Lodz University of Technology
Faculty of Proces & Environmental Engineering

Visegrad Fund

From biofuels to high added value bioproducts in the development of 3G biorefineries

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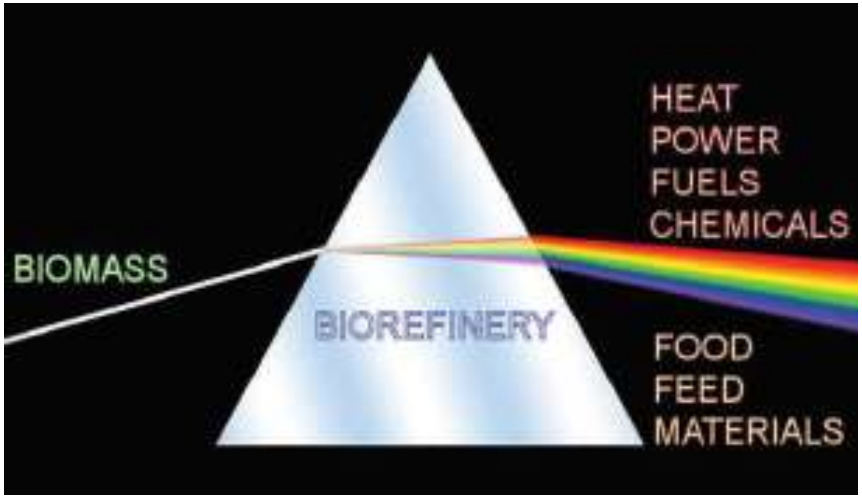
Biorefinery - definition

The concept of biorefineries is derived from oil refineries, with biomass as the starting material, not a crude oil.

International Energy and Bioenergy Agency (IEA) task 42 defines a biorefinery as **"the *sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, and chemicals) and energy (fuels, power, and heat)*"**.

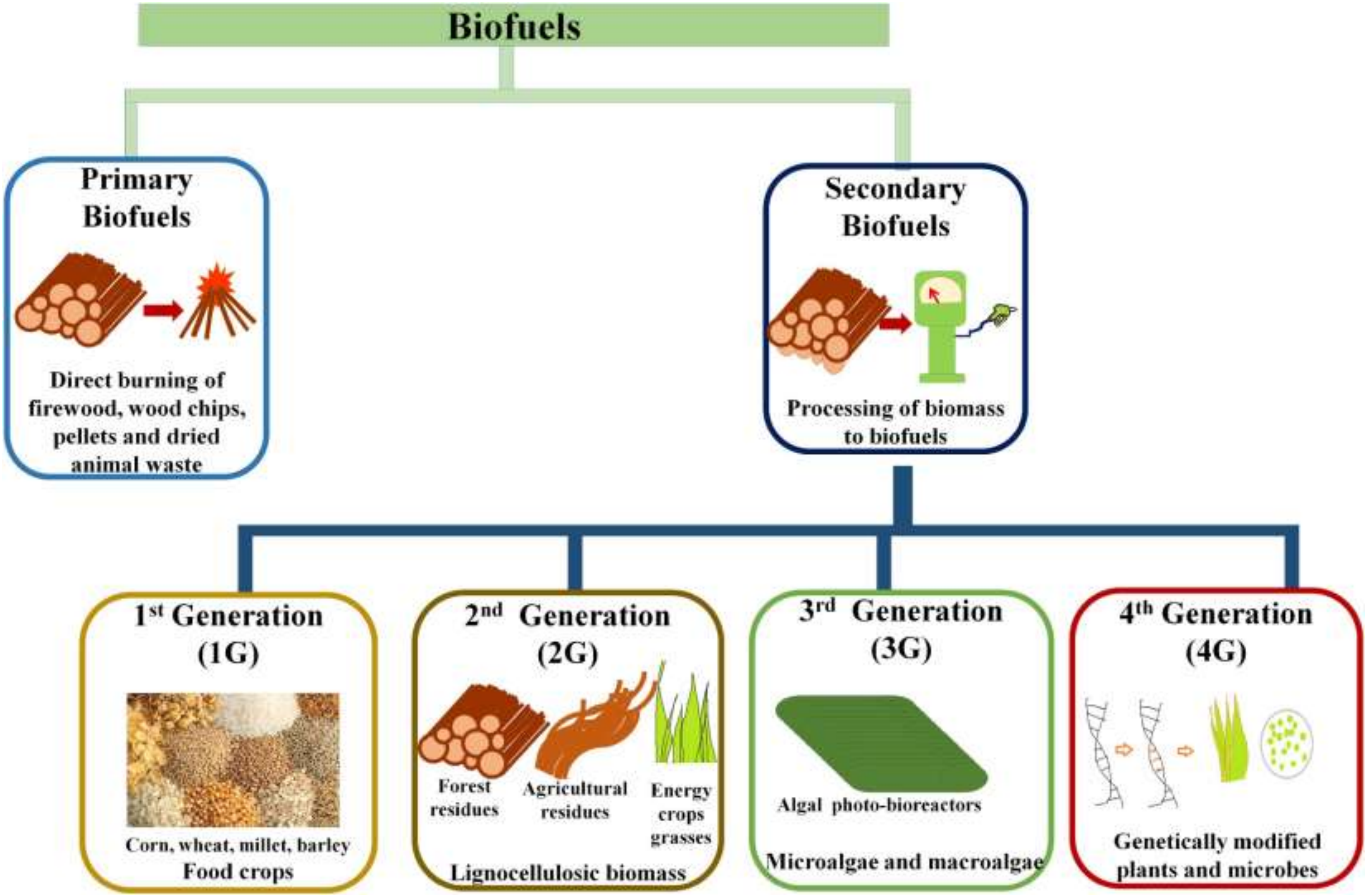


Industrial Biorefineries and White Biotechnology
http://dx.doi.org/10.1016/B978-0-444-63453-5.00001-X

another versions:

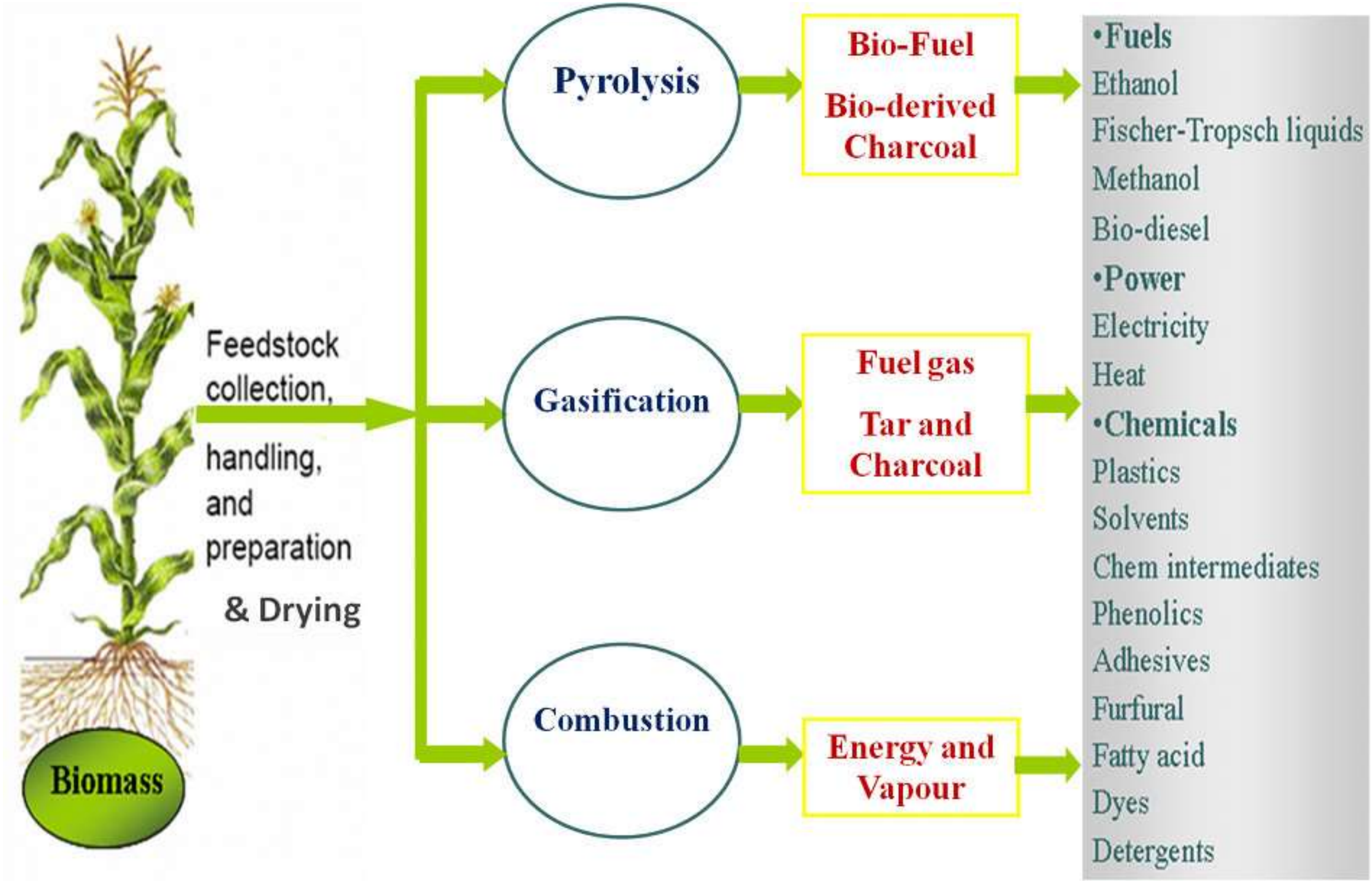
"***a facility (or network of facilities) that integrates conversion processes and equipment to break down biomass resources (wood, grasses, corn, etc.) into their building blocks (carbohydrates, proteins, triglycerides, etc.) and subsequently converted them into value-added products such as biofuels and chemicals***".

Biorefinery classification based on feedstock

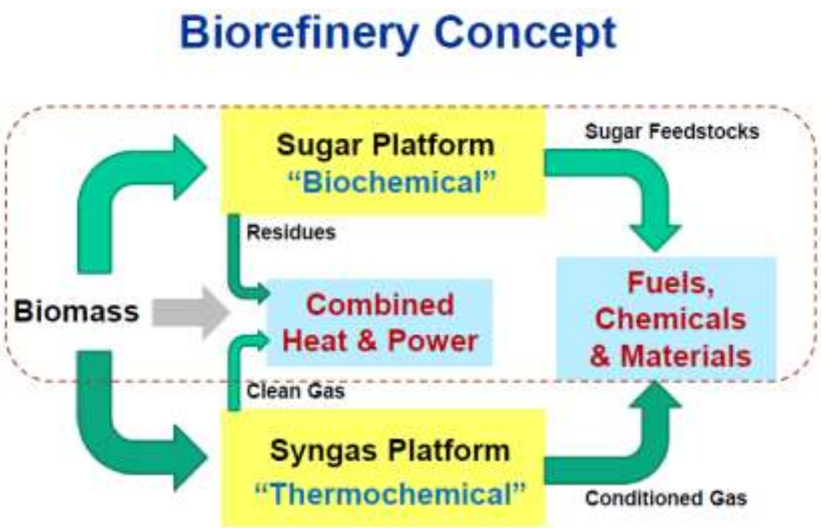


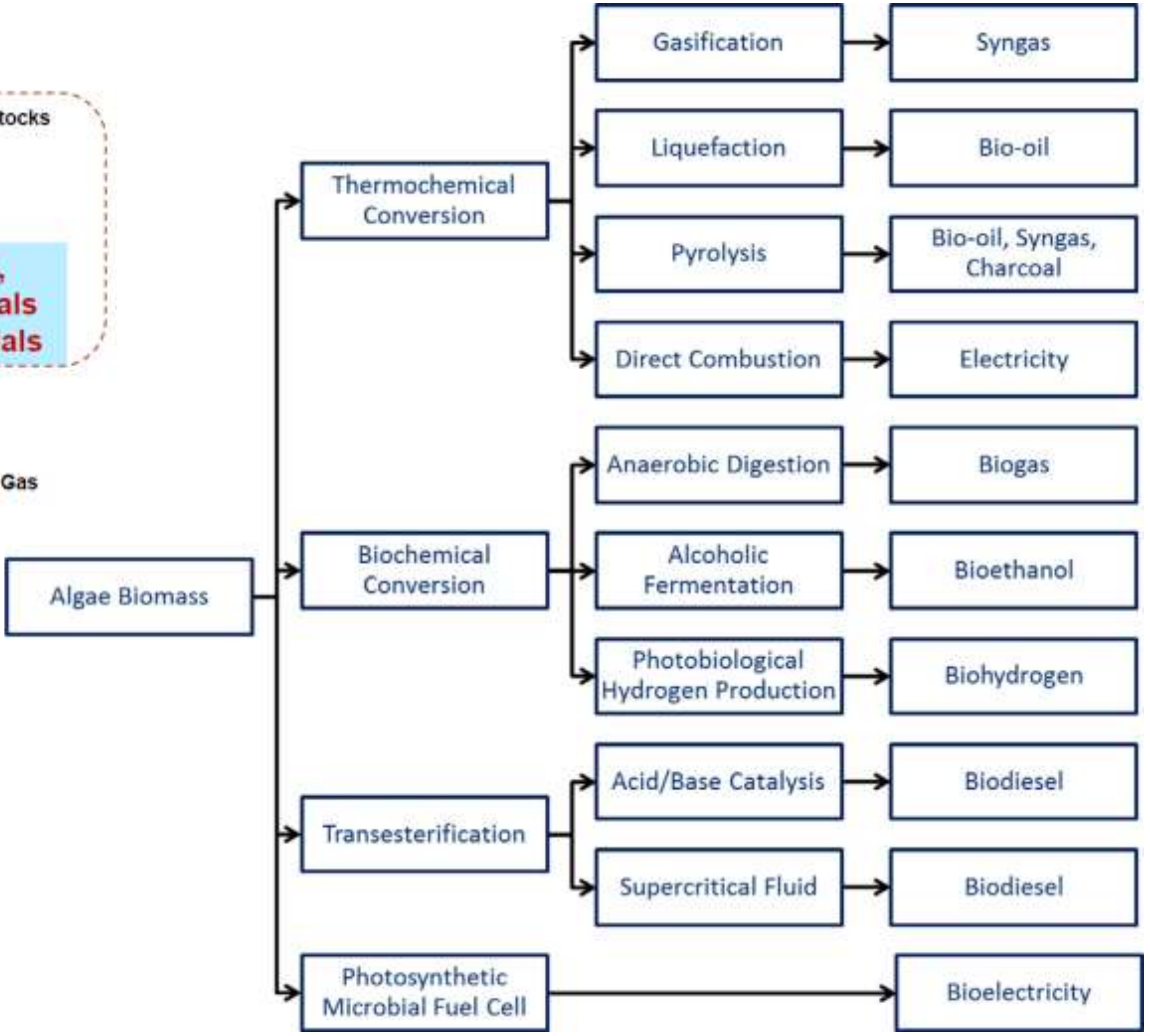
4th Generation biorefinery uses genetically modified plants and microorganisms with high CO_2 absorption capacity to produce biofuels and bio-chemicals

Various processes of thermo-chemical conversion of biomass



Integration of bio- & thermo-chemical platforms for biorefinery





Biorefineries in Europe 2020

There are approx. 300 commercial and demonstration biorefineries in the EU. Most biorefineries in Europe are based on chemical pulping processes. The second largest group are biorefineries, which mainly use by-streams of existing pulp and paper mills. There are several completely new types of biorefineries that are based on developing methods of wood processing.

New biorefineries 2-nd generation:

- The refinery operated by Veolia, will be built in Finland with production capacity of 12.000 t/a biomethanol, the plant, come on stream by 2024.
  www.veolia.com/sites/g/files/dvc4206/files/document/2022/04/pr-CO2-neutral-biofuel-veolia-Finland.pdf
- The Finnish company **UPM** turns waste streams (woody biomass, liquid waste) into bio-fuels and other residual materials. The planned refinery in Rotterdam will have a capacity of 500,000 t/a.
- **UPM** builds a biorefinery in Leuna, Germany, converting 100% birch wood into mono-ethylene and mono-propylene bio-glycols and functional lignin-based fillers - 220,000 t/a
  https://www.industryandenergy.eu/chemcycling/upm-chooses-rotterdam-for-construction-of-biorefinery/
- **Clariant** completes construction of first commercial sunliquid® cellulosic ethanol (production of 50,000t/a from 250,000 t of straw) plant in Podari, Romania
  www.clariant.com/en/Corporate/News/2021/10/Clariant-completes-construction-of-first-commercial-sunliquid-cellulosic-ethanol-plant-in-Podari-Rom

Third-generation biorefineries

Third-generation (3G) biorefineries aim to utilize microbial cell factories to convert renewable energies and atmospheric CO_2 into fuels and chemicals in a carbon-neutral manner. The 3G biorefineries aim to use a microbial cell factory to transform renewable energy and CO_2 into algae biomass that can be used to produce a wide variety of food, feed, pharmaceuticals and chemicals, and biofuels. This is the transition from lignocellulosic raw materials (2G biorefinery) to the use of CO_2 for the production of biofuels and bio-chemicals (3G biorefinery).

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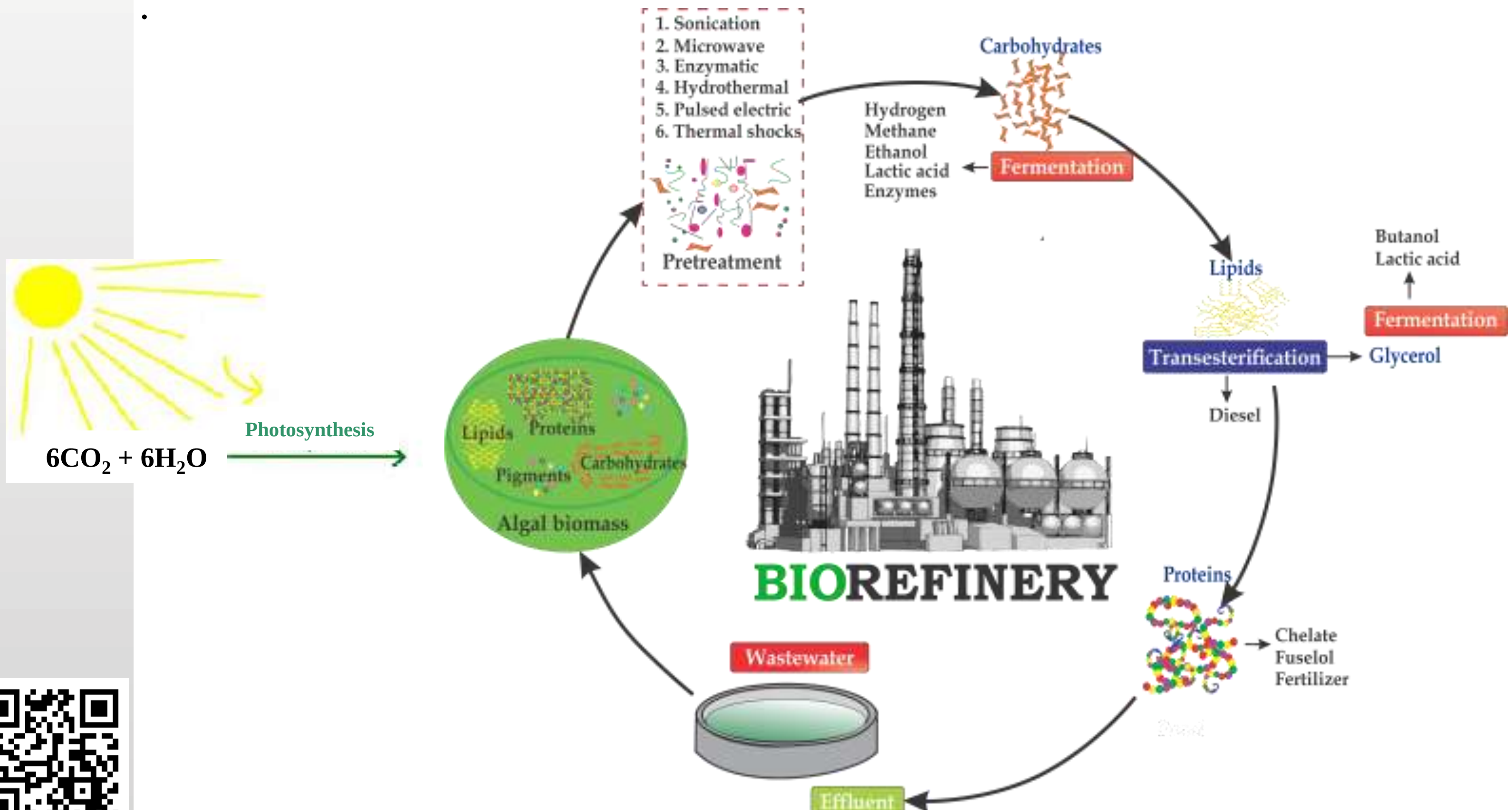

Production stages in biorefinery 3G

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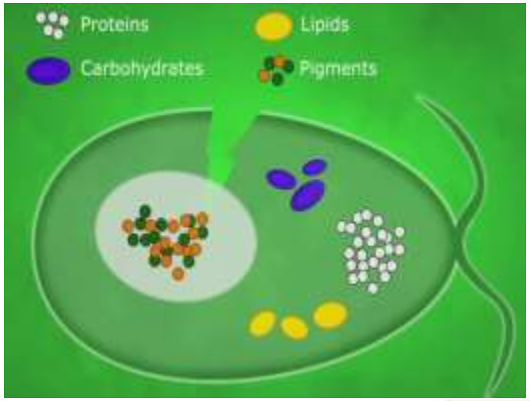

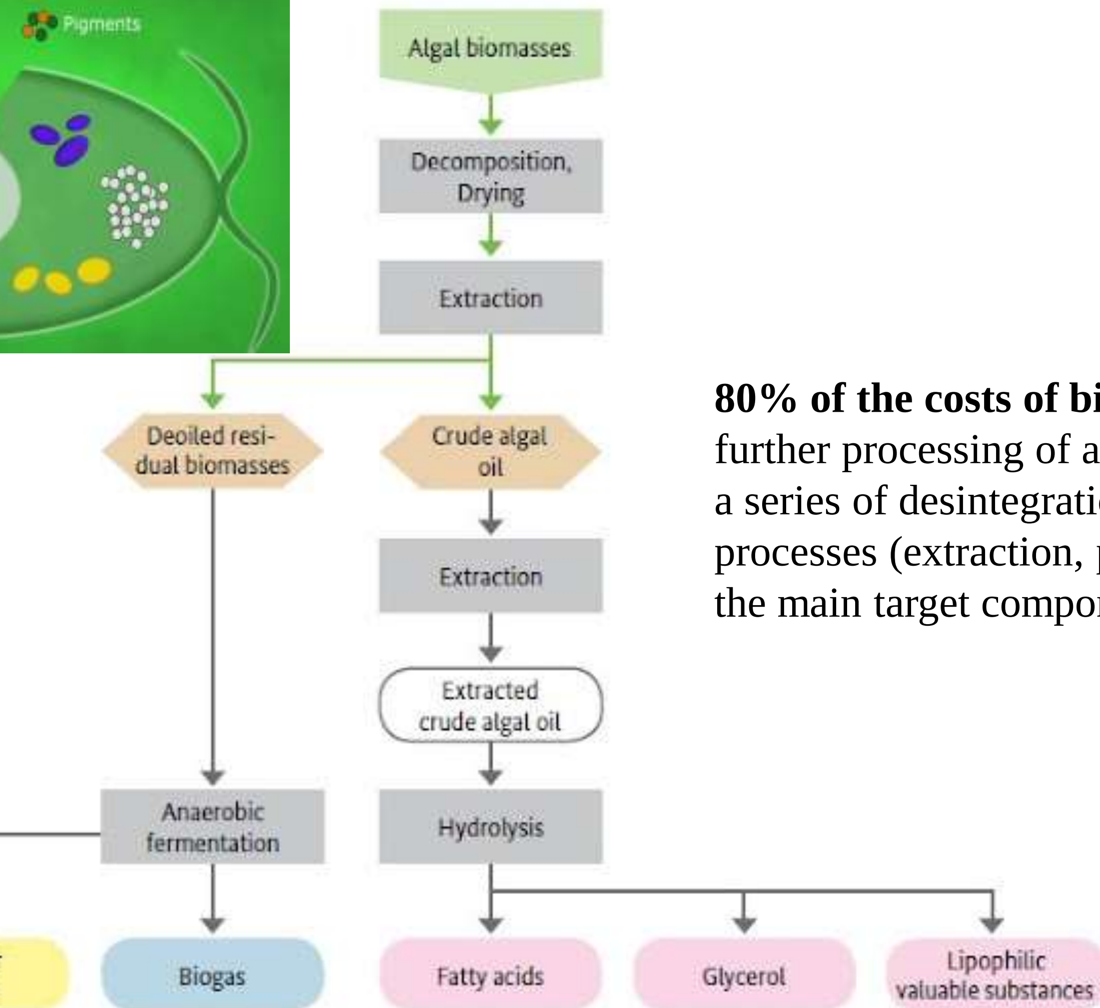

80% of the costs of biorefining - further processing of algae result from a series of desintegration and separation processes (extraction, purification of the main target component).

The algae bioproduct is a **crude oil** that is hydrolyzed to obtain **fatty acids, glycerol & lipophilic** substances. The de-oiled residual biomass undergoes anaerobic digestion to produce **biogas** fertilizer digestate.

Viridos & ExxonMobil scale-up algae biofuels production

Synthetic Genomics now **Viridos**

was founded in 2005 by leaders in synthetic biology, Craig Venter and Hamilton Smith. With the support of **ExxonMobil**, they developed the California Advanced Algal Facility (**CAAF**) in the **Imperial Desert** as the initial pilot facility to test algae strains to move us toward commercialization. In the past 5 years, Viridos has achieved greater than 5x oil productivity growth by increasing both the oil content in the algae and the algae yield, due to discovery of the "**lipid switch**" in microalgae (publication in 2017) that enabled both the growth and production of lipids - a seminal breakthrough.

https://www.viridos.com/technology/

Potential of the G4 biorefinery examples include:

•Optimization of use of the residual carbohydrate and protein rich biomass after algae oil extraction – a valuable and sustainable resource with uses as fuel, feedstock & animal feed.

•Bioengineering of microalgae to suppress CH_4 emissions in ruminants

•Replacement of seafood protein with sustainably grown microalgae protein

•Replacement of fish oils, which are really algae oils, with sustainably grown algae oils

•Bio-polymers from microalgae

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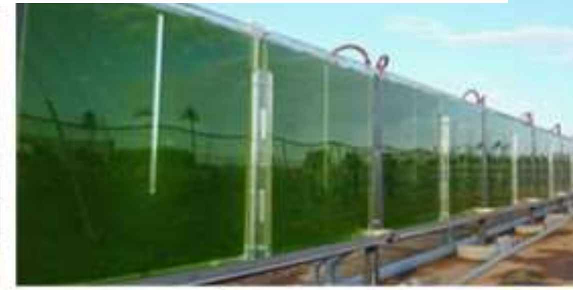
Photobioreactor configurations

Tree-shape PBRs

A pilot cultivation including 20 bags (20 cm in width and 2 m in length and a volume of 16 L) made from PE and fixed on a metal stand.
Huang et al. Engineering 3 (2017)318

(a) Open raceway pond ORP

(b) Plastic plate photobioreactor



(c) Inclined tubular *PBR*

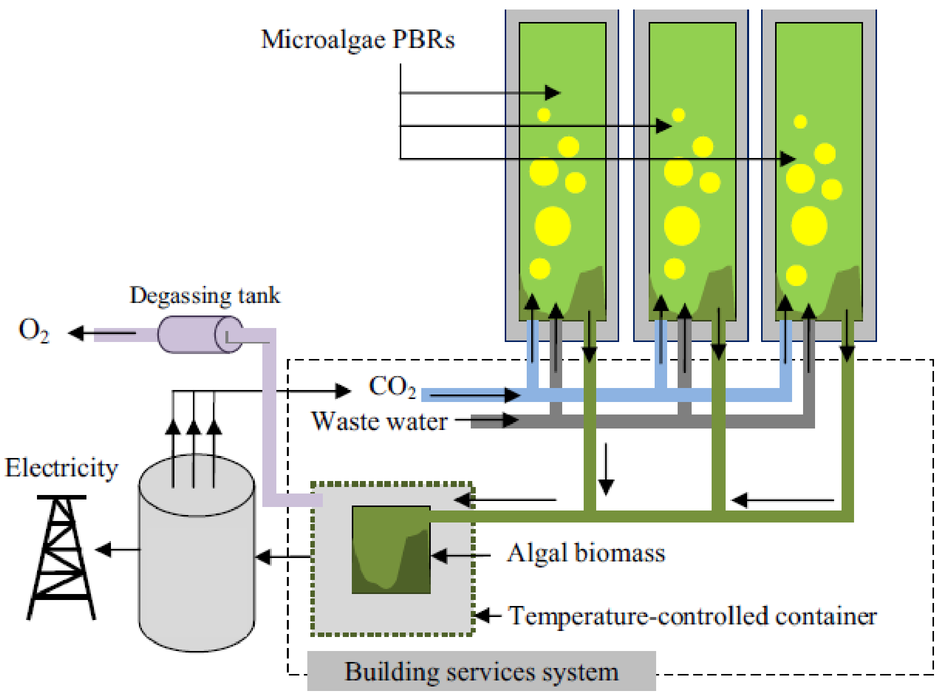
(d)Tubular glass PBR

Algae cultivation includes open, closed and hybrid systems.
The open system is a circulation pond (with circulation pumps and distribution pipelines).
A closed photobioreactor (PBR) system is a transparent bioreactor (pipes, panels, bags or columns, flat plates).
PBR provides better breeding control with regard to nutrient dosing, water supply, gas mixing, protection from contamination and evaporation loss.
Higher algae productivity in PBR compared to ORP systems.

World's First Algae Bioreactor Facade

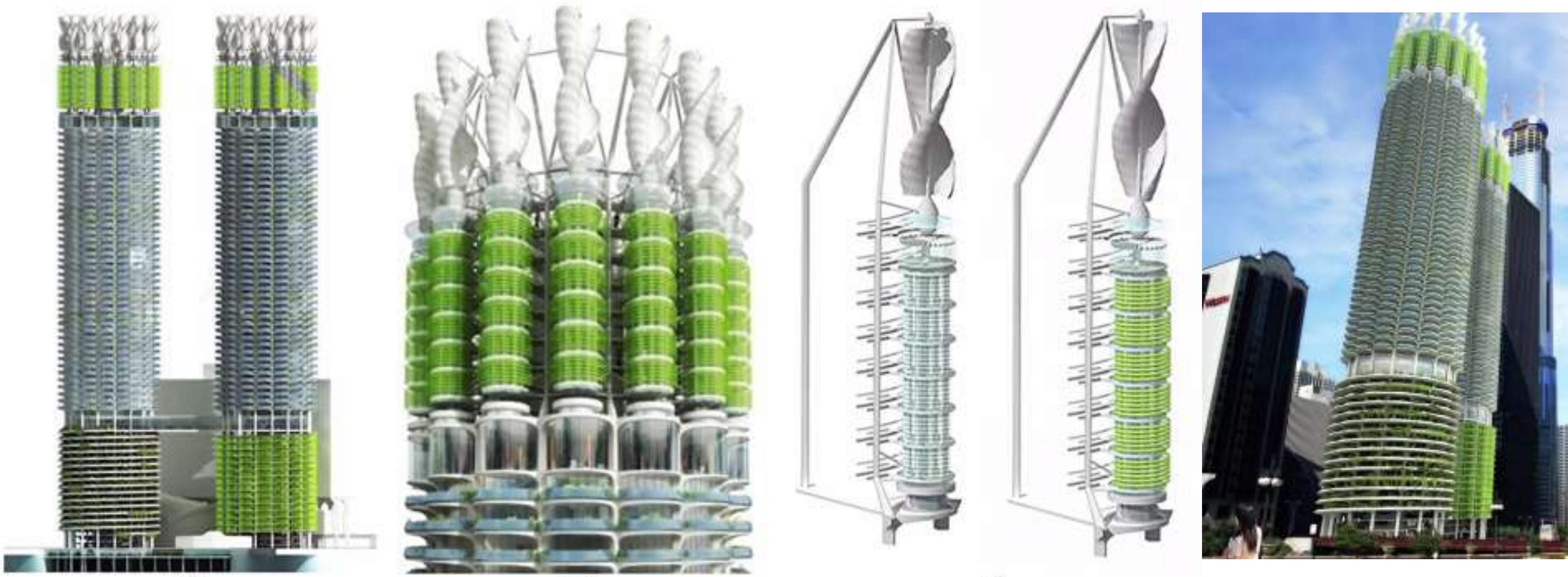
Bio-Intelligent Quotient (BIQ) *w Hamburgu*, (129 PBR panels of Volume 24L each)

https://www.dezeen.com/2013/04/15/arup-unveils-worldsfirst-algae-powered-building/



Infrastructure:
1. CO_2 inflow;
2. Nutrients dosing;
3. Filtration and harvesting of biomass
4. Monitoring i regulation of temperature and circulation of medium
5. Heat recovery and distribution;
6. Biomass transport to biogas plant and coversion to biogas and electricity

Algae Green Loops as Bio-facade

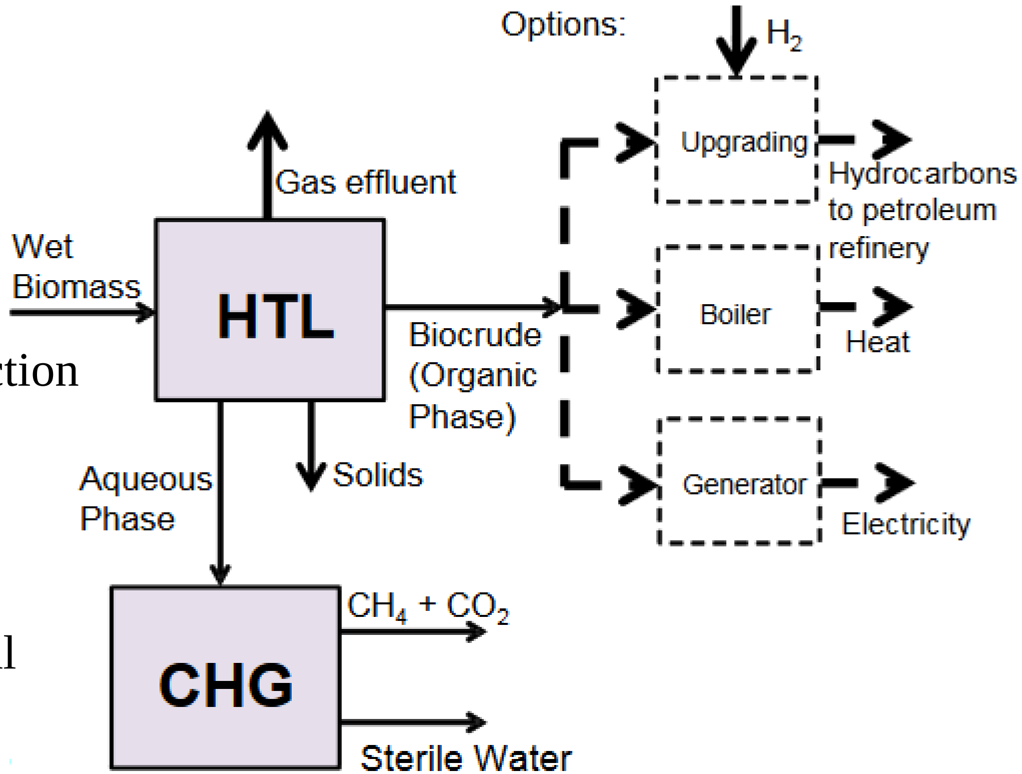


a) b) c) d)

a) **The Marina City's Towers -** Chicago DeCarbonization Plan;
b) Installation of photobioreactor panels
c) The energy-efficient capture of CO2 from ambient air by wind power turbines (enhance the CO_2-scrubbing device air flow and providing with electricity power
d) Photo

http://www.archdaily.com/191229/algae-green-loop-influx-studio

Hydrothermal process technology



HTL = Hydrothermal Liquefaction

CHG = Catalytic Hydrothermal Gasification

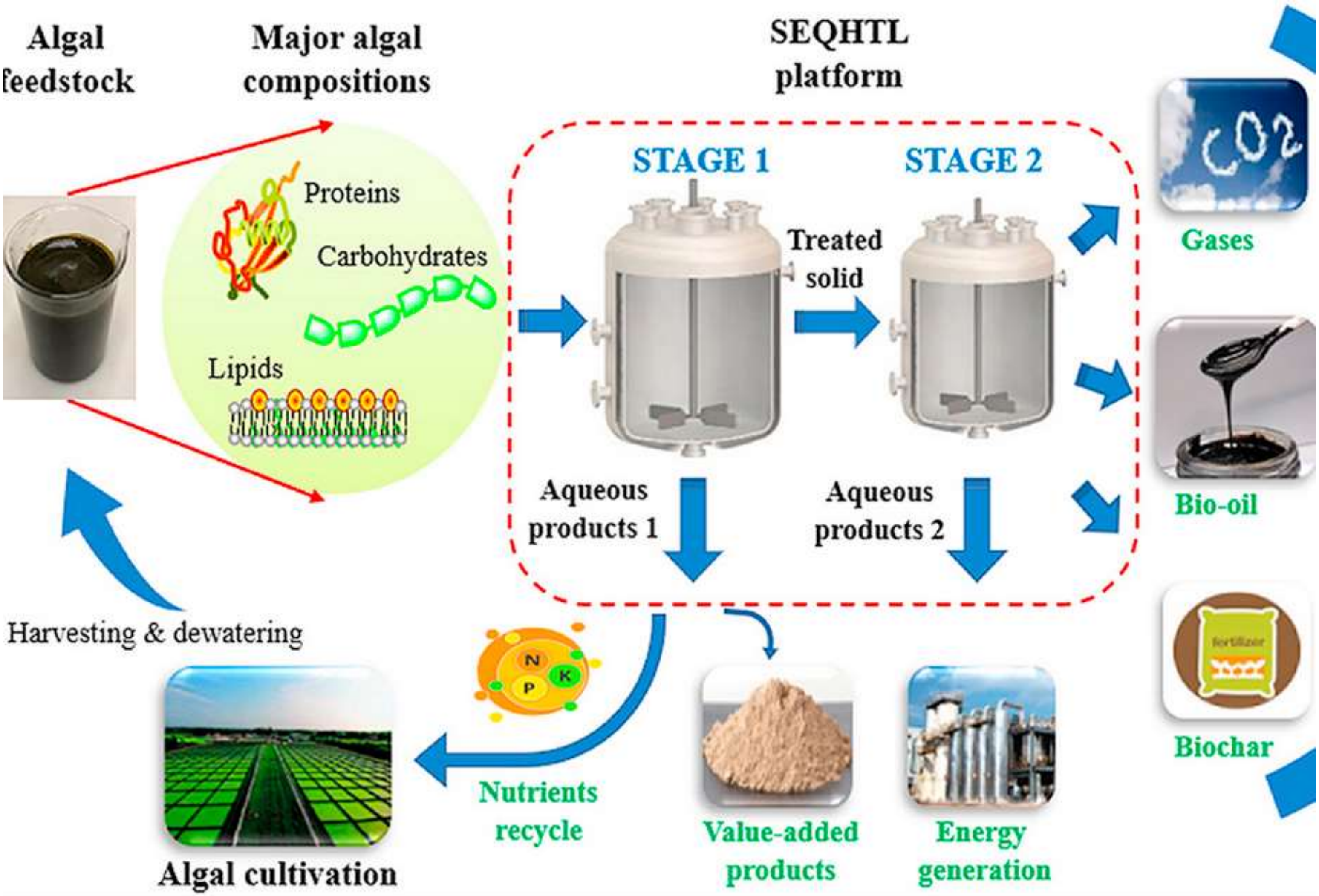
HTTP is a thermochemical process where water is used as the Medium for the breakdown and reconstituting of organic matter into relatively simpler chemical compounds at **elevated temperatures (350ºC) and pressures above 20MPa**.

– No drying of feed needed

– Utilizes all components of feed

Two-stage sequential HTL

Temp. 220–240 °C Pressure 0.8–3.5 MPa



X. Gu et al Recent development of hydrothermal liquefaction for algal biorefinery Renewable Sustainable Energy Reviews 121 (2020) 109707
https://doi.org/10.1016/j.rser.2020.109707

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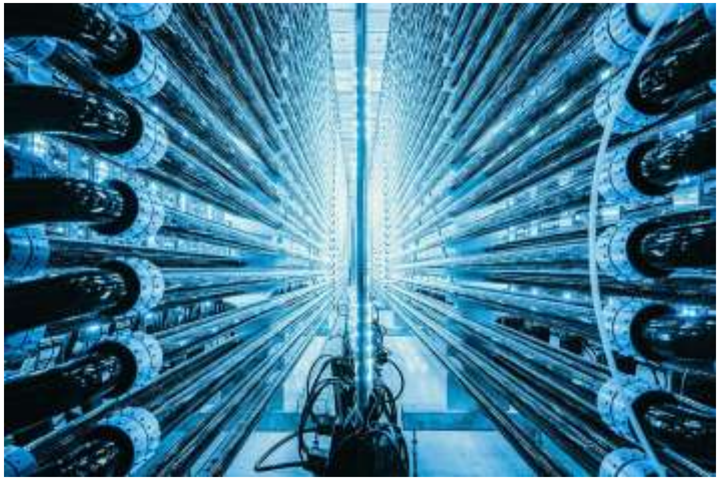
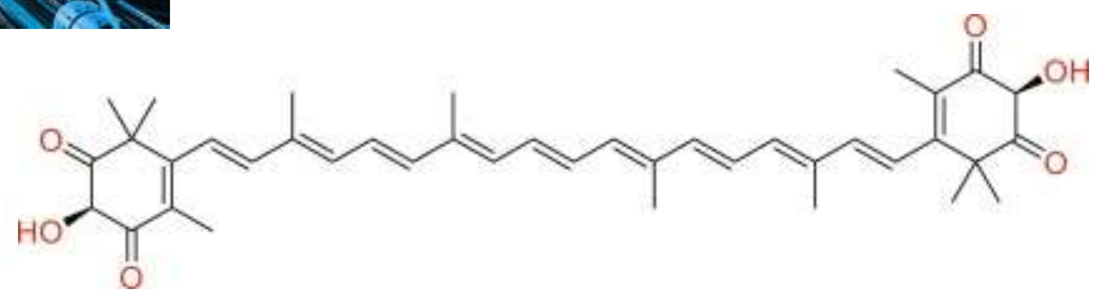
https://www.wplgroup.com/aci/event/european-algae-industry-summit/

Algalif Iceland –astaxanthin

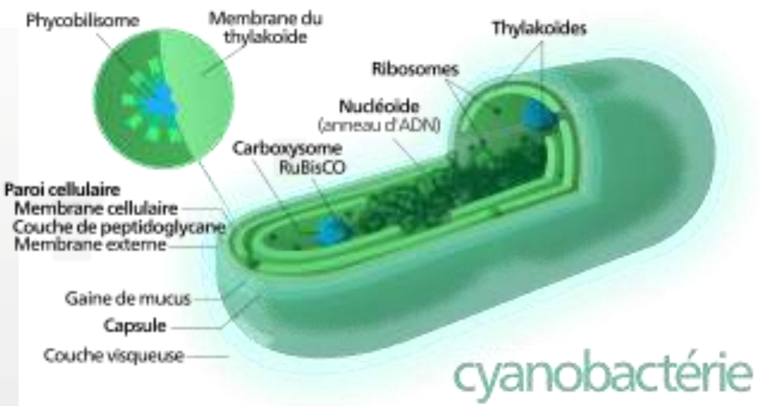


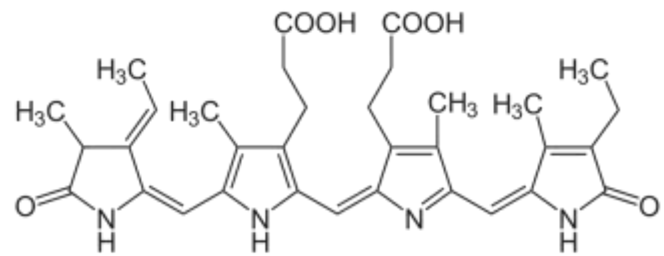
At AlgalifTM, Iceland, astaxanthin is obtained from the cultivation of the microalgae *Hematococcus pluvialis* (green algae) in tubular photo-bioreactors using LED lamps.
https://algalif.is/a-photo-taken-at-algalifs-microalgae-plant-in-iceland-wins-international-awards/



Astaxanthin ($C_{40}H_{52}O_4$)) is classified as xanthophyll - oxygen derivatives of carotenoids, responsible for the red color of salmon and shrimp meat. For humans, astaxanthin is one of the most powerful natural antioxidants known, (free radical scavenger), hepato- and cardio-protective properties, reduces the level of pro-inflammatory cytokines in neurophiles that are supported by extensive scientific research, including human clinical trials. Astaxanthin is produced by the microalgae *Haematococcus pluvialis* as a defense mechanism against harsh environmental conditions.

Phycocyanin (C-PC) its role & application





Phycocyanin

C-Phycocyanin

C-PC occurs as the major phycobiliprotein in many cyanobacteria and as a secondary phycobiliprotein in some red algae. The pigment has a single visible absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~650 nm.

Phycocyanin (C-PC) is a soluble phycobiliprotein in cyanobacteria and is considered a high value product due to its brilliant blue color and fluorescent properties. The factor limiting the wider use of C-PC (from *Spirulina*, *Arthrospira platensis*) is the narrow range of its thermal and - pH stability. C-PC from *A. platensis* is denatured at > 45 ° C and outside the pH range 4–7, which causes loss of blue color, fluorescence and antioxidant properties and limits its use in the food, cosmetic and textile industries.

Price (EUR) €106/mg https://www.aatbio.com/products/c-pc-c-phycocyanin-cas-11016-15-2

Thermostability of C-PC

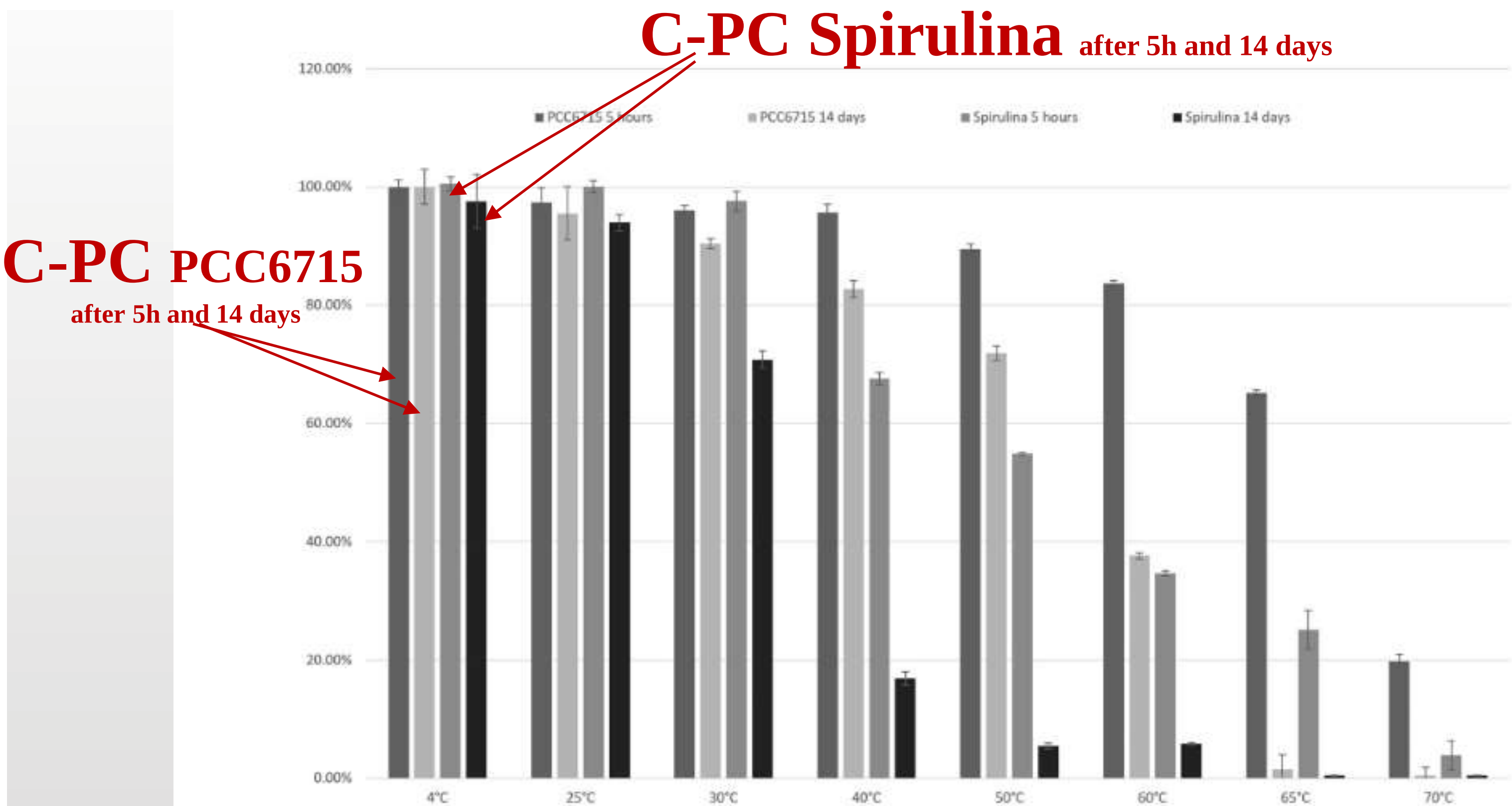


Fig. 6. Thermostability of purified PCC 6715 C-phycocyanin and control Spirulina C-phycocyanin after 5 h and 14-day incubations at selected temperatures. Data series are indicated in the figure legend.

Y. Liang, M.B. Kaczmarek, A.K. Kasprzak, J. Tang, M.M.R. Shah, P. Jin, A. Klepacz, J.J. Cheng, S. Ledakowicz, M. Daroch *Algal Research 2018:* Thermosynechococcaceae as a source of thermostable -phycocyanins:sources, properties and molecular insights. doi.org/10.1016/j.algal.2018.08.037

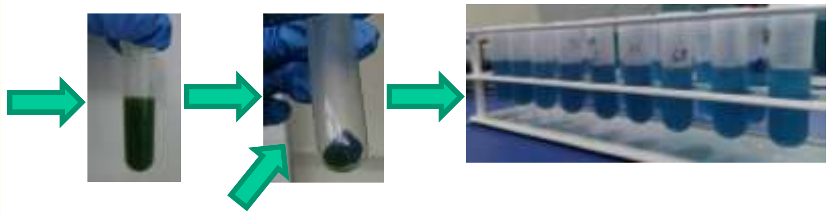
Photo-biosynthesis and down-stream processing of thermostable phycocyanin C-PC

Project NCN, No. 2018/31/B/ST8/00822

- Optimisation of microalgae growth
- Biomass disintegration & recovery of bioproducts (Distraction)
- Downstream processing (DSP) of C-PC
- Characteristics of purified final product

Bioreactor Labfors 5 Lux

Synechococcus sp. PCC6715



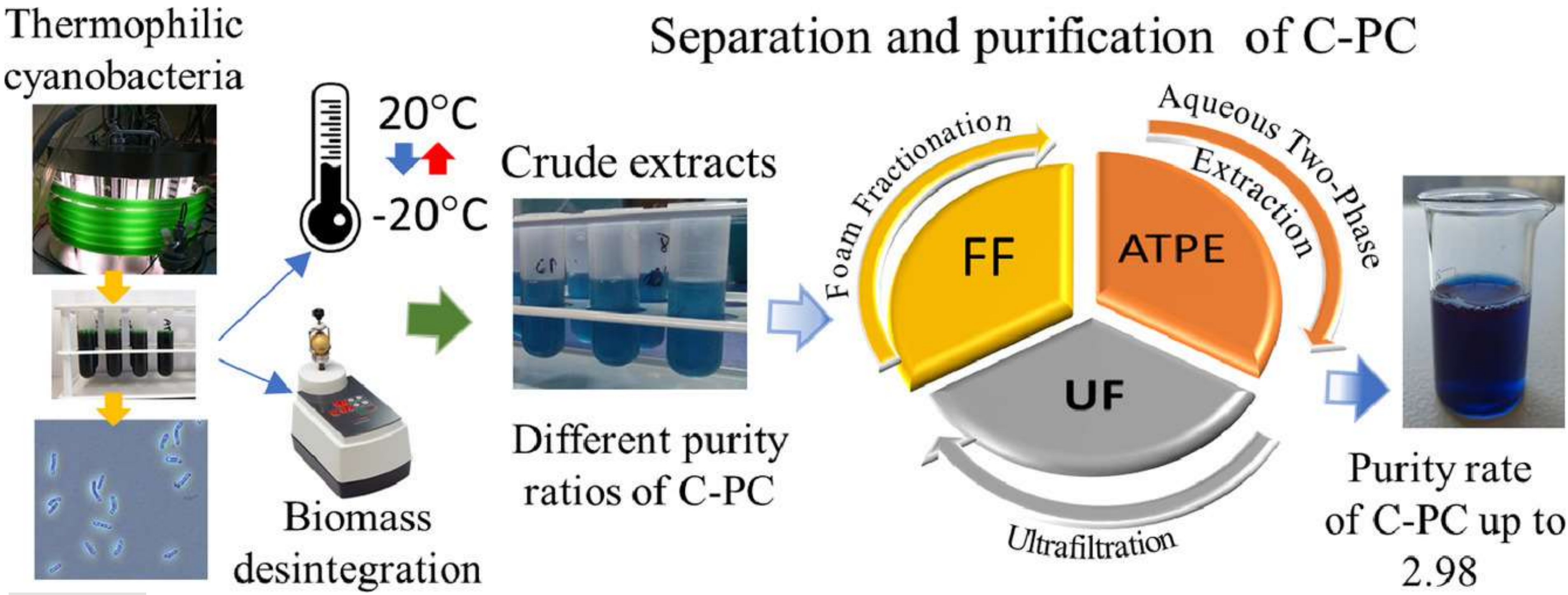
PBS

Helicoid - BIOSTAT® PBR 2S
Sartorius Stedim Biotech

P. Głuszcz, A. Klepacz, S. Ledakowicz: Chemical & Process Engineering 2018, 39 (4), 457 Experimental evaluation of a helical laboratory photobioreactor for the cultivation of thermophilic cyanobacteria – hydrodynamics and mass transfer studies.

A. Klepacz-Smółka et al.: Bioresource Technology 313 (2020) 123700 Effect of light colour and photoperiod on biomass growth and phycocyanin production by *Synechococcus* PCC 6715

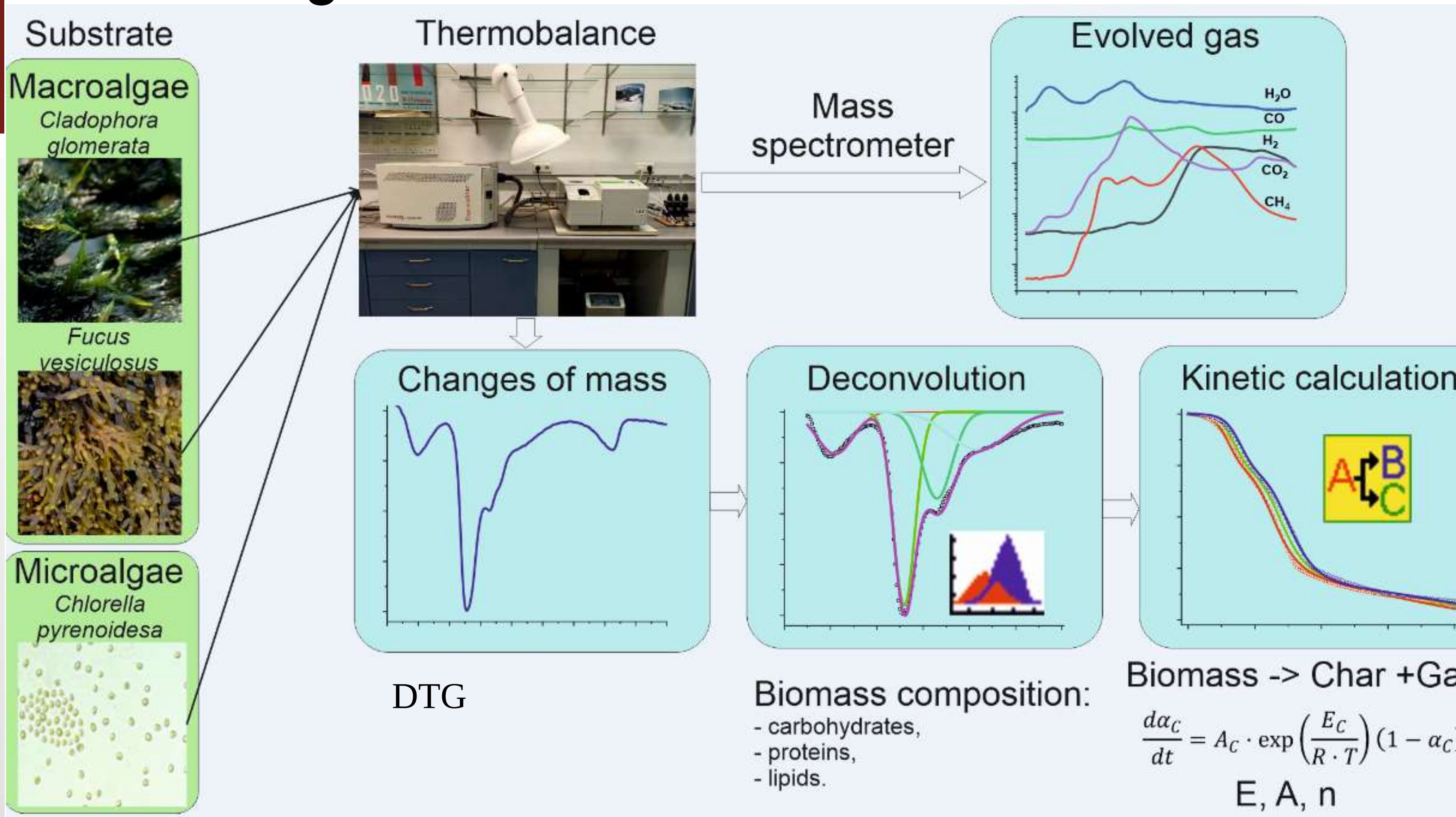
Comparison of phycocyanin separation and purification methods



Dextraction of crude C-PC is the most effective method of freezing and thawing biomass in BG 11 buffer.
The following techniques were used to purify thermostable C-PC from Synechococcus sp. PCC6715:
Foam fractionation (FF): recovery efficiency 49%, purification factor PF = 1.472
Aqueous two-phase extraction (ATPE) PEG 6000 - phosphate salt: recovery efficiency 97%, PF=1.466
Ultrafiltration (Hydrosart 10 kDa membrane): recovery efficiency 92%, PF=1.472
Fast FPLC protein liquid chromatography gave PF = 3.4 pharmaceutical purity

A. Antecka et al. Comparison of three methods for thermostable C-phycocyanin separation and purification *CEP - Process Intensification 171, 2022, 108563* https://doi.org/10.1016/j.cep.2021.108563

Pyrolysis and gasification of micro- & macro-algae



The deconvolution of DTG curves allows to follow the change in the composition and the rate of thermochemical decomposition of individual components of various algae species and, along with the composition of gaseous products (from MS) to determine 3 stages of the process: **evaporation, pyrolysis and auto-gasification**, and to identify the parameters of the kinetic model, which facilitate the design of 3rd generation biorefineries .

R. Ślęzak , S. Ledakowicz. *Algal Research*, *2022* Pyrolysis of micro- and macro-algae in thermobalance coupled with MS. *in print.*
Financial suport of Research Centre for Low- carbon Energy Technologies Project Nr.CZ.02.1.01/0.0/0.0/16_019/0000753
Czech Technical University in Prague, Faculty of Mechanical Engineering

Conclusions

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1. **The profitability of 3G biofuels production is questionable**. There is no commercial installation for the production of biofuels from algae. In order to be profitable the algae production efficiency must be 3 times higher and the costs 10 times lower.

2. Currently, microalgae are the most economically promising source of **nutraceuticals**, bioactive medical and food products such as proteins, pigments, along with vitamins etc. **The high added value products can improve the economics of 3G biorefineries**.

3. **Biofasades** clean the atmosphere in cities from harmful CO_2 and generate O_2. The integration of biofasides into the biorefinery system is the main concern. Flat plate photobioreactors proved to be advantageous in architectural design, but the **payback period ranged from 9 to 13 years.**

4. **Algae should be grown in non-agricultural areas (deserts).** The production of biodiesel and bioproducts from algae will be profitable in the future when the **expectations of geneticists and bioengineers from Viridos will come true.**

5. **Shifting the focus of study on algae from biofuels to products with high added value**, (e.g. phycocyanin or astaxanthin), and **integration** of **bio- & thermo-chemical platforms of** can **ensure the profitability of 3G biorefineries.**

6. **Optimization of photobiosynthesis** of thermophilic microalgae *Synechococcus* sp. Separation and purification of phycocyanin allowed to achieve **pharmaceutical purity level of thermo - and pH - stable phycocyanin**.

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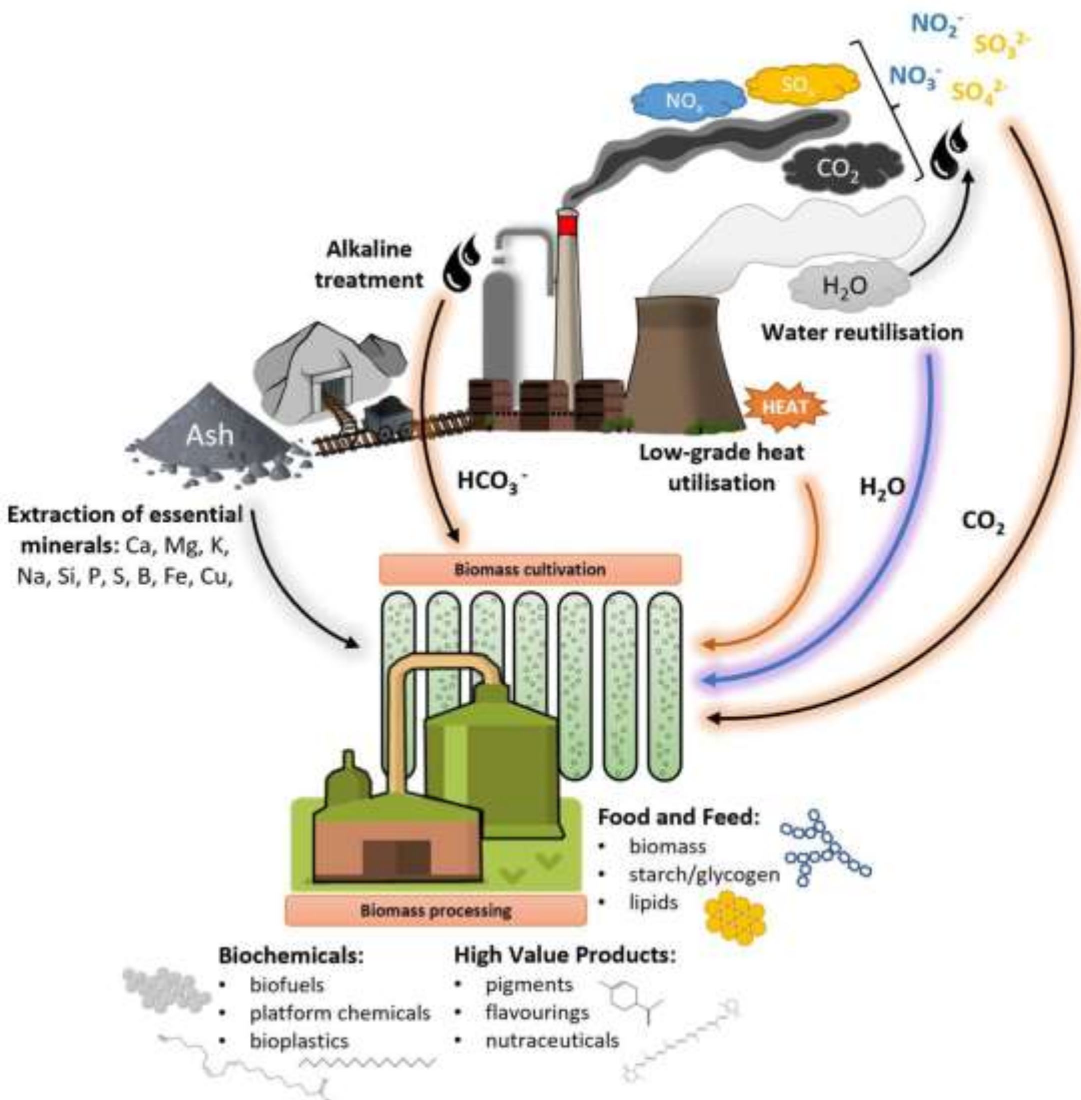
Acknowledgements

to my coworkers realising the the NCN project No. 2018/31/B/ST8/00822

Photo-biosynthesis, extraction and purification of thermostable phycocyanin

Acknowledgement
National Science Centre Poland Project NCN, No. 2018/31/B/ST8/00822
Green Deal strategies for V4 countries: The needs and challenges to reach low-carbon industry. The International Visegrad Fund's Strategic grate No.22120032 (1.1.2022 - 31.12.2023)

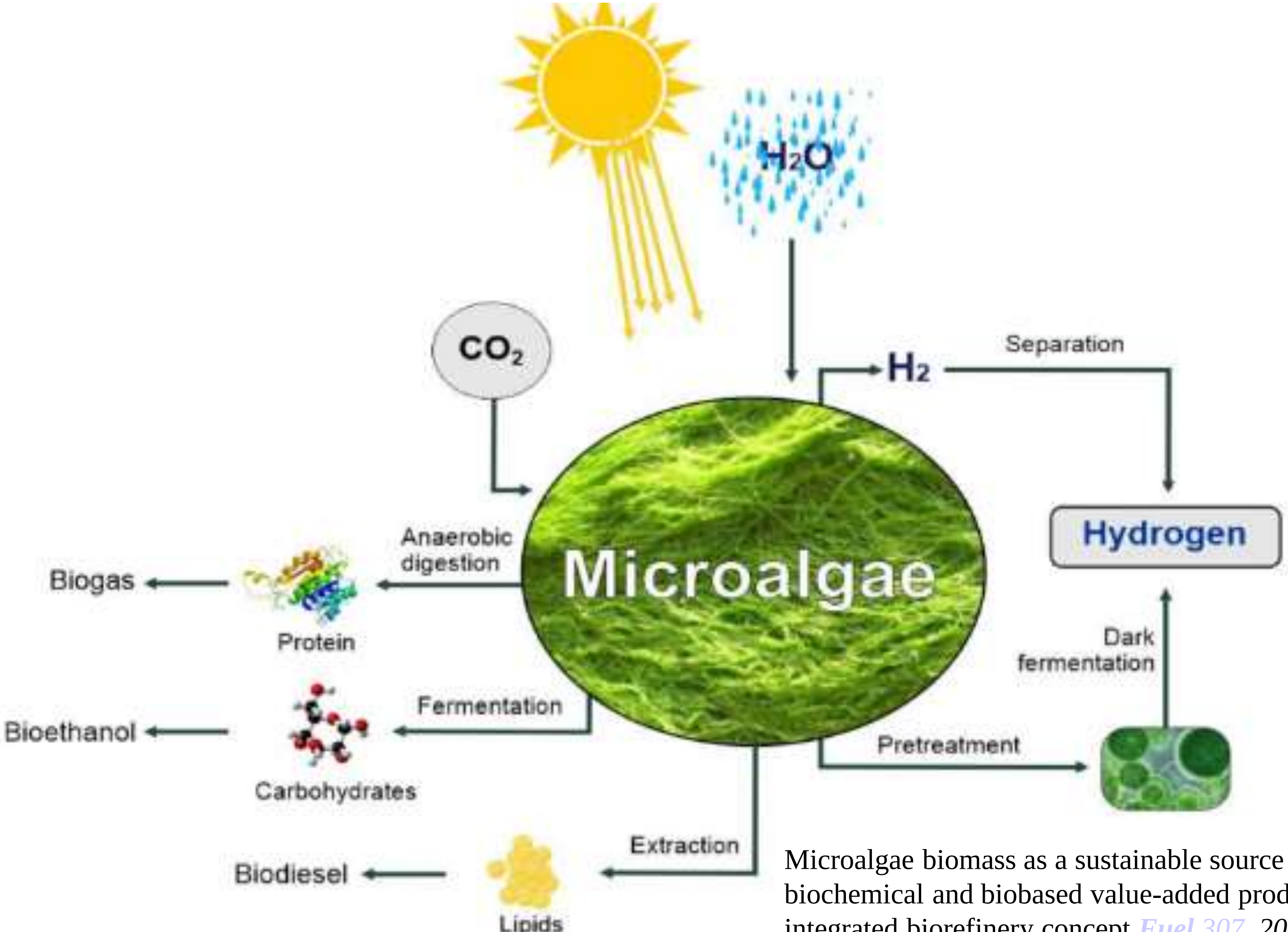
Power industry waste microalgal valorisation scheme



„Integrating microalgal technology for waste stream valorisation and closing biorefinery loops in the power industry"
A. Klepacz-Smółka et al. submitted to Critical Reviews in Biotechnology 2022 ,

The application of microalgae to close potential power industry biorefinery loops. **Flue gasses** utilization, **combustion ashes**, and **cooling water** were analyzed for their **applicability** as **feedstocks and supplementary streams** to assist the **cultivation of microalgae**. Using dissolved inorganic carbon such as **bicarbonate** mitigates these three challenges: first CO2 can be stored for a long time as a bicarbonate solution, second, even CO2 emissions from intermittent sources can be captured, and third, the issues of temperature and carbon delivery are decoupled in the bicarbonate system.

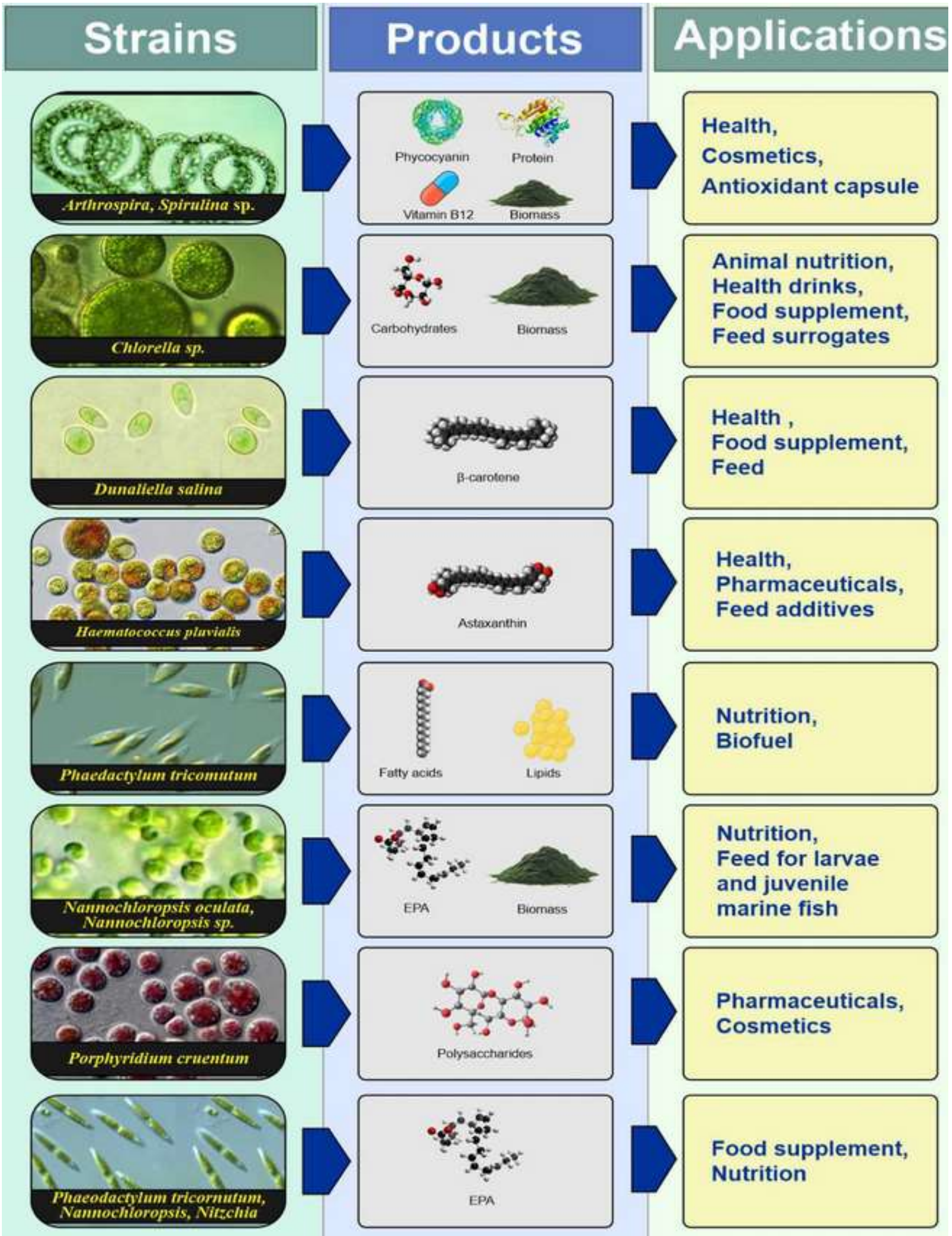
Potential application of microalgae in producing biofuel through anaerobic digestion, fermentation, transesterification process



Microalgae biomass as a sustainable source for biofuel, biochemical and biobased value-added products: An integrated biorefinery concept *Fuel 307, 2022, 121782*

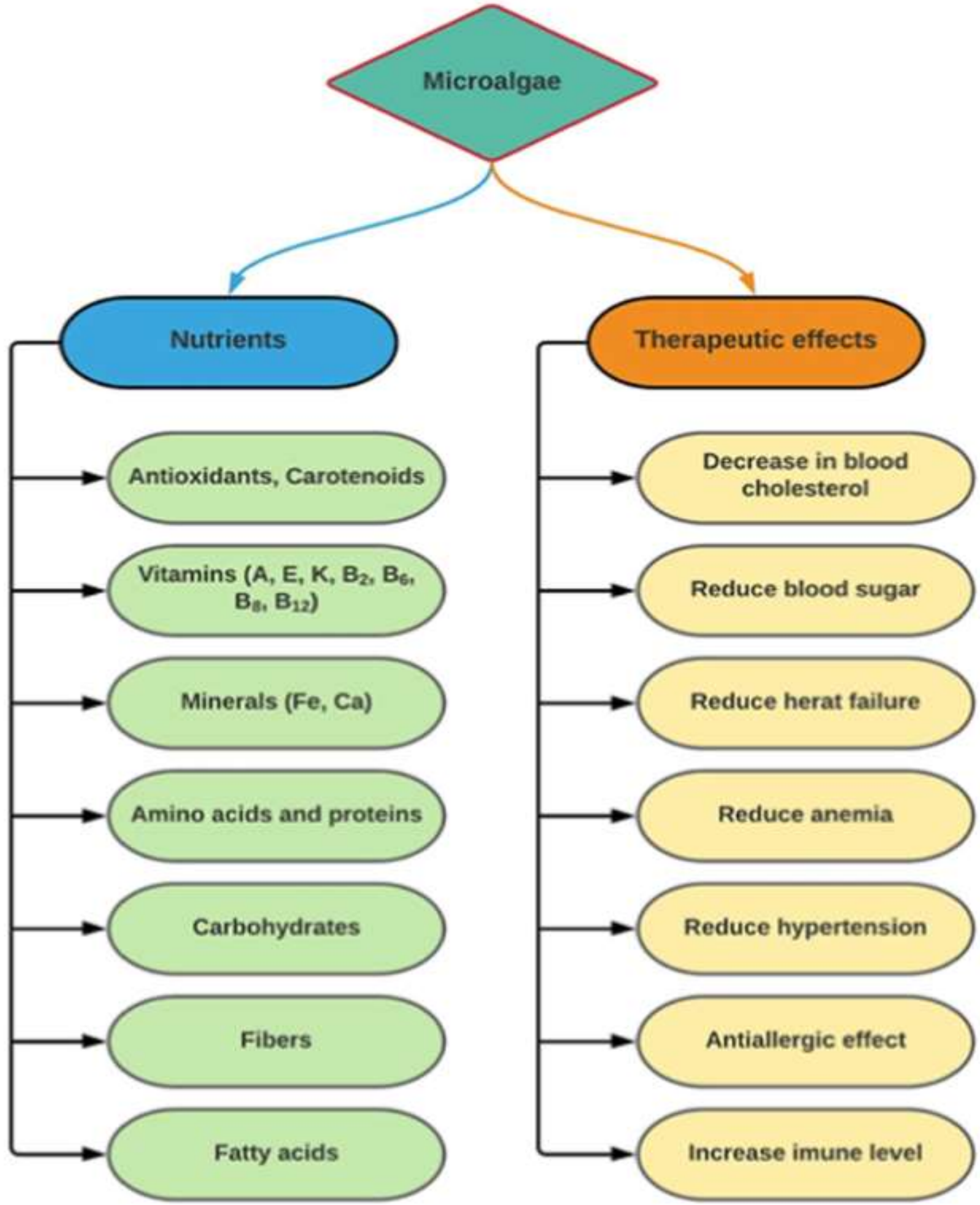
Microalgae species have oil content. There is up to 50% lipids in *Chlorella*, with *Botryococcus braunii* having the highest oil content at ca. 80%, they produce various bioactive compounds that are used in nutraceuticals, the chemical and food industries, and pharmaceuticals.

Application of microalgae to generate value-added products



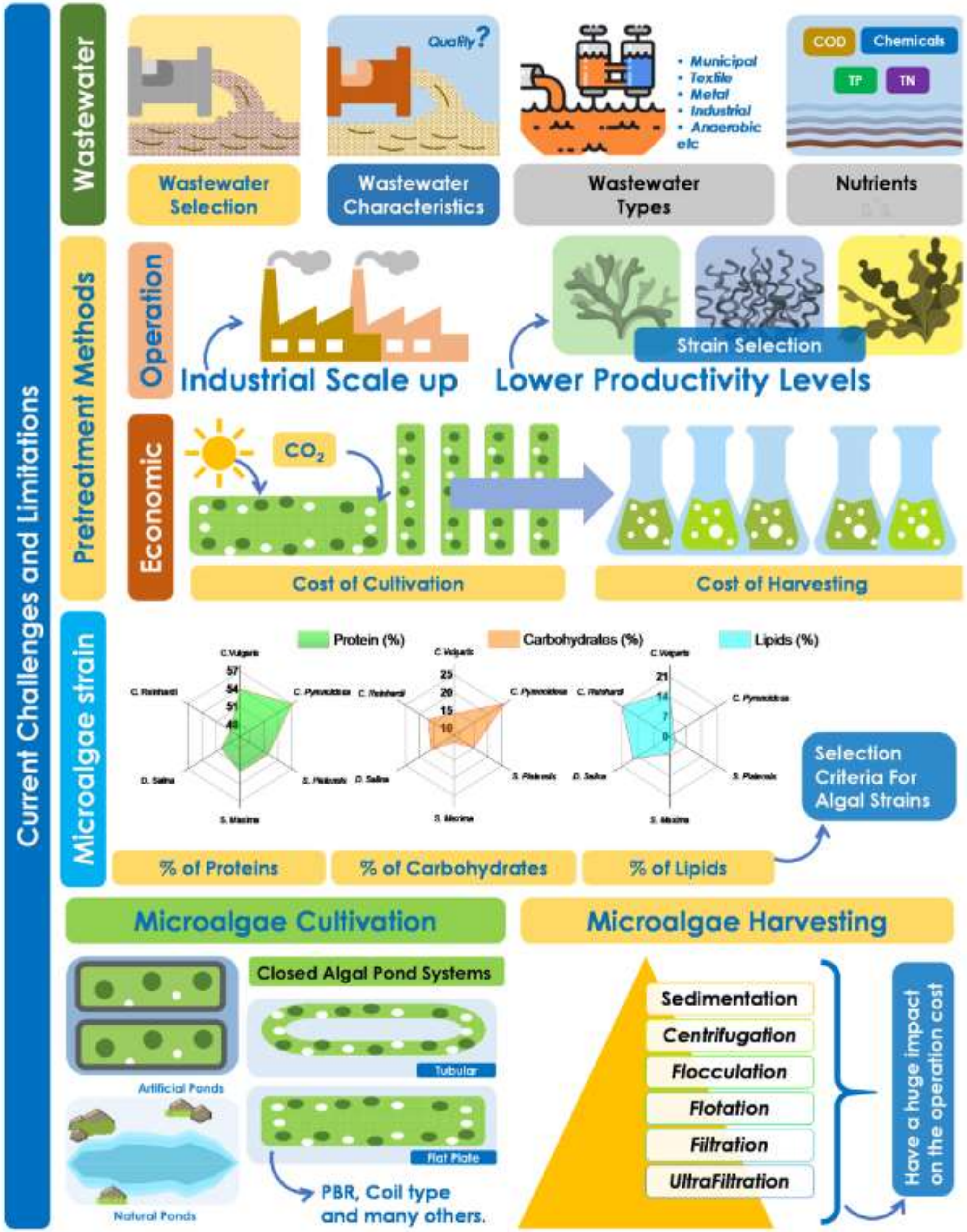
Fuel 307, 2022, 121782

Microalgae offer several benefits as medicine including immune level improvement, reduce anemia, heart failure, hypertension etc.



Fuel 307, 2022, 121782

Challenges & limitations in algae use as wastewater treatment pathway



The estimated break-even selling price of biofuel from the process was estimated to be **0.58 $/kg**.

Wastewater treatment and biofuel production via algal cultivation resulted in the replacement of 4.6 million kg of CO2 –equivalent.

The authors discussed the potential cost reductions using **open ponds** for mass cultivation of microalgae in comparison to **PBR** using wastewater and concluded that a raceway treating wastewater and using monochromatic wavelengths can reduce costs by up to 73.0% from an initial cost of **2.71 $/kg** biomass. There is a lack of literature that can provide an economic outlook on wastewater treatment via algae and further full-scale studies are required to analyze the potential gains from this method.

Valorization of algal cells for biomass and bioenergy production from wastewater: Sustainable strategies, challenges, and techno-economic limitations
Ren. Sust. Energy Rev. 157 (2022) 112024