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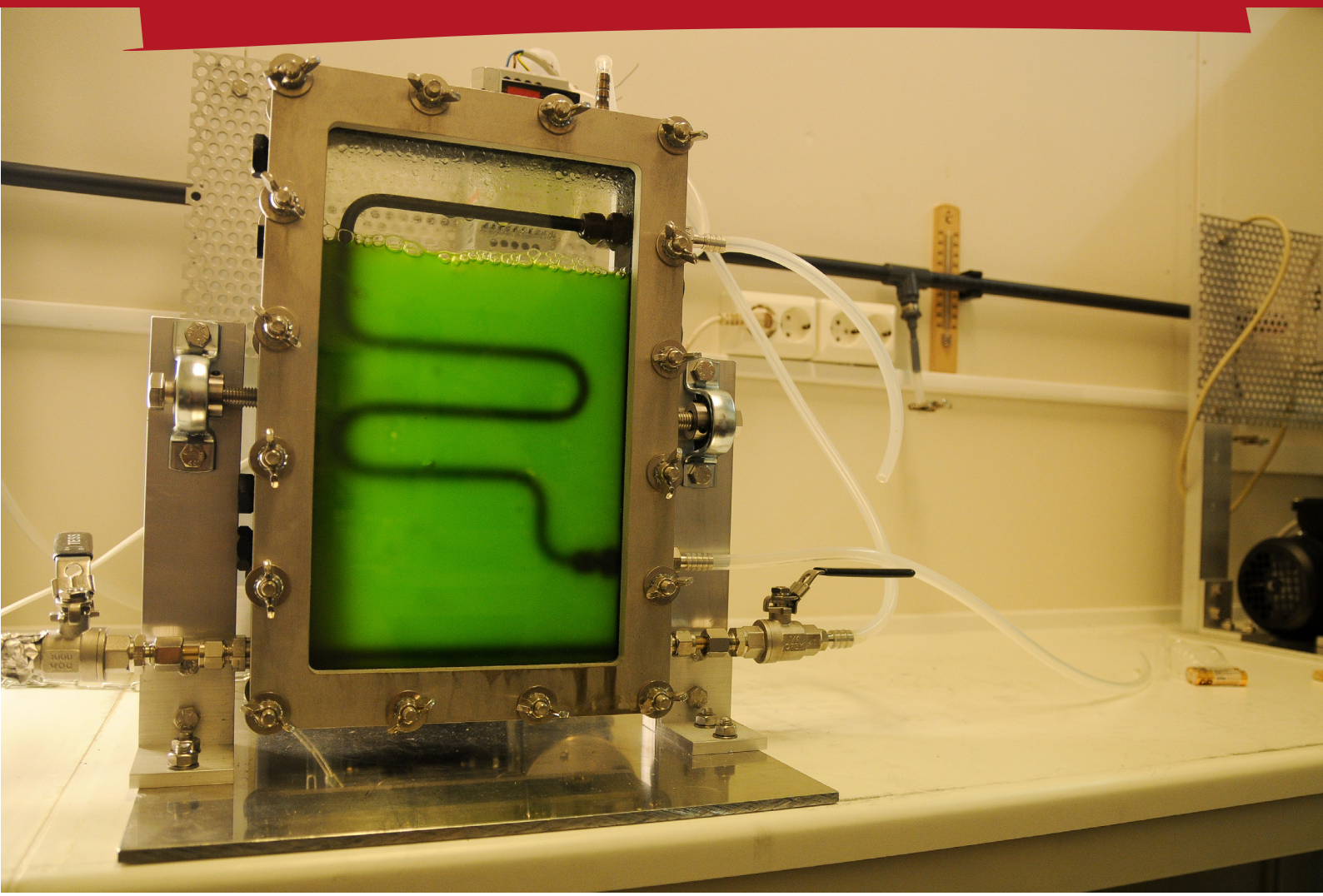

International Workshop on Algae Technology, Hydrogen Production and Use of Algae Biomass

Kolkata, India
17-18 October 2011

Book of abstracts

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Editor: Stig A. Borgvang

BioForsk

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Introduction

Research plays a key role in the Norwegian Government's new strategy for relations with India. The Research Council of Norway's (RCN) has now a dedicated Programme to facilitate Indian-Norwegian Cooperation, the INDNOR-programme. One can read that the RCN wants to promote research collaboration between the two countries that will help to expand research cooperation between India and Norway. "India's increasing economic and geopolitical significance creates new opportunities and poses new challenges. The country is rapidly gaining stature as an important research and knowledge nation," states Arvid Hallén, Director General of the Research Council of Norway.

The research programme is one component of the Norwegian Government's strategy for cooperation between Norway and India. The Government's strategy is designed to facilitate increased investment from Norwegian trade and industry in India. Norway also seeks to strengthen, broaden and further develop its contacts in several other important areas, such as climate change, the environment, research and international and cultural issues. "Cooperation with India within areas such as knowledge development, research and higher education are crucial for strengthening Norway's relations with Indian society," says Hallén.

Our BioCO_2 project fits well into the thematic priority areas of the Research Council which are:

- International political issues
- Climate change
- The environment
- Clean energy
- Social development

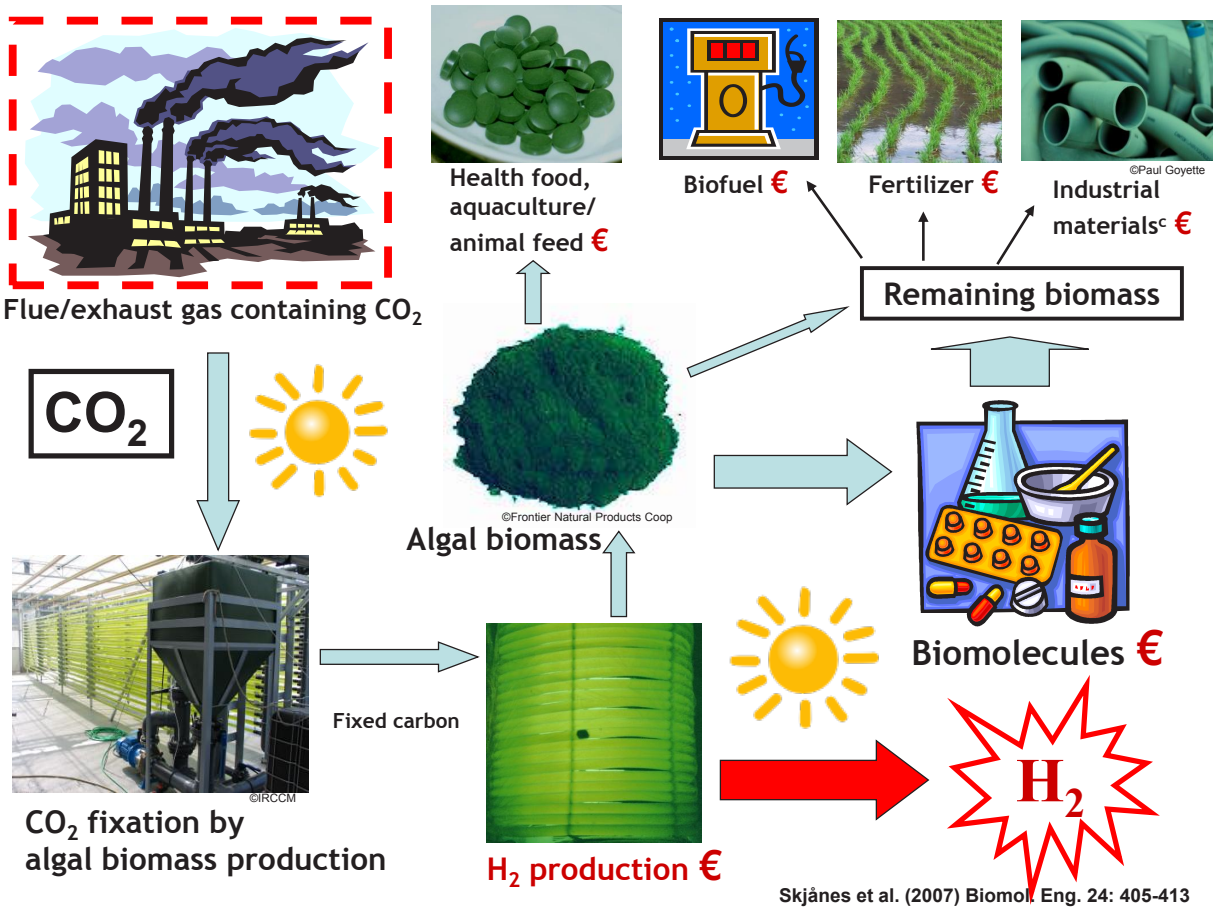
Over the last three years, the Norwegian Institute for Agricultural an Environmental Research (Bioforsk) in Norway, IIT Kharagpur in India and Uppsala University in Sweden have worked together within the framework of the BioCO_2 project, i.e. "An integrated multidisciplinary project using solar energy and algae for production of renewable hydrogen combined with CO_2 capture, to address global warming and energy production", November 2008-November 2011.

This project has been funded 100% by the Norwegian Ministry of Foreign Affairs and facilitated by the Royal Norwegian Embassy in New Delhi.

The project had as main objective to of establishing a Scientific and Technological Platform for the Development of new, Commercially Competitive and Environmentally friendly H_2 Production Systems by converting Solar Energy to H_2 using Photosynthesis in Algae, combined with Capture of CO_2 from Flue Gas and Production of high Value Products.

The project concept is illustrated in the figure below where project partners have mainly focused 6 first steps of the process, i.e.:

- Capture of flue gas as C-source for the photosynthesis
- CO_2 fixation by algae biomass production
- H_2 production by green algae and cyanobacteria
- Production of secondary metabolites
- Use of algae biomass for health food



The co-operation between the three partners have:

- faced and overcome a number of challenges of technical/scientific and cultural character
- based on project objectives and agreed working procedures/methods, achieved a number of important results both in terms of practical outcome, i.e. manufactured project photobioreactors and published a number of project articles in renowned International Journals, and theoretical outcome linked to a number of project activities such as function, characteristics and regulation of hydrogenases, improvement of carbon dioxide biofixation in photobioreactors and maximisation of CO_2 sequestration

At the Annual Project meeting with representatives of the Royal Norwegian Embassy in New Delhi in March 2011 the substantial progress made within the project was discussed, including the considerable interest shown for project activities, in particular at the Delhi International Renewable Energy Conference (DIREC 2010, http://direc2010.gov.in/), elements that could provide the platform for a broader audience Final Project Workshop than originally planned. The Embassy was positive to such an extended Workshop as long-term impacts of the project largely depend on interaction with the private sector. It was also underlined that it would be important to include representatives of relevant government institutions and policy makers in order to disseminate the project findings with respect to potential policy implications.

The co-operation between the three partners therefore reaches an important milestone end of October this year at the organisation of the Project Final Workshop in Kolkata, India, 17-18 October as an *International Workshop on Algae Technology, Hydrogen Production and Use of Algae Biomass*.

Together with the Royal Norwegian Embassy, project partners have taken the opportunity to invite fellow researchers, students, industrial interests and relevant authorities to share results and experiences, and have a joint "look into the future of solar energy microalgae, biohydrogen and secondary metabolites" in Kolkata. The interest in the Workshop has been considerable and the Workshop now integrates participants:

- with main fields of research linked to:
  - green algae and cyanobacteria-biomass production
  - photobioreactor design and operation
  - CO_2 capture, use of flue gas for algae cultivation
  - green algae and cyanobacteria- H_2 production
  - secondary metabolites, health food from microalgae
- from industrial plants interested in CO_2 capture and algae cultivation
- from commercial interests linked to hydrogen production and/or use of secondary metabolites
- from the BioCO_2 project
- from the Royal Norwegian Embassy in New Delhi

Project results will be presented by representatives from all three project partners at all of the four Workshop sessions. However, the main interest of the Workshop remains the interaction between project scientists and scientists from 14 Indian states, 4 European countries and Thailand, with representatives of a number of industrial organisations related to the four Workshop themes and sessions, i.e.:

Photobioreactors: Construction of algae bioreactors: design and development. Scale-up.
Cultivation of Green algae and Cyanobacteria, effects of flue gas: Growth optimisation. Use of flue gas as CO_2 source.
Hydrogen production from Green algae and Cyanobacteria: Novel processes. H2 production mechanisms at molecular level. Nutrient formulation/optimisation.
Secondary metabolites and health food: Health promoting chemicals, feed additive, food additive.

My wish as BioCO_2 Project Co-ordinator and Workshop Chairman is that the Workshop will:

1. shed further insight into the scientific and technical challenges we face with regard to the four Workshop themes
2. provide fruitful discussions between Workshop participants
3. provide each Workshop participant with added knowledge and experience
4. make progress as regards to the elements necessary to achieve viability of the process by making steps towards bridging of the gap between research and commercial interests

BUT MOST OF ALL I wish that the Workshop be remembered as a useful, interesting and enjoyable event where people from different cultures and backgrounds met to discuss vital aspects for future life on this globe that we share.

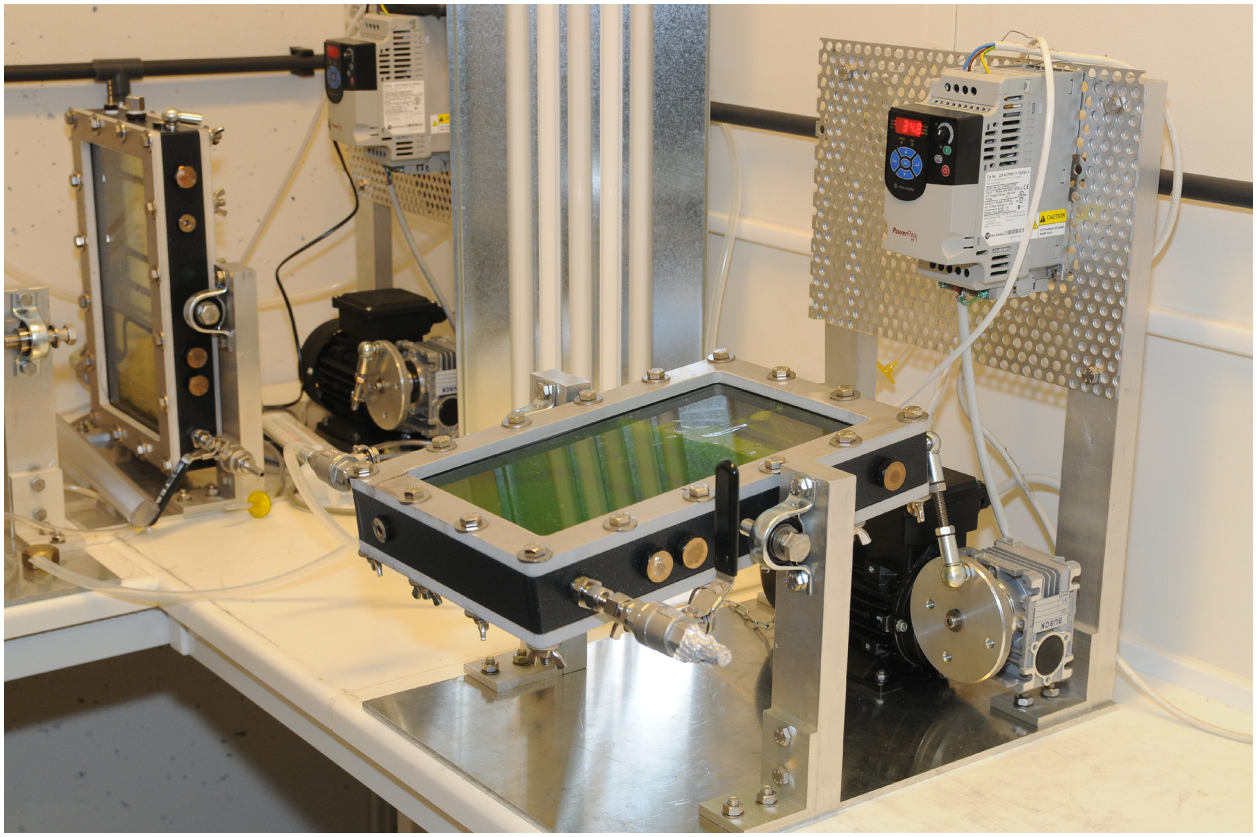
This Workshop represents the end of a project that, in a way, started with a presentation of a concept for hydrogen production from solar energy using flue gas at the Royal Norwegian Embassy in New Delhi in April 2008, followed by a concept presentation at the Indo-Norwegian co-operation meeting in Oslo end of May 2008 (although the contractual start was in Kharagpur in November 2008) and now ends in Kolkata October 2011.

However, the end of the project is not the end of India-Norwegian co-operation on energy related issues. I do believe I can state that all three project partners, i.e. scientific/technical personnel, have appreciated the engagement and enthusiasm for environmental and energy related issues emanating from within the Royal Norwegian Embassy in New Delhi, made possible by H.E. Ms. Ann Ollestad. We have met fellow researchers in India, Norway and Sweden, we have created a platform for future work, not only between researchers and research institutes, but for efforts of research, commercial interests and policy.

This Book of Abstracts provides you with a first insight into Workshop presentations, both oral and posters. More than 20 posters and almost the same number of oral presentations will be presented during the two days of Workshop. I hope you will enjoy the oral presentations from a large number of renowned scientists, the interesting poster sessions with a panoply of poster presentations related to all Workshop themes, the Panel discussion where we wish to involve not only Panel Members, but also where the remaining participants should voice their opinion and share their experiences and, not to underestimate, the possibilities providing during breaks and joint meals to more unofficially share experiences and ideas.

Ås, 11 October 2011

Stig A. Borgvang



Background, challenges, implementation and main outcome of the BioCO_2 project within the Indo-Norwegian co-operation framework

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Project background: A Joint Indo-Norwegian-Swedish Scientific Research Collaboration Project , facilitated by the Royal Norwegian Embassy in Delhi, funded by the Norwegian Ministry of Foreign Affairs with the aim of establishing a Scientific and Technological Platform for the Development of new, Commercially Competitive and Environmentally friendly H_2 Production Systems by converting Solar Energy to H_2 using Photosynthesis in Algae, combined with Capture of CO_2 from Flue Gas and Production of high Value Products.

Challenges include making the process economical and energetically efficient, producing H2 from algal cultures grown on different carbon sources, including using flue gas from industry as a CO2 source., designing and manufacturing functional and optimised photobioreactors for H2 production, understanding better functions, characteristics and regulation of hydrogenases, as well as the enzyme's role in the energy metabolism of the cell and analysing the algae biomass for production of valuable components.

Implementation: All three partners have carried out research work in their respective institutes, Bioforsk on green algae, Uppsala University on cyanobacteria and IIT Kharagpur on both green algae and cyanobacteria. The latter has carried out a large proportion of the practical work. Bi-annual meetings, all but one in India, were held to discuss research results, exchange experiences and agreement on next year's work.

Main Outcome include the design, construction and testing of a flat panel, rocking photobioreactor for algae cultivation (non-rocking mode) and hydrogen production (rocking mode), optimisation of the H_2 production from green algae and cyanobacteria by improved insight into function, characteristics and regulation of hydrogenases, including discoveries of transcriptional regulation of Hox-hydrogenase genes in the cyanobacteria *Synechocystis*, introduction of foreign hydrogenase into bacteria and discovery of three distinct HydA hydrogenase genes in the green algae *Chlamydomonas noctigama*, Improvement of carbon dioxide biofixation in photobioreactor by using *Anabaena sp. PCC 7120*, maximisation of CO_2 sequestration by *Chlorella sorokiniana*, studies and analysis of biomass production and characterisation of *Scenedesmus MJ11/18* with respect to total protein, carbohydrate, lipid and carotenoids content under different culture conditions. Furthermore, the project has provided the resources and research framework for five PhD thesis (already defended or soon to be defended).

Main future non-scientific challenge is bridging of the gap between research and commercial interests in order to make the whole process economically viable.

Microalgal cultivation in closed photobioreactors: design and scale-up

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Although tubular reactors represent a promising technique for mass production of microalgae, information concerning large-scale unit is scanty. At the present, to the best of our knowledge, only very few industrial tubular reactors have been constructed and are in the stage of reaching a full commercial operation, i.e. *Chlorella* in Germany, and *Heamatococcus* in Hawaii and Israel.

One of the main drawbacks in the use of industrial scale photobioreactors is due to the lack of a reliable systematic scale-up method. Many small-scale reactors have been proposed and operated during the last 20 years with fairly impressive productivity data. Yet, only lately initial attempts have been made that may represent the first indication for a breakthrough in enabling scale up of the laboratory scale prototypes to a real industrial operational system that still has to be proven to be of economic feasibility. In general, scale-up can be realized by increasing the tube length and/or the tube diameter. Simulations based on the mass transfer model indicate that increasing tube length for a constant diameter will alter the culture pH at the tube exit, and in particular the oxygen concentration in the culture, as well as the CO_2 losses. The major problem is not how to develop efficient photobioreactors, in term of productivity and light utilization efficiency, but rather how to develop large-scale photobioreactors which are cost effective.

Mass cultivation of useful microalgae in Indian Scenario

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Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications. Several microalgae are potentially promising for the food industry as a valuable source of LCPUFAs in alternative to fish oil, supplying sterols, tocopherols, colouring pigments and other neutraceuticals. Recently microalgae have been considered as a suitable feedstock for biodiesel production in substitution for oil from vegetable crops and also for hydrogen production. A number of microalgae have been reported having medicinal properties like preventive actions against atherosclerosis, hypercholesterolemia by glycolipids and phospholipids, and antitumor actions by glycoproteins, peptides and nucleotides.

Microalgae are also active as immune-stimulators, free-radical scavengers and reducer of blood lipids. Therefore mass cultivation of potential algal genera represents an important field of research. Mass cultivation of useful biomass using photobioreactors started in the 1960s in Taipei, Taiwan by 'Nihon Chlorella'. In India, *Spirulina* cultivation started in the 1970s at CFTRI, Mysore; and Muragappa Chettiar Research Institute, Chennai followed by ICAR,PUSA. Since then several companies and research institutes have initiated cultivation programmes of different algae. Still only a few algal taxa have been commercialised in India, as the production cost of most of the algal biomass remains very high in the Indian market.

The main obstacle of microalgal cultivation system is to provide congenial system of particular microalgae to grow in pure form with cost effective methods. In the present study, the constraints of mass cultivation of a few potential micro-algae will be discussed in an Indian scenario in different systems, providing examples. As it is well known for algologists that the problem is not making bioactive compounds from algae, it is making algae with bioactive compounds; actually it's just making algae.

Suitability of different photobioreactors for CO_2 sequestration and biohydrogen production using green algae and cyanobacteria

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Hydrogen production through biological routes is promising because they are environmentally friendly. Hydrogen production through biophotolysis or photofermentation is usually a two stage process. In the first stage, CO_2 is utilised for biomass production, followed by hydrogen production in the second stage under anaerobic/sulfur-deprived conditions. In addition, one-stage photobiological hydrogen production process can be achieved using selected cyanobacterial strains (*Anabaena*, *Synechocystis* and *Nostoc punctiforme*).

The green algae used in the present studies were *Chlamydomonas reinhardtii*, *Chlamydomonas noctigama* and *Chlorella sorokiniana*. All the cyanobacterial cultures were supplied by Uppsala University, Sweden, and the green algal cultures were supplied by Bioforsk, Norway. The major challenges confronting the large scale production of biomass/hydrogen are limited not only to the performance of the photobioreactors, in which light penetration in dense cultures is a major bottleneck, but also to the characteristics of the organisms. Other dependable factors include area/volume (A/V) ratio, mode of agitation, temperature and gas exchange.

Photobioreactors of different geometries were used for biomass and biohydrogen production: Airlift, Bubble column, Tubular, Flat plate, Controlled fermentor type etc. Every reactor has its own advantages and disadvantages. Biological fixation of CO_2 was greatly affected by the characteristics of the microbial strains, their tolerance to temperature and the CO_2 present in the flue gas including SO_x,NO_x. However, there are additional factors like the availability of light, pH, O_2 removal, culture density and the proper agitation of the reactor, influencing significantly the CO_2 sequestration process. Present research work is also focused on the hybrid types of reactors (integrating two reactors), which can be used for overcoming the bottlenecks of a single photobioreactor. Airlift reactors were found most suitable with respect to biomass production, whereas the flat panel rocking reactor was found suitable for the hydrogen production. The bioreactors were employed for hydrogen production with necessary modifications to overcome the existing bottlenecks like gas hold up, oxygen toxicity and poor agitation.

Technical and scientific challenges to overcome to design and construct an efficient, combined photobioreactor at lab-scale for microalgae cultivation and hydrogen production

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The research co-operation project between The Norwegian Institute for Agricultural and Environmental research in Norway, Uppsala University in Sweden and IIT Kharagpur in India, the BioCO_2 project, has designed, constructed and tested a flat panel, rocking photobioreactor for algae cultivation (non-rocking mode) and hydrogen production (rocking mode). It consists of two glass plates fixed between an inner frame made of stainless steel and outer frames made of aluminium, an air bubbling tube and a tube designed for temperature regulation.

All parts in contact with the algae culture are coated with a layer of non-toxic Teflon (PFA) (inner frames, screws and connections, air tube, temperature tube). Hydrogen leakage free sealants are used in all contact points and connections. The specially designed stand and engine/gear box allow a smooth and well-balanced rocking motion for optimised hydrogen production.

Several alternatives have been tried for all parts in contact with the algae culture- change of material e.g. different types of SS, change of coating substance, change of dimensions of screws and sealants. This study shows the pros and cons of the alternatives having been tested and demonstrates the strengths of the selected solutions.

Photobioreactors for Microalgal Cultivation vis-à-vis Biofuel Production: Design Considerations and Complications

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In recent years, microalgae have occupied the centre stage in biofuel research mainly for their use as lipid rich feedstock for biodiesel and as polysaccharide producing cellulose containing biomass feedstock for bioethanol production. Particularly for biodiesel, marine microalgae have emerged as a potentially viable source of the lipid feedstock in terms of very high neutral lipid productivity as opposed to the non-edible, non-conventional energy crops like Jatropha and Karanja, the mass cultivation of which requires huge amount of land area, fertiliser and water.

On the other hand, microalgae can be easily grown to high biomass density in openraceway pond system and with closed photobioreactors. While open raceways are preferred for robust microalgae strains with contamination-proof culture conditions, photobioreactors are favoured for their greater flexibility with less vulnerability to contamination, varied configurations and higher efficiencies in terms of controlling the media and process parameters for achieving maximum biomass and product productivities. While designing a photobioreactor, certain parameters are given utmost importance such as growth rate and characteristics of the culture, whether filamentous or unicellular, the culture light distribution and penetration, liquid circulation, CO_2 transfer and consumption, dissolved O_2, pH, temperature, nutrient composition, superficial gas and liquid velocities, gas hold up and mass transfer characteristics.

Among various designs, the tubular / horizontal, column / vertical and flat plate / panel reactor configurations have been very popular for algal cultivation. The factors critically affecting the mixing performance or the mass transfer characteristics of the reactor are geometric design, operational variables, fluid and hydrodynamic properties. The biggest challenge is to scale up these reactors and optimise each parameter without affecting the overall process. Despite having number of advantages, the photobioreactor designs need to address certain complications that contribute to system cost and energy demands, which are very high when compared to any other traditional reactor designs. They require large amount of space and their scalability is difficult. Due to photoinhibition and lower gas velocities, growth of the culture may be adversely affected. Other major complications include low photosynthetic efficiency, shear damage of cells due to aeration, high oxygen concentration and improper settling of algal culture. Proper design considerations for addressing these complications adequately will surely make photobioreactors a promising device for mass algal cultivation with high cell density.

Reduction CO_2 in thermal power plant by Algae based carbon capture technology

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Nearly 73% of India's total installed power generation capacity is thermal, of which coal based generation about 90%. A 1000 MW pulverized coal-fired power plant emits 6 to 8 Mt/yr of CO_2. Increasing of greenhouse gases and their effect on global warming, is a major environmental problem today. Much research has been made to reduce CO_2 emissions from different industries. Among them Algae based carbon capture technology has a good future in thermal power plants of India to reduce greenhouse gas emissions. Flue gas of thermal power plants contains N_2 (82%), CO_2 (14%), CO (80 ppm), O2 (4%), NO_x (70 ppm), SO_x (it is very small quantity (0,03%) because, Indian coal has low sulfur content) and soot dust (about 50mg/m^3). Indian coal also has low carbon content (25% to 30%) and high ash content (46% to 80%).

Algae can be grown on high concentrations of CO_2, NO_x and at temperatures from freezing point to about 80 ^oC. Also high concentration of ash in the flue gas cannot affect the algae growth. Via photosynthesis, CO_2 and water are basic requirement for algae growth; O_2 and water vapour are the by-products. NO_x or SO_x can be effectively used as nutrients for microalgae. Thermal power plants emit nearly 1T of CO_2 for every MWh, by which nearly 0.5T algae can be grown.

Flue gas temperature of a thermal power station (after induced draft fan or Electrostatic Precipitator (ESP)) is about 120 ^oC. It can be decreased to 60 ^oC by direct contact with ambient water. In this process heavy ash particle are also being removed. Then it can be directly fed to the photobioreactor, in which algae can grow. Some species of algae (*Chlorella* sp.) can grow in the effluent water. There are different types of photobioreactors viz. open pond, closed pond, tubular and plastic bag. This process is more effective because it reduces the greenhouse gas CO_2, as well as providing by-products such as bio-diesel, ethanol, fertilizer for agriculture use, high protein animal food, biopolymer / bio-plastic.

Use of CO_2 in Microalgal Biomass Production Enhancement

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The increased interest in biofuels driven by the anticipated global fuel demand and environmental security has raised the issue of food vs. fuel. Aquatic biofuel feedstocks, to a great extent, can overcome this issue. In general, aquatic plants are more efficient in fixing CO_2 than the terrestrial plants. Again among the aquatic plants, the microalgae are very efficient in nutrient uptake and biomass production. They can also be cultivated on otherwise non-productive lands or in brackish, saline, and wastewater that has little competing demand.

Besides the nutrient requirements, it has been found that CO_2 as a source of carbon has enhancing effect on biomass production. Addition of CO_2 in culture medium also helps in easier harvesting of the algal biomass. In this connection use of wastewater and flue gases for the cultivation of algal biomass are good options for reducing the environmental burden.

While high lipid yields can be obtained under nutrient limitation, this is generally at the expense of reduced biomass yields. Nevertheless, the possibility that microalgae could generate considerable amount of biomass from wastes is an exciting opportunity. Although the land requirement for algal cultivation is non-competing, its water requirement and other environmental aspects need a life cycle assessment for sustainability of this option.

Studies on Growth of Microalgae for CO_2 Sequestration—A Review

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In view of increasing energy costs and the global impact of petroleum based fuels, focus on developing alternative energy solutions from renewable resources are growing. Microalgae are remarkable and efficient biological factories capable of taking a waste (zero-energy) form of carbon (CO_2) and converting it into a high density liquid form of energy (natural oil).

Microalgae's direct utilisation of CO_2 for growth also facilitates CO_2 sequestration and helps reduce greenhouse gases responsible for global warming. However, algae are not as well understood as other organisms like bacteria, fungi etc., which are extensively used in today's biotechnology industries. In-depth studies on growth characteristics of oil-rich microalgae are necessary to exploit the benefits of micro-algal technology fully.

Our group investigates the growth kinetics of two microalgae, namely, *Scenedesmus sp*. and *Phormidium sp*. both in small flasks, as well as in cost-effective reactors. Effects of CO_2 feeding rate, dark and light cycles etc. on the overall growth characteristics of microalgae have been studied. Optimum temperature, maximum specific growth rate, activation energy, yield coefficient on the basis of CO_2, algae and lipid production rates of both the strains have been determined. FTIR analysis of algal oils have been performed. Pyrolysis characteristics of microalgal biomass have been determined. CHN analysis of algal oils, pyrolysis char and tar have also been performed. Present review focuses on the results of the investigation of the present group, as well as on the present status of international research from the same perspective.

Improvement of carbon dioxide biofixation in photo-bioreactor by using *Anabaena* sp. PCC 7120

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The biological photosynthetic process is found suitable and sustainable under both environmental and social considerations as compared to other CO_2 mitigation options like oceanic or geologic injection.

In the present study, *Anabaena* sp. PCC 7120 was used for the carbon dioxide sequestration processes. A customized airlift photobioreactor, having internal draft tube, was found to give higher light utilisation and higher rate of CO_2 biofixation in comparison to that of bubble column. The maximum biomass concentrations were 0.71 and 1.13 g L^{-1} in bubble column and airlift photobioreactor respectively, using BG110 medium under aerated condition. The specific growth rates of the cell were 0.78, 1.15, 1.17, 1.18, and 1.13 day^{-1} at 30, 40, 50 60 and 70 mg L^{-1} of phosphate concentration respectively in the exponential growth phase.

The respective CO_2 biofixation rates of *Anabaena* sp. PCC 7120 were 0.461, 0.581, 0.583, 0.677, 0.52 g L^{-1} day^{-1} respectively under similar phosphate concentrations, light intensity of 120 µE m^{-2} s^{-1} and 5 % (v/v) CO_2 enriched air. However, it was observed that with the increase in light intensity there was an extension of the linear growth phase without any significant change in the specific growth rate. The present research work shows that *Anabaena* sp.PCC 7120 may be considered as a suitable candidate for bio-CO_2 sequestration because it can tolerate higher concentration of CO_2. Furthermore, the study showed that increased light intensity, phosphate and CO_2 concentrations could enhance the CO_2 biofixation efficiency.

Maximization of CO_2 sequestration by *Chlorella sorokiniana*

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The threat of global warming is becoming more and more alarming as the time passes due to increased CO_2 concentration in the atmosphere. It is imperative to identify and improve the process of CO_2 sequestration. The present paper explores the possibility of use of the green algae *Chlorella sorokiniana* for the same.

Studies were conducted using different percentage of carbon dioxide with air in order to find out how to maximize the algal biomass production. The pH of the growth medium plays an import role for the algal biomass production. The use of $NaNO_3$ in place of NH4Cl in growth medium improved the algal growth significantly. The standard TAP [-acetate] medium was modified by replacing NH_4Cl by $NaNO_3$ to control the drastic decrease in pH of the medium not only due to NH_4Cl, but also due to higher percentage of CO_2 input in the reactor. In addition, the performance of the bubble column and airlift reactors was compared with respect to KLa, mixing time and growth profile in different percentages of CO_2.

Algal strain selection and characterisation: A key to successful algae feedstock production

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Photosynthetic algae and cyanobacteria are an extremely diverse, yet highly specialized group of microorganisms that live in different ecological habitats with varied climatic conditions and nutrient availabilities. Besides their known contribution in food, feed, agriculture, industry, environment and pharmaceuticals, algae have nowadays received lot of attention as a new biomass for alternate fuels, as well as their role in CO_2 sequestration to safeguard environment. Many algae accumulate a significant amount of lipids, mainly in the form of triacylglycerides (TAGs,) which can be converted into biodiesel.

While algae may provide the natural raw material in the form of lipid rich feedstock, the immense diversity among algal species for biofuel production makes it imperative to select the best strains for the purpose. For a successful and promising bio-feedstock for biofuel, there is a need to isolate algal strain/s with higher oil yielding efficiency, faster growth rate, higher CO_2 fixation and wide range of tolerance to varying environmental conditions so that using algae for biofuel production is commercially viable and economically feasible.

This can be achieved by exploration of the natural algal biodiversity followed by strain improvement using modern biotechnological tools to increase their effectiveness. Characterisation of selected algal strains based on growth behaviour, lipid and fatty acid profile as influenced by abiotic and biotic factors will further help in optimizing the conditions for maximizing biomass production and specific lipid class abundance.

Algal species are also known to accumulate large amounts of lipids when exposed to some form of stress. Modification of biochemical pathways, which trigger this accumulation, may further enhance the ability of these organisms to produce more targeted lipids throughout the growth cycle.

Progress in the photobiological hydrogen production with *Chlamydomonas reinhardtii* cultures in laboratory and outdoor photobioreactors

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A D1 protein mutant of *C. reinhardtii* has been screened for its H_2 evolution capacity. The L159I-N230Y mutant carries a double amino acidic substitution. The L159 leucine residue was replaced by isoleucine, and the N230 residue asparagine replaced by tyrosine. This mutant has proved to be 5 times more productive as compared to the cc124 strain, and is one of the most productive strains described in the literature, so far.

A preliminary phenotypic characterization has identified some important features, such as: (i) a reduced amount of chlorophyll per cell) (ii) high photosynthesis and respiration rates, (iii) higher D1 content per cell, and (iv) higher accumulation of starch. Results of hydrogen production experiments carried out with *C. reinhardtii* cultures in a 50-liter horizontal tubular photobioreactor under both artificial and direct solar light are also presented. In both cases, the H_2 output attained was 18-20% of what was obtained in the laboratory. It was concluded that the reduced H_2 output achieved in the 50-L photobioreactor was due to the different illumination pattern to which the cultures were exposed (one-sided vs. a two-sided illumination provided in the laboratory), as well as to the great difference in the mixing times (60 min vs. 15 s achieved in the lab-scale photobioreactor).

Evidence for transcription of three genes with characteristics of hydrogenases in the green alga *Chlamydomonas noctigama*

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The production of hydrogen in green algae is catalyzed by FeFe- hydrogenases, which have high conversion efficiency and high oxygen sensitivity. Most green algae analyzed to date where hydrogenase genes are detected, have been shown to contain two distinct hydrogenases. However, very little is known about which functions the two different enzymes represent.

The presented study focuses on the possibility for presence of more than two hydrogenases in a single green alga. A large number of degenerate primers were designed and used to produce 3'-RACE products, which in turn were used to design gene specific primers used for PCR and 5'-RACE reactions. The sequences were aligned with known algal hydrogenases to identify products which had homology to these. Products where homology was identified were then explored further. A high number of clones from each band were sequenced to identify products with similar lengths which would not show up as separate bands on a gel. Sequences found to have homology with algal hydrogenases were translated into putative amino acid sequences and analyzed further to obtain detailed information about the presence of specific amino acids with known functions in the enzyme. This information was used to evaluate the likelihood of these transcripts coding for true hydrogenases, versus hydrogenase-like or narf-like proteins.

Conclusion: Evidence showing that *Chlamydomonas noctigama* is able to transcribe three genes which share a significant number of characteristics with other known algal FeFe-hydrogenases is presented. The three genes have been annotated *HYDA1*, *HYDA2* and *HYDA3*.

Transcriptional Regulation of Cyanobacterial Hydrogenases

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In cyanobacteria three enzymes are directly involved in the hydrogen metabolism: a nitrogenase producing H_2 as a by-product of nitrogen fixation, an uptake hydrogenase (HupSL, encoded by *hup*SL) that recaptures H_2 and oxidize it, and a bidirectional hydrogenase (encoded by *hox*EFUYH, forming an enzyme with a hydrogenase part, HoxYH, and an electron transfer partner protein, HoxEFU) that can both oxidize and produce H_2 (1). Generally, little is known about the transcriptional regulation of cyanobacterial *hup* genes, wheras the regulation of the *hox* genes have been characterised in some more detail.

Results obtained, with a strain containing a not fully segregated inactivation mutation of the *abrB*-like gene and a strain overexpressing the same *abrB*-like gene, suggested that CalA functions as a regulator of *hox* gene expression (2). In addition, LexA interacts with the promoter region (3). Further studies using the filamentous, heterocyst-forming, nitrogen-fixing strain *Nostoc* PCC 7120 demonstrated that CalA interacts with the upstream region of the *hypC* operon (4). HypC is one out of the auxiliary gene products required for synthesis of a functional hydrogenase. The bidirectional hydrogenase activity was significantly downregulated when CalA was overexpressed, demonstrating a correlation with the transcription regulator, either directly or indirectly (4). The same strain showed a bleaching phenotype with lower growth rate and truncated filaments two days after induction of overexpression (5).

Using gel-based quantitative proteomics, the induced overexpression of CalA was shown to downregulate the abundance of FeSOD, one of two types of superoxide dismutases in *Nostoc* 7120. The change in protein abundance was also accompanied by lower transcript, as well as activity levels. Purified recombinant CalA from *Nostoc* 7120 was shown to interact with the promoter region of *alr2938*, encoding FeSOD, indicating a transcriptional regulation of FeSOD by CalA (5). The bleaching phenotype is in line with a decreased tolerance against oxidative stress and indicates that CalA is involved in regulation of cellular responses in which FeSOD has an important and specific function in the filamentous cyanobacterium *Nostoc* 7120.

Significant advances have been made in the understanding the transcriptional regulations of cyanobacterial hydrogenases, needed knowledge when designing cells for a direct production of renewable H_2 from solar energy and water.

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Studies on heterologous expression of *Enterobacter cloacae* IIT-BT 08 hydrogenase in cyanobacteria

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Fossil Fuels such as coal and gasoline provide most of the energy needs of the world today. However, because of their limited reserves, high prices and, most importantly, their devolving effect on the environment, alternative sources of energy and environmental friendly fuels are now being developed.

Biohydrogen is currently regarded as the most promising future energy source as it shows clean combustion and can easily be converted into electricity via fuel cells. In keeping with the same, hydrogen evolution by cyanobacteria has been extensively studied. However, it has been found that nitrogenase-dependent hydrogen production is theoretically less efficient than hydrogenase-dependent production, this because of ATP requirements.

Enterobacter cloacae IIT-BT 08, a dark fermentative gram negative bacteria, has been previously reported as a high hydrogen producing organism. It has [FeFe] hydrogenase as the key enzyme responsible for hydrogen production. Though it is a short sequence of 444 bp, it includes the functionally important and conserved motif, the H-cluster of [FeFe]-hydrogenase which incorporates the catalytic site. Thus, the present study investigated the expression of *E.coli*-cyanobacteria codon optimised [FeFe] hydrogenase in *Nostoc punctiforme* ATCC 29133 and *Synechocystis sp*. PCC 6803 using synthetic biology tools and techniques.

The effect of co-expression of codon optimised maturation systems of *Clostridium acetobutylicum* or *Chlamydomonas reinharditti* along with hydrogenase construct was also studied. Although the construct could not be expressed in cyanobacteria, it could be successfully expressed in its homologue, *E. cloacae*, having its own native hydrogenase maturation system. The recombinant *E. cloacae* IIT-BT 08 expressing [FeFe]-hydrogenase actively produced H2 (58.29 mmol H_2/(h•L) in 300 mL in glucose medium under anaerobic condition, whereas the wild type was found to produce 35.62 mmol H_2/h•L At the operational pH 6.5, the volatile fatty acid concentration of the recombinant showed 1.2 times higher acetate to butyrate ratio (A/B ratio) as compared to that of the wild type. The study thus concluded that the hydrogenase from *Enterobacter cloacae* may be novel and hence could not be maturated in cyanobacteria by either of the maturation systems used in this study.

Improvement of H_2 production by redirecting electrons to bidirectional Hox-hydrogenase in *Synechocystis* sp. strain PCC 6803

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Nitrate reductase (*narB*) and nitrite reductase (*nirA*) are the enzymes involved in the nitrate assimilation pathway. This pathway is a competing pathway that reduces the electron flow to the bidirectional Hox-hydrogenase, resulting in a decrease of H_2 production. The genetic engineering of the nitrate assimilation pathway was used to improve H_2 production in unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. We constructed Synechocystis mutant strains disrupted in either nitrate reductase (*ΔnarB*) or nitrite reductase (ΔnirA) or both nitrate reductase and nitrite reductase (*ΔnarB:ΔnirA*), and tested for their ability to produce hydrogen.

Higher H_2 production and bidirectional Hox-hydrogenase activity were found in all mutant strains compared with the wild type. Highest H_2 production was observed in the *ΔnarB:ΔnirA* strain. Small changes of Hox-hydrogenase enzyme activities and only minor changes in transcript levels of *hoxH* and *hoxY* were observed. The data showed no correlation between activity, transcriptional level and H_2 production. These results clearly demonstrated that the increased H_2 production in the *Synechocystis* sp. strain PCC 6803 could be achieved by using metabolic engineering to inactivate the nitrate assimilation pathway so that more electrons could be redirected towards to the Hox-hydrogenase.

Potential for use of green microalgae to produce hydrogen from solar energy, with subsequent use of algal biomass for pharmaceutical or industrial products

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Green microalgae can be used for a number of commercial applications, including health food for human consumption, aquaculture and animal feed, coloring agents, cosmetics and pharmaceuticals. Several products from green algae that are in use today consist of metabolites which can be extracted from the algal biomass. The most well-known examples are the carotenoids astaxanthin and ß-carotene, which are used as coloring agents and for health promoting purposes. Many species of green algae are able to produce valuable components for different uses, examples are antioxidants, several different carotenoids, polyunsaturated fatty acids, vitamins, anticancer and antiviral drugs. In many cases these components are secondary metabolites, produced when the algae are exposed to stress conditions induced by environmental factors like for example nutrient concentration, light intensity, temperature, salinity, pH and other. In other cases the components have been detected in algae grown under optimal conditions, and little is known about how an optimal production of each product could be induced and how their production would react to stress conditions.

Some green algae have shown the ability to produce significant amounts of hydrogen gas during sulfur deprivation, a process which is currently extensively studied. Currently there is scarce information available regarding the possibility for producing hydrogen and other valuable components in the same process.

This study explores stress conditions which are known to induce production of the different valuable products in comparison with stress reactions leading to hydrogen production. This information is explored with the goal of forming a basis for a future multidisciplinary process, where hydrogen production from solar energy is combined with production of valuable metabolites and other commercial uses of the algal biomass.

Extraction & Characterization of Biochemicals from Microalgal Biomass and its Use in Preparation of RTS Beverage

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Spirulina is well known for its protein supplements and pigments. Optimization of an ultrasound assisted solvent extraction (hexane: acetone and water: ethanol) process from *Spirulina platensis* biomass was carried out using response surface methodology. Central composite rotatable design (CCRD) was used for designing the experiments using 4 independent variables (solvent volume, extraction temperature, extraction time and sonicator amplitude) with 5 levels each. The extract obtained under optimized extraction condition was freeze dried and characterized for different Invitro biological assays viz., protein digestibility index, Invitro antioxidant activity, Invitro antidiabetic activity (α-amylase and α-glucosidase inhibition) and Invitro anticancer activity.

A ready-to-serve (RTS) beverage was prepared by adding 0.1 % w/v of freeze dried extract powder. The optimization of extraction process variables showed that maximum yield of ß-carotene (0.18 mg/g) and phycocyanin (30.72 mg/g) was obtained at solvent volume 20 ml, and extraction time 4 min. The optimum level of the sonicator amplitude and temperature was 48 % and 74 %, and 35 ^{O}C and 15 ^{O}C, for ß-carotene and phycocyanin, respectively.

Results of various Invitro biological assays of the extract showed that the protein digestibility index was 79.40 %, IC_{50} value of antioxidant activity, α-amylase inhibitory assay, α-glucosidase and anti-cancer activity were 155.80 µg/ml, 58.47 µg/ml, 51.28 µg/ml and 91.43 µg/ml, respectively. The overall quality of the developed RTS beverage was good under refrigerated (5±2 ^{O}C) storage for 2 months. However, at ambient (30±2 ^{O}C) and accelerated (45±2 ^{O}C) conditions the overall quality was better up to 30 days.

Biotechnological Potentials and Role of Cyanobacteria in Agriculture and Industry

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Cyanobacteria, commonly known as blue green algae, form an unusually well-defined group of organisms of undoubted antiquity showing oxygenic photosynthesis. Their prokaryotic cellular organization is characterized by the lack of membrane bound organelles. The structural kinship with bacteria on one hand and functional proximity to eukaryotic algae and higher plants on the other hand has formed the framework of increased attention devoted to these organisms. A number of important advances have occurred in cyanobacterial biotechnology in the recent years and worldwide attention is drawn towards these organisms for their possible use in food, feed, biofertilizer, wastewater management, colorant, production of secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, lipids and other high value molecules of industrial applications.

Extensive research on different fundamental and applied aspects of these organisms has demonstrated that the biomass can be used for the diverse applications. There can be utilization of whole biomass or certain valuable constituents are extracted, including metabolites and enzymes. Large number of processes has been developed for mass cultivation and isolation of valuable constituents from these organisms. Despite the well-known potentials reported in literature, these are not well studied still from a biotechnological point of view. Out of several thousands of species known to exist, few have been studied for biochemical profile and handful is cultivated for industrial purpose. Production systems have to be improved in these organisms in order to become more competitive and economically feasible. Heterotrophic and mixotrophic cultivation could be a possible avenue of research. Genetic improvement also poses a challenge, and use of transgenic strains for commercial applications may hold significant promise. In view of this, the possible biotechnological potentials and role of cyanobacteria in agriculture and industry will be discussed.

Biomass production & characterization of *Scenedesmus* MJ11/18 with respect to total protein, carbohydrate, lipid and carotenoids content under different culture conditions

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Algae have become a key research interest as a potential source of food, feed, biofuels and biochemicals. *Scenedesmus* is a small, nonmotile colonial green alga capable of producing important metabolites under different culture conditions. In the present study the growth response of *Scenedesmus* MJ11/18 was monitored in batch culture in an air lift fermenter using different culture conditions viz. initial pH (4 – 9), salinity (0.25 % - 2 %), and nitrogen content (0.85 – 2.0 g/l). After harvesting the biomass, it was also analyzed for total protein, carbohydrate, lipid and carotenoid content. The optimum pH was found to be 7.0 for the biomass production (10.4 µg/ml) as well as total protein (0.42mg/mg), carbohydrate (0.15mg/mg), lipid (0.12 mg/mg) and carotenoid production (5.14 µg/mg). Biomass and carotenoid production increased to 14.13 µg/ml & 9.24 µg/mg respectively, with increase in salinity upto 0.5%. There was no significant effect of salinity on total protein carbohydrate & lipid content. Although the biomass (16.4 µg/ml), protein (0.51 mg/mg), carbohydrate (0.17 mg/mg) and carotenoid (11.31 µg/mg) content increased at high nitrogen concentration but the total lipid increased to 0.23 mg/mg under nitrogen starved condition. The results indicate the importance of different culture conditions for production of suitable high value products from this algal culture.

Small and medium scale production and consumption of *Spirulina*

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Introduction:

Spirulina blue-green algae (Cyanobacteria) can serve as vital source of nutrition. Its important features are cheap, effective and produced with simple and well-known technology. More suitable to produce and process from decentralized small rural farms, thereby creating income for poor women in rural villages.

Rationale:

There is lacking awareness on nutritional benefits and the simple production techniques of *Spirulina* in developing nations.

Materials and Methods:

WHO confirmed *Spirulina* is able to be administered to children without any risk and isconsidered as a very suitable food. SPRTC-Antenna's contribution is to produce *Spirulina* locally in a cost effective manner with the aim to combat malnutrition and micronutrient deficiencies, and also to enable the rural women to generate reasonable income. *Spirulina* grows in an alkaline medium in the tropical regions. The cultivation, harvest and the processing techniques are easily mastered by village women and the quality test has been supported from the nearby laboratories. For easy consumption the value additions (candies-for children, pills-for adults) are carried out and promoted to the community, thus ensure the consumption through awareness programs. *Spirulina* offers remarkable health benefits, especially in children

- One to three grams reduces Iron deficiency anaemia significantly.
- ß-carotene overcomes sufficiently eye defects caused by VAD.
- According to recent clinical trials cognitive ability of children is improving.

Results and conclusion:

The local small scale production centers can be ensured by adapting SPRTC-antenna's simple production model. Substantial health improvement can be achieved by recommendations of micronutrients rich Spirulina and Spirulina enriched products to the rural children and women.

1: Development of suitable Photobioreactor for Algae production – A Review

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Microalgal species are recently in the spotlight for Biofuels production like biodiesel, bioethanol and biohydrogen. Algae are also used as a biofertiliser, source of nutrient and for controlling pollution. Microalgae being a photosynthetic organism are produced in photobioreactors. Hence the design and development of photobioreactors for maximum production of algae is very important. Apart from maximum production, other factors such as design, cost effectiveness of the bioreactor, purity of the microalgae produced, user friendly, low maintenance and space convenience need to be optimised.

The bioreactors that are used for the purpose of growing microalgae are Bubble Column Photobioreactor, Airlift Photo Bioreactor, Flat panel Bioreactor, Horizontal Tubular Photobioreactor, Stirred Tank Photobioreactor etc. These bioreactors have their specific advantages and disadvantages. Work is ongoing for developing a hybrid type of bioreactors which may overcome the limitations of the photobioreactors developed to date. This paper covers the salient features, limitations of developed photobioreactors and recent developments in the field of photobioreactors.

2: Photobioreactors: Design and performance aspects

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General design considerations for photobioreactors include lighting, mixing, water consumption,CO_2 consumption,O_2 removal, nutrient supply, temperature and pH. As compared to other parameters, light distribution is a technical challenge for biomass production. In contrast to area intensive open pond technology, space saving closed cultivation systems could be gradually established in the market of industrial photobioreactors. Closed systems may out-perform raceway ponds by about 300%, which represents productivities over 100 g dry algal biomass per m^2 per day. The growth potential is increased to 100% due to internal illumination arrangements. Thus, economic aspects for the cultivation of phototrophs, even in moderate climates, are improved. All parameters are maintained more accurately in photobioreactors with aseptic condition with a product value which justifies the expense. However, in photobioreactors oxygen build-up and low carbon dioxide storage capacity represent a challenge, although a number of design alternatives are being developed.

3: Microbial carbon capture cell using algae for simultaneous power generation, carbon dioxide sequestration and wastewater treatment

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Microbial carbon capture cell (MCC) is considered as a promising approach for simultaneous carbon fixing and electricity generation during wastewater treatment without aeration. MCCs were constructed with blue green algae grown photo biocathode sparged with CO_2 in dual chambered stack type mediator-less MFCs, using different ion exchange membranes and pure culture of *Shewanella putrefaciens* as anolyte. In the present study, the effect of different cathodic experimental conditions like light intensity, CO_2 concentration etc. were studied and the performance of the MCC with *Anabaena* and *Chlorella* sparged with CO_2-air mixture in photo biocathode was evaluated and compared with that of the conventional cathode having normal air sparging. The performance of MCC using *Chlorella* and *Anabaena* was found suitable using cation and anion exchange membrane respectively.

The results obtained were in the following order: *Anabaena* with CO_2-air mixture sparging (57.8mW/m^2) > CO_2-air mixture sparging (39.2mW/m^2) > *Anabaena* with air sparging (29.751 mW/m^2) > air sparging (19.6 mW/m^2). Cathodic pH was monitored along with the cathodic half-cell potential. It was found that as the pH of the cathode containing *Anabaena* gradually increased, the power generation decreased. The effect of operational parameters like CO_2 concentration and light intensity were considered to assess the performance of MCC. Furthermore, it was observed that the power density obtained in MCC increased by 31 % when BG11 was used instead of $BG11_o$ since BG11 contained nitrate as additional electron acceptor. These findings suggest that flue gas (CO_2 air mixture with little amount of NO_X and SO_X) can be used as a sustainable source for operating MCC.

4: Fermented Fatty acids as carbon source for lipid accumulation in mixotrophic microalgae (IICT-Algae-ABST1).

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Biologically produced fuels like biodiesel, biohydrogen and bioethanol have been identified as potential alternative energy sources which can mitigate greenhouse gas emissions. In spite of the advantages, the main challenges encountered with fermentative biohydrogen (H_2) production process are low substrate conversion efficiency and residual substrate present in acid-rich effluents generated from the acidogenic process. Persistent accumulation of acidogenic by-products (soluble acid intermediates/volatile fatty acids (VFA)) leads to lesser substrate conversion efficiency and low H_2 yields attributing to the inhibition of fermentation process. Even at optimum conditions, about 60–70% of the original organic matter remains as residue in the wastewater. Economic concerns suggest that it is advisable to utilize the residual carbon fraction of acidogenic outlet for additional energy generation in the process of its treatment.

Recently, microalgae have received much attention for its rapid growth and photosynthetic mechanism that can convert atmospheric CO_2 into carbohydrates, biomass, lipids, etc. In this realm, we have intended to use acidogenic effluents rich in acid metabolites as substrate for photosynthetic organisms which can store lipids in the form of triacylglycerides (TAGs) that can be transesterified to biodiesel. Main focus of the study is to use mixed microalgae to operate in mixotrophic mode. An initial attempt was made to evaluate the role of synthetic volatile fatty acids (VFAs) like Acetic acid (A), Butyric acid (B), Propionic acid (P), A+B, B+P, A+P, A+B+P and organic carbon source on both biomass and lipid accumulation.

Experiments were operated as sequential growth and starvation phase. Among all the conditions operated, 'A' registered higher biomass concentrations in growth phase with maximum of 0.9 mg/ml and higher lipid productivity in starvation phase (19.7%). Fatty acid profile of the lipid content showed high prevalence of saturated fatty acids over unsaturated ones. Experimental data documented the efficiency of microalgae to utilise VFAs for lipid accumulation, suggesting the possible route for the utilisation of acid rich effluents as carbon source. In this context, it can be inferred that algae growth and lipid accumulation can be achieved with acid rich effluents generated during acidogenic dark fermentation process of hydrogen production which makes the process economically viable and sustainable.

5: Effect of temperature on growth of *Scenedesmus* sp & Modeling by RSM technique

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Response surface methodology was used to relate the growth conditions as a function of carbonate concentration (0.25 to 0.75 g/L) and light intensity (175- 225 µmol Photons/m^2/s) for *Scenedesmus sp* at two different temperature (30 & 35˚ C). Three level factorial design with two factors was employed to optimize the independent variables to achieve the maximum biomass yield. A second order polynomial regression model was developed.

The results were analyzed using Pareto analysis of variance (ANOVA) to check the adequacy and accuracy of the fitted models. It fits with experimental results within ±10% error. The response surface and contour plots represent the actual relationship between the independent variables and the responses. Biomass yield increases with increasing carbonate concentration and light intensity. The maximum biomass yield was obtained at 35 ˚C, rather than at 30 ˚C. Since this species has shown sustainability at 35 ˚C, it may be chosen as species for open pond cultivation and climatic conditions corresponding to India.

6: Effect of salt concentrations on growth and lipid production of *Scenedesmus* species of north-east Assam

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The microalgae, *Scenedesmus* sp. were cultivated in various salt concentrations to assess their growth and lipid production. In this study, the microalgae species were cultivated in batch mode for 11 days in BG11 medium containing a series of sodium chloride salts, ranging from 10 mM to 100 mM. The species survived at high salinity of 100 mM with maximum specific growth rate of 0.973 d^{-1} and a minimum doubling time of 16.8 h.

From various salt concentrations experimented in this study, the species grown in 30 mM, 50 mM and 80 mM showed maximum specific growth of 1.137 d^{-1}, 1.102 d^{-1}, 1.128 d^{-1} respectively. However, the microalgae grown in normal BG11 medium, which were used as a control of the experiment, showed maximum specific growth of 1.106 d^{-1} with minimum doubling time of 15.04 h. The lipid production was found to be maximal in 30 mM salt containing medium with 35.8 % comparative to species grown in BG11 medium with 36.2 %. From the study, the *Scenedesmus* species showed its potentiality towards the various salt concentrations with maximum growth rate and lipid production. Nevertheless, it may show its tolerance beyond the studied concentrations.

7: Biohydrogen production from photosynthetic algae: the roadblocks, their probable solutions and the prospects of O_2 tolerant [Fe]-Hydrogenase

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The predicted exponential depletion of fossil fuel reserves in the coming years and the resulting energy crisis could be avoided, in part, through building of a sustainable Hydrogen economy. To make hydrogen fuel commercially viable, Biohydrogen production from photosynthetic algae and cyanobacteria must be utilised. Hydrogen evolution in green algae commences upon reversible activation of Hydrogenase genes under anaerobic condition. However, the enzyme activity is short lived.

Ongoing research continues to focus on understanding the road blocks preventing the realisation of large scale hydrogen production, as well as the solutions to bypass those. For a green alga like *Chlamydomonas reinhardtii*, there are several cellular machineries which limit Hydrogen production. These include low sunlight utilisation owing to larger chlorophyll antennae, non-dissipation of proton gradient across the thylakoid membrane or inhibition of [Fe]-Hydrogenase enzyme by molecular oxygen. The Hydrogenase enzyme has a complex initiation, maturation and activity, all of which are inhibited by oxygen. Although research is ongoing, the complete processes and roles of all components are yet to be known. This presentation throws some light on the molecular mechanism of hydrogen evolution and the road blocks with an emphasis on recent research activities involving Hydrogenase maturation; role of enzymes HydE, HydF, HydG ; and molecular engineering to make the enzyme oxygen tolerant.

8: Biohydrogen production by thermophilic mixed culture using green algal biomass

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Algal biomass is gaining importance as high value product for biofuel production as these are produced by capturing and sequestering atmospheric CO_2 in the form of biomass rich in carbohydrates. In the present study, biohydrogen production was carried out by the thermophilic mixed culture using algal biomass as substrate. The advantage of using thermophiles in dark fermentation is that unautoclaved raw materials can be directly used for hydrogen production. Furthermore, thermophiles are known for their vast repertoire of hydrolytic enzymes that may be useful for utilization of algal biomass for the hydrogen production. The thermophilic mixed culture used in the present studies was an enriched culture developed from an anaerobic digester grown at a temperature range of 60 $^{O}C \pm 2\ ^{O}C$ capable of producing 2.69 mol H_2 mol^{-1} glucose.

The study showed that *Chlorella sorokiniana* when subjected to thermophilic fermentation produced 0.0168 mol H_2 g COD^{-1}reduced. The effect of various physical and chemical pre-treatments processes were studied for the improvement of hydrogen production from the algal biomass. It was observed that the pre-treatment with heat and 1.5 % (v/v) HCl gave higher yield of hydrogen. The study thus showed the potential of algal biomass as substrate for biological hydrogen production.

9: Acidogenic fermentation of de-oiled algal cake for biohydrogen production (IICT-Algae-ABST3)

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The ability of photosynthetic microalgae to fix atmospheric CO_2 through intracellular starch accumulation associated with oxygen release makes them unique. Microalgae have the capability to metabolize the synthesized starch into reserve lipid materials, which upon processing can be used as biodiesel. After extracting lipids, the residual de-oiled algae biomass can be used for other value added biorefinery products like bioethanol, biohydrogen, proteins, energy, etc. The main objective of this communication is to evaluate the potential of de-oiled algal cake as substrate for production of biohydrogen through dark fermentation process using anaerobic mixed consortia as biocatalyst at acidophilic conditions. De-oiled algal biomass (lipid extracted residual biomass) consists of lignin free cellulose along with accumulated starch, which makes it ideal substrate for H_2 production.

Chemical characteristics of the de-oiled algae cake used in this study comprised of 22.45% of carbon, 2.80% of nitrogen, 3.05% of hydrogen and 0.48% of sulfur. Experimental methodology involves pre-treatment of algae followed by dark-fermentation of extracted carbon to H_2 at ambient room temperature. The solid and liquid pre-treated extracts along with slurry were evaluated separately for hydrogen production. Maximum hydrogen production rate (HPR) and cumulative hydrogen production (CHP) were observed with liquid extract (11.65 mmol/h; 28.5 mmol) followed by slurry (11.43 mmol/h; 25.04 mmol), solid (1.3 mmol/h; 7.8 mmol) and control (0.1 mmol/h; 0.3 mmol). Likewise substrate degradation was also observed to be higher in liquid extract (61.8%) over solid, slurry (57.7%) and control (40%). Drop in pH (6 to 5) was observed in all the conditions studied indicating the generation of fatty acids. Higher VFA production was observed in liquid extract (3996 mg/l) than slurry (2054 mg/l), solid (1854 mg/l) and control (1583 mg/l). Cyclic voltammetry (CV) profiles showed more reduction than the oxidation, which supports the anaerobic fermentation of H_2 production. Experiments illustrated the feasibility of utilizing de-oiled microalgal biomass as feed stock for biohydrogen production.

10: Photo-biocatalytic fuel cell: Utilization of acidogenic effluents from hydrogen production process as substrate for bioelectricity generation (IICT-Algae-ABST2)

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It is evident that civilization is progressively dependent on energy with the advancement of science and technology. Increasing energy demand creates unbalanced energy management and requires power sources that are able to sustain for longer periods. In this aspect, biofuels have been recognized as a potential renewable energy sources which can compensate raising energy demands. Biologically produced hydrogen (H_2) is recently recognized as environmentally sustainable fuel. In spite of the advantages, the main challenges encountered with fermentative H_2 production process are low substrate conversion efficiency and accumulation of volatile fatty acids (VFA). This hampers the fermentation process and consequently lower substrate conversion and low H_2 yields. Under optimum conditions, about 60–70% of the organic matter remains as residue in the wastewater.

Therefore, an attempt was made in this report to utilize acid rich effluents for bioelectricity generation through a photo-biocatalytic fuel cell (PhFC) operated with anoxygenic photosynthetic bacteria (APB) as biocatalyst in mixotrophic mode. A single chambered fuel cell with non-catalyzed electrodes and open-air cathode was operated in fed batch mode for a period of 90 days, using VFA rich wastewater as substrate. The power generated in this PhFC directly relates the light intensity and ability of the microorganisms to produce electrons and protons by oxidation of VFA and transfer of electrons to the anode. Improvement in power output was observed with increasing biomass concentrations, i.e. from 1.55 g/l (37±5 mW/m^2) to 3.24 g/l (110±10 mW/m^2), which later got stabilized???. The stable open circuit voltage of 550±20 mV and current of 3.51±0.10 mA (100 Ω) were maintained after 10 days operation along with 96% of substrate (COD) degradation efficiency. Bio-electrochemical analysis of PhFC was evaluated based on polarization behaviour, cell potential and cyclic voltammetry. The voltammetric profiles supported high redox abilities of photosynthetic consortia for power generation. Bioprocess evaluation evidenced that basic pH (8.5) and higher bacteriochlorophyll supports higher electrogenic activity of APB. This process provides triple benefit of harnessing biohydrogen, bioelectricity and efficient treatment for the effluents rich in fatty acids generated during fermentative hydrogen production process.

11: Potential of selected cyanobacteria for natural antioxidants, phenoloics and lipids

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Cyanobacteria are simple, but primitive and diverse group of microorganisms with characteristics common to both bacteria and algae. Their success as a group in a wide range of habitats has been attributed to their unique physiological characters and high adaptive ability under a wide range of environmental conditions. The potential of cyanobacteria as a source of food, feed, natural colors, biofuel, biofertilizer, enzymes, pharmaceuticals and other fine chemicals is well recognized. Besides these, cyanobacteria are also considered as a rich source of natural antioxidants and phenolic compounds that are able to scavenge highly reactive free radicals causing tissue damage. Cyanobacteria constitute a vast potential resource, but only a few species have been studied intensively and used commercially. In order to identify new sources of safe and inexpensive natural compounds, 20 cyanobacterial strains isolated from different parts of North-West India were evaluated with respect to phenolics, antioxidants, and lipids. The cyanobacterial strains studied showed great variation among each other with respect to production of antioxidants, phenolic and lipids.

Out of the 20 strains, *Nostoc sp.* (Strain no. 14) recorded maximum production of antioxidants followed by *Anabaena sp.* (Strain no. 7), whereas *Chroococcus sp.* (Strain no. 16) showed the least production of antioxidants. *Westiellopsis sp.* (Strain no. 9) recorded maximum phenolics, while *Lyngbya sp.* (Strain no. 17) showed the minimum phenolic production. In case of lipids, *Anabaena sp.* (Strain no.7) accumulated maximum lipids expressed as percentage dry weight basis, while *Chroococus sp.* (Strain no. 19) had minimum percentage of lipids. Although, the above strains showed variation with respect to the biosynthesis of these valuable compounds, the potential strains can be exploited commercially for the production of natural antioxidants and phenolics for their possible use as nutraceuticals. *Anabaena sp.*, which accumulated maximum lipids, can be a potential resource for algal biofuel or biodiesel.

12: Identification of suitable microalgal strains as the source of food, feed or biofuel

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Micro-green algae represent an exceptionally diverse and highly specialized group of microorganisms adapted to various ecological habitats. These are also reported as the promising source of biofuels and important biochemicals for food and feed.

Water samples were collected from different aquatic habitats (lake, ponds, estuaries, salt marshes, swamps) of north-eastern and western part of India. Strains were isolated by using the micro-capillary method and were purified by repeated subculturing using appropriate medium. Identification was done on the basis of standard morphometric keys and pure strains of *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Dictyosphaerium*, *Dunaliella* and *Kirchneriella* were used for further studies. Standard procedures were followed for studying growth, pigment profile (chlorophyll and carotenoids) lipids, total soluble proteins, carbohydrates and phenolics. Distinct variations were recorded in these attributes of selected microalgal strains. The chlorophyll content declined at 30th day of incubation and highest was recorded in *Chlorella* isolated from the Katitirtha temple pond, Bhubaneshwar, Odisha.

Carotenoids exhibited variable pattern with respect to time of incubation and the same *Chlorella* strain showed maximum carotenoids at 30th day of incubation. Carbohydrates enhanced invariably with incubation time and *Dictyosphaerium* strain isolated from pond at Khurda (way to Chlika Lake), Odisha, showed maximum carbohydrates at 30th day of incubation. Total soluble proteins exhibited variable pattern with incubation time and *Scenedesmus* strain isolated from pond, Balasore, Odisha, showed highest level of total soluble proteins at 30th day of incubation. Total phenolic exhibited distinct variation with the incubation time and *Chlorella* strain isolated from Sambhar Lake, Rajasthan, depicted highest phenolics content at 21st and 30th day of incubation.

Based upon the results, the efficient microalgal strains for their possible role in the development of food and feed have been identified. Total lipids also showed distinct variation with different incubation time in selected microalgal strains and the efficient lipid producer has been identified for future interventions of biofuel programme.

13: Utilization of cyanobacteria for bioplastics production

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Biologic polyhydroxyalkanoates (PHAs) are a well-known class of biodegradable polyesters that offer much potential for significant contributions as ''bioplastics'' and have gained tremendous attention in agriculture, medicine and pharmaceutical industries as a biocompatible substitute for conventional petroleum-based plastics. PHAs are accumulated as carbon and energy storage materials by a number of microorganisms under growth-limited condition. High cost of polymer production, due to high carbon requirement and oxygen demand during fermentation, has inhibited the replacement of synthetic plastics by PHAs. Cyanobacteria, however, are indigenously the sole prokaryotes that accumulate PHAs by oxygenic photosynthesis. In addition to their photoautotrophic nature and minimal nutrient requirements, cyanobacteria can be viewed as attractive hosts for low-cost PHA production. Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate). [P(3HB-*co*-3HV)] is a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate units, having superior thermal and mechanical properties over poly-3-hydroxybutyrate (PHB, the best characterized PHA).

In the present study, the main objective was to investigate P(3HB-*co*-3HV) co-polymer production using a N_2-fixing cyanobacterium, *Nostoc muscorum* Agardh, in a two stage cultivation. Polymer production by *Nostoc muscorum* was conducted in two phases: biomass production in fructose supplemented medium in the first phase, and nitrogen-deficiency was employed in the second phase for co-polymer accumulation under valerate supplementation. Results showed that *N. muscorum* accumulated the homopolymer of PHB under mixotrophy with fructose. However, synthesis of 3-hydroxyvalerate monomer was observed, when a secondary carbon source (e.g. valerate) was supplemented in the second phase. In this report, P(3HB-*co*-3HV) co-polymer accumulation in *N. muscorum* was boosted up to 42-48% of dry cell weight (dcw) when cultures pre-grown in fructose supplementation were subjected to nitrogen-deficiency with valerate supplementation for 6 days (6 fold rise against control condition). Therefore, two stage cultivation practise can be efficiently utilized for biomass and P(3HB-*co*-3HV) co-polymer production from *N. muscorum* Agardh.

14: LC-MS/MS and GC-MS/MS mediated profiling of novel healthcare metabolites of a new cyanobacterial isolate of microalga *Lyngbya* sp.

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Microalgae are well known rich natural sources of many biologically active human healthcare metabolites. A filamentous blue green alga have been enriched and isolated from fresh water by growing in synthetic medium. The alga was grown in a bubble column bioreactor (working volume 8L) for 2 weeks and then harvested for product analysis. The bioreactor was placed in an ambience having maximum illumination ~ 9000 lux and diurnal temperature in the range 28-32 ^{O}C.

The dried algal biomass was extracted with different solvents, and antimicrobial activity was checked against *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans*. Acetone extract showed positive antimicrobial activity against bacteria, but no activity was found against Candida albicans. LC-MS/MS and GC-MS/MS based profiling revealed the presence of several metabolites, including a few phenolics, canrenone and its derivatives. Canrenone is an aldosterone antagonist and is used in combination with drugs for inhibition of chronic heart failure. In addition, trehalose and a UV-absorbing peptide could be detected.

15: Cyanobacterial diversity from rice field soils of Northern Orissa and evaluation of antibacterial activity of selected species

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Cyanobacterial diversity from rice field soils of Northern Orissa was studied during 2006 to 2009 both in rabi and kharif crop seasons at 3 study locations. Altogether 168 species belonging to 44 genera were encountered, including 70 heterocystous and 98 non-heterocystous forms. Out of these some selected cyanobacterial species were used for extraction of crude metabolites. Crude metabolites were extracted using organic solvents of different polarity viz. chloroform, methanol and ethyl acetate. The metabolites were tested against clinically significant microorganisms. The results showed that 9 cyanobacteria (*Oscillatoria sp.*, *Oscillatoria curviceps*, *Phormidium sp.*, *Aphanothece microscopica*, *Nostoc piscinale*, *Anabaena variabilis*, *Anabaena spiroides*, *Cylindrospermum muscicola* and *Microcystis sp.*) were active in inhibiting the test pathogens with chloroform extracts displaying considerable antibacterial activity. Cyanobacteria like *Phormidium* sp., *Aphanothece microscopica* and *Cylindrospermum muscicola* effectively inhibit at least 1 of the test pathogens, indicating that these cyanobacteria might have active antibacterial metabolites. Minimum inhibitory concentration (MIC) was carried out in selected cyanobacterial strains that showed good zone of inhibition against the test pathogens during agar cup diffusion assay. MIC ranged from 500µg/ml to 250µg/ml, with highest values recorded against *E. coli* and *S. aureus*, and lowest value against *B. subtilis*. Findings revealed that the crude extract showed higher activity against Gram positive bacteria than Gram negative bacteria. This study illustrates that cyanobacteria from rice fields could be a potent source of antibacterial agents.

16: Mechanisms of oxidative-stress tolerance in the diazotrophic cyanobacterium *Nostoc punctiforme* ATCC 29133 and consequences relevant to biofertilizer technology

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Nostoc punctiforme ATCC 29133 is a heterocystous, nitrogen-fixing cyanobacteria that is highly sensitive to oxidative stress imposed by the herbicide methyl viologen (MV), used widely in agricultural fields to contain weeds. Upon entering the cell, MV mediates production of various cell-toxic reactive oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals that damage proteins, nucleic acids and lipids. Here, we report construction and characterization of a mutant of *Nostoc punctiforme* that is highly resistant to MV. The mutant showed unaltered levels of growth and nitrogen-fixing activity, whether growing in presence or absence of MV. The hydroperoxide level in the mutant was much lower compared to its parent. This was a result of increased expression of the gene encoding iron superoxide dismutase (*sodB*) that correlated well with superoxide dismutase activity, Also, a concomitant increase in catalase activity conferred tolerance to hydrogen peroxide in the mutant. More importantly, the mutant was found to adapt better to high salinity and thermal-stress that both presumably induce oxidative stress in cyanobacteria.

Taken together, these results suggest that resistance to MV-induced oxidative stress in the cyanobacterium *Nostoc punctiforme* may be engineered by enhancing the ability to detoxify superoxide radicals and hydrogen peroxide efficiently and promptly. Furthermore, an added advantage of using such oxidative-stress resistant mutants in biofertilizer technology is that they can grow and fix nitrogen in an unhindered manner even under many other types of environmental stress often prevalent in natural ecosystems, which otherwise is a major limitation of using cyanobacteria as biofertilizers.

17: Survival strategy in a cyanbacterium facing salt stress: role of intracellular osmoticum

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Cyanobacteria accumulate a wide range of osmolytes depending on the nature of stress. Trehalose is one of such solute accumulated in cyanobacterial cells during salt or osmotic stress. The di-saccharide rescues membranes by preventing phase transitions of dehydration (drying) and the associated leakage upon rehydration the gene cluster comprising trehalose synthesizing enzymes, maltooligosyltrehalose synthesae (MTSase, *allo*167) and maltooligosyltrehalose hydrolase (MTHase, *allo*168) is worked out in *Anabaena* 7120.

Our study indicated maximum expression of such enzymes in cells facing 150mM NaCl. Further, the MTSase gene was amplified and cloned in pGEMT easy vector (Promega) and sub-cloned in pET -19b (Noragen) expression vector. The recombinant *E.coli* BL-21 (DE-3) had optimum expression in IPTG induced cells. The recombinant MTSase showed maximum transglycosylation activity at pH 6 (40 ^{O}C) in case maltohexaose was the substrate. This presentation could be taken as the first of its type on purification and expression of MTsase from a mesophilic cyanobacterium.

18: Algae – A Sustainable Alternative Fuel for Conventional Fuels

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The biofuel from microalgae is a sustainable alternative for fossil fuels. Algae represent a very promising source of biomass in this context as they sequester a significant quantity of carbon from atmosphere and industrial gases, and are also very efficient in utilizing the nutrients from industrial effluents and municipal wastewater. Therefore cultivation of algal biomass provide dual benefit, it provides biomass for the production of biofuels and also save our environment from air and water pollution. Microalgae are photosynthetic microorganisms that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and useful chemicals.

Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels. Microalgae can be converted to biodiesel, bioethanol, bio-oil, biohydrogen and biomethane via thermochemical and biochemical methods. Algae can be grown almost anywhere, even on sewage or salt water, and do not require fertile land or food crops. Processing of algae requires less energy than the algae provides. Microalgae have much faster growth rates than terrestrial crops. The per unit area yield of oil from algae is estimated to be from 20,000 to 80,000 liters per acre, per year; this is 7– 31 times greater than the next best crop, palm oil. Dry algae contain about 50% of oil by weight.

19: Sustainable biodiesel from marine algae of Indian Sundarban

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Sundarban is the largest tiger inhabiting mangrove forest of the world, situated in southeast part of Asia including India and Bangladesh. During our recent survey screening of algal genera from different salinity level and their fatty acid profiling for biodiesel production were done extensively. Total lipid and fatty acid composition of 10 algal genera isolated from different aquatic habitat of Indian Sundarban delta have been analysed by Gas chromatography – mass spectrometric (GCMS) study to explore the suitable source for biodiesel. The GC-MS analysis showed variation in n-saturated, unsaturated and long chain branched fatty acids with respect to location and habitat.

Among the fatty acids detected most of them belong to polyunsaturated fatty acids (more than 60%). The study also revealed that palmitic acid C16:0 and oleic acid C18:1 were found in all the algal samples in higher amount followed by Stearic acid C18:0 and linoleic acid C18:2. In some, the long chain fatty acids (C20:1, C20:2, C22:6 and C24:0) were found in lower concentrations. C16:2 and C16:3 were also found in very lower concentration among the algal genera. Variation in fatty acid compositions was also remarkable. In the present communication morphotaxonomic description of the algal genera and their total lipid and fatty acid composition would be discussed in detail.

20: Potential of algae as utilizable biofuels

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Biofuel, a biodegradable, and nontoxic less CO_2 and NO_x emissioning alternative fuel that is obtained from renewable sources. Algae have emerged as one of the most promising sources for biofuel production. Microalgae can provide several different types of renewable biofuels like biodiesel, ethanol and biohydrogen. Biodiesel derived from microalgal oil/fatty acid through trasesterification is one of them. At an assumed recovery rate of 30% of the weight of algae, 45.6 tonnes of oil/hectare/year can be produced from algae. Rate of biomass production in algae can be increased using thermal power exhaust as a source of CO_2. Unicellular eukaryotic green algae like *Chlorella*, *Chlorococcum* or *Scenedesmus* are suitable for algae to biofuel journey with necessary nutrient manipulation to optimize oil content. According to growth pattern experiment mixotrophy is more suitable than phototrophy and heterotrophy using 10% hydrolised molasses. *Chlorella* is the hardiest strain followed by *Chlorococcum* and *Scenedesmus*. Oil content (Total lipid) content is much less in *Chlorella* in compare to other. Contamination by rotifer and paramecium in open cultivation system was the major threat in this context. Oxygenic photosynthesis splits water to release oxygen gas and uses the hydrogen atoms to drive the reduction of carbon dioxide to sugars. Under some circumstances, cyanobacteria are able to release the reductant as hydrogen gas. Hydrogen is an excellent fuel for fuel cells and has some attractive features like three times more potentiality than ethanol from corn as a transportation fuel. Cyanobacteria can produce H_2 through three main routes: 1) H_2-production directly from the native bidirectional hydrogenase; 2) H_2-production from a native nitrogenase; and 3) H_2-production from an introduced hydrogenase. The present communication will mainly explore the hydrogen production potential of algae.

21: ITS-Region characterization of selected microalgae

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Oxygenic photosynthetic organisms use solar energy to split H_2O into H+, e-, and O_2. A selected group of photosynthetic microorganisms, which include green microalgae like *Chlamydomonas reinhardtii*, *Scenedesmus obliquus* and *Chlorella fusca*, can also produce biohydrogen. Hydrogen production can be initiated by including purging with inert gas, providing exogenous reductants, and/or allowing cellular respiration to metabolize dissolved O_2.

In the present study these different algae are collected from natural sources and include *Chlorella* sp, *Scenedesmus* sp and *Chlamydomonas* sp. They were isolated using dilution plating, and single cell isolation by micropipette technique and Phototaxis in TAP agar medium respectively. The broad spectrum antibiotic Cefotaxime of 500 µg/ml was sufficient to remove all the bacterial contamination. DNA was isolated from all the collected microalgae using CTAB method and DNA amplification of ITS1, 5.8S, and ITS2 regions of the ribosome was performed for their phylogeny analysis. The highest cell density was found to be 110 × 106 in *Chlorella* sp among all the collected microalgae in TAP medium. Hydrogen production from the isolated algae is currently under progress.

22: Molecular diversity of phytoplankton assemblages in a tidal creek of Indian Sundarbans

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The phylogeny and diversity of a key functional gene was investigated as the basis for improved understanding of the community structure and temporal variations of natural microscopic phytoplankton assemblages in a tidal creek of the Indian Sundarbans, the world's largest mangrove ecoregion. Phytoplankton community composition from the creek was elucidated based on *rbcL* (encoding the large subunit of the ribulose-1, 5- bisphosphate carboxylase/oxygenase) sequences. Diatoms (Bacillariophyceae) were by far the most frequently detected group in the creek, consistent with their importance as a major bloom-forming group. The diatoms accounted for more than 70% of the sequences detected in the *rbcL* clone libraries. The *rbcL* libraries contained sequences representing cosmopolitan and key bloom forming taxa, including *Thalassiosira*, *Ditylum* and *Phaeocystis*. Other chromophytic algal groups, including Cryptophyceae, Eustigmatophyceae, Pelagophyceae and Haptophyceae, were also detected as part of this study. Temporal variations in the natural phytoplankton assemblages were observed as part of this study, with occurrence of specific phytoplankton functional groups in the creek during certain times of the year. Further investigations are presently underway to elucidate spatio-temporal variations in community assemblages and expression profile of key genes involved in carbon and nitrogen metabolism of dominant phytoplankton genera in the creek, in link with changing aquatic biogeochemistry.

23: Species Diversity of the Freshwater Algae of the Pathanamthitta District of Kerala

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Biodiversity provides the basis of life on earth. Algae are very important as a source of food and serve as an early step in the food chain of larger aquatic animals, especially fish. Systematic studies on the freshwater algal flora of Kerala are scanty. The present investigation is the first systematic study on the Species Diversity of the freshwater algae of the Pathanamthitta district of Kerala. This project is a part of our attempt to enlist and taxonomically evaluate the algal components of the Kerala state and to report any algal species of economic and ecological importance.

Pathanamthitta is a true tropical diversity with respect to geographical, topographical and climatic conditions. It has evergreen forests, rivers, hills and plains furnishing freshwater, wetland, and sub-aerial habitats for the growth of algae.

This study is being carried out through extensive field visits to various parts of the district. The coordinates of the locations of collections were measured with the help of a G.P.S. The temperature and pH of water are recorded using digital equipment. Algal specimens were observed, and digital photomicrographs made with the help of a trinocular Research Microscope and advanced digital camera. Standard manuals, monographs and journals are referred apart from various web-sites for the identification of various taxa.

In addition to providing and reporting a comprehensive data base on the freshwater, wetland, and subaerial algal flora of the Pathanamthitta District, the work also expects to report any endemic species. It is also expected to assess any positive and negative environmental impacts of algal growth in the ecosystems of Pathanamthitta and report those species useful for mankind as source of biofuels, biofertilizers, biohydrogen etc.

Our investigation revealed the occurrence of various kinds of algae. The chlorophycean microalgae include 12 taxa of *Pediastrum* Meyen, 13 taxa of *Scenedesmus* Meyen and the hydrocarbon rich *Botryococcus braunii* from chlorococcales, as well as twenty six taxa from nine genera of the filamentous desmids four taxa of *Desmidium* Agardh; three taxa of *Hyalotheca* Ehrenberg; four taxa of *Spondylosium* Brebisson; two taxa of *Groenbladia* Teiling; two taxa of *Teilingia* Bourrelly and one taxa each of *Sphaerozosma* Corda; *Streptonema* Wallich; *Onychonema* Wallich and *Babusina* Kutzingand.

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