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Anaerobic conversion of microalgal biomass to sustainable energy carriers – A review

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HIGHLIGHTS

- Microalgal biomass is amenable to anaerobic energy carrier production.
- The highest energy yields have been reported for ethanol and CH_4.
- The highest butanol and H_2 fermentation yields are still relatively low.
- Simultaneous and sequential production of several energy carriers is also considered.
- Energy yields from microalgae are similar to those from other feedstocks.

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ABSTRACT

This review discusses anaerobic production of methane, hydrogen, ethanol, butanol and electricity from microalgal biomass. The amenability of microalgal biomass to these bioenergy conversion processes is compared with other aquatic and terrestrial biomass sources. The highest energy yields (kJ g^{-1} dry wt. microalgal biomass) reported in the literature have been 14.8 as ethanol, 14.4 as methane, 6.6 as butanol and 1.2 as hydrogen. The highest power density reported from microalgal biomass in microbial fuel cells has been 980 mW m^{-2}. Sequential production of different energy carriers increases attainable energy yields, but also increases investment and maintenance costs. Microalgal biomass is a promising feedstock for anaerobic energy conversion processes, especially for methanogenic digestion and ethanol fermentation. The reviewed studies have mainly been based on laboratory scale experiments and thus scale-up of anaerobic utilization of microalgal biomass for production of energy carriers is now timely and required for cost-effectiveness comparisons.

1. Introduction

Biomass-based energy produced in microbial processes is one prospect of the sustainable supplements and alternatives to fossil fuels but has yet to reach its full potential. Advantages of photosynthetic biomass-based feedstocks (i.e., terrestrial plants, microalgae) include their carbon neutral CO_2 emissions and increased energy security and independence in regions without fossil fuel reserves. Microalgae have several advantages over terrestrial plants such as higher photosynthetic efficiencies, lower need for cultivation area, higher growth rates, more continuous biomass production, no direct competition with food production, and possibility to use saline waters and wastewater streams for biomass production (Schenk et al., 2008). Microalgae, like terrestrial crops, can be used in energy and fuel production in several ways. Microalgal biomass can be anaerobically processed to gaseous (methane, hydrogen) or liquid (alcohols) biofuels. Chemical and physical processes at high temperatures and in the absence of oxygen can produce bio-oil and bio-syngas. Further, dewatered biomass can be incinerated, and lipids can be extracted from the cells to produce biodiesel or renewable diesel. Biodiesel is considered by many to be an ideal fuel that can be derived from microalgal biomass because areal productivities of microalgal lipids are substantially higher compared to the most efficient terrestrial crops and because biodiesel can be used with little or no modification in diesel engines of motor vehicles (Schenk et al., 2008; Lam and Lee, 2012). However, recent life-cycle assessments indicate that microalgal biomass cultivation and use for biodiesel production consume more energy than can be harvested from the process (Lardon et al., 2009; Beal et al., 2012). For example, Lardon et al. (2009) demonstrated that the requirement to dry microalgal biomass prior to lipid extraction significantly reduces overall energy efficiency. Anaerobic conversion of algal biomass to energy carriers

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does not require cost-intensive drying of the biomass. In addition, high content of lipids, starch and proteins and the lack of recalcitrant lignin make microalgal biomass a promising substrate for anaerobic microorganisms (Schenk et al., 2008).

Different microalgal biofuel production processes and especially microalgal use in diesel production have been thoroughly reviewed (for reviews, see Chisti (2007), Schenk et al. (2008), Brennan and Owende (2010) and Lam and Lee (2012)). Previous reviews have not focused on many anaerobic processes such as dark fermentative hydrogen production and microbial fuel cells. The purpose of this review is to focus on recent research on anaerobic processes for conversion of microalgal biomass to sustainable energy carriers all of which are within the sustainable biorefinery concept. The current status of each anaerobic process is considered and possible integration of these processes is discussed. For comparison, selected examples of energy yields from other aquatic and terrestrial biomasses are presented.

2. Anaerobic digestion for methane generation

Anaerobic digestion of organic matter in the absence of terminal electron acceptors such as sulfate, nitrate or ferric iron produces methane (55–75 vol%), CO_2 (25–45 vol%) and fermentative metabolites. Anaerobic degradation is carried out by heterogeneous microbial populations involving multiple biological and substrate interactions. Anaerobic biodegradation can be divided into four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Anaerobic digestion (sometimes also called methanogenic fermentation) is widely applied in digestion of manure, sewage sludge and organic fraction of municipal solid wastes in industrial and agrarian societies.

Anaerobic digestion of microalgal biomass has been studied from many freshwater and marine microalgae in various combinations. Ranges of process temperatures, reactor configurations, pretreatment methods as well as use of co-substrates are summarized in Table 1. The digestibility of microalgal biomass varies significantly even between closely related species (Mussgnug et al., 2010). CH_4 yields from microalgae vary due to variation in cellular protein, carbohydrate and lipid content, cell wall structure, and process parameters such as the bioreactor type and the digestion temperature. Theoretically, proteins, carbohydrates and lipids yield 0.851, 0.415 and 1.014 L CH_4 per g of volatile solids, respectively (Sialve et al., 2009). Chemical composition of microalgal biomass varies among microalgal species and even within the same species under different growth conditions (Sheehan et al., 1998). Thus, CH_4 production from microalgae should be examined under different experimental conditions to seek high growth yields and to determine optimal biomass composition for anaerobic digestion.

Rigid eukaryotic cell walls of microalgae can limit the anaerobic digestion of the biomass (Golueke et al., 1956; Chen and Oswald, 1998). Rates and yields of CH_4 formation from microalgal biomass often increase with digestion temperature. They can also be enhanced with pretreatment of microalgal biomass prior to digestion (Table 1). For example, Golueke et al. (1956) reported 5–10% increase in digestibility of microalgal biomass, when the digestion temperature was increased from 35 to 50 °C. Chen and Oswald (1998) increased the CH_4 yield by 33% by heat pretreating microalgal biomass at 100 °C for 8 h. In both examples, however, the amount of energy consumed in the heating and pretreatment was higher than the corresponding energy gain from increased CH_4 production (Yen and Brune, 2007; Sialve et al., 2009). Drying of microalgal biomass prior to digestion would also increase energy consumption and has been reported to reduce CH_4 yields (Mussgnug et al., 2010). These findings together with data on terrestrial plant materials (Lakaniemi et al., 2012b) indicate that pretreatment of microalgal biomass does not increase the energy gain of CH_4 production.

Biomass slurries of salt water algae contain sodium, calcium and magnesium ions that are inhibitory to anaerobic digestion at high concentrations. Methanogens are sensitive to excessively high salt levels but the susceptibility varies. Lakaniemi et al. (2011a) reported significantly lower CH_4 yields from NaOH-flocculated marine microalga *Dunaliella tertiolecta* than from chitosan-flocculated freshwater microalga *Chlorella vulgaris*. Mussgnug et al. (2010) reported similar or higher CH_4 production from marine microalga *Dunaliella salina* than from freshwater species. Factors affecting the level of inhibition of methanogenesis include biomass feedstock type and concentration, and source and previous growth history of microbial consortia in anaerobic digestion. Potential salt inhibition can be reduced by using cultures from saline environments and by successive enrichment at incremental concentrations of salt ions (Feijoo et al., 1995).

Microalgae cultivated under optimal growth conditions often contain high proportion of proteins. Consequently, the biomass has a relatively low C/N ratio, which may reduce digestibility and cause ammonium accumulation (Yen and Brune, 2007). C/N ratio can be adjusted to more optimal values with C-rich co-substrates such as cellulose (e.g., waste paper) (Yen and Brune, 2007) or glycerol (Ehimen et al., 2009). The C/N ratios of algal biomass can also be modified by selecting growth conditions that reduce cellular protein synthesis and favor lipid or carbohydrate production; an example is nitrogen limitation (Sheehan et al., 1998). High lipid content would increase the theoretical CH_4 yield, whilst it can also cause problems in the digestion due to adhesion of fat on cell surfaces. This leads to mass transfer limitations and unwanted flotation of digester biomass. Nitrogen limited cultivation would be useful for energetic balance and sustainability of microalgal biomass production because nitrogen fertilizer production consumes significant amount of energy (Hulatt et al., 2012). When normalized to surface area, microalgal biomass production requires substantially more nitrogen as compared to most terrestrial plants (Sialve et al., 2009).

Retention times required to obtain high CH_4 yields from untreated microalgal biomass are relatively long, 20–30 days (Ras et al., 2011; Zamalloa et al., 2011). Anaerobic digestion of microalgal biomass has been investigated in batch and fed-batch systems as well as in continuously stirred tank reactors (De Schamphelaire and Verstraete, 2009; Sialve et al., 2009). Zamalloa et al. (2011) suggested that anaerobic sludge blanket reactors, anaerobic filter reactors and anaerobic membrane bioreactors should be tested due to their high volumetric conversion rates. These processes have, however, been designed for wastewater treatment and high solids content of microalgal slurry may interfere with generation of anaerobic biomass and clog the membranes.

3. Dark fermentative hydrogen production

Many microalgae produce H_2 via photobiological pathways (for a review, see Levin et al. (2004)). Photosynthetic H_2 production, $2H_2O + \text{light} \rightarrow 2H_2 + O_2$, does not generate CO_2 and provides direct conversion of microalgal biomass to H_2. The hydrogenases involved in this reaction are relatively sensitive to high partial pressure of H_2 and O_2 and their activity is contingent on intact photosynthetic apparatus. The rates and conversion efficiencies of H_2 synthesis are considerably higher with dark fermentation than with the photobiological pathway. Dark fermentative H_2 production of biomass in anaerobic digestion is also more amenable for practical application (Levin et al., 2004; Levin and Chahine, 2010). The rates and yields of the photobiological H_2 production by microalgae are not comparable to anaerobic digestion systems because the process parameters are quite different. Therefore, only H_2 production studies where microalgal biomass has been used as a substrate for fermentative organism are included in this review.

Table 1
Methane production yields obtained from various microalgal species and other aquatic and terrestrial feedstocks.

Feedstock	Feedstock pretreatment	Reactor type	Temp (°C)	CH_4 yield (mL g^{-1})	Reference
Cyanobacterium *Arthrospira maxima*[a]	None	Digester flasks, continuous operation	35	160–310	Samson and LeDuy (1983a,b)
Cyanobacterium *Arthrospira maxima*[a]	Ultrasonication	Digester flasks, continuous operation	35	170	Samson and LeDuy (1983a)
Cyanobacterium *Arthrospira maxima*[a]	Heat treatment (50 °C, pH 11)	Digester flasks, continuous operation	35	210	Samson and LeDuy (1983a)
Cyanobacterium *Arthrospira maxima*[a]	Heat treatment (100 °C, pH 11)	Digester flasks, continuous operation	35	220	Samson and LeDuy (1983a)
Cyanobacterium *Arthrospira maxima*[a]	Heat treatment (150 °C, pH 11)	Digester flasks, continuous operation	35	240	Samson and LeDuy (1983a)
Cyanobacterium *Arthrospira maxima*[a] with domestic sewage sludge	None	Digester flasks, continuous operation	35	360	Samson and LeDuy (1983b)
Cyanobacterium *Arthrospira maxima*[a] with peat hydrolyzate	None	Digester flasks, continuous operation	35	280	Samson and LeDuy (1983b)
Cyanobacterium *Arthrospira maxima*[a] with spent sulfite liquor	None	Digester flasks, continuous operation	35	250	Samson and LeDuy (1983b)
Cyanobacterium *Arthrospira platensis*[a]	None	Batch fermenter	38	293	Mussgnug et al. (2010)
Macroalga *Ulva lactuca*	Chopping	Batch bottle	52	174	Bruhn et al. (2011)
Macroalga *Ulva lactuca*	Chopping and maceration	Batch bottle	52	271	Bruhn et al. (2011)
Microalga *Chlamydomonas reinhardtii*	None	Batch fermenter	38	387	Mussgnug et al. (2010)
Microalga *Chlorella kessleri*	None	Batch fermenter	38	218	Mussgnug et al. (2010)
Microalga *Chlorella* spp.	Drying and grinding	Batch bottle	37	>400	Ehimen et al. (2009)
Microalga *Chlorella* spp.	Lipid extraction with 1-butanol[e]	Batch bottle	37	268	Ehimen et al. (2009)
Microalga *Chlorella* spp.	*In situ* transesterification[e]	Batch bottle	37	222	Ehimen et al. (2009)
Microalga *Chlorella vulgaris*	None	Batch bottle	37	286	Lakaniemi et al. (2011a)
Microalga *Chlorella vulgaris*	None	Continuous reactor	35	147–240	Ras et al. (2011)
Microalga *Dunaliella salina*	None	Batch fermenter	38	323	Mussgnug et al. (2010)
Microalga *Dunaliella tertiolecta*	None	Batch bottle	37	24	Lakaniemi et al. (2011a)
Microalga *Euglena gracilis*	None	Batch fermenter	38	325	Mussgnug et al. (2010)
Microalga *Phaeodactylum tricornutum*	None	Batch bottle	33	350	Zamalloa et al. (2012)
Microalga *Phaeodactylum tricornutum*	None	Hybrid flow-through reactor	33	270	Zamalloa et al. (2012)
Microalga *Phaeodactylum tricornutum*	None	Hybrid flow-through reactor	54	290	Zamalloa et al. (2012)
Microalga *Scenedesmus obliquus*	None	Batch bottle	33	210	Zamalloa et al. (2012)
Microalga *Scenedesmus obliquus*	None	Hybrid flow-through reactor	33	130	Zamalloa et al. (2012)
Microalga *Scenedesmus obliquus*	None	Hybrid flow-through reactor	54	170	Zamalloa et al. (2012)
Microalga *Scenedesmus obliquus*	None	Batch fermenter	38	178	Mussgnug et al. (2010)
Microalga *Scenedesmus* spp.	Lipid extraction and alkaline heat treatment (100 °C 8 h)	Batch bottle	37	323	Yang et al. (2011)
Mixed microalgal culture with *Scenedesmus* and *Chlorella* spp.	None	Fed-batch operated digester	35	248	Golueke et al. (1956)
Mixed microalgal culture with *Scenedesmus* and *Chlorella* spp.	None	Fed-batch operated digester	50	314	Golueke et al. (1956)
Mixed microalgal culture	None	Fed-batch operated digester	38	240	Chen and Oswald (1998)
Mixed microalgal culture	Heat treatment (100 °C 8 h)	Fed-batch operated digester	38	320	Chen and Oswald (1998)
Mixed microalgal culture[b]	Heat treatment (70 °C 60 h)	Semi-continuous plug-flow type sequential digester setup	40	335[f]	De Schamphelaire and Verstraete (2009)
Mixed microalgal culture[c]	None	Fed-batch operated digester	45	402	Golueke and Oswald (1959)
Mixed microalgal culture[d]	None	Semi-continuous digester	35	143	Yen and Brune (2007)
Mixed microalgal culture[d] with waste paper (1:1)	None	Semi-continuous digester	35	293	Yen and Brune (2007)
Reed canary grass	Chopping	Batch bottle	35	340–430	Lehtomäki et al. (2008)
Timothy-clover grass	Chopping	Batch bottle	35	370–380	Lehtomäki et al. (2008)
Tops of sugar beet	Chopping	Batch bottle	35	340	Lehtomäki et al. (2008)
Water hyacinth	Sun-drying and pulverization	Batch bottle	55	230	Chuang et al. (2011)

[a] *Arthrospir*a is also known as *Spirulina*.
[b] Culture from hydroponic growth system supplemented with *Chlamydomonas reinhardtii* and *Pseudokircheneriella subcapitata*.
[c] *Chlorella*, *Scenedesmus*, *Euglena* and *Oscillatoris* spp.
[d] *Scenedesmus*, *Chlorella* spp. and others.
[e] Microalgal biomass residue after lipid extraction.
[f] CH_4 yield was calculated from the biogas yield by assuming that 2/3 of the biogas was CH_4.

H_2 is a key intermediate produced to maintain the electron balance during anaerobic digestion. H_2 does not generally accumulate in nature, because it is rapidly used by methanogens and other H_2 utilizing microorganisms. Dark fermentation pathways leading to H_2 formation are found in numerous bacterial genera including obligate anaerobes such as *Clostridium* and rumen bacteria, and facultative anaerobes such as *Escherichia*, *Enterobacter*, *Citrobacter*, *Alcaligenes* and *Bacillus* spp. (for a review, see Li and Fang (2007)). Hydrogen can be produced with pure and mixed cultures. For complex feedstocks such as microalgal biomass and for large scale bioprocess systems, feedstocks cannot be sterilized and, inevitably, mixed cultures are used. H_2 production can be encouraged over CH_4 production with pH control and using short hydraulic retention times, by inhibiting non-spore-forming H_2 consumers with heat treatment, or by addition of specific methanogen inhibitors such as 2-bromoethanesulfonic acid (BESA), acetylene or chloroform (Li and Fang, 2007). H_2 production is thermodynamically favorable only when H_2 partial pressure is low. Thus, when growth

of H_2-consuming microorganisms is inhibited, continuous H_2 production requires continuous or intermittent H_2 removal from the system. The theoretical H_2 yield maximum is 4 mol-H_2 mol-glucose^{-1} with acetate as the sole soluble product of dark fermentation (Levin et al., 2004). In general, previous studies on H_2 production by dark fermentation have focused on using simple substrates such as glucose or sucrose, as well as biopolymers, plant materials and waste streams (for a review, see Li and Fang (2007)).

Dark fermentative H_2 production from microalgal biomass has received increasing attention over the last few years (Table 2). Variable H_2 yields have been reported and they have been in the same range as those from other aquatic and terrestrial biomass feedstocks (Table 2). Microalgal biomass slurries concentrated from cultivation units may also contain H_2-producing bacteria (Carver et al., 2011; Lakaniemi et al., 2011a). Authenticity of H_2 fermentation by microalgae under dark and anaerobic conditions as reported in the previous literature (Gfeller and Gibbs, 1984; Miura et al., 1986) is impossible to evaluate in hindsight because microbial communities were not characterized in those studies. Only recently it has been recognized that H_2 is probably produced by heterotrophic bacterial satellites present in the microalgal biomass slurries.

The inhibition of H_2-consuming organisms in complex microbial consortia required to decompose microalgal biomass for H_2 production is challenging. For example, H_2 was produced from green algae *C. vulgaris* and *D. tertiolecta* biomasses by anaerobic digester enrichment cultures containing BESA, but H_2 was subsequently consumed by non-methanogenic microorganisms (Lakaniemi et al., 2011a). Similar H_2 consumption, although to a lower extent, was reported by Yang et al. (2010) for H_2 production by heat treated anaerobic digester sludge that was fed lipid extracted *Scenedesmus* biomass. Hydrogen consumption also occurs with other complex substrates. For example, Dong et al. (2009) reported H_2 consumption in a mixed culture fermenting rice, potato or lettuce although methanogens were inhibited by thermal inactivation.

Pretreatment of complex feedstocks (Zhang et al.,2007; Lakaniemi et al., 2011b) and thermophilic fermentation can increase H_2 yields (Valdez-Vazquez et al., 2005). Pretreatment seems to be more effective for H_2 production than for methanogenic digestion (Tables 1 and 2; Lakaniemi et al., 2011b). However, the pretreatment expends energy and needs to be optimized for low energy consumption. In general, studies with waste materials and lignocellulosic biomass have shown higher H_2 generation from carbohydrate-rich materials than from lipid- or protein-rich materials (Dong et al., 2009). These reports indicate that microalgal biomass with high carbohydrate content should be favorable for dark fermentative H_2 production.

4. Ethanol fermentation

In ethanol fermentation, organic feedstock is first hydrolyzed (saccharified) to the corresponding sugars (pentoses and hexoses depending on the feedstock), which are fermented to ethanol and CO_2 (for reviews, see Lin and Tanaka (2006) and John et al. (2011)). With starch-rich substrates, such as corn, cassava and potatoes, pretreatment for ethanol fermentation typically first consists of hydrolysis with acid or α-amylase and then cooking at high temperatures, approx. at 140–180 °C, for liquefaction and sometimes followed with glucoamylase reaction to complete starch hydrolysis to glucose (Lin and Tanaka, 2006). Pretreatment of complex lignocellulosic material such as woody plants, energy crops and forest residues requires a harsher pretreatment due to the lignin fraction. In these cases, ozonolysis, organosolv process, steam explosion, liquid hot water treatment and ammonia fiber explosion have been tested with variable success (for a review, see Alvira et al. (2010)). Several organisms including yeasts, fungi and bacteria can produce ethanol through fermentation, and the most

Table 2
Hydrogen production yields obtained from various microalgal species and other aquatic and terrestrial feedstocks.

Feedstock	Feedstock pretreatment	Reactor type	Source inoculum	Temp (°C)	H_2 yield (mL g^{-1})	Reference
Cornstalk	Grinding	Batch bottle	Cow dung compost	36	3	Zhang et al. (2007)
Cornstalk	Grinding and NaOH pretreatment	Batch bottle	Cow dung compost	36	57	Zhang et al. (2007)
Cornstalk	Grinding and HCl pretreatment	Batch bottle	Cow dung compost	36	150	Zhang et al. (2007)
Macroalga *Laminaria japonica*	Drying (at room temp) and grinding	Batch reactor	WWTP[a] digester sludge	35	71	Shi et al. (2011)
Microalga *Chlamydomonas* spp.	None	Batch tube	None[b]	37	48	Miura et al. (1986)
Microalga *Chlorella* spp.	None	Batch bottle	Anaerobic digested sludge	35	7	Sun et al. (2011)
Microalga *Chlorella vulgaris*	None	Batch bottle	Compost	60	114	Carver et al. (2011)
Microalga *Chlorella vulgaris*	None	Batch bottle	None[c]	60	82	Carver et al. (2011)
Microalga *Chlorella vulgaris*	None	Batch bottle	None[c]	37	11	Lakaniemi et al. (2011a)
Microalga *Dunaliella tertiolecta*	None	Batch bottle	Compost	60	58	Carver et al. (2011)
Microalga *Dunaliella tertiolecta*	None	Batch bottle	None[c]	60	39	Carver et al. (2011)
Microalga *Dunaliella tertiolecta*	None	Batch bottle	None[c]	37	13	Lakaniemi et al. (2011a)
Microalga *Scenedesmus* spp.	Lipid extraction	Batch bottle	Anaerobic digested sludge	37	17	Yang et al. (2010)
Microalga *Scenedesmus* spp.	Lipid extraction and alkaline pretreatment (27 °C 24 h)	Batch bottle	Anaerobic digested sludge	37	17	Yang et al. (2010)
Microalga *Scenedesmus* spp.	Lipid extraction and heat treatment (100 °C 8 h)	Batch bottle	Anaerobic digested sludge	37	35	Yang et al. (2010)
Microalga *Scenedesmus* spp.	Lipid extraction and heat treatment (121 °C 4 h)	Batch bottle	Anaerobic digested sludge	37	36	Yang et al. (2010)
Microalga *Scenedesmus* spp.	Lipid extraction and alkaline heat treatment (100 °C 8 h)	Batch bottle	Anaerobic digested sludge	37	46	Yang et al. (2010, 2011)
Microalga *Scenedesmus* spp.	Lipid extraction and alkaline heat treatment (121 °C 4 h)	Batch bottle	Anaerobic digested sludge	37	37	Yang et al. (2010)
Water hyacinth (*Eichornia crassipes*)	Sun-drying and pulverization	Batch bottle	Pig slurry	55	27	Chuang et al. (2011)

[a] WWTP = wastewater treatment plant.
[b] H_2 generation was attributed to intracellular fermentation.
[c] H_2 was likely produced by bacteria present in microalgal biomass slurry.

Table 3
Ethanol production yields obtained from various microalgal species and other aquatic and terrestrial feedstocks.

Feedstock	Pretreatment/hydrolysis/saccharification	Fermentation	Ethanol yield (mg g^{-1})	Reference
Corn (*Zea mays*) grain	Drying, milling, hydrolysis with α-amylase at 105 °C for 15 min and saccharification with glucoamylase at 90 °C for 60 min	Batch growth of *Saccharomyces cerevisiae* at 30 °C for 60 h	311-364[a]	Mangat et al. (2010)
Macroalga *Gracilaria salicornia*	Homogenization, dilute acid hydrolysis with 2% H_2SO_4 at 120 °C for 30 min and cellulase treatment at 40 °C for 4 h	Batch growth of recombinant *Escherichia coli* KO11 at 30 °C	79	Wang et al. (2011)
Microalga *Chlamydomonas reinhardtii*	Acid treatment with 3% H_2SO_4 at 110 °C for 30 min	Batch growth of *Saccharomyces cerevisiae* at 30 °C for 24 h	292	Ngyuen et al. (2009)
Microalga *Chlamydomonas reinhardtii*	Hydrolysis with 0.005% α-amylase at 90 °C for 30 min, saccharification with 0.2% amyloglucosidase at 55 °C for 30 min	Batch growth of *Saccharomyces cerevisiae* at 30 °C for 40 h	235	Choi et al. (2010)
Microalga *Chlamydomonas reinhardtii*	Washing and resuspension to fresh buffer solution	Intracellular ethanol fermentation	10	Hirano et al. (1997)
Microalga *Chlorella vulgaris*	Disruption with ultrasonic radiation, hydrolysis with α-amylase at 100 °C and saccharification with glucoamylase at 60 °C	Batch growth of *Saccharomyces cerevisiae*	2	Hirano et al. (1997)
Microalga *Chlorococcum humicola*	Drying, milling and acid treatment with 3% H_2SO_4 at 160 °C for 15 min	Batch growth of *Saccharomyces cerevisiae* at 30 °C	520	Harun and Danquah (2011)
Microalga *Chlorococcum infusionum*	Drying, milling and alkaline treatment with 0.75% NaOH at 120 °C for 30 min	Batch growth of *Saccharomyces cerevisiae* at 30 °C for 72 h	260	Harun et al. (2011)
Microalga *Chlorococcum littorale*	Washing and resuspension to fresh buffer solution	Intracellular ethanol fermentation	21	Ueno et al. (1998)
Microalga *Chlorococcum* spp.	Lipid extraction with supercritical CO_2 at 60 °C and drying	Batch growth of *Saccharomyces bayanus* at 30 °C for 60 h	383	Harun et al. (2010)
Microalga *Chlorococcum* spp.	Drying at 60 °C	Batch growth of *Saccharomyces bayanus* at 30 °C for 60 h	160	Harun et al. (2010), Harun and Danquah (2011)
Microalga *Dunaliella* spp.	Rethawing, drying, autoclaving and saccharification with Glucozym AF6 at 58 °C for 1 day	Batch growth of *Saccharomyces cerevisiae* at 25 °C for 5 days	11	Shirai et al. (1998)
Water hyacinth (*Eichornia crassipes*)	Drying, powdering, alkaline oxidative treatment with 1% NaOH for 12 h, followed by addition of 1% H_2O_2 and incubation for another 12 h	Batch growth of recombinant *Escherichia coli* KO11 at 37 °C with cellulase for SSF[b]	170	Mishima et al. (2008)

[a] Calculated from the given values.
[b] SSF = simultaneous saccharification and fermentation.

commonly used microorganism in ethanol production is *Saccharomyces* yeast, especially *S. cerevisiae*. Wild-type *S. cerevisiae* ferments only hexose sugars, hydrolysis products of cellulose, but not pentoses, hydrolysis products of hemicelluloses. Therefore, ethanol fermentation has also been studied with other organisms such as a genetically modified *Escherichia coli* KO11, which can degrade both hexose and pentose sugars (Ohta et al., 1991; Mishima et al., 2008; Wang et al., 2011). The product of ethanol fermentation is dilute, 10–15% ethanol with yeasts and even more dilute with bacteria. For final product, distillation is required before ethanol can be used as a gasoline substitute or supplement (Lin and Tanaka, 2006).

Some microalgae, especially green algae (phylum *Chlorophyta*), have relatively high content of carbohydrates such as cellulose and starch as cell wall constituents and storage products making them potentially good candidates for ethanol fermentation (Metting, 1996; John et al., 2011). However, interest in using microalgal biomass for fermentative ethanol production has emerged only during last few years (Table 3). Photosynthetic microalgal cultures (Lakaniemi et al., 2012a,b) and harvested microalgal biomass slurries (Carver et al., 2011; Lakaniemi et al., 2011a) often contain diverse bacterial populations that may contribute to the formation and consumption of alcohols. Endogenous alcohol fermentation by live microalgal biomass, if it occurs, is of minor significance as compared to yields and rates in anaerobic digestion processes. Pretreatment of microalgal biomass is required for efficient ethanol production and to date dilute acid hydrolysis seems most promising (Table 3). Ethanol yields obtained from microalgal biomass are similar to those obtained from other aquatic feedstocks and terrestrial crops (Table 3). Yields such as 520 mg ethanol g^{-1} dry wt. biomass obtained from dilute acid pretreated *Chlorococcum humicola* (Harun and Danquah, 2011) indicate reasonable potential for using microalgal biomass feedstock for commercial ethanol fermentation. All the reviewed studies have been conducted in batch mode and evaluation of continuous ethanol fermentation from microalgal biomass is needed. Acid pretreatment may also result in transformation of glucose and xylose into furfural and hydroxymethylfurfural (Mussatto and Roberto, 2004). These compounds may be inhibitory to ethanol fermentation and methods of pretreatment of microalgal biomass should be further refined in an effort to eliminate the formation of these compounds.

5. Microbial fuel cells for electricity generation

Microbial fuel cells (MFCs) convert chemically bound energy into electricity via anaerobic microbial respiration that couples with anode as the terminal electron acceptor. MFCs under optimized conditions have relatively high coulombic efficiencies in the energy conversion, which makes them potentially attractive for current generation from organic waste and biomass. MFC applications have mostly been tested at mesophilic temperatures but designs are now available for the thermophilic range. Because current is produced by microbial metabolism, the rate increases with temperature. Current generation and coulombic efficiency vary with MFC configurations, feedstocks, and types of electrogenic microbial consortia.

MFCs have been operated with pure and mixed anode cultures. Pure culture studies provide useful information of a given species and its exoelectrogenic properties, but cannot be used with complex feedstocks such as microalgal biomass. They are prone to process disturbances and tend to have relatively low power outputs (Pham et al., 2006). Numerous obligate and facultative anaerobes can use the anode as the e^- acceptor in batch, fed-batch and continuous flow applications of MFCs. For practical applications with photosynthetic biomass or cellular polymer feedstocks mixed microbial populations are employed to ensure metabolic diversity. Some photosynthetic organisms such as cyanobacteria *Synechococcus* spp. also generate electricity in photo-electrochemical cells (Tsujimura et al., 2001). The role of eukaryotic microalgae in anaerobic decomposition and respiration in MFCs remains unexplored. Strik et al. (2008) reported a solar-powered MFC with live, but not photosynthetically active microalgae and electrogenic bacteria co-operating on the anode. The role of the microalgae in current production was not established, however.

Microalgal biomass has been used as a feedstock for the exoelectrogenic bacteria in the anode chamber (Reimers et al., 2007; Velasquez-Orta et al., 2009; Lakaniemi et al., 2012c). Microalgae can also act as the electron acceptor at the cathode (De Schamphelaire and Verstraete, 2009; Powell et al., 2009). The flow of electrons in the microalgae at the cathode is not fully understood. According to Powell et al. (2009), microalgae can act as the electron acceptor and utilize the electrons for cell growth. In contrast, De Schamphelaire and Verstraete (2009) reported that the actual electron acceptor is oxygen generated in oxygenic photosynthesis. Untreated microalgal biomass does not rank well for electricity production based on the low power densities reported thus far (Table 4). However, Velasquez-Orta et al. (2009) reported relatively high current generation from dry, pulverized *C. vulgaris* biomass. Their results are comparable to or even higher than those reported for pretreated terrestrial crops and macroalgal biomass (Table 4). In general, substantially higher power densities have been reported for soluble substrates, but that is to be expected because of improved mass transfer kinetics.

High salinity of salt water microalgal biomass has not been reported to inhibit electrogenic activity in MFCs. Provided that the anodic culture is adapted to or derived from a saline environment, elevated salinity of anode and cathode solutions often increases maximum power density of an MFC due to increased solution conductivity (Zuo et al., 2006). Depending on the chemical makeup of the salinity, ions such as Mg^{2+} and Ca^{2+} may precipitate especially as phosphates, as reported by Lakaniemi et al. (2012c) for the cathodes in two-chamber MFCs fed with marine *D. tertiolecta* biomass slurry. Cathodic precipitates in MFCs can decrease current generation as they interfere with electron transfer and other electrochemical reactions.

6. Other possible fuels from fermentative processes

Alkanes and longer-chain alcohols, such as butanol, have several advantages over ethanol as transport fuel. These include a higher energy content, lower absorption of water and possibility to be used as such or as mixtures with petroleum in conventional combustion engines. Ellis et al. (2012) reported acetone, butanol and ethanol (ABE) fermentation by *Clostridium saccharoperbutylacetonicum* from mixed microalgal biomass that had been pretreated sequentially with acid and base hydrolysis. In batch mode fermentation the yield reached 0.244 g ABE/g microalgal biomass, of which the bulk (0.201 g/g) was butanol. Ethanol was not detected as a major product. Addition of xylanase and cellulase enzymes to the fermentation broth increased ABE production to 0.311 g/g, of which 0.249 g/g was butanol (Ellis et al., 2012). Lakaniemi et al. (2012c) reported concurrent electricity and butanol generation in MFCs fed with non-pretreated microalgal biomass. A butanol yield of 0.042 g per g of volatile solids was reported from non-pretreated *C. vulgaris* (Lakaniemi et al., 2012c).

7. Process integration

It is important to integrate sustainable bioprocesses to increase overall yields of microalgal biomass conversion to energy carriers.

Table 4
The maximum power densities and coulombic efficiencies obtained with microalgal biomass using different feedstock pretreatments and MFC configurations. Results obtained with terrestrial crop and macroalgal biomass feedstocks are given as comparison.

Feedstock	Feedstock pretreatment	MFC configuration	Max power (mW m−2)	CE (%)[a]	Reference
Corn stover	Neutral steam explosion (liquid product)	Single chamber MFC with air–cathode and carbon paper anode	810	10–30	Zuo et al. (2006)
Corn stover	Acidic steam explosion (liquid product)	Single chamber MFC with air–cathode and carbon paper anode	861	10–30	Zuo et al. (2006)
Corn stover	Pulverization, drying	Bottle MFC with air–cathode and carbon paper anode	331	n.a.[b]	Wang et al. (2009)
Corn stover	Neutral steam-explosion (residual solids)	Bottle MFC with air–cathode and carbon paper anode	406	n.a.	Wang et al. (2009)
Macroalga *Ulva lactuca*	Pulverization, drying	Single chamber MFC with air–cathode and brush type anode	760	23	Velasquez-Orta et al. (2009)
Manure (solid)	None	Single chamber MFC with air–cathode and brush type anode	67	1.3–5.2	Zheng and Nirmalakhandan (2010)
Microalga *Chlorella vulgaris*	Pulverization, drying	Single chamber MFC with air–cathode and brush type anode	980	28	Velasquez-Orta et al. (2009)
Microalga *Chlorella vulgaris*	None	Two-chamber MFC with ferricyanide cathode and graphite plate anode	15	1.7	Lakaniemi et al. (2012c)
Microalga *Dunaliella tertiolecta*	None	Two-chamber MFC with ferricyanide cathode and graphite plate anode	5	8.1	Lakaniemi et al. (2012c)
Mixture of marine phytoplankton and zooplankton	None	H-type two chamber MFC with graphite and carbon impregnated rod electrodes and oxic seawater circulated cathode	3–17	11–16	Reimers et al. (2007)

[a] CE = coulombic efficiency.
[b] n.a. = Data not available.

Residual microalgal biomass after lipid extraction for biodiesel production is a potential substrate for production of CH_4 (Ehimen et al., 2009; Yang et al., 2011), H_2 (Yang et al., 2010) and ethanol (Harun et al., 2010). Mussgnug et al. (2010) produced H_2 photosynthetically with *Chlamydomonas reinhardtii* and the biomass was disposed of through methanogenic digestion. H_2 production effluents contain volatile fatty acids that can be utilized for CH_4 or bioelectricity production. For example, Shi et al. (2011) produced CH_4 using effluent from H_2 fermentation of macroalga *Laminaria japonica*. Yang et al. (2011) reported sequential fermentative H_2 and CH_4 production from lipid-extracted microalga *Scenedesmus* biomass with H_2 and CH_4 yields of 46 and 394 mL g^{-1}, respectively. Yang et al. (2011) also reported that H_2 production stage increased subsequent CH_4 production by 22%. Sequential production of CH_4 and bioelectricity from microalgal biomass has also been reported (De Schamphelaire and Verstraete, 2009; Lakaniemi et al., 2012c). CH_4 production from ethanol production residues (saccharification and fermentation residue) has been demonstrated by Park et al. (2012) using macroalga *Gelidium amansii*. Lakaniemi et al. (2012c) also reported concurrent electricity and butanol production in MFCs fed with microalgal biomass. Electricity production in these MFCs fed with *C. vulgaris* and *D. tertiolecta* biomass was 9.8 and 12.9 J g^{-1} volatile solids, respectively. When the energy content of produced butanol was taken into account energy production increased to 1400 and 270 J g^{-1} volatile solids, respectively (Lakaniemi et al., 2012c).

To facilitate efficient nutrient recycle and full use of the energy content of algal biomass, closed loop systems have also been suggested. Golueke and Oswald (1959) combined a microalgal growth unit to an anaerobic digester and an "activated sludge" unit. In this system, microalgal biomass was first cultivated in the growth unit and then used for the anaerobic digester. The effluent of the anaerobic digester was treated in the "activated sludge" unit and finally circulated back to the microalgal growth unit. In this manner solar energy was converted to CH_4 with a single closed loop system (Golueke and Oswald, 1959). De Schamphelaire and Verstraete (2009) modified this process slightly by combining microalgal growth unit to an anaerobic digester and an MFC. They digested microalgal biomass and further polished the digester effluent in an MFC. Oxygen produced by the microalgae was used in the MFC cathode and the MFC effluent was recycled to the algal growth unit.

8. Comparison of the energy conversion processes

MFCs are in research and development stage (De Schamphelaire and Verstraete, 2009) and fermentative H_2 production is in pilot stage (Kim et al., 2010). Progress with MFCs is hampered by the relatively low power outputs and high maintenance and material costs. R&D in this area needs to address design, material and scale-up optimization. Based on the present state-of-the-art MFC technology, it is difficult to envision that these systems will be developed to large commercial scale systems that feed electricity to grids. Economic, technological feasibility and energy balance analyses have yet to suggest that this technology will have a role in the global energy consumption. The MFC technology may be at best in solid and liquid waste treatment where the remaining energy content would otherwise be lost untapped. Such applications will certainly be also feasible in biorefinery approaches where photosynthetic biomass is converted to fuels, heat, electricity and perhaps even value-added chemicals.

CH_4 and ethanol are produced commercially from various feedstocks for use as energy carriers. The feedstocks, technology, production schemes, and market in both cases are well established and of global significance. Butanol production via ABE fermentation is of great interest for industrial applications and has already been reduced to practice in some countries (Ni and Sun, 2009). Butanol is generally used as a solvent in chemical industry and the most efficient producer strains are *Clostridia*. Axenic cultivation conditions are required for high yields, which make the process less cost-effective for large-scale production as compared to CH_4 and ethanol. Butanol tolerance of known alcohol producing microorganisms is limited to 1–3%, whereas ethanol is tolerated up to 18% (for a review, see Liu and Qureshi (2009)).

To compare energy yields obtained as CH_4, H_2, ethanol and butanol, the highest conversion yields of each energy carrier were

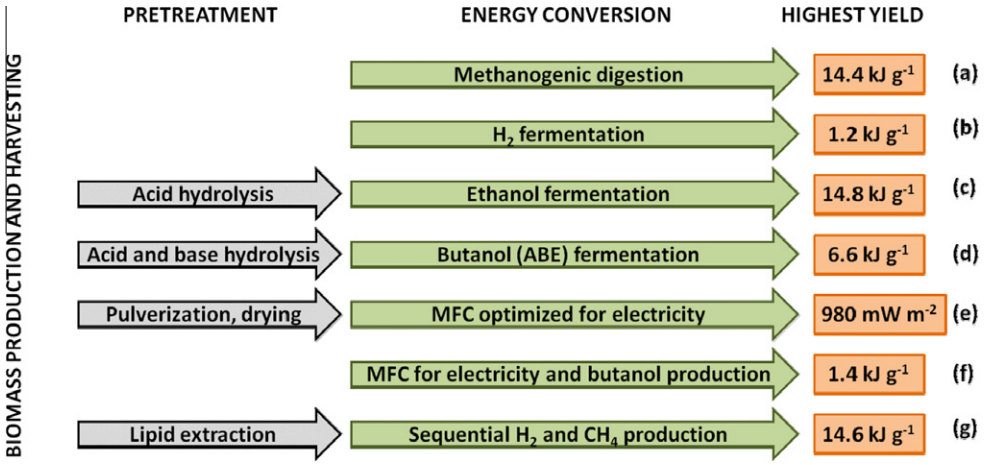

Fig. 1. Highest energy yields (kJ g^{-1} dry wt. microalgal biomass) from the different anaerobic energy conversion processes calculated from published studies using the lower heating values presented in Table S1. Pretreatments are included for the specific published examples included in the schematic but, in general, pretreatments are not specific and they may not be needed for the different energy conversion steps. Electricity yield per g of substrate is generally not reported in MFC studies and therefore highest electricity production was given as power density (mW m^{-2}). References for the pretreatments, energy carrier yields and process steps are: (a) Golueke and Oswald (1959); (b) Carver et al. (2011); (c) Harun and Danquah (2011); (d) Ellis et al. (2012); (e) Velasquez-Orta et al. (2009); (f) Lakaniemi et al. (2012c); (g) Yang et al. (2011).

normalized to kJ per g of microalgal biomass using the lower heating values (Table S1, Supplementary Material). Electricity yield per g of substrate is generally not reported in MFC studies and its calculation was not possible with the reported results. Therefore, the highest electricity production is expressed as power density (mW m^{-2}) in Fig. 1. Based on the reviewed literature, the highest energy yield from microalgal biomass to date has been obtained with ethanol fermentation using acid pretreated microalgal biomass (Fig. 1). The highest energy yield with methanogenic digestion was very close to the value obtained for ethanol fermentation and was obtained from non-pretreated microalgal biomass. The highest H_2 and butanol yields obtained from microalgal biomass were about 10% and 50% of the yields obtained as ethanol, respectively. Simultaneous production of electricity and butanol in MFCs yielded similar levels of energy as compared to H_2 fermentation (Fig. 1). It is possible that MFCs would work better as a polishing step after H_2, CH_4 or ethanol production because MFCs perform best at low concentrations of readily biodegradable organic material (Pham et al., 2006). Based on the technological status and conversion yields, methanogenic digestion and ethanol fermentation can most efficiently convert microalgal biomass to energy carriers. Very promising is also high energy yield obtained from lipid extracted microalgal biomass residue that has been sequentially fermented to H_2 and CH_4 (Fig. 1). In many studies microalgal biomass has been dried and then again suspended into water prior to use. Drying is energy intensive and is not needed in applications discussed in this review. In addition, drying may reduce energy carrier yields (Mussgnug et al., 2010).

CH_4 and ethanol yields (normalized to g dry wt. of feedstock) from microalgal biomass are comparable to those obtained with other aquatic feedstocks and terrestrial crops (Tables 1 and 3). Areal productivity of 10–50 g m^{-2} d^{-1} (i.e., 36.5–183 ton ha^{-1} - $year^{-1}$) has been used as a reference value when microalgal biomass production is compared to that of terrestrial crops (Chisti, 2007; Schenk et al., 2008). As high microalgal biomass productivity as 98 g m^{-2} d^{-1} (358 ton ha^{-1} $year^{-1}$) has been reported (Pulz, 2007). For comparison, areal productivities of 13–24, 44 and 73–87 ton ha^{-1} $year^{-1}$ have been reported for corn stover, sweet sorghum and sugar cane, respectively (Huber et al., 2006). Areal productivities reported for macroalga *Ulva lactuca* and water hyacinth (*Eichhornia crassipes*) are 45 and 100 ton ha^{-1} $year^{-1}$, respectively (Bruhn et al., 2011; Chuang et al., 2011). These estimates further highlight the competiveness of microalgal biomass as feedstocks for anaerobic energy production. Full comparison of energy conversion processes and energy feedstocks requires comparison of energy balances and life cycle assessments of the entire energy production chains, which are out of scope of this review. Most energy carrier production studies included in this review have been conducted in laboratory scale and scale-up to larger and pilot scale digester/fermentor operation with microalgal biomass as the substrate is still needed to provide a credible basis for fundamental life-cycle assessments and cost analyses.

9. Conclusions

At present the highest ethanol, methane, butanol and hydrogen yields from anaerobic conversion of microalgal biomass have been 14.8, 14.4, 6.6 and 1.2 kJ g^{-1}. The highest reported electricity generation from microalgal biomass in MFCs has been 980 mW m^{-2}. Combination of different energy production processes can increase the overall energy yields but also maintenance and material costs. Based on the technological status and conversion yields, methanogenic digestion and ethanol fermentation can most efficiently convert microalgal biomass to energy carriers. The energy balances, environmental impacts and cost efficiency of different microalgal biomass-fed anaerobic conversion processes remain to be carefully analyzed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2012.08.096.

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