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MarineBiotech

Novel bioprocessing

what are the future processing challenges?

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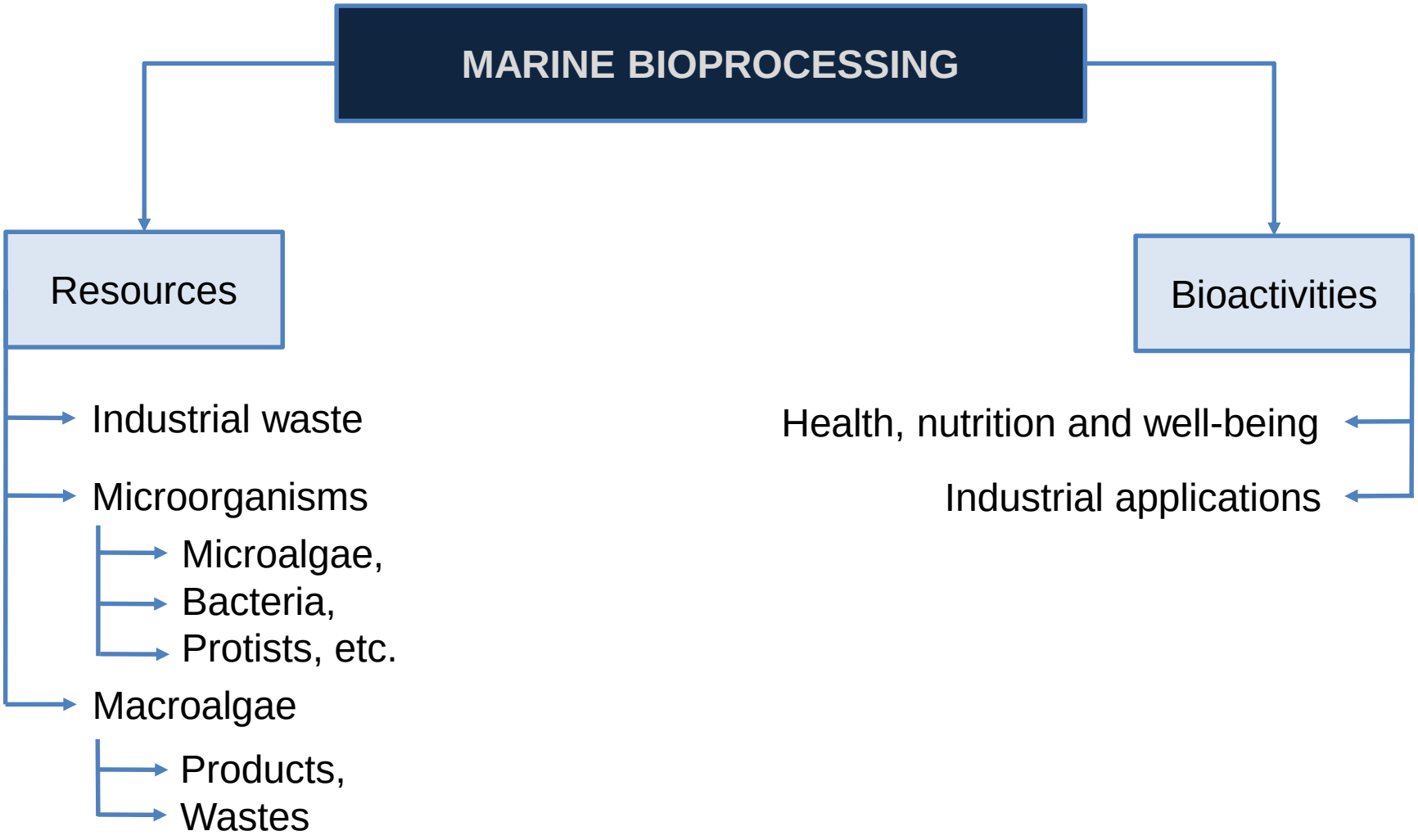
Bioprocessing



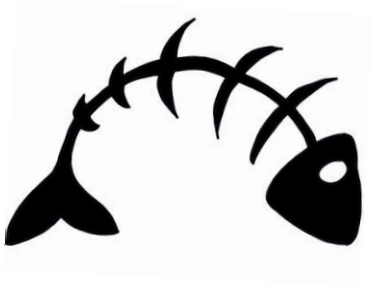
Industrial waste

Largely untapped biomass

Fishery Production: EU-28 was more than 9.5 million ton live weight

2 million ton/year of Marine Rest Raw Material generated in Europe [1]

Thousands of ton of fish are discarded within European waters, due to low commercial value of the catches, lack of quota or because it has been required by law in the old common fisheries policy. New EU Directives introduce significant changes. [2]



1: Richardsen, et al. 2015. Analyse av tilgang og anvendelse for marint restråstoff i Norge, SINTEF Fisheries and Aquaculture, Trondheim.
2: EU: Fisheries, Facts and figures on the Common Fisheries Policy 29/04/2015.

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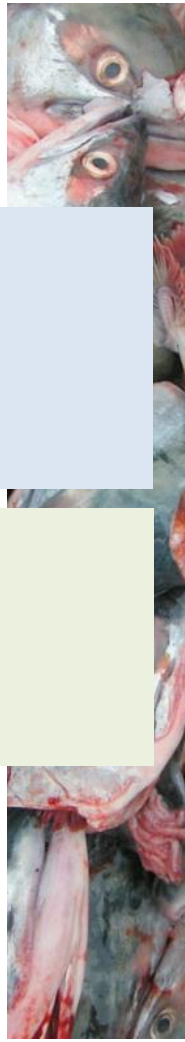


State of play

Today	Trimmings Heads Backbones Skin	→	Marine oils Proteins Collagen Gelatin
Future	Viscera, ~30-40% waste	→	Proteins Enzymes (ex. proteases) Nucleic acids

Development:

- Improvement of fractionation steps
- Processes that cope with the use of multiple species
- Use of potentially new, endogenous enzymes to extract bioactive peptides

DAFIA

Horizon 2020 Programme

Macroalgae

Largely untapped biomass

Carbohydrate-rich

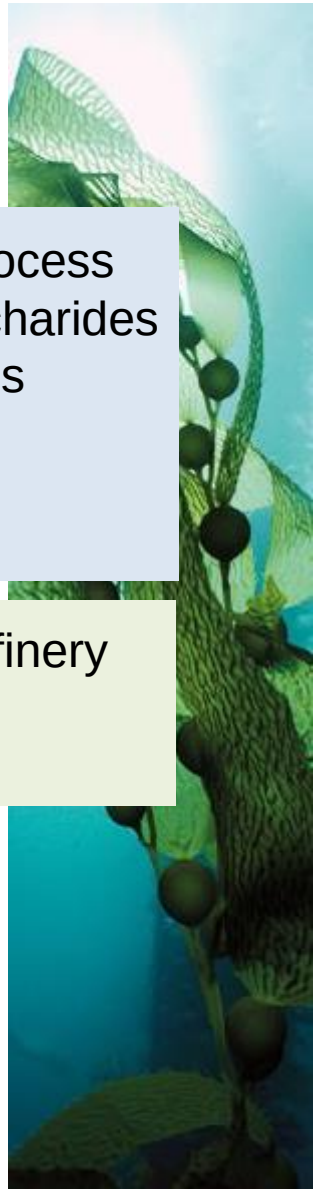
Does not contain recalcitrant lignin of terrestrial plants

Component (%dry weight)	Seaweeds		
	Green	Red	Brown
Carbohydrates	30-60	30-50	20-30
Polysaccharides	Ulvan, starch, cellulose, mannan	Agar, carrageenan, cellulose, lignin	Laminarin, alginate, mannitol, fucoidan, cellulose
Monosaccharides	Glucose, mannose, rhamnose, xylose, galactose, uronic acid, glucuronic acid	Glucose, galactose	Glucose, galactose, xylose, uronic acid, glucuronic acid, fucose, mannuronic acid, guluronic acid
Proteins	10-20	6-15	10-15
Lipids	1-3	0.5-1.5	1-2
Ash	13-22	5-15	14-28

Macroalgae

State of play

Today	250 000 ton produced in Europe [1]	→	Industrial operation, mostly to process high volumes of marine polysaccharides for the production of hydrocolloids
	New bio-based products of higher value		Initiatives manly driven by a new generation of SMEs
Future	Use of marine macro-algae as source of biobased carbon	→	Use of polysaccharides as biorefinery feedstock to be transformed by microbial fermentation



Development:

- Improvement of large scale cultivation and fractionation
- New microbial strains, mostly marine, able to break down macroalgal polysaccharides (enzymes and sugar uptake)

1: FAO Fishstat

Microorganisms

State of play – Microbe as final product

Today	Mostly autotrophic microalgae biomass produced for feed and nutrition application	→	Typically higher end applications. Scalability of operations remains a challenge, particularly for higher volume and lower value products.
Future	Heterotrophic or mixotrophic strains amenable to very large scale production	→	A much wider range of nutrition and feed products will emerge, including lower cost protein sources.

Development:

- Sourcing of microbes at the basis of the trophic chains
- Microbe adaptation and development of large scale and optimized low cost production processes

Microorganisms

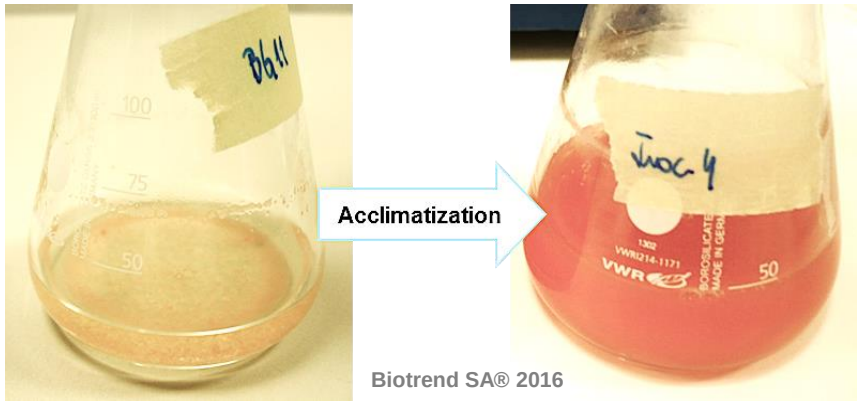
Challenges impacting the ability to culture novel marine microorganisms

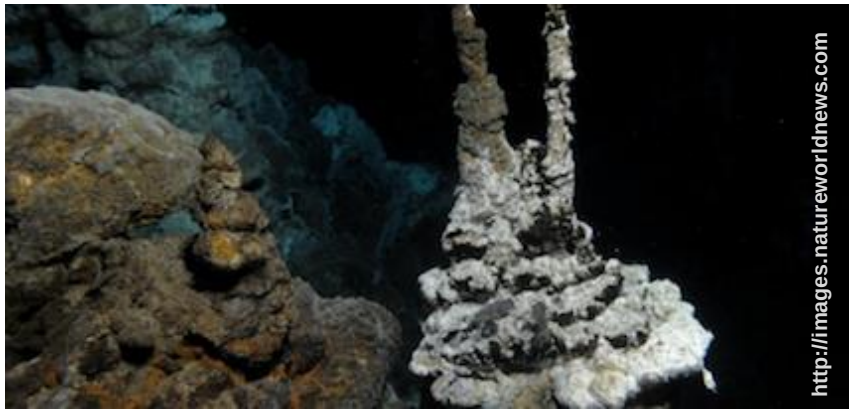
Organism level: Fundamental lack of knowledge of the organisms and their physiology.

Habitat level: Lack of knowledge of the chemistry of the natural habitats.

Interaction level: Lack of knowledge the natural biotic and abiotic interactions.

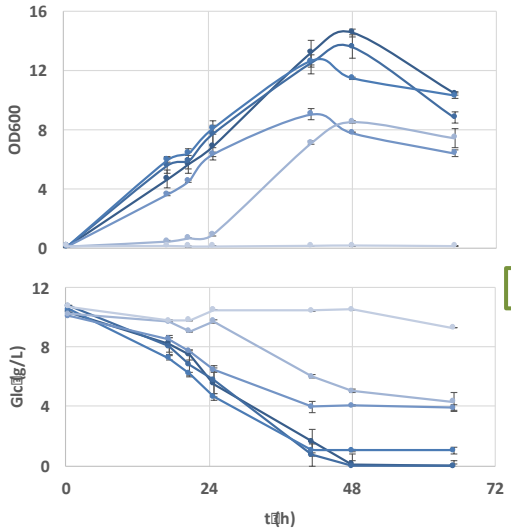
Cultivation level: Conventional *in vitro* cultivation techniques break intra-species communication.

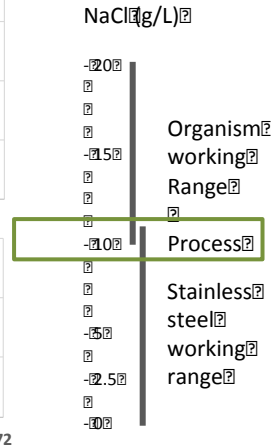


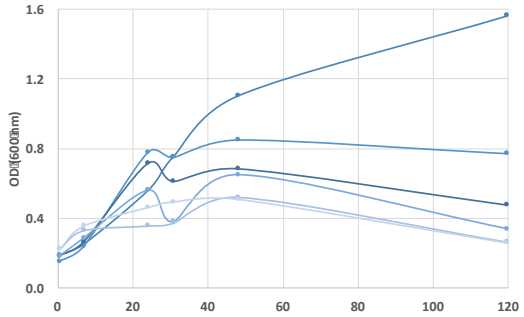


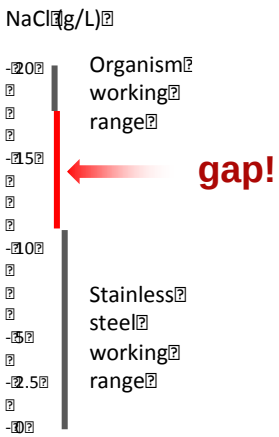
Microorganisms

Coping with industry-standard equipment

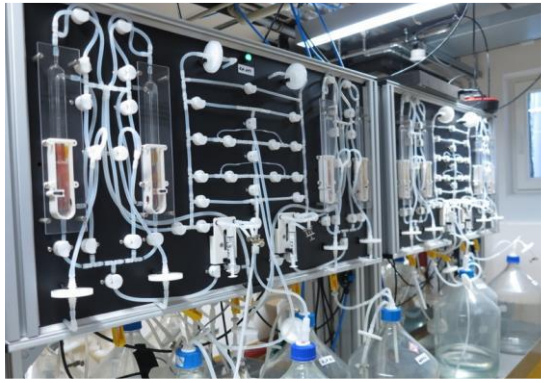








Microorganisms


www.heurisko-inc.com

Evolution of marine organisms (non-GMO)

- Higher growth rates
- Lower salt concentration
- Culture medium engineering and fermentation strategy optimization

Using heterologous hosts

Allows

- Production of molecules from “unculturable” marine organisms
- Use of hosts with proven industry use

Requires:

- Improved annotation of the genomic information from marine microorganisms
- Expand the available molecular biology tools enabling the engineering of a wider range of marine microorganisms

Marine enzymes

Examples of novel activities

- Verenium/BASF: Mext generation alpha-amylase for starch liquefaction originating from *Thermococcus* sp. isolated from a deep-sea hydrothermal vent. The product is a highly evolved, high-performance, thermostable enzyme that is effective over an exceptionally wide temperature and pH range allowing greater operational flexibility.

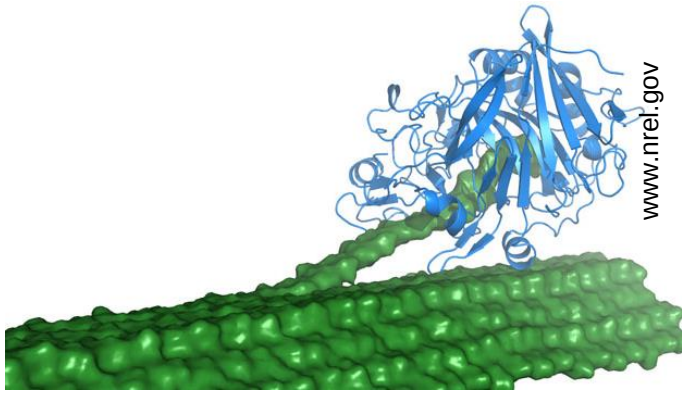
- ArcticZymes: Produces recombinantly expressed enzymes originating from various arctic fish or shrimps living in the cold Arctic sea. The cold-adaption allows the use of simple heating steps for their irreversible inactivation. This unique property allows us to introduce novel, simple and efficient workflows to a range of applications in molecular biology.

- Novozymes: Patented an isolated organophosphorous hydrolase activity from sea slug useful for decontamining an area or a device contaminated with at least one harmful or undesired organophosphorous compound, including poisonous nerve agents and pesticides.

Marine enzymes

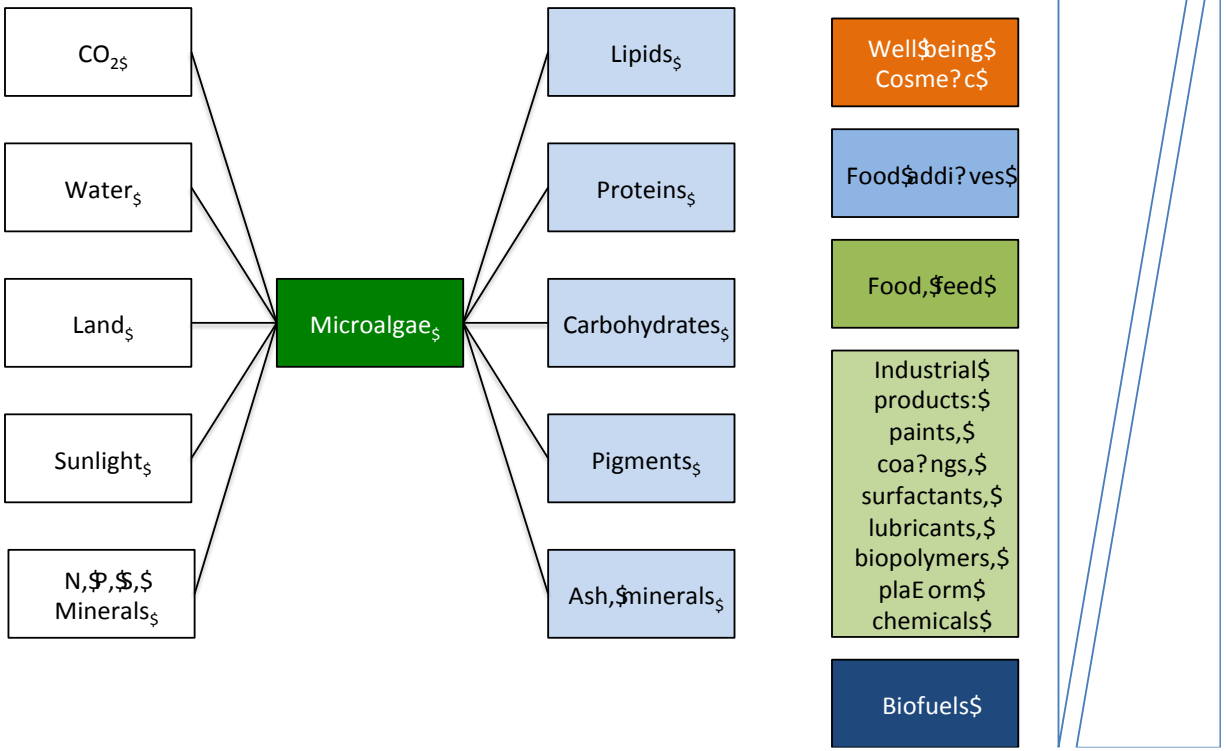
Enhanced diversity allows accessing biorefinery-relevant materials

Compatibility with pre-processing	
Acid hydrolysis:	Enzyme active at low pH, from acidophiles
Heat:	Enzyme active and stable at high temperature, from thermophiles
Ionic liquids:	Enzyme active at high salinities, from halophiles
Compatibility with biomass	
Biomass from halophytes:	Enzyme active at high salinities, from halophiles
Macroalgal biomass:	Enzyme with new functions, new carbohydrases



Marine Biorefineries

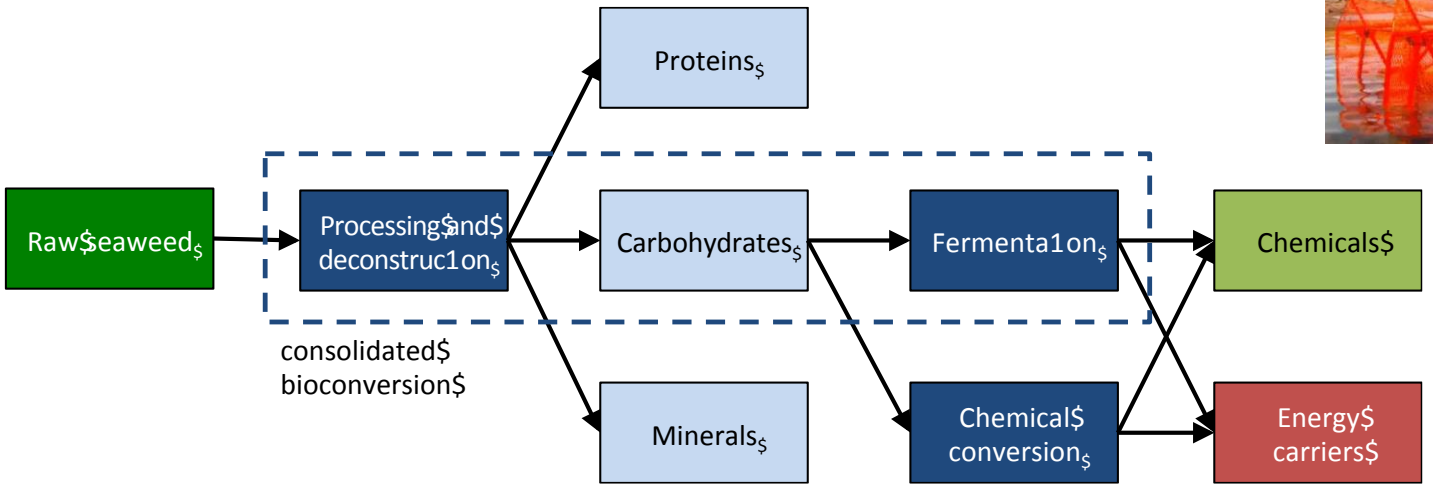
Microalgae biorefinery



Knowledge:	- Improve knowledge of basic biological functions, metabolic engineering tools
Cultivation:	- New cultivation methods of marine microalgae to improve the photosynthetic efficiency and enhance productivity (of biomass and/or specific product or products).
Reactors:	- Develop innovative photobioreactors able to support high photosynthetic efficiency.
Harvest:	- Develop efficient harvest, fractionation and purification processes for microalgae.

Marine Biorefineries

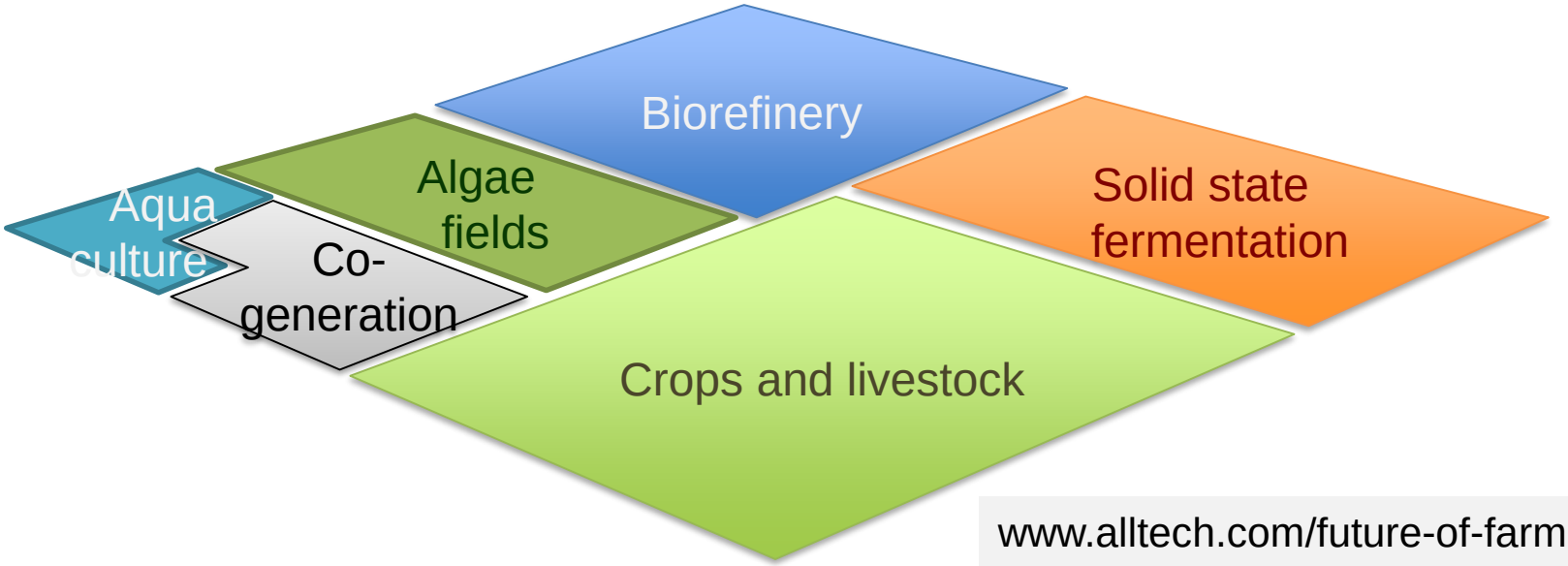
Macroalgae biorefinery



Cultivation:	- Improve methods for the effective off-shore macro-algal farming.
Harvesting:	- Develop efficient harvest of macroalgae.
Fractionation:	- Develop fractionation, including enzymatic, processes of macroalgae.
Fermentation:	- Develop microorganisms able to ferment the range of sugars obtained from the hydrolysis of macroalgal polyssacharides.
Logistics and LCA:	- Study the logistics of supply of biomass and life cycle analysis of the biorefinery concept.

Integration opportunities

Integrated farming



www.alltech.com/future-of-farming

Integration opportunities

Integrated farming

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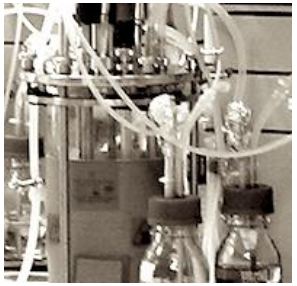

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Advanced services

Conceptual studies

- Literature studies, freedom to operate, competitive intelligence.
- Bioprocess simulation and conceptual design.
- Preliminary economic assessment.

Microbial strain screening

- High-throughput platform for preliminary assessment of process conditions.
- Typical screening activities include:
  - Production of a specific compound
  - Ability to cope with specific medium requirements (ex. use specific sugars, presence of inhibitors, osmotolerance vs. low salt concentrations).

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Advanced services

Process development and optimization

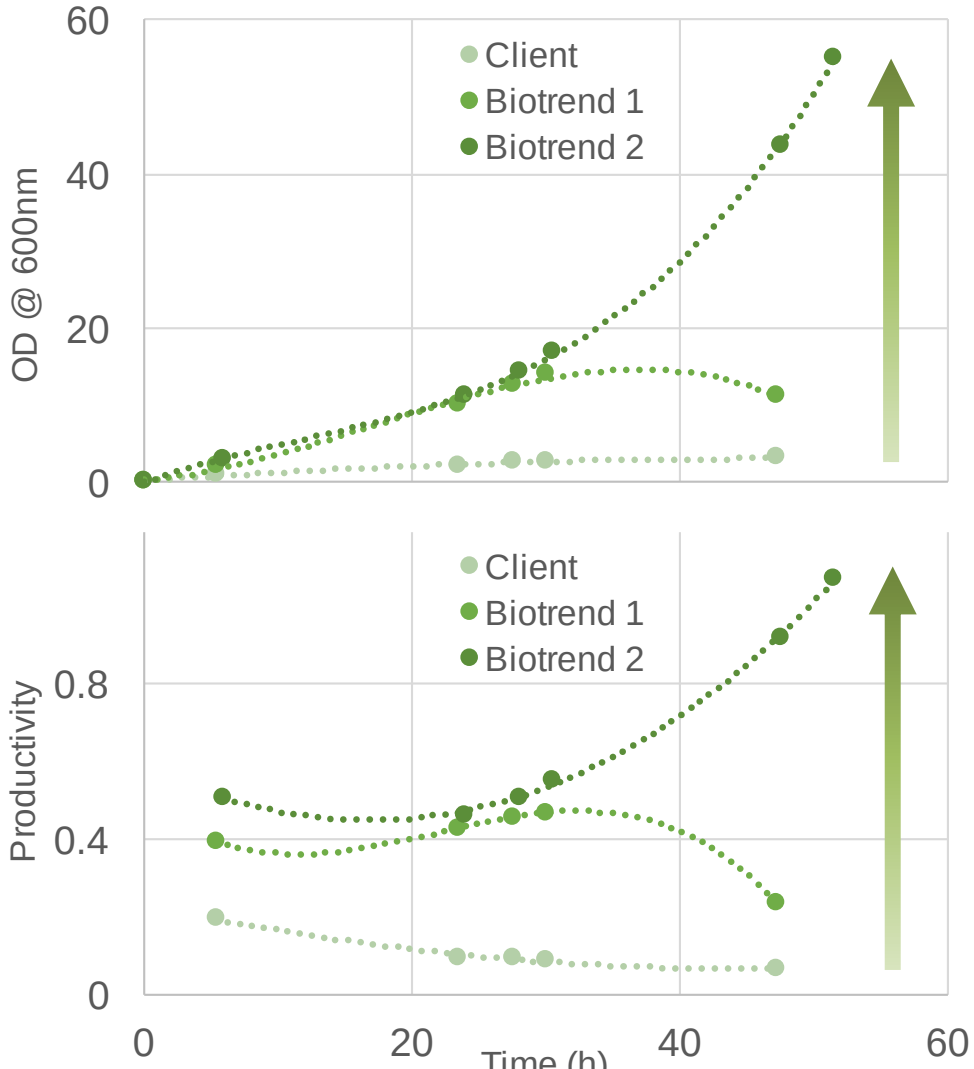
- Parallel fully controlled bioreactors, to devise cultivation strategies aiming at maximizing productivities, yields and concentrations.
- Advanced process optimization strategies.
- Process integration with raw material pre-processing and with downstream processing.

Process scale-up, de-risking and validation

- 2L, 10L, 50L and 250L bioreactors available for thorough scale-up studies and process de-risking.
- Direct access to facilities from 1,000L to 100,000L fermentation capacity.

Core competency

Process intensification: obtain more from less



- Marine bacteria extract production for a marine biotech start-up

Problem: Need to produce larger amounts of biomass for bioactivity tests.

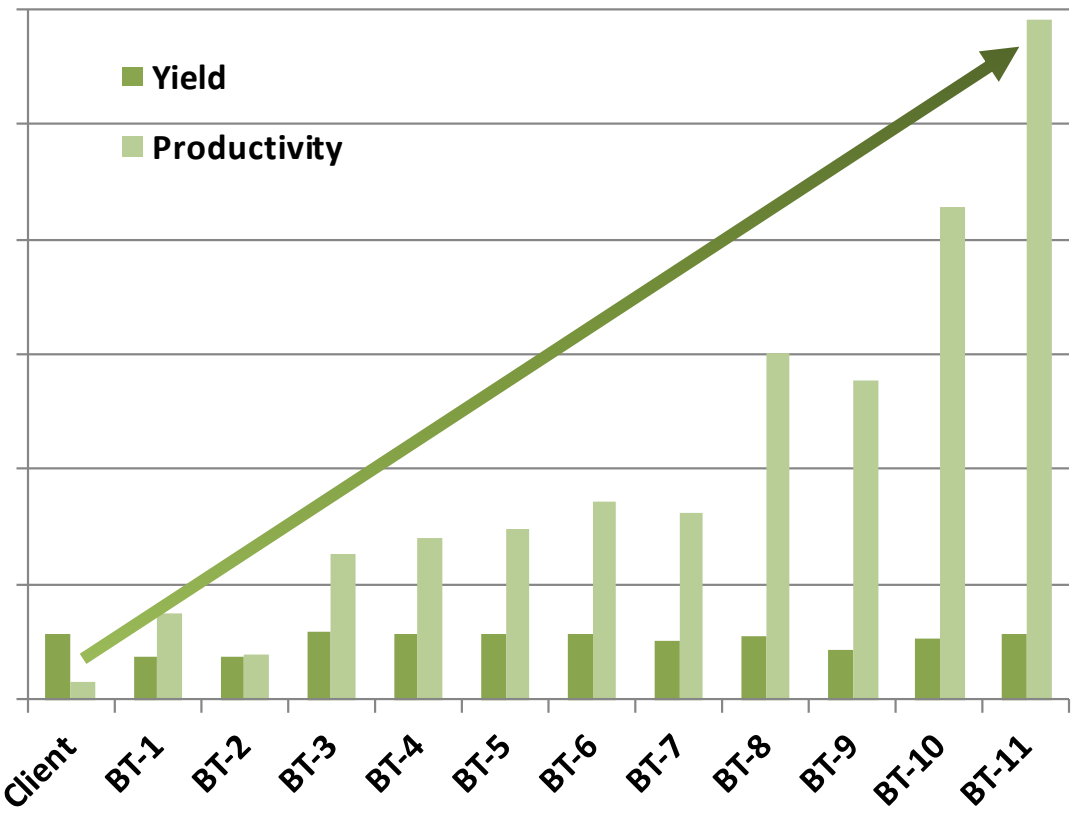
Approach: Implement fed-batch strategy to improve titres and productivities without compromising bioactivity profile.

Result: 50-fold production increase achieved.

Core competency

Process intensification: Technology validation

- Cell-extract formulation



Problem: Provide the proof of concept beyond bench-scale discovery.

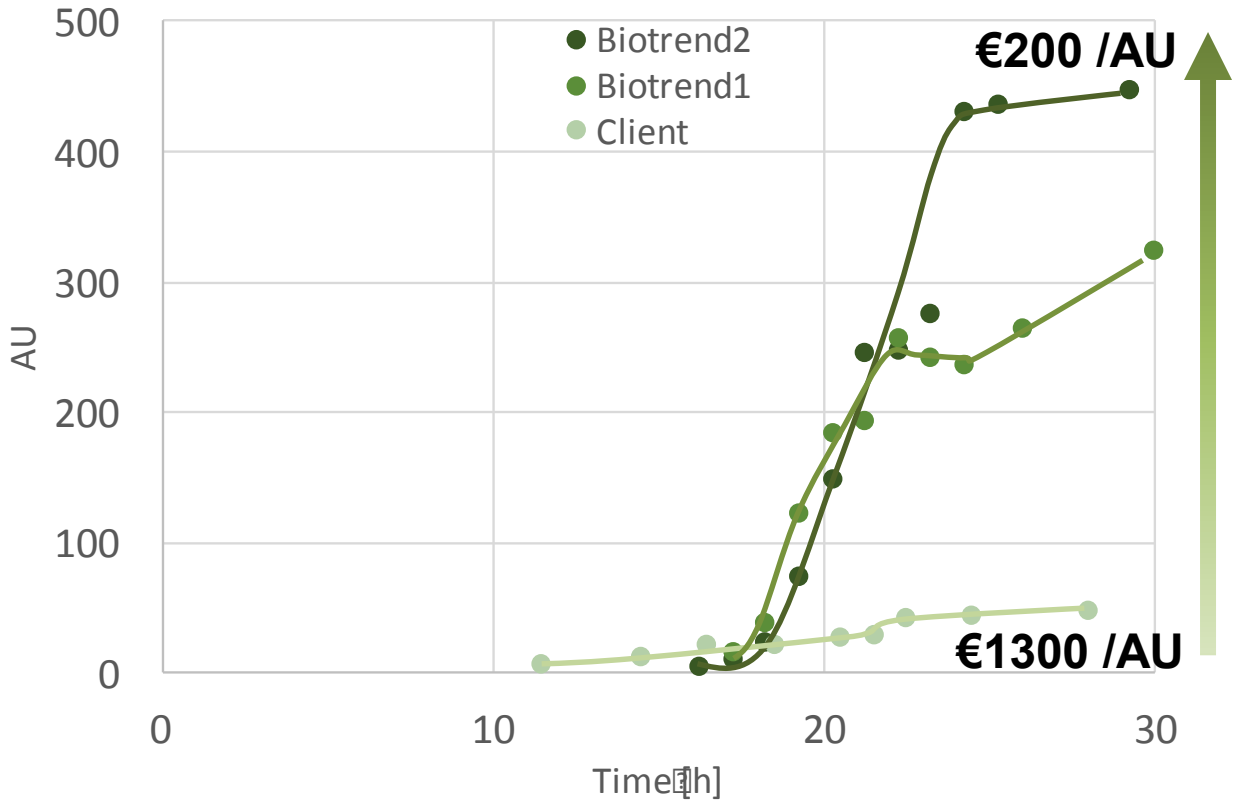
Approach: Develop fed-batch fermentation protocol from lab flask to 200L industry-standard bioreactor.

Result: >35-fold increase in productivity. Client successfully met investor milestone 6 month earlier than expected.

Core competency

Process intensification: Improve the economics of the process

- Ex. Industrial enzyme production



Problem: Reduce the production cost for an existing product produced at 50000L scale.

Approach: Implement fermentation strategy with improved feeding regimes of nutrients and inducer.

Result: Higher cell densities and higher specific activities allowed reduction of raw materials cost of over 80%.

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Example projects for clients

Marine biotechnology:

- Reduce cost of production of cell extracts for cosmetic use.
- Increase the productivity and scale-up of the production of ingredients with anti-fouling properties.
- Reduce cost of production of marine microorganism biomass for protein and PUFAs. Very high cell density fermentation.
- Develop process for heterotrophic fermentation of microalgae.

Innovative industrial enzyme engineering company:

- Increase the productivity and reduce the production cost of an enzyme.

Large pulp and paper company, world leader in premium office paper:

- Fermentation of carbon-rich waste streams from pulp and paper mills.

Leading confectionery multinational:

- Produce biomaterials for packaging from carbohydrate-rich residues.

Beverage company:

- Scale-up of the production of an innovative fermented beverage.

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Experienced with biodiversity

Microorganisms

Bacteria:

Sphingomonas sp.
Pseudomonas sp.
Cupriavidus necator
Cupriavidus taiwanensis
Alcaligenes latus
Sphyngopyxis macrogoltabida
Burkholderia sacchari
Lactobacillus casei
Escherichia coli
Pseudoalteromonas sp.
Mycobacterium sp.
Vibrio sp.
Rhodothermus marinus
Rhodococcus sp.
Sulfitobacter sp.
Actinobacillus succinogenes
Basfia succiniciproducens
Corynebacterium glutamicum

Yeasts and Fungi:

Saccharmoyces cerevisiae
Kluyveromyces lactis
Schizosaccharomyces pombe
Aureobasidium pullulans
Cryphonectria parasitica

Protists:

Schyzochytrium sp.

Microalgae:

Chlorella vulgaris

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Bioprocess development

Highly qualified and experienced team

- Combined experience of +1000 fermentation runs at various scales
- Staff with international experience

  (Portugal, The Netherlands, Canada, France, Germany, Switzerland)

State-of-the-art process development facilities

- Process development, optimization and integration
- Process scale-up, de-risking and validation

Collaborative R&D

Thermofactories: Thermophilic cell factories for efficient conversion of brown algae biomass to high-value chemicals

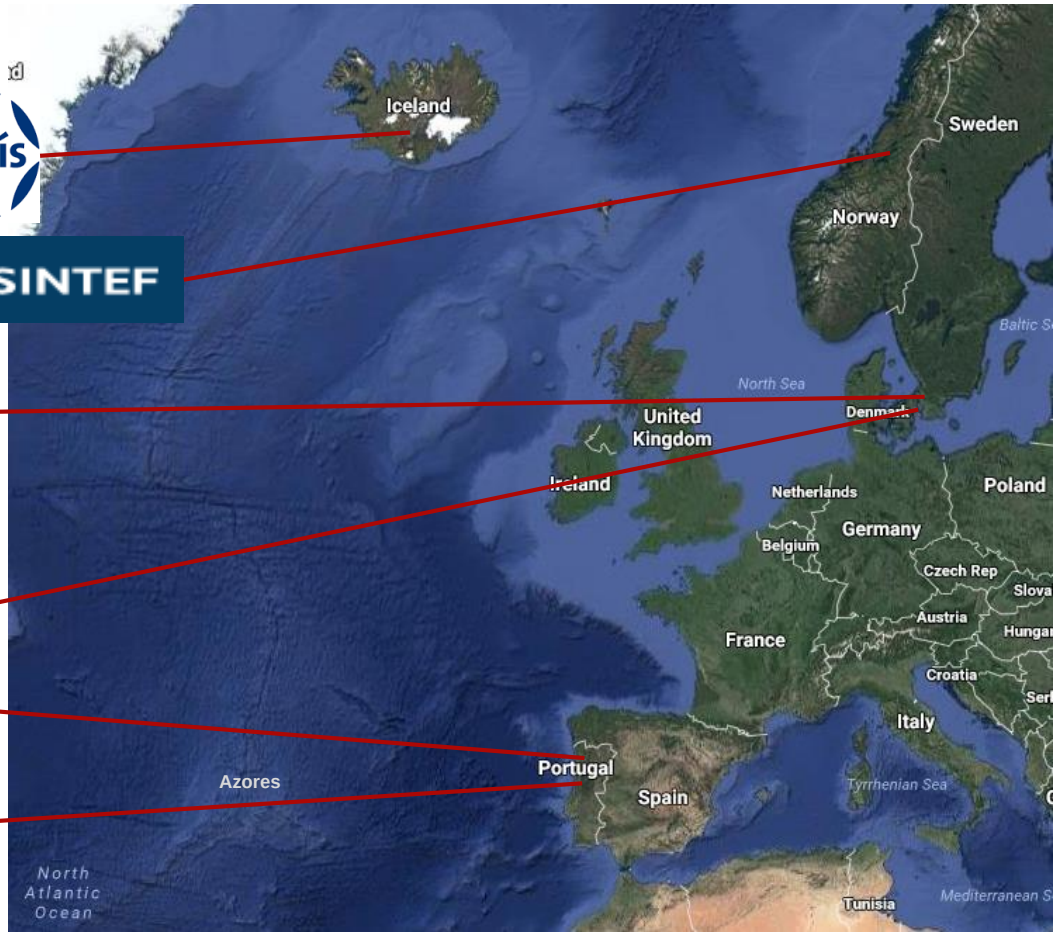
MarineBiotech

Brown algae

Carbohydrate fractions

Thermophilic bacteria

Bioproducts



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