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Environmental Impacts of Experimental Production of Lactic Acid for Bioplastics from *Ulva Spp.*

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Abstract: An exploratory Life Cycle Assessment was carried out to provide insight into the environmental impacts of using the green seaweed *Ulva spp.* as a feedstock for production of bioplastic. The study focused on the production of lactic acid as a precursor of polylactic acid. The studied production process consists of (1) the cultivation of *Ulva spp.* in Integrated Multitrophic Aquaculture, (2) the processing of the biomass for solubilisation of sugars, (3) the fermentation of the sugars to lactic acid and (4) isolation of lactic acid from the fermentation broth. The study identifies environmental hotspots and compares the experimental seaweed production chain with conventional feedstock. The main hotspot identified is electricity consumption during seaweed cultivation. This impact of electricity consumption can be lowered by reducing energy use and sourcing renewable energy, and by improving the material efficiency in the product chain. To improve understanding of the environmental impacts of the production process, future studies can broaden the system boundaries and broaden the scope of sustainability issues included in the environmental assessment.

Keywords: bioplastics; seaweed; lactic acid; Life Cycle Assessment; *Ulva spp.*

1. Introduction

Europe produced 58 million of tonnes of plastics in 2015 [1], corresponding to 18% of the global production. The most common polymers used for plastic production are derived from petrochemicals. Concerns regarding depleting fossil fuel resources and recyclability and biodegradability of these conventional plastics have led to considerable effort to produce plastics from renewable biomass.

Terrestrial crops such as corn, wheat, sugar beets and sugar cane are currently the main feedstocks for the production of bioplastics [2-4]. One type of bioplastic is polylactic acid (PLA), a highly versatile, biodegradable, aliphatic polyester derived from renewable resources, such as corn and sugar beets [5, 6].

There is a growing concern that the use of these feedstocks for bioplastics will compete with food, feed or fuel production, which will subsequently increase the raw material costs and negatively impact the environment [7, 8]. To overcome the food, feed and fuel versus bioplastics predicament, novel feedstocks must be sought.

One potential source is seaweed. Seaweeds – also known as macro-algae – are photosynthesis-driven macro-organisms able to convert carbon dioxide (CO₂) into carbohydrates, lipids, proteins, and other cellular components with potential biofuel, foods, feeds, intermediate compounds, and high-value bio-actives applications [9]. The use of seaweed as feedstock for bioplastic applications can have benefits over land-based feedstocks like sugarcane and maize. Seaweed growth rate as well as the productivity per land surface area is higher and their carbohydrate yields per surface area are higher than traditional feedstocks [10]. Particularly green seaweeds have been shown to contain

comparatively high levels of accessible sugars, including starch and cellulose [11]. When grown on land in ponds, seaweeds can be induced to produce more carbohydrates through manipulation of their production factors.

1.1 Objective of the paper

The goal of this study is to estimate the environmental impacts of producing lactic acid and its coproducts from the green seaweed *Ulva spp.*, identifying environmental hotspots and opportunities for improvement. The study also investigates which environmental impacts would be avoided if the coproducts would be used to substitute useful products, which benefits the development of this chain as well.

1.2 System Description

1.2.1 Seaweed cultivation

Ulva spp. was selected as feedstock for production of bioplastic precursors due to its composition, being rich in sugars and proteins [12] and relatively low salt content. Several studies have shown the success of cultivation of *Ulva spp.* under IMTA conditions [13, 14].

Ulva spp. was grown in a land-based IMTA system, where nutrient enriched water (nitrate, CO₂ and ammonia) from the adjacent semi-intensive seabream and seabass farm provided essential nutrients for the cultivation of the seaweed. This allowed for consistent high biomass yields without the use of artificial fertilizers or addition of CO₂ to the water. In order to maximize starch yields the IMTA-conditions were followed by conditions of N-deprivation. During this period, water flows are cut and electricity is only used for bottom-aeration of the seaweed basins. After a pilot trial in 1000L tanks, starch content in *Ulva spp.* biomass of up to 25% of dry weight was achieved in the commercial scale IMTA system of ALGAplus (20.000-liter tanks) under nitrogen-depleted conditions.

1.2.2 Seaweed processing

The produced *Ulva spp.* was hydrolysed in the two-step approach developed in the SEABIOPLAS project. Enzymatic and acidic hydrolysis are common approaches in the treatment of land-based feedstock for starch hydrolysis to produce first generation bioethanol production [15-17]. *Ulva spp.* biomass was subjected to enzymatic hydrolysis with commercially available starch degrading enzymes (spirizyme®, liquozyme®), preventing sugar degradation and inhibitor formation. Subsequently, an acid hydrolysis step (pH 0.88) using sulphuric acid (96%) hydrolysed *Ulvans* and decreased viscosity. The hydrolysate contained glucose (23 g/L), rhamnose (6.4 g/L) and xylose (1.1 g/L), reflecting a yield of 0.75 g fermentable monosaccharides/g starch available. The hydrolysis of *Ulva spp.* yields the hydrolysate and the hydrolysate residue.

The hydrolysate of *Ulva spp.* was fermented by *Lactobacillus plantarum*, obtaining yields of 0.9 g lactic acid/g sugar consumed. The hydrolysate fermented well and did not require the addition of nutrient supplements. Lactic acid produced in the seaweed-derived growth medium was extracted using standard basic precipitation on lab scale with calcium sulphate (gypsum) as a coproduct. The depleted fermentation broth is considered bio-waste and is treated by anaerobic digestion. Anaerobic digestion could be considered a useful application, since it provides energy and digestate, while other treatments might extract higher-value products from the fermentation broth (e.g. fish feed).

2. Materials and Methods

2.1 Goal and Scope

The aim of this paper is to identify the environmental hotspots of the conversion of the seaweed *Ulva spp.* into lactic acid (LA) in the current experimental setup. For this purpose, the Life Cycle Assessment methodology was used with primary data collected in the SEABIOPLAS project. SEABIOPLAS was a European Commission funded research project that aimed to prove the feasibility of producing lactic acid from *Ulva spp.* cultivated in an Integrated Multitrophic Aquaculture (IMTA) systems [18, 19]. The standard four-step Life Cycle Assessment (LCA) approach [20] was followed in this study.

The functional unit was defined as “1 kg of purified lactic acid (LA) and its coproducts, at the bioprocessing laboratory”, abbreviated to “1 kg of LA”. The lactic acid produced is an intermediate product, which can be applied to the production of several functional materials. When polymerized into PLA, it can be used in packaging films.

To investigate the current experimental supply chain, the base case scenario (LA1) models all steps from cultivation of *Ulva spp.* up to lactic acid purification as done in the experimental supply chain. The base case scenario is compared with two other scenarios (see Table 1). The piloted optimizations of *Ulva spp.* cultivation and of lactic acid purification were modelled in scenario LA2. The influence of the feedstock was tested with scenario LA3 by modelling maize grain input in the experimental hydrolysis and fermentation steps. In discussing the results, a comparison between lactic acid from seaweed and lactic acid from land-based feedstocks is made, based on prior studies.

Table 1 Main characteristics of the three scenarios studied with LCA

Scenario:	LA1	LA2	LA3
Species used (and part):	<i>Ulva spp.</i> (whole)	<i>Ulva spp.</i> (whole)	<i>Zea mays</i> (grains)
Biomass production	IMTA (experimental)	IMTA (experimental, optimized for starch enrichment)	Conventional agriculture
Processing	Experimental	Experimental (optimized)	Experimental (theoretical)

2.2 System boundaries

The scope of this assessment was limited to the experimental chain from land-based cultivation to lactic acid isolation, with the aim to investigate the state of the technology utilized within the SEABIOPLAS project. No extrapolations were made assuming higher efficiencies due to larger volumes or technical development. It was assumed that all production steps of the *Ulva spp.* production took place as one uninterrupted chain of unit operations in Ílhavo, Portugal, where the seaweed cultivation took place. In this supply chain set-up, periods that equipment was idle, as well as cooling and transportation of intermediate materials does not occur. The machinery, equipment and other capital goods were excluded from the assessment since the properties and use intensity of the experimental equipment in which the biomass conversions were done could not be estimated. All raw materials and energy that were needed from outside the experimental production chain were included in the study.

PLA film was not chosen as functional unit for two reasons: (1) polymerization to alkyd resins was piloted for coatings applications in the SEABIOPLAS project, hence no primary data is available on PLA, and; (2) secondary industrial data of lactic acid polymerization as a separate step was not available, only in aggregated cradle-to-gate format [3, 4].

Effects of human activities at or near the sea on marine biodiversity and on marine biotic resource availability have been described qualitatively [21], but a directly applicable quantitative method is yet to be developed.

2.3 Multifunctionality

Multifunctionality is the phenomenon that a process operation provides multiple products. It occurs at three places in the system studied: (1) the IMTA system produces fish and *Ulva spp.*, and during biomass processing (2) hydrolysis residue and (3) gypsum are produced in addition.

In the IMTA system, both fish and seaweed is grown in one system with some linked resource flows. For the IMTA system, only the flows that directly go to the seaweed production were included in the LCA (a subdivision approach was taken). Water flows and electricity use for fish basins and nutrients from wasted or digested fish feed were not included, since these inputs and outputs are not influenced by the seaweed production activity.

A system expansion approach was taken for the coproducts from biomass processing, compliant with EC-JRC-IES [22]. The hydrolysis of fresh *Ulva spp.* results in a liquid hydrolysate for fermentation, and a solid nutrient-rich residue. This residue contains all components of the whole

Ulva spp. except for the extracted polysaccharides. In this study, it was assumed that the hydrolysis residue can partially replace protein soy in European seabass feed, based on studies that confirm that *Ulva spp.* proteins can contribute up to 15% of the total protein requirement without compromising growth performance [23]. The amount of replaced soy was determined by using the nitrogen contents of *Ulva spp.*, maize and soy as a proxy for their protein contents.

After the fermentation of the *Ulva spp.* hydrolysate, lactic acid is purified from the solution by precipitation with calcium hydroxide, yielding calcium sulphate (gypsum). The gypsum was assumed to substitute gypsum as a building material on a one-to-one basis.

2.4 Data collection and LCI modelling

Primary data on the resources used in the production process was provided by the project partners involved in the experimental processes (see Table 2). The amounts of electricity required for hydrolysis, fermentation and lactic acid purification were estimated as conservative industrial usages by the researchers of each process and cross-checked with [24].

SimaPro 8.4.0.0 (Pré Consultants, Amersfoort, the Netherlands) was used to derive the life cycle inventory (LCI) and characterize the impacts. Cultivation, hydrolysis, fermentation and purification were modelled as four separate unit processes using the primary data reported by the project partners. So-called system models, which contain partial LCIs for the reference flow they cover, were taken from EcoInvent 3.3 [25] for the used resources, including the global average impact of maize cultivation. The Portuguese electricity production was modelled separately using information from the International Energy Agency [26] and the system models from EcoInvent 3.3 of individual electricity generation technologies, and connected to the four unit processes. The enzyme production impacts were taken from [27]. This procedure results in a supply chain model consisting of several linked processing steps linked with partial LCIs for each supplied resource. The SimaPro software was used to translate this model to one overall LCI for the production of 1 kg of LA and its coproducts, for each scenario.

Table 2 Data sources for each step of the production process.

Description	Data source
Cultivation of <i>Ulva spp.</i>	ALGAplus
Hydrolysis of <i>Ulva spp.</i> , yielding hydrolysate and residue	CEVA
Fermentation of hydrolysate and purification of lactic acid from fermentation broth	Wageningen UR
Chemicals (cultivation operation, salts, acids, bases), maize cultivation	[25]
Electricity	[25-26]
Enzymes for hydrolysis	[27]

2.5 Life Cycle Impact Assessment

To identify hotspots, the impact characterization method ReCiPe 2008 [28] was used as it has weighting and normalization factors that enable comparison of different environmental impacts. The commonly used Hierarchist version was used for midpoint characterization, with European normalization factors and average weighting factors for endpoint characterization.

3. Results

3.1 Inventory of resource use

Table 3 gives an overview of resource use, yields and mass ratios for biomass production, biomass processing and residues in each of the production step, as provided by project partners (for LA1 and LA2) and derived from literature (for LA3). All mass amounts are expressed per 1 kg lactic acid produced. Dry matter and starch content of maize were taken from [4]. Electricity and diesel use for maize cultivation (LA3) are the total consumptions for the life-cycle of dried maize from EcoInvent 3.3 [25], while direct electricity and diesel use are listed for the *Ulva spp.* cultivation (LA1 and LA2). Nitrogen contents of maize and soy were taken from [29].

Table 3 Inventory of resource, energy and biomass consumptions and byproduct productions, relative to 1 kg of LA production for each of the three scenarios (WM = Wet Mass, DM = Dry Mass)

Scenario:		LA1	LA2	LA3
Biomass production	Dry matter content (%WM)	8.5	15	85
	Starch content (%WM)	1.7	6.0	58
	Starch content (%DM)	20	40	68
	Direct land occupation (m ² *yr)	0.07	0.07	0.7
	Electricity use (kWh)	8.2	8.2	1.1
	Diesel use (kWh)	0.012	0.012	0.022
	On-site use of pick-up truck (km)	2.1	0.33	n.a.
Biomass processing	Biomass used (kg WM)	158	25	4.6
	Biomass used (kg DM)	13	3.7	3.9
	Hydrolysis yield (%)	75	75	75
	Fermentation yield (%)	90	90	90
	LA purification yield (%)	56	99	56
	Starch used (kg)	2.0	1.2	2.0
	Direct electricity use (kWh / kg LA)	13.7	13.5	13.7
	Sulphuric acid, concentrated (kg)	3.4	0.80	3.4
	Hydrochloric acid (1 M, kg)	1.7	0.27	1.7
	Calcium carbonate (kg)	57	9.0	57
	Enzyme mix for hydrolysis (kg)	0.38	0.060	0.38
	Monoammonium phosphate (kg)	0.058	0.033	0.058
	Ammonium sulphate (kg)	0.10	0.057	0.10
	Calcium hydroxide (kg)	0.82	0.46	0.82
	Purified water (also waste water, L)	21	18	21
	Biowaste produced (kg)	20	20	20
	N content in biomass (%DM)	1.5	4.9	2.3
Hydrolysis byproduct	N in residue (kg)	0.20	0.19	0.089
	N content in soy (%DM)	7.5	7.5	7.5
	Substituted soy (kg)	2.7	2.4	1.2
Purification byproduct	Gypsum (kg)	0.95	0.95	0.95

3.2 ReCiPe environmental score

The aggregated environmental impact is expressed as the ReCiPe score expressed in mPt (millipoints). The score is a weighted aggregation of 17 environmental impact categories covering the three areas: human health, ecosystems and resource depletion. Human health damage due to climate change contributes 42% to the total impact score of scenario LA1, as shown in Figure 1a, and is caused by the electricity consumption during cultivation and to a lesser extent by direct emissions in the hydrolysis. Other major impacts are exclusively caused by electricity consumption: 32% of the impact score of LA1 is determined by fossil resource depletion, and 14% is due to particulate matter formation. Minor impacts are human toxicity and damage to ecosystems due to climate change (contributing 5% and 4% respectively). The remaining impact ("Rest" in Figure 1a) amounts to 3% and is determined by agricultural land occupation and metal depletion. The hydrolysis residue and gypsum from purification would replace soy in fish feed and conventional gypsum, respectively. The impacts of these replaced products amount to less than 1% of the total impact and are not shown in the results.

Optimisation of *Ulva spp.* cultivation and lactic acid purification (LA2) reduce the electricity demand significantly, as less *Ulva spp.* is needed to yield the same amount of lactic acid. The ReCiPe environmental score then decreases considerably. LA3 shows a lower ReCiPe environmental score, emphasizing the large influence of, currently experimental, *Ulva spp.* cultivation on the total impact.

3.3 Global warming potential

The global warming potential (GWP, Figure 1b) demonstrates that electricity use is dominant in climate change impacts. 99% of the electricity consumption occurs during cultivation, for water pumping (2-3 weeks) and aeration of the tanks (4-5 weeks). A secondary contribution to climate change is due to direct emissions of CO₂ from the use of carbonate as a neutralization base in the hydrolysis of the biomass. Other resources add up to a climate change contribution of 1% in LA1 and were grouped in the category "Rest". The substitution of soy in fish feed by the hydrolysis residue would save 6.7 kg CO₂eq / kg LA, which is not a substantial reduction compared to the total GWP (not shown in the Figure 1b). Substitution of conventional gypsum with gypsum from the LA purification has no significant effect on the GWP. When the *Ulva spp.* cultivation and lactic acid purification is further optimized (LA2), the electricity demand can also be reduced significantly, as less *Ulva spp.* is needed to yield the same amount of lactic acid. The direct emission in the hydrolysis is also reduced because the starch is more concentrated in the *Ulva spp.*, while the same reaction conditions are used. When maize (*Zea mays*) is used as a feedstock instead of *Ulva spp.* (LA3), the GWP is significantly lower.

3.4 Land Occupation

While urban land occupation is limited (6.1 m²a for scenario LA1), the agricultural land occupation is substantial with 206 m²a (Figure 1c) for LA1. This land is largely used for the growing biomass used for electricity generation. The *Ulva spp.* cultivation facility is the second largest contribution to agricultural land occupation, since the *Ulva spp.* is cultivated on land in the current production setup. The land required for *Ulva spp.* cultivation was classified as "agricultural", because of its rural location although the current production site, consisting of abandoned salt pans and aquaculture ponds on land, is more suitable for aquaculture than for agricultural purposes.

Substitution of soy in fish feed by the hydrolysis residue would save land in scenario LA1 (6.3 m²a, Figure 1c) and to a lesser extent in LA2 (5.7 m²a). In scenario LA2, less *Ulva spp.* is hydrolysed for the same amount of sugar for the fermentation, which is partially compensated by a higher protein content in the *Ulva spp.* of LA2. Less land would be saved by using the residue from maize hydrolysis (LA3) because the protein content of maize is lower than that of *Ulva spp.* and less maize is needed to provide the same amount of sugar for the fermentation. Substitution of conventional gypsum with gypsum from the LA purification would hardly reduce land occupation.

3.5 Marine Eutrophication

Marine eutrophication impacts were characterized by using the ReCiPe midpoint method. The largest contribution to the nitrogen emissions causing marine eutrophication comes from electricity consumption (LA1 and LA2) and maize cultivation (LA3) (Figure 1). Under optimized *Ulva spp.* cultivation (LA2), the total impact of producing soy for fish feed alone is almost the same as that of producing the hydrolysis residue for fish feed as a by-product of lactic acid. Substitution of soy in fish feed by the hydrolysis residue would reduce nitrogen emissions: the nitrogen emissions from electricity production in Portugal would be compensated by avoiding emissions from soy production for fish feed. Elaborating this consequential line of thought would require a more detailed assessment. Substitution of conventional gypsum with gypsum from the LA purification would hardly reduce nitrogen emissions.

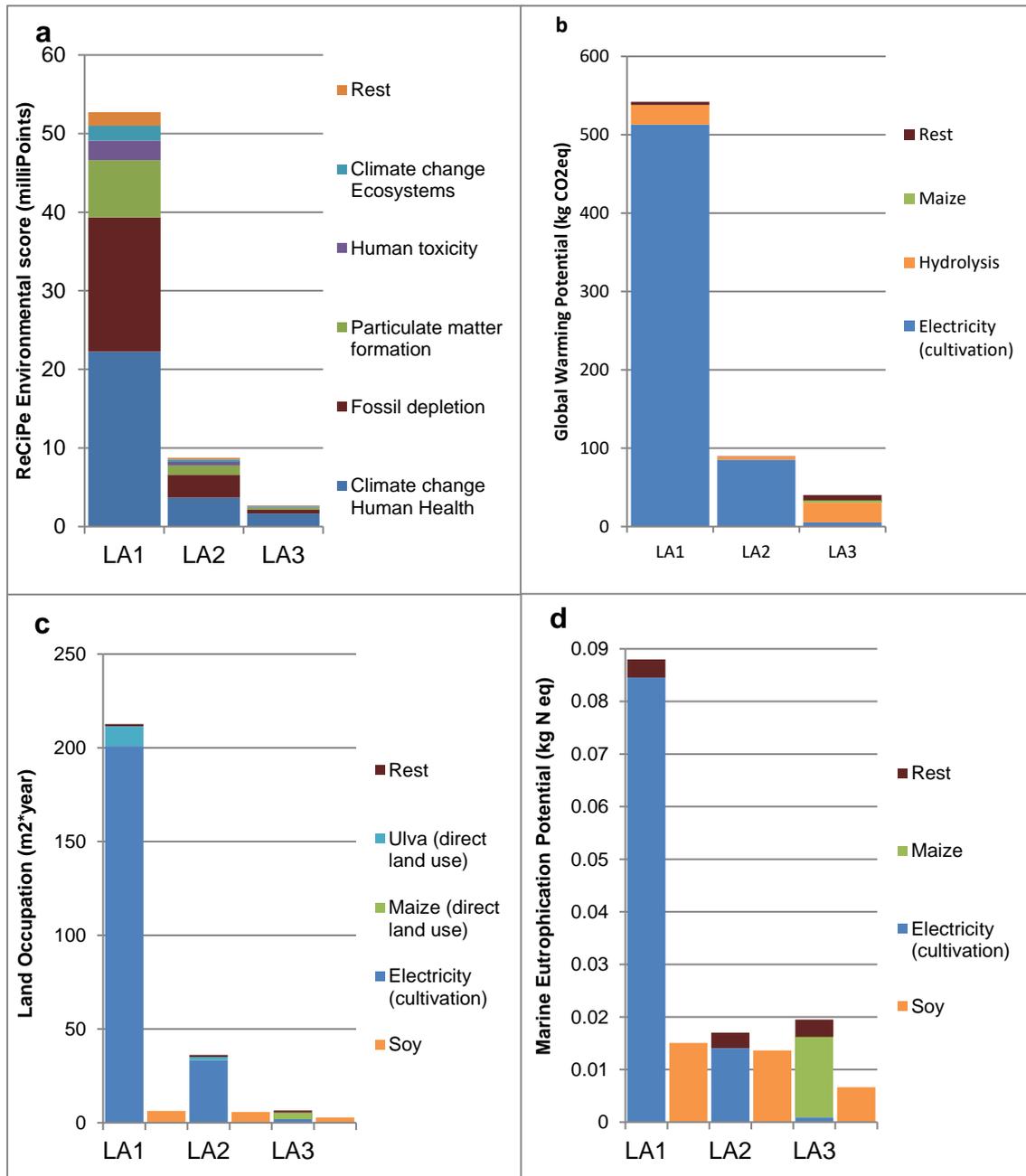


Fig. 1 Life Cycle Impact Assessment results for the three scenarios for (a) Weighted and aggregated environmental impact using the ReCiPe method (b) Climate Change, (c) Land Use, and (d) Marine Eutrophication. Every second column in Figures 1c and 1d indicates the impact of the soy production avoided, if the hydrolysis residue is used in fish feed to partially replace soy, for each scenario.

3.6 Hotspot identification and sensitivity analysis

The electricity consumption during the *Ulva spp.* cultivation is the most important environmental hotspot with a 97% contribution to the ReCiPe score of scenario LA1. Because electricity use during cultivation drives the environmental impact, the results are very sensitive to the electricity use and to the electricity impact. The direct CO₂ emission from the hydrolysis contributes 2% and is a secondary hotspot. The remainder of all processes in the life cycle contributes approximately 1% in which the treatment of bio-waste from the fermentation has the largest contribution.

Electricity use can be reduced in two ways. First, an electricity use reduction of 10% in LA1 results in a similar reduction in the ReCiPe score. Furthermore, optimized cultivation and purification in scenario LA2 reduces the amount of *Ulva spp.* required for 1 kg WM of lactic acid by a factor six, which reduces the required amount of electricity with the same factor.

The impact of electricity can also be reduced and this was studied by replacing the total electricity supply in the production chain with the Portuguese mix renewable electricity technologies, consisting of wind, solar, biomass and hydro-energy. The ratios between the renewable technologies were kept the same as in the overall grid mix, following [26]. Since the fossil based generation technologies (i.e. hard coal, natural gas and oil) contribute 80% of the electricity's impact, their exclusion would strongly reduce the environmental impact of lactic acid. The GWP is reduced from 0.11 kg CO₂eq/kWh to 0.013 kg CO₂eq/kWh, while the agricultural land occupation increases from 0.043 m²a/kWh to 0.071 m²a/kWh. Because electricity use has a large contribution to several impact indicators in scenarios LA1 and LA2, such as fossil resource depletion and marine eutrophication, a substitution of electricity source results in very strong changes, as shown in Figure 2.

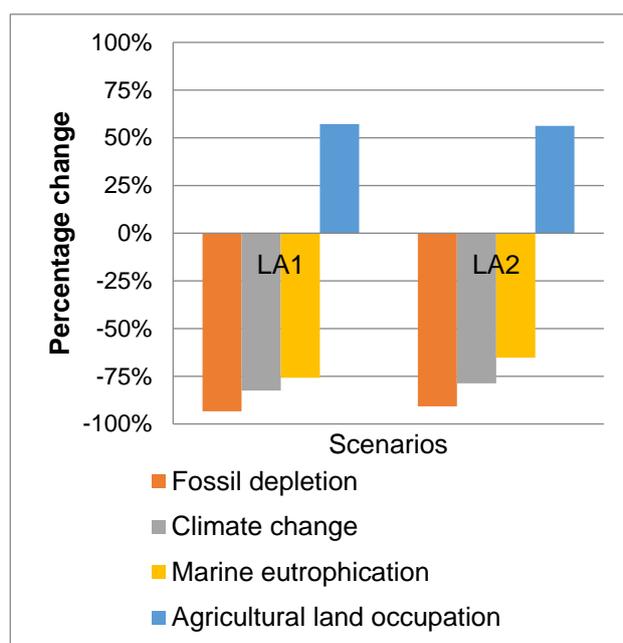


Fig. 2 Percentage change in four impact categories in scenarios LA1 and LA2 due to switch from average Portuguese electricity to renewable electricity for the three scenarios

3.7 Comparison with other feedstocks

The use of *Ulva spp.* can be compared with other feedstocks for lactic acid production. The results of scenario LA3 (Figure 1) show that, under the same processing conditions, maize has a significantly lower environmental impact than *Ulva spp.* because of the large electricity consumption used in producing the latter. LA2 demonstrates that the piloted improvements can achieve a feedstock efficiency gain from 158 kg WM / kg LA to 25 kg WM / kg LA, bringing the environmental impact of *Ulva spp.*-based LA closer to that of maize-based LA.

The contribution of *Ulva spp.* cultivation to agricultural land occupation is reduced from 11 m²a/kg LA in LA1 to 1.7 m²a/kg LA in LA2, compared with 3.3 m²a/kg LA for maize production in LA3. The contribution of electricity in the *Ulva spp.* cultivation remains substantial for both LA1 and LA2 (140 and 23 m²a/kg LA respectively), while electricity use hardly plays a role in maize cultivation.

4. Discussion

4.1 Uncertainties and improvements

Due to the experimental character of the production chain, technical and methodological uncertainties complicate the assessment of the current and future production systems. These are discussed below.

An influential uncertainty in this study is how the *Ulva spp.* residue from the hydrolysis is applied. The current assumption is that the residue partially replaces soy in fish feed. An alternative

use of different components of the residue, as pigments or active pharmaceutical ingredient, could have a greater economic and environmental potential.

The carbon dioxide fixed in the end product represents a second uncertainty. It is subtracted from the total greenhouse gas emissions, under the assumption that the carbon from the atmosphere is stored in the end-product. This is a common approach, followed by [30] and [4], which assumes that if 1 kg of bioplastics degrades at the end of its life and releases its carbon to the atmosphere, another kg of bioplastics is produced elsewhere that stores the carbon from its biomass feedstock. In this study, the effects are limited as the carbon credit is 1.47 kg CO₂eq / kg LA when compared to the overall GWP of 513 kg CO₂eq / kg LA for scenario LA1. Studies that include the end-of-life phase of the product elaborate how the bioplastics are distributed over waste flows and subsequent release of carbon to the atmosphere [31].

The third uncertainty is about the benefits of nitrogen and phosphorus fixed in the *Ulva spp.* By assuming that all nutrients end up in the residue during hydrolysis, the benefits are reflected in the substitution of soy in fish feed. In contrast to carbon, no benefits were granted to nutrient fixation in the *Ulva spp.*, as nutrients are not stored away from the sea for a significant period: Because the fish digest the residue supplemented in the fish feed, the nutrients are excreted back into the sea. If the nitrogen would be fixated for a longer period, this would be a significant reduction of the marine eutrophication. Storing phosphorus for a longer period would not translate into less marine eutrophication, since the current LCA characterization method assumes that marine biotic production is limited by nitrogen in general.

4.2 Expected effects of upscaling to industrial production

The path from an experimental production chain to a large-scale industrial chain is uncertain but improvements in the individual steps can result in substantial improvements. [31] makes predictions based on micro-algae. By forecasting optimization from small-scale to large-scale and improved recycling and reuse of waste streams, they predict that a difference of two orders of magnitude can be bridged between small-scale microalgae technology and the reference of large-scale soy production. Also open seaweed farming systems for bioenergy seem very favourable with net carbon and nutrient uptake and positive energy balances, when early stage results are extrapolated to an upscaled scenario [33].

4.3 Improvement opportunities for technology

Several improvements not yet implemented in the production chain can alter the outcome of the LCA. Material efficiency can be improved by adjusting the hydrolysis and the fermentation. In the hydrolysis, reducing the excess of acid used would reduce the amount of neutralization base needed, thus reducing the direct CO₂ emissions from hydrolysis. In addition, use of an alternative base to sodium carbonate for neutralization after hydrolysis would eliminate these emissions. The fermentation yield of *Ulva spp.* hydrolysate can be improved further by increasing the starch content and by lowering the salt content in the hydrolysate. Washing the *Ulva spp.* directly after harvest would reduce the salt content and consequentially the salt content in the hydrolysate. Washing would increase water and energy use in the production chain but may be beneficial from an environmental point of view.

The hydrolysis residue could also be used for higher value applications, especially if the hydrolysis of the *Ulva spp.* is part in a cascading biorefinery approach. Such an approach would maximize the total value of the *Ulva spp.* biomass [34]. Whole seaweed has been historically used as a fertilizer or to improve general soil conditions [35, 36] and the residue could fulfil the same role [36]. Seaweed can be used as a feed additive due to its prebiotic compounds that may help to enhance livestock production and health [38]. A recent *Ulva spp.* based biorefinery process has been described, where the carbohydrates are fermented to acetone, butanol, ethanol and 1,2-propanediol, and the protein-rich fraction is used as animal feed [39]. The nutrients taken from the sea would be stored in a livestock production chain, more permanently than in fish production, thus reducing marine eutrophication. Seaweeds and their derivatives may also be used for high-value applications such as pharmaceuticals [40], food and food additives [41].

While the improvement opportunities can be clearly identified, a best-case-scenario beyond scenario LA2 cannot be quantified as too little is known about technical scaling effects and the potential for radical innovation in both *Ulva spp.* production and further processing of all coproducts. The potential reduced impacts and positive effects of improved *Ulva spp.* cultivation and application can be proven with an assessment of the complete life cycle of all coproducts.

4.4 Broadening the sustainability assessment

Many interactions between seaweed cultivation and the status of the marine ecosystems are still hard to quantify with LCA. Existing characterization methods need to be improved to include exchanges with the sea, such as toxicity impacts and the role of phosphorus in marine eutrophication. Although assessment of benthic impacts, by-catch, emissions from anti-fouling paints and biotic resource use have been studied in LCAs on fisheries and aquaculture, it is common to focus on traditional environmental impact categories [42]. Novel characterization methods should be developed for less-studied impacts, such as those described by [21], including biotic resource use, invasive species, seafloor destruction, artificial habitat creation, shading, turbidity and sedimentation, noise and original habitat loss.

The cultivation in this system has a strong relation with the sea, because water and substances in the water are exchanged by the change of tides with the bordering coastal area. The effluent from the seaweed system flows directly to the coastal lagoon, reducing the nutrient and sediment load in the lagoon. The seaweed cultivation does not consume industrial auxiliary chemicals, so that none are released into the water basin. The intensification of seaweed farming, might favour disease outbreaks, similar to what is seen with agricultural plagues, or even a decrease in the genetic diversity of local seaweed stocks [43]. By using only local species, there is no risk of biological contamination or invasive species.

In the current study, the impact of fish aquaculture was not included in the assessment, because of the subdivision approach. Fishmeal, sourced from fishery-for-feed, is a common component in sea bass and sea bream [44] which may cause biotic resource extraction from the sea and seafloor damage, depending on the fishing technology but is included in fish diets on variable levels. The suggested future research on the potential uses of the *Ulva spp.* residue should include how the *Ulva spp.* cultivation contributes to a reduction of fishmeal usage. [23] also recommends to study the application of seaweed components in the fish diet, instead of the whole *Ulva spp.*

The improvements suggested in the previous paragraph and the issues presented above call for a broader sustainability assessment: the system boundaries and the scope of sustainability themes could be expanded. A broadened assessment puts greater demands on data collection efforts and possibly increases the uncertainty of the study. In this early innovation stage, the current approach already provides some intuitive predictors of the overall sustainability, such as biomass and starch efficiency, land occupation and global warming potential.

5. Conclusions

The study indicates that most of the environmental impact is due to electricity consumption during *Ulva spp.* cultivation. In addition to directly reducing electricity impacts, improvements in the material efficiency in the production chain will also reduce the impacts of the electricity use, as illustrated with scenario LA2 and the suggested improvements. This study helps to identify both major and minor improvement opportunities during the proof of concept phase of experimental research – along the complete production chain.

This study is a first analysis of the environmental performance of the green seaweed *Ulva spp.* in polymer applications. Coproduct and waste flows with their nutrient and carbon contents should be tracked in more detail, and other environmental impacts should be assessed in addition to common LCA impact categories. These steps would further improve the understanding of the current scenarios and the proposed improvements, including alternative usages for the hydrolysis residue.

Author Contributions: All authors have contributed to the preparation of this article. The following contributions were provided: Conceptualization, R.H. and S.B.; Methodology, R.H.; Software, R.H.; Data

Collection, R.H., A.C., H.A. and M.B.; Validation, A.C., H.A. and M.B.; Formal Analysis, R.H.; Writing-Original Draft Preparation, R.H., S.B. Writing-Review & Editing, F.M., S.B.; Supervision, JM, S.B.

Funding: This work was supported by the European Commission, through the Seventh Framework Program, SEABIOPLAS (grant number 606032).

Conflicts of Interest: Helena Abreu is co-owner and director R&D and Business Development at AlgaPlus Lda.

References

1. PlasticsEurope, 2016. *Plastics – the Facts 2016*. Available at: <http://www.plasticseurope.org/application/files/4315/1310/4805/plastic-the-fact-2016.pdf>. Accessed 11 March 2018
2. Belboom, S. & Léonard, A. Does biobased polymer achieve better environmental impacts than fossil polymer? Comparison of fossil HDPE and biobased HDPE produced from sugar beet and wheat. *Biomass and Bioenergy* **2016**, 85:159-167
3. Groot, W.J. & Borén, T. Life cycle assessment of the manufacture of lactide and PLA biopolymers from sugarcane in Thailand. *Int. J. Life Cycle Assess.* **2010**, 15:970-984
4. Vink, E.T.H. & Davies, S. Life Cycle Inventory and Impact Assessment Data for 2014 Ingeo Polylactide Production. *Industrial Biotechnology* **2015**, 11:167-180
5. Drumright, R.E., Gruber, P.R. & Henton, D.E. Polylactic acid technology. *Adv. Mater.* **2000**, 12:1841-1846
6. Masutani K., Kimura Y. Present Situation and Future Perspectives of Poly(lactic acid). In: Di Lorenzo M., Androsch R. (eds) *Synthesis, Structure and Properties of Poly(lactic acid)*. Advances in Polymer Science, vol 279. 2017, Springer, Cham
7. Juturu, V. & Wu, J.C. Microbial production of lactic acid: the latest development. *Crit. Rev. Biotechnol.* **2015**, 1-11
8. Changwichan, K., Silalertruksa, T. and Gheewala, S.H. Eco-Efficiency Assessment of Bioplastics Production Systems and End-of-Life Options. *Sustainability*, **2018**, 10(4), p.952.
9. Holdt, S.L. & Kraan, S. Bioactive compounds in seaweed: functional food applications and legislation. *J. Appl. Phycol.* **2011**, 23:543-597
10. Hughes, A.D., Kelly, M.S., Black, K.D. & Stanley, M.S.. Biogas from Macroalgae: is it time to revisit the idea? *Biotechnol. Biofuels* **2012**, 5:1
11. Wei, N., Quarterman, J. & Jin, Y.S.. Marine macroalgae: An untapped resource for producing fuels and chemicals. *Trends Biotechnol.* **2013**, 31:70-77
12. van der Wal, H., Sperber, B.L., Houweling-Tan, B., Bakker, R.R., Brandenburg, W. & López-Contreras, A.M.. Production of acetone, butanol, and ethanol from biomass of the green seaweed *Ulva lactuca*. *Bioresource Technol.* **2013**, 128:431-437
13. Korzen, L., Abelson, A. & Israel, A.. Growth, protein and carbohydrate contents in *Ulva* spp. and *Gracilaria bursa-pastoris* integrated with an offshore fish farm. *J. Appl. Phycol.* **2016**, 28:1835-1845
14. Marinho, G., Nunes, C., Sousa-Pinto, I., Pereira, R., Rema, P. & Valente, L.M.P.. The IMTA-cultivated Chlorophyta *Ulva* spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. *J. Appl. Phycol.* **2013**, 25:1359-1367
15. Aehle, W.. *Enzymes in industry: production and applications*. 3rd ed. 2007, John Wiley & Sons, Weinheim
16. Gupta, R., Kumar, S., Gomes, J. & Kuhad, R.C.. Kinetic study of batch and fed-batch enzymatic saccharification of pretreated substrate and subsequent fermentation to ethanol. *Biotechnol. biofuels* **2012**, 5:16.
17. Nigam, P.S. & Singh, A.. Production of liquid biofuels from renewable resources. *Prog. Ener. Combust.* **2011**, 37:52-68
18. Abreu, M.H., Pereira, R., Yarish, C., Buschmann, A.H. & Sousa-Pinto, I. IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture* **2011**, 312:77-87
19. Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H. & Fang, J.-G.. Ecological engineering in aquaculture—Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture* **2009**, 297:1-9
20. ISO Technical Committee ISO/TC 207. Environmental management : life cycle assessment : principles and framework ISO 14040. 2006 ISO, Geneva
21. Langlois, J., Fréon, P., Steyer, J.-P., Delgenès, J.-P. & Hélias, A.. Sea-use impact category in life cycle assessment: state of the art and perspectives. *Int. J. Life Cycle Assess.* **2014**, 19:994-1006
22. EC-JRC-IES 2010. *International Reference Life Cycle Data System (ILCD) Handbook - General guide for Life Cycle Assessment - Detailed guidance*. 1st ed. Publications Office of the European Union, Luxembourg, 417 pp
23. Peixoto, M.J., Svendsen, J.C., Malte, H., Pereira, L.F., Carvalho, P., Pereira, R., Gonçalves, J.F. & Ozório, R.O.. Diets supplemented with seaweed affect metabolic rate, innate immune, and antioxidant responses,

- but not individual growth rate in European seabass (*Dicentrarchus labrax*). *J. Appl. Phycol.* **2016**, *28*:2061-2071
24. Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B. & Worley, M.. *Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: dilute-acid pretreatment and enzymatic hydrolysis of corn stover*. 2011, National Renewable Energy Laboratory (NREL), Golden, CO
 25. Wernet, G., Bauer, C., Steubing, B., Reinhard, J., Moreno-Ruiz, E. & Weidema, B.. The ecoinvent database version 3 (part I): overview and methodology. *Int. J. Life Cycle Assess.* **2016**, *21*:1218-30
 26. IEA 2016. Energy Policies of IEA Countries: Portugal. Paris, France. Retrieved from https://www.iea.org/publications/freepublications/publication/Energy_Policies_of_IEA_Countries_Portugal_2016_Review.pdf
 27. Nielsen, P.H., Oxenboll, K.M. & Wenzel, H.. Cradle-to-Gate Environmental Assessment of Enzyme Products Produced Industrially in Denmark by Novozymes A/S. *Int. J. Life Cycle Assess.* **2007**, *12*:432-438.
 28. Goedkoop, M., Heijungs, R., Huijbregts, M.A.J., Schryver, A.d., Struijs, J. & van Zelm, R.. *ReCiPe 2008, A Life Cycle Impact Assessment method which comprises harmonised category indicators at the midpoint and the endpoint level, Report I Characterization*. 2009. Ministry of Housing, Spatial Planning and the Environment (VROM), Den Haag, 132 pp
 29. Hansen, B.. Determination of nitrogen as elementary N, an alternative to Kjeldahl. *Acta Agr. Scand.* **1989**, *39*:113-118
 30. Vink, E.T., Glassner, D.A., Kolstad, J.J., Wooley, R.J. & O'Connor, R.P.. Original research: the eco-profiles for current and near-future NatureWorks® polylactide (PLA) production. *Industrial Biotechnology* **2007**, *3*:58-81
 31. Hottle, T.A., Bilec, M.M. & Landis, A.E.. Sustainability assessments of bio-based polymers. *Polym. Degrad. Stabil.* **2013**, *98*:1898-1907
 32. Taelman, S.E., De Meester, S., Van Dijk, W., da Silva, V. & Dewulf, J.. Environmental sustainability analysis of a protein-rich livestock feed ingredient in The Netherlands: Microalgae production versus soybean import. *Resour. Conserv. Recy.* **2015**, *101*:61-72
 33. Aitken, D., Bulboa, C., Godoy-Faundez, A., Turrion-Gomez, J.L. & Antizar-Ladislao, B.. Life cycle assessment of macroalgae cultivation and processing for biofuel production. *J. Clean. Prod.* **2014**, *75*:45-56
 34. van Hal, J.W., Huijgen, W. & López-Contreras, A.. Opportunities and challenges for seaweed in the biobased economy. *Trends Biotechnol.* **2014**, *32*:231-233
 35. Blunden, G.. Agricultural uses of seaweeds and seaweed extracts. In Blunden, G., Guiry, M.D. [Eds.] *Seaweed resources in Europe: uses and potential*. 1992. J. Wiley, Chichester, pp. 63-81
 36. Chapman, V.. *Seaweeds and their uses*. 2012. Springer Science & Business Media
 37. Alvarado-Morales, M., Boldrin, A., Karakashev, D.B., Holdt, S.L., Angelidaki, I. & Astrup, T.. Life cycle assessment of biofuel production from brown seaweed in Nordic conditions. *Bioresour. Technol.* **2013**, *129*:92-99
 38. Makkar, H.P., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F. & Ankers, P.. Seaweeds for livestock diets: a review. *Animal Feed Science and Technology* **2016**, *212*:1-17
 39. Bikker, P., Krimpen, M.M., Wikselaar, P., Houweling-Tan, B., Scaccia, N., Hal, J.W., Huijgen, W.J., Cone, J.W. & López-Contreras, A.M.. Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. *J. Appl. Phycol.* **2016**, *1-15*
 40. Yang, Y., Chai, Z., Wang, Q., Chen, W., He, Z. & Jiang, S.. Cultivation of seaweed *Gracilaria* in Chinese coastal waters and its contribution to environmental improvements. *Algal Res.* **2015**, *9*:236-244
 41. Radulovich, R., Umanzor, S., Cabrera, R. & Mata, R.. Tropical seaweeds for human food, their cultivation and its effect on biodiversity enrichment. *Aquaculture* **2015**, *436*:40-46
 42. Pelletier, N.L., Ayer, N.W., Tyedmers, P.H., Kruse, S.A., Flysjo, A., Robillard, G., Ziegler, F., Scholz, A.J. & Sonesson, U.. Impact categories for life cycle assessment research of seafood production systems: review and prospectus. *Int. J. Life Cycle Assess.* **2007**, *12*:414-421
 43. Cottier-Cook, E., Nagabhatla, N., Badis, Y., Campbell, M., Chopin, T., Dai, W., Fang, J., He, P., Hewitt, C. & Kim, G.. *Safeguarding the future of the global seaweed aquaculture industry*. 2016. United Nations University and Scottish Association for Marine Science Policy Brief, p. 12

44. Kousoulaki, K., Sæther, B.S., Albrektsen, S. & Noble, C. 2015. Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: a practical guide for optimizing feed formulation and farming protocols. *Aquacult. Nutr.* 21:129-151