

International Society for
Applied Phycology
NEWSLETTER



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Message from the President, Dr. Roberto De Philippis

Dear ISAP Members,

I am happy to introduce the second Issue of ISAP Newsletter for 2016, where you will find two contributions on topics of interest to our scientific community.

In the time between the previous and the current issue of the Newsletter the following developments occurred:

1. On behalf of ISAP, I signed Memoranda of Understanding (MoU) with three important scientific associations. In May 2016, I signed an MoU with the European Aquaculture Society (EAS, <http://easonline.org/>) fostering interactions between the two societies and the dissemination of information on topics of common interest. More recently, I signed an MoU with the Algae Biomass Organization (ABO, <http://algaebiomass.org/>) pertaining to the 2016 Algae Biomass Summit Media Sponsorship Agreement. These agencies look forward to discussions on furthering partnership opportunities. Finally, few days ago I signed in Florence an MoU with Dr Vitor Verdelho, President of the European Algae Biomass Association (EABA; <http://www.eaba-association.org/en>) and an ISAP Member. I wish to reiterate here that we already have very good connections with the EABA, which has been co-sponsoring Training Courses together with ISAP for some years.
2. The Training Course organized in Nigeria by Prof James Ogbonna was a success and I am very glad that ISAP support was utilised effectively for such a well-organized course. I would also like to acknowledge that the contribution received by ISAP from EABA in 2015 was utilized to increase the funds given in support to this Course. You will find a detailed report on the conduct of this training course both in the Newsletter and on the ISAP website. I take this occasion in thanking Prof James Ogbonna and all his colleagues involved in the organization of this course.
3. **Our 6th ISAP Congress to be held in Nantes, France in June 2017 is fast approaching. You will receive more detailed information on this event in the immediate future. I do however suggest that you block off the week of the 18th to 23rd June in your calendar. The URL of the Congress is <https://isap2017.sciencesconf.org/>**
4. I would like to remind you that we now have a Facebook page and a LinkedIn group for ISAP. ISAP recently added to these two opportunities for receiving information from the Society also a Yahoo group for communications only for ISAP Members. Please, register yourselves on them through the links you will find on ISAP Website and in this newsletter. This way, you will be in touch with developments within the Society.

https://au.groups.yahoo.com/neo/groups/Applied_Phycology/
5. I would like to remind you also that ISAP Members who are in good standing with their dues have free access to the electronic version of the Journal of Applied Phycology through the ISAP Website. Take advantage of this offer by Springer.

Finally, I would like to thank the Members of the EC Committee that is in charge of editing the Newsletter, Amha Belay (Chair), Céline Rebours, Sasi Nayar, and Stephen O'Leary for the hard work done in preparing this Issue.

With my warm regards

Roberto De Philippis

President, International Society for Applied Phycology

Message from the Editor – Amha Belay

The last 10 years has seen a tremendous resurgence of interest in applied algal research. This was initially fuelled by the vision to produce sustainable and affordable biofuel from algae. While affordable algae biofuel is not in sight yet, there have been tremendous advances in algal biotechnology research generating hundreds of patents and thousands of jobs. Meanwhile, almost every algae biofuel company has now shifted direction to producing foods, feeds, nutraceuticals, cosmetics and other bio-products as a path to developing the technology to produce affordable fuel while generating revenue on the way. This new attempt to obtain diversified products will certainly help accelerate the effort in algae biofuel production. In this regard the continuing support of algal biofuel research by the United States Department of Energy and US Department of Agriculture despite recent failures is very exemplary and shows how committed the government is to replacing fossil fuels with sustainable energy, algae biofuel being one component of this effort. Such efforts are also being made by several other countries in the world.

The rapid development of algae-based products as a result of the efforts mentioned above calls for the training of future algal biotechnologists and mid-level technicians that would be required when the vision is materialized. There have been some efforts as in the programs by CALCAB (the California Center for Algal Biotechnology) but one that is set out to develop curricula for grass roots training (K-12) is the initiative by the Algae Foundation. This is the subject of the first article in this newsletter.

The production of a diverse group of algae products calls for analytical methods that are fairly simple, rapid, cost-effective and suitable for online process control. In this regard, the efforts of the Arizona Center for Algae Technology and Innovation (AzCATI) and the Algae Biomass Organization (ABO) in the USA and other organizations in Europe and elsewhere are worth mentioning. In this issue of the newsletter, we present an article on Near Infrared Spectroscopy (NIRS) that has gained popularity in the food and pharmaceutical industries recently. The article highlights the advantages and drawbacks of this method. Of particular interest is the ability to use this method for on-line monitoring of products and processes.

The Editorial Committee of ISAP continues to appeal to members of ISAP in particular, and the algae community in general, to submit articles of interest that may be as simple as summarizing their current published material or some interesting reports that they may have written for one purpose or other but have not been disseminated. We hope that the recent attempt to link ISAP with other algae organizations like the Algae Biomass Organization may help us tap interesting articles from a wider horizon.

Promoting the Power of Algae in K-12 Classrooms: An Algae Foundation Initiative

JAKOB O. NALLEY^{1,2,3*}, TIFFANY CANNIS^{1,4} AND IRA A. LEVINE^{1,5}

¹ The Algae Foundation, Preston, MN

² Kellogg Biological Station, Michigan State University

³ Department of Integrative Biology, Michigan State University

⁴ Global Algae Innovations, San Diego, CA

⁵ University of Southern Maine, Lewiston, ME

*corresponding author:

Mailing Address: 3700 Gull Lake Road, Hickory Corners, MI 49060

Email: nalleyja@msu.edu

Phone: (269) 671-2242

Fax: (269) 671-2351

Introduction

In March 2016, over three hundred young phycologists wrapped up a weeklong exploration of the power of algae. Throughout the week, these scientists cultivated and monitored algal biomass accumulation, explored the many uses of algae, zoomed in to see the diversity of algae, and even designed hypothetical algal species tailored to a range of adverse environments (Fig. 1 & 2). These 6th and 7th grade science students in San Diego, CA were part of the initial pilot launch of the newest educational initiative from The Algae Foundation, the K-12 STEM Algae Initiative. This effort aims to educate, engage and excite young scientists about the many applications of algae to significantly reduce greenhouse gases while providing a sustainable source of biomass for food, feed, and fuel for our ever-growing population.

Is There a Need?

Over the next decade, as the algae industry continues to grow, thousands of jobs for educated and technically trained workers will be generated. The Algae Foundation has placed a strong emphasis on developing this workforce. To achieve these goals, the Foundation has adopted a “K to Gray” approach for education, focusing on providing and promoting lifelong education and learning opportunities. The K-12 STEM Algae Initiative is the beginning step in exciting and fostering what will be the next generation of algae-related talent.

Curriculum Development

In mid-2015, The Algae Foundation launched the K-12 STEM Algae Initiative and immediately began assembling a team of teachers, scientists, project managers, business leaders, and philanthropists to plot the path for project success. In October 2015, at the annual Algal Biomass Summit, a successful round of fundraising led to fully funding an initial effort to develop and distribute 50 algae-related “drop-in” ready kits at no cost to participating school districts (Fig. 3).

After securing the adequate funding, the curriculum development team began outlining the desired curriculum framework. Through the collaboration of K-12 educators, scientists and business leaders, it was determined that the curriculum would span a 5-day period and focus on exposing students to real world algal cultivation techniques, strengthening data interpretation proficiency, developing microscopy skills and employing engineering capabilities (for specifics, see “Kit Day-to-Day Overview”). During development, we placed an emphasis on conforming to the newly adopted Next Generation Science Standards (NGSS), specifically satisfying the Human Impacts and the Ecosystems: Interactions, Energy and Dynamics components (MS-ESS3-2, 3, 4 and MS-LS2.1, respectively). To satisfy these standards and immerse the students in science and engineering practices, we structured our curriculum within the framework of the 5 E’s that was developed by the Biological Sciences Curriculum Study (BSCS): Engage, Explore, Explain, Elaborate, and Evaluate.

Kit Day-to-Day Overview

- **Day One – Algae 101 Introduction:** To begin the week, the first day consists of a brief lecture highlighting basic living requirements for algae, algal diversity, and the top uses (industrial and commercial) of algae. After this brief overview, students break into groups to try some edible forms of algae (seaweed chips and algae drink) and also inspect ingredient labels of common food items to identify whether algae bioproducts are present. (“Think, Pair, Share”)
- **Day 2 – Establishing an Algae Culture:** Students will work with a small group (max 4 students) to inoculate an algae culture and then quantify the initial dry weight of the culture through filtration and drying. Groups will have different inoculation volumes of algae to investigate biomass differences depending on starting population densities.
- **Day 3 – Exploring the Diversity of Algae:** Within the same small groups from the previous day, students will create their own wet mounts of 3 functionally distinct algae and observe them under the microscope. They will draw what they are seeing, noting the level of magnification and quantitative findings.
- **Day 4 – Sampling and Growth Rates:** Students will return to their algae cultures they established 2 days ago, and following the same protocol, filter a portion of their culture and mass the dry weight of algae. After collecting this data, students will then calculate the growth rate of the algae in their group culture.
- **Day 5 – Plotting and Analyzing Data, and Design-Your-Own Algae:** With the biomass data that was collected on Day 2 and 4, students will create a bar plot of the biomass data and then follow the “Claim, Evidence, Reasoning” framework for analysis/interpretation (this can be scaled to meet the students’ level of plotting proficiency). To conclude the week, students will be presented with an extreme environment and they will be tasked to apply their experiences throughout the week to design an optimal alga for that environment.

Future Plans

To date, we have piloted in three classrooms, at three grade levels (6th, 7th and 8th). We are currently in the process of stocking 50 total kits that will be distributed to our two targeted markets, the greater San Diego region and southwestern Michigan. Then, after a second round of fundraising, we plan to distribute 100-125 additional kits nationwide as we identify more partner districts. It is important to note that we will continue to restock “consumable” materials within all kits year-to-year in an effort to increase the longevity of each individual kit. We will also encourage partner teachers to share kits across grade levels within their own districts to also increase kit exposure.

Currently, the kits are tailored to the 6th-8th grade levels due to our initial pilot program involving strictly upper-middle school educators and students. The intention is to scale the kit activities and worksheets to higher and lower grade levels as we begin to expand our targeted audience. At an elementary level, we would like to expose students to algae as an organism that they interact with on a daily basis, whether that be in the environment or in their food products. Scaling up to a high school level would simply

require tweaking the current curriculum to include more advanced mathematical and graphical approaches. This full K-12 coverage would be projected to be ready for the 2017-2018 school year.

Conclusions

The Algae Foundation strives to promote the power of algae to transform human society and the environment upon which it depends. This K-12 STEM Algae Initiative is an integral step in harnessing the full transformative power algae offers through energizing the next generation of algae experts, innovators, and entrepreneurs.

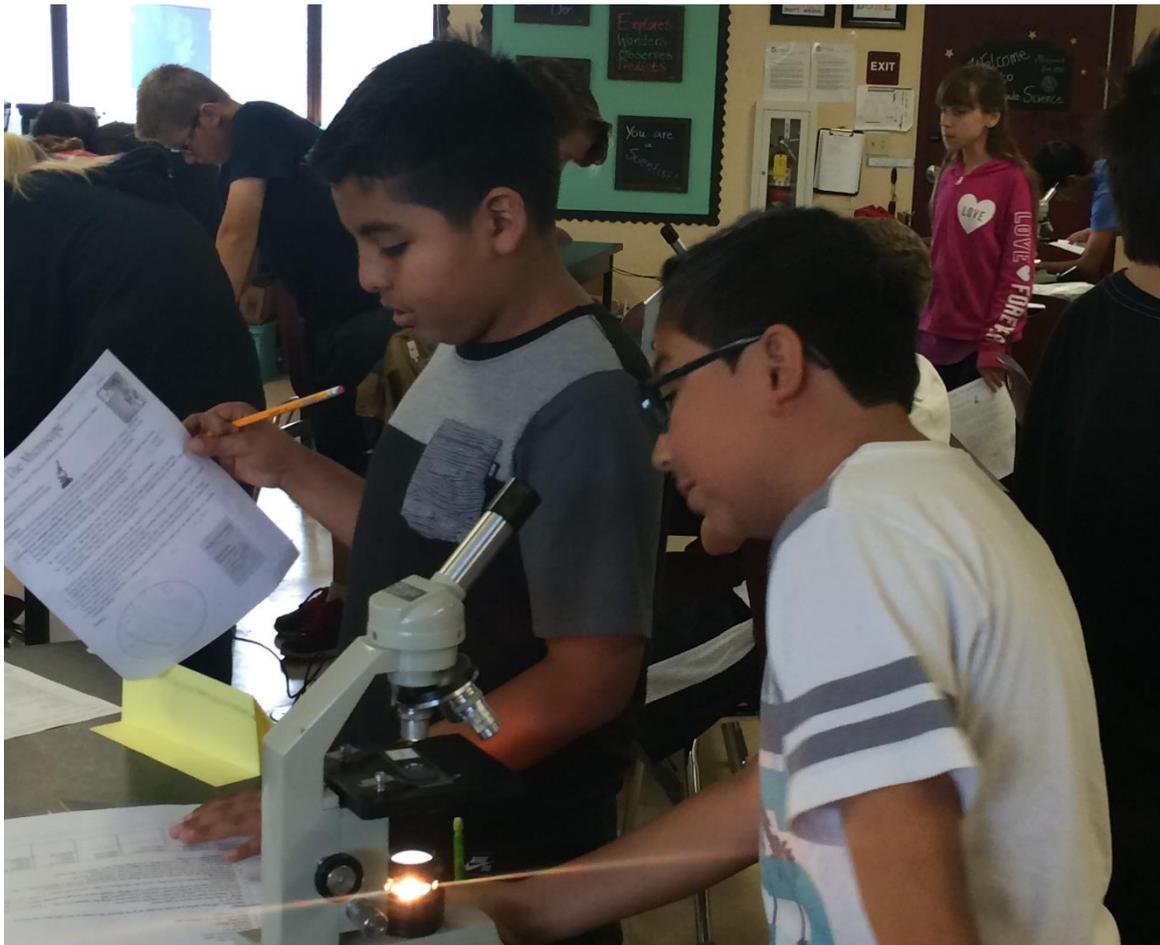


Figure 1: Students developing microscopy skills as they explore algal diversity at the microscopic level.



Figure 2: Students filtering their algal cultures after 48 hours of growth.

Use of NIR technique in applied algal biofuels research

VINEELA CHALLAGULLA

International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telengana 502324, India
dr.vinela@gmail.com

Overview

Algal biomass has been recognized as a potential source of biofuels largely due to the high growth rates and ease of cultivation of algae and their ability to grow in a wide range of environments. Various biofuel pathways from algal biomass include transesterification of lipids to biodiesel, fermentation of sugars to bio-ethanol and bio-hydrogen production. For the algal biofuels production processes to be viable techno-economically, analytical and process management tools need to be improved significantly. The analytical methods currently in use are based on conventional techniques, which are not only time consuming but may use hazardous chemicals. Very often, the analytical outputs from these techniques take time to be processed, and consequently do not assist with any improvements to the process during the cultivation run. These conventional methods also generate large amounts of waste during the analytical process and require large amount of samples for analysis (da Silva et al., 2012). Therefore, a model analytical technique would ideally be rapid, cost effective, non-destructive and less time consuming in ascertaining various parameters relevant to algal biofuel research.

Techniques such as Near Infrared Spectroscopy (NIRS) not only provide qualitative or quantitative outcomes, but are high throughput when compared to conventional techniques. If appropriate models are developed, NIRS can be used inline in bioprocess monitoring to provide realtime data as the processes are running. This article explores and introduces NIRS as a potential technique to optimize production and conversion processes utilized in algal biofuels R&D with the option of extending the technique for other applications as well.

An overview of Near-Infrared Spectroscopy (NIRS)

Near infrared radiation between the wavelengths of 1100 and 2500 nm is strongly absorbed by water, with an operative path length of around 1 mm. The short wavelength NIR region of 700–1000 nm has an effective path length of a few centimeters. However, absorbance features are wider and more overlapped in the NIR than the Infrared (IR) region, thus needing chemometrics for data analysis. This process involves the collection of spectra and reference analyses of a large number of samples, using an appropriate optical geometry for the attribute and sample type under consideration, i.e. reflectance, interactance or transmittance modes (Bokobza, 1998).

The use of NIR region has its merits given the lower absorptivity of water in this region of the spectra compared to IR, while still holding relevant information such as the second and third overtones of the fundamental absorption features seen in IR (Challagulla et al., 2016). Relative to other analytical techniques, NIRS also offers the advantage of rapid and in situ quantitative assessment of compounds rich in O–H, C–H, N–H or S–H bonds, which determine absorption related to the stretching of these bonds (Thyholt and Isaksson, 1997). The CH, CH₂ and CH₃ absorption bands in NIR spectra are related to lipids and carbohydrates in biological materials, and the method has been extensively used in assessment of hydrocarbon content of both natural products and petrochemicals (Hidajat and Chong, 2000).

NIRS is a well-established technique used since the 1990s to analyze biochemical constituents (Barton, 2002). In recent years, NIRS has gained popularity for accurate determination of biochemical constituents such as carbohydrates, lipids and proteins in biological materials and food products such as

meat, oils seeds, edible oils and dairy products (Huang et al., 2008, Prieto et al., 2009). Very often NIRS has also been applied in process control, with inline measurements made using fiber optic probes in the reactor vessels or in piping to monitor quality of the final product (Owen-Reece et al., 1999). It offers great potential and promise as a high throughput technique that can be used to estimate biochemical components in algae (Table 1). As such, the technique has relevance in the assessment of biomass content and composition of biological materials; however, there is a paucity of literature relevant to its application in algal biofuels research.

Table 1: An overview of the application of NIR in microalgal biomass research

| Species | Sample presentation | Analysis | Resolution nm | Spectral range nm | Spectral processing | Regression | R ² | Reference method | Source |
|---|--|---|---------------|------------------------|--|------------|--|--|----------------------------|
| <i>Nannochloropsis</i> sp. <i>Chlorococum</i> sp. <i>Spirulina</i> sp. | Lyophilised biomass | Spiked triglyceride | 2 | 400–2500 | MSC 1 st and 2 nd derivatives (S.Golay) | PLS | 0.91-0.98 across species | Biomass spiked with known concentration of lipids | Laurens and Wolfrum (2011) |
| <i>Chlorella vulgaris</i> <i>Navicula</i> sp. <i>Nitzschia pusilla</i> | Culture samples filtered through GF/C filters | Biomass and lipid content | 4 | 300–1100 and 1100–2500 | 2 nd derivatives (S.Golay), SNV and MSC | PLS | 0.63-0.97 across species and cultivation conditions | Biomass dry weight and solvent extracted total lipids | Challagulla et al. (2014) |
| <i>Chlorella</i> sp. <i>Scenedesmus</i> sp. <i>Nannochloropsis</i> sp. | Freeze-dried biomass (ring cup format and 96-well format) | Biomass and lipid | - | 400–2500 | S.Golay derivatives, SNV and MSC | PLS | 0.81-0.92 across species and sample presentation methods | solvent extraction | Laurens and Wolfrum (2013) |
| <i>Kirchneriella</i> sp. <i>Nannochloropsis</i> sp. | Culture samples filtered through GF/C 25 mm filter papers | Biomass and lipids (SFA, MUFA and PUFA) | - | 1100–1900 | MSC | PLS-1 | Biomass 0.85-0.96 and lipid 0.84-0.97 across species | Biomass- cell counts (Coulter Counter) Lipid-GC | Brown et al. (2014) |
| <i>Rhopalosolen saccatus</i> | Culture samples filtered through GF/C 2.5 cm filter papers | Biomass, lipid and fatty acids | 4 | 1100–2500 | 2 nd derivatives (S.Golay), SNV and MSC | PLS | Biomass-0.78-0.86 Total lipid-0.46-0.74 Fatty acids- 0.63-0.90 across cultivation conditions and sample presentation methods | Biomass –dry weight, total lipids- solvent extraction and fatty acids-GC | Challagulla et al. (2015) |
| <i>Chlorella vulgaris</i> <i>Chlorella protothecoides</i> <i>Chlorella zofingiensis</i> | Freeze-dried biomass packed into a 1.5-mL Eppendorf tube | Fatty acids | 1 | 1000–2499 | S.Golay derivatives and MSC | PLS-1 | 0.96-0.98 across species and fatty acids | Fatty acids assessed using GC-MS | Liu et al. (2015) |

MSC- multiplicative scatter correction, SNV-standard normal variant, PLS- Partial Least Square Regression, GC-Gas Chromatography, SFA-Saturated Fatty Acids, MUFA- Monounsaturated Fatty Acids, PUFA-Polyunsaturated Fatty Acids, GC-MS- Gas Chromatography attached to mass spectroscopy.

Source: Challagulla et al. (2016)

Potential applications of NIRS in algal biofuels R & D

NIRS can be applied to measure biomass concentrations and biochemical composition in algal cultures or in processed samples (Challagulla et al., 2015, Challagulla et al., 2014). The spectrum can be acquired *in situ* of algal cultures using a fiber optic probe. In such cases the effect of light scattering due to culture density and cell size will need to be considered (Owen-Reece et al., 1999).

However, to date there have been very few publications on the use of NIRS in the assessment of microalgae. As an example, single species partial least square regression (PLS) models were used to estimate triacylglycerides and phospholipids in lyophilized microalgal cultures. However, the model was based on addition of exogenous lipids to algal biomass. Dry extract method for collection of NIR spectra was attempted with success on *Rhopalosolen saccatus* and three other species (Figure 1). The technique developed required minimal preparation and used dry filtrates for NIR reflectance spectroscopy, requiring just 1–20 mL of the sample depending on biomass concentrations.

A common failing of studies reporting on NIRS assessment is the use of validation samples that are drawn from the same population as the calibration samples (Challagulla et al., 2016). For NIRS to be precise in the assessment of algal biomass, it is important that the predictive model is validated using samples that are independent of the calibration set. Other important factors are sample presentation and the mode of NIR measurement such as transmittance, diffused transmittance and trans reflectance (Owen-Reece et al., 1999). The choice of the measurement mode is dictated by the optical properties of the analyte. In the case of algal cultures, parameters such as biomass, lipids, proteins, carbohydrates and other constituents such as pigments can be assessed *in situ* and in real time using fiber optic coupled spectrometers (Sandnes et al., 2006). However, changes in light path due to culture density, cell size, pigment composition and biochemical make up must be considered in ensuring accuracy and precision. For increased precision, the measurements can be made on processed biomass samples such as lyophilized culture or culture deposited on to a solid support matrix (Challagulla et al., 2015, Challagulla et al., 2014, Laurens and Wolfrum, 2011, Laurens and Wolfrum, 2013).

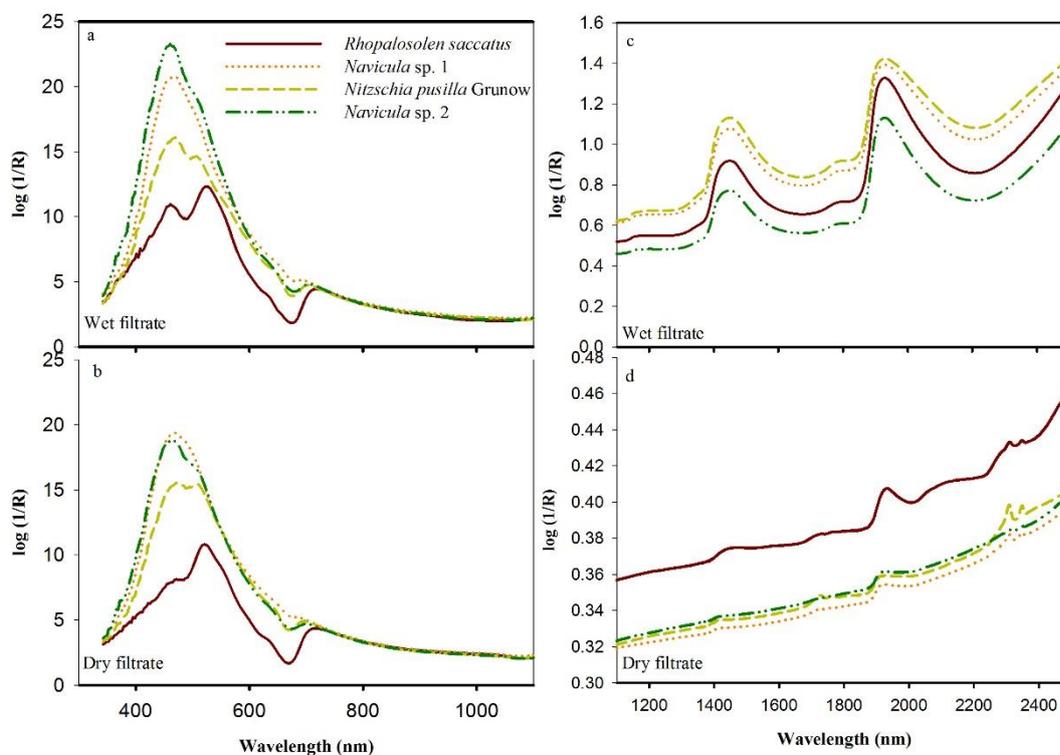


Fig. 1. Absorption spectra of four microalgal species grown under nutrient sufficient conditions at 300-1100 nm of (a) wet filtrate and (b) dry filtrate, and at 1100-2500 nm of (c) wet filtrate and (d) dry filtrate nm (Challagulla et al. 2014).

Conclusions

Non-invasive techniques such as NIRS have gained popularity over conventional wet chemistry analytical techniques for assessment of algal species for high biomass accumulation and the management of cell cultures for biofuel production. NIR spectra can provide data on the cellular content of microalgal lipids in a single step, however further work is required to validate the robustness of the prediction models that are an integral part of the NIRS data analysis.

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| News and Views |
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Workshop Report

‘Training course on microalgae biotechnology: Harnessing the potential of microalgae for economic development of Nigeria’

Date: 26 June – 1 July 2016

Venue: National Biotechnology Development Agency, University of Nigeria Nsukka, Nigeria

Sponsors: International Society for Applied Phycology & National Biotechnology Development Agency. European Algae Biomass Association was a partial sponsor.

Course facilitator: Professor James Ogbonna, University of Nigeria Nsukka

Resource personnel:

1. Dr. Nkechi Nweze, University of Nigeria, Nsukka
2. Dr. Innocent Ogbonna, University of Agriculture, Makurdi
3. Dr. Ikechukwu Onah, University of Nigeria, Nsukka
4. Mr. Emeka Nwoba, Ebonyi State University, Abakaliki
5. Prof Jerry Ugwuanyi, University of Nigeria, Nsukka

The primary objective of the training course was to train young scientists and academics on techniques adopted for isolation, characterization, identification and cultivation of microalgae.

The training course was attended by 48 researchers from 11 institutions and comprised 12 lectures, 6 hands-on training sessions and a field trip to Opi Lake and other water bodies adjoining the lake. Water samples collected by the participants were brought back and utilized for the training course.

The lecture topics included:

1. Microalgae and its significance
2. Metabolites of commercial significance
3. Applications in aquaculture
4. Microalgae as a source of oil for biodiesel
5. Applications in waste water treatment
6. Methods of isolating microalgae from different environments
7. Identification of microalgae using morphological and molecular techniques
8. Culture media
9. Cultivation systems including photobioreactor concept and design

The technical hands-on sessions included:

1. Different culture media recipes
2. Identification of microalgae using morphological characteristics
3. Determining biomass concentrations
4. Determining chlorophyll and other pigments in microalgae
5. Extraction and quantification of lipids in microalgae
6. Molecular techniques for the identification of microalgae



Participants from the workshop



Resource personnel from the workshop



A lecture in progress



Participants at the field trip



Isolating microalgal cells from the water samples



Microscopic analysis of the microalgal sample

Conferences



After Kunming - China in 2005, Galway - Ireland in 2008, Halifax - Canada in 2011 and Sydney - Australia in 2014, the International Society of Applied Phycology (<http://www.appliedphycologysoc.org/>) entrusted a scientific team from Western France to organize the 6th congress of the society in Nantes at La Cité Nantes Events Center from June 18th to 23rd 2017. Indeed, with over 300 people working in its universities and research centers and more than 100 businesses, Western France is a European hotspot for the transfer and industrial development of marine biotechnology (Marine Biotech in Western France).

Previous ISAP Congresses have seen the role of applied algal biotechnologies and their potential developed in a commercial, remedial or regulatory context. In 2014, the theme of ISAP was chosen to reflect on the actual successes of algae applications as they already represent a sustainable and relevant field of biotechnology.

In 2017, the scope of the 6th edition of ISAP congress is to appreciate the huge phycological biodiversity and the diversity of its biotechnological applications through the prism of a new and promising industrial sector in full development. The Congress will include speakers and poster presentations, exhibitors and for the first time a BtoB session to meet the right partners.

For details visit <https://isap2017.sciencesconf.org/>



Details at <http://www.algaebiomasssummit.org/?page=AboutSummit>

The 10th annual Algae Biomass Summit will take place October 23 - October 26, 2016 at the Renaissance Glendale Phoenix Hotel & Spa in Phoenix, Arizona. This dynamic event unites industry professionals from all sectors of the world's algae utilization industries including those involved financing, algal ecology, genetic systems, carbon partitioning, engineering & analysis, biofuels, animal feeds, fertilizers, bioplastics, supplements and foods.

Produced by the Algae Biomass Organization, this event brings current and future producers of biobased products and energy together with algae crop growers, municipal leaders, technology providers, equipment manufacturers, project developers, investors and policy makers. It's a true one-stop shop – the world's premier educational and networking junction for all algae industries.

Courses



SECOND LISBON MICROALGAE BIOTECHNOLOGY ADVANCED COURSE 2016

2nd Advanced Course 2016: Microalgae Biotechnology

A unique opportunity for hands-on experience with microalgae Pilot Scale production

Aim

The LiMBAC 2016 aims to familiarize participants with advanced concepts of microalgae cultivation paying special attention to scale-up processes, large scale cultivation and downstream processing. The course will take an interdisciplinary approach, presenting lectures covering the whole microalgae value chain as well as lab and pilot plant practical sections where participants will have hands-on experience in culturing microalgae.

Who should attend?

MSc, PhD or Post-Docs in the biology, chemistry and biochemical engineering fields.

The course is also intended for new and established industry professionals seeking to deepen their knowledge and network with other stakeholders in the field.

The deadline to enroll is October 21st!

Details at <<http://limbac2016.wixsite.com/limbac2016>>

International Societies that signed MoUs with ISAP



ABO website - , <http://algaebiomass.org/>

Algae Biomass Summit - <http://algaebiomasssummit.org>



EABA website - <http://www.eaba-association.org/en>

Conference ALGAE Europe, Madrid December 13th-15th 2016 - <http://algaecongress.com/>



EAS website - <http://easonline.org/>

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|----------------------------|

President: Prof. Roberto De Philippis

Department of Agrifood Production and Environmental Sciences (DISPAA)
 Florence University, Piazzale delle Cascine 24; I-50144 Firenze - ITALY
 Tel: +39 0552755910
 E-mail: roberto.dephilippis@unifi.it
<http://www.dispaa.unifi.it/>

Vice President (Outgoing President): Dr. Susan Blackburn

Australian National Algae Culture Collection
 CSIRO Marine and Atmospheric Research
 GPO Box 1538, Hobart, TAS7001, AUSTRALIA
 Tel: +61-(0)3-62325307
 Fax: +61-(0)3-62325000
 E-mail: susan.blackburn@csiro.au
<http://www.csiro.au/ANACC>

Vice President (President-elect) and web coordinator: Dr. Céline Rebours

Møreforsking Ålesund AS
 Postboks 5075, Larsgården, 6021 Ålesund, NORWAY
 E-mail: celine.rebours@moreforsk.no
<http://www.moreforsk.no/>

Secretary/Treasurer: Dr. Pia Winberg

School of Medicine, University of Wollongong and Venus Shell Systems
 c/o UOW Shoalhaven Campus, Mundamia NSW 2540, AUSTRALIA
 Tel: +61-429-338846
 E-mail: pia@uow.edu.au
<http://www.venusshellsystems.com.au>

Editor, ISAP Newsletter: Dr. Amha Belay

Earthrise Nutritionals LLC
 113 E Hooper Rd, Calipatria, CA 92233, USA
 Tel: +1-760-348-5027
 Fax: +1-760-348-2895
 E-mail: abelay@earthrise.com
<http://earthrise.com/>

Social media administrator: Dr. Sasi Nayar

Algal Production Group
 South Australian Research and Development Institute - Aquatic Sciences
 2 Hamra Avenue, West Beach, SA 5024, AUSTRALIA
 Tel : +61 8 8207 5321
 Fax : +61 8 8207 5481
 E mail : sasi.nayar@sa.gov.au
<http://pir.sa.gov.au/research>