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Applied Phycology
NEWSLETTER



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Message from the President, Dr. Susan Blackburn

Dear Friends and Colleagues

I am delighted to introduce this 2013 Newsletter for the International Society for Applied Phycology (ISAP). ISAP brings together scientists, industry representatives, students and educators to promote research, preservation of algal genotypes, and the dissemination of knowledge concerning the utilisation of algae. ISAP's importance is increasing with the current interest globally in algae and their applications.

I am very grateful to Amha Belay and the members of the ISAP Newsletter Working Group of the Executive Committee for bringing us this newsletter. We are fortunate to have articles from Amos Richmond and John Benemann, without doubt two important leaders in algal applications research and development over many years. I anticipate that their articles will make this newsletter a valuable reference for many years to come, as we move forward in a century in which the value of algae as a renewable resource for a wide range of applications will be increasingly realized.

For recent ISAP activities and information please visit the web site <http://www.appliedphycologysoc.org>. Particular thanks to Céline Rebours and Mike Guiry for looking after the web site.

Of importance is the planning of the next ISAP Congress next year. We are very excited to be holding ISAP 2014 in Sydney, Australia. Pia Winberg, Convener of ISAP 2014 and the Local Organizing Committee have been meeting regularly for some time and plans are well advanced for an enjoyable and interesting conference that will provide a wonderful venue for sharing our science and approaches to algal applications. If you have not already been included in the communications on ISAP2014 please visit the conference web site <http://www.isap2014.com/> and register your interest.

Please take the time to read and enjoy this newsletter and share it with your colleagues and friends. We welcome your ideas and feedback on ISAP. Please contact either myself, the ISAP Secretary Treasurer Alan Critchley, or anyone of the Executive Committee (see web site for details). We look forward to hearing from you.

With warm regards,

Susan Blackburn

President, International Society for Applied Phycology

Message from the Editor – Amha Belay

I am happy to introduce the first Newsletter of 2013. I am also happy to serve you as Editor of the Newsletter together with my other colleagues in the editorial group. The Newsletter will act as a medium of communication among members of ISAP and all other interested professionals at large, and will highlight the latest news, views and developments in the field of applied phycology. To do this we need the cooperation of every member of the society in particular and the phycological community in general in supplying information of relevance to the newsletter.

One recent development in applied phycology is biofuels production with microalgae which is rapidly expanding into animal feeds, chemicals, and many other products and co-products, low and high value. It has been recently estimated that as many as ten thousand researchers are now employed on such endeavors globally, in private enterprise and academic and government laboratories with over a billion US\$ spent annually. What are we to make of this enormous increase in interest in applied phycology?

We must admit, applied phycology has developed rather slowly over the past few decades, with only about one algal new specie introduced commercially per decade. Production has also increased rather slowly, from a rather small base of less than 1000 tons annually, by not much more than about 10% per year over the past thirty years. Thus we estimate that currently autotrophic production is not much more than about 15,000 tons, perhaps twice as much if we include dark fermentation.

Major advances in technology and commercial production seem overdue: advances that reduce production costs and increases scales of production by a substantial factor, even ten-fold. Even though economic production of biofuels from algae may be 10-15 years away by some estimates, significant strides have been made in the areas of genomics, metabolomics and proteomics of select algal species as well as fundamental knowledge on growth physiology and pest control, photobioreactor design, harvesting and dewatering of biomass and downstream refining of final products. Moreover, numerous young phycologists have been trained and are being trained through this endeavor.

ISAP, and our Newsletter, should, and can, play a role in this great effort, through our members' roles as researchers, and by providing a forum for discussion of the salient issues, and by providing an unbiased, peer review-based evaluations of contending views and arguments.

Let a hundred, or more, contending technologies compete for their place in the sun or the fermenter to advance the technology of production of biomass for food, feed, biofuel and biochemicals. This is a great and challenging call for all applied phycologists.

Professor Amos Richmond: Up Close and Personal on Algal Biomass Production

BASIC ASPECTS CONCERNING MASS CULTIVATION OF PHOTOAUTOTROPHIC MICROALGAE

Amos Richmond, PhD. Professor Emeritus, BGU. The Blaustein Institutes, Sede-Boker, Israel

Interest in this field of research culminated in the late forties and early fifties but soon subsided, Burlew (1953), providing a key to a basic difficulty: "we face the problem of arranging matters so that algal cells can utilize high intensity sunlight just as efficiently as they can utilize weak light." To this day this dilemma is at the root of the challenge to develop cost effective microalgal alternatives for food and feed and perhaps fuel, although chances for success with the latter being, in my opinion, exceedingly dim. In the past few decades some commercial microalgal enterprises had been nevertheless developed, all confined however to small volume, high-value specialty markets and the ambitions to mass produce microalgae for a commercial highly nutritious cost-effective product are as yet far from fulfillment. The body of information amassed to date concerning mass-culture physiology is however quite significant, permitting a few basic generalizations which follow: *A basic principle in mass-production is that the out-put rate of light-limited continuous cultures is a function of both cell-density and the photosynthetic flux density (pfd) impinging on the culture*, first shown by Myers & Graham (1958). They provided identical irradiance to continuous laboratory cultures differing only in their cell densities and discovered that photosynthetic efficiency (PE) varied very significantly with culture density, the optimal cell concentration - identified empirically - yielding 66% more cell mass than that maintained farthest from the optimal concentration.

Clearly, light is most effectively used by culture cells as the interaction between the intensity of the light source and the extent of mutual shading (affected by cell density) reaches an optimal balance. It follows that the specific growth rate in such light-limited continuous cultures may greatly vary, being so sternly depended on culture density. The principle unfolding is that light applied on continuous cultures is most effectively used at the optimal interaction between the intensity of the light source and the extent of mutual shading in the culture. The efficiency of photosynthesis at which point is maximal, reflected in peak primary productivity of cell mass.

Therein emerges the basic parameter in mass cultivation of light-limited photoautotrophic microorganisms *i.e.* the light regime to which the average cell in the culture is exposed. This interaction is clearly evidenced in photo-inhibitory environment unfolding under conducive ambient conditions. Photoinhibition would occur, accordingly, due to a gross departure from the suitable interaction between the intensity of the light source and culture cell density. A straight forward demonstration for this was provided by Hu and I, having observed continuous *Arthrospira platensis* cultures exposed to a photoinhibitory environment (strong morning light coupled with close to freezing culture temperature). One culture, maintained in a relatively low density was severely photo-damaged while the identical culture placed next to it and differing only in having a 3 fold higher cell density showed no ill effects.

A significant practical outcome of this is that careful maintenance of the optimal cells' light regime, permits effective use of very high light (e.g. an intensity of some 4 suns): a culture grown in the proper photobioreactors therefore, could produce very high out-put rates (e.g. over 100 g m⁻² day⁻¹). This was demonstrated in 1.3 cm light-path flat plate reactors exposed to progressively increased irradiance, up to 8000 μM photons /m²/sec, resulting with a corresponding progressive increases in out-put rates of up to 1.2 g. dry wt per L/h. The high growth response to very strong light (ca. 4 suns) was only attainable providing culture cell densities were raised to match the increasing photon flux densities. The optimal light regime could thus be maintained even as radiation intensity quadrupled, becoming some 20 to 40 times higher than the cells' light-saturation energy.

Reduction of the optic path exerts a significant effect on increasing the optimal cell density and areal productivity. This is due to the rise in the light-dark cycle frequency culture cells which significantly elevates the efficiency of photosynthesis. Greenwald, Gordon and Zarmi came recently with an explanation for this course of events. Accordingly, when algal cultures of high cell density are cultured in reactors of very narrow optical path, the time scales (i.e. periods of exposure to light and darkness) evolving in the random motion of cells in the reactor, approach the time scales involved in photosynthesis. Synchronization of these time scales enhances light-use efficiency and facilitates the observed increase in bio-productivity.

A major obstacle in harnessing strong solar light to cell-mass productivity stems from the wide discrepancy existing between the major solar irradiance available during some 6 to 7 hours along daylight

(averaging some 1500 to 2000 $\mu\text{mol}/\text{m}^2/\text{s}^2$), and that which saturates the photosynthetic mechanism of the cell which, as a rule, is very significantly (ca. 5 to 10 times) lower. If solar light is not diluted along the hours of peak irradiance on mid-day, therefore, most of it is not available for micro-algal photosynthesis and light use of e.g. strong sunshine is realized to only a small fraction of its overall potential. Hence dilution of strong solar light, using various tactics, must be used in operating mass microalgal cultures outdoors. These may take several forms; one involves an increase in culture cell density which dilutes the light dose by distributing the light falling on culture surface to a larger number of cells. Mixing clearly dilutes the impinging radiation by increasing the frequency of the light-dark cycle the mixed cells undergo. Cells are more evenly exposed to light and the shortening of cycle time permits a more efficient light-use in providing a higher rate of light impulses per reaction center. Narrowing the optic path works in essentially the same manner and likewise, departure from horizontal to vertical culture layout increases the irradiated culture area per foot-print, diluting in effect direct beam irradiance. As a generalization, dilution of a strong light source (e.g. solar) implies an improvement in the mode light is available for culture cells, enhancing photosynthetic productivity of light-limited cultures.

The most meaningful practical effect in improving the efficiency of light-use and in which micro-alga culture departs from the cultivation mode of land plants is obtained by setting the conventional horizontal algal culture device in a vertical position, an idea pioneered in the 1980s by Mario Tredici and Otto Pulz. Vertical setting doubles the exposed surface area per culture volume and foot print, offering endless combinations to optimize the cells' light regime and thereby enhance productivity.

Otto Pulz was first to suggest arranging flat plate reactors short (e.g. 20 cm) distances apart, affecting practically full, cross shading, in the reactors setting. In this fashion, inefficient direct strong solar light of mid-day impinges on the plate surfaces as weak, highly efficient, diffused light. A daily output of some 100g dry-wt per m^2 of reactor foot- print is therefore approachable in this fashion. Using improved microalgal strains and providing nutrients, temperature and growth inhibition are not rate limiting, such vertical devices would enable photosynthesis to approach its maximal theoretical limits outdoors, resulting in areal productivities significantly higher than those obtainable in horizontal culturing devices.

The background is now set for the current major question concerning micro-algal biotechnology: would a very significant increase in culture productivity, resulting from improved growth protocols and algal strains, make a microalgal – fuel commodity become an economic reality? While much enhanced productivity should improve significantly the general production economy of microalgae, cost –effective microalgal fuel would yet remain, in my opinion, unattainable. This for several reasons, among which is the sobering recognition of leading scientists that the present microalgal production cost running, at the lowest, ca. 3500 \$/t, is some 15 to 20 times higher than the permissible cost of oil- rich cell mass as raw material for fuel. The time period estimated by leading scientists to develop the biotechnology required for an economic mass production of algal-fuel lies, if at all possible, in the range of some 15 years.

Along this time period however, a global demand for food and feed rich in protein and poly-unsaturated fatty acids is rapidly unfolding, one key for easing it is seen in microalgae providing feed e.g. as partial replacement of fish meal. The anticipated large expansion in aquaculture accompanied by rising demands for fishmeal (a resource of depleting supply), sets a potentially strong demand of several million tons of selected microalgal strains as animal and aquaculture feed. Once improvements in algal biotechnology would cut, in the foreseeable future, current production costs by ca. one half, substantial new venues for nutritious microalgae rich in PUFA, carotene, and protein will be opened for fish and animal feed. It is worth noting that the idea to extract from algal cell mass oil for fuel and turn the residue to feed, would not produce the high value feed required to replace fish meal.

Shortages of nutritious food and feed resulting from increasing world populations and rising living standards drive up their demand and prices. The economic gain from growing micro-algae for PUFA and protein - rich animal feed as well as human food supplements, would be therefore many times greater than growing it for fuel. This issue is further amplified by the rapid developments currently attained in cost effective alternative energies. Chances of producing a cost-effective fuel based on photoautotrophic microalgae rapidly wane in a world facing revolutionary developments related to alternative energies and posing an ever greater demand for nutritious animal feed and human food.

Note. All citations relate to References shown in Chapter 11, p.201 -204. Handbook of Microalgal Cultures (2nd Edition), A. Richmond & Q. Hu (editors), Wiley Blackwell, 2013.

A SHORT HISTORY OF ALGAE R&D IN THE FORMER SOVIET UNION AND SATELLITE COUNTRIES, AND ITS LEGACY.

John Benemann, CEO, Microbio Engineering Inc. USA

After WWII the industrialization of agriculture, and particularly of animal feed production, became a vexing problem - it did not fit the model of centralized command and control economy. A major, secret (CIA, 1977; Rimmington, 1985) effort was started in the 1950s to develop an industrial process based on the conversion of oil into feeds by bacterial and yeast fermentations. The single-cell protein (SCP) approach was also tried in the West, in particular by British Petroleum and Shell Oil (the latter using natural gas). Over the following decades large production systems were set up all over the Soviet Union producing millions of tons of animal feed, a great success for the five year plans. Unfortunately consumption by the animals (they refused it!) was not as hoped for, and contamination with residual hydrocarbons was also a problem. Failure of the system to produce sufficient feed was a significant contributory reason for its collapse.

In parallel with the fermentation program, a major effort was also carried out to produce SCP with microalgae with both closed photobioreactors (PBRs) and open systems deployed on a production scale in favorable locations of the Soviet Union such as, for example, in Tashikistan (see Figure 1). It can also be noted that the Soviet Union had a microalgae program initiated in the 1950s to support cosmonauts in space, something the USA then copied, and that the first industrial production of beta-carotene from *Dunaliella salina* was in the Ukraine in the 1960s.



Figure 1. Example of Soviet Union Algae Projects: PBRs in Tashikistan (ca. 1988)

Technology leaders in East Germany and Czechoslovakia also helped develop the algae feed production effort. The Hydrobiological Institute in Trebon in the Czech Republic developed an open culture system of very shallow inclined trays (Figure 2).



Figure 2. Trebon, Czech Republic. Shallow Cascade System Algae Cultivation (ca. 1995)

This system was used to produce *Scenedesmus* biomass, and the Trebon group published many important scientific papers. By the 1970s a 1 ha production plant was built in Bulgaria. With the end of the Soviet Union, the Trebon group wanted to become a commercial enterprise, but the market demanded *Chlorella*, not *Scenedesmus*. Eventually they switched over, as did the plant in Bulgaria, but nothing much has come of it commercially. The Trebon group continues to do excellent research and has also started a small company to supply research equipment to algae companies. Their cultivation system has, however, significant limitations (CO₂ losses, pumping energy, etc.) that would limit scale-up and thus commercial development of this technology.

In East Germany, the Institut fuer Getreideverarbeitung, Gmbh (IVG, Institute for Cereal Processing), a food processing research institute, was assigned to develop PBRs, but its work during the Soviet period is somewhat of a mystery (perhaps it also was a state secret). With the fall of the Berlin Wall, IGV was taken over by the German government, which privatized it, funding it initially with government R&D grants, but then it had to also seek private contracts. IGV has five divisions, the algae division headed, until this past month, by Dr. Otto Pulz, seems to be most successful. It concentrated on developing tubular fence type PBRs, located, due to local weather conditions, in greenhouses, with artificial lighting as needed (and for N. Germany it is needed). It has a several hundred square meter system at its headquarters near Potsdam, and its signature project was the first large-scale commercial photobioreactor in Kloetze, Germany built a dozen years ago with a total area of about one hectare, and 700 m³ of algae culture (Figure 3).

The Kloetze facility was for commercial production of *Chlorella*, with an anticipated output of 100 t/y with a competitive advantage over Asian producers. The competitive advantage was that by growing the algae in an enclosed system there would not be contamination by insects, bird droppings, etc. However, this advantage came at a several-fold higher cost, and the company soon enough went bankrupt. It was then purchased (cheaply) by a new venture, which lasted for a little while, and a couple of years ago was purchased by Roquette, whose deep pockets can keep it running. A similar, smaller plant was built a few years ago Ritschenhausen, Germany, owned by Salata, Gmbh, it remains to be seen how long it remains in business. IGV has also sold several dozen research PBRs around the world, a rather successful business model, used by several other companies. However, none of these PBRs have been proven commercially. That is of course the experience of almost all PBR projects (with one exception: the tubular PBRs of Algatechnologies in Israel, for *Haematococcus pluvialis* production). One small addendum: one project in Russia gave microalgae cultures (grown in ponds) directly to cows to drink, claiming outstanding even astounding results. We hear of such projects again now. Overall it seems that not much was learned from the Soviet experience.



Figure 3. IVG Photobioreactors in Kloetze, Germany (appx. 1 ha of greenhouses)

REFERENCES:

- CIA (1977) The Soviet Hydrocarbon-based Single Cell Protein Program, http://www.foia.cia.gov/sites/default/files/document_conversions/89801/DOC_0000498552.pdf
Rimington, A., "Single-cell protein: The Soviet Revolution? New Scientist June 27, 1985 12-16

FROM INSIGHT TO EYESIGHT

The student with the discovery was none other than our own Ami Ben-Amotz, the discovery an organism with a high *cis* β -carotene content, *Dunaliella bardwali*. Now it has been shown to improve sight in people affected by a rare genetic eye disease, Retinitis Pigmentosa (PR) with no known cure. As Ami put it in his message to all of us “reward comes many years after the scientific discovery

Details at: <http://www.algaeindustrymagazine.com/algae-researchers-develop-treatment-for-child-blindness/>

MEETINGS

• Algae Biomass Summit

The 7th annual Algae Biomass Summit took place September 30 - October 3, 2013 at the Hilton Orlando in Orlando, Florida. This dynamic event unites industry professionals from all sectors of the world's algae utilization industries including, but not limited to, financing, algal ecology, genetic systems, carbon partitioning, engineering & analysis, biofuels, animal feeds, fertilizers, bioplastics, supplements and foods. Organized by the Algae Biomass Organization and coproduced by BBI International, 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. Details at: http://algaebiomasssummit.org/ema/DisplayPage.aspx?pageId=Block_Agenda

• ISAP 2014 Conference 22-27 June 2014, Sydney, Australia www.isap2014.com/

Save the date! Call for abstracts now open. Proposed Conference Themes:

- *Photobiology* – uptake systems in algae and adaptation in extreme environments
- *Health & nutrition* – multidisciplinary approaches
- *Biodiscovery & bioresources* – metabolites from lab to market
- *Selecting the strain* – application of molecular and traditional phylogenetics
- *Strain improvement* – genetic, mutagenetic & environmental approaches
- *Agricultural applications* – benefits, economics and scale
- *Developing nations* – harnessing resources and value adding the future
- *Novel industries* – polymer and nanotechnology research and applications
- *Advances in biofuels* – status, collaboration, multidisciplinary approaches, achieving market diversity, current impediments
- *Industry showcase session* – the latest phycolgical tools and technologies
- *Phycological networks* – alignment, services and membership

• Training Courses on Algal Biotechnology supported by ISAP in 2013

In February 2013, ISAP launched a “Call for proposals for support from ISAP to organize training courses in Algal-Biotechnology”. ISAP received four proposals, which were checked by the Committee for ISAP Sponsored Courses, composed by Avigad Vonshak (President), Roberto De Philippis, David Lewis, Emilio Molina Grima, Joao Navalho and Mario Tredici (Members). The funds for supporting the Courses came from ISAP balance (9500 CAN \$) and from a kind contribution of 2500 € given by the European Algae Biomass Association (EABA) <http://www.eaba-association.eu/>.

The Committee decided to support the following four Courses:

1) Training Course: “The use of microalgae for the production of biofuels and for the removal of pollutants”, organized at the Institute of Ecology, Xalapa, Vera Cruz, Mexico, September 2nd-6th, 2013 (tentative date)

Objectives of the course are: i) to provide an overview of the general aspects of algal growth and cultivation, ii) to get to know the environmental friendly algal biotechnologies as modern tools for pollution control, remediation of wastewater and biofuels generation; iii) to review the critical factors related to the production of biodiesel from microalgae.

Name of coordinator and contact details:

Dr. Eugenia J. Olguín, Institute of Ecology, Carretera Antigua a Coatepec #3, El Haya, Xalapa, Veracruz, 91070, México, E-mail: eugenia.olguin@inecol.edu.mx

Research Assistant: Dra. Gloria Sánchez-Galván, E-mail: gloria.sanchez@inecol.edu.mx

2) Training Course: “Algal Biotechnology”, organized at the Department of Plant Biology & Biotechnology, University of Benin, Benin City, Nigeria., November 3rd - 8th, 2013 (tentative date)

Objective of the course is: providing hands-on training to young generation of lecturers of Phycology and Algal Biotechnology who did not have opportunity of overseas training.

Name of Coordinator and contact details:

Professor (Mrs) Medina Kadiri, Department of Plant Biology & Biotechnology, University of Benin, Benin City, Nigeria. Email: mokadiri@hotmail.com

3) Training Course: “Lisbon microalgae biotechnology – first advanced course”, organized by: A4F, Algafuel, Lisbon, Portugal, November 18 – 22 , 2013.

Objective of the course is to promote more advanced and specialized courses in this research field. A4F believes that several graduated students will be interested in the course not only because it is a unique opportunity but also because there is a lot of research needs in this area

Name of Coordinator and contact details:

Vitor Verdelho, vvv@a4f.pt and João Navalho, E-mail: jnavalho@a4f.pt

4) Training Course: “Algaculture for Biotechnology & Molecular Methods for Algae Research”.

This course was held at Scottish Marine Institute (SAMS), Oban, Scotland, June 17th- 20th, 2013. (see the Report in the following pages)

Objectives of the Course are investigating ‘Algaculture for Biotechnology’ and learning ‘Molecular Methods for Algae Research’.

Name of Coordinator and contact details:

Dr John Day, Scottish Marine Institute, Oban, Scotland, E-mail: john.day@sams.ac.uk

The next call for proposals for support from ISAP to organize training courses in Algal-Biotechnology” will be launched in December 2013.

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