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Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion

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ABSTRACT

The biomass production potential at temperate latitudes (56°N), and the quality of the biomass for energy production (anaerobic digestion to methane and direct combustion) were investigated for the green macroalgae, *Ulva lactuca*. The algae were cultivated in a land based facility demonstrating a production potential of 45 T (TS) ha^{-1} y^{-1}. Biogas production from fresh and macerated *U. lactuca* yielded up to 271 ml CH_4 g^{-1} VS, which is in the range of the methane production from cattle manure and land based energy crops, such as grass-clover. Drying of the biomass resulted in a 5–9-fold increase in weight specific methane production compared to wet biomass. Ash and alkali contents are the main challenges in the use of *U. lactuca* for direct combustion. Application of a bio-refinery concept could increase the economical value of the *U. lactuca* biomass as well as improve its suitability for production of bioenergy.

1. Introduction

The green macroalgae *Ulva lactuca* (Chlorophyceae) has been considered as a potential aquatic energy crop as early as in the Aquatic Species programme in the USA back in 1978–1996, thanks to its high potential growth rates and high content of carbohydrates. The conclusion then was that use of *U. lactuca* as aquatic energy crop was not economically sustainable (Ryther et al., 1984). However, the climate change agenda has caused a growing interest in renewable and CO_2 neutral energy including biofuel for combined heat and power production, which has increased the pressure on traditional biomass resources. Land based resources are limited and used for many applications including food production, energy and materials, and therefore the identification of alternative, sustainable resources such as aquatic biomass has become increasingly important. This brings macroalgae such as *U. lactuca* back in focus for production of bioenergy.

U. lactuca is common from tropical to polar climates, although the strains most likely vary among regions. Despite reports of natural growth rates up to 30% d^{-1} in northern temperate regions (Pedersen and Borum, 1996), cultivation has yet only taken place in warmer regions of lower latitudes. The species has previously been harvested from natural populations in shallow coastal areas (Cecchi et al., 1996) or cultivated in land based systems, alone or as part of integrated multitrophic aquaculture systems, see for instance Ryther et al. (1984), Neori et al. (1991), Msuya and Neori (2008) and Robertson-Andersson et al. (2008). The biochemical composition of macroalgae depends strongly on the growth conditions and thereby season (Black, 1950; Lamare and Wing, 2001). *U. lactuca* has a total solid (TS) content between 9.6% (Msuya and Neori, 2008) and 20.4% (Lamare and Wing, 2001). (TS is hereafter used as equivalent to dry weight and dry matter). Approximately, the TS consists of 27% protein, 0.3% lipids and 62% carbohydrates (Ortiz et al., 2006), but under high external nitrogen loads the protein content can exceed 40% (Msuya and Neori, 2008). The carbohydrates of *U. lactuca* are predominantly in the form of the complex hydrocolloid ulvan (8–29% of TS), a sulphated glucuronoxylorhamnan, which together with cellulose are structural components of the cell wall (Lahaye and Robic, 2007) and starch, which is used as intracellular energy storage.

Today most of the naturally produced and harvested *U. lactuca* biomass is an unused resource. The algae can be incorporated into compost and spread on fields as enriching agents, but is mainly dumped or left stranded to decompose on the shore creating waste

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problems (Morand et al., 2006). Conversion of *U. lactuca* into bioenergy has only been described to a limited extent and primarily as substrate for anaerobic digestion to biogas. Fermentation of *U. lactuca* carbohydrates into bioethanol for automobiles would be advantageous as the transport sector has problems with reducing its CO_2-emmisions. However, preliminary results on fermentation of *U. lactuca* and nine other species of green macroalgae to ethanol show relatively poor yields (Isa et al., 2009). Anaerobic digestion of *U. lactuca* to methane seems more suitable and yields have been reported in the range of 180–330 ml CH_4 g^{-1} Volatile Solids (VS) depending on the treatment procedure (Habig et al., 1984; Ryther et al., 1984; Briand and Morand, 1997; Morand and Briand, 1999; Morand et al., 2006). In addition to fermentation technologies, a number of thermochemical options for conversion of macroalgae into bioenergy are available. These include direct combustion as used in combined heat and power plants as well as gasification and pyrolysis where the biomass is converted to gas or liquid (tar) before further processing. The gas and tar can be utilised in engines or turbines, as biofuels for transportation or it can be used as building blocks in bio-refineries, producing high value products.

Compared to traditional terrestrial biomasses, such as wood and straw, information about aquatic biomass as a feedstock for thermochemical conversion is scarce. Thermal behaviour of macroalgae for combustion and pyrolysis is described for a few species of brown algae, primarily *Laminaria* and *Fucus* (Ross et al., 2008, 2009). The ash and calorific contents of 28 species of macroalgae from New Zealand are described, including the seasonal variation in ash and calorific content for *U. lactuca* and *Macrocystis porifera* (brown algae) (Lamare and Wing, 2001).

In this paper we aim at characterising the green macroalgae *Ulva lactuca* as feedstock for bioenergy production. We examine the production potential of *U. lactuca* biomass under temperate, northern conditions and compare the potential for energy conversion via two established technologies: anaerobic fermentation to methane and as feedstock for the production of heat and power through combustion. Results are compared to relevant terrestrial and aquatic biomass resources.

2. Methods

2.1. Biomass production

Ulva lactuca was sampled late April at Seden Beach (Odense Fjord), Denmark. Cultivation experiments were carried out from May to September 2008 in a land based facility at the Danish Shellfish Centre, Nykøbing Mors (56°47′16″N. 8°52′36″E). The cultivation tanks had light opaque walls, a surface area of 1 m^2 and a water depth of 60 cm, with the water level approximately 10 cm below the rim. To keep the algae in the tanks, the outflow from each tank was fitted with plastic pipes (55 cm long and with a diameter of 5 cm) equipped with a fine mesh at the top. Natural sunlight supplied the sole input of light with a daily dose in the range of 7–59 mol photons m^{-2} d^{-1} and a mean ± SD of 38.7 ± 12.9 mol photons m^{-2} d^{-1} (Fig. 1). Unfiltered, untreated surface water from 2 m depths in the adjacent estuary (Limfjorden) was continuously supplied during daytime at a rate of 5 l min^{-1}. Salinity ranged between 25‰ and 28.5‰ and water temperature between 7 and 23 °C, respectively, during the experimental period (Fig. 1). Concentrations of ammonium-N (NH_4^+–N) in the natural seawater were below 2 μM throughout the experimental period. Concentrations of nitrate-N (NO_3^-–N) decreased from 30 μM in early April to below the detection limits (0.1 μM) in early June and remained at this level until the end of the experimental period. Concentrations of inorganic phosphorous were below 1 μM until mid August where concentrations increased to 1–3 μM. Nutrients were added to the cultivation tanks continuously over 2 h every evening (8–10 p.m), to approximate concentrations of 15 μM of NO_3^-–N, 5 μM of NH_4^+–N and 2.5 μM ortho-phosphate (ortho-P) in the tanks, using a solution of liquid green house fertiliser (Blaakorn Drivhusgoedning, Bauer, Germany). The nutrient additions corresponded to loadings of 0.17 g dissolved inorganic nitrogen (DIN) m^{-2} d^{-1} and 0.048 g dissolved inorganic phosphorus (DIP) m^{-2} d^{-1}. After addition of nutrients, the water flow was stopped for 8 h (10 p.m.–6 a.m.). During these hours, the algae assimilated the added nutrients, and nutrient concentrations in the tanks returned to background levels. Gentle aeration of the water using a centrifugal blower (Bora, RIETSCHLE Thomas Denmark SAH95) continuously circulated the algae between surface and bottom of the pond with approximately one minute intervals. Stocking densities of algae were 1, 2, 4, 6 or 8 kg fresh weight (FW) m^{-2}, distributed in triplicate between randomly selected tanks. Algae biomasses were harvested and manually adjusted to initial stocking densities at least once a week. The specific growth rates were calculated from the FW of the algae:

$$SGR = 100 \times [\ln(W_t/W_0)]/t,$$

where W_0 corresponded to the initial stocking density, and W_t to the biomass after t days of cultivation. The fresh algae were thoroughly drained before determination of FW, leaving approximately 3% of external water content in the bulk FW measurements. This water was corrected for when calculating TS from FW. The biomass yields in TS are calculated based on the actual TS% of the algae measured during the experimental period. At every harvest event, the tanks were brushed clean and algae were sampled for analysis of intracellular molar carbon:nitrogen (C:N) ratio and total solid content. Samples were oven dried at 60 °C for a minimum of 24 h before used for further analysis. C and N content was analysed on an elemental analyser (Roboprep C/N, Europa Scientific Ltd., UK) in line with a triple collector isotopic ratio mass spectrometer (Tracermass, Europa Scientific Ltd., UK). Concentrations of monosaccharides were determined according to Vanhande (1972).

Water samples for analysis of nutrient concentrations (NO_3^-–N, NH_4^+–N and ortho-P) were taken at mid day once a week in every tank. Concentrations of NO_3^-–N were determined using a NO–NO_2–NO_x analyser (Thermo Environmental Instruments Inc. 42C). Concentrations of ortho-P and NH_4^+–N was determined spectrophotometrically according to standard methods. Data on local surface irradiance during the cultivation period were kindly supplied by the Faculty of Agricultural Sciences at Aarhus University.

2.2. Methane production

2.2.1. Preparation of substrates

U. lactuca was sampled at Seden Beach (Odense Fjord), Denmark and exposed to different treatments resulting in 8 different batch series (Table 1). Batch 1 served as control. Here the algae were only roughly chopped (≈2 × 2 cm) to facilitate the distribution of the algae in the batch vials. Batch 2 was a homogenized paste obtained by maceration of the algae. Batches 3 and 4 were made by washing the algae in order to dilute the concentration of salts and to remove sand and gravel. Two hundred grams of algae were suspended in 10 l of water for 24 h. After washing, the batch 3 substrate was roughly chopped as in batch 1 and in batch 4 the algae were macerated as in batch 2. In batches 5 and 6 the algae were treated as in batch 3 and subsequently exposed to thermal treatment at 110 °C/20 min and 130 °C/20 min, respectively. For preparation of batch 7, the algae were dried at 45 °C until a constant weight was obtained. The substrate was subsequently grounded (<1 mm). In batch 8, the algae were treated as in batch 1 but digested at mesophilic temperatures (37 °C) instead of at thermophilic temperatures (52 °C) (see later). TS and volatile solids (VS) were determined after each

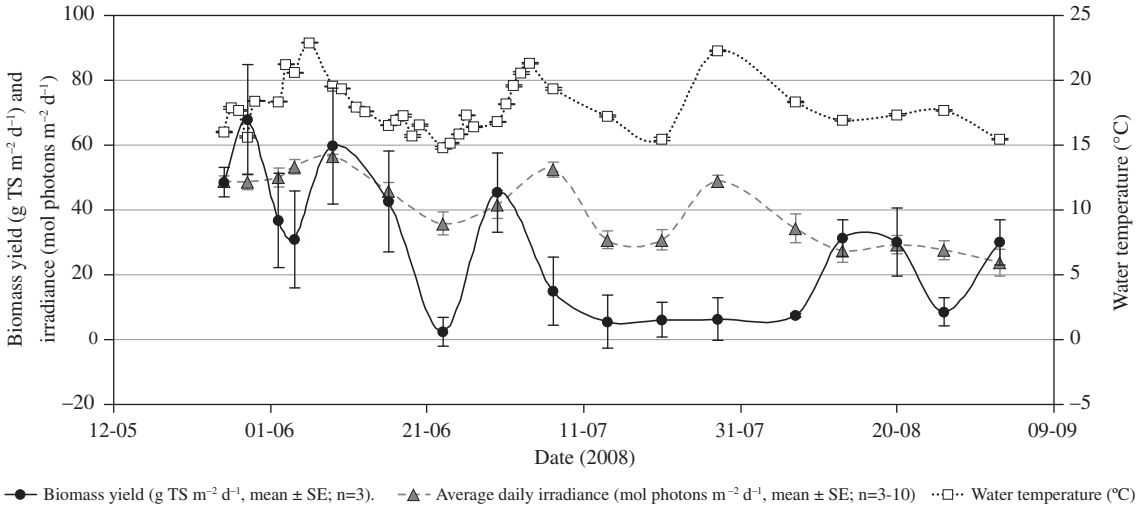

Fig. 1. Biomass production (●) (g TS m^{-2} d^{-1}, mean ± SE; $n = 3$) in tanks with a biomass density of 4 kg FW m^{-2}, average daily irradiance (▲) (mol photons m^{-2} d^{-1}, mean ± SE; $n = 3$–10) and water temperature (□) (°C, mean ± SE; $n = 3$) as a function of time. The low biomass yield recorded on the 23rd of June was the result of sporadic sporulation events in some of the tanks.

Table 1
Description of substrates used in batch experiments for estimation of the methane potential of *Ulva lactuca*.

Experiment	Pre-treatment	Incubation temp. (°C)	TS/VSn (%)
Batch 1, control	Unwashed algae, roughly chopped.	55	12.79/7.34
Batch 2	Unwashed algae, macerated.	55	12.79/7.34
Batch 3	Washed algae, roughly chopped.	55	9.84/7.16
Batch 4	Washed algae, macerated.	55	9.84/7.16
Batch 5	Washed algae, pre-treatment at 110 °C/20 min	55	9.84/7.16
Batch 6	Washed algae, pre-treatment at 130 °C/20 min	55	9.84/7.16
Batch 7	Dried algae, grounded	55	90.4/54.4
Batch 8	Unwashed algae, roughly chopped	37	9.84/7.16

treatment according to standard methods by drying of the biomass at 102 °C (24 h) and incineration at 550 °C (3 h).

2.2.2. Setup of batch experiments

Substrates 1–7 were distributed in 500 ml serum bottles in amounts of 0.4 g VS (≈5 g FW and ≈0.7 g TS). The bottles were supplemented with water to reach a total volume of 20 ml and inoculated with 60 g of effluent from a 3.0 litre lab-scale reactor treating cattle manure (5.4% VS) at a temperature of 52–53 °C. Substrate from batch 8 was distributed in the same way as substrates 1–7 but inoculated with biomass from a 37 °C full-scale centralized biogas plant (Hashøj biogas plant, Denmark) treating cattle/pig manure (≈80%) and industrial waste (≈20%).

All bottles were flushed with N_2/CO_2 (80%/20%), to obtain anaerobic conditions, closed with butyl rubber stoppers and aluminium crimps and incubated at either 52 °C (batch 1–7) or 37 °C (batch 8). Methane production was measured by gas chromatography using flame ionization detection (Hewlett Packard 6890 G1530A, Agilent Technologies Inc., Hewlett Packard Inc., UK) and the production from the substrates was corrected for the amount of methane produced by the reactor effluents (inoculums) incubated in separate bottles. The vials were incubated until the methane production died out (i.e. 42 days for vials incubated at 52 °C and 58 days for vials incubated at 37 °C). All experiments were performed in at least triplicates.

2.3. Elementary analyses

For combustion analyses, samples of *U. lactuca* from the cultivation experiment as well as samples of natural material from Roskilde Fjord, Denmark were used. Prior to drying, samples of the cultivated *U. lactuca* were rinsed with freshwater to reduce the salt content. The washing was performed stepwise in batches of freshwater until reaching a salinity below 2‰ in the rinsing water.

Each sample (Roskilde Fjord and cultivated samples) was dried for 24 h at 105 °C and subsequently the brittle material was crumbled manually to fine particles. The samples, now consisting of fine particles, were divided, using a rotational splitter, into ten representative sub-samples used for the following analyses.

Ash content of the different *U. lactuca* samples were determined by combusting the dried samples at 550 °C according to CEN/TS 14775 standard for analyses of biomass.

The remaining ash was pressed in a tablet press and subsequently analysed for its chemical content by means of WD-XRF analyses (Philips PW2400/UNIQUANT ver 5.49).

The analyses were made on both sides of the tablet and the results are an average of the two sides. The analysis provides data for elements from fluorine to uranium during the same round of analysis. Only data for the main elements were obtained in the analysis results due to a detection limit of typically 10 ppm of the method. The Net Calorific Value (NCV) and Gross Calorific Value (GCV) were determined according to CEN/TS 14918 standard for analyses of biomass.

3. Results and discussion

3.1. Biomass production

3.1.1. Growth and production

The biomass yields obtained in this experiment demonstrated that a substantial amount of *U. lactuca* biomass can be cultivated at latitudes as far north as Denmark (56°N). Extrapolation of the

results obtained in this study leads to an estimated annual biomass production of 45 t TS ha^{-1} y^{-1}. This is 2–20 times the production potential of conventional terrestrial energy crops (McKendry, 2002; Lehtomaki et al., 2008) and three times the production of brown algae in temperate waters (Kelly and Dworjanyn, 2008). The highest area specific biomass yield was achieved with a stocking density of 4 kg FW m^{-2} (Fig. 2). Thus, the presented annual production estimate is based on the correlation between average incoming light and biomass production for this stocking density (Fig. 3a), assuming a seven month growth season (mid March to mid October) and using a 30 year average of daily incoming light. Comparing to the production results from the US Aquatic Species Programme, energy intensive cultivation yielded 74 t TS ha^{-1} y^{-1} (18.8 g TS m^{-2} d^{-1}, equivalent to 30 t TS ac^{-1} y^{-1}) whereas non-energy intensive cultivation yielded 26.7 t TS ha^{-1} y^{-1} (6.8 g TS m^{-2} d^{-1} corresponding to 10.8 t TS ac^{-1} y^{-1}) assuming a 250 days growth period (Ryther et al., 1984). The photosynthetic efficiency during the experimental period was estimated as mol carbon (C) incorporated into the harvested biomass (average C content of 29.4% TS), and the number of incident photons. The annual biomass yield observed corresponded to an average photosynthetic efficiency of 16 ± 2 mmol C incorporated $(\text{mol photons})^{-1}$ (1.6%) and a maximal photosynthetic efficiency of 32 ± 8 mmol C incorporated $(\text{mol photons})^{-1}$ (3.2%). These efficiencies are comparable to or higher than of high productive terrestrial crops (McKendry, 2002). Still the efficiencies are much below the theoretical maximal efficiency for gross photosynthesis of 125 mmol C incorporated $(\text{mol photons})^{-1}$. This could be due to respiratory losses, shading from tank rims and walls, surface reflection and that some of the irradiance was received at levels above light saturation of photosynthesis. Thus, the efficiency might be increased by optimal manipulation of the stocking density in order to reduce respiratory losses and in larger tanks where shading effects from walls are lower. The TS increased over the period from 18.6 ± 1.0% to 25.2 ± 1.4%, with an average of 23.3 ± 2.7%, which is in range with results (20.4%) reported by Lamare and Wing (2001), but higher than the 11.6–11.7% reported by Habig et al. (1984). The highest biomass yield was obtained with a stocking density of 4 kg FW m^{-2} (Fig. 2 and Table 2a) resulting in an average yield per day of 25.1 ± 3.6 g TS m^{-2} d^{-1} (156.3 ± 21.2 g FW m^{-2} d^{-1}). For this stocking density the biomass yield over the experimental period was highly variable and fluctuated between a maximal yield of 67.9 ± 17.0 g TS m^{-2} d^{-1} (433.3 ± 108.4 g FW m^{-2} d^{-1}) (mean ± SE) and negative production rates (Fig. 1). However, the highest specific growth rates, 18.7 ± 1.5% d^{-1}, were found in the tanks with the lowest stocking density (1 kg FW m^{-2}) (Table 2b) and there was a significant negative correlation between stocking density and specific growth rates ($p < 0.01$ (Fig. 2 and Table 2b). The daily biomass yields and growth rates presented here are in range with a maximal yield of 55 g TS m^{-2} d^{-1} and maximal specific growth rates of 18% d^{-1} reported from studies with integration of *U. lactuca* in multitrophic aquaculture using similar size tanks and using a stocking density of 1 kg FW m^{-2} (Neori et al., 1991), but lower than the biomass yields reported under cultivation of *U. lactuca* with high nitrogen loads: 37.6 ± 8.6 g TS m^{-2} d^{-1} (mean ± SD) reported by Msuya and Neori (2008) (calculated from the FW:TS ratio

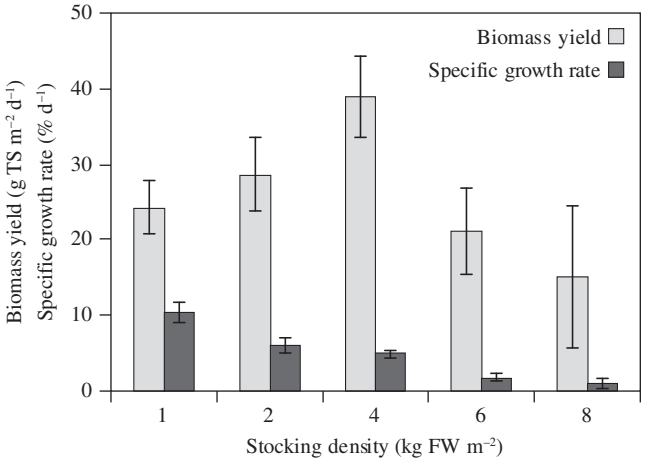

Fig. 2. Average biomass yield (light grey bars, g TS m^{-2} day^{-1}; mean ± SE, $n = 3$) and specific growth rate (dark grey bars, % d^{-1}; mean ± SE, $n = 3$) for the period 26th of May-7th of July 2008 in tanks with different biomass densities.

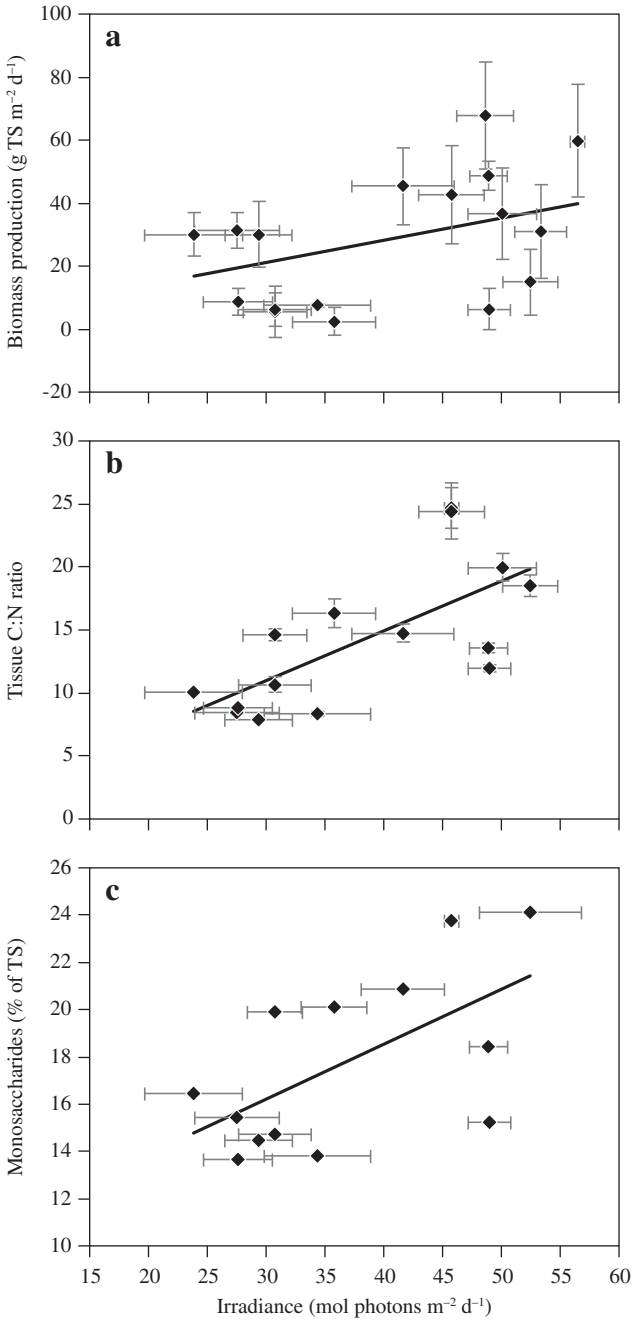

Fig. 3. The correlations between average daily irradiance and (a) the biomass production (g TS m^{-2} d^{-1}. $R^2 = 0.74$, $p < 0.01$; mean ± SE, $n = 3$). The open symbols represent data from production periods where the water temperature exceeded 20 °C. (b) the C:N ratio ($R^2 = 0.54$, $p < 0.01$; mean ± SE, $n = 3$) and (c) the monosaccharide content (% of TS. $R^2 = 0.40$, $p < 0.05$; mean ± SE) of the *U. lactuca* biomass (mean ± SE).

Table 2a
Biomass yields (g TS m^{-2} day^{-1}; mean ± SE; $n = 3$). Highest biomass yield in every period is marked in bold.

Experimental period (date of harvest, 2008)	Biomass density (kg FW m^{-2})				
	1	2	4	6	8
1 (26.05)	43.8 ± 4.8		**48.5 ± 4.6**		−3.1 ± 15.7
2 (29.05)	9.7 ± 5.2		**67.9 ± 17.0**		−1.7 ± 14.5
3 (02.06)	16.6 ± 5.0		**36.8 ± 14.5**		26.0 ± 13.7
4 (04.06)	16.6 ± 11.3		31.0 ± 15.0		**39.8 ± 36.7**
4 (09.06)	26.1 ± 3.4		**59.7 ± 17.8**	12.9 ± 6.9	
5 (16.06)	32.7 ± 3.6		**42.7 ± 15.6**	29.3 ± 7.1	
6 (23.06)		**27.3** ± 4.9	2.4 ± 4.5		
7 (30.06)		41.0 ± 7.3	**45.4 ± 12.2**		
8 (07.07)		**17.4 ± 8.0**	14.9 ± 1.05		
Average (26.05–07.07)	24.5 ± 3.5	28.5 ± 4.9	**38.8 ± 5.4**	21.1 ± 5.7	15.2 ± 9.4

Table 2b
Growth rates (% d^{-1}; mean ± SE, n = 3). Highest growth rate in every period is marked in bold.

Experimental period (date of harvest, 2008)	Biomass density (kg FW m^{-2})				
	1	2	4	6	8
1 (26.05)	**18.7 ± 1.5**		6.7 ± 0.6		−0.3 ± 1.2
2 (29.05)	5.5 ± 2.9		**9.2 ± 2.1**		−0.2 ± 1.2
3 (02.06)	**8.2 ± 2.1**		4.9 ± 1.7		1.8 ± 1.0
3a (04.06)	**8.5 ± 5.5**		4.4 ± 2.1		2.8 ± 2.6
4 (09.06)	**10.9 ± 1.1**		6.8 ± 1.8	1.1 ± 0.6	
5 (16.06)	**11.3 ± 0.9**		4.6 ± 1.4	2.4 ± 0.5	
6 (23.06)		**6.0 ± 0.9**	0.3 ± 0.6		
7 (30.06)		**8.3 ± 1.1**	5.2 ± 1.2		
8 (07.07)		**3.9 ± 1.6**	1.8 ± 1.3		
Average (26.05–07.07)	**10.5 ± 1.4**	6.1 ± 0.9	4.9 ± 0.7	1.8 ± 0.5	1.0 ± 0.8

reported in the reference). In larger tanks (5 m^2) using a stocking density of 2 kg FW m^{-2}, specific growth rates of 6.3 ± 3.4% d^{-1} have been reported, which is lower than what obtained in this study (8.3 ± 1.1% d^{-1}) at a similar stocking density, whereas the broad range of biomass yields (0.12–2 kg FW m^{-2} d^{-1}) are comparable to our results (up to 0.23 ± 0.04 kg FW m^{-2} d^{-1}) (Robertson-Andersson et al., 2008). In this study, the biomass production was significantly correlated to the incoming irradiance (R^2: 0.74, $p < 0.01$). However, when high incoming light caused water temperatures to exceed 20 °C, a decrease in the production rates was observed (Fig. 3a). This corresponds to reports of optimal temperatures for growth of temperate strains of *U. lactuca* of approximately 15 °C (Fortes and Luning, 1980). The lower specific growth rates and biomass yields in the tanks with higher stocking densities could be explained by light limitation due to self shading. In tanks with stocking densities of 4 kg FW m^{-2}, 11% of the light available at the surface was penetrating to the middle of the tank, and at the bottom of the tank only 2% of the incoming light was available. In the tanks with maximal stocking density (8 kg FW m^{-2}) 2% of the surface light was penetrating to the middle of the tank, whereas less than 0.01% of the incoming light was available at the bottom. The light compensation point for growth for *U. lactuca* has been determined to between 0.33 and 1.87 μmol photons m^{-2} s^{-1} (Markager and Sand-Jensen, 1996) at 7 °C and a 14:10 h light:dark cycle. With an approximate mean value of 500 μmol m^{-2} s^{-1} during the cultivation experiment, the light compensation point would correspond to about 0.31% of surface light, which is close to the 0.1% estimated as light compensation point for foliose macroalgae (Markager and Sand-Jensen, 1992). Thus, our observation of a maximum biomass yield at densities of about 4 kg FW m^{-2} corresponded well with these findings, indicating a light compensation point in the tanks close to 2% of the surface light measured below 4 kg FW m^{-2}, whereas, shading by the dense biomass in tanks with biomass exceeded 4 kg FW m^{-2} would cause a dark zone where respiratory losses exceed the photosynthesis.

3.1.2. Biochemical composition

The C:N ratio of the cultivated algae ranged from 7.9 to 24.4 and was positively correlated to incoming irradiance ($R^2 = 0.54$, $p < 0.01$. Fig. 3b). This is in agreement with the findings of Neori et al. (1991), who described how light limitation caused by stocking densities of 6 kg FW m^{-2}, caused specific growth rates as well as C:N ratios to decrease. In this study, the incoming irradiance also correlated positively to the total content of monosaccharides ($R^2 = 0.4$, $p < 0.05$. Fig. 3c). This is in accordance with previous findings showing that macroalgae tend to accumulate carbon, and therefore in many cases also carbohydrates, when growing at light levels above their light saturation point (Chapman and Lindley, 1980; Markager and Sand-Jensen, 1992, 1994, 1996). Habig et al. (1984) found a higher content of soluble carbohydrates and neutral fibres, and a lower content of protein and crude fibres, in nitrogen starved *U. lactuca* (C:N ratio of 30.71). Nitrogen starved *U. lactuca* biomass proved superior to nitrogen replete biomass, regarding production of methane. In this study, the increasing C:N ratio with increasing light levels is probably not indicating nitrogen limitation but rather that carbon fixation exceed the maximum growth rate for the algae resulting in a temporary storage of carbohydrates. We observed that the internal N pools are only occasionally below the critical value of 2.17% of TS reported as limiting for maximal growth (N_C). The N pools are never near or below the subsistence quota (N_Q) of 0.71% of TS (Pedersen and Borum, 1996). This has important implications for the optimal strategy for cultivating algae for bioenergy purposes. Presumably, the best strategy is to maximize light exposure and balance the nutrient addition in order to achieve the optimal balance between overall growth rate and accumulation of carbohydrates.

3.2. Methane production

The washing procedure of the algae (sampled directly from the field) resulted in a 23% decrease in TS content but only in a 2.5% decrease in VS concentration, due to the removal of gravel and sand

(Table 1). However, washing had no effect on the methane yield as illustrated in Fig. 4 (batch 3 compared to batch 1). Maceration of unwashed algae resulted in a significant boost (56%) in methane yield from 174 ml g VS^{-1} (substrate 1) to 271 ml g VS^{-1} (substrate 2). A more moderate increase (17%) as a consequence of the maceration was observed for washed algae (substrate 4 compared to substrate 3). Thermal treatment at 110 °C (batch 5) negatively affected the methane yield and treatment at 130 °C (substrate 6) only gave a 7% increase. The methane yield of the dried algae (batch 7) was in the same range as for the wet algae (batch 1). A decrease of the digestion temperature from 52 °C to 37 °C (batch 8) lowered the final methane yield by 7%.

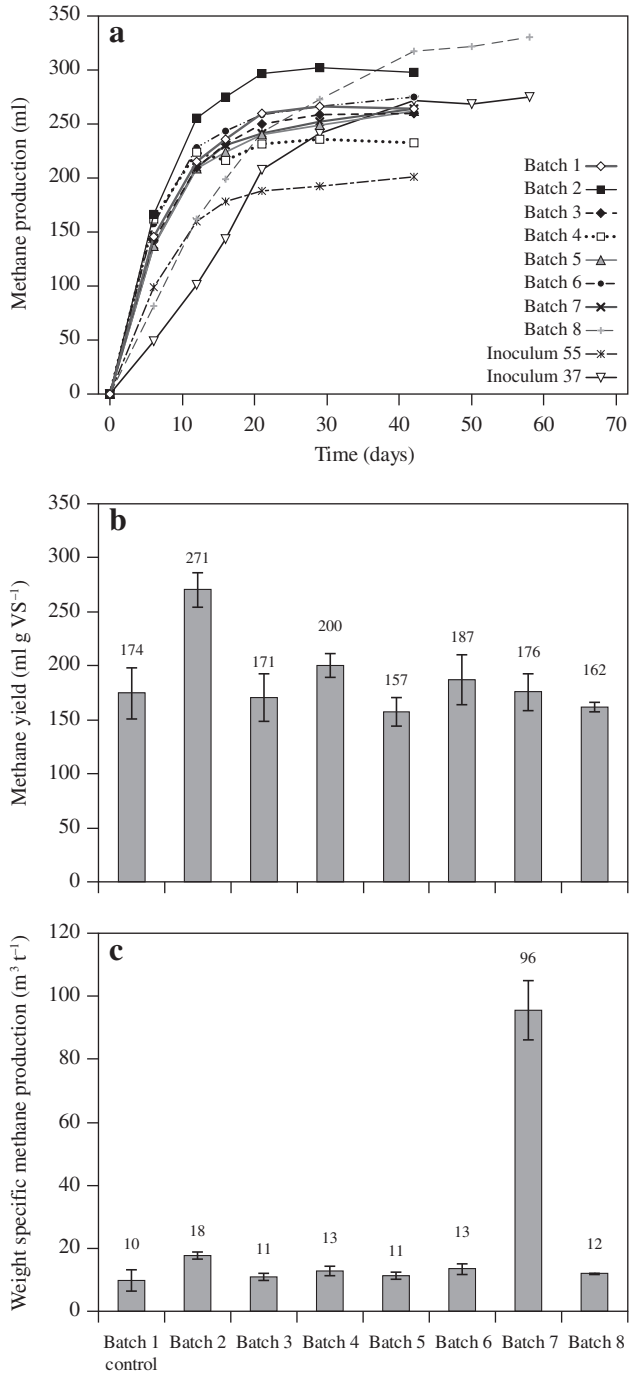

Fig. 4. (a) Methane production profiles of the batch vials. (b) Methane yield of *U. lactuca* (mean ± SD, $n \geqslant 3$). (c) Weight specific methane production of *U. lactuca* (mean ± SD, $n \geqslant 3$). The different batch setups are described in Table 1 and the text.

A previous batch study evaluated the methane potential of three different samples of *Ulva sp.* differing in thallus nitrogen content (Habig et al., 1984). The specific methane yield was estimated to be between 190–270 ml g VS^{-1} following 38 days of incubation and 220–330 ml g VS^{-1} after 58 days. In the present study, the yields of non-pre-treated *U. lactuca* were in the lower range of these numbers and also lower than the yields of other macroalgae such as *Gracilaria* and *Macrocystis* and various terrestrial energy crops and crop residues (Table 3). However, the total methane potential (m^3 ha^{-1}) of *U. lactuca* is considerably higher than for many terrestrial energy crops when taking the high growth yield of the algae into account. In addition to this, optimised biochemical composition of *U. lactuca* via manipulation of light and nitrogen conditions during growth can increase the methane yield, and the methane potential of *Ulva sp.* has been estimated to be between 400–421 l CH_4 g VS^{-1} (Habig et al., 1984; Briand and Morand, 1997) based on the chemical composition. Development of efficient pre-treatment methods to exploit the full potential of *U. lactuca* would make anaerobic digestion of the algae even more favourable. In this context, the thermal pre-treatments at 110 °C and 130 °C in the present study had a negative or only minor positive impact on the methane yield and confirmed previous observations where hydrothermal treatment of dried *U. lactuca* (195 °C for 10 min with and without oxygen) resulted in 15–34% decrease in methane yield (Nielsen et al., 2009). However, it is notable, that a simple maceration of *Ulva* (batch 2) had a significant positive impact on the methane yield. This was also observed by Otsuka and Yoshino (2004) who estimated the methane potential of *Ulva* species sampled at Rinku Park in Osaka bay. Here a combined washing and grinding (dry algae) gave a methane yield of approximately 180 ml g VS^{-1} while only 70% of this value was obtained with non-pre-treated, washed or grinded material.

A disadvantage of anaerobic digestion of macroalgae compared to for instance terrestrial energy crops is the high water content of algae. In this part of the present study the TS and VS content of fresh *U. lactuca* were 12.8% and 7.3%, respectively, which will not allow a loading rate in a continuously fed system to be more than approximately 4–5 g VS l^{-1} d^{-1} at 15–18 days hydraulic retention time. In addition, the weight specific methane yield of *U. lactuca* was low (10–18 m^3 t^{-1}) and in the same range as of cattle and pig manure, due to the high water content. Therefore in its raw form, *U. lactuca* cannot be used to boost the methane yield of manure based biogas plants like industrial waste is used today (Angelidaki and Ellegaard, 2003, Table 3). Storage of wet *Ulva* is also a challenge when compared to terrestrial plants since the high water content (>85%) speeds up the decay of the algae. Drying is an efficient technique to avoid this problem, but is associated with a cost and in many cases also energy consumption, which will lower the overall efficiency of the use of macroalgae in a CO_2-reduction context. Our data, showed that the drying procedure reduced the volume of the biomass, increased the TS/VS content and resulted in a 5–9 times higher weight specific methane production than when compared to wet biomass (batch 7 versus batch 1 and 2, Fig. 4). Furthermore, the higher TS/VS content in dried biomass will allow a higher organic loading rate in a continuous system without lowering the hydraulic retention time and thereby increase the methane production rate of the facility.

3.3. Thermal conversion

The analyses of marine biomass performed in this study as well as in other studies (Ross et al., 2008; Lamare and Wing, 2001) reveal that seaweeds in general contain significantly higher amounts of ash than do typically utilised terrestrial biomass fuels such as straw and wood. While ashes from terrestrial biomass typically are dominated by Si- and Ca-oxides, the ash composition in marine biomass

Table 3
Methane potential of selected macro algae and boreal energy crops and crop residues and organic wastes.

Substrate	Growth yield (t TS ha^{-1} y^{-1})	Methane yield		Methane potential (m^3 ha^{-1})
		(m^3 t TS^{-1})	(m^3 t VS^{-1})	
Macro algae				
Ulva lactuca	45[a]	93–155[a]	162–271[a]	4200–7000
Ulva energy intensive	74[b]	–	–	–
Ulva non-energy intensive	27[b]			
Gracilaria	87.5[b]	–	280–400[c]	–
Sargassum	–	–	120–190[d]	–
Sargassum	–		260–380[e]	–
Laminaria[a]	15[f]	–	260–280[e]	–
Macrocystis	–	–	390–410[e]	–
Crops and crop residues				
Timothy clover grass[b]	8–11[g]	306 [g]	333[g]	2600–3600[g]
Vetch–oat mixture[b]	5–7[g]	329[g]	365[g]	1600–2300[g]
Jerusalem artichoke[b]	9–16[g]	306[g]	333[g]	2800–4900[g]
Tops of sugar beet[b]	3–5[g]	255[g]	299[g]	700–1300 [g]
Maize	9–18[h]	–	–	4000–8000[h]
Straw, wheat	7[i]			–
Miscanthus	12–30[i]	–	–	–
SCRC Willow	10–15[i]	–	–	–
Wastes				
Flotation sludge	–	–	540[j]	–
Fish oil	–	–	600–800[j]	–
Meat and bone flour	–	–	570[j]	–
Source sorted house hold	–	–	400[j]	–

[a] *Saccharina latissima*
[b] 50 days incubation

[a] This study.
[b] Ryther et al. (1984).
[c] Habig et al. (1984).
[d] Chynoweth et al. (2001).
[e] Chynoweth (2005).
[f] Kelly and Dworjanyn (2008).
[g] Lehtomaki et al. (2008).
[h] Seppala et al. (2008).
[i] McKendry (2002).
[j] Angelidaki and Ellegaard (2003).

(based on *U. lactuca*) consists for a large part of chlorine and sulphur salts (Table 4). By washing the *U. lactuca* in fresh water the majority of the chlorine can be removed together with sodium and some of the K, while the main part of K and S remain in the biomass (Table 4).Washing with freshwater will result in a considerable water consumption. However, the fresh water does not need to be tap water but could be e.g. outflow from sewage treatment plants.

The amount of alkali (Na and K) has a large impact on the melting behaviour of ash: The higher the amount of alkali, the lower is the melting temperature and the more problematic is the fuel in thermal conversion units. This has previously been described by the formulation of the alkali index (Jenkins et al., 1998) which relates the amount of alkali to the heating value (sum of Na and K on an oxide basis divided by the Gross Calorific Value (GCV (MJ/kg TS)). Index values above 0.17 imply risk for slagging and fouling while values exceeding 0.34 would mean significant risks. The alkali index of macroalgae like *U. lactuca* as well as species of brown algae have index values in the range of 3–6, significantly exceeding the critical value of 0.34 (Ross et al., 2008). Washing of the cultivated *U. lactuca* biomass with fresh water decreases the index to around 1.2. However, this value is still significantly higher than 0.34, as well as higher than the index values for known biomass fuels such like straw and wood pellets (Table 4).

By analyzing *U. lactuca* harvested by hand from the Roskilde Fjord compared to analyses of *U. lactuca* cultivated in tanks, the potential contamination with sand from harvest in the sea becomes obvious. Another important factor which could significantly impact the ash content and thereby subsequently the calorific value of the seaweed of temperate regions is in what time of the year it was harvested. According to Lamare and Wing (2001) the ash content in *U. lactuca* could vary between 14% and 35% of TS during a year. The contamination with sand was not discussed by Lamare and Wing (2001), but even at 14% of TS, which is similar to the ash content in the cultivated biomass from this study, the ash content and presumably the alkali index, is still very high.

Contamination of *U. lactuca* with sand significantly increases the ash content of the biomass by adding large amounts of SiO_2 to the ash composition and also lowers the heating value (Table 4). Sand containing SiO_2 in combination with high concentrations of alkali (K and Na) is an extra dangerous combination as this is the perfect basis for forming low temperature melting ash in combustion or gasification units. Thus, considering the ash chemistry and what is known from combustion of straw, which is a very challenging fuel, these first analyses reveal *U. lactuca* to be quite challenging for direct thermal conversion in conventional combustion or gasification units and would very likely cause significant problems with molten ash, fouling, corrosion and also particle emissions.

3.4. Evaluation of U. lactuca as a future bioenergy crop

The principal selection criteria for selection of promising energy crops are (McKendry, 2002):

- high growth rate/yield,
- low cost (low energy input/low nutrient requirements/ease of management),
- intrinsic material properties (moisture/ash/alkali content).

Table 4
Characteristics and ash composition of *Ulva lactuca* - influence by harvest and comparison to brown seaweed and terrestrial biomass.

	Ash	K_2O	Na_2O	MgO	CaO	Fe_2O_3	SiO2	Al_2O_3	SO_3	Cl	P_2O_5	GCV	GCV	Alkali index[e]
	Wt% of fuel (TS)	Ash composition (Wt% of ash)										MJ kg^{-1} (TS)	MJ kg^{-1} (VS)	kg alkali GJ^{-1} (TS)
Ulva lactuca (pond)[a]	16.5	19.3	13.5	18.2	6.3	1.1	0.2	1.1	30.0	9.6	2.2	14.9	17.8	3.64
Ulva lactuca (pond. washed)[a]	14.0	13.3	1.0	26.0	13.0	3.6	0.4	0.0	30.0	1.7	9.5	16.8	19.5	1.20
Ulva lactuca (Roskilde fjord)[a]	35.0	10.4	7.8	12.4	8.3	1.9	23.5	0.0	19.0	9.0	3.4	12.7	n.a.	*5.03*
Ulva lactuca[b]	14–35	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13.6–17.6	n.a.
Laminaria digitata[c]	25.8	14.8	19.6	5.2	5.0	1.0	0.4	0.1	n.a.	n.a.	6.7	17.6	23.7	5.02
Laminaria hyperborea[c]	18.0	40.2	16.5	6.1	7.9	0.5	0.5	1.5	n.a.	n.a.	5.4	16.5	20.2	6.16
Danish wheat straw[d]	6.0	22.4	1.6	2.2	7.0	0.8	51.6	2.4	8.9	6.0	2.4	19.1	20.3	0.80
Danish wood pellets[d]	0.4	23.2	1.0	5.5	29.8	0.6	7.1	0.5	9.8	2.0	4.8	20.8	20.8	0.05

n.a: Not analysed. TS: total solids (=dry matter content). VS: volatile solids (=dry ash free matter). GCV: Gross Calorific Value (=higher heating value). Wt%: weight %.
[a] This study.
[b] Lamare and Wing (2001).
[c] Ross et al. (2008).
[d] Biolex.
[e] Jenkins et al., 1998

U. lactuca gets good scores at the first criterium in demonstrating high growth rates and yields that are 2–20 times higher than for land based energy crops and other cultivated algae (Table 3). Second comes the criterion of low production costs. The biomass yield as well as the cost efficiency of the biomass produced will depend on the cultivation method applied (Ryther et al., 1984). The cultivation method here applied is energy intensive and thus, the cost efficiency of the cultivation process is doubtful in case the sole outcome product is energy. However, energy costs could be reduced considerably by reducing the aeration of the cultivation tanks. It has been demonstrated that aeration can be decreased up to 12-fold with minimal impact to the productivity (Ryther et al., 1984). Aeration may even be omitted if nutrient concentrations are sufficient (Msuya and Neori, 2008). Cultivation of *U. lactuca* with a high yield will require a high input of nutrients. These nutrients do not have to be (energy) expensive mineral fertiliser, but could be e.g. nitrogen-rich effluents from aquaculture of fish or shellfish (Neori et al., 1991; Msuya and Neori, 2008; Robertson-Andersson et al., 2008) or potentially agricultural or municipal waste water, depending on the concentrations of contaminants. Nutrients supplied in this form will not induce a major cost, and may even add to the value of the biomass by taking advantage of the bioremediation capacity of *U. lactuca*. Considering the management of the crop, *U. lactuca* cultivated at stocking densities of 4 kg FW m^{-2} and above have not been observed to suffer from problematic bio-fouling of the algae or the tanks. The high biomass densities seem to capture the available light, and the smooth and sheet-like morphology of *U. lactuca* seems to repel bio-fouling. Avoidance of sporulation, causing disintegration of the fronds of the algae is a challenge that needs to be dealt with. Harvest every one of 2 weeks to keep the cultures at the optimal stocking density will need to be part of the management. However, this procedure could be mechanised.

An alternative way of obtaining a low cost biomass of *U. lactuca* could be by harvest from natural resources. "Green tides" caused by eutrophication are a significant problem at many coast lines. Dense populations of *U. lactuca* and other macroalgae do occur in e.g. Denmark and Italy where they have a negative effect on the ecological state of estuaries and are a nuisance for outdoor activities. In Denmark, the potential for harvesting *U. lactuca* green tides has been estimated to 100 t TS y^{-1}, which is a relatively limited resource. Moreover, the harvesting process is potentially so damaging for the ecosystem that it is not beneficial in all but the most eutrophic estuaries (Cecchi et al., 1996). Disturbance of other macro vegetation, in particular seagrasses, bird life and fish habitats are likely to be major problems. Open sea cultivation of *U. lactuca* is not yet taking place. However, technology does exist for open sea cultivation of foliose macroalgae and is being used in large-scale in Asia for cultivation of the red algae known as Nori (*Porphyra* sp.). Cultivation at sea would have the further advantage of not competing with agriculture for land resources; however it may be a problem to ensure sufficiently high nutrient concentrations for maximal growth (Pedersen and Borum, 1996).

Production of *U. lactuca* biomass in temperate regions will, due to the irradiation, result in a seasonally fluctuating delivery of biomass, and hence pre-treatment and storage that adds to the cost will have to be considered. In the conditioning process, washing, drying and other pre-treatments will increase the costs and consume energy and freshwater, lowering the benefit in terms of green values, unless waste water and waste heat can be used. Regarding storage, preliminary tests have demonstrated that *U. lactuca* biomass is easily pelletised (Jonas Dahl, unpublished). As an alternative option for storage, ensilation of the wet biomass is presently being considered and tested.

Summarising the first and second criteria, *U. lactuca* seems a promising candidate for a bioenergy crop. The challenges arise concerning the third criterion, the intrinsic material properties, moisture, ash and alkali content. *U. lactuca* represents an intrinsic wet biomass and is following better suited for wet processing techniques such as fermentation or anaerobic digestion than for combustion, pyrolysis or gasification (McKendry, 2002). Combustion as well as pyrolysis of brown algae has been tested concluding that this type or biomass was not suited for these conversion technologies due to the high contents of ash as well as alkali components (Ross et al., 2008, 2009). This study demonstrates that *U. lactuca* biomass has both an ash content and an alkali index in the same range as brown algae (Table 4). In order to be able to use seaweeds for bioenergy via combustion, some pre-processing of the seaweed which separates ash and alkali salts from the biomass has to be conducted prior to drying. However, alternative thermochemical conversion technologies such as pyrolysis, low temperature gasification, or direct liquefaction at high temperature and pressure, may prove better suited for fuel with this high content and composition of ash. Regarding the wet processes of energy conversion the technologies for energy conversion of *U. lactuca* are at the moment not mature and face various challenges that need to be solved. The challenges lie primarily in the salt and moisture content as well as the carbohydrate composition of the biomass. Fermentation of *U. lactuca* to ethanol has not yet achieved high yields (Isa et al., 2009) compared to fermentation of the sugars of brown algae (Adams et al., 2009). This most likely has to do with the relatively high content of the C5 sugar rhamnose in *U. lactuca* (Lahaye and Robic, 2007). However, the algae can relatively easily be converted to methane by anaerobic digestion. The most important parameter in choosing crops for methane production is net energy yield per hectare (Lehtomaki et al., 2008). *U. lactuca* has a potential for net energy yield per hectare, that compares to maize (Seppala et al., 2008) (Table 3) but due to the high water content of the biomass, anaerobic digestion will require a digester of too significant size to be economically feasible; as also stated by Briand and Morand (1997). Thus, development of cost-efficient methods for concentrating the organic content is necessary. Furthermore, obstacles such as competition between methanogens and sulphate reducing bacteria and increased proportions of H_2S in the biogas might be observed as a consequence of the high S concentrations in *Ulva* and should be evaluated in the future (Briand and Morand, 1997).

The economic, as well as the environmental, sustainability of the biomass production could be improved by taking advantage of the bioremediation capacity of the macroalgae, using nutrient rich waste streams as N, P and C sources for algae growth (Neori et al., 1991; Gao and Mckinley, 1994; Msuya and Neori, 2008). Furthermore, utilisation of the produced *U. lactuca* biomass could be optimised in order to extract high value products, such as pigments, plant growth hormones, proteins, dietary fibers and other food additives before using the remaining biomass for energy purposes (Ortiz et al., 2006; Lahaye and Robic, 2007). This approach would increase the relative content of carbohydrates in the end biomass, which would be an advantage in the energy conversion process.

4. Conclusions

The green macroalgae *U. lactuca* demonstrates a high biomass yield and a high photosynthetic efficiency compared to terrestrial crops. Use of the biomass for combustion represents a challenge due to high contents of moisture, ash and alkali. Anaerobic digestion of the wet biomass to methane seems more promising and several roads for further improving this conversion technology are indicated. The economical and environmental sustainability of using *U. lactuca* for production of bioenergy would benefit from exploiting the bioremediation capacity of *U. lactuca* during production, as well as from extracting of high value products from the biomass prior to energy production.

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