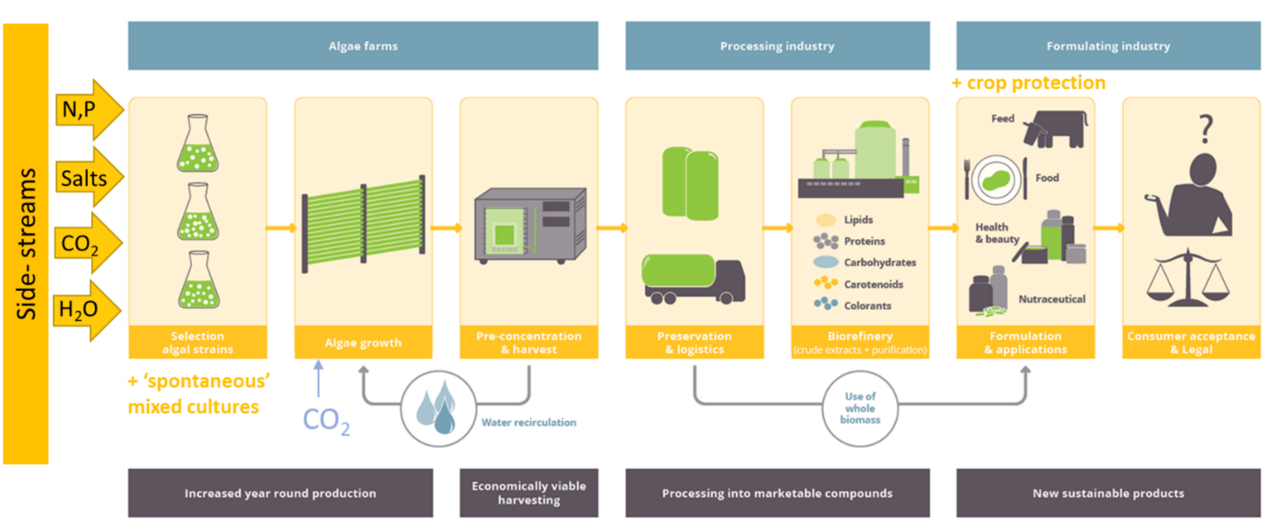
September 28th, 2023

Programme
&
posters abstracts

Organised by VITO as lead partner of the Interreg NWEurope IDEA project

Venue: VITO, Boeretang 200, 2400 Mol, Belgium – Conference room



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1 INTRODUCTION

Dear participant,

This international microalgae event is organized as a dissemination happening for the Interreg NWEurope project IDEA.

IDEA is a NORTH-WEST EUROPE INTERREG project (grant number NWE 639) that envisions the development and enrolment of economic viable value chains based on micro-algae in NWEurope. Partners from Belgium (VITO, Thomas More, Innovatiesteunpunt), Germany (Forschungszentrum Jülich), France (Central Supélèc), The Netherlands (Feed Design Lab, University of Twente) and Ireland (TEAGASC) joined forces and are collaborating since end 2017 with VITO as lead partner.

In 2021 the IDEA consortium was granted an extension by Interreg NWEurope to capitalize on the initial project findings in other application fields. One of the new challenges considered in the capitalisation part (IDEA+) was to evaluate to which extent side-streams such as residual heat, nutrients, water and CO_2 can be used to cultivate algae biomass. To evaluate the use of digester outputs (nutrients, CO_2, residual heat) two new full partners joined the IDEA consortium, being Swansea university (UK) and Heirbaut ALgriculture (B). Besides algae cultivation in closed photobioreactors, also open ponds operating on process water were being considered, where VITO's MAF-technology was used to harvest the algae. The application areas studied before (feed, food, cosmetics) were extended to crop protection, for which PCfruit (B) was involved as a new IDEA+ partner.

Project partners are presenting their findings via oral presentations and multiple posters.

During the event, different aspects of the algae value chain are presented and discussed, comprising:

- Algae growth on low organic carbon containing process water
- Algae growth on digestate as a nutrient source
- Algae cultivation on recycled CO_2
- Wet preservation of algae biomass
- Processing of algae biomass into products
- Algae-based product evaluation
- Whole value chain assessments
- Opportunities/barriers for algae in (NW)Europe.

The event offers opportunities to share results and exchange thoughts on algae value chains in Europe and on the use of side-streams for algae cultivation. This is relevant for all stakeholders of the algae value chain, comprising (potential) algae growers, algae biomass processors, companies that (potentially) produce algae-based products, equipment suppliers, authorities, investors, and scientists.

The IDEA consortium is pleased to welcome you to the event!

The organising Committee:

- Joran Verspreet (VITO)
- Ewoud Beirlant (VITO)
- Heidi Hensen (VITO, administration)
- Leen Bastiaens (VITO, Chair)
- Supported by Jolien Deroost & Kristof Severijns (Innovatiesteunpunt)

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2 ORAL PROGRAMME

9h00 Coffee, registration & poster display

9h50 Welcome
Bruno Reyntjens, Commercial Director (VITO, Belgium)

10h00 Algae value chains in NWEurope from an IDEA+ project perspective - Leen Bastiaens (VITO, Belgium) - IDEA coordinator

10h25 Keynotes

- Commercial microalgae production for aquaculture applications – Marco La Russa (Algaspring, The Netherlands)
- Potential of algae for crop stimulation and protection – Stefania Lupinelli (ILSA, Italy)

11h00 Algae biomass production on low organic carbon-containing process water from a demineralization plant

- Removal of nitrogen from process water originating from a demineralization plant by algae grown in open ponds - Dimitri Overmeire (YARA, The Netherlands)
- Harvesting and processing of mixed algae biomass from an open pond - Leen Bastiaens (VITO, Belgium)
- Cultivation of specific algae species on process water from a demineralization plant in closed systems - Floris Schoeters (Thomas More Radius, Belgium)

11h30 Digestate as a nutrient source for algae cultivation

- Pretreatment requirements for using digestate in algae cultivation - Alla Silkina (Swansea University, UK)
- Establishing growth conditions for unialgal growth on pre-treated digestate via lab scale trials - Behnam Taidi (CentraleSupélec – University Paris-Saclay, France)
- Pilot scale growth of *Scenedesmus* on pre-treated digestate - Alla Silkina (Swansea University, UK)

12h00 Algae growth on CO_2 from (burned) biogas

- Capture and separation of CO_2/CH_4 gases from biogas - Wim Brilman (University of Twente, The Netherlands)
- Algae growth on recycled CO_2 from biogas at larger scale - Kris Heirbaut (Heirbaut Algriculture, Belgium)

12h20 Lunch & poster session

13h20 Visit to VITO facilities

14h30 Impact of side-streams on algae biomass use

- Algae biomass quality and safety aspects - Maria Hayes (TEAGASC, Ireland)
- First screening of application potential of algae biomass - Joran Verspreet (VITO, Belgium)
- Potential of algae biomass for food/feed applications Maria Hayes (TEAGASC, Ireland)
- Potential of algae biomass extracts for agro-applications – Yana De Ruyter (PCFruit, Belgium)
- Techno-economic considerations - Mohammed El Ibrahimi (VITO, Belgium)

15h20 Algae value chains - Company perspective

- Farmers diversifying towards algae growth: opportunities & challenges - Kristof Severijns (Innovatiesteunpunt, Belgium)
- Innovative approach for algae growth on residual heat - Arthur Boven – (GRO_2, Belgium) - VITO4starter
- Application of algae in Petfood – Leonard Greene (Puremeatsnax/Tonitreat, Ireland)
- High-performance nutrition with fresh microalgae - Zakaria Grevisse (Astrofood, Belgium)
- Vlaamse microalgen (grouping of Flemish companies & start-ups) – Yves Vande Velde (PROVIRON, Belgium)

16h20 Conclusions & policy recommendations

16h30 Networking reception & poster session

3 ABSTRACTS POSTER SESSION

3.1 POSTER SESSION 1: ALGAE GROWTH & HARVEST

Algae biomass harvesting from an open pond using the membrane-based MAF technology: longer-term field operations

L. Bastiaens [1], S. Van Roy [1], F. Vanhoof [1], H. Sterckx [1], B. Van den Bosch [1] P. Van Elslande [2] & D. Overmeire [2]

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At Yara Sluiskil (The Netherlands), two 100 m^3 algae ponds are operational for reducing the nitrogen content in the process water from a demineralisation unit. Efficient harvesting of the algae biomass from open pond systems is challenging as processing of larger amounts of low-density algae culture is required. Within IDEA, the MAF submerged membrane-based technology (VITO) was evaluated for realizing a first dewatering of such a mixed low-density algae culture. As good dewatering results were obtained in off-site MAF trials performed (up to > 200 times volume reduction), continuous on-site harvesting trials were performed for evaluating longer-term performance.

End of September 2022, a pilot MAF unit was installed next to open pond under a shelter and was connected to the open pond. During two test periods, lasting 70 days (till mid-December) and 50 days (January-March), the PLC controlled system was operated continuously (24/24 and 7/7). The functioning was monitored remotely, and biomass was collected from the system twice a week. During the first weeks of the trial, the focus was on collecting algae biomass (> 10 kg DM) for further use in the IDEA projects. Afterwards, the robustness & reliability of the MAF-technology received special attention. In total, more than 240 m^3 of algae culture was pumped from the pond and dewatered in the MAF-unit. In respect to the degree of dewatering, Volume Concentration Factors (VCF) up to 100 were reached. This implies that the algae were concentrated up to 100 time. About 98% of the extracted algae culture was converted into algae-free permeate that can be discharged or used for other application. Thanks to the backwashable membranes, maintenance needs could be reduced from a weekly maintenance to an intervention every 2-3 weeks. In conclusion, a stable and robust operation of the MAF algae harvesting technology was observed. The impact of the harvesting approach on the algae production yield in the pond is a topic that remains to be explored. It is expected that with efficient harvesting the nitrogen removal capacity of the pond can be improved.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

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European Regional Development Fund

Growth of microalgae on process water from a demineralisation unit: a lab trial test

A. De Cuyper [1], F. Schoeters [1], E. Swinnen [1], L. Bastiaens [2] & S. Van Miert [1]

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Increasing the sustainability of the cultivation of microalgae is an important factor to increase the potential of microalgae as a future feedstock. In many processes large amounts of wastewaters are produced that often still contain interesting nutrients for microalgae cultivation. In the process of a demineralisation unit, a nitrogen (N) rich water containing up to 50 mg/L mainly in the form of nitrate is produced, termed process water hereafter. This process water holds potential to be used for microalgae cultivation as N is one of the two major nutrients, together with phosphorus, for microalgae cultivation. To assess the potential of this water several laboratory tests were performed with multiple algae species. Two streams of process water were evaluated: PW, process water as such and PER, process water collected and MAF-treated from an open pond in which algae grow. In total 6 algae strains were grown on the PW and PER: *Nannochloropsis gaditana*, *Porphyridium purpureum*, *Chloromonas typhlos*, *Chlorella sorokiniana*, *Acutodesmus obliquus* and *Desmodesmus armatus*. The latter three strains showed similar growth on the PW water compared to control medium, while the former three (IDEA strains) showed less good growth compared to the control medium, however, still acceptable growth. On PER both *N. gaditana* and *C. typhlos* showed potential. However, it was crucial to add nutrients, similar to the control medium, to achieve the best growth. Future research will be needed to fully elucidate the nutritional potential of both PW and PER to cultivate algae without or limited addition of extra nutrients such as Fe, N and P. Furthermore, cells cultivated in PW and PER showed aberrant morphologies, indicative of cell stress. A more in-depth analysis of the cell biomass will be required.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Use of cooked brown crab process water to cultivate microalgae: an exploratory study

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The cultivation of microalgae can be done in a more sustainable way than many other alternative foods currently produced. Microalgae, when cultivated autotrophically, require in essence only five major components: (sun) light, CO_2, phosphorus (P), nitrogen (N) and water. Production of brown crab for human consumption often involves a boiling step and resulting water is ordinarily discarded after use. However, this "waste-water" still contain nutrients, such as P and N that have potential for use in the cultivation of microalgae. The sustainability of microalgae cultivation can be improved by using such wastewaters. In this exploratory study, the potential of brown crab process water, which results from boiling of brown crab, was evaluated as a potential nutrient source for microalgae cultivation.

The undiluted crab water proved to be rich in protein (27.3%+/-0.5%), low in nitrate (2.48 mg/L), but high in ammonia (668 mg/L), had a neutral pH (6.8) and a high salt concentration (EC = 52.9 mS/cm). Because of the high salt concentration, five species of salt tolerant algae were selected for growth trials: *Porphyridium purpureum*, *Nannochloropsis gaditana*, *Phaeodactylum tricornutum*, *Dunaliella salina* and *Isochrysis galbana*. A 50% dilution of the crab water was used for the experiment, giving a final EC of 33 mS/cm, similar to the EC of brackish water. Prior to diluting the crab water, a sedimentation step was performed as this water contained a lot of debris. Aside from the latter, also a fatty like layer was visible on top of the water. After sedimentation, a filtration step was performed using 20 and 10 μm filters to remove large particles. The final dilution step aimed to maximize the chance of microalgal growth by decreasing the turbidity and colour of the crab water.

All five microalgae strains failed to grow substantially in the crab water. One reason for this could be the low amount of nitrate and the high protein and ammonia concentrations. Furthermore, the water proved to be rich in microorganisms (total aerobic and anaerobic count >3.0 x 10^3), which could also have negatively influenced the microalgae. Future tests are necessary to fully elucidate the potential of crab water as a water source for microalgae cultivation.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Growth of microalgae on process water from a demineralisation unit: a pilot-scale test

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While ample studies and literature is available on lab-scale tests there is less information available on larger-scale tests regarding the cultivation of microalgae. Even less information is available when using side streams, such as process water from a demineralisation unit, to cultivate microalgae. Within the IDEA project, the possibility to cultivate microalgae on N-containing process water from a demineralisation unit was evaluated. This water is low in organic carbon content (< 0.9 w/v %), has an elevated salt level (EC 9-13 mS/cm) and contains nitrogen (up to 50 mg N/L), mainly present as nitrate. By using such process water one can increase the sustainability of microalgae cultivation. Two species of microalgae were evaluated, at pilot-scale, for their potential to grow on this process water: *Nannochloropsis gaditana* and *Chloromonas typhlos*. The latter is a snow algal species that can still grow at low temperatures and could thus be used to bridge the colder periods in Northwestern European countries. Both species were successfully cultivated in a 1500 L pilot-scale photobioreactor located in a greenhouse in Belgium. Undiluted and MAF-treated process water, enriched with nutrients was used for the growth tests. Growth rates of 0.19 and 0.17 d^{-1} were obtained for *N. gaditana* and *C. typhlos* respectively in process water. While the growth rate for *C. typhlos* in process water is similar to the growth rate in regular medium, the growth rate of *N. gaditana* was 50% lower compared to regular medium. However, this lower growth rate was potentially due to contamination in the process water by a diatom, causing competition. Adding an extra filtration step prior to using the water solved this problem. Aside from this higher risk of contamination, both *C. typhlos* and *N. gaditana* showed stress when cultivated in the process water. However, the stress did not necessarily lead to worse growth. To fully elucidate this stress reaction, more in-depth trials will be needed and a comparison of the harvested biomass to algae grown in regular medium will be required. For *C. typhlos* this was already partly done and e.g., a higher lipid content was observed in cells grown in the process water compared to in regular medium. While the complexity of algae cultivation increases and extra steps are needed to fully utilize its potential, these pilot-scale tests showed that using MAF-treated process water can increase the sustainability of algae cultivation.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Wastewater-born algae for wastewater treatment

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Selecting effective algal species for nutrients removal is paramount for microalgae-based wastewater treatment [1]. Wastewater-born species might be a good choice when purifying the local wastewater as the algae growing in the associated water bodies might be resilient to their complex environments, such as the high concentrations of nutrients, possible presence of heavy metals (Cadmium, Mercury), oxygen and pesticides, etc [2].

With the goal of selecting algal species/consortia that can highly remove nitrogen (N) and/or phosphate (P) in process waters (PWs), mixed cultures were collected from an open pond (with PW1). Due to the fact that PW1 has a high level of nitrogen, the selection and enrichment of the targeted algal species/consortia from PW1 could be carried out using an N-enriched medium (Zarrouk's) in lab conditions. By measuring the growth characteristics and nutrients removal efficiency of the targeted species, the potentials of PWs as nutrients source and the capacity of selected alga in purifying PWs were then estimated.

Results showed that microbial compositions in PW1 varied with seasons, and cyanobacteria (untitled filamentous cyanobacterium and *Synechocystis*), green microalgae (*Chlorella*, *Desmodesmus* and *Ettlia*), and diatom (*Pseudostaurosira*) were dominant phototrophic microorganisms in PW1. After a lab selection procedure, a wastewater-born alga, *Picochlorum eukaryotum*, was enriched and identified. This alga exhibited high biomass accumulation (8.87 g DW L^{-1}) and nutrients removal capacity (99.9% N-NO_3^-; 95.1% P-PO_4^{3-}).

In conclusion, pilot or large-scale microalgae-based wastewater treatment are sources of nutrients-removing microalgae germplasm from the associated polluted water bodies, like the alga, *P. eukaryotum*,identified in this work.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

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[1] Z. Chen, Y. Xiao, T. Liu, M. Yuan, G. Liu, J. Fang, B. Yang, Exploration of Microalgal Species for Nutrient Removal from Anaerobically Digested Swine Wastewater and Potential Lipids Production, Microorganisms. 9 (2021) 2469. https://doi.org/10.3390/microorganisms9122469.

[2] S. Maryjoseph, B. Ketheesan, Microalgae based wastewater treatment for the removal of emerging contaminants: A review of challenges and opportunities, Case Stud. Chem. Environ. Eng. 2 (2020) 100046. https://doi.org/10.1016/j.cscee.2020.100046.

Optimizing the combination regimes of process waters for microalgae production

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Both nitrogen (N) and phosphorus (P) are essential elements for algal growth, metabolism, and reproduction. In order to determine the possibility of two process waters (PWs) as N- (PW1) or P-source (PW2) as nutrients sources for algal cultivation, the growth characteristics of 4 microalgae (2 fresh-water algae, *Chloromonas typhlos* and *Picochlorum eukaryotum* as well as 2 marine algae, *Nannochloropsis Gaditana* and *Porphyridium purpureum*) in combined PW1 and PW2 solutions were determined. PW1 contained up to 50 mg nitrogen/L, mainly present as nitrate, while the P-content in PW2 was 1.8-8 mg/L. The N/P ratio in the growth solution has been widely reported to impact the biomass production and nutrient removal efficiency of microalgae [1], therefore the PWs combination regime must be optimised prior to large-scale cultivations.

Small-scale experiments were first carried out in 96-well microplates to identify the best combination regime of PWs based on the maximum growth rate and final biomass density. The optimal combinations for microalgae were species-specific (PW1:PW2 by volume): 1:1 for *C. typhlos* (maximal OD_{800} = 0.71), 9:1 for *P. eukaryotum* (maximal OD_{800} = 1.42), 7.5:2.5 for *N. Gaditana* (maximal OD_{800} = 0.89) and 2.5 PW1:PW2 for *P. purpureum* (maximal OD_{800} = 0.95). Under the best combination regimes, *C. typhlos* and *P. eukaryotum* also presented high nutrient removal capacities, and over 80% of N or P were bio-remediated from PWs-combined growth solutions.

This work confirmed that the process waters can be adopted for feeding microalgae and after optimizing their combination regimes, the PWs could be used as an economic alternative to the cost-intense media for microalgae large-scale cultivation.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

[1] D.S. Wágner, C. Cazzaniga, M. Steidl, A. Dechesne, B. Valverde-Pérez, B.G. Plósz, Optimal influent N-to-P ratio for stable microalgal cultivation in water treatment and nutrient recovery, Chemosphere. 262 (2021) 127939. https://doi.org/10.1016/j.chemosphere.2020.127939.

Dewatering and desalting of different algae species using submerged membranes (MAF-technology)

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An integrated technology for algae pre-concentration and medium re-use was developed and examined by VITO. It concerns a submerged membrane-based technology named MAF (Membrane based Algae Filtration). The technology has been developed focusing on the robust microalgae species *Nannochloropsis gaditana*, but meanwhile its application field has been extended to other species and larger scale MAF-devices. Other species like *Chloromonas*, *Chlorella*, *Scenedesmus* and *Pavlova* (Cultivated by Thomas More in Belgium, Forschungszentrum Jülich in Germany and the University of Lille in France) were concentrated successfully with volume concentration factors up to 50 and more. One of the features of the MAF-technology, is the low shear force that is implied on the algae during the concentration. For this reason, the MAF-technology offers potential for harvesting fragile algae species, like species without a cell-wall. Within the Interreg 2seas Valgorize project, the MAF-technology was applied to *Rhodomonas* cultures, being a fragile algae species without a cell wall. Batches of a *Rhodomonas* culture, cultivated by Hogeschool Zeeland (The Netherlands) in closed bioreactors with a density of 0.2-0.4 g/L, were concentrated up to 100 times using the MAF-technology. Permeation fluxes reached at that time were still good, suggesting higher concentration factors are likely to be within reach. The cell integrity during the process was monitored. Microscopic analyses proved that cells remained intact during the concentration process. For all algae species it was observed that the storage time and conditions of the algae prior to the MAF-concentration influence the quality of the culture and as such the filtration fluxes. In addition, the MAF technology has also been applied to desalt preconcentrated biomass, allowing its application in feed formulation for animal that are sensitive to high salt concentrations.

More recently, the performance of the MAF-technology for two additional biomass types was evaluated, being, mixed algae biomass from an open pond and Spirulina grown in closed bioreactors. Both trials were successful.

This research was co-funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe) & Interreg 2Seas project ValgOrize.

The wet preservation of algae concentrates obtained by membrane filtration

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To bridge the time period between algae harvest and processing, algae preservation is needed. A new preservation approach is presented here: First, algae are preconcentrated by membrane filtration to obtain so-called preconcentrates. Next, preconcentrates are stored at 4°C and finally centrifuged to obtain a final concentrate ready for processing.
In this study, the cooled storage of *Nannochloropsis gaditana* and *Chlorella sp.* preconcentrates was evaluated. Preconcentrates were stored for 10 days and sampled regularly to monitor organic matter levels and lipids levels. Moreover, the yield of centrifuging was determined after batch centrifugation of the stored preconcentrates.
It was shown that the organic matter level of the preconcentrates remained rather stable or decreased only slightly during storage. Similarly, when stored preconcentrates were centrifuged, the centrifugation yield was either stable or decreased only slightly. Also lipids remained stable.
In conclusion, membrane filtration of algae followed by cooled storage seems to be a promising approach for temporary algae storage allowing storage with minimal organic matter and lipid losses while minimizing the algae volumes that require cooling.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

AlgaeBrew: Unlocking the potential of microalgae for the valorisation of brewery waste products into omega-3 rich animal feed and fertilisers

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As one of the largest agri-food industries, beer production generates large amounts of nutrient-rich wastewater and spent grain. The conventional linear "collect-treat-discharge" way of handling waste is costly and environmentally unsustainable. AlgaeBrew will use microalgal biotechnology to convert these wastes into useful products, thereby creating new revenue streams for breweries, decreasing their environmental impacts, and promoting a circular bioeconomy.

Eicosapentaenoic fatty acid (EPA) is essential for the immune system and widely used in dietary supplements for humans and animals. Commercial EPA production relies on fish oil derived from wild-caught fish, thereby putting enormous strain on the fish stock and the ocean ecosystem. A group of microalgae known as Nannochloropsis produces EPA naturally and can be exploited as an alternative source of EPA. By recapturing waste nutrients, Nannochloropsis can help breweries treat their waste products while producing sustainable EPA. This will be a win-win solution for both breweries and EPA producers.

AlgaeBrew aims to develop scalable processes that use Nannochloropsis to upgrade brewery wastewater and spent grain into high-value EPA for the feed industry. The residual *Nannochloropsis* biomass after EPA extraction will be developed into biofertiliser to achieve a zero-waste goal. The project will address technical challenges associated with *Nannochloropsis* cultivation on brewery waste, EPA extraction, feed formulation, and socio-economic analysis.

The project is undertaken by 7 universities, a beer producer, and an animal feed producer (Lambers Seghers) across 4 EU (Ireland, Belgium, Italy, Romania) and 3 associated countries (Morocco, Turkey, and the UK). Our estimation suggests that the brewery-microalgae system proposed by AlgaeBrew has a future potential to treat up to 26.8% of spent grain and 19.3% of brewery wastewater produced globally, while replacing the global demand for 21.6% of fish oil.

Large-scale production of Scenedesmus obliquus biomass and nutrients removal from Heirbaut Anaerobic digestate

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Nowadays, algal cultivation and produced biomass is considered a very important source used in different human applications and waste remediation. The cost of microalgae biomass production medium is the effective factor that has been studied by many projects. Consequently, usage of rich nitrogen and phosphorus wastewater sources as microalgae cultivation medium will affect the products final cost. So, a nutrient rich digestate appears as an ideal and cost-effective substrate for the large-scale production and cultivation of microalgae. In the Idea plus project the mass scale production (using photobioreactor) of algal biomass using Agri based digestate from Heirbaut Anaerobic digestion plant was used.

Scenedesmus obliquus (CCAP276/6A) obtained from Swansea University CSAR culture collection was adapted and grown in mass scale (up to 1,000L) in heated greenhouse. Large-scale growth trial took place over 70 days in February, March and April 2023. The Phyco-Flow bioreactor (Varicon Aqua manufacturer) of 1,000L capacity was inoculated with 100L of inoculum (10%) and supplied with 40 mg/L of NH_4^+ (31 mg/L of TNC) at day 0.

The successful growth of S. obliquus was maintained during the period of 70 days, with a daily supplementation of treated digestate (equivalent to 1.5 mg/L TNC) to prevent run out of culture growth nutrients. 75% of the culture was harvested at D24 and D50 and replaced by bleached and neutralized water supplied by the same amount of nutrients like in day 0. As a result ~20 kg of wet algal dewatered paste were produced and used for the downstream experiments for the Idea plus project and new algal biomass products creation.

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This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Evaluation of Scenedesmus obliquus growth performance on different treated digestate.

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Digestate as a nutrient rich waste stream coming after Anaerobic digestion process needs to be remediated, as excess of this material spread to the land could cause the creation of Nitrate Vulnerable Zones (NVZ) around North-West Europe and beyond. Several solutions were proposed to tackle this problem, as a use as a biofertilizer, however algae it seems represent a very applicable solution for all a year around remediation. Additionally, the production of valuable algal biomass, bring to validation of circular bioeconomy concept.

Not all algal species could effectively grow, using waste nutrients. In the digestate composition, a part of valuable Nitrogen and Phosphorus, many different other compounds could be presented that will affect the growth of algal cultures. Also, even N and P could be presented in the different forms and usually very concentrated and provide some toxicity for the algal cultures. So, the selection of right species for the remediation is essential. Previous studies demonstrated the good adaptation of species isolated from the AD site and one of these was *Scenedesmus* species.

The cultivation of *Scenedesmus obliquus* (CCAP276/6A) obtained from Swansea University CSAR culture collection using pre-treated and diluted digestate was studied. *Scenedesmus obliquus* cultures were grown in 1L bottles using three digestates as a source of nutrients, tested at 3 different concentrations, and in triplicate against F/2 medium as a control. The ammonium concentrations of 50, 25, and 10 $mg.L^{-1}$ were used for algal cultivation.

The best growth performance of *Scenedesmus* cultures was using the Heirbaut digestate, at concentrations of 25 and 10 $mg.L^{-1}$ of ammonium. The culture was optimised to grow on these nutrients from this digestate source. The plan for scale up was undertaken after these results.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Microalgae cultivation as a key enabling technology for Circular Green Biorefineries

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Green Biorefineries aim to sustainably produce chemicals, materials, proteins and energy by processing green biomass into a solid fraction (fibers) and a liquid fraction (juice) for further refining. While most Green Biorefineries rely on cultivated biomass, such as alfalfa, clover or ryegrass, the use of residual streams, such as roadside grass clippings, could further improve the sustainability of the process and enable the establishment of Circular Green Biorefineries. However, the green juice produced from residual streams might have a lower nitrogen (N) content and result in an economically unviable process for protein production in the current Green Biorefinery configuration. To address this, microalgae cultivation could be a more robust alternative for protein production from low N biomass. Here, we studied if the liquid fraction of residual grass clippings (grass juice) can be used as a nutrient source for microalgae cultivation, and if the produced algal biomass meets the safety requirements for animal feed applications. *Chlorella sorokiniana* and *Acutodesmus obliquus* were cultivated on multiple dilutions and after different pre-treatments of the grass juice. An initial experiment resulted in comparable growth to mineral commercial medium when algae were cultivated in 10% grass juice after a sedimentation step followed by 0.2-µm filtration and pH adjustment to 7. Sedimentation was necessary to reduce the presence of particles and improve the light penetration of the medium, but the microbial load of contaminants was still high even after the 0.2-µm filtration. Therefore, other combined treatments were tested, i.e., pH adjustment to 8.5 to inhibit fungal growth in association with 5-µm filtration and heat treatment. The addition of the 5-µm filtration and heating steps to reduce the microbial load did not increase algal productivity, while the sole increase of pH to 8.5 resulted in a significant reduction of yeast contaminants and promoted good algal growth. The produced biomass had a 41% protein content, and most microorganisms quantified complied with safety norms for feed production. Overall, these findings offer new perspectives to sustainably manage plant waste and convert it to a protein source in a Green Biorefinery.

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Algae cultivation on recycled CO_2 from biogas and recycled medium in a 9000 L PBR

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Heirbaut LV is an agricultural company (dairy cattle) that envisions circular farming. The manure produced by cows is converted in a digester into biogas (CO_2/methane mixture) and digestate rich in nutrients (like nitrogen and phosphorus). Photobioreactors were installed near the digester to grow microalgae on recycled CO_2. Some technical challenges were encountered to connect the CO_2 rich gas to the algae system as these are usually designed to be operated with 99.9 percent pure gaseous carbon dioxide from cylinders. In autumn 2022, as part of the IDEA project, a *Chlorella* culture was grown on recycled CO_2 in a 9000 L photobioreactor. The CO_2 originated from the off gas after burning of pre-treated biogas. The nice weather in September/October stimulated the algae growth well, although growth rates and culture densities were lower compared to summer cultivations.

The VITO MAF-filtration technology was used for continuous pre-harvesting of the algae biomass while recycling the medium. More specifically, a MAF unit (3 m² submerged membranes) was installed next to the photobioreactor (9000 L) for continuous harvesting of the algae. The algae-free MAF permeate (containing water & salts) was recycled via immediate re-injection into the photobioreactor system. Compounds that are consumed by the algae (like N, P) were added at regular timepoints. For 4 weeks, 5-8 % of the reactor volume was dewatered daily with an average medium recycling of 97 %. Technically, the growth and MAF-harvesting systems were operating synchronised, and the daily harvested amounts were adjusted to the growth rate. Twice a week, the MAF-concentrated algae biomass was further dewatered using centrifugation.

Based on the results obtained, it can be concluded that algae cultivation on recycled CO_2 and recycled medium is possible. Some technical adaptations were required to enable this new approach of algae production. Points for further optimisation of the approach were identified.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

CO_2 from Biogas for microalgae cultivation – using a supported amine sorbent with air purge regeneration

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For autotrophic microalgae cultivation the carbon dioxide supply is a significant contributor to the operational cultivation cost. Using a local sourced, sustainable and affordable CO_2 source as ambient air or CO_2 from biogas is therefore of interest. In the IDEA project the Direct Air Capture option was studied leading to a small-scale pilot unit demonstrating the concept. In this work the separation of CO_2 from biogas is studied.

Amine-functionalized ion exchange resins (IERs)have proven to be succesful in capturing CO_2 from concentrated- and from diluted streams as in Direct Air Capture. Due to significant CO_2 adsorption capacity difference under biogas feed conditions (40-45% CO_2) vs. DAC conditions (ca. 0.042% CO_2 in air), it is possible to operate under 'concentration swing operation' conditions.

The strong affinity of the supported amine sorbent towards CO_2 causes a significant temperature rise during adsorption, and consequently high heat supply requirement during regeneration. To do this in a short time, each cycle, a large specific heat transfer area is needed. The polymeric nature of the sorbents used caused a low thermal conducitivity, resulting in long cycle times for heating and cooling alone. To avoid this, it is proposed here to operate more or less adiabatically, storing the heat of adsorption in the sorbent bed during adsorption and using it during desorption.

In this study the concept of cyclic operation of a fixed bed for adsorption from (synthetic) biogas and regeneration with air/nitrogen was experimentally validated. Preventing- and compensating for heat losses to the environment (e.g. with heat tracing) was found to be essential for efficient operation. A conceptual design was made, and optimized, for a farm-scale biogas separation unit for a 10 m3/h capacity. An optimized design was found for near isothermal operation at 40°C and ambient pressure. The fixed bed can handle ca. 47 Nm3/h of biogas per m3 of sorbent. During the adsorption phase biogas is upgraded to biomethane of ca. 98% purity, with a limited methane loss of less than 0.3%. In the sorbent regeneration phase a CO_2 enriched air stream with ca. 2.4% CO_2 is produced, sufficiently concentrated and suitable for microalgae cultivation.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

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CO_2 for microalgae cultivation by biogas upgrading with cation-exchanged bentonite clay

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Cation-exchanged bentonite clay can rapidly adsorb/desorb CO_2 in its interlayer spacing, provided the size of the cation separating the layers is sufficiently larger. By selecting the cation-size such that CO_2 *can* access the interlayer spacing, but the slightly more voluminous CH4 molecule *can not*, a selective CO_2 sorbent can be created. This material was tested for suitability as regenerative sorbent for biogas upgrading and a conceptual process design is created.

For the modified clay fixed bed breakthrough experiments are carried out for proof of concept, using simulated biogas (50/50 mixtures of CO_2 and CH_4) as feed for a labscale unit. Exposure of the clay materials to real biogas did not lead to degradation in capture performance.

A conceptual process model was developed to investigate the effect of process configurations and conditions that lead to a biomethane product gas concentration with more than 90% v/v of methane and a methane recovery above 99.5%. Initial optimisation work showed that a two-stage process, operating at a slightly elevated pressure (2-3 bar) is able to achieve these goals. The process configuration designed can treat ca. 25 Nm3/h of biogas per m3 sorbent and produces, next to the biomethane, a 99.3% pure CO_2 product gas, which can be used for (e.g.) microalgae cultivation.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

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3.2 POSTER SESSION 2: ALGAE BIOMASS PROCESSING & APPLICATIONS

Processing of IDEA+ algae biomass harvested during the different pilot trials

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Within IDEA, the potential of side-stream use in algae-value chains was investigated. In this context, several pilot scale algae growth and harvesting trials were performed. More precisely, an on-site continuous harvesting of a mixed algae culture grown on low carbon process water in an open pond was operated for 130 days to evaluate the robustness and reliability of the membrane based MAF technology. The permeate generated in the trial was subsequently used to cultivate *Nannochloropsis gaditana* and *Chloromonas typhlos* in closed bioreactors (1500 L). Further, *Chlorella* sp. was grown in a 9000 L photobioreactor that was fed with recycled CO_2. Finally, *Scenedesmus* sp. was cultivated on pre-treated digestate as source of nutrients in pilot scale closed bioreactors.

Each of the algae growth trials described above aimed at producing at least 3 kg of algae biomass (on dry matter basis) for further processing and testing in the project. More specifically, after harvesting, the wet biomass was subjected to a cell disruption step via beadmilling. The disrupted biomass was subsequently freeze-dried for storage. In total, more than 10 m^3 of concentrated biomass was generated, of which more than 750 L and 800 L was beadmilled and freeze-dried, respectively. In a next step, part of the biomass was defatted (vi solvent extraction with recuperation of solvent) to generate an algae oil and a partially defatted algae meal.

In this way, > 14 different IDEA+ algae biomass types (whole biomass & fractions) were generated, of which the composition was determined in terms of the organic matter, ash content, total lipids, total proteins, total carbohydrate, carotenoids and the fatty acid profile and amino acid profiles. The biomass was subsequently distributed among partners to evaluate their potential as ingredients for cosmetics, feed, food and agro applications.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Assessment of the safety of microalgal biomass grown on either nutrient-rich process water or carbon dioxide side-streams: Steps used and caveats concerning algal utilization

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Growth of microalgae on nutrient-rich waste or carbon dioxide (CO_2) side-streams generated from food or energy production is a promising and sustainable approach for biomass & ingredient generation. If products are for sale in the EU27, microalgae for use in food and feed applications must comply with food and feed safety laws. The level of contaminants, allergens, or hazardous substances including pathogenic bacteria, viruses and fungi generated during microalgae recovery, collection and processing must be documented and within limits outlined by the European Food Safety Authority (EFSA). Five microalgae-derived bioactive ingredients - astaxanthin, β-carotene, phycocyanin, omega-3 fatty acids (EPA and DHA), and selected algal biomass including *Spirulina* and *Chlorella* species are currently approved for use as food/feed ingredients in the EU27. However, research has demonstrated the potential of several other microalgae for use as foods, functional foods and feed ingredients.

As part of IDEA+, safety parameters including the microbial load, heavy metal content (specifically the content of cadmium, lead, mercury, and arsenic) and iodine content of several microalgae (>15 species) were determined using developed Standard operating procedures (SOPs) at Teagasc. The presence and quantity of pathogenic bacteria including Coagulase positive *Staphylococcus* sp., Coliforms, *Bacillus cereus* and *Salmonella* sp. were determined using developed SOPs. Heavy metal content was determined according to a published method (Napan et al., 2015). The iodine content of the biomass was determined using the Iodine Colorimetric Assay Kit (BioVision, California, USA), according to the manufacturer's instructions. The content of pathogenic bacteria, heavy metals and iodine was measured in each of the 15+ algal biomass samples and a review of the legislation determined their future potential for use in ingredient generation.

Reference: Napan, et al., (2015). Impact of heavy metals from flue gas integration with microalgae production, Algal Research, Volume 8, 83-88, https://doi.org/10.1016/j.algal.2015.01.003.

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Microalgal use as ingredients for potential methane abatement and health benefits in cows and dogs

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The anti-methane (CH_4) effect and heart health benefits of two microalgal species –*Chlorella sp.* and *Nannochloropsis* sp. produced on side-streams and supplied by VITO was determined. The safety of these algae was assessed at Teagasc and microbial load, heavy metal and iodine content values were obtained prior to use in trials. *Chlorella* sp. was included at 2% of dry matter intake (DMI) in a closed gas system used to monitor CH_4 and ammonia production in the presence of a grass silage concentrate. The trial ran over a 48 hr period and included the red seaweed *Asparagopsis taxiformis* (known to inhibit CH_4 production) as a positive control. At the 3.5 hr time-point post-incubation, the 2% inclusion rate of *Chlorella* sp. in grass silage significantly reduced CH_4 ($P<0.01$) production. At the 24 h time-point, the microalgae significantly reduced CH_4 across all inclusion rates ($P<0.001$) in comparison to the grass silage control containing no algae. However, at the 48 h time-point, the microalga and silage concentrate test significantly increased CH_4 production at all inclusion rates (2, 4 and 8% inclusion rates, $P<0.001$). *Asparagopsis* species included as a positive control in the presence of grass silage showed significant CH_4 reduction across all time points and inclusion levels.

Chlorella sp. along with *Nannochloropsis* sp. produced using a bioreactor were identified previously in IDEA as antihypertensive agents in an *in vivo* animal trial using ten elderly dogs. The potential of the same algae produced on side-streams in IDEA+ to inhibit the Angiotensin-1-converting enzyme (ACE-1; EC 3.4.15.1) and produce an anti-hypertensive effect was determined using *in vitro* and *in vivo* trials using elderly dogs to validate initially observed results.

This work shows the potential uses of microalgae for use as feed ingredients to reduce CH_4 and hypertension from cattle and dogs, respectively. However, further *in vivo* cattle trials are required to validate the CH_4 mitigating effects observed. Bioactive identification is also necessary so that ingredients for use in animals can be concentrated to produce more beneficial, targeted effects.

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Cultivation of *Chloromonas* on medium and regeneration water from a demineralisation unit: impact on algae biomass

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Algae biomass cultivation is a process that requires water and nitrogen, besides other nutrients (like P, Fe, Zn, etc.), CO_2 and light. Within the IDEA project, the possibility to grow algae on N-containing regeneration water from a demineralisation unit was evaluated. The water is low in organic carbon content (< 0.9 w/v %), has an elevated salt level (EC 9-13 mS/cm) and contains nitrogen (up to 50 mg N/L), mainly present as nitrate. During larger scale growth and harvest trials with *Chloromonas typhlos* (snow algae species), clear differences were observed, not only in cell shape but also oily substances were observed that reduced filtration fluxes during MAF-dewatering. Harvesting with centrifuge was also less efficient. In lab scale growth trials on amended process water similar differences were noticed. Microscopically, lipid-like globules could be noticed.

For this reason, during pilot scale growth trials, the algae biomass composition was studied at different time points while growing *C. typhlos* on culture medium, as well as at different time points after transfer of the algae culture to process-water based growth conditions. The biomass of subsequent harvests was freeze-dried and the organic matter, ash content, total lipids, total proteins, total carbohydrate, and the fatty acid profile and amino acid profiles were quantified.

Lipid levels for algae grown on permeate-based medium (26-32% lipids on an organic matter basis) tended to be slightly higher than those grown on the regular medium (24-27%) but differences were small. According to some studies (Hounslaw et al., 2016 and Bazzani et al., 2021) an accumulation of lipids in the algae biomass can be caused by stress factors like higher salt concentration in the medium. In the current case, loses of lipids from the algae biomass during centrifugation might have occurred. Further research is required to unravel the biochemical processes and link them to the biomass composition.

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Impact of gastrointestinal digestion on bioactive properties of algae extracts

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Algae biomass is a source of multiple known and unknown bioactive compounds. Once a promising bioactive property has been identified, it is important to evaluate whether the bioactive biomass is suitable for being formulated into a product as a bioactive ingredient. An important question in this respect, is whether the bioactive property is not modified or completely degraded during gastrointestinal digestion. Algae4IBD is a bioprospecting project aiming at bioactive compounds (small sized – 0.1-10 kDa) that can be useful to prevent and cure IBD (Inflammatory Bowel Disease). More than 150 algae strains are being screened for bioactivity by preparing solvent extracts and screening the extracts for a set of bioactive properties.

A procedure was elaborated by VITO for evaluating the impact of digestion on bioactivity of solvent extracts of algae biomass. Points of attention were the hydrophobic nature of the extracts and the minimization of the risk that compounds added during the in vitro digestion procedure impact the subsequent bioactivity tests. The procedure was validated using different bioactive algae extracts and comparison of in vitro bioactivity before and after digestion. Anti-inflammatory properties (IL-6 and TNFα expression levels by cellular reporter gene assays and ELISA; inhibition of COX-1 & COX-2) and antioxidant properties (DPPH, ORAC, ABTS, Fe reducing and copper chelating activity) were considered. The results indicated that the digestion of extracts may impact the bioactivity level in some extracts and reductions as well as increases were observed for some samples. Yet, no indications were found that components added during the digestion drastically influenced the bioactivity. An important observation in this respect was that no cytotoxicity was observed in the samples nor in the blanks. The results lead to the conclusion that the digestion procedure, including the post-treatment steps, are suitable for the targeted evaluation.

Based on the first results, most of the bioactive extracts seem to remain bioactive after the digestion, which is encouraging for the use of algae extracts as bioactive ingredients for functional food & pharma applications.

Algae4IBD
Health from aquatic resources

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The effect of microalgae extracts on biotic and abiotic stress in fruit crops

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In recent years, it has become increasingly evident that the intensive use of chemical management practices in agriculture, such as synthetic pesticides and fertilizers, is yielding detrimental impacts on both the environment and human health. Therefore, the search for alternative methods to mitigate these negative effects has become a topic of great interest. At the core of these new strategies are biopesticides, biofertilizers and biostimulants. In line with this, during the IDEA+ project the potential of microalgae extracts was investigated to determine their efficacy against biotic and abiotic stress factors affecting fruit crops. Water and ethanol extracts were prepared from IDEA+ algae biomass and were subsequently tested towards their potential for crop,protection.

Apple scab (*Venturia inaequalis)* and crown rot (*Phytophthora cactorum)* are prevalent fungal diseases in the fruit industry, capable of inducing significant losses if left untreated. *In vitro* trials utilizing microalgae extracts showed promising results for apple scab. These extracts demonstrated a partial inhibition of ascospore germination. Regarding crown rot, trials were conducted to assess the inhibition of mycelium growth. Based on the *in vitro* results, *in vivo* trials were performed to further investigate the potential of the microalgae extracts.

Additionally, pests, such as pear psylla (*Cacaopsylla pyri)* and thrips, also play an important role in quality and harvest losses. Therefore, *in vitro* trials were performed to determine the mortality rate caused by different microalgae extracts on pear psylla and thrips.

Not only biotic stressors but also abiotic stressors such as drought stress and nutrient deficiency are often managed using chemical interventions. Within the scope of this project, the biostimulatory potential of these microalgae extract was investigated. The experiments were designed to determine whether these extracts possess the capability to mitigate the growth constraints induced by drought stress or, alternatively, function as growth enhancers similar to fertilizers for fruit cultivation.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Evaluation of the digestibility and the antimicrobial activity of algae cultivated on sidestreams

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Food and feed are some of the potential application areas for algae cultivated on sidestreams. Apart from the safety and legal requirements, the digestibility of the produced algae biomass should be assessed to evaluate this potential. Another potential application area of algae and fractions thereof is their use as an antimicrobial agent.

The goal of this work was twofold: (i) to study the digestibility of algae cultivated on different sidestreams, and (ii) to perform a first evaluation of the antibacterial activity and antifungal activity of algae grown on sidestreams. Disrupted biomass form different algae species grown on two different sidestreams were tested; either a medium based on the permeate from a low organic carbon containing process water or CO_2 from a biogas combustion. For each biomass type, also a defatted fraction was prepared and tested.

The digestion in the upper part of the gastrointestinal tract was simulated *in vitro*. The rather high N (57%-69%) and organic matter (51-64%) solubility after digestion suggested that most of the proteins and organic matter are accessible for enzymatic degradation in the gut.

In the second part of this study, the antibacterial activity was explored by monitoring the bacterial growth of both Gram-positive and Gram-negative bacteria in the presence or absence of algae extracts. Most ethanol extracts of the algae had a significant impact on the growth of *E. coli* and *S. aureus.* Finally, the anti-fungal activity of algae extracts was assessed. Applying certain algae fractions on *Botrytis cinerea*-infected tomato leaves slowed down or prevented the growth of *B. cinerea*. In conclusion, several algae fractions had promising antimicrobial activities. More research is now needed to confirm the activity and to identify the active compounds.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

Impact of including defatted *Chlorella*, *Chloromonas* and *Nannochloropsis* biomass in broiler diets on intestinal morphology and histology

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Algae are an attractive nutrient source for broiler chickens. To reduce costs, one can use defatted algal biomass for feed and extract lipids for other, high-value applications like nutraceuticals. Yet, the effect of defatted algae on broiler gut health needs further investigation, which is the focus of our *in vitro* and *in vivo* studies.

Firstly, defatted algae were digested *in vitro*, the soluble digestate was analyzed and the growth potential of *Lactobacillus amylovorus* on the soluble digestate was evaluated. Next, an *in vivo* trial was executed with 105 broilers randomly distributed over 7 treatments: *Chlorella* 1 and 2%, *Chloromonas* 1 and 2%, and *Nannochloropsis* 1 and 2% and a control standard broiler feed. Performance parameters and intestinal health parameters were evaluated.

After i*n vitro* digestion, the highest level of indigestible and soluble carbohydrates was observed for defatted *Nannochloropsis*. *In vitro* growth trial data suggested that *L. amylovorus* is able to use the digested *Chloromonas* and *Chlorella* fractions as growth substrates. The *in vivo* trials showed that ileum length tended to increase in broilers fed with algae, with the highest increase for *Nannochloropsis* 2% and *Chloromonas* 1% compared to the control group. Jejenum length increased slightly for all treatment groups compared to the control group. Villi width tended to increase for all algae-supplemented broilers, except for *Nannochloropsis* 2%. Villi length tended to increase with *Chloromonas* diets. Crypt depth seemed to increase especially for *Chlorella* 2% and *Nannochloropsis* 2%. Crypt width increased for all treatment groups compared to the control group, only *Chlorella* 1% did not differ from the control group. The thickness of the *Tunica muscularis* tended to decrease for all algae-supplemented broilers. In summary, including defatted algae biomass in broiler diets had clear and varying effects on gut morphology and histology, depending on the type of algae used.

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Aqueous antimicrobial extracts of *Ascophyllum nodosum* using microwave technology

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In the ongoing transition towards sustainable feedstocks, brown seaweeds have garnered attention, particularly for their wide array of antimicrobial constituents, such as polysaccharides and polyphenols [1]. This study focuses on the solubilization of these compounds from the brown seaweed *Ascophyllum nodosum* via microwave-assisted treatment. A main benefit of this method is the unique phenomenon where heating above the solvent's boiling point causes an internal pressure surge, facilitating the release of the compounds of interest [2], [3]. Differing from the literature, general solubilization was investigated as the combined solubilized compounds are expected to contribute to the overall antimicrobial traits. The optimal solubilization conditions (temperature of 120°C; treatment time of 15 min and solid to liquid ratio of 1.5% SW) were determined using Response Surface Methodology (RSM) and the desirability approach by Derringer & Suich (1980). The study aimed to maximize solubilization efficiency while minimizing energy input per mass of solubilized seaweed biomass (252.3 kJ/g SSW) to obtain maximal biomass feedstock usage (67.2%), without resorting to energy-intensive conditions. Moreover, this research is the first to correlate the zeta potential and antimicrobial properties in seaweed biomass. While the literature suggested a positive correlation between zeta potential values and antimicrobial characteristics for chitosan solutions [4], no such correlation was observed for these complex extracts. Nevertheless, the extracts exhibited robust antimicrobial properties with growth inhibition by up to 97% within the 8h experiment. A significant inhibitory effect was found for the gram-positive *S. aureus*, but not for the gram-negative *E. coli*. The more pronounced effect against gram-positive bacteria might be attributed to the better accessibility of the extracted compounds through the peptidoglycan layer compared to the outer membrane of their gram-negative counterparts [5].

[1] H. U. Dahms and S. Dobretsov, "Antifouling compounds from marine macroalgae," Marine Drugs, vol. 15, no. 9. MDPI AG, Sep. 01, 2017. **[2]** S. Saji, A. Hebden, P. Goswami, and C. Du, "A Brief Review on the Development of Alginate Extraction Process and Its Sustainability," Sustainability (Switzerland), vol. 14, no. 9. MDPI, May 01, 2022. **[3]** D. B. Stengel and S. Connan, "Natural Products From Marine Algae Methods and Protocols Methods in Molecular Biology 1308," 2015. [Online]. Available: http://www.springer.com/series/7651 **[4]** S. H. Chang, H. T. V. Lin, G. J. Wu, and G. J. Tsai, "pH Effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan," Carbohydr Polym, vol. 134, pp. 74–81, Dec. 2015. **[5]** T. F. L. Vicente, C. Félix, R. Félix, P. Valentão, and M. F. L. Lemos, "Seaweed as a Natural Source against Phytopathogenic Bacteria," Mar Drugs, vol. 21, no. 1, p. 23, Jan. 2023.

3.3 POSTER SESSION 3: ALGAE VALUE CHAINS – OVERARCHING ASPECTS

Legislation concerning the use of microalgae in agriculture, food, feed, pharma and cosmetics

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Valuable components can be extracted from microalgae and used in food supplements, food additives, nutraceuticals or functional foods. It is important that microalgae producers and users are knowledgeable on the legislation governing the use of microalgae relevant to their use for agricultural, food, feed, pharma, and cosmetic applications. Both groups must be compliant with what is required to obtain ecological footprint certification and with the Nagoya Protocol. Concerning microalgae use in food and feed applications, aspects related to food safety namely the presence of contaminants, allergens, or hazardous substances generated during microalgae processing must receive due attention. Three main EU regulations apply to the marketing of microalgae or their components as foods in the EU. These regulations relate to novel foods, food safety and the nutrition and health claims for food. Food safety legislation is of great importance. The consumption history of the alga affects their regulatory status. The Novel Food Regulation regulates entry of the species, or extracts from the species, to the market. This states that, "species having not been used as food to a significant degree in any of the EU member countries before 15th May 1997 need to undergo authorization procedures in order to ensure their safety for human consumption (Regulation (EC) No 258/97)". In the New Novel Food Regulation (EC) 2015/2283, an additional notification system is provided for species that have demonstrated a history of safe use for at least 25 years in a country outside of the EU. The notification system may provide an easier route to the EU market for some microalgal species. The novel food catalogue is a useful resource for algal growers, processors and users. It contains the unions list of all authorised novel foods. This legislation applies to microalgae intended to be used as food (https://ec.europa.eu/food/safety/novel-food/novel-food-catalogue_en). Several microalgae are included on this list. Our poster collates the relevant regulations for microalga use in foods, feeds, agriculture, cosmetics and pharma.

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Techno-Economic and Sustainability Assessment of Side Streams Utilization in Microalgae Cultivation

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This work focuses on the sustainability impacts associated with the utilization of side streams, specifically process water, digestate, and CO2, in microalgae cultivation. First, we investigated the feasibility of using low carbon-process water, sourced from a demineralization unit, as a sustainable replacement to freshwater. Our findings indicate that this substitution can offer both economic and environmental benefits. The economic gains stem from potential revenue generation (through gate fees collection) and reduced operating expenses (owing to lower freshwater and ammonia needs). From an environmental perspective, reusing process water in algae cultivation can contribute to alleviating the strain on freshwater resources and preserving natural ecosystems and biodiversity.

Regarding the use of digestate as a nutrient source for algae cultivation, several implications were identified. This practice was found to introduce additional expenses, primarily related to digestated pre-treatment, which were not totally offset by the potentially generated revenues. Moreover, several environmental repercussions were highlighted, notably the excessive water consumption, high electricity needs, and considerable methane emissions. Additionally, given that the algae-based products are intended for human consumption (either directly or indirectly), it is recommended to adopt systematic quality control measures and regular testing of the digestate, microalgae biomass, and end products.

Finally, we assessed the economic and carbon footprint impacts associated with using CO2 from different sources in microalgae cultivation. The economic assessment showed that changing the source of CO2 used in cultivation would result in marginal impacts on the microalgae cultivation costs. Nevertheless, the carbon footprint assessment revealed a much more significant impact on the associated GHG (greenhouse gas) emissions. To keep these emissions as low as possible, CO2 sourced from biogas plants close to the cultivation site should be preferred over CO2 from liquid DAC (direct air capture) or industrial flue gases.

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe).

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IDEA roadmap towards implementation of economic viable algae value chains in NWEurope

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The Interreg NWEurope project IDEA envisions the development and enrolment of economic viable value chains based on micro-algae in NWEurope. More particularly, Belgium, Germany, France, The Netherlands and Ireland are represented by the IDEA consortium, which are countries exposed to a similar climate that is significantly different from the south-European climate. The project focusses on phototrophic algae growth in closed reactors targeting higher value applications of the algae biomass (feed, food, cosmetics).

IDEA envisioned to formulate concepts of an algae value chain implementation plan. This implies interlinking the different parts of the value chain, taking into account (1) the needs of different actors along the value chain comprising algae growth, algae processing and formulating industry of algae-based ingredients, (2) the reality of spatial distributions (logistic aspect), (3) quantities of biomass (fractions) to be processed, (4) product specific requirements, and (5) economic aspects.

The IDEA roadmap serves two purposes. First and foremost, it is a plan or strategy intended to achieve a particular goal. It gives an overview of challenges and opportunities and explains which routes to be followed. It includes the steps to be taken or milestones to be met and hurdles to be overcome to reach the goal. Further, the roadmap is also a communication tool and articulates the strategy behind the goal and the way to reach it. The most updated version of the IDEA roadmap will be presented which also included some key-message related to value chain development with algae grown on side-streams.

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