

Biorefinery

Recovery of valuable biomolecules

Prof.dr Michel Eppink

Inaugural lecture upon taking up the post of Special Professor of Biorefinery for mild separation technologies of complex biomolecules at Wageningen University on 23 April 2015



WAGENINGEN UNIVERSITY

WAGENINGEN **UR**

Biorefinery

Recovery of valuable biomolecules

Prof.dr Michel Eppink

Inaugural lecture upon taking up the post of Special Professor of Biorefinery
for mild separation technologies of complex biomolecules at Wageningen University
on 23 April 2015



WAGENINGEN UNIVERSITY
WAGENINGEN **UR**

Biorefinery

Recovery of valuable biomolecules

I wish everybody welcome to this inaugural lecture with title “Biorefinery: Recovery of valuable Biomolecules” and subtitle “from single towards multiple products” for my appointment as special professor at March 1st 2014 at Bioprocess Engineering.

Two words are already striking in the title, ‘Biorefinery’ and ‘Complex biomolecules’ what do these difficult words mean?

Biorefinery

‘Biorefinery’ is a facility that integrates cell disruption, extraction, conversion and separation technologies of biomass [1]. The biorefinery strategy is analogous to today’s petroleum refinery, in which multiple fuel products and chemicals are produced from crude petroleum [2].

Biorefinery includes the selective isolation of products (proteins, carbohydrates, lipids) from crude biomass. Biorefinery needs to be mild and efficient at the same time in order to maintain the functionality of the products (e.g. native protein conformation) and thus value. For the development of a sustainable biobased economy it is essential to use, when feasible, all biomass ingredients efficient for bulk and specialty product applications. Biorefinery will result in ingredients (Figure 1) for a variety of applications (e.g. food, feed, fuel, pharma, chemicals) to cope with the worldwide scarcity of food, medicines and fuel in the coming decades.

Biomolecules

“Biomolecules” are complicated structures (Figure 2) and include proteins (consisting of amino acids), lipids (consisting of fatty acids, sterols, etc.) and carbohydrates (consisting of sugars) that are worldwide used in food / feed / materials / chemicals / pharma / commodities, etc. Just have a look onto the labels of the products you buy normally in the supermarket that contains proteins, lipids and carbohydrates as well [3].

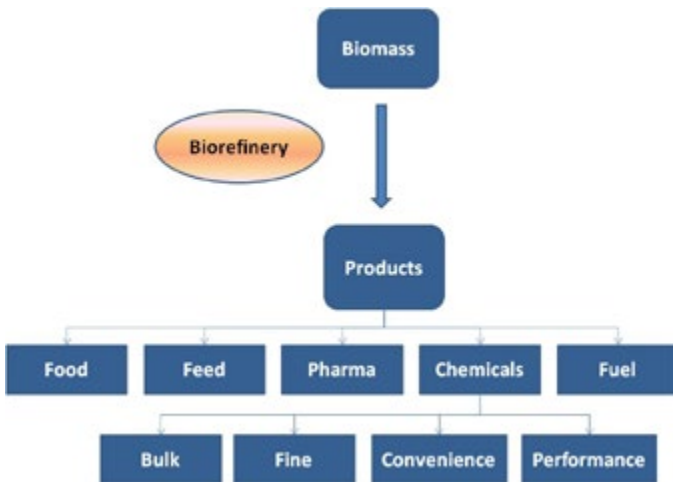


Figure 1: Ingredients from biomass streams

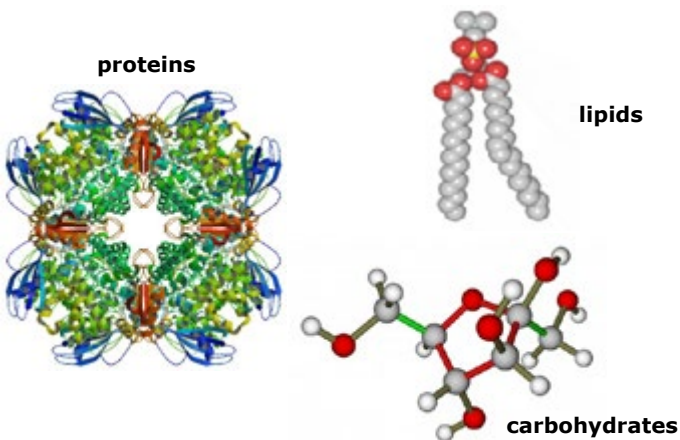


Figure 2: Complex Biomolecules

Biobased Economy

Today, we are facing complex and difficult challenges that will shape our future, such as sustainable supply of food and energy, the need for new biomedicines, climate change and environmental degradation, aging populations, etc. The current economic downturn has made finding solutions to these issues even more critical. The global economy needs to be based on a cradle to cradle principle, and for this, production processes need to be developed without accumulation of waste or depletion of resources. The economy will become bio-based. Biorefinery can make an important contribution in meeting these grand societal challenges and contribute towards economic recovery and growth [2].

Although biorefinery is considered as an important area, most biorefinery processes are based on traditional cell disruption and separation techniques on which basis it is impossible to refine all functional high value ingredients. Effective mild disruption and separation techniques are at its infancy today.

From single towards multiple products

The current approach to obtain products from biomass streams is mainly focused on the single product isolation such as lipids from yeast (Figure 3), discarding the other products as waste [4]. If the focus is on lipids you could use organic solutions to recover the products, but this will destroy the other products, especially the proteins which are very valuable and fragile. A lot of products and thus value is lost.

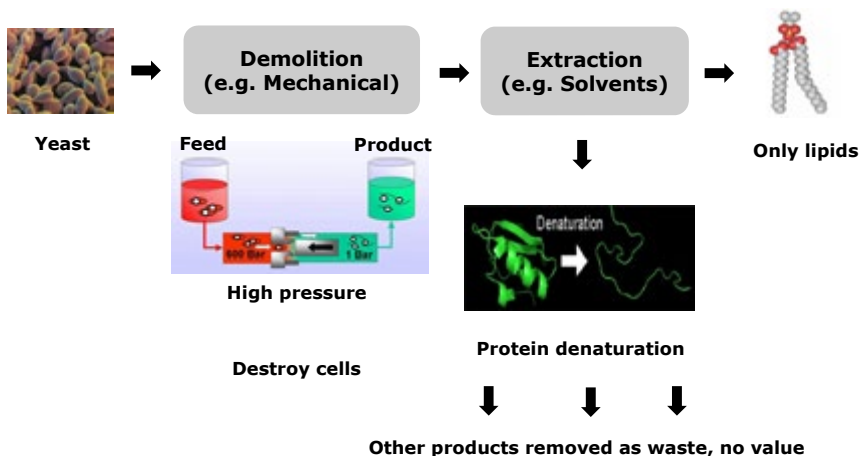


Figure 3: Single product isolations

A few examples are given:

Starch from potatoes / sugar from sugar beet / beer from barley, whereas the rest is discarded as waste such as leaves (Figure 4). However, in the past years the industry/institutes are working hard to recover more products from waste such as these plants (one success story is protein recovery from potatoes (AVEBE), currently one of their top products [5], as no protein is discarded as waste stream into the canals of Groningen anymore). Further research on products from leaves is in progress, but this takes a long time and unknown whether it will become economical feasible as leaves are not easily degraded to recover functional biomolecules [6]. A drawback is that the conditions used to get the main products might affect the other products (mentioned above) as well and the value will become less, but these products are still suitable for e.g. chemical and fuel applications. Recently, the Bioprocess Pilot Facility (BPF) in Delft has started up for the conversion of biobased residues into useful chemicals and biofuels [7].

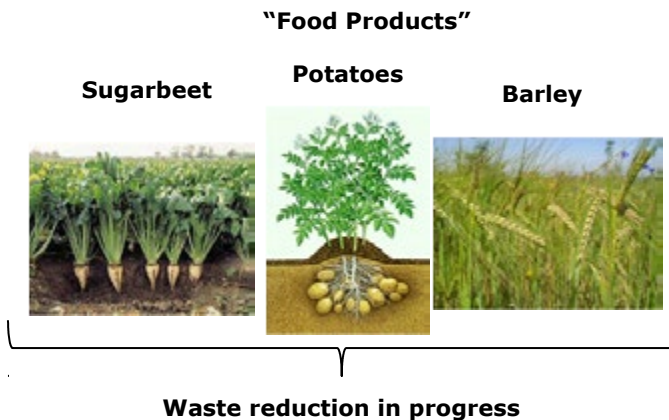


Figure 4: Food products

Another example includes the isolation of insulin from animals in the early years (1923 at Organon) whereby the rest of the animals is discarded as waste, during the years these products were more and more produced as recombinant proteins (e.g. for pharmaceutical applications). Unfortunately, all the other products from these organisms are destroyed and removed as from a regulatory/ethical point of view it is not allowed to use the other products from these Genetic Modified Organisms (GMO's) for other applications. This would otherwise give additional value. Maybe you have read the newspapers if we get a good agreement between USA and Europe (Transatlantic Trade and Investment Partnership (TTIP)) other GMO like material might enter Europe and people are afraid of GMO like material in e.g. food [8].

The solution to this will be to develop another philosophy to obtain all products from biomass streams (no GMO's) going from one/two product isolation towards multiple product isolation directly from the start and keeping **full product functionality** and no waste. This would also indicate that new manufacturing plant concepts need to be developed, opening up a new area of different range of products which need to be captured in a functional state and not as degraded product. On the other hand, this is quite a complex operation as the current technologies are not adapted for that which will take time to develop, as new technologies and skilled personnel are needed as well when constructing such new biorefinery plants.

From single towards multiple products

Novel biorefinery concepts for bulk products and specific biomedicines are being developed. For bulk products such as proteins, carbohydrates and lipids from the 3rd generation eukaryotic sources (e.g. microalgae, seaweeds, duckweed) the refinery should be cheap, efficient and selective [9]. Refinery approaches need to be developed to obtain functional proteins, carbohydrates and lipids for the different markets in the food / feed / chemical / commodity / energy areas. This in relation to the biomedicines were more sophisticated and expensive refinery approaches are needed to obtain high quality functional biomedical products from eukaryotic sources (e.g. animal cells, sponges, yeast). These complex proteins, vaccines or bioactive peptide products need to be highly purified from a complex protein mixture in its full functional state and applied as therapeutic medicine [10].



Single product (demolition)

Biomass



One product range
(e.g. only lipids)



Multiple products (disentanglement)

Biomass



Multiple products
(e.g. proteins, lipids, carbohydrates)



Complexer, but creates more value

Figure 5: From single towards multiple product isolations

Combining this all together would result in the biorefinery of biomass streams into a range of different products (proteins, lipids, carbohydrates) [11]. Keep in mind that it would not mean that one manufacturing plant concept is able to process all different biomass streams towards functional biomolecules.

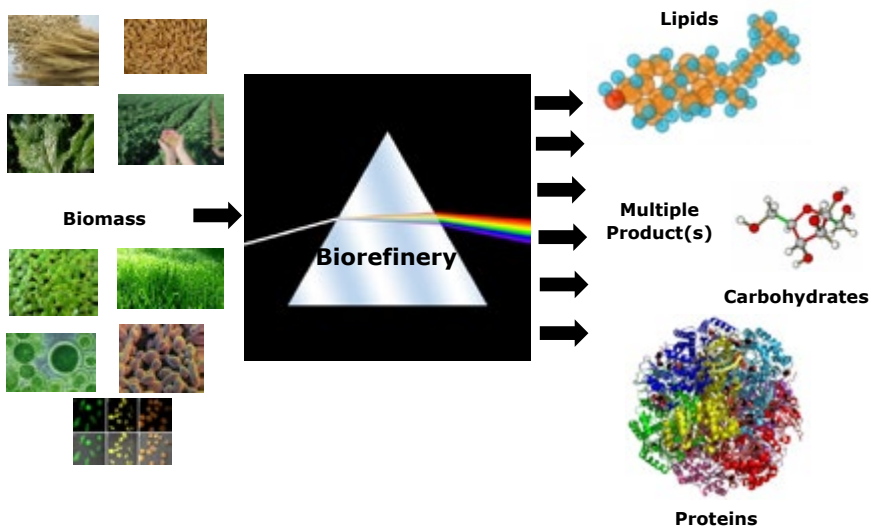


Figure 6: Multiple ingredients from different biomass streams

Which biomasses are we talking about?

Table 1 categorizes the biomasses into cereal crops, oil crops, lignocellulosic crops, marine crops, green crops and other eukaryotic organisms from which different products can be obtained with biorefinery approaches [12]. Some of these Crops are already being used in a biorefinery approach for the generation of biofuels with the intention to replace fossil fuels in the next decades. These crops are 1st generation feedstock (Cereal Crops), 2nd generation feedstocks (Lignocellulosic Crops) and most recent the 3rd generation feedstocks (Marine Crops) which will be explained in the next slides.

Currently, biorefinery is mainly focused on biofuel (biogas, biodiesel, bioethanol) products and not on more complex functional biomolecules as these molecules are destroyed during the biorefinery according the harsh conditions (e.g. temperature, organic solutions).

Table 1: Feedstocks for biorefinery

Biomass	Feedstock	Products	Remark
Cereal Crops (1st)*	Maize, Wheat, Straw, Corn	Monomers, polymers	commercialized
Oil Crops	Rape Seed, Soy Bean, Oil Palm	Chemicals, monomers / lipids	commercialized
Lignocellulosic Crops (2nd)*	Lignocellulose products, Wood	Monomers, sugars	Close to commercial scale
Green Crops	Grass, Leaves (e.g. sugar beet, tea)	Proteins, sugars, fibers	R&D Phase
Marine Crops (3rd)*	Microalgae, Macroalgae	Proteins, sugars, lipids	R&D Phase
Others	Yeast, Fungi, Bacteria, Mammalian	Proteins, sugars, lipids	Commercialization, R&D phase

**1st, 2nd and 3rd generation biofuels*

The first and second generation feedstocks are already being explored for biofuel (bioethanol, biogas, biodiesel) production (Figure 7). However, there are drawbacks as the 1st generation feedstocks has to compete with food applications keeping in mind the growing population in the world that needs food. Furthermore, high energy costs are needed to recover biofuels from the 1st and 2nd generation feedstocks and harsh conditions are needed to decompose especially lignocellulose. Finally, the 3rd generation feedstocks might have a more promising future as these marine organisms can produce lipids (up to 50%), do not compete for land or food and need less energy for recovery of the lipids [13]. Interestingly, the recovery of other valuable products (proteins, carbohydrates) is feasible under mild conditions keeping full product functionality which will increase the value [11].

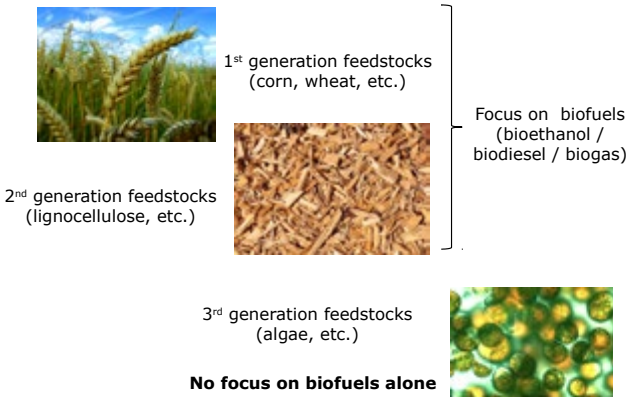
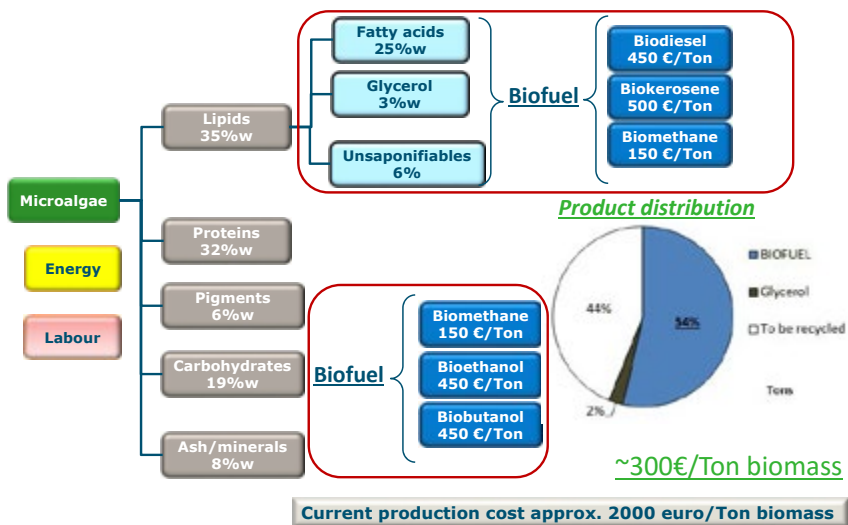
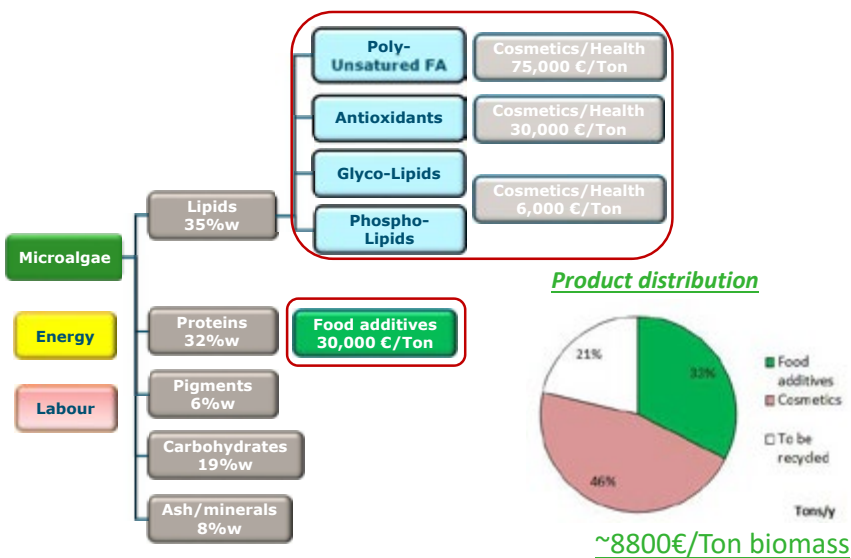


Figure 7: 1st, 2nd and 3rd generation biofuel biomasses



Scheme 1: Biofuel production from microalgae



Scheme 2: Specialties production from microalgae

To produce only lipids from marine organisms for e.g. fuel production (look at the selected area's in Scheme 1) the earnings are 300 euro/Ton whereas the production costs could be around 2000 euro/Ton up to now (Scheme 1), so much higher indicating that the current process is economically not feasible.

When recovery of a large range of products including proteins the specialties sector has much higher earnings (Scheme 2), approx. 8800 euro/Ton (maybe higher) and with this process current economic production is feasible.

So when looking back to the biomasses as given in Table 1 the focus will be on 'marine' and 'others' unicellular eukaryotic organisms (mainly fermentable organisms) which contain valuable biomolecules and should be recovered in a mild and efficient way.

What is the approach?

The approach is biorefinery of the functional biomolecules and with this orange as example a little bit is explained as everybody who would like to have the fruits from an orange will peel it gentle and obtain in this way the fruits in an intact form. More or less the same occurs with biorefinery of selected eukaryotic organisms but is much more complex.

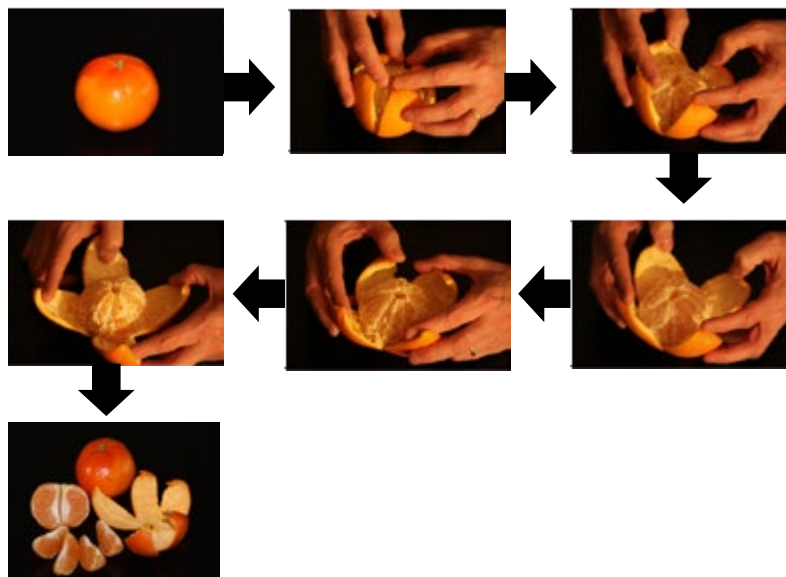
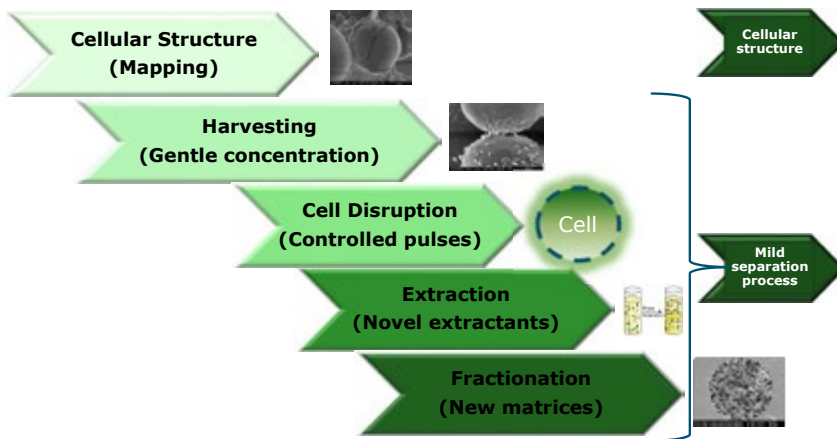


Figure 8: Schematic picture showing the gentle peeling of an orange

The Biorefinery Strategy

Here we explain the steps to recover these functional biomolecules as shown in scheme 3:

- 1 Cellular knowledge (e.g. cell wall strength, localization of components in the cells)
- 2 Harvesting (working with fermentable streams needs reduction of large scale volumes with gentle methods to small scale needed for further processing)
- 3 Cell disruption (controlled release of products out of the cells preventing damaging of products)
- 4 Extraction (separation of the hydrophilic from hydrophobic components in a mild procedure and keeping full functionality)
- 5 Fractionation (separation of specific components (e.g. proteins, carbohydrates) for different applications or separation of lipids)

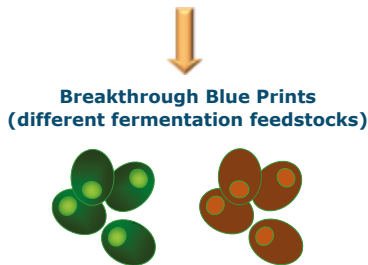


Scheme 3: Schematic overview of biorefinery approach

Combining these techniques together might yield a biorefinery process able to recover a broad range of functional biomolecules [14].

Moreover, it might open up new biorefinery processes for different feedstocks in the near future with recovery of multiple valuable biomolecules for different market applications. For GMO's this might be difficult, but certain biorefinery steps (e.g. extraction, fractionation) can be implemented for recovery of specific products (e.g. proteins).

Cellular structure
+
Mild separation process



Scheme 4: Blue print for different fermentation feedstocks

How complex are the eukaryotic cells to get the products out?

This Scanning Electron Microscopy picture (Figure 9) of the microalgae *Dunalliella salina* gives a more realistic picture showing the different organelles embedded in a more fluidized cellular structure and clearly indicates that it is not easy to gradually fractionate the different products or able to fractionate certain organelles which contain enriched products (e.g. oil bodies contain lipids) [15].

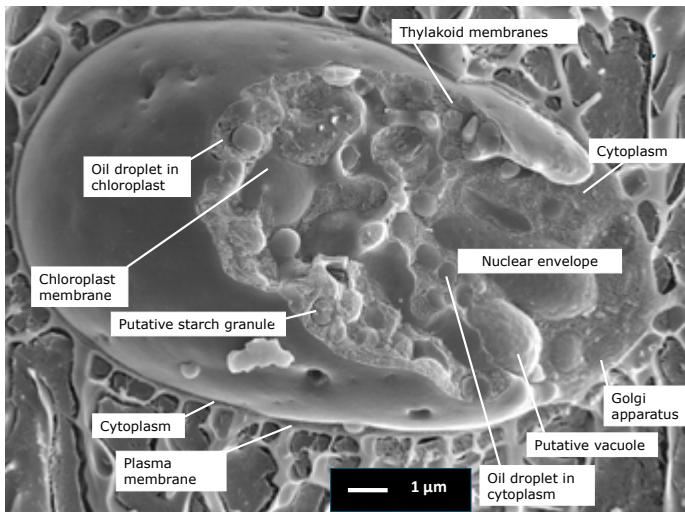
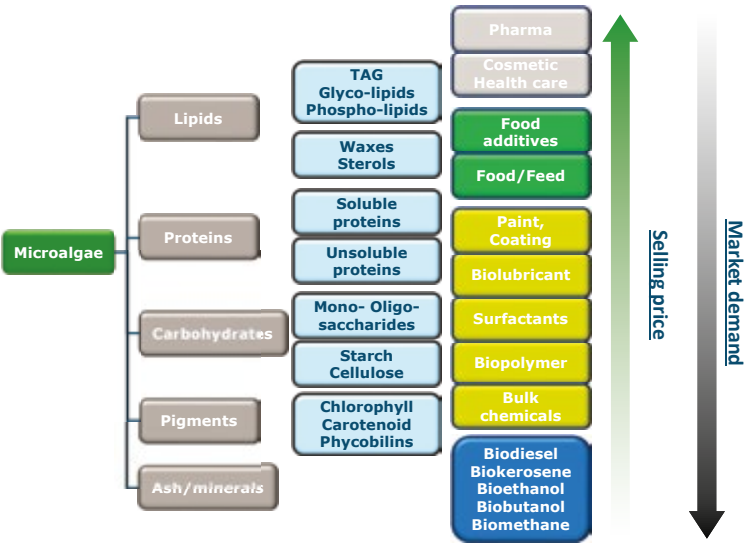


Figure 9: Cross section of the microalgae Dunaliella salina

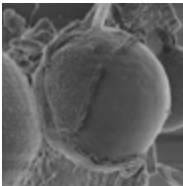
In this marine species (microalgae) a range of products (not specified in detail yet) can be obtained and be used for diverse applications (Scheme 5), which will be the focus for the coming years for biorefinery so that we learn and implement novel technologies and able to translate these technologies towards the biorefinery for other eukaryotic organisms as well.



Scheme 5: Ingredients from microalgae

What is the current progress, what has been done yet when going back to the platform approach (Scheme 3) where we displayed the different steps, detailed studies on each topic will be explained. Step by step the different biorefinerys steps including cellular structure, harvesting, cell disruption, extraction and fractionation will be explained in more detail and a future outlook presented on each topic showing the potential and complexity of biorefinery in the coming years.

Cellular Stucture (mapping)



As shown above in Figure 9 this SEM picture of the microalgae is presented detecting different organelles in the cytoplasm.

Microalgae consists of complex cells containing a cell wall with different organelles, such as mitochondria, lysosomes, endoplasmatic reticulum, golgi apparatus, etc. (see figure), each with a specific composition, some rich in nucleic acids, others in proteins or lipids or carbohydrates. The complexity of the biomass structure provides an opportunity for efficient biorefinery.

A detailed structural approach for identifying the polysaccharide composition of the cell wall will be performed during the different growth phases (lag phase, log phase, stationary phase, starvation phase) of specific microalgae strains so that more information about the cell wall structures would become explored making it feasible to select in this way the best cell disruption strategy. The visual changes (Figure 10) are mainly due to changes in the carbohydrate composition of the cell wall and that influences the cell wall strength as well [16]. Identification of the cell wall composition is a prerequisite which is being studied in collaboration with Plant Breeding.

Labeling studies (e.g. fluorescence) to identify the location of the different components in the cytoplasm might indicate that only cell wall rupture should be enough to collect the valuable components discarding the cell walls and the different organelles. On the other hand, when specific proteins are stored in special organelles (e.g. Rubisco in pyrenoids) enrichment of these organelles would be another strategy to efficiently obtain the products of interest.

Specific cell shear studies need to be developed dealing with fragile cells (e.g. microalgae), to unravel and understand the mechanisms of cell disruption of microalgae at various conditions (e.g. cell wall composition and structure, cell size, medium composition and microalgae growth condition). In micro-shear devices rupture studies in shear fields can be made for the same algae species at similar environmental conditions.

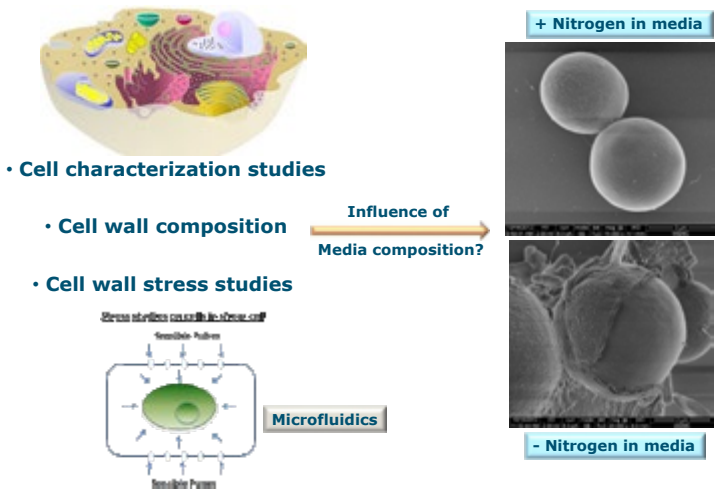
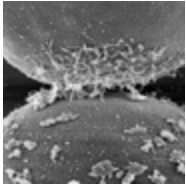


Figure 10: Cellular characterization studies

Harvesting (Gentle concentration)



Biomass concentration is an important step before cell disruption can be carried out as the efficiency of cell disruption is improved in concentrated biomass streams as compared to highly diluted biomass streams and decrease in overall biomass volume is reached as well. Different methods are available dependent on the energy requirements such as filtration and centrifugation which

are quite well known as scalable techniques but not energy efficient processes whereas flocculation (Figure 11) remains low in energy consumption but the overall concentration factor of the biomass is lower. On the other hand flocculation is mild and prevents cell lysis which normally induces autolysis of the microalgae.

Focus will be directed towards flocculation of microalgae (Figure 11) for which the mechanism is quite unknown responsible for inducing the flocculation of the microalgae cells. Research has been carried out on (electro)chemical flocculation [17, 18, 19] and auto/bio-flocculation [20, 21, 22] whereby the bio-flocculation approach is quite intriguing as Extracellular PolySaccharides (EPS) are excreted by the microalgae and plays an important role in the flocculation mechanism of microalgae. Therefore, with respect to flocculation it would be interesting to know what happens at the molecular level? Moreover, recycling of these polymers might be necessary as well by adapting salt or pH conditions preventing contamination after cell disruption.

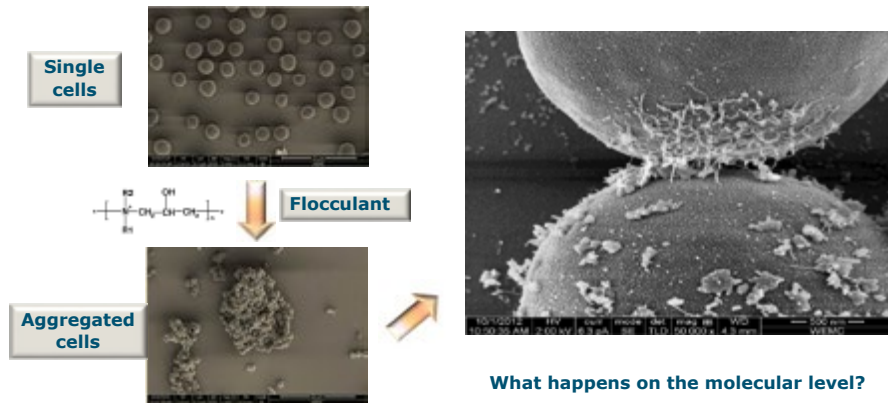


Figure 11: Flocculation studies of microalgae cells

Cell Disruption (Controlled pulses)



Cell disruption is the most crucial technology to harvest high quality biomolecules in their functional conformation, because often the methods used are based on complete disruption of the cells or intended to focus on one specific product by using the more general techniques like mechanical (e.g. homogenizers, bead milling, high pressure) or non-mechanical (e.g. ultrasonic, autoclaving, microwaves, osmotic shock, chemicals, enzymatic) cell disruption methods for isolation of specific components. These processes do not only require high amounts of energy but also a complex mixture of ingredients is obtained from which the different components are difficult to separate and additionally may lead to denaturation, degradation or irreversible modification of the functional compounds. Cell lysis normally triggers the cellular mechanism to release proteolytic enzymes from different organelles (e.g. lysosomes) and (irreversibly) damage the high valuable bio-based products. Therefore, to protect and recover important biomolecules sophisticated and mild technologies are needed to lyse the cells and efficiently fractionate intracellular components [23]. Occasionally valuable components are already extractable from the outer cell wall/membranes (e.g. proteins, sugars) which can be reached using mild detergent methodologies before cells are being destroyed releasing the cellular content. On the other hand, techniques are already in progress expressing cellulytic enzymes preventing the formation of thick cell walls in crops which would simplify the cell disruption of e.g. algae. Real breakthrough technologies might be the development of novel enzymes or the use of continuous flow techniques.

Continuous flow techniques (Figure 12) are receiving more and more attention in research the last decade and all are focussed on perforating the outer cell wall [24, 25, 26]. Possible applications are; 1) analysing single cellular content, 2) transfection of cells, 3) inactivating cells in the food industry and 4) analysing cellular properties e.g. blood samples. The major advantage of these techniques is that they do not harm the intracellular content, and the low sample volumes required for analyses due to effective separation of the intracellular content and the effective cell inactivation. Cell inactivation occurs due to the perforation of the cell wall or cell membrane causing irreversible damage and the cell dies [27, 28]. Moreover, due to the perforation the intracellular components will be easily extractable. The challenge for biorefinery of microalgae is to apply these principles at large scale and low cost. An advantage of these types of techniques is that they can handle low concentrated streams, therefore no or less concentration is necessary and this will lower energy use significantly. However, it will require larger volume processing in the continuous flow technique.

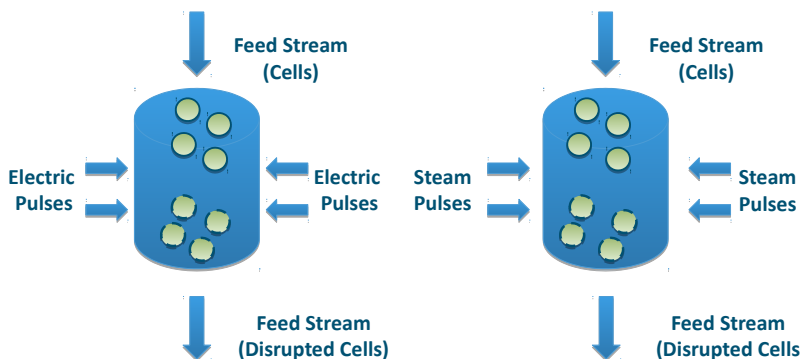


Figure 12: Cell Disruption Techniques

Pulsed Electric Field, Supersonic Fluid Flow Processing, and Ultrasound are techniques that perforate cell walls or membranes (electroporation), the actual perforation is done using different mechanisms. Depending on the energy requirement and the efficiency of the different perforation techniques the best technology can be chosen. These continuous flow technologies can also be used in combination with enzymes, which work very specifically on cell wall compounds able to weaken the cell wall so that the continuous flow techniques are able to perforate the cells. However, the effectiveness of each technique should be studied before clear conclusions can be drawn and breakthroughs are achieved [24, 25, 26, 27].

A nice overview is given in Figure 13 whereby pulses can be used to make controlled holes in the cells so that products can be captured out of the cells, but this kind of technologies is still in its infancy and needs more time to develop in coming years.

The benefits/advantages of this approach are scalability, controlled cell disruption by fine tuning energy release and omitting biomass concentration by centrifugation before cell disruption. It might be that a pre-concentration step is needed, the best opportunity for that would be flocculation whereby the energy consumption is kept low.

Moreover, a stepwise approach in cell disruption could be developed as presented in Figure 14 whereby a schematic impression of the cell disruption strategy is proposed. At first, small holes are pierced in the cells with low energy so that the different components (proteins, carbohydrates, lipids) from cytoplasm are easily extracted out of the damaged cells (1st Cycle). Secondly, the punctured cells are concentrated by filtration and further exposed to increased energy input generating larger holes in the cells excreting the organelles (2nd Cycle). Finally, dependent on the value of the

components, the separated organelles and/or cell wall fragments can be further concentrated and disentangled by fine tuning the supersonic flow fluid processing or pulsed electric field technologies to isolate the compounds from these organelles and/or fragments with higher energy input (3rd Cycle). All three technologies will be investigated in detail.

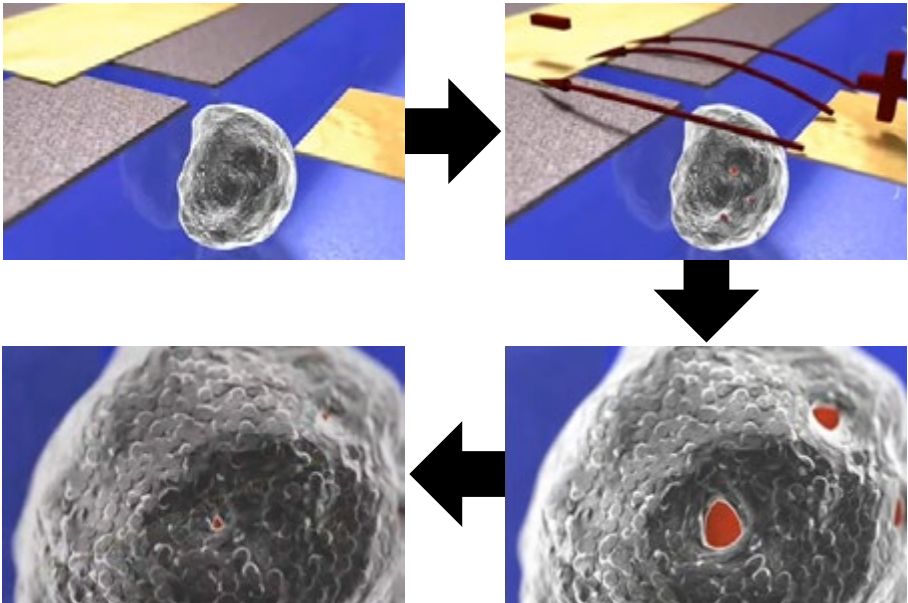


Figure 13: Controlled pulses for microalgae cell disruption

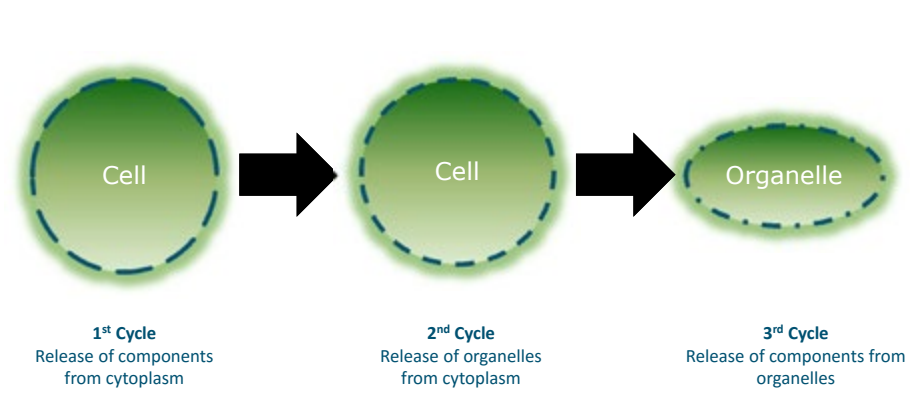


Figure 14: Cell Disruption of microalgae

Extraction (Novel extractants)

After cell lysis, primary separation of biomass components (lipids, proteins and oligosaccharides) is the next step. Lipid recovery methodologies (e.g. supercritical CO₂, continuous centrifugation and solvent extraction methods) are the prime technologies. However, it remains unclear if the proteins and carbohydrates as a concentrated cell extract can be used for further refinement technologies. The 'green' solvent supercritical carbon dioxide offers great potential for the extraction of biomolecules and will be explored. Continuous aqueous recovery process for oils, fats and waxes with centrifugation offers another technology free from organic solvents. Finally, for solvent extraction the amount and impact of residual solvent on the remaining cell components is the most important parameter and the most optimal solvents such as surfactants (e.g. Polysorbate) and polymers (e.g. Polyethylene glycol) are used as well. Next to that the new classes of liquid salts 'Ionic Liquids' offer a great potential as well in the near future by solubilization of the hydrophobic and/or hydrophilic biomolecules in a mild separation procedure [29]. Exploring mild extraction technologies increases the valorization of the fragile remaining components (proteins and oligosaccharides) as well.

It would therefore be much more pioneering to extract the hydrophilic and hydrophobic components simultaneously in one step (figure 15).

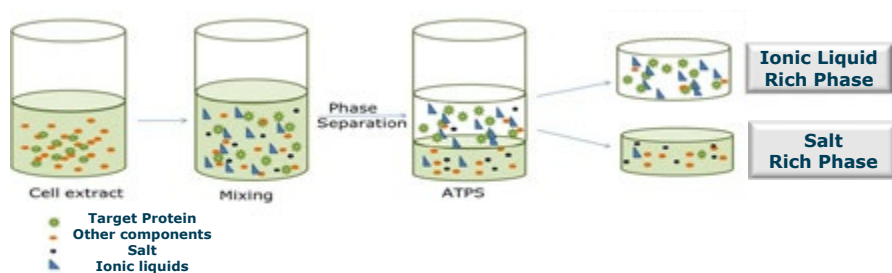


Figure 15: Extraction of valuable components

The intention will be to develop extraction methods to solubilize both the hydrophobic (lipids) and hydrophilic (proteins) compounds with the use of 'Ionic Liquids' and compare with polymers (e.g. polyethylene glycol). Ionic Liquids, the 'green chemistry' molecules, are able to extract hydrophilic from hydrophobic components and very useful as a mild extraction method. A large range of ionic liquids are available depending on their charged state for solubilization of hydrophilic or hydrophobic components (Imidazolium or Phosphonium based ionic liquids). It should however be

taken in mind that the ionic liquids are new so the effects on the structural integrity of the compounds (e.g. proteins) need to be investigated and ionic liquids are still in an exploration phase and not yet used for large scale applications. Extraction by using the polymer polyethylene glycol for the solubilization of hydrophobic or hydrophilic components is used as a reference as quite some research is available for these polymers. Although it should be kept in mind that also for these extraction methods the combined approach is novel so it will require quite some effort to define optimal extraction methods for the selective separation of hydrophilic from hydrophobic components. In Figure 15 the phase separation is shown by using ionic liquids separating specific proteins in one phase and other components in the other phase, it should be kept in mind that quite some studies are needed to develop new extraction technologies with these selective reagents (they are not cheap, recycling is needed and toxicity might play a role as well, however, a large range of extractants can be used and they are very effective). Moreover, for some proteins (e.g. BSA, IgG) no effect on functionality/stability is observed but for some other proteins (e.g. Rubisco) the stability (decreased activity) is affected although the separation seems good for the proteins as determined in recent studies [30]. In the coming time more studies will be elaborated on disrupted biomass.

A nice technology shows the use of specific ionic liquids to get the products out of fresh cells as is clearly indicated by the change in color of the cell from orange/brown towards colorless by extracting the pigment astaxanthin from fresh cells (Figure 16). In this way more hydrophobic compounds could be extracted out of the cells leaving the hydrophilic more fragile proteins in the cells which can be recovered later after cell breakage. This is a so-called pre-fractionation step and interesting to know what is the mechanism behind [31].

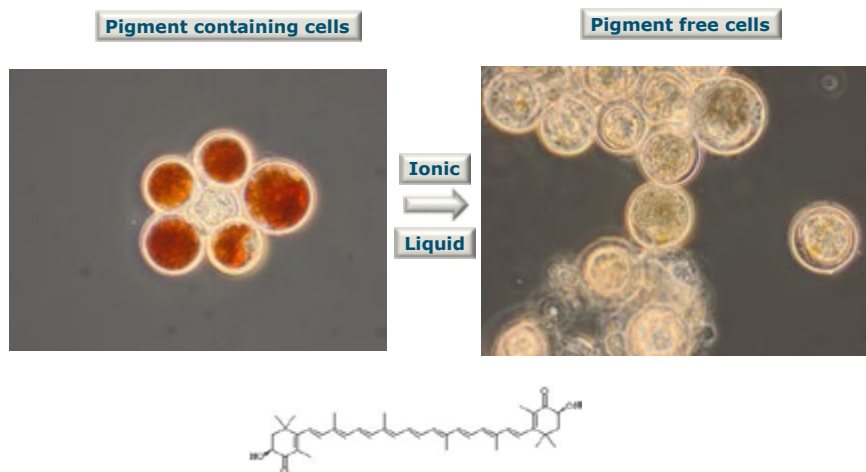
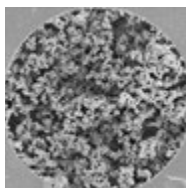


Figure 16: Astaxanthin extraction from microalgae with ionic liquids

Fractionation (New matrices)



The final step in the biorefinery, when needed, is the selective separation of especially proteins/carbohydrates [32, 33] as products with (non)chromatographic techniques. It might be that high valuable products could be isolated from the pool of proteins or carbohydrates or lipids with special functions, such as Rubisco from the protein fraction as food ingredient or phospho/sphingolipids from the triacylglycerides (TAG's) as pharmaceutical ingredients.

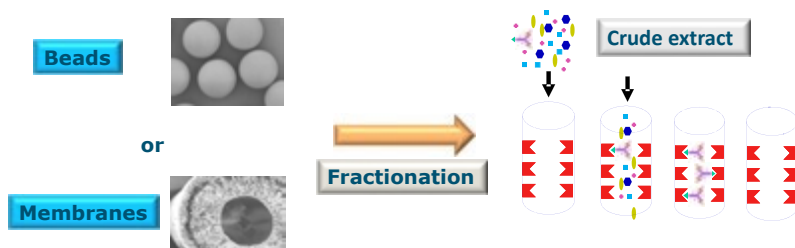
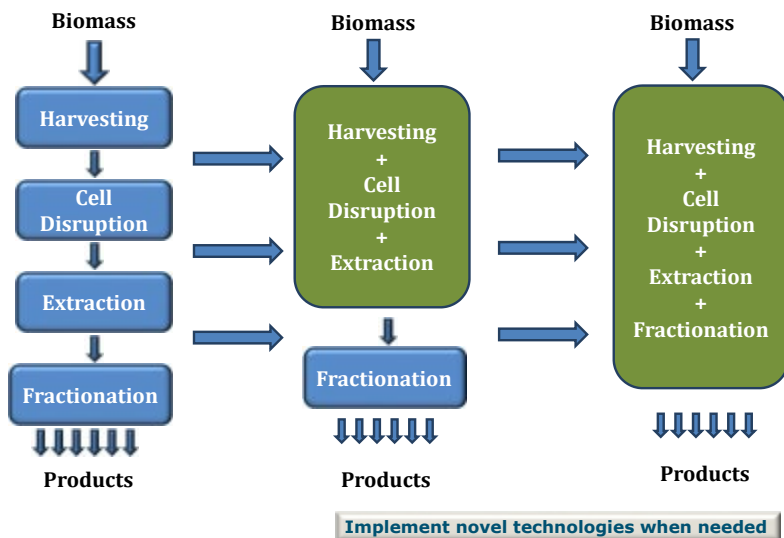


Figure 17: Functional matrices for fractionation of valuable products

The focus will be on developing novel responsive surfaces by functionalizing beads and/or membranes for further purification of proteins from polysaccharides and when feasible further differentiation of different lipids as well (Figure 17). The idea would be to use polymers that can fold/unfold by other triggers as salt or pH such as light, towards temperature, potential differences, etc (socalled responsive polymers). For example protein binding to the polymers occurs at a somewhat higher temperature (37°C) and release occurs at low temperature (25°C) due to unfolding/folding of these responsive polymers.

How will the future look when combining these steps?

Combining the different unit operations step by step would finally end up in a Continuous Biorefinery Concept that could be laid down in newly to be developed biorefinery plants (scheme 6). Of course we do not have to forget to develop new separation technologies in coming time and whether these can be implemented as well as continuous improvement is needed.



Scheme 6: Overview biorefinery approach from single towards continuous

In this way we might develop in the future biorefinery plants for functional biomolecules obtained with mild separation technologies and products for further processing for different market applications (figure 18). This concept or specific steps can be further explored for other fermentative biomass streams as well.

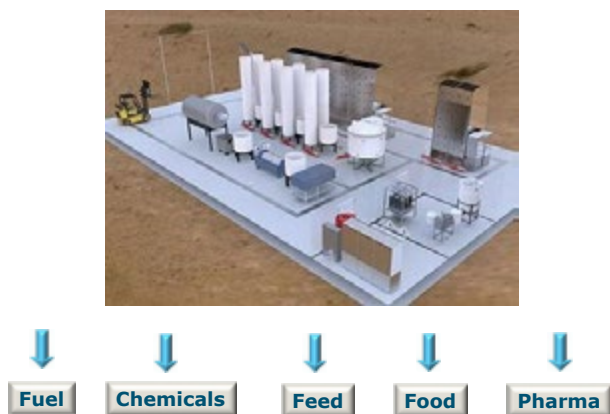


Figure 18: Schematic overview of products from biorefinery plant of the future

What about education?

- Bachelor (BSc)
 - Biorefinery
 - Life Sciences (TU Delft)
 - Biotechnology 1/2

} Physical Separations
(unit operations)
- Master (MSc)
 - Advanced Biorefinery
 - Bioprocess Design

} Integration
(unit operations)
- Advanced (Ph.D, PostDoc, etc.)
 - Biorefinery of Complex Biomolecules
 - Downstream Processing (TU Delft)
 - Bioprocess Design (TU Delft/WUR)

} Separation /
Integration /
Scale Up

For the Bachelor, Master and Ph.D/Postdocs education has been setup in close collaboration with other departments (e.g. Biobased Commodity Chemistry, Environmental Technology) to train the students more and become prepared in the future when applying for positions in the industry/institutes supporting transition towards biorefinery plants.

- *Modern education needed to raise biorefinery graduates*
- *Understand the disentanglement approach (single to multiple product approach)*
- *PROCESS, MOLECULAR and ANALYTICAL understanding*
- *Explain from fundamental and applied point of view*

Nawoord

Als eerste wil ik mijn ouders bedanken, moeder overleden in 1981 en vader in 1992, maar nog steeds denk ik vaak aan ze en daarom ben ik ook zeer verheugd dat mijn familie aanwezig is. De jaren 80 waren een zeer moeilijke periode maar met inzet en doorzettingsvermogen is alles mogelijk.

Willem van Berkel: op het juiste moment uit het ziekenhuis gehaald voor de omslag van klinische chemie naar de biochemie / biotechnologie en mij gemotiveerd voor de wetenschap samen met X-Ray Kristallografie ingebracht door Herman Schreuder vanuit Universiteit Groningen/Sanofi was dit een krachtige combinatie in de jaren 90 voor structuur/functie relatie onderzoek aan eiwitten. Gijs van Dedem niet alleen mij op het juiste moment bij Diosynth/Organon en Synthon getrokken maar ook gestimuleerd in opzetten van samenwerkingen met externe instituten metname met TU Delft (Luuk v/d Wielen en Marcel Ottens) wat in de afgelopen jaren duidelijk zijn vruchten heeft afgeworpen waarbij DSM Delft ook nauw bij betrokken is geweest en nog steeds voortduurt.

Jacques Lemmens (eigenaar Synthon) / Aad van de Leur (COO) mijn leidinggevendenden met het hart op de juiste plaats (om mij de mogelijkheid te geven om een dag in de week aan de Wageningen Universiteit te verdiepen in het onderzoek) en verder DSP (de club mensen die ondanks mijn afwezigheid op vrijdag goed geolied draait daar ben ik jullie zeer dankbaar voor).

Bioprocess Engineering (BPE): Ik wil Rene Wijffels (leerstoelhouder Bioprocess Engineering) bedanken dat ik op het juiste moment begonnen ben binnen BPE en dat je mij de mogelijkheid gegeven hebt om het Biorefinery/Downstream Processing deel op te zetten wat ook uitstekend past binnen de filosofie van Bioprocess Engineering. Verder het Biorefinery team (de gemotiveerde onderzoekers die het werk doen en speciaal aandacht voor Giuseppe Olivieri die als tenure tracker in September 2013 is begonnen veel van de dagelijkse gang behartigd en daarin ook zijn weg zal vinden daar ik met een dag per week dit niet allemaal meer kon behappen en 24 uur per dag werken zit er nog niet in). Ik wil verder ook alle mensen bedanken waar ik mee heb samengewerkt en nog mee samenwerk want jullie allemaal hebben daar een bijdrage in gehad maar teveel om jullie allemaal op te noemen.

Met deze dubbele functie is een goede thuisbasis essentieel en daar heb ik mijn vrouw Hanneke in gevonden vanaf het begin. Ookal heeft zij aan de zijlijn gezien dat het altijd maar werken, werken, werken en nog eens werken was en je veel van de dagelijkse gang van zaken regelt zijn we toch een goed team met een stel jonge kerels erbij. Verder heb ik en zal ik tijdens de lange schaats/fietstochten (200 km of meer)

alles nog eens goed overdenken en zijn het ook de juiste momenten voor uitdenken van nieuwe vindingen.

Tot slot “eens een TUKKER altijd een TUKKER”

Ik dank u voor uw aandacht.

References

- [1] <http://www.nrel.gov/biomass/biorefinery.html>
- [2] <http://www.iea-bioenergy.task42-biorefineries.com/en/icabiorefinery.htm>
- [3] <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Labeling/Nutrition/ucm064880.htm>
- [4] Espinosa-Gonzalez, Parashar and Bressler (2014) Hydrothermal treatment of oleaginous yeast for the recovery of free fatty acids for use in advanced biofuel production. *J. Biotechnology* 187, 10-15.
- [5] <http://www.avebe.com/products/>
- [6] <http://pulp2value.eu/>
- [7] <http://bpf.eu/>
- [8] <https://ustr.gov/ttip>
- [9] Wijffels, Barbosa and Eppink (2010) Microalgae for the production of bulk chemicals and biofuels. *Biofuels Bioproducts & Biorefining-Biofpr.* 4, 287-295.
- [10] Shukla and Thommes (2010) Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends in Biotechnology* 28, 253-261.
- [11] Vanthoor-Koopmans, Wijffels, Barbosa and Eppink (2013) Biorefinery of microalgae for food and fuel. *Bioresource Technology.* 135, 142-149.
- [12] Langeveld, Dixon, Jaworski (2010) Development perspectives of the biobased economy: A review. *Crop Sci.* 50, S-142 – S-151.
- [13] Wijffels and Barbosa (2010) An outlook on microalgae biofuels. *Science* 329, 796-799.
- [14] Eppink, Barbosa and Wijffels (2012) Biorefinery of Microalgae: production of high value products, bulk chemicals and biofuels. *Microalgal Biotechnology* (C. Posten and C. Walter), De Gruyter.

- [15] Lamers, van der Laak, Kaasenbrood, Lorier, Janssen, De Vos, Bino and Wijffels (2010) Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. *Biotech. Bioeng.* 106, 638-648.
- [16] Gerken, Donohoe, and Knoshaug (2013) Enzymatic cell wall degradation of *Chlorella vulgaris* and other microalgae for biofuels production. *Planta* 237, 239-253.
- [17] Vandamme, Pontes, Goiris, Foubert, Pinoy and Muylaert (2011) Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. *Biotechnol. Bioeng.* 108, 2320-2329.
- [18] Vandamme, Foubert, Fraeye, Meesschaert and Muylaert (2012) Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. *Bioresource Technology* 105, 114-119.
- [19] Vandamme, Foubert, Meesschaert and Muylaert (2010) Flocculation of microalgae using cationic starch. *J. Appl. Phycol.* 22, 525-530.
- [20] Salim, Bosma, Vermue and Wijffels (2011) Harvesting of microalgae by bio-flocculation, *J. Appl. Phycol.* 23, 849-855.
- [21] Salim, Vermue and Wijffels (2012) Ratio between autoflocculation and target microalgae affects the energy-efficient harvesting by bio-flocculation, *Bioresource Technology* 118, 49-55.
- [22] Salim, Kosterink, Tchetskoua Wacka, Vermue and Wijffels (2014) Extracellular polymeric substances; the key factor in autoflocculation of *Ettlia texensis*. *J Biotechnol.* 174, 34-38.
- [23] Gunerken, d'Hondt, Eppink, Garcia-Gonzalez, Elst and Wijffels (2015) Cell disruption for microalgae biorefineries. *Biotechnol. Adv.* 33, 243-260.
- [24] Gottel, Eing, Gusbeth, Frey, Muller (2011) Influence of Pulsed Electric Field (PEF) treatment on the extraction of lipids from the microalgae *Auxenochlorella protothecoides*. In International Conference On Plasma Science, Proceedings 1-1.
- [25] Goettel, Eing, Gusbeth, Straessner and Frey (2013) Pulsed electric field assisted extraction of intracellular valuables from microalgae. *Algal Res.* 2:401-408.
- [26] Postma, Capitoli, Barbosa, Wijffels, Eppink, Olivieri and Pataro (2016). Selective extraction of intracellular components from the microalgae *Chlorella vulgaris* by combined Pulsed Electric Field - Temperature treatment. *Bioresour. Technol.* 203, 80-88.
- [27] Zbinden, Sturm, Nord, Carey, Moore and Shinogle (2013) Pulsed electric field (PEF) as an intensification pretreatment for greener solvent lipid extraction from microalgae. *Biotechnol. Bioeng.* 110, 1605-1615.
- [28] Parniakof, Barba, Grimi, Marchal, Jubeau, Lebovka and Vorobiev (2015) Pulsed electric field and pH assisted selective extraction of intracellular components from microalgae *Nannochloropsis*. *Algal Research* 8, 128-134.

- [29] Kim, Choi, Park, Lee, Yang, Kim, Park, Hwan Kim and Lee (2012) Ionic liquid-mediated extraction of lipids from algal biomass. *Bioresource Technology* 109, 312-315.
- [30] Desai, Streefland, Wijffels and Eppink (2014) Novel astaxanthin extraction from *Haematococcus pluvialis* using cell permeabilising ionic liquids. *Green Chem.* 18, 1261-1267.
- [31] Desai, Streefland, Wijffels and Eppink (2016) Extraction and stability of selected proteins in ionic liquids based aqueous two phase systems. *Green Chem.* 16, 2670-2679.
- [32] Urzu, Marcati, Sayd, Sante-L houtellier, Djelveh and Michaud (2014) Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella Vulgaris*. *Bioresour. Technol.* 157, 134-139.
- [33] Cuellar-Bermudez, Aguilar-Hernandez, Cardenas-Chavez, Ornelas-Soto, Romero-Ogawa and Parra-Salvidar (2015) Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. *Microbial Biotechnology* 8, 190-209.
- [34] Schadock-Hewitt, Pittman, Stevens and Marcus (2013) Extrusion-Based Differences in Two Types of Nylon 6 Capillary-Channeled Polymer (C-CP) Fiber Stationary Phases as Applied to the Separation of Proteins via Ion Exchange Chromatography. *J.Appl. Polymer Science.* 128, 1257-1265.
- [35] Podgornik (2013) Fast separation of large biomolecules using short monolithic columns. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 927, 80-89.
- [36] Avramescu, Borneman and Wessling (2003) Mixed-matrix membrane adsorbers for protein separation. *Journal of Chromatography A* 1006, 171-183.
- [37] Weaver, Husson, Murphy and Wickramasinghe (2013) Anion exchange membrane absorbers for flow-through polishing steps: Part II. Virus, host cell proteins, DNA clearance and antibody recovery. *Biotechnol. Bioeng.* 110, 500-510.



Prof.dr Michel Eppink

'Biorefinery is the recovery of products from biomass with less or no waste production analogous to the oil refinery which has evolved in the last 100 years developing a defined set of products. However, as the fossil fuels will decrease in the next decades other solutions have to be developed to replace these oil refineries and with that biorefinery will become important in the near future not only for fuel but more for other applications (chemicals, feed, food, pharma, etc.).'