AQUACOMBINE

Final conference, Esbjerg, Denmark, November 21, 2023

Funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 862834. Any results of this project reflect only this consortium's view and the European Commission is not responsible for any use that may be made of the information it contains.

PANEL SESSION II: BIOREFINERY CONCEPT

How to extract, fractionate and purify bio-active compounds of halophyte plants in a cheap, common, easy to handle and environmentally friendly biorefinery.

PROF. PAUL CHRISTAKOPOULOS
LULEÅ UNIVERSITY

PROF. HINRICH UELLENDAHL
FLENSBURG UNIVERSITY OF APPLIED SCIENCES

DR. JOB TCHOU-MTCHOUA
CELABOR

MALTHE FREDSGAARD
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PROF. IWONA CYBULSKA
UNIVERSITE CATHOLIQUE DE LOUVAIN

DR. LAURA HULKKO
AALBORG UNIVERSITY

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Plan for the panel

- 10 MIN INTRO TO THE AQUACOMBINE BIOREFINERY
- 5 MIN ON PHYTOCHEMICALS EXTRACTION IN LAB AND PILOT SCALE
- 5 MIN ON GREEN PROTEIN FROM HALOPHYTES
- 5 MIN ON PROBIOTIC FEED FROM HALOPHYTES
- 5 MIN ON XOS FROM HALOPHYTES
- 5 MIN ON BIOGAS FROM HALOPHYTES
- 5 MIN ON BIOREFINERY SCALE UP
- 20 MIN DISCUSSIONS

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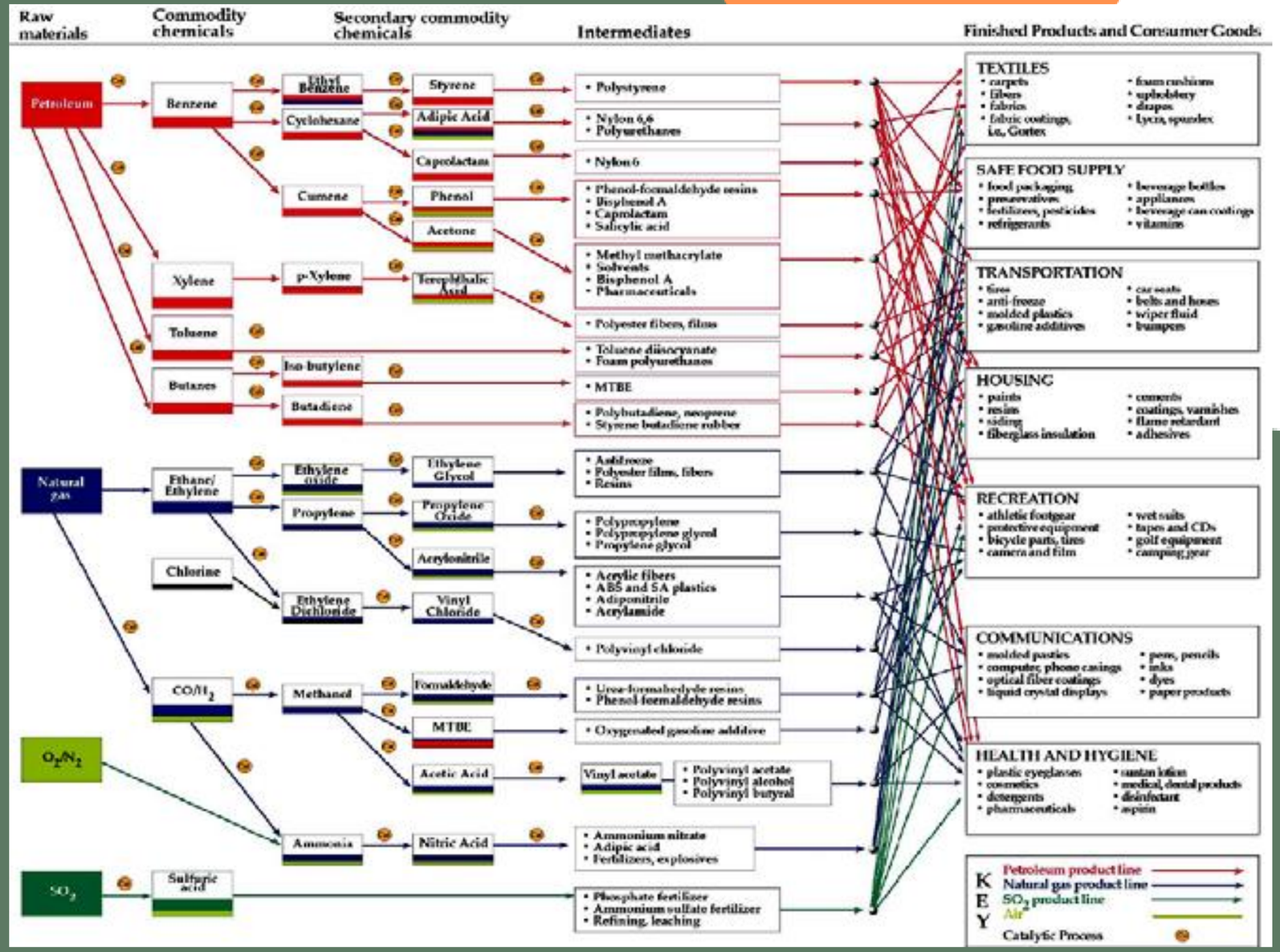
What is a Bioefinery

PETROLEUM REFINERY



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What is a Bioefinery

BIO-REFINERY

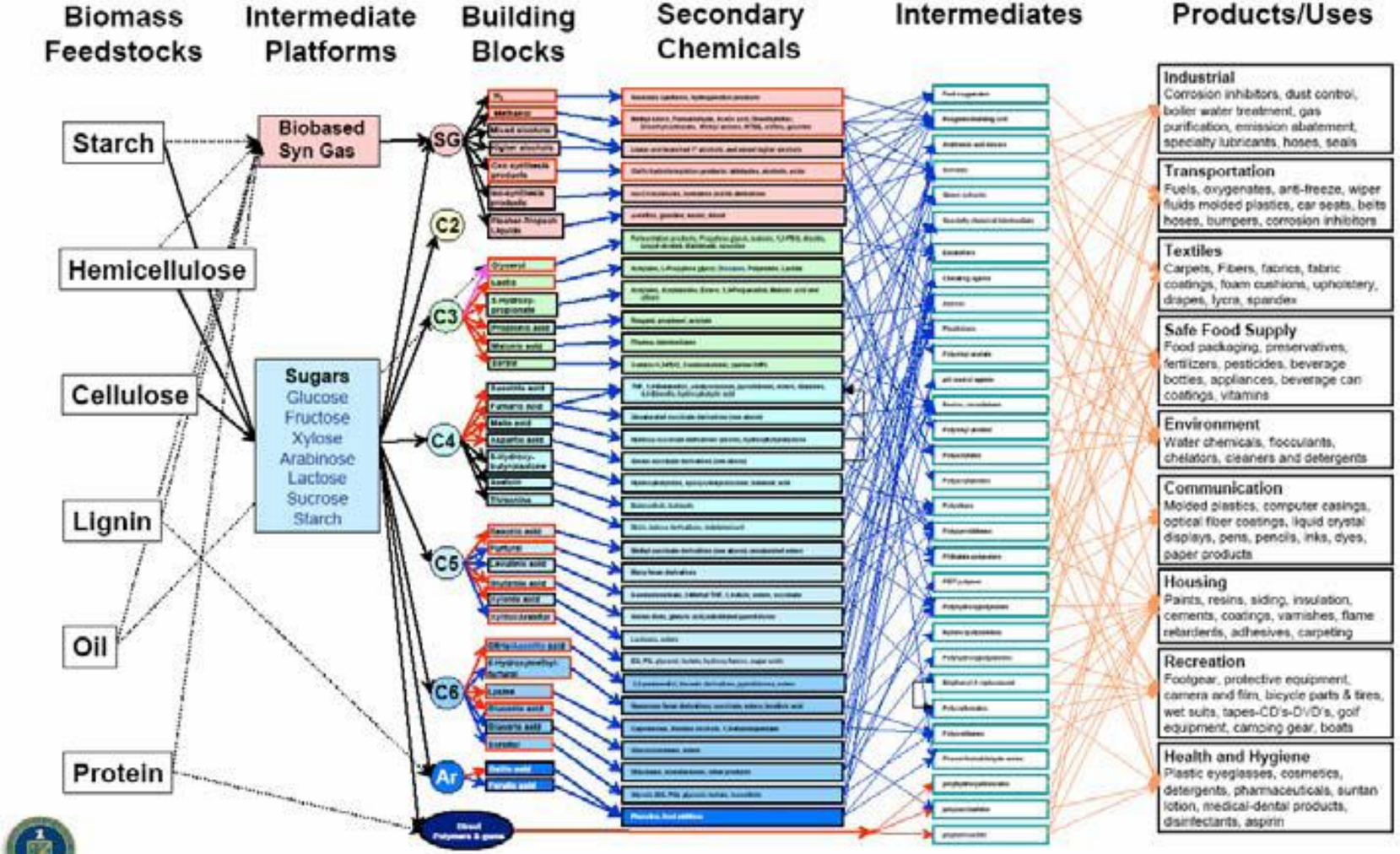

Figure 3 – Analogous Model of a Biobased Product Flow-chart for Biomass Feedstocks

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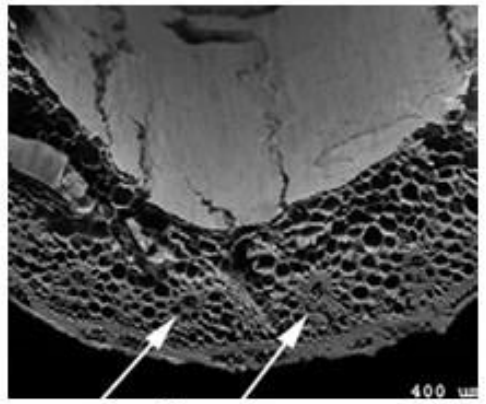
Traditional lignocel. Bioefinery

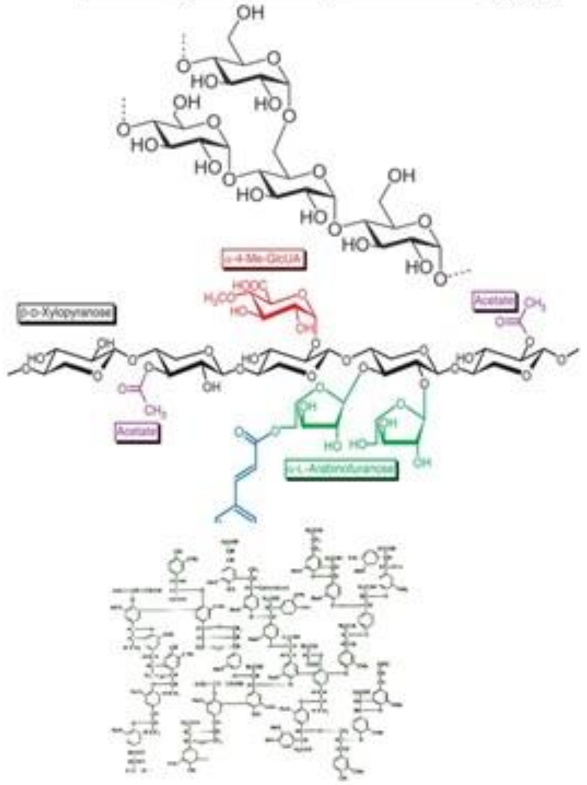
TRADITIONAL BIOREFINERIES LIGNOCELLULOSIC BIOMASSES

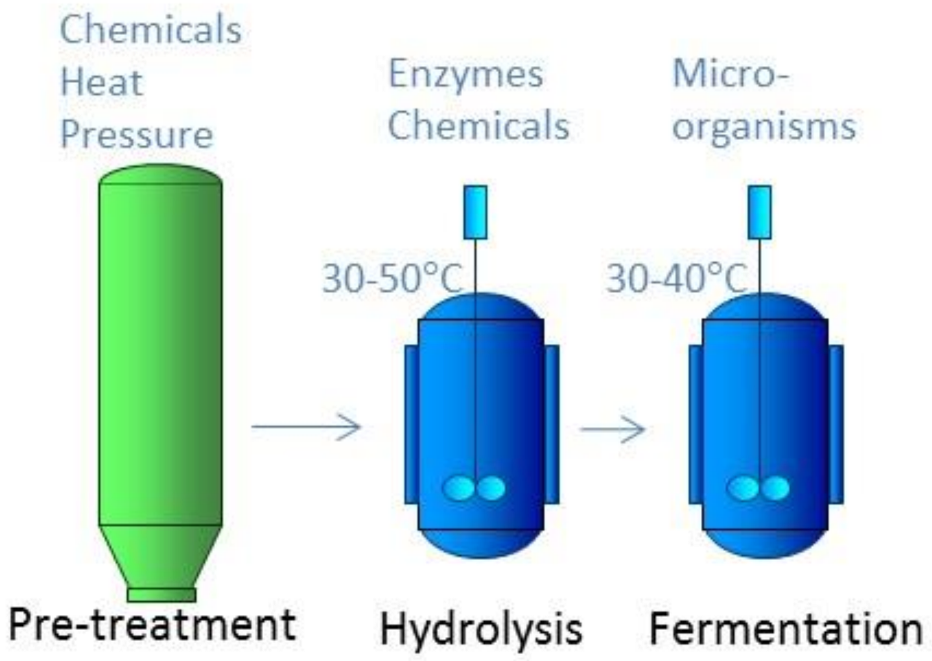
1. Detailed knowledge of biomass composition

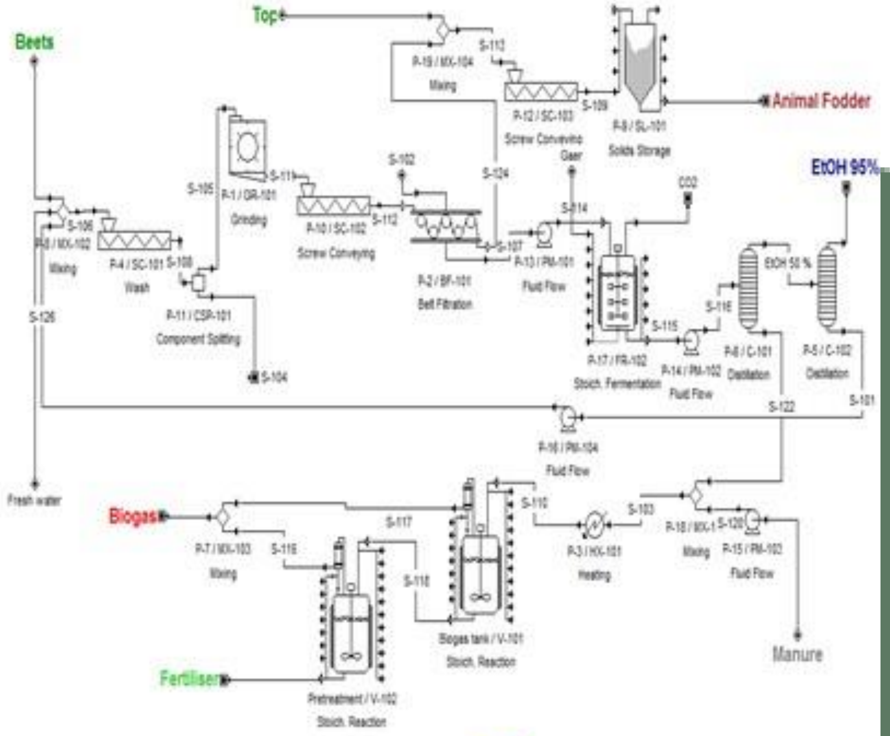
2. Biomass processes (low temperature biological conversion)

3. Bioprocess evaluation (computer design and modeling)









Lack of feasibility!
Solution: Value added products.

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The Aquacombine Bioefinery

Halophyte are old medicinal plants and offer health benefits that are highly sought after in today’s society, where consumption of purpose bred crops and refined food are causing an epidemic in lifestyle diseases.

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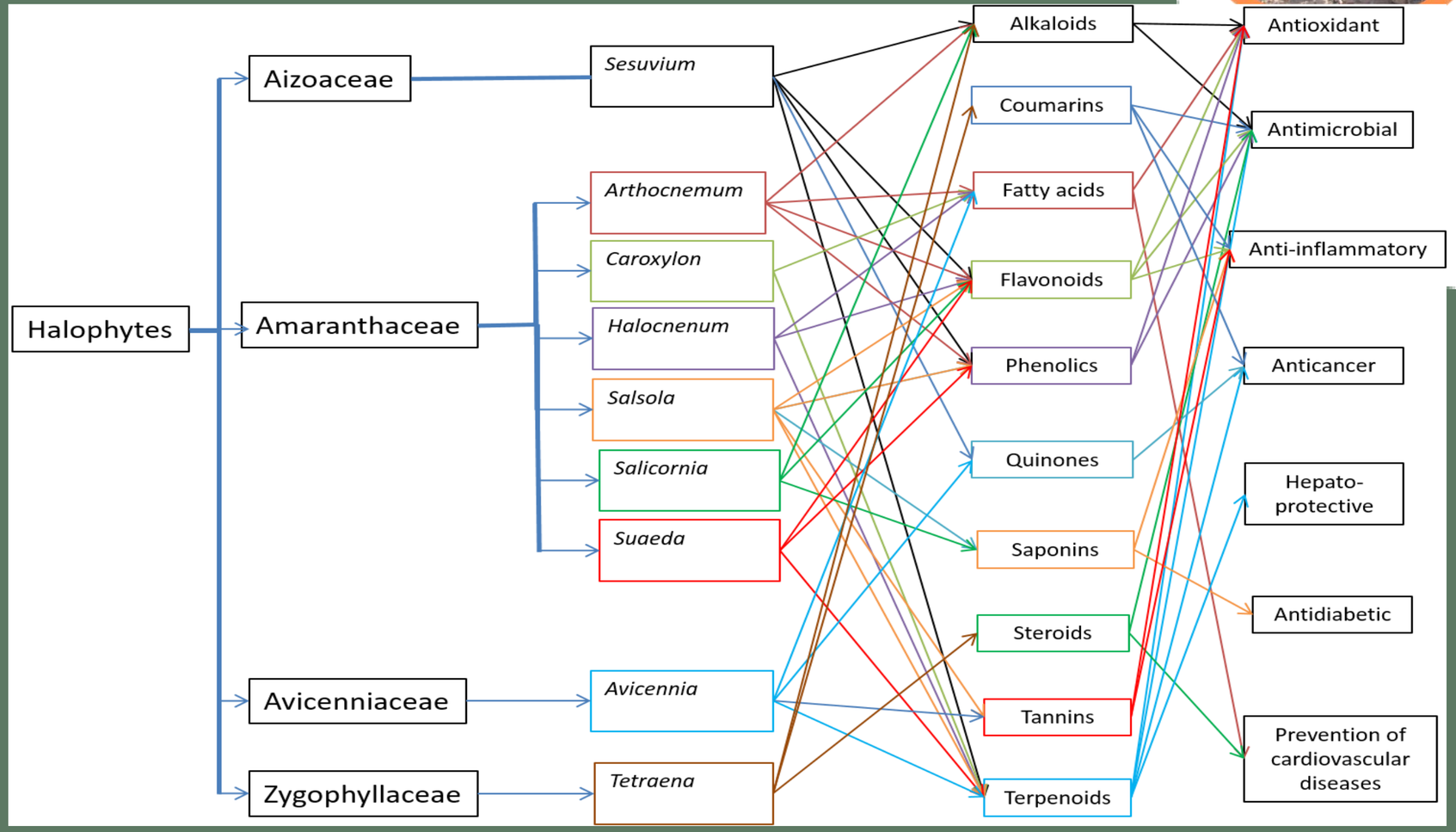
The Aquacombine Bioefinery

30-50% of the dry plant is extractives!

Some phenolic acids and flavonoids have very high value!

Possible production of high value products in the biorefinery!



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AQUACOMBINE

Fresh tips for food

Dry Halophyte straw

Green succulent halophyte biomass

Extractives fraction

Bioactive compounds

Antioxidants

Anti-inflammatory compound

Antimicrobials

Extractives free fibres

Fibres for biogas

Fibres for feed products (dietary fibres)

Green juice

Protein

Lipids

Carotenoids

Chlorophyll

Green pulp

Fibres for biogas

Fibres for feed products (dietary fibres)

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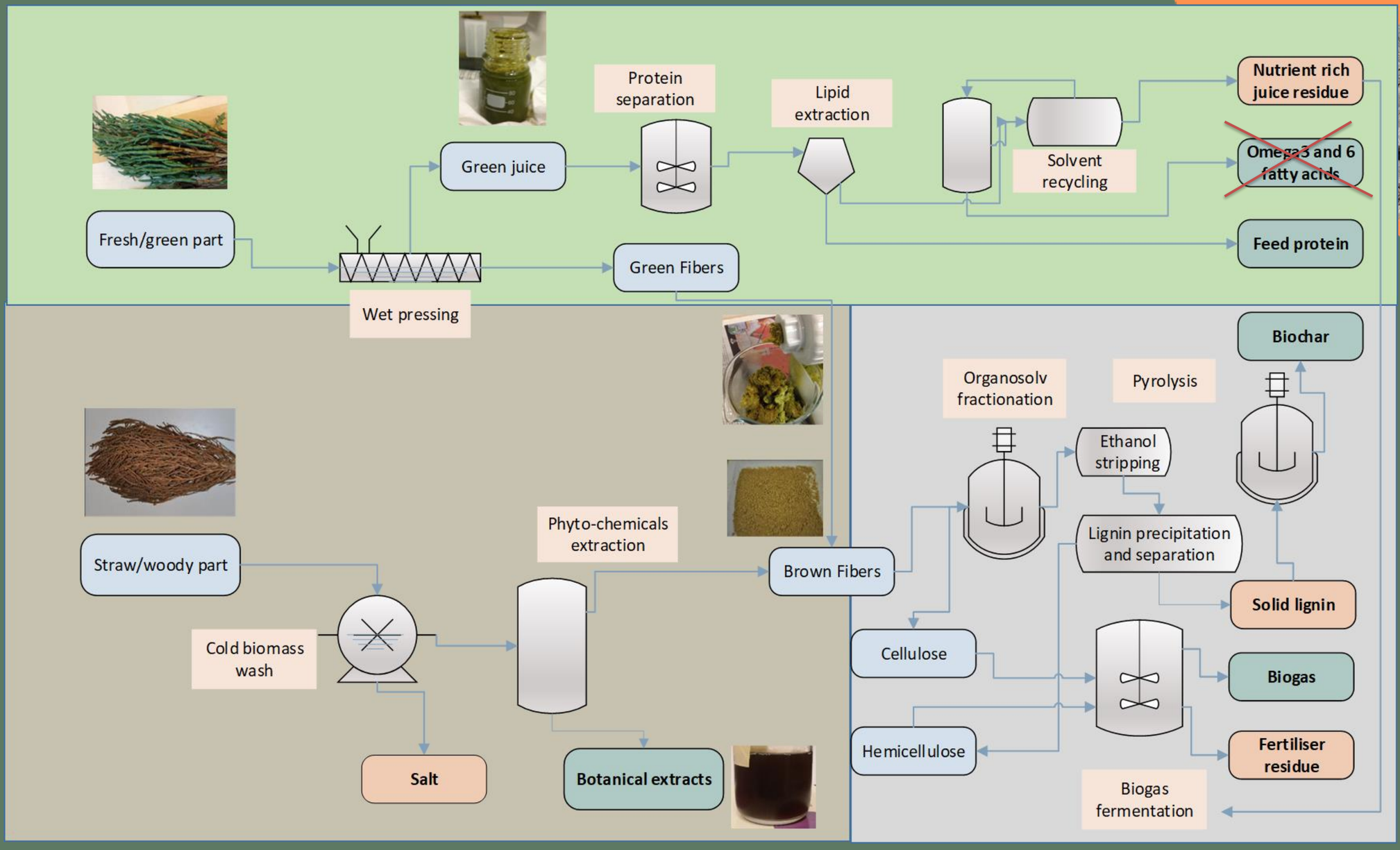

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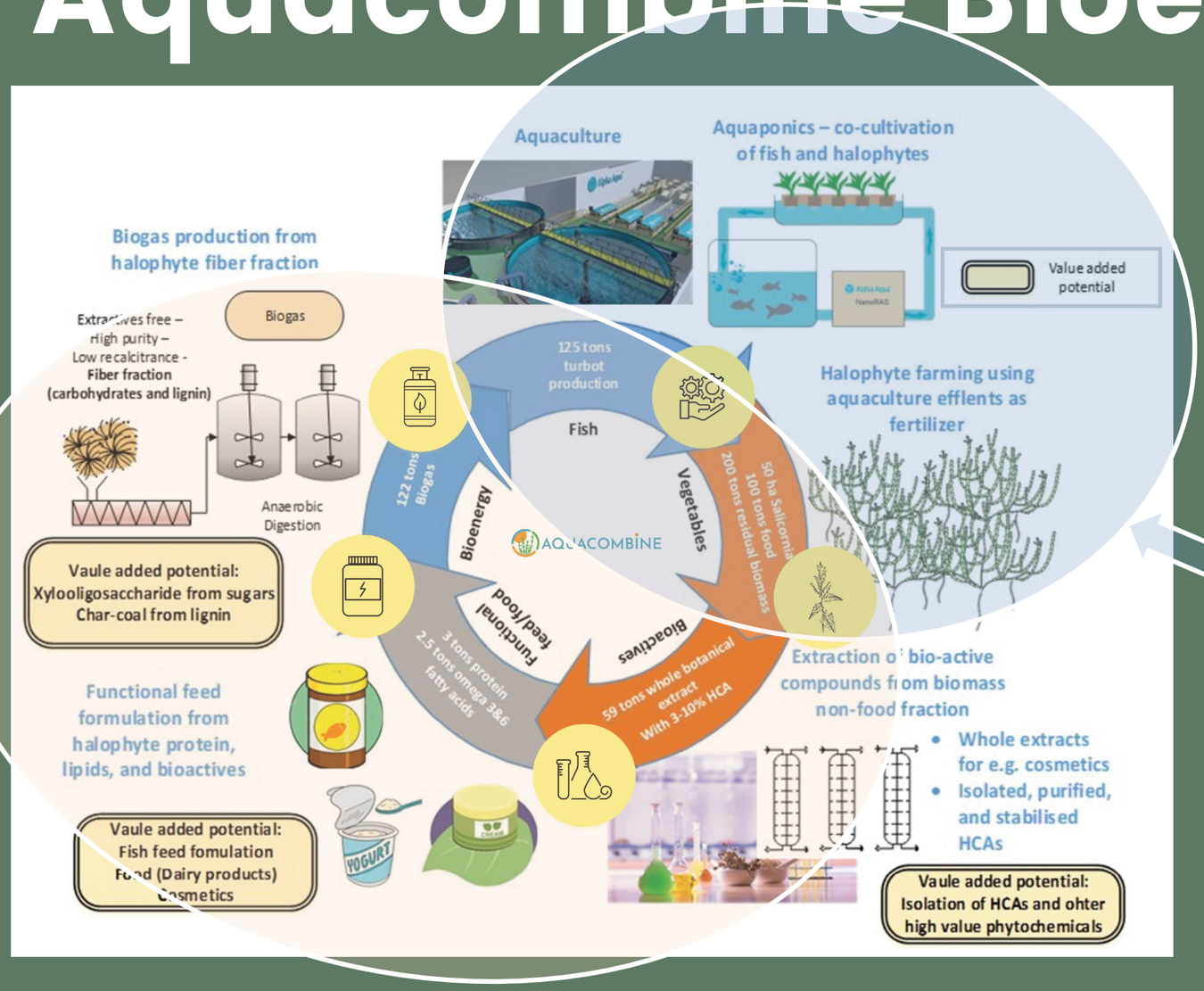
The Aquacombine Bioefinery

Biorefinery part



Food part

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The Aquacombine Bioefinery

DR. JOB TCHOU-MTCHOUA
CELABOR

EXTRACTION
STABILIZATION

PROF. IWONA CYBULSKA
UNIVERSITE CATHOLIQUE DE LOUVAIN

GREEN PROTEIN FRACTIONATION

DR. LAURA HULKKO
AALBORG UNIVERSITY

GREEN BIOREFINERY

PROF. PAUL CHRISTAKOPOULOS
LULEÅ UNIVERSITY

FIBER CONVERSION XOS

PROF. HINRICH UELLENDAHL
FLENSBURG UNIVERSITY OF APPLIED SCIENCES

BIOGAS
BIOCHAR

MALTHE FREDSGAARD
AALBORG UNIVERSITY

BIOREFINERY SCALE UP

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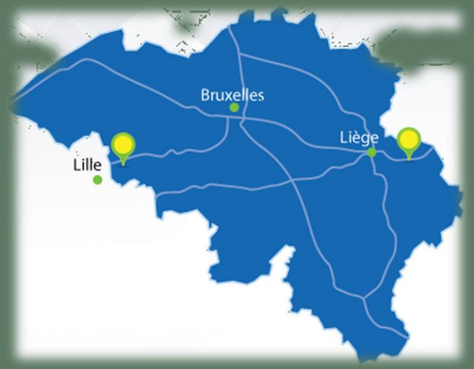
Phytochemicals extraction in lab and pilot scale

JOB TCHOUMTCHOUA

R&D Project Leader - Celabor



CELABOR is a Belgian private **research** and **innovation** center composed of a multidisciplinary team of around **50 people** involved in the **bioeconomy** sector

EXTRACTION | **FOOD** | **MATERIALS** | **ENVIRONMENT**

ISO 17025 certified routine analysis laboratory

- ❑ 400+ customers in Belgium and Europe
- ❑ 200+ analyses in our catalog
- ❑ 20+ analytical equipment
- ❑ Possibility of personalised development

Participation in innovative projects

- 20+ National projects
  - ➢ Win2wal, Win4collective, Feder,…
- 20+ European projects
  - ➢ Cornet, CBE, Interreg, Horizon Europe…

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Phytochemicals extraction in lab and pilot scale

Scaling-up of bioactives recovery by green solvent extraction technology in Celabor's ATEX zone

- ✓ Enrichment by **adsorption resin** or **membrane filtration**
- ✓ Purification by **CPC-CPE** or **flash chromatography**

- ✓ Global content evaluation by **spectrophotometry**
- ✓ Metabolites quantification by **UHPLC/HPLC/GC**
- ✓ Contaminants determination by **GC/ICP**

Pre-treatment & Extraction

Fractionation & Purification

Post-treatment & Drying

Characterisation & Quantification

Evaluation & Stability Study

- ✓ Evaluation of the **grinding** and **drying** steps
- ✓ Evaluation of **different types** of extraction
- ✓ Optimisation of an extraction process with **DoE**

- ✓ Separation by **centrifugation** or **filtration**
- ✓ Solvent elimination by **rotavapor**
- ✓ Drying by **freeze drying** or **spray drying**

- ✓ Specific **activity evaluation** (anti-ageing, antioxidant,…)
- ✓ Stability studies in climatic chamber for **conservation assessment**

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Phytochemicals extraction in lab and pilot scale

Green Salicornia

Brown Salicornia

Press

Juice
(AAU)

Residues

Supercritical
CO2
extraction

Carotenoids

Subcritical
water
extraction

Polyphenols

Residues

SWE
alkali
extraction

Polyphenols

Residues

Supercritical
CO2
extraction

Lipids ?

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Phytochemicals extraction in lab and pilot scale

Workflow of brown salicornia

Pre-treatment of feedstocks

Drying & Grinding at 4mm

Subcritical H2O extraction

100 – 160°C 1 – 3 cycles (+) or (-) NaOH

Concentration & Enrichment

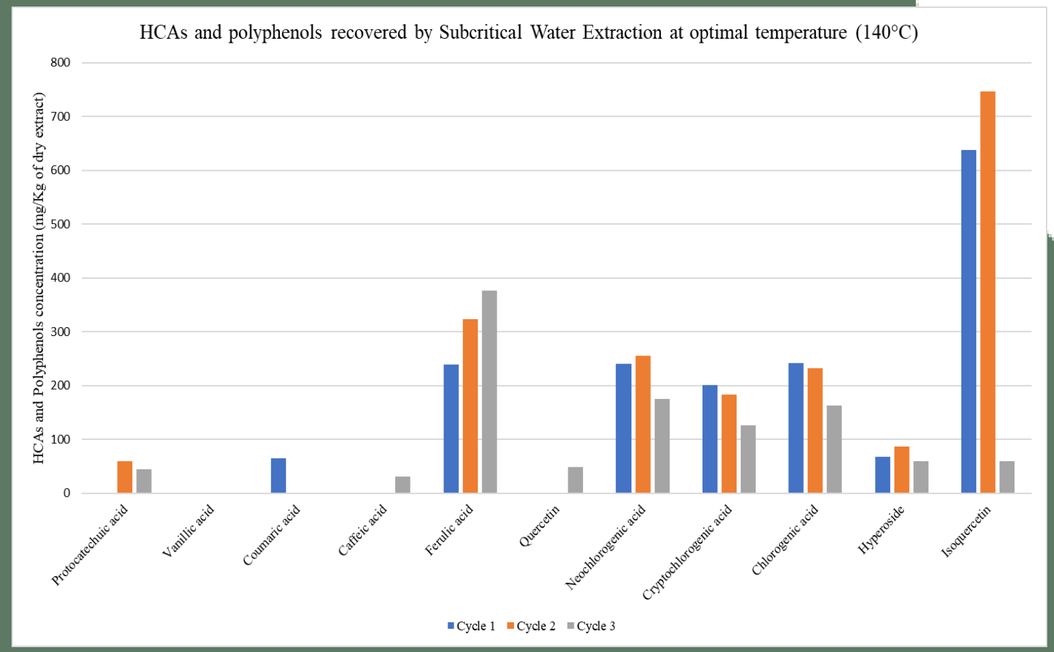
Ultrafiltration or Xad Resin

Isolation of bioactives

Fast Centrifugal chromatography

Post-treatment

Freeze-drying or Spray drying



Subcritical water extraction allowed the recovery not only of bioactive HCAs but also high value flavonoids such as hyperoside and isoquercetin

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Phytochemicals extraction in lab and pilot scale

Subcritical water extraction - A scalable process

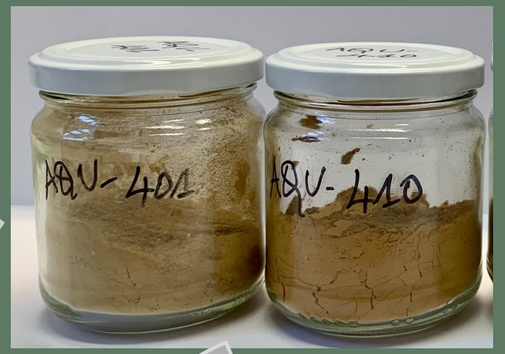
Sub-critical Water Extraction
- High temperature
- High pressure
- Water in liquid state
- But with lower polarity
- And high solubility of phenolics
- Short extraction time

Optimisation
- Duration
- Temperature
- S/L ratio
- Pressure

Lab-scale
(1 – 10g)

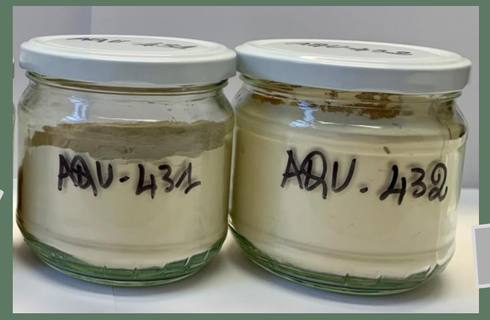
Initial scale-up
(300 – 500g)

Optimised method



Freeze-dried and concentrated
- ✓ Folin >> High TPC
- ✓ LC-MS >> Concentrated phenolics
- ✓ FRAP >> Highly anti-oxidant
- ✓ DPPH >> Highly anti-oxidant

Encapsulation in maltodextrin



Dairy, Feeds & Cosmetics Products

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Green Protein from halophytes

IWONA CYBULSKA

PROF. IWONA CYBULSKA

UNIVERSITE CATHOLIQUE DE LOUVAIN

Iwona Cybulska is a scientist with many years of experience in the topics of biorefineries, biomass fractionation to generate intermediates, their characterization and downstream processing to target bio-based commodity and high-value chemicals as well as processing of halophytes and medicinal plants: botanical extracts isolation, purification and characterization for application in the production of food, nutraceuticals and cosmetics. In the Aquacombine project, she is the leader of WP5 with the main focus on protein and xylooligosaccharides recovery and purification.

Contact data
Email: Iwona.Cybulska@uclouvain.be
LinkedIn: Iwona Cybulska

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Green Protein from halophytes

IWONA CYBULSKA

PROF. IWONA CYBULSKA

UNIVERSITE CATHOLIQUE DE LOUVAIN

[20231116_Iwona_Video.mp4 (sharepoint.com)]

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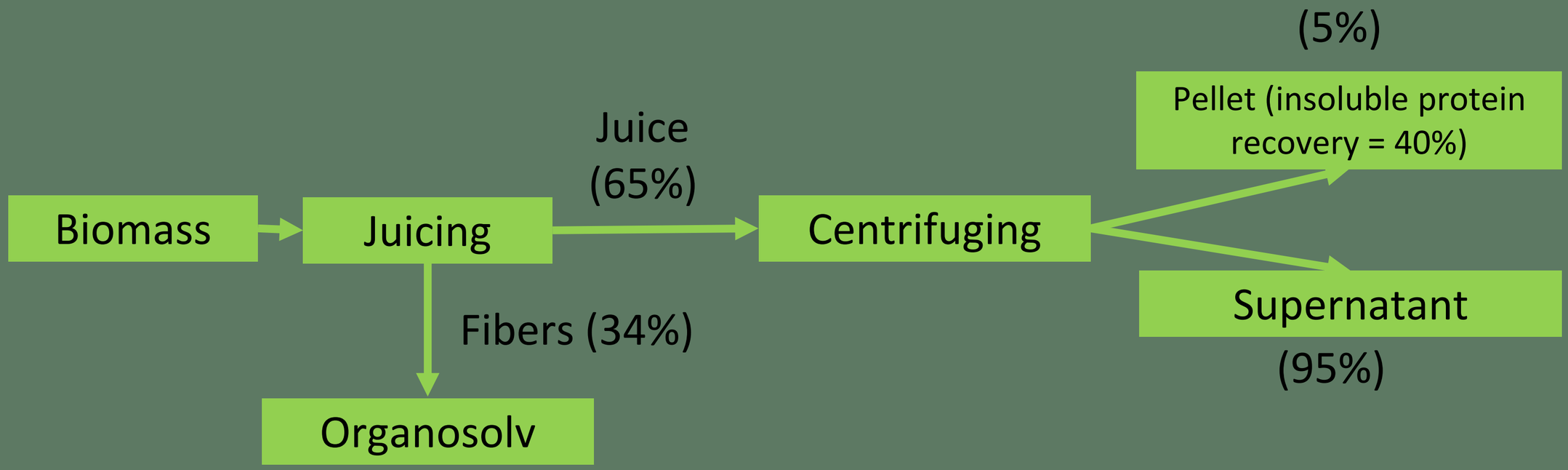
Green Protein from halophytes

MAIN RESULTS

- Protein recovery from fresh plants in a processable form
- Method – centrifugation of the green juice => further use of the fractions



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Probiotic feed from halophytes

LAURA HULKKO

- PhD project carried out within AQUACOMBINE project
- Focused on green biorefinery processing of halophyte biomass
  - Feedstock characterisation
  - Functional feed (protein precipitation)
  - Nutraceuticals and cosmetics (bioactive extracts)

Contact data
Email: lssh@energy.aau.dk
LinkedIn: Laura Hukko

https://vbn.aau.dk/da/persons/148747

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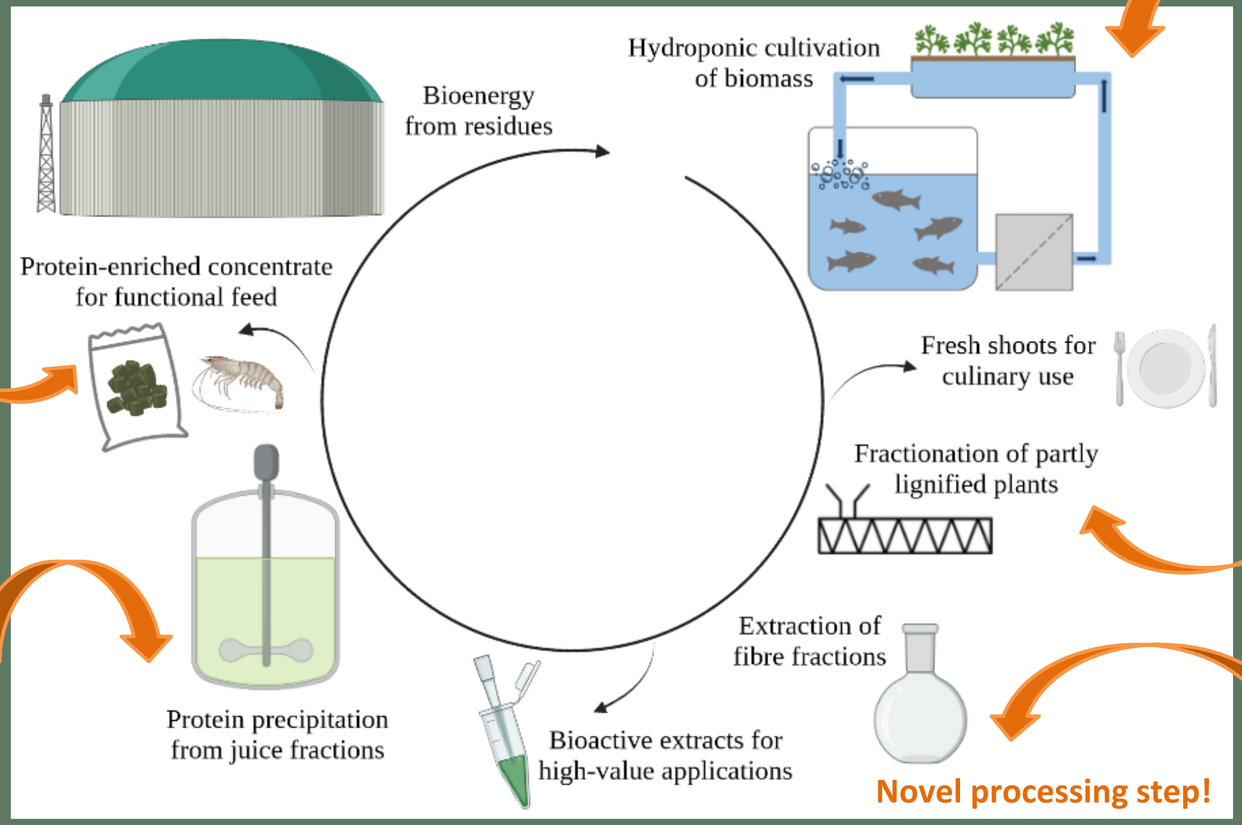
Probiotic feed from halophytes

LAURA HULKKO



Feeding trials with shrimp

Protein precipitation from juice:

- **Protein recovery comparable traditional green biorefineries**
- **No significant differences between precipitation methods**
- **Lactic acid bacteria capable to survive in saline juice**
- **More value from juice residue?**

Cultivation conditions (e.g., salinity) affect the biomass composition

Fractionation process tested on demonstration scale

Novel processing step!

High concentration of bioactive extractives in fibre residue:

- **Phenolic compounds**
- **Antioxidant properties**
- **Enzyme inhibition activity**

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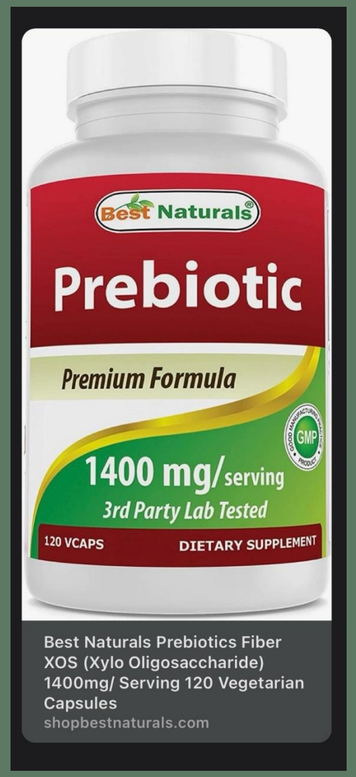
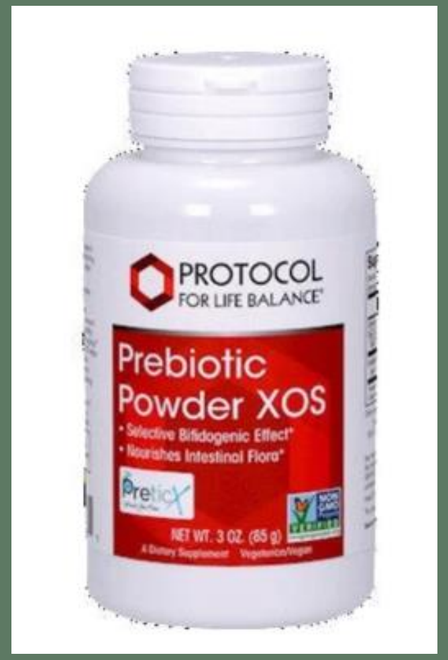
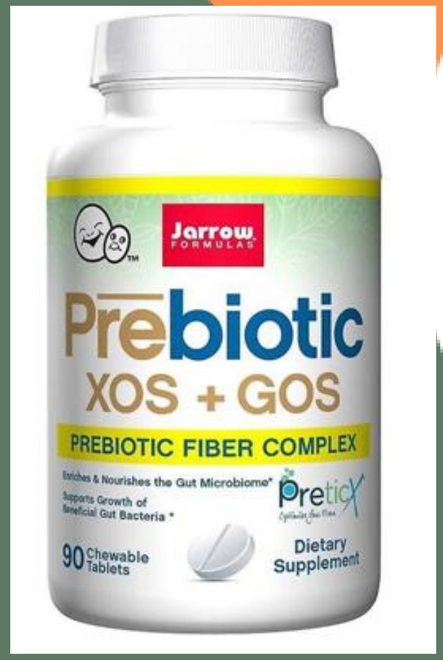
XOS from halophytes

PAUL CHRISTAKOPOULOS







"Prebiotic" describes substances that, while indigestible by humans, act as food for probiotics, the advantageous bacteria in the human gut.

The global prebiotic ingredients market was valued at USD 7.15 billion in 2022 and it is predicted to be worth around USD 22.71 billion by 2032, with a compound annual growth rate (CAGR) of 12.30% from 2023 to 2032.

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Market drivers

Expanding utilization of prebiotics for stomach wellbeing

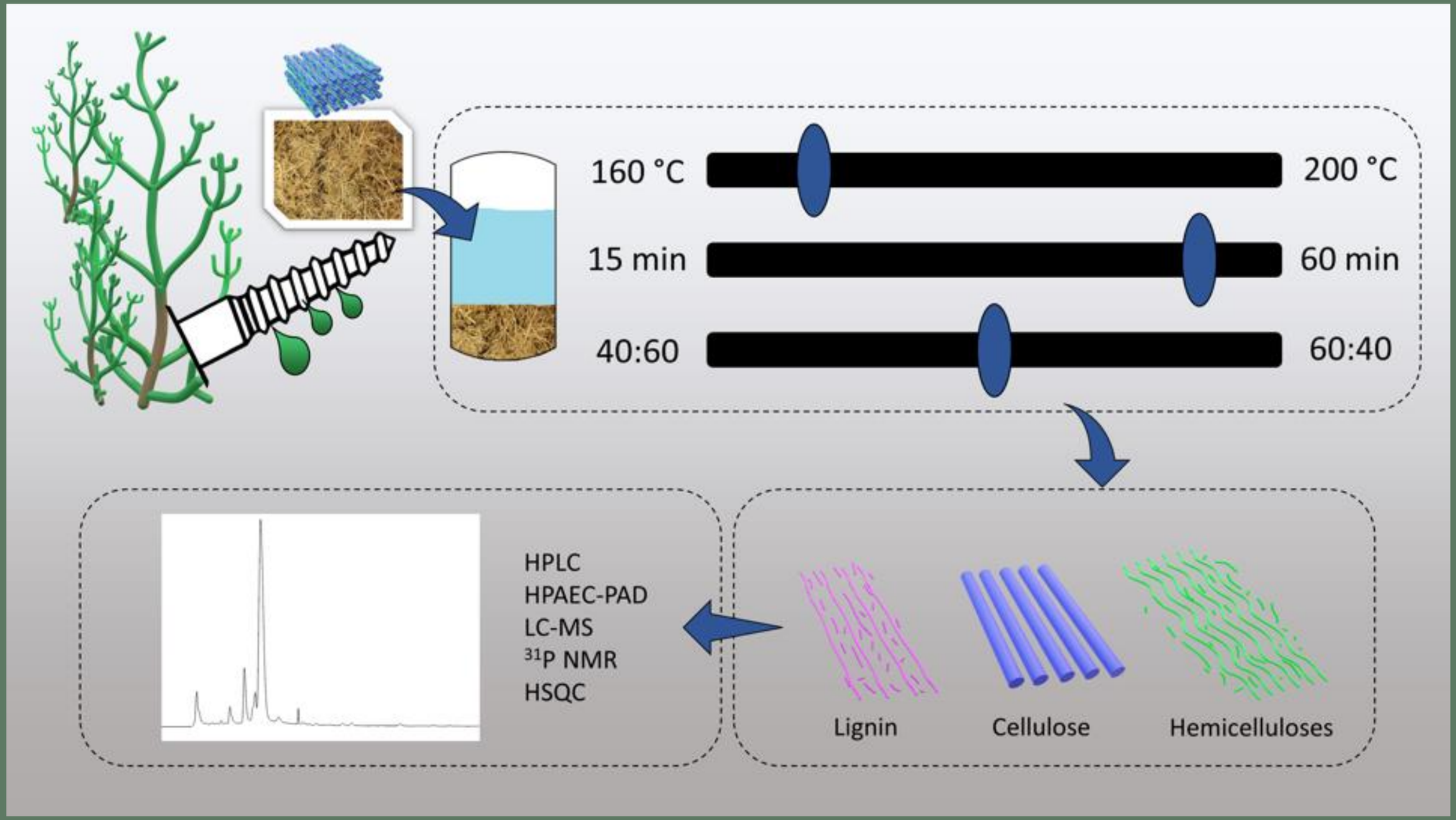
New lifestyle
Maximise wellbeing
Reduce consumption

Market Restraints

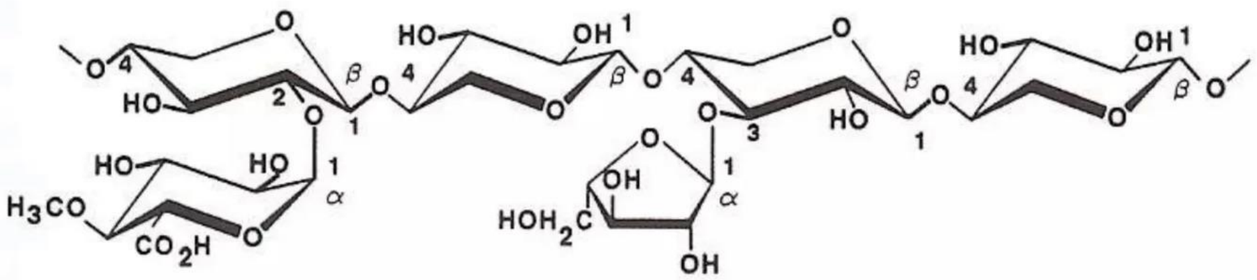
Lack of awareness about the health benefits of prebiotic products among officials and the public.

Current situation in EU

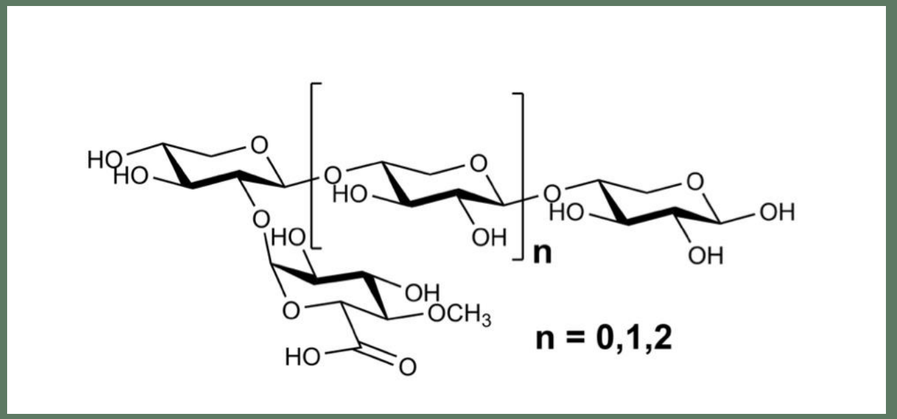
The European Commission (EC) is working to introduce a legislative proposal to find a solution in the scientific substantiation of botanical preparations health claims. While this is not yet resolved, 2,078 health claims on botanical substances can be used.

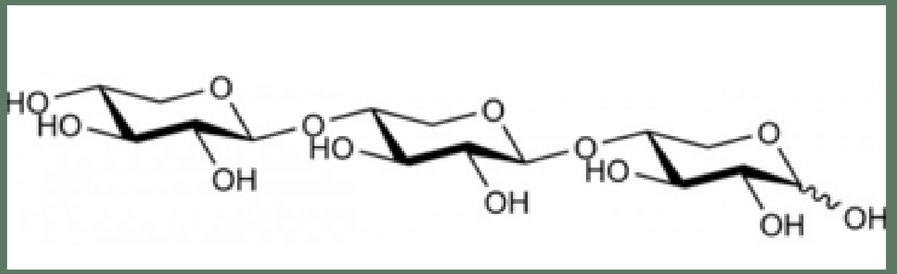

160 °C
200 °C
15 min
60 min
40:60
60:40
HPLC
HPAEC-PAD
LC-MS
31P NMR
HSQC
Lignin
Cellulose
Hemicelluloses

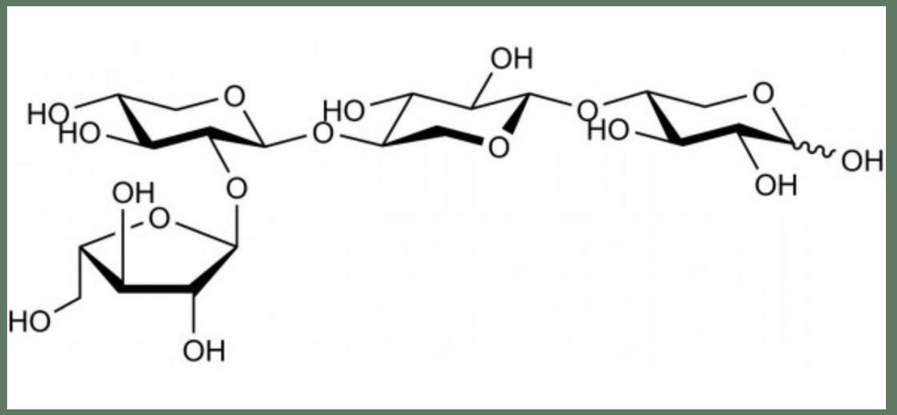
Principal Structure of Arabinoglucuronoxylan



ENZYMES







Biotechnology Letters **24:** 1413–1416, 2002.

Production of acidic xylo-oligosaccharides by a family 10 endoxylanase from *Thermoascus aurantiacus* and use as plant growth regulators

P. Katapodis[1], A. Kavarnou[1], S. Kintzios[2], E. Pistola[2], D. Kekos[1], B.J. Macris[1] & P. Christakopoulos[1,*]

ELSEVIER

International Journal of Biological Macromolecules 41 (2007) 584–589

Biological Macromolecules

www.elsevier.com/locate/ijbiomac

In vitro three-stage continuous fermentation of wheat arabinoxylan fractions and induction of hydrolase activity by the gut microflora

Maria Vardakou [a,b], Carmen Nueno Palop [b], Michael Gasson [b], Arjan Narbad [b], Paul Christakopoulos [a,*]

Aquaculture 578 (2024) 740002

Contents lists available at ScienceDirect

Aquaculture

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

Effects of dietary supplementation of lignocellulose-derived cello-oligosaccharides on growth performance, antioxidant capacity, immune response, and intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*)

Aprajita Singh [b,*], Aleksandar Vidakovic [c], Bernt Hjertner [b], Eleni Krikigianni [c], Anthi Karnaouri [c], Paul Christakopoulos [c], Ulrika Rova [c], Johan Dicksved [c], Kartik Baruah [b], Torbjörn Lundh [b]

Available online at www.sciencedirect.com

ScienceDirect

International Journal of Food Microbiology 121 (2008) 166–170

INTERNATIONAL JOURNAL OF Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short communication

Evaluation of the prebiotic properties of wheat arabinoxylan fractions and induction of hydrolase activity in gut microflora

Maria Vardakou [a,b], Carmen Nueno Palop [a], Paul Christakopoulos [b], Craig B. Faulds [a], Michael A. Gasson [a], Arjan Narbad [a,*]

Eur J Nutr 42: 55–60 (2003)
DOI 10.1007/s00394-003-0400-z

ORIGINAL CONTRIBUTION

Petros Katapodis
Maria Vardakou
Emanuel Kalogeris
Dimitris Kekos
Basil J. Macris
Paul Christakopoulos

Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan

Molecules **2007**, *12*, 1367-1375

molecules

ISSN 1420-3049

www.mdpi.org/molecules

Full Paper

Structural Characterisation by ESI-MS of Feruloylated Arabino-oligosaccharides Synthesised by Chemoenzymatic Esterification

Christina Vafiadi [1], Evangelos Topakas [1], Edwin J. Bakx [2], Henk A. Schols [2] and Paul Christakopoulos [1,*]

Karnaouri et al. Biotechnol Biofuels (2019) 12:285
https://doi.org/10.1186/s13068-019-1628-z

Biotechnology for Biofuels

RESEARCH Open Access

Valorization of waste forest biomass toward the production of cello-oligosaccharides with potential prebiotic activity by utilizing customized enzyme cocktails

Anthi Karnaouri, Leonidas Matsakas, Eleni Krikigianni, Ulrika Rova and Paul Christakopoulos*

Hrůzová et al. Biotechnol Biofuels (2021) 14:32
https://doi.org/10.1186/s13068-021-01875-4

Biotechnology for Biofuels

RESEARCH Open Access

Valorization of outer tunic of the marine filter feeder *Ciona intestinalis* towards the production of second-generation biofuel and prebiotic oligosaccharides

Kateřina Hrůzová[1], Leonidas Matsakas[1], Anthi Karnaouri[1], Fredrik Norén[2], Ulrika Rova[1] and Paul Christakopoulos[1*]

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

Effects of dietary supplementation of lignocellulose-derived cello-oligosaccharides on growth performance, antioxidant capacity, immune response, and intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*)

Aprajita Singh [b,*], Aleksandar Vidakovic [c], Bernt Hjertner [b], Eleni Krikigianni [c], Anthi Karnaouri [c], Paul Christakopoulos [c], Ulrika Rova [c], Johan Dicksved [c], Kartik Baruah [b], Torbjörn Lundh [b]

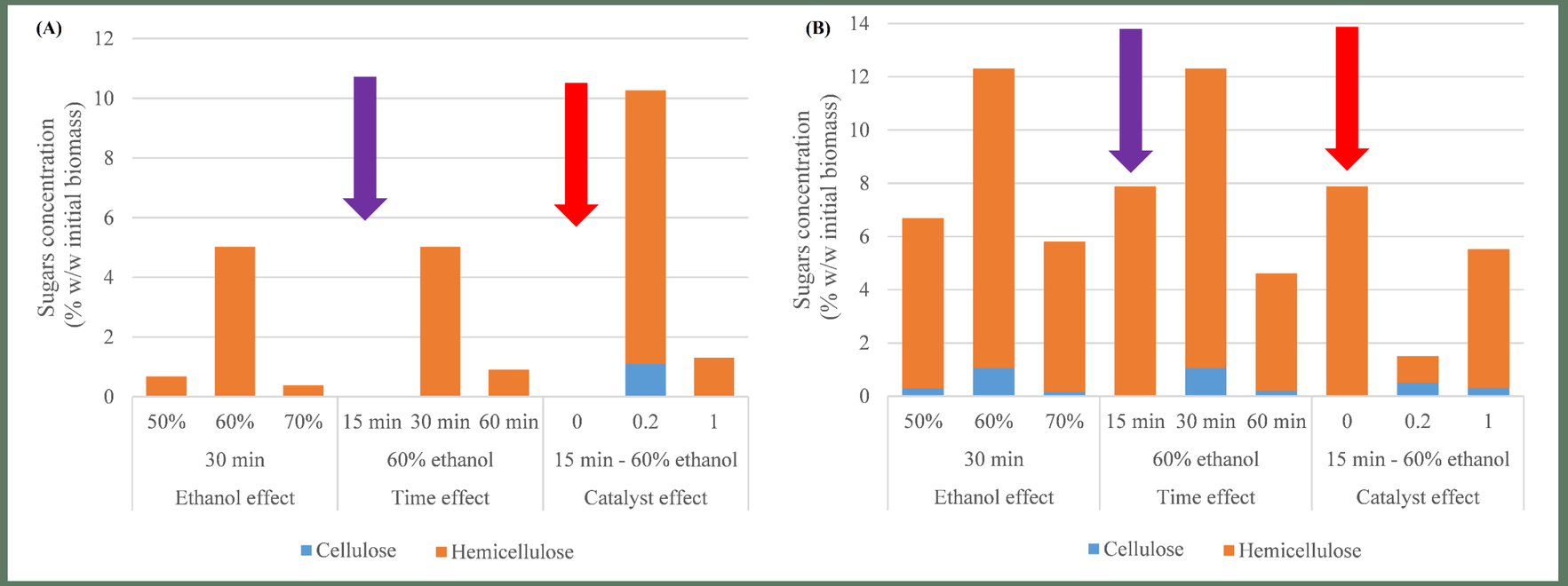
Matsakas *et al. Biotechnol Biofuels (2018) 11:160*
https://doi.org/10.1186/s13068-018-1163-3

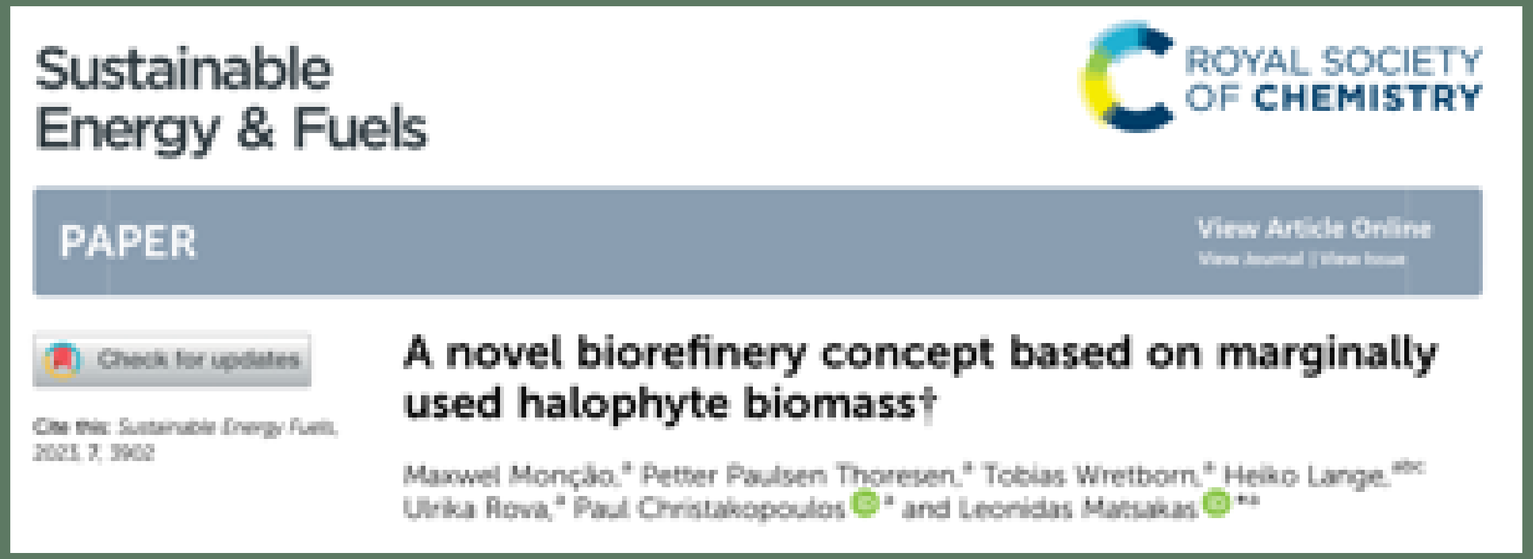
Biotechnology for Biofuels

RESEARCH Open Access

A novel hybrid organosolv: steam explosion method for the efficient fractionation and pretreatment of birch biomass

Leonidas Matsakas[1], Christos Nitsos[1], Vijayendran Raghavendran[2,4], Olga Yakimenko[1], Gustav Persson[3], Eva Olsson[3], Ulrika Rova[1], Lisbeth Olsson[2] and Paul Christakopoulos[1*]





Sustainable Energy & Fuels

ROYAL SOCIETY OF CHEMISTRY

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Cite this: Sustainable Energy Fuels, 2023, 7, 3602

A novel biorefinery concept based on marginally used halophyte biomass†

Maxwel Monção,[a] Petter Paulsen Thoresen,[a] Tobias Wretborn,[a] Heiko Lange,[abc] Ulrika Rova,[a] Paul Christakopoulos[a] and Leonidas Matsakas[a]*

Organosolv Conditions: Temperature 160 °C – Time 30 min -Ethanol content 40 % v/v

Total hemicellulose sugars (XOS) 8.2 g per 100 $g_{biomass}$

XOS 6.23 g Xylose 0.8 Arabinose 0.9 Glucose 0

67% Xylose 33% Arabinose

Biogas from halophytes

HINRICH UELLENDAHL

Professor at Flensburg University of Applied Sciences (FUAS)

WP leader of **WP9:**

Develop and scale up of processes for conversion of residual fractions from extraction processes into biogas (from carbohydrates) and biochar (from lignin)

Task 9.6-9.8

Conversion of residual fibers and juice fractions into biogas

Task 9.1 -9.3

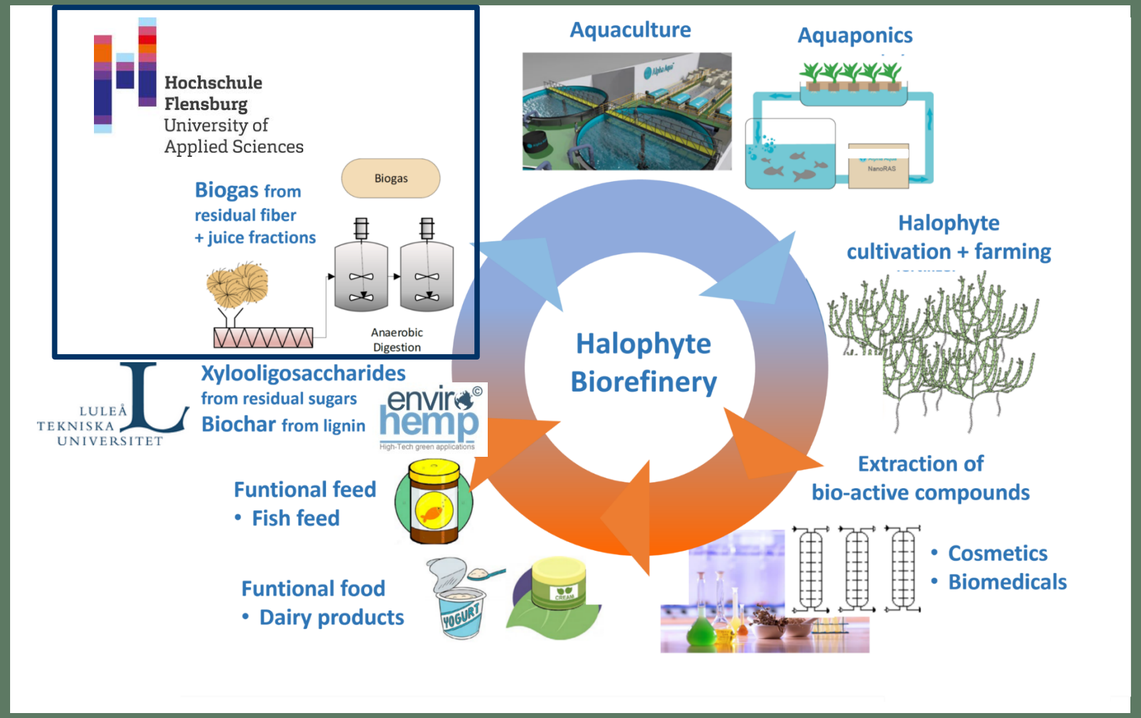
Organosolv pretreatment

Task 9.4 -9.5

Conversion of lignin into biochar

Task 9.9

Scale-up of biogas process



Biogas from halophytes within the biorefinery

Aadila Cayenne

Siddiq Tariq

Hinrich Uellendahl

AQUA-COMBINE

Aquaculture and halophyte Biorefinery

Hochschule Bremerhaven

Assessment of the integrated system

FOOD PROCESSING INITIATIVE

Whole plant **samples** from **AAU (*S. dolichostachya*), LUH (*S. europaea*), RSR (*S. ramossissima*)**

Juice and green fibers after fractionation performed by **FUAS**

Fibers before & after organosolv treatment from **LTU**

Task 9.4 -9.5

Conversion of lignin into biochar

Task 9.1 -9.3

Organosolv pretreatment

Task 9.6-9.8

Conversion of residual fibers and juice fractions into biogas

Task 9.9

Scale-up of biogas process

Biogas from halophytes within the biorefinery

Aadila Cayenne

Siddiq Tariq

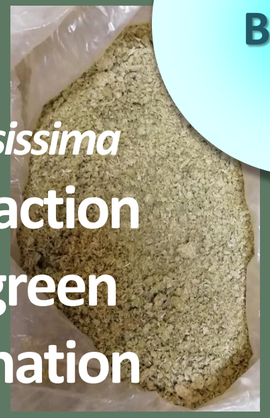
Hinrich Uellendahl

Biogas

S. ramosissima
whole plant

- ***salt content is lowered***

S. ramosissima
fiber fraction after green fractionation



Biogas

Task 9.6-9.8
Conversion of residual fibers and juice fractions into biogas

Hochschule Flensburg
University of Applied Sciences

Biogas from halophytes within the biorefinery

Aadila Cayenne

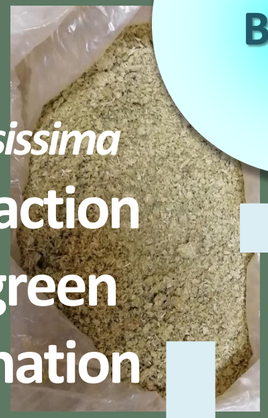
Siddiq Tariq

Hinrich Uellendahl

Biogas

S. ramosissima
whole plant

- *salt content is lowered*
- *lignocellulosic structure is broken*



Biogas

S. ramosissima
fiber fraction after green fractionation

Biogas

S. ramosissima
fibers after organosolv treatment

Task 9.4 -9.5
Conversion of lignin into biochar

Task 9.1 -9.3
Organosolv pretreatment

Task 9.6-9.8
Conversion of residual fibers and juice fractions into biogas

Biogas from halophytes within the biorefinery


Aadila Cayenne


Siddiq Tariq


Hinrich Uellendahl

Biogas

S. ramosissima
whole plant

- *Salt content is lowered*
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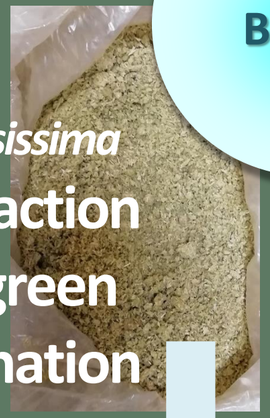


Biogas

S. ramosissima
fiber fraction after green fractionation

Biogas

S. ramosissima
fibers after organosolv treatment

Benefits from biorefining:

- *Lower risk of process inhibition*
- *Higher biogas yield*
- *Easier technical handling*
- *Successful co-digestion with manure in 45:55 (VS/VS) ratio in pilot-scale*

Biorefinery Scale up

MALTHE FREDSGAARD

Research assistant and PhD fellow at AAU

WP leader of **WP3:**

Develop optimized extraction methods for bioactive compounds in lignified biomass

Task 3.1-3.3

Extraction of bioactive polyphenols

Task 4.1

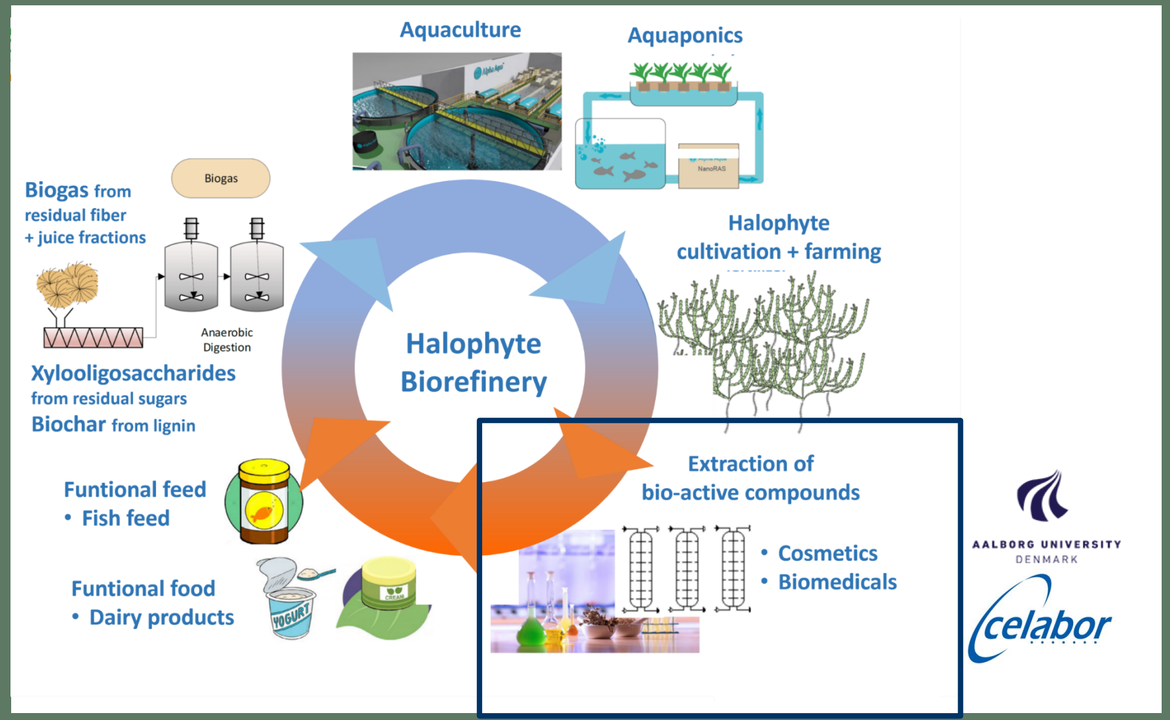
Isolation of polyphenols from extract

Task 6.1

Chemical characterization of the botanical extracts

Task 11.3

Demonstration of biomass processing



Biorefinery Scale up

MALTHE FREDSGAARD

Main considerations for scale-up:

- ***Process flexibility***
- ***Solvent recycle***
- ***Environmental impact***
- ***Extraction time***
- ***Cost of operation***
- ***Scalability***
- ***Simple process – should be operated on a farm site***

200 ml 125x 25 L

10-100 ml 100x 10 L 10x 100 L

Funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 862834. Any results of this project reflect only this consortium's view and the European Commission is not responsible for any use that may be made of the information it contains.

Biorefinery Scale up

MALTHE FREDSGAARD

The scaled AQUACOMBINE extraction method has been:

- *Scaled to 100 L*
- *Made in a 4-skid system in stainless steel in a 40 foot container (30 m^2 footprint)*
- *The process plant can produce 73 m^3 extract per year from 4.3 ton lignified Salicornia*
- *The process plant is modular, can be mass produced, and placed in rural areas for Salicornia extraction*

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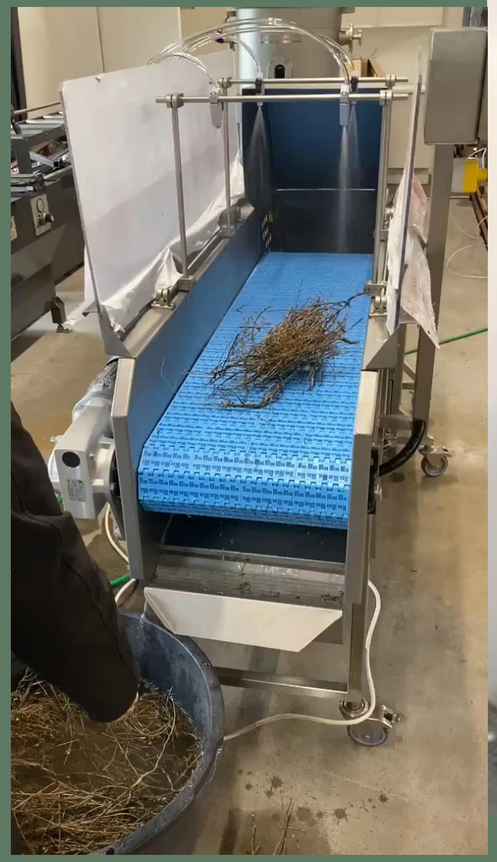
Biorefinery Scale up

MALTHE FREDSGAARD

Green biomass fractionation, fermentation, and pro-biotic protein separation scale up to 100 liter.



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Discussions

QUESTIONS BY MODERATOR:

- MOST PROMISING RESULTS/INNOVATIONS
- NEW INSIGHTS
- CHALLENGES FACED
- SUGGESTIONS HOW TO MOVE FORWARD

QUESTIONS FROM THE AUDIENCE

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Main conclusions

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Thank you!

NAMES AND CONTACTS OF SPEAKERS:

- Mette Hedegaard Thomsen: mht@energy.aau.dk
- Please add!!!

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