Red Alga Dixoniella grisea

Subjects: Plant Sciences | Biochemical Research Methods

Contributor: Imke Lang

There is an increasing interest in algae-based raw materials for medical, cosmetic or nutraceutical applications. Additionally, the high diversity of physicochemical properties of the different algal metabolites proposes these substances from microalgae as possible additives in the chemical industry. Among the wide range of natural products from red microalgae, research has mainly focused on extracellular polymers for additive use, while this study also considers the cellular components.

extracellular polymeric substances | Dixoniella grisea | bio-additive | polysaccharides | proteins | fatty acids | red algae | culture conditions | ecotoxicological effect

1. Introduction

As blue biotechnology has become an emerging field globally, marine resources such as algae are now being targeted for biotechnological applications [1][2]. The need to identify algae-derived molecules is evident given their bioactive potential as, for example, proven anti-inflammatory, antioxidant or antimicrobial effects [3]. With an increasing global market of 7.4% per year, microalgae products reached a market of 1 billion euros during the period of 2016–2018. Moreover, the projected growth of up to 80% by 2024 is sufficient reason to continue the exploration of microalgae as a sustainable and bio-based source for a rich plethora of effective molecules [4].

Besides the already established production of microalgae for food, feed and cosmetic industries, microalgae display alternatives to replace fossil fuel-derived chemicals, for example as surfactants, emulsifiers or lubricants [5][6][7].

Lubricants are a mixture of an oily or watery base liquid and additives. The most important property of lubricants is viscosity, which determines the thickness of the lubricating film and thus the performance of the lubricant [8]. Moreover, viscosity changes as a function of temperature, pressure and shear rate. The lubricant's additives can be active in the lubricant itself, i.e., improving dispersion and viscosity and functioning as an antioxidant. They can also be surface active as anticorrosive, anti-wear or extreme pressure additives [8]. Current bio-based target molecules as lubricants are polymers such as polysaccharides (PS) and proteoglycans, which are regarded as biodegradable and generally as non-toxic. At present, the food and cosmetic industry widely use polymers as thickeners, stabilizers and hydrogels [9][10][11][12][13]. More recently, biogenic polymers have also received attention as potential lubricants in oil recovery and drilling processes as well as tribological applications [14][15][16][17][18][19].

The success of lubricity seemed to depend directly on the adsorption of the polymers to metal surfaces and was interlinked with the molecular weight, the molecule's structure and its functional groups. Particularly, sulfur groups are identified to mediate anti-wear protection and enhance lubricity [8][20].

Many microalgae and cyanobacteria, in particular those living in the benthic zone, excrete large amounts of polymeric mucilage which cover their cells. In liquid culture, a minor fraction of the excreted polymers dissolve into the surrounding medium, whereas the majority of polymers remain attached to the cell. Presumably, these polymers render osmo-protection and protect cells from predators [21][22] and viral infections [23].

A biotechnologically important source of algae-based PS are species of cyanobacteria, diatoms and green and red microalgae. Exopolysaccharides of the cyanobacterium *Cyanothece epiphytica* showed excellent potential as a biolubricant [14]. This was related to the similarity of the measured visco-elastic properties of the EPS to conventional grease, showing a high storage modulus compared to the loss modulus (G' >> G"). These properties are supposed to stabilize the lubricant film thickness when high pressures occur, e.g., in rolling bearings of a high load [14]. High viscosities are considered favorable for lubrication as viscosity controls the lubrication film thickness [24]. Arad et al. [25] state that at high pressures, high loads and low sliding velocities, the main friction mechanism is boundary lubrication. They also stressed that the adhesion of red microalgae polysaccharides, that was related to also present glycoproteins, was an important advantageous influence on the lubrication compared to the properties of hyaluronic acid alone. Interestingly, strong lubricating boundary layers were reported by Lin et al. [26] when using hyaluronic acid together with phosphatidyl choline lipids for tendons. This strong effect was related to the also-present glycoprotein lubricin. Borah et al. [14] also corroborated the versatility of exopolysaccharides, showing their great potential as emulsifiers, flocculants and dispersers. Gasljevic and coauthors [15] evaluated the polysaccharides of several marine microalgae as suitable drag-reducing additives for naval applications. They found that the red microalgae species *Porphyridium cruentum* and *Rhodella maculata*, and the green microalgae species *Schizochlamydella capsulata* and *Chlorella stigmatophora* exhibited the best drag-reducing ability among the strains tested. They also included cellular polysaccharides into their study, which revealed similar properties than the extracellular polymers (EPS), and when applied together, increased drag-reducing ability by fourfold [15]. The potential of PS from red microalgae in tribological processes was superior to the conventional hyaluronic acid as a lubricant in terms of friction reduction, adsorption and stability [25][27]. Notably, only low polymer concentrations were necessary to result in high viscosity [28].

The EPS of red microalgae comprise mainly sulfated PS, and several species from fresh and brackish water as well as seawater habitats have been studied in detail [29]. The unique properties of sulfated PS initiated many research activities to find out more about their chemical composition, physicochemical properties and biosynthesis [28][30][31][32][33]. In general, sulfated PS are negatively charged, and the prominent monosaccharides were found to be xylose, glucose and galactose, which fall into different ratios, depending on growth conditions [20][34][35][36]. In addition to other sugars in smaller proportions and the sulfate content (1–9% *w*/*w*) already mentioned, proteins covalently and non-covalently bound to the PS represent an important component [37][38][39][40]. The protein moiety in microalgal EPS can either have a structural function or act as extracellular enzymes that are involved in the

degradation of polymers [21]. However, functional data of proteins within microalgal extracellular polymers are scarce.

With regard to tribological processes, proteins function well as bio-additives. In the food industry, they are not only applied as nutritional additives, but in particular as techno-functional additives, where they fulfil roles as solvents, emulsifiers and thickeners, among other things [41]. Amino acid side chains, particularly sulfur groups, result in differences in various chemical properties such as polarity or reactivity and improve, mixed into a lubricant, the interaction with the surface of the friction partners in the contact zone [42]. Current funded research initiatives are evaluating crop-plant-derived proteins as bio-additive in lubricants. However, even though it is a renewable resource, the sustainability of using lubricant additives derived from crops is questionable in the light of circular economies. Therefore, microorganisms, and especially microalgae are favored. Recently, a study on the use of amphiphilic fungal hydrophobins as aqueous lubricant additives showed effective reduction in friction forces [43]. However, the use of microalgae proteins as lubricant additives represents a widely unexplored field with high potential.

Beside molecules that render lubricity, dispersion and viscosity index improvement, other synthesized and often environmentally toxic substances are used as additives in tribological processes [8]. To name a few of them, biocides, anti-corrosives, antioxidants and extreme pressure additives are commonly supplemented to improve the lubricant performance and extend equipment lifetime. Structural similarities of these conventional additives are found in fatty acids (FA), pigments and amino acid derivatives, among other things. These compounds are synthesized de novo by microalgae, which advances them a suitable resource for the exploitation of bio-additives to replace mineral-oil based additives.

With regard to the use of microalgae fatty acids (FAs) as lubricant additives, only little is known. However, the use of algae oil as lubricant has been described in a recently filed patent [6]. Thus, it can be assumed that FAs synthesized by algae are functionally similar to the FAs already used from vegetable oil [44]. Both water-soluble and lipophilic pigments from microalgae can act as antioxidant and anti-corrosive agents, and are widely applied in different industries, especially in the food, cosmetic and health sectors [45][46]. Plant-based carotenoids and chlorophyll have successfully been tested as octane-boosting additives in gasoline, demonstrating the possibility of using plant-based pigments as additives in chemical industry processes [47]. Nonetheless, little information is available on the use of microalgal pigments as lubricant additives.

Irrespective of the substance group, the replacement of conventional lubricant additives by algal-based ones is also related to the possibility to replace toxic or non-degradable substances by non-toxic degradable ones. In general, red algae are not reported to produce toxins [48], and *D. grisea* EPS was reported to promote cell growth, and possess antimicrobial, antiviral and antioxidant activity [49][50][51][52]. Even though this represents potentially interesting properties for additives, it also indicates a high reactivity, which in turn could induce unwanted side effects when released in the environment.

2. Discussions on Red Alga Dixoniella grisea

2.1. Challenges for Large-Scale Production of D. grisea

To address the need for low salt load for the analytical studies (preventing any damage to GC-MS and other instruments), we aimed at reducing the salinity of the culture medium. The genus *Dixoniella* appears euryhaline, as species distribution is described from freshwater to seawater habitats [53][54][55][56]. In the case of strain UTEX 2320, the best growth was obtained with a salinity of 8.25 psu and no growth was observed at salinities higher than 16.5 psu, approving its brackish water origin. Additionally, freshwater cultivation was not possible with that particular strain.

As previously described, *D. grisea* UTEX 2320 was found to be a microalga with a high potency to produce very viscous cultures [20][37][53]. This kind of behavior is common in high molecular weight polymers and physical gels and other structured interconnected systems [20]. The viscosities were a little lower but in the same order of magnitude, as reported by Liberman and coworkers [20]. This deviation may be a result of our more direct 2-propanol extraction method to produce the tested EPS-P.

The growth behavior of this red microalga challenges the culturing and handling, as well as the down-streaming process for the extraction of valuable compounds. To obtain purified polysaccharides, different membrane filtration techniques have been proposed, but this separation process poses some challenges, such as biofouling and the loss of valuable components in the permeate [57]. Additionally, the centrifugation procedure used in other studies [20] was not always successful in our case. Consequently, alternative separation methods will also be required. The separation into three fractions after direct extraction with 2-propanol may also be used for production on a larger scale. Still, it represents an additional working step and as discussed below, the separation procedure may affect the composition of the obtained fraction itself.

Even though overall biomass accumulation in our air-sparged cultures was comparably low, the degree of viscosity affected the monitoring of biomass dry weight and cell numbers. Furthermore, the aeration flow rate and bubble size impacted the handling of the cultures: *D. grisea* UTEX 2320 formed superficial compact foam with increased flow rates (>250 mL min^{-1}) in our system. Thus, for initial characterization of the molecular composition, we focused on a stable and constant biomass and EPS production rather than on high biomass productivity, keeping in mind that the molecular ratio might change with improved growth conditions as previously described [35][58].

Enhanced productivity of *D. grisea* was already achieved by using a sleeve-type reactor system [59], but the biomass obtained from these cultivations was not used for the presented data here. Nevertheless, the production enhancement of the overall biomass productivity of *Dixoniella* is mandatory in order to achieve our goals for the production of multiple products as bio-additives for tribological processes.

Considering the overall challenges for large-scale microalgae production, such as production costs and capacity, a high overall productivity is required to be competitive with other bioproducts [60]. Current photobioreactor systems in use might not be suitable for large scale production of highly viscous cultures, as the mass transfer capacity is

significantly lowered. Alternatively, a continuous harvest or a biofilm reactor might be more applicable. This would also provide solutions to the still very high costs of microalgae harvesting and product extraction [32][61][62][63].

2.2. Molecular Composition of Dixoniella

To evaluate whether *D. grisea* is a suitable microalga to gain bio-additives for tribological processes, the molecular culture composition was examined. This was done in several independent cultivations and by varying culture parameters, such as temperature, time of harvest (culture age) and addition of a supplementary carbon source (sodium bicarbonate). The impossibility to centrifuge large volumes of *D. grisea* due to its viscosity, resulted in two different sampling procedures that were analyzed for their molecular composition: small volumes (up to 1 mL of sample) were centrifuged (resulting in cells-C and medium-C), and big volumes were extracted by 2-propanol and split into three different fractions EPS-P, cells-P and medium-P.

Looking into the detailed analysis of the different compound groups that was used for the larger volumes (EPS-P, cells-P, medium-P), the initial experiments were dedicated to finding a suitable analytical method that enables the identification of a broad variety of molecules. The sample preparation using the corresponding FAMEs [64][65][66][67] was successful for all kind of our alga fractions (data not shown). The faster silylation to produce trimethylsilyl (TMS)-esters gave stable results and was well suited for sugar analysis. As sugars have been frequently considered the most important fraction in EPS [68][69][70], we used this method for the detailed analysis of all algal culture fractions. GC-MS after derivatization has also been used by other researchers to identify the monosaccharides in EPS [20]. In our case, it also worked well for pellet and media samples.

In general, the data obtained for medium-C revealed a high content of released polysaccharides and proteins that were secreted to the medium in all cultures that were analyzed. The same was observed for medium-P in the GC-MS analysis, while EPS-P samples showed a higher proportion of lipids. These differences suggest that the separation process may have affected the distribution of the different substance groups into the different fractions, in this case, medium-C vs. medium- and EPS-P. However, it is also possible that the glycerol also included in the lipid content came from different sources: it can result from bacterial degradation of sugars, as well as from lipid metabolism [71]. To differentiate these two sources, additional measurements and experiments would be required. Still, an effect of the separation process on EPS composition would allow modifications of the gained product in accordance with the needs of the targeted application. In this case, it would represent an additional advantage for the commercial use of *D. grisea*.

The secretion of released polysaccharides (and other metabolites) and its accumulation in the surrounding medium was concomitant with increasing cell density in the growth experiments. This is a commonly observed phenomenon with polymer-secreting microalgae and cyanobacteria, and is well-studied [33][69]. Especially in this late growth phase, carbon flux is directed to the EPS rather than to other cellular processes. We found extracellular proteins associated with released polysaccharides with a relative percentage of 22–30% of the total medium-C secreted. The ratio changes of released polysaccharides and proteins in the extracellular matrix can be explained by differences in cultivation conditions. As previously described by Ivanova and coworkers, released polysaccharide content seems to be strongly correlated with an increase in temperature [72]. In another study, Soanen and

coworkers showed that temperature affected the production of released polysaccharides in the red microalga *P. marinum* [73]. They demonstrated that already a small rise in temperature by 4 °C increased the production of released polysaccharides by almost 2-fold, and with culture media modifications up to 4-fold.

Analyzing the cellular *D. grisea* fraction, we found high amounts of PS and proteins, whereas lipids were less prominent in cells-C samples. When cells are centrifuged after the precipitation step (cells-P), they contain about one third of sugar, proteins, and lipids, each. Taking also the faster separation process of centrifugation and the shorter time period until harvesting for younger, so less viscous, cultures into account, these two major fractions, PS and proteins, should be the focus for developing a biorefinery process, whereas the lipophilic fraction might be a valuable side product.

Even though the relative proportion of sugars varied with the culture component analyzed, the dominant monosaccharides remained galactose and glucose for EPS-P, cells-P and media-P. The highest diversity of monosaccharides was observed in cellular samples while media samples were composed of glucose and galactose and their related oximes only. Aside from monosaccharides, glucose and galactose and their oximes, traces of arabinose, xylose and mannose were also detected. Additionally, methylated sugars, sugar alcohols and sugar acids were found to a smaller extend.

In addition, after static dialysis, most of the water-soluble components could not be detected in the remaining sample, while this was possible for extracted EPS in other studies [74]. As the dialysis was run with the media fraction after EPS extraction by precipitation with 2-propanol, it is possible that the larger polysaccharides were included in the precipitate and the remaining ones were too small to be retained. According to Patel et al. [74] ultrafiltration/diafiltration through a 10 kDa NMWCO membrane at constant pressure of two bars increased purity of extracted EPS in terms of total sugar content, so it is possible that smaller saccharides or attached proteins were removed by dialysis in their experiments as well.

Unlike Libermann and coworkers [20], who identified xylose and rhamnose as major components and did not report either glucose or galactose to be present in their samples, we did not find these sugars in large proportions in *D. grisea* EPS. As the same strain (UTEX 2320) was used in both studies, this difference is either caused by a difference in culture media and conditions or in the EPS extraction method. As a brackish water culture medium was used in both studies, aeration mode or temperature regime remain influencing factors being responsible for the difference in EPS composition. In our case, variations of light intensity and carbon supply (batch 5 and 6, respectively) did not alter EPS composition. As reported elsewhere [75], alterations of temperature, irradiance, pH, nitrogen or phosphate content of the media could change the amount of EPS produced by the red algae *Rhodella violacea*, but had no effect on EPS composition. Thus, it is not very likely that culture medium or culture conditions were the cause for the different EPS composition in *D. grisea* observed in the two studies. Thus, it is more likely that the method used for EPS extraction caused the difference in sugar composition observed here. A similar effect has been observed for the extraction of fucoidan [13]. Libermann and coworkers [20] used a protocol to separate EPS that focuses on a physical separation of EPS and cells by centrifugation [76]. Centrifugation of cultures to separate cells from EPS was not possible in the present study due to the high viscosity of the harvested medium.

The use of 2-propanol and cooling to induce precipitation of the extracellular polymers is thus very likely to have altered the measured polymer composition. This is further supported by the observed differences in molecular composition of medium-C and EPS- medium-P discussed above. For the monosaccharides in fucoidan, an effect of the extraction procedure on the detected monosaccharide composition was reported as well [13]. A more precise description of the extraction procedure is thus important for future comparisons in EPS composition.

As mentioned previously, the protein content in EPS-P was slightly lower compared to the cells and clearly lower compared to the medium-P. Interestingly, its composition was also different from the cells, indicating that proteins present in the EPS-P expectedly differ from those in the cells (cells-P).

In summary, the analytical results indicate that (a) EPS and biomass differ significantly in their molecular composition and may thus be used for different purposes, (b) PS and proteins are the main components in all fractions and might represent the main raw materials for bio-additives and (c) further work on the extraction process is needed to develop a simple, but more uniform method that is able to extract target substances while removing the salt from the culture media from the product.

2.3. Ecotoxicological Impact of D. grisea Fractions

The replacement of conventional and often toxic lubricant additives by algae-based additives requires the ecotoxicological reviews according to the REACh recommendations. Therefore, we conducted standardized tests with all algae fractions using the waterflea *D. magna*, the potworm *E. crypticus*, the springtail *F. candida*, and the soil bacterium *A. globiformis*, respectively.

Overall, the two soil organisms, *F. candida* and *E. crypticus* were more sensitive towards the lyophilized algal fractions compared to the aquatic test organism and the bacteria. In addition, enchytraeids were most affected by EPS, while Collembola were more frequently affected by algal fractions containing algae cells or parts of them.

The toxicity to *F. candida* varied strongly between the different batches, but was mostly zero when test medium was used as solvent. This suggests a lipophilic substance of the protocol being responsible, as these would only be present in the case of DMSO-solved samples. However, we were not able to identify a single lipophilic substance present in most culture samples that was not found in EPS samples. Still, two fatty acids were found most frequently: palmitic and stearic acid. Unsaturated fatty acids are especially known to act toxic to various aquatic organisms [77]. Additionally, saturated fatty acids can be toxic, even though most studies show a lower toxicity compared to their unsaturated counterparts [78][79]. Ikawa et al. [77] suggest interruption of cell membrane functioning and/or metabolic malfunction as potential reasons for fatty acid toxicity. It is possible that palmitic and stearic acid were able to interrupt the integrity of *F. candida* cuticles and thus caused immobilization when *F. candida* were exposed to cellular-based samples solved in DMSO. On the other hand, DSMO itself could be responsible for the toxicity by enabling hydrophilic substances to enter the cells of *F. candida.* For silver nitrate, a detergent mixture of Tween 20 and TAGAT®TO enhanced the silver toxicity to *F. candida* [80]. In this case, a hydrophilic substance which is present in cellular based samples acts toxic to *F. candida* once it enters its tissues. A direct toxicity of DMSO as also described for the detergent mixture [80] can be excluded in the present study, as

only tests with valid solvent control, i.e., no additional mortality, were considered for this article. Still, also two EPS samples were toxic to *F. candida*. Therefore, it remains possible that the same substance or substance group is responsible in all cases of *F. candida* toxicity.

Toxic effects to other organisms have only been reported for EPS, not for algal cell samples: for example, microbial pathogens were affected by *D. grisea* EPS, but not by cellular components [50]. However, not all pathogens were affected by *D. grisea* and the effect differed with species. This is also similar to the differences observed in this study: EPS is most toxic to *E. crypticus*, partly toxic to *F. candida*, and not toxic to *D. magna* or *A. globiformis*. Additionally, *Porphyridium* sp. polysaccharide showed moderate cell growth inhibition at a concentration of 1 g L^{-1} [51]. In addition, the red macroalgae *Champia parvula* was able to inhibit the growth of larvae of the dengue mosquito vector *Aedes aegypti* [81]. However, none of these studies linked the observed effects to specific EPS components or some related effect such as altered pH, salinity changes, osmolarity problems or similar. We could rule out effects by remaining bacteria, salinity or pH by additional controls or control experiments (data not shown). Consequently, we expect one component of the EPS or its combination to be responsible for the negative effects observed here.

Overall, lyophilized algal fractions obtained from *D. grisea* were not toxic, if EPS were not included and the sample did not contain DMSO. When EPS is used as an additive for lubricants, further investigation on the toxicity to enchytraeids and other soil organisms is required. This information is especially important, as REACh recommends tests with soil organisms only for high amounts and substances of high lipophilicity [82]. This study does not only contribute to the ecotoxicological evaluation of microalgae-based products, it also illustrates the importance of soil organisms for this evaluation.

2.4. Evaluation of Dixoniella for a Biorefinery Approach

Prior work has documented the success of using of bio-based additives for tribological processes, mainly in food and cosmetic industry. Proteins, for example, are techno-functional components in many food products by enhancing dispersion and functioning as emulsifier [21][41]. Additionally, for polysaccharides, in particular those with functional groups, and lipids, the potential as lubricant and additive is evident [14][15][25][44][57]. However, the majority of these studies have either been focused on other organisms, such as bacteria or macroalgae, or have only focused on secreted polymers. In the study by Gasljevic and co-authors, the advantage of combined use of cellular and extracellular polysaccharides to increase the effectiveness as additive has already been shown [15].

In this study, we aimed at expanding the potential product range of *D. grisea* UTEX 2320 by following the biorefinery concept. With this concept, the initial algae biomass is fractionated into multiple intermediates, such as proteins, sugars and lipids, and then further converted to the targeted products [19]. The need for such approaches is obvious in the light of a circular economy and economical challenges faced by algae biotechnology.

The richness of *Dixoniella* in EPS is well documented [20][36]; however, the potential of its biomass for the co-production of multiple products for a biorefinery approach has, to our knowledge, not yet been implemented. Our results show that *Dixoniella* represents a valuable bioresource for multiple products potentially useful as additives

for lubricants: the EPS and cellular fraction, both rich in sugars and proteins, but different in their molecular composition of monosaccharides and amino acids. As a valuable side-product, we identified the lipid fraction, which harbors fatty acids and carotenoids. The data also show high variations between the ratios of the individual components and a clear dependency between the obtained product and the extraction technique used, which makes a standardized production process necessary. These findings are in accordance with several other studies, confirming that changes in culture conditions can influence the release and molecular composition of microalgae metabolites [83][84][85]. It is a common practice to induce the biosynthesis of target metabolites, such as pigments, EPS and lipids by changes in nutrient, salinity and light availability [22][63][68][86][87][88]. However, the production of multiple products would require further fine-tuning in order to meet the requirements of a multiple-product approach. Recent studies that focused on a biorefinery approach with different microalgae highlighted the interplay of nutrients, light and temperature to yield lipids, pigments and polysaccharides in a biorefinery concept [83][85][89]. The application of this process to *D. grisea* could be suitable for the harvesting of these promising metabolites to use as lubricants additives.

Although our hypothesis on finding multiple products as bio-additives in *D. grisea* is supported, further research is needed to generate a cost-effective process. Future work should therefore include follow-up cultivations to evaluate optimal culture condition for maximum multiple-product yield, which includes the examination of interactive effects of cultivation conditions on the synthesis of commercially relevant molecules. Furthermore, follow-up experiments are necessary to test the different culture fractions with regard to their physico-chemical properties in tribological processes.

References

1. de Vera, C.R.; Crespín, G.D.; Daranas, A.H.; Looga, S.M.; Lillsunde, K.E.; Tammela, P.; Perälä, M.; Hongisto, V.; Virtanen, J.; Rischer, H.; et al. Marine Microalgae: Promising source for new bioactive compounds. Mar. Drugs 2018, 16, 317.
2. Aslam, A.; Fazal, T.; Zaman, Q.; uz Shan, A.; Rehman, F.; Iqbal, J.; Rashid, N.; Ur Rehman, M.S. Biorefinery of Microalgae for Nonfuel Products. Microalgae Cultiv. Biofuels Prod. 2020, 197–209.
3. Chu, W.L.; Phang, S.M. Bioactive Compounds from Microalgae and Their Potential Applications as Pharmaceuticals and Nutraceuticals. In Grand Challenges in Algae Biotechnology; Hallmann, A., Rampelotto, P.H., Eds.; Springer Nature: Cham, Switzerland, 2019; pp. 429–469.
4. European Union. Blue Bioeconomy Last Update: 2018WWW.EUMOFA.EU Situation Report and Perspectives; European Union: Luxembourg, 2018; ISBN 978-92-79-96713-9.
5. Quinchia, L.A.; Delgado, M.A.; Valencia, C.; Franco, J.M.; Gallegos, C. Natural and synthetic antioxidant additives for improving the performance of new biolubricant formulations. J. Agric. Food Chem. 2011, 59, 12917–12924.

6. Vijayendran, B.; Randall, M.; Schmid, E. Algal Oil Based Bio-Lubricants. U.S. Patent 9.458.407 B2, 4 October 2016.
7. Koch, T.; Gläbe, R.; Sakka, Y.; Nentwig, N.; Filser, J.; Siol, A.; Köser, J.; Thöming, J.; Mesing, S.; Larek, R.; et al. Alternative additives for lubricants based on microalgae. Tribol. Schmier. 2020, 67, 41–46.
8. Ahmed, S.N.; Nassar, A.M. Lubricating Oil Additives. In Tribology-Lubricants and Lubrication; IntechOpen: London, UK, 2011; pp. 249–268.
9. Barcelos, M.C.S.; Vespermann, K.A.C.; Pelissari, F.M.; Molina, G. Current status of biotechnological production and applications of microbial exopolysaccharides. Crit. Rev. Food Sci. Nutr. 2020, 60, 1475–1495.
10. Rhein-Knudsen, N.; Ale, M.T.; Meyer, A.S. Seaweed Hydrocolloid Production: An Update on Enzyme Assisted Extraction and Modification Technologies. Mar. Drugs 2015, 13, 3340–3359.
11. Viana, A.G.; Noseda, M.D.; Duarte, M.E.R.; Cerezo, A.S. Alkali modification of carrageenans. Part V. The iota–nu hybrid carrageenan from Eucheuma denticulatum and its cyclization. Carbohydr. Polym. 2004, 58, 455–460.
12. Cicinskas, E.; Kalitnik, A.A.; Karetin, Y.A.; Saravana, M.; Mohan, G.; Achary, A.; Kravchenko, A.O. Immunomodulating Properties of Carrageenan from Tichocarpus crinitus. Inflammation 2020, 43, 1387–1396.
13. Ale, M.T.; Mikkelsen, J.D.; Meyer, A.S. Important determinants for fucoidan bioactivity: A critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. Mar. Drugs 2011, 9, 2106–2130.
14. Borah, D.; Nainamalai, S.; Gopalakrishnan, S.; Rout, J.; Alharbi, N.S.; Alharbi, S.A.; Nooruddin, T. Biolubricant potential of exopolysaccharides from the cyanobacterium Cyanothece epiphytica. Appl. Microbiol. Biotechnol. 2018, 102, 3635–3647.
15. Gasljevic, K.; Hall, K.; Chapman, D.; Matthys, E.F. Drag-reducing polysaccharides from marine microalgae: Species productivity and drag reduction effectiveness. J. Appl. Phycol. 2008, 20, 299–310.
16. Spinelli, L.S.; Aquino, A.S.; Lucas, E.; d'Almeida, A.R.; Leal, R.; Martins, A.L. Adsorption of Polymers Used in Drilling Fluids on the Inner Surfaces of Carbon Steel Pipes Luciana. Polym. Eng. Sci. 2008, 1886–1891.
17. Wijffels, R.H.; Barbosa, M.J.; Eppink, M.H.M. Microalgae for the production of bulk chemicals and biofuels. Biofuels Bioprod. Biorefining 2010, 4, 287–295.
18. Lee, J.; Yoo, C.; Jun, S.; Ahn, C.; Oh, H. Bioresource Technology Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 2010, 101, S75–S77.

19. Bastiaens, L.; Van Roy, S.; Thomassen, G.; Elst, K. Biorefinery of algae: Technical and economic considerations. In Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products; Gonzalez-Fernandez, C., Munoz, R., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 327–345. ISBN 9780081010273.
20. Netanel Liberman, G.; Ochbaum, G.; Mejubovsky-Mikhelis, M.; Bitton, R.; Arad, S.M. Physico-chemical characteristics of the sulfated polysaccharides of the red microalgae Dixoniella grisea and Porphyridium aerugineum. Int. J. Biol. Macromol. 2020, 145, 1171–1179.
21. Xiao, R.; Zheng, Y. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol. Adv. 2016, 34, 1225–1244.
22. Kumar, D.; Kaštánek, P.; Adhikary, S.P. Exopolysaccharides from cyanobacteria and microalgae and their commercial application. Curr. Sci. 2018, 115, 234–241.
23. Keeling, P.J. Diversity and evolutionary history of plastids and their hosts. Am. J. Bot. 2004, 91, 1481–1493.
24. Paul, A.K.; Borugadda, V.B.; Goud, V.V. In-Situ Epoxidation of Waste Cooking Oil and Its Methyl Esters for Lubricant Applications: Characterization and Rheology. Lubricants 2021, 9, 27.
25. Arad, S.; Rapoport, L.; Moshkovich, A.; Van Moppes, D.; Karpasas, M.; Golan, R.; Golan, Y. Superior biolubricant from a species of red microalga. Langmuir 2006, 22, 7313–7317.
26. Lin, W.; Mashiah, R.; Seror, J.; Kadar, A.; Dolkart, O.; Pritsch, T.; Goldberg, R.; Klein, J. Lipid-hyaluronan synergy strongly reduces intrasynovial tissue boundary friction. Acta Biomater. 2019, 83, 314–321.
27. Eteshola, E.; Karpasas, M.; Arad, S.M.; Gottlieb, M. Red microalga exopolysaccharides: 2. Study of the rheology, morphology and thermal gelation of aqueous preparations. Acta Polym. 1998, 49, 549–556.
28. Singh, A.; Corvelli, M.; Unterman, S.A.; Wepasnick, K.A.; Mcdonnell, P.; Elissee, J.H. Enhanced lubrication on tissue and biomaterial surfaces through peptide-mediated binding of hyaluronic acid. Nat. Mater. 2014, 13, 988–995.
29. Arad, S.M.; van Moppes, D. Novel Sulfated Polysaccharides of Red Microalgae: Basics and Applications. Handb. Microalgal Cult. Appl. Phycol. Biotechnol. 2013, 406–416.
30. Raposo, M.F.D.J.; De Morais, A.M.M.B.; De Morais, R.M.S.C. Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from Porphyridium cruentum. Life Sci. 2014, 101, 56–63.
31. Singh, S.; Kate, B.N.; Banecjee, U.C. Bioactive compounds from cyanobacteria and microalgae: An overview. Crit. Rev. Biotechnol. 2005, 25, 73–95.

32. Singh, G.; Patidar, S.K. Microalgae harvesting techniques: A review. J. Environ. Manag. 2018, 217, 499–508.
33. Arad, S.; Levy-Ontman, O. Red microalgal cell-wall polysaccharides: Biotechnological aspects. Curr. Opin. Biotechnol. 2010, 21, 358–364.
34. Li, S.-Y.; Shabtai, Y.; Arad, S.M. Production and composition of the sulphated cell wall polysaccharide of Porphyridium (Rhodophyta) as affected by CO2 concentration. Phycologia 2000, 39, 332–336.
35. Arad, S.M.; Lerental, Y.B.; Dubinsky, O. Effect of nitrate and sulfate starvation on polysaccharide formation in Rhodella reticulata. Bioresour. Technol. 1992, 42, 141–148.
36. Dubinsky, O.; Simon, B.; Karamanos, Y.; Geresh, S.; Barak, Z.; Arad, S.M. Composition of the cell-wall polysaccharide produced by the unicellular red alga Rhodella reticulata. Plant Physiol. Biochem. 1992, 30, 409–414.
37. Capek, P.; Matulová, M.; Combourieu, B. The extracellular proteoglycan produced by Rhodella grisea. Int. J. Biol. Macromol. 2008, 43, 390–393.
38. Shrestha, R.P.; Weinstein, Y.; Bar-Zvi, D.; Arad, S. A glycoprotein noncovalently associated with cell-wall polysaccharide of the red microalga Porphyridium sp. (Rhodophyta). J. Phycol. 2004, 40, 568–580.
39. Solymosi, K.; Böddi, B. Optical properties of bud scales and protochlorophyll(ide) forms in leaf primordia of closed and opened buds. Tree Physiol. 2006, 26, 1075–1085.
40. Heaney-Kieras, J.; Chapman, D. Structural studies on the extracellular polysaccharide of the red alga Porphyridium cruentum. Carbohydr. Res. 1976, 52, 169–177.
41. Luyten, H.; Vereijken, J.; Buecking, M. Using Proteins as Additives in Foods: An Introduction. In Proteins in Food Processing; Yada, R.Y., Ed.; Woodhead Publishing Limited: Cambridge, UK, 2004; ISBN 9781855737235.
42. Minami, I.; Mori, S.; Isogai, Y.; Hiyoshi, S.; Inayama, T.; Nakayama, S. Molecular Design of Environmentally Adapted Lubricants: Antiwear Additives Derived from Natural Amino Acids. Tribol. Trans. 2010, 53, 713–721.
43. Lee, S.; Røn, T.; Pakkanen, K.I.; Linder, M. Hydrophobins as aqueous lubricant additive for a soft sliding contact. Colloids Surf. B Biointerfaces 2015, 125, 264–269.
44. Karmakar, G.; Ghosh, P. Green additives for lubricating oil. ACS Sustain. Chem. Eng. 2013, 1, 1364–1370.
45. Novoveská, L.; Ross, M.E.; Stanley, M.S.; Pradelles, R.; Wasiolek, V.; Sassi, J.-F. Microalgal Carotenoids: A Review of Production, Current Markets, Regulations, and Future Direction. Mar. Drugs 2019, 17, 640.

46. Saini, D.K.; Pabbi, S.; Shukla, P. Cyanobacterial pigments: Perspectives and biotechnological approaches. Food Chem. Toxicol. 2018, 120, 616–624.
47. Yuliarita, E.; Zulys, A. Utilization of natural compounds (chlorophyll and carotene extracts) as an octane-boosting additive in gasoline. IOP Conf. Ser. Mater. Sci. Eng. 2019, 496, 012048.
48. Carmichael, W.W. Algal toxins. Adv. Bot. Res. 1986, 12, 47–101.
49. Chen, B.; You, W.; Huang, J.; Yu, Y.; Chen, W. Isolation and antioxidant property of the extracellular polysaccharide from Rhodella reticulata. World J. Microbiol. Biotechnol. 2010, 26, 833–840.
50. Najdenski, H.M.; Gigova, L.G.; Iliev, I.I.; Pilarski, P.S.; Lukavský, J.; Tsvetkova, I.V.; Ninova, M.S.; Kussovski, V.K. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. Int. J. Food Sci. Technol. 2013, 48, 1533–1540.
51. Talyshinsky, M.M.; Souprun, Y.Y.; Huleihel, M.M. Anti-viral activity of red microalgal polysaccharides against retroviruses. Cancer Cell Int. 2002, 2, 14–17.
52. Toshkova, R.; Gardeva, E.; Minkova, K.; Panova, T.; Zvetkova, E. Immunopotential and mitogenic properties of marine microalgal polysaccharides extracted from Porphyridium cruentum and Dixoniella grisea (Rhodophyta). Comptes Rendus L'Academie Bulg. Sci. 2009, 62, 589–594.
53. Eggert, A.; Raimund, S.; Michalik, D.; West, J.; Karsten, U. Ecophysiological performance of the primitive red alga Dixoniella grisea (Rhodellophyceae) to irradiance, temperature and salinity stress: Growth responses and the osmotic role of mannitol. Phycologia 2007, 46, 22–28.
54. Geitler, L. Beitrage zur epiphytischen Algenflora des Neusiedler Sees. Osterr. Bot. Ztg. 1970, 118, 17–29.
55. Deason, T.R.; Butler, G.L.; Rhyne, C. Rhodella reticulata sp. nov.: A new coccoid rhodophytan alga (Porphyridiales). J. Phycol. 1983, 19, 104–111.
56. Fresnel, J.; Billard, C.; Hindak, F.; Pekárková, B. New observations on Porphyridium griseum Geitler=Rhodella grisea (Geitler) Comb-Nova (Porphyridiales, Rhodophyceae). Plant Syst. Evol. 1989, 164, 253–262.
57. Bernaerts, T.M.M.; Kyomugasho, C.; Van Looveren, N.; Gheysen, L.; Foubert, I.; Hendrickx, M.E.; Van Loey, A.M. Molecular and rheological characterization of different cell wall fractions of Porphyridium cruentum. Carbohydr. Polym. 2018, 195, 542–550.
58. Li, S.; Ji, L.; Chen, C.; Zhao, S.; Sun, M.; Gao, Z.; Wu, H.; Fan, J. Efficient accumulation of high-value bioactive substances by carbon to nitrogen ratio regulation in marine microalgae Porphyridium purpureum. Bioresour. Technol. 2020, 309, 123362.
59. Cohen, E.; Arad, S.M. A closed system for outdoor cultivation of Porphyridium. Biomass 1989, 18, 59–67.

60. Fernández, F.G.A.; Reis, A.; Wijffels, R.H.; Barbosa, M.; Verdelho, V.; Llamas, B. The role of microalgae in the bioeconomy. New Biotechnol. 2021, 61, 99–107.
61. Christenson, L.; Sims, R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 2011, 29, 686–702.
62. Muylaert, K.; Bastiaens, L.; Vandamme, D.; Gouveia, L. Harvesting of microalgae: Overview of process options and their strengths and drawbacks. In Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products; Gonzalez-Fernandez, C., Munoz, R., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; ISBN 9780081010273.
63. Pierre, G.; Delattre, C.; Dubessay, P.; Jubeau, S.; Vialleix, C.; Cadoret, J.P.; Probert, I.; Michaud, P. What is in store for EPS microalgae in the next decade? Molecules 2019, 24, 4296.
64. Araujo, G.S.; Matos, L.J.B.L.; Fernandes, J.O.; Cartaxo, S.J.M.; Gonçalves, L.R.B.; Fernandes, F.A.N.; Farias, W.R.L. Extraction of lipids from microalgae by ultrasound application: Prospection of the optimal extraction method. Ultrason. Sonochem. 2013, 20, 95–98.
65. Van Wychen, S.; Laurens, L.M.L. Determination of Total Carbohydrates in Algal Biomass-Laboratory Analytical Procedure (LAP); National Renewable Energy Lab(NREL): Golden, CO, USA, 2015.
66. Mathimani, T.; Mallick, N. A comprehensive review on harvesting of microalgae for biodiesel–Key challenges and future directions. Renew. Sustain. Energy Rev. 2018, 91, 1103–1120.
67. Niemi, C.; Lage, S.; Gentili, F.G. Comparisons of analysis of fatty acid methyl ester (FAME) of microalgae by chromatographic techniques. Algal Res. 2019, 39, 101449.
68. Gaignard, C.; Gargouch, N.; Dubessay, P.; Delattre, C.; Pierre, G.; Laroche, C.; Fendri, I.; Abdelkafi, S.; Michaud, P. New horizons in culture and valorization of red microalgae. Biotechnol. Adv. 2019, 37, 193–222.
69. Gaignard, C.; Laroche, C.; Pierre, G.; Dubessay, P.; Delattre, C.; Gardarin, C.; Gourvil, P.; Probert, I.; Dubuffet, A.; Michaud, P. Screening of marine microalgae: Investigation of new exopolysaccharide producers. Algal Res. 2019, 44.
70. Aguilera, A.; Souza-Egipsy, V.; San Martín-Úriz, P.; Amils, R. Extraction of extracellular polymeric substances from extreme acidic microbial biofilms. Appl. Microbiol. Biotechnol. 2008, 78, 1079–1088.
71. Paidi, M.K.; Agarwal, P.; More, P.; Agarwal, P.K. Chemical Derivatization of Metabolite Mass Profiling of the Recretohalophyte Aeluropus lagopoides Revealing Salt Stress Tolerance Mechanism. Mar. Biotechnol. 2017, 19, 207–218.
72. Ivanova, J.G.; Kabaivanova, L.V.; Petkov, G.D. Temperature and Irradiance Effects on Rhodella reticulata Growth and Biochemical Characteristics. Russ. J. Plant Physiol. 2015, 62, 690–695.

73. Soanen, N.; Da Silva, E.; Gardarin, C.; Michaud, P.; Laroche, C. Improvement of exopolysaccharide production by Porphyridium marinum. Bioresour. Technol. 2016, 213, 231–238.

74. Patel, A.K.; Laroche, C.; Marcati, A.; Ursu, A.V.; Jubeau, S.; Marchal, L.; Petit, E.; Djelveh, G.; Michaud, P. Separation and fractionation of exopolysaccharides from Porphyridium cruentum. Bioresour. Technol. 2013, 145, 345–350.

75. Villay, A.; Laroche, C.; Roriz, D.; El Alaoui, H.; Delbac, F.; Michaud, P. Optimisation of culture parameters for exopolysaccharides production by the microalga Rhodella violacea. Bioresour. Technol. 2013, 146, 732–735.

76. Adda, M.; Merchuk, J.C.; Malis, S. Effect of Nitrate on Growth and production of Cell wall polysaccharide by the unicellular red alga Porphyridium. Biomas 1986, 10, 131–140.

77. Ikawa, M. Algal polyunsaturated fatty acids and effects on plankton ecology and other organisms. UNH Cent. Freshwat. Biol. Res 2004, 6, 17–44.

78. McCracken, M.D.; Middaugh, R.E.; Middaugh, R.S. A chemical characterization of an algal inhibitor obtained from chlamydomonas. Hydrobiologia 1980, 70, 271–276.

79. Bosma, R.; Miazek, K.; Willemsen, S.M.; Vermuë, M.H.; Wijffels, R.H. Growth inhibition of Monodus subterraneus by free fatty acids. Biotechnol. Bioeng. 2008, 101, 1108–1114.

80. McKee, M.S.; Köser, J.; Focke, O.; Filser, J. A new test system for unraveling the effects of soil components on the uptake and toxicity of silver nanoparticles (NM-300K) in simulated pore water. Sci. Total Environ. 2019, 673, 613–621.

81. Yogarajalakshmi, P.; Venugopal Poonguzhali, T.; Ganesan, R.; Karthi, S.; Senthil-Nathan, S.; Krutmuang, P.; Radhakrishnan, N.; Mohammad, F.; Kim, T.J.; Vasantha-Srinivasan, P. Toxicological screening of marine red algae Champia parvula (C. Agardh) against the dengue mosquito vector Aedes aegypti (Linn.) and its non-toxicity against three beneficial aquatic predators. Aquat. Toxicol. 2020, 222, 105474.

82. Echa. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint Specific Guidance November 2012; European Chemical Agency: Helsinki, Finland, 2012; Volume 2012, ISBN 9789292447618.

83. Guihéneuf, F.; Stengel, D.B. Towards the biorefinery concept: Interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in Porphyridium purpureum. Algal Res. 2015, 10, 152–163.

84. Silva, M.B.F.; Azero, E.G.; Teixeira, C.M.L.L.; Andrade, C.T. Influence of culture conditions on the production of extracellular polymeric substances (EPS) by Arthrospira platensis. Bioresour. Bioprocess. 2020, 7, 47.

85. Aussant, J.; Guihéneuf, F.; Stengel, D.B. Impact of temperature on fatty acid composition and nutritional value in eight species of microalgae. Appl. Microbiol. Biotechnol. 2018, 102, 5279–5297.

86. Li, S.; Ji, L.; Shi, Q.; Wu, H.; Fan, J. Advances in the production of bioactive substances from marine unicellular microalgae Porphyridium spp. Bioresour. Technol. 2019, 292, 122048.

87. Gujar, A.; Cui, H.; Ji, C.; Kubar, S.M.; Li, R. Optimizing culture system to promote cell growth and optimizing culture system to promote cell growth and polysaccharides contents of Porphyridium cruentum. Fresenius Environ. Bull. 2020, 29, 6738–6747.

88. Liu, L.; Pohnert, G.; Wei, D. Extracellular metabolites from industrial microalgae and their biotechnological potential. Mar. Drugs 2016, 14, 191.

89. Gilbert-lópez, B.; Mendiola, J.A.; Fontecha, J.; van den Broek, L.A.M.; Sijtsma, L.; Cifuentes, A.; Herrero, M.; Ibáñez, E. Downstream processing of Isochrysis galbana: A step towards microalgal biorefinery. Green Chem. 2015, 17, 4599–4609.