ALGAE BIOREFINERY: PROTEIN FOR NON-FOOD APPLICATIONS

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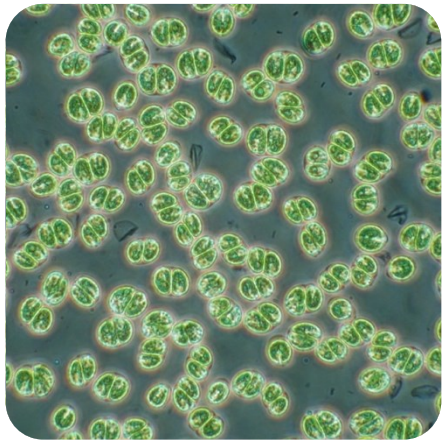


MIRACLES
SPECIALTIES FROM ALGAE

FOOD & BIOBASED RESEARCH
WAGENINGEN UR

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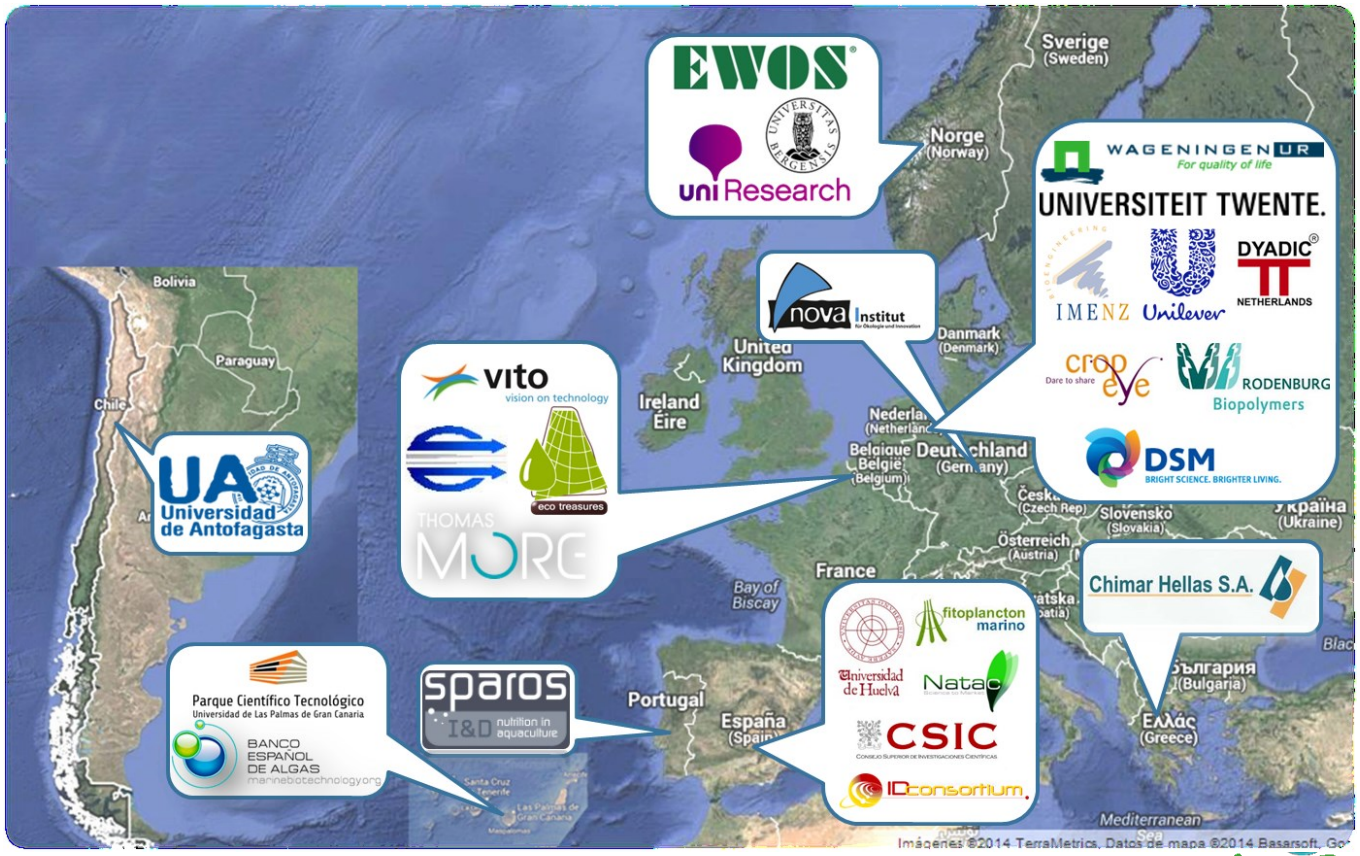
Introduction

- **M**ulti-product **I**ntegrated bio**R**efinery of **A**lgae: from **C**arbon dioxide and **L**ight **E**nergy to high-value **S**pecialties: **MIRACLES**



Introduction

- Wageningen UR Food & Biobased Research

- Food & Biobased Research
  - Fresh, Food & Chains
  - Biobased Products

- Biobased Products
  - Biobased Chemicals
  - Biobased Materials
  - Biorefinery & Bioenergy

Introduction

- Biobased Chemicals
  - Chemicals derived from biomass (e.g. plants)

- Biobased Materials
  - Research and development of materials and products like paper, construction material and plastics based on renewable resources

- Biorefinery & Bioenergy
  - Chemical, thermal and enzymatic fractionation of biomass for the production of biobased intermediates
  - Production of biofuel and chemicals by fermentation

Introduction

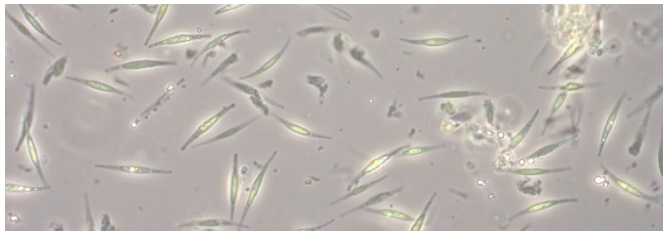
- Why protein?
  - Non-stressed algae contain 30-60% (w/w dw) protein

- Non-food applications
  - Adhesives
  - Coatings
  - Surfactants
  - Chemical building blocks

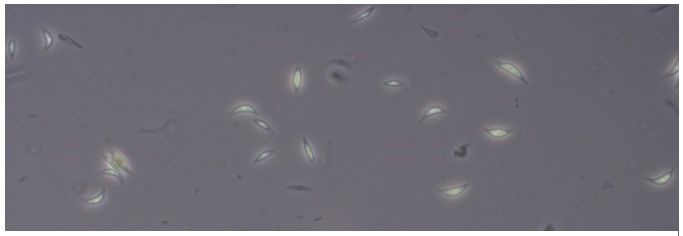
- Aim
  - Purification and characterization of algae protein

Characterization algae strains

- Algae strains (non-stressed)
  - *Isochrysis galbana*
  - *Nannochloropsis gaditana*
  - *Phaeodactylum tricornutum*
  - *Scenedesmus obliquus*



P. tricornutum 400×

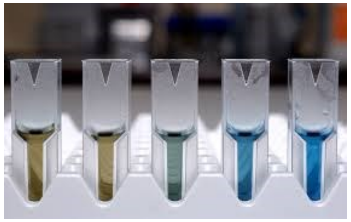


S. obliquus 400×

- Biochemical characterization
  - Proteins
  - (Poly)saccharides
  - Lipids/oil
  - Ash

Characerization algae strains

- Protein content can be determined by the total amino acid content
- Total protein: Kjeldahl or DUMAS (N-to-protein factor)
- Soluble protein: Lowry, BCA, Sedmak, Bradford etc.

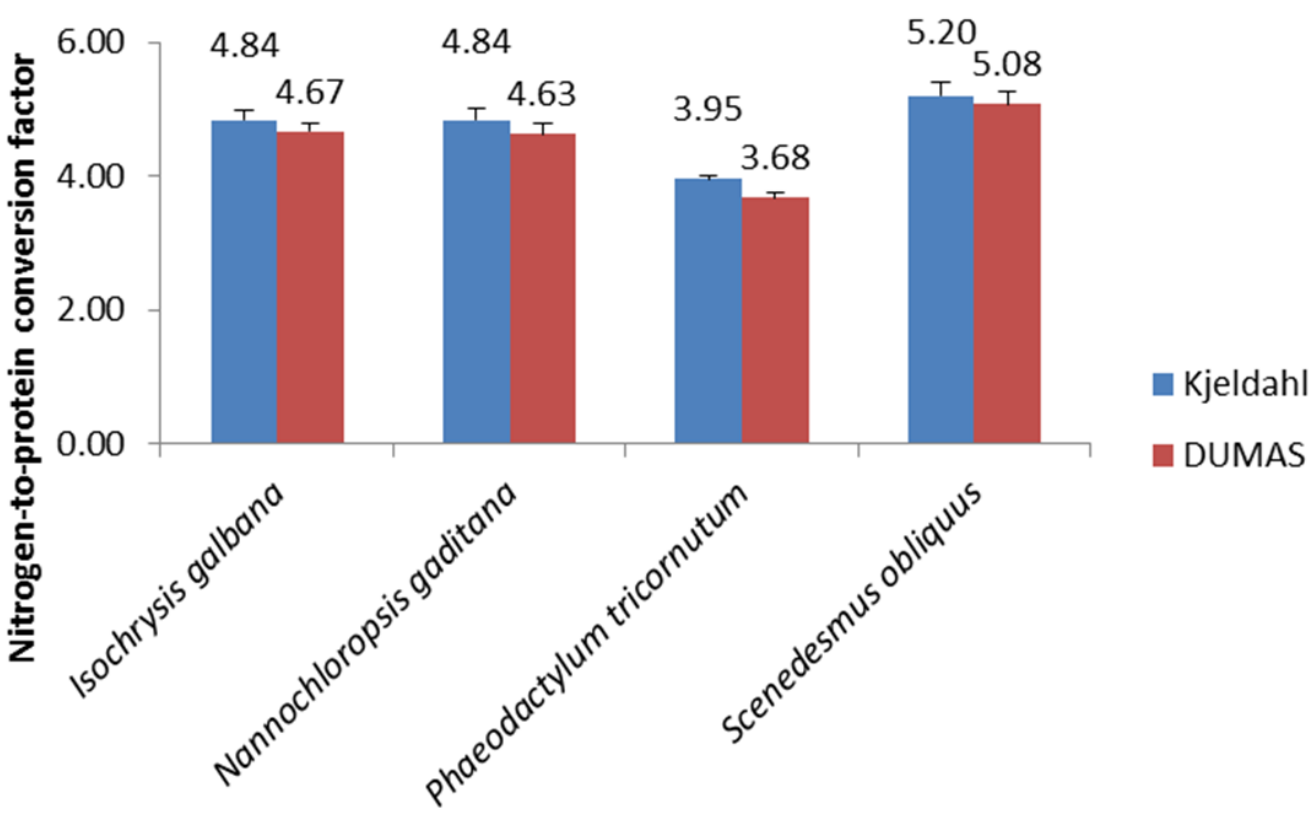


Characterization algae strains

- Amino acid composition

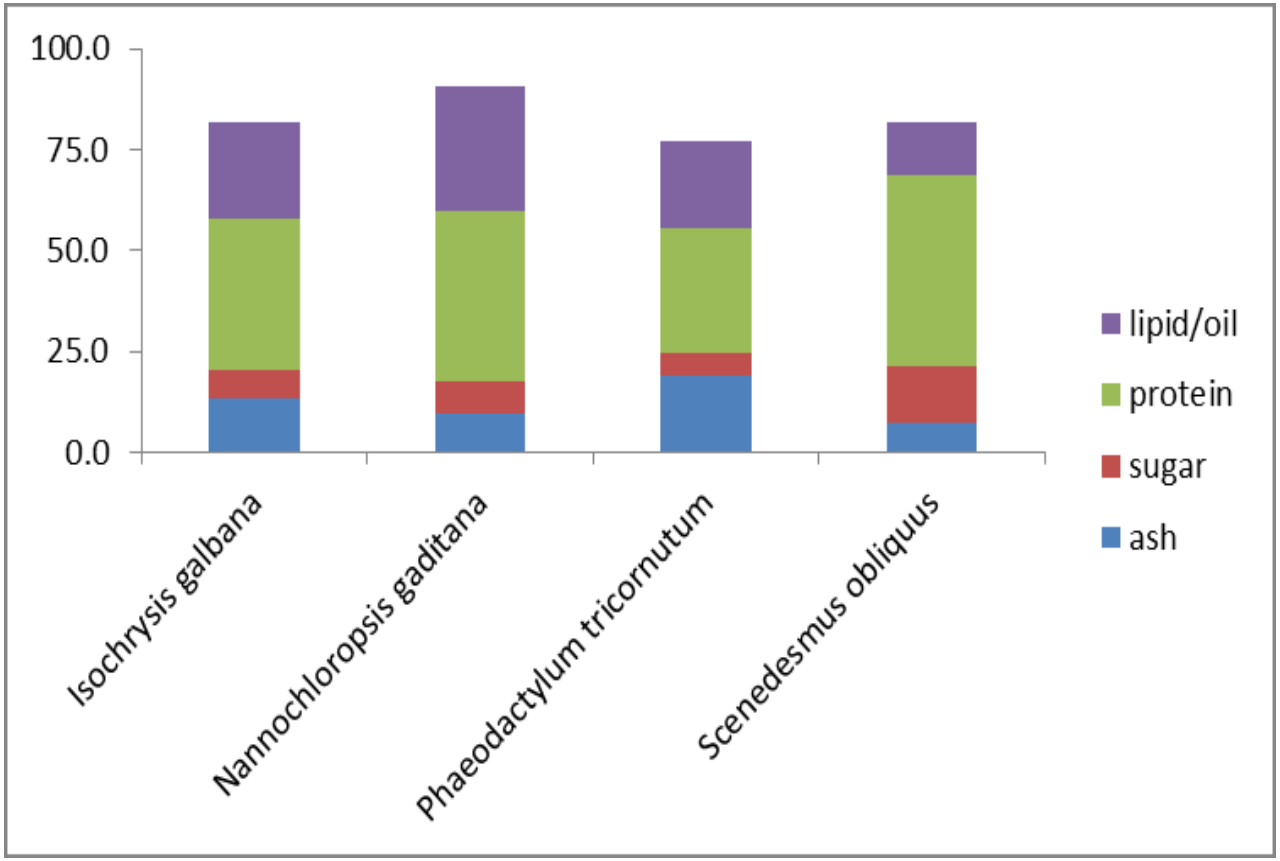
Characterization algae strains

- N-to-protein conversion factors



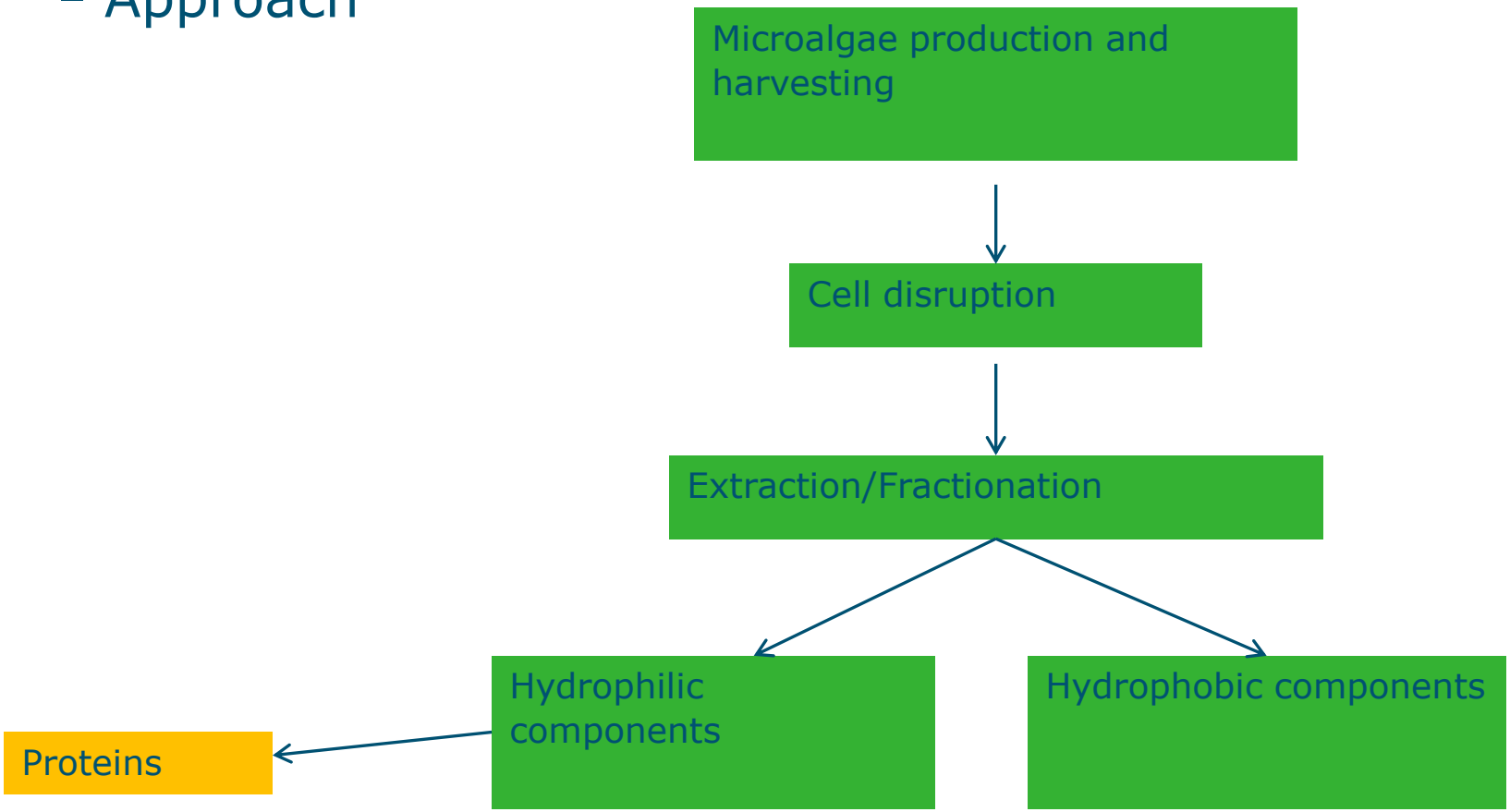
Characterization algae strains

- (Bio)chemical composition



Biorefinery

- Approach



Biorefinery: cell disruption

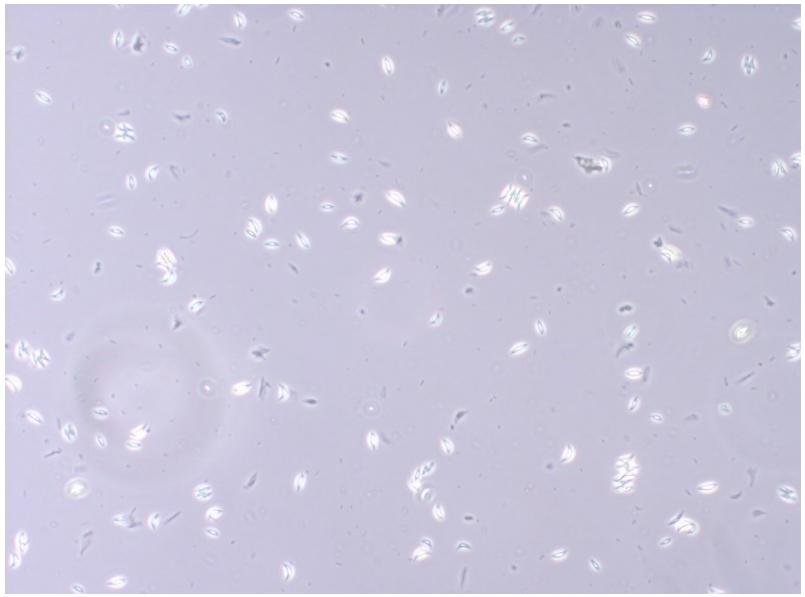
- Mechanical disruption techniques

Bead mill

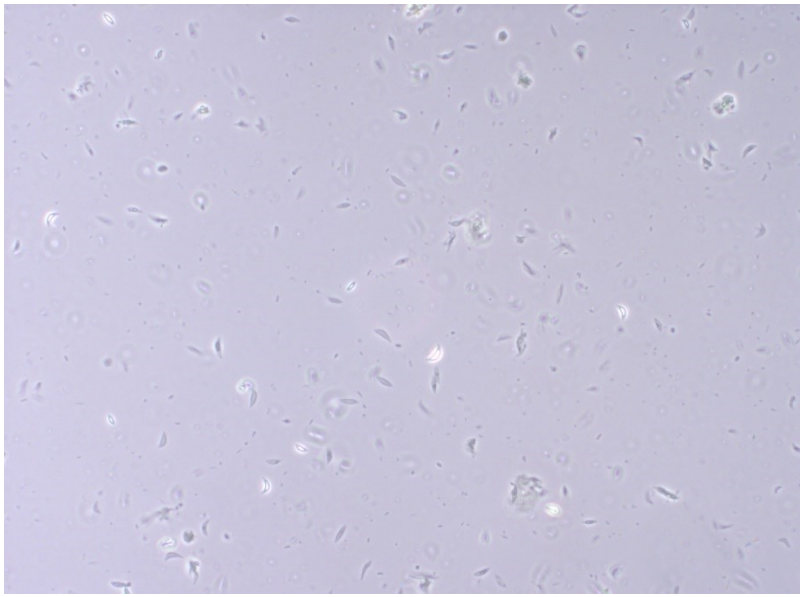
High pressure homogenizator

Biorefinery: cell disruption

Cells before bead milling

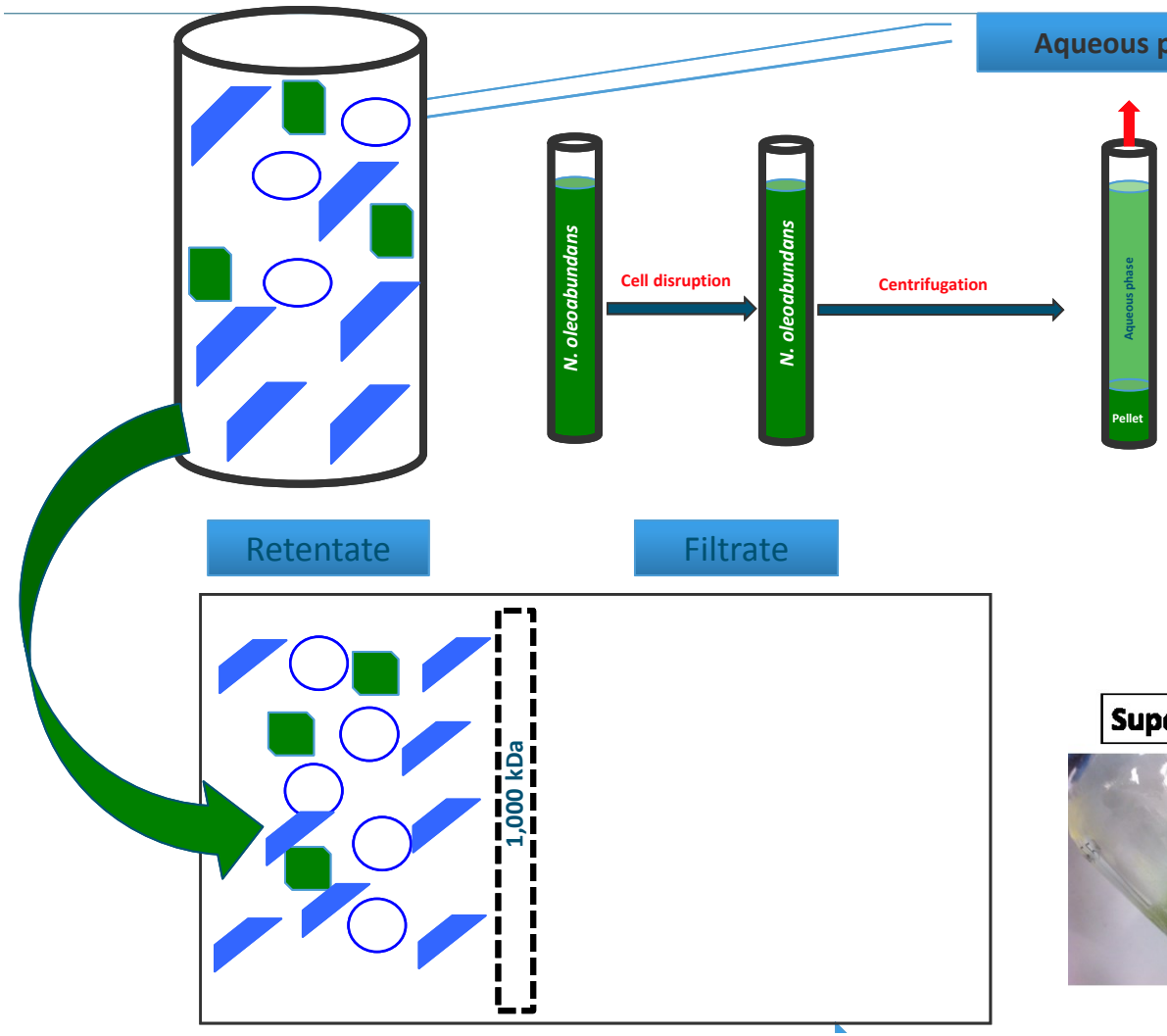


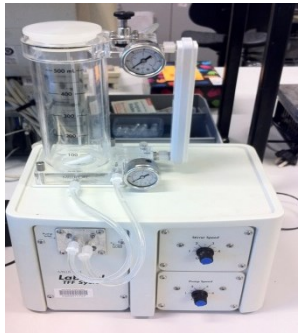
Cells after bead milling

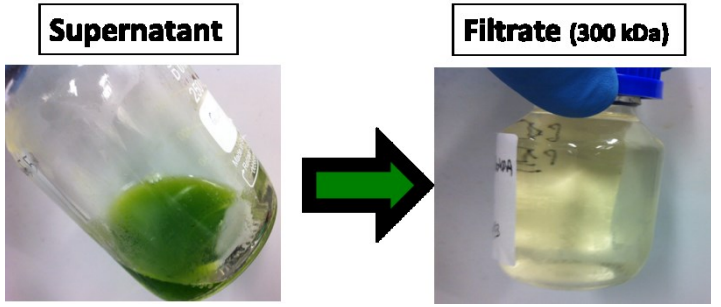


S. obliquus 200×

Biorefinery: filtration

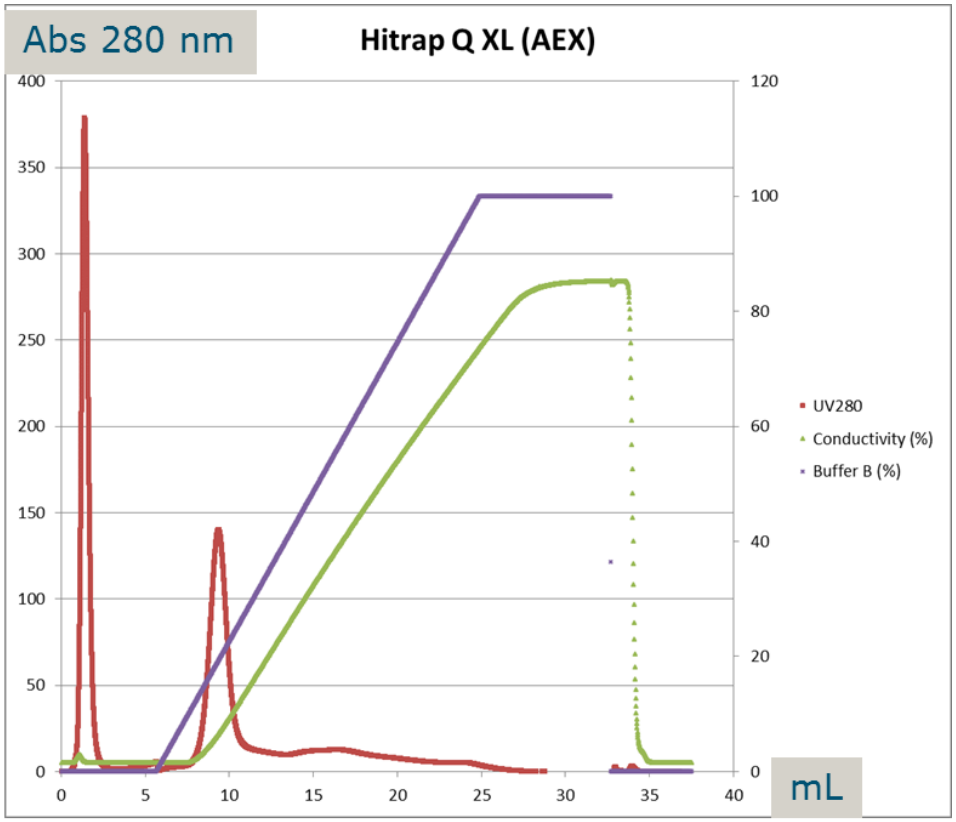






Biorefinery: column chromatography

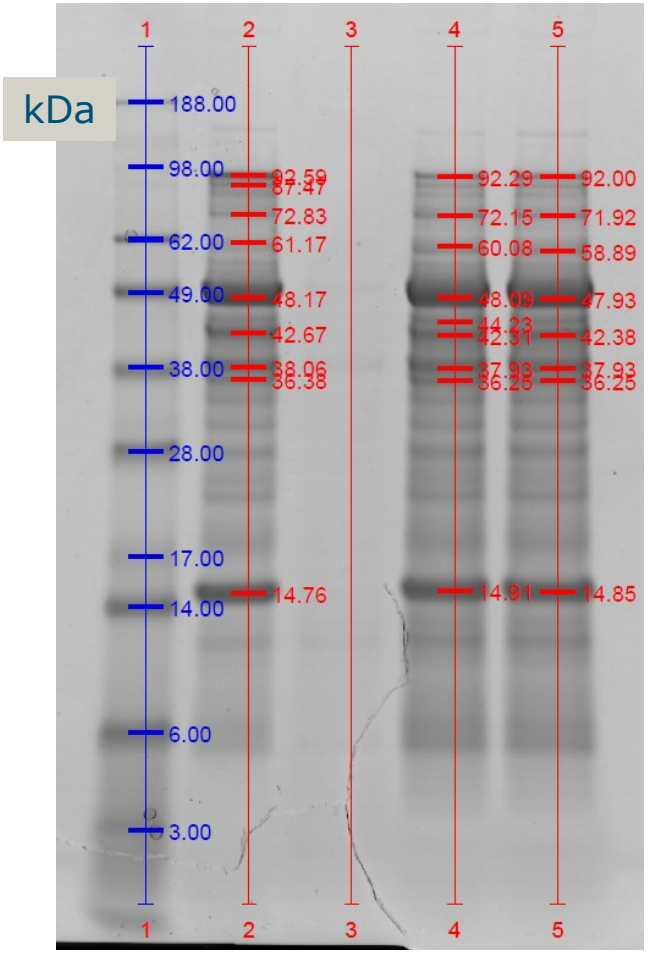
- Ammonium sulphate precipitation
- Anion exchange chromatography



Characterization protein

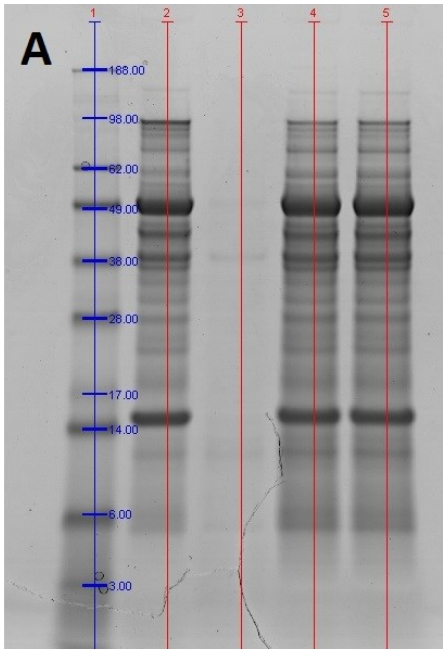
- SDS-PAGE of *I. galbana* protein extract after cell disruption
  - 1: Marker
  - 2, 4, 5: protein extract *I. galbana*

- Rubisco has two sub-units
  - ~48 kDa (L)
  - ~15 kDa (S)



Characterization protein

- Identification proteins
  - In-gel tryptic digestion (IGD)
  - Peptide extraction and sequencing



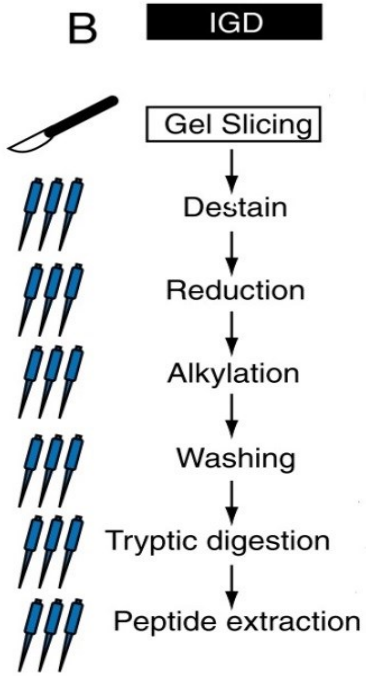
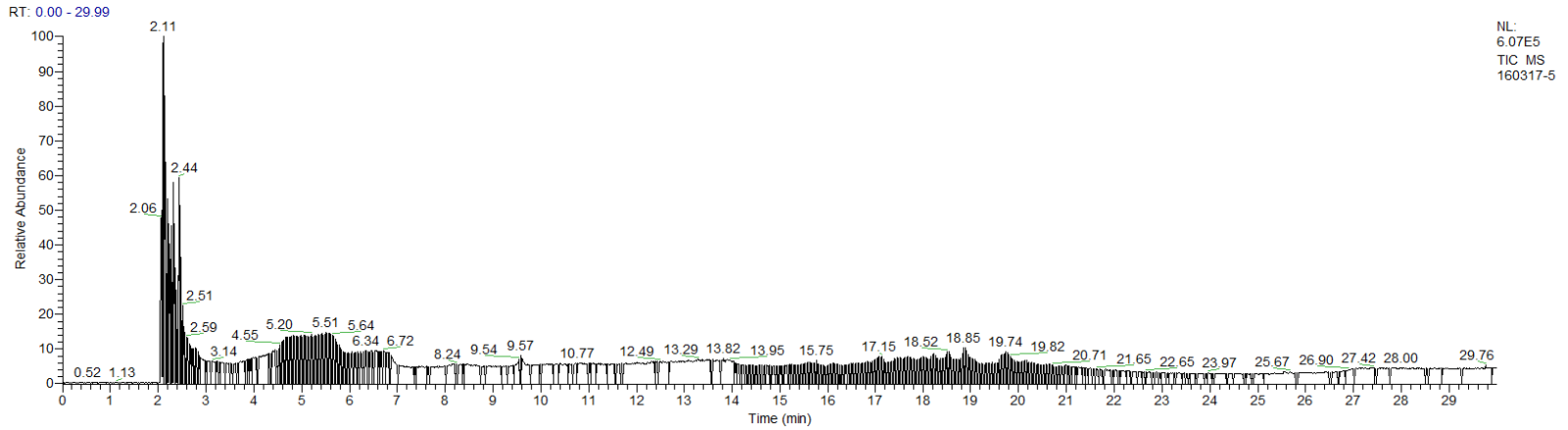


Figure B adapted from Piersma, S. R., et al. (2013). "Whole gel processing procedure for GeLC-MS/MS based proteomics." Proteome Science 11: 8

Characterization protein

- Hypothesis: Rubisco (L) ~48 Da

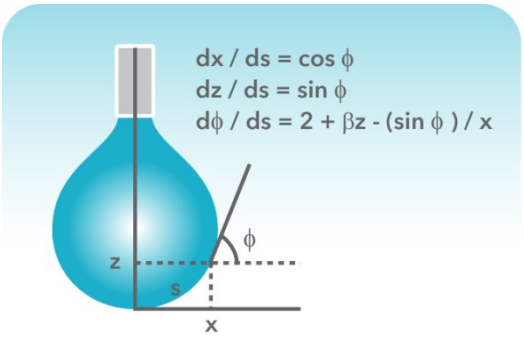
LC-MS



Database	OS	Description	Score
NCBInr	*I. galbana*	RuBisCO (L)	90
NCBInr	*G. longissima*	RuBisCO (L) (partial v. chloroplast)	88
NCBInr	*D. dichotoma*	RuBisCO (L)	73

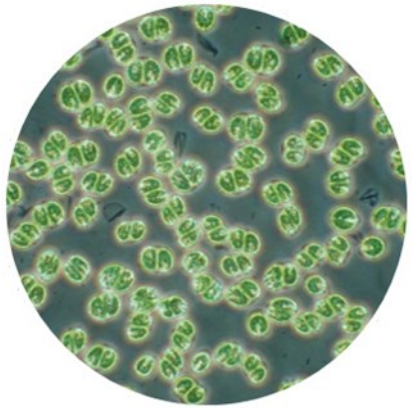
Characterization protein

- Techno-functional properties
  - Solubility
  - Emulsification
  - Foaming
  - Surfactant
  - Gelation

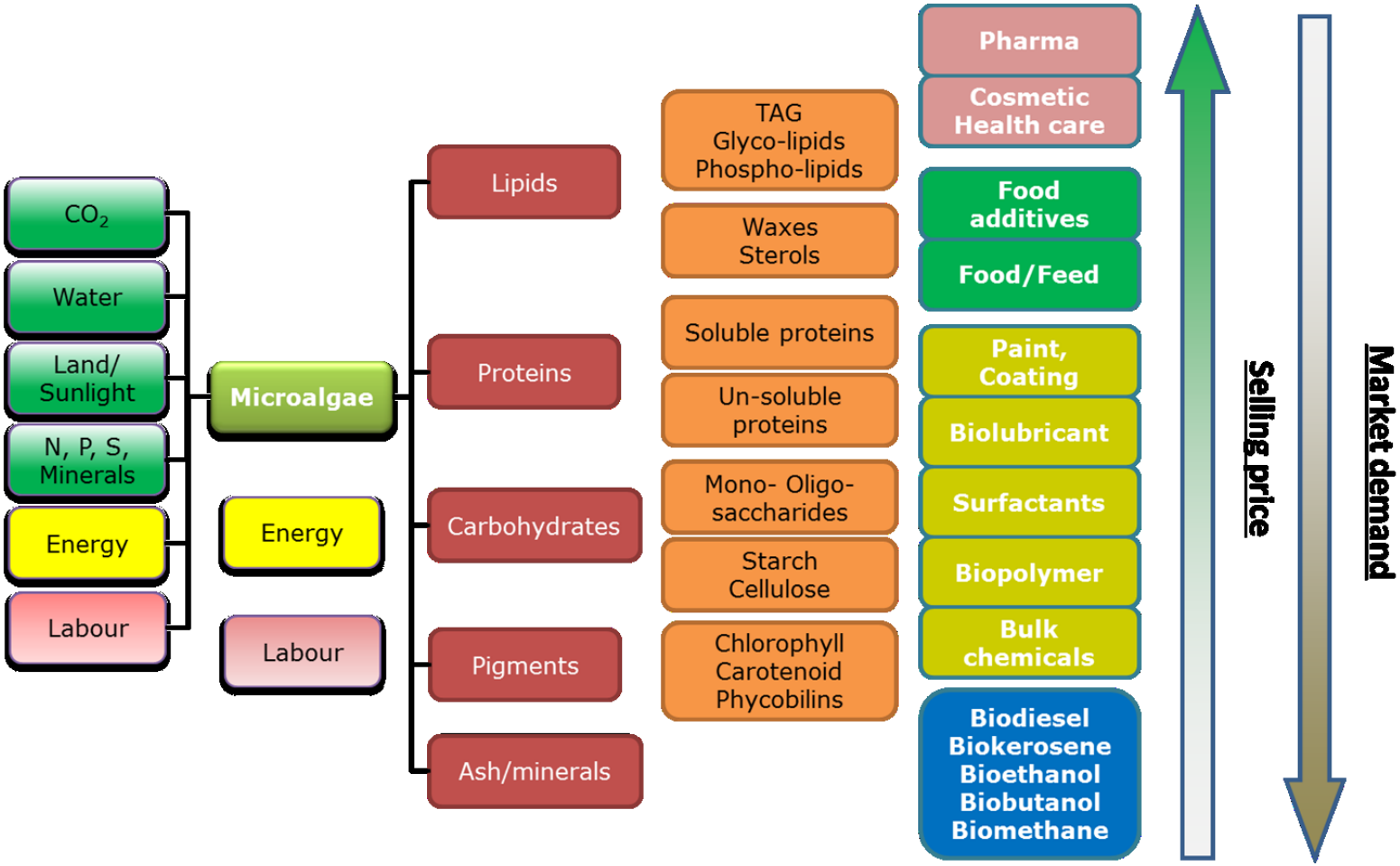


Conclusions

- Non-stressed microalgae have a high protein content
- Different approaches can be used to fractionate protein
- Information is scarce about the techno-functional properties of algae protein



Future


CO_2
Water
Land/ Sunlight
N, P, S, Minerals
Energy
Labour
Microalgae
Energy
Labour
Lipids
Proteins
Carbohydrates
Pigments
Ash/minerals
TAG Glyco-lipids Phospho-lipids
Waxes Sterols
Soluble proteins
Un-soluble proteins
Mono- Oligo-saccharides
Starch Cellulose
Chlorophyll Carotenoid Phycobilins
Pharma
Cosmetic Health care
Food additives
Food/Feed
Paint, Coating
Biolubricant
Surfactants
Biopolymer
Bulk chemicals
Biodiesel Biokerosene Bioethanol Biobutanol Biomethane
Selling price
Market demand

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- Bwee Houweling Tan

Thank You!

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