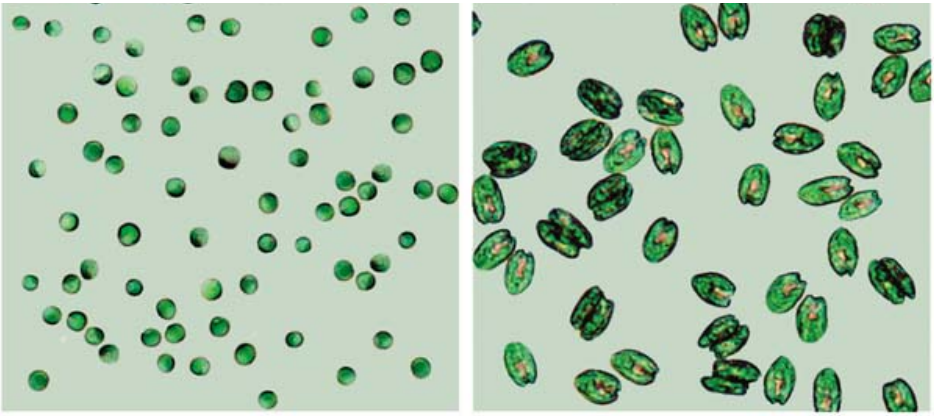


# Do microalgae biorefineries really exist? Concept, applications and future directions

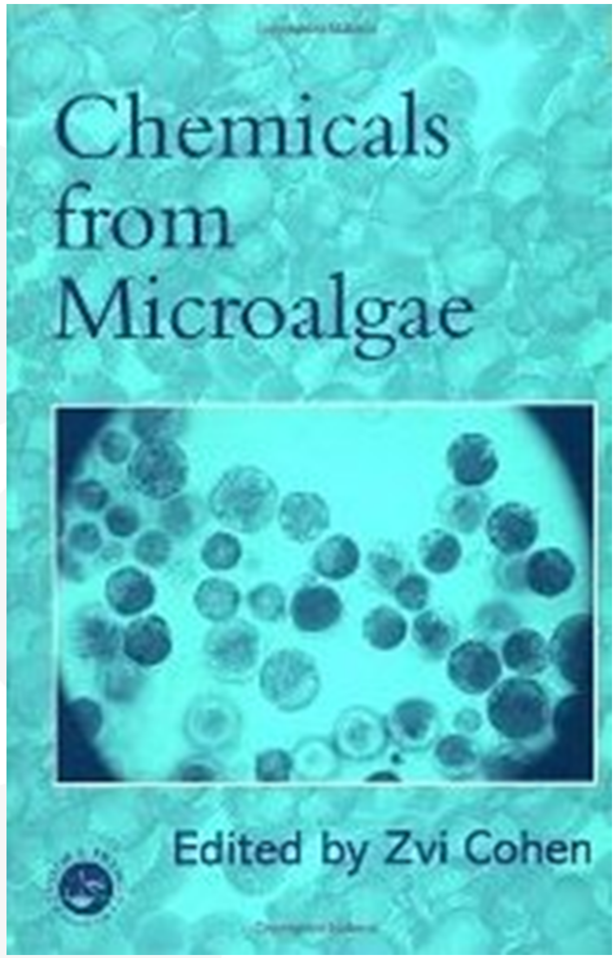
**Alberto Reis**

Laboratório Nacional de Energia e Geologia, I. P.

GOVERNO DE PORTUGAL | MINISTÉRIO DA ECONOMIA E DO EMPREGO

# the PASS

# the chemical composition of microalgae is well known since a long time ago

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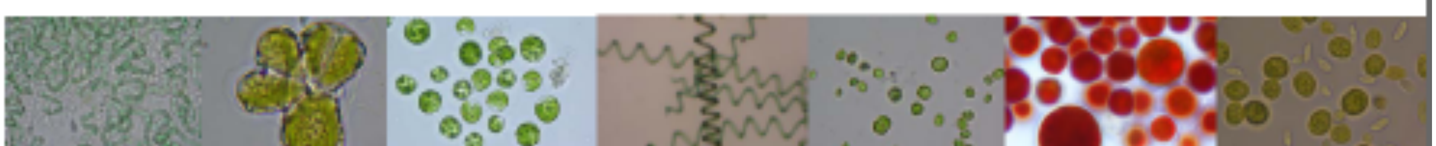
# Main research fields of Microalgal Biotechnology

| 1940ies | Research topic | Algae | Reference |
|---|---|---|---|
| 1970ies | Fertilizers ($N_2$ fixing) | *Anabena, Nostoc* | Venkataraman 1972<br>Wantanabe et al. 1977 |
| | Waste Water Treatment | *Scenedesmus, Chlorella* | Abeviolich & Azov 1976<br>Oswald et al. 1978 |
| | Protein Source | *Spirulina, Chlorella* | Soeder 1978; Becker 1984<br>Richmond 1986 |
| 1980ies | β-carotene production | *Dunaliella* | Ben Amotz et al. 1982<br>Borowitzka & Borowitzka 1988 |
| | Investigations on secondary metabolites | *Cyanobacteria* | Metting & Pyne 1986 |
| 1990ies | Astaxanthin production | *Haematococcus* | Boussiba et al. 1991 |
| | Analysis of algal toxins | *Cyanobacteria, Dinoflagellates* | Falconer 1993 |
| | Aquaculture | *Nannochloropsis etc.* | Borowitzka 1997 |
| | PUFA production | *Crypthecodinium* | Kyle et al. 1998 |
| 2000s | Bioenergy | *Botryococcus* | Banerjee et al. 2002 |
| present | | | |



(Gouveia, 2011)

III Congreso Latinoamericano Biorrefinerías
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19 al 21 de noviembre de 2012, Pucón, Chile

# 1 product →1 process

# **NO** integration

# Nov, 20th, 2012 query

**16,687 microalga**

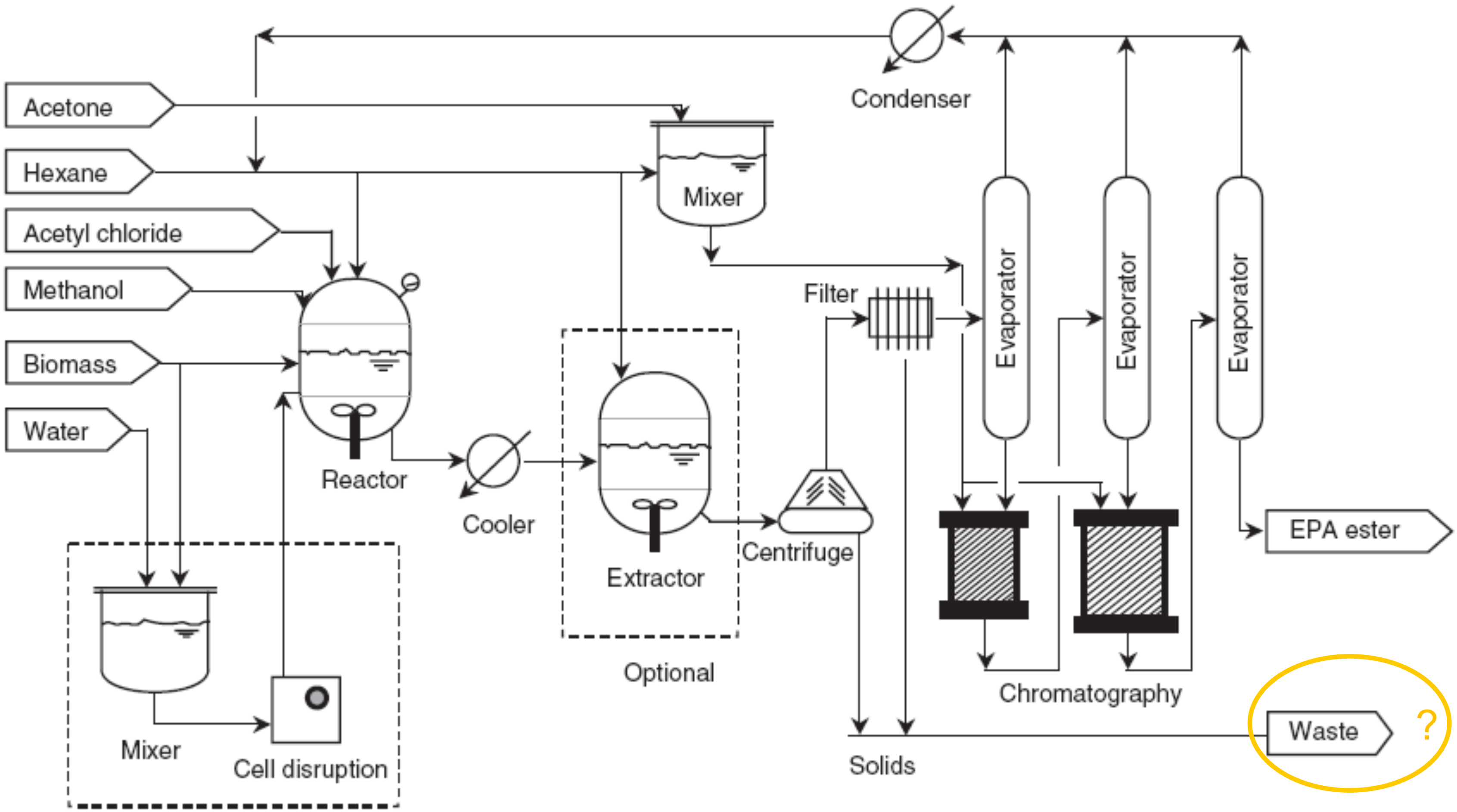
**2,577 biorefinery**

**269 microalga + biorefinery**

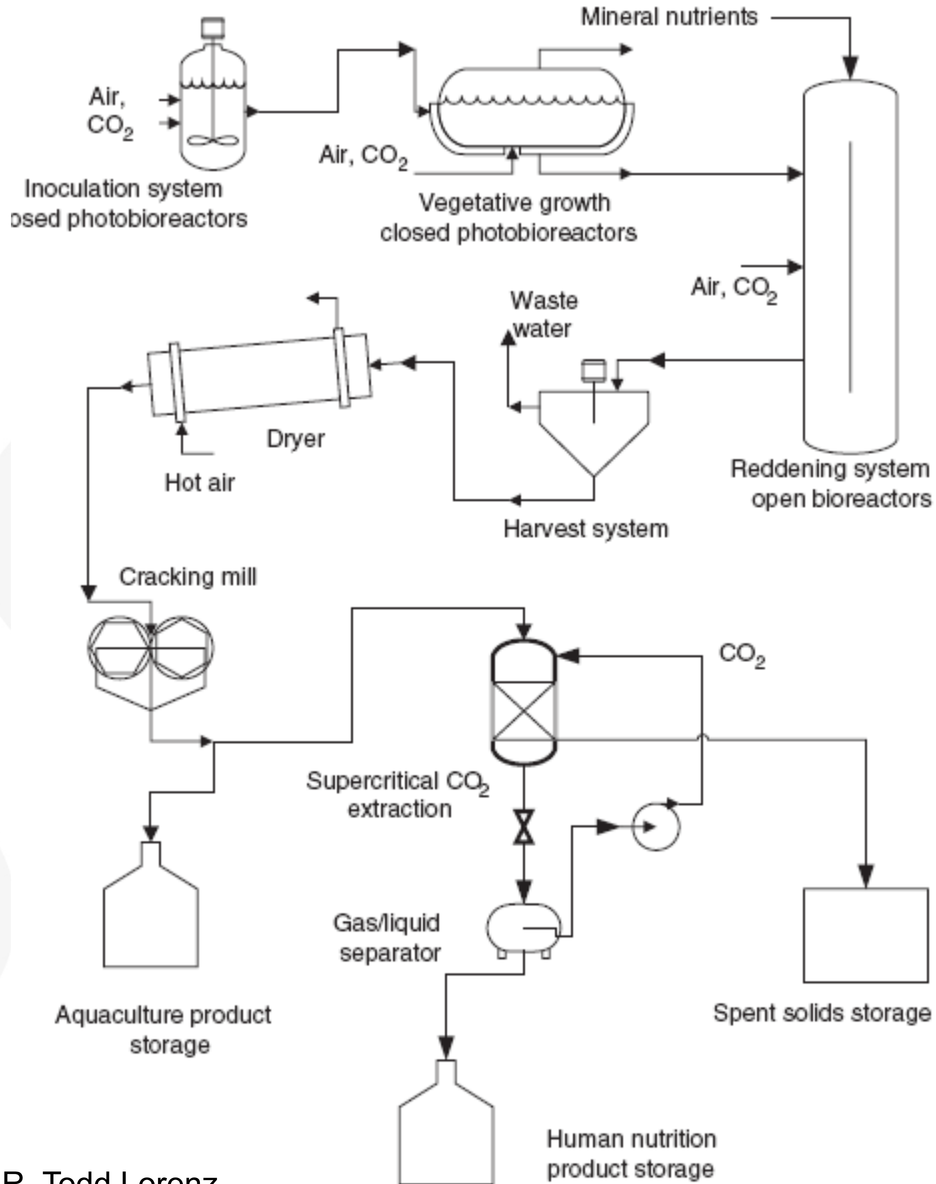
disappointing results !!!

ScienceDirect

# EPA Production from *Phaeodactylum tricornutum*




(Belarbi *et al*.,
Enzyme Microb Technol. 2000;
26(7):516-529.)

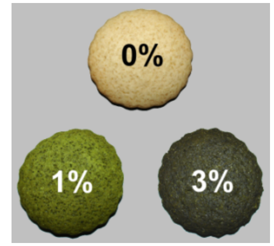



# Astaxanthin Production from *Haematococcus*

(G.R. Cysewski and R. Todd Lorenz, Trends Biotechnology) previous own lab experience

# PIGMENTS, ANTIOXIDANTS AND POLYUNSATURATED FATTY ACIDS FROM MICROALGAE IN FOOD PRODUCTS

FCT - PTDC/AGR-ALI/65926/2006


cookies



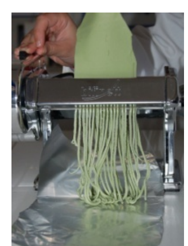

mayonnaises

Gouveia *et al*

puddings


caviar






pasta, spaghetti

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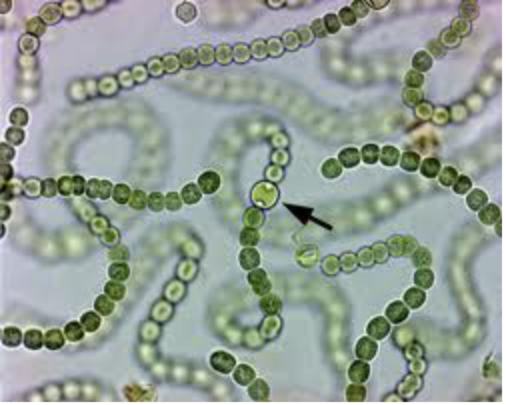

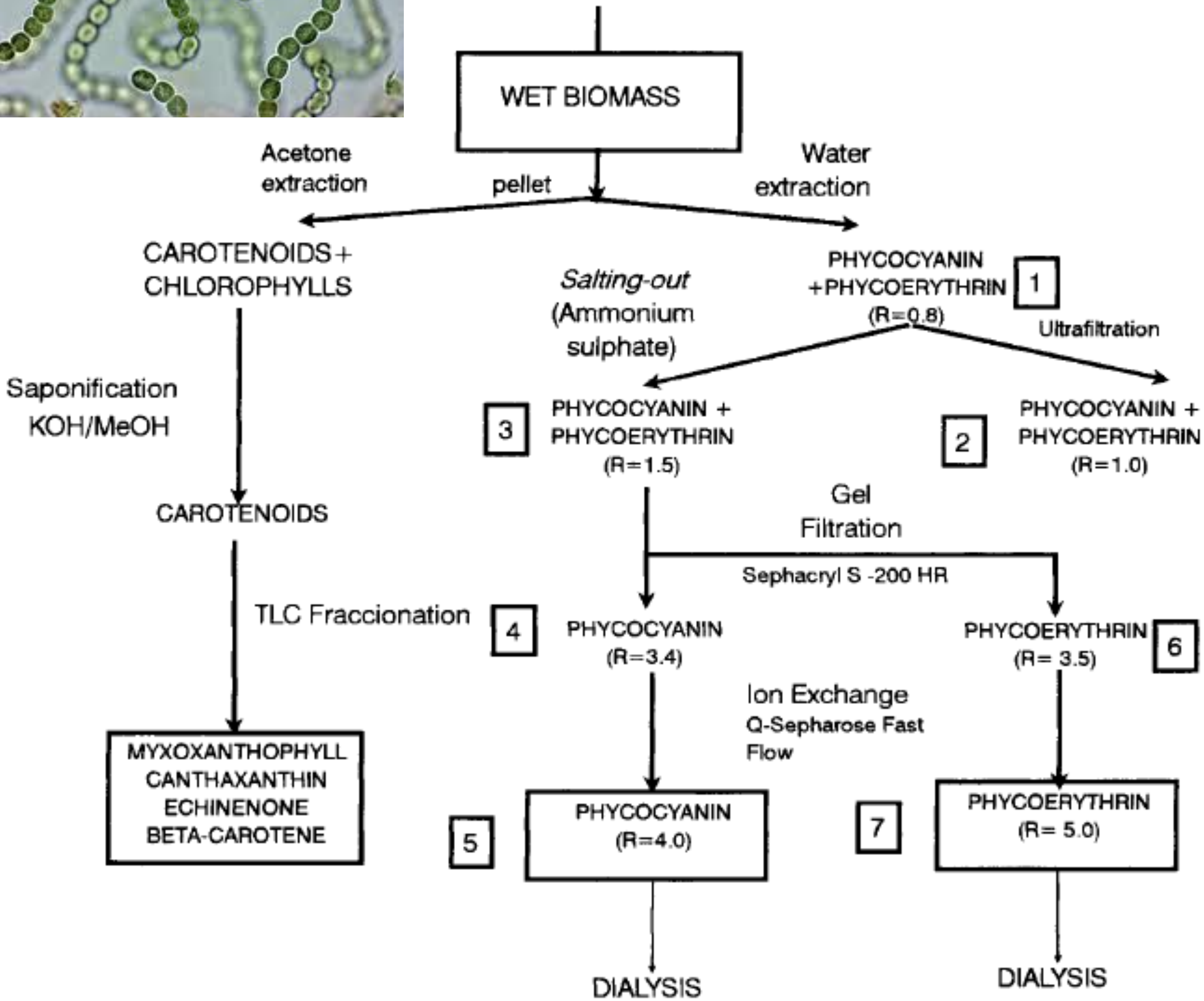


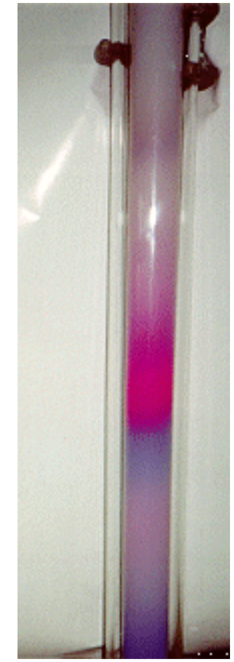

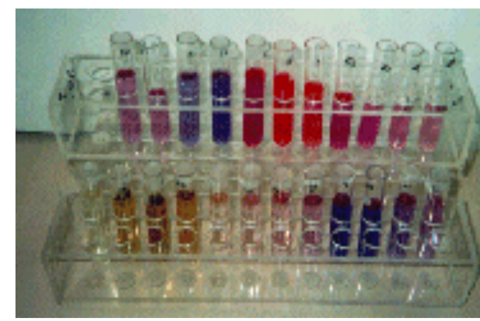

Reis *et al*

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Bioresource Technology 66 (1998) 181–187

Printed in Great Britain
0960-8524/98 $—see front matter

PII:S0960-8524(98)00064-9

# PRODUCTION, EXTRACTION AND PURIFICATION OF PHYCOBILIPROTEINS FROM *NOSTOC* SP.

A. Reis,[a]* A. Mendes,[b] H. Lobo-Fernandes,[a] J. A. Empis[b] & J. Maggiolly Novais[b]

[a]*Departamento Energias Renováveis, I.N.E.T.I./ITE, Estrada Paço do Lumiar, 1699 Lisboa Codex, Portugal*
[b]*Laboratório Engenharia Bioquímica, I.S.T., Av. Rovisco Pais, 1096 Lisboa Codex, Portugal*

(Received 9 March 1998; revised version received 8 April 1998; accepted 14 April 1998)

**Abstract**

*One strain of nitrogen-fixing cyanobacterium (*Nostoc *sp. PCC 9202) was grown indoors in 1 litre glass air-lift reactors as well as 17 litre polyethylene bags. Temperature dependence of growth–kinetic parameters, as well as of biomass and phycobiliprotein productivities, were determined. Harvesting and phycobiliprotein extraction methodologies are presented. The purification of crude extracts was performed by means of ultrafiltration or* $(NH_4)_2SO_4$ *precipitation followed by gel filtration and ion-exchange chromatography. Final phycocyanin and phycoerythrin preparations were characterized by purity ratios above 4 and 5, respectively. Phycobiliprotein production cost determination for each purity grade and cumulative phycobiliprotein weight losses along purification steps are shown. Production cost for high purity phycoerythrin (30 US$ per g) seems to be far below the market price. © 1998 Elsevier Science Ltd. All rights reserved.*

*Key words:* Cyanobacteria, phycobiliprotein, phycocyanin, phycoerythrin, allophycocyanin, *Nostoc* sp.

phycoerythrins (red). Whereas phycocyanin and allophycocyanin are always present in Cyanophyceae and Rhodophyceae, phycoerythrin may be absent in the former (Moreno *et al*., 1995). Phycobiliproteins are organized in supramolecular aggregates — phycobilissomes — in order to maximize energy transfer to the chlorophyll–protein complexes located at the thylakoid membrane. No such aggregates have ever been detected in Cryptophyceae (Rowan, 1989).

Due to their limited distribution, and to the difficulties in their purification, these pigments are rather expensive, and obtaining them as pure compounds is a potentialy attractive endeavour. Phycobiliproteins are used as a natural protein dye in the food industry (C-phycocyanin) and in the cosmetic industry (C-phycocyanin and R-phycoerythrin), as tracers in fluorescence immunoassays, and in microscopy for diagnostic and biomedical research, due to their high absorbance and reddish fluorescence (CQVB, 1988; Hill, 1988; Tandeau de Marsac, 1993). Glazer (1994) published an extensive review about the uses of phycobilipro-

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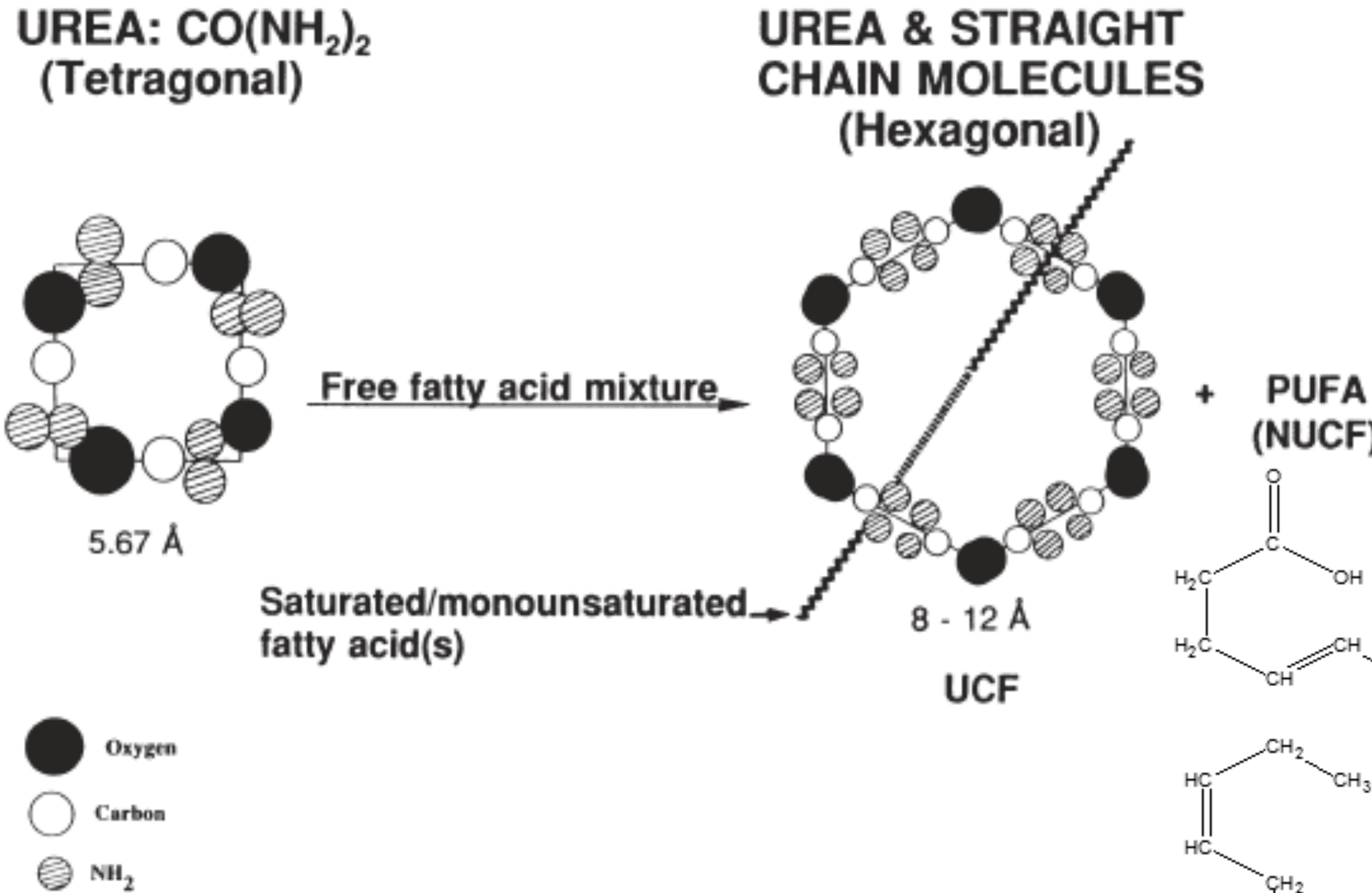
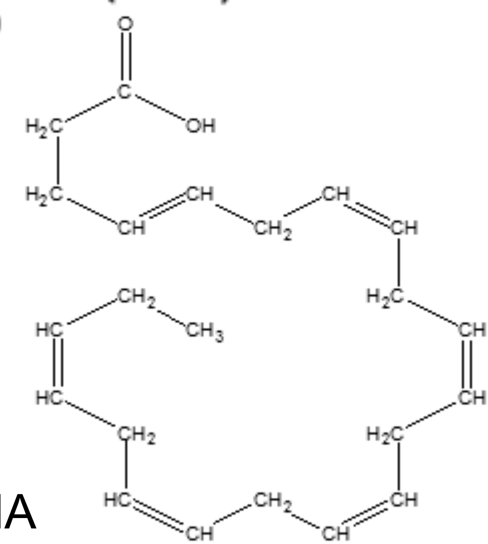
19 al 21 de noviembre de 2012, Pucón, Chile

STEARIC ACID


Reis *et al*

DHA

# saponification, winterization and urea complexation



ISSN 1330-9862 *original scientific paper*
(FTB-1609)

## DHA Concentration and Purification from the Marine Heterotrophic Microalga *Crypthecodinium cohnii* CCMP 316 by Winterization and Urea Complexation

*Ana Mendes, Teresa Lopes da Silva* and Alberto Reis*

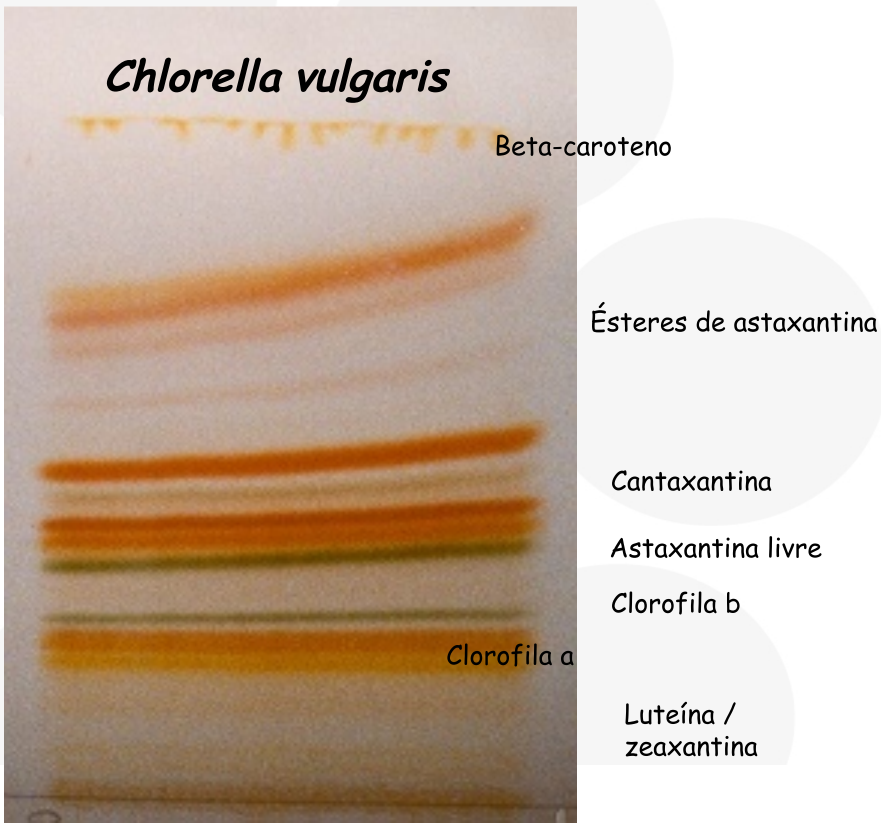
Instituto Nacional de Engenharia, Tecnologia e Inovação, Departamento de Biotecnologia, Unidade de Bioengenharia e Bioprocessos, Estrada do Paço do Lumiar 22, P-1649-038 Lisboa, Portugal

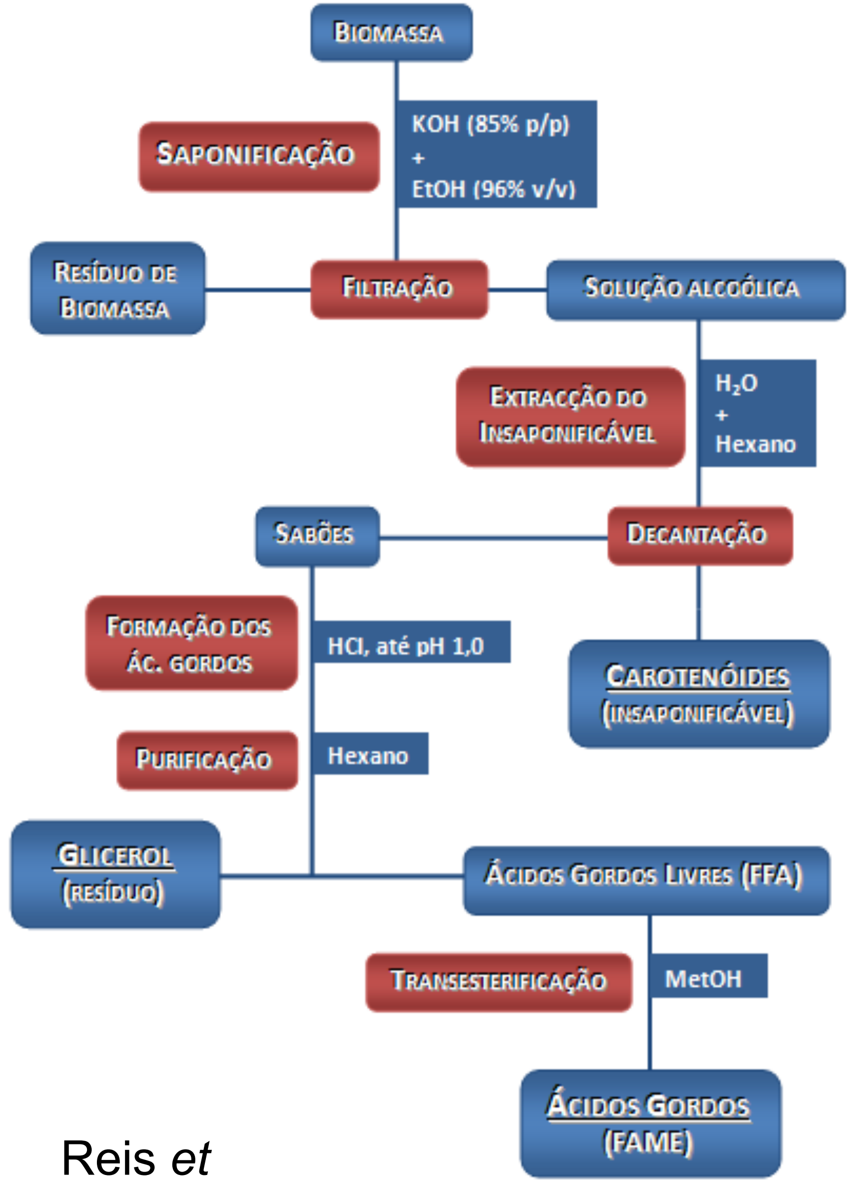
Received: January 9, 2006
Accepted: April 6, 2006

**Summary**

A simple and inexpensive procedure involving saponification and methylation in wet biomass, winterization and urea complexation in a sequential way has been developed in order to concentrate docosahexaenoic acid (DHA) from *Crypthecodinium cohnii* CCMP 316 biomass. Different urea/fatty acid ratios and crystallization temperatures were tested in the urea complexation method. ANOVA test revealed that, in the studied range, the tempera-

# BIOREFINARIA de MICROALGAS




Reis *et al*

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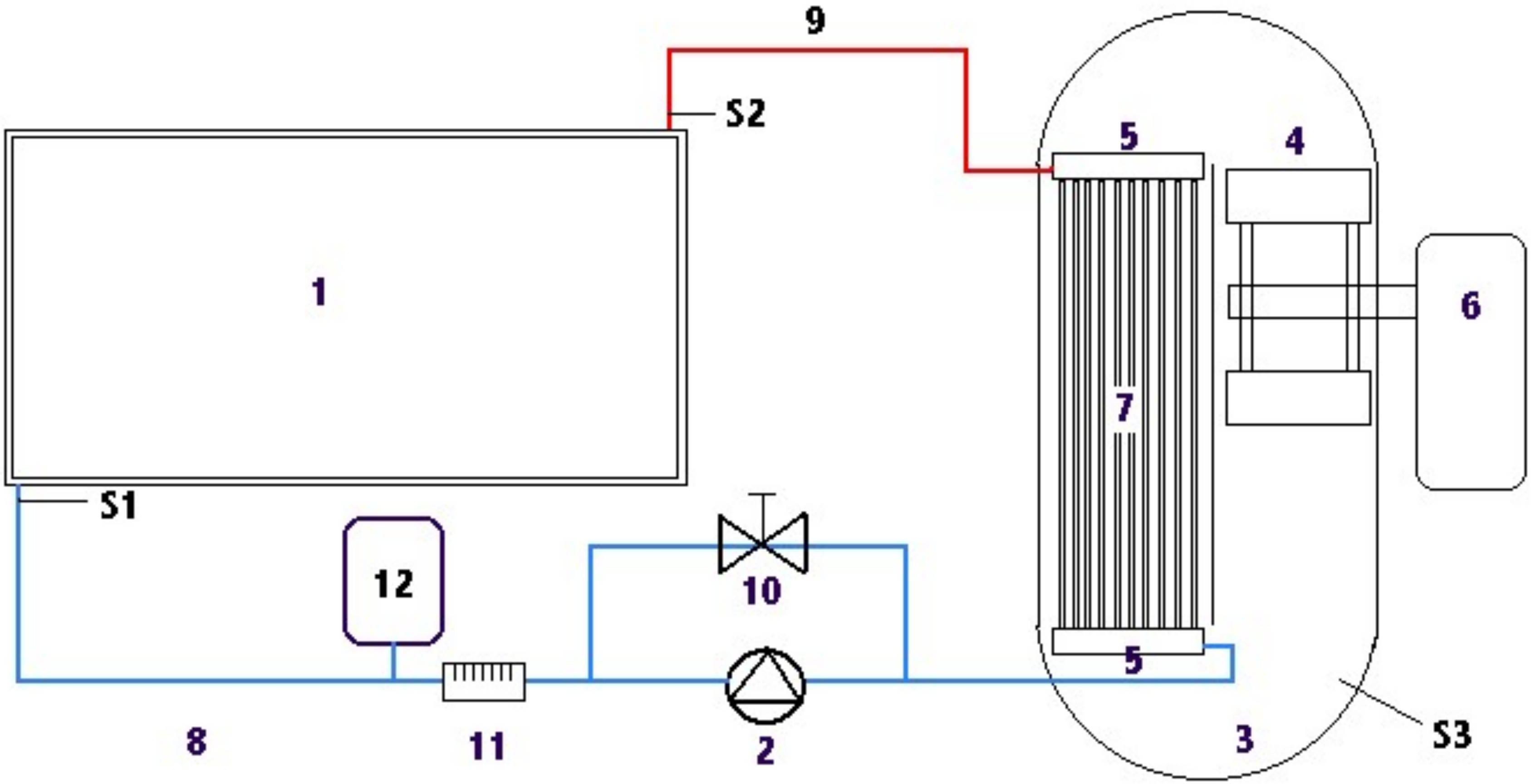
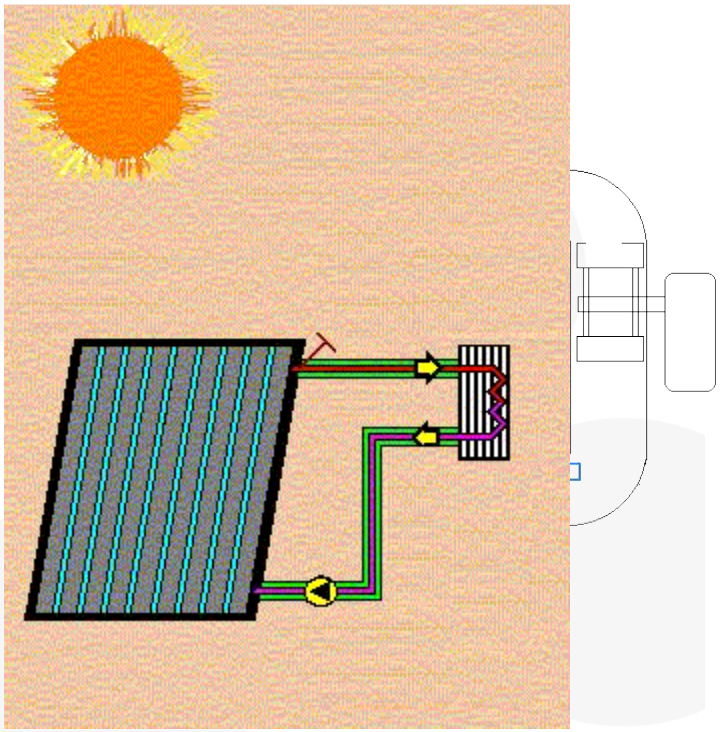
19 al 21 de noviembre de 2012, Pucón, Chile

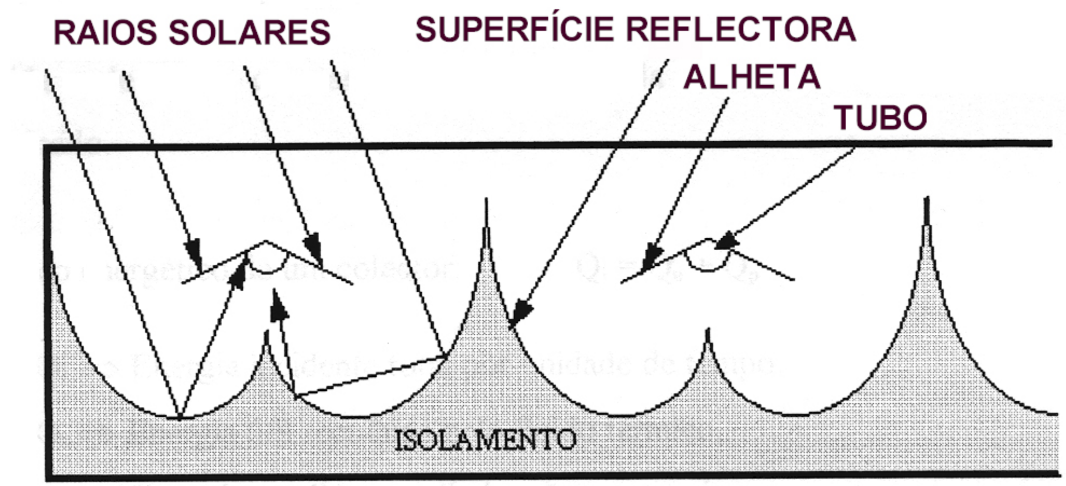
# Integrating raceways and solar collectors

improving energy balances and
productivities → energy efficiency



# INTEGRATING RACEWAY AND SOLAR COLLECTOR






improving energy balances and productivities → energy efficiency

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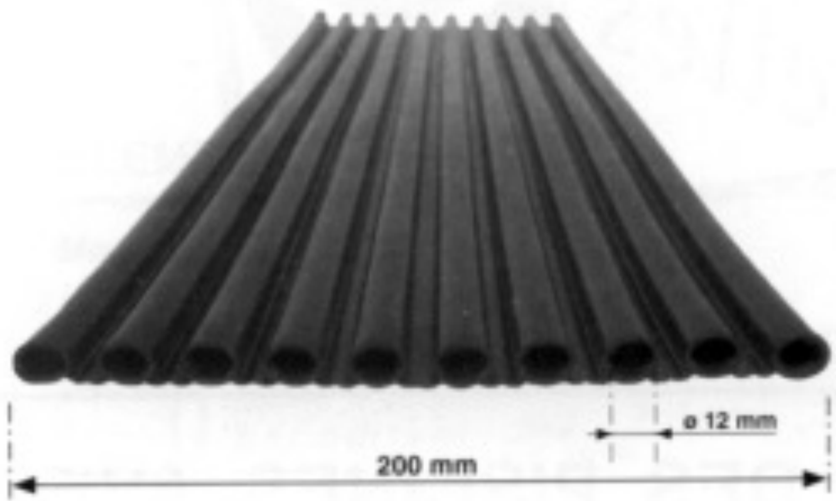
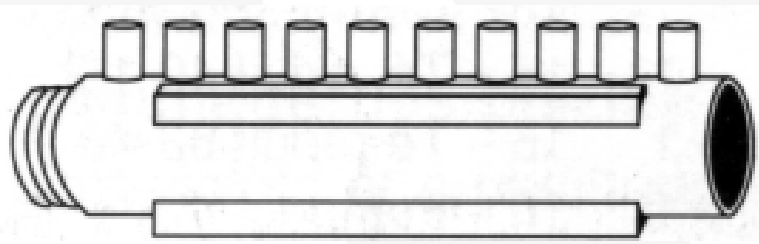
# Integrating raceway and solar collector

heat exchanger



improving energy balances and productivities → energy efficiency

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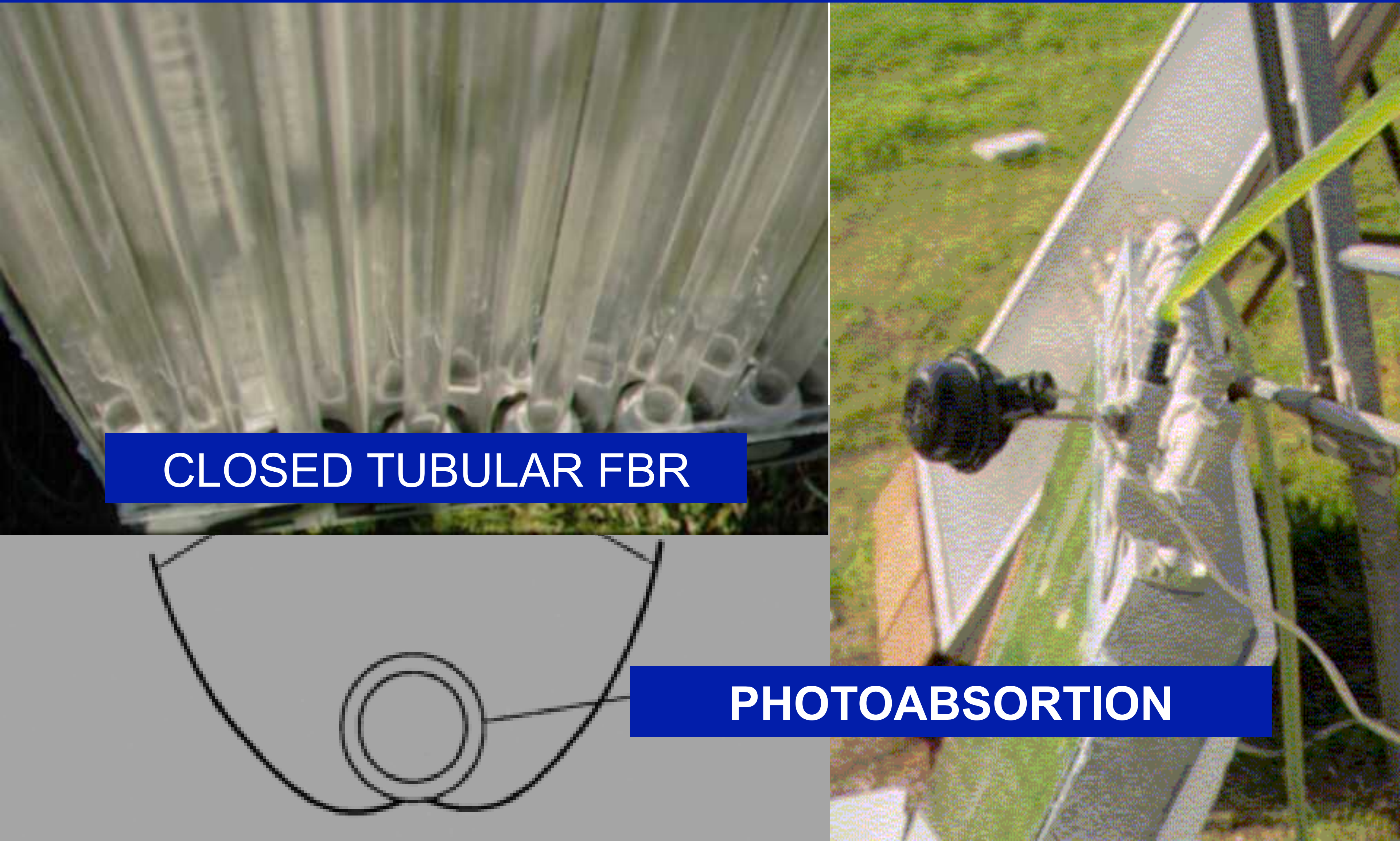
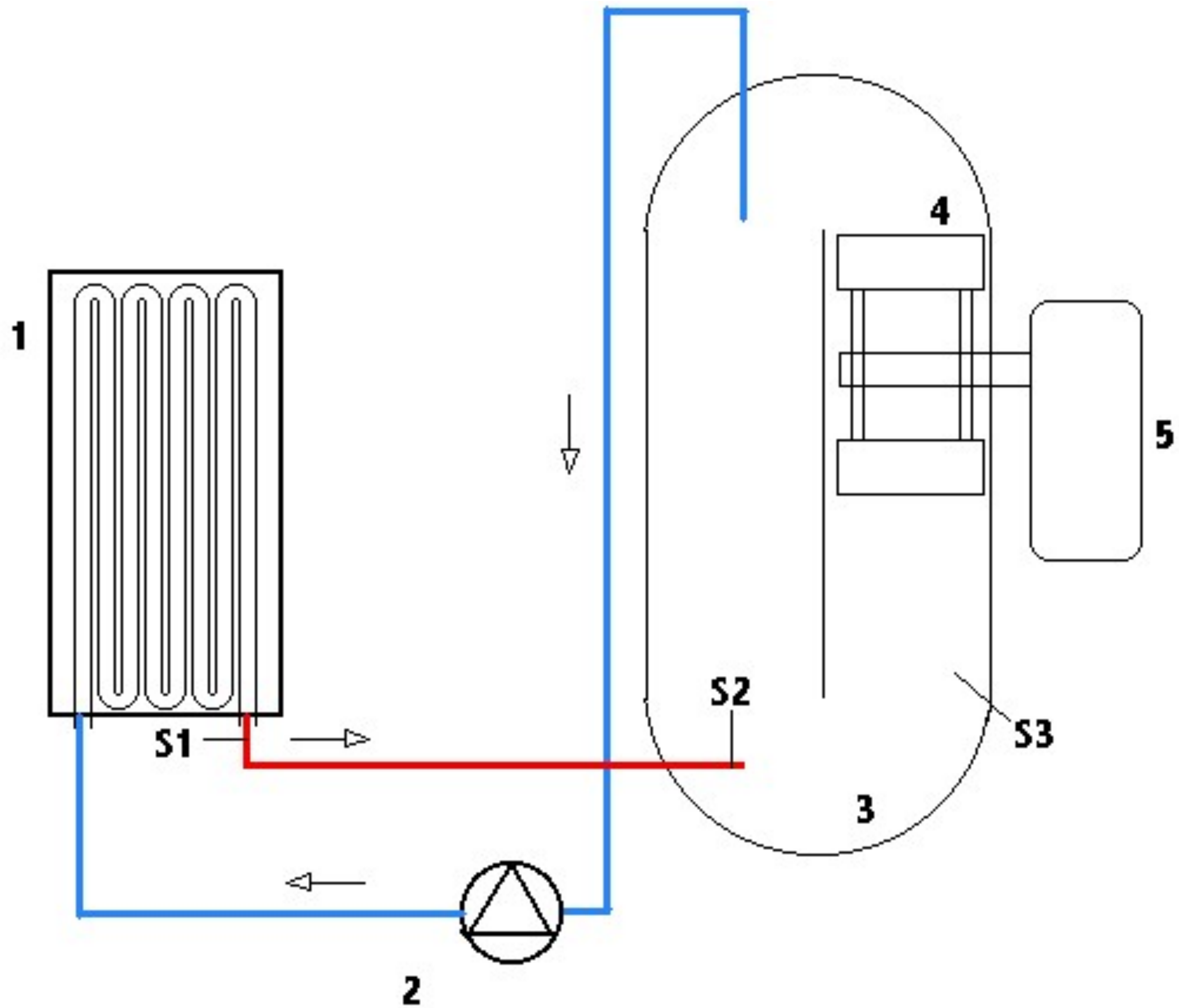
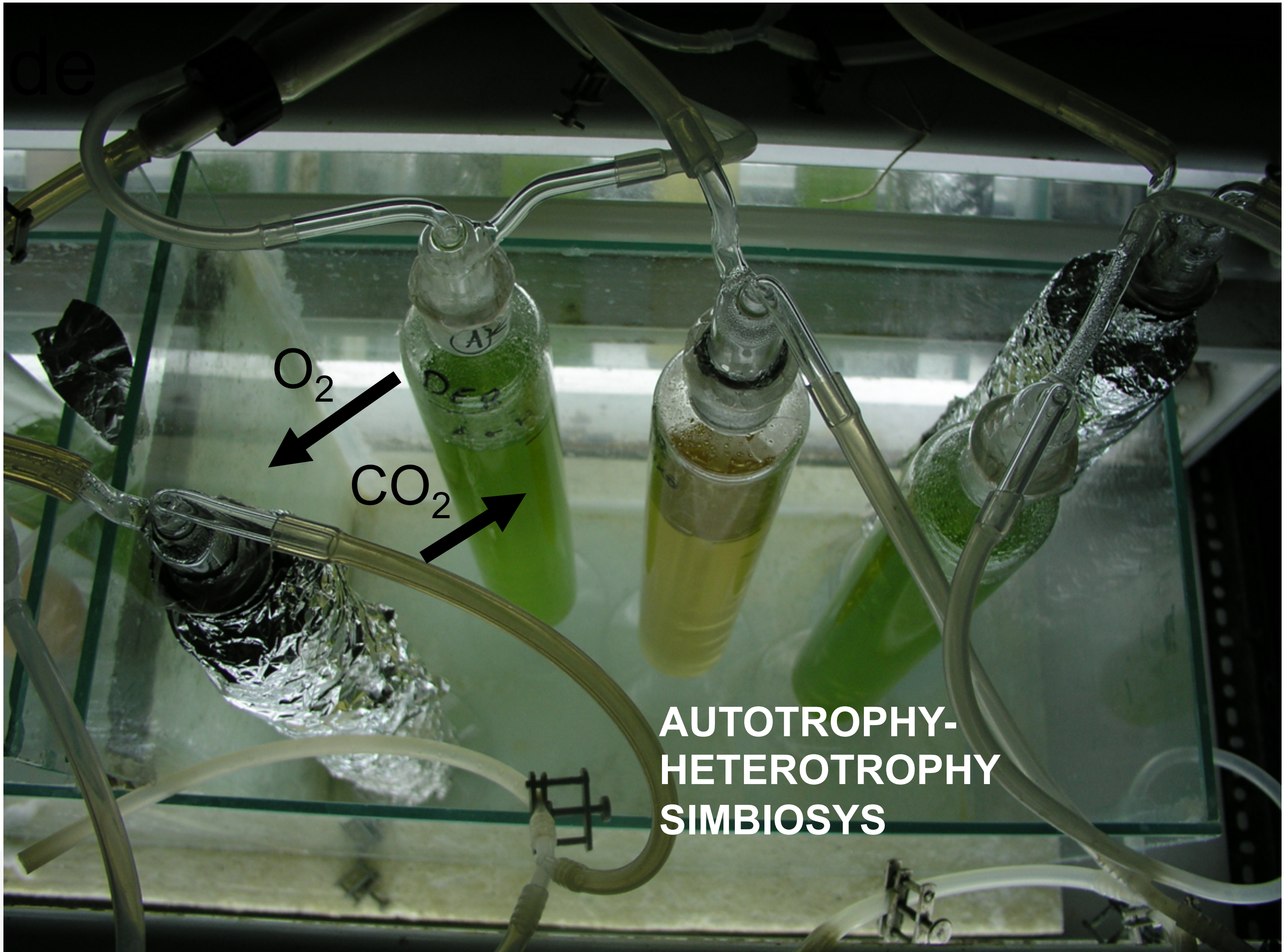
# INTEGRATING CLOSED FBR AND RACEWAY REACTOR



# INTEGRATING CLOSED FBR AND RACEWAY REACTOR



# new challenges for microalgae reactors




Reis
*et al*

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19 al 21 de noviembre de 2012, Pucón, Chile

J Ind Microbiol Biotechnol (2011) 38:909–917
DOI 10.1007/s10295-010-0860-0

ORIGINAL PAPER

# A symbiotic gas exchange between bioreactors enhances microalgal biomass and lipid productivities: taking advantage of complementary nutritional modes

C. A. Santos · M. E. Ferreira · T. Lopes da Silva ·
L. Gouveia · J. M. Novais · A. Reis

Received: 13 April 2010 / Accepted: 24 August 2010 / Published online: 8 September 2010


**Abstract** This paper describes the association of two bioreactors: one photoautotrophic and the other heterotrophic, connected by the gas phase and allowing an exchange of $O_2$ and $CO_2$ gases between them, benefiting from a symbiotic effect. The association of two bioreactors was proposed with the aim of improving the microalgae oil productivity for biodiesel production. The outlet gas flow from the autotrophic ($O_2$ enriched) bioreactor was used as the inlet gas flow for the heterotrophic bioreactor. In parallel, the outlet gas flow from another heterotrophic ($CO_2$ enriched) bioreactor was used as the inlet gas flow for the autotrophic bioreactor. Aside from using the air supplied from the auto- and hetero-trophic bioreactors as controls, one mixotrophic bioreactor was also studied and used as a model, for its claimed advantage of $CO_2$ and organic carbon being simultaneously assimilated. The microalga *Chlorella protothecoides* was chosen as a growth achieved the highest biomass productivity and lipid content (>22%), and furthermore showed that these lipids had the most suitable fatty acid profile in order to produce high quality biodiesel. Both associations showed a higher biomass productivity (10–20%), when comparing the two separately operated bioreactors (controls) which occurred on the fourth day. A more remarkable result would have been seen if in actuality the two bioreactors had been inter-connected in a closed loop. The biomass productivity gain would have been 30% and the lipid productivity gain would have been 100%, as seen by comparing the productivities of the symbiotic assemblage with the sum of the two bioreactors operating separately (controls). These results show an advantage of the symbiotic bioreactors association towards a cost-effective microalgal biodiesel production.

# the PRESENT

MICROALGAL BIOREFINERIES

are **still** in the **infancy**

are **far to be mature**

however
increasingly concern of
multiproduct process
driven approach

*Energy Sources, Part A*, 30:754–764, 2008

ISSN: 1556-7036 print/1556-7230 online
DOI: 10.1080/15567030600817795

Taylor & Francis
Taylor & Francis Group

# Towards Producing a Truly Green Biodiesel

A. B. CHHETRI[1] and M. R. ISLAM[1]

[1]Faculty of Engineering, Dalhousie University, Halifax, Nova Scotia, Canada

**Abstract** *The production of biodiesel has received considerable attention throughout the world in the past few years. As an alternative to petrodiesel, biodiesel is a renewable fuel that is derived from vegetable oils and animal fats. However, the existing biodiesel production process is neither completely "green" nor renewable because it utilizes fossil fuels, mainly natural gas as an input for methanol production. Also the catalysts currently in use are highly caustic and toxic. The purpose of this article is to propose a new concept that uses waste vegetable oil and non-edible plant oils as biodiesel feedstock and non-toxic, inexpensive, and natural catalysts that overcome the limitation of the existing process. The economic benefit of the proposed method is also discussed. The new concept will render the biodiesel production process truly green.*

**Keywords** biodiesel feedstocks, green biodiesel, natural catalysts

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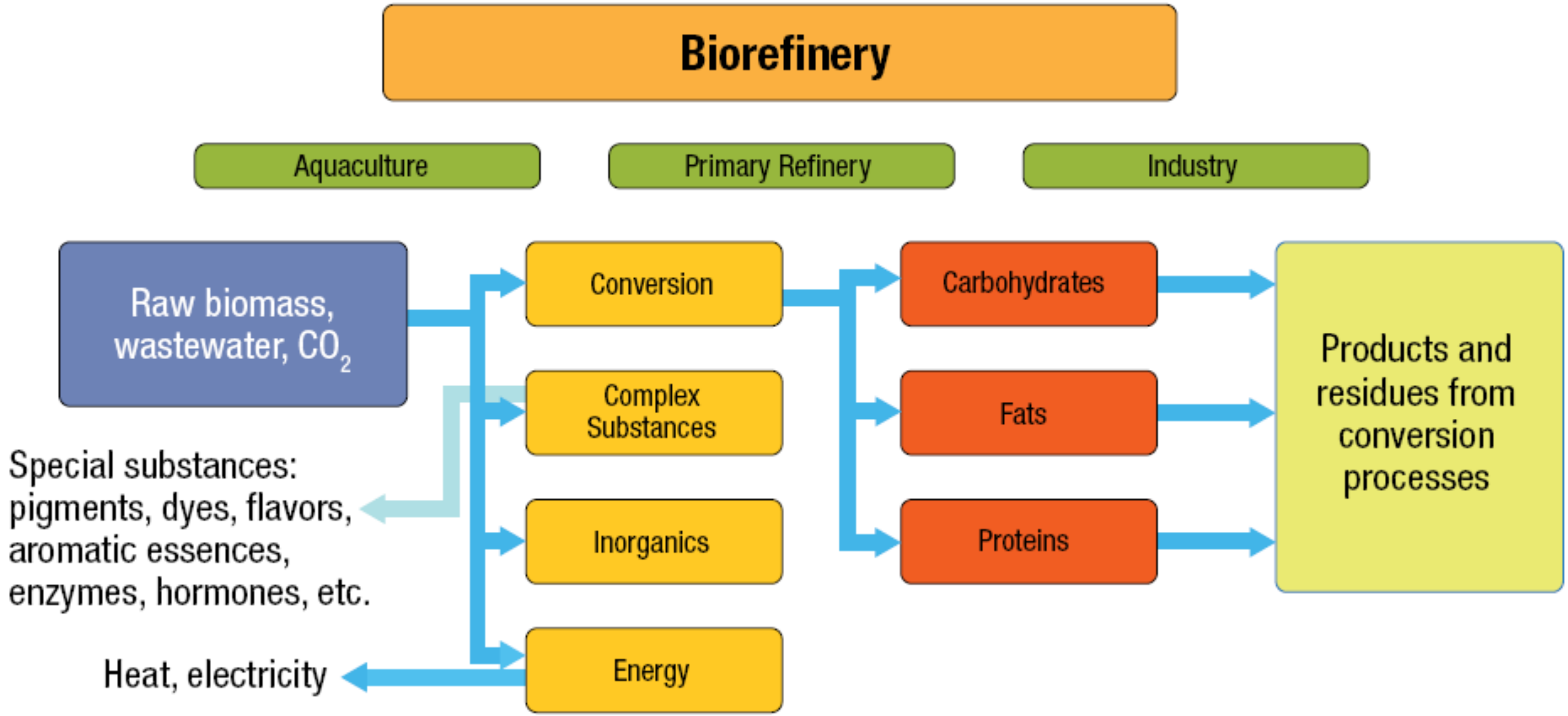
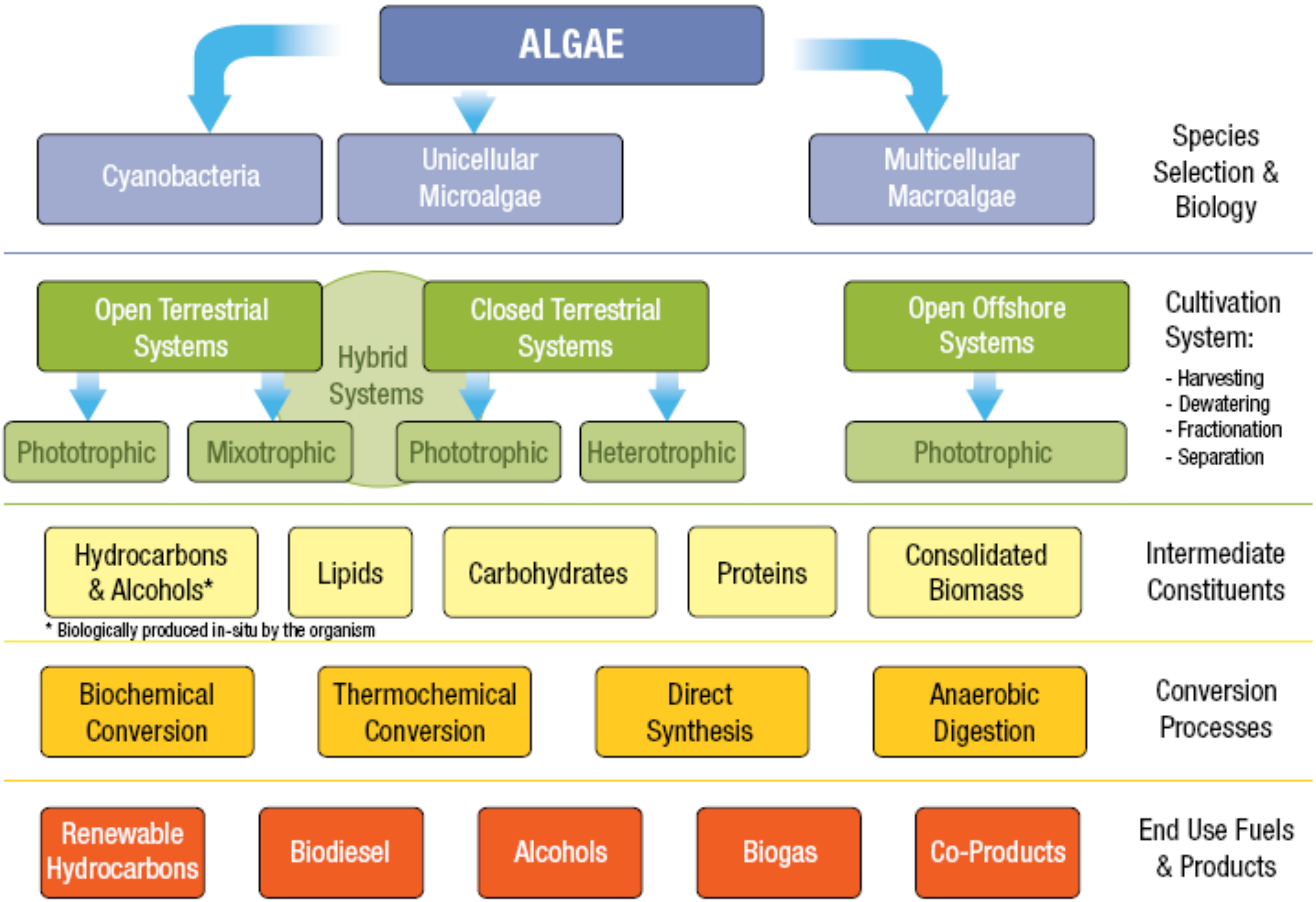
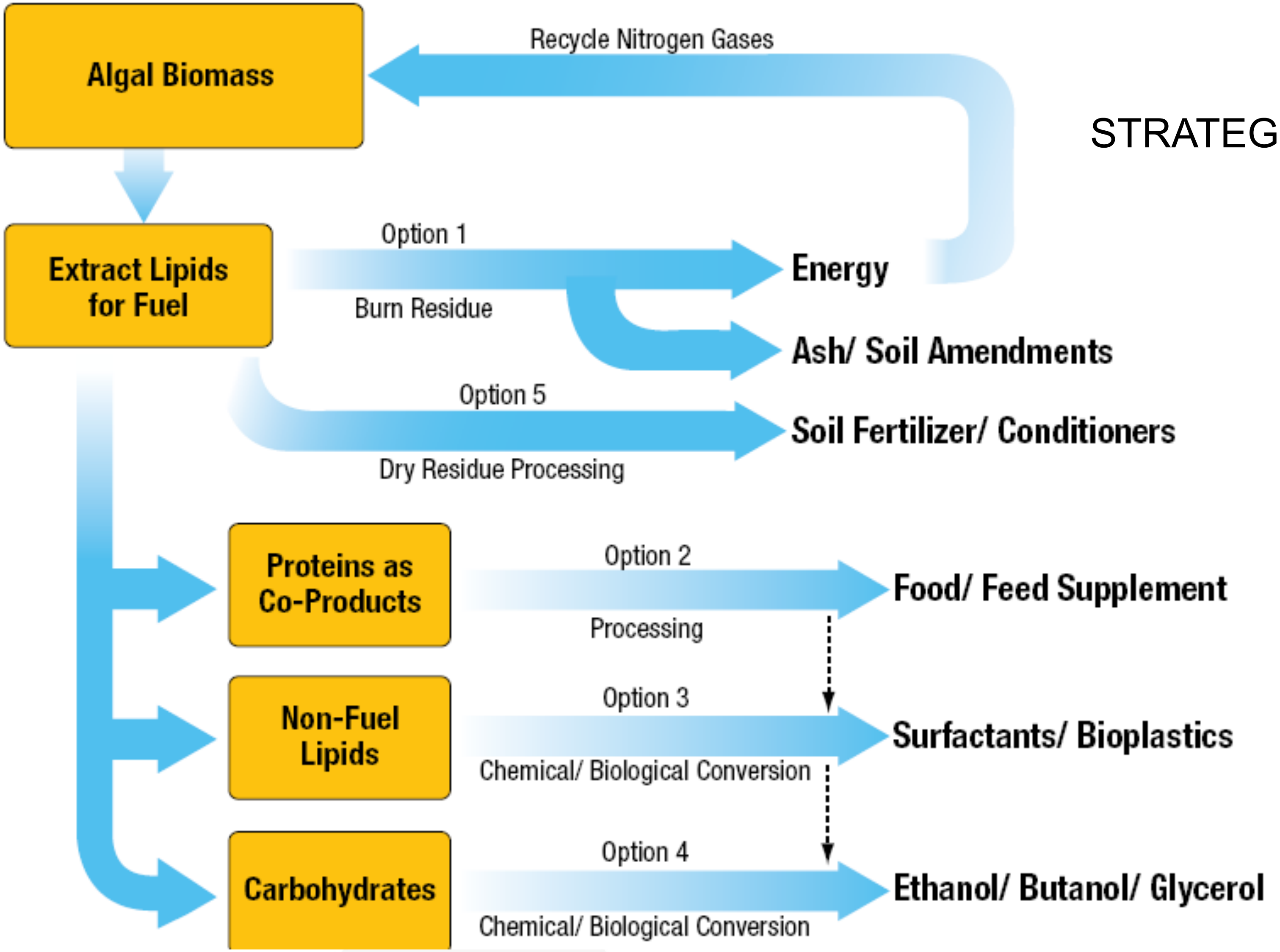



National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

various approaches and pathways to developing algae-derived biofuels and co-products



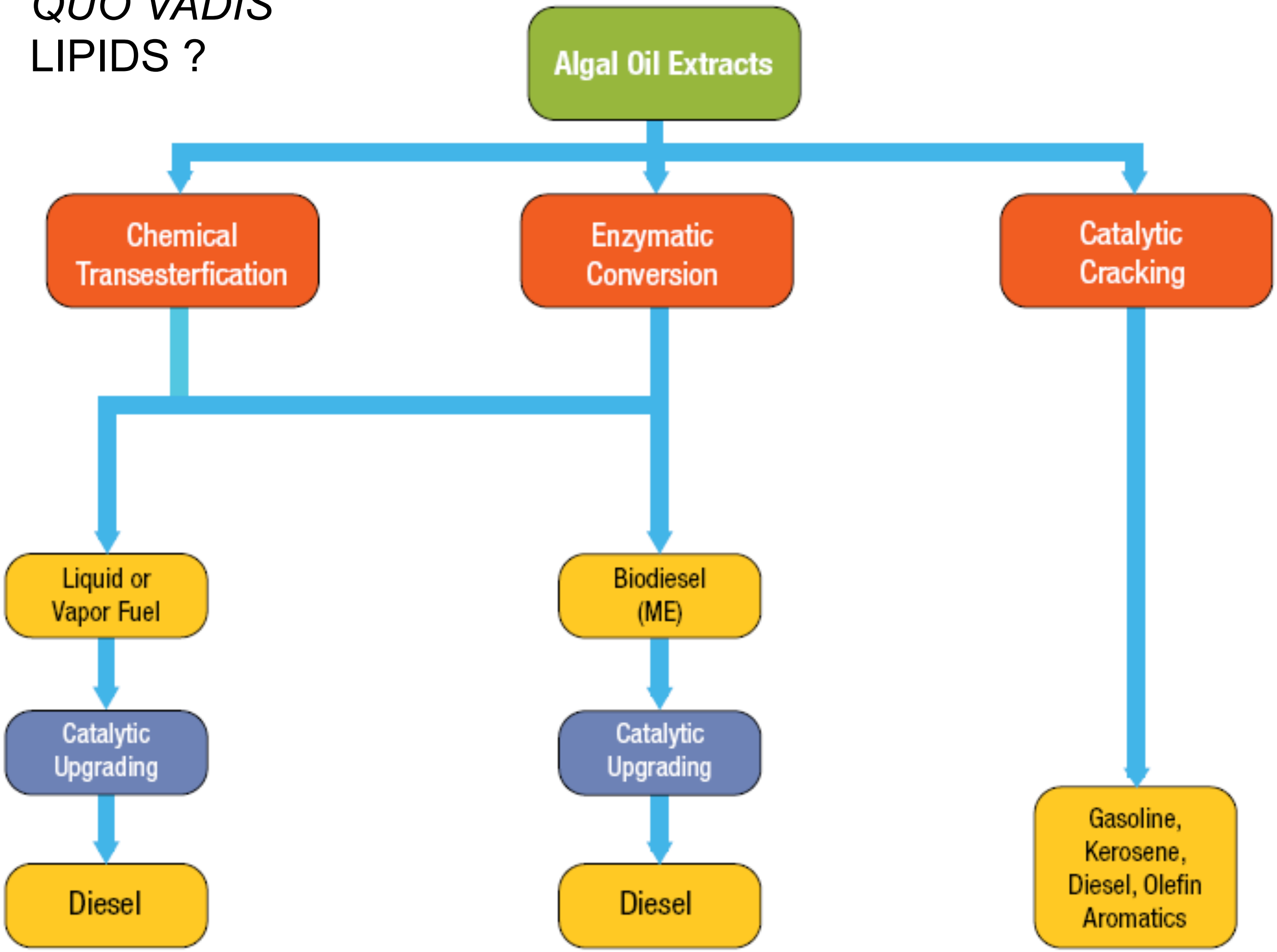

National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)




STRATEGIES?

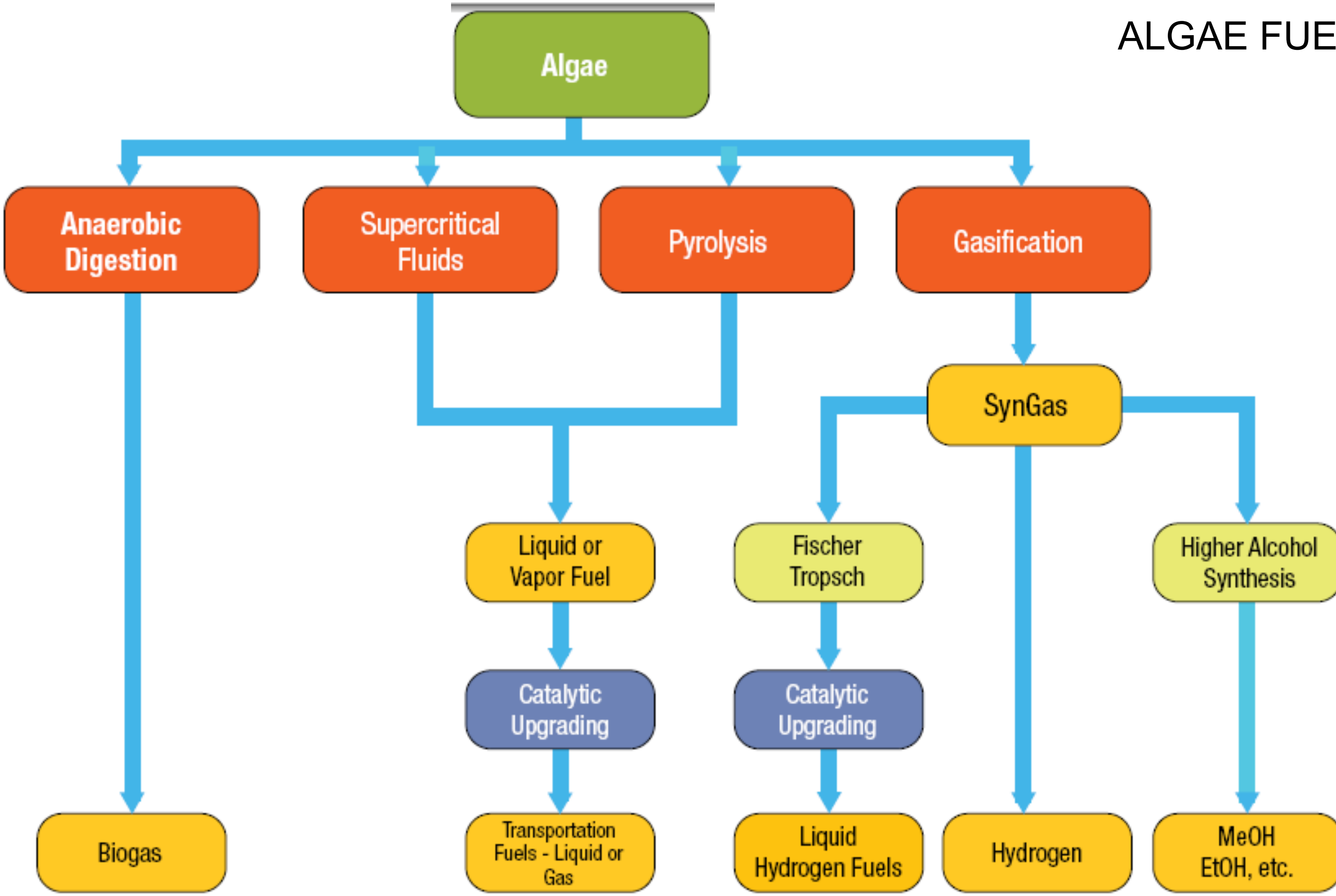
National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

*QUO VADIS*
LIPIDS ?



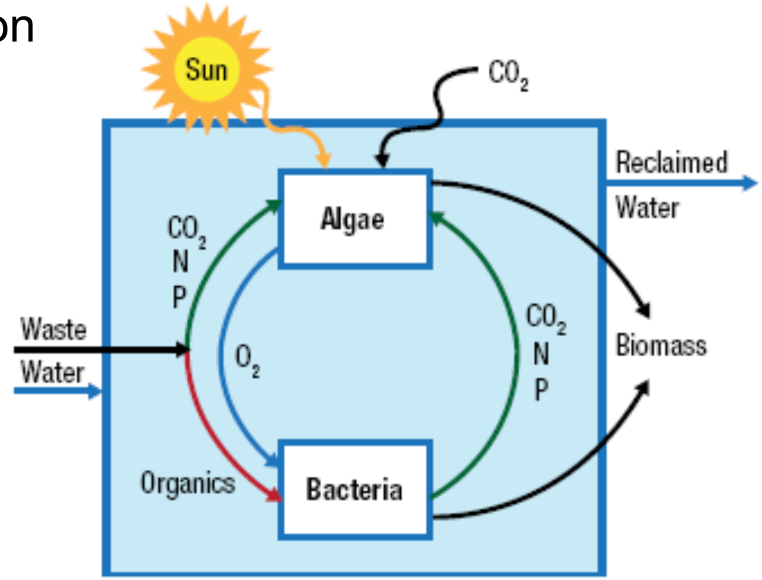

National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

# ALGAE FUELS



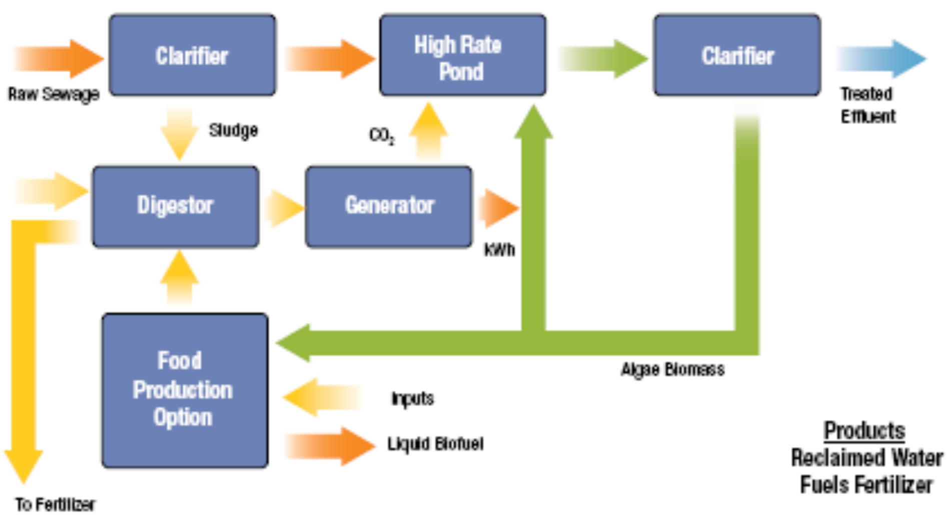

National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

Integration of algae production with wastewater treatment for nutrient removal and biomass production



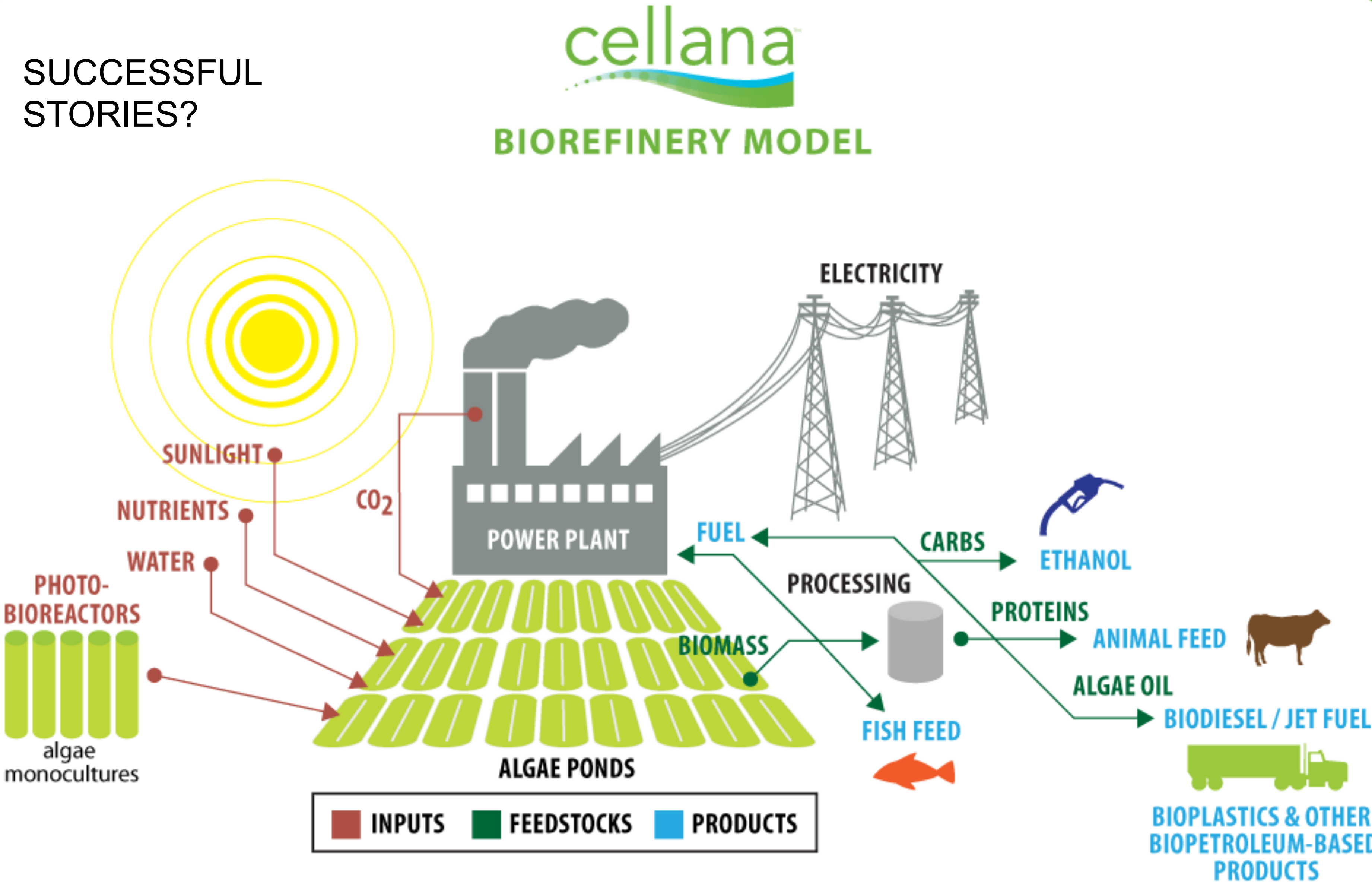

a) Basic principles of operation;

WASTES for FUELS and useful PRODUCTS

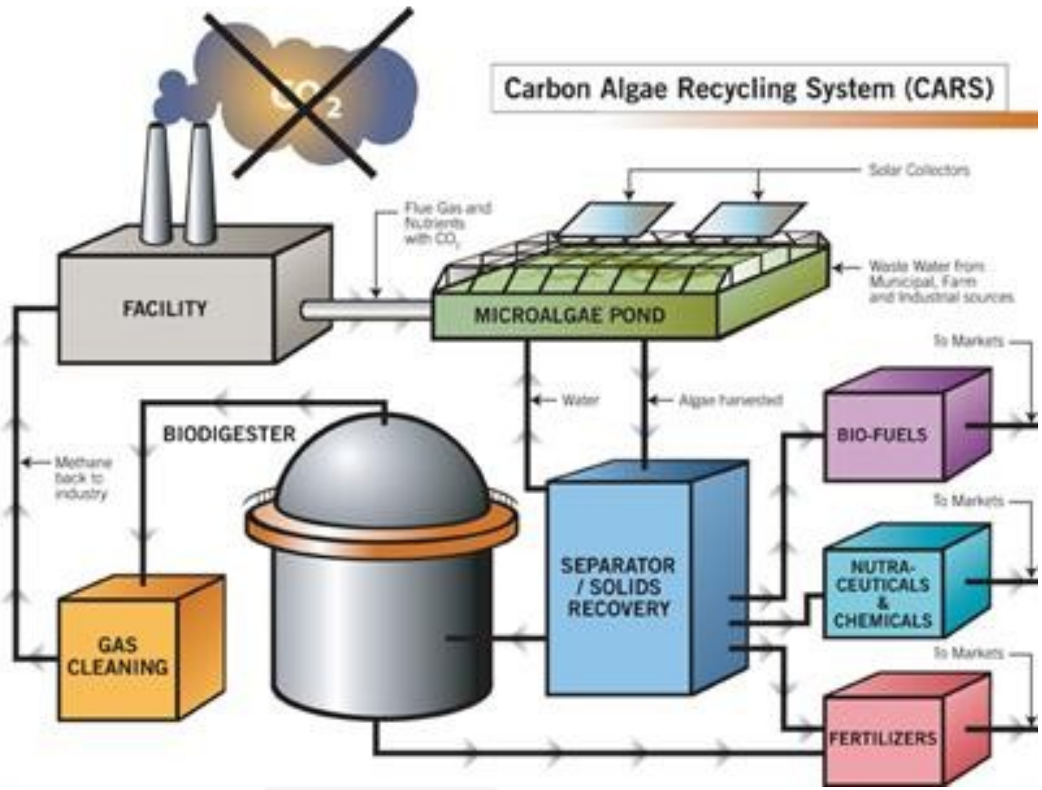



National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

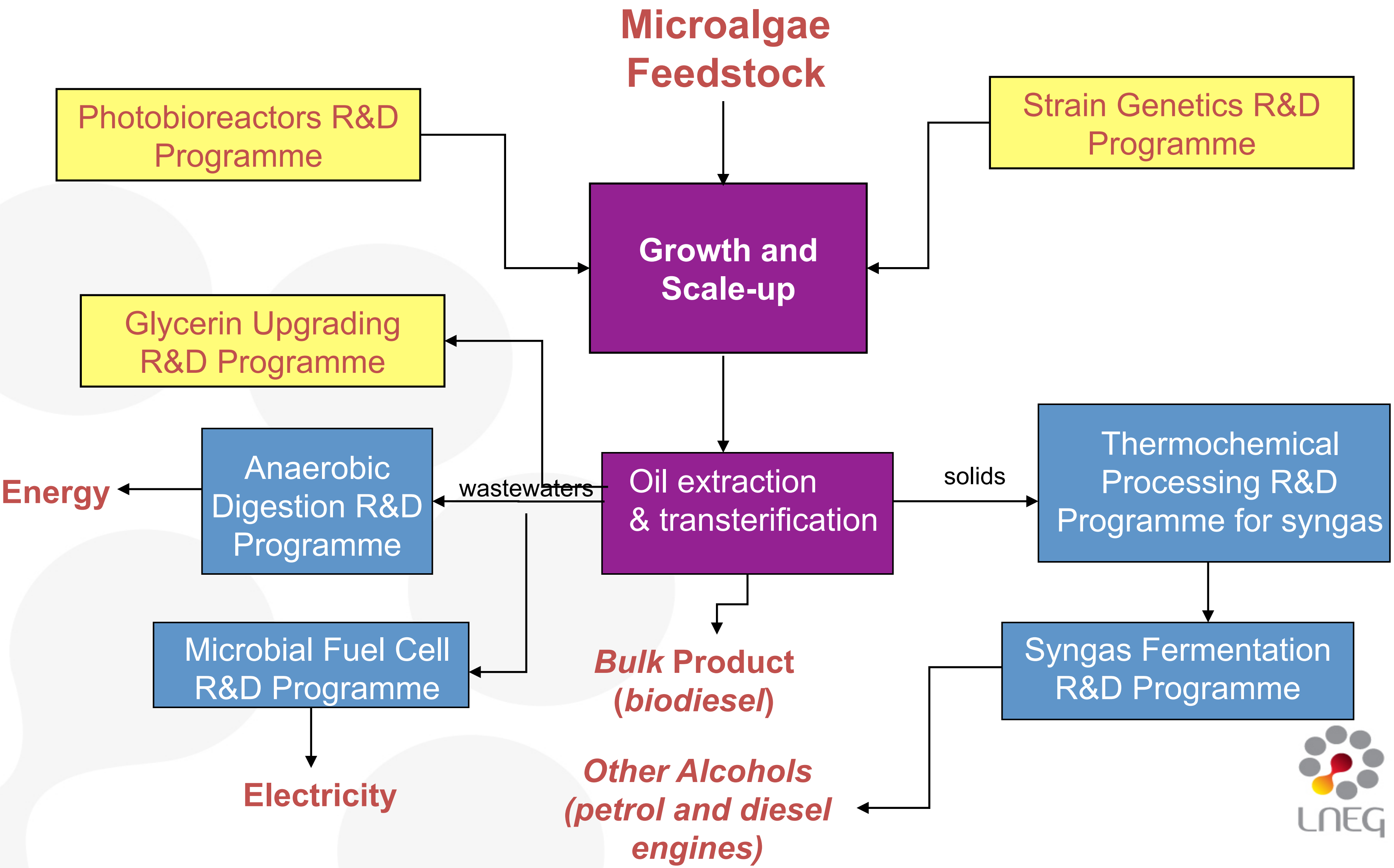
SUCCESSFUL STORIES?



# SUCCESSFUL STORIES?



# Bioenergy Programme: The Microalgae studies at LNEG



http://www.clean-energy.cl

**Producción de biocombustibles y captura de $CO_2$ mediante cultivo de microalgas.**

Actualmente, Clean Energy es asesorado por un equipo de investigación de primer nivel, con décadas de experiencia en la investigación y desarrollo de sistemas de microalgas.

- Inicio
- Quienes Somos
- Proyecto
- Prensa
- Galerías
- Partners
- Contacto

### Noticias

**11 de Noviembre 2011.**

Clean Energy inauguró su Planta Piloto con presencia de altas autoridades del país, vea los artículos de prensa [Acá].

**Mayo 2010.**

Clean Energy y el equipo del

## Clean Energy ESB S.A.

**Clean Energy ESB S.A.**, es una empresa dedicada a desarrollar Proyectos y Negocios Energéticos, utilizando para ello los más modernos conceptos de biotecnología ecosustentable para la producción integrada de biomasa de microalgas, biocombustibles de segunda generación (2G) y compuestos de alto valor agregado en simultáneo con la captura de anhídrido carbónico ($CO_2$) y de otros gases de efecto invernadero (GEI).

Conocer mas »


MEC

STRAIN SELECTION

- locally existing (Ventanas, Chile)
- high biomass output rates under the prevailing conditions existing at the thermal plant including untreated flue gases (15% $CO_2$, no stripping)

PROCESS DEVELOPMENT, INTEGRATION AND OPTIMIZATION AS **A WHOLE**

- MULTI-PRODUCT STRATEGY
- MULTI-PURPOSE STRATEGY

COST PRODUCTION SAVINGS
TIME SAVINGS

FROM THE HARSH OUTDOOR ENVIRONMENT TO THE LAB…

# the FUTURE ?

a long way needs to be done

# **REAL MICROALGAL BIOREFINERIES** ARE NEEDED

- ✓ FOR **TECHNO-ECONOMICAL** REASONS
- ✓ FOR **ENVIRONMENTAL** REASONS
- ✓ FOR **SUSTAINABILITY** ISSUES
- ✓ FOR **SOCIETAL** REASONS

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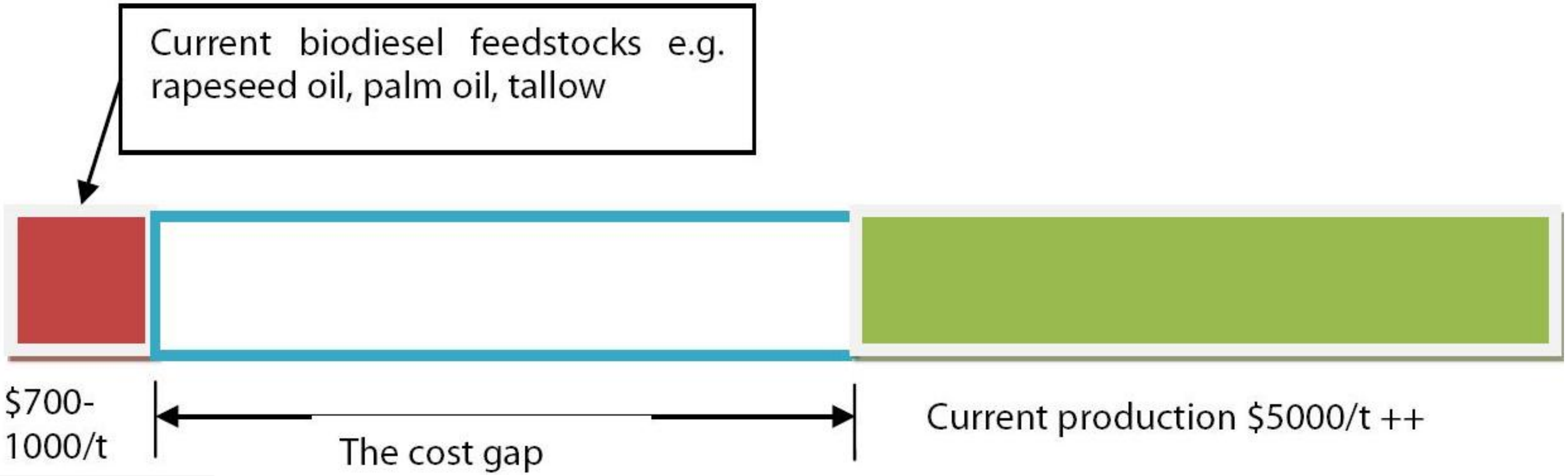
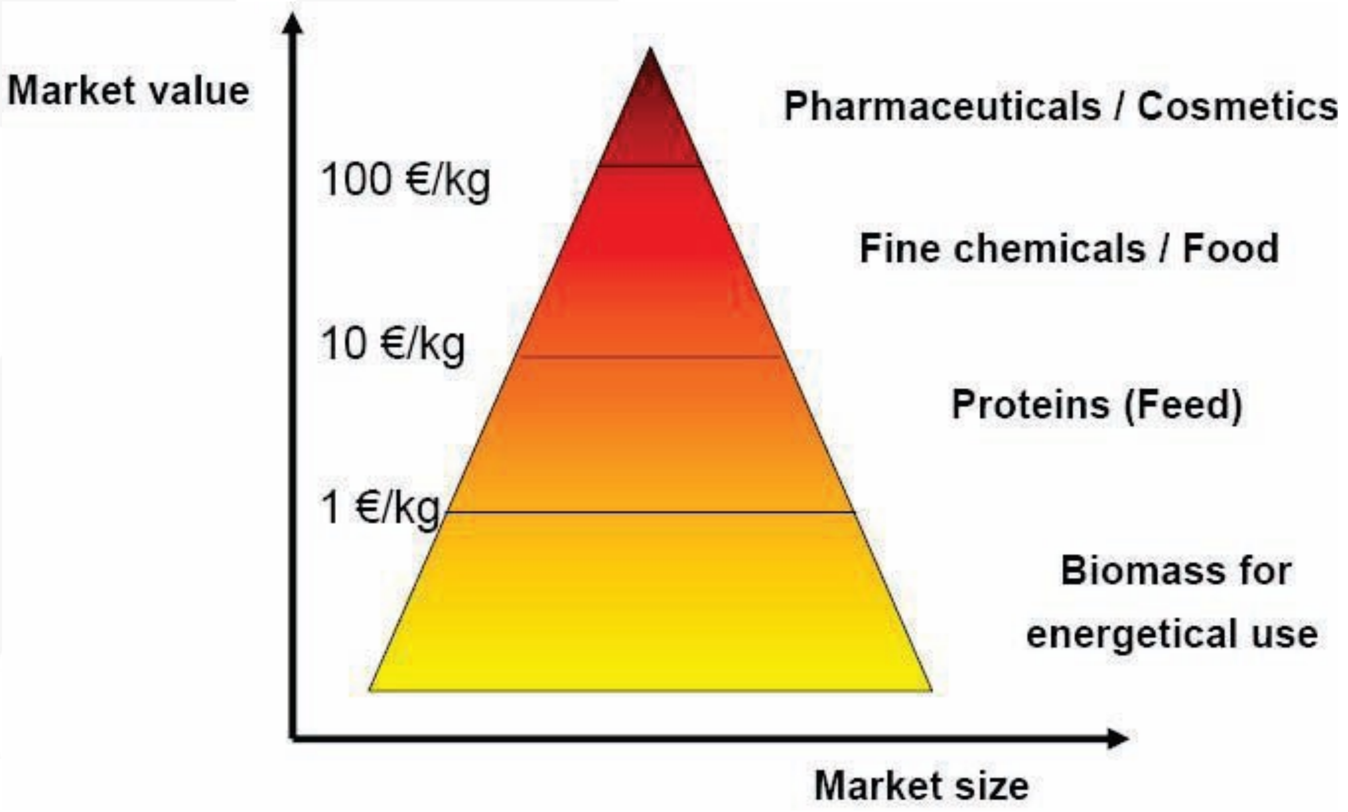
# still prohibitive costs?



the co-production of **high value-low market size** products is mandatory in order to pay the technology

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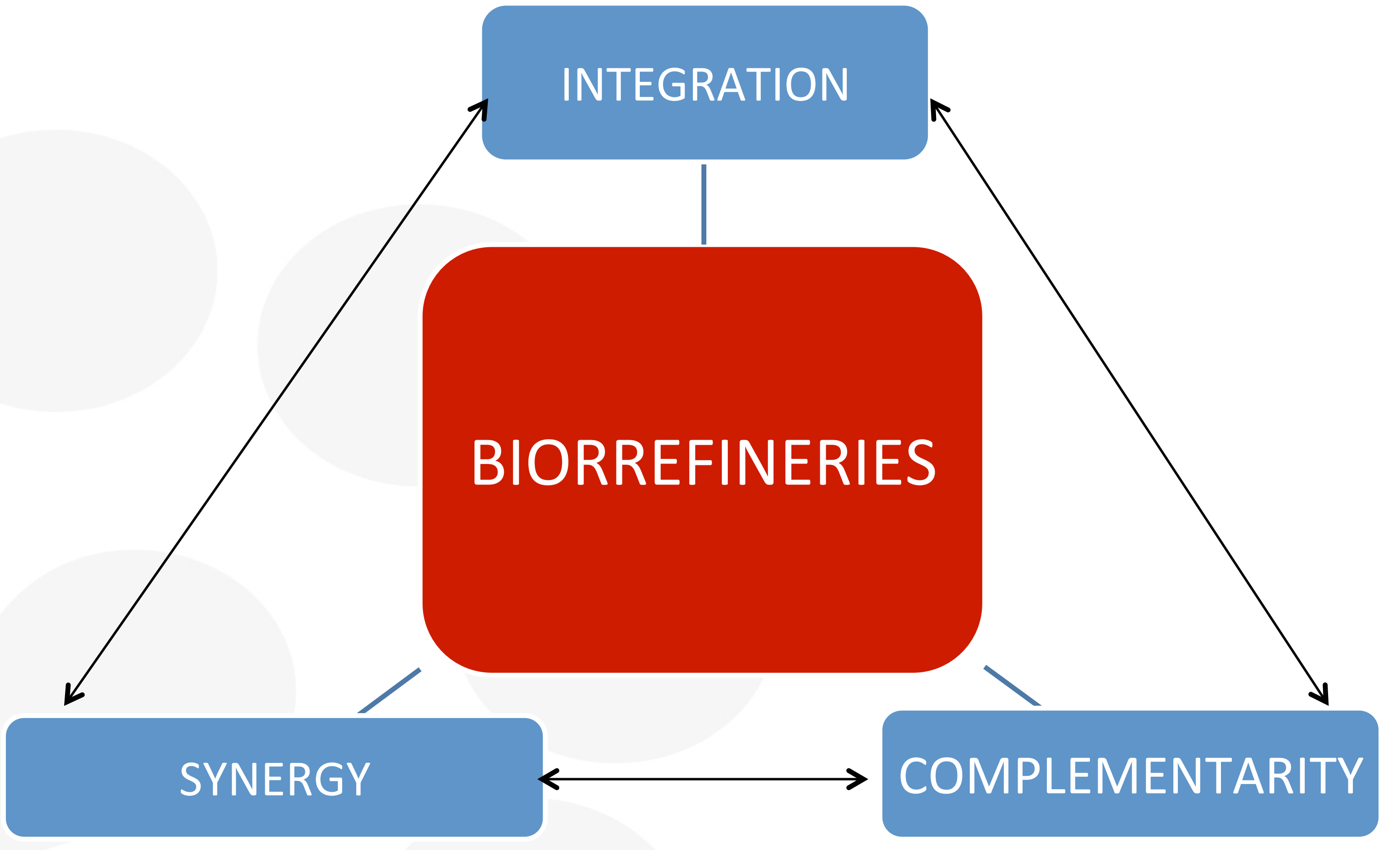

INTEGRATION
BIORREFINERIES
SYNERGY
COMPLEMENTARITY

# Implementation
# of a real fully integrated
# **Multiproduct**
# **Multipurpose**
# Process

Biotechnology Advances 27 (2009) 409–416

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

# Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable

Bruno Sialve [a,*], Nicolas Bernet [a], Olivier Bernard [b]

[a] INRA, UR050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, Narbonne F-11100, France
[b] INRIA-COMORE, 2004 Avenue des lucioles, BP93, Sophia-Antipolis F-06902, France

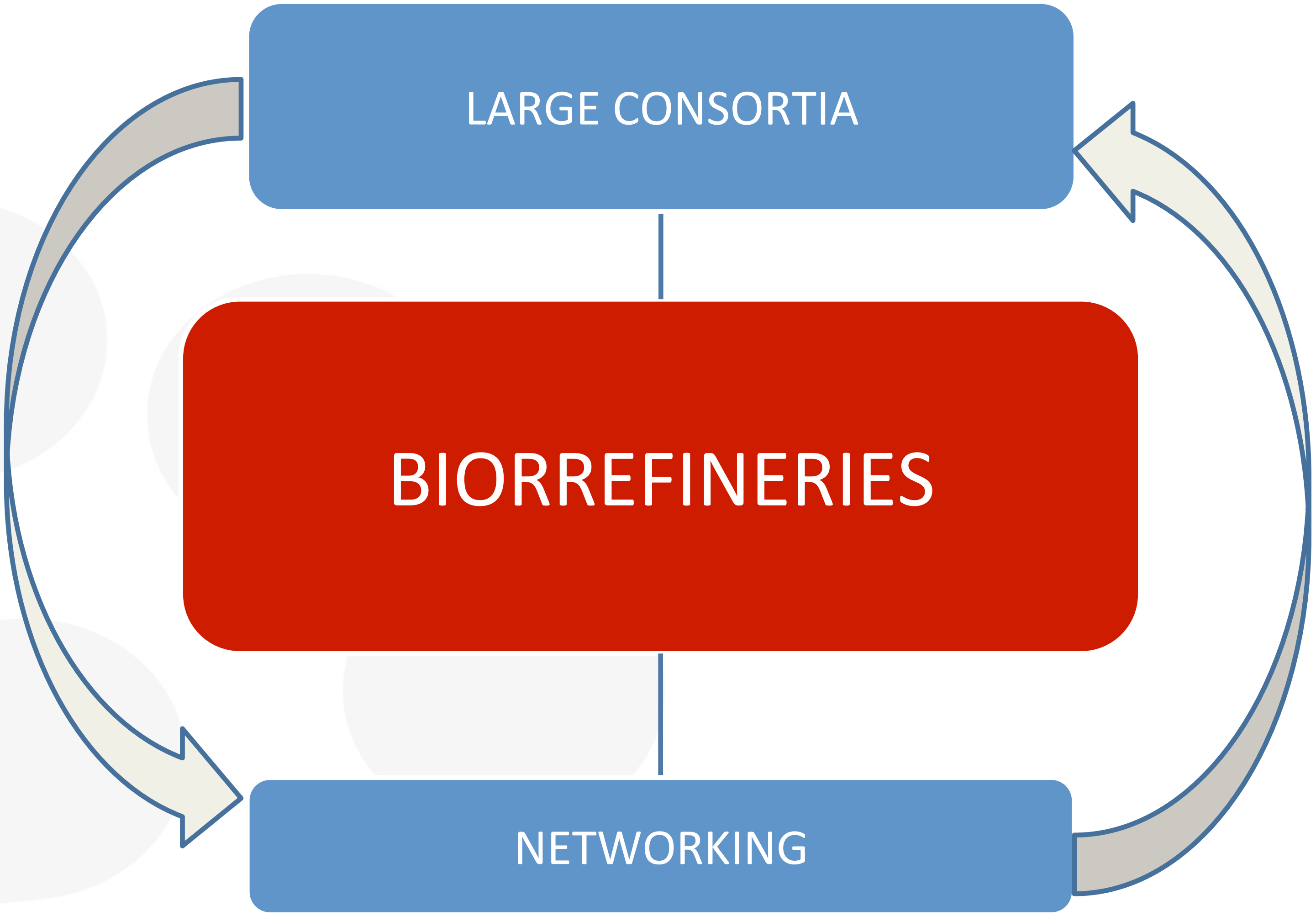
ARTICLE INFO

*Article history:*
Received 28 October 2008
Received in revised form 28 February 2009
Accepted 8 March 2009
Available online 14 March 2009

*Keywords:*
Anaerobic digestion
Microalgae
Biochemical methane potential
Codigestion
Pretreatment

ABSTRACT

The potential of microalgae as a source of biofuels and as a technological solution for $CO_2$ fixation is subject to intense academic and industrial research. In the perspective of setting up massive cultures, the management of large quantities of residual biomass and the high amounts of fertilizers must be considered. Anaerobic digestion is a key process that can solve this waste issue as well as the economical and energetic balance of such a promising technology. Indeed, the conversion of algal biomass after lipid extraction into methane is a process that can recover more energy than the energy from the cell lipids. Three main bottlenecks are identified to digest microalgae. First, the biodegradability of microalgae can be low depending on both the biochemical composition and the nature of the cell wall. Then, the high cellular protein content results in ammonia release which can lead to potential toxicity. Finally, the presence of sodium for marine species can also affect the digester performance. Physico-chemical pretreatment, co-digestion, or control of gross composition are strategies that can significantly and efficiently increase the conversion yield of the algal organic matter into


LARGE CONSORTIA
BIORREFINERIES
NETWORKING

# LARGE CONSORTIA

Consortium Desert Bioenergy S.A.

Algae Fuels Consortium

Balfuels Consortium

Consortium Bioenercel

# NETWORKING

Iberian-Latin-American Thematic CYTED Network
P711RT0095

Iberian-Latin-American Society of Applied Algology SI3A

162 researchers

29 groups/institutions

10 countries

# NETWORKING

## Iberian-Latin-American Society of Applied Algology SI3A

| INSTITUTION | TYPE | PERSON IN CHARGE | COUNTRY |
|---|---|---|---|
| CENTRO DE INVESTIGACIÓN CIENTÍFICO TECNOLÓGICO PARA LA MINERIA-CICITEM | *Non-Profit Private Research Centre* | *MARIELLA RIVAS ALVAREZ* | *CL* |
| CLEAN ENERGY ESB S.A. | SME | *ANDREA IRARRÁZAVAL O.* | CL |
| *I-MAR,* UNIVERSIDAD DE LOS LAGOS | University | *MARÍA CARMEN HERNÁNDEZ-GONZÁLEZ* | CL |
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| UNIVERSITY OF ANTOFAGASTA | *University* | *CARLOS RIQUELME SALAMANCA* | *CL* |

**6** CHILEAN GROUPS

# LCA-LIFE CYCLE ASSESSMENT STUDIES

will offer attractive possibilities
to **develop** and **expand**
microalgal biorefineries

- ✓ data were **scarce** in the **past** and many times **conflicting**

- ✓ data are getting more and more **reliable**

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III Congreso Latinoamericano
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19 al 21 de noviembre de 2012, Pucón, Chile

# Policy Analysis

## Life-Cycle Assessment of Biodiesel Production from Microalgae

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*Received March 10, 2009. Revised manuscript received June 8, 2009. Accepted June 18, 2009.*

This paper provides an analysis of the potential environmental impacts of biodiesel production from microalgae. High production yields of microalgae have called forth interest of economic and scientific actors but it is still unclear whether the production of biodiesel is environmentally interesting and which transformation steps need further adjustment and optimization. A comparative LCA study of a virtual facility has been undertaken to assess the energetic balance and the potential environmental impacts of the whole process chain, from the biomass production to the biodiesel combustion. Two different

photosynthetic yields; about 3−8% of solar energy can be converted to biomass whereas observed yields for terrestrial plants are about 0.5% (*2, 3*). These interesting properties lead to potential productivities (in terms of oil production per ha and per year) which are far higher than those of rapeseed or sunflower (*4*). This high productivity combined with both the moderate competition with feed crop and the possibility to uptake industrial sources of $CO_2$ has motivated studies depicting microalgae as an alternative source of vegetal oil for biodiesel (*2, 4*).

Despite strong interest from economic and scientific actors, up to now, there is to our knowledge no industrial facility producing biodiesel from microalgae. The studies undertaken on the subject have been restricted to lab and pilot scales. Hence, no thorough Life Cycle Assessment of the production chain from microalgae culture to biodiesel is currently available, with the exception of LCA studies about the cofiring of microalgae with coal (*5*). The aim of this study is therefore to assess the environmental impacts of this technologically immature process. To do so, we extrapolated laboratory observations combined with known processes developed for first generation biofuel to design a realistic industrial facility. The potential pollution transfers are computed for various scenarios and guide the choice of selected steps in the process chain. In addition to the overall energetic balance of the production chain, the impacts of the combustion of algal biodiesel are compared to those produced by first generation biofuel and diesel fuel. The considered functional unit of the LCA is the combustion of

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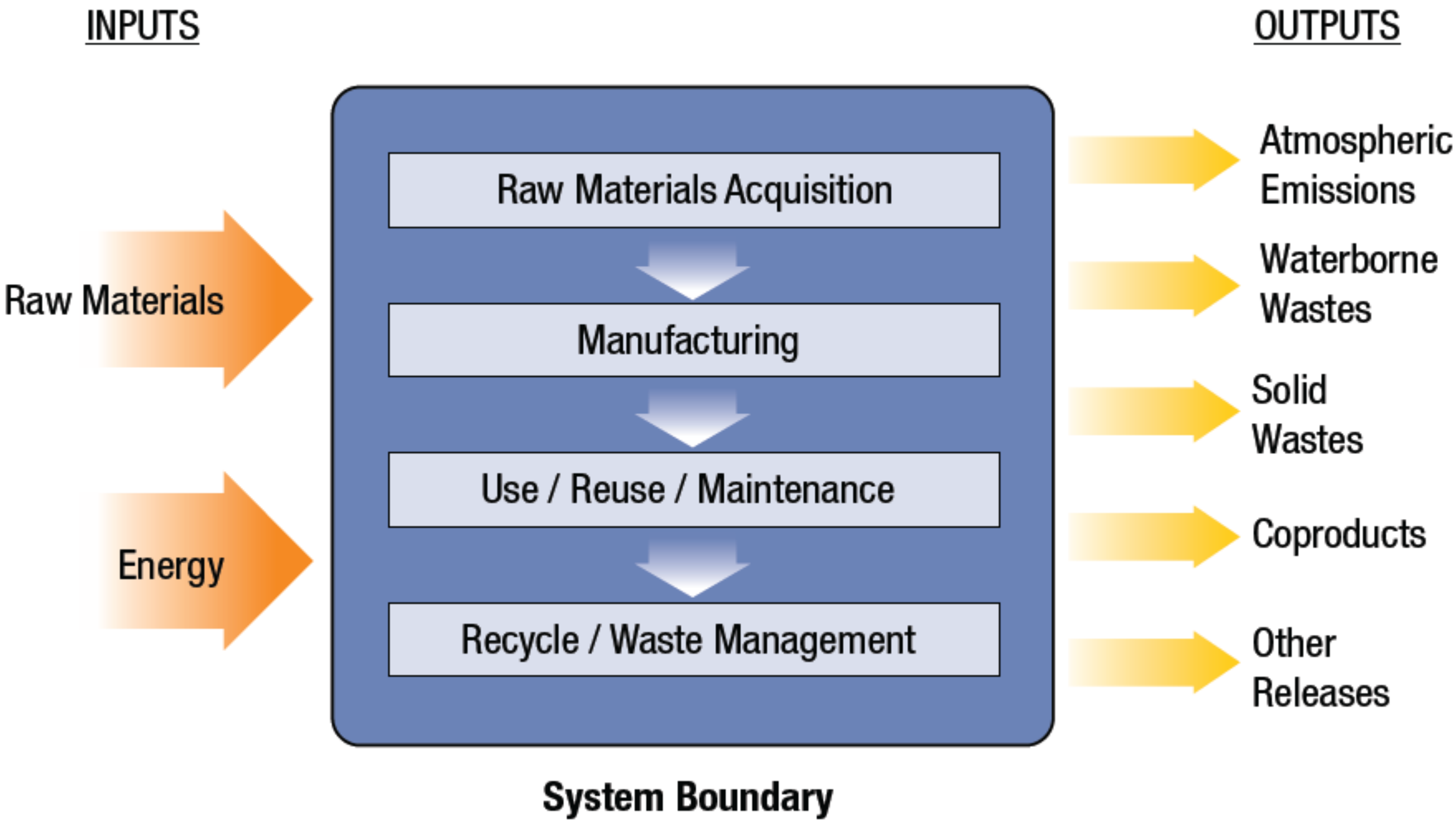
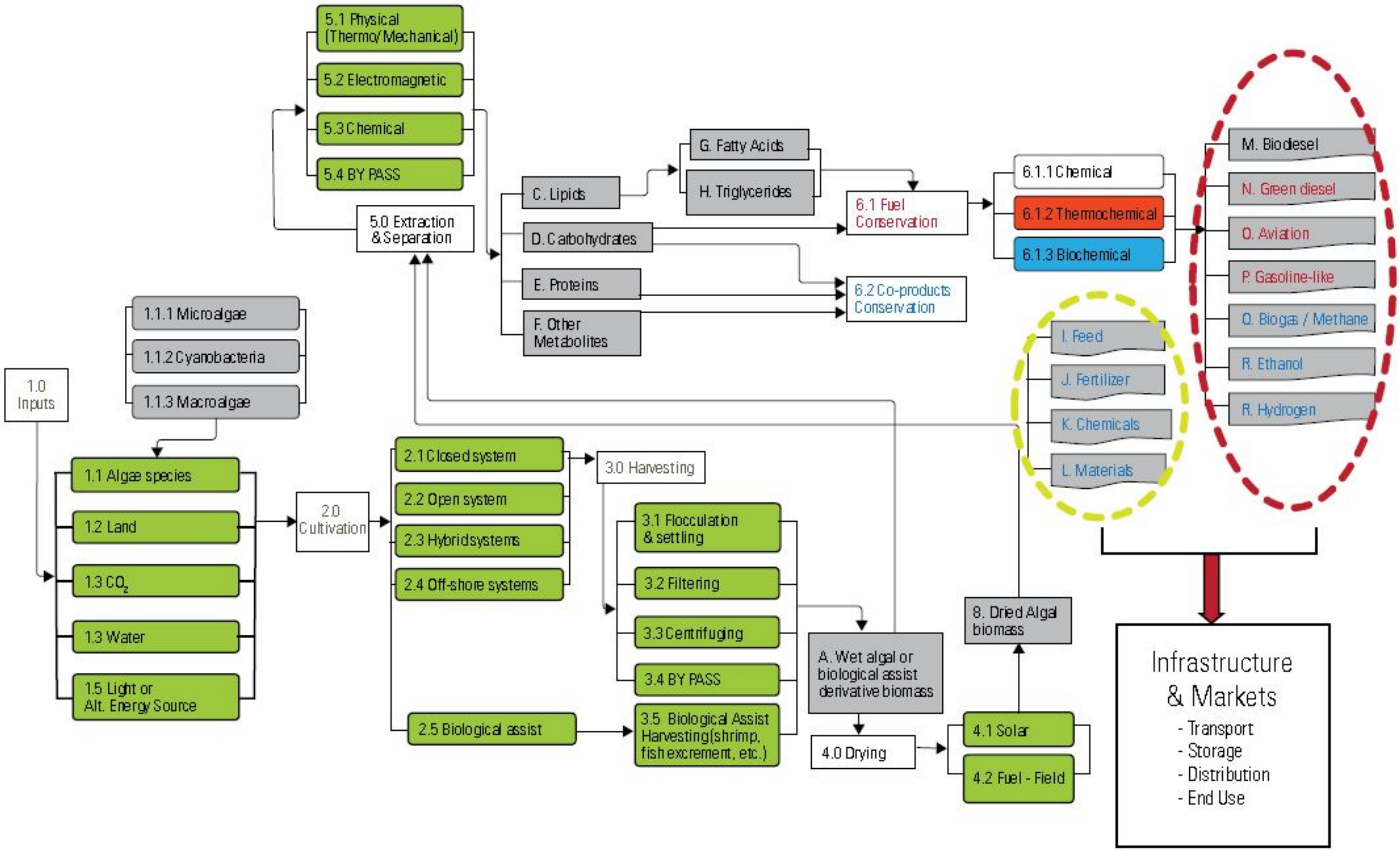
# Scope of Life Cycle Analysis/Potential Directions for R&D Effort




National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

- ✓ INFRASTRUCTURE
- ✓ LOGISTICS
- ✓ DISTRIBUTION
- ✓ STORAGE
- ✓ TRANSPORTATION
- ✓ END USE




National Algal Biofuels Technology Roadmap, (USA Department of Energy, 2010)

**Acknowledgments**

Portuguese Foundation for Science and Technology supported studies presented here through the SIMBIOALGA Project (PTDC/AAC-AMB/112954/2009) FCOMP-01-0124-FEDER-013935 (also supported by FEDER funding through COMPETE- Programa Operacional Factores de Competitividade).

The financial support of CYTED (Thematic Network SI3A P711RT0095) is also acknowledged.

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**thank you !**

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Nov, 20th 2012