From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Lisbon, Portugal

May 23-24, 2023

Ulva
The Future Wheat of the Sea

ORGANIZERS

Dr Rui Pereira, A4F – Algae for future, WG2 leader
Dr Sylvia Strauss, The Seaweed Company, WG3 leader

The COST Action SEAWHEAT (https://seawheatcost.haifa.ac.il/) and the companies A4F and The Seaweed company organized a 2-day Workshop on the technical aspects of *Ulva* cultivation (WG2) and its utilization for food and feed (WG3), from May 23-24, 2023. It was hosted by A4F, Algae for Future at the ALGATEC Eco Business Park (https://algatec.eu/), in Lisbon, Portugal.

RATIONAL AND OBJECTIVES

This workshop intended to promote a closer cooperation between *Ulva* experts and end users in the food and feed sector. Despite being one of the most common seaweed species in nature (with world-wide distribution) one of the most studied and a genus already accepted as a food ingredient in Europe, *Ulva* production and utilization is almost insignificant. Part of that can be due to a lack of coordination between the producers and the specialized end-users of food and feed biomass, with a much closer knowledge of the market needs. In other words, there is a sort of gap between what the SMEs are producing and what the market needs. The all process is often a vertical chain, with the *Ulva* producers also doing product development and commercialization.
This workshop is thought to bring together seaweed experts in the *Ulva* production and product development with experts in the food and feed sectors in general. Specific objectives are to identify bottlenecks and gaps and draft a common strategy to overcome those. The outcome would be a significant contribution towards the overall SEAWHEAT goal, which is the development of *Ulva*-based blue-biotech industries and the promotion of *Ulva* as a model organism in European algaculture.




ORGANIZATION

The workshop was part of the planned list of events for the current grant period, a joint organization between the leaders of WG2 and WG3, "*Ulva* in Aquaculture" and "*Ulva* in feed and food". The Workshop program was discussed and decided among a group of experts that constituted the scientific committee. These were (listed alphabetically): Anna Fricke (Germany); Bela Buck (Germany), Muki Shpigel (Israel); Raquel Quintã (Portugal); Rui Pereira (Portugal) and Sylvia Strauss (The Netherlands).
In addition to the organizing committee, it is also important to highlighting the role of the Chair Sessions. Anna Fricke (Leibniz Institute of Vegetable and Ornamental Crops) and Alex Golberg (Tel Aviv University) chaired the session on "*Ulva* Post-Harvesting and other emerging issues"; Mariana Doria (A4F) chaired the session on "Algae Biorefinery; Raquel Godinho (Algikey) and Sylvia Strauss (The Seaweed Company) chaired the sessions on "*Ulva* in Food and Feed" and Raquel Quintã (S2AQUA - Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture) and Erick Malta (CTAQUA - Centro Tecnológico de Acuicultura de Andalucía) chaired the session on "Engineering and Production).

PARTICIPANTS *IN SITU*

The workshop was participated by 38 experts (5 of which presented online) from 15 different countries (including 32% of participants from ITC countries), affiliated to SMES (34%) or

Academic institutions (66%). In figures 1 and 2 we present more statistical details of the participants.

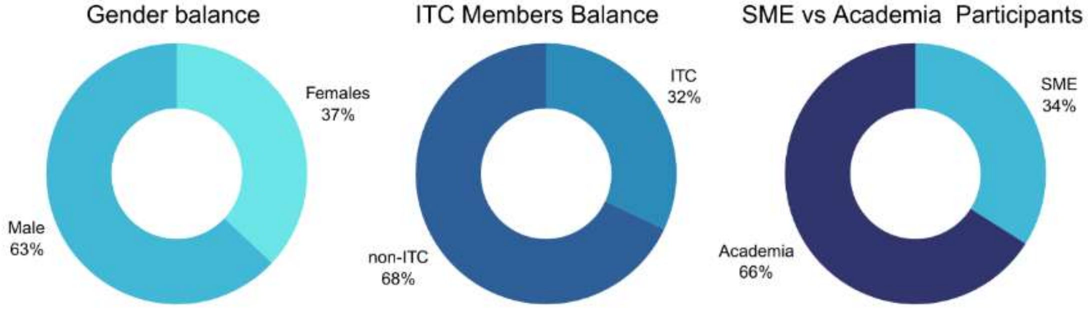

Figure 19 Distribution of the Workshop participants by gender, members of ITC countries and affiliation.

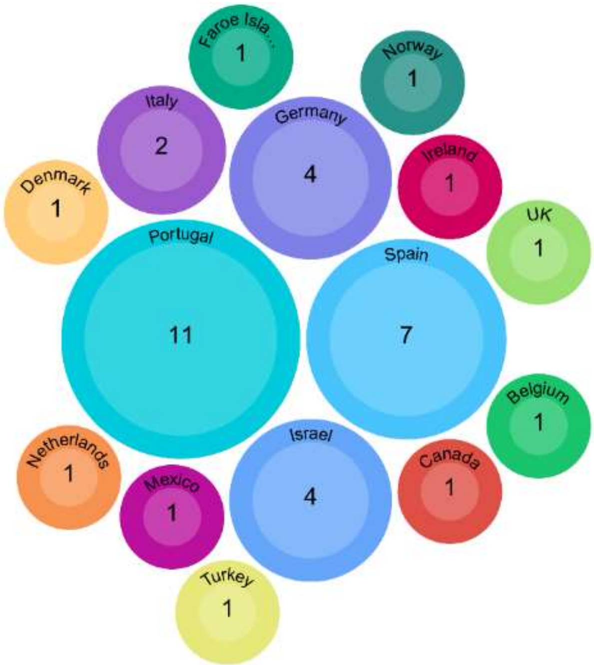

Figure 20 Distribution of the workshop participants by country according to their affiliation.

Figure 21 Group photo of the participants of the workshop held in Algatec Eco Business Park

In addition to the main group of 38 participants with interaction possibilities (including 5 online speakers), it was also possible to organize the live streaming of the presentations and final discussion moments. The access to that was completely free but required a registration. A total of 133 people registered for the live streaming during the 5 initial sessions, divided into 79 that are already Cost Members and 54 people that were not registered Cost Members at least at that time.

WORKSHOP DESIGN AND FUNCTIONING

The workshop was designed to include presentation moments by topic, including 30 minutes for discussion of each topic, half day of discussions in parallel breakout groups spread in two 1hr sessions and a technical visit to Green Aqua's algae production facility. In summary, there were 7 sections, divided in presentation sessions and breakout groups for discussions. Topics covered during the sessions, in line with WG2 and WG3 and decided by the scientific committee were: Session *1 – Post-Harvesting processes and other emerging issue; Session 3 – Ulva in food; Session 4 – Ulva in Feed; Session 5 – Engineering and Production*. In addition, Session 2 of the program was a side-event on "*Algae Biorefinery*", organized by partners of the European Project MULTI-STR3AM and with representatives of the projects CIRCALGAE, MULTISTR3AM, SEAMARK as well as from SINTEF. For more details on the program, please see Annex I at the end of this report.

Each session comprised between 4-6 presentations and included 30 minutes for discussion of the correspondent topic. A total of 22 talks, including 2 plenary talks by our international guest speakers (Dr José Zertuche from Mexico and Dr Alan Critchley from Canada) set the stage for the discussion moments. Also relevant to note the role of the sessions chairs. Each session had 2 designated chairs, with the mission to a) keep time and moderate the discussions during the presentation moments and b) animate and manage the discussions during the group breakout sessions for each topic, including organizing and presenting the final outcome of those discussion.

Prior to the beginning of the workshop, the designated Session Chairs received an information package for their session, including a short-bio of each of the speakers as well as the abstracts for each presentation.

The afternoon of Day 2 was fully dedicated to discussions and brain storming in breakout groups. Two parallel sessions were ongoing in different rooms and the participants were free to travel between groups and participate as much as possible. After approximately 1 hour the discussion topics changed and gave room to two more parallel sessions with the same functioning.

The workshop concluded with each session chairs presenting the main conclusions for their topic. The document summarizing those conclusions is still an "in process document", but a draft can be found in Annex II of the present report.

Finally, last but not least, the participants were taken on a guided tour to Green Aqua's algae production facility. Dr Luis Costa (A4F Board Member) explained the functional of the entire site, the technologies involved, the capacities and the synergies with other industrial partners at the site.

Figure 22 Different moments of the breakout groups for discussion.

Figure 23 Different moments of the coffee break with seaweed products degustation.

Figure 24 Different moments of the visit to Green Aqua's Algae production facility.

CONCLUSIONS

The workshop "From *Ulva* aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps" was a very enriching event for all participants, judging from the lively discussions (that sometimes extended beyond the planned time) and from the feedback of the participants at the end of the event. The level of participation of the online viewers was also very unexpected, since that was not in the initial plans and, therefore, was not announced but a few days before the event. An e-Book of Proceedings is also under preparation, with extended abstracts provided by the speakers and students that presented posters.

Most important, we feel that the objectives of the workshop were attained. The SME participants represented 34% of the workshop population assuming relevant roles during the different sessions (both presenting their experiences and chairing sessions) and participating actively in the discussions, thus clearly contributing to reinforce the interaction between the former and the Academia partners.

In terms of the specific objectives defined, the discussion sessions resulted in information regarding "Bottleneck & Challenges" and "Proposals for solutions" in all 4 discussion topics.

As already mentioned, Annex II of the present report, it is possible to find the draft documents produced by the session chairs, with the main conclusions of the discussion groups. Very briefly, in Session 1 (**Post-harvesting processes and other emerging issues**), the participants elected and classified 5 main topics – **Scale and price**; **Feedstock quality**;

Technology; **Logistics**; **Market**. In addition, 2 main gaps were identified (**Technical Economic Analysis** and **Knowledge transfer**). For possible solutions, the group pointed to **Partnerships**, **Specific *Ulva* solutions**, **Research in pilot scale** and **Mobile Processing Units**.

In sessions 3 & 4 the participant started by dividing the topics related to *Ulva* in Food into 1. Cultivation and Processing and 2. Product Development and Consumer Acceptance. For each sub-topic they identified several Bottlenecks and Challenges and proposed solutions. Some of the issues identified in the first sub-topic overlap with issue also identified in the other sessions (*e.g.* **Unstable quality**, **Production Costs**, **Investors** and **Subsidies**, **Regulation**, **Upscaling**). In the Product Development and Consumer Acceptance, the main bottlenecks identified were **Valorization** of biomass, Lack of **consumer acceptance**, Lack of **knowledge & information** about seaweed as food, **Food neophobia**, Lack of **Marketing + Communication** strategies, with proposals for solutions for all of those.

Finally, in session 5, the main problem identified was the relatively high price per biomass unit. There was also a consensus that there is a need to increase productivity. Based on this starting point, two groups of solutions are proposed, one classified as Biological Solutions (*e.g.* **strain selection**, **specialization** of the companies, ***Ulva* hatcheries**, **modelling**, bioengineering and application of **holobiont**, **optimization** of **production based** on **market study**) and Engineering Solutions (e.g. **technological solutions**, **mechanization**, strategical **partnerships**, **large scale** cultivation, **energy-saving** solutions for cost reduction, data collection and processing for **site selection**).

From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Lisbon, Portugal

PROCEEDINGS

Ulva
The Future Wheat of the Sea

INTRODUCTION

This document compiles the information presented by the speakers and poster presenters (students) at the SEAWHEAT workshop organized by WG2 and WG3 in Lisbon, Portugal, during 23-24 May, 2023.

The workshop intended to promote a closer cooperation between *Ulva* experts and end users in the food and feed sector. Despite being one of the most common seaweed species in nature (with world-wide distribution) one of the most studied and a genus already accepted as a food ingredient in Europe, *Ulva* production and utilization is almost insignificant. Part of that can be due to a lack of coordination between the producers and the specialized end-users of food and feed biomass, with a much closer knowledge of the market needs. In other words, there is a sort of gap between what the SMEs are producing and what the market needs. The all process is often a vertical chain, with the *Ulva* producers also doing product development and commercialization.

The workshop was thought to bring together seaweed experts in the *Ulva* production and product development with experts in the food and feed sectors in general. Specific objectives were to identify bottlenecks and gaps and draft a common strategy to overcome those. The outcome intends to be a significant contribution towards the overall SEAWHEAT goal, which is the development of *Ulva*-based blue-biotech industries and the promotion of *Ulva* as a model organism in European algaculture.

The abstracts are organized in their order of presentation at the workshop.

The poster abstracts are grouped at the end and organized in alphabetic order of the first author.

PROGRESS AND CHALLENGES IN *ULVA* BIOREFINERY. HOW TO SCALE UP?

Alexander Golberg[1]*

[1]Department of Environmental Studies, Porter School of Environment and Earth Sciences, Faculty of Exact Sciences. Tel Aviv University, Israel.

*agolberg@tauex.tau.ac.il

Biorefinery, as used in the Biorefinery Euroview report, is: “Integrated bio-based industries, using a variety of different technologies to produce chemicals, biofuels, food and feed ingredients, bio materials (including fibers) and power from biomass raw material“ (Euroview, 2010). Biorefineries are the manufacturing units of bioeconomies. In a biorefinery, one or several biomass feedstocks (crops, lignocellulosic biomass, seaweed, microalgae, insects, etc.) are processed into a wide range of products targeting zero waste and minimal green house gases emissions. Furthermore, the key advantage of biorefineries is the capacity to produce multiple products from the same raw materials not only it increases the revenue per mass of feedstock, but also diversify the applications thus making the whole system economically resilient (Golberg, Liberzon, et al., 2020). This economic analysis of co-production has been recently demonstrated in seaweeds (Palatnik et al., 2023).
The co-production of multiple products usually refers to the use of multiple subsequent processing steps. Each step leads to the production of one or several different products (Golberg, Robin, et al., 2020). Since each step removes a fraction of the biomass, concentrating the leftover, the subsequent steps are simpler and more efficient. For example, in the case of *Ulva sp.* biomass, by extracting protein and water soluble molecules in a first step, the content and purity of residual water-soluble polysaccharides such as ulvan increases (Golberg, Robin, et al., 2020). This is critical since the processing to achieve a certain level of concentration and purity is a major economical and technological hurdle of biorefinery. In addition, certain processes such as crushing, milling, and other cell and tissues disruptions will usually benefit most the subsequent processes by improving the contact surface of the biomass and the accessibility of intracellular components without drying as the intermediate process. Therefore, the energy and cost of such process benefit the entire processing chain instead of a single product (Zollmann et al., 2019).
The major current challenge resides in designing the biorefinery, i.e to chose the processes, equipment and to integrate the processes into one optimized process flow (Golberg, Robin, et al., 2020). This is a challenge because biomass processes and equipment are usually designed for the sole production of one product and not the preservation of the left over for subsequent processing targeting zero waste. Their integration required the understanding of the effect of each process on each constituent of the biomass, which are the product target of the subsequent processing steps. As for today, there is no widespread and successful standard for industrial seaweed biorefinery design. Although a considerable effort is being deployed to answer those two technological gaps (better bioprocesses and integration of those processes) at the research level (Table 1), there are still gaps in industrial research and development and large scale production to fill.

Table 1 Ulva biorefinery. Co-production of various ingredients from Ulva and their transformation to additional products

Species	Directly co-extracted products	Products produced by transformation of *Ulva* derived ingredients	Reference
Ulva lactuca	water-soluble proteins and carbohydrates		(Postma et al., 2017)
U. lactuca	protein and carbohydrates	glucose, rhamnose and xylose, acetone, butanol, ethanol, and 1,2-propanediol	(Bikker et al., 2016)
U. fasciata	mineral rich liquid extract, lipid, ulvan, and cellulose	ethanol	(Trivedi et al., 2016)
U. lactuca	mineral rich liquid, lipid, ulvan, protein, and cellulose		(Gajaria et al., 2017)
U. lactuca	water-soluble carbohydrates	acetone, butanol, and ethanol (ABE)	(van der Wal et al., 2013)
U. rigida	carbohydrate, salt, concentrated protein		(Pezoa-Conte et al., 2015)
U. ohnoi	salt, pigment, ulvan, and protein		(Glasson et al., 2017)
U. lactuca	mineral rich liquid extract, ulvan, protein	methane	(Mhatre et al., 2019)
U. ohnoi	salts, starch, lipids, ulvan, proteins, and cellulose.		(Prabhu et al., 2020)
Mix of U. rigida and *U. fascia*	hydrochar, 5-HMF, monosaccharides, proteins, peptides.	ethanol	(Polikovsky et al., 2020)
U. ohnoi	Mix of monosaccharides	ethanol	(Jiang et al., 2016)
U. ohnoi	Starch, Proteins, and minerals		(Prabhu et al., 2019)
U. ohnoi	hydrochar	polyhydroxyalkanoates	(Ghosh et al., 2021)
U. lactuca	polysaccharides, proteins		(Andrade et al., 2022)
Mix of *Ulva* species	monosaccharides		(Robin et al., 2017)
Ulva sp. not defined	Polysaccharides	biodiesel	(Ruangrit et al., 2023)
U. ohnoi	hydrochar, monosaccharides	polyhydroxyalkanoates	(Steinbruch et al., 2020)
U. lactuca	antioxidants and phenolic compounds		(Rashad et al., 2021)
Ulva sp. not defined	ulvan	biogas, polyhydroxyalkanoates	(Arul Manikandan & Lens, 2023)
Ulva rigida and Ulva ohnoi.	Water soluble and insoluble protein		(Robin et al., 2018)
U. lactuca	Bio-oil, hydrochar	bioethanol	(Sharmiladevi et al., 2021)

When processing seaweeds using biorefinery approach it is possible to define two types of products (Golberg et al., 2014). The first type includes extracts directly separated from the seaweed biomass. In *Ulva* biorefinery, for example, separation of salt, cellulose, ulvan, starch, proteins, lipids, simple monosaccharides and peptides has been reported in various process configurations. The second type includes products produced by transformation of the first type using biological, chemical or thermal catalyzes. The summary of the published works which address co-production of various ingredients from *Ulva* and their transformation to additional products appears in **Table 1**.

The next critical steps in the development of *Ulva* biorefinery is demonstration of processes using common to food and chemical industry process steps and equipment, which can be scaled. The must to get output of these efforts will be a mass and energy balances for each step as well as decision on scalable equipment. Next steps should include process simulation using common industry tools such as AspenPlus or SuproPro process design software which also enable economic and life cycle analysis of processes and plants. Following steps could include integrated process demonstration for determination of products safety, stability, final applications and complete economic and environmental analysis. This is a significant effort that would require joint expertise of biomass producers, process engineers and environmental economists.

References

Andrade, C., Martins, P. L., Duarte, L. C., Oliveira, A. C., & Carvalheiro, F. (2022). Development of an innovative macroalgae biorefinery: Oligosaccharides as pivotal compounds. *Fuel*, *320*. https://doi.org/10.1016/j.fuel.2022.123780

Arul Manikandan, N., & Lens, P. N. L. (2023). Sustainable Biorefining and Bioprocessing of Green Seaweed (*Ulva sp.*) for the Production of Edible (Ulvan) and Non-Edible (Polyhydroxyalkanoate) Biopolymeric Films. *SSRN Electronic Journal*. https://doi.org/10.2139/SSRN.4377216

Bikker, P., van Krimpen, M. M., van Wikselaar, P., Houweling-Tan, B., Scaccia, N., van Hal, J. W., Huijgen, W. J. J., Cone, J. W., & López-Contreras, A. M. (2016). Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology*, *28*(6), 3511–3525. https://doi.org/10.1007/s10811-016-0842-3

Euroview, E. U. (2010). *Euroview Biorefinery : Current situation and potential of biorefinery concept in the EU-Strategic framework and guidelines for its development, 2007*.

Gajaria, T. K., Suthar, P., Baghel, R. S., Balar, N. B., Sharnagat, P., Mantri, V. A., & Reddy, C. R. K. (2017). Integration of protein extraction with a stream of byproducts from marine macroalgae: A model forms the basis for marine bioeconomy. *Bioresource Technology*, *243*, 867–873. https://doi.org/10.1016/j.biortech.2017.06.149

Ghosh, S., Greiserman, S., Chemodanov, A., Slegers, P. M., Belgorodsky, B., Epstein, M., Kribus, A., Gozin, M., Chen, G.-Q., & Golberg, A. (2021). Polyhydroxyalkanoates and biochar from green macroalgal *Ulva sp.* biomass subcritical hydrolysates: Process optimization and a priori economic and greenhouse emissions break-even analysis. *Science of the Total Environment*, *770*. https://doi.org/10.1016/j.scitotenv.2021.145281

Glasson, C. R. K., Sims, I. M., Carnachan, S. M., de Nys, R., & Magnusson, M. (2017). A cascading biorefinery process targeting sulfated polysaccharides (ulvan) from *Ulva* ohnoi. *Algal Research*, *27*, 383–391. https://doi.org/10.1016/j.algal.2017.07.001

Golberg, A., Liberzon, A., Vitkin, E., & Yakhini, Z. (2020). Design and Analysis of Offshore Macroalgae Biorefineries. In *Methods in Molecular Biology* (Vol. 1980). https://doi.org/10.1007/7651_2018_127

Golberg, A., Robin, A. N., Zollmann, M., Traugott, H., Palatnik, R. R., & Israel, A. (2020). Macroalgal biorefineries for the blue economy. *Macroalgal Biorefineries for the Blue Economy*, 1–288. https://doi.org/10.1142/11937/SUPPL_FILE/11937_PREFACE.PDF

Golberg, A., Vitkin, E., Linshiz, G., Khan, S. A., Hillson, N. J., Yakhini, Z., & Yarmush, M. L. (2014). Proposed design of distributed macroalgal biorefineries: Thermodynamics, bioconversion technology, and sustainability implications for developing economies. *Biofuels, Bioproducts and Biorefining*, *8*, 67–82. https://doi.org/10.1002/bbb.1438

Jiang, R., Linzon, Y., Vitkin, E., Yakhini, Z., Chudnovsky, A., & Golberg, A. (2016). Thermochemical hydrolysis of macroalgae *Ulva* for biorefinery: Taguchi robust design method. *Scientific Reports*, *6*(27761). https://doi.org/doi:10.1038/srep27761

Mhatre, A., Gore, S., Mhatre, A., Trivedi, N., Sharma, M., Pandit, R., Anil, A., & Lali, A. (2019). Effect of multiple product extractions on bio-methane potential of marine macrophytic green alga *Ulva lactuca*. *Renewable Energy*, *132*, 742–751.

Palatnik, R. R., Freer, M., Levin, M., Golberg, A., & Zilberman, D. (2023). Algae-Based Two-Stage Supply Chain with Co-Products. *Ecological Economics*, *207*, 107781. https://doi.org/10.1016/J.ECOLECON.2023.107781

Pezoa-Conte, R., Leyton, A., Anugwom, I., von Schoultz, S., Paranko, J., Mäki-Arvela, P., Willför, S., Muszyński, M., Nowicki, J., Lienqueo, M. E., & Mikkola, J. P. (2015). Deconstruction of the green alga *Ulva rigida* in ionic liquids: Closing the mass balance. *Algal Research*, *12*, 262–273. https://doi.org/10.1016/j.algal.2015.09.011

Polikovsky, M., Gillis, A., Steinbruch, E., Robin, A., Epstein, M., Kribus, A., & Golberg, A. (2020). Biorefinery for the co-production of protein, hydrochar and additional co-products from a green seaweed *Ulva sp.* with subcritical water hydrolysis. *Energy Conversion and Management*. https://doi.org/10.1016/j.enconman.2020.113380

Postma, P. R., Cerezo-Chinarro, O., Akkerman, R. J., Olivieri, G., Wijffels, R. H., Brandenburg, W. A., & Eppink, M. H. M. (2017). Biorefinery of the macroalgae *Ulva lactuca*: extraction of proteins and carbohydrates by mild disintegration. *Journal of Applied Phycology*, 1–13. https://doi.org/10.1007/s10811-017-1319-8

Prabhu, M. S., Israel, A., Palatnik, R. R., Zilberman, D., & Golberg, A. (2020). Integrated biorefinery process for sustainable fractionation of *Ulva* ohnoi (Chlorophyta): process optimization and revenue analysis. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-020-02044-0

Prabhu, M. S., Levkov, K., Livney, Y. D., Israel, A., & Golberg, A. (2019). High-Voltage Pulsed Electric Field Preprocessing Enhances Extraction of Starch, Proteins, and Ash from Marine Macroalgae *Ulva* ohnoi. *ACS Sustainable Chemistry and Engineering*, *7*(20). https://doi.org/10.1021/acssuschemeng.9b04669

Rashad, S., El-Chaghaby, G., Lima, E. C., & Simoes dos reis, G. (2021). Optimizing the ultrasonic-assisted extraction of antioxidants from *Ulva lactuca* algal biomass using factorial design. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-021-01516-8

Robin, A., Chavel, P., Chemodanov, A., Israel, A., & Golberg, A. (2017). Diversity of monosaccharides in marine macroalgae from the Eastern Mediterranean Sea. *Algal Research*, *28*, 118–127. https://doi.org/10.1016/j.algal.2017.10.005

Robin, A., Kazir, M., Sack, M., Israel, A., Frey, W., Mueller, G., Livney, Y. D., & Golberg, A. (2018). Functional Protein Concentrates Extracted from the Green Marine Macroalga *Ulva sp.*, by High Voltage Pulsed Electric Fields and Mechanical Press. *ACS Sustainable Chemistry & Engineering*, *6*(11), 13696–13705. https://doi.org/10.1021/acssuschemeng.8b01089

Ruangrit, K., Chaipoot, S., Phongphisutthinant, R., Duangjan, K., Phinyo, K., Jeerapan, I., Pekkoh, J., & Srinuanpan, S. (2023). A successful biorefinery approach of macroalgal biomass as a promising sustainable source to produce bioactive nutraceutical and biodiesel. *Biomass Conversion and Biorefinery*, *13*(2). https://doi.org/10.1007/s13399-021-01310-6

Sharmiladevi, N., Swetha, A., & Gopinath, K. P. (2021). Processing of Gracilaria edulis and *Ulva lactuca* for bioethanol and bio-oil production: an integrated approach via fermentation and hydrothermal liquefaction. *Biomass Conversion and Biorefinery*, *1*, 1–9. https://doi.org/10.1007/S13399-021-01925-9/FIGURES/8

Steinbruch, E., Drabik, D., Epstein, M., Ghosh, S., Prabhu, M. S., Gozin, M., Kribus, A., & Golberg, A. (2020). Hydrothermal processing of a green seaweed *Ulva sp.* for the production of monosaccharides, polyhydroxyalkanoates, and hydrochar. *Bioresource Technology*, *318*. https://doi.org/10.1016/j.biortech.2020.124263

Trivedi, N., Baghel, R. S., Bothwell, J., Gupta, V., Reddy, C. R. K., Lali, A. M., & Jha, B. (2016). An integrated process for the extraction of fuel and chemicals from marine macroalgal biomass. *Scientific Reports*, *6*, 30728. https://doi.org/10.1038/srep30728

van der Wal, H., Sperber, B. L. H. M. H. M., Houweling-Tan, B., Bakker, R. R. C. C., Brandenburg, W., & López-Contreras, A. M. (2013). Production of acetone, butanol, and ethanol from biomass of the green seaweed *Ulva lactuca*. *Bioresource Technology*, *128*, 431–437. https://doi.org/10.1016/j.biortech.2012.10.094

Zollmann, M., Robin, A., Prabhu, M., Polikovsky, M., Gillis, A., Greiserman, S., & Golberg, A. (2019). Green technology in green macroalgal biorefineries. *Phycologia*. https://doi.org/10.1080/00318884.2019.1640516

PILOT-SCALE BIOREFINING.

Jessica M.M. Adams[1]*

[1]Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Gogerddan, Aberystwyth SY23 3EE UK

*jaa@aber.ac.uk

In 2010 Aberystwyth University, alongside Bangor and Swansea Universities, won a large European regional development fund grant to set up BEACON, a biorefining centre of excellence in west Wales and the valleys, a regionally deprived area within the UK. This five-year project was followed by a second five-year project, BEACON+, with subsequent later funding which included east Wales and the University of South Wales before BEACON ended in early 2023. The biorefining centre of excellence at Aberystwyth has moved from strength to strength and is now based within Aberystwyth's Innovation and Enterprise Centre adjoining the University's institute, IBERS.

There has been macroalgae processing at IBERS, Aberystwyth University, since 2007, with initial studies producing biofuels before developing into more higher value products and latterly biorefining to multiple products. Alongside the development of BEACON, the macroalgae processing has also increased from bench scale (*e.g.* 10 g wet weight feedstock) and there are now several processes that have been conducted using seaweed at pilot-scale (up to 300 kg wet weight). Following the first macroalgae pilot-scale biorefining process at IBERS, a joint publication was produced between IBERS staff and the commercial partner, Oceanium Ltd, containing pre-processing considerations, observations and recommendations when working at pilot-scale. The process conducted was multi-layered and generated three liquid products and one solid product, with the process overview shown in Figure 25.

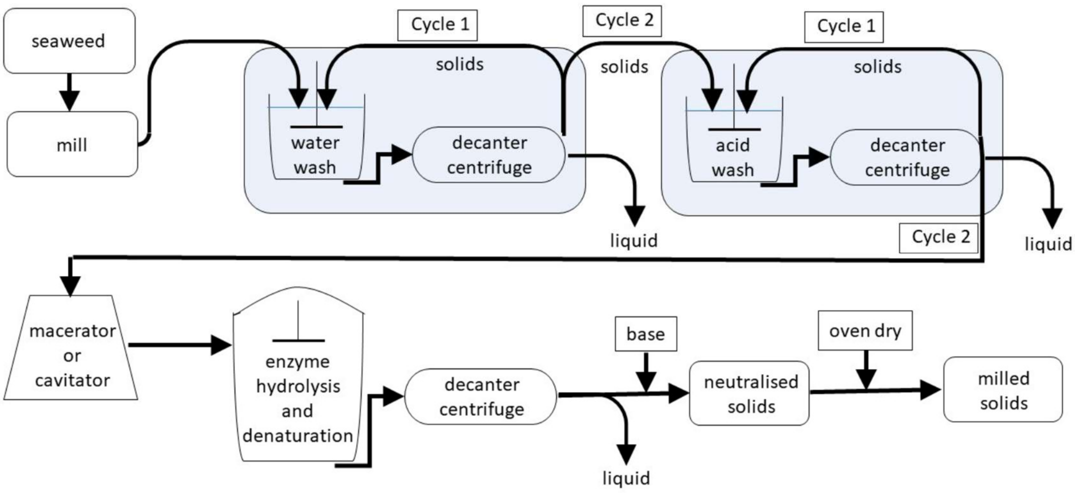

Figure 25. Schematic of the main steps involved with the macroalgae processing. Inverted 'T' indicates stirred mixtures. Material stored at 4°C overnight between steps or at -20°C longer term. From Adams et al., 2021

Key findings reported in this work include the following: the requirement for exceptionally clean macroalgae feedstock, as only a few snails or stones can damage milling machinery. Considerations should be made regarding the composition of larger equipment material, as pilot-scale is generally conducted in plasticware or stainless steel rather than glassware as in lab-scale processing. This has implications for cleaning and heating processes. Relatedly, the acid used in the process needs greater consideration as stainless steel does not have resistance to hydrochloric acid and even higher-grade steel only has a limited resistance to it. Conversely, most stainless steel has resistance to sulphuric acid at high or low concentrations, though is non-resistant at intermediate concentrations. Acids also react with metals within the macroalgae, with industrial research showing that sulphuric acid increases the lead (Pb) content in remaining solids compared to that by hydrochloric acid by forming low-solubility lead sulphate which is thought to become trapped in the biomass rather than the more soluble lead chloride or chloro-complexes which are partly removed in the wash steps.

This presentation will reflect on this and other pilot-scale processing of macroalgae including previous pilot-scale work using *Ulva* at scale. It will discuss some of the differences between macroalgae and terrestrial biomass and how macroalgae can handle differently – not all biomass is the same. The presentation will also include the use of the pilot-scale batch steam explosion and the potential of this process to yield single sugars for subsequent bioprocessing.

References:

Adams, J.M.M., Morris, S.M., Steege, L., Robinson, J., Bavington, C. (2021) Food-Grade Biorefinery Processing of Macroalgae at Scale: Considerations, Observations and Recommendations Journal of Marine Science and Engineering 9(10)1082 DOI: 10.3390/jmse9101082

APPLICATION OF *ULVA SP.* IN PACKAGING.

Laurie C. Hofmann*[1,2], Isabel Cardoso[1], Bela Buck[1,2]

[1]Alfred Wegener Institute Helmholtz Center for Polar and Marine Research

[2]Bremerhaven University of Applied Sciences

*Laurie.c.hofmann@awi.de

The use of single-use packaging materials has increased dramatically in recent decades in parallel with increasing trends in convenience and fast-food. Most of these packaging materials are made of non-biodegradable, petroleum-based polymers that have degradative impacts on the environment and contribute to the global plastic pollution crisis. Rethinking and redesigning packaging materials using natural resources sustainably to replace single-use plastics will therefore be an essential step to achieving the European Commission's new circular economy action plan (European Commission, 2020). Marine Resources, including seaweed, provide an enormous pool of yet unexplored and potentially valuable resources for the production of diverse biomaterials, including packaging, and interest is growing in the use of macroalgae in the packaging industry. The global seaweed-based packaging market is expected to account for $613.42 million by 2029 (Data Bridge Market Research). Screening of local macroalgae species revealed that *Ulva sp.* are good candidates for packaging materials, considering their functional qualities (*e.g.* antioxidant and antimicrobial activity) high growth rates, broad geographic range, mild taste, low iodine content, and the high abundance of biomass produced during summer green tides in many coastal areas. Therefore, several packaging films and prototypes have recently been developed using *Ulva sp.* biomass. Nevertheless, the source of biomass for packaging production continues to be a major limitation due to the risk of heavy metal contamination in harvested material, as well as the knowledge and technology gaps associated with land-based production. Here we present recent developments in the use of *Ulva sp.* in packaging materials, discuss current limitations and knowledge gaps, and provide recommendations for future research needs.

LIPIDS FROM *ULVA SP.* WITH ADDED-VALUE AS NUTRIENTS AND BIOACTIVE PHYTOCHEMICALS FOR HEALTHY FOOD AND FUNCTIONAL INGREDIENTS.

Rosário Domingues*[1,2], Francisca Marques[1,2], Diana Lopes[1,2], Ana Moreira[1,2], Tânia Melo[1,2], Margarida Martins[3], Maria H. Abreu[3], Pedro Domingues[1]

[1]Mass Spectrometry Center, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[2]CESAM, Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal

[3]ALGAplus SA, PCI · Creative Science Park Aveiro Region, Via do Conhecimento, 3830-352 Ílhavo

*mrd@ua.pt

Ulva species are green seaweeds that are found abundantly in the marine environment, and can also be produced in large scale in sustainable aquaculture, namely in integrated multitrophic aquaculture (IMTA) systems. They are some of the most widely-traded edible seaweeds with high market value. These popular green seaweeds are often commercialized as fresh or dried products, but also as extracts, either for direct or processed consumption worldwide. In fact, *Ulva* species has a lot of potential as a versatile ingredient that can be incorporated in various dishes, as salads, soups, and smoothies. In addition, it can be processed into different products like *Ulva* chips, *Ulva* pasta, and *Ulva* powder, which makes it an attractive option for both food manufacturers and consumers. The demand for plant-based foods and sustainable food sources is increasing, and *Ulva* fits the bill perfectly as it is a promising food source for healthy and sustainable diets.

The biochemical composition of *Ulva* has been extensively studied, revealing that these seaweeds are rich sources of healthy nutrients and various bioactive compounds, including carbohydrates, proteins, lipids, vitamins, and minerals, making them a valuable ingredient not just for food but also feed, nutraceuticals and pharmaceuticals (Simon et al., 2022). The major components of *Ulva* are carbohydrates, accounting for up to 70% of dry weight. These include polysaccharides as ulvan, which showed anti-inflammatory and anticancer properties, as well as various mono- and disaccharides, such as glucose, mannose, and xylose, which can serve as a primary source of energy.

On the other hand, protein also account for a significant portion of *Ulva*'s composition, making up to 20% of dry weight. Essential amino acids, like lysine and methionine, can be found in high quantities, which are vital for maintaining human health. Lipids can account for up to 5% of dry weight, comprising a large diversity of molecules such as polyunsaturated fatty acids (PUFA). Studies have shown that PUFA lipids have several health benefits, including reducing the risk of heart disease. *Ulva* also contains a wide range of micronutrients, such as vitamin C, vitamin E, iron, and calcium, that are indispensable for a good health and for preventing nutrient deficiencies. This benefits targeted populations such as vegetarians, vegans, and sports enthusiasts as *Ulva* is characterized by a high nutritional value and low-calorie content. *Ulva* can be a valuable addition to the food industry, as a sustainable source of nutrients and a versatile ingredient, and it can also help promote sustainable food system. Moreover, the consumption of *Ulva* has been linked to various health benefits, including anti-inflammatory, antioxidant, antidiabetic, and anticancer properties, that can help prevent and manage non-communicable diseases (NCDs), such as cardiovascular disease, diabetes, and cancer.

Recently algae lipids and lipidome are gaining increasing interest and thus the lipidome of some *Ulva* species has analyzed using modern OMICs approaches (Lopes et al., 2019; Moreira et al., 2020). Distinct *n*-3 fatty acids with benefits for human health were detected, such as alpha-linoleic acid (ALA) and docosapentaenoic acid (DPA). These fatty acids are mostly esterified to more complex lipids that were identified in the lipidome of lipidome, including glycolipids, betaine lipids, and phospholipids. These lipids possessed bioactive properties of interest, such as antioxidant and anti-inflammatory properties. Moreover, the lipidome of *Ulva sp.* was found to be influenced by environmental factors, as season and geographical location, which makes it a promising tool for traceability that is very usefull for the authentication and geographic origin certification of the algal product (Moreira et al., 2020; da Costa et al., 2020; Monteiro et al., 2020). Effectively, the plasticity of the lipidome and unique lipidomic features of *Ulva* species highlight its potential to ensure quality control and prospect biomass for target bioactive compounds.

Overall, the lipids of *Ulva sp.* have a great potential as functional ingredients. The knowledge on the composition and lipid plasticity of this green seaweed is relevant to optimize culture condition and and valorize specific wild production sites, as well as, maximaze production of lipids of interest.

References:

Simon, C., McHale, M., Sulpice, R. Applications of Ulva Biomass and Strategies to Improve Its Yield and Composition: A Perspective for Ulva Aquaculture. Biology, 11 (2022). https://doi.org/10.3390/biology11111593

Lopes, D., Moreira, A.S.P., Rey, F. et al. Lipidomic signature of the green macroalgae Ulva rigida farmed in a sustainable integrated multi-trophic aquaculture. J Appl Phycol 31, 1369–1381 (2019). https://doi.org/10.1007/s10811-018-1644-6

Moreira, A.S.P., da Costa, E., Melo, T., et al. Seasonal plasticity of the polar lipidome of Ulva rigida cultivated in a sustainable integrated multi-trophic aquaculture. Algal Res 49 (2020). https://doi.org/10.1016/j.algal.2020.101958

da Costa, E., Ricardo, F., Melo, T., et al. Site-Specific Lipidomic Signatures of Sea Lettuce (Ulva sp., Chlorophyta) Hold the Potential to Trace Their Geographic Origin. Biomolecules 10, (2020). https://doi.org/10.3390/biom10030489

Monteiro, J.P., da Costa, E., Melo, T., et al. Lipidome in-depth characterization highlights the nutritional value and species-specific idiosyncrasies of different Ulva species. Algal Res 64 (2022). https://doi.org/10.1016/j.algal.2022.102694

Acknowledgments: Authors are also grateful to FCT/MCTES (Portugal) for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020UID/AMB/50017/2019), and RNEM (LISBOA-01-0145-FEDER-402-022125), and COST Action CA 20106 Seawheat

SEAWEEDS/SEAWHEAT CULTIVATION ONLAND – VIA COMMODITY TO SPECIALTY PHYCONOMY (TO OUTER SPACE).

Alan T. Critchley[1]*

[1]Verschuren Centre for Sustainability in Energy and Environment, Sydney, Cape Breton, Nova Scotia, Canada

*alan.critchley2016@gmail.com

Relative to the open water cultivation of seaweeds, with a history of a few centuries, on land cultivation is in its infancy. However, this intensive and relatively expensive form of algal aquaculture has made many leaps and bounds within the past five to six decades.
Notwithstanding the on land cultivation of microscopic stages of laver and kelps and perhaps the co-cultivation of multiple-species, in coastal dugout ponds, of red and green seaweeds with shrimp and fish, pioneered in eastern countries, the west has embraced on land practices using various types of ponds for specialist applications of macroalgal biomass, and in particular products with high values.
On land cultivation of seaweeds is an expensive undertaking given the land, infrastructure and necessary technical expertise required by the operators and scientists involved. Such activities are not to be entered into lightly! First define the value of the biomass and its costs of production. It is not a field of dreams and a “build it and they will come” attitude will not cut it. A “just do it” approach does not work in the case of applied phycology, called here precision phyconomy. Indubitably the end product needs to have an inherent high value and clear path to market to bear the costs of on land operations (both capital payback and operational outlays).
There has been an evolution of ponds and structures used to produce various types of seaweeds for specific applications. Consequently, the range of seaweeds currently grown on land cultivation is limited. It needs to be expanded in terms of species, cultivars, nurseries and libraries; not to do so would be a critical flaw.
There are success stories and there are those where the facility has changed hands and purposes as a crop of higher value has been identified to warrant the embedded costs of operating on land. When successful, precision phyconomy of some seaweeds provides multiple opportunities for exceptional levels of control and manipulation. Notably, on land farming of macroalgae has learned much from the operations of microalgal facilities, from staged production in the lab. and ponds, via small paddle wheel raceways to large, field-like operations of ponds relying on spargers and compressed gases for circulation of biomass. This talk presents some of the challenges, but highlights successes and provides an optimistic future for the phyconomic production of a wide range of candidate seaweed species.

DEVELOPMENT OF *ULVA* FOOD PRODUCTS AND THEIR CONSUMER ACCEPTANCE.

Sylvia Strauss[1]*

[1]The Seaweed Company, The Netherlands

*sylvia.strauss@theseaweedcompany.com

Lately, seaweed is becoming recognised as an important sustainable food alternative for land crops. Although seaweed cultivation and consumption is predominant in Asia, where eating seaweed has been part of the culinary heritage for many centuries, this is not the case in Europe, where macroalgae are generally not considered as attractive food source. Until recently, the low consumer acceptance of seaweed products in the Western societies has been a hindrance for the food industry to invest in the development of new seaweed products. Although the majority of global seaweed products consist of brown and red algae species, green algae from the genus *Ulva sp.* have been identified as the most suitable macroalgae for biomass production and innovative blue biotech industries in European mariculture. The advantageous nutritional profile of *Ulva* and its high content of beneficial dietary fibres and other bioactive ingredients make this a valuable food item exhibiting not only health benefits but also functional improvements. Despite these favourable qualities, the share of *Ulva* in European mariculture and in food products is still marginal and many efforts need to be taken to exploit *Ulva*'s potential to the best.

The success or failure of a newly developed *Ulva* product is determined by several steps along the whole *Ulva* value chain. First, the choice of suitable strains, a scalable cultivation and food grade processing conditions assure sufficient quantity and quality of the biomass. As the European *Ulva* cultivation is still in its infancy, demand can be higher than the production capacity by one farmer. Furthermore, certification of the *Ulva* biomass is mandatory for the food industry to assure compliance with European food safety and quality standards. Finally, the development of the end product and its marketing strategy strongly influences consumer's purchase decision and enduring acceptance.

To date, only few *Ulva* products are existing on the European market that are scarcely available in specialised shops or online. Thereby, *Ulva* is mainly offered as algae itself for a small niche market. However, to profit from *Ulva*'s versatile potential, to better valorise the raw biomass in food and to increase it's sustainable impact, *Ulva*'s most promising applications are as enriching ingredient in familiar, everyday staples and snacks.

Cereal-based products such as bread, pasta or crackers are mainly produced from refined flours, being poor in fibres and micronutrients. Several studies have shown that enrichment of bread or pasta with *Ulva* powder or dried flakes in concentrations up to 4% can improve nutritional profiles without affecting taste. Furthermore, *Ulva* can also serve as suitable ingredient in gluten-free products when applied in concentrations up to 5%.

Another promising application of *Ulva* as sustainable ingredient could be the inclusion in processed meat and fish products, e.g. in burgers and sausages. The substitution of a certain percentage of meat content with *Ulva* can result in a healthier product with less meat, increased fibres and reduced CO_2 imprint. Additionally, due to the hydrocolloidal properties of ulvan and other polysaccharides, *Ulva* supplementation improves the water binding capacity, resulting in juicier texture with less cooking loss.

An increasing demand for alternative protein sources as meat-free analogues opens up further opportunities for *Ulva* as ingredient. Suitable strains high in protein could at least partially substitute less sustainable plant-based protein sources such as soy. Furthermore, *Ulva* can enrich dairy products such as cheese and seasoned butter as well as sauces, spreads and probiotic products. Fermentation of *Ulva* is still an underdeveloped yet efficient method to add nutritional value by enhancing digestibility and the bioavailability of bioactive compounds. Marketable applications remain marginal to date despite the fact that pre-treated *Ulva* can be a suitable fermentation substrate. Another important outcome of seaweed fermentation is the improvement of organoleptic qualities by removing unwanted sea smell which could help to increase consumer acceptance.

To increase the impact of *Ulva* as food ingredient, the current niche market needs to be extended for a broader range of consumer types. Recent food trends, such as the search for new sustainable food sources and alternative proteins, awareness for healthier diets and openness to a world cuisine are favourable conditions that might help to generally increase acceptance of seaweed in our daily diet and to promote *Ulva* products.

However, consumer groups are very heterogeneous and resistance to food innovation is a function of personality, values, attitudes and socio-economics. The most promising target group for seaweed marketers are food innovative consumers who can mostly be found among young, urban, well educated communities who are also health and environmentally conscious and appreciate new taste experiences. This group is normally highly committed and likely to spread positive feedback on choosing seaweed food. In contrast, conventional, more careless or uninvolved consumers could be targeted mostly with convenience products and snacks including seaweed more inconspicuously.

The necessity to understand the motivation of consumers to eat seaweed in order to build up a successful market is reflected by numerous studies that have been conducted in recent years in different consumer groups in the UK, Canada, Sweden, Norway and Australia. As a general tendency, these studies showed that the awareness of health benefits has a stronger influence on seaweed consumption than sustainable responsibility. However, the feeling of environmental obligation to eat seaweed can also be activated by the perception of health consequences. Interestingly, among those who are already familiar with consuming seaweed products (mainly snacks), the most important driver to eat seaweed is good flavour. In contrast, the strongest barriers to seaweed consumption is food neophobia, the fear of the unknown, as well as the lack of knowledge and unfamiliarity, followed by poor availability and choice of products. Unsustainable packaging is also a purchase barrier among eco-conscious consumers.

In order to increase the impact of *Ulva* in European food products, the hitherto niche sector should become more mainstream. After assuring constant supply and quality biomass, this can primarily be best achieved by a product co-development of seaweed farmers with the food industry, developing business cases for defined products and taking different consumer types into account. Secondly, the low acceptance in many consumer groups can be increased by providing more information about seaweed, increasing exposure in stores, media and restaurants by spreading the sustainability and health story and giving inspiration through recipes, tastings, cookbooks, etc. More trust in new products can be achieved by transparent labelling and including *Ulva* as ingredient in familiar or convenient products, for example in bread, pasta or snacks. Generally, the incorporation of *Ulva* in products will have a higher acceptance and ability to overcome food neophobia more easily than consuming the whole algae itself which most people are not familiar to handle in the kitchen. Ultimately, nowadays, the role of trendsetters and social exposure should not be underestimated. Ambassadors could be celebrity chefs, food bloggers and influencer in social media, promoting seaweed consumption not only in gastronomy and cooking shows but also in everyday life.

ULVA FOR INTEGRATIVE URBAN FARMING – THE FOOD4FUTURE APPROACH.

Anna Fricke*[1,2], Monika Schreiner[1,2], Vogt J.[1,2], Fitzner M.[1,2,3], Psarianos M.[4], Schadow D.[4], Schulz J.[5], Dreyer C.[5,6], Schlüter O.[4], Susanne Baldermann[1,2,7]

[1]Food4Future (F4F), c/o Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany

[2]Department of Plant Quality and Food Security, Leibniz Institute of Vegetable and Ornamental Crops, Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany

[3]Institute of Nutritional Science, Food Chemistry, University of Potsdam, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

[4]Department of Systems Process Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany

[5]Fraunhofer Institute for Applied Polymer Research IAP, Research Division PYCO

[6]Technical University of Applied Sciences Wildau

[7]University of Bayreuth, Food Metabolome, Faculty of Life Science: Food, Nutrition and Health, Fritz-Hornschuch-Straße 13, Kulmbach, Germany

*fricke@igzev.de

Driven by the growing awareness in macroalgal products and the interest to use edible macroalgae as food, rather than only as source of food additives, macroalgal cultivation is expanding in different ways. Next to coastal-based farming approaches, there is also a rising interest to provide these fresh and healthy sea vegetables in inland areas to diversify the local food supply. In this context land-based cultivation approaches are fostering a stable and controlled production. To meet the current challenge of integrating cultivation systems within existing urban infrastructure, transdisciplinary approaches are required. Under the umbrella of the "Agricultural Systems of the Future" funding line of the German Federal Ministry of Education and Research (BMBF), the joint project "food4future" (funding code IGZ: 031B0730A, www. https://www.food4future.de) brings together novel technologies, such as lightweight materials and new LED irradiation concepts, for the co-cultivation of marine and terrestrial organisms in a saline environment. Among other promising alternative crops (*e.g.* halophytes) and animal breeds (e.g. crickets, jellyfish), food4future also considered macroalgae as ideal candidates for an integrative urban farming approach.
In this context, two species, *Ulva compressa*, formerly known as Enteromorpha sp. and *U. fenestrata*, formerly known as *U. lactuca*, were identified as suitable for the intended approach. After isolation from field material the macroalgae were exposed to different cultivation conditions to define the potential cultivation range (*e.g.* temperature, salinity and light) and to identify the most suitable cultivars. Next to testing different light regimes and intensities, the project also focused on the effect of applied construction materials and the use of different cultivation media. In addition to development, growth and biomass production, strong emphasis was given to the metabolic composition and nutritional value of the cultivated species, like pigments (chlorophylls and carotenoids), fatty acids (SFA, MUFA, PUFA), or mineral compositions of cultivation media and algal biomass.
Aiming to construct viable and flexible cultivation units, the project focused on modern lightweight composite materials, able to serve for several construction purposes of the fostered cultivation systems and tested their feasibility for macroalgae (*U. compressa*, *Ectocarpus* sp.) and crickets (*Achaeta domesticus*). Considering material resistance, rigidity, and direct material-organism interactions, from the 10 initially chosen composites, the bio-based polylactic acid (PLA) was identified as most suitable material for joint farming. For macroalgae cultivation, PLA sustained the corrosive cultivation conditions and provided a suitable settlement substrate without affecting the macroalgal physiology or nutritional composition (carotenoids and chlorophylls) (Fricke et al., 2022).
With growing distance from the coast, the access to and choice of cultivation media provides a crucial and often cost-intensive aspect in marine aquaculture. Taken this in account we focused on alternative cultivation media and identified regional saline brine water sources as suitable

cultivation medium for marine macrophytes (Fitzner et al., 2021). First results clearly showed that not only water treatment but also taxa identity played a role for cultivation success in the new media (Fricke et al., 2023, under review). *Ulva compressa* was successfully grown and cultivated in the brine water based medium. In addition, the project aimed to use the valuable water source in long-term experiments, cultivating *U. compressa* in artificial seawater under greenhouse conditions over several months. This study not only showed alterations in the media composition but also differences in the carotenoid concentrations, showing seasonal alterations, as well as changes over cultivation time.

Bringing the marine algae into our artificial environment and exposing them to so far unexperienced conditions potentially not only affected the macroalgae itself, but also seem affect their associated microbiota as we experienced in different contamination events. To further study this issue and also ensure the biological safety of our developing systems we recently started to study the bacterial community associated with our cultivated organisms.

In parallel to all research activities, food4future always aims for further outreach to the public. In this respect, the project provides a modern information platform (https://www.food4future.de/en/news-events/news), using different online and also offline channels to inform different stakeholders on the project content to foster transparency and creating trust and interest in the new technologies and related products.

After four years of the project, the species *U. compressa* and *U. fenestrata* can be considered as feasible candidates to become an integrative part of urban agriculture, providing a valuable nutritional profile, able to grow in different alternative media and cope with alternative construction materials. Thus, our study shows that these sea vegetables are promising candidates for environmental controlled inland cultivation.

References:

Fricke, A., Harbart, V., Schreiner, M., and Baldermann, S. (under review). A pilot study for inland production of “sea-vegetables” in Brandenburg (Central Europe) using regional saline groundwater. Algal Research.

Fricke, A., Psarianos, M., Sabban, J., Fitzner, M., Reipsch, R., Schlüter, O.K., et al. (2022). Composite materials for innovative urban farming of alternative food sources (macroalgae and crickets). Frontiers in Sustainable Food Systems 6. doi: 10.3389/fsufs.2022.1001769.

Fitzner, M., Fricke, A., Schreiner, M. & Baldermann, S. 2021. Utilization of regional natural brines for the indoor cultivation of Salicornia europaea. Sustainability, 13, 12105.

THE USE OF *ULVA SP.* IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS (IMTA). AN ECOLOGICAL APPROACH FOR A SUSTAINABLE MARICULTURE

Muki Shpigel[1]*

[1]Morris Kahn Marine Research Station, The Leon H. Charney School of Marine Sciences, University of Haifa, Israel.

*shpigelm@gmail.com

Scarcity of fresh water, overfishing and decline of ocean biodiversity, marine eutrophication by anthropogenic activities, and the increasing demand for seafood has all required attention from a more comprehensive, global perspective. Moving from conventional aquaculture toward an ecological approach to developing and managing a sustainable aquaculture that cares for environmental and sociological aspects can relieve at least some of these problematic issues. Nutrient assimilation in aquaculture using Integrated Multi-Trophic Systems (IMTA) is a promising ecological approach for sustainable aquaculture. The rationale behind the IMTA systems is to convert the excretions of the organism cultured upstream into valuable food for the organism cultured downstream. Seaweed has a high capacity for nutrient uptake per unit of culture area and can be some important additional valuable products. *Ulva sp.* (sea lettuce) Macroalgae are important for marine ecology and have been identified as ideal candidates for biofiltering fishpond effluents. In particular, the worldwide distribution of *Ulva* in many ecological settings indicates that this is a suitable species for cultivation globally. The rapid growth of *Ulva* is attributed to its high photosynthetic activity and uptake rate of nitrogenous nutrients. In addition to using *Ulva* as a biofilter, their integration into a diet, either as feed for macroalgivores (abalone, sea urchins, shrimp, and fish) or as a supplement for human consumption, can increase the overall profitability. *Ulva* assimilation rate ranged between 1-5 g N m^{-2} d^{-1}, and *Ulva* yield ranged between 150-300 g (WW) N m^{-2} d^{-1} or 55 -110 kg N m^{-2} d^{-1}. We have to take into consideration that *Ulva* growth is light-dependent, and the production is not uniform throughout the year. During summer, when the days are longer, production reaches 300 g N m^{-2} d^{-1}; in winter, production is as low as 150 g m^{-2} d^{-1}. The area required in IMTA for seaweed production must consider the lowest yields. Excess *Ulva* in summer can be dried and incorporated into pellet feed. In the biochemical composition of Ulva, we can see that protein levels are about 34% (DW) compared to 10-12% in the wild. Although *Ulva* is an efficient biofilter, it has a low commercial value. Using this species as a biofilter alone would be expensive, as running such a system requires electricity, space, and labor. Therefore, adding macroalgivores such as sea urchins to the fish seaweed or using it as a feed supplement for marine fish is an attractive option to increase the overall profit of the integrated system. Protein derived from fishmeal is the most expensive ingredient in fish feeds. Therefore, reducing and replacing this ingredient with a less expensive protein source yielding the same fish growth performances will contribute to reducing production costs. Protein-rich *Ulva*, used as a biofilter in an IMTA system, was evaluated as a dietary ingredient for gilthead seabream (*Sparus aurata*) at a high (up to 30%) replacement of fishmeal ratio. By removing 100% of the fishmeal (i.e., replacing 260 g kg^{-1} of fishmeal with 291 g kg^{-1} less expensive animal meal and 146 g kg^{-1} Ulva), the total cost of feed containing *Ulva* was reduced by \$0.25 kg-1; with an FCR of 1.7, the saving was \$0.45 per 1 kg fish produced. Since fish feeds represent over 50% of the operating costs in intensive aquaculture, with protein being the most expensive dietary source, about 10% saving on the feed cost is economically significant. The somatic growth rates of two species of Abalone (*Haliotis discus hannai* and *Haliotis fulgens*) and two species of sea urchins (*Paracentrotus lividus* and *Tripneustes gratilla*) were significantly faster when fed with IMTA-based *Ulva* compared to wild *Ulva* exhibited high gonad somatic index (SGI), and high quality, bright-orange gonads.

Land Based IMTA systems will increase profit for the farmer, improve food conversion rate (FCR), diversify the mariculture products, often create additional jobs, and, most importantly, reduce environmental pollution.

USE OF *ULVA SP.* IN ANIMAL DIETS: OPPORTUNITIES AND CHALLENGES.

Umair Ahsan*[1,2]

[1]Department of Plant and Animal Production, Burdur Vocational School of Food, Agriculture and Livestock, Burdur Mehmet Akif Ersoy University, İstiklal Campus, 15030, Burdur, Turkey

[2]Center for Agriculture, Livestock and Food Research, Burdur Mehmet Akif Ersoy University, İstiklal Campus, 15030, Burdur, Turkey

*uahsan@mehmetakif.edu.tr, *umair.asawar46@gmail.com

A considerable augmentation in the demand for food is foreseen as the world population is poised to a continual increase estimated to grow more than 9 billion by the year 2050. Concurrently, growing urban and richer population due to enhanced income levels will drive the demand for animal-origin foods especially meat and milk. These factors will impact the land use to feed the animals for food production since more affordable feedstocks and biofuel will be required. Consequently, a competition for land use might arise between agricultural production for human and animal populations. Increased human activity over the past decades has resulted in global warming, increased greenhouse gas emissions, unusual precipitation, growth of weeds, pest attacks, and disease load. These will eventually impact the global food production. Furthermore, the use of synthetic and conventional feed supplements has been discontinued or banned in whole or parts of the world. Therefore, the use of new and alternative feeds and feed additives in animal diets is necessitated to spare the conventional counterparts. Algae and algae-derived products are among these non-conventional feed components. *Ulva sp.* are chlorophytes (green algae) belonging to the Ulvaceae family that are commonly referred to as sea lettuces, marine algae, or seaweeds. Although *Ulva sp.* can grow naturally, human activity releasing nutrients to pollute the oceans has made substantial contribution in the growth of *Ulva sp.* as environmental hazard. However, seaweeds comprise of useful nutrients like protein, fats, and carbohydrates, and functional components that provide opportunity for use in animal diets. *Ulva sp.* may account for the partial replacement of major plant-based feedstuffs competing with the food crops intended for expanding global population. In addition, bioactive molecules extracted from *Ulva sp.* can be used to produce functional animal diets to improve animal health and performance. Thus, *Ulva sp.* can ensure the food security by serving as a component of diets either as a feedstuff or as feed additive. Nonetheless, the presence of certain components in *Ulva sp.* may act as antinutrients and the use of bioactive molecules of *Ulva sp.* in animal diets might be expensive thus limiting the use of these seaweeds in animal diets. This paper critically presents the opportunities and challenges of the use of *Ulva sp.* in animal diets from feedstuff and feed supplement perspectives.
Ulva sp. as feedstuff/feedstock: *Ulva sp.* possess very low content of dry matter/mass (DM), a considerable amount of cellulose and starch along with complex carbohydrate ulvan (carboxy-sulfated group; $COOH^{+}$ $SO3^{-}$), lower quantities of mostly saturated lipids, and substantial amounts of valuable micro-, macro-, and trace minerals. Given the nutrient content, the use of *Ulva sp.* looks promising for use in animal diets at higher inclusion levels. Given the protein and cellulose contents, *Ulva sp.* seem suitable for the replacement of alfalfa in ruminant diets which was further confirmed in an in vitro gas production test of *Ulva lactuca*. In situ study conducted in cows suggested that a large portion of the crude protein fraction of *Ulva lactuca* was rumen escape/undegradable protein. The use of *Ulva sp.* in poultry diets is potentially limited. Previous studies have reported that *Ulva sp.* can be used up to 3% in poultry diets without any adverse effect on the growth performance of chickens. However, recent studies have shown that the inclusion of *Ulva sp.* up to 15% does not pose harmful effects on the growth performance of broiler chickens. Although the use of *Ulva sp.* is possible to some extent in cows, it seems challenging in poultry as the digestive system of poultry neither possesses the cellulose digesting enzymes nor the big fermentation compartment like rumen. Keeping this in view, the use of fibrolytic, cellulolytic, and proteolytic enzymes is imperative to allow for greater inclusion levels of *Ulva sp.* in poultry diets. Few recent studies have reported that the inclusion of

enzymes in diets containing *Ulva sp.* do improve the growth performance of broiler chickens attributable to several factors.

Ulva sp. as a source of bioactive molecules: Besides the use of *Ulva sp.* directly as feedstuff in animal diets, *Ulva sp.* can provide various bioactive molecules such as ulvan and a range of different pigments. These bioactive molecules can be extracted from *Ulva sp.* using different techniques and biorefinery approaches. Ulvan can act as a potential prebiotic that can be used in monogastric animals whereas pigments can be used to enhance the color of the animal-origin foods and antioxidant status of cells and tissues. Biorefinery approach can also yield other high-end products like proteins and carbohydrates that have the potential to be used in monogastric and polygastric animals alike.

While *Ulva sp.* present various opportunities for use in animal diets to reduce the competition between humans and animals to use the plant-based cereals and grains as food or feed resources, it poses challenges that need to be addressed before the routine use of *Ulva* can be made possible. Since green tides and algal blooms have appeared due to man-made disaster of pollution and release of effluents to water and oceans, there are risks of heavy metals and undesired impurities in Ulva. Additionally, *Ulva sp.* harvested from the ocean generally contains greater ash content that creates hinderance in the use of *Ulva sp.* in animal diets. Over the past few years, tank cultivation has seen an upward trend, however, most of tank cultivated *Ulva sp.* goes for human consumption. Therefore, scaling-up of *Ulva* production is a major challenge as animals consume large amount of feed and *Ulva* will be required in larger quantities. Studies have shown that protein fraction in *Ulva sp.* might have been overestimated because most studies used the nitrogen-to-protein conversion factor of 6.25 that is not true. Standardization of analysis of *Ulva* is imminent. Either a universal conversion factor of 5 or a conversion factor of 4.6 specific to *Ulva* has been proposed in recent studies. This will allow greater transparency in the use of *Ulva* to balance the nutrient requirements of animals. Moreover, seaweeds in general and particularly *Ulva sp.* are not a part of the reference feed databases of different countries and world-wide popular organizations. This should be taken into consideration after careful studies to enhance the use of *Ulva sp.* in animal diets. A recent study has reported that the fermentation of ulvan in the gut can liberate the hydrogen sulphide that affects the mitochondrial function of the cells and tissues. Hence, it is necessary to address this question before proposing the use of *Ulva sp.* in animal diets.

COMMERCIAL CULTIVATION OF *ULVA* IN MEXICO: FROM THE LABORATORY TO COMMERCIAL PONDS.

José A. Zertuche-González[1]*

[1]Universidad Autónoma de Baja California. Instituto de Investigaciones Oceanológicas. Ensenada, Baja California. México.

*zertuche@uabc.edu.mx

From the late fifties to the early eighties, the Mexican seaweed industry was concentrated on the utilization of seaweeds for the production of phycocolloids and more recently, the use of seaweeds as fodder in abalone aquaculture and seaweed extracts for agriculture. All the seaweeds used in these activities are harvested from natural beds. In the mid 90´s, the first studies to develop the cultivation of seaweeds in ponds took place at the Instituto de Investigaciones Oceanologicas (IIO) of the Universidad Autonoma de Baja California (UABC). These studies were focused on the cultivation of carregenophytes in ponds following the principles of "tumbling culture" published in the 80´s by JS Craigie, RGS Bidgell, and collaborators. Although the results for the commercial cultivation of carragenophytes at a pilot scale were technically successful, the reduction of carrageenan demand worldwide and the abundance of commercial natural beds halted its commercial scalation. These studies, however, provided the opportunity to develop essential infrastructure at the UABC-IIO that included an outdoor twelve 1m^3 tanks facility and pilot ponds (40 to 100 m^2) to continue seaweed aquaculture research at a commercial pilot scale.

In the Spring of 2015, efforts to develop the commercial cultivation of *Ulva* spp were initiated at the IIO-UABC. This was the first effort for land cultivation of seaweed focused on human consumption. *Ulva* strains were collected from Bahia San Quintin on the Pacific coast of the Baja California Peninsula, 200 km south of Ensenada and 300 km south of the US-Mexican Border. Basic studies for light and temperature tolerance, optimal initial density, and pilot culture in 100 m^2 ponds were performed on these strains for six months including winter and summer. The best strain was selected to be cultivated in ponds for two and a half years in 100 m^2 ponds (1 m depth). Cultures were initiated with 3 kg per m^2 and fully harvested after 21 days and re-seeded with the initial density. Seaweed production was maximum in the Spring (290 g m^{-2} d^{-1} ww) and minimum in Winter (40 g m^{-2} d^{-1} ww). The annual average production throughout the study was 174 g m^{-2}d^{-1} (equivalent to 24.8 g m^{-2} d^{-1} dw considering a ratio of 7:1 ww:dw).

These studies provided the bases for the development of the first commercial *Ulva* farm in Mexico. The farm presently has 1920 m^2 of ponds with a capacity of 100 to 121 wet tons per year. Studies now in progress have been focusing in testing the possibility for improving the productivity per unit area following three lines of research; increasing light and temperature in winter and cooling water in summer by artificial means; adding CO2 and; a strain selection. The increase in temperature and light in winter can increase the Dec-Feb yield by 44 % while maintaining the water temperature in summer at 23^{o}C and 20^{o}C can increase the yield by 55 and 72 %, respectively. Although the pH in the pond cultures often reach values above 9.3 indicating a lack of bicarbonate, the addition of CO_2 does not increase productivity significantly due to the alternative mechanisms of *Ulva* to concentrate CO_2. Finally, a more comprehensive selection strain program, now in progress, indicates the feasibility to find a strain, even of the same species, that can perform better as a cultivar providing consistently, higher yield and properties.

Due to its high growth rate, and the feasibility to grow vegetatively, *Ulva* cultivation has been considered for multiple uses including; biofuels, bioplastics and, as a CO_2 sink. These markets require huge amounts of biomass at a very low cost. Certainly, onshore cultivation by tumble culture, regardless of the scale, may never be economically feasible for these uses. Ulva, however, has also been considered for its nutritional, nutraceutical, and pharmacological uses. The main challenge to reach these markets is to provide seaweed with consistent properties and quality. Pond cultivation, where fertilization can be controlled and, to some extent, environmental conditions of light and temperature can be influenced, or partially controlled (by managing biomass density, water exchange or other means) may be the proper cultivation approach. Although these markets can afford seaweed at a higher cost, production cost is still a major challenge. The overhead cost of producing seaweed for human consumption, for example, where sanitary regulations required expensive infrastructure and multiple certifications, is one of the main barriers for the industry to succeed. Productivity per unit area is one of the main factors that influence cost in onshore seaweed cultivation. Therefore, studies to increase productivity are essential in order to have financial success.

Proper infrastructure design and engineering are also essential to optimize seaweed productivity. Farm operation can be improved by mechanization and automatization of processes such as fertilization and harvesting. However, the investment necessary for installing or improving mechanization and automatization is tied to an increasing scale of production. So, even if the technology is available, the necessary investment may not be justified or affordable by small farms. On the other hand, the increase in productivity by the development of improved strains can increase productivity at any scale. This is the reason for the importance of proper

ALGAPLUS: TEN YEARS OF PRODUCTION AND EVOLUTION.

Helena Abreu[1], Inês Oliveira[1], Andreína Azevedo[1], Madalena Mendes[1], Rosa Melo[1], Andreia Rego[1], Margarida Martins[1]*

[1]ALGAplus, Produção e Comercialização de Algas e seus Derivados Lda

*inovacao@algaplus.pt

ALGAplus is a private Portuguese company that initiated its activity in 2012, in a property located at Ria de Aveiro with 14 ha. ALGAplus produces customized seaweed and seaweed-based products mainly for the food and well-being markets, reaching the B2B (companies & restaurants) and B2C (retail) sectors with different brands. ALGAplus seaweed are organic certified, assuring warranties of sustainability, quality standards through time and traceability to the customers. At ALGAplus, the production is done under the Integrated Multi-Trophic Aquaculture (IMTA) concept, with a strong focus in R&D and continuous support to the customers.

All seaweed production phases are carried out in-house: biomass production in a land-based modular IMTA system, processing (washing, drying, salting, cutting, milling), and packaging. Besides, ALGAplus works with their customers on tuning the seaweed biomass to the desired characteristics or on the domestication of new species of interest, giving them the product differentiation needed in highly competitive markets. The main species in production at this Portuguese company is *Ulva sp.*, however, other species native from the Atlantic Ocean are also farmed and commercialized year-round, such as *Porphyra dioica*, *Porphyra umbilicalis*, *Gracilaria gracilis*, *Codium tomentosum*, among others.

In 2016, the company reached a milestone with 600 m2 of surface of production, however, this meant still a small production in comparison with the market demand and the upscaling production/processing was mandatory. Since 2020, ALGAplus is actively working on the process's optimization and upscaling, that goes from the nursery to outdoors tanks, including the update of protocols of cultivation. In 2022, ALGAplus reached another milestone with 0.4 ha of production surface due to the implementation of 15 raceways for seaweed production under the same IMTA concept, that were designed together with INEGI in the frame of the European project GENIALG. In 2023, the company expects a production of 80 tons of seaweed (fresh weight), however, this production can reach 165 tons fresh weight with the raceways fully operational.

Currently, ALGAplus is optimizing the system in terms of energy and labour costs, manipulation of production factors according to downstream and upscaling needs. These keystone tasks are being carried out in the frame of different national and international collaborative projects, such as SeaMarK (Horizon Europe) and Pacto da Bioeconomia Azul (02/C05-i01/2022); and also together with strategic partners with expertise in the field of macro- and microalgae, such as GreenCoLab.

ALGAplus works on the development of new seaweed-based food products in partnership with already established food companies. Some products already in the market are: a seaweed bread with no salt, a salted honey, a ready-to-eat canned meal, frozen ready-meal, and a mayonnaise with sea-lettuce that adds an umami flavour to the sauce, having also the claim of 50% less salt. In terms of outreach, the company is strongly related with the local community (associations, schools, governmental bodies), is part of national associations of the sector (BlueBioAlliance, PROALGA, Associação Portuguesa de Aquacultures) and integrated in international networks (International Seaweed Association, Global Seaweed Coalition, EU4Algae). The company has an open-door policy, being also partner of the Portuguese program Escola Azul, since education part of its strategy.

TECHNICAL CONSIDERATIONS FOR THE INTENSIVE TANK CULTIVATION OF ULVA: THE ROLE OF NITROGEN AVAILABILITY.

Juan Luis Gómez Pinchetti*[1], Carlos Almeida Peña[2], Manuel Serrano Crespo[2], Antera Martel Quintana[1].

[1]Banco Español de Algas, Instituto de Oceanografía y Cambio Global, Universidad de Las Palmas de Gran Canaria, Muelle de Taliarte s/n, 35214 Telde, Canary Islands, Spain

[2]Banco Español de Algas, Fundación Parque Científico Tecnológico, Universidad de Las Palmas de Gran Canaria

*juan.gomez@ulpgc.es

During the last 30 years, native *Ulva* species were the main assayed organism for the development of intensive seaweed aquaculture in the Canary Islands. In particular, biomass of *Ulva rigida* C. Agardh (nowadays identified as *Ulva lactuca* Linnaeus) have been produced year-round from laboratory experimental setups to tubular photobioreactors, tanks and raceways at pilot plant scale in our facilities at Taliarte (east coast of Gran Canaria). As far as successful outdoor productivities, reaching seasonal values higher that 100 g DW m^{-2} d^{-1}, and biomass qualities were obtained during the first experimental trials, integrated multi-trophic aquaculture methodologies were evaluated and established as sustainable and environmental-friendly principles for the semi-industrial development of the sector in the region.

In some of our studies, the availability of inorganic nitrogen and dynamics on tank-cultivated biomass, grown under N-enriched and N-depleted conditions, deeply affected growth, physiological and biochemical characteristics of *Ulva*.

Chlorophylls, ash, caloric content, fatty acids, dietary fibres and colouration varied significantly depending on the nitrogen conditions. C:N ratios (and protein contents) correlated significantly with biochemical parameters. Fatty acid (FA) synthesis continued during the N-starvation period; saturated and mono-unsaturated FA increased to a maximun of 72.2 %, while poly-unsaturated fatty acids (PUFA) decreased to 27.7 %. During the N-enriched recovery period, the reverse was found. C:N ratios above 10 correlated with carbohydrate synthesis as shown by the dietary fibre level. Under nitrogen enriched conditions, C:N ratios decreased along with a decrease in fibre level. Under controlled conditions, nitrogen represents a major influence on the development of intensive tank cultivation of *Ulva rigida*, not only by affecting parameters closely related to nitrogen metabolism but also some clearly influenced by carbon uptake.

Ulva has been widely used as a biofilter because of its high efficiency to remove nitrogenous inorganic compounds (up to 90 % in the form of ammonium) from wastewaters. *Ulva* species show the capacity to utilize, quickly absorb and metabolize different forms of inorganic nitrogen, mainly nitrate and ammonium, depending on their availability. However, ammonium ($N\text{-}NH_4^+$), which can be toxic or inhibitory for some seaweeds at concentrations higher than 30–50 µm, is the preferred nitrogen form for *Ulva* and other species with interest in seaweed aquaculture. Moreover, some authors demonstrated that $N\text{-}NH_4^+$ availability controls ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and carbonic anhydrase (CA) activities in *Ulva rigida*, confirming that there is a close relationship between inorganic nitrogen, photosynthesis and carbon metabolism. Therefore, excess and depletion of nitrogen sources in the culture medium causes important cellular responses in both wild and cultivated algae.

More recently, intensive tank produced *Ulva* biomass have been evaluated for multiple applications related to sectors as food and feed or those related with the use of high value metabolites as the cosmeceutical or nutraceutical. It is particularly relevant the characterization

of biomass with different biochemical profiles, obtained under specific growth-controlled conditions, as feed for mollusks such as the abalone or as novel dietary ingredients for aquafeeds improving the immune response in aquaculture.
All this knowledge on how *Ulva* intensive cultivation and biomass quality are strongly related will be reviewed and considered in the near future as the basis for the development of new projects with the aim to evaluate the carbon sequestration and footprint reduction in a scenario of global change.

ULVA PRODUCTION; CAN WE IMPROVE ENGINEERING SOLUTIONS TO CULTIVATE AND PROCESS AT LOWER PRICES?

Stefan Kraan[1]*

[1]The Seaweed Company

Mausrevagh Kilcoona, Headford, Ireland
*Stefan.kraan@theseaweedcompany.com

Over the last 2 decades large blooms of the opportunistic macroalgae *Ulva sp.* has been witnessed with the epiphany in 2009 in the yellow sea with a recorded 1.9 million tonnes caused by eutrophication (Chuanmin Hu et al, 2010). Several companies have tried to capitalise on this, drying the biomass using solar and wind to make fertilisers (high N and P content in tissue) and animal feed or process it as fresh. However, this brings many issues from contamination with sand and plastics to high metal content or other impurities which are a signature of the site harvested. However, for food production these issues severely limit the use of algae blooms and cultivation of *Ulva* is the only viable alternative, either through rope culture (Sweden), tank cultivation (e.g., Israel, France and others) or pond cultivation (Netherlands) or in IMTA systems using tanks and ponds as side product of a higher value species like shrimp. Nevertheless, food grade material also means a stringent drying and processing protocol, all factors making cultivated *Ulva* extremely expensive and not a main stream product. This presentation will discuss the current status in cultivation and processing and will suggest options to make the process cheaper by lowering the carbon footprint and energy input. If we truly want to make *Ulva* the wheat of the sea, we need to improve or cultivation ad processing protocols in a more economic and sustainable way.

References

Hu, Chuanmin; Li, Daqiu; Chen, Changsheng; Ge, Jianzhong; Muller-Karger, Frank E.; Liu, Junpeng; Yu, Feng; and He, Ming-Xia, "On the Recurrent *Ulva* Prolifera Blooms in the Yellow Sea and East China Sea" (2010). *Marine Science Faculty Publications.* Paper 58. http://scholarcommons.usf.edu/msc_facpub/58

ALTERNATIVE AND POTENTIAL APPROACHES IN THE MAINTENANCE OF VEGETATIVE GROWTH IN *ULVA* AQUACULTURE AND ENHANCED DELIVERY OF ALGAL PRODUCTS.

Thomas Wichard[1]*

[1]Institute for Inorganic and Analytical Chemistry, Jena School for Microbial Communication, Friedrich Schiller University Jena, 07743 Jena, Germany

*Thomas.Wichard@uni-jena.de

Many *Ulva* species proliferate in nature and form green algal blooms ("green tides"), which can occur when nutrient-rich wastewater from agricultural or densely populated areas is flushed into the sea. The seaweed growth rate and chemical content are hardly predictable and are affected by environmental factors, including epiphytic bacteria. Bacteria are necessary for the adhesion of *Ulva* to its substrate, its growth, and the development of its blade morphology. *Ulva mutabilis* develops into a callus-like morphotype in the absence of certain bacteria. However, with the addition of the necessary marine bacteria, the entire morphogenesis can be restored. Surprisingly, just two bacteria isolated from *U. mutabilis* are sufficient for inducing morphogenesis and establishing the reductionist system of a tripartite community such as *Ulva mutabilis*- *Roseovarius* sp.-*Maribacter* sp. (Wichard, 2023). The reductionist approach has enabled the study of the bacteria-induced morphogenesis and the life cycle of *Ulva under standardized conditions: The presentation will highlight two approaches relevant to Ulva´s aquacultures.*

(1) Control of changes in the life cycle: Blade cells of the green macroalga *Ulva mutabilis* Føyn (Chlorophyta) excrete regulatory factors into their cell wall and growth medium to control the status of the cell. These unknown sporulation inhibitors, a glycoprotein (SI-1) and a small molecular weight compound (SI-2) located within the bilayer of *Ulva* control the vegetative status of the cell. Upon removal of the sporulation inhibitors by mincing the mature thallus into small single-layered fragments and washing intensively, the gametogenesis of the thalli can be artificially onset. The gametogenesis is subdivided into a determination phase (36 h), within vegetative growth could be resumed by the addition of SIs, and a differentiation phase afterwards, insensitive to SIs, when gametogenesis occurred (Figure 1) (Stratmann et al., 1996; Wichard and Oertel, 2010; Vesty et al., 2015). At this time of no return, the formation of progametes starts, and the gametes are duplicating up to 32 gametes in one gametangium during the last night. The swarming phase occurs on the third day, when the pore caps are opening, and the gametes can leave the gametangia upon the removal of an additional swarming inhibitor.

It is tempting to assume that those inhibitors allow us to manipulate the life cycle of land-based aquacultures applying a non-genetic approach. A protocol is suggested for the direct application in bioassays and aquacultures (Kessler et al., 2017; 2018). As sporulation inhibitors are produced and released depending on the age of *Ulva* cultures, mixed-age populations might help to prevent sporulation events in aquacultures (Obolski et al., 2022).

(2) Applications of microbiome leveraging and engineering for promoting macroalgal growth and bioactives in aquaculture: Our study found that IMTA conditions significantly affected the structure and composition of the *U. rigida* microbial community (Califano et al., 2020) Morphogenetic activities were detected in sterile-filtered water samples tested in bioassays with *U. mutabilis* because of the clustering of species known for their morphogen production (e.g., *Roseovarius* sp., *Sulfitobacter* sp., and *Maribacter* sp.). We hypothesized that microbiome engineering could control the chemical composition of *Ulva* biomass and constituents. We demonstrated that the engineered *Maribacter* sp. and *Roseovarius* sp. consortium modulated *Ulva mutabilis* photosynthate content such as amino acids, polyols (Polikovsky et al., 2020) and fatty acids. Further microbes colonising macroalgae should be isolated under controlled, reproducible conditions. Hereby, multiple omics technologies should be used to determine the phenotypes of the designed holobiont and its new traits. As bacteria can influence algal development and other bacteria in many ways, a diverse inoculum may improve aquaculture

development. Therefore, functional groups of bacterial strains should be defined based on a suite of similar traits (Wichard 2023).

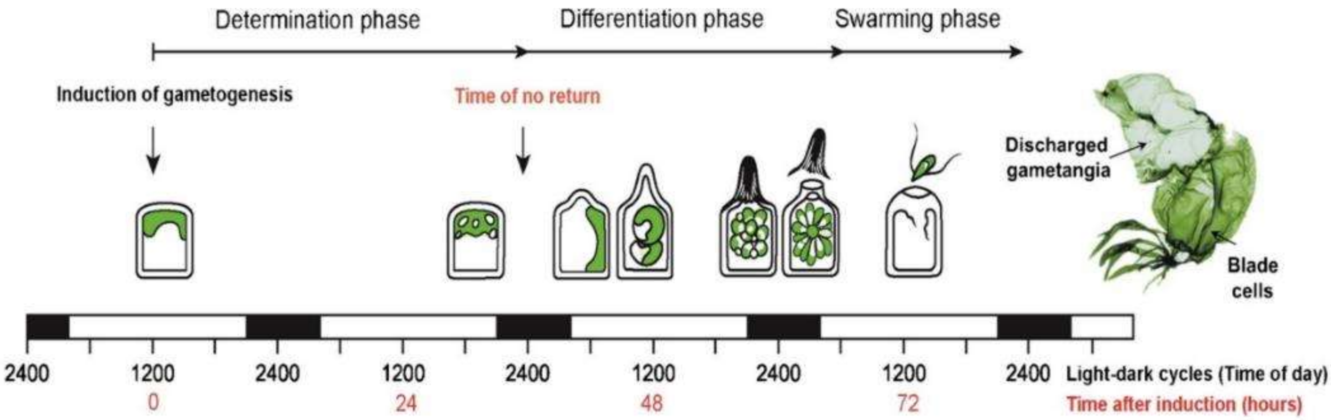

Figure 26 Regulation of gametogenesis in Ulva mutabilis under the control of sporulation and swarming inhibitors (adopted from Wichard and Oertel (2010) and Kessler et al. (2017))

In summary, understanding the chemical ecology of *Ulva* and its controlled life cycle will improve the maintenance of aquaculture and improve their biorefinery products.

References

Califano G., Kwantes M., Abreu M.H., Costa R., Wichard T. (2020) Cultivating the macroalgal holobiont: effects of integrated multi-trophic aquaculture on the microbiome of *Ulva rigida* (Chlorophyta). *Frontiers in Marine Science.* 7:52.

Kessler, R.W., Crecelius, A.C., Schubert, U.S., Wichard, T. (2017) In situ monitoring of molecular changes during cell differentiation processes in marine macroalgae through mass spectrometric imaging. *Analytical Bioanalytical Chemistry* 409: 4893-4903.

Kessler, R.W., Alsufyani, T., Wichard, T. (2018) Purification of sporulation and swarming inhibitors from *Ulva*: application in algal life cycle controlling. In Protocols for Macroalgae Research, eds. B. Charrier, T. Wichard, C.R.K. Reddy, Chapter 8. Boca Raton: CRC Press, Francis & Taylor Group.

Obolski, U., Wichard, T., Israel, A., Golberg, A., and Liberzon, A. (2022) Modeling the growth and sporulation dynamics of the macroalga *Ulva* in mixed-age populations in cultivation and the formation of green tides. *Biogeosciences* 19, 2263–2271.

Polikovsky M., Califano G., Dunger N., Wichard T., Golberg A. (2020) Engineering bacteria-seaweed symbioses for modulating the photosynthate content of *Ulva* (Chlorophyta): Significant for the feedstock of bioethanol production. Algal Research 49: 101945.

Vesty, E.F., Kessler, R. W., Wichard, T., Coates, J.C. (2015) Regulation of gametogenesis and zoosporogenesis in *Ulva linza* (Chlorophyta): comparison with *Ulva mutabilis* and potential for laboratory culture. Frontiers in Plant Science 6:15.

Wichard, T., Oertel, W. (2010) Gametogenesis and Gamete release of *Ulva mutabilis* and *Ulva lactuca* (Chlorophyta): Regulatory effects and chemical characterization of the "swarming inhibitor". Journal of Phycology 46, 248-259.

Wichard, T. (2023) From model organism to application: Bacteria-induced growth and development of the green seaweed *Ulva* and the potential of microbe leveraging in algal aquaculture. *Seminars in Cell & Developmental Biology* 134: 69-78.

From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Lisbon, Portugal

POSTER SESSION

Ulva
The Future Wheat of the Sea

NATURALLY OCCURRING PROTOPLASTS IN A "GREEN TIDE" STRAIN OF *ULVA LACINULATA*.

Isabel Cardoso *[1,2], Bela H. Buck [1,3], Laurie C. Hofmann [1,3]

[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany,

[2]University of Bremen, Germany,

[3]Bremerhaven University of Applied Sciences, Bremerhaven, Germany

*isabel.cardoso@awi.de

The *Ulva* genus has proved to be a suitable candidate for large-scale cultivation and can serve multiple purposes in several industries (e.g., pharmacy, cosmetics, food, and feed) (Mantri et al., 2020; Leyva-Porras et al., 2021). However, to guarantee the success of its large-scale production, it is important to find solutions to the currently existing biological and technical limitations. Some *Ulva* species reproduce in predictable ways, and their reproduction can be induced by following established methodologies (see Mantri et al. 2020 for recent review). However, attempts at inducing the reproduction of the commonly cultivated species *Ulva lacinulata* (Kützing) have been unsuccessful, and large-scale cultivation of this species requires re-stocking from the wild or limiting the harvest to maintain a starting stock of biomass. While this species is often sought for cultivation because it grows well unattached and it does not sexually reproduce, which results in the loss of biomass, this species often degrades, which also contributes to a loss of biomass. The cause of this biomass degradation has until now not been understood. Here, we present evidence that tissue degradation in *U. lacinulata* occurs as a result of natural protoplast production and release. These protoplasts can then develop in several different directions. New blades can be formed, which either develop into adult thalli or undergo gametogenesis very early in their development, resulting in the release of gametes and the germination of new germlings. These results contribute to a new understanding of the life cycle of *U. lacinulata*, and provide the first evidence of natural protoplast production in this species. Because the induction of protoplasts in *Ulva sp.* is time-consuming and expensive, finding the trigger for this process will be an important step to accelerating the success of large-scale cultivation of this species.
The biological material (previously molecularly identified as *Ulva lacinulata* by Cardoso et al. 2023) was collected in 2021 in a "green tide" area in Lagoa de Óbidos, Portugal (following the Nagoya Protocol) and then brought to the Alfred Wegener Institute, Germany where it has been cultivated in a closed system. Throughout its cultivation, it was kept in 5 L glass vessels at 15 °C (± 1 °C) and an irradiance of 70 μmol photons m^{-2} s^{-1} with a 16:8 h light:dark photoperiod (LD) in pasteurized artificial seawater (ASW) (30 PSU) supplemented with half-strength Provasoli in a concentration of 10 mL L^{-1}. The medium was replaced once per week and an aeration system guaranteed the continuous tumbling of the material inside of the vessels.
Upon observation of the onset of biomass degradation, fresh biomass (0.72 g) was collected. The biomass was distributed equally into four 1 L beakers. The experiment ran for four weeks under the previously mentioned cultivation conditions. Every week, the water in each beaker was filtered and centrifuged to collect the protoplasts. After filtering the water, the debris and small pieces of the original biomass were collected, weighed, and placed back into the beaker with newly added culture media. Calcofluor white (CFW) was used to observe the presence/absence of cell walls and disposable hemacytometers were used to quantify the protoplasts yields obtained in each beaker each week. A defined amount of the collected protoplasts were isolated in individual Petri dishes and their development was observed under the microscope. The number of protoplasts that germinated into new blades were counted after five weeks.
Our observations under the fluorescent microscope confirmed the absence of cell walls in the cells dyed with CFW, thus confirming that these cells are naturally occurring protoplasts. The total protoplast yield obtained in our first experiment was 3.21×10^7 cells per gram of fresh biomass, which is comparable to those reported in studies where the protoplast formation of

different *Ulva* species was enzymatically induced (Reddy et al. 2018). Approximately half of the protoplasts regenerated (40-60 %) and grew into discs or germlings in a similar fashion to what has been described in the literature for induced protoplasts of *Ulva* spp (Reddy et al. 2018; Figure 27). Moreover, sexual reproduction was found to occur during gametogenesis in protoplasts, rather than in adult blades.

Additionally, by measuring the weight of the initial biomass each week, we observed that the original tissue did not completely degrade after protoplast formation and release, and a final total fresh weight of 5.79 g resulted in a 7 % daily relative growth rate. Furthermore, there was a clear recovery in the last week of the experiment with the average RGR reaching 12.3 % day^{-1} (Figure 28). Thus, revealing that degradation does not necessarily result in a total loss of the culture.

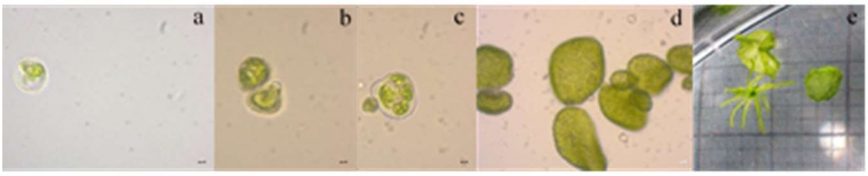

Figure 27 Development of the regenerated protoplasts from U. lacinulata obtained during a "degradation" event. a: single protoplast with no visible cell wall; b: first cell division; c: second cell division; d: Ulva discs originated from cell division of protoplasts; e: discs and germlings originated from protoplasts. A, b and c at a 400 x amplification, d at an amplification of 100 x, e taken with a binocular (each square = 1 x 1 mm).

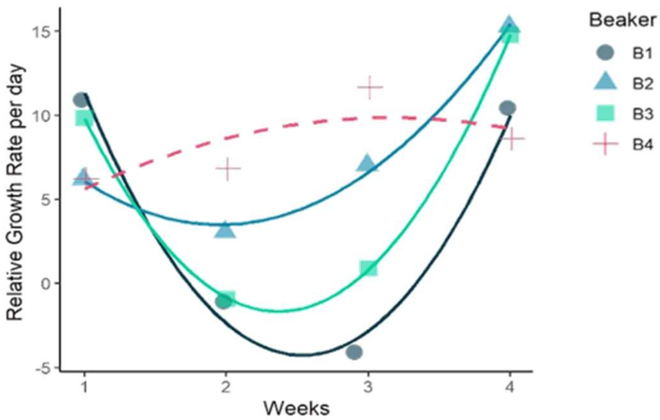

Figure 28 Relative growth rates (RGR day-1) of the degraded/protoplast-producing pieces of Ulva lacinulata in each beaker over a period of 4 weeks.

Our observations of natural protoplast production in *U. lacinulata* close an important knowledge gap in understanding this species' reproductive cycle. This new knowledge can be beneficial when trying to understand the formation of "green tides" and the differences between "green tide" strains and non-"green-tide" strains. Additionally, the production of natural protoplasts can potentially be exploited to improve the efficiency of *Ulva* cultivation methods in the future and remove the bottlenecks existing today for safe and profitable large-scale production.

References

Cardoso I, Meissner A, Sawicki A, Bartsch I, Valentin K-U, Steinhagen S, Buck BH & Hofmann LC (2023) Salinity as a tool for strain selection in recirculating land-based production of *Ulva sp.* from germlings to adults. J Appl Phycol (Accepted, under review).

Leyva-Porras C, Román-Aguirre M, Cruz-Alcantar P, Pérez-Urizar JT & Saavedra-Leos MZ (2021) Application of Antioxidants as an Alternative Improving of Shelf Life in Foods. Polysaccharides, 2(3): 594–607. https://doi.org/10.3390/polysaccharides2030036.

Mantri VA, Kazi MA, Balar NB, Gupta V & Gajaria T (2020) Concise review of green algal genus *Ulva* Linnaeus. J Appl Phycol 32(5): 2725–2741. https://doi.org/10.1007/ s10811-020-02148-7.

C RK & Seth A (2018) Manual for protoplast preparation from *Ulva* and their seeding for cultivation. Biowaste and algae knowledge for the production of the 2nd generation biofuels: BioWALK4Biofuels. CSIR- CSIR-Central Salt and Marine Chemicals Research Institute. https://doi.org/10.13140/RG.2.2.20466.15048.

USING *ULVA LACTUCA* AS A FEED INGREDIENT IN WEANED PIGLET DIETS: EFFECTS ON NUTRIENT AVAILABILITY AND GUT HEALTH.

David Miguel Ribeiro*[1], Daniela Filipa Pires Carvalho[1], Mónica Costa[2,3], Cátia Martins[1,2,3], Federico Correa[4], Paolo Trevisi[4], Miguel Mourato[1], André Martinho de Almeida[1], João Pedro Bengala Freire[1], José António Mestre Prates[2,3]

[1]LEAF - Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

[2]CIISA - Centre for Interdisciplinary Research in Animal Health, Faculdade de Medicina Veterinária, Universidade de Lisboa, 1300-477 Lisboa, Portugal

[3]Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS)

[4]DISTAL - Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

*davidribeiro@isa.ulisboa.pt

Seaweeds are novel feed ingredients with the potential to at least partially replace conventional feedstuffs. *Ulva lactuca* is a green seaweed with bioactive properties that can enhance animal gut health and product composition. The objective of this study is to evaluate the effect of 7% dietary *U. lactuca*, with or without carbohydrases supplementation, on nutrient availability and gut health of piglets. Growth performance was not affected by dietary treatments. Dry and organic matter digestibility decreased with dietary seaweed inclusion and ulvan lyase supplementation. Hemicellulose digestibility increased with seaweed inclusion, suggesting a high fermentability of this cell wall fraction, independent of carbohydrases supplementation. Some beneficial microbial populations increased as a consequence of enzymatic supplementation (*e.g. Prevotella*), while seaweed diets as a whole led to increased abundance of *Shuttleworthia*, *Anaeroplasma* and Lachnospiraceae_NK3A20_group, all related with a healthier gut.

Seaweeds such as *Ulva lactuca* are potential sources of nutrients in animal diets, while providing important bioactive substances, such as n-3 polyunsaturated fatty acids and fermentable polysaccharides. Weaned piglets are a particularly sensitive group of animals that could benefit from such feeding. They endure severe changes, mostly nutritional, that cause dysbiosis and compromise their immune system. They require high-quality and highly digestible feedstuffs, preferably with health-promoting properties. This is where seaweeds could be advantageous. However, the cell wall of these novel feedstuffs can have antinutritional effects for monogastrics. Our team has previously reported how a single recombinant ulvan lyase can break their recalcitrant bonds, in *in vitro* conditions. The objective of this study is to evaluate how dietary *U. lactuca*, and the supplementation with either commercial carbohydrase mixture or ulvan lyase, can impact nutrient availability of weaned piglet diets and their microbiota.

The experimental trial took place at the Animal Production Department of the School of Agriculture (ISA) of the University of Lisbon, Portugal. Forty weaned piglets (Large White × Duroc) were randomly divided across four experimental diets: control (standard diet, wheat-maize-soybean meal based), UL (with 7% *U. lactuca* replacing the control diet), ULR (UL + 0.005% Rovabio® Excel AP, a commercial carbohydrase mix bought from Adisseo, Antony, France) and ULU (UL + 0.01% ulvan lyase). Each piglet was allocated to a metabolic cage. After an adaptation period of five days to the experimental conditions, the trial lasted two weeks (14 days). Piglets were weighed at the beginning and end of each week and faeces were collected daily. At the end of trial, piglets were slaughtered and samples of colon contents were sampled for microbiota analysis.

The experimental diets had no effect on the growth performance of piglets. The total tract apparent digestibility (TTAD) of dry matter and organic matter were reduced in the ULU diet compared to control. The TTAD of the cell wall fractions, namely neutral detergent fibre and acid detergent fibre, were increased and reduced by seaweed incorporation, respectively (Figure 1). This could demonstrate that enzymatic supplementation was not required at this level of incorporation, and that the hemicellulose fraction is easily fermented by the piglets' microbiota, with positive repercussions on the gut ecosystem. In fact, the piglets fed with *U. lactuca* had a higher relative abundance of *Prevotella* in the colon, in addition to others including *Shuttleworthia*, *Anaeroplasma* and Lachnospiraceae_NK3A20_group, which have been related with beneficial fermentation in weaned piglets. The lack of major negative effects could be attributed to the pre-treatments of the seaweed biomass (low particle size - <250 μm, and micronization). Furthermore, the high availability of minerals should be considered given possible negative repercussions of mineral imbalances, that include osmotic diarrhoea.

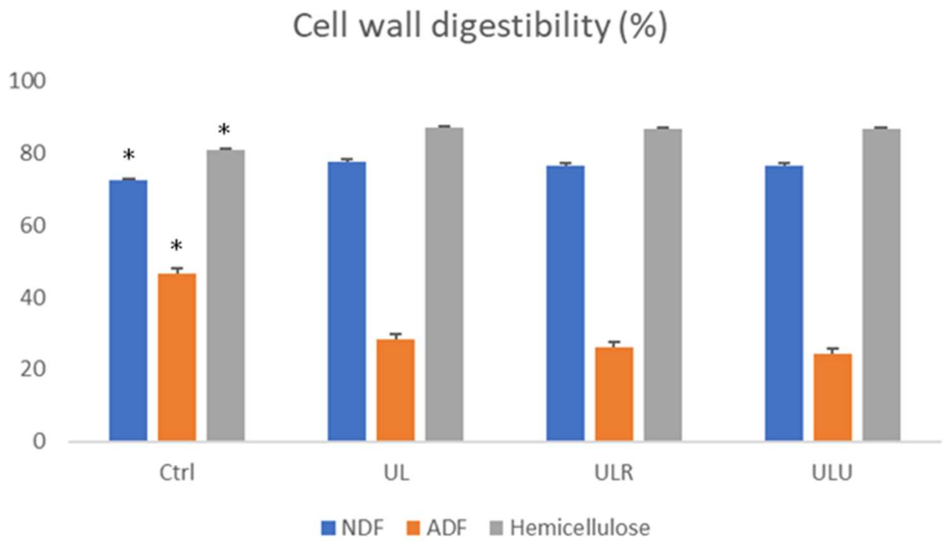

*Figure 29 Total tract apparent digestibility of neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose of four experimental diets: control (standard diet, wheat-maize-soybean meal based), UL (with 7% Ulva lactuca replacing the control diet), ULR (UL + 0.005% Rovabio® Excel AP, a commercial carbohydrase mix bought from Adisseo, Antony, France) and ULU (UL + 0.01% ulvan lyase). * indicates statistically significant differences from other groups (P<0.05).*

Ulva lactuca shows potential to be used as a feed ingredient at this level of incorporation in weaned piglet diets, without the need for enzymatic supplementation. In the future, testing starch digestibility would be highly interesting given its relevance as a storage polysaccharide in green seaweeds.

Keywords: *Ulva lactuca*, piglet, digestibility, microbiome

Acknowledgements

This research was funded by Fundação para a Ciência e a Tecnologia (FCT), Portugal, through PTDC/CAL-ZOO/30238/2017 grant, associated with a post-doc contract to M.C., and PhD fellowship to D.M.R. (SFRH/BD/143992/2019). CIISA (UIDB/00276/2020), AL4AnimalS (LA/P/0059/2020) and LEAF (UIDB/04129/2020) grants, also from FCT, are acknowledged.

PRODUCTION OF FOOD GRADE LIPID EXTRACTS FROM *ULVA SP.* AND A *ULVA SP.* BASED-ALGAE BLEND USING ULTRASOUND-ASSISTED EXTRACTION.

Francisca Marques*[1,2], Marisa Pinho [1,2], Tiago Conde [1,2], Inês Guerra [1,2], Joana Silva [3], Helena Abreu [4], Maria Rosário Domingues [1,2]

[1]CESAM— Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal

[2]Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal

[3]Allmicroalgae Natural Products S.A., R&D Department, Rua 25 de Abril 19, 2445-287 Pataias, Leiria, Portugal

[4]ALGAplus— Production and Trading of Seaweed and Derived Products Ltd., 3830-196 Ílhavo, Aveiro, Portugal

*francisca.marques@ua.pt

Algae are a rich source of bioactive compounds, such as healthy lipids with potential commercial uses (Djuricic et al. 2021; Ullmann et al, 2021). Lately, the interest in lipids from algae for application in food, nutraceutical, and feed industries, due to their great nutritional quality and attractive bioactivities, is rising. This has sparked the hunt for innovative food-grade and sustainable extraction approaches in alternative to the traditional lipid extraction that use toxic chlorinated-solvents. Instead, ethanol (EtOH) and ethyl acetate (EtOAc) is a non-toxic green extraction solvent approved to produce algae lipid extracts to be incorporated into food products and supplements. However, EtOH and EtOAc have associated with low lipid yield and/or low lipid purity (Lopes et al, 2021). To overcome this drawback, the extraction assisted by ultrasound has been investigated showing encouraging results (Zhou et al., 2021).
The purpose of this study was to evaluate EtOH and EtOAc as food-grade extraction solvent in combination with ultrasound (UAE) to obtain lipid extracts from *Ulva sp.* and a *Ulva sp.* based-algae blend.
While in *Ulva sp.* the lipid content was low (≈40 %), EtOH+UAE extract from blend was lipid-rich (≈60 %). Both *Ulva* and blend EtOH extracts showed very low lipid yield, possibly due to the non-disruption of cells walls. However, better results were obtained with EtOAc. All extracts obtained by EtOAc+UAE and EtOAc showed to be almost exclusively lipids, purity ≈90 %, while EtOAc+UAE has the advantage of provided a higher yield compared with EtOAc. The FA profile of Folch and EtOH+UAE extracts was comparable, but EtOH extract from blend was significantly different. Highest content (%) of *n*-3 and *n*-6 FA were achieved in *Ulva sp.* extracts. Fatty acid analysis of EtOAc+UAE and EtOAc extracts is still in progress. The results of DPPH antioxidant assay revealed that the extracts with the higher scavenging capacity were obtained by Folch (65.5±1.8 %) for *Ulva sp.* and by EtOH+UAE (93.2± 03%) and EtOH (94.0±0.4 %) for blend. Concerning anti-inflammatory potential, *Ulva* EtOH+UAE extract (71.7±3.1 %), and blend EtOH+UAE (77.6±1.4 %) and EtOH (82.9±1.8 %) extracts showed the best ability to inhibit COX-2. Evaluation of bioactivities of EtOAc and EtOAc+UAE extracts is ongoing.
Overall, our results showed that EtOH or EtOAc assisted with UAE is more promising to obtain food-grade extracts enriched in *n*-3 and *n*-6 lipids with health promoting properties for food, feed, and nutraceutical applications.

References

Djuricic, I.& Calder, P.C. BeneficialOutcomesofOmega-6andOmega-3Polyunsaturated Fatty Acids on Human Health: An Update for 2021. *Nutrients* 13,1–23(2021).

Ullmann, J.& Grimm, D. Algae and their potential for a future bioeconomy, landless food production and the socio-economic impact of an algae industry.*Org Agr* 11,261–267(2021).

Lopes, D.et al. Bioactivities of Lipid Extracts and Complex Lipids from Seaweeds: Current Knowledge and Future Prospects. *Mar Drugs* 19,1–24(2021).

Zhou, J. et al. Extraction of lipids from microalgae using classical and innovative approaches. *Food Chem* 384,1–15(2022).

PHAEOBACTER AND *ULVA* INTERACTIONS WITH IMPLICATIONS IN INTEGRATED MULTITROPHIC AQUACULTURE.

Gonzalo Del Olmo*[1], Patricia Ruiz[1], Jadranka Nappi[2], Zujaila Qui[1], Ryan Marchington[1,3], Javier Cremades[4], Torsten Thomas[2], Suhelen Egan[2], José Pintado[1]

[1] Marine Ecology and Resources Group. Institute of Marine Research (IIM-CSIC), Galicia, Spain

[2] Centre for Marine Science and Innovation. School of Biological, Earth and Environmental Sciences. The University of New South Wales, Sydney, Australia

3 The Scottish Association for Marine Science, Oban, United Kingdom.

[4] Coastal Biology Research Group (BioCost). Centro de Investigaciones Científicas Avanzadas (CICA), Universidad de A Coruña, Galicia, Spain

*gdelolmo@iim.csic.es

The biofiltration of fish effluents using macroalgae has gained significant interest in Integrated Multi-Trophic Aquaculture (IMTA) systems. *Ulva* species, such as *Ulva ohnoi*, possess favourable characteristics such as high nutrient uptake rates or adaptability to various environmental conditions. Moreover, its high surface/volume ratio provides a niche for the development of epiphytic bacteria (Oca et al., 2019). These bacterial communities play crucial roles in the growth, development, and defence mechanisms of *Ulva* (Egan et al., 2008). The composition and activity of macroalgal microbial communities are strongly influenced by biological, chemical, and physical processes occurring on the surface of macroalgae. Light, in particular, plays a crucial role due to the photosynthetic nature of macroalgae, which strongly influences their metabolism and, consequently, their microbiome (Coelho-Souza et al., 2017).

Understanding the interactions between macroalgae, their associated microbial communities, and environmental factors like light intensity provides insights into the physiological processes and ecosystem roles of algae. It also opens up possibilities for developing biotechnological applications. By manipulating the microbiota of macroalgae, it may be possible to improve aquaculture practices by identifying microbial communities that promote robust growth of the algae and enhance overall health (Li et al., 2022). Moreover, microbial manipulation of algae in IMTA systems can also have an effect on water quality or the health of the organisms in co-culture (*e.g.* fish).

Among the bacteria associated with *Ulva*, *Phaeobacter* spp. stands out for its ability to inhibit fish pathogens. Some members of *Phaeobacter* genus produce tropodithietic acid (TDA), an antimicrobial compound effective against fish pathogens like *Vibrio* spp (Bruhn et al., 2007). This has led to the isolation and investigation of *Phaeobacter* as a potential probiotic in aquaculture systems. Studies have shown that introducing *Phaeobacter* to fish larvae, either through live prey or biofilters, can significantly reduce mortality caused by *Vibrio* infections (Planas et al., 2006; Prol-García et al., 2014; Prol-García & Pintado, 2013). In the context of IMTA, recent research (Pintado et al., 2023) has assessed the possibility of co-culturing of the algae *Ulva ohnoi* with *Phaeobacter* to mitigate mortality in fish larvae during *Vibrio anguillarum* infections. However, the study also suggested that the persistence of *Phaeobacter* on the surface of *Ulva ohnoi* may be influenced by light intensity.

Our research aimed to investigate the effect of light intensity on the colonization and maintenance of *Phaeobacter* sp. 4UAC3 on *Ulva ohnoi* surfaces under controlled in vitro conditions. We hypothesize that light intensity may influence epiphytic bacterial communities of *U. ohnoi* (*e.g.* in terms of diversity or functionality), and may also influence the colonization and maintenance of *Phaeobacter* sp. 4UAC3. The effect of light could be due to i) a different response of *Phaeobacter* bacteria to light, that could also have an indirect effect on algae physiology and bacterial communities, or ii) the response of the algae to light intensity, promoting growth and the production different metabolites that would influence the microbial communities and *Phaeobacter* or iii) a combination of both factors.

The results showed that the inoculated *Phaeobacter* disappeared from the surface of the *Ulva* as the light intensity increased. However, light itself did not have a detrimental effect when *Phaeobacter* was cultured on inert surfaces (glass and autoclaved *Ulva* discs) (Figure 30).

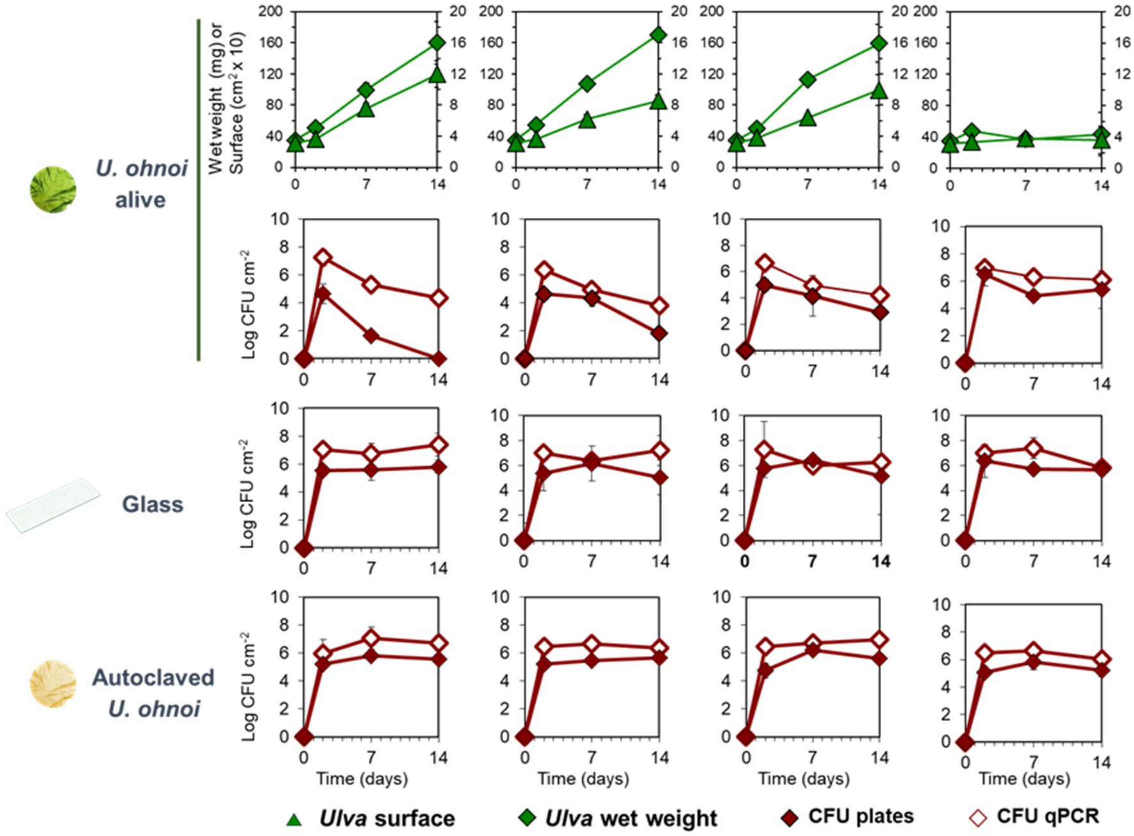

Figure 30 Phaeobacter maintenance on diferenternt surfaces under different light intensities. The concentration of Phaeobacter on different surfaces (live Ulva disc, glass and autoclaved Ulva disc) is shown under different light intensities (143, 75, 45 and 0 µmo/m2 s-1). Phaeobacter concentrations are expressed in CFU/cm2 and quantified by qPCR and Marine Agar culture plates. Also, it is shown the growth of Ulva throughout the experiment expressed in wet weight (mg) and disc surface area (cm2).

Considering this, the negative effect of light could be due to the development of bacterial taxa that would compete with *Phaeobacter*. However, bacterial communities on *Ulva* surfaces did not significantly differ for the different light intensities tested (high light, 143 µmol m^{-2} s^{-1}, medium light, 75 µmol m^{-2} s^{-1}, low light, 45 µmol m^{-2} s^{-1} and darkness), although high light intensity favoured higher species richness initially. It is possible that larger differences in light intensity are required to observe significant effects on bacterial communities. The addition of *Phaeobacter* altered bacterial communities on *Ulva* surfaces initially, but this effect diminished after a week. We observed a slight increase in the growth of *Ulva* when *Phaeobacter* was inoculated compared with control situations, nevertheless, this was not statistically significant. Certain bacterial taxa correlated positively with *Phaeobacter* (like *Litorimonas*, *Henriciella* or *Deinococcus*), suggesting potential synergy or interdependence, while others showed negative correlations (such as *Alteromonas*, *Caldicellulosiruptor* or *Roseobacter*), indicating possible antagonistic or competition interactions. Previous studies have reported similar correlations between light intensity and specific bacterial taxa (Paix et al., 2020; 2021). Additionally, we can consider the possibility that the disappearance of *Phaeobacter* could be influenced by competition and cooperation dynamics involving non-bacterial taxa and the production of specialized metabolites by the *Ulva* holobiont. However, those specific factors contributing to the disappearance of *Phaeobacter* and the interactions between different microbial taxa will require further investigation.

Based on these results, we designed an IMTA-RAS with a tripartite system Sole-*Ulva*-*Phaeobacter* and evaluated the capacity of *Phaeobacter* to control *Vibrio anguillarum* outbreaks. A small-scale prototype system was developed, consisted of three 3L-tanks connected in series: the first contained *Ulva* exposed to light with actively growing algae, the second contained *Ulva* maintained in darkness with the algae being inoculated with *Phaeobacter* (10^7 CFU/mL), and the third tank contained seawater with nutrients added to a level corresponding to the ones in the presence of fishes (Oca et al., 2019). This last tank was inoculated with *Vibrio anguillarum* (10^4 CFU/mL) to simulate an infection in the system. During the experiment, the presence of *Phaeobacter*, *Vibrio* and bacterial communities in water and algal surface were assessed by colony counting in Marine Agar plates, qPCR and 16S rRNA gene sequencing. The results indicated that *Phaeobacter* colonised the entire system, and as in the previous experiment a slight improvement of algal growth was observed. The presence of *Phaeobacter* reduced *Vibrio* concentration in water, being undetectable at the end of the experiment, compared to the control with non-inoculated *Ulva*. Considering this, a foundation has been laid for the design of a larger-scale system for industrial production of sole an IMTA-RAS which is currently been developed.

This research paves the way for the implementation of sustainable aquaculture strategies, based on the use of *Ulva sp.*, that harness the potential of beneficial microbial communities to improve fish health and production.

References

Bruhn, J. B., Gram, L., & Belas, R. (2007). Production of antibacterial compounds and biofilm formation by Roseobacter species are influenced by culture conditions. *Applied and Environmental Microbiology*, *73*(2), 442–450. https://doi.org/10.1128/AEM.02238-06/SUPPL_FILE/AEM2238_06SUPPL.ZIP

Coelho-Souza, S. A., Jenkins, S. R., Casarin, A., Baeta-Neves, M. H., Salgado, L. T., Guimaraes, J. R. D., & Coutinho, R. (2017). The Effect of Light on Bacterial Activity in a Seaweed Holobiont. *Microbial Ecology*, *74*(4), 868–876. https://doi.org/10.1007/S00248-017-0995-X/FIGURES/5

Egan, S., Thomas, T., & Kjelleberg, S. (2008). Unlocking the diversity and biotechnological potential of marine surface associated microbial communities. *Current Opinion in Microbiology*, *11*(3), 219–225. https://doi.org/10.1016/J.MIB.2008.04.001

Li, J., Weinberger, F., de Nys, R., Thomas, T., & Egan, S. (2022). A pathway to improve seaweed aquaculture through microbiota manipulation. *Trends in Biotechnology*. https://doi.org/10.1016/J.TIBTECH.2022.08.003

Oca, J., Cremades, J., Jiménez, P., Pintado, J., & Masaló, I. (2019). Culture of the seaweed *Ulva* ohnoi integrated in a Solea senegalensis recirculating system: influence of light and biomass stocking density on macroalgae productivity. *Journal of Applied Phycology*, *31*(4), 2461–2467. https://doi.org/10.1007/S10811-019-01767-Z/FIGURES/5

Paix, B., Carriot, N., Barry-Martinet, R., Greff, S., Misson, B., Briand, J. F., & Culioli, G. (2020). A Multi-Omics Analysis Suggests Links Between the Differentiated Surface Metabolome and Epiphytic Microbiota Along the Thallus of a Mediterranean Seaweed Holobiont. *Frontiers in Microbiology*, *11*, 494. https://doi.org/10.3389/FMICB.2020.00494/BIBTEX

Paix, B., Potin, P., Schires, G., Le Poupon, C., Misson, B., Leblanc, C., Culioli, G., & Briand, J. F. (2021). Synergistic effects of temperature and light affect the relationship between Taonia atomaria and its epibacterial community: a controlled conditions study. *Environmental Microbiology*, *23*(11), 6777–6797. https://doi.org/10.1111/1462-2920.15758

Pintado, J., Del Olmo, G., Guinebert, T., Ruiz, P., Nappi, J., Thomas, T., Egan, S., Masaló, I., & Cremades, J. (2023). Manipulating the *Ulva* holobiont: Co-culturing *Ulva* ohnoi with Phaeobacter bacteria as a strategy for disease control in fish-macroalgae IMTA-RAS aquaculture. *Journal of Applied Phycology*, *1*, 1–13. https://doi.org/10.1007/S10811-023-02986-1/FIGURES/6

Planas, M., Pérez-Lorenzo, M., Hjelm, M., Gram, L., Uglenes Fiksdal, I., Bergh, Ø., & Pintado, J. (2006). Probiotic effect in vivo of Roseobacter strain 27-4 against Vibrio (Listonella) anguillarum infections in turbot (Scophthalmus maximus L.) larvae. *Aquaculture*, *255*(1–4), 323–333. https://doi.org/10.1016/j.aquaculture.2005.11.039

Prol-García, M. J., Gómez, M., Sánchez, L., & Pintado, J. (2014). Phaeobacter grown in biofilters: a new strategy for the control of Vibrionaceae in aquaculture. *Aquaculture Research*, *45*(6), 1012–1025. https://doi.org/10.1111/ARE.12046

Prol-García, M. J., & Pintado, J. (2013). Effectiveness of Probiotic Phaeobacter Bacteria Grown in Biofilters Against Vibrio anguillarum Infections in the Rearing of Turbot (Psetta maxima) Larvae. *Marine Biotechnology*, *15*(6), 726–738. https://doi.org/10.1007/s10126-013-9521-4

USE OF SEAWEED BIOMASS IN FOOD PRODUCTS BASED ON TECHNO-FUNCTIONAL, NUTRITIONAL AND SENSORIAL PROPERTIES

Lomme J. Deleu*[1,2,3], Ilse Fraeye[1], Yves De Bleecker[2], Imogen Foubert[3]

[1]KU Leuven Ghent, Meat Technology & Science of Protein-rich Foods (MTSP), Gebroeders Desmetstraat 1, B-9000 Gent, Belgium

[2]VIVES University of Applied Sciences, Centre of Expertise Agro- and Biotechnology, Wilgenstraat 32, B-8800 Roeselare, Belgium

[3]KU Leuven Kulak, Food & Lipids, E. Sabbelaan 53, B-8500 Kortrijk, Belgium

*Lomme.Deleu@kuleuven.be

Seaweed has interesting nutritional and techno-functional properties for the use in food, which are currently undervalued. An intelligent use of seaweed should be targeted: making product-dependent, well-informed choices for specific seaweed species based on their techno-functional, nutritional and sensorial properties. The research groups from KU Leuven and VIVES have a strong track record on the use of innovative raw materials like microalgae, insects, duckweed and brewer's spent grain in food products, with focus on techno-functional, nutritional and sensorial properties related to proteins, lipids and other constituents.
We combine expertise from three research groups:

I. Meat Technology and Science of Protein-rich Foods (MTSP) from KU Leuven: with knowledge and expertise on meat products and its vegetarian or vegan alternatives, covering ingredients quality, additive functionality and processing in relation to end product quality;
II. Centre of Expertise Agro- and Biotechnology from VIVES: with knowledge and expertise on development and industrial implementation of new food products and their processes;
III. Food & Lipids from KU Leuven: with knowledge and expertise on the nutritional and stability properties of lipids, antioxidants and color components relaying on 15 years of research on microalgae for food applications.

The collaboration between the research groups allows to cover the whole innovation chain, translating fundamental insights into applied research and covering a broad TRL scale to target optimal and diverse valorization trajectories. Exploiting and combining the strengths of the research groups allows intelligent use of full biomass of raw materials in food products. The expertise on proteins of MTSP is combined with that of lipids and antioxidants of F&L and merged with the recipe development expertise of VIVES. This is supported by the analytical infrastructure of KU Leuven and VIVES allowing a complete characterization of composition, quality and stability of seaweed and food products. Pilot equipment for preparing protein-rich food products (such as vegan meat analogs) is available at MTSP. VIVES has full access to industrial kitchen pilot infrastructure in which real conditions of cooking, packaging, cooling and regeneration in (semi) industrial environments are simulated, and to microwave technology which can be combined with vacuum for drying at low temperatures. Taste labs to perform sensorial analysis with both trained or consumer panels are used to evaluate the food products.
We are looking for partnerships aiming at an increased use of seaweed in food products. We want to achieve this by using seaweed not only for its nutritional and sensorial quality, but also for its techno-functional contributions to food products, e.g. water binding, emulsification, gelation. By focussing on using the full biomass rather than extracted components, we want to valorize the full potential of the seaweed while limiting side streams. This way, we can lower or even exclude the need for additives while maintaining the quality of the food product.