

**Munyawera, James**

***University of Rwanda***

**Contribution to the Optimization of Algal Production as Biomass for Generating Biofuel**

*Clement Ahishakiye ,University of Rwanda Birungi Martha Mwiza, University of Rwanda*

Algae are photosynthetic organism including macro and microalgae species and they mostly live in moist environment. Algae especially microalgae species are important due to high nutritional content mainly oil that can be used for production of biofuel as an alternative to petroleum products. Biofuel is a fuel produced through biological processing of photosynthetic matter. Aim of this study was to identify the algal species present in sample collected from Rwamamba marsh land and to determine their optimal growth conditions for biomass production. in order to produce biofuels in Rwanda. The isolation of algae strains was performed by using appropriate culture media and the identification by phenotypic characteristics based by microscopic observation. During the biomass production, a culture has been supplied with CO<sub>2</sub> from a reaction produced by calcium carbonate and hydrochloric acid, another one was remained naturally with no CO<sub>2</sub> supp. The effect of pH (6.5, 7.5, 8.0) on the growth of algae species was also evaluated. In the samples collected from different sites. Two different algal species were identified namely Chlorella sp. and Botryococcus sp. The result showed that Chlorella sp. grows better than Botryococcus sp. the optimum growth temperature for isolation was around 25oC. The culture of Chlorella sp in the medium with additional CO<sub>2</sub> grow better than when there is no CO<sub>2</sub> supply. The both Chlorella and Botryococcus species were grows best at the pH 7.5 and 8.0. These culture conditions could be used for the rational production of these algae strains as biomass for generating biofuels in Rwanda. However, further studies on the artificial fluorescent light on the growth of these algae species, and the use of other culture media are needed.

**Yu, Jianping*****NREL*****Glycogen Synthesis and Metabolite Overflow Contribute to Energy Balancing in Cyanobacteria***Melissa Cano and Jianping Yu, NREL*

There is a lack of systematic study on the regulation of high energy metabolites such as ATP levels that drive the metabolism in living organisms. Using light as the energy input, we found that the energy charge (ratio of ATP over ADP+ATP) in the cyanobacterium *Synechocystis* 6803 vary through different growth stages, with a peak upon entry into the rapid growth phase, as well as a positive correlation with light intensity. In contrast, a mutant that can no longer synthesize the main carbon storage compound glycogen showed higher energy charge. The overflow of organic acids in this mutant under nitrogen depletion could also be triggered under high light in nitrogen-replete conditions, with an energy-input level dependency. These findings suggest that energy charge in cyanobacteria is tightly linked to growth and carbon partition, and that energy management is of key significance for their application as photosynthetic carbon dioxide assimilating cell factories.

**Pudney, Alex**

*Algenuity*

**Multiparametric Optimization of Commercial Microalgal Strains for Improved Productivity Using the Algem and Algem HT24 Family of Photobioreactors**

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Introduction: Commercial microalgae strains are often cultivated under controlled artificial lighting and temperature environments as well as tightly controlled nutrients, pH, CO<sub>2</sub> and mixing. Optimization of biomass productivity and product content is often carried out through trial and error approaches, in many cases from historical media formulations. An increase of even a few percentage points in relative growth rate and product content is highly commercially desirable. In this study we present examples of how we have used the Algem family of lab-scale photobioreactors in a multiparametric approach to rapidly identify improved cultivation conditions. Methods: We used a statistical design of experiments (DoE) approach with a suite of Algem PBRs and Algem HT24 (24 x 50ml flask) PBRs to co-optimize several process factors during mixotrophic cultivation of two commercially grown microalgae, including: light intensity, light wavelength, temperature, pH, and carbon source. Results: Optimal carbon sources and their levels were identified for each strain using the Algem HT24 system. Multifactorial DoE experiments also identified additional process factors (and their interactions) having a significant effect on biomass productivity, and were subsequently refined using successive rounds of DoE. Relative improvements in strain productivity as well as content of key desirable bioproducts were obtained. Discussion: The algal industry is reliant upon consistent production of biomass and bioproducts with production platforms operating on tight margins. We have demonstrated that, depending upon the strain and/or product, significant improvements can be made using a multiparametric optimization approach. The key next step is pilot scale validation of the relative impact of these improved cultivation conditions. We believe this is a generally applicable approach that can and should be applied widely across the algal industry to drive success stories.

**Bell, Tisza*****Los Alamos National Laboratory*****Temperature Perturbation Alters the Transcriptomic and Metabolomic Signatures in a Production Strain of *Scenedesmus***

*Tisza Ann Szeremy Bell*<sup>1</sup>, *Igor V. Grigoriev*<sup>2</sup>, *Trent Northern*<sup>2</sup>, *Michael Guarnieri*<sup>3</sup>, and *Shawn Robert Starkenburg*<sup>1</sup> <sup>1</sup>*Los Alamos National Lab, Los Alamos, NM U.S.A.*; <sup>2</sup>*Department of Energy Joint Genome Institute, Walnut Creek, CA U.S.A.*; <sup>3</sup>*National Renewable Energy Lab, Golden, CO U.S.A.*

Improving the productivity and robustness of algal strains against environmental perturbations is pivotal to the success of the mass cultivation strategies required for algal biofuel scalability. Successful production in large outdoor raceways begins with appropriate strain selection and "crop" rotation strategies that utilize different strains at certain times of year in order to maintain maximum productivity. When assessing suitable production strains for a given region, temperature tolerance is a critical factor, but this basic information is lacking for most algal species and screening each strain can be costly and time consuming. Here, we conducted high and low temperature perturbation studies on strain 46D-B3 (*Scenedesmus* sp.) based on temperatures observed in Mesa, AZ during open pond cultivation in May. Following temperature perturbation, samples were collected for complete transcriptomic and metabolomic analysis identifying differential expression and chemical signatures for three temperature conditions. With this information we can facilitate strain improvement and utilize chemical indications of culture health to harvest and rotate strains prior to declines in productivity. Ultimately this will contribute to the standardization of functional genomics experimentation critical for minimizing the time required for strain development and improvement while accelerating the identification of both conserved and strain-specific pathways for enhancements.

**Kamalanathan, Manoj**

***Texas A&M University at Galveston***

**Use of a Stoichiometric Metabolic Model to Optimize Astaxanthin Production: A Laboratory and In-silico Study**

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Astaxanthin produced by *Haematococcus pluvialis* is one of the most promising high value algal products, with a commercial profit of \$200 million/year because of its anti-oxidative, anti-inflammatory, and anti-apoptotic properties. The current market supply bottle neck in astaxanthin production is the lack of knowledge about the life cycle, cellular and biomolecular processes necessary to regulate synthesis in *H. pluvialis*. Therefore, innovative approaches are needed to allow for better understanding of the regulation and optimization of astaxanthin production in cells. We tested a novel approach of using a stoichiometric metabolic model of *H. pluvialis* in conjunction with laboratory experiments to analyze and understand the carbon flow through various biochemical pathways under different nitrogen sources. As predicted by the metabolic model, growth rates were lower and astaxanthin content was higher under ammonium chloride compared to nitrate. Further analysis of the carbon flow through biochemical pathways are underway using this approach in order to develop methods which will enhance cost-effective the astaxanthin production.

**Sudasinghe, Nilusha**

***Los Alamos National Laboratory***

### **Saline Adaptation of Freshwater Microalga *Chlorella sorokiniana***

*Nilusha Sudasinghe<sup>1</sup>, Claire Sanders<sup>1</sup>, Irene Kwon<sup>1</sup>, Elisa Cirigliano<sup>1</sup>, Kimberly Wright<sup>1</sup>, John McGowen<sup>2</sup>, Taraka Dale<sup>1</sup>#. 1- Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87545. 2- Algae Testbed Public Private Partnership (ATP3), Arizona State University, Mesa, AZ 85212.*

The microalgae species, *Chlorella sorokiniana*, holds great potential for biofuel production due to its high biomass productivity and reasonably high levels of carbon storage in the form of carbohydrates and triglycerides. However, *C. sorokiniana* is a freshwater species, which reduces its utility as a potential algae production strain due to limited sustainability and competition with potable water uses such as agriculture and drinking water. In this study, three *Chlorella sorokiniana* strains, *C. sorokiniana* UTEX 1228 (Cs1228), *C. sorokiniana* UTEX 1230 (Cs1230) and *C. sorokiniana* DOE 1412 (Cs1412) were screened for their tolerance to cultivation at varying levels of salinities, with the aim of maintaining high biomass productivity while increasing sustainability and reducing competition with other human needs. Cs1228 was successfully adapted to grow in a traditional f/2 medium, containing full strength (“100%”) seawater salinity from Instant Ocean Sea Salt. Cs1412 and Cs1230 strains were also adapted to an f/2 medium, containing salinities that were 70% and 75% of full strength seawater, respectively. The adapted strains were cultivated in their corresponding adapted growth media in an advanced flask system consisting of a 16:8 light:dark cycle at 800  $\mu\text{mol}/\text{m}^2\text{-sec}$  light intensity and pH control using CO<sub>2</sub> sparging. The growth and lipid accumulation of the cultures were monitored and compared to the unadapted Cs cultures grown in a freshwater HS medium. The saline adapted Cs1230 and Cs1412 strains showed similar linear growth rates and higher final biomass productivities compared to the Cs strains grown in the HS media. Also, increased neutral lipid accumulation and larger cells were observed by flow cytometry for all saline adapted Cs strains. Triglyceride quantification by gas chromatography with flame ionization detection (GC/FID) revealed 1.6-fold higher lipid accumulation for the saline adapted Cs 1230, and similar FAME analysis is ongoing for the other two strains. Our results thus far indicate that freshwater *Chlorella* strains can be adapted to improve sustainability metrics of algae production, while still maintaining, or even improving, strain productivity. We will cultivate the saline adapted Cs strains in 50 L mini-ponds to further evaluate their performance and lipid accumulation relative to the freshwater grown Cs strains.

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Wei, Dong

*South China University of Technology*

### **Enhanced Production of Astaxanthin by *Chromochloris zofingiensis* in a Microplate-based Culture System Under High Light Irradiation**

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Astaxanthin (3, 3'-dihydroxy- $\beta$ ,  $\beta$ -carotene-4,4'-dione) is a bright red ketocarotenoid with high commercial value used in functional foods, dietary supplements, beverages, cosmetics and the nutraceutical industry because of its super-high anti-oxidative activities. In the current bio-based industry, the green microalga *Haematococcus pluvialis* and the red yeast *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) are regarded as ideal producers of commercial natural astaxanthin, but they have faced serious challenges. The green microalga *Chromochloris zofingiensis*, formerly named *Chlorella zofingiensis*, is a promising alternative producer of natural astaxanthin. In the present study, *C. zofingiensis* was first cultivated in shake flasks under low light irradiation and then subjected to continuous high light irradiation, which effectively promoted astaxanthin production. In addition, a microplate-based culture system in concert with high light irradiation from blue light and white light above  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  was constructed and applied to improve astaxanthin production. Blue light exerted more positive influences on astaxanthin accumulation, but when the light intensity was increased to  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , astaxanthin biosynthesis was substantially inhibited. Conversely, in a nitrogen-deprived culture under white light, the highest astaxanthin content for *C. zofingiensis*, 7.1 mg/g, was obtained. The highest astaxanthin yield achieved was 38.9 mg/L in a culture with 0.1 g/L nitrate under the same culture conditions. This study demonstrates that *C. zofingiensis* has great potential for natural astaxanthin production.

**Tyler, Christina**

***Los Alamos National Laboratory***

### **Mining the Algal Genome for Epigenetic Machinery**

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All eukaryotic organisms contain factors that regulate the organization, structure, and accessibility of their genomes. These factors collectively constitute chromatin remodeling mechanisms that, when inherited after mitotic activity, are deemed epigenetic in nature. Epigenetic machinery is responsible for posttranslational modification of amino acids in histone proteins or nucleic acids in RNA and DNA. For many algal species, these modifications are uncharacterized; further, only a handful of genetic signatures of epigenetic machinery have been identified in select species. Utilizing bioinformatics approaches, we determined the presence of epigenetic machinery responsible for DNA modifications and potential mechanisms of epigenetic transcriptional repression that *Chlorella sorokiniana* strains 1230, 1228, and 1412 use to regulate their genomes. We identified multiple orthologs of DNA methylation machinery, suggesting that these algal strains maintain the ability to methylate DNA in the same nucleic acid contexts as plants; however, there was high sequence variability for potential methyltransferases utilized by each strain suggesting divergent strategies for heterochromatin formation. Additionally, *C. sorokiniana* strains lack sequences for epigenetic machinery responsible for de novo and RNA-directed methylation but encode for DNA glycosylases, thereby demonstrating the potential for dynamic DNA methylation that may play a role in gene expression. We found many components of the RNA-induced silencing complex (RISC) and enzymes for RNA modification. Thus, *C. sorokiniana* likely employs RNAi machinery capability coupled with DNA maintenance methylation to inhibit transcriptionally active RNA and perpetuate the methylome during mitosis. These maintenance methyltransferases, while different among each strain, all perform the same function, and may have dual functionality in de novo methylation as the presence of transposable elements requires epigenetic protective genomic structure. Analysis of RNA and DNA methylation machinery in *Nannochloropsis salina* and *Aureococcus anophagefferens*, a harmful algal bloom species, demonstrates that eukaryotic algae contain highly diverse genomic signatures for epigenetic machinery and thus may have evolved distinctive mechanisms for chromatin maintenance and gene expression.

**Douchi, Damien***nrel***Engineering the Central Carbon Metabolism for the Optimization of Energy Harvest and Utilization***National renewable energy laboratory*

Critically needed improvements in algal carbon sequestration and biomass productivity can be made by addressing fundamental inefficiencies in carbon conversion efficiency (CCE). Photosynthesis is the fundamental process that powers inorganic carbon uptake and assimilation, and biomass accumulation. Understanding photosynthesis regulation and control will ultimately help to design metabolic engineering strategies that will help to increase biomass productivity rates. Metabolic carbon sinks (starch, lipids, molecules of interest) have been demonstrated as playing a major role in this regulation but this mechanism is not fully understood. Central carbon metabolism, pathways toward the production of storage compounds, cell physiology in response to stresses, and light harvesting appear to be the main targets in current metabolic engineering efforts as part of the Rewiring Algal Carbon Energetics (RACER) project consortium. Of many photosynthetic algae, only a few are routinely genetically modified in laboratories. Unfortunately, many of the laboratory model species do not thrive in outdoor conditions at industrial scale for the production of molecules of interest. We directed our efforts toward the domestication and engineering of both a model cyanobacterium, *Synechocystis* sp. PCC 6803 and an industrially-relevant species *Desmodesmus armatus* SE00107, specially focusing on the modification of the Calvin Benson Basham (CBB) cycle and on the starch biogenesis pathway. The work presented here covers the establishment of a laboratory method to measure inorganic carbon uptake and fixation applied to understand effects of the manipulation of the CBB cycle and the production of photo-assimilates in both organisms. This will be tied with carbon partitioning between the different metabolic sinks present at different times during cultivation.

Tyus, Chelsea

*Louisiana State University*

### **Discovering Potential Protein-based Food Ingredients in a Co-culture of Microalgae**

*Chelsea Tyus, Zhimin Xu, Maria Teresa Gutierrez-Wing, Joan King\* (all authors institution is Louisiana State University)*

Microalgae is a source of functional ingredients with positive health effects due to high PUFAs, polysaccharides, pigments, essential minerals, vitamins, enzymes and bioactive peptides. Better understanding of the proteins present in algae strains encourages use in food products, and applications such as biosensors, drug delivery, foam-stabilizers and emulsifiers. Protein levels in *Spirulina Platensis* (571.4ug/mg) and *Chlorella* species (580ug/mg) have been previously determined, but protein content varies based on algae culture type and growth conditions. Also very little is known about algal protein chemical properties. This study focused on *Chlorella vulgaris* (Chlorophyta) /*Leptolyngbya* sp. (Cyanobacteria) co-culture microalgae (CCA) protein characterization, information that is imperative for application of these proteins in the future. CCA obtained from the LSU Aquatic Resources Engineering was lyophilized prior to protein assays. Total protein concentration (TPC) was analyzed using the Pierce BCA method. Amino Acids (AA) were quantified using ion chromatography. SDS-PAGE provided protein molecular masses. MALDI-TOF-MS provided peptide sequences and identified two proteins. FTIR provided a molecular fingerprint of the CCA. TPC was 123.8ug/mg of CCA. 18 out of 21 AA were detected. CCA contains all the essential AA. 168.0ug/mg total AA; the most prevalent being Ala (20.8ug/mg), glutamic acid (17.3ug/mg), and Arg (16.3ug/mg). The molecular mass of extracted proteins was determined under denaturing conditions by SDS-PAGE; bands were identified at 100, 52, 32, 25, 15, 13kDa. The 52, and 15kDa peptides are hypothesized to be subunits of the L8S8 Rubisco enzyme. FTIR identified peaks at amide I wavenumbers 1575-1705cm<sup>-1</sup> and amide II at 1480-1575cm<sup>-1</sup>. Amide linkages are a defining molecular feature of proteins. MALDI-TOF-MS identified two centriole, cilia and spindle-associated partial proteins (N321\_10891, and N312\_00929) and several peptide sequences. There is a growing demand for healthy, tasty, sustainable, low impact, high-protein foods. Microalgal products need to become more diversified and economically competitive. Algal chemical composition and bioactivity levels are species and sample-specific. These co-culture algal proteins could possibly be used as a source of green or vegan proteins and nutraceuticals.

**Laurens, Lieve**

***National Renewable Energy Laboratory***

**Renewable Bioenergy from Algae; Tying Together Biochemistry of Storage Carbon Metabolism with Biomass Productivity**

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Integrating biochemistry of storage carbon metabolism of algae and plants to produce biofuels and bioproducts, is needed for removing key barriers that currently limit overall carbon efficiency, photosynthetic efficiency and ultimately productivity in the context of limiting resources. Addressing critically needed improvements in biomass, bioproduct and biofuel productivity is a priority and one that is guided by supporting economic and sustainability principles. Exploiting pathways for the integration of engineering approaches with fundamental biochemistry of photosynthetic organisms may help to unravel the contentious nexus of growth rates, biomass productivity and composition, and nutrient load. This presentation will cover progress towards the uncoupling of growth rate and biomass composition in the context of serving as a feedstock for biochemical conversion to fuels and products. We have established an experimental set up that allows us to test at small, but relevant, scale the impact of different dilution rates of cultures, with varying nutrient loadings for two different species of algae; *Chlorella vulgaris* and *Desmodesmus armatus*. The biomass composition, in particular the carbon storage sink accumulation rates, and photon efficiency were studied and placed in direct context to implemented growth and harvesting regimes in outdoor cultures. The data was used to establish a preliminary economical and cost trade off of the growth and productivity measures for both species. Biochemical characterization of the underlying metabolic status of the cells indicates that the storage carbohydrates are rapidly accumulating at the higher density cultures, but at a cost of productivity. This trade-off is largely compensated for by the increased value of the biomass

**Gutierrez-Wing, Maria Tera**

*Louisiana State University*

### **Strategy to Develop Cryopreservation Methods for Recalcitrant Microalgae**

*Maria Teresa Gutierrez-Wing, Louisiana State University Terrence Tiersch, LSU Agricultural Center  
Michael Benton, Louisiana State University*

The maintenance of microalgal cultures is essential for commercial and research operations. Most seed cultures are maintained as live organisms. Several problems have been observed for long-term live cultures, including selection for traits adapted to stable laboratory environments. The time, cost and risk of maintaining these as live cultures, as well as the routine loss of traits, have prompted repositories to cryopreserve strains. Although this approach has advantages, including reducing the cost for end users, not all species and strains have been successfully cryopreserved. At present, there are no high-throughput, standardized methods with industrial quality controls for microalgal cryopreservation. Most of the methods used are based on protocols developed for cattle sperm preservation. The diversity of cell types, sizes, cell wall characteristics and growth strategies of microalgae, requires different approaches to overcome the hurdles in the development of successful cryopreservation methods. In this work, we developed a basic framework to define the range of relevant variables in microalgal cryopreservation, such as freezing rates and temperatures, cryoprotectant type, concentration and exposure times, and physiological condition, including growth stage, size, lipid and carbohydrate content relative to culture environment. Based on this information, the use of high throughput strategies developed in the multimillion dollar industry of cattle sperm can be adapted for the commercialization of microalgal strains with lower costs and higher reliability. The mechanisms for this development depend not only on the collection and preservation of the cultures of interest, but also on the proper and complete data basing of the phenotypic and genotypic characteristics of each strain. This approach can be advantageous not only for centralized facilities, with highly trained personnel, but also by end users, that will be able to recover the valuable genetics of their cultures even after catastrophic losses of active production units.

**Wright, Kimberly**

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**Overexpression of the Vacuolar Proton-pumping Pyrophosphatase, AVP1, Increases Starch Accumulation in *Picochlorum soloecismus***

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*Picochlorum soloecismus* (hereafter *Picochlorum*) is a green halotolerant microalga with fast growth rates, moderate starch/lipid accumulation, and is amenable to genetic engineering. We set out to increase carbon storage in *Picochlorum* by overexpressing its native vacuolar proton-pumping pyrophosphatase, AVP1, which has been shown to increase biomass in plants. We hypothesized that by overproducing AVP1 in microalgae, the consequent increase in the vacuolar proton gradient, and the decrease in inhibitory pyrophosphate, would increase ATP levels and photosynthetic efficiency. Ultimately, this results in an increase in carbon storage molecules. The expression of AVP1 and Sh-ble was driven by native RbcS and TELF promoters/terminators, respectively, and were inserted into the genome via electroporation. Gene integration and expression were confirmed by PCR, sequencing, and RT-PCR. AVP1 overexpression lines were compared to wildtype by growth, light and electron microscopy, biochemical composition, and oxygen evolution. Although we continue to characterize these strains, our preliminary data shows that AVP1 mutants accumulate more starch than the wild type, without significantly affecting growth. These results suggest that AVP1 overexpression can benefit algal biofuel production strains by increasing the overall carbon storage of *Picochlorum*. LA-UR: 18-22167

**Gao, Song*****Pacific Northwest National Laboratory*****High Biomass Productivity On Air CO<sub>2</sub> Using a Fast Growing, Alkaliphilic Cyanobacterium***Michael Huesemann\*, Scott Edmundson, Song Gao Pacific Northwest National Laboratory John Benemann\* and Braden Crowe MicroBioEngineering, Inc.*

The AlgaeAirFix™ process enhances CO<sub>2</sub> mass transfer from air into the culture medium, thereby reducing the logistical and economic constraints of large-scale microalgae cultivation on industrial CO<sub>2</sub> sources. A microalgae strain, *Anacystis* sp. (SSL1), was isolated from Soap Lake, WA which exhibited relative high growth rates under brackish water, high alkalinity, and high pH culture conditions. High pH, e.g., above 10, and high alkalinity are known to increase chemically enhanced CO<sub>2</sub> transfer from air into the medium. These conditions also lower the risk of culture crashes caused by invading species. The strain was tested in the LEAPS (Laboratory Environmental Algae Pond Simulator). Light and water temperature conditions from an outdoor raceway pond operated at 20 cm depth in July in Mesa, Arizona were used to grow the alga. The experiment was conducted with ambient air flowing over the culture surface. No other sources of concentrated CO<sub>2</sub> were supplemented. Modified BG-11 medium was used, and the salinity was maintained at 25 PPT. The result showed that SSL1 was able to achieve biomass productivity at 16 g/m<sup>2</sup>-day using only CO<sub>2</sub> from ambient air. Further experiments under high pH, high alkalinity condition indicated that SSL1 grew faster at 10 PPT than 25 PPT salinity. Therefore, the strain could potentially achieve higher biomass productivity if lower salinity were to be used. An indoor climate-simulation raceway pond experiment was conducted to confirm that a productivity of at least 16 g/m<sup>2</sup>-day could be achieved at 10 PPT salinity. If such high biomass productivities on air CO<sub>2</sub> can repeatedly be demonstrated in raceway ponds, large scale microalgae cultivation can be decoupled from stationary industrial sources of CO<sub>2</sub>, thereby increasing microalgae biofuels production potential by at least 10 fold.

**Bharati, Nikita*****Basha High School*****A Novel Approach to Optimizing Algae Biofuel Production by Using Naturally Occurring Extracellular Polymeric Substances (EPS) Through Bioflocculation***Nikita Bharati\* (Institution: Basha High School) (ABO Member)*

Extensive fossil fuel use has been linked to climate change and is increasingly viewed as unsustainable. Microalgae offer an intriguing alternative, carbon-neutral energy source, as they can produce biofuels at large scale. The cost of microalgae harvesting is a major contributor to production cost. Chemical flocculation, although widely used, has relatively high-energy requirements and is potentially toxic to microalgal biomass restricting reuse of the culture medium. Extracellular Polymeric Substances (EPS) (substances secreted from aggregated forms of bacteria/biofilms) offer exciting potential to induce bioflocculation in algae harvesting and reduce biofuel production cost, as well as optimize algal biomass recovery for more efficient algal harvesting. Herein, EPS secreting bacteria are extracted from their natural environment and evaluated for their ability to facilitate microalgae flocculation and lower process cost. 36 bacterial colonies were isolated from crude petroleum oil, which is known to contain EPS producing bacteria. Several strains facilitated recovery of large amounts of algal biomass and the all-important lipids that are used in biofuel production. Three strains with flocculation activities exceeding 90% were selected for further investigation. 16s rDNA sequencing and PCR Amplification showed that the three bacteria strains are from the species *Bacillus Subtilus* and *Psuedomonas* sp. Spectra analysis (FT-IR and <sup>1</sup>H NMR) confirmed that 1) the bacteria have preferred functional groups for flocculation and 2) specific molecular structures that indicate the bacteria produce glycoproteins, the molecules that induce algal flocculation. The bioflocculants investigated herein have been found to have relatively broad temperature and pH stability, with dosing rates comparable to those of chemical flocculants to flocculate suspended solids, indicating they have strong potential for application as algal flocculants in biofuel production.

**Mettler, Jackie**

***Los Alamos National Laboratory***

**Targeted Knockout and Knock-in of Photoreceptor Genes to Improve Biomass Accumulation in Microalgae**

*Jackie Mettler<sup>1</sup>, Blake Hovde<sup>1</sup>, Sangeeta Negi<sup>2</sup>, Taylor Britton<sup>3</sup> (1) Los Alamos National Laboratory, (2) New Mexico Consortium, (3) University of New Mexico Contact info for Jackie Mettler: 10 Verde Ridge St., Apt. C, Los Alamos, NM 87544 (602) 828-2891 [jmettler@lanl.gov](mailto:jmettler@lanl.gov)*

The current estimate of algal biofuel production costs, utilizing all current best practices and scaled to industrial levels, are \$8.50 per Gallon of Gasoline Equivalent (GGE). However, greater than 75% of that cost comes from the algal feedstock production and harvest, whereas only a minimal portion of the cost comes from processing and refinement of the biocrude. By increasing algal yields and rates of harvest for algal biofuel feedstocks, the production costs can be significantly reduced. To achieve a significant yield increase in algal biomass, we propose carrying out both a targeted gene knockout of an algal photoreceptor (phototropin) and targeted gene replacement with a chimeric plant photoreceptor (neochrome). Phototropins are a class of highly conserved photoreceptors used by photosynthetic organisms to sense blue light and, when activated by light, enable plants to maximize the capture of photosynthetically active radiation. Preliminary data shows that knocking out phototropin alone can increase biomass production in the model green alga *Chlamydomonas reinhardtii* two-fold. Therefore, knockout of phototropin in a commercial production level algal species may greatly improve yields. The gene proposed for targeted insertion, neochrome, is a chimeric photoreceptor that contains both phototropin and phytochrome photoreceptor domains and is able to perceive blue/red/far red light exhibited in shaded conditions. We anticipate that photosynthetic efficiency will be improved and result in an additive increase in the biomass accumulation rate in rapidly changing light environments. Both of the methods described have the potential to greatly improve the biomass production or photosynthetic efficiency of production level strains.

**Qari, Huda**

***KING ABDULAZIZ UNIVERSITY***

### **Fatty Acid Synthesis by *Chlamydomonas reinhardtii* in Phosphate Limitation**

*NAME: Huda A Qari INSTITUTIONS: associate professor*

The importance of nutrient factor and its limited availability lead to a variable changes in fatty acid and phospholipid composition of the green alga. These fatty acids and phospholipids are playing main role in biofuel production. The production of phospholipids in alga might be naturally enhances by the optimized supplied of specific key nutrient like Phosphate. In this study, green alga *Chlamydomonas reinhardtii* was cultivated in phosphate stress condition to obtain maximum phospholipid. This stress condition exhibited variable changes in chlorophyll, fatty acid and phospholipid compositions. These parameters were analyzed by biomass, X-ray, GC and TLC. Interestingly, saturated fatty acids, monounsaturated, and di-unsaturated fatty acids amounts, increases, while polyunsaturated fatty acids to decrease markedly. Maximum fatty acid content was observed at 0.4 mg l<sup>-1</sup> P content in growing media. A broad peak area 56% for hexadecanoic acid (C 16:0) and followed by 28.8% linolenic (C18:3) was observed during GC analysis as a highest content of fatty acid. These results indicate the essential fatty acid accumulation maximized at particular phosphate concentration in growing media. Such type essential and non-essential fatty acid production from green algae in sustainable manner is inexpensive and best way for commercialization and biofuel production.

Zhang, Chengwu

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**A Novel Strategy for Ultrahigh Cell Density of Photoautotrophic Culture and High Astaxanthin Productivity of *Haematococcus pluvialis* JNU35**

*Chengwu ZHANG, Baoyan GAO, Feifei WANG, Luodong HUANG, Manman WU*

Effective photoinductive plays an important role in *Haematococcus pluvialis* biomass and astaxanthin accumulation, but no reports have evaluated the combined effects of light-paths (LPs, 3 or 6 cm) and illumination modes (unilateral or bilateral high light illumination, UHLI or BHLI). In this paper, mBBM medium containing 18 mM of initial urea concentration were firstly proved to be more suitable for *H. pluvialis* JNU35 strain vigorous growth. Most importantly, an optimum LP combination was determined by changing LP from 6 cm (green stage) to 3 cm (red stage) under continuous BHLI and resulted in 20.13 0.21 g/L of biomass concentration and 18.32 mg/L/d of astaxanthin yield. Furthermore, the biomass concentration was further increased to 27.34 0.58 g/L by transferring 15 days of the green vegetative cells in fed-batch culture on the basis of the optimal LP combination. The time-resolved (two step cultures: green stage (nitrogen sufficient) and red stage (nitrogen free) comparative transcriptome was analyzed. The astaxanthin biosynthesis pathway and the central carbon metabolism were reconstructed. The synthesis of IPP in JNU-35 mainly through the MEP pathway, some of the branch path in astaxanthin biosynthesis pathway was significantly down regulation. During the rapid accumulation stage of astaxanthin, glycolysis and starch degradation metabolism were up-regulation. Through the TCA cycle metabolic pivot, it combined the energy metabolic pathways of pentose phosphate and oxidative phosphorylation to produce more energy, which drove Calvin cycle for enhancing carbon fixation. Meanwhile, the operation of cycle electronic flow was strengthen for maintaining high photosynthetic performance, and promoting the growth of *Haematococcus pluvialis*.

**Mokhtar, Noor Azlin**

*King Abdullah University of Science and Technology*

### **Generation of a Red Sea Industrial Microalgal Strain Library**

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The climate, geography and industrial infrastructure of Saudi Arabia make it one of the most promising global locations for algal biotechnology: its desert climate, cloudless skies and intense sunlight (1559 - 2200 photons.s<sup>-1</sup>.m<sup>-2</sup>) combined with the low-lying 1,800 km Gulf of Aqaba-Red Sea and 840 km Persian Gulf coastlines that provide land and access to marine waters (Red Sea 36.0 - 41.0 PSU) which can be used as a culture medium for halotolerant strains, as well as feedstock CO<sub>2</sub> from 23 massive coastal industrial complexes (emitting >400 million tons p.a. CO<sub>2</sub>) provide unparalleled support for algal technology. To capitalize on this potential, however, the production strains used must tolerate high light, temperature and salinity. Unfortunately, of those model and commercial microalgae/cyanobacterial strains available, none are suitable for applications in Saudi Arabia. To address this problem, we carried out a multi-year bioprospecting survey across 105 Red Sea locations applying a high-throughput isolation pipeline that targeted physiologically robust microalgae and cyanobacteria that would be suitable for industrial applications in Saudi Arabia. Using chemically defined marine media under a range of temperature, salinity and light regimes, we generated high density biofilms from which unialgal cultures were generated to create a biobank of robust marine photoautotrophs. Isolate strains were subjected to quantitative physiological profiling to provide a baseline from which to select promising strains that have been further characterized by light and transmission electron microscopy, 16S/18S rRNA phylogenetic and full genomic sequence determination. From 1197 biofilm cultures 51 unialgal strains including members of Ulvophyceae, Porphyridiophyceae, Synechococcaceae, Cyanobacteriaceae, Chroococcaceae and Coleofasciculaceae with process-friendly characteristics have been identified. This strain library provides the fundamental basis for the development of product-specific bioproduction strains for sustainable industrial application in Saudi Arabia.

**Mills, Bryan*****AkzoNobel*****Chelated Micronutrients for Algae***Bryan Mills<sup>1</sup>, Mark Kean<sup>2</sup> and Marcel Bugter<sup>2</sup>*

Chelating agents such as EDTA are important solubilizing agents for essential metal ions like Fe used in starter nutrient broths as well as to maintain metal ion levels during growth. Changes in pH and algal harvesting can deplete these essential metal ions. While there are many forms of EDTA the use of highly soluble salts of EDTA allow easier preparation of these chelated metal nutrient salts while high quality EDTA is critical for production of food and food additives. An improvement over reaction of EDTA salts with metal salts is use of pre-chelated nutrients such as [EDTA-Fe] or [EDTA-Mn]. Pre-chelated [EDTA “ metal] complexes not only provide convenience for preparation of nutrient solutions, but also ensure exact stoichiometric match between the EDTA and metal ions. Additionally these pre-chelated products are highly pure, have high solubility allowing high % metal concentrations and are completely compatible with other nutrients such as phosphates. A further improvement in the delivery and maintenance of metal nutrients particularly Fe during algal growth and harvesting is use of HBED (Bis(2-hydroxybenzyl)ethylenediamine diacetic acid, ferric potassium complex). HBED not only provide the benefits of a pre-chelated nutrient but also maintain their solubility at high pH and during algal harvesting. Fe levels therefore experience less fluctuation and overall less Fe needs to be added to maintain proper growth conditions. In addition HBED is less susceptible to photo degradation, which allows it to maintain its stability in chelated iron under UV light for extended periods of time.

**Goemann, Hannah**

***Montana State University***

**Impact of a Cyanobacterial Biofertilizer on Switchgrass Production and the Soil Microbiome.**

*Hannah Goemann, Dr. Brent Peyton, Dr. Paul Stoy*

As global agricultural demands are predicted to increase exponentially in coming years, there is an expected concurrent demand for increased sustainability in agricultural practice and production. Chemical fertilizer production for agriculture is an extremely energy intensive process and is among the greatest sources of greenhouse gas emissions. As such, innovative, alternative fertilization practices are needed to reduce environmental impacts while maintaining agricultural productivity. Biological soil crusts comprised of nitrogen-fixing cyanobacteria have been shown to increase nutrient availability for cropped plants, and improve soil water holding capacity, while also reducing erosion and nutrient runoff. These effects have been shown in recent greenhouse and arid, uncultivated land studies but little is known regarding the viability or productivity of exogenous cyanobacterial biofertilizers in agricultural field settings. In addition, the impact of a living biofertilizer on the resident soil microbial community is relatively unstudied yet vital to the sustainable management of soil microbial ecosystems. Therefore, this study aims to bridge this knowledge gap by coupling biofertilizer treatment to switchgrass crop production and monitoring the soil microbial community through metagenomic analysis and changes in soil physicochemical characteristics.

**Cicha, Calvin**

***Montana State University***

**Genome Annotation of Coal Bed Methane Production Water Isolate PW95**

*Calvin Cicha: Montana State University Luisa Corredor Arias: Montana State University Matthew Fields\*: Montana State University*

Algal isolate PW95 belonging to the family Chlorococcaceae has recently been isolated from coal bed methane (CBM) production waters of the Powder River Basin in Montana. This native alga has shown potential in remediation of CBM waste water and produces high levels of lipid (27%) in CBM water amended with nitrate. Currently, this organisms genome is unannotated and poorly understood. Therefore, it is of interest to annotate the PW95 genome to better understand the genetics behind these desirable phenotypes. Herein, we used the ab initio prediction software MAKER to annotate PW95 genomic assemblies. Optimized annotations were then blasted against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to confirm predicted genes and provide insight into the metabolic capabilities of algal isolate PW95. This research will provide valuable data that can be used to optimize this algae for CBM waste water remediation and biofuel production.

**A. Badr, Amal**

***The American University in Cairo***

**Evaluating Culturing Conditions on Freshwater Microalgae Diversity Collected from the River Nile.**

*Amal A. Badr, Walid Fouad The American University in Cairo*

Microalgal biomass enhancement on a lab scale is essential for the initiation of any microalgal based research. Microalgal biomass can be utilized in biodiesel, feedstock and food production. Maintaining algal diversity obtained from an environmental sample at the lab scale is impacted by various factors including but not limited to the nutrient, carbon dioxide enrichment and light intensity. The aim of the study is to examine factors that impact growth and microalgae diversity from a freshwater source. Two different samples were collected from the River Nile at the same location within the Cairo governorate. In this study, we investigated the effect of media composition and concentration, along with aeration on the microalgae diversity and growth rate. The cell density and microalgae diversity was analysed for each treatment every four days using a haemocytometer. Four different types of media varying in nitrogen, potassium and calcium compositions that ranged from low, moderate to high was used. Across, the different culturing conditions a total of 36 species was morphologically identified. Most of the strains observed were green microalgae and cyanobacteria. The predominant species observed in all treatments were *Scenedesmus* sp. and *Chlorella* sp., together they present 33-80% of the population. In exception, high nitrogen, low potassium (media 1) supported the growth of *Merismopedia* sp. and the media containing high nitrogen and high potassium enhanced the growth of *Botryococcus* sp. with both species representing 50% of the population. The highest cell density was observed in the media containing moderate nitrogen, potassium and calcium (media 3), followed by media 1. Molecular identification of the diversity using 16S and 18S rRNA will also be presented. These data indicate the significance of optimizing culturing conditions for environmental samples for the purpose of maintaining and isolating maximum diversity of microalgae species.

**Loftus, Sarah*****Duke University*****Effect of Cultivation Water Reuse on the Accumulation of Dissolved Compounds and Algae Growth***Sarah E. Loftus, Duke University Marine Lab Zackary I. Johnson, Duke University Marine Lab*

Reusing growth medium (i.e. water) for microalgae cultivation is required for economical and environmentally sustainable production of algae-based fuels and other products. However, recycled medium contains microbes and dissolved algal-based compounds that may affect algae growth. While other studies have measured the accumulation of dissolved organic carbon (DOC) in recycled medium, it is unclear whether the DOC concentration in recycled medium correlates with algae growth response, and whether accumulated DOC in recycled medium affects subsequent algal DOC excretion. To address these unknowns, lab-scale experiments were conducted with three marine algae strains, two diatoms and one green alga, grown in medium reused up to four times. Recycled medium was created by harvesting algae via vacuum filtration (0.45  $\mu$ m) and replenishing nutrients. DOC was produced and accumulated at approximately the same rates across each reuse, indicating that the accumulated DOC did not suppress DOC excretion. However, one algae strain, a diatom, became completely inhibited in recycled medium by the second reuse. This diatom had the lowest accumulated DOC concentration, indicating that bulk DOC concentration alone cannot predict algae growth response. Additionally, recycled media alone supported bacteria growth but DOC concentrations did not decrease, indicating that DOC in the recycled medium was mostly refractory to the associated microbial community. Overall, these results suggest that strain-specific compounds may be responsible for algae growth response in recycled medium, and that the concentration of recalcitrant DOC will linearly increase with each medium reuse.

**Renuka, Nirmal**

***Institute for Water and Wastewater Technology, Durban University of Technology, Durban, South Africa***

**Collective Effect of Phytohormones on Biomass and Lipid Production in *Acutodesmus obliquus* Under Nitrogen Limitation for Biofuel Application**

*Nirmal Renuka, Abhishek Guldhe, Poonam Singh, Faizal Bux Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban, 4000, South Africa. Tel: +27 31 373 2346, Fax +27 31 373 2777*

Nitrogen-limitation is considered as the most feasible strategy for lipid enhancement in microalgae, although it leads to significant reduction in biomass production, which affects overall lipid productivity. The use of phytohormones is an emerging trend for biomass and lipid enhancement under standard and nitrogen-limited conditions for microalgal biofuel production. Present study was conducted to evaluate the combined effect of selected phytohormones viz. cytokinin - zeatin (Z), auxin - indole acetic acid (IAA), and gibberellin - gibberellic acid (GA) to optimize biomass and lipid production in microalga *Acutodesmus obliquus* under nitrogen-limitation (ON) using response surface methodology (RSM). Supplementation of combination of Z (0.5 mg L<sup>-1</sup>), IAA (1.0 mg L<sup>-1</sup>), and GA (5.0 mg L<sup>-1</sup>) under nitrogen-limitation significantly increased the biomass and lipid productivity by 49.07% and 77.20% respectively as compared to optimized nitrogen control (ON). Zeatin was found to be the most significantly influencing phytohormone followed by GA in RSM data analysis. The present study demonstrated the effect of combination of phytohormones for enhancing lipid productivity under N limited conditions with suitable lipid profile for biofuel applications. Developed strategy increased carbohydrate and protein productivity by 18.89% and 56.02% respectively as compared to ON control. Exogenous phytohormones also improved the photosynthetic activity of *A. obliquus*. The synergistic effect of exogenous phytohormones on biomass and lipid enhancement in microalgae under N-limitation showed its efficacy for the development of scalable strategy using phytohormones for microalgal biofuel production.

**Mahan, Krissy**

***Sandia National Laboratories***

**PonDx: A Novel Method for Rapid and Sensitive Detection of Deleterious Species in Microalgae Cultivation**

*Kristina M. Mahan, Chung-Yan Koh, Laura T. Carney, Pamela D. Lane, and Todd W. Lane Systems Biology, Sandia National Labs, PO Box 969, MS 9671, Livermore, CA 94551, USA*

The primary challenge facing commercial algae cultivation is cost effective crop protection and maintenance of high productivity in large-scale algae ponds. Since algae deleterious species are very diverse, multiple approaches have to be developed for detection and treatment. Current detection techniques including qPCR are expensive, requires laboratory to prepare samples, and requires specialized expertise. PonDx provides a simple, low cost option for all algal researchers and companies to quantify the algae pond microbiota. This pond-side diagnostic system is capable of detecting major species of algal predators, such as rotifers, at concentrations of one individual per 10 mL of culture. This detection limit is about 1000-fold better than that for microscopy, and detects biocontaminants prior to being detectable by microscopy. The PonDx platform detects biocontaminants using a device that rotates disposable PonDx discs for separation from matrix components. Beads functionalized with a fluorescent oligonucleotide probe specific for the target analyte bound to a quencher are mixed with nucleic acids extracted from lysed samples of interest, heated and allowed to anneal. This mixture is layered on top of a medium less dense than the beads, but denser than the sample on average. In the presence of the target sequence, the quencher is displaced. When the disc is spun the beads pellet at the bottom of the channel, while other components remain suspended above the density medium. The labeled oligonucleotide probe can then be detected in proportion to the concentration of the target molecule in the sample by fluorescence. As a proof of concept, this bench-top PonDx system was tested with whole organism lysates derived from several known algal predators including rotifers and ciliates. All tests were performed within a background of *Microchloropsis salina* lysate in order to simulate a dense algal culture. Probes were used against the eukaryotic small subunit ribosomal RNA (SSU rRNA) gene for the predator organisms. The total sample-to-answer time for our system is less than 30 minutes. Using the PonDx disk and hybridization assay, lysed rotifers, ciliates and other known algal predators were successfully detected within a dense algal lysate at densities relevant for early detection.

**Browne, Daniel**

***Texas A&M University***

**Systems Analyses of Metabolism and Physiology in the Oil-producing Green Alga *Botryococcus braunii***

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The green microalga *Botryococcus braunii* is well known for its ability to produce petroleum-like liquid hydrocarbons. Geochemical studies have identified fossil remains of the species in petroleum source rocks dating as far back as the Precambrian. Thus *B. braunii* is an established source of petroleum throughout the geological ages and presents an interesting model to study hydrocarbon metabolism. To better understand the biochemical and genetic systems that underpin the properties of *B. braunii*, we first sequenced and analyzed its genome. Using comparative genomics, we identified gene functions that are unique in *B. braunii* among the Viridiplantae. These include parts of the photosynthetic apparatus, the ubiquitin system, the cytoskeleton, cytochrome P450s, peptidases, and more. To further understand the active interpretation of the genome, we sequenced the transcriptome of *B. braunii* every six hours over the course of three days. The goal of this experiment was to determine the gene expression patterns associated with light/dark transitions. Interestingly, we found several coexpression modules that cycle with time of day, in addition to modules that cycle with light/dark conditions. To determine the impact of light/dark and time of day on metabolism, we generated metabolomics data for each of the samples utilized in the transcriptome analysis. Targeted and untargeted analyses of polar and nonpolar metabolites revealed that unlike transcription, the metabolome does not cycle with time of day, nor with light/dark conditions. These data add great value to our fundamental understanding of holistic *B. braunii* metabolism and physiology.

**Khandual, Sanghamitra****CIATEJ****Efficient Extraction and Conversion Process of Phycocyanin to Get end Products from *Spirulina platensis* Collected from Mexico.**

*Sanghamitra Khandual<sup>1</sup>, Lorena Amaya Delgado<sup>1</sup>, Rosa Maria Camacho<sup>1</sup>, Maria Dolores Garcia Para<sup>1</sup>. El Centro de Investigaci3n y Asistencia en Tecnolog3a y Dise±o del Estado de Jalisco, A.C. (CIATEJ).*

Phycocyanin is an important blue natural food colorant which can be used again as functional food ingredient for several health benefits. In Mexico there are very few reports on native spirulina species and their bioactive compounds. Herein we compared various phycocyanin extraction methods from *Arthrospira platensis* collected from Texoco, Mexico with dry biomass. Two different solvents i.e. distilled water, phosphate buffer, at 3 different pH (6, 7, and 8) with two different times of agitation (1hr and 24 hr) were applied for the extraction process. Process variables of Freeze-thaw, ultrasonic and sonication extraction methods were optimized as experimental design to enrich phycocyanin in the extraction solution. The highest amount of phycocyanin was found 38.5mg/g which was extracted from dry biomass using water at neutral pH. The optimum conditions of extraction methods determined at 1:50 % biomass/solvent ratio for dry biomass, with freeze thaw method for 20 min alternatively for 2 times and then agitated with 120rpm for 24 hours. The phycocyanin content was 38.5mg/g biomass with a purity 0.48. The other similar methods of extraction like sonication and ultrasonic method can be recommended for scalable phycocyanin production as there is no difference statistically neither on quantity of production nor purity of phycocyanin as it was found 34-36.5 mg/g biomass with purity. But freeze thaw method looks attractive for large scale phycocyanin extraction without much energy use and easy handling. Again freeze thaw method shown relatively better purity. So to convert phycocyanin as a functional food ingredient we are precipitating with ammonium sulphate to get more purity like 0.7-2 which can be a food grade product and evaluating microencapsulation of this extract with gelatin and maltodextrin with gum arabic to give more stability and control release for functional food and nutraceutical purpose. These studies are under process.

**Koh, Hyun Gi**

*Korea Advanced Institute of Science and Technology*

**Increasing Photosynthetic Efficiency in *Nannochloropsis salina* by Introducing a New Light Harvesting Pigment**

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Photosynthesis is one of the most fundamental process in microalgae that enables the conversion of light energy into chemical energies<sup>1</sup>. Increased photosynthetic efficiency, which is highly dependent on the light harvesting complex such as antenna size or pigment compositions, can therefore directly enhance the overall productivity of both biomass and lipids. In this research, we have targeted the complex plastid of *N. salina* for the heterologous expression of chlorophyllide a oxygenase (CAO) from *Chlamydomonas reinhardtii*<sup>2</sup>, which resulted in the synthesis of chlorophyll (Chl) b for the first time in a Chl b-lacking eukaryote in the nature. The introduction of Chl b in *N. salina* resulted in not only the absorption of Chl b specific spectrums, but also in accumulation of light harvesting complex binding (Lhcb) proteins and increased light harvesting efficiencies, demonstrated by enhanced maximum electron transfer rate by 7.5 ~ 15.8 % and quantum yields by 3 ~ 17 %. This increment has led to enhanced growth rate and lipid synthesis under both high light and moderate lights, but the effects were larger under lower light conditions. These results suggest heterologous expression of CAO in Chl b-lacking microalgae can be applied for the industrial production of biomaterials.

**Dwivedi, Vinay*****Reliance Industries Limited*****Optimizing Growth Parameters of *Chlorella sorokiniana* under Controlled Conditions**

*Vinay Dwivedi, Chandra Shekharaiah PS, Nishant Saxena, Debanjan Sanyal\*, and Santanu Dasgupta  
Reliance Industries Limited*

*Chlorella sorokiniana* is a microalgae and in nature it is found to grow both in fresh as well as sea water environment. This microalgae has been widely used as a model system to study enzymes involved in higher plant metabolism and also considered as one of the super food because of its high nutritional content. *C. sorokiniana* is a fast growing robust strain and could be used as an alternative feed stocks for production of biofuels. To optimize the cultivation of *C. sorokiniana* with minimum operational expenses, impact of agricultural grade, industrial grade and lab grade nutrient sources was evaluated. The culture was found to grow equally good in all types of N sources without any growth inhibition, and hence the least expensive nutrient source can be utilized without any compromise on productivity. Hypochlorite, H<sub>2</sub>O<sub>2</sub> and lizol can be used to control the major grazers in open pond algae cultivation. Minimum inhibitory concentration (MIC) of hypochlorite, H<sub>2</sub>O<sub>2</sub> and lizol were studied on *C. sorokiniana*. MIC of H<sub>2</sub>O<sub>2</sub>, lizol & hypochlorite were found to be 5 ppm & 2.5 respectively. These concentrations were successfully used to kill grazers in open pond cultivation. Attempt was also made to determine the effect of light duration on biomass productivity of *C. sorokiniana*. In this study 12/12 light: dark cycle and 24hours continuous light illumination at 250  $\mu$ L of light intensity were studied. The growth results at 12:12 light: dark cycles was statistically at par with 24 hours continuous light illumination. This shows that the same productivity can be achieved in less illumination time (12:12 light: dark) as compared to continuous illumination of 24h. The overall study helps in designing a low opex cultivation package for *C. sorokiniana* without compromising productivity. Also, the tolerance level to multiple biocides, helps to define biocide dosage for the cultivation package.

**Avila, Nickolas**

***Montana State University***

**Thermophilic, High Alkalinity Microalgal Enrichment from a Yellowstone National Park Hot Spring Outflow Cyanobacterial Mat**

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Yellowstone National Park (YNP) has long been a hotspot for biotechnology innovations. This raises interest for the enrichment of microalgae that naturally occur in YNP. In this study, samples were taken from a cyanobacterial mat from the outflow of a hot spring (Five Sisters 5) in the Midway Geyser Basin. The water temperature and pH of the hot spring were 48.5°C and 7.6, respectively. Enrichments for phototrophic microbes were set up at high and low alkalinity (100 mM and 0 mM HCO<sub>3</sub><sup>-</sup>) and high and low temperature (40°C and 22°C). The culturing media contained no bioavailable nitrogen to select for nitrogen-fixing microorganisms. Cultures appeared to grow stably after several enrichment steps, and microscopic examination revealed a unique, approximately 5 μm long, 1 μm diameter rod-shaped cell morphology. 16S rRNA gene analysis from the enrichment at 40°C and 100 mM HCO<sub>3</sub><sup>-</sup> indicated a single organism in the enrichment, which appears to belong to the genus *Synechococcus*. The organism appears to be phylogenetically most similar to organisms that have been isolated previously from other hot spring systems. Physiological characterizations of this and other isolates are currently underway.

Jackson, Matthew

*Montana State University*

### **Bicarbonate Amendment at Nitrogen Depletion for Enhanced Lipid Accumulation Under Different Nitrogen and Carbon Regimes**

*Matthew Jackson, Center for Biofilm Engineering and Department of Chemical and Biological Engineering, Montana State University; Robin Gerlach, Center for Biofilm Engineering and Department of Chemical and Biological Engineering, Montana State University*

Biodiesel from algae can reduce global dependence on fossil fuels, however production is currently limited for a variety of reasons including nutrient and water limitations, as well as high costs. One method of reducing costs is improving lipid production in algal cultures. The application of a timely bicarbonate amendment prior to nitrogen depletion for enhanced lipid accumulation has been demonstrated for several algal species. To understand how different culturing conditions impact the effectiveness of this amendment, two *Chlorella* species, *C. vulgaris* strain UTEX 395 and *C. sorokiniana* strain SLA-04, were cultured using a variety of nitrogen and carbon regimes. As cultures approached nitrogen depletion a 50mM NaHCO<sub>3</sub> amendment was applied to half of the replicates of each condition. Both organisms were cultured using four different nitrogen regimes (nitrate, ammonium, urea, and a combination of the three) in Bold™s Basal Medium under standard media conditions and at increased alkalinity. In addition, both organisms were cultured under mixotrophic conditions to evaluate the effect of organic carbon availability. An increase in lipid accumulation was observed for all nitrogen conditions that received a bicarbonate amendment. The byproducts from nitrogen utilization had varying effects on the growth medium pH. The generation of hydroxyl ions during nitrate assimilation increased culture alkalinity, potentially improving the mass transfer of CO<sub>2</sub> into solution. This increased the available inorganic carbon pool and as a result allowed for the generation of more biomass and possibly additional storage molecules, such as lipids. In contrast, the generation of protons during ammonium assimilation decreased culture pH, lowering the amount of inorganic carbon available. As a result, growth rates in the ammonium-amended treatments might have been limited, however increased lipid accumulation after bicarbonate amendment at nitrogen depletion was still observed. The effects of the bicarbonate amendment in the presence of organic carbon were less consistent between the different organisms. The findings from this research serve as a preliminary guide for predicting the applicability of a bicarbonate amendment prior to nitrogen depletion for different types of growth media and under different environmental conditions.

**Guymon, Nathan**

***Utah State University***

**Effects of Photoperiods on the Performance of an Algal Biofilm Reactor for Wastewater Remediation & Biomass Production**

*Nathan Guymon - Utah State University Dr. Ronald Sims - Utah State University*

Algae-based treatment processes have been investigated for use in municipal, dairy, petroleum, fracking, and other types of wastewater. Conventional suspended-growth methods have many limitations including the cost to separate algae from water, the large area required, and limited light availability as the depth of media increases. The Rotating Algal Biofilm Reactor (RABR) directly addresses these limitations by operating as a semi-submerged bioreactor with a rotating substrate on which an algal biofilm culture develops. A primary challenge of using a Rotating Algal Biofilm Reactor to remediate wastewater in different geographical locations is changes in light intensity and availability. Little information currently exists in the literature about the effect of seasonal photoperiods on biofilm formation and productivity in RABR technology. The objective of this experiment was to first design and build rotating algal biofilm reactors (RABRs) at a bench scale. The RABRs were then inoculated them with a mixed culture of algae and cyanobacteria sourced from local municipal wastewater treatment plants. These were then used to investigate the effect of photoperiods on biomass yield, productivity, and resultant wastewater treatment. The microalgae and cyanobacteria species were also identified to find those that grow best under different photoperiods. The initial results indicate significant differences in algal productivity with an increase in light from 10 to 16 hours of light and 13 to 16 hours of light. The information obtained from the experiment will aid in the future design of RABRs used in wastewater remediation.

**Eustance, Everett**

***Swette Center for Environmental Biotechnology***

**Enhanced CO<sub>2</sub> delivery via membrane carbonation for sustained *Scenedesmus* cultivation with various CO<sub>2</sub> concentrations**

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Microalgal-derived biofuels are promising renewable fuels, but the development encounters several challenges, including the efficient use of nutrients to minimize cultivation costs. Membrane carbonation (MC) has been proved as a great alternative for delivering CO<sub>2</sub> without bubble formation and captures nearly 100% of the CO<sub>2</sub> when supplying pure CO<sub>2</sub>. However, most industrial CO<sub>2</sub> sources are composite gas mixtures with a CO<sub>2</sub> content ranging 10% to 90%. Directly delivering mixed CO<sub>2</sub> is a desirable option. However, the compatibility of MC with various CO<sub>2</sub> compositions has not been investigated. The goal of the studies was to grow *Scenedesmus* using a wide range of CO<sub>2</sub> concentrations from 10% to 100% CO<sub>2</sub> via MC. Although CO<sub>2</sub> fluxes significantly declined (up to two orders of magnitude lower) when supplying 10% CO<sub>2</sub> at the same pressure as 100% CO<sub>2</sub> supply, adjusting the MC operating conditions provided similar biomass productivities to sparging with pure CO<sub>2</sub>. The greatest advantage of MC was the increased CO<sub>2</sub> capture efficiency, which was at least 3 times higher than for sparging. This evaluation demonstrates that MC is highly promising to work with various CO<sub>2</sub> streams from industrial resources.

Alvarez De la Hoz, Adriana

*University of Minnesota*

### **Effect of microalgal biomass on soil nutrient and microbial dynamics under simulated rain**

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Agriculture has global environmental impacts, including nutrient pollution and soil degradation, and microalgal biofertilizers are promising options in the urgent challenge of sustainable agriculture. Croplands are naturally exposed to rain and more extreme rainfall is projected to occur with climate change. This study describes the effects of the nitrogen-fixing cyanobacteria *Anabaena cylindrica* on soil nutrient and microbial dynamics under high intensity rain. Fresh algal biomass was applied to the surface of an arable soil on 6-in pots at 70 lb N acre<sup>-1</sup>. Inoculated and non-inoculated pots (controls) were maintained in a growth chamber in a 14:10 light-dark cycle. Pots were incubated for 2 weeks and then subjected to rain for 40 min at 2.0-2.5 in hr<sup>-1</sup> twice in one week, followed by one rain a week for 4 weeks, for a total of 6 rains. Soil samples from the surface, middle and bottom of the pots were taken 1 day after inoculation, 2 weeks after inoculation (before the 1st rain), after the 1st rain, and after 6 rains. Soil was analyzed for relative algal abundance (chlorophyll a), nutrients, microbial biomass carbon (MBC) and nitrogen (MBN), and microbial activity (fluorescein diacetate hydrolysis). Runoff and leaching water samples were analyzed for nutrient washout. Inoculation improved soil fertility, significantly increasing nutrient levels and microbial dynamics, mainly on surface soil. One day after inoculation, most of the algal biomass (81%) was on the surface, and soluble organic carbon (SolC) and soluble nitrogen (SolN) increased. Soil chlorophyll a decreased throughout the experiment. After 2 weeks, surface soil had higher nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>), indicating a rapid mineralization of the algal biomass. This was coupled to higher SolC, SolN and microbial activity. After the 1st rain, the primary NO<sub>3</sub><sup>-</sup> loss occurred and a third of surface NO<sub>3</sub><sup>-</sup> still remained. After 6 rains, surface soil had significantly increased NH<sub>4</sub><sup>+</sup>, available phosphorus, microbial activity, MBC and MBN. This study demonstrates that inoculations with *A. cylindrica* had positive effects on soil nutrient dynamics, nitrogen (N) cycling, microbial pools, and carbon and N microbial fluxes that persisted after high intensity rain. These results add value to microalgal biofertilizers for preserving and improving the soil function in the search of a sustainable agriculture.

**Hanifzadeh, Matin***University of Toledo***Production of lipid and carbohydrate from microalgae without compromising biomass productivities: Role of Ca and Mg***Matin Hanifzadeh, Elena Cerdan Garcia, Sridhar Viamajala*

We report the cultivation of *Chlorella sorokiniana* str. SLA-04 in media containing trace amounts of Ca and Mg. The differences in productivities of biomass, lipids and carbohydrates were assessed relative to cultures grown in standard media (BG-11) that contain approximately 8 $\mu$ M— higher concentration of Ca and 30 $\mu$ M— higher concentration of Mg. Culture performance in N-limited standard media was also investigated. In addition to growth and accumulation of storage products (lipid and carbohydrate), we measured the utilization of N, Ca and Mg and monitored changes in cell size and photosynthetic activity. Our results showed that limitation of Ca or Mg did not inhibit cell replication and culture growth. On the contrary, Ca-limited (CaL) limited cultures had ~30% higher biomass productivity relative to the control with excessive nutrients possibly due to improvement in cell wall flexibility and cell division. We also observed that CaL and Mg-limited (MgL) cultures had nearly 3-fold higher lipid concentration (measured as fatty acid methyl ester) and 50% higher carbohydrate concentration than the nutrient excess control cultures. Simultaneous culture growth and lipid accumulation in CaL and MgL cultures suggest that de novo synthesis was the primary mechanism for lipid accumulation in Ca/Mg-limited media. Overall, our study demonstrates that micronutrient optimization, in addition to optimization of macronutrients, could significantly improve microalgal biorefinery yields.

Kumar, Roshan

*Central University of Punjab*

### **Lipid Production and Molecular Dynamics Simulation for Regulation of Acc Gene(s) in Cyanobacteria Under Different N and P Stress Condition**

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Bio-energy production has in recent past become a topic of intense interest due to increased concern over limited petroleum-based fuels supplies and the contribution of their use to atmospheric CO<sub>2</sub> levels. It is further envisaged that with development of new growing economies, like India and China, the global energy consumption will rise leading to more environmental damage. Above all, continued use of petroleum based fuels and limited supplies has raised a question on its sustainability in near future. Total lipids in selected cyanobacteria viz. *Oscillatoria* sp. (SP8), *Anabaena* sp. (SP12), *Anabaena* sp. (SP13), *Microcoleus* sp. (SP18) and *Nostoc* sp. (SP20) showed gradual increase with maximum accumulation at 35 days of incubation. The isolates varied in their ability to accumulate lipids which ranged from a lowest of 0.13 percent in *Anabaena* sp. (SP13) to the maximum of 7.24 percent in *Microcoleus* sp. (SP18). *Microcoleus* sp. (SP18) also recorded highest lipid accumulation with 270% and 153% increase over control at both N (6mM NaNO<sub>3</sub>) and P (0.20 mM K<sub>2</sub>HPO<sub>4</sub>) limiting conditions. The ACC carboxylase (acc D) gene amplification and sequencing from *Oscillatoria* sp. (SP8) and *Microcoleus* sp. (SP18) showed similarity with representatives of same genera i.e. *Oscillatoria acuminata* PCC 6304 and *Microcoleus* sp. PCC 7113 respectively. The overall expression of acc D was found to be upregulated in both the cultures for all limited nitrogen concentrations but was differentially regulated with both positive and negative induction under limited phosphorus stress conditions. Maximum induction was observed in *Microcoleus* sp. (SP 18) at 0.20 mM K<sub>2</sub>HPO<sub>4</sub>. The obtained 3D structure of SP8 protein (21.8 kDa) showed 6 alpha helices, while SP18 protein (16.7 kDa) exhibited 4 alpha helices and 4 beta sheets. There was no beta sheet present in the 3D structure of the SP8 protein. The instability index of SP 18 was 30.47 compared to 37.21 for SP 8 but the phi ( $\phi$ )/psi ( $\psi$ ) angles of the amino acid residues observed in Ramachandran plot analysis showed that both proteins, SP8 and SP18 were highly stable and having more than 90 % amino acids in allowed regions. The ZDOCK score obtained was 1324.398 for SP8-1C2Q and 1217.939 for SP18-1C2Q. Further molecular dynamics simulation of SP8 was performed and the results indicated the stability of ligand-bound protein complexes during MD simula

**Yelagiri Munisamy, Purushothaman**

***Proteogen Biosciences (India) Pvt. Ltd***

**Genetic and Metabolic Engineering of Microalgae for Enhanced Production of Bio-diesel: An Alternative to Sustainable Energy Source.**

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In recent times, microalgae have gained attention due to the depletion of non-renewable fossil fuel. Biofuel produced from microalgae has benefit to reduce 78% emission of carbon dioxide, 98% decline in sulfur emissions and 50% decline of particulate matter after combustion (Brown and Zeiler, 1993). Biomass produced from microalgae has excellent prospects to convert into biofuel due to the low emission of CO<sub>2</sub> compared to other biomass sources. Microalgae grow rapidly and generally tolerate a wide range of environments. The major disadvantage of microalgae for biofuel production is the low biomass concentration in the microalgal culture due to the light penetration limit, and also insufficient oil contents of microalgae cells. In addition to this, the small size of algal cells makes the harvest of algal biomasses relatively costly. The most efficient strategy suggested for enhancing biofuel production from microalgae seems to be the genetic engineering of metabolic pathways associated with fatty acids production. Genetic and metabolic engineering of particular microalgal sp. are likely being an economical choice for biofuel production, because of its availability and low cost. Additionally, the role that genetic engineering may play to enhance algal lipid and biofuels production and increase its competitiveness through a biorefinery approach. Advancements in the omics technologies are generating information to allow design and creation of super algal strains for producing biofuels. The application of these modern metabolic engineering tools in photosynthetic microalgae has the potential to create important sources of renewable fuel that will not compete with food production or require fresh water and arable land. There is scope for both small-scale and large-scale algal farming, which the algae are a fundamental source of energy, nutrition products and ecological services for sustainable societies globally. Keywords: Biodiesel, Microalgae, Metabolic Engineering, Biomass.

**Stephen, Dayana Prihadharshini**

***National Institute of Technology- Tiruchirappalli***

**Strain Improvement of *Chlorella pyrenoidosa* for Phenol Biodegradation by Adaptive Laboratory Evolution**

*Dayana Priyadharshini Stephen, Bakthavatsalam kannappan Ayalur National Institute of Technology- Tiruchirappalli*

Cultivation of microalgae in wastewater is a potential method for the treatment of wastewater as well as biomass production. Phenol is one of major environmental contaminant present in water. Microalgae normally have a low tolerance for and a low degradation rate to, high concentration of phenol. Adaptive Laboratory Evolution (ALE) was performed for the treatment of phenol by *Chlorella pyrenoidosa*. The resulting strains provide appropriate variants for selection by the stress involved, thus accelerating evolution. The improved strain was obtained after 10 cycles (about 70days) under 200mg/L to 1600mg/L of phenol as environmental stress. It could grow under local ambient conditions up to 800 mg/L of phenol without significant inhibition. The maximal biomass concentrations of the resulting strain at day 5 were 3.8g /L under 600mg/L phenol and 4.20g/L under 800mg/L phenol, respectively. Increase in rate of growth of *C. pyrenoidosa* noticed with increase in phenol concentration up to 800mg/L. They were more than two times of those of the original strain. In addition, 800mg/L phenol was fully removed by the resulting strain in 5 d when the initial cell density was 1g/L.

Sandoval Salazar, Ivan Alberto

*Servicio Nacional de Aprendizaje-SENA Nodo Pitalito*

**Metabolic Manipulation as a Strategy for Optimization of Biomass Productivity and Oil Content in the Microalgae *Desmodesmus* sp.**

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*Silvia Fernanda Valderrama Centro de Gestión y Desarrollo Sostenible Surcolombiano-SENA Nodo Pitalito*

The microalgae oil is emerging as a promising source of raw materials in the long term for the biodiesel production. This study had as objective cultivate a species of *Desmodesmus* sp. microalgae in laboratory scale with the purpose of maximize the biomass production and the triglycerides content in the lipid fraction. Initially, the culture conditions were selected to optimize the biomass production which later was submitted to nutritional stress varying the concentrations of nitrate and phosphate to increase the content and the productivity of fatty acids. In the first stage the medium selected was Bold 3N. In this step the nitrate and the phosphate consumption was evaluated to determine the time required for the beginning of the second phase of culture. In the second phase the culture was carried out at 23 C through the variation of the light intensity (250, 500 and 1000 mol photons.m<sup>2</sup>.s<sup>-1</sup>) photoperiod 12:12. In the best conditions of the tests a maximum cell division of 1,13 d.d<sup>-1</sup> was obtained in the 6th day of culture, at the beginning of the exponential phase and a maximum concentration of 8,42x10<sup>7</sup> cel.mL<sup>-1</sup> and dry biomass 3,49 g.L<sup>-1</sup> at 20 days in the stationary phase. The lipids concentration in the first culture stage was approximately 8% then of 12 days and at the end of the culture in the stationary phase varied from 12% to 16% (20 days). In the cultured microalga at 250 mol photons.m<sup>2</sup>.s<sup>-1</sup>, the fatty acids profile was mostly polyunsaturated (52%). The total of unsaturated fatty acids identified in this species of microalgae reached values between 70 and 75% being qualified for the use in the food and pharmaceutical industries. Further, this study showed that the growing conditions influenced mainly the PUFA production, in the majority the acid linolenic. However the saturated and monounsaturated fatty acids present a greater oxidative stability and were mostly identified (60 to 70%) in the cultures submitted to higher light intensity (1000 mol photons.m<sup>2</sup>.s<sup>-1</sup>) lower concentrations of nitrate and phosphate, which were qualified for the production of biodiesel and the oleo chemicals. This research highlights the potential of the microalgae as raw material for different industrial purposes.

**Collins, Lowell**

***Florida A&M University***

**Wastewater Remediation Roupled with Fuel Production from the Cultivation of Oleaginous Algal Micro-organisms Native to Tallahassee, FL**

*Lowell Collins Dr. Ashvini Chauhan Devin Alvarez Florida A&M University*

This study sought to identify novel species of algae native to the microbial ecology of wastewater in Tallahassee Florida for producing biofuels and materials from algal lipids. The study tests selected strains for neutral lipid production, growth rates and their ability to compete in the wastewater environment. The end goal is to produce neutral lipids for domestic fuel production and phytoremediation benefits to the water treatment process. In conventional algal cultivation, algal producers face barriers that include; a steady and cost-effective supply of nutrient, freshwater and an algal strain that endures the environmental pressures associated with open-air production methods. The application of algal sourced biofuel and the co-production of material is limited by the ability to produce algal biomass in sufficient commercial quantities. Industrial production of algal oil that would be competitive with the current petroleum oil model has not yet been achieved. This research isolated both eukaryotic algal strains and prokaryotic cyanobacterial strains from wastewater treatment plant holding tanks. The strains were characterized by growth rates, cellular pigment, and genetic sequencing as well as neutral lipid profiling. Following characterization, the strains selected for their ideal qualities were cultivated with water from two main aspects of the water reclamation process: raw influent and treated effluent. The strains were monitored during a ten-day trial to determine their ability to remediate nutrients and maintain a stable colony under open-air conditions and weather changes.

Ribeiro, Dagon

*Universidade de Bras lia/Embrapa*

**Final Evaluation of the Biomass in Prolonged Growth of Different Brazilian Microalgae of the Order Chlorococcales in Culture with Aeration and Shaker in a Medium Composed by Fertilizers**

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The production of biomass algae to supply the chains of bioeconomics, such as food and energy, is also linked to the use of great biodiversity present in own land. Chlorella is an important candidate for biomass production and accumulation of values components such as lipids, carbohydrates, proteins and pigments. Here we evaluated four microalgae isolated in different regions of Brazil, LBA 29 (Atlantic Forest), LBA 32 (Amazon), LBA 39 (Cerrado), and LBA 50 (Pantanal) and the quantification and characterization of fatty acid profile grown in fertilization medium BGUF. Cultures were carried out in duplicate in 250 ml Erlenmeyer flasks with a medium volume of 150 ml, and were maintained on a rotating orbital shaker and in duplicate in 500 ml Erlenmeyer flasks with a medium volume of 350 ml, were maintained on constant aeration with 6 Lh<sup>-1</sup> both with a photoperiod of 12h/12h with 40 lux at 25  C. After 30 days, the LBA 29 cultivated in constant aeration reach 1.9 g/L based on dry weight, followed by LBA 32 and LBA 50 with 1.7 g/L (dw) and LBA 39 with 1.2 g/L (dw) in cultures with constant aeration, in general the cultures maintained in shakes obtain less biomass with no statistic difference between the species varying 0.4 g/L to 0.7 g/L (dw), the higher amount of FA was found in the cultures with constant aeration, among the microalgae tested the best results was founded in LBA 29, 32 and 39, with in average 10% (w/w) of FAME accumulated. In conclusion, based on measurements of biomass production and oil accumulation, the LBA 29 growned in fertilization medium BGUF shows a promising choice for use in the production of algal biomass.

Ribeiro, Dagon

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### **Evaluation of Microalga *Chlorella sorokiniana* LBA 39 Isolated in the Brazilian Savanna (Cerrado) Cultivated in Different Culture Media**

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The characterisation of new strains of microalgae represents an important and continuous activity as part of the analysis of algal biodiversity in Brazil and search potential candidates for biotechnological applications. Here we evaluated the effects of different culture media on growth of *Chlorella sorokiniana* | LBA39, a strain isolated in the Brazilian Savanna (Cerrado) and identified by Hadi et al. (2016). Three media previously described in the literature were used; the Basal Bold medium described by Bischoff and Bold (1963), the BG 11 medium described by Stanier, Kunisawa, Mandel, & Cohen-Bazire, (1971) and the NitrU medium described by Dragone, Fernandes, Abreu, Vicente, & Teixeira (2011). Cultures were carried out in triplicate in 250 ml Erlenmeyer flasks with a medium volume of 150 ml, and were maintained on a rotating orbital shaker with a photoperiod of 16/8 with 40 lux at 25 ± 0.5 °C and 150 rpm. After 7 days growth *Chlorella sorokiniana* | LBA39 grown in NitrU medium produced more biomass with 583.3 mg/L based on dry weight, followed by BG11 with 400 mg/L (dw) and BBM with 166.67 mg/L (dw). This difference may be related to different amounts of nitrogen in the medium as NitrU contains more than double that of BG11, and ten times more than BBM. Algae grown in NitrU medium accumulated greater levels of fatty acids especially the C18:3 and was detected no difference in protein content between BG11 and NitrU. In conclusion, based on measurements of biomass production and oil accumulation, the NitrU medium appears a promising choice for use in the production of the microalga *Chlorella sorokiniana* LBA 39.

**Estipular, Noriel**

***IMAHINASYON Architecture + Research Group***

**SIBOL Project An Urban Living Generator : 360 Degrees Algae Photobioreactor Window**

*Noriel M. Estipular BS Architecture - Nueva Ecija University of Science and Technology Director-  
IMAHINASYON Architecture + Research Group Design Architect- Palafox Associates*

The project envisions a climate risk resilient Philippines with healthy, safety, prosperous, self-reliant communities, productive ecosystem, simultaneously grow the economic development of the country in renewable use of energy. And to fulfill the human needs while maintaining the quality of the natural environment for current and future generation. SIBOL Project is a new form of residential building in which adaptable architectural designs can be combined with intelligent technologies and construction materials. As one of the main themes of the A building as a living thing for living being these constitute an architectural pilot project, using four exemplary building types to show how new technological approaches can be translated into a forward looking architectural language, and traditional techniques reinterpreted. As its starting point for the SIBOL Project theme, the researcher presented the following basic ideas. Smart materials are active, with a transformative character. They respond to changing environmental conditions. In an intelligent interaction with smart technologies•, this process can be extended to the level of networked building services, and can monitor and optimize the energy and material maintenance. For this purpose, the existing categories of materials must be considered afresh, because smart materials, being active, take on opposing properties and functions at different times. Material and technological innovations in architectural history were always associated with a fundamental change in what architecture could and should be. These days, it can be observed that sustainability is the background to many design decisions.

**Enwereuzoh, Uzochukwu**

***University of the Witwatersrand***

**Algae Biomass Cultivation in Fish Farm Effluent for Biodiesel Production**

*ENWEREUZOH, UZOCHUKWU Harding Kevin, Low Michelle School of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg, South Africa.*

Micro-algae research is driven by need for alternative fuels for reasons such as climate change mitigation, depleting fossil fuel reserves, increased energy demand and economic growth. High cost of microalgae biomass production is a major setback of microalgae biodiesel production. Furthermore, high cost of inorganic nutrients and large volumes of water required for microalgae contribute to increased cost of microalgae cultivation. The use of fertiliser for the production of microalgae may be counterproductive, as production of fertiliser through conventional technologies release carbon dioxide. The main aim of this research is to reduce cost of microalgae biomass cultivation for biodiesel production by recycle nitrogen and phosphorus in nutrient rich fish farm effluent which are released in a form that is preferred by microalgae (that is NH<sub>3</sub> for nitrogen and PO<sub>4</sub>-3 for phosphate) for microalgae cultivation. Therefore, instead of purchasing fertilizer, those nutrients from farm effluent can be exploited in the cultivation of microalgae for biodiesel production. As consequence, this will not only supply nutrients and large volumes of water required for microalgae cultivation, but will also mitigate environmental challenges of discharging nutrient rich effluents. Growth rate, lipid productivity, carbohydrate and protein content will be determined in addition to production and analysis of biodiesel produced from selected microalgae species cultivated in fish effluent and will be compared with same species cultivated in standard growth medium.

**Leader, Sara**

***MicroBio Engineering Inc.***

**Annual Average Lipid, Carbohydrate and Protein Outdoor Pond Productivities of Uni-algal and Polycultures.**

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The productivities and composition of carbohydrates, lipids, and proteins of algal cultures will vary seasonally. Six 4.5m<sup>2</sup>, 0.3m deep, raceway ponds were operated continuously outdoors in San Luis Obispo, CA in replicate for a full year starting May 8, 2017, with both spontaneous native polycultures that were diluted with either primary clarified wastewater (CWW) or reclaimed wastewater (RWW) at a 2-day hydraulic residence time (HRT), and *Scenedesmus obliquus* (DOE 0152z) diluted with RWW with a seasonally adjusted 2 to 4-day HRT. The ponds were sampled three times per week for ash-free dry weight (AFDW), and weekly for lipid, carbohydrate, and protein content. Biochemical composition data and pond productivity data were combined to determine the areal productivity (g/m<sup>2</sup>-day) of carbohydrates, lipids, and proteins. The annual average productivities for the CWW and RWW polycultures and *S. obliquus* cultures were, respectively, 26.7, 15.1, 10.4 g AFDW/m<sup>2</sup>-day, 12.8, 7.1 and 4.7 g protein/m<sup>2</sup>-day protein, 4.3, 2.7 and 1.9 g carbohydrate/m<sup>2</sup>-day, and 1.5, 1.0 and 0.7 g lipid as FAMEs/m<sup>2</sup>-day. There were no significant seasonal variations in the biochemical profiles (% protein, carbohydrate and lipids), except for somewhat lower carbohydrate composition in the winter by all three cultures. Outdoor biochemical composition was also measured for six other pure culture algae strains cultivated in the outdoor ponds for shorter periods: *Desmodesmus* sp. DOE 1051, *Chlorella sorokiniana* DOE 1412, *Chlorella antartica* UTEX 1959, *Desmodesmus armatus* UTEX 2533, and two strains isolated from the RWW polyculture, *Tribonema* sp. RWW Isolate 4 and *Pseudopediastrum* sp. RWW Isolate 8, allowing a comparison between the different species for biochemical composition and productivity.

Hennig, Emma

*California Polytechnic State University San Luis Obispo*

**Productivity in raceways of a filamentous *Tribonema* sp. isolated from a reclaimed wastewater polyculture**

*Authors: Emma Hennig (a), Sara Leader (a,b), Ruth Spierling (a,b), Aubrey Davis (a,b), John Benemann (b), Tryg Lundquist (a,b) Affiliations: California Polytechnic State University, San Luis Obispo (a), MicroBio Engineering Inc (b) Presenter contact: emmahennig8@gmail.com, 425-923-0043*

Compared to colloidal algal monocultures, filamentous algae are generally easier to harvest and dewater. Additionally, native polycultures tend to exhibit higher productivity in raceway ponds compared to pure cultures from culture collections. In this research, we attempted to capture these advantages with a filamentous alga (*Tribonema* sp) isolated from a long-standing spontaneous polyculture grown outdoors on reclaimed wastewater in San Luis Obispo, California. After isolation, the *Tribonema* species was cultivated as a monoculture alongside the original polyculture ponds and a monoculture of *Scenedesmus obliquus* (DOE strain 0152-Z) from a culture collection. All ponds were 1000-L raceway ponds, with 30-cm depth and 2-day hydraulic residence time. Areal productivities of the *Tribonema* and the polyculture ponds were comparable from September 15 to November 18, 2017; the *Tribonema* ponds were 14.8 +/- 0.24 g AFDW/m<sup>2</sup>-day (mean +/-SD among duplicate raceways) and the polyculture ponds were 14.7 +/- 1.01 g AFDW/m<sup>2</sup>-day. In the same period the areal productivity of *Scenedesmus obliquus* (DOE strain 0152-Z) was 6.8 +/- 0.35 g AFDW/m<sup>2</sup>-day. In the spring and summer of 2018, the *Tribonema* ponds demonstrated higher areal productivity values than that of the polyculture and *Scenedesmus*. From March 2 to March 30, 2018, *Tribonema* ponds were 19.5 +/- 0.11 g AFDW/m<sup>2</sup>-day, while the polyculture was 12.7 +/- 0.96 g AFDW/m<sup>2</sup>-day, and the *Scenedesmus* ponds were 6.4 +/- 0.26 g AFDW/m<sup>2</sup>-day. From July 16 to August 22, 2018, areal productivity in the *Tribonema* ponds measured 26.5 +/- 3.08 g AFDW/m<sup>2</sup>-day, the polyculture ponds averaged 19.4 +/- 2.51 g AFDW/m<sup>2</sup>-day, and the *Scenedesmus* measured 5.88 +/- 1.79 g AFDW/m<sup>2</sup>-day. The high productivity values exhibited by the outdoor pure cultures of *Tribonema* relative to the *Scenedesmus* indicate the importance of adaptation to place for highly productive pure cultures. Results will be reported on the *Tribonema* biochemical composition and on experiments aimed at using the carbohydrates in the biomass for ethanol production through dilute acid hydrolysis followed by yeast fermentation.

Mendel, Hannah

*Colorado State University*

**Acoustic Harvesting: Implications of Algal Composition on Performance**

*Alyssa Aligata, Hannah Mendel, Peter Chen, Esteban Hincapi Gomez, Jessica Tryner, Anthony Marchese, Jason C. Quinn Mechanical Engineering, Colorado State University, Fort Collins, CO 80523-1374*

Substantial technoeconomic challenges exist across the value chain for microalgae-based biofuels and bioproducts. Acoustic harvesting, or ultrasonically enhanced settling, could dramatically reduce harvesting costs and directly address current energy barriers to separating algae from growth media. This technology utilizes ultrasonic standing waves to create an acoustic radiation force that, due to differences in the acoustic properties of the cells and media, causes the microalgae cells to agglomerate and settle out of the solution. The magnitude of the acoustic radiation force is directly related to the cell radius and acoustic contrast factor (ACF), the latter of which is a function of the density and sound velocity of the cell. These properties can vary widely depending on the algae species, cultivation conditions and growth stage all of which affect the composition of the microalgae cells (e.g., lipid, carbohydrate, protein content). In this work, two methods were used to determine the ACF of microalgal cells. The first method involved experimentally measuring the density and sound velocity of the cells and calculating the ACF. The second method utilized particle tracking velocimetry and a COMSOL Multiphysics model to estimate the ACF. The ACF was characterized, using both techniques, for three species *Chlamydomonas reinhardtii*, *Nannochloropsis salina*, and *Tetraselmis chuii* as a function of dynamic cellular composition over a 2-week growth period. The ACFs of *C. reinhardtii* and *N. salina* decreased 40% and 50%, respectively, over this period. ACF decreases as lipid content increases because lipids have a negative ACF, whereas carbohydrates and proteins have a positive ACF. Selective acoustic harvesting of one species in a polyculture could be possible if one species has a negative ACF (high lipid) and the other has a positive ACF (low lipid). This research shows that acoustic harvesting can be a technological advancement in the algal biofuels value chain.

Cheng, Jun

*Zhejiang University*

**Mutation of *Spirulina* sp. by Nuclear Irradiation to Improve Growth Rate Under 15% CO<sub>2</sub> in Flue Gas**

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*Spirulina* sp. was mutated by  $\gamma$ -rays from <sup>60</sup>Co nuclear irradiation to improve growth and CO<sub>2</sub> fixation rate under 15 vol.% CO<sub>2</sub> (in flue gas from a power plant). Mutants with enhanced growth phenotype were obtained, with the best strain exhibiting 310% increment in biomass yield on day 4. Ultrastructure of *Spirulina* sp. shows that the fractal dimension of *Spirulina* cells decreased by 23% after mutation. Chlorophyll synthesis and energy provision were both enhanced. The mutant was then domesticated with elevated CO<sub>2</sub> concentration, and the biomass yield increased by 500% after domestication under 15 vol.% CO<sub>2</sub>, with stable inheritance. Pore size in the cell wall of *Spirulina* mutant increased by 33% after 15 vol.% CO<sub>2</sub> domestication. This characteristic facilitated the direct penetration of CO<sub>2</sub> into cells, thus improving CO<sub>2</sub> biofixation rate. After CO<sub>2</sub> domestication, more pyruvate flowed towards TCA cycle and fatty acid biosynthesis. ATP synthesis pathway was also enhanced.

**Damle, Ashok**

***Techverse, Inc.***

**Advanced, Low-cost, System for Algae Dewatering**

*Dr. Ashok Damle Techverse, Inc.*

Algae represents a promising next generation bioenergy source for producing energy from renewable sources because of the fast growth and high oil content of certain algae strains. In the near-term, algae derived products are being developed for a variety of applications in nutraceutical and food industries. Algae grown in open ponds or in closed PBRs are very dilute with < 1 gm/liter solids concentration. The whole cell algae concentration must be increased by more than 200 times to >20 % w/w for optimal downstream processing and conversion to produce algal products. Because of very dilute algae concentrations and consequently very large volumes of water that must be processed, harvesting algae and extracting the lipids are significant cost drivers of algal products. Reduction of capital and operating costs of algae harvesting and concentration step is essential to make algal products economical. This presentation will describe a two-step algae dewatering process combining a low cost and high volume gravitational settling based pre-concentration and an advanced high-performance membrane filtration based algae concentration to produce >20% w/w algae paste. Low-cost, low-energy, bio-flocculation-based gravitational settling process is used to remove bulk of water, increasing the algae concentration to about 1 % w/w solids. Advanced membrane Tangential Flow Filtration (TFF) technology with open channel membrane module design is used to dewater algae further to produce algae paste. A pilot scale membrane filtration system was successfully demonstrated to continuously dewater pre-concentrated algae of ~1% w/w concentration at ~100 liters/hour feed rate to produce >20% w/w algae paste stream. Observed steady state membrane system performance data will be presented for several algae strains processed along with expected power consumption in a commercial scale system. The overall two-step algae dewatering process will lower capital costs and reduce energy consumption significantly compared to the baseline dissolved air flotation/centrifugation technology.

McDowell, Daniel

*Queen's University Belfast*

### **From Anaerobic Digestate to Microalgal Animal Feeds**

*Authors; Daniel McDowell<sup>1</sup>, Gary Sheldrake<sup>1</sup>, Katerina Theodoridou<sup>2</sup>, Jaimie Dick<sup>3</sup>, Matt Julius<sup>4</sup>, Pamela Walsh<sup>1</sup> <sup>1</sup>School of Chemistry & Chemical Engineering, Queen's University Belfast <sup>2</sup>The Institute for Global Food Security, Queen's University Belfast <sup>3</sup>The School of Biological Sciences, Queen's University Belfast, <sup>4</sup>Biological Sciences, St. Cloud State University, St. Cloud, MN, USA*

Growing microalgae with a waste source is an area of research that is attracting greater interest, as responsible and sustainable management of our resources is becoming a greater priority. In recent years, anaerobic digestion (AD) has proliferated across Northern Ireland due to a fixed price for energy production and an abundance of animal slurry which makes up a large part of the AD feedstock. The waste digestate produced after digestion can be used as an effective fertiliser on the land, however the quantity produced is more than the farm land requires. The excess digestate produced from AD presents an opportunity to add value to the farming system. This could be achieved if microalgae were to be produced using digestate as a nutrient source. Microalgae contain many nutritional compounds including fatty acids, proteins, minerals and antioxidants. Incorporating microalgae into animal feedstock would have the advantage of improving nutrition, boosting production and strengthening food security. This research investigates the possibilities for growing native microalgae with AD digestate and analysing their nutritional composition paying specific attention to fatty acids and protein content. In this study *P. tricornutum* microalgae was grown in photobioreactors with a range of nutrient sources. The nutrient sources included AD from three different plants and a synthetic F/2 media control. Preliminary results have shown that *P. tricornutum* can be grown with the three different digestates at concentrations of 1% and 3% digestate in seawater. The *P. tricornutum* grown with the control media accumulated 120mg/g sample of total fatty acids. The amount of total fatty acids accumulated in *P. tricornutum* varied with digestate type and concentration. The lowest was 39mg/g while the highest was 121mg/g which exceeds the levels found in the control (algae grown on F/2 media). The type of fatty acids accumulated was dependent on the media. The high value fatty acid *cis*-5,8,11,14,17-eicosapentaenoic acid, was found to be present at levels of 25mg/g in 1% digestate media and 16mg/g in the control. Research is currently being carried out to expand this study, this will include protein analysis of microalgae and investigation into higher digestate media concentrations. Further research plans to investigate the pathogen profile of the microalgae grown using digestate media.

Gross, Martin

*Gross-Wen Technologies*

**Revolving Algal Biofilm Systems: A Versatile Wastewater Treatment Platform**

*Martin Gross, Gross-Wen Technologies Xuefei Zhao, Gross-Wen Technologies Zhiyou Wen, Iowa State University*

Revolving algal biofilm systems have been shown to be efficient microalgae cultivation systems. In recent years they have been evaluated for the treatment of a variety of wastewater streams. In municipal wastewater they have been tested to treat primary influent, final polishing, side stream treatment. They have also been used to treat a variety of industrial wastewaters ranging from food/feed/biofuel/animal processing, fermentation and mining. Rotating biofilm systems have four distinct advantages over suspension based algae treatment systems. These include: (1) high biomass productivity, (2) simple and inexpensive harvest, (3) natural separation of hydraulic and solids retention time and (4) presence of extracellular polymeric substances which allow for enhance adsorption on the algae cells surface.

Chi, Zhanyou

*Dalian University of Technology*

**Massive Scale Cultivation with Floating Photobioreactor for Bicarbonate-based Integrated Carbon Capture and Algal Production Systems on Ocean (BICCAPSO) and its Roadmap for Commercial Application**

*Zhanyou Chi, Chenba Zhu, Yunpeng Zhao, He Zhu, Yimei Xi, Ruolan Zhang, Chunwei Bi Dalian University of Technology*

With supplying carbon as bicarbonate, floating photobioreactor (PBR) without gas bubbling and/or agitation system was developed, with wave as the only energy for fluid mixing. This system was named as Bicarbonate-based Integrated Carbon Capture and Algae Production System on Ocean (BICCAPSO). Without gas bubbling and/or agitation system, plastic bag with inflatable arch structure can be used as horizontal floating PBR. This significantly reduced the cost for PBR™s manufacturing and installation. Also, this system almost saved all operating cost, since its temperature is controlled by sunlight heating and surrounding water cooling, dissolved oxygen is controlled with free wave energy, pH is controlled by buffering effect of bicarbonate/carbonate, and no interval feeding is necessary. With these advantages, no labor on duty is necessary during the whole algae cultivation process, except inoculation and harvesting. This innovative mode for microalgae cultivation is similar to agriculture production, and will significantly reduce production cost of microalgae biomass. Single photobioreactor in this system has been scaled-up to 100 m<sup>2</sup> or 10 m<sup>3</sup> with few challenge. It has been applied in production of *Dunaliella*, *Spirulina*, *Chlorella* with large volume commercial production currently, as well as *Neochloris* with high lipid content for biofuel production in future. Commercial scale production of *Dunaliella* has been launched, with high productivity of 2.5 g/m<sup>2</sup>/day. Also, productivity of *Spirulina* with this system was almost two times as that in open pond system. It is notable that bicarbonate is only a more efficient approach for CO<sub>2</sub> supplying in this system, and there is no net consumption of it, since spent medium enriched with carbonate can be used to absorb more CO<sub>2</sub> and used in recycling culture. Feasibility of this recycling culture has been proved with our recent study on *Neochloris*. With high productivity, plus recycling culture integrated with carbon capture, this system is promising to reduce production cost of *Spirulina* from current \$3/kg to \$1.5/kg. This would make it economic viable to replace fish meal, which has a market volume of \$7.5 billion. In addition, application of this system in oleaginous microalgae such as *Neochloris* would significantly reduce its production cost, and greatly shorten the road of commercialization for algal biofuel.

Samaratunga, Ashani

*Old Dominion University*

### **Acid-assisted Flash Hydrolysis of *Scenedesmus* sp. for Recovery of Sugars and Lipids**

*Ashani Samaratunga<sup>1</sup>, Ali Teymouri<sup>1\*</sup>, Mason Martin<sup>1</sup>, Tao Dong<sup>2\*</sup>, Nick Nagle<sup>2\*</sup>, Philip T. Pienkos<sup>2\*</sup>, Sandeep Kumar<sup>1\*</sup>. 1 Civil and Environmental Engineering, Old Dominion University, Norfolk, Virginia, United States. 2 National Renewable Energy Laboratory, Golden, CO, United States.*

Sugars as value added products and intermediates for chemicals and fuels will help commercialize algal biorefineries and reduce production costs of algal biofuels. In recent times, Flash Hydrolysis (FH) at 280°C for 9 s in continuous process under subcritical water medium has shown promising results for enhancing lipid recovery along with proteins. Similarly, dilute acid hydrolysis has been studied extensively to enhance the recovery of sugars from algae and lignocellulosic biomasses. Therefore, dilute acid was incorporated into FH for the first time to enhance monomeric sugar recovery at low temperature, using a continuous flow reactor. In this study, acid-assisted FH of *Scenedesmus* sp. biomass was carried out to maximize sugar recovery in the aqueous phase while preserving the lipids in solids. The study focused on optimizing the monomeric and oligomeric sugar yields in the range of 150-280°C, sulfuric acid concentrations from 0% to 5% (w/w), and residence time of 10-20 s under subcritical water conditions in a continuous flow reactor. The preliminary data on FH at 200°C and 4.5% acid resulted in algae hydrolysate with more than four times the oligomeric and monomeric sugars concentration to 200°C without any acid. Furthermore, the ratio of oligomers to monomers decreased to 1:1 at 200°C with 4.5% acid, compared to the 3:1 ratio under the similar process conditions without acid. The algae hydrolysate recovered from acid-assisted FH could be tuned to produce monomeric sugars (mainly glucose) as a valuable product from carbohydrate-rich algae species while preserving the lipids in solids termed as biofuels intermediate (BI). These BIs contained more than 90 wt% of the total lipids content available in the initial algae biomass. Comprehensive lipid profiles including poly-unsaturated fatty acid (PUFAs) analyses on both microalgae and its BIs were performed to study the lipid recovery and quality under the acid-assisted FH conditions.

**Dong, Tao**

***National Renewable Energy Laboratory***

**Improving Biofuel Intermediate Yield and Quality by Tuning Algal Composition**

*Tao Dong, Nick Sweeney, Wei Xiong, Jianping Yu, Philip T. Pienkos National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401*

Hydrothermal liquefaction (HTL) can convert wet biomass into liquid biofuel intermediate (BFI) under elevated temperatures and pressure. Recently, it has been realized that the composition of biomass might significantly affect the BFI yield and quality. Algenol and its partners have proposed a pathway to increase cyanobacteria-based BFI yield and quality by optimizing the composition of biomass. Our overarching hypothesis is that variations in cyanobacterial biomass composition have significant impact on BFI yield and quality. To test the hypothesis wild type *Synechocystis* sp. PCC 6803 biomass was doped with various typical cellular storage compounds, such as glycogen, polyhydroxybutyrate (PHB), and middle chain-length polyhydroxyalkanoate (mcl-PHA), performing lab-scale HTL to determine optimal BFI yield and quality. Our results indicate that doping of glycogen in the biomass negatively affects the BFI yield. BFI yield can be dramatically increased using glycogen knockout mutant biomass. PHB was converted into propylene under our HTL condition and did not contribute to the BFI yield; while the addition of mcl-PHA significantly increased BFI yield and reduced nitrogen content in the BFI product. Moreover, partial mcl-PHA was converted into middle chain-length linear alpha-olefin (mcl-LAO), which can be easily separated after the HTL to be used as a value-added co-product. In summary, the experimental results support our hypothesis that the composition of biomass can remarkably affect the BFI yield and quality. Suppressing the accumulation of glycogen can increase the BFI yield. The most important observation is that mcl-PHA is a promising bio-storage compound that can increase BFI yield and quality and produce considerable amount of value-added mcl-LAO. \*This work is funded by DOE BETO.

Wendt, Lynn

*Idaho National Laboratory*

### **Management of Ash in Benthic Microalgae**

*Lynn M Wendt, Idaho National Laboratory John E Aston, Idaho National Laboratory Bradley D Wahlen, Idaho National Laboratory Michelle Walton, Idaho National Laboratory Derek Hess, Colorado State University Jason C Quinn, Colorado State University Ryan W Davis, Sandia National Laboratory Hongqiang Hu, Idaho National Laboratory*

The algal turf scrubber technology can effectively remediate impaired waterways by removing nutrients that contribute to eutrophication, meanwhile also producing algae biomass for biofuel production. However, the benthic biomass obtained from an algal turf scrubber can contain greater than 50% ash as a consequence of the growth environment (sand, silt and other suspended solids) and the contribution of biogenic silica from diatoms. Ash represents non-convertible material and complicates downstream conversion processing by incurring wear and abrasion to handling and conversion equipment, necessitating additional separations, and increasing the overall throughput required to achieve overall conversion targets. Physical and chemical approaches to removing ash can improve biomass quality as well as conversion yields. Previous results have shown that water washes can reduce ash content from 64 to 40 wt%, representing an economically viable pretreatment. However, ash levels of 40 wt% may still be too high for compatibility with many biochemical and thermochemical conversion methods. The high silica content in the benthic biomass may lend itself well to a chemical approach to solubilize ash, and in fact, up to 84 wt% of ash reduction has been obtained with alkaline extraction using up to 2 wt% NaOH in this study. However, techno-economic assessment revealed that economic viability of alkaline extraction, or any other chemical preprocessing, as a deashing mechanism will require significant chemical recovery and reuse to reduce chemical and associated waste water treatment costs. While water washing and alkaline extraction were both found to have limitations on their own, combining these two approaches showed promise. Fractional separation of the biomass from sediment by size prior to dewatering was combined with both water washes and chemical treatment. While a sequential two-stage deashing approach was found to increase costs, a parallel water wash and chemical treatment step prior to dewatering was shown to reduce the impact of ash removal costs. Overall, management of ash in benthic biomass produced using a turf scrubber shows promise for increasing the applicability of this biomass for bioenergy production.

**Spierling, Ruth**

***Cal Poly San Luis Obispo***

### **A Comparison of Pure Culture and Polyculture Productivity and Culture Health in Raceway Ponds**

*Ruth Spierling, Cal Poly and MicroBio Engineering Inc. Aubrey Davis, Cal Poly and MicroBio Engineering Inc. Sara Leader, Cal Poly and MicroBio Engineering Inc. Mike Scott, Cal Poly Tryg Lundquist, Cal Poly and MicroBio Engineering Inc. John Benemann, MicroBio Engineering Inc.*

Spontaneously occurring algal polycultures are well adapted to their environments and highly productive when compared to pure cultures under the same environmental conditions. In this study six 4.5 m<sup>2</sup> and four 3.3 m<sup>2</sup> ponds were operated in duplicate sets in San Luis Obispo California from 5/8/17 to 3/1/18. All ponds were operated continuously at 0.3 m depth and fed reclaimed treated municipal wastewater (RWW), or clarified primary wastewater (CWW). For this entire period, two ponds were operated at a 2-day residence time on RWW with a naturally occurring algal polyculture, two ponds were operated at a 2-day residence time on CWW with a natural algal polyculture, and two ponds were operated on a 2 to 4-day variable residence time on RWW with a pure culture of *Scenedesmus Obliquus* (DOE 0152z). In the remaining four ponds, six other pure culture strains were cultivated in replicate over short time periods at a 2 to 4-day variable residence time on RWW. Species studied included *Desmodesmus* sp. (DOE 1051), *Chlorella sorokiniana* (DOE 1412), *Chlorella antarctica* (UTEX 1959), *Desmodesmus armatus* (Utex 2533), and a filamentous strain isolated from the RWW polyculture ponds. Productivity results from these shorter cultivation periods were compared to the productivity of the polyculture ponds and the *Scenedesmus obliquus* (DOE 0152z) ponds for the same time period. Over the six-month period from 5/8/17 to 11/8/17 all ponds were operated at a 2-day hydraulic residence time, with the following result: The productivity of the polyculture on CWW was 32.1 g AFDW/m<sup>2</sup>-day with a standard deviation between the replicates of 1.8 g AFDW/m<sup>2</sup>-day. The productivity of the polyculture on RWW was 20.5 g AFDW/m<sup>2</sup>-day with a standard deviation of 1.2 g AFDW/m<sup>2</sup>-day. The productivity of the pure culture of *Scenedesmus obliquus* (DOE 0152z) on RWW was 14.6 g AFDW/m<sup>2</sup>-day with a standard deviation of 0.3 g AFDW/m<sup>2</sup>-day. Overall the polyculture ponds on RWW were roughly 30 to 50% more productive than the pure cultures and exhibited no downtime due to pond crashes caused by contamination or predation.

**Itsygin, Simon*****Czar Salt*****Innovative Sea Salt Products Infused with Algae and Other Natural Sources***Simon Itsygin, Ph.D, Czar Salt*

Salt (sodium chloride) is the most essential compound in the human body. Sodium chloride is involved in regulating the fluid balance and also absorbs and transport nutrients, maintains blood pressure, and transmits electrical signals throughout the nervous system. Meanwhile too much sodium in our diet can have negative health consequences (high blood pressure, swelling, etc.). Review of publications related to salt daily usage is provided; a tendency for reducing a daily salt usage was specified. Methods of reducing consumed sodium chloride without a taste sacrificing are discussed. Rock salt and sea salt comparison is shown, and recommendations for use are done. Sea salts from the ecologically protected Lagoon of Cuyutlājn on the Pacific Coast of Mexico were selected as the healthiest products. These salts are harvested and manually produced according to ancient technologies. The certified organic sea salts have a lower concentration of sodium chloride (85-95%) and don't contain plastic and other poisonous substances. The innovative method of enrichment of the organic sea salts with algae products and other natural sources was suggested. The technology, based on the creation of diverse salt-substance combinations, provides for an increasing line of innovative salt products. The final salt products (branded as Czar Salt) contain beneficial substances integrated into salt crystals, resulting in vivid colors and high stability. Infused into salts crystals, the healthy natural ingredients (vitamins, minerals, carbohydrates, proteins, flavonoids, oils, etc.) partly or completely cover the human demand for these substances and provide new health effects. These benefits come with daily consumption of salt enriched with our healthy nutrients. The main health benefits of Czar Salt products include supporting the immune system, maintaining a healthy circulatory system; managing blood pressure and cholesterols in healthy ranges; improving digestion, vision, skin and bone health; enhancing cognitive functions, etc. Various examples of the new healthy sea salts are presented.

**Teymouri, Ali**

***Old Dominion University***

### **Holistic Approach in Microalgae Conversion to Bioproducts and Biofuels Through Flash Hydrolysis**

*Ali Teymouri<sup>1\*</sup>, Eleazer P. Resurreccion<sup>2\*</sup>, and Sandeep Kumar<sup>1\*</sup> <sup>1</sup>Civil and Environmental Engineering, Old Dominion University, Norfolk, Virginia, United States <sup>2</sup>Civil Engineering Technology, Montana State University Northern, Havre, Montana, United States \*ABO members*

In recent years, the demand for renewable energy has increased. The U.S. Energy Information Administration reported that more than 13.3% of the total energy production in the first seven months of 2017 was produced from a biomass source. Among all resources, microalgae has proved its potential to contribute to the renewable fuels portion. However, its development is far behind industrial production due to both technical and economic barriers. In this study, an integrated biorefinery approach has been proposed and implemented on microalgae slurry to exploit the biomass by producing value-added bioproducts in addition to the biofuels main stream. To this aim, *Chlorella vulgaris* was fractionated using flash hydrolysis (FH) technique at 280 C and 10 s of residence time to extract macro-/micronutrients in aqueous phase (hydrolysate) and retained more than 90 wt% of the lipids of the initial biomass in the solid fraction termed biofuels intermediates (BI). These lipid-rich BIs were subjected to hydrothermal liquefaction (HTL) process which resulted in almost 70 wt% low-nitrogen content biocrude yields with HHV and H/C ratio of 40.0 MJ/kg and 1.74 respectively. Comprehensive lipid analysis has confirmed the higher quality of the biocrude for biofuels applications. On the other hand, hydrothermal mineralization (HTM) process applied on the phosphorus-rich hydrolysate where more than 97 wt% phosphorus recovery occurred through hydroxyapatite formation with numerous biomedical applications. Life Cycle Impacts and Techno-economic Implications for this integrated approach were also evaluated using a "well-to-pump" system boundary. Through this study, we developed an integrated pathway that optimized yields and reduced the production costs.

**Sabri, Laith**

*student in Missouri university of science and technology*

### **Local Hydrodynamics for Internal Loop Photobioreactor During Microalgae Culturing**

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The airlift photobioreactors are widely used for microorganisms cells growth. Due to perfect mixing and mass transfer are crucial for culturing system. The scope of this work, a green microalgae, *Scenedesmus* was growing in split airlift photobioreactor. In these culture systems, local hydrodynamics can enormously impact the reactor conduct. Where the multiphase reactor design dependence entirely on these hydrodynamics parameters. Consequently, the deep understanding of the local flow dynamics for multiphase flow is required for efficient design and successful scale-up. The properties of the culturing media during the microalgae growth could frequently change, then these fluid flow factors necessary to be further evaluated and developed to account for this variation, which is unfortunately unclear yet. Thus, the advanced non-invasive Radioactive Particle Tracking (RPT) and Dual-Sources Gamma Ray Computed Tomography (CT) measurement techniques have been utilized to investigate flow features. This kind of powerful techniques has capable of measuring cross-section distribution and their radial profile holdup, liquid velocity field, shear stress, and turbulence parameters for any multiphase system including culturing phases. The experimental work carried out with a Plexiglas split column in 5.5-inch (0.14m) inner diameter and 59 inches (1.5m) high including green algae (*Scenedesmus*) at various superficial gas velocities (1, 2, and 3 cm/sec) and through a different time of microalgae growth segments. As a conclusion, the experimental outcome will provide benchmark data for design, scale-up, and simulation to understand the performance of split airlift photobioreactor. The preliminary results and findings will present at the conference.

**Pererva, Yehor**

***Utah State University***

**Reuse / Recycling of Produced Water with Halotolerant Microalgae**

*Igor Pererva : Utah State University Ronald Sims: Utah State University*

The present study addresses the problem of the oil extraction industry wastewater streams bioremediation at site in the Utah's Uintah Basin. Produced Water (PW) is the dominant wastewater at the oil extraction site and is characterized by high salinity and oil-derived chemicals, which are mostly toxic and are required to be removed for public health purposes. The goal of the project described is to evaluate the potential application of halotolerant and halophile microalgal species for bio-remediation and salinity removal from PW. Ultimate goal is to made remediated water suitable for site reuse, or agricultural application, and/or water discharge. Evaluation of bio-remediation capabilities was performed for 5 species, including confirmed halophile green microalgae *Dunaliella salina*, and locally isolated strains of cyanobacteria from wastewater treatment facility and Great Salt Lake. Cultures were tested for cultivability in PW under lab conditions. The growth dynamics and salinity removal rates (as sodium and potassium ions, and electrical conductivity) are investigated in batch system with further transfer to continuous flow system known as Rotary Algal Biofilm Reactor, developed at Utah State University. Preliminary data demonstrate feasibility of cultivating the microalgal species listed above on PW, and the potential for bioremediation. However, additional quantitative tests are being used to determine a more precise measure of bioremediation with this technology.

Han, Yang

*Desert Research Institute*

### **Hydrothermal Liquefaction of Marine and Freshwater Algae Biomass Using Co-solvents**

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Hydrothermal liquefaction (or HTL) of microalgae biomass is a promising conversion technology in which wet biomass is treated under high temperature (280-350 °C) and pressure (700-3000 psi), and the associated water in the wet biomass forms the reaction medium. A significant fraction of the microalgae cell is converted into biocrude, which separates from the aqueous layer by gravity and/or chemical/mechanical methods. Since the HTL process uses the whole biomass, increasing lipid content during microalgae cultivation is not essential. With an overall goal to utilize highly productive algal strains as liquid hydrocarbon fuel precursors, the current research investigates HTL conversion of a marine algae feedstock (Tetraselmis) and a freshwater algae (Spirulina) to biocrude in the presence of co-solvents. HTL was performed using a 1.2 L Parr reactor at 275-350 °C for 30 min in the presence or absence of a co-solvent (ethylene glycol and isopropyl alcohol). The results showed that conversion of Tetraselmis algae was promoted by severe conditions: biocrude yield increased from 25% to 33% as the temperature increased from 275 °C to 350 °C. On the contrary, Biocrude yield produced from Spirulina decreased from 47% to 38% with the severity of HTL conditions. For both HTL treatments of these two algal feedstocks, addition of 10% isopropyl alcohol as co-solvent promoted biocrude conversion. The biocrude yield did not change significantly when using ethylene glycol as co-solvent. More gases were produced by addition of isopropyl alcohol than that of ethylene glycol.

**Nagle, Nick**

***National Renewable Energy Laboratory***

**Assessing Impact of Reactor Scaling on Carbohydrate and Lipid Yields from Pretreatment of Saline and Fresh Water Algae**

*Nick Nagle<sup>1</sup>, Tao Dong<sup>1</sup>, Stefanie Van Wychen<sup>1</sup> Lieve Laurens<sup>1</sup> <sup>1</sup> National Renewable Energy Laboratory, Golden, CO, United States*

Pretreatment of terrestrial feedstocks using similar reaction conditions, identical feedstock and pretreatment chemistry has reported wide ranges in total sugar yields. This has been attributed to differences in reactor size or scale, type of operation (batch vs continuous), type of heating and reactor geometry. To understand how total sugar yields in algal biomass are affected by different dilute acid pretreatment configurations we evaluated total sugar and lipid release at three reactor sizes ranging from 10ml to 500 ml, operated under mixed and non-mixed regimes. Three different methods of heating, fluidized sand bath, microwave and steam, were employed using identical pretreatment chemistry and reaction conditions. All three of the process configurations were in a vertical orientation, solids loading ranged from 7.5% (w/w) for the microwave pretreatment and 20% (w/w) for the sand bath and the steam injected, mixed Zipperclave reactor. *Scenedesmus acutus* and *Desmodesmus* sp. were pretreated using a modified central composite design to explore and identify areas of similar yields resulting from different pretreatment configurations. The results from these pretreatment experiments comparing total sugar and lipid yield from the three pretreatment configurations will demonstrate the impact of scale, differences in reactor geometry and method of heating on pretreatment performance. These findings can be used to understand the differences in yields using similar reaction conditions and chemistry while providing guidance for scaling bench scale to pilot and demonstration-scale reactors which is of interest to researchers, commercial technology developers.

**Kesner, Stephanie**

*University of Kentucky Center for Applied Energy Research*

### **Optimization of Flocculation-sedimentation for Algae Harvesting**

*John G. Groppo, Stephanie Kesner, Mark Crocker; University of Kentucky*

Previous work has shown that under quiescent conditions, algae cells will settle when biomass is flocculated in large batches. However, operation of a continuous harvesting system requires some degree of mixing in order to achieve adequate and consistent flocculant dosage, thus negating the quiescence necessary to achieve settling. To overcome this challenge, a lamella clarifier was designed and constructed to facilitate the continuous harvesting of algal biomass by means of flocculation and sedimentation. Flocculated feed slurry is pumped into a cylindrical mixing chamber, from which biomass settles into a conical-shaped settling chamber in the lower portion of the vessel. The conical shape promotes compaction as the flocculated biomass settles to the base of the conical section where it is periodically removed through a discharge valve. As clarified water rises above the cylindrical section, it passes through an array of lamella plates inclined at 45° to provide a settling surface for residual solids. After rising through the plates, clarified water overflows the device at a rate equivalent to the feed rate. In trial runs, solids capture efficiency was determined by measuring algae concentrations in the feed suspension and overflow water using three different analysis methods: dry mass measurements, spectrophotometry, and cell count. Results showed that solids recovery efficiency of >95% could be obtained for *Scenedesmus acutus* algae that had first been flocculated using a polymeric cationic flocculant (5-7 ppm). In separate experiments, it was shown that the combined use of cationic (5 ppm) and anionic (2 ppm) polymeric flocculants has the potential to give even better results. These findings demonstrate that a high degree of solids capture is possible using flocculation-sedimentation under continuous harvesting conditions.

Ajala, Sheriff

*Texas A&M University-Kingsville*

**Microalgae Growth and Lipid Accumulation Parameter Optimization for Biofuels Production Using Response Surface Methodology: A Case of Municipal Wastewater as Growth Medium**

*1 Sheriff Olalekan Ajala, 2 Matthew Alexander 1 Sustainable Energy System Engineering, Texas A&M University-Kingsville 2 Chemical and Natural Gas Engineering, Texas A&M University-Kingsville Contact: +14695791185, sheriffajalao@gmail.com*

The quest for alternative fuels has not only been borne out of the desire to develop environmental friendly, renewable and sustainable fuels that can replace conventional fuels, but also the need for most nations of the world to be energy independent. Microalgae-biomass based fuels are recently gaining attention due to numerous advantages like CO<sub>2</sub> sequestration, less competition for agricultural land for food crops, elimination of food vs fuels crisis associated with current biofuels feedstocks, wastewater bioremediating agents, high biomass and lipid productivities and, above all, the possible integration of biorefining with wastewater treatment. In this work, response surface methodology (RSM) was used to optimize the microalgae growth and lipid accumulation of *Neochloris conjuncta* using municipal wastewater supplemented with nitrate and phosphate as the growth medium. A three-factor-three-level Box-Behnken design of RSM was employed for the optimization studies and the independent variables considered include nitrate, phosphate and CO<sub>2</sub> concentrations, whereas biomass concentration and lipid content are the dependent variables. Based on the RSM predictions, the combined optimum values for biomass concentration and lipid content were 1445.53 mg dw/L and 197.37 mg/ g wt. biomass respectively, under the culture conditions of 0.36 g/L of nitrate concentration, 0.20 g/L of phosphate concentration and 4.00 % v/v CO<sub>2</sub> supply. Also, a separate analysis of the model gave biomass concentration of 1788.34 mg dw/L with nitrate, phosphate and CO<sub>2</sub> concentrations of 0.13 g/L, 0.20 g/L and 3.98 % v/v respectively. Similarly, lipid content of 253.70 mg/g wt. biomass was obtained as optimum at nitrate, phosphate and CO<sub>2</sub> concentrations of 0.38 g/L, 0.19 g/L and 4.00 % v/v respectively. This work shown that biomass and lipid productivities can greatly be enhanced by supplementing municipal wastewater with essential growth nutrients, thus lowering the cost of medium and the freshwater demand for algal cultivation.

Vieira Costa, Jorge Alberto

*Federal University of Rio Grande*

**Effect of Pentoses and Light Intensity on Carbohydrate Production and Protein Profile of *Chlorella minutissima* Grown in Tubular Photobioreactors**

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Pentoses have been considered potential carbon sources for microalgae cultivation due to the great interest in the search for alternative sources to remodeling the culture media and the great availability of lignocellulosic residues. The objective of this work was to evaluate if the addition of D-xylose and L-arabinose and the light variation affect the carbohydrate and the protein profile of *Chlorella minutissima* grown in tubular photobioreactors. The highest cellular concentration was 1.55 g.L<sup>-1</sup> for the control cultures exposed to the light intensity of 40.50 μmol.m<sup>-2</sup>.s<sup>-1</sup>. The variation of the luminous intensity was able to interfere in pentoses utilization as a carbon source, generating modifications in biomass composition and in the protein profile of *Chlorella minutissima* grown in tubular photobioreactors. Lower light intensities caused lower degradations for Rubisco, D1 and D2 proteins. The higher content of carbohydrates (66.4%) was obtained for cultures with the use of xylose and arabinose and 40.50 μmol.m<sup>-2</sup>.s<sup>-1</sup>, which can represent a theoretical ethanol production of 43.0 mL.100 g<sup>-1</sup>. The results presented the high potential of *Chlorella minutissima* to collaborate with the reduction of environmental impacts and biofuels production.

Greque de Morais, Michele

*Federal University of Rio Grande*

### **Chlorella fusca LEB 111 Cultivated with CO<sub>2</sub> and Thermoelectric Ashes for Production of Macromolecules**

*Vagner da Silva Braga, Federal University of Rio Grande Luana Pereira da Silva Marques, Federal University of Rio Grande Duna Joanol da Silveira Mastrantonio, Federal University of Rio Grande Jorge Alberto Vieira Costa, Federal University of Rio Grande Michele Greque de Morais, Federal University of Rio Grande*

Microalgae are microorganisms capable of converting inorganic carbon into organic carbon by photosynthesis, producing the carbonic backbone chain of proteins, carbohydrates and lipids. Production of carbohydrates and lipids has been widely studied when nitrogen source is limited. The use of thermoelectric ashes is a new possibility of maximizing the production of these compounds. These ashes are residues that aggravate environmental problems when discarded incorrectly, however recent studies prove that the minerals present in the ashes can influence the microalgal cultivation. In this sense, the objective of this study was to verify if the reduction of the nitrogen source along with the use of CO<sub>2</sub> and thermoelectric ashes alter the production of macromolecules in *Chlorella fusca* LEB 111. Every 20 min during 12 hours lighting-photoperiod, 10% (v/v) of CO<sub>2</sub> was injected at a flow rate of 0.3 vvm for 5 min. In the assays, the nitrogen concentrations in the culture medium were 1.5 and 0.75 g/L. In addition, 0, 40 and 120 ppm of ashes from the Presidente Médici coal power plant were added in the experiments. Cultures with CO<sub>2</sub> added of 40 ppm of ash presented the highest specific growth rates ( $0.33 \pm 0.01$ ) and the shortest generation times ( $2.12 \pm 0.01$ ). Additionally, 0.75 g/L of NaNO<sub>3</sub> resulted in protein content equal or greater than the tests with 1.5 g/L of this nitrogen source. In this study, it was also observed that the reduction of the nitrogen source is a strategy to accumulate up to 35.2% of carbohydrates, which results in 25.5 mL of theoretical bioethanol production for each 100 g of biomass. An approach for further increasing carbohydrate content in the biomass would be the addition of lower concentrations NaNO<sub>3</sub> in the culture medium. Thus, the use of CO<sub>2</sub>, ashes and reduction of the nitrogen source represent an alternative to minimize the costs with nutrient sources for microalgae cultivation, as well as the reduction of environmental problems caused by these effluents.

Shirazi, Yaser

*The University of Toledo*

**Thermochemical Conversion of Algae to Biofuels and Chemicals: A Study on Integration of Algae Fractionation and Ex-situ Catalytic Pyrolysis**

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Microalgae are attractive feedstocks for biofuel production and are especially suitable for thermochemical conversion due to the presence of thermally labile biomass constituents - lipids, starch and protein. However the presence of starch and proteins also poses challenges and microalgae pyrolysis produces water as well as other O- and N-compounds that are mixed-in with energy-dense lipid pyrolysis products. We developed a two-step fractional pyrolysis approach integrated with vapor phase catalytic upgrading. This approach allows (1) the separate recovery of energy-dense lipid pyrolysis products and the lower calorific value bio-oils from the degradation of starch protein and (2) tailored vapor phase upgrading of the resulting fractions. To evaluate the validity of this approach, *Chlorella* sp. was first pyrolyzed at 320 °C to volatilize and degrade the biomass starch and a majority of the protein. Then, the residual biomass was pyrolyzed again at 450 °C to recover products from lipid decomposition. The volatiles from each fraction were passed through an ex-situ zeolite catalyst bed to assess hydrocarbon yields. In presence of ex-situ catalyst, high yield of aromatics, in particular benzene, toluene and xylene was achieved. Besides, the bio-char was N-rich and contamination-free and can be used as soil amendment or fertilizer.

**Meng, Fanxu**

***Houston Advanced Research Center (HARC)***

**Combined Heat and Power (CHP) for Algae Product Systems**

*Fanxu Meng (Houston Advanced Research Center (HARC)) Gavin Dillingham (Houston Advanced Research Center (HARC)) Marina Badoian-Kriticos (Houston Advanced Research Center (HARC))*

Combined Heat and Power (CHP) perfectly fits for sustainable development of algae product systems, because it supports electricity demands, heat requirements and nutrient needs (e.g. CO<sub>2</sub>) to enable an energy efficient and environmental friendly production of algae. CHP can provide heat requirements for many purposes by different heat recovery applications: 1) Exhaust gas can be used directly for dryers or process heating; 2) fluid heating can be applied with specific heat exchangers; 3) steam can be produced by a heat recovery steam generator (HRSG); 4) absorption chillers and desiccant dehumidifiers can be utilized to convert heating to cooling and drying capacities. Systematic study need to be conducted to address technical and economic issues. DOE Southcentral Combined Heat and Power Technical Assistance Partnership (CHP TAP) will discuss different heat recovery applications that are applicable and practical to algae product systems and identify the attractive opportunities for the emission-free and less-costly energy sources. The match between quantity and quality of recovered heat and demands, the capital and operational costs and the sensitivity analysis will be discussed based on successful case studies and proof-of-principle studies. This presentation will serve for better understanding the heat recovery benefits to commercialization of algae product systems.

Kim, Junho

*Inha University*

**Cultivation of *Tetraselmis* sp. MBEyh04Gc in Floating Algal Culture Systems in the Ocean**

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The production of biofuels using microalgae can reduce inappropriate usage of fossil fuels to results in environment problems such as air pollution and global warming. Conventionally, microalgae have been cultivated on land using raceway ponds and photobioreactors (PBRs), but algal cultivation in land is required for energy, nutrients, and cultivable area. Developments on ocean-based algal culture systems started with possible benefits from large cultivable area, relatively stable temperature, restless waves, direct access to seawater, nutrients in seawater, etc., that could help ease resource requirements for large-scale microalgal cultivations. In this study, floating algal culture systems were developed, and *Tetraselmis* sp. MBEyh04Gc isolated from the coastal water of Young-Heung Island, Incheon, Korea was cultivated in both Tubular Module-PBRs (TM-PBRs) and floating culture ponds. The effects of solar irradiance, seawater temperature, and wave conditions on algal biomass productivity were investigated. Algal biomass productivity was positively correlated with solar irradiance, seawater temperature, and wave energy. The highest biomass productivity of 25.6 g/m<sup>2</sup>/d was achieved in July with the one-month average of 12.9 g/m<sup>2</sup>/d in floating culture ponds. As opened floating culture systems were more favorable for heat and CO<sub>2</sub> exchanges with surrounding environment than closed TM-PBRs, algal biomass productivity of floating ponds was higher than that of TM-PBRs. Also, floating culture systems have higher space utilization than TM-PBRs required spacing in between to prevent colliding each other. In order to achieve high biomass productivity not only high solar irradiance but also high wave energy was crucial. Development of floating algal culture systems with efficient conversion of wave energy into culture mixing will be needed to improve biomass productivity in ocean-based algal cultivation.

Kang, Sungmo

*Inha University*

**Effect of Nutrient Supply Rates of *Tetraselmis* sp. MBEyh01L in Floating Culture Systems with Selective Permeable Membranes on Biomass and Fatty Acid Productivity**

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Nutrient requirement for algal cultivation could be alleviated by using wastewater, recycling of nutrients via hydrothermal treatment, and utilization of nutrients dissolved in seawater. In this study, Selectively Permeable Membranes (SPMs) for cultivating microalgae were developed to supplying nutrients for microalgal cultivation. Nutrients for producing biomass enter in algal culture while containing the cell inside through SPMs. Various SPMs were measured for their nutrient supply rates, and *Tetraselmis* sp. MBEyh01L isolated from costal water of Youngheung Island, Incheon was cultivated in a variety of SPMs. As the results, maximum nutrients supply rates of SPMs varied depending on SPM properties such as materials, fiber density, pore size, etc. Higher algal biomass productivities were considerably correlated with higher nutrient transfer rates of SPM systems. Maximum biomass productivity of 15.7 g/m<sup>2</sup>/d was obtained without any fertilizer application. The linear growth curve of microalgal culture showed that nutrient supply of SPMs is the major growth limiting factor. Higher fatty acid contents of green algae cultivated in SPM systems had a negative association with higher nutrient supply rates. However, biomass produced in SPMs with higher mass transfer rates showed higher fatty acid productivity, by having higher biomass productivity. This study demonstrated that it is possible to produce algal biomass using nutrients dissolved in seawater through SPM systems. Improvements of SPM systems for higher biomass productivity might be needed to be successful algal biomass production not any fertilizer uses but nutrient supply in seawater.

Jeong, JeongHo

*Inha University*

**Influence of Reaction Parameters on Biofuel Production from Lipid-extracted *Tetraselmis* sp. Using Hydrothermal Liquefaction**

*Young-Jin Ryu<sup>1,2</sup>, Jeongho Jeong<sup>1,2</sup>, Hee-Yong Shin<sup>2</sup>, Ji-Hyun Yang<sup>2</sup>, Donggu Lee<sup>2</sup>, and Choul-Gyun Lee<sup>1,2\*</sup>* *1 Department of Marine Science & Biological Engineering, Inha University, Incheon 22212, Korea 2 National Marine Bioenergy R&D Consortium, Inha University, Incheon 22212, Korea Tel.: +82-(32)-872-7518, \*E-mail address: leecg@inha.edu*

Hydrothermal liquefaction of lipid-extracted *Tetraselmis* sp. feedstock containing 80 wt.% water was conducted in a batch reactor at different temperatures (300, 325, and 350°C) and reaction times (5, 10, 20, 40, and 60 min). The biofuel yield, elemental composition and higher heating value obtained at various reaction conditions were used to predict the optimum conditions for maximizing energy recovery of biofuel with good quality. A maximum energy recovery of 67.6% was obtained at 325°C and 40 min with a high energy density of 31.8 MJ/kg and lower contents of nitrogen and oxygen. Results showed that reaction conditions of 325°C, 40 min was most suitable for maximizing energy recovery while at the same time achieving improved quality of biofuel.

**Nagle, Nick**

**NREL**

**High Productivity Conversion of Algal Biomass to Ethanol Using Immobilized Cell Technology**

*Xing-Feng Huang<sup>1</sup>, Nicholas J. Nagle<sup>2</sup>, Wylie P. Borden<sup>1</sup>, Zachary M. Dwyer<sup>1</sup>, Lieve Laurens<sup>2</sup>, and Kenneth F. Reardon<sup>1,2</sup>*

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One strategy to maximize the areal yield of fuels and chemicals from algal cultivations is to convert carbohydrates in residual biomass via fermentation. Immobilized-cell technology in a continuous cultivation system has the potential to achieve significantly higher productivities than those from standard batch fermentation using free cells. The goal of this project was to determine the productivity gain from using immobilized cells and to evaluate the influence of key bioconversion parameters.

Hydrolysates of the alga *Desmodesmus armatus* were prepared using a dilute acid treatment at elevated temperature and pressure. *Saccharomyces cerevisiae* JAY 270 cells were immobilized in alginate to produce ethanol from algal hydrolysates of *Desmodesmus armatus*. The immobilized cells were packed in a column that was used in a system that mimicked chemostat operation. The rates of immobilized-cell production of ethanol were determined, along with the effects of pH, temperature, and residence time in the continuous immobilized-cell bioreactor system. In addition, the effects of post-hydrolysis treatments on the fermentation were evaluated.

No added nutrients are required for ethanol production using the algal hydrolysate of *D. armatus* in the continuous immobilized-cell bioreactor system. It was found that pH 4 and 35 °C are the optimum conditions for the immobilized yeast fermentation. The productivity of the chemostat-like continuous immobilized-cell bioreactor system could be more than ten times that of free-cell bioreactors. Furthermore, shorter residence times led to higher ethanol productivities but lower glucose conversion rates. Using these data, strategies for obtain high productivity and high conversion were evaluated. A sequence of a continuous well-mixed bioreactor and a plug-flow bioreactor has the potential to achieve both goals.

Lin, Yanxia

*Stevens Institute of Technology*

### **Optimization of an Attached-growth System for Harvesting of Microalgae**

*Yanxia Lin, Han Cao, Abhishek RoyChowdhury, Juliana Abraham, Tsan-Liang Su, Washington Braida, Christos Christodoulatos*

One of the major economic constraints associated with the microalgae industry is the efficiency of the harvesting technology employed. Growing microalgae as a biofilm on an attached-growth substratum has a significant potential to reduce the cost of the dewatering process, the most expensive part of harvesting microalgae from suspended-growth systems. The disadvantages of a suspended-growth system include high water demand and the requirement of a large operational facility; attached-growth algae cultivation systems are a promising alternative that does not present these issues. The main objective of the present study was to select an appropriate attached-growth substratum for microalgae and optimize the microalgae attachment parameters. A Flood-and-Drain System (FDS) was set up to test the catch capability of the microalga *Scenedesmus obliquus* ATCC®11477 on the surface of 12 different materials including cotton duct, PVC foam, ceramic, and plastic board. It was found that cotton duct had the highest biomass productivity 5.05 g·m<sup>-2</sup>·day<sup>-1</sup> followed by cotton rope (biomass productivity of 4.65 g·m<sup>-2</sup>·day<sup>-1</sup>). Although cotton duct produced the highest biomass, it showed many disadvantages, e.g., low durability over extended run-time periods (over a month). Other conditions such as running time, the addition of bacteriostatic agents, and total cost must also be considered before implementing cotton duct in a scaled-up system. An adjustable inclined plane system was built to test biomass productivity on the best performing materials by controlling parameters such as media flow rate, nutrient ratios and algae species. Based on the inclined system, optimum conditions for the growth of representative species were determined.

Choi, Hong Il

*Korea University*

### **Direct Combustion Provides New Possibilities for Microalgae-based Fuel Systems**

*\*Hong Il Choi (Presenter & Corresponding author), Sang Jun Sim, Min Eui Hong - Department of Chemical and Biological Engineering, Korea University \*Won Seok Chang - Research Institute, Korea District Heating Corp.*

As interest in climate change has increased, bio-energy with carbon capture and storage (BECCS) has recently gained popularity owing to its ability to deliver negative CO<sub>2</sub> emissions. Along with the growing interest in BECCS, direct combustion of diverse biomass has been actively studied since it is a core element of the bio-based energy system. Although microalgae have been recognized as the most promising renewable energy sources owing to their superlative productivities and energy densities, an aspect of microalgae as a direct combustion fuel has been overlooked until now. This is because of the inherently high water content of microalgae which not only requires energy-intensive, complicated dehydration processes for their end-uses but also hinders the combustion of the biomass. However, previous studies have shown that the problems concerning the high moisture can be overcome by well-designed dewatering paths. Thus, it is time to seriously consider the practical use of dried microalgal solid fuel to make microalgae actively contribute to effective reduction in CO<sub>2</sub> emissions. To verify the practicability of microalgae as a direct combustion fuel, herein, we holistically scrutinized the potential of diverse microalgae-based energy systems through experimentally-grounded life cycle assessments (LCAs). The LCAs were conducted mainly based on the self-produced database which is made of the productivity of microalgal biomass measured from pilot-scale photobioreactors and the various physicochemical properties of the resulting biomass (e.g., heating values and cellular composition, etc.). According to the assessments, using the raw fuel appears to provide significant benefits over conventional microalgae-based energy systems, such as various extraction- and hydrothermal treatment-based liquefaction routes, regarding their energy return on investments (EROIs) and net CO<sub>2</sub> emissions in the fuel production processes, thanks to the simple production process and the entire biomass-to-energy nature. We additionally confirmed that for a preferably designed biofuel production layout, EROI of microalgal solid fuel is increased to 2.59 which can compete with that of commercialized woody biomass. This result suggests that the microalgal solid fuel can practically serve as a platform for BECCS thereby contributing to the CO<sub>2</sub> reduction in the near-term with profitability.

Hong, Min Eui

*Korea University*

### **Development of Advanced Microalgae-based Biomass-Biomineralization Hybrid System for Improved Biological CCU Technology**

*\*Min Eui Hong(presenter), Sang Jun Sim (alt. presenter & Corresponding author) - Department of Chemical and Biological Engineering, Korea University \*Won Seok Chang - Research Institute, Korea District Heating Corp.*

Previous studies on microalgae-based CO<sub>2</sub> capture and utilization (CCU) technology have been mainly focused on increasing production of biomass and accumulation of intracellular high-value byproducts over two-stage process. Biomass production in 1-stage directly contributes to providing high-titer CO<sub>2</sub> conversion, but relatively inefficient for the production of secondary metabolites in 2-stage. Herein, we introduce biomass-biomineralization hybrid system for the first time in order to improve CO<sub>2</sub> removal rate during 2-stage via extracellular CaCO<sub>3</sub> (calcite) generation. Two microalgae species of *Neochloris oleoabundans* and *Chlorella vulgaris* were selected to show maximal biomass production in the conditions up to pH 8.0, 40 mM HCO<sub>3</sub><sup>-</sup>, 30–50 μM CaCl<sub>2</sub>, and 4 mM CaCO<sub>3</sub>, and were very CaCO<sub>3</sub>-friendly to facilitate extracellular CaCO<sub>3</sub> production. Two strains were examined by comparing performance on the production of biomass, lipid and calcite. Fully-grown cell biomass (1.25 g/L) in 1-stage was used along with 50 μM of CaCl<sub>2</sub> to induce both lipid accumulation and photosynthetic biomineralization without direct exchange of old medium into N-depleted medium. As a result, during the second induction stage, the productivities of biomass (inductive growth), lipid and CaCO<sub>3</sub> in *N. oleoabundans* and *C. vulgaris* were 0.32 and 0.31 g/L/day for biomass, 0.45 and 0.35 g/L/day for calcite, 145 and 140 mg/L/day for lipid in the range of pH7.4–7.9, respectively. In particular, lipid productivity of each cell was increased by 93% and 100% to stimulation of inductive growth and lipid accumulation compared to control cells induced in the absence of Ca<sup>2+</sup> supplementation, respectively. Eventually, during the whole culture period (10 days), total CO<sub>2</sub> removal rates of *N. oleoabundans* and *C. vulgaris* were increased by 52% (0.81 gCO<sub>2</sub>/L/day) and 44% (0.64 gCO<sub>2</sub>/L/day) via Biomass-Biomineralization hybrid system compared to control cells cultured without Ca<sup>2+</sup> supplement, respectively. CaCO<sub>3</sub> is normally used as an excipient in pharmaceutical industry, in this case, the combined dried mixture of omega3-enriched biomass and CaCO<sub>3</sub> will be consumed as the functional feed ingredients for livestock like pig and poultry. The Biomass-Biomineralization hybrid system could be a reliable strategy to improve CO<sub>2</sub> removal capacity for the photobioreactor-driven cultivation system using microalgae in the future.

Quinn, J.C.

*Colorado State University*

**Economic Viability of Multiple Algal Biorefining Pathways and the Impact of Public Policies**

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This study makes a holistic comparison between multiple algal biofuel pathways and examines the impact of co-products and methods assumptions on the economic viability of algal systems. Engineering process models for multiple production pathways were evaluated using techno-economic analysis (TEA). These pathways included baseline hydrothermal liquefaction (HTL), protein extraction with HTL, fractionation into high-value chemicals and fuels, and a small-scale first-of-a-kind plant coupled with a wastewater treatment facility. The impact on economic results from policy scenarios was then examined. The type of depreciation scheme was shown to be irrelevant for durations less than 9 years, while short-term subsidies were found to capture 50% of the subsidy value in 6 years, and 75% in 12 years. Carbon prices can decrease fuel costs as seen by the production facility through carbon capture credits. TEA tradeoff assessments determined that \$7.3 of capital costs are equivalent to \$1 yr<sup>-1</sup> of operational costs for baseline economic assumptions. Comparison of algal fuels to corn and cellulosic ethanol demonstrates the need for significant co-product credits to offset high algal capital costs. Higher value co-products were shown to be required for algal fuel economic viability.

Quinn, Jason

*Colorado State University*

**Environmental Impacts of Producing BioFuels from Long-Line Cultivated Macroalgae**

*Jonah Greene<sup>1</sup>, Michael Huessemann<sup>2</sup>, Thomas Mumford<sup>3</sup>, Jascha Gulden<sup>4</sup>, Jason C. Quinn<sup>1</sup>* *1Colorado State University 2Pacific Northwest National Laboratory 3Reliance LLC 4Marin Agronomics*

Marine macroalgae holds the potential to provide large amounts of renewable energy in the form of biofuels. With emerging technologies, it is possible to construct cultivation systems that can withstand natural forces and produce biomass in the open ocean, eliminating the need for farmland and irrigation systems that consume fresh water. This work evaluates a system that would launch seeded carbon fiber long-lines off the coast of Washington and retrieve them 3 months later off the coast of California. Advantages of this system include limited infrastructure requirements for the cultivation system and minimal energy and nutrient inputs. Through Life Cycle modeling, the environmental impacts of this system quantified through greenhouse gas emissions can be determined and compared to existing fuel production systems. Foundational assumptions include a biomass yield of 2 dry tonnes per km of line over the 135 day growth period, carbon fiber growth lines 5 km in length, an average harvest retrieval distance of 200 km from shore, biomass harvested at 10% solids, and a biomass conversion efficiency of 30% to bio-crude through thermochemical processing. Alternative technologies are investigated for comparison including the incorporation of autonomous drone vessels and adhesive seeding techniques.

**Erturk, Berrak**

***Montana State University***

**Sodium Bicarbonate Amendment for Enhanced Astaxanthin Production from *Haematococcus pluvialis***

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*Haematococcus pluvialis* is a freshwater green microalga which is widely considered to be the best natural source for high value keto-carotenoid astaxanthin. Under certain environmental stress conditions *H. pluvialis* starts to accumulate astaxanthin up to 4% (w/w) on a dry weight basis. It is known that *H. pluvialis* stores astaxanthin inside lipid bodies as esters. Previous studies have shown that the addition of high concentration bicarbonate salts near nitrogen depletion immediately ceased cellular replication and resulted in high lipid content. Combining these observations, we hypothesized that a bicarbonate amendment near nitrogen depletion would also enhance the production of high value compounds found in microalgae. In this study, *H. pluvialis* was investigated for growth and astaxanthin accumulation with sodium bicarbonate amendment. *H. pluvialis* (UTEX 2505) was cultured in stirred flask reactors containing MES-Volvox medium continuously shaken at 120 rpm with a 14:10 light dark cycle. Different gas applications were used to ensure a sufficient carbon delivery as carbon dioxide. In a second phase, the cultures were triggered with 50mM sodium bicarbonate for enhanced astaxanthin accumulation. Cultures with bicarbonate addition responded immediately with higher astaxanthin content compared to controls. Results indicated that astaxanthin content of bicarbonate treated cultures was 2.53 times higher than controls, and gave an overall reduced cell mortality, indicating that sodium bicarbonate amendment could improve astaxanthin productivity significantly.

Du, Niu

*Phitec, LLC*

### **Simultaneous quantum yield measurements of carbon uptake and oxygen evolution in microalgal cultures**

*Niu Du*<sup>1,2</sup>, *Pardis Gholami*<sup>1,2</sup>, *David I. Kline*<sup>1,3</sup>, *Christopher L. DuPont*<sup>2</sup>, *Andrew G. Dickson*<sup>1</sup>, *Dominick Mendola*<sup>1</sup>, *Todd Martz*<sup>1</sup>, *Andrew E. Allen*<sup>1,2</sup>, *B. Greg Mitchell*<sup>1</sup> 1. *Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093* 2. *J. Craig Venter Institute, San Diego, La Jolla, CA 92037* 3. *Smithsonian Tropical Research Institute, Apartado 0843-03092, 36 Balboa, Ancon, Republic of Panama*

The photosynthetic quantum yield ( $\phi$ ), defined as carbon fixed or oxygen evolved per unit of light absorbed, is a fundamental but rarely determined biophysical parameter. A method of estimating  $\phi$  for both carbon uptake and oxygen evolution simultaneously can provide significant insight into energy and mass fluxes. Here we present details for a novel system that allows quantification of carbon fluxes using pH oscillation (pHOS) and simultaneous oxygen fluxes by integration with a membrane inlet mass spectrometer (MIMS). The pHOS system was validated using *Phaeodactylum tricornutum* (CCMP 2561) cultured with continuous illumination of 110  $\mu\text{mole quanta m}^{-2} \text{s}^{-1}$  at 25°C. Furthermore, simultaneous measurements of carbon and oxygen flux using the pHOS-MIMS and photon flux based on spectral absorption were carried out to explore the kinetics of  $\phi$  in *P. tricornutum* during its acclimation from low to high light (40 to 750  $\mu\text{mole quanta m}^{-2} \text{s}^{-1}$ ). Comparing 0 and 24 hours we observed strong decreases in, cellular chlorophyll a (Chla, 0.58 to 0.21  $\text{pg cell}^{-1}$ ), Fv/Fm (0.71 to 0.59) and maximum  $\phi_{\text{CO}_2}$  (0.019 to 0.004) and  $\phi_{\text{O}_2}$  (0.028 to 0.007), confirming the transition toward high light acclimation. The  $\phi$  time-series indicated a non-synchronized acclimation response between carbon uptake and oxygen evolution, which has been previously inferred based on transcriptomic changes for a similar experimental design without physiological data. The integrated pHOS-MIMS system can provide simultaneous carbon and oxygen measurements accurately, and at the time-resolution required to provide high-resolution carbon and oxygen physiological dynamics.

Zhang, Xuezhi

*Institute of Hydrobiology, Chinese Academy of Sciences*

**A novel low cost microalgal harvesting technique with coagulant recovery and recycling**

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Coagulation based microalgal harvesting has been widely used in the biomass production. However, the contamination of harvested biomass coagulant and the separated media limited its application for the production of microalgal feedstock for food and feed. Moreover, the cost of coagulant limited its application for the biofuel production. In this study, a novel low cost and sustainable microalgal harvesting technique was developed using the concept of coagulant recovery concentration and recycling. Al<sup>3+</sup> can be recovered from harvested *Scenedesmus acuminatus* biomass with 0.1 M HCl, at an acid solution-biomass ratio of 250 ml g<sup>-1</sup>. The residual Al<sup>3+</sup> content in the purified biomass was reduced to 0.11 ± 0.0006 mg g<sup>-1</sup>, while a much higher content of 59.74 ± 3.11 mg g<sup>-1</sup> was found in the coagulation harvested biomass. The recovered Al<sup>3+</sup> solution was concentrated 25 times by alkaline sedimentation and then reused for the harvesting of *S. acuminatus*. The Al<sup>3+</sup> recovery and reuse were repeated 5 times, and the harvesting efficiencies were found higher than the fresh Al<sup>3+</sup> as a result of the presence of extracellular polymeric substances in the recovered coagulant solution which aided the coagulation process. According to the technical-economic analysis, the cost of chemicals decreased 50% after 5 times recycling, this coagulant recovery and recycling technique enables reuse of coagulant and purification of biomass, which makes a leap forward for low cost and sustainable microalgal harvesting.

Rickman, William

*TSD Management Associates*

**Zobi Harvester Results from Large-scale, Continuous Operations in Commercial Algae Production Facilities**

*William Rickman, TSD Management Associates David K Hazlebeck and David A Hazlebeck, Global Algae Innovations*

The Zobi harvester® is a major breakthrough in algae harvesting and dewatering that was announced at a previous algae biomass summit. The technology attains the capture efficiency of membrane systems and dewatering level of centrifuges. The energy use is 1/10th to 1/30th the energy use of conventional crossflow membrane or dissolved air floatation systems and 1/100th the energy use of typical centrifuges. The process does not utilize any flocculants, and the system has very low maintenance and control requirements. The capture efficiency is 100%, so the permeate is crystal clear. Many species of cyanobacteria, green, red, and diatom algae have been harvested and dewatered using the technology, so the Zobi® system provides a universal solution to algal harvesting and dewatering. There are now large-scale Zobi® systems being utilized in continuous harvesting and dewatering for commercial algae production - including systems with throughputs of one to two million gallons per day. This presentation will cover operational data and a discussion of the results and lessons-learned from these initial large-scale commercial installations of the Zobi® technology.

**Vaeth, Hans**

***Algoliner GmbH & Co. KG***

**New Method to Realize Horizontal Photo Bioreactors by Using Hanging Profiles Which are Produced on Site.**

*Hans Vaeth, founder and CEO of Algoliner GmbH & Co. KG. Hans has a degree in engineering and business administration. He has 30 years' experience in the field of mass production, especially of parts made out of polymeric materials.*

Beside OPEX the CAPEX is an important issue to make algae cultivation profitable. Therefore Algoliner developed a revolutionary new method to realize horizontal PBRs. Instead of using pipe sections which will be put together with joints, the pipes are being produced on site in any required length. Additional to this we are hanging the pipes instead of laying them on racks. Consequently the walls of the pipes are much thinner which saves about 50% of the raw material. The pipes will be placed immediately after their production into their final position by using the same staff which is in charge of the pipe production itself. All those advantages lead to much lower costs compared to all other methods to realize PBRs. However we are using polymeric raw materials which have the best UV stability and scratch behaviour of all available transparent materials on the market.

Deliismail, Ozgun

*Izmir Institute of Technology*

### **Biofuels from Microalgae Without Dewatering**

*Erol SEKER, Izmir Institute of Technology Ozgun DELIISMAIL, Izmir Institute of Technology Bertan OZDOGRU, Izmir Institute of Technology*

Aquatic biomass has been regarded as the next generation path to the production of biofuels. Marine microalgae is very viable feedstock for economical and sustainable fuel production, since they do not require any landfill or fresh water and could sequester carbon dioxide. In the production of biofuels, most common way is to increase the weight percent of microalgae biomass in water from 3-10 wt.% to 20 wt.%. Unfortunately, this way of microalgae harvesting and dewatering are cost/energy intensive processes which results in high OPEX and CAPEX. After harvesting/dewatering steps, pyrolysis and liquefaction are used to convert microalgae to biofuel. Pyrolysis generally works with dry microalgae at 400-600 °C under atmospheric pressure, while hydrothermal liquefaction requires sub/super-critical water to produce biocrude oil. The requirements of high temperature and pressure make these biofuel production processes unviable for continuous large-scale biofuel production. Another alternative common process is sulfuric acid treatment to disrupt algal cell wall by hydrolysis and breaking carbohydrates to monosaccharides. But sulfuric acid is not reusable and also corrosive. The key challenge is to find economical and applicable way to produce biofuel/oil from microalgae. The conversion of 3-10 wt.% marine microalgae over heterogeneous catalysts into hydrocarbons/saccharides under atmospheric pressure at relatively low temperature, such as <90 °C, would eliminate harvesting/dewatering steps. To the best of our knowledge, there are no studies in the literature on conversion of 3-10 wt.% marine microalgae into hydrocarbons/saccharides over heterogeneous catalysts under atmospheric pressure at 80 °C. In this study, 6 wt.% *Nannochloropsis oculata* in sea water was used to obtain hydrocarbons/saccharides at 80 °C and 1 atm over single step sol-gel made 30% Al<sub>2</sub>O<sub>3</sub>-70% SiO<sub>2</sub> supported 10% Ni catalysts. The highest *N. oculata* conversion, 86.5 %, were observed on the catalyst prepared with H<sub>2</sub>SO<sub>4</sub>. The product distribution consisted of various types of esters, triglycerides, mono/poly-saccharides consisting mainly glucose, and hydrocarbons ranging from C<sub>6</sub> to C<sub>30</sub>.

Deliismail, Ozgun

*Izmir Institute of Technology*

### **Novel Hybrid Process to Produce Bio-based Ethylene for Petrochemical Industry Through Digestion and Oxidative Coupling Units**

*Erol SEKER, Izmir Institute of Technology Ozgun DELIISMAIL, Izmir Institute of Technology Okan AKIN, Izmir Institute of Technology*

The conventional production process of ethylene uses naphtha as feedstock and then, it is used in many processes to produce variety of products as HDPE, and LDPE. Unfortunately, consumer products based on petroleum polymers are not biodegradable; i.e. they will not decompose naturally in the environment which results in air, soil and water pollutions. Even if they are incinerated, this will create another environmental concern, such as emission of CO<sub>2</sub> and NO<sub>x</sub>. The key challenge is hereby to develop alternative processes using feedstock other than petroleum to achieve sustainable production. In fact, marine microalgae is viable choice because they do not require fresh water and energy during growth, and they also use CO<sub>2</sub> as carbon source during growth, so CO<sub>2</sub> emitted from coal power plants or incineration of biopolymers, could be used to grow marine microalgae; thus, resulting almost zero net CO<sub>2</sub> emission in the whole cycle from microalgae to bio-ethylene to biopolymers. Thermochemical and biochemical conversion are the main methods to convert algal aqua-biomass to chemicals. Thermochemical methods are energy and cost intensive processes due to high temperature and sub-/supercritical water requirements. In contrast, biochemical conversion can be more feasible since it does not require any pretreatment steps, such as lipid extraction, and harvesting. Thus, the project involves preliminary conceptual design and simulation of hybrid process for bio-ethylene production. To the best of our knowledge, there is no process simulation on bio-ethylene production from microalgae using UniSIM. Microalgae conversion to bio-ethylene was achieved through a path in which methane was first synthesized in a digester and then, oxidative coupling of methane (OCM) was carried out to produce bio-ethylene. ADM1 model was used for anaerobic digestion of microalgae, and unit operation in UniSIM was linked to MATLAB for dynamic simulation of anaerobic digestion to produce CH<sub>4</sub>. Also, since UniSIM has a feature of registering OLE code written in Visual Basic environment, we used this feature to write extension for OCM kinetics. The plant layout was designed with the consideration of heat integration between streams to economize the process. In addition, the chase flow analysis was applied to find out the profitability of bio-ethylene production using marine microalgae.

**Kuo, Chih-Ting**

*Illinois Sustainable Technology Center*

**Techno-Economic Analysis of Large Scale (100 MGD) LED Enhanced Fixed-film Algal Wastewater Treatment System for Phosphorus Removal**

*Chih-Ting Kuo, Illinois Sustainable Technology Center, University of Illinois at Urbana-Champaign Lance Schideman, Illinois Sustainable Technology Center, University of Illinois at Urbana-Champaign*

The increasing use of nitrogen (N) and phosphorus (P) in industry and agriculture as well as increased protein consumption in the human diet has led to greater loading of N & P nutrients to municipal wastewater and to the aquatic environment. This has also led to other undesirable consequences, such as eutrophication and hypoxia, as excess N & P from municipal wastewater, industries, fertilizer, and animal manures eventually enter surface waters. One promising approach to enhance nutrient removal at wastewater treatment plants is to encourage the growth of photosynthetic algal biomass, which can continue to uptake nutrients after organic carbon is depleted. As a result, it is possible to remove nutrients to much lower levels with biological processes that include algal biomass. We have developed and tested a bench-scale LED enhanced fixed-film algal wastewater treatment for secondary wastewater at the local wastewater treatment plant. The system was seeded with mixed culture algae harvested locally and incorporated submerged LED tubes, floatable plastic media and regenerable adsorbents. These features allow the system constantly removes the nutrients in the water at the much lower HRT compares to other algal treatment systems. The results showed that the system is able to reduce the ortho phosphorus concentration from 2 mg/L to below 0.5 mg/L at 6 hour HRT. While feeding with 1% (v/v) CO<sub>2</sub>, the P removal was further increased by 10%. A photosynthetic efficiency of 16% was achieved when the lighting intensity was 720 mol/m<sup>2</sup>-s and flashing at 100 Hz. A techno-economic analysis of the LED enhanced algal wastewater treatment system for large scale (100 MGD) plant was conducted. The results showed that the phosphorus removal cost for the system is \$29.4/lb-P removed and \$20.3/lb if selling the algal biomass as animal feed. This P removal cost is competitive with other biological removal technologies.

**Dayrell, Ivan**

***Alcom***

**Integrated System to Produce Algae Biomass**

*Alcom Combustiveis Alternativos Ltda*

Alcom has designed a new system to produce Algae biomass deployed at sea. The system is composed of a new type of photobioreactor, an integrated dewatering system and a CO<sub>2</sub> extractor that recovers CO<sub>2</sub> from the sea water. The photobioreactor is hydraulic powered and features distributed pumping, distributed nutrient dispersal and precise control of the CO<sub>2</sub> concentration throughout the reactor. The reactor is made of positive buoyant plastics and can be submerged to a safe depth to escape severe weather. The algae remain fully contained at all times: in case of a leak the reactor automatically seals the leaking section and opens a bypass to allow operations to continue. Faults are reported to a central monitoring system and faulty units are identified in the field to facilitate the work of maintenance teams. Capital and operational costs are minimized allowing the production of Algae Biomass at an estimated cost lower than U\$0,50 / kg.

**Zaglio, Francesco**

***Zaglio S.r.l.***

**Algae Improvement Production Efficiency**

*Prof. Ing. Enrico Zaglio*

Zaglio S.r.l. Italy developed an innovative System that allow to encrease the algae production both for open pond and bioreactors of 4 times more than the normal. We developed an innovative system with optoelectronic technology with a magnetic integrated system. The single unit can worz for 1square meter. The cost of one unit is very low.

**Blaylock, Myra**

***Sandia National Labs***

**Effects of Viscosity on Turbulence Mixing in Pilot Scale Open Raceway Ponds**

*Myra Blaylock, Eric Monroe, Ryan Davis Sandia National Laboratories, Livermore, CA 94550, USA Carlos Quiroz-Arita Mechanical Engineering, Colorado State University, Fort Collins, CO 80524, USA*

One of the fundamental assumptions for most algae growth models is that the medium is well mixed so that the algae experience the same nutrient conditions everywhere and the concentration of algae is also homogeneous throughout. While this is a good first order assumption, it is likely that for some scenarios (larger ponds, slower mixing velocities, etc.) that this might not be the case. One example where this assumption might not hold is when the algae has a very high viscosity. We have measured the viscosity of a cyanobacteria consortium that produces extra cellular polymeric sugars which produces a very large dynamic viscosity (up to 10 mPa-s, which is ~10 times more viscous than water) through-out one growing cycle. Velocity measurements for the pilot scale open raceway pond used to grow this cyanobacterium were made for the conditions at the beginning (low viscosity) and at the end (high viscosity) of the cycle. We compare the velocity and the turbulence for these two conditions, which give insight into the well mixed assumption. To further understand how the viscosity affects the mixing, a 3D computational fluid dynamics (CFD) model is run with the two viscosities implemented. The experimental data is used to validate these simulations. Other secondary topic: Modeling and Simulation

Kolapalli, Jayachandra

*The University of Toledo*

### **Microalgae Cultivation in Alkaline Solutions Supplemented with Borate Promoter for Enhanced CO<sub>2</sub> Capture**

1) Jayachandra Kolapalli\* *Chemical Engineering, University of Toledo* 2) Agasteswar Vadlamani *Chemical Engineering, University of Toledo* 3) Sridhar Viamajala *Chemical Engineering, University of Toledo* 4) Sasidhar Varanasi *Chemical Engineering, University of Toledo*

A major barrier to economic viability of microalgae biofuels is the high cost of concentrated CO<sub>2</sub> supply cost. Furthermore, global algae biofuel generation would be seriously restricted if micro-algae farms are restricted to the vicinity of CO<sub>2</sub> sources along with the additional limitations of low slope lands and favorable climate. One potential solution to decrease CO<sub>2</sub>-related costs is to cultivate microalgae cultures in high pH media (pH >10) that can uptake ambient/atmospheric CO<sub>2</sub> at high rates. In addition, adding high concentrations of carbonate/bicarbonate to the media (i.e. media with high carbonate alkalinity) provides high dissolved inorganic carbon (DIC) for photosynthesis. The presence of rate promoters like boric acid further aids in CO<sub>2</sub> hydration and absorption and can lower the requirements for physical mass transfer and mixing costs in high pH media. In this study, we report results from studies that compare rates of DIC uptake in borate-promoted and promoter-free alkaline solutions exposed to the atmosphere. The experiments were set up by maintaining constant system parameters including the initial pH and total alkalinity in both the systems. Along with the initial and final DIC content, pH was continuously measured until both the system reached equilibrium with the atmosphere. A mathematical model, developed from chemical equilibria first principles, was fit to the measured pH values to determine the rate of DIC increase, mass transfer coefficients and the enhancement factor. Our results showed that the rate of DIC increase in the borate promoted systems was at least 50% higher than the non-promoted carbonate/bicarbonate solutions. Our ongoing studies are investigating the cultivation of the alkaliphilic *Chlorella sorokiniana* str SLA-04 in borate promoted carbonate/bicarbonate systems to achieve high biomass productivity without concentrated CO<sub>2</sub> inputs.

Le Thai, Hang

*University of Yamanashi*

### **Effects of Sodium Chloride and Calcium Chloride on the Lipid Productivity of Microalgae**

*Hang Thai Le\* - Integrated Graduate School of Medicine, Engineering and Agricultural Sciences, University of Yamanashi, Japan. Tadashi Toyama - Graduate Faculty of Interdisciplinary Research, University of Yamanashi, Japan. Kazuhiro Mori - Graduate Faculty of Interdisciplinary Research, University of Yamanashi, Japan.*

Microalgal biomass are considered as renewable and sustainable biofuel. Microalgae have a high lipid production ability and accumulate lipid in their biomass, which is the main source for biofuel. Enhancement of microalgal lipid productivity is critical to achieving a highly efficient microalgal biofuel production system. In this study, our objectives were to understand the effects of Sodium Chloride (NaCl) and Calcium Chloride (CaCl<sub>2</sub>) on microalgal lipid productivity. We used two freshwater microalgae: *Chlamydomonas reinhardtii* and *Chlorella sorokiniana*. We cultured each microalgae in C medium and wastewater supplemented with NaCl/CaCl<sub>2</sub> concentration at 0.005, 0.010, 0.020, 0.040 M in 3 days. Lipid content in the microalgal biomass was increased up to 20% in the presence of NaCl/CaCl<sub>2</sub>. The results demonstrate that the use of NaCl/CaCl<sub>2</sub> in microalgal culture can improve the lipid productivity.

Luzzi, Siane

*University of Minnesota*

### **Algae Biomass Production Using Dairy Wastewater for Livestock Feed Application**

*Authors: Siane C. Luzzi<sup>1</sup>, Robert D. Gardner\*<sup>1</sup>, Bradley J. Heins<sup>2</sup> Institutions: <sup>1</sup>University of Minnesota, St. Paul, MN, USA; Department of Bioproducts and Biosystems Engineering; University of Minnesota, St. Paul, MN, USA; Department of Animal Science.*

The removal of nutrients – in particular N and P – from wastewater is a growing regulatory need, and the use of algae may create a unique combination between dairy wastewater treatment and livestock feed application. This study aims to identify a wastewater dilution capable of producing considerable amounts of algae biomass and also to determine if additional CO<sub>2</sub> influences algal biomass productivity. The reactions described in this study occurred in hanging bags of 70L. The treatments were: control (only AM6 media and water), 1:30 (2.33L of wastewater and 67.66L of water) and 1:10 (7L of wastewater and 63L of water), and they were made in duplicates. The algae strain used in this study was isolated from the same wastewater lagoon where the wastewater for the dilutions was collected. Temperature and pH were monitored using an Apex system. Daily samples were collected from day 0 to 6 for analysis of cell growth, biomass, ammonium, and nitrate. Harvest occurred on day 4 for 1:30 and on day 6 for the control and 1:10. The biomass was centrifuged for 2 min at 6500xg. The statistical analysis was performed using SAS 9.4. Cell growth was significantly higher ( $p < 0.05$ ) on days 5 and 6 when CO<sub>2</sub> was added. Cell growth was not different between treatments until day 4, when the control showed a higher growth than 1:10 and 1:30. On day 5, the control was only higher than 1:10. For biomass yield, there was no significant difference in adding CO<sub>2</sub>. However, when comparing dilutions, 1:10 showed a significantly lower biomass yield than control on days 0, 1, 4, 5 and, 6. Biomass yield was higher for 1:10 on day 4 compared with 1:30. Bags with CO<sub>2</sub> added produced 333% more algae biomass in control, 14% more in 1:10 and 50% more in 1:30, compared with the bags without additional CO<sub>2</sub>. Ammonium removal rates varied between 67.1% and 94.4% on both treatments (CO<sub>2</sub> added or not). Nitrate removal was higher for 1:30 (more than 90%), followed by 1:10 and control. The 1:10 dilution showed satisfactory algae biomass yield, which may be used for livestock feeding. Biofilm formation was observed, which caused variation in biomass measurements. Furthermore, the nutrient removal rates show that algae are capable of reducing the amount of nutrients in dairy wastewater. Keywords: algae; wastewater; algae biomass production; nutrient removal.

Sabri, Laith

*Missouri University of Science and Technology*

**Investigation of Local gas holdup and bubble dynamics during microalgae culturing in a split airlift photobioreactor**

*Aastha Ojha<sup>1</sup>, Laith S. Sabri<sup>1</sup>, Muthanna H. Al-Dahhan<sup>1,2</sup> aovc4@mst.edu, lssf25@mst.edu, aldahhanm@mst.edu <sup>1</sup>Chemical and Biochemical Engineering Department, Multiphase Flow and Reactors Engineering Applications laboratory (mFReal), Missouri University of Science and Technology, Rolla, MO 65409-1230, USA <sup>2</sup>Cihan University-Erbil, Iraq*

In this study, the variation of local parameters such as local gas holdup and bubble dynamics properties like bubble passage frequency, bubble chord length and velocity, and interfacial area in real culturing have been investigated. These properties were studied inside a split- airlift photobioreactor at superficial gas velocities of 1.0, 2.0, and 2.8 cm/s while culturing microalgae *Scenedesmus* sp. These measurements were made at the radial center of the riser and the downcomer sections of the split airlift photobioreactor along the axial height. The viscosity of the medium was seen to increase with the optical density of the culture, while the surface tension remained the same throughout the experiment. Bubble passage frequency, gas holdup, and interfacial area were seen to increase with an increase in superficial gas velocity, and decrease with an optical density which is related to the growth of microalgae. The bubble chord length and bubble velocity distributions became wider at higher superficial gas velocities and higher optical densities; while no significant axial variation in the bubble properties was observed in the riser, an axial variation in these properties was observed in the downcomer due to a decrease in the number of bubbles descending through the downcomer. New correlations, accounting for the change in optical density as well as superficial gas velocity, to predict the gas holdup in the riser and at different axial locations in the downcomer were also developed.

Sabri, Laith

*Missouri University of Science and Technology*

### **Estimating the Local Volumetric Mass Transfer Coefficient for Microalgae *Scenedesmus* in a Split Airlift Photobioreactor**

*Aastha Ojha*<sup>1</sup>, *Laith S. Sabri*<sup>1</sup>, *Muthanna H. Al-Dahhan*<sup>1,2</sup> [aovc4@mst.edu](mailto:aovc4@mst.edu), [lssf25@mst.edu](mailto:lssf25@mst.edu), [aldahhanm@mst.edu](mailto:aldahhanm@mst.edu) <sup>1</sup>*Chemical and Biochemical Engineering Department, Multiphase Flow and Reactors Engineering Applications laboratory (mFReal), Missouri University of Science and Technology, Rolla, MO 65409-1230, USA* <sup>2</sup>*Cihan University-Erbil, Iraq*

In this study, a plug flow model without axial dispersion was applied to evaluate the liquid-side mass transfer coefficient in a *Scenedesmus* culture grown in a split airlift photobioreactor at superficial gas velocities of 1.0, 2.0, and 2.8 cm/s. At each superficial gas velocity, the liquid-side mass transfer coefficient, assumed to be constant throughout the reactor, was seen to increase with an increase in superficial gas velocity. Using the local interfacial area, local volumetric mass transfer coefficient was also calculated. The local volumetric mass transfer coefficients in the riser and the downcomer were dominated by gas holdup and interfacial area, and were favored by higher superficial gas velocities and lower optical densities of the microalgae culture. The effect of optical density on the liquid side and the local volumetric mass transfer coefficient was analyzed over the growth period divided into three zones; Zone I for optical density up to 0.08, Zone II, optical density between 0.08 and 0.19, and Zone III for optical density between 0.19 and 0.30. While the local volumetric mass transfer coefficient was constant axially in the riser, it decreased axially on moving down the downcomer. The estimated local volumetric mass transfer coefficient was always found to be higher than that estimated from the available correlations.

**Qin, Jennifer**

**Ovivo**

**BioAlgaNyx A Breakthrough Technology for Biosolids Management**

*Jennifer Qin, Ovivo Li Cong, Ovivo Hiren Trivedi, Ovivo*

Majority of current focus on use of algae for wastewater treatment involves photosynthetic algae. However, providing a sustainable light source for photosynthesis at a wastewater treatment plant site and achieving effective phase separation for algae harvesting remain major challenges. To overcome these problems, Ovivo, partnering with University of Akron, has developed a breakthrough solution based on a phagotrophic algal species. The algae can ingest small microbial, viral and other organic particulates through its phagotrophic capability; it can consume dissolved organics through its heterotrophic capability and can also grow with light using the phototrophic capability as their survival mechanism. Digestion forces microorganisms to starve, feed off themselves and subsequently die in absence of any externally available food. United States Environmental Protection Agency has well defined criteria for Class A biosolids which can be reused and Class B biosolids which can be land applied. Aerobic digestion usually aims for reduction of volatile solids, specific oxygen uptake rate and pathogen present in the sludge. Class B biosolids aerobic digestion process is often constrained by long retention time requirement. This phagotrophic algae technology accelerates sludge digestion process to 50% of the retention time. In this approach, sludge is first being pretreated so individual microorganism is released from sludge flocs. Then individual microorganism is fast consumed by phagotrophic algae for growth. Then, after the concentration of these small microorganisms has been rapidly reduced, the phagotrophic algae themselves are starved. Being eukaryotic without cell walls, to help them maintain cell integrity the phagotrophic algae require much higher energy. Hence, in the dark digestion processes, they quickly die off. The combined sequence of destroying the small microorganisms including pathogens in sludge followed by the rapid death of phagotrophic algae, leads to faster volatile solids (VS) reduction and earlier attainment of treatment objectives. The commercial benefits of this technology is attaining sludge treatment objective with less retention time and less energy.

**Dong, Tao**

***National Renewable Energy Laboratory***

**Fully Renewable Polyurethane Produced from Microalgal Lipids and Amino Acids-derived Diamines**

*Tao Dong 1, Lieve Laurens 1, Ivan Javni 2, Olivera Bilic 2, Philip T. Pienkos 1 1. National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401 2. Kansas Polymer Research Center, Pittsburg State University, Pittsburg, KS 66762*

The bio-based polymer markets are influenced heavily by increasing preference for eco-friendly and sustainable solutions. At NREL, we have begun to develop a series of novel polyurethanes that are polymerized from polyamines and carbonated fatty acids to eliminate the highly toxic isocyanate-based crosslinkers. These non-isocyanate polyurethane (NIPU) replacements have a sustainability advantage and are predicted to exhibit performance improvements for large-scale applications. Microalgae are rich in polyunsaturated fatty acids (PUFAs) which are preferred to produce polyurethane foams. Novel biopolymers from a fraction of the lipid stream can dramatically reduce the modeled minimal fuel selling price of algal biofuels. Bio-based polyamines, such as 1,4-butanediamine (BDA) and 1,5-pentanediamine (PDA), can be produced from waste protein streams, which are generated in substantial amounts as agricultural waste, and in the future, as by-products from biofuel production using such feedstocks as algal biomass. These diamines can react with other amino acids to produce novel polyamines via peptide bonds. We have demonstrated the use of amino acid and peptide-derived polymer crosslinkers for NIPU production. In this research three oil samples extracted from different algal biomass were used to produce carbonated lipids via epoxidation and carbonation. The carbonated oil was used to react with bio-derived diamine crosslinkers to produce novel NIPU products. The properties of the algae-derived NIPU products were characterized and will be presented.

Rue-Zusman, Zabrina

*University of California Berkeley*

**Closing the Loop on Algae Biomass Production Using Biofilm Cultivation for Water Remediation**

*Zabrina Rue-Zusman ; University of California Berkeley*

Algae biofuels and coproducts have the potential to revolutionize green industries. From renewable energy to sustainable materials, algae's value chain is multifunctional and cutting-edge. Commercialization is limited by conventional methods for growing and harvesting algae, preventing industrial operations from generating profits and competing with fossil fuels. This project reviewed the potential of co-locating algae farms with waste streams to integrate phycoremediation with biofilm cultivation. A techno-economic analysis of different immobilized growing systems was performed to identify the most cost-effective way to scale up production. The findings suggest that collaborating with municipalities and upgrading suspended algal wastewater treatment basins with heterogeneous attached-media filtration systems can drastically reduce the baseline cost of biofuel production by 70%. This project demonstrates how Alga-culture can resolve multiple environmental problems at once in a process that is not only sustainable, but has a minimally disruptive environmental impact.

**Pankratz, Stan**

*University of Alberta*

**The Economics of Producing Algae Biomass in Canada for Biofuels Via Open Pond Raceways and Photo-bioreactors**

*Stan Pankratz, University of Alberta Adetoyese Olajire Oyedun, University of Alberta Amit Kumar, University of Alberta*

Research into the potential of using microalgae for production of biofuels continues amid studies that conclude that this is unsustainable under the majority of conventional cultivation practices both from a techno-economic analysis (TEAs) perspective. To overcome this challenge, more recent TEAs consider a biorefinery approach. In Canada, climatic factors exacerbate this challenge limiting cultivation in open raceway ponds (ORPs) to periods of the year when pond surface water temperatures and ambient light conditions enable optimal culture growth. This presentation predicts, evaluates, and compares the TEA results from three autotrophic algae cultivation scenarios as part of a platform to produce algae biomass. The first is a modeled ORP site located in the southern US and based on conventional cultivation practices. A second identical ORP system is co-located in at a municipal landfill site near Fort Saskatchewan, AB, and uses flue gas CO<sub>2</sub> from a combined heat and power (CHP) system run on landfill gas and chicken manure for other key algae cultivation nutrients. A third photo-bio-reactor (PBR) cultivation system is modeled as co-located at the same central Alberta site. Each system is scaled to produce 515 tonne day<sup>-1</sup> ash-free dry weight of algae biomass. The results of the study indicate that PBR systems deployed at this scale may achieve significant production cost savings over ORP systems in a similar Canadian siting despite high initial capital costs associated with the PBR construction.

**Kakinada, Niveditha**

**NATIONALCOLLEGE OF IRELAND**

**Bringing Algae into Data Centers**

*Niveditha Kakinada and Sai Guna Ranjan; Emani National College of Ireland*

The main problem faced by both modern and traditional data centres is carbon usage efficiency (CUE) and power usage efficiency. As per a report, over 10 year, data centres will waste over 0.5M in power charges and emit 80% of co2 but the chances of increasing the percentage in coming years are more. Thus creating Data centre a Villain- Destroyer of Earth. This paper illustrates and explains about the new Algae concept for carbon usage efficiency and power usage efficiency. Algae unicellular microorganism is a billionaire solution for all the current data centres. It can absorb tons of carbon dioxide in few minutes whereas a single tree can't even do in its life time. Algae undergo photosynthetic reaction and gives biogas and biofuel as a by-product. Thus turning CO2 into valuable products lead to significant changes in Data centers. This new Co2 controlling technique to save the environment with the help of algae panels can give us the most profitable business. By Moving to Algae Centered Power Plant(ACPP) Model, we can achieve the sustainability, energy efficiency and global conservation. Data Center Operators are no longer a pure Consumer, but also they are become a Producing Consumer- a Prosumer. The Opportunities to change a date center from the consumer to prosumer are more diverse here. This technology is a pioneer in green algae power and carbon neutrality. Using this algae technology, data centre can change its image from CO2 villain to an Environment Saviour •.

Hasan, Jafrul

*US Environmental Protection Agency*

**Development of the Algae Supplement to the Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms**

*Jafrul Hasan<sup>1</sup>, Gwendolyn McClung<sup>1</sup>, Stephan M. Cameron<sup>2</sup>, Carolina Peñalva-Arana<sup>1</sup>, Khoa Nguyen<sup>1</sup>  
<sup>1</sup>Risk Assessment Division, <sup>2</sup>Chemistry, Economics, and Sustainable Strategies Division, Office of  
Pollution Prevention and Toxics, U.S. Environmental Protection Agency*

Through the Biotechnology Program in OPPT, EPA regulates intergeneric microorganisms intended for commercial use or commercial research and development under the Toxic Substances Control Act (TSCA) which was recently amended by the Frank. R. Lautenberg Chemical Safety for the 21st Century Act. Motivated by a growing number of biotechnology submissions employing genetically engineered (GE) eukaryotic microalgae and cyanobacteria, hereafter referred to as algae and the advent of recent biotechnological developments, EPA acknowledged the need to update its 1997 guidance document, Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms (PtC). Given the recent developments in emerging algal industry for biofuels and bioproducts, EPA decided to first update the PtC specifically for GE algae. EPA solicited input from the regulated community and the public by holding two public meetings, one in 2015 and the other in 2016, regarding data and information that warrant consideration when evaluating potential hazards and exposures of field testing and commercial production of GE algae. Both meetings were followed by a public comment period. Using the algae documents developed for both meetings and comments received at both workshops and in their respective dockets, EPA has consolidated the relevant information specifically for GE algae which is now being finalized as the Algae Supplement to the PtC. This information ensures EPA efficiently conducts risk assessments for GE algae notifications. Additionally, simultaneous issuance of the Agency Response to Public Comments on EPA's Draft Algae Guidance for the Preparation of TSCA Biotechnology Submissions is planned. The Algae Supplement addresses these novel microorganisms not previously considered for TSCA applications and the different design and manufacturing systems used for GE algae by expanding on the data and information elements already outlined in the PtC for other microorganisms. Information elements in the Algae Supplement specific for GE algae include potential human health effects from phycotoxins, the potential for formation of harmful algal blooms, the use of outdoor photobioreactors and open ponds, and the associated worker and environmental exposures of GE algae.

Neori, Amir

*Israel Oceanographic & Limnological Research, National Center for Mariculturel*

**Algal Protein and Bio-fuel: Inseparable**

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A large gap develops between demand for protein and supply from agriculture. Mariculture remains the last undeveloped food protein frontier, which will inevitably expand to the scale of agriculture, on the High Seas and on coastal deserts. Algal biomass for food, feed and bio-fuels, as well as for the higher-value products of phyco-colloids, cosmetics and specialty bio-chemicals, can be produced without arable land, freshwater or fertilizer. Mariculture production growth by orders of magnitude will happen with minimal engineering, by just helping nature do the work. Its current growth rate, a doubling/decade, will bring the seaweed production to 8 billion ton in 2100 (!). Micro-algae can do the same in 'green water'. As agriculture lags behind the demand, biomass prices should rise, and with them the economic drive for the production of billions tons of algal biomass. Furthermore, it is argued here, that production of algae for food and for fuel may depend on each other. Only the bio-fuel market is large enough to absorb the uneaten fractions of billion-ton quantities of algal biomass for protein, and only the revenues from algal food- protein can make seaweed bio-fuel economically-competitive with other renewable energy options, benefiting from the economy of scale.

**Blume, David**

***Blume Distillation***

**BioFuel and Diverse High-return Co-products Biorefined from Localized Algae and Regenerative Ag Systems**

*David Blume - Blume Distillation Author of the Critically acclaimed book "Alcohol Can Be A Gas!"*

BioFuel and High-Return Co-Products Produced from Algae: Leveraging new programs to produce fuel and hi-protein animal feed from Algae and regenerative Ag production. Appropriate scale alcohol fuel is an inexpensive to produce commodity and a channel for high-value high-return co-product development. The keys to profitability in a well run small-scale alcohol operation are getting good margins, and producing value-added co-products. These co-products are actually worth much more than the fuel itself and provide income stability when alcohol or feedstock prices are volatile. This talk provides detailed and unique insight into the design and development of lucrative new products related to fuel production as well as myriad non-exportable jobs related to development and distribution for small-scale biofuel businesses and presents proven methods for market development and communications. The presentation will be based on interactive discussion and MultiMedia PowerPoint support. Starting from a strong case assertion that Millions of non-exportable jobs can be generated through proper application of biofuel production. Subtopic focuses will be on: 1) Agriculture (looking at Algae innovation and regenerative agricultural process that support best practices appropriate scale biofuel development 2) Production technology including Aquaponics and non-farmland harvesting, 3) Distribution development Understanding the value of appropriate scale biofuel production Defining the myriad co-products and jobs derived from this fuel production methodology Gaining insight into new market channel and outlet development for biofuel and co-products Suited to Ag market, finance agencies, biofuel production engineers, biofuel contractors, Government Agencies Appropriate scale biofuel production provides a means of optimizing local resources and repurposing waste products to deliver sustainable localized resources

Zhou, Wenguang

*Nanchang University*

### **Life Cycle Assessment of Industrial Scale Production of Spirulina Tablets in China**

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*Spirulina platensis* has been successfully commercialized as functional food ingredients, animal feed and medicine due to its high contents of protein, beta-carotene, vitamins, and minerals. In this study, we investigated the environmental performance (cradle-to-gate) of edible *Spirulina* tablets using Life Cycle Assessment (LCA). A comparative analysis with other three traditional foods or diets was conducted by using various nutrient values as functional units (e.g., protein content and a composite nutrient score) in the analysis. This research showed that *Spirulina* tablets production for protein caused environmental impacts mainly in fossil fuel use, acidification, climate change, smog formation, and eutrophication. The impact of the cultivation stage was the highest environmental impacts among all production stages resulting from the extensive use of chemicals, nutrients, and energy. The impacts of algae food production are around 2-5 times to algae production for biofuels. In terms of protein production, algae tablet cause higher impacts than traditional terrestrial crops but lower impacts than protein from animal products. However, as the algae contain a wide variety of nutrients, especially high micronutrients such as the beta-carotene, the environmental impacts of producing the same nutrient combinations of protein and beta-carotene from carrot + tofu were higher than producing *Spirulina* tablets. The results in this work can be used to assess edible algae production inventories and provide reliable information for development of more sustainable products and processes.

**Itsygin, Simon**

***Czar Salt***

**New Products with Algae-Derived Substances for Foods and Wellness**

*Simon Itsygin, Ph.D Czar Salt, San Diego, CA, USA*

Recent publications show the increasing use of algae-derived products as additives to foods and essential substances in dietary supplements. A list of algae-derived products available on the market is presented, including Astaxanthin, Phycocyanin, Omega-3 fatty acids, etc. Comparison analysis of the products is carried out and shown. There are many expensive dietary supplements and few foods with algae-derived products. Sales volume of such foods is still not sufficient. Original, new healthy foods are developed. These foods are combinations of sea salts with algae products. Certified organic sea salts with sodium chloride concentration (85-95%) are the basic components of the foods. The lower sodium concentration decreases fluid retention in the human body allowing for blood pressure and swelling reduction. These salts are harvested and manually produced in an ecologically protected area, and don't contain plastics or other poisonous substances. An innovative method was suggested for the sea salts enrichment with algae and other natural sources. Final salt products (branded as Czar Salt) are listed. These new products contain beneficial substances integrated into salt crystals, resulting in vivid colors and high stability. Infused into salts crystals are healthy, natural ingredients (vitamins, minerals, carbohydrates, proteins, flavonoids, oils, etc.) that partly or completely cover the human demand for these substances. Benefits come with the daily consumption of salt enriched with the healthy nutrients. Health benefits of Czar Salt products include supporting the immune system, maintaining a healthy circulatory system, managing blood pressure and cholesterol levels, improving digestion, vision, skin and bone health, enhancing cognitive functions, etc. Samples of 3 sea salts with the algae derived- products (Astaxanthin, Phycocyanin and Seaweed) are presented in the Algae Product Showcase and lunch buffet. Further development prospects of the innovative foods with algae are discussed.

Dach, Baruch

*AlgaeMor*

### **Overcoming Industrialization Challenges in Biotech**

*Baruch Dach, CTO and co-founder, AlgaeMor*

Industrialization has been one of the highest hurdles to overcome since the beginning of algae production, mostly in aspects of scale, stability and cost effectiveness. Looking at the basic principle of industrialization, which is transitioning to new technology based manufacturing processes, reveals the heart of the problem: algae experts get very uncomfortable when it comes to speaking of control and automation, while engineers have lots of missing pieces in the understanding of algae behavior, biology and physiology. Adding dynamic factors like environmental conditions, water quality, contaminating agents and strain specific constraints increases complexity of the required functional design of both cultivation and downstream processes, and makes the gap even larger. In fact, this issue is very common throughout the entire biotech sector, whether it is fish farming, bio-pharmaceutical production, water treatment or integration of new food ingredients or technologies into traditional lines. AlgaeMor has struggled with automation and industrial control scale up. We worked with an external control system vendor for over 3 months, trying and failing to communicate our needs effectively across the science-to-engineering discipline. I found a solution in a serendipitous way, completely by accident. With this solution, I was able to design my OWN system, with my own people, in about 8 hours. Eight hours! Wonderlogix's game-changing platform eliminates these challenges by empowering applicative life science practitioners to design and engineer their own large scale, automated systems by themselves. By taking control of their own control systems design, companies will be able to create tailor-made and engineer-grade solutions directly from the lab, as early as establishment of a POC, without compromising IP by outsourcing, or even having to depend on outside service providers. In later stages, the algae experts will be able to evolve and optimize their designs, integrate new technologies and solve problems in an autonomous way.