

SAHYOG Mini-SYMPOSIUM and TWINNING WORKSHOP Developments in Sustainable Biomass Valorisation EU-India R&D collaboration on Biomass and Biowaste 28-29 October 2013, Utrecht, The Netherlands



Algal Bio-fuels -Indian Scenario

Dr. V Sivasubramanian, Director- Tech, Phycospectrum Environmental Research Centre (PERC), Chennai



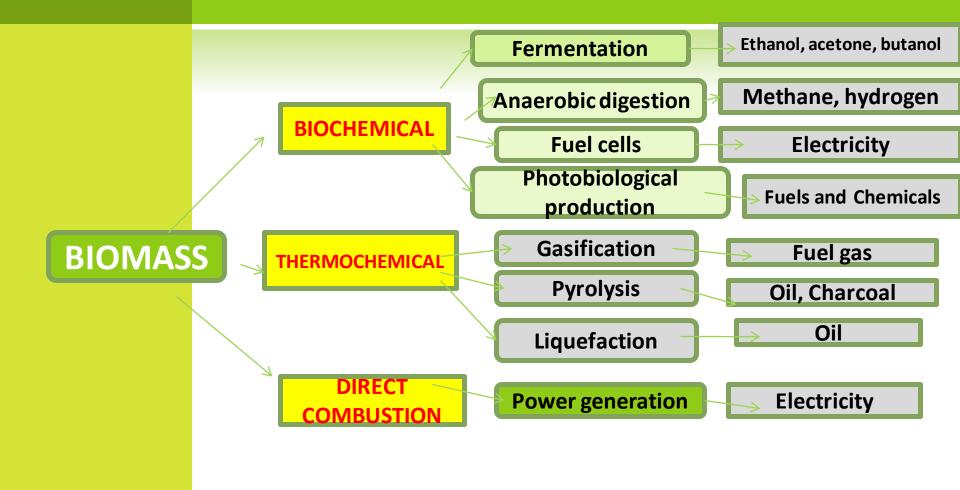
CHALLENGES

- Isolation and characterization of algae
- Lab-scale cultivation and selection of robust species
- Large scale cultivation technologies and challenges
- Harvesting and processing
- Bio-refinery
- LCA and EIA
- Energy balance

Seconomics



ENERGY CONVERSION OPTIONS FROM ALGAL BIOMASS





ECONOMIC VIABILITY IS STILL IN ITS NASCENT PHASE

- There are no commercial scale examples producing only bioenergy.
- Energy and biofuels tend to have a low value.
- Algae cultivation and processing systems require a high capital input (higher than agriculture).
- Biomass output is potentially high, but still under development.
- At least in the short term, and probably in the medium term, higher value co-products are needed for economic viability.

ALGAE-BASED BIOFUELS: A Review of Challenges and Opportunities for Developing Countries – report by Food and Agriculture Organization of the United Nations (FAO), Environment, Climate Change and Bioenergy Division Viale delle Terme di Caracalla, Rome, Italy , 2009



KNOWLEDGE GAPS EXIST FOR SEVERAL CRITICAL FACTORS:

- Due to a lack of industrial scale experiments, there is insufficient knowledge to adequately judge the economic viability.
- Productivity data is often extrapolated from small experiments, and not always presented clearly and consistently.
- Overall analysis of energy balances, GHG balances and CO₂ abatement potential are lacking.

ALGAE-BASED BIOFUELS: A Review of Challenges and Opportunities for Developing Countries – report by Food and Agriculture Organization of the United Nations (FAO), Environment, Climate Change and Bioenergy Division Viale delle Terme di Caracalla, Rome, Italy, 2009



INDIAN SCENARIO

- Projects funded by MNRE, DBT, CSIR etc.
- Projects supported by private industries.
- Indian capabilities
- Work to be done



MACRO ALGAE ADVANTAGE

Macro algae - an attractive renewable source for bio-energy molecules

- Higher biomass production rate per unit area.
- Easier depolymerization as they contain less complex cell wall metrics.
- Carbon dioxide fixation rate is much higher than by terrestrial plants.
- \Box Attractive option for CO₂ sequestration and recycling
- □ No diversion of food from the human food chain.



rðir faल्लो ∰New Delhi. Kathmandu I anioenisi v ∰Thimphu

1.2 billion population

India

Continental shelf	372, 424 sq. km
EEZ	2,103,415 sq. km
Coastline	7500 km
Total seaweed flora	841 species ^a
Total standing stock	600,000 tons fresh wt.

^a Oza and Zaidi 2001

Some marine facts



Pakistan



Macro algae:

- Extensive work has been done by Indian scientists on utilization of seaweeds for food and pharmaceutical applications. In India, seaweeds collected from natural vegetation are used for the production of phycocolloids such as agar and alginates.
- SMCRI, Bhavnagar, has long been working on the cultivation of various seaweeds and recently forayed into value addition for seaweed products. Seaweeds like Gracilaria, Gelidium, Kappaphycus etc are being cultivated in large scale.



Macro algae:

- Source ethanol using a seaweed polysaccharide.
- Rengasamy and his team have successfully developed a technology to produce biogas from seaweeds.

• More work has to be done before these can be commercialized.

Mahesh R. Gandhi, K Eswaran, K H Mody, H. C. Bajaj, Parimal Paul, Assessment of technoeconomic feasibility of large scale seaweed cultivation integrated with biofertilizer and ethanol production. 2009. **MNRE** R. Rengasamy - Potential of Seaweed and Seagrass for biogas Production. **Aguagri**, New Delhi. **2008 – 2009** CSIR CSMCRI's initiative on seaweed biomass for bio-energy and CSIR CSMCRI Chemicals

whiskas

edigree

Sap

Pet foods

Biodegradable

film

Soil Application

Potash

Energy

Ash

Gasifier

Bioethanol

Carrageenan

Integrated process for utilization of Kappahycus for biopotash, bioethanol and energy





CSMCRI - Bioethanol from seaweeds -Challenges

- Development of innovative cost effective technology for setting up offshore seaweed farms.
- Development of bio-catalytic conversion routes for biomass hydrolysis.
- Innovative process for simultaneous saccharification and fermentation process.
- Salt tolerant yeasts capable of fermenting sugars in marine broths.
- Yeasts for fermenting mannitol and galactose sugars into alcohol.





CSMCRI - Bioethanol from seaweeds - MNRE project Achievments

Development of enzymatic saccharification process

Enzymatic saccharification requires enzymes to hydrolyze carbohydrates prior to fermentation. This would require desalinization if terrestrially sourced enzymes are to be used. Therefore, development of a system utilizing enzymes like **cellulase**, **alginase**, **carrageenase** and **agarase** derived from a marine source would be beneficial.





CSMCRI - Bioethanol from seaweeds - Achievments

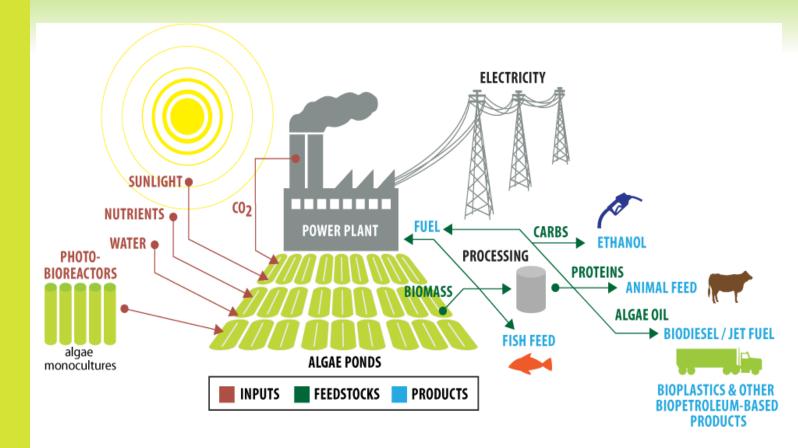
Patent:

•Mody KH, Ghosh PK, Barindra S, Gnanasekaran G, Shukla AD, Eswaran K, Brahmbhatt HR, Shah BG, Thampy, S, Jha B. A process for integrated production of ethanol and seaweed sap from *Kappaphycus alvarezii* WO2011/027360A1 dated 10.03.11

- 1. Isolation and utilization of marine yeast for converting *K. alvarezii* biomass into bioethanol as a by-product, was conducted to avoid a step of desalting. this is the first report on utilization of marine yeast for the production of seaweed-based ethanol.
- 2. Various polysaccharide degrading bacteria were isolated and used for hydrolysis of granules. Though the process worked, the concentration of sugar generated was too low. Detailed work needs to be done to utilize these bacteria for the same to achieve higher yield of monosaccharide.



MICRO ALGAE ADVANTAGE





Micro algae: Freshwater algae

Ravishankar and his team from Plant Cell Biotechnology Department, Central Food Technological Research Institute, Mysore have done extensive work on isolation and characterization of hydrocarbon producing micro alga Botryococcus braunii from Indian waters.

B. Braunii produces and accumulates - hydrocarbons (hydrococcens C30 - C36 - 30 to 70% DRY WEIGHT

Chandrappa Dayananda, Ravi Sarada, Vinod Kumar and Gokare Aswathanarayana Ravishankar 2007. Isolation and characterization of hydrocarbon producing green alga Botryococcus braunii from Indian freshwater bodies. Electronic Journal of Biotechnology, Vol.10 No.1.

DAYANANDA, C.; SARADA, R.; BHATTACHARYA, S. and RAVISHANKAR, G.A. Effect of media and culture conditions on growth and hydrocarbon production by Botryococcus braunii. Process Biochemistry, September 2005, vol. 40, no. 9, p. 3125-3131

DAYANANDA, C.; SARADA, R.; SRINIVAS, P.; SHAMALA, T.R. and RAVISHANKAR, G.A. Presence of methyl branched fatty acids and saturated hydrocarbons in botryococcene producing strain of Botryococcus braunii. Acta Physiologiae Plantarum, 2006, vol. 28, no. 3, p. 251-256

TRIPATHI, U.; SARADA, R. and RAVISHANKAR, G.A. 2001. A culture method for micro algal forms using two-tier vessel providing carbon-dioxide environment: studies on growth and carotenoids production. World Journal of Microbiology and Biotechnology, June 2001, vol. 17, no. 4, p. 325-329



Micro algae: Freshwater algae

Recently, Rengasamy and his team from University of Madras have successfully cultivated Botryococcus braunii in open raceway pond without any contamination - supported by ABAN

R Rengasamy - Development of germplasm of *Botryococcus braunii* strains isolated from South Indian water bodies for hydrocarbon production, 2007-2008, **Aban** Informatics, Pvt., Ltd.

Mass culture of *Botryococcus braunii* under open cultivation system for bio diesel production. Aban Informatics, Pvt., Ltd. 2008- 2009.

Optimization of conditions for mass culture of *Botryococcus braunii* under open race way ponds. Aban Informatics Pvt. Ltd. Chennai, 2008 – 2009

Mass culture of *Botryococcus braunii* under open cultivation system for biodiesel production.- Phase I. Aban Informatics Pvt. Ltd. Chennai. 2009

Mass culture of *Botryococcus braunii* under open cultivation system for biodiesel production – Phase II. Aban Informatics Pvt. Ltd. Chennai. 2010

Mass culture of Botryococcus *braunii* under open cultivation system for biodiesel production – Phase III. Aban Informatics Pvt. Ltd. Chennai. 2010

Mass culture of *Botryococcus braunii* under open cultivation system for biodiesel production – Phase IV. Aban Informatics Pvt. Ltd. Chennai. 2010 - 2011



Nannochloropsis

Memorandum of Agreement Signed University of Madras and Aban Infrastructure Pvt. Ltd., Chennai On 28th September 2011 Harvesting done by electroflocculation, chemical flocculation and filter press



Development of Next Generation Algal Biofuel Technology

International Center for Genetic Engineering & Biotechnology, ICGEB – New Delhi

- Synthetic Biology & Biofuel Group (ICGEB, New Delhi) has been working on genetic modification of marine and fresh water green algae (*Chlamydomonas* and *Chlorella* sp) and cyanobacteria for enhancement of growth rate and biofuel production.
 - The group is in the process of establishing genetic manipulations of chloroplasts and nuclear genome of algae. Further work involves enhancement of lipid production beyond **35% in** *Chlorella* sp. using various approaches of genetic engineering.
 - Metabolic engineering of **isoprenoids** pathway in algae is also under progress for production of **isopentenol** (a novel additive for gasoline).
 - **Protoplast isolation** is being optimized in green algae for the purpose of generating robust algae by **hybridization**.

Development of Bioscience & Biotechnologies for Next Generation Biofuels. Funded by DBT in 2010; Pls: Shams Yazdani and Shashi Kumar



Micro algae:

DBT-ICT Centre for Energy Biosciences, Institute of Chemical Technology, Mumbai and ICGEB, New Delhi- Development of Bioscience and Biotechnology for next generation biofuels – DBT 2010

Algal strain improvement

Algal strain Improvement by adaptation and/or hybridization of selected candidates.

Genetic manipulation of algal strain to incorporate desired traits)

Algal growth engineering

•Algal metabolic engineering for lipid production

Modeling and design of Photobioreactors

Studies in design of Raceway ponds with varying design parameters like water depth, stirrer design, velocity of circulation, sparging of gas and gas compositions, and rate and type of algal harvesting with recirculation of media and partial replenishment of media/media components



INDIAN CONTRIBUTION TOWARDS ALGAL BIO-FUELS AND CO₂ SEQUESTRATION RESEARCH

Micro algae:

Freshwater algae

Solution Clean Energy, Ahmedabad - Design and development of dual operating pilot scale bio-reactor system for comparative simulation studies on algal cultivation. MNRE, 2011 – To develop an indigenous PBR which is also capable of sequestering waste CO₂.



INDIAN CONTRIBUTION TOWARDS ALGAL BIO-FUELS AND CO₂ SEQUESTRATION RESEARCH



We are delighted to share with you that we have commissioned a first of its kind in India, large scale closed Algae Bio-reactor system, with support from the **Ministry of New & Renewable Energy (MNRE), Govt. of India**.

Algae based bio-fuel technology is one of the most researched topics world-over. Many companies are working in this field and it is poised to become a major industry in coming years. With the depletion of fossil fuels and increasing prices in the world market, it has become even more important for us to find an alternative solution to replace fossil fuels.



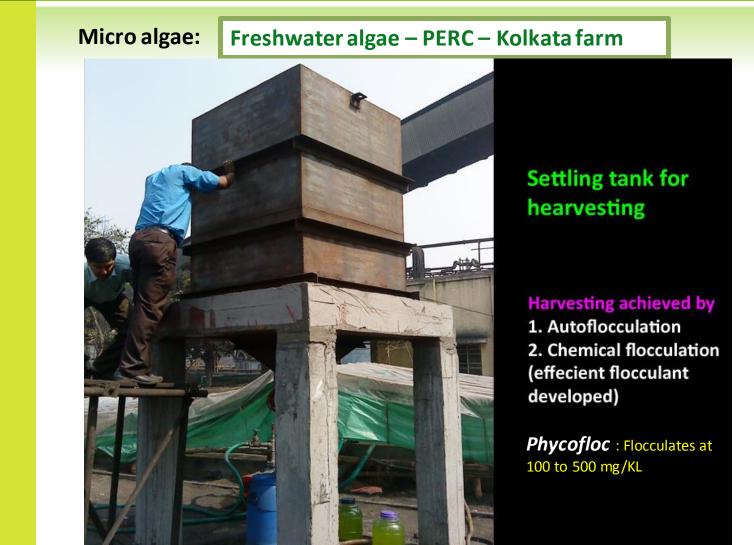
Micro algae:

Freshwater algae

PERC– Kolkata algal farm – Open raceway cultivation

- Nutrient inputs optimized and productivity stabilized
- Contamination controlled
- Harvesting accomplished by a combination of autoflocculation and Chemical flocculation (PHYCOFLOC)







Micro algae:

Freshwater algae – PERC– Kolkata farm



Harvested biomass being sundried

Harvesting done daily (50%) 1.5 to 2 g /L dryweight biomass achieved

Dewatering using muslin cloth





Micro algae:

Freshwater algae – PERC– Kolkata farm





Micro algae:

Freshwater algae – PERC– Kolkata farm

Economics based on Kolkata farming experience

Assumptions

Capex/acre for tanks= 40lac Tank depth 46cm Total water 18 lac litre Nutrient mix 0.1g urea/phosphate per litre 0.008g MgSO4/litre 0.005g bicarbonate/litre Power cost for paddle and pump 500/day/acre Drying,oil extraction and admin costs to be determined Flocculant cost@Rs2/kilo dry biomass Int&dep 10lac/acre

In the given situation known costs/acre/yr are 6 lac for nutrients 1.83 lac for power 10 lac for int and dep plus flocculant,drying and oil extraction costs which will be Rs 5 at least/kilo considering biomass price at 20/kilo we get a net of 15/kilo therefore to cover cost and breakeven we must produce 20 lac/15 kilos of biomass/year=134000 kg this means 447 kilos/day(300 working days) harvesting one third tank is 6 lac litre

so we need to achieve 0.75 gram/litre



PERC - Heterotrophic cultivation – Bio-reactor

To increase productivity

•To enhance oil content



Algae are grown with sugars – glucose/molasses/confectionery effluent/sugar industry waste



Micro algae: Freshwater algae

O Application of Pulsed magnetic field in improving the quality of algal biomass – MNRE Project

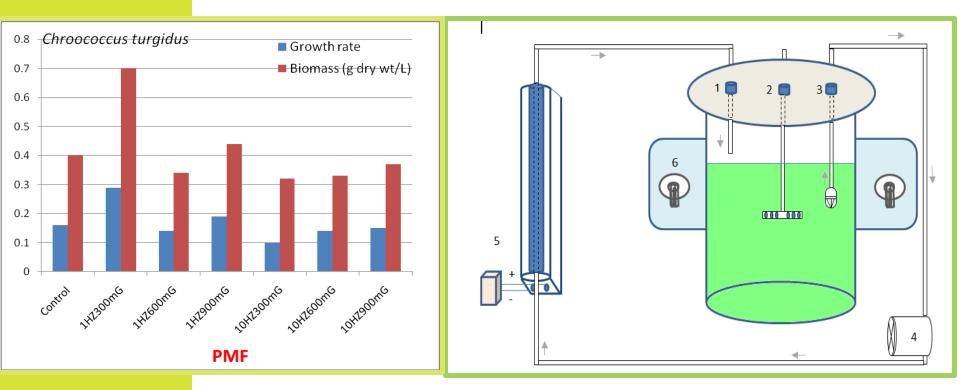
• Pulsed magnetic field (PMF) can be suitably integrated with the existing mass cultivation technology to enhance the bio-fuel quality of algal biomass. The present investigation has revealed that PMF increases oil content as well as *FAME* % in *Desmococcus olivaceous*

V Sivasubramanian, V V Subramanian, Leela Priya, T, and R Murali. 2010. Application of Pulsed Magnetic Field in improving the quality of algal biomass. *J. Algal Biomass Utln*. Vol 1 No 4: 1 – 9 VV Subramanian and Leela Priya 2011. Development of a hybridized bioreactor – open pond cultivation system integrating Sinusoidal Magnetic Field technology to enhance the qualitative and quantitative efficacy of algal biomass production – **MNRE project**



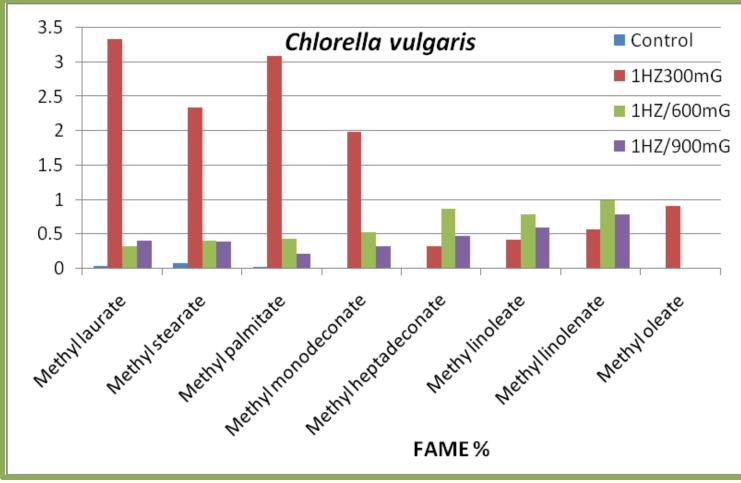
Micro algae: Freshwater algae

Application of Pulsed magnetic field in improving the quality of algal biomass – PHYCOSPECTRUM & MIM – MNRE Supported





Application of Pulsed magnetic field in improving the quality of algal biomass – PHYCOSPECTRUM & MIM





Algal biomass production integrated with Phycoremediation

Algae visiting Industries

- Sivasubramanian and his team from PERC, Chennai, have been involved in developing algae based technology to treat industrial effluents and wastewater. Algal technology for treating effluents has been implemented in a number of industries by PERC for the past 10 years.
- Solution has been delivered to alginate industry, leather processing chemicals industry, detergent industry, electroplating industry, confectionery industry, textile dyeing industries, oil drilling effluent treatment plant and more recently copper smelting industry



Algal biomass production integrated with Phycoremediation

Say NO to Chemicals

The main advantage of phycoremediation is complete avoidance of chemicals normally employed by various industries to correct pH, remove colour and odour, remove sludge etc. The industries save lot of chemicals and huge amount of energy. Algal technology involves maintenance of the critical level of algal biomass for effective remediation of effluents

Sivasubramanian V. 2010. Gaining an edge with algal technology. Search - The Industrial Sourcebook, Vol 13, No. 3 pp : 76 - 80



Algal biomass production integrated with Phycoremediation

First Step

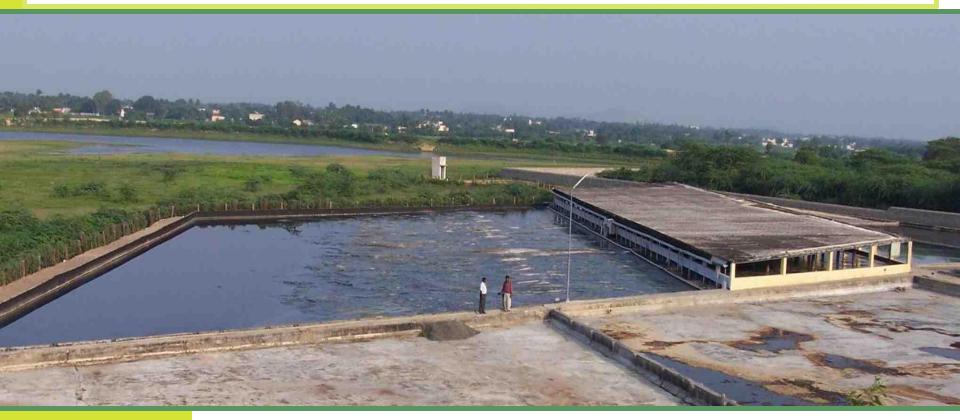
- World's First Phycoremediation plant to treat industrial effluent was commissioned in SNAP Natural and Alginate products at Ranipet, Tamilnadu based on the research support from PERC This industry generates huge volume of highly acidic effluent with a high TDS. Algal technology is being effectively employed to correct pH and reduce sludge with lots of benefits to industry as well as to the environment.
- In the process this industry also generates huge amount of algal biomass which is being incorporated into a bio-fertilizer product. PERC is working on other possibilities of utilizing algal biomass including **biogas**, **bio-ethanol and biodiesel**. Studies conducted by PERC have shown that algae grown in the effluent is highly suitable for **biodiesel application**.

V. Sivasubramanian, V.V. Subramanian, B.G. Raghavan and R. Ranjithkumar 2009 Large scale phycoremediation of acidic effluent from an alginate industry. *ScienceAsia* 35: 220-226



First Phycoremediation plant at Ranipet, India

Large-scale phycoremediation plant is in operation at SNAP Natural and Alginate Products, Ranipet, India from September 2006





Algal biomass production potential of SNAP effluent

Algal cell density is maintained at 2300 x 10⁴ / ml (0.75 g/L on dry weight basis).

Excess algal biomass is regularly harvested by the industry to produce two important products viz., Bio-fertilizer and aquaculture feed. Both these products (06 EMMA and PLANK-10) are produced @ 2 tons per month (\$ 2 million / year). Bio-fertilizer and aquaculture product produced by SNAP industry





Algal biomass production integrated with Phycoremediation

Following Steps

- Other industrial effluents which PERC has found favourable for algal biomass production are textile dyeing industry effluent and effluents from confectionery industries.
- PERC has successfully grown *Chlorella* species in confectionery industry effluent to correct pH and remove sugars. *Chlorella* sp grown in confectionery effluent produces higher amount of lipids.
- One of the textile dyeing industries which employs algal technology to remove dyes and reduce BOD and COD, is harvesting algae, dry the slurry and the dried algal cakes are being used in boilers along with firewood. The calorific value of algal cake has been analyzed by PERC and it was found to be superior.

P Hanumantha Rao, R Ranjith Kumar, BG Raghavan, VV Subramanian and V Sivasubramanian. 2011. Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility. *Water SA* Vol. **37** (1)



Algal biomass production integrated with Phycoremediation

Welcome Sludge!

Sludge produced by various industries could be also used as nutrient source for growing certain types of micro algae. PERC has investigated the biochemistry of sludge grown algae and found highly suitable for biodiesel production.

V Sivasubramanian, V V Subramanian and M Muthukumaran. 2010. Bioremediation of *chrome-sludge* from an electroplating industry using the micro alga *Desmococcus olivaceus* – A pilot study. *J. Algal Biomass Utin.* Vol 1 No 3: 104.128 R. Ranjith Kumar, P. Hanumantha Rao, V.V. Subramanian & V.Sivas ubramanian. 2011. Enzymatic and non-enzymatic antioxidant potentials of *Chlorella vulgaris* grown in effluent of a confectionery industry. *J Food Sci Technol*, DOI 10.1007/s13197-011-0501-2. P Hanumantharao, R Ranjith Kumar, B Govindaraghavan, V V Subramanian, V Sivas ubramanian 2011. Is phycovolatilization of heavy metals a probable (or possible) physiological phenomenon? - An in situ pilot-scale study at a leather-processing chemical industry. Water Environment Research, 83(4):291-297(7)



Leather processing chemical industry, Tamilnadu

The industry manufactures dyes, binders and pigments. The effluent and sludge generated has heavy metals and residual chemicals used in production. Algal technology is employed in treating the effluent and sludge generated by the industry (60 to 70 KL/day)

Algal growth (450 cells x 10^4 /ml of *Chlorella vulgaris*) in effluent

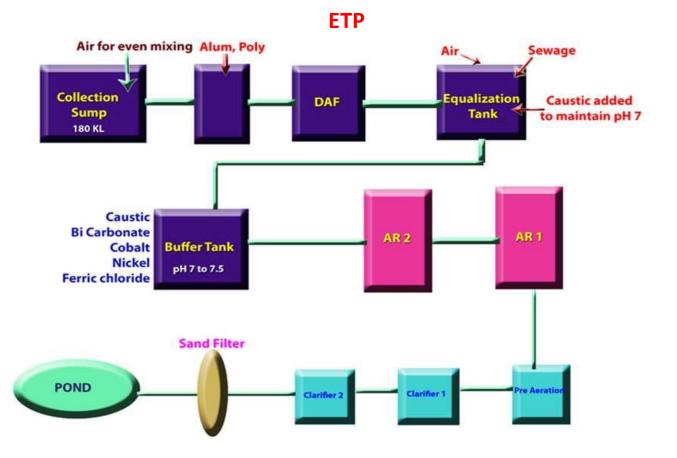


P Hanumantha Rao, R Ranjith Kumar, BG Raghavan, VV Subramanian and V Sivasubramanian. 2011. Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility. *Water SA* Vol. **37** (1)



Effluent from a confectionery industry, Tamilnadu

The total effluent generated per day amounts to an average of 50 – 70 kilo litters. The plant effluent generated is divided into two streams viz., industrial effluent stream from the production process and sewage effluent stream from the human activities.





Growth of *Chlorella vulgaris* . (Circulation flow rate 900 L/h) – Pilot tank at Confectionery industry, Chennai

Lab Study lad traca ted estim Perfetti PH 3.8 Lab Study lad traca ted estim (perfetti) PH 7.9 Chionestia vulgaritis (Nerseco curra Sp) 19.8		Initial Dip in cm	Addition of raw effluent in cm (whole day)	Evaporation in cm	Final Dip in cm	cell <i>Chlorell</i> VIA Ini	int x 10 ⁴ Is/ml <i>a vulgaris</i> T027 itial nal	cells Yeast) 4 s/ml t cells tial
19.6 LIPID		1 cm = 40 lits				rma		Fila	
19.4	1	20.0	5.0	1	24	230	262	35	5
9 19.2	2	17.5	7.5	0.5	24.5	262	273	56	21
et X	3	12.5	12.5	1	24	273	285	120	15
9 19.4 9 19.2 9 19.2 19 19 19 19 19 19 19 19 19 19 19 19 19 1	4	7.5	17.5	1	24	285	305	131	19
	5	5.0	20.0	1	24	305	320	152	10
	6	1.0	24.0	0.7	24.3	320	338	160	2
Control Medium culture Effluent treated microalgae microalgae	7	0.0	25.0	1.1	23.9	338	362	165	Nil



Growth of *Chlorococcum humicola* in confectionery effluent from Trivandrum, India

Productivity 3160 x 10⁴ cell/ml 68 g/L wet weight 4.8 g /L dry weight





List of microalgae identified in electroplating industrial ETP site – WHEELS INDIA, Chennai Chlorophyceae

Ankistrodesmus convolutus Corda Chlamydomonas pertusa Chod.v.indica.lyengar. Chlorella vulgaris Beijerinck Desmococcus olivaceus (Persoon et Acharius) J.R. Laundon Bacillariophyceae Navicula pupula Kuetz.



Occurrence of Algae in Dyeing Industrial ETP Waste and Effluents - Gumidipoondi(Tamil Nadu)

Chlorophyceae

Chlamydomonas pertusa Chod.v.indica Iyengar. Chlorella vulgaris Beijerinck Cosmarium tetraophthalmum Breb. Chlorococcum humicolo(Naegeli) Rabenhorst Coela strum cambricum Archer Desmococcus olivaceus (Persoon et Acharius) J.R. Laundon Pandorina morum (Muller) Bory. Tetraedron minimum (A.Braun) Hansgirg Ulothrixelongatum Hodgetts

Bacillariophyceae Cyclotel la meneghiniana Kuetz. Gomphonema olivaceum (Lyng.) Kuetz. Na vicula pupula Kuetz. Pinnularia viridis (Nitzsch) Ehr.

Euglenineae Euglena acusEhrenb. Euglena polymorpha Dang. Phacus longicauda (Ehrenb.) Dujardin

Myxophyceae Anabaenopsis arnoldiivar.indica Ramanathan. Arthrospira platensis (Nordst.) Gom., Aphanocapsa biformisA.Br. Chroococcus minutus (Kuetz.) Nag. Chroococcus turgidusv.maximus Nygaard Merismopedia aeruginea Breb. Microcystis robusta (Clark) Nygaard. Nostoc calcicola Breb. Oscillatoria curvicepsAg. ex Gomont Oscillatoria salina Bis was Oscillatoria subbrevis Schmidle Oscillatoria willei Gardner em. Drouet SUNTEX PROCESSING MILLS, Tamilnadu



SUNTEX Processing Mills

ANALYSIS OF OIL FROM Dye bath effluent GROWN ALGAE

The GC FAME ANALYSIS shows the presence of

- Heneicosanoic acid methyl ester (C21:0) 75.8%
- Arachidic acid methyl ester (C20:0) 10.7%
- cis-11,14,17-Eicosapentanoic acid methyl ester (C20:5n3)
 6.3%
- Lignoceric acid methyl ester (C24:0) 6.7%
- The total amount of saturated oil content comes to around 91%



Occurrence of Algae in soft drink Industrial ETP Waste and Effluents - Ahmedabad, Gujrat.

Chlorophyceae

Chlamydomonas pertusa Chod.v.indica.lyengar. Chlorella conglomerata (Artari) Oltmanns. Chlorella vulgaris Beijerinck Chlorococcum humicolo (Naegeli) Rabenhorst Desmococcus olivaceus (Persoon et Acharius) J.R. Laundon Coelastrum cambricum var. intermedium (Bohlin) G.S.West Oocystis borgei Snow, Scenedesmus dimorphus (Turpin) Kuetz Scenedesmus arcuatus (Lemm.) Lemm. Selenastrum minutum (Nageli) Collins. Staurastrum stellinum WB Turner

Bacillariophyceae

Fragilaria pinnata Ehr. *Gomphonema vibrio* Ehrenb. *Navicula fluminitica* Cambarn. *Navicula phyllepta* Kuetz. *Navicula pupula* Kuetz.

Myxophyceae

Chroococcus turgidus v. maximus Nygaard Lyngbya dendrobia Bruhl et Biswas. Microcystis robusta (Clark) Nygaard. Merismopedia aeruginea Breb. Nostoc calcicola Breb. Osillatoria tenuis Ag. ex Gomont Oscillatoria terebriformis Ag. Phormidium fragile (Meneghini) Gomont



Chlorophyceae

Synechocystis aquatilis Sauv.

Ankistrodesmus convolutusCorda Chlamydomonas pertusa Chod.v.indica.lyengar. Chlorella vulgaris Beijerinck Chlorococcum humicolo (Naegeli) Rabenhorst Coelastrum cambricum Archer Closterium decorum Breb. Cosmarium undulatumCorde exRalfs. Crucigenia tetrapedia (Kirchner) W.et G.S. West. Desmococcus olivaceus (Persoon et Acharius) J.R. Laundon Eudorina elegans Ehrenb. Kirchneriella lunaris (Kirchn.) Moebius Pandorina morum Bory. Scenedesmus quadricauda (Turp.) Breb. var. quadrispina Chodat. Scenedesmus bijugatus (Turp.) Kuetz. Scenedesmus obliquus (Turp) Kuetz. Scenedesmus opoliensis Richter Selenastrum minutum (Naegeli) Collins. Staurastrum coroniferum WB Turner. Staurastrum pinnatum Turner Tetraedron muticum (A.Braun) Hansgirg Tetraedron enormevar. pentaedricum Prescott. Oocystis elliptica W. West Ulothrix elongatum Hodgetts *Microcystis robusta* Nygaard. Nostoc calcicola Breb. Oscillatoria proboscidea Gomont. Oscillatoria curviceps Ag. ex Gomont. Oscillatoria salina Biswas Oscillatoria subbrevis Schmidle Oscillatoria terebriformis Ag. Phormidium tenue (Menegh.) Gomont Scytonema bohneri Schmidle Spirulina gigantea Schmidle

Bacillariophyceae

Cymbella affinis Kuetz. Fragilaria virescens Ralfs. Navicula viridula (Kuetz.) Ehr. Navicula pupula Kuetz. Nitzschia stagnorum Rabenh. Pleurosigma javanicum Grun. **Euglenineae** Euglena acus Ehrenb. Euglena polymorpha Dang. Phacus longicauda (Ehrenb.) Dujardin

Myxophyceae

Anabaena circinalis Rabenh. Aphanocapsa biformis A. Braun. Calothrix marchica Lemmermann Chroococcus turgidus v.maximus Nygaard Gloeotrichia intermedia v. kanwaensis C.B. Rao. Merismopedia aeruginea Breb.

List of micro algae isolated from Oil drilling industrial ETP site



Occurrence of Algae in Dyeing Industrial ETP Waste and Effluents – Ahmedabad, Gujrat

Chlorophyceae

Ankistrodesmus convolutes Corda Chlorella vulgaris Beijerinck Chlorococcum humicolo (Naegeli) Rabenhorst Coelastrum cambricum var. intermedium (Bohlin) G.S.West Oocystis crassa Wittrock Tetraedron muticum (A.Braun) Hansgirg Ulothrix elongatum Hodgetts

Bacillariophyceae

Amphora ovalis Kuetz. Cyclotella meneghiniana Kuetz. Cymbella cistula (Hemp.) Grun. Cymbella ehrenbergii Kuetz. Eunotia amphioxys Ehrenb. Fragilaria viresceus Ralfs. Gomphonema parvulum (Kuetz.) Grun. Navicula cuspidata Kuetz. Navicula microsporae Kant et Gupta. Navicula pupula Kuetz. Pleurosigma javanicum Grun. Synedra ulna (Nitz.) Ehrenberg var. amphirhynchus (Ehrenb.) Grun.

Euglenineae

Euglena polymorpha Dang.

Myxophyceae

Aphanocapsa littoralis Hansg. Chroococcus turgidus v.maximus Nygaard Merismopedia aeruginea Breb. Microcystis robusta (Clark) Nygaard. Osillatoria tenuis Ag. ex Gomont Oscillatoria salina Biswas Phormidium fragile (Meneghini) Gomont

ARVIND MILLS, Ahmedabad



Chlorococcum humicola growing luxuriantly in Sulphonation effluent

ULTRAMARINE and PIGMENTS TAMILNADU

Cell No. 450 x 10⁴/ml

Phycoremediation plant - Pilot





The cell density reaches up to 450×10^4 /ml (1.5 g dry weight/L) showing thereby detergent effluent can be a very good medium for growing algae.



List of micro algae identified in detergent industrial ETP site Chlorophyceae

Ankistrodesmus convolutus Corda. Chlorella vulgaris Beijerinck. Chlorococcum humicolo (Naegeli) Rabenhorst Scenedesmus incrassatulus Bohlin. Scenedesmus quadricauda (Turp.) Breb. var. quadrispina Chodat. Tetrastrum punctatum (Shmidle.) Ahlstrom et Tiffany Bacillariophyceae

Navicula microsporae Kant et Gupta.

Myxophyceae

Calothrix viguieri Fremy. Chroococcus turgidus v.maximus Nygaard Merismopedia aeruginea Breb. Nostoc calcicola Breb. ULTRAMARINE and PIGMENTS TAMILNADU



Cultivation of micro algae with Piggery waste at Barranquilla, Colombia



Scenedesmus sp grown with piggery waste as nutrient source





PHYCORE : Algae Power: A joint technology implementation programme between <u>CORE BIOTECH</u>, Colombia and Phycospectrum has been initiated with the successful installation of a 20 KL integrated tanks system (Pilot demonstration plant) at <u>Pacific Rubiales</u> oil drilling site near Bogota to treat petrochemical wastewater by employing micro algae.



350 x 10⁴ cell/ml; 0.7 to 1 g/L dry biomass/day









Algal biomass production integrated with Phycoremediation

Don't Waste "WASTE"

- Algal biomass production integrated with remediation is the best option since it will not encroach upon agricultural land and water. Growing algae in waste water will make the whole process very cost-effective and economically viable.
- Senthil Chinnasamy et al (2009) from Laboratory of Soil Microbiology, Division of Soil Science and Microbiology, Central Rice Research Institute, Cuttack, Orissa has done investigations on biomass production potential of waste water alga Chlorella vulgaris
- Ruma Pal, University of Calcutta, has worked on utilization of industrial effluent for cultivation of *Spirulina*

Senthil Chinnasamy, Balasubramanian Ramakrishnan, Ashish Bhatnagar and Keshav C. Das. 2009. Biomass Production Potential of a Wastewater Alga *Chlorella vulgaris* ARC 1 under Elevated Levels of CO2 and Temperature. Int. J. Mol. Sci. 2009, 10, 518-532

Ruma Pal and Chatterjee, P.1988. Use of Industrial Effluents for the cultivation of Spirulina. Bangladesh J. Bot. 17 (1): 89 - 93.

Ruma Pal 1990. Reclamation of Industrial waste for useful biomass production and lessening pollution level using Spirulina platensis In Ecology and Environment. Today and Tomorrow Publ. Eds.S. Mondal and M. Roy. Chattopadhyay, P. and Ruma Pal. 1995. A procedure for low cost outdoor mass production of Spirulina platensis using industrial waste at rural West Bengal. J. Natl. Bot.Soc. 49 : 35 - 41.



Advantages of integration of algal biomass production with phycoremediation - PERC

Effluent/Sludge	Type of		Algal biomass			
	effluent/sludge/waste	pH correction	Reduction of BOD, COD	Sludge reduction	Reduction in Operation cost	production potential (Kg dry biomass/KL/day)
SNAP Natural & Alginate products, Ranipet	Acidic effluent with high TDS	Н	Н	H	Н	0.75
Leather processing Chemical Industry, Ranipet	Effluent and sludge	L	Н	Н	Н	1.0
Confectionery industry 1 - Chennai	Acidic effluent	Н	Н	Н	Н	1.5
Confectionery industry 2 - Trivandrum	Acidic effluent	Н	Н	М	Н	4.8
Soft drink industry, Ahmedabad	Neutral effluent	L	L	L	Н	0.5
Textile dyeing industry, Chennai	Effluent and sludge	L	Н	Н	Н	0.75
Oil drilling industry, Kakinada	Effluent and sludge	L	Н	Н	Н	0.5
Detergent industry, Ranipet	Acidic effluent with high TDS	Н	Н	Н	Н	1.5
Electroplating Industry, Chennai	Effluent and sludge	L	Н	Н	Н	1.5
Chemical industry, Ranipet	Acidic effluent with high TDS	L	Н	Н	Н	0.75
Petrochemical, Colombia	Effluent with nutrients	L	Н	Н	Н	



Marine phytoplankton

- Many research laboratories in India are also involved in developing biodiesel technology based on marine phytoplankton species including diatoms.
 - Rengasamy and his team from University of Madras are in the process of isolation and characterization of suitable marine phytoplankton for biodiesel production.
 - Ramachandra, Durga Madhab Mahapatra, and Karthick Energy & Wetlands Research Group, Centre for Ecological Sciences/Centre for Sustainable Technologies, Indian Institute of Science, Bangalore, have done studies on bio-fuels production from species of diatoms

R Rengasamy - Isolation of Marine Phytoplankton for Biodiesel Production Products. ABLF— Associates of Biotechnology Ltd. Enterprises. Bangalore. **July 2008 – January 2009** T. V. Ramachandra, Durga Madhab Mahapatra, and Karthick B. Milking Diatoms for Sustainable Energy: Biochemical Engineering versus Gasoline-Secreting Diatom Solar Panels. Ind. Eng. Chem. Res



Marine phytoplankton

- N. Thajuddin DBT Project 2009 -11. "Pilot scale demonstration of algal oil production up to 100 litres per month" - Bharathidasan University, Tiruchirappalli, Tamilnadu has started working on algal biodiesel production from marine micro algae. A total of 78 micro algal cultures in pure form are being maintained. Optimization of selected cultures (with lipid content > 25%) for mass cultivation (25 – 75 L) under semi continuous mode is underway. The most suitable cultures will be ready for pilot scale trials (Up to 5 KL) by the end of November 2011.
- Ruma Pal, Calcutta University Bio-fuel from marine micro-algae and
- Kirubakaran, NIOT, Chennai Tubular Photobio-reactor (All India Coordinated project in CSIR- NMITLI (New Millennium Indian Technology Leadership Initiative), 2010-2013.



Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)



MASS CULTIVATION AT NIOT

Chlorella sp (Strain code - NIOT- 74/POSA) was cultivated in modified Bold Basal medium Axenic batch cultures was grown in 500mL Erlenmeyer flasks containing 250mL of modified Basal medium at 24°±1°C under 16:8 light / dark regime.The cultures were then scaled up in 3L Haufkins flask with an initial inoculumn of 10%.Step 3 was done in 12 or 20L Nalgene carbuoys. Step 4 was attempted in 320L Bubble column photobioreactors. Aeration was provided from step 3 onwards using aeration tubes connected to an aeration pump



Marine phytoplankton

Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)



MASS CULTIVATION OF CHLORELLA SP. AT CSMCRI EXPERIMENTAL SALT FARM

Mass cultivation of *Chlorella sp.* – 20 KL salt pans were employed for mass cultivation of micro algae and harvesting done by autoflocculation.



Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)



Biomass harvested from 11 ponds with a volume of 1,00,000 L through auto-settling.at CSMCRI

Mass cultivation of *Chlorella* sp.at CSMCRI

Summary: Micro algal culture: Chlorella variablis

- Type of cultivation : Open pond in salt pan (experimental salt farm)
- Total lipid : 22.5%
- Non-polarlipid: 10-12%
- Specific Growth rate: 0.09 g/day
- Scale: upto 1.1TPA dry biomass in 500 m²
- Productivity: 32.4 g/m²/day
- In first such development, road worthiness of B20 and B100 biodiesel from marine micro algal source was established through test drive of a regular diesel vehicle under full load condition.
- Cost of biodiesel using the above process is calculated to be approximately *Rs* 146/litre.



Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)

Marine phytoplankton - CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)

OUTDOOR MASS CULTIVATION AT ANDHRA UNIVERSITY: Initially mass scale culture was done in 100L, 200L Rectangular tanks. Later it was done in 1, 2 Tons in outdoor and 200L indoor in vertical Fibre cylinders for the following microalgal species *Isochrysis galbana*, *Platymonas convolutae*, *Tetraselmis gracilis*, *Tetraselmis chuii*, *Pavlova lutheri*. *Tetraselmis gracilis* was later cultured in 5 ton and 10 ton out door cement tanks. Tetraselmis Sp. was cultured in the open air farm of 50m² area on an experimental basis . *Tricodesmium* sp, was also cultured in the farm. 200 g of dry biomass was obtained in the open air tank (1500L). 25kgs of dry biomass of mixed microalgae (*Nitzschia*-40%, *Tetraselmis gracilis*-30%, *Navicula*-20%, other diatoms-10%) was also obtained.



Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)



Marine phytoplankton – CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)

NATIONAL INSTITUTE FOR INTERDISCIPLINARY SCIENCE & TECHNOLOGY, KERALA

- Development of auto-flocculating algal cultivation technology with CO_2 fertilization, settler for algal biomass separation; determination of brackish water nutrient status for algal cultivation and determination of biomass productivity in scalable reactor - CSIR - NMITLI project

NIIST - Trivandrum has perfected raceway pond technology to grow autosettleable marine micro algae with consistent oil content and great control of all parameters and excellent economy achieved. The work has been carried out on a bench scale (300 L) and scaling up as well as revalidation in another site has been planned for phase2.



Marine Micro algae

Institute of Bioresearches and Sustainable Development (IBSD), Takyelpat, Imphal

-Creation of fresh water cyanobacterial repository at IBSD, Imphal, Manipur – DBT 2010

1228 unialgal cyanobacterial/micro-green algae have been purified and maintained in the freshwater Cyanobacterial and Microalgal Repository with accession numbers.

Institute of Minerals and Materials Technology IMMT, Bhubaneswar, Indian Institute of Chemical Technology, Hyderabad, Department of Agronomy, Institute of Agricultural Science, Kolkata - Collection, characterization and screening of potential microalgae from West Bengal & Orissa Coast and Pilot Scale Demonstration of Algal Oil Production – DBT 2010

University of Pune, Pune - Collection, Identification, and Screening of Algal Biodiversity from the Western Maharashtra for Quality and Quantity of Lipids – DBT 2011



Institute of Minerals and Materials Technology IMMT, Bhubaneswar-Collection, characterization and screening of potential microalgae from West Bengal & Orissa Coast and Pilot Scale Demonstration of Algal Oil Production – **DBT** 2010



30 KL Raceway ponds (8) at IMMT

V. Aishwarya et al. 2012. Enhanced inorganic carbon uptake by Chlorella sp IMMTCC-2 under autotrophic conditions for lipid production and CO2 sequestration. J Appl Phycol. DOI 10.1007/s10811-012-9801-9



Marine phytoplankton – CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI) IIT Kharagpur: CSIR - NMITLI Project

An efficient, cost-effective and eco-friendly process for dewatering of algal biomass

SUMMARY:

Process : Dewatering using ALGORBENT

Scale : Lab-scale for processing 2 Kg biomass

Features

- An inexpensive natural adsorbent based efficient dewatering system; Recovery of 85% moisture in thickened biomass slurry after bulk water removal; and
- Patent application filed
- Using the combination of above processes for dewatering, estimated cost for biodiesel was calculated to be Rs. 200 per litre biodiesel.



National Chemical Laboratory (NCL)- Pune - CSIR _NMITLI Project

Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)

NCL Pune, is also exploring bio-oil production from marine micro algal biomass through hydrothermal liquefaction



Marine phytoplankton -CSIR - New Millennium Indian Technology Leadership Initiative (NMITLI)

CSIR -NMITLI Project : Achievements of Phase 1

- 1. Identification of a few micro algal species (marine) for bio-diesel production, cultivation protocols optimized.
- 2. Harvesting procedure for these species standardized and Some harvesting strategies tried which need to be validated.
- 3. Extraction, conversion and testing of biodiesel done (http://www.indianexpress.com/news/suv-that-runs-ofbiodiesel-unveiled/930603/).
- 4. NCL Pune, is exploring bio-oil production from marine micro algal biomass through hydrothermal liquefaction..
- 5. NIO, Goa has started on an extensive as well as intensive EIA which would go hand in hand with pilot scale development of the entire process and will be finalized at the end of Phase 2..
- 6. CMCRI has successfully developed a low cost technology of cultivation and harvesting using marine Chlorella in salt pan area. Cultivation area is planned to be expanded for scale up purposes during phase 2.
- 7. IIT Karagpur Patent application filed for easy, low cost dewatering of algal slurry.
- 8. NIIST Trivandrum has perfected raceway pond technology to grow autosettleable marine micro algae with consistent oil content and great control of all parameters and considerable economy achieved. The work has been carried out on a bench scale (300 L) and scaling up as well as revalidation in another site has been planned for Phase 2.
- 9. With all these achievements in Phase I now CSIR is ready to go for Phase II which requires industry partners for validating these technologies and taking it to really large-scale.





Dr Carole Llewellyn



Dr Daniel White





SUSTAINABLE BIOENERGY AND BIOFUELS (SuBB): CASE FOR Department of Biotechnology

Using flow cytometry and genomics to characterize and optimize **micro algal-bacterial consortia** cultivated on Wastewater to produce biomass for Biofuel - An Indo UK Joint Project Under **BBSRC – DBT** Sponsorship

Plymouth Marine Laboratory (UK) and BDU, PERC, India

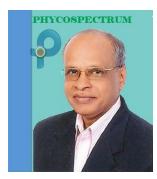


Dr Karen Tait





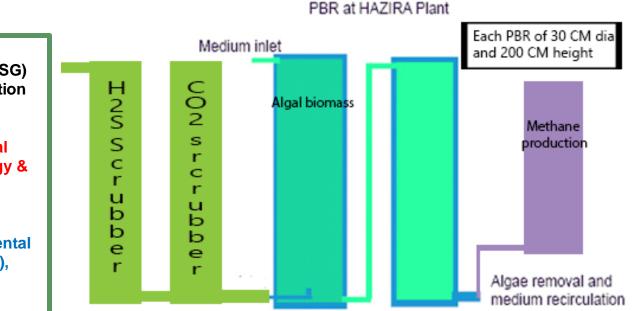








Algal technology to fix CO₂ from flue gas and convert algal biomass into methane



Carbon Management & Sustainability Group(CM&SG) Oil & Natural Gas Corporation Ltd(ONGC)

Department of Biological Birla Institute of Technology & Science, Pilani K K Birla Goa Campus

Phycospectrum Environmental Research Centre (PERC), Chennai



Marine phytoplankton

PERC has been working on cultivation of selected diatoms species in seawater and extraction of oil. Work on standardization of nutrient supplement, harvesting and extraction is complete. Further work is on progress to optimize other parameters to enhance the production of oil by diatoms in pilot scale open race way pond. **Oil percentage of 30 to 40 %** could be achieved.



Cultivation of Marine diatom in open raceway pond with seawater -PERC





INDIAN CAPABILITIES

- Valuable culture collection of algae isolated from a variety of ecological habitats
- Robust species of algae isolated from hostile environments including industrial wastes, wastewaters etc – heterotrophic capabilities and ability to adapt to various environmental conditions
- Of the second second
- Open raceway pond cultivation
 - Optimization of nutrient inputs and harvesting schedule
 - Control of contamination
 - Harvesting
 - Autoflocculation
 - Chemical flocculation (PHYCOFLOC)
- Seaweed cultivation and processing



INDIAN INSTITUTES INVOLVED IN ALGAL BIOFUELS RESEARCH

- Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar
- Department of Marine Living Resources, Andhra University (AU), Vishakapatnam
- International Center for Genetic Engineering & Biotechnology, ICGEB – New Delhi"
- Calcutta University (CU), Kolkata
- Indian Institute of Chemical Technology (IICT), Hyderabad
- Indian Institute of Technology, Khargpur(IIT-KGP)
- **National Chemical Laboratory (NCL), Pune**
- National Institute of Oceanography (NIO), Goa



INDIAN INSTITUTES INVOLVED IN ALGAL BIOFUELS RESEARCH...

- National Institute of Ocean Technology (NIOT), Chennai
- National Institute of Interdisciplinary Science & Technology (NIIST), Thiruvananthapuram
- Institute of Chemical Technology ICT Mumbai
- CAS in Botany, University of Madras
- **Bharathidasan University, Tiruchirappalli**
- Energy & Wetlands Research Group, Centre for Ecological Sciences/Centre for Sustainable Technologies, Indian Institute of Science, Bangalore



INDIAN INDUSTRIES AND OPPORTUNITIES FOR COLLABORATION

- Shree Cements, Rajasthan Algal biomass
- Reliance Industries Algal biofuels
- **Solution** Tata Power, Mumbai CO₂ mitigation and biomass production
- **Solution** Abellon Clean energy, Ahmedabad Remediation and biomass production
- **KGDS, Coimbatore Algal biomass Bioenergy**
- Jubiliant Life Sciences, Noida Effluent treatment and biomass production, bioenergy
- In NFCL, Kakinada Effluent treatment and algal biomass production
- Jain Irrigation Systems Ltd. And Jain Plastic Park, Bambhori, Jalgaon remediation and biomass production
- Biocon, Bangalore Remediation and algal biomass production
- HPCL, Gujrat Remediation and biomass production
- **© GNE-India Algal biomass for fish feed and oil**
- Output AGNI BIO ENERGY Chandigarh Remediation and algal biomass production
- **ONGC CO₂ mitigation and biomass production**



THANK YOU

