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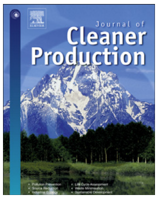

Life cycle assessment of macroalgal biorefinery for the production of ethanol, proteins and fertilizers – A step towards a regenerative bioeconomy

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ABSTRACT

Seaweed is a key biomass for the development of a biobased economy because it contains valuable components such as proteins, sugars, nitrogen and phosphorus. This paper analyses innovative offshore seaweed cultivation for the production of biorefinery feedstock. The biomass is converted into three products: bioethanol, liquid fertilizer and protein-rich ingredient for fish feed. We performed comparative life cycle assessment of a base case and six alternative production scenarios in order to maximize the benefits and minimize the trade-offs in environmental performance of future macroalgal biorefineries (MABs). The results show that the base case provides a net reduction in climate change factors, i.e. $-0.1 \cdot 10^2$ kg CO_2 eq. per ha of sea cultivated despite a cumulative net energy demand of $3.9 \cdot 10^4$ MJ/ha, 13% of which originates from fossil sources. Regarding the environmental performance of the system, we obtained a reduction in marine eutrophication of −16.3 kg N eq./ha, thanks to bioextraction of nitrogen. For the base case the net impact on human toxicity (carcinogenic effects) was $2.1 \cdot 10^{-4}$ comparative toxic units per ha of cultivation. The increase in human toxicity is seven times greater than the system can deal with, however reduction of materials for the cultivation lines, i.e. iron ballast, reduces human toxicity to $0.2 \cdot 10^{-5}$ comparative toxic units. Externalities from the use of biofertilizer affect the non-carcinogenic effects of the system, resulting in $20.3 \cdot 10^{-4}$ comparative toxic units per ha. Hotspots in the value chain show that biomass productivity is the main constraint against being competitive with other energy and protein producing technologies. Minor changes in plant design, i.e. use of stones instead of iron as ballast to weight the seeded lines, dramatically reduces human toxicity (cancer). Including engineered ecosystem services in the LCA significantly improves the results. As such, an increase in soil carbon stock represents 15% of the climate change mitigation provided by the MAB system. The study shows that MABs can contribute to a regenerative circular economy through environmental restoration and climate mitigation.

1. Introduction

Our fossil-based society is not environmentally sustainable, because it produces waste at a higher rate than nature is able to absorb. A biobased economy is proposed by the scientific community and the European Parliament as a way to move towards a more sustainable society. The European Commission argues that securing growth and jobs in Europe can be achieved only by increasing resource efficiency, creating a circular economy able to reduce waste generation and using waste as a resource (EC, 2011; EC, 2015). The biorefinery concept is a technical application of this principle in which exploitation of biomass is enhanced beyond bioenergy production. In the biorefinery, individual steps

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of the value chain mimic natural stepwise exploitation of biomass in order to exploit all available compounds in the biomass. If all of the bioresource is used, zero waste production is achieved.

Offshore cultivation of seaweed (macroalgae) relies on unexploited biomass to overcome the problems of first generation biofuels in terms of land occupation and competition with food (Naik et al., 2010). The fact that it grows in the sea makes it unique among energy crops. During growth, seaweed acts as a bio-filter, bioextracting excess nitrogen, phosphorus, carbon dioxide and pollutants, such as heavy metals. When harvested, such substances are removed from the aquatic system, thereby improving the quality of coastal and marine waters. In this way, manmade emissions are used as a resource for biomass production, engineering ecosystem services such as support for nutrient cycling and regulation of climate (Millennium Ecosystem Assessment, 2005). Nitrogen, phosphorus and biomolecules may replace fossil-based products; however, the flow of micropollutants in the seaweed value chain from the raw harvest to valuable bioproducts needs to be secured to avoid recirculation of technical nutrients, i.e. micropollutants, into the natural environment. Only by holistic analysis can the pathways and quantities involved be appreciated. Life Cycle Assessment (LCA) is one of the available scientific methods for understanding the impacts of bioextractive production systems on the environment and human health.

There are currently several projects to develop and explore designs for seaweed production and biorefinery systems (e.g. www.MAB3.dk, www.seafarm.se) but so far no company has upscaled the concept. At lab-scale, different kinds of seaweed have been successfully converted into bioethanol or biogas (Jang et al., 2012; van der Wal et al., 2013) and have shown potential for ethanol fermentation due to their high carbohydrate content. Besides sugars, seaweed contains biological molecules with special value for biobased production systems and potential as substitutes for scarce resources such as proteins and fertilizers. Protein can be marketed as optimal protein meal for aquaculture, 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 substitutes for animal proteins (FAO, 2010). Soy bean and pea-derived proteins are already marketed in significant amounts in the feed and food sectors. Proteins extracted from seaweed may therefore be an attractive alternative protein resource on the green market.

Very few LCA studies have been performed on macroalgal biorefinery systems focused on energy production (Alvarado-Morales et al., 2013; Fry et al., 2012; Aitken et al., 2014). The present study takes a step towards more inclusive and complete assessment through a cradle-to-grave approach, including the use and end-of-life phases of the products and enabling assessment of the net positive or negative impact of macroalgal biorefinery systems on the environment and human health. Positive impact and benefits are obtained from process-engineered ecosystem services such as circular nutrient flows from seaweed production and harvest. The LCA is based on the latest data on seaweed cultivation and processing in northern Europe.

2. Materials and methods

2.1. Goal and scope definition

The goal of the analysis is to evaluate the impacts and/or benefits of an innovative macroalgal biorefinery concept. The macroalgal biorefinery presents an integrated production of bioethanol, liquid fertilizers and protein-rich ingredients for fish feed. The study aims at identify the critical features influencing resource efficiency and environmental performance of the system, in order to provide decision support for macroalgal biorefinery industries. Focus of the study is on reduction of environmental impacts, such as climate change mitigation and bioremediation, human health and energy consumption.

2.2. System description

The system modeled includes the whole value chain of bioresource flows from production of biomass, through conversion in the biorefinery, to production of protein-rich ingredients for fish feed, bioethanol, and liquid fertilizer (Fig. 1).

The system represents Danish conditions and includes a production cycle based on cultivation of seaweed. Using the latest reliable data on macroalgae biorefining under Danish conditions, five keys characteristics can be considered to define the base case scenario (BC):

1- Productivity: average productivity of 10 Mg fresh weight (FW) per hectare;
2- Species: *Laminaria digitata* harvested in Denmark;
3- Conversion technology: bioethanol production using separate hydrolysis and fermentation (SHF);
4- Season: seaweed harvested in summer;
5- Cultivation design: seeded lines 8 mm in diameter.

We varied the five key characteristics of our BC scenario and modeled six alternative scenarios to explore ways to improve the environmental performance of the system:

- A1 and A2 – Productivity: evaluating low (A1) and high (A2) productivity scenarios;
- A3 – Species: *Saccharina latissima* cultivation instead of *Laminaria digitata*;
- A4 – Conversion technology: simultaneous saccharification and fermentation (SSF) instead of SHF;
- A5 – Season: spring harvest instead of summer harvest;
- A6 – Cultivation design: hollow rope filled with stones instead of 8 mm seeded line;

Key features of the BC and alternative scenarios A1 to A6 are summarized in Table 1.

It was not possible to obtain biomass characterization on *Saccharina latissima* harvested in summer, therefore scenario A3 varies both species and harvest season compared to the base case scenario.

The system is modeled on 208 km^2 of Danish marine water which could be occupied by seaweed cultivation. This is a short term estimation of large-scale seaweed production systems based on a 20-year trend in offshore wind farming development (Seghetta et al., 2016a) (Supplementary material, Fig. S1). The results refer to a functional unit of 1 ha of sea under cultivation.

LCA is performed according to international standard ISO 14040-44 (ISO, 2006) using system expansion to include multiple products according to a consequential approach (Ekvall and Weidema, 2004) and services provided by the biobased production system. 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).

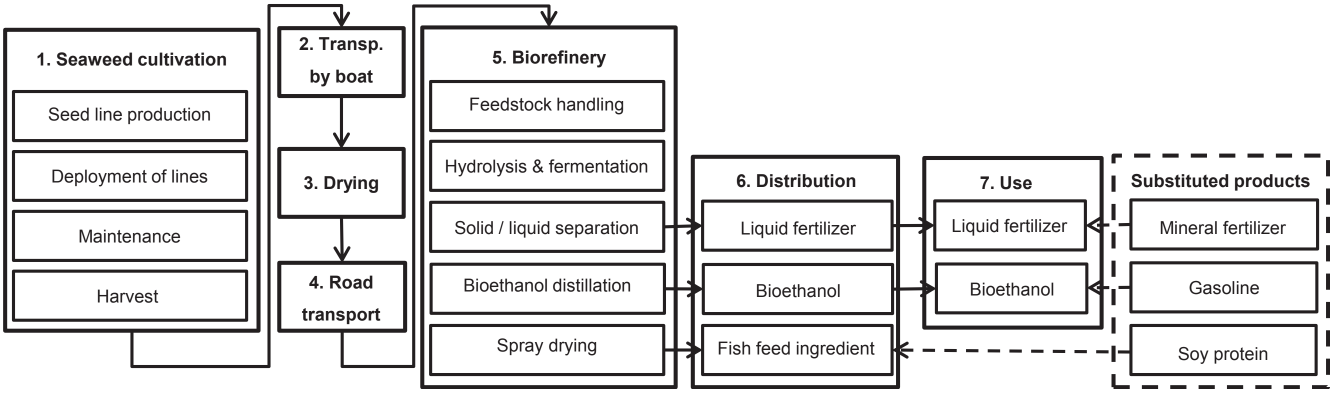


Fig. 1. Scheme of the seven-phase system analyzed: 1) seaweed cultivation composed of seed line production, deployment of lines, maintenance during the growth phase and harvest, 2) water transport of seaweed biomass from cultivation sites to harbor, 3) drying of biomass, 4) road transport from harbor to biorefinery, 5) biorefinery consisting of 5 main steps, i.e. feedstock handling, hydrolysis and fermentation, separation of solid and liquid phases from fermentation broth, distillation of liquid phase to obtain 99.5% (w/w) ethanol, and spray drying of the solid phase for protein recovery, 6) distribution of bioethanol, liquid fertilizer and fish feed ingredient, 7) use of liquid fertilizer and bioethanol. The dashed line encloses substituted products, i.e. gasoline, mineral fertilizer and soy protein.

Table 1
Summary of the key characteristics used for the base case scenario and the variations applied in the six alternatives marked in bold.

Key characteristics	Scenarios						
	BC-Base case	A1- Low prod	A2- High prod	A3- Species	A4- Conversion	A5- Season	A6- Cult. design
Productivity	Average	**Low**	**High**	Average	Average	Average	Average
Species	*L. digitata*	*L. digitata*	*L. digitata*	***S. latissima***	*L. digitata*	*L. digitata*	*L. digitata*
Biorefinery	SHF	SHF	SHF	SHF	**SSF**	SHF	SHF
Cultivation design	8 mm rope	8 mm rope	8 mm rope	8 mm rope	8 mm rope	8 mm rope	**Stone rope**
Season	Summer	Summer	Summer	**Spring**	Summer	**Spring**	Summer

2.3. Life cycle inventory

The Life Cycle Inventory (LCI) for the system includes seven main phases as shown in (Fig. 1):

1- offshore cultivation of seaweed;
2- water transport from cultivation site to harbor;
3- drying of harvested biomass;
4- road transport from harbor to biorefinery;
5- conversion of biomass into protein-rich fish feed ingredient, bioethanol and liquid fertilizer;
6- distribution of bioethanol, fish feed ingredient and liquid fertilizer;
7- use of the products:
    a. Use of bioethanol;
    b. Use of liquid fertilizer.

The BC scenario converts 60,285 Mg DW of biomass into 12,053 Mg ethanol, 7676 Mg protein-rich fish feed ingredient and 29 Mg phosphorus in the form of liquid fertilizer (Table 2). The data used for LCI is presented in Tables 3 and 4.

2.3.1. Phase 1. Offshore cultivation of seaweed

The first phase of the value chain of the seaweed production and biorefinery system 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 very similar to the one currently used in Ireland for cultivating *Palmaria palmata* (Watson and Dring, 2011).

The seaweed production system proceeds in four steps: 1a seed line production, 1b deployment of lines, 1c maintenance during the growth phase and 1d harvest (Fig. 1). In the first phase, kuralon twines are seeded with seaweed spores and incubated in a cold room where nutrients and sterile water support their growth. In the second phase 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. The combination of seeded kuralon twines, support ropes and weights constitutes

Table 2
Total production of seaweed DW, bioethanol, protein-rich fish feed and liquid fertilizer for 208 km^2 of sea cultivated with seaweed.

	Scenarios						
	BC- Base case	A1- Low prod	A2- High prod	A3- Species	A4- Conversion	A5- Season	A6- Cult. design
Seaweed (Mg DW)	60,285	40,821	79,748	30,766	60,285	60,285	60,285
Bioethanol (10^6 MJ)	301	204	399	20	216	46	301
Protein-rich fish feed (Mg)	7,676	5,198	10,154	10,906	7,676	20,486	7,676
Liquid fertilizer (Mg P)	29	20	39	137	29	211	29

Table 3
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	Value	Unit/208 km^2	Life time	Material
Seed line production				
Collection of fertile material				
Fuel for car	83,332	L	1	Diesel
Tank for seawater	1,042	kg	10	PET
Spore release				
Plastic jug	2,083	kg	5	PET
Autoclave	312,494	kWh	1	Electricity mix DK
Refrigerator	97,482	kWh	1	Electricity mix DK
Preparation of collectors				
Block of collectors	83,332	kg	5	PEHD
Kuralon twine	223,954	kg	1	Polyvinyl alcohol
Gas	19,791	kg	1	Natural gas
Seeding of lines				
Spray bottle	833	kg	5	PET
Nursery phase				
Electricity – Air pump	30,624	kWh	1	Electricity mix DK
Sand	46,295	kg	1	Sand
Mechanical filter (1–5 μm)	10,800	kg	1	Polypropylene
UV filter (Bulb)	18.7	Piece	1	Light emitting diode
Electricity – Water pump	7,650	kWh	1	Electricity mix DK
Electricity – Sand filter	4,612	kWh	1	Electricity mix DK
Electricity – UV filter	34,374	kWh	1	Electricity mix DK
F2 medium	49,999	L	1	Mix of substances
Deployment of lines				
Screw anchor	56,249	kg	20	Iron
Black buoys	890,608	kg	8	Polyethylene
Thin rope	481,241	kg	1	Nylon
Concrete block	8,333,170	kg	20	Concrete
Iron bars[a]	1,874,963	kg	5	Iron
Cable ties[a]	56,249	kg	1	Polyamide
Ropes for buoys	187,496	kg	1	Polypropylene
Headline rope (HL)	202,720	kg	13	Polypropylene
8 mm rope[a]	687,487	kg	5	Polypropylene
Concrete block rope	312,494	kg	1	Concrete
Boat use	145,830	tkm		
Alternative – rope for spores (SL)[b]				
Stone rope	1,774,965	kg	5	Polypropylene
Maintenance				
Boat use	145,830	tkm		
Harvest				
Industrial bags	22,321	kg	1	Polypropylene

[a] These inputs are not considered in alternative scenario A6.
[b] This input is only considered in alternative scenario A6.

the seeded lines (SL) (Fig. 2). In the alternative scenario A6, the seeded lines are designed differently, being hollow ropes filled with stones instead of 8 mm ropes weighted with iron bars (Fig. 2). In scenario A6 the seeded kuralon twine is coiled around the stone-filled rope.

The seeded lines are deployed in the sea in September and harvested in summer for the BC and the alternative scenarios A1, A2, A4 and A6, whereas the seaweed is harvested in spring in A3 and A5. The data necessary to model the engineered cultivation was obtained from a pilot cultivation site at Limfjorden, Denmark, during the growth season 2012–2013. Table 3 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 for the BC scenario (Table 5). The alternatives A1 and A2 consider low productivity, 6.8 Mg WW/ha, and high productivity, 13.2 Mg WW/ha (Table 5). The productivity data was measured at the pilot scale cultivation in Limfjorden (Denmark) and the industrial scale cultivation in Horsens Fjord (Denmark) (Seghetta et al., 2016a, 2016b).

The composition of the macroalgal biomass is based on literature studies of *Saccharina latissima* and *Laminaria digitata* harvested in Denmark; see supplementary materials (Table S1).

According to the IPCC guidelines (IPCC, 2006), 0.5% of nitrogen emissions in freshwater are naturally converted into N_2O. In this study we sustain that 0.5% of the nitrogen bioextracted from the seawater during seaweed growth should be considered avoided emission of N_2O.

2.3.2. Phase 2. Transport by boat

In large scale cultivation sites, seaweed is harvested and transported by boat. We consider the distance from the cultivation sites to the closest industrial harbor. The distances and amount of seaweed transported from each cultivation site are shown in the supplementary material (Table S2 and Fig. S1). The sum of transport by boat from cultivation sites to harbours is $12.6 \cdot 10^6$ tkm for the BC scenario (Table 4).

2.3.3. Phase 3. Drying

In order to prevent seaweed breakdown and to reduce the mass to be transported from harbor to biorefinery, a drying phase occurs

Table 4
LCI of the base case scenario BC and the six alternative systems showing inputs and outputs of one cultivation cycle in 208 km^2 of sea cultivated with seaweed.

Scenario	BC-Base case	A1- Low prod	A2- High prod	A3- Specie	A4- Conversion	A5- Season	A6- Cult. design	Unit
Item	Value							
Offshore cultivation of macroalgae								
Cultivated area	20,833	20,833	20,833	20,833	20,833	20,833	20,833	ha
Water transport								
Transport by boat	12,672,201	8,580,851	16,763,551	12,672,201	12,672,201	12,672,201	12,672,201	tkm
Drying process								
Water evaporated	132,523	89,736	175,309	169,421	132,523	132,523	132,523	Mg
Road transport								
Transport by lorry	9,229,744	6,249,827	12,209,661	4,710,352	9,229,744	9,229,744	9,229,744	tkm
Biorefinery								
Feedstock Handling – Electricity	1,827	1,237	2,417	1,548	3,032	1,827	1,827	MWh
Hydrolysis & Fermentation – Heat	1,906	1,290	2,521	973	1,810	1,906	1,906	MWh
Enzyme Production – Electricity [a]	3,861	2,614	5,107	1,970	3,861	3,861	3,861	MWh
Distillation – Heat	1,536	1,040	2,031	784	1,459	1,536	1,536	MWh
Storage – Electricity	8	5	11	4	8	8	8	MWh
Utilities – Electricity	2,271	1,538	3,004	1,159	2,271	2,271	2,271	MWh
Spray drying	2,952	1,999	3,905	4,195	1,365	7,879	2,952	Mg
Process Water	106,380	72,034	140,726	54,290	106,380	106,380	106,380	Mg
Ethanol distribution								
Transport by tanker truck	602,648	408,077	797,219	39,299	432,774	92,011	602,648	tkm
Protein distribution								
Road transport	1,003,144	1,481,443	1,959,742	2,104,849	1,481,443	3,953,849	1,003,144	tkm
Liquid fertilizer distribution and use								
Road transport	1,300,400	724,594	1,978,554	1,493,718	1,357,802	3,739,963	1,300,400	tkm
Application	150,493	101,905	199,081	80,305	157,136	157,652	150,493	Mg
Substituted products								
Light fuel oil (Consumed within the drive)	89,093,548	60,328,784	117,858,313	5,809,889	63,979,931	13,602,602	89,093,548	km
Soy proteins	2,354	1,594	3,115	3,418	2,354	6,422	2,354	Mg
Mineral fertilizer	28	19	37	130	28	211	28	Mg P
Avoided emissions								
CO_2 – Carbon stock (100yr)	3,976	2,692	5,260	1,980	4,950	3,998	3,976	Mg
N_2O from seawater	2.4	1.6	3.1	6.3	2.4	12.8	2.4	Mg
Substances bioextracted								
NO_3^-	1,328	899	1,756	3,523	1,328	7,169	1,328	Mg
P	29.47	19.96	38.99	136.57	29.47	222.15	29.47	Mg
Ba	0.55	0.37	0.73	1.21	0.55	3.09	0.55	Mg
Cr	0.04	0.02	0.05	0.18	0.04	0.36	0.04	Mg
Cu	0.10	0.07	0.13	0.07	0.10	0.24	0.10	Mg
Pb	0.02	0.01	0.02	0.05	0.02	0.05	0.02	Mg
Zn	1.77	1.20	2.34	1.37	1.77	2.97	1.77	Mg
Cd	0.01	0.00	0.01	0.04	0.01	0.01	0.01	Mg
As	1.98	1.34	2.62	0.66	1.98	3.31	1.98	Mg
Spreading of liquid fertilizers								
P	29.47	19.96	38.99	136.57	29.47	222.15	29.47	Mg
Ba	0.27	0.19	0.36	0.60	0.27	1.55	0.27	Mg
Cr	0.02	0.01	0.02	0.09	0.02	0.18	0.02	Mg
Cu	0.05	0.03	0.06	0.04	0.05	0.12	0.05	Mg
Pb	0.01	0.01	0.01	0.02	0.01	0.02	0.01	Mg
Zn	0.88	0.60	1.17	0.68	0.88	1.49	0.88	Mg
Cd	0.00	0.00	0.00	0.02	0.00	0.00	0.00	Mg
As	0.99	0.67	1.31	0.33	0.99	1.66	0.99	Mg

[a] Enzyme production. Energy consumption for enzyme production is considered equal to corn stover bioethanol production (NREL, 2011). It was not possible to retrieve specific information about production of alginate lyase, which is used to hydrolyse alginate in the laboratory.

in the port. The water content is reduced to 20%, which is the standard for commercial dried seaweed (CP kelco, Danish carrageenan production company, personal communication). Consumption of matter and energy in the drying process is based on grass drying as modelled in the Ecoinvent v3 database (Weidema et al., 2013).

2.3.4. Phase 4. Road transport

Road transport is calculated considering the distance from the harbours to the biorefinery in Kalundborg, Denmark. Kalundborg has an interconnected exchange network of energy and materials from different industrial processes, i.e. the symbiosis concept (Chertow, 2007). Moreover, Kalundborg Symbiose includes an industrial-scale bioethanol plant, Inbicon, coupled with a power plant (Larsen et al., 2012), which could increase production by including parallel macroalgal biomass fermentation. Transport routes are shown in supplementary materials (Table S2 and Fig. S1).

2.3.5. Phase 5. Biorefinery

The biorefinery model presented in this study consists of a series of consecutive steps necessary for the production of bioethanol, protein-rich fish feed and liquid fertilizer. Since this is the first industrial scale seaweed based biorefinery, energy consumption in the biorefinery system is adapted to the process design of cornstover bioethanol production (NREL, 2011).

The main processes that take place at Kalundborg are: 5a-feedstock handling, 5b hydrolysis and fermentation, 5c separation of solid and liquid phases from fermentation broth, 5d distillation

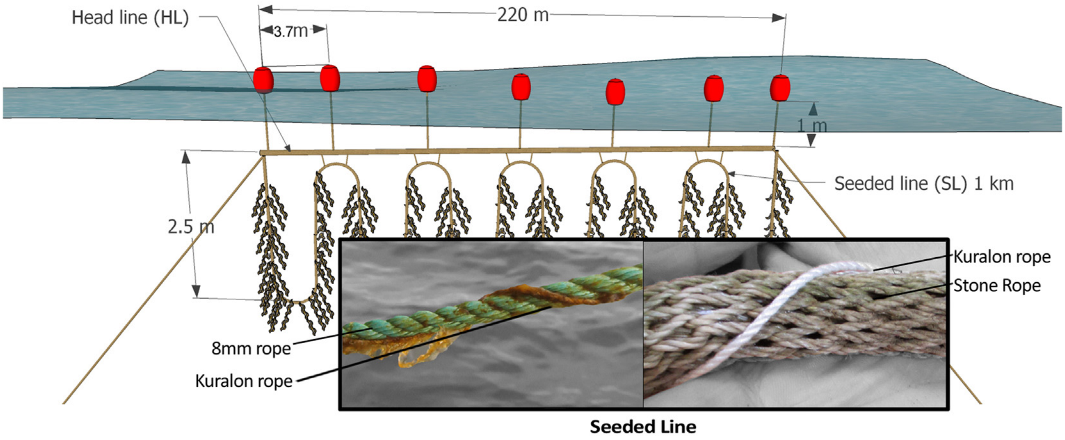


Fig. 2. Diagram of a floating seaweed cultivation system. Each cultivation line consists of a seeded line (SL) festooned in U-shaped loops on a horizontal head line (HL) (Stone rope photo courtesy of Mette Møller Nielsen, DTU Aqua).

Table 5
Summary of cultivation productivity of *Laminaria digitata* and *Saccharina latissima* in Denmark. HL = Head line, SL = Seeded line, WW = Wet weight, DW = Dry weight.

Species	Productivity	kg WW/m HL	kg WW/m SL	kg WW/line	Mg WW/ha	kg DW/m HL	kg DW/m SL	kg DW/line	Mg DW/ha
L. digitata	Average	9.1	2.0	1,996	10.0	2.6	0.6	579	2.9
	Low	6.1	1.4	1,351	6.8	1.8	0.4	392	2.0
	High	12.0	2.6	2,640	13.2	3.5	0.8	766	3.8
S. latissima	Average	9.1	2.0	1,996	10.0	1.3	0.3	295	1.5

of liquid phase to obtain 99.5% (w/w) ethanol, 5e spray drying of the solid phase for protein recovery.

Process 5a consists of simple transfer of biomass from the storage to the hydrolysis reactor. Enzymatic hydrolysis releases all available glucose without high temperature acid pre-treatment (Adams et al., 2009) or milling (Manns et al., 2015). However a chopper is used to chop up the biomass so that it can be conveyed through pipelines by water.

Process 5b consist of a separate hydrolysis and fermentation process (SHF) according to Hou et al. (2015). The electricity consumption for reactor stirring and enzyme production for hydrolysis are indicated in Table 4. A conversion efficiency of 75% of maximum theoretical yield (0.511 g ethanol/g glucose) is obtained for SHF (Hou et al., 2015). An alternative conversion process based on simultaneous saccharification and fermentation (SSF) was included in the analysis (Cardona Alzate and Sánchez Toro, 2006). Energy consumption is 5% lower than for SHF due to the lower saccharification temperature (30 °C instead of 50 °C) but bioethanol yield falls to 54% of maximum theoretical yield (Hou et al., 2015). After fermentation, the solid and liquid phases are separated, 5c, assuming that 50% of heavy metals remain with the solid while the rest remains with the liquid phase. Distillation phase 5d separates water from bioethanol which is purified by filtering to 99.5% w/w concentration through a molecular sieve (NREL, 2011). The remaining aqueous liquid fraction contains minerals, phosphorus and carbon which constitute liquid biofertilizer. The solid fraction undergoes a process of spray drying to reduce water content from 35% (NREL, 2011) to 10%, which is the average moisture content in fish meal (Masoum et al., 2012). The energy consumption of the spray drying process is estimated from dry milk protein as modeled in the Ecoinvent v3 database (Weidema et al., 2013).

The mass balance for the BC is shown in Fig. 3, and the other scenarios are included in supplementary material (Fig. S2).

2.3.6. Phase 6. Product distribution

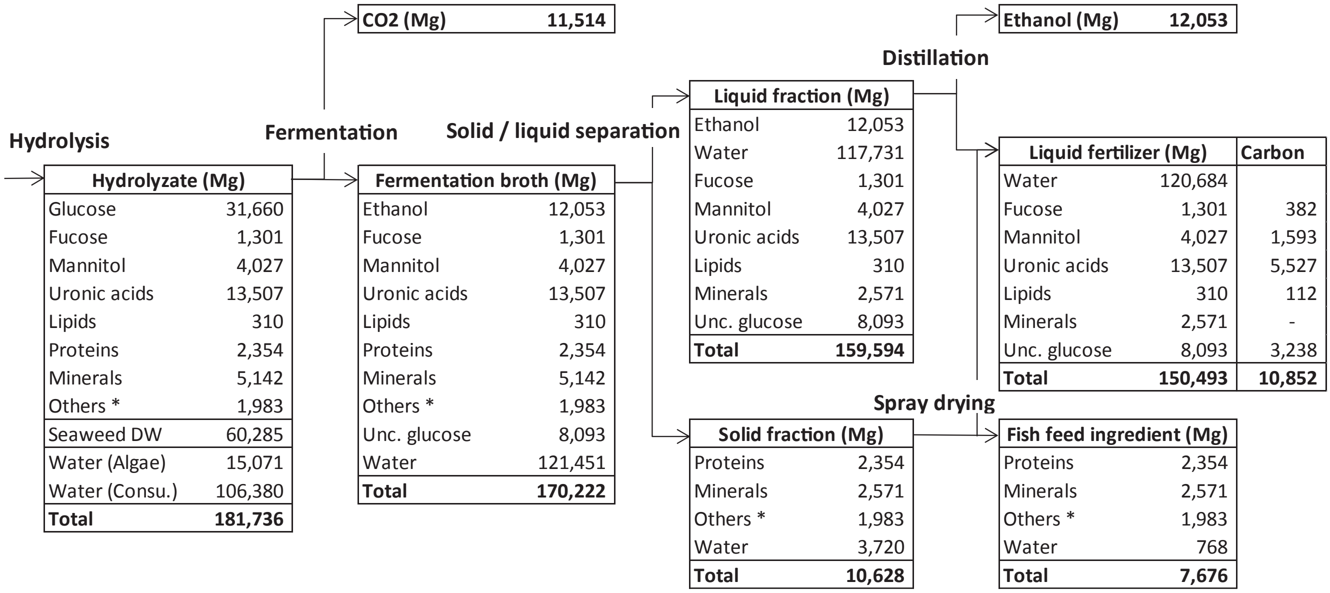
The transport distance of liquid fertilizer from biorefinery to field is proportional to the area on which P can be applied. It is 8.6 km for the BC and varies between 7.1 km (A1) to 23.7 km (A5) (supplementary materials, Table S3). The bioethanol is distributed by tanker truck considering an average distance of 50 km (supplementary materials Table S3). The end user of the protein-rich ingredient is a fish feed production facility 193 km from the biorefinery, i.e. Aller Aqua A/S in Christiansfeld, Denmark.

2.3.7. Phase 7. End users and substituted products

The liquid biofertilizer is applied to fields, substituting mineral fertilizers. The mineral fertilizer substitution ratio is 0.95 corresponding to the content of bioavailable P in sewage sludge produced using P-accumulating bacteria (Jensen et al., 2015). When applying biofertilizers, 10% of the carbon is considered undecomposed after 100 years, increasing the carbon stored in soil (Mogensen et al., 2014; Petersen et al., 2013). We set protein recovery at 100% in the biorefinery process, so there is no nitrogen in the liquid fertilizers (Fig. 3; supplementary material, Fig. S2).

The bioethanol is 99.5% w/w purity grade, substituting fossil gasoline. The gasoline substitution ratio is 0.54 kg gasoline/kg ethanol, estimated from the heating value of the two fuel types: 25 MJ/kg for bioethanol, 47 MJ/kg for gasoline. For the base case we substitute 6,480 Mg of gasoline, while for the alternative scenarios it varies from 423 Mg (A3) to 8,572 Mg (A2). We considered avoided emissions based on the consumption of 0.07 kg/km of a medium size EURO 5 car as reported in the Ecoinvent database (Weidema et al., 2013).

The protein-rich fish feed ingredient is considered to substitute plant-based proteins, i.e. soy protein, the second most widely used ingredient in the production of fish feed after fish meal. The protein substitution ratio is 1:1, the ratio of protein content of the two ingredients. The system boundaries exclude the use phase of the protein-rich fish feed ingredient, and therefore do not include fish farm consumption and waste production.



CO2 (Mg)	11,514

Ethanol (Mg)	12,053

Hydrolyzate (Mg)	
Glucose	31,660
Fucose	1,301
Mannitol	4,027
Uronic acids	13,507
Lipids	310
Proteins	2,354
Minerals	5,142
Others *	1,983
Seaweed DW	60,285
Water (Algae)	15,071
Water (Consu.)	106,380
Total	**181,736**

Fermentation broth (Mg)	
Ethanol	12,053
Fucose	1,301
Mannitol	4,027
Uronic acids	13,507
Lipids	310
Proteins	2,354
Minerals	5,142
Others *	1,983
Unc. glucose	8,093
Water	121,451
Total	**170,222**

Liquid fraction (Mg)	
Ethanol	12,053
Water	117,731
Fucose	1,301
Mannitol	4,027
Uronic acids	13,507
Lipids	310
Minerals	2,571
Unc. glucose	8,093
Total	**159,594**

Liquid fertilizer (Mg)		Carbon
Water	120,684	
Fucose	1,301	382
Mannitol	4,027	1,593
Uronic acids	13,507	5,527
Lipids	310	112
Minerals	2,571	-
Unc. glucose	8,093	3,238
Total	**150,493**	**10,852**

Solid fraction (Mg)	
Proteins	2,354
Minerals	2,571
Others *	1,983
Water	3,720
Total	**10,628**

Fish feed ingredient (Mg)	
Proteins	2,354
Minerals	2,571
Others *	1,983
Water	768
Total	**7,676**

Fig. 3. Mass balance of seaweed in the biorefinery. The scheme illustrates the conversion of glucose by fermentation and distribution of the compounds throughout the process to the three final products. *Unc. glucose* stands for unconverted glucose, *water consu*. indicates the amount of water consumed by the process per year and not recycled within the biorefinery. * The category *others* includes unhydrolyzed cell wall polymers.

2.4. Selected impact categories and methodologies

We selected a series of impact categories in order to analyze 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 CO_2 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., 2016a, 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., 2011). 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 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 bio-extracted carbon is released in the value chain. Mass balance identifies accumulation of carbon in soil lasting more than 100 years and therefore causing a net reduction in atmospheric CO_2 (Seghetta et al., 2016b).

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., 2016a).

Human toxicity was selected to identify critical flows of micro-pollutants 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 of the BC scenario and the alternative scenarios are shown in Table 6.

Net positive performance indicates that the production system has environmental impact, whereas net negative performance indicates that the system avoids impacts and offers environmental services. A net positive value of Cumulative Energy Demand means that energy consumption exceeds energy production, but scenarios BC, A2 and A6 nevertheless provide climate change mitigation (net negative CC values). The human toxicity impact categories are positive for all scenarios and marine eutrophication decreases in all scenarios.

Fig. 4a–f are explained and discussed in detail in the following sections.

3.1. Climate change- Fig. 4a

For climate change the BC scenario scores $-0.1 \cdot 10^2$ kg of CO_2 eq./ha, meaning that the system has less impact on climate than a situation without seaweed cultivation and processing (Fig. 4a). The main positive contribution to climate change is the cultivation of seaweed (65% of the impact) but its impact is compensated by avoided fossil emissions obtained by substituting fossil products with biobased products. The second highest contribution in the BC scenario (25% of positive impacts) is the biorefinery phase; the third highest is the drying of seaweed (6% of the positive impacts)

Table 6
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.ple

	CC	CED-T	CED-F	ME	ME-PLim	HT-C	HT-NC
FU – 1 ha	10^2 kg CO_2 eq	10^4 MJ	10^4 MJ	kg N eq.	kg P eq.	10^{-4} CTUh	10^{-4} CTUh
BC – Base case	−0.1	3.9	0.5	−16.3	−1.1	2.1	20.3
A1 – Low prod	2.6	3.2	0.9	−11.0	−0.7	2.1	14.4
A2 – High prod	−2.8	4.6	0.2	−21.7	−1.5	2.2	26.2
A3 – Species	6.6	6.2	1.7	−41.5	−6.3	2.2	29.3
A4 – Conversion	2.3	4.5	1.0	−16.3	−1.1	2.1	20.4
A5 – Season	3.2	4.6	1.7	−84.6	−10.4	2.0	29.4
A6 – Cult. design	−1.9	4.0	0.6	−16.4	−1.2	0.2	18.5

(Fig. 4a). Bioethanol, which can replace gasoline production and use, is the most significant benefit of the production system, amounting to 70% of all negative values, followed by carbon stock (15%) and substituted proteins (12%).

The productivity of seaweed has a significant influence on the net value of impact category CC. The A1 low-productivity scenario produces a net impact of $2.6 \cdot 10^2$ kg CO_2 eq./ha compared to $-2.8 \cdot 10^2$ kg CO_2 eq./ha for A2: high productivity transforms the seaweed production and biorefinery system into a CO_2-negative system providing CC mitigation service. A doubling in productivity from 7 (A1) to 13 (A2) Mg WW/ha results in a threefold improvement in system performance due to a combined increase in ethanol, protein and liquid fertilizer production.

If *Saccharina latissima* (A3) is grown instead of *Laminaria digitata* (BC), the total impact on climate change increases dramatically due to the lower sugar content of *Saccharina latissima* (Manns et al., 2014), which translates into lower production of bioethanol.

The 5% reduction in energy consumption for SSF (A4) is insignificant compared to the 28% reduction in bioethanol production (Hou et al., 2015), giving a net impact, i.e. $2.3 \cdot 10^2$ kg of CO_2 eq./ha, and A4 becomes CO_2-positive contributing to impact on CC (Fig. 4a).

In scenario A5, harvest in spring results in reduced sugar and increased protein content of seaweed and a shift in the main product from ethanol to protein. However, the CO_2 footprint of gasoline production is higher than the substituted soybean protein and the net value obtained for A5 is positive, i.e. $3.2 \cdot 10^2$ kg CO_2 eq./ha, meaning a burden on CC. In this scenario, N_2O emissions avoided by assimilating N emissions to water are higher than in BC, due to the higher N content of the spring harvest. Nevertheless, the performance of A5 is the second worst in terms of contribution to CC.

The impact of the cultivation phase on CC is largely affected by the materials composing the cultivation lines. In A6, consumption of materials is reduced by replacing 8 mm ropes and iron with hollow ropes filled with stones (Fig. 2) resulting in a 21% reduction in impact on CC by this phase (Fig. 4a).

3.2. Total cumulative energy demand – Fig. 4b

Total CED shows that BC has a net consumption of $3.9 \cdot 10^4$ MJ/ha, meaning that the system consumes more energy than it can substitute (Fig. 4b). The energy requirement for the system is almost three times greater than the energy saved: $6.2 \cdot 10^4$ MJ/ha and $-2.2 \cdot 10^4$ MJ/ha, respectively. The main contribution is the drying process, which consumes 63% of the energy used, followed by the cultivation phase (28%). In the latter, plastics production is the most energy intensive process. The greatest energy substitution is that of gasoline (76%), followed by protein (23%) (Fig. 4b).

Considering the alternatives, in A2 the gap between consumption and substitution increases due to higher consumption of heat during the drying process, since more biomass is produced. *Saccharina latissima* cultivation (A3) has the highest CED due to significantly lower production of ethanol. Energy saving in SSF in the biorefinery, A4, does not reduce energy consumption significantly and decreases the ethanol produced, resulting in an overall worsening of system performance compared to the BC. Similarly, A5 increases protein production but is counterbalanced by a decrease in ethanol and consequently performs worse than BC. The change in cultivation design, A6, is not significantly different from BC.

3.3. Cumulative fossil energy demand – Fig. 4c

The fossil energy fraction used by the system is 13% of total energy consumption. Net CED-F is $0.5 \cdot 10^4$ MJ per ha for the base case scenario (Fig. 4c). In this case the gap between energy used and energy substituted is reduced, but the balance is still positive (Fig. 4c). The cultivation phase is the most energy intensive process (77%), due to the energy required to produce the materials of which the cultivation lines are made; the biorefinery phase is in second place (16%). In this case the drying process is not a major contribution due to the fact that most of the heat used for drying comes from combustion of biomass. The high productivity scenario A2 is the only one that performs better than BC, and this is due to higher production of ethanol and therefore increased gasoline substitution.

3.4. Marine eutrophication – Fig. 4d

The major contribution to reduction in ME, 87–93% of the negative values, is bioextraction of nitrogen during seaweed growth (Fig. 4d). Avoided production of soybean proteins contributes a 6–13% reduction in impact on ME. No scenario has processes resulting in significant positive values for this impact category. The environmental benefit of all processes across scenarios is proportional to the nitrogen content of seaweed biomass. The best performing scenario is A5, i.e. harvest of *Laminaria digitata* in spring, which has a nitrogen content five times higher than the summer harvest. This increases the environmental benefits on marine eutrophication to a maximum of −84.6 kg N eq. (Table 6). The second best alternative is A3, explained by cultivation of *Saccharina latissima* which has a higher N content than *Laminaria digitata* harvested in August. As expected, the low productivity scenario A1 provides less benefit than BC, whereas high productivity scenario A2 provides more benefits in terms of stronger reduction of ME. Alternatives A4 (energy efficiency of saccharification and fermentation) and A6 (lower material input of cultivation design) do not significantly change environmental performance.

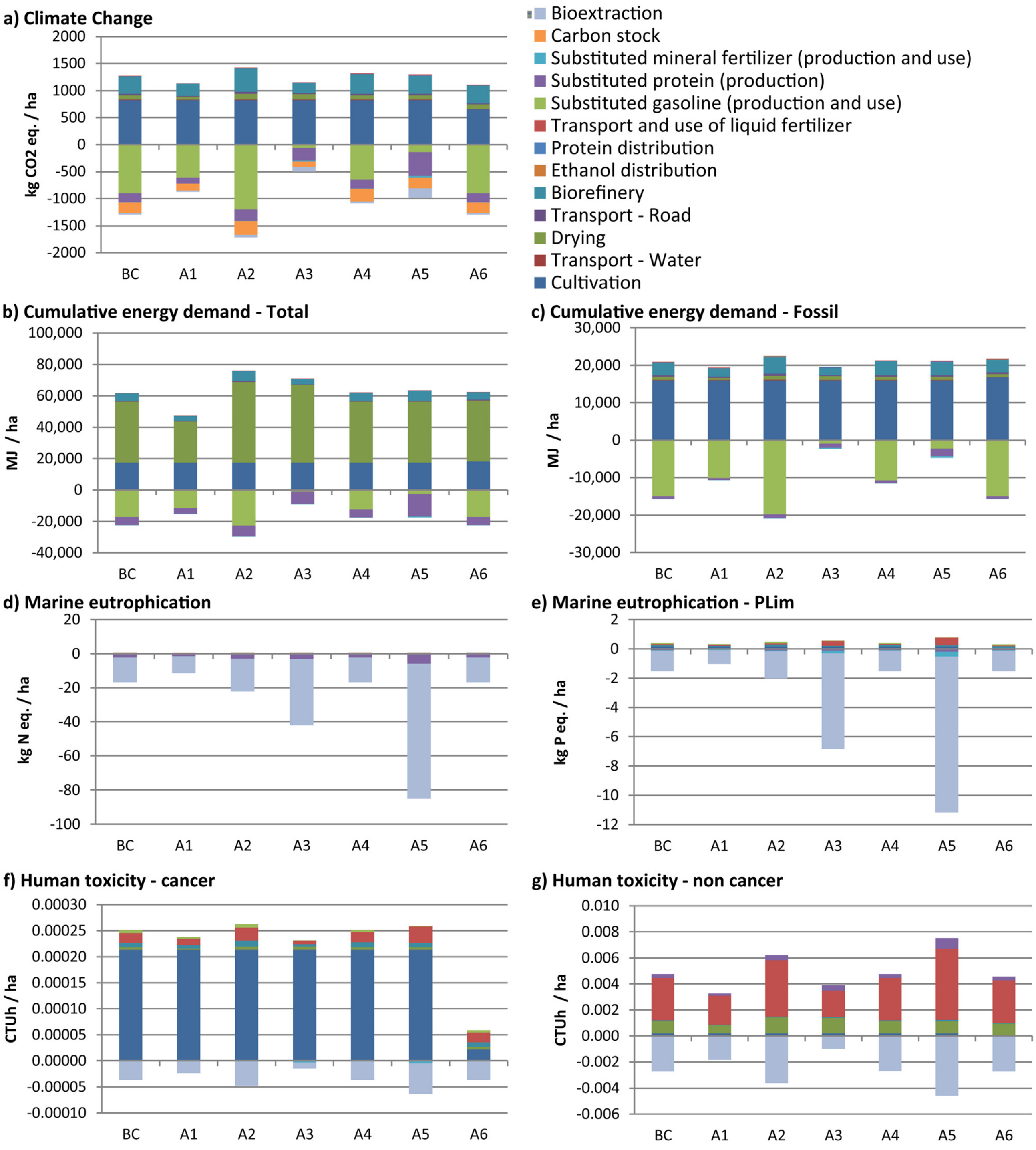


Fig. 4. Results of life cycle impact assessment of the base case scenario (BC) and the six alternatives: A1 Low productivity, A2 High productivity, A3 Species, A4 Conversion technology, A5, Seasonality, A6 Cultivation design. The functional unit considered is 1 ha of sea cultivated with seaweed.

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

The BC scenario results in a net negative impact quantified as −1.1 kg P eq./ha (Fig. 4e). The most significant contribution is bioextraction of P during seaweed growth, which varies among the scenarios from 95% to 97% of the negative values. Among the positive values, the cultivation phase contributes 18–53%, the drying phase 1–3% and biorefinery 11–47%.

The total positive contribution is 7–26% of the negative one. For ME-PLim, the impact of the different alternatives is related to biomass composition. The A5 scenario shows that *Laminaria digitata* harvested in spring has the highest potential for mitigation of marine eutrophication, quantified as −10.4 kg P eq., performing nine times better than BC. The second best alternative is *Saccharina latissima* cultivation in A3, which has a higher P content than *Laminaria digitata* harvested in summer (BC). Reduced energy consumption in the saccharification process (A4) and reduced material consumption during the cultivation phase (A6) do not significantly affect the results with respect to BC.

3.6. Human toxicity – cancer – Fig. 4f

In the human toxicity – cancer category, the base case scenario has a net value of $2.1 \cdot 10^{-4}$ CTUh/ha (Table 6). The main contribution is the cultivation phase (Fig. 4f), in particular use of iron bars to keep the lines submerged. According to the 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 positive contribution is emissions due to use of liquid fertilizer (8%), where 50% of the heavy metals bioextracted during seaweed growth is applied to soil together with the phosphorus (Pizzol et al., 2014). Arsenic has particular impact in this phase. Bioextraction during seaweed growth is the main contributor to reduction in the net value of this impact category for all scenarios, ranging from $-0.1 \cdot 10^{-4}$ to $-0.6 \cdot 10^{-4}$ CTUh/ha. The method identifies arsenic as the major heavy metal bioextracted.

The alternatives from A1 to A5 are similar to the BC scenario, since the material input for cultivation is the most significant contributor. However, the alternative design A6 presents a significant reduction of the positive values resulting in just $0.2 \cdot 10^{-4}$ CTUh/ha (Fig. 4f). This is explained by the replacement of the iron bars with the alternative hollow rope filled with stones. In this case, the impact from the material input to the cultivation design is about 10 times less than in BC scenario.

It should be mentioned that when considering the 208 km^2 cultivation area in Danish marine waters, the method quantifies an increase in risk of cancer of 0.5 CTUh corresponding to 0.5 more cases of cancer in the total world human population.

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

Human toxicity – non cancer shows that the impact of BC is $20.3 \cdot 10^{-4}$ CTUh/ha (Fig. 4g). The cultivation of 208 km^2 with seaweed in Denmark results in 42.4 additional morbidities in the human population compared to a situation without seaweed.

For BC, the most significant contribution is emissions during and after spreading of liquid fertilizer, corresponding to the 69% of the positive impacts. In particular, emission of heavy metals to soils caused by micropollutants in the biofertilizer is the main burden on human health in this impact category. Zinc and arsenic are the main contributors to health impact: 57% and 42%, respectively. The second most significant process is drying (19% of positive impact); combustion of wood to produce heat releases zinc to soil and air according to the Ecoinvent v3 database (Weidema et al., 2013). Among negative values (benefits), remediation of the marine system by bioextraction reduces the impact on human health by $-27.0 \cdot 10^{-4}$ CTUh/ha in the base case scenario.

The variations in scenarios A1 and A2 are related to the total amount of biofertilizer applied with respect to BC, giving A2 a higher impact than A1. The seasonal variation in composition of *Laminaria digitata* (A5) is observed in the higher bioextraction of arsenic compared to BC. However, it is followed by higher emissions of zinc and arsenic to soil which result in a net positive value for HT-NC. *Saccharina latissima* (A3) has a lower As/Zn ratio than *Laminaria digitata*. Since zinc has a lower impact in water than soil, the balance between bioextraction and biofertilizer application results in a net positive HT-NC, i.e. $18.5 \cdot 10^{-4}$ CTUh.

4. Discussion

4.1. Limitations regarding the heavy metal composition of seaweed

Directive 2002/32/EC sets thresholds for the concentrations of undesirable substances in animal feed. Among heavy metals, it regulates concentrations of lead (10 mg/kg), cadmium (1 mg/kg) and arsenic (40 mg/kg). We compared estimated concentrations in protein-rich fish feed for BC, A3 and A5 (Supplementary material, Table S4). In the case of lead, all the scenarios are below the threshold: 1.2 mg/kg, 2.1 mg/kg and 1.2 mg/kg for BC, A3 and A5, respectively. For cadmium only A3 is over the threshold (1.8 mg/kg) while BC and A5 are below (0.4 mg/kg and 0.2 mg/kg, respectively). For arsenic, BC and A5 are above the threshold while A3 is below: 128.9 mg/kg for BC, 80.8 mg/kg for A5 and 30.5 mg/kg for A3. The comparisons show that *Saccharina latissima* produces a fish feed ingredient with a lower concentration of heavy metals.

4.2. Energy production

The base case scenario shows an energy production of 301 TJ from bioethanol (Table 2) from 208 km^2 of sea cultivated with seaweed. In Denmark, BC would provide 0.5% of the gasoline consumed for road transport, which according to the latest statistics is 57,731 TJ (DEA, 2013). The percentage varies from 0.03% in the case of A3 to 0.7% for A2. When compared with the quantity of biofuels consumed for road transport, i.e. 8,710 TJ, BC would provide 3.5%, with variations from 0.2% for A3 to 4.6% for A2.

In our scenarios, we considered a cultivated area of 208 km^2, which is currently the area of sea occupied by offshore wind farms. Recent statistics show that offshore wind farms provided 14,381 TJ of energy in 2013 (DEA, 2013). The BC scenario can provide 301 TJ of energy from the same area of sea, equal to 2.1% of the wind energy, and ranging from 0.1% (A3) to 3% (A4). We therefore recommend coupling offshore wind farming with seaweed cultivation, rather than competing to exploit the full potential of both technologies. This solution has already proved feasible in The Netherlands (Reith et al., 2005).

4.3. System improvement perspectives

Cumulative energy demand showed that technical improvements in the drying process are required to significantly reduce total energy consumption. The drying process used in our analysis was not optimized for seaweed. Grass drying is the most similar process available in the Ecoinvent database v3 but this could lead to an overestimation of the energy consumption. At the moment, large scale production of seaweed occurs mainly in tropical areas, where it is possible to dry the biomass by leaving it in the sun. This option is not available in the Danish climate. However, small companies in Nordic countries collect beach-cast seaweed and use handmade desiccators to dry the biomass, with minimum energy consumption (cornishseaweed.co.uk). The energy consumption of the industrial grass drying process could be reduced by adapting methods to seaweed biomass, for example by setting different temperature and air flow. Since impacts from transport by boat and road are insignificant and drying can be left out in periods when it is possible to avoid storage, the environmental performance of the system could be considerably improved. Alternatively, improvements could be obtained by using a biorefinery with a process capacity equal to the daily harvest of seaweed, and/or flexible design enabling processing of different types of feedstock according to seasonal variations.

Overall, the production system lends itself to improvement with respect to environmental sustainability by increasing productivity, thus providing more bioethanol, proteins and biofertilizers to a future Danish green market. Increases in productivity are expected for *Laminaria digitata* and *Saccharina latissima* since the cultivation of seaweed in northern Europe is still in its infancy and the species have therefore not undergone genetic improvement to highlight characteristics with market demand (Robinson et al., 2013) as in the case of land crops. There is presumably significant margin for improvement since productivity in Chile (Aitken et al., 2014) and the Faroe Islands (Bak et al., 2016) is 10 times greater than in Denmark.

5. Conclusions

This study assessed seaweed production and biorefinery systems producing bioethanol, liquid fertilizer and protein-rich fish feed. Life cycle assessment identifies the ability of the system to provide climate change and marine eutrophication mitigation services. The environmental performance of the circular resource system contributes to climate change mitigation by substitution of gasoline and soybean proteins, while returning excess atmospheric and marine carbon (HCO_3^-) into soil carbon stock. Marine eutrophication mitigation is achieved by N and P bioextraction and returned to economic system in terms of biobased products.

The analysis of the five keys characteristics shows that by increasing productivity (A2) it is possible to improve the performance of the system for the CC, ME, ME-Plim, CED-T and CED-F impact categories, due to the increased amount of substituted products.

System performance improvement is also observed when the cultivation technology uses stone ropes (A6). In this case CC, HT-C and HT-NC impact categories show improved results compared to base case scenario. The present design is an innovative application for seaweed production, imitating current technology for mussel cultivation (Nielsen et al., 2015); the life time of the materials is therefore uncertain. An extended study of resistance to salt water could improve the accuracy of the analysis.

For the three net negative CO_2 performing scenarios, the results vary between $-0.1 \cdot 10^2$ (BC) and $-2.8 \cdot 10^2$ (A2) kg CO_2eq./ha. All scenarios provide water quality restoration services. As such, a net reduction of the aquatic N and P load of 11–84 kg N/ha and 1–11 kg P/ha, respectively, is obtained. The MAB3 concept represents a unique opportunity to use emissions as a resource for seaweed production: a key to a regenerative circular economy.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jclepro.2016.07.195.

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