Microalgal Cultivation on Wastewaters for Energy and Environmental Sustainability

Ashok Pandey

PhD, DSc (*h.c.*), *FBRS, FNASc, FRSB, FIOBB, FAMI, FISEES, FICT, FWSSET*

Distinguished Scientist

Centre for Innovation and Translational Research

CSIR-Indian Institute of Toxicology Research

Lucknow, India

Executive Director (Hon)

Centre for Energy and Environmental Sustainability

Lucknow, India

ashok.pandey1@iitr.res.in

ashokpandey1956@gmail.com

Distinguished lecture series, Hong Kong Baptist University; 25th May 2021

This lecture will cover:

- **Introduction - Centre for Biofuels, CSIR-NIIST & CSIR-IITR**
- **Environmental challenges**
- **Biofuels for energy & environmental sustainability**
  - **Algal biofuels**
    - **Mixotrophic algal biofuels**
    - ***Chorococcum* sp – potential candidate for biofuels**
- **Conclusions**

C S I R   M I S S I O N

To conduct scientific and industrial R&D that maximizes the economic, environmental and societal benefits for the people of India.

Map of India indicating locations of CSIR institutes

AMPRI	Advanced Materials and Processes Research Institute Bhopal-462 026, www.ampri.res.in
CBRI	Central Building Research Institute, Roorkee-247 667 www.cbri.org
CCMB	Centre for Cellular and Molecular Biology Hyderabad-500 007, www.ccmb.res.in
CDRI	Central Drug Research Institute, Lucknow-226 001 cdriindia.org
CECRI	Central Electrochemical Research Institute Karaikudi-623 006, www.cecri-india.com
CEERI	Central Electronics Engineering Research Institute Pilani-333 031, www.ceeri.res.in
CFTRI	Central Food Technological Research Institute Mysore-570 020, www.cftri.com
CGCRI	Central Glass and Ceramic Research Institute Kolkata-700 032, www.cgcri.res.in
CIMAP	Central Institute of Medicinal & Aromatic Plants Lucknow-226 015, www.cimap.res.in
CIMFR	Central Institute of Mining & Fuel Research Dhanbad-828 108, www.cmriindia.nic.in
CLRI	Central Leather Research Institute, Chennai-600 020 www.clri.org
CMERI	Central Mechanical Engineering Research Institute Durgapur-713 209, www.cmeri.org
CRRI	Central Road Research Institute, New Delhi-110 020 www.crridom.org
CSIO	Central Scientific Instruments Organisation Chandigarh-160 030, www.csio.nic.in
CSMCRI	Central Salt & Marine Chemicals Research Institute Bhavnagar-364 002, www.csmcri.org
IGIB	Institute of Genomics & Integrative Biology Delhi-110 007, www.igib.res.in
IHBT	Institute of Himalayan Bioresource Technology Palampur-176 061 (HP), www.ihbt.org
IICB	Indian Institute of Chemical Biology Kolkata-700 032, www.iicb.res.in
IICT	Indian Institute of Chemical Technology Hyderabad-500 007, www.iictindia.org
IIIM	Indian Institute of Integrative Medicine Jammu-180 001, www.iiim.org
IIP	Indian Institute of Petroleum, Dehradun-248 005 www.iip.res.in
IMMT	Institute of Minerals and Materials Technology Bhubaneshwar-751 013, www.rrlbhu.res.in
IMTECH	Institute of Microbial Technology, Chandigarh -160 036 www.imtech.res.in
IITR	Indian Institute of Toxicology Research Lucknow-226 015, www.itrcindia.org
NAL	National Aerospace Laboratories, Bangalore-560 017 www.nal.res.in
NBRI	National Botanical Research Institute Lucknow-226 001, www.nbri-lko.org
NCL	National Chemical Laboratory, Pune-411 008 www.ncl-india.org
NEERI	National Environmental Engineering Research Institute Nagpur-440 020, www.neeri.nic.in
NEIST	North-East Institute of Science and Technology Jorhat-785 006, www.neist.res.in
NGRI	National Geophysical Research Institute Hyderabad-500 007, www.ngri.org.in
NIO	National Institute of Oceanography, Goa-403 004 www.nio.org
NIIST	National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram-695 019 www.niist.csir.res.in
NISCAIR	National Institute of Science Communication And Information Resources, New Delhi-110012 www.niscair.res.in
NISTADS	National Institute of Science Technology And Development Studies, New Delhi-110012 www.nistads.res.in
NML	National Metallurgical Laboratory, Jamshedpur-831 007 www.nmlindia.org
NPL	National Physical Laboratory, New Delhi-110 012 www.nplindia.org
SERC	Structural Engineering Research Centre Chennai-600 113, www.sercm.org

India's largest R &D organization with 38 laboratories, 50 field stations and with 17000 employees

CSIR

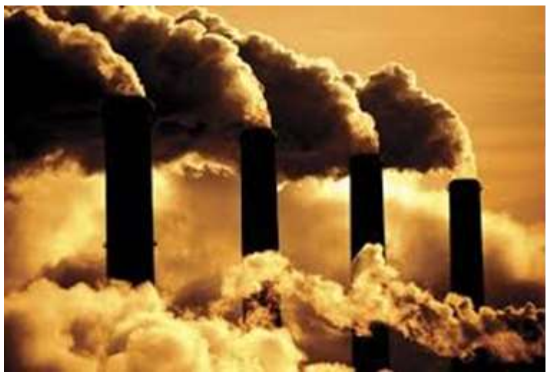
India's largest R&D organization with 38 laboratories, 50 field stations and with 17000 employees

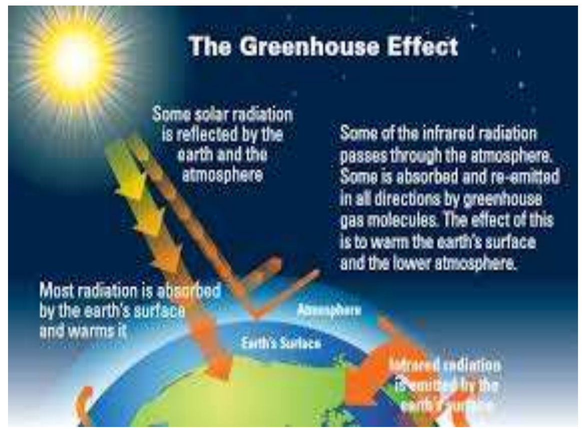
CSIR- Indian Institute of Toxicology Research

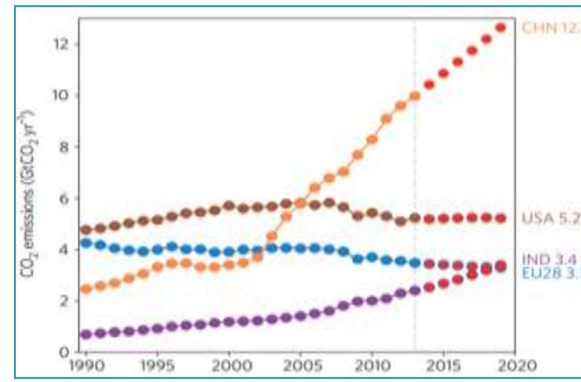
CSIR- National Institute for Interdisciplinary Science and Technology

Today's environmental challenges

- **Increase of greenhouse gas (GHG) in the atmosphere**







- Global CO_2 emissions
  : CO_2 emission 36Gt in 2013
  : CO_2 emission 45Gt in 2020

- The CO_2 emissions from the top four emitters (China, USA, EU, India)
  - China 12.7 Gt, USA 5.2Gt in 2020

Biofuels for Energy and Environmental Sustainability

- Worldwide interest in renewable fuels, especially biofuels are intensified due to concerns about short age of fossil fuels, increasing crude oil price, energy security and accelerated global warming.
- Biofuels are the fuels derived from organic biomass
- Biofuels can be categorized into four generations

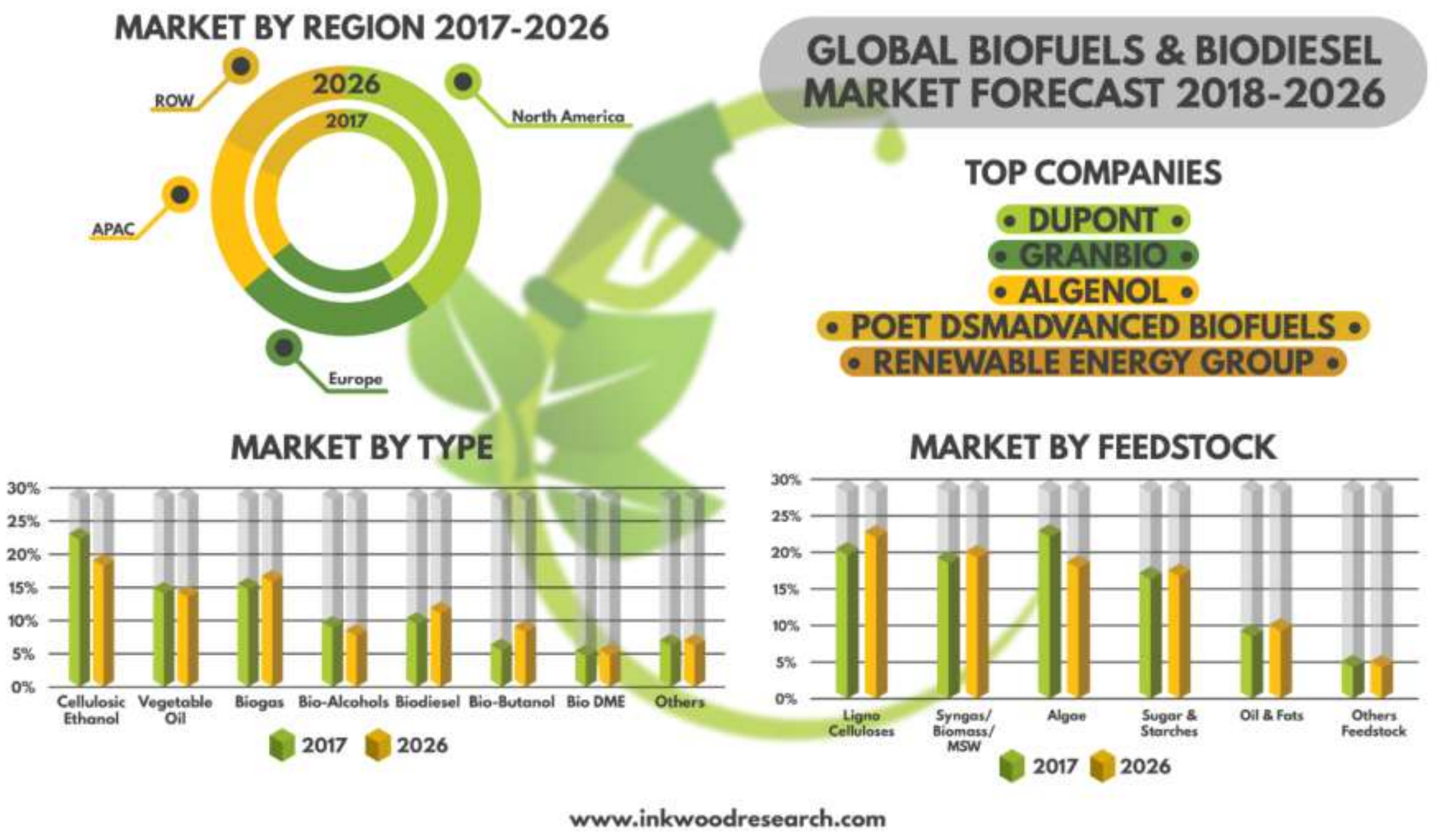
1st Generation of biofuels: ethanol from sugar, corn, molasses, starchy biomass, etc

2nd Generation of biofuels: biodiesel from vegetable oils and bioethanol from lignocellulosic biomass

3rd Generation of biofuels: fuels from algal biomass

4th Generation of biofuels: biohydrogen

Global biofuels market



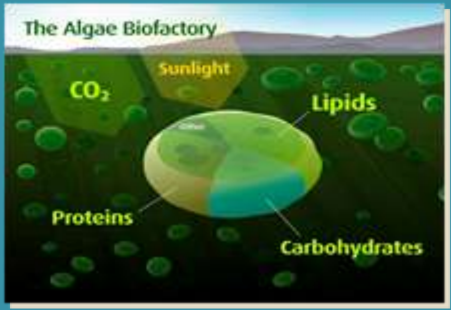
Current environmental challenges and algae

- **Various waste streams generated from industrial processes affect to the environment**

Glycerol waste from biodiesel

Milk Industry waste (whey)

Brewing industry wastes



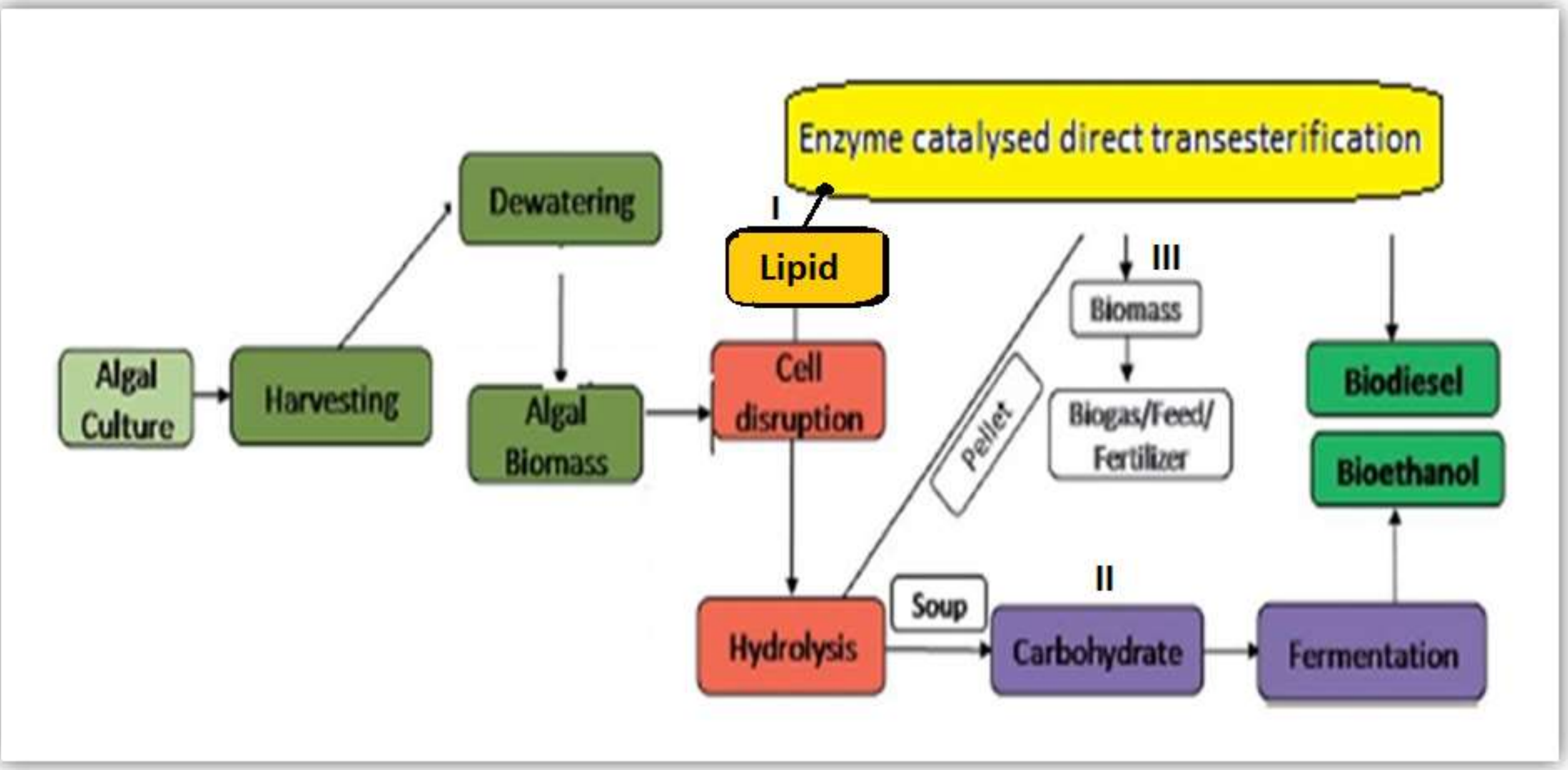
Autotrophic cultivation of microalgae significantly Reduce the GHGs emission via CCU technology

Later Heterotrophic cultivation of microalgae found to be Cost-effective however not Environmental-friendly

Recent trend: Mixotrophic cultivation of microalgae not only **Environmental-friendly** but also have potential for **cost-effectiveness**

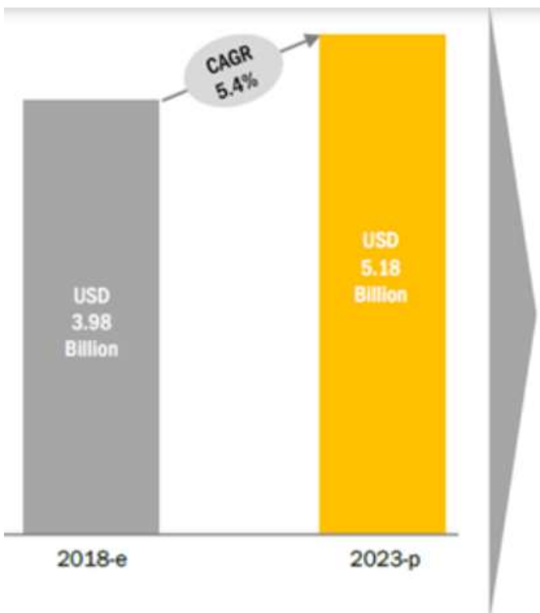
Algal biofuels

Schematic view of algal biofuels production process

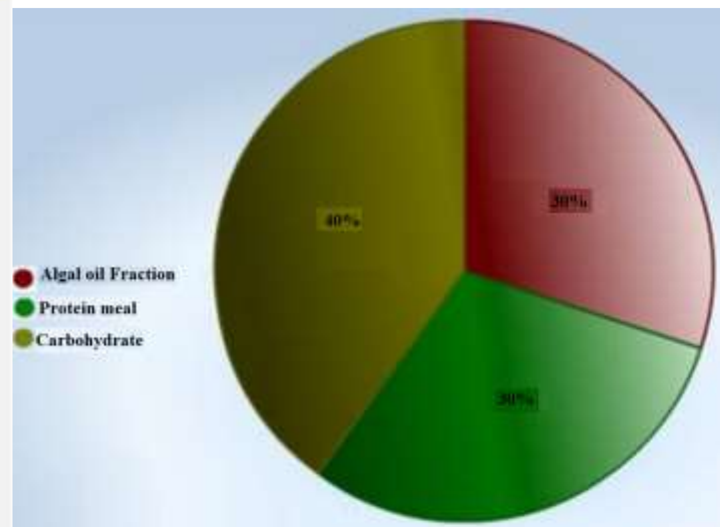


Market potential of algae

The report "Algae Products Market”
by Type (Lipids, Carrageenan, Carotenoids, Alginate, and Algal Protein),
by Application (Food & Beverages, Nutraceuticals & Dietary Supplements, Personal Care, Feed, Pharmaceuticals),



- The global algae products market is estimated at USD 3.98 billion in 2018. It is projected to grow at a CAGR of 5.4% from 2018 to 2023.
- The key driving factors of the algae products market include the following:
  - Multiple industry coverage
  - Growth in consumer awareness regarding the health benefits of algae-based products
  - Alternate food source and food ingredient
- Developing economies such as China, India, and Brazil offer high growth opportunities for the market.



Greentech Media Research has predicted that algae biofuels could be produced at a rate of 6 bln gallons a year by 2022.

Source Global Forecast to 2023 : https://www.marketsandmarkets.com/PressReleases/algae-product.asp

Algal biofuels: economics?

Algal biodiesel for sustainable commercial production: Largely depends on biomass and lipid yields.

Can we reach theoretical lipid yields in microalgae?

It can significantly reduce the gap between current and theoretical maximum yield.

A realistic maximum is approx 0.5 g TAG per mol photons, about **5t higher** than current outdoor yield (Remmers et al., 2018)

Heading towards improved yields in microalgal lipid production, current research is moving towards advanced cultivation strategies: **Mixotrophic cultivation could be one of the important approach**

Economic analysis of biodiesel from microalgae

Variable	Photobioreactor
Biomass production (kg yr^{-1})	100,000
Biomass productivity (g L^{-1} d^{-1})	1.535
Biomass productivity (kg m^{-2} d^{-1})	0.048
Biomass concentration (kg m^{-3})	4.00
Space requirements (m^2)	5,861
Reactor size	132 parallel tubes/unit 80 m long tubes 0.06 m tube diameter
Reactor number	6
Oil yield (m^3 ha^{-1})	58.7

Biomass productivity 1.5 g L^{-1} d^{-1} (CO_2 uptake rate 2.8 kg m^{-3} d^{-1})

Production cost of biomass $2.95 kg^{-1}

Scale-up to 10,000 ton

Production cost of biomass $0.47 kg^{-1}

If biomass contains 30% oil by weight

Production cost of oil $2.8 L^{-1}

Production cost of petrodiesel $0.66~0.79 L^{-1}

Competitive price of biodiesel

$0.86 L^{-1}

(attainable target with biorefinery based production)

Chisti et al., 2007, 25, 294-306

If the biomass productivity and lipid contents of microalgae can be >1.5 g/L/d and 70%, it can produce algal biofuels with economic feasibility.

Mixotrophic Algal Biofuels

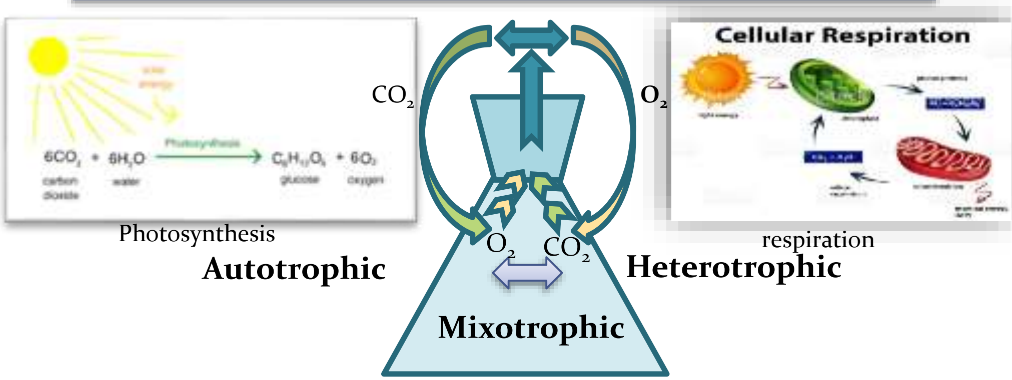
Algal cultivation

Concept of mixotrophy

Primarily algae are efficient in photosynthesis for their growth.
Some can assimilate organic carbon either **alternatively** or **simultaneously**

Mixotrophic is such a growth mode in which algae can assimilate inorganic and organic carbon for their growth (via light/organic energy).

Complementing to each other ➡ **Increased Biomass**



Improved growth conditions

(1) Mixotrophic is simple combination of Auto + Hetero

(2) Improve DO and DIC concentrations

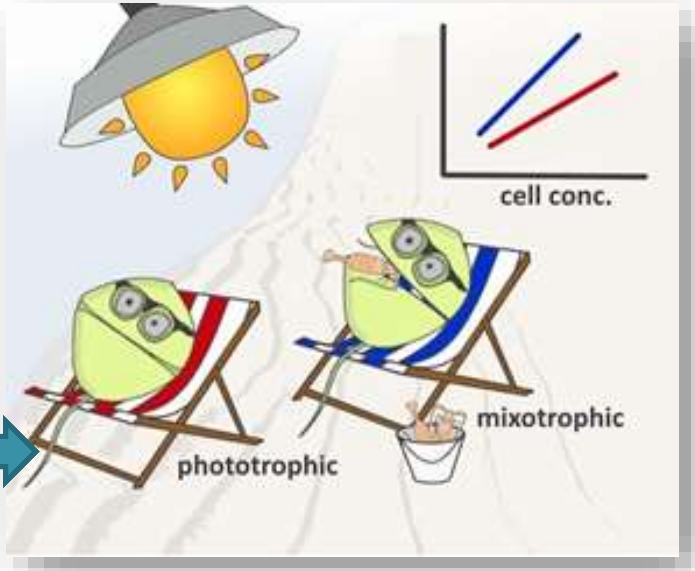
Algal cultivation

Comparative account of other growth modes with respect to Mixotrophy

Table 1 Growth modes of algae (microalgae) cultivation

Growth mode	Energy source	Carbon source	Light availability requirements	Metabolism variability
Photo-autotrophic	Light	Inorganic	Obligatory	No switch between sources
Heterotrophic	Organic	Organic	No requirements	Switch between sources
Photoheterotrophic	Light	Organic	Obligatory	Switch between sources
Mixotrophic	Light and organic	Inorganic and organic	No obligatory	Simultaneous utilization

Ref: Perez Garcia & Bashan, 2015



Mixotrophic mode of microalgae cultivation looking promising not only for organic waste removal but also for inorganic waste removal

Mixotrophy benefits

Benefits

(i) Higher growth rates

(ii) Extended exponential phase

(iii) Decreasing biomass loss during dark

(iv) Decrease in photo-inhibition

(v) Flexibility to switch of growth modes

(vi) Shield from photo-ox damage (O_2 accum. in closed system)

Offer better **carbon footprint** than that of heterotrophy due to sequestering of CO_2 simultaneously

Mixotrophic condition favors **better lipid yield** and **desired lipid fractions** for qu ality biodiesel

Challenges and opportunities

Challenges and opportunities for mixotrophic cultivation

Challenges	**Opportunities**
Carbon source costs	Investigate new source of cheap organic carbon Bioprospecting/metabolic eng. (able to uptake)
Competition by fast growing bacteria	Develop strategies to overcome contaminations Develop strain able to thrive in that condition Immobilization
Bioreactor implementation and operation costs	Cheaper material and methods Alternative strategies of mixing, sterilization, axenic capability
Downstream processing cost and product transformation	EPS mediated flocculation Immobilization Spontaneous secretion of desired products

Challenges of mixotrophic cultivation

Contamination

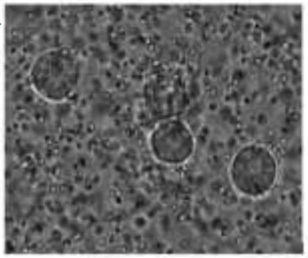
Type of Contamination

Cilliates contamination:

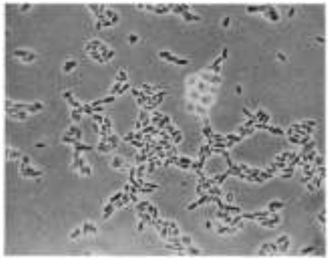
Begins as Trophozoites (cysts)

Yeast contamination

They all can grow in lower pH 5.0



Sarcodina/ameoba



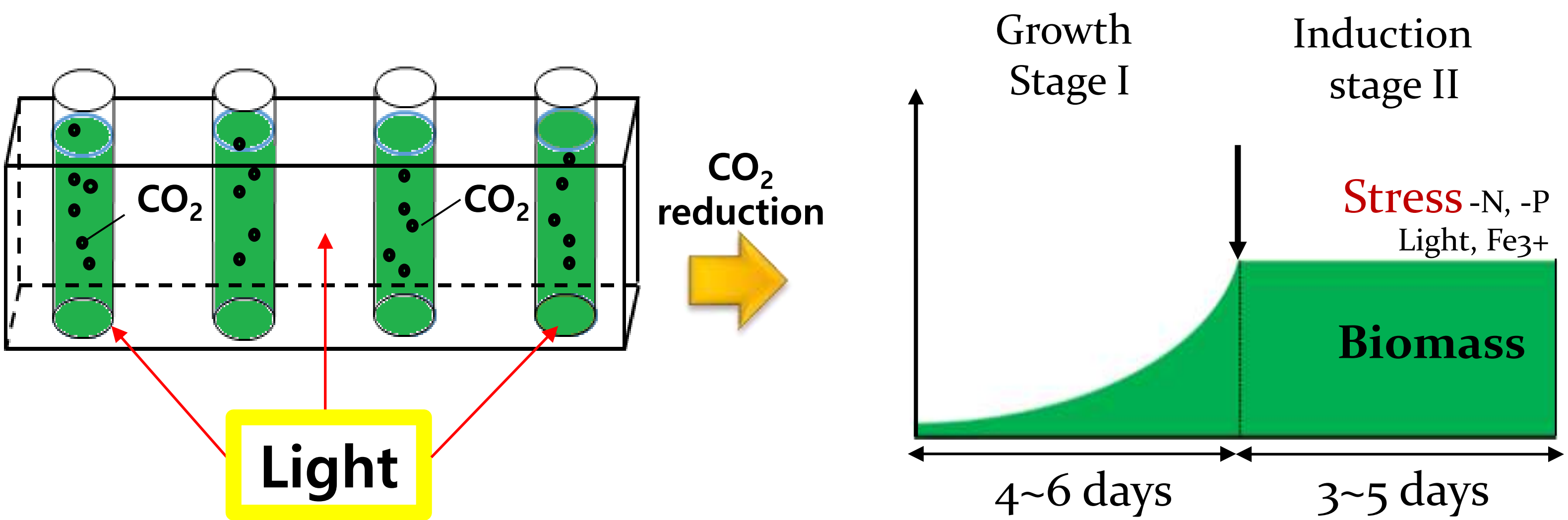
Yeast/cysts

paramecium

Rotenone, quinine sulfate are used to prevent contamination of Protozoa

Mixotrophic lipids production

Two stage bioprocess scheme for growth



CO2 Purging is reported to be best condition for higher accumul ation of lipid than the flask (diffusion)

Cultivation of *Chlorococcum* sp. RAP13 under various mode s and conditions

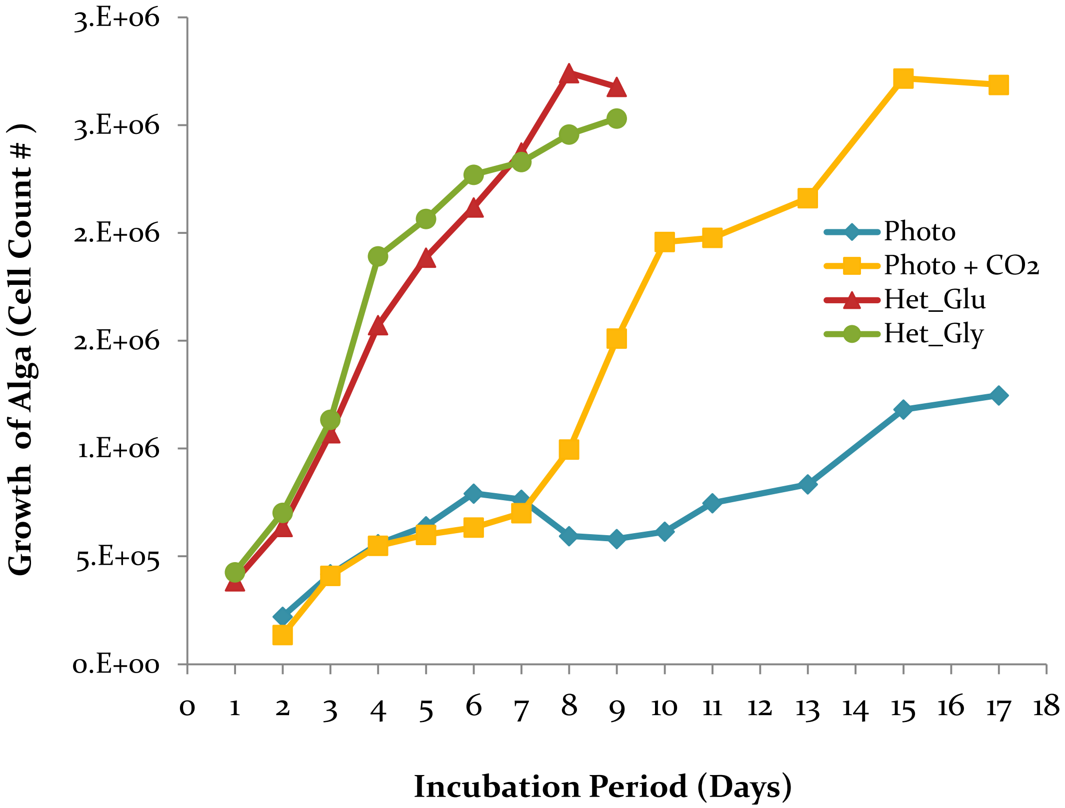
Crude Glycerol as Carbon Source

Utilization of waste for economic viability of algal process
We chose to utilize glycerol waste from biodiesel industry
Generated from biodiesel Plant ~10% of oil during lipid transesterification.
It has 30-75% **GLYCEROL** content which is good for lipid bioprocess

(1) Biodiesel-derived glycerol considered as potential substrate for **mixotrophic** cultivation of microalgae to reduce the process cost
(2) Still only a few reports examined the crude glycerol for biomass and lipid production under **mixotrophic** conditions.

Cultivation of the fresh water microalga *Chlorococcum* sp. RAP13 in sea water for producing oil suitable for biodiesel

Growth of alga in 50% natural seawater medium



Het _ Glu: Heterotrophic with glucose supplementation, Het_ Gly - Heterotrophic with glycerol supplementation

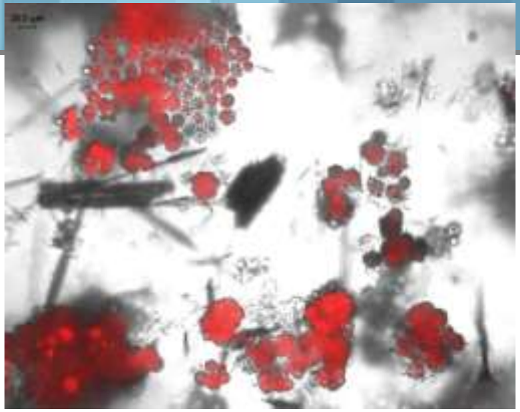
Culture condition

○50% sea water medium was supplimented with 5% of glucose or waste glycerol.

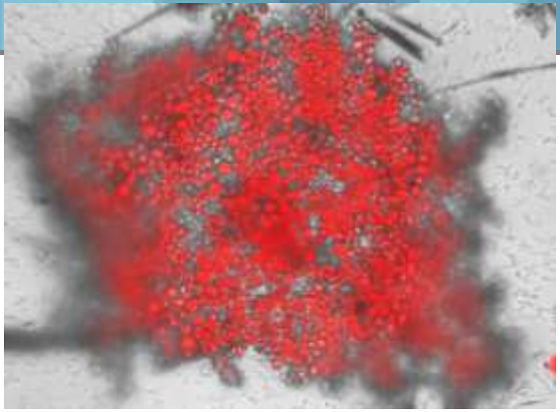
○5% (v/v) algal suspension containing 3 ×10^6 cells/ml was used as inoculum

○Incubated at 30°C with 100rpm agitation. For autotrophic condition, medium was bubbled with 0.8 vvm of CO_2

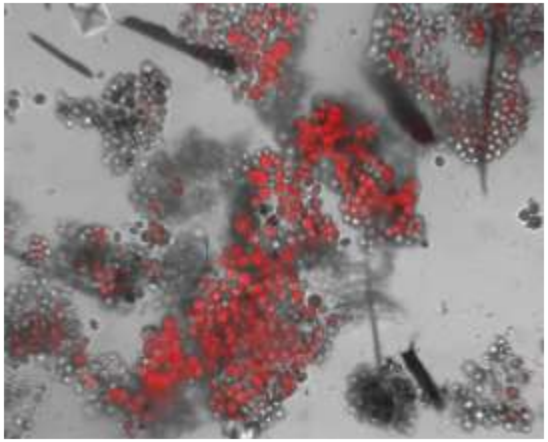
Nile red staining of *Chlorococcum* sp grown under phototrophic or heterotrophic conditions



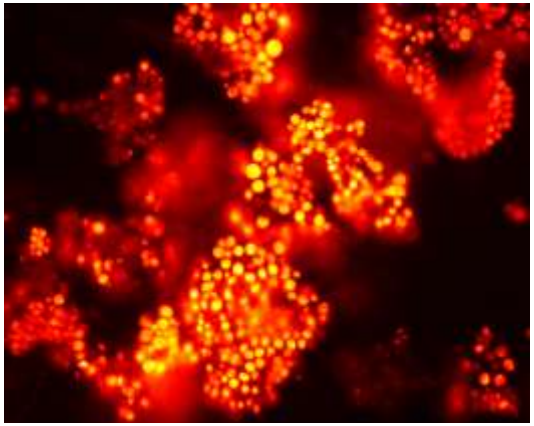
Phototrophic with CO_2 bubbling



Heterotrophic on crude glycerol



Heterotrophic on glucose



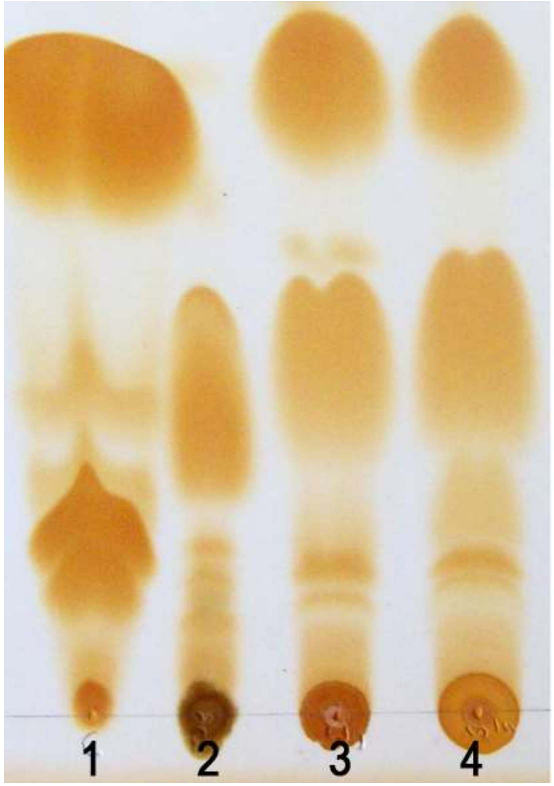
Orange yellow fluoresc ence indicate the prese nce of intra cellular lipi d droplets

Appl. Phycol 27(1), 141-147

Biomass and lipids production potential of *Chlorococcum* sp R-AP13 grown phototrophically or heterotrophically on sea water medium

Cultivation conditions	Biomass conc, mg/l	Lipids yield, mg/l	% DCW of lipids
Phototrophic without carbon	152.5 ± 0.7	31.0 ± 0.65	20.8 ± 2.6
Phototrophic/with CO_2 bubbling	301.0 ± 0.3	72.5 ± 0.4	24.0 ± 0.84
Heterotrophic with waste glycerol	850.0 ± 7.0	330.0 ±1.0	38.9 ± 1.9
Heterotrophic with glucose	1.008 ± 7.7	304.0 ± 2.0	30.5 ± 0.35

Lipid profiling of *Chlorococcum* oil from phototrophic and heterotrophic culture by TLC

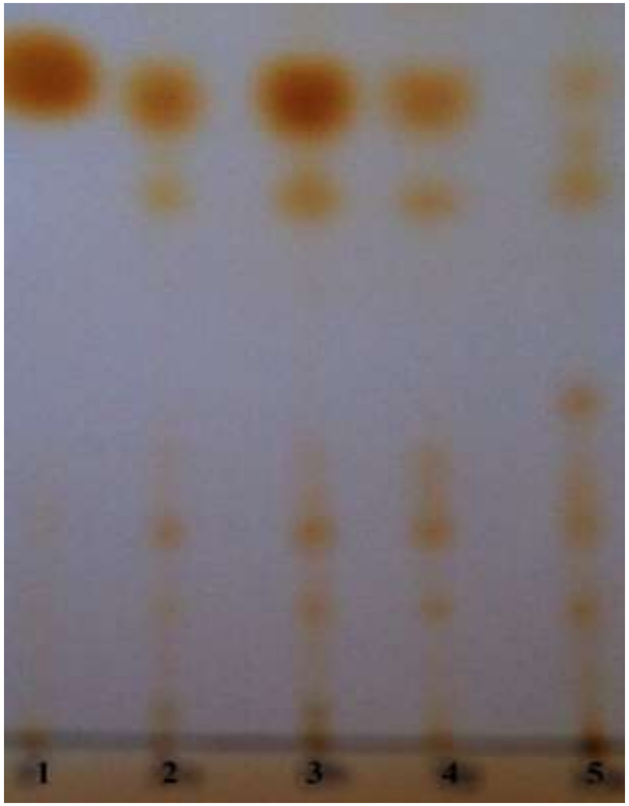


TAG

DAG

FFA

FAME



1-Control (trioleate), 2. Phototrophic lipids, 3 heterotrophic lipids from waste glycerol
4-heterotrophic lipids from glucose

Fatty acid profile of phototrophic and heterotrophic lipids of *Chlorococcum* sp R-AP13 grown in sea water medium

Fatty acid (wt %)	Heterotrophic on Glucose	Heterotrophic on glycerol	Phototrophic
C14:0	0.3	0.7	1.9
C15:0	0.4	-	2.5
C16:0	12.4	16.4	36.1
C16:1	7.9	9.0	4.6
C17:0	0.8	0.6	2.6
C18:0	11.0	8.7	12.8
C18:1	54.0	41.1	11.7
C18:2	9.9	8.1	4.4
C18:3	-	6.3	19.3
C20:0	0.8	5.4	2.9
C22:0	0.7	2.7	1.4
C22:1	-	-	-
C24:0	2.1	1.6	-
SFA	28.5	45	60.2
USFA	71.8	64.5	40

Summary

- Maximal biomass production was recorded with cells grown heterotrophically in sea water medium containing glucose (1.0 g/l), followed by waste glycerol (0.850 g/l). Lipid content was high in heterotrophic growth with waste glycerol (330 mg/l)

- Heterotrophic lipids contained triglycerides as major lipids

- Fatty acid profiling of lipids indicated that major fraction was oleic acid (C18:1), followed by palmitic acid (16:0), stearic acid (18:0), palmitoliec acid (16:1) linoleic acid (18:2), linolenic acid (18:3), and longer chain fatty acids were also produced in very low percentages. Monounsaturated fatty acids such as 18:1 elevated in heterotrophic condition.

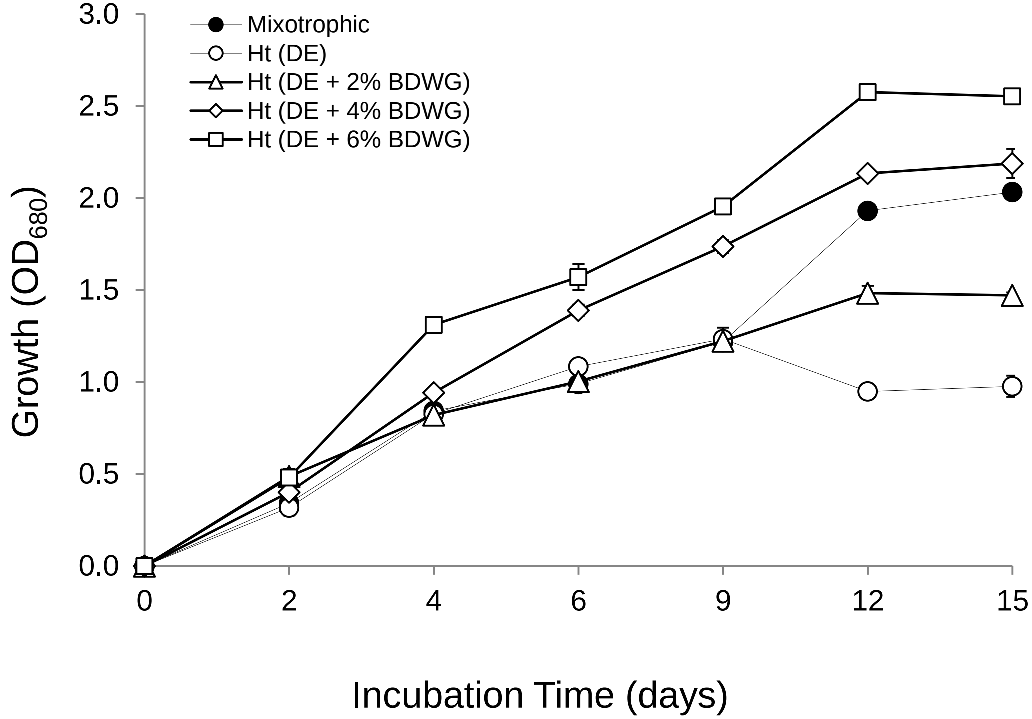
- Heterotrophically grown *Chlorococcum* sp. produced oil rich in fatty acids that could be ideal for biodiesel production and also contained polyunsaturated fatty acids, indicating potential applications in nutraceutical industry.

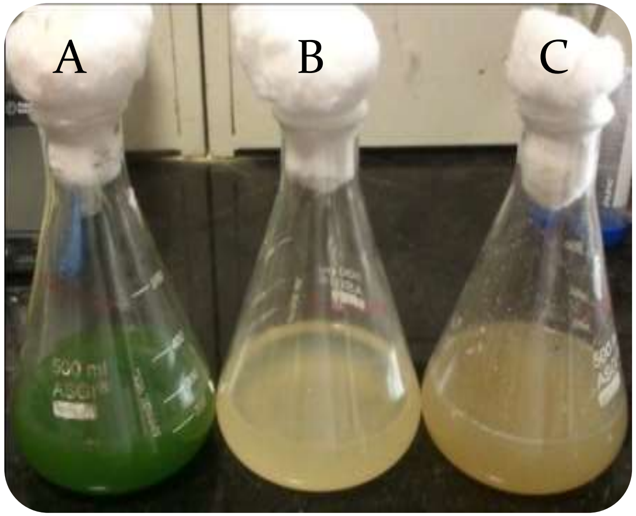
- The fatty acid profile of the alga could be altered by the mode of cultivation and this offered an advantage for enriching the desired type of fatty acids in the biomass for specific application.

- Also since the alga could grew well in 50% seawater, it would be advantageous for mass cultivation since less fresh water would be required.

Bioremediation of Dairy effluent by microalgae *Chlorococcum* sp RAP-13

Growth response of *Chlorococcum sp RAP13* dairy waste water

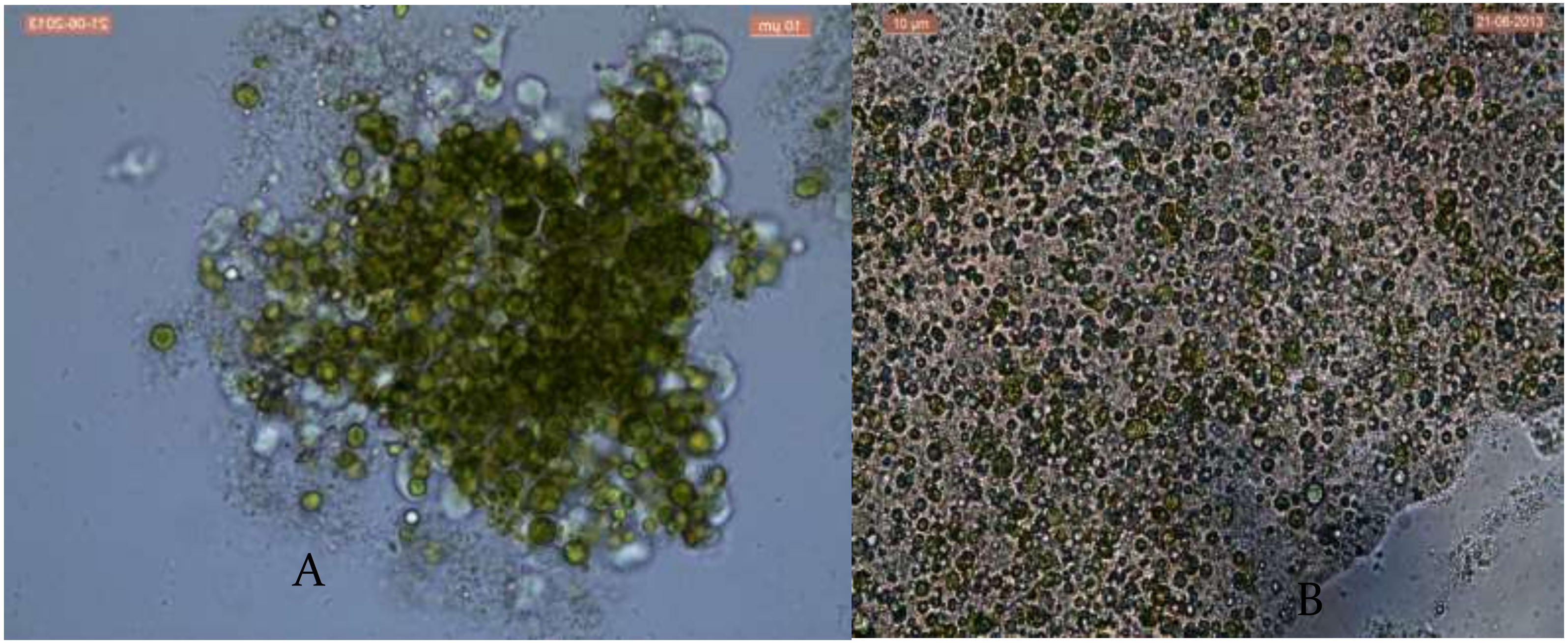




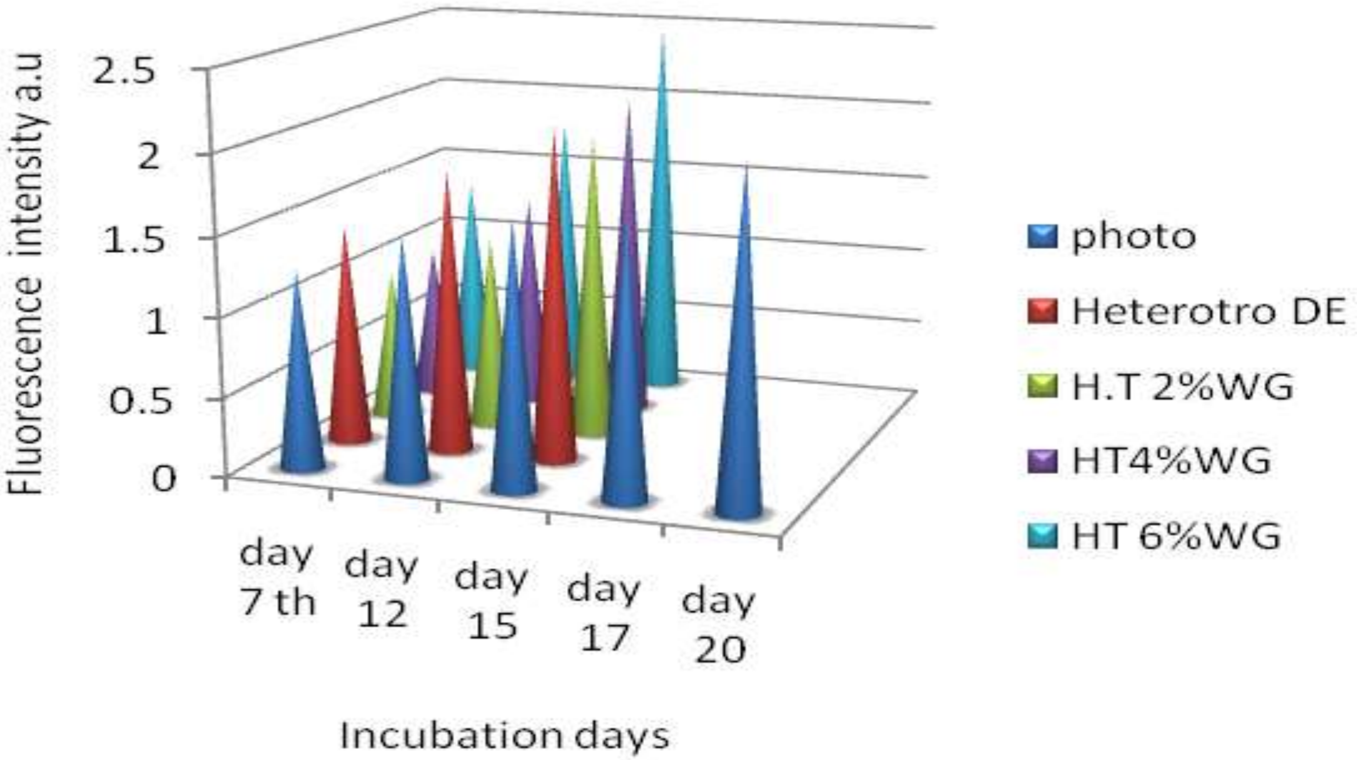
A- Mixotrophic
B-Heterotrophic DWW alone
C-DWW + 6% waste glycerol

Ht(DE): Heterotrophic with Dairy wastewater alone
Ht(DE)+2% Biodiesel derived waste glycerol (BDWG)
Ht(DE):4% Biodiesel derived waste glycerol (BDWG)
Ht(DE):6% Biodiesel derived waste glycerol (BDWG)

Microscopic observation of *Chlorococcum* sp. cells cultivated in mixotrophic (A) and heterotrophic (B) modes in DWW



Accumulation of neutral lipids by the alga under various modes of growth monitored as Nile Red fluorescence



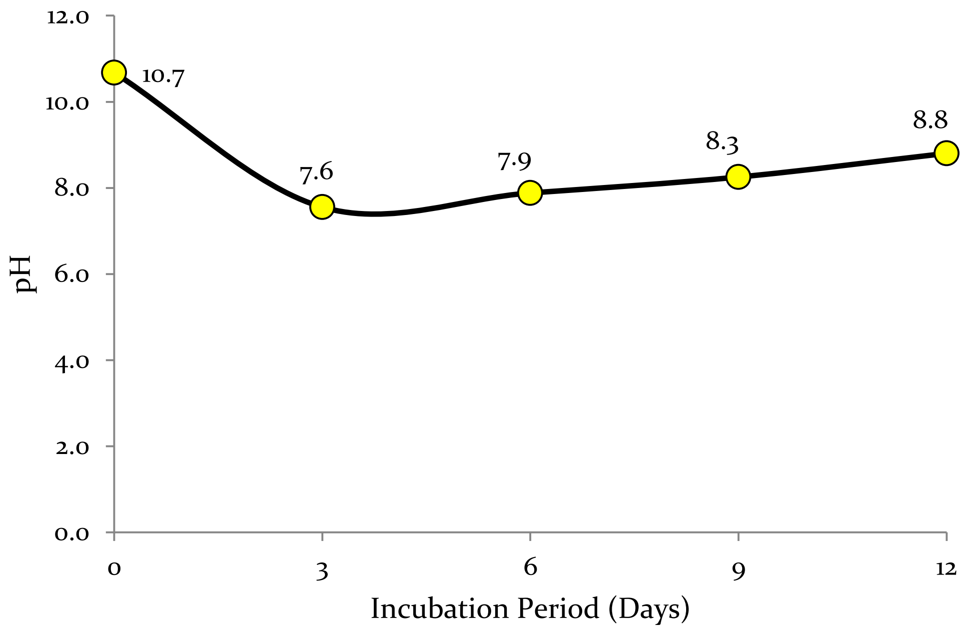
Biomass and lipids production by *Chlorococcum* sp R-AP13 grown in dairy waste water (DWW)

Cultivation condition	Biomass conc. (g/l)	Yield of lipids (g/l)	% DCW of lipids
Mixotrophic DWW	0.870 ± 0.06	0.255 ± 0.02	29
Heterotrophic DWW	0.586 ± 0.04	0.217± 0.05	37
Heterotrophic 2%waste glycerol	1.005 ± 0.01	0.360 ± 0.04	36
Heterotrophic 4%waste glycerol	1.475 ± 0.02	0.579 ± 0.07	39
Heterotrophic 6%waste glycerol	1.935 ± 0.04	0.818 ± 0.04	42

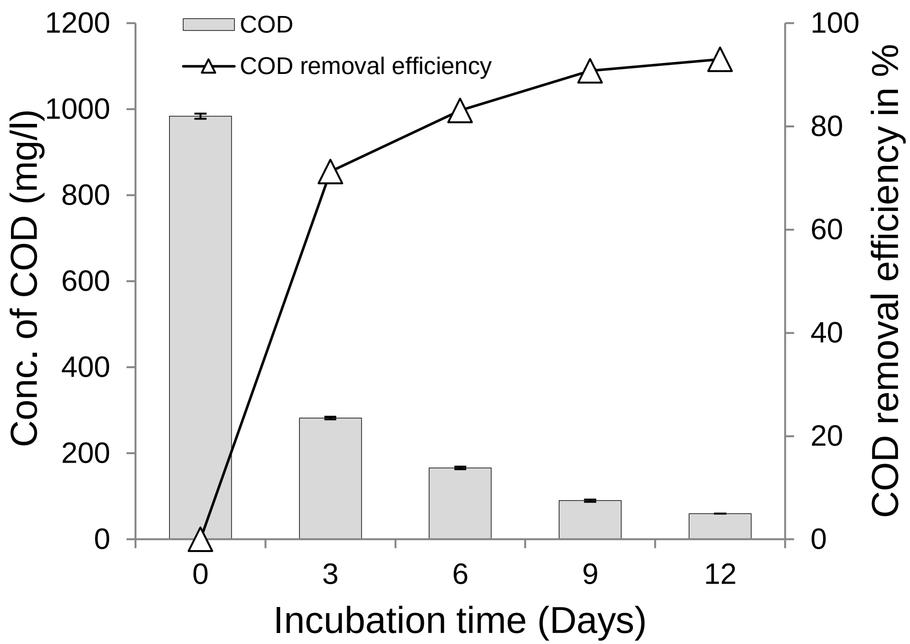
Fatty acids profile of *Chlorococcum sp.* grown in DWW

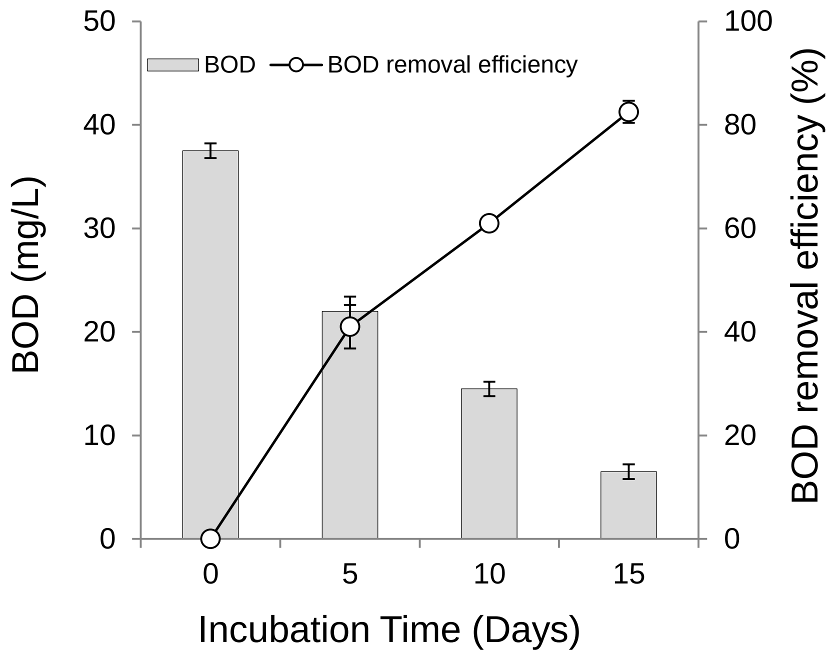
Fatty acids(wt%)	Phototrophic	Heterotrophic	Hetero with 6.0 % BDWG
C12	6.91	9	5.9
C14	2.4	3.96	1.82
C16	53.9	46.2	44.3
C16:1	-	1.59	1.23
C16:2	-	-	1.56
C18	2.35	2.3	16.9
C18:1	27	22.5	20.9
C18:2	3.1	10.8	-
C18:3	-	-	4.7
SFA	65.61	61.51	68.92
USFA	30	33.3	28.39

Changes in pH of the DE during mixotrophic cultivation of *Chlorococcum* sp R-AP 13



Removal of the organic pollution load of DWW by mixotrophic cultivation of *Chlorococcum* sp R-AP13





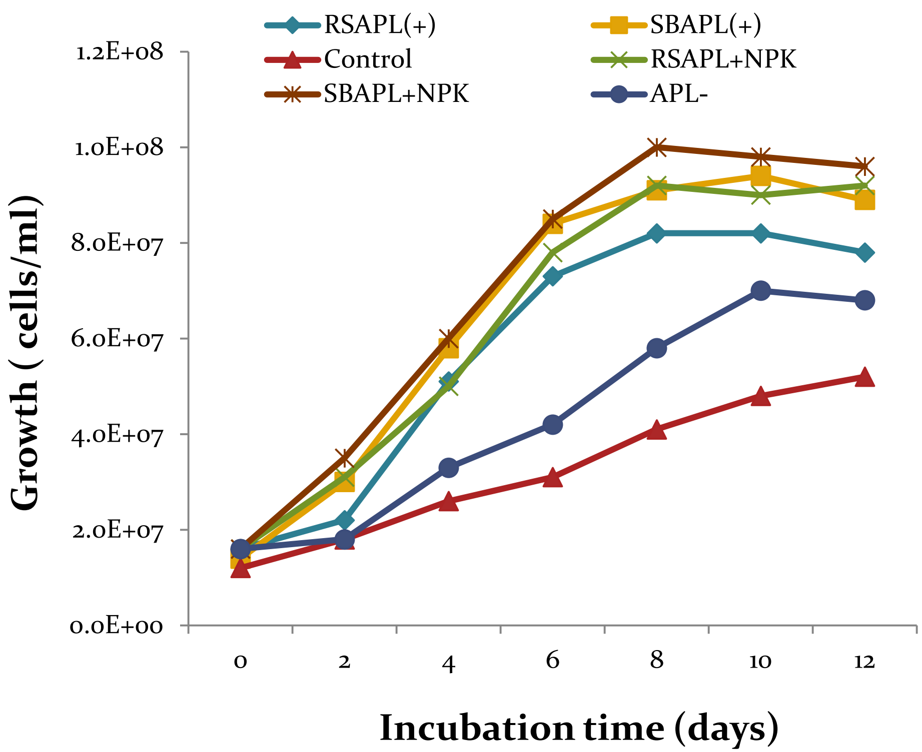
***Ummalyma SB**, Sukumaran RK, 2014 **Bioresour. Technol**. 165: 295-301*

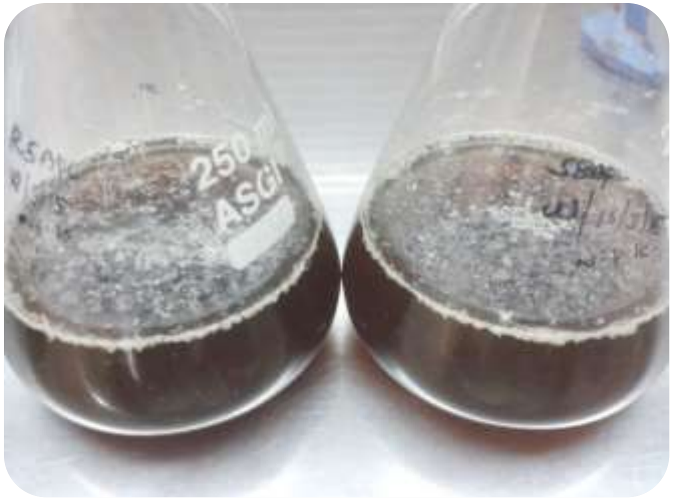
Summary

- Mixotrophically grown cells produced more biomass and lipid (0.87 and 0.25 g/l respectively), when DWW alone was used as medium. In comparison, heterotrophic conditions resulted in 0.586 and 0.217g/l of biomass and lipids yield.
- Biomass and lipids production was enhanced in heterotrophic condition, when the waste glycerol percentage increased. Maximum biomass and lipid production obtained in DWW with 6% waste glycerol was 1.94 and 0.82 g/l, respectively.
- Major fatty acids present in the oil were palmitic acid, oleic acid and linolenic acid: saturated fatty acids production was enhanced in waste water medium.
- Algal growth in DWW reduced the organic pollution load. BOD and COD removal were 82 and 93 %, respectively.
- Dual use of microalgae cultivation for wastewater treatment and production of value- added compounds / biofuel could be an attractive option, in terms of reducing the energy cost, and the nutrient and freshwater resource costs.
- The high biomass productivity of *Chlorococcum* sp R-AP13 on dairy effluent suggests that this cultivation method offer real potential as a viable means for algal biomass generation along with phycoremediation and value- addition of this waste stream..

Mixotrophic cultivation of *Chlorococcum* sp RAP13 in effluent from biomass processing industry : Acid Pretreatment Liquor (APL)

Growth of *Chlorococcum sp-RAP-13 in APL medium*

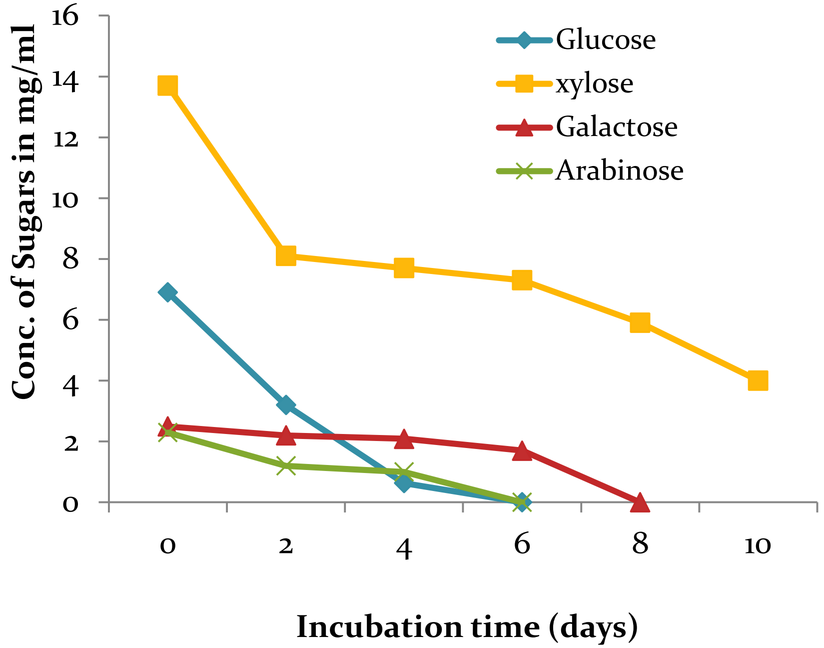




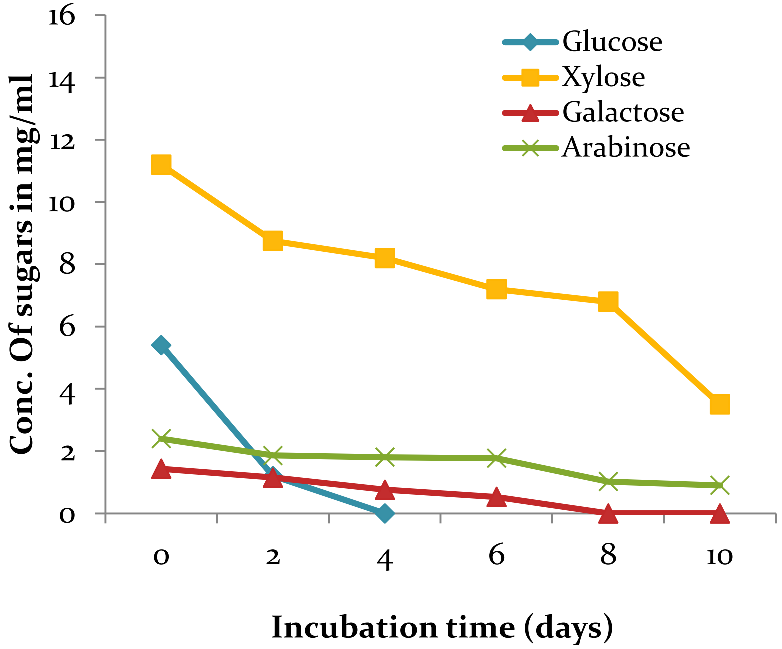
RSAPL(+) Rice straw with medium supplementation
AB APL(+) Sorghum with medium supplementation
RSAPL+NPK: Rice straw with supplementation of N.P.K alone
SBAPL+NPK: Sorghum with supplementation of N.P.K
APL-:Acid pretreated liquor without any supplementation
Control: Phototrophic MA medium

Sorghum biomass APL: Utilization of C6 and C5 sugars

Sorghum biomass APL

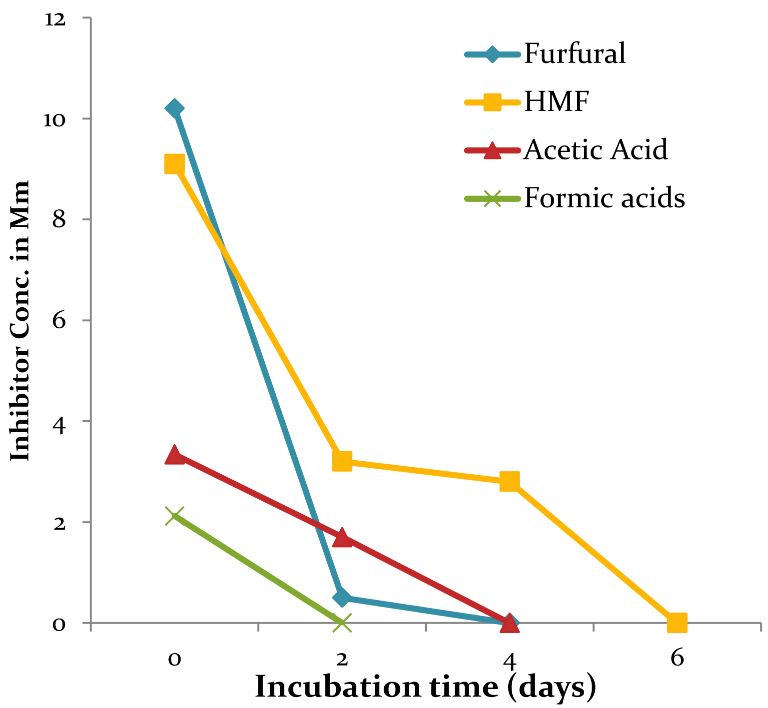


Rice straw APL

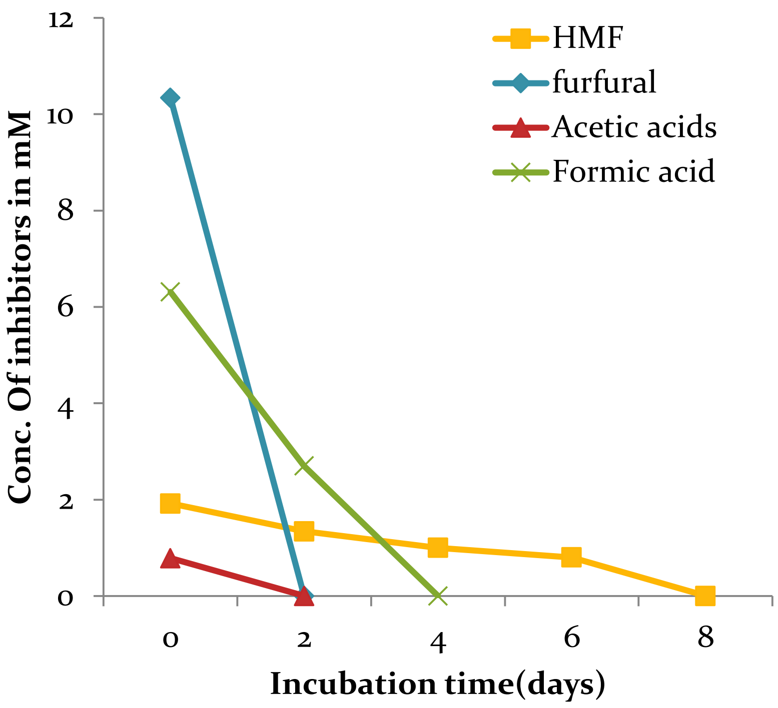


Removal of Inhibitors present in the APL

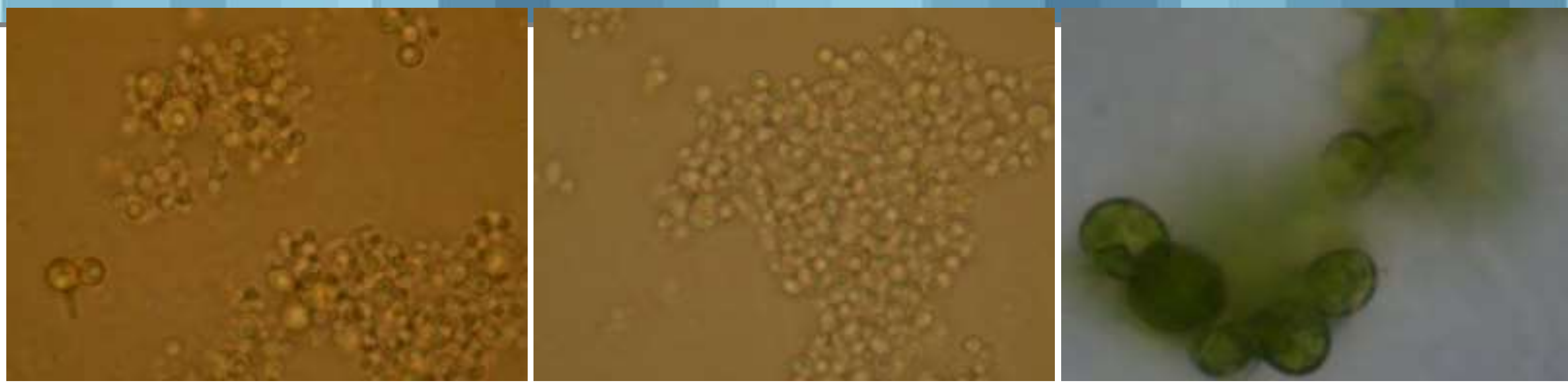
SB-APL



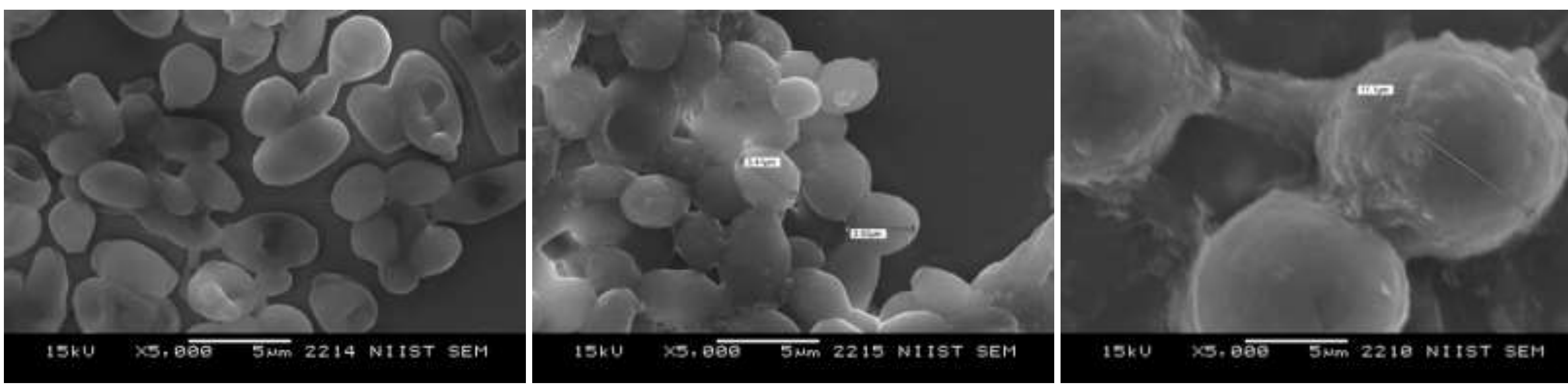
RS-APL



Microscopic and SEM analysis of algal cells grown in APL medium



RS-APL SB-APL Control



SEM and microscopic images showed that size of the algal cells was reduced when cultivated in APL medium co mpared to control medium and pigmentation pattern of the cells is altered in APL medium

Biomass and lipids production of *Chlorococcum* sp. R-AP13 grown on APL medium

Conditions	Biomass conc. (mgL^{-1})	Yield of lipids (mgL^{-1})	Cellular Lipid Accumulation (% DCW)
APL-	1088 ± 33.9	298 ± 0.9	27.4 ± 0.7
SBAPL+	1752 ± 36.1	485 ±11	28 ± 0.8
RSAPL+	1630 ± 20	443 ±12	27 ± 0.4
SBAPL(NPK)	1832 ± 24.7	568 ±15.2	30.8
RSAPL(NPK)	1795 ± 21.2	538 ± 20.5	29.5 ± 2.12
Control	816 ± 34	190 ± 14	23 ± 0.7

Fatty acids profile of *Chloro coccum* sp.R-AP13 grown on APL medium

Fatty acid Type (wt%)	MA medium	RS-APL	SB-APL
C12	1.6	0.5	0.4
C14	1.5	0.4	0.2
C15	6.8	-	-
C16	52	31.4	30.1
C16:1	-	1.83	2.7
C17	-	1.9	-
C18:0	-	15.84	15.7
C18:1	20	41.5	44.7
C18:2	7	2.7	2.6
C18:3	7.6	4.8	2.2
C20:1	0.4		
C20:2	0.4	-	-
C22:0	0.5	-	-
C24	-		
SFA	**62.4**	**47.8**	**46.7**
MUFA	**20.4**	**43.2**	**47.4**

Biodiesel properties of algal oils assessed by *in-silico* analyses of the FAME profile of lipids by Biodiesel Analyzer®

	CN	SV (mg KOHg-1)	IV(gI² 1 oog-1fat)	DU (wt %)	LCSF(wt%)	CFPP (° C)	CP (°C)	APE	BAPE	υ mm²s1 -	P (gm- ³)	HHV (MJ K g-1)
EN14214	≥51	-	≤120	-	-	≤5/-20	-	-	-	3.5-5.0	0.86	NA
ASTM-D6 751-02	≥47	-	NA	-	-	NA	-	-	-	1.9-6.0	0.9	NA
IS-15607	≥51	-	NA	-	-	6/18	-	-	-	2.5-6	0.86	NA
RS-APL	60.47	202.49	56.83	58.01	11.02	18.14	11.31	56	12.11	1.36	0.85	38.87
SB-APL	61.06	203.25	53.77	57.16	10.89	17.7	5.46	54.46	7.08	1.38	0.86	39.08
Control	60.9	207.88	51.79	49.6	5.95	2.22	22.36	49.2	22.2	1.27	0.85	38.21

DU: Degree of unsaturation, CN: Cetane Number , SV: Saponification value, IV: Iodine value, LCSF: Long-chain saturated factor , CFPP: cold filter plugging point, CP: cloud point , HHV: Higher heating value υ:kinematic viscosity, ρ :Density , APE: Allylic position equivalents, BAPE: Bisallylic position equivalent

Summary

- The alga utilized C5 (xylose, arabinose, galactose) and C6 (glucose) sugars present in APL as carbon sources, and could survive in the presence of inhibitors like furans and sugar breakdown products.

- *Chlorococcum sp.* R-AP13 removed almost completely the sugars, furfural and HMF in the APL during its growth and produced 1.8 g/L of biomass. The lipids yield was 0.568 g/L when supplemented with agricultural grade NPK (18:18:18) mixture.

- FAME profile of fatty acids indicated that the oil was rich in oleic acid (18.1) with~ 45 % of the oil being this fatty acid, followed by palmitic acid (16.1) and stearic acid (18.0) which formed respectively ~30 and 15 % and of the oil.

- Fatty Acid Methyl Ester (FAME) was synthesized from the algal oil using acid catalysis and was evaluated for its physicochemical properties and suitability as biodiesel using BiodieselAnalyzer® software which indicated its compliance to EN 14214 and ASTM D6751.

- Ability of the alga to grow in biomass acid pretreatment liquor is particularly interesting for value addition of this byproduct which is otherwise difficult to process. Cultivation of microalgae that can grow and produce oil in this medium would be immensely beneficial to the biorefineries since it will address both waste water treatment and value addition of the resource.

Summary

- *Chlorococcum sp* R-AP13 cultivated in APL- a major effluent from lignocellulosic biomass processing plant, dairy effluent produced high amount of biomass(~2g/l) and accumulated significant amount of lipids (42%), particularly under mixotrophic/heterotrophic condition. Cutivation of freshwater algae in seawater based medium also enhancing the biomass and lipids. The cells displayed different growth characteristics, lipid content and fatty acid profiles, based on differences in the growth conditions, substrates and supplementation of nutrients in various effluents. The lipid profile of the oil produced from algal biomass grown in waste waters/seawater indicated interesting features like high triglycerides content and fatty acid profile with high proportion of oleic acids. These features project the alga as a potent source of oil which can be used as feedstock for biodiesel and also as a food or feed supplement.
- The results showed that fatty acids compositions of algal oil may be modulated by adjustment with growth mode and conditions. While the use of sea water reduces the requirement of mineral supplementation, dairy wastewater and APL reduces the carbon cost and saves fresh water, the ability to form oil from waste glycerol indicates the potential to recycle this waste and the alga can be considered as efficient in growth and lipid production using no-potable waters and holds potential for future exploitation as an economic means of oil production for biodiesel and nutraceutical industries.

Conclusions

- Mixotrophic microalgae cultivation established **2-5 t better** biomass yield depending on substrate and growth conditions
- **Effect of light** on mixotrophic growth governed by **substrate types**
- **Glucose** found to be a **best substrate** for mixotrophic cultivation
- Glycerol did not exhibit biomass yield improvement as compare to autotrophic yield however showed **effect on improved lipid** yield
- Mixotrophic cultivation mode exhibited **better growth conditions** in terms of **DIC** and **DO** concentrations
- To obtained maximum benefits from glycerol supplementation **combination of growth modes** looking promising

Thank You

Thank you!