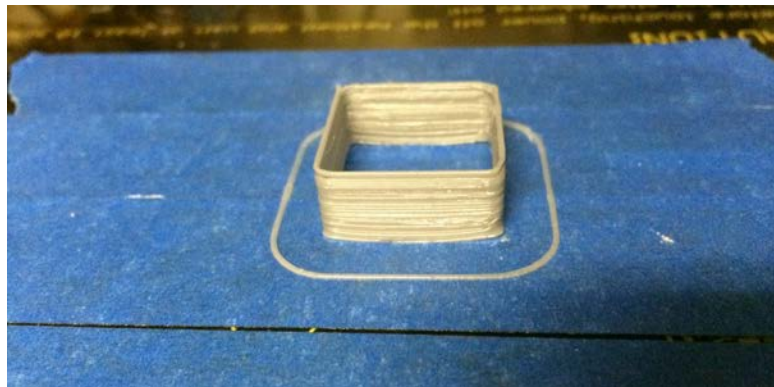
Students Supported: Asif Rahman (PhD), Ryan Putman (BS), Ashik Sathish (MS), Ranil Anthony (PhD), and Terence Smith (MS)

Results/Products/Outcomes: Grand Prize presentation at the National Annual Conference of the Institute of Biological Engineering (IBE), Lexington, KY, March 6-8, 2014.

Dissemination of Results:



Growth of bacteria (*E. coli*) in aqueous phase with different waste carbon sources was scaled from shaker flasks (left) to 10 liter fermenters (middle) to 100 liter fermenters (right). The media supported growth of genetically engineered *E. coli* and the production of bioplastic material (polyhydroxybutyrate).



Algae-based bioplastic biomaterial was successfully manufactured with 3-D printing.

Project: Anaerobic co-digestion of algal biomass and rich carbon source material to produce methane for bioenergy

HERC Theme: Biodegradation, Recycling (nutrients)

Collaborators: Central Weber Sewer Improvement District, Wes-Tech Engineering

Students Supported: Yousef Soboh (PhD)

Results/Products/Outcomes: Presentation at the National Annual Conference of the Institute of Biological Engineering (IBE), Lexington, KY, March 6-8, 2014.

Dissemination of Results: Algae that are grown in wastewater treatment lagoons could be an important substrate for biofuel production; however, the low C/N ratio of algae is not conducive to anaerobic digestion of algae with economically attractive methane production rates. Increasing the C/N ratio in anaerobic, laboratory scale, batch reactors by blending algal biomass with sodium acetate resulted in increased methane production rates as the C/N ratio increased. The highest amount of methane was produced when the C/N was 21/1. When the C/N was 24/1, the biogas production rate decreased.

Biogas production with different carbon-to-nitrogen (C/N) ratios provided by algae

C/N Ratio	Biogas Volume (ml) (methane + carbon dioxide)	Methane (%)
5	567	62
12	2,147	80
18	2,486	84
21	2,858	82
24	1,928	83