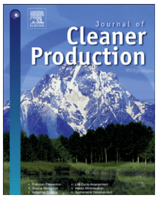
Journal of Cleaner Production 150 (2017) 1–15

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro



Seaweed as innovative feedstock for energy and feed – Evaluating the impacts through a Life Cycle Assessment

Michele Seghetta [a], Daina Romeo [b], Martina D'Este [c], Merlin Alvarado-Morales [c], Irini Angelidaki [c], Simone Bastianoni [b], Marianne Thomsen [a, *]

[a] *Research Group on EcoIndustrial System Analysis, Department of Environmental Science, Aarhus University, Frederiksborgvej 399, DK-4000 Roskilde, Denmark*
[b] *Ecodynamics Group, Department of Physical Sciences, Earth and Environment, University of Siena, Pian dei Mantellini 44, 53100 Siena, Italy*
[c] *Department of Environmental Engineering, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark*

ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form
28 January 2017
Accepted 3 February 2017
Available online 20 February 2017

Keywords:
Seaweed
Macroalgae
Biogas
Proteins
Life Cycle Assessment
Biobased economy

ABSTRACT

Offshore cultivation of seaweed provides an innovative feedstock for biobased products supporting blue growth in northern Europe. This paper analyzes two alternative exploitation pathways: energy and protein production. The first pathway is based on anaerobic digestion of seaweed which is converted into biogas, for production of electricity and heat, and digestate, used as fertilizer; the second pathway uses seaweed hydrolysate as a substrate for cultivation of heterotrophic microalgae. As a result the seaweed sugars are consumed while new proteins are produced enhancing the total output. We performed a comparative Life Cycle Assessment of five scenarios identifying the critical features affecting resource efficiency and environmental performance of the systems with the aim of providing decision support for the design of future industrial scale production processes. The results show that all scenarios provide environmental benefits in terms of mitigation of climate change, with biogas production from dried *Laminaria digitata* being the most favorable scenario, quantified as $-18.7*10^2$ kg CO_2 eq./ha. This scenario presents also the lowest consumption of total cumulative energy demand, $1.7*10^4$ MJ/ha, and even resulting in a net reduction of the fossil energy fraction, $-1.9*10^4$ MJ/ha compared to a situation without seaweed cultivation. All scenarios provide mitigation of marine eutrophication thanks to bioextraction of nitrogen and phosphorus during seaweed growth. The material consumption for seeded lines has 2–20 times higher impact on human toxicity (cancer) than the reduction achieved by energy and protein substitution. However, minor changes in cultivation design, i.e. use of stones instead of iron as ballast to weight the seeded lines, dramatically reduces human toxicity (cancer). Externalities from the use of digestate as fertilizer affect human toxicity (non-cancer) due to transfer of arsenic from aquatic environment to agricultural soil. However concentration of heavy metals in digestate does not exceed the limit established by Danish regulation. The assessment identifies seaweed productivity as the key parameter to further improve the performance of the production systems which are a promising service provider of environmental restoration and climate change mitigation.

1. Introduction

Offshore cultivation of seaweed, also known as macroalgae, is an innovative business in northern Europe and constitutes a promising technology to support blue growth and biobased economy (EC, 2012; EC, 2015). The development of a biobased economy is encouraged by the European Commission as a means for achieving a sustainable society (EC, 2015). Seaweed contains a variety of valuable commercial substances such as sugars (glucose and mannitol), proteins, alginate and fucoidans. The development of seaweed cultivation in Europe would be beneficial for several industrial sectors: agriculture, pharmaceutical, food, aquaculture and energy (Draget et al., 2005; Adams et al., 2011; Horn et al., 2000; Van Hal et al., 2014).

---

* Corresponding author.
E-mail addresses: mise@envs.au.dk (M. Seghetta), daina.romeo@student.unisi.it (D. Romeo), mdest@env.dtu.dk (M. D'Este), meal@env.dtu.dk (M. Alvarado-Morales), iria@env.dtu.dk (I. Angelidaki), bastianoni@unisi.it (S. Bastianoni), mth@envs.au.dk (M. Thomsen).

http://dx.doi.org/10.1016/j.jclepro.2017.02.022
0959-6526/© 2017 Elsevier Ltd. All rights reserved.

Brown algae cultivation in northern Europe, i.e. *Saccharina latissima* and *Laminaria digitata*, is demonstrated to be feasible and the market for sea vegetables is expected to increase 7–10% per year (BIM, 2014). The high sugar content, i.e. 51–55% of dry weight (Manns et al., 2014; Adams et al., 2009), makes seaweed a suitable feedstock for bioethanol production (Seghetta et al., 2016a; Aitken et al., 2014) and biogas production (Alvarado-Morales et al., 2013; Kaspersen et al., 2016) as a form of renewable energy that could support society in reaching the carbon reduction goals and in mitigating climate change (DG, 2013). A breakthrough in the provision of biomass as feedstock for industrial scale processes is represented by the innovative offshore cultivation of seaweed (Nielsen et al., 2016). This technique avoids the competition for land occupation typical of first generation biofuels based on land energy crops which generate problems in terms of land-use change and deforestation (Pimentel, 2003; Havlík et al., 2011). Moreover, when combined with aquaculture, it can reabsorb the excess emissions of nitrogen and phosphorus providing mitigation of eutrophication service (Marinho et al., 2015).

Several studies have been performed at lab scale (Jard et al., 2013; Bruhn et al., 2011) but still only a limited number of large scale seaweed-based biogas plants have been developed. In Denmark a new biogas plant is co-digesting beach-cast seaweed, manure and residues from a pectine production industry (Kaspersen et al., 2016).

Extraction of proteins from seaweed is a second promising business scenario which can tackle the problem of feed shortage. Proteins can be marketed as optimal ingredient for fish feed, a sector that conventionally uses proteins sourced from fish, i.e. fish oil and fish meal (ground bones and offal from processed fish) (Tacon et al., 2006). The increased demand for fish meal coupled with depletion of wild fish has raised the price of protein, leading feed producers to use plant as feedstock (Tacon et al., 2006; FAO, 2010). Use of vegetable proteins as a total or partial replacement of fishmeal has been studied with positive results for marine and freshwater fish species (Kaushik et al., 1995, 2004). Soybean and pea-derived proteins are already marketed in significant amounts in the feed and food sectors. The world production trend for soybean is predicted to increase 2.2% annually by 2030 leading to a restriction of the cultivated area due to competition for arable land with other marketed crops (Masuda and Goldsmith, 2009).

Products for carnivorous fish feed, e.g. *Oncorhynchus mykiss*, requires high protein supply with concentration higher than fresh seaweed (Aller aqua A/S personal communication). Maximization of protein production can be performed by genetic improvement of seaweed species (Robinson et al., 2013) or by conversion of organic compounds, such as sugars into proteins enhancing the total production. Heterotrophic microalgae are able to support the latter process using the sugars as a source of energy and structural material to support biomass growth. Autotrophic microalgae have been widely studied for production of energy and proteins (Becker, 2007; Lam and Lee, 2012; Reis et al., 2014) while heterotrophic microalgae represent a relatively unexplored field. The latter has the advantage of being able to grow in the absence of light, reducing the production costs dramatically (Chen et al., 2011).

Energy and protein production from seaweed is still in its infancy, therefore the sustainability assessment performed so far on offshore cultivation of seaweed and conversion is affected by uncertainty (Alvarado-Morales et al., 2013; Aitken et al., 2014; Cappelli et al., 2015). This study moves a step forward the sustainability assessment of industrial production of energy and proteins using the most recent studies obtained in the MacroAlgae Biorefinery 3 project (www.mab3.dk). This is also the first sustainability assessment on protein production based on combined exploitation of seaweed and microalgal biomass. The analysis focuses on environmental impacts, such as climate change mitigation and bioremediation, and impacts on human health and energy demand. This preliminary evaluation can highlight positive and critical aspects of these innovative productions and therefore provide information for future research and development on this topic.

2. Materials and methods

2.1. Goal and scope definition

The goal of the analysis is to assess the environmental performance of offshore seaweed production and two alternative exploitation pathways: energy and protein production. Critical features influencing resource efficiency and environmental performance of the systems are evaluated with the aim of providing decision support for future industrial scale production processes.

2.1.1. System description

We designed two alternative systems using offshore cultivated seaweed as feedstock for energy or protein production. The first pathway for energy production is based on offshore cultivation of macroalgae in 208 km^2 of marine water surface, harvest and transport to the closest harbour. The 208 km^2 of Danish marine water occupied by offshore seaweed cultivation is a short term estimation based on a 20-year trend in offshore wind farming development (Seghetta et al., 2016b) (Supplementary material, Fig. S1). Seaweed is then partially dried and transported to the biogas plant where anaerobic digestion converts organic matter into biogas and digestate. Biogas is combusted in a cogeneration engine producing electricity and heat. Digestate is transported and applied to fields. We model three scenarios combining two different brown algae species, i.e. *Saccharina latissima* and *Laminaria digitata* and two seaweed storage methods, i.e. drying and ensilage:

- BioS1: biogas production from the species *Saccharina latissima* dried;
- BioS2: biogas production from the species *Saccharina latissima* ensilage;
- BioL1: biogas production from the species *Laminaria digitata* dried.

The second pathway, protein production, as displayed in Fig. 1, is based as well on the offshore cultivation of macroalgae in 208 km^2 of marine water surface (Fig. 1). Seaweed is harvested and transported to the closest harbour where it is partially dried to guarantee conservation of the biomass. The biomass is transported to the protein production plant where it is chopped; the carbohydrates are hydrolyzed and used as substrate for growth of microalgae. After 7 days, the microalgae are dewatered and the biomass is recovered constituting a protein-rich ingredient which is distributed to fish feed production industries. Two scenarios are modeled based on two seaweed species, i.e. *Saccharina latissima* and *Laminaria digitata*, and one storage method, i.e. drying:

- ProS1: protein production from *Saccharina latissima* dried;
- ProL1: protein production from *Laminaria digitata* dried.

We considered a functional unit of 1 ha of offshore cultivation area. This means that the impacts or benefits generated by the systems analyzed are referred to the biomass produced and converted from 1 ha of cultivation area. LCA is performed according to international standard ISO 14040-44 (ISO, 2006) using system expansion to include multiple products according to a

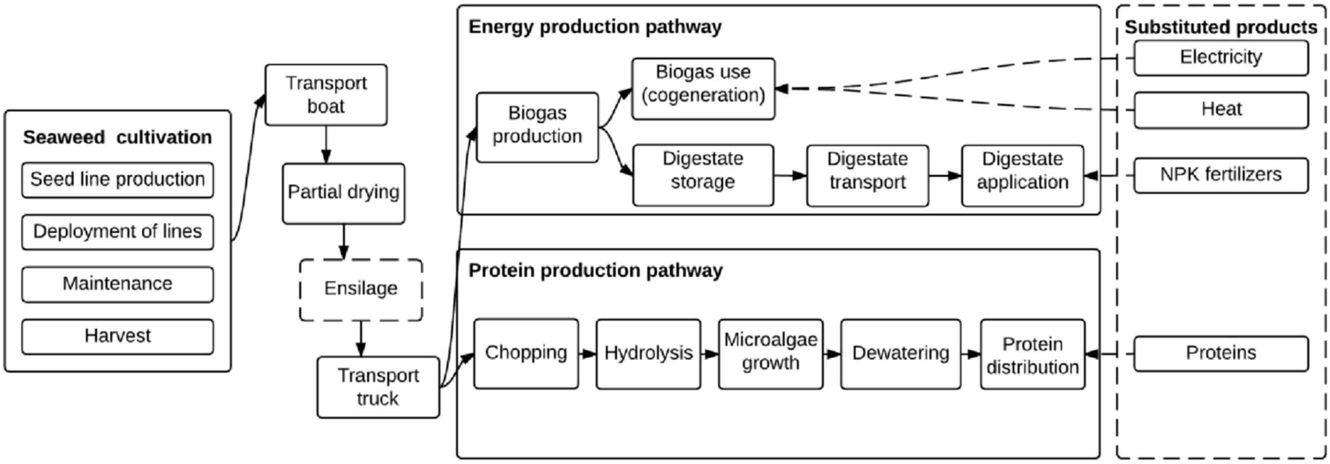


Fig. 1. Scheme of the system analyzed starting from seaweed cultivation composed of seed line production, deployment of lines, maintenance during the growth phase and harvest; water transport of seaweed biomass from cultivation sites to harbor; partial drying of biomass; ensilage of seaweed only for scenario BioS2; road transport from harbor to biogas/protein production plant. Two alternative pathways are modeled after the transport: energy production and protein production. The energy production pathway is composed of biogas production through anaerobic digestion, biogas use in a cogeneration engine producing electricity and heat, digestate storage, transport and application on soil. The protein production pathway is composed of chopping, hydrolysis of sugars contained in seaweed, microalgae growth and dewatering producing a protein-rich fish feed ingredient. The dashed line encloses substituted products, i.e. electricity, heat and NPK fertilizers for the first pathway and proteins for the second.

consequential approach (Ekvall and Weidema, 2004). The system expansion includes the substitution of products generated by the modeled systems, i.e. electricity, heat, fertilizers and proteins. The calculation was performed using SimaPro 8.0.4 software (PRé Consultants, 2008) and the integrated inventory Ecoinvent v3.1 (Weidema et al., 2013). Analysis is based on the latest available literature and information from the MAB3 project (www.mab3.dk).

2.2. Life cycle inventory

The Life Cycle Inventory (LCI) for the two systems includes the phases described in Fig. 1. Seaweed cultivation is common to all the modeled scenarios therefore data are presented in Table 1.

2.2.1. Seaweed cultivation

The first phase of both pathways is a bioengineered cultivation system as visualized in Fig. 2. The main feature of the cultivation system is an artificial growth substrate, i.e. the seeded lines, allowing seaweed to grow and be harvested isolated from the natural habitats of the marine ecosystem. The cultivation technology can be applied to *Laminaria digitata* or *Saccharina latissima* and is similar to the one currently used for cultivating *Palmaria palmata* (Watson and Dring, 2011).

The cultivation starts with the seed line production (Fig. 1) where kuralon twines are seeded with seaweed spores and incubated in a cold room where nutrients and sterile water support their growth (Tørring and Nielsen, 2014). Afterwards the seeded kuralon twines are coiled around 8 mm diameter ropes that act as support and provide the necessary surface for the seaweed to attach to during its growth phase. In order to prevent the lines from floating, weights are tied to the support rope, i.e. small iron bars (0.3 kg each) every 3.2 m (Tørring and Nielsen, 2014). The combination of seeded kuralon twines, support ropes and weights constitutes the seeded lines (SL) (Fig. 2).

The seeded lines are deployed (Fig. 1) in the sea in September and harvested either in spring, scenarios BioS1, BioS2 and ProS1, or in summer, scenario BioL1 and ProL1. The data necessary to model the engineered cultivation were obtained from a pilot cultivation site at Limfjorden, Denmark, during the growth season 2012–2013. Table 1 shows the quantities of input-output flows of matter and energy in the seaweed production phase.

Since the model considers the cultivation of seaweed in 10 different locations (Supplementary materials, Fig. S1), we use an average productivity of 10 Mg ww/ha based on pilot scale cultivation in Limfjorden (Denmark) and industrial scale cultivation in Horsens Fjord (Denmark) (Seghetta et al., 2016b) (Table 2). The composition of seaweed biomass is based on literature studies of *Saccharina latissima* and *Laminaria digitata* harvested in Denmark (see supplementary materials, Table S1). Composition is used to quantify the amount of macro- and micro-elements bioextracted from seawater.

According to the IPCC guidelines (IPCC, 2006), 0.5% of nitrogen emissions in freshwater are naturally converted into N_2O. As the yearly harvest of nitrogen in macroalgal biomass does not exceed the yearly land-based emission, we considered 0.5% of the nitrogen bioextracted from the seawater during seaweed growth as avoided N_2O emissions (Seghetta et al., 2016a).

2.2.2. Transport by boat

Harvest and transport for the offshore cultivation were assumed operated by boat, i.e. barge. We considered the distance from the cultivation sites to the closest industrial harbour. The distances and amount of seaweed transported from each cultivation site are shown in the supplementary material (Table S2 and Fig. S1) while the sum is provided in Tables 3 and 4. The most productive sites (cultivated area greater than 2100 ha) are located at a distance between 13 km and 117 km from the harbour (Table S2) and between 9 and 32 km from shore. The smallest sites (less than 2100 ha) are located at a distance between 68 km and 3 km from the closest industrial harbour (Table S2) and between 5 km and 40 km from shore.

2.2.3. Partial drying

The partial drying phase reduces the water content in the seaweed in order to avoid degradation of biomass. A moisture content of 20% is achieved in scenarios BioS1, BioL1, ProS1 and ProL1 which is the standard for commercial dried seaweed (CP Kelco personal communication). For BioS2 75% of moisture is required to ensilage the biomass. Energy consumption is modeled based on grass drying; a process included in the Ecoinvent v3

Table 1
LCI of the offshore seaweed cultivation phase showing input of energy and material necessary to cultivate 208 km^2 of sea. All values in column 2 are already divided by their lifetime. Column 5 indicates the composition of the material or type of energy used in the inventory.

Phases	Amount	U.M./208 km^2	Life time	Material
Seed line production				
Collection of fertile material				
Fuel for car	833	L	1	Diesel
Tank for seawater	10	kg	10	PET
Spore release				
Plastic jug	21	kg	5	PET
Autoclave	3125	kWh	1	Electricity mix DK
Refrigerator	975	kWh	1	Electricity mix DK
Preparation of collectors				
Block of collectors	833	kg	5	PEHD
Kuralon twine	2240	kg	1	Polyvinyl alcohol
Gas	198	kg	1	Natural gas
Seeding of lines				
Spray bottle	8	kg	5	PET
Nursery phase				
Electricity - Air pump	306	kWh	1	Electricity mix DK
Sand	463	kg	1	Sand
Mechanical filter (1–5 μm)	108	kg	1	Polypropylene
UV filter (Bulb)	0.2	Piece	1	Light emitting diode
Electricity - Water pump	76	kWh	1	Electricity mix DK
Electricity - Sand filter	46	kWh	1	Electricity mix DK
Electricity - UV filter	344	kWh	1	Electricity mix DK
F2 medium	500	L	1	Mix of substances
Deployment of lines				
Screw anchor	562	kg	20	Iron
Black buoys	8906	kg	8	Polyethylene
Thin rope	4812	kg	1	Nylon
Concrete block	83,332	kg	20	Concrete
Iron bars	18,750	kg	5	Iron
Cable ties	562	kg	1	Polyamide
Ropes for buoys	1875	kg	1	Polypropylene
Headline rope (HL)	2027	kg	13	Polypropylene
8 mm rope	6875	kg	5	Polypropylene
Concrete block rope	3125	kg	1	Concrete
Boat use	1458	tkm		
Maintenance				
Boat use	1458	tkm		
Harvest				
Industrial bags	223	kg	1	Polypropylene

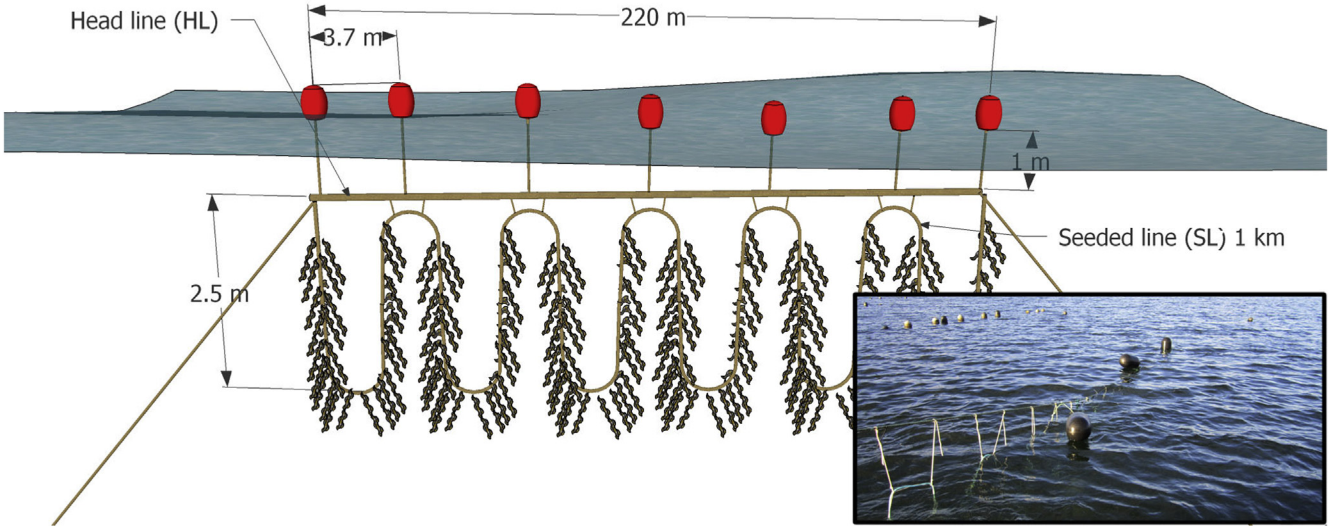


Fig. 2. Offshore seaweed cultivation system. Each cultivation line consists of a seeded line (SL) festooned in U-shaped loops on a horizontal head line (HL).

database (Weidema et al., 2013). A chopper is used to chop up the biomass prior ensilage.

2.2.4. *The ensilage process*

Ensilage is a process for storage of biomass based on the creation

Table 2
Summary of cultivation productivity of *Laminaria digitata* and *Saccharina latissima* in Denmark.

	Species		Unit
	Laminaria digitata	*Saccharina latissima*	
Productivity wet weight			
Referred to Head Line	9.1	9.1	kg WW/m HL
Referred to Seeded Line	2.0	2.0	kg WW/m SL
Referred to one cultivation line	2.0	2.0	Mg WW/line
Referred to hectares	10	10	Mg WW/ha
Productivity dry weight			
Referred to Head Line	2.6	1.3	kg DW/m HL
Referred to Seeded Line	0.6	0.3	kg DW/m SL
Referred to one cultivation line	0.6	0.3	Mg DW/line
Referred to hectares	2.9	1.5	Mg DW/ha

of an anaerobic condition. In this way, the microorganisms naturally present in the biomass use the water-soluble carbohydrates to create lactic acid. Accumulation of acids results in a pH reduction to 4–5, which inhibits the growth of spoilage microorganisms. Ensilage is proved to enhance the methane yield of the biomass (Alvarado-Morales and Angelidaki, 2016; Herrmann et al., 2015).

2.2.5. *Transport by truck*

Road transport considers the distance from the harbours to three hypothetical biogas/protein production plants located in Kalundborg, Blåvand and Maribo. Transport routes are shown in supplementary materials (Table S2 and Fig. S1). The locations were selected based on the nearest town with sufficient infrastructure to integrate a biogas/protein production plant with the local facilities. Kalundborg has an interconnected exchange network of energy and materials from different industrial processes, i.e. the symbiosis concept (Chertow, 2007). Maribo already has a biogas plant, Nysted Bioenergy LLC, which could potentially increase the process capacity to utilize seaweed as feedstock.

2.2.6. *Biogas production and use*

Biogas production is based on anaerobic fermentation of the biomass which is converted into a gas fraction (containing 60% methane, 38% carbon dioxide 0.5% ammonia, 1.2% hydrogen sulfide and 0.3% water (supplementary materials, Table S4) and a digestate comprised by unconverted slurry material. Biogas yields are based on lab analysis of *Saccharina latissima* dried, ensilage and *Laminaria digitata* dried (Table 5) (Alvarado-Morales and Angelidaki, 2016). The total production of biogas and digestate is shown in Table 6. Detailed calculations for reactor dimensioning and biogas production are presented in the supplementary material (Tables S3 and S4).

Energy and material consumption for the biogas process is based on literature data of full scale biogas plants (Naegele et al., 2012; Langlois et al., 2012). Combustion of biogas takes place at the biogas plants, in a cogeneration engine with 38% electricity production efficiency, 42% heat production efficiency and 20% lost as heat (Thomas and Wyndorps, 2012; Reichhalter et al., 2011). Electricity and heat are delivered to the grid substituting production of Danish electricity mix and district heating according Ecoinvent v3 database (Weidema et al., 2013).

2.2.7. *Digestate*

The digestate is temporarily stored at the biogas plant for later transportation to fields (Table 6). The distance from the three biogas plants to the fields is based on the area necessary for digestate application according to the Nitrate Directive (91/676/EEC), which establishes a limit of 170 kg N/ha. The average distance varies from 3 to 6 km (supplementary material, Table S5). Ammonia emissions during storage and application phase are accounted considering an emission factor of 0.013 during storage and 0.006 during spreading (DCE, 2014). The use of digestate as soil fertilizer substitutes the production and use of mineral fertilizers NPK in a ratio equal to bioavailable NPK in digestate. Only 9.2% of N and 2.5% of P applied to soil is transferred to marine water (Seghetta et al., 2016b). Of the carbon contained in digestate 10% is considered undegraded, i.e. conserved after 100 years, increasing the carbon stored in soil (Morgensen et al., 2014; Petersen et al., 2013) (Table 3).

2.2.8. *Chopping*

In the protein production pathways the dried seaweeds are temporarily stored. At the plant a chopper is used to chop up the biomass so that it can be conveyed through pipelines with water. Electricity consumption for the chopper is based on Ecoinvent v3 database (Weidema et al., 2013) (Table 4).

2.2.9. *Hydrolysis and microalgae growth*

The first step in the processing plant is the hydrolyzation of seaweed biomass; this process breaks the carbohydrates into sugar monomers used by microalgae as growth substrate. Enzymatic hydrolysis releases all available glucose without high temperature acid pre-treatment (Adams et al., 2009) or milling (Manns et al., 2016) with 80% conversion efficiency (Hou et al., 2015). The hydrolysis occurs in a heated tank where water and enzymes (cellulase and alginate lyase) are added to seaweed and stirred for 24 h (Supplementary materials, Table S6). It was not possible to retrieve specific information about production of alginate lyase and cellulase, therefore the impact of their production is excluded from the analysis. The hydrolysate is transferred to a reactor where the heterotrophic microalga *Chlorella protothecoides* grows. Mannitol and glucose are converted to proteins with a conversion factor of 0.18 g of proteins/g of sugar (D'Este et al., 2016). Protein mass balance is visualized in Fig. 3 exemplified considering 1000 kg seaweed DW and composition based on scenario BioS1.

2.2.10. *Dewatering*

After 7 days, microalgae and unconverted compounds in the growth reactor are dewatered through a sequence of processes based on the best available technology (O'Connel et al., 2013). Firstly a spiral plate centrifuge concentrates the dry matter to 31.5%; secondly a heat assisted rotary pressure filter increases dry matter concentration to 56% and finally a heat integrated dryer allows a 95% concentration to be reached (O'Connel et al., 2013). The final product is a mixture of proteins and unconverted compounds that can be used as a fish feed ingredient (Table 6).

2.2.11. *Protein distribution*

The end user of the protein-rich ingredient is assumed the fish feed production facility Aller Aqua A/S located in Christiansfeld, Denmark (supplementary material, Fig. S1).

Only the protein contained in the fish feed ingredient is considered substituting soy-based proteins. We do not take into consideration the different amino acid composition of the proteins due to lack of information both in seaweed proteins and in Ecoinvent Database.

2.3. *Selected impact categories and methodologies*

We selected a series of impact categories in order to analyze

Table 3
LCI of the three scenarios modeled for the energy production pathway showing inputs and outputs of one cultivation cycle in 208 km^2 of sea cultivated with seaweed.

Scenario	BioS1	BioS2	BioL1	
Item	Amount			U.M.
Seaweed cultivation				
Cultivated area	20,833	20,833	20,833	ha
Transport by boat				
Transport	12,672,201	12,672,201	12,672,201	tkm
Partial drying				
Water reduction	169,421	84,815	134,342	m^3
Ensilage				
Electricity consumption		2,461,285		kWh
Storage		123,064		m^3
Transport by truck				
Transport	259,905	831,695	496,980	tkm
Biogas production				
Water to be added	171,812	87,205	462,790	Mg
Lubricant oil	3	3	5	Mg
Agitators - Electricity	403,225	403,225	1,028,493	kWh
Feeding system - Electricity	82,012	82,012	209,185	kWh
Pump - Electricity	11,391	11,391	29,053	kWh
Heat	1,921,725	1,921,725	4,901,681	kWh
Biogas production - Emissions to air				
CH_4 loss	50,939	62,478	154,712	kg
NH_3 loss	439	538	1333	kg
H_2S loss	2040	2502	6197	kg
H_2O loss	264	324	802	kg
Biogas use (cogeneration)				
CHP control unit + aux power	41,917	41,917	106,917	kWh
CHP compartment fan	20,959	20,959	53,458	kWh
Heating circuit pump	39,297	39,297	100,235	kWh
Emergency cooler fan	23,578	23,578	60,141	kWh
Emergency cooler pump	41,917	41,917	106,917	kWh
Biogas mixture cooler fan	23,578	23,578	60,141	kWh
Biogas mixture cooler pump	36,678	36,678	93,552	kWh
Gas compressor	26,198	26,198	66,823	kWh
Gas cooler	7859	7859	20,047	kWh
Biogas use - Emissions to air				
NOx	43,529	53,390	132,208	kg
UHC	71,759	88,014	217,947	kg
NMVOC	2155	2643	6545	kg
CH_4	93,524	114,709	284,050	kg
CO	66,803	81,935	202,893	kg
N_2O	345	423	1047	kg
Digestate storage and transport				
NH_3 emissions	12	12	3	Mg
Transport	1,108,005	1,090,379	1,458,398	tkm
Digestate application				
Application	199,788	197,416	504,521	Mg
Digestate application - Emissions to air				
NH_3	5.4	5.4	1.5	Mg
NO_2	12.0	11.9	3.3	Mg
Digestate application - Emissions to soil				
K	785	785	516	Mg
Zn	1.37	1.37	1.72	Mg
As	0.66	0.66	1.93	Mg
Pb	0.05	0.05	0.02	Mg
Cd	0.04	0.04	0.01	Mg
Cu	0.07	0.07	0.09	Mg
Cr	0.18	0.18	0.04	Mg
Digestate application - Transfer to marine water				
N	69.3	68.8	18.8	Mg
P	3.2	3.2	0.7	Mg
Substituted products				
Electricity	22,196,499	27,224,630	67,415,385	kWh
Heat	24,532,973	30,090,381	74,511,741	kWh
Fertilizer N	472	469	128	Mg
Fertilizer P_2O_5-P	130	130	27	Mg
Fertilizer K_2O-K	785	785	516	Mg
Avoided emissions				
Avoided CO^2 carbon sequestration	1253	869	2832	Mg CO_2 eq.
Avoided emissions from mineral fertilizer application				
To soil				
As	0.011	0.011	0.002	Mg
Cd	0.019	0.019	0.004	Mg
Cr	0.162	0.162	0.034	Mg
Cu	0.032	0.032	0.007	Mg

Table 3 (*continued*)

Scenario	BioS1	BioS2	BioL1	
Item	Amount			U.M.
Pb	0.005	0.005	0.001	Mg
Zn	0.284	0.284	0.060	Mg
K	785	785	516	Mg
To marine water				
N	69.3	68.8	18.8	Mg
P	3.2	3.2	0.7	Mg
Seaweed bioextraction				
P	137	137	29	Mg
N	796	796	293	Mg
K	785	785	516	Mg
Zn	1.37	1.37	1.72	Mg
As	0.66	0.66	1.93	Mg
Pb	0.05	0.05	0.02	Mg
Cd	0.04	0.04	0.01	Mg
Cu	0.07	0.07	0.09	Mg
Cr	0.18	0.18	0.04	Mg
Avoided N_2O	6.28	6.28	2.31	Mg

different aspects with a view to a circular regenerative economy:

- Climate Change (CC), midpoint category calculated by the ReCiPe methodology v.1.06 (Goedkoop et al., 2013); impacts quantified in kg CO2 eq.
- Cumulative Energy Demand, total (CED-T) and fossil (CED-F) (Frischknecht et al., 2007) expressed in MJ.
- Marine eutrophication (ME), midpoint category calculated by ReCiPe v.1.06 (Goedkoop et al., 2013), impacts quantified in kg N eq.
- Phosphorus-limited marine eutrophication (ME-PLim), midpoint category based on freshwater eutrophication (Seghetta et al., 2016b), impacts quantified in kg P eq.
- Human toxicity, cancer (HT-C) and non-cancer (HT-NC), calculated by the USEtox methodology v1.01 (Rosenbaum et al., 2008). Impacts quantified in comparative toxic units (CTUh), namely the estimated increase in morbidity in the total human population per unit mass of chemical emitted, assuming equal weighting between cancer and non-cancer (Rosenbaum et al., 2008).

The impact category Climate Change was selected to address the possibility of the system becoming CO_2 neutral or negative, thus providing climate change mitigation as a service. Bioextraction of carbon during seaweed growth reduces atmospheric CO_2 through a high exchange rate at the water surface. However, part of the bioextracted carbon is released during the conversion processes for biogas or protein production. In the energy pathway, digestate application results in an accumulation of carbon in soil lasting more than 100 years and therefore causing a net reduction in atmospheric CO_2 (Seghetta et al., 2016c).

Cumulative Energy Demand was selected to evaluate the energy efficiency of the biobased production system. CED-T is direct and indirect energy used throughout the life cycle and sums the six energy categories: 1) non-renewable, fossil, 2) non-renewable, nuclear, 3) non-renewable, biomass (i.e. primary forests), 4) renewable, biomass, 5) renewable, wind, solar, geothermal, 6) renewable, water. CED-T is the sum of the six categories, and CED-F is the fossil fraction of CED-T.

N- and P-limited Marine Eutrophication was selected to evaluate seaweed production with a view to circular nutrient management (Seghetta et al., 2016b).

Human toxicity was selected to identify critical flows of micropollutants in order to underpin the need of upcycling technologies as a risk management tool to avoid externalities in a circular economy.

3. Life cycle impact assessment

The results for the five scenarios are shown in Table 7.

A scenario showing a value greater than zero indicates an increase of the environmental/health impacts compared to a situation where no seaweed is cultivated and processed. A scenario showing a value lower than zero indicates environmental/health improvement compared to a situation without seaweed cultivation and processing. In case of cumulative energy demand, a positive value corresponds to energy consumption while negative value is avoided energy consumption. The five scenarios present a net impact reduction on CC, ME and ME-PLim. All scenarios have a net consumption of energy showed by CED-T while BioL1 is the only scenario that has a net negative CED-F (Table 7). All scenarios increase the impact on human health for both HT-C and HT-NC except scenario ProL1 which reduces HT-NC (Table 7).

3.1. *Climate change - Fig. 4a*

Biogas production from *Laminaria digitata* dried (BioL1) is the best performing scenario, scoring $-18.7*10^2$ kg CO_2 eq./ha, followed by ProL1 scenario, $-12.3*10^2$ kg CO_2 eq./ha (Table 7 and Fig. 4a). However all scenarios present an impact reduction on climate change.

In the energy production pathway the most relevant contribution is substitution of energy corresponding to 67–92% of avoided impacts, with electricity more relevant than heat (71% of the avoided impact) due to the fraction of the electricity mix in Denmark produced by coal combustion. The second most relevant is the substituted NPK fertilizer due to digestate application on fields, 3–24% of avoided impacts. Among the impacts, the most relevant is the cultivation phase which is mainly affected by the plastic material composing the cultivation lines (Fig. 4a). The impact of cultivation is the same for the five scenarios since the material and energy required is independent of the selected species. *Laminaria digitata* achieves a higher impact reduction than *Saccharina latissima* mainly due to two reasons: 1) almost double the content of dry matter, i.e. 28.3% vs 14.8% and 2) higher methane yield (Table 5) (Alvarado-Morales and Angelidaki, 2016). Higher production of biogas in BioL1 leads also to higher emissions in the production and use phase of biogas compared to BioS1 and BioS2,

Table 4
LCI of the two scenarios modeled for the protein production pathway showing inputs and outputs of one cultivation cycle in 208 km^2 of sea cultivated with seaweed.

Scenario	ProS1	ProL1	U.M.
Item	Amount		
Seaweed cultivation			
Cultivated area	20,833	20,833	ha
Transport by boat			
Transport	12,672,201	12,672,201	tkm
Partial drying			
Water reduction	169,421	134,342	m^3
Transport by truck			
Transport	259,905	496,980	tkm
Chopping			
Chopping machine	3	3	p
Electricity consumption	769,152	1,470,743	kWh
Storage	38,458	73,537	m^3
Hydrolysis			
Agitators consumption - Electricity	28,591	54,671	kWh/yr
Feeding system consumption - Electricity	5815	11,120	kWh/yr
Slurry pump consumption - Electricity	808	1544	kWh/yr
Heat consumption	136,264	260,558	kWh/yr
Microalgae growth			
$CaCl_2$	0.8	1.6	Mg
$Ca(NO_3)_2$	1.7	3.2	Mg
$MgSO_4$	4.2	0.4	Mg
EDTAFeNa	0.2	0.4	Mg
H_3BO_3	0.2	0.4	Mg
$NaNO_3$	6.6	12.7	Mg
Na_2HPO_4	3.0	5.7	Mg
Agitators consumption - Electricity	223,046	426,500	kWh/yr
Feeding system consumption - Electricity	45,365	86,746	kWh/yr
Slurry pump consumption - Electricity	6301	12,048	kWh/yr
Net water consumption	61,870	118,305	Mg
Dewatering			
Spiral plate centrifuge - Electricity	315,703	603,675	kWh/Mg DW
Rotary pressure filter - Electricity	1,934,504	3,699,085	kWh/yr
Heat dryer - Electricity	13,094,263	25,038,353	kWh/yr
Protein distribution			
Transport - truck	6,658,279	12,731,710	tkm
Substituted producs			
Protein - soy	4535	8259	Mg
Services			
CO_2 sequestration	25,720	48,762	Mg CO_2 eq.
Seaweed Bioextraction			
P	137	29	Mg
N	796	293	Mg
K	785	516	Mg
Zn	1.37	1.72	Mg
As	0.66	1.93	Mg
Pb	0.05	0.02	Mg
Cd	0.04	0.01	Mg
Cu	0.07	0.09	Mg
Cr	0.18	0.04	Mg
Avoided N_2O emissions	6.28	2.31	Mg

Table 5
Parameters utilized for calculation of methane yield in the three scenarios.

Scenario	Species	Storage method	Methane yield	Total solid	Volatile solid
			mL CH4/g VS	% of FW	% of TS
BioS1	*S. latissima*	Dry	258.82	14.8%	62%
BioS2	*S. latissima*	Silage	317.45	14.8%	70%
BioL2	*L. digitata*	Dry	308.19	28.3%	91%

mainly due to the emissions of uncombusted methane in the cogeneration process. The ensilage process in scenario BioS2 results in a better performance of scenario BioS1 due to a higher methane yield (+23%, Table 7) showing a promising application of this technology for biogas production.

In the protein production pathway the most relevant contribution is the temporary sequestration of carbon in the protein-rich fish ingredient. Here the balance between CO_2 uptake during seaweed growth and CO_2 released during heterotrophic growth of microalgae is accounted for, plus organic compounds accumulated in microalgae biomass (e.g. proteins). Not all compounds present in the microalgae biomass have been quantified by the laboratory analysis so this value has to be considered conservative. The carbon sequestration in scenarios BioS1, BioS2 and BioL1 is much lower

Table 6
Total production of seaweed in dry weight (DW) and wet weight (WW), biogas, methane content, digestate, fish feed ingredient (5% moisture content) and protein content for 208 km^2 of sea cultivated with seaweed.

	Seaweed	Seaweed	Biogas	Methane	Digestate	Fish feed ingredient	Proteins
	Mg FW	Mg DW	E+3 m^3	E+3 m^3	Mg WW	Mg WW	Mg DW
BioS1	207,879	30,766	9070	5442	199,798	–	–
BioS2	207,879	30,766	11,125	6675	197,426	–	–
BioL1	207,879	58,830	27,548	16,529	504,524	–	–
ProS1	207,879	30,766	–	–	–	33,939	4535
ProL1	207,879	58,830	–	–	–	64,896	8259

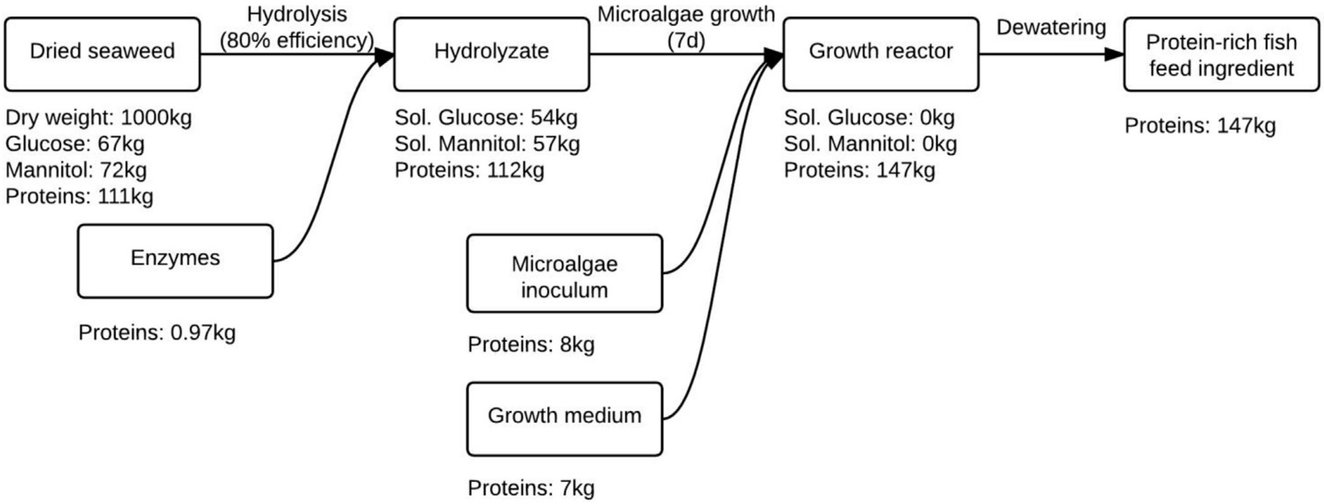


Fig. 3. Mass balance for protein and sugars within the protein production plant based on lab scale analysis (D'Este et al., 2016). Sol. Glucose and Sol. Mannitol represent the sugar fraction in solution after the hydrolysis process.

than ProS1 and ProL1 since in the first case the microbial degradation of the organic matter during 100 year after the application of digestate on fields is considered. The second most relevant contribution in ProS1 and ProL1 scenarios is the substitution of soy protein accounting for 27% of the total negative values. Among the impacts, dewatering of microalgae is the most significant (35–50%). The significant energy consumption in this phase is a known constraint for microalgae industry (O'Connel et al., 2013) which limits the sector development.

Biogas production is a better exploitation strategy than protein production for *Laminaria digitata* dried (BioL1) due to higher avoided impacts related to energy compared to protein. For *Saccharina latissima* there is not a significant difference between the energy and protein production pathways, however BioS2 is the best performing scenario.

3.2. Total cumulative energy demand - Fig. 4b

The total cumulative energy demand shows that all scenarios are net energy consumers with the best energy balance achieved by scenario BioL1, $1.7*10^4$ MJ/ha, followed by BioS2, 2.5*104 MJ/ha, and ProL1, $4.3*10^4$ MJ/ha (Table 7). All scenarios show the significant impact of seaweed drying contributing for 56–73% of energy consumption (Fig. 4b). Scenarios BioS1 and ProS1 are the highest consumer due to lower organic matter content compared to BioL1 and ProL1. Ensilage (BioS2) represents an alternative storage method able to reduce energy consumption for drying of 50% due to higher moisture content in ensilage than in dried seaweed, i.e. 75% vs 20%. The avoided energy consumption in ProS1 is comparable to BioS1 and BioS2, but the dewatering of microalgae increases the total energy consumption creating a significant disadvantage for the protein production pathway.

3.3. Cumulative fossil energy demand - Fig. 4c

The fossil energy fraction is 26–41% of total energy consumption. BioL1 has the best balance ($-1.9*10^4$ MJ/ha) between fossil energy consumed ($-3.8*10^4$ MJ/ha) and fossil energy saved ($1.9*10^4$ MJ/ha). In BioS1 and BioS2 the gap between energy used and energy substituted is reduced because the drying process consumes mainly energy from biomass combustion (Fig. 4c). Also in ProS1 and ProS2 the fossil energy consumption from drying is negligible, but they result as net energy consumers because avoided soy protein production is mostly based on renewable sources. The cultivation phase is the most energy intensive process in all scenarios (58–89%), due to production of materials of which the cultivation lines are made. In the protein production pathway, the dewatering phase has a significant contribution since it consumes electricity form the Danish mix. Our results are based on ecoinvent v3 (Weidema et al., 2013) which considers the Danish electricity production and supply mix referred to 2011, i.e. about 70% of electricity is based on fossil fuels (Itten et al., 2014). The fossil share increases to 84% when considering the total cumulative demand. The development of wind energy in Denmark is expected to improve the results in scenarios ProS1 and ProS2.

3.4. Marine eutrophication - Fig. 4d

The major contribution to reduction in ME, 57–92% of avoided impacts, is bioextraction of nitrogen during seaweed growth

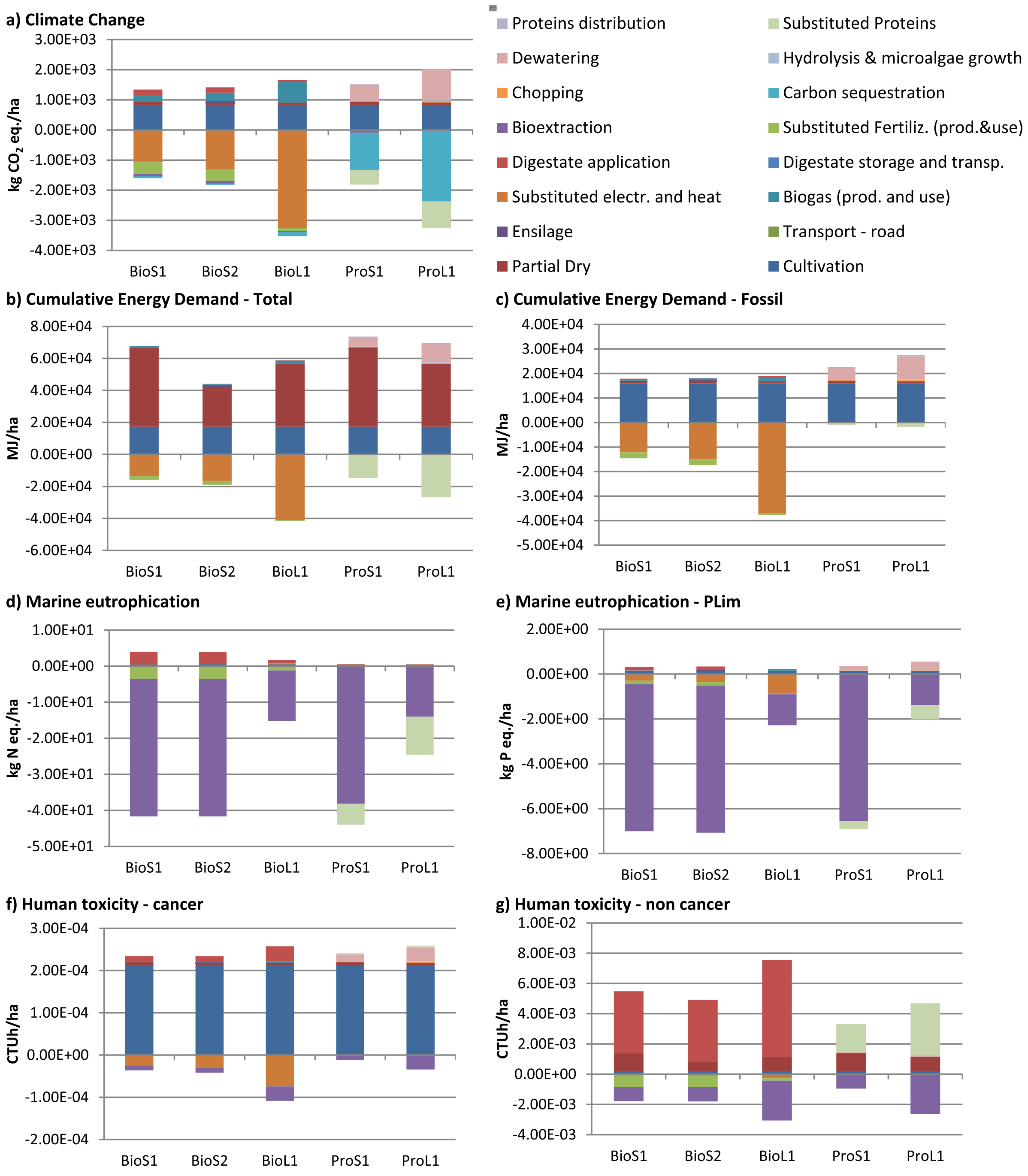


Fig. 4. Results of life cycle impact assessment of the biogas production from *Saccharina latissima* dried (BioS1) and ensilage (BioS2), biogas production from *Laminaria digitata* dried (BioL1), protein production from *Saccharina latissima* dried (ProS1) and *Laminaria digitata* dried (ProL1). The functional unit considered is 1 ha of sea cultivated with seaweed.

Table 7
Results of Life Cycle Impact Assessment for seven impact categories: climate change (CC), Cumulative Energy Demand - total (CED), Cumulative Energy Demand – fossil energy (CED-F), Marine eutrophication (ME), P-limited Marine eutrophication (ME-PLim), Human toxicity – cancer (HT-C), Human toxicity – non cancer (HT-NC). The results concern a functional unit of 1 ha of sea cultivated with seaweed.

FU 1ha	CC	CED-T	CED-F	ME	ME-PLim	HT-C	HT-NC
	E+2 kg CO_2 eq.	E+4 MJ	E+4 MJ	kg N eq.	kg P eq.	E-4 CTUh	E-4 CTUh
BioS1	−2.6	5.2	0.3	−37.7	−6.7	2.0	37.1
BioS2	−4.0	2.5	0.1	−37.8	−6.7	1.9	31.1
BioL1	−18.7	1.7	−1.9	−13.6	−2.1	1.5	45.0
ProS1	−3.0	5.9	2.2	−43.4	−6.5	2.3	23.8
ProL1	−12.3	4.3	2.6	−24.0	−1.5	2.2	20.6

(Fig. 4d). BioS1, BioS2 and ProS1 perform significantly better than BioL1 and ProL1 due to the higher content of nitrogen in *Saccharina latissima* compared to *Laminaria digitata*, i.e. 2.6% of DW and 0.5% of DW respectively (Manns et al., 2014). In the energy pathway the avoided production of electricity and heat is negligible while in the protein pathway the avoided production of soy proteins has a significant contribution, i.e. 13% in ProS1 and 43% in ProL1. The contribution of the avoided production of soy proteins results in the best performance of scenario ProS1 scoring −43.4 kg N eq./ha. In scenarios BioS1, BioS2 and BioL1 the avoided production and use of NPK fertilizers is counterbalanced by the transfer of N from agricultural fields, where digestate is applied, to marine waters.

3.5. Phosphorus-limited marine eutrophication - Fig. 4e

All scenarios present a mitigation of eutrophication of phosphorus limited marine waters, BioS1 and BioS2 were determined to be best scenarios scoring −6.7 kg P/ha. The major contribution to the avoided impacts is the bioextraction phase during seaweed growth. Higher content of phosphorus in *Saccharina latissima* compared to *Laminaria digitata* (0.44% and 0.05% of DW respectively (Supplementary Material Table S1)) makes scenarios BioS1, BioS2 and ProS1 two times better than scenarios BioL1 and ProL1. Substitution of electricity and heat is particularly relevant for scenario BioL1 (38%) due to the substituted share of coal-based electricity. In scenarios ProS1 and ProS2 the avoided production of soy proteins is offset by the electricity consumption for dewatering microalgae.

3.6. Human toxicity – cancer – Fig. 4f

In the human toxicity – cancer category, the best performing scenario is BioL1 which scores $1.5*10^{-4}$ CTUh/ha. All scenarios are equally affected by the impacts of cultivation phase, mainly due to iron bars that keep the lines submerged. According to Ecoinvent v3 database (Weidema et al., 2013) iron production emits chromium VI to the water compartment, increasing human toxicity – cancer as calculated by USEtox. The second most significant contribution is digestate application, $0.1–0.3*10^{-4}$ CTUh/ha, which increases human toxicity due to spreading of heavy metals on fields. Arsenic is more relevant in BioL1 than BioS1 and BioS2, since *Laminaria digitata* has a higher As content than *Saccharina latissima,* i.e. $3.3*10^{-5}$ kg/kg DW and $2.2*10^{-5}$ kg/kg DW seaweed (Manns et al., 2014).

Bioextraction during seaweed growth reduces the impacts for all scenarios, ranging from $-0.1*10^{-4}$ to $-0.3*10^{-4}$ CTUh/ha. Arsenic is the heavy metal with the highest impact on human toxicity cancer.

The protein production pathway is affected mainly by seaweed cultivation and microalgae dewatering, while substitution of proteins has a negligible effect on the final result. Among the two scenarios for protein production, ProL1 provided the best results. However in this assessment we do not quantify the effect of fish feed consumption. We qualitatively discuss the concentration of heavy metals in fish feed in Section 4.1.

It should be mentioned that when considering 208 km^2 of cultivation in Denmark, an increase in risk of cancer of 3–5 CTUh, i.e. 3–5 more cases of cancer in the total world human population, is obtained.

3.7. Human toxicity – non cancer – Fig. 4g

Human toxicity – non cancer shows that the best scenarios are from protein production pathway: ProL1 $20.6*10^{-4}$ CTUh and ProS1 $23.8*10^{-4}$ CTUh/ha. Cultivation of 208 km^2 of Danish water results in 43–50 more morbidities in the human population than a scenario without seaweed.

In the energy production pathway digestate application is the most relevant contribution (75–85% of the impacts) due to arsenic and zinc release on agricultural soil. The amount of metals bioextracted from seawater and released on soil is the same; however an emission of arsenic on agricultural soil has a higher impact on human toxicity since the population is more exposed through different pathways (Rosenbaum et al., 2008). As a result the digestate application has a higher impact than what bioextraction can avoid (Fig. 4g).

The substitution of soy proteins has an impact on human toxicity explained by the fact that the soy plant extracts zinc from soil during the cultivation, similarly to the bioextraction process of seaweed in seawater. Avoiding this process turns the protein substitution in a contribution to the total impact. As mentioned before, the impact of heavy metal through fish feed ingestion is not quantified in the analysis. However we discuss it qualitatively in Section 4.2.

In all scenarios the second most significant process is partial drying (12–35% of impact): the combustion of wood to produce heat releases zinc to soil and air according to the Ecoinvent v3 database (Weidema et al., 2013).

4. Discussion

4.1. Limitations regarding the heavy metals content in fish feed ingredient

Maximum concentration of heavy metals in animal feed is regulated by Directive 2002/32/EC: lead 10 mg/kg, cadmium 1 mg/kg and arsenic 40 mg/kg. We compared estimated concentrations in protein-rich fish feed for scenarios ProS1 and ProS2 (Supplementary material, Table S7). In the case of lead, all the scenarios are below the threshold: 1.4 mg/kg for ProS1 and 0.3 mg/kg for ProL1. For cadmium, ProS1 is just above the threshold (1.1 mg/kg) while ProL1 is below (0.1 mg/kg). For arsenic, both scenarios are below the threshold: 19.6 mg/kg for ProS1 and 29.8 for ProL1. The comparison show that both scenarios produce a fish feed ingredient that can be used without endangering human health according to directive 2002/32/EC.

4.2. Limitations regarding the heavy metal content in digestate

The Danish Statutory Order no. 1650 (86/278/EEC) sets thresholds for concentration of heavy metals in sewage sludge when used in agriculture. We utilize these as an indication about the feasibility of applying seaweed digestate on Danish agricultural soil. Among the regulated heavy metals in the Danish statutory order (Pb, Cd, Zn, Cu and Cr) only Cd is over the thresholds (0.8 mg/kg DW) in scenario BioS1 (1.9 mg/kg DW) and BioS2 (2.2 mg/kg DW) (Supplementary material, Table S8). However, when considering the directive 86/278/EEC the cadmium is below the threshold, 40 mg/kg DW. A method to reduce the heavy metals concentration (in particular Cd) in the digestate is the technology Bionorden (Thomsen and Johansen, 2012). Through oxidation and sedimentation processes, this technology separates the digestate into a liquid humic fertilizer and a silicate fraction containing between 79 and 96% of the heavy metals of the digestate. This technology dramatically reduces impact on human toxicity non cancer of scenario BioS1 (−74%), BioS2 (−89%) and BioL1 (−71%). However the increased energy consumption is a drawback that increases the impacts in all the other categories analyzed (Supplementary Material Table S9). For example the scenario BioS1 would increase the impact on climate change from $-2.6*10^2$ kg CO_2 eq. to

$4.1*10^2$ kg CO_2 eq (Supplementary Material Table S9) losing the mitigating service provided.

4.3. Cultivation technology improvements

The systems analyzed are still in the developmental stage therefore major improvements are expected also in the cultivation phase. A critical parameter highlighted in the analysis is the dry matter produced in 1 ha. An increase in productivity would reduce the relative impacts of the cultivation phase due to increased output of energy or proteins (Seghetta et al., 2016a). Values reported in literature estimate a production varying from 16.7 Mg WW/(ha*yr) (Hansen, 2013) to 40 Mg WW/(ha*yr) (Peteiro and Freire, 2013) for *Saccharina latissima* cultivation in Denmark and Spain which means from 2 to 4 times higher than the one considered in this analysis. Genetic improvement of seaweed biomass is another available method to enhance the characteristics with market demand (Robinson et al., 2013) for example the concentration of sugars or proteins.

The design of seeded lines can be further improved substituting the 8 mm ropes and iron weight with a stone-filled rope tested in Limfjorden, Denmark. This results in a significantly lower impact on human toxicity cancer thanks to avoided emissions of chromium from the iron production: BioS1 $0.1*10^{-4}$CTUh, BioS2 $0.002*10^{-4}$CTUh, BioL1 $-0.4*10^{-4}$CTUh, ProS1 $0.4*10^{-4}$CTUh, ProL1 $0.3*10^{-4}$CTUh (Supplementary Material Table S10).

4.4. Protein concentration

The protein concentration in the fish feed ingredient is approximately 14–15% of the total biomass input. Other studies have shown that microalgae can achieve higher protein yield than the present study (Reis et al., 2014). This can be seen as a drawback of the modeled system unless we put the results in perspective. The aim of the lab scale experiment is to demonstrate the feasibility of increasing the protein production from seaweed biomass. This result was achieved successfully. The second aim was to produce a protein rich fish feed ingredient. This can be further optimized by adding a series of separation steps to concentrate the protein in the final product. At the moment there are no available data to model such scenario. Moreover, tests should be performed on fish in order to evaluate whether the seaweed compounds can act as health enhancer. Therefore, given the innovative nature of the analysis, the results of this paper show that protein production from seaweed can be enhanced and the environmental performance of the process is comparable with other vegetable sources.

4.5. Land use change

A peculiar feature of offshore seaweed cultivation is the avoided occupation of productive land. This is a significant advantage compared to first and second generation biofuels. However, in this paper we did not integrate the land use change in the LCA analysis. Such integration is expected to improve the performance of scenarios ProS1 and ProL1, which consider the substitution of soy-based proteins. Given the innovative nature of the present paper, we decided not to include the analysis to avoid introducing a further element of uncertainty affected both by lack of data and by lack of standard methodology to quantify the impact on marine areas occupation (Taelman et al., 2014).

4.6. Sensitivity analysis

A sensitivity analysis was performed in order to evaluate the variation of the impact scores when applying a perturbation on key parameters. The selected parameters were: seaweed productivity, sugar-to-protein conversion factor (PCF) and methane yield (CH_4 yield). A perturbation of ±25% on the three parameters was applied. The results are shown in Fig. 5.

The performances of all scenarios in the climate change impact category are improved when considering a increase of the key parameters. Increase of methane yield or seaweed productivity provide the same result in scenarios BioS1, BioS2 and BioL1; the latter showing a more marked improvement due to the higher amount of seaweed dry weight produced per hectare compared to BioS1 and BioS2. Increase in PCF does not produce significant results variation in scenario ProS1. In ProL1 instead, since the amount of organic matter per hectare is higher than ProS1, the perturbation is more marked. As expected, an increase in PCF results in an improved performance of scenario ProL1.

The score of the cumulative energy demand – total increases with higher seaweed productivity. This is related to increased energy used in the drying phase that offsets the higher methane production. Only in scenario BioL1 a balance between increased energy consumption and increased energy saving is obtained, resulting in a negligible difference with the base case score. When increasing the methane yield, more energy can be substituted, therefore an improved performance of scenarios BioS1, BioS2 and BioL1 is observed. The variation in PCF does not produce significant variations in scenarios ProS1 and ProL1.

The cumulative energy demand – fossil is less dependent on the drying phase, which is based on renewable sources, therefore the increase of methane yield and seaweed productivity produces similar variations on the final score. Regarding scenarios ProS1 and ProS2, a variation of seaweed productivity and PCF does not produce significant perturbation in the final score.

In the marine eutrophication impact category an increase in seaweed productivity improves the performance of all scenarios since more nitrogen is bioextracted during the seaweed growth. Methane yield does not affect significantly the scores. Increased PCF generated more proteins resulting in a better performance of scenarios ProS1 and ProL1 compared to the base case. Identical pattern is observed for the marine eutrophication – PLim impact category.

The human toxicity – cancer impact category is not affected significantly by any perturbation of key parameters. This is due to the dependence on the material requirements in the cultivation phase, as explained in Section 3.1.6.

In the human toxicity – non cancer impact category, an increased seaweed productivity generates more digestate production which is spread on soil. As explained in Section 3.1.7, the increased heavy metals spreading results in a worsening of the performance for all scenarios. Methane yield variation does not provide significant perturbation in the score. PCF variation is significant in scenario ProL1, where an increased substitution of soy proteins decrease the performance of the scenario.

5. Conclusions

We analyzed two pathways for production of energy or proteins consisting of five scenarios based on exploitation of offshore cultivated seaweed. The energy pathway, i.e. scenario BioL1, showed better performance in climate change, total and fossil cumulative energy demand. However, all scenarios provided services in terms of mitigation of climate change. There is not a significant difference among the five scenarios regarding marine eutrophication and marine eutrophication phosphorus limited, and they all provide services in terms of mitigation of eutrophication. Human toxicity cancer identifies BioL1 as the best scenario but all scenarios can further reduce their impact (84–128%) by technical

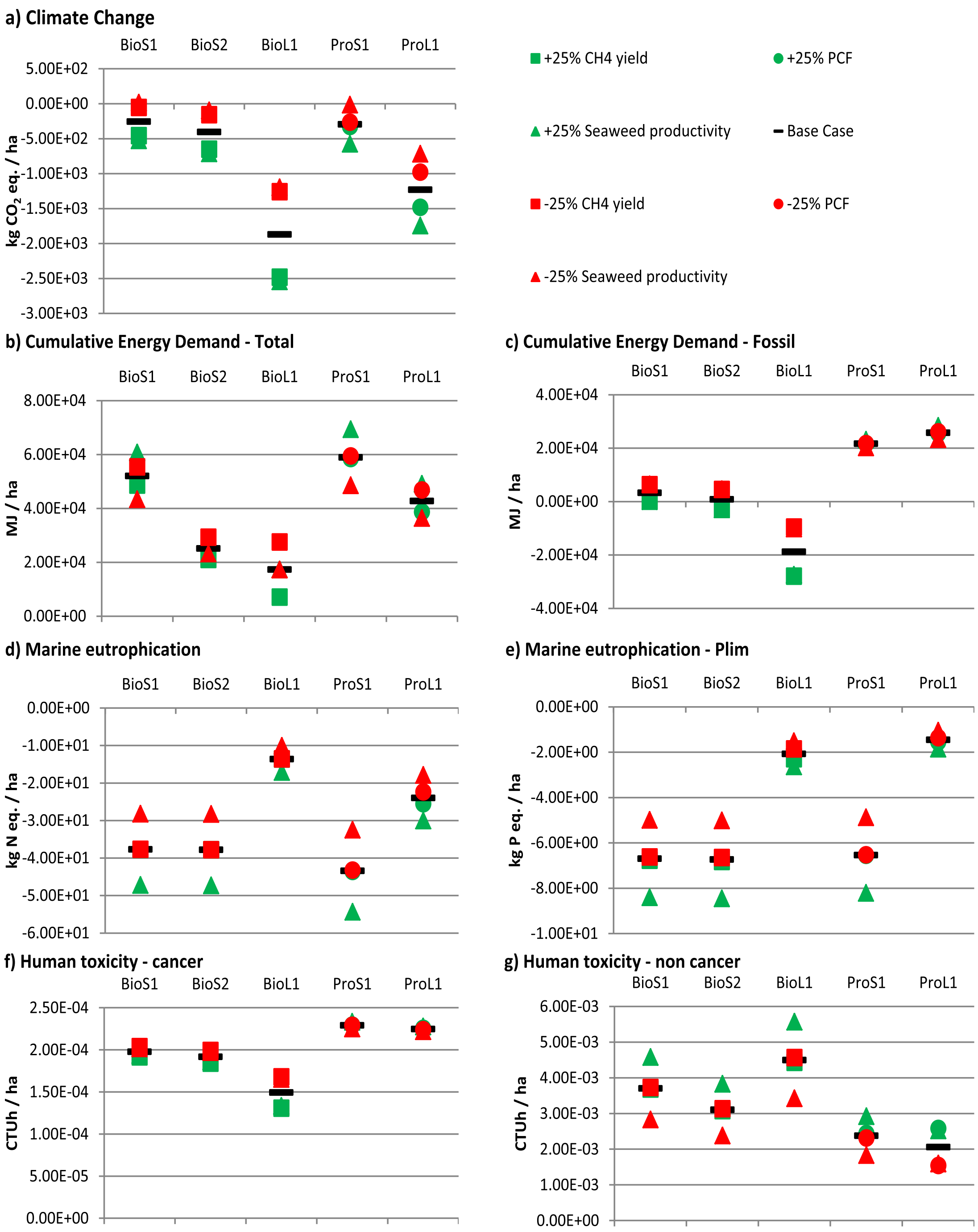


Fig. 5. Results of life cycle impact assessment of the biogas production from *Saccharina latissima* dried (BioS1) and ensilage (BioS2), biogas production from *Laminaria digitata* dried (BioL1), protein production from *Saccharina latissima* dried (ProS1) and *Laminaria digitata* dried (ProL1) when applying a variation of ±25% on methane yield (CH_4 yield), sugar-to-protein conversion factor (PCF) and seaweed productivity per hectare. The functional unit considered is 1 ha of sea cultivated with seaweed.

improvement in seeded line design. Human toxicity non cancer shows advantage in protein production pathway, scenarios ProL1 and ProS1. Even if human toxicity cancer and non-cancer categories show a net impact, the heavy metals concentration in digestate and protein-rich fish feed ingredient are below the thresholds of Danish regulations, therefore should not represent a significant threat to human health. Further research is needed to quantify the impact on human toxicity when fish is fed with the seaweed based ingredient, identifying the assimilated fraction of the heavy metals and the bioaccumulation. Special focus should be dedicated to arsenic speciation analyses.

At a scenario level, ensilage of seaweed (BioS2) performs better than drying (BioS1) in all impact categories due to higher methane yield and lower electricity consumption in drying phase, suggesting use of ensilage also for *Laminaria digitata* in order to improve performance of scenario BioL1. Seaweed drying and microalgae dewatering are two processes that need further optimization in order to reduce impacts in all categories analyzed. Upon improvement of the dewatering phase, the innovative protein production from microalgae can compete with conventional production of soy protein and reduce pressure on wild fish catches for production of fish meal. It has to be mentioned that we did not analyze the lipid content in the microalgae biomass, which could represent a valuable co-product of protein production in a similar fashion to soy protein industries.

Acknowledgments

The research was funded by the Danish Council for Strategic Research via the MacroAlgaeBiorefinery project (www.mab3.dk, project no. 11–116872) and the Graduate School of Science and Technology at Aarhus University.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jclepro.2017.02.022.

References

Adams, J.M., Gallagher, J.A., Donnison, I.S., 2009. Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. J. Appl. Phycol. 21, 569–574.

Adams, J.M.M., Toop, T.A., Donnison, I.S., Gallagher, J.A., 2011. Seasonal variation in *Laminaria digitata* and its impact on biochemical conversion routes to biofuels. Bioresour. Technol. 102, 9976–9984.

Aitken, D., Bulboa, C., Godoy-Faundez, A., Turrion-Gomez, J.L., Antizar-Ladislao, B., 2014. Life cycle assessment of macroalgae cultivation and processing for biofuel production. J. Clean. Prod. 75, 45–56.

Alvarado-Morales, M., Boldrin, A., Karakashev, D.B., Holdt, S.L., Angelidaki, I., Astrup, T., 2013. Life cycle assessment of biofuel production from brown seaweed in Nordic conditions. Bioresour. Technol. 129, 92–99.

Alvarado-Morales, M., Angelidaki, I., 2016. Biogas Production from Macroalgal Biomass and Pretreatment Effect on Their Biodegradability. In preparation.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25, 207–210.

BIM, Bord Iascaigh Mhara, 2014. The European Market for Sea Vegetables, p. 36. Report for "Farmed Irish Seaweed: An ocean wonder food?" conference.

Bruhn, A., Dahl, J., Nielsen, H.B., Nikolaisen, L., Rasmussen, M.B., Markager, S., Olesen, B., Arias, C., Jensen, P.D., 2011. Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. Bioresour. Technol. 102, 2595–2604.

Cappelli, A., Gigli, E., Romagnoli, F., Simoni, S., Blumberga, D., Palerno, M., Guerriero, E., 2015. Co-digestion of macroalgae for biogas production: an LCA-based environmental evaluation. Energy Procedia 72, 3–10.

Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour. Technol. 102, 71–81.

Chertow, M.R., 2007. "Uncovering" industrial symbiosis. J. Ind. Ecol. 11 (No. 1), 11–30.

D'Este, M., Alvarado-Morales, M., Angelidaki, I., 2016. *Laminaria Digitata* as Potential Carbon Source in Heterotrophic *Clorella Protothecoides* Cultivation. In preparation.

Danish Statutory Order no. 1650 based on Council Directive 86/278/EEC on the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge Is Used in Agriculture, OJ L 181/6.

DCE, Danish Center for Environment and Energy, 2014. Denmark's National inventory report 2014: emission inventories 1990-2012 submitted under the United Nations framework convention on climate change and the Kyoto protocol. Sci. Rep. No. 101, 1226.

DG, Danish Government, 2013. The Danish Climate Policy Plan, towards a Low Carbon Society. The Ministry of Climate, Energy and Building, Stormgade 12, DK-1220 Copenhagen K, p. 64. ISBN 978-87-93071-29-2.

Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture, 1986. OJ L 181/6.

Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources, 1991. OJ L 375/1.

Draget, K.I., Smidsrød, O., Skjåk-Bræk, G., 2005. Alginates from algae. Biopolym. Online 6, 30.

EC, European commission, 2012. Third Interim Report. ECORYS, Deltares, OCEANIC développement. Call for tenders No. MARE/2010/01. Rotterdam/Brussels. Blue Growth, Scenarios and Drivers for Sustainable Growth from the Oceans, Seas and Coasts, vol. 13, p. 126. March 2012.

EC, European commission, 2015. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Closing the Loop - an EU Action Plan for the Circular Economy. COM, Brussels (2015) 614/2.

Ekvall, T., Weidema, B.P., 2004. System boundaries and input data in consequential life cycle inventory analysis. Int. J. Life Cycle Ass. 9 (3), 161–171.

FAO Fisheries and Aquaculture Department, 2010. World Aquaculture 1010. FAO Fisheries and Aquaculture Department. Technical Paper. No. 500/1. Rome, FAO. 105 pp.

Frischknecht, R., Jungbluth, N., Althaus, H., Bauer, C., Doka, G., Dones, R., Hischier, R., Hellweg, S., Humbert, S., Köllner, T., 2007. Implementation of Life Cycle Impact Assessment Methods. Ecoinvent report.

Goedkoop, M., Heijungs, R., Huijbregts, M., De Schryver, A., Struijs, J., van Zelm, R., 2013. ReCiPe 2008, a Life Cycle Impact Assessment Method Which Comprises Harmonised Category Indicators at the Midpoint and the Endpoint Level, p. 133. Report 1, characterization. Ministry of housing, special planning and the environment.

Hansen, S.C.B., 2013. Dyrkning Og Anvendelse af Alger I Danmark. The Ocean Center Denmark [In Eng: Cultivation and use of algae in Denmark].

Havlík, P., Schneider, U.A., Schmid, E., Böttcher, H., Fritz, S., Skalsky, R., Aoki, K., De Cara, S., Kindermann, G., Kraxner, F., Leduc, S., McCallum, I., Mosnier, A., Sauer, T., Obersteiner, M., 2011. Global land-use implications of first and second generation biofuel targets. Energ. Policy 39.10, 5690–5702.

Herrmann, C., FitzGerald, J., O'Shea, R., Xia, A., O'Kiely, P., Murphy, J.D., 2015. Ensiling of seaweed for a seaweed biofuel industry. Bioresour. Technol. 196, 301–313.

Horn, S., Aasen, I., Østergaard, K., 2000. Production of ethanol from mannitol by Zymobacter palmae. J. Ind. Microbiol. Biot. 24, 51–57.

Hou, X., Hansen, J.H., Bjerre, A.B., 2015. Integrated bioethanol and protein production from brown seaweed *Laminaria digitata*. Bioresour. Technol. 197, 310–317.

IPCC, 2006. Wastewater Treatment and Discharge in Guidelines for National Greenhouse Gas Inventories, 6.1-6.28.

ISO, 2006. Environmental Management. Life Cycle Assessment. Requirements and Guidelines. ISO 14044:2006. International Organization for Standardization (ISO), Geneva, CH.

Itten, R., Frischknecht, R., Stucki, 2014. Life Cycle Inventories of Electricity Mixes and Grid. Paul Scherrer Institut (PSI), p. 230.

Jard, G., Marfaing, H., Carrère, H., Delgenes, J.P., Steyer, J.P., Dumas, C., 2013. French Brittany macroalgae screening: composition and methane potential for potential alternative sources of energy and products. Bioresour. Technol. 144, 492–498.

Kaspersen, B.S., Christensen, T.B., Fedenslund, A.M., Møller, H.B., Butts, M.B., Jensen, N.H., Kjaer, T., 2016. Linking climate change mitigation and coastal eutrophication management through biogas technology: evidence from a new Danish bioenergy concept. Sci. Total Environ. 541, 1124–1131.

Kaushik, S., Cravedi, J., Lalles, J., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, Oncorhynchus mykiss. Aquaculture 133.3, 257–274.

Kaushik, S., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, Dicentrarchus labrax. Aquaculture 230.1, 391–404.

Lam, M.K., Lee, K.T., 2012. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol. Adv. 30, 673–690.

Langlois, J., Sassi, J.F., Jard, G., Steyer, J.P., Delgenes, J.P., Hélias, A., 2012. Life cycle assessment of biomethane from offshore-cultivated seaweed. Biofuel Bioprod. Bior 6, 387–404.

Manns, D., Deutschle, A., Saake, B., Meyer, A.S., 2014. Methodology for quantitative determination of the carbohydrate composition of brown seaweeds (Laminariaceae). Roy. Soc. Chem. 4, 25736–25746.

Manns, D., Andersen, S.K., Saake, B., Meyer, A.S., 2016. Brown seaweed processing: enzymatic saccharification of Laminaria digitata requires no pre-treatment. J. Appl. Phycol. 28 (2), 1287–1294.

Marinho, G.S., Holdt, S.L., Birkeland, M.J., Angelidaki, I., 2015. Commercial cultivation and bioremediation potential of sugar kelp, Saccharina latissima, in Danish waters. J. Appl. Phycol. 27, 1963–1973.

Masuda, T., Goldsmith, P.D., 2009. World soybean production: area harvested, yield, and long-term projections. Int. Food Agribus. Manag. Rev. 12 (issue 4), 143–162.

Morgensen, L., Kristensen, T., Nguyen, T.L.T., Knudsen, M.T., Hermansen, J.E., 2014. Method for caclulating carbon footprint of cattle feeds – including contribution from soil carbon changes and use of cattle manure. J. Clean. Prod. 73, 40–51.

Naegele, H.J., Lemmer, A., Oechsner, H., Jungbluth, T., 2012. Electric energy consumption of the full scale research biogas plant "Unterer Lindenhof": results of long term and full detail measurements. Energies 5, 5198–5214. http://dx.doi.org/10.3390/en5125198.

Nielsen, M.M., Canal-Vergés, P., Petersen, J.K., Rasmussen, M.B., Bruhn, A., 2016. Co-cultivation of sugar kelp (*Saccharina latissima*) and blue mussels (*Mytilus edulis*) in Limfjorden, Denmark, using mussel long line technology. Submitted to J. Appl. Phycol.

O'Connel, D., Savelski, M., Slater, C.S., 2013. Life cycle assessment of dewatering routes for algae derived biodiesel processes. Clean. Techn. Environ. Policy 15, 567–577.

Peteiro, C., Freire, Ó., 2013. Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in the coastal bay in the Atlantic coast of Spain. J. Appl. Phycol. 25, 205–213.

Petersen, B.M., Knudsen, M.T., Hermansen, J.E., Halberg, N., 2013. An approach to include soil carbon changes in life cycle assessments. J. Clean. Prod. 52, 217–224.

Pimentel, D., 2003. Ethanol fuels: energy balance, economics, and environmental impacts are negative. Nat. Resour. Res. 12 (No.2), 127–134.

PRé Consultants, February 2008. Introduction to LCA with SimaPro. Product Ecology Consultants.

Reichhalter, H., Bozzo, A., Dal-Salvio, S., Guerra, T., 2011. Analisi energetica, ambientale ed economica di impianti a biogas in Provincia di Bolzano [In English: Energetic, environmental and economic analysis of biogas plants in Bolzano province], Relazione conclusiva. TIS innovation park, Area Energia & Ambiente, p. 106. http://www.provincia.bz.it/agricoltura/download/Bilancio_ecologico_di_impianti_a_biogas.pdf.

Reis, C.E.R., Amaral, M.D.S., Loures, C.C.A., da Ros, P.C.M., Hu, B., Filho, H.J.I., de Castro, H.F., Gianesella, S.M.F., Silva, M.B., 2014. Microalgal Feedstock for Bioenergy: Opportunities and Challenges. Chapter 17 of Biofuels in Brazil. Springer International Publishing Switzerland, pp. 367–392, 2014.

Robinson, N., Winberg, P., Kirkendale, L., 2013. Genetic improvement of macroalgae: status to date and needs for the future. J. Appl. Phycol. 25, 703–716.

Rosenbaum, R.K., Bachmann, T.M., Gold, L.S., Huijbregts, M.A.J., Jolliet, O., Juraske, R., Koehler, A., Larsen, H.F., MacLeod, M., Margni, M.D., McKone, T.E., Payet, J., Schuhmacher, M., van de Meent, D., Hauschild, M.Z., 2008. USEtox - the UNEP-SETAC toxicity model: recommended characterisation factors for human toxicity and freshwater ecotoxicity in life cycle impact assessment. Int. J. Life Cycle Ass. 13, 532–546.

Seghetta, M., Hou, X., Bastianoni, S., Bjerre, A.B., Thomsen, M., 2016a. Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers – a step towards a regenerative bioeconomy. J. Clean. Prod. 137, 1158–1169.

Seghetta, M., Tørring, D., Bruhn, A., Thomsen, M., 2016b. Bioextraction potential of seaweed in Denmark – an instrument for circular nutrient management. Sci. Total Environ. 263–264, 513–529.

Seghetta, M., Marchi, M., Bjerre, A.B., Thomsen, M., Bastianoni, S., 2016c. Modelling biogenic carbon flow in a macroalgal biorefinery system. Algal Res. 18, 144–155.

Tacon, A.G.J., Hasan, M.R., Subasinghe, R.P., 2006. Use of Fishery Resources as Feed Inputs for Aquaculture Development: Trends and Policy Implications. FAO fisheries circular. No. 1018. Rome, FAO. 99p.

Taelman, S.E., De Meester, S., Schubroeck, T., Sakshaug, E., Alvarenga, R.A.F., Dewulf, J., 2014. Accounting for the occupation of the marine environment as a natural resource in life cycle assessment: an exergy based approach. Res. Conserv. Recycl. 91, 1–10.

Thomas, B., Wyndorps, A., 2012. Efficiencies and emissions of a 192 kWelOtto engine CHP-unit running on biogas at the research station “Unterer Lindenhof”. Eng. Life Sci. 12, 306–312.

Thomsen, M., Johansen, A., 2012. From Waste to Energy, Bionorden – a Resource Efficient Technology. Danida Fellowship Center – Green Energy and Carbon Markets. Presentation.

Tørring, D., Nielsen, K.O., 2014. Manual for Cultivation of Sugar Seaweed (Saccharina Latissima) in Limfjorden, Denmark [Available in Danish: Manual til dyrkning af sukkertang (Saccharina latissima) I Limfjorden, Danmark]. Danish shellfish center, pp 12. http://www.skaldyrcenter.aqua.dtu.dk/-/media/Centre/DSC_Forskning/Forskning/De-lokale-dyder/Manual-for-dyrkning-af-sukkertang.ashx?la=da.

Van Hal, J.W., Huijgen, W.J.J., López-Contreras, A.M., 2014. Opportunities and challenges for seaweed in the biobased economy. Trends Biotechnol. 32 (5), 231–233.

Watson, L., Dring, M., 2011. Business Plan for the Establishment of a Seaweed Hatchery and Grow-out Farm, p. 41. Bord Iascaigh Mhara, Irish Sea Fisheries Board.

Weidema, B.P., Bauer, Ch., Hischier, R., Mutel, Ch., Nemecek, T., Reinhard, J., Vadenbo, C.O., Wernet, G., 2013. The Ecoinvent Database: Overview and Methodology, Data Quality Guideline for the Ecoinvent Database Version 3. www.ecoinvent.org.

Web references

HYPERLINK “http://www.MAB3.dk” \o “http://www.MAB3.dk”http://www.MAB3.dk [Last accessed: 06-09-2016]