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Biohythane production from marine macroalgae *Sargassum* sp. coupling dark fermentation and anaerobic digestion

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HIGHLIGHTS

- Biohythane production from *Sargassum* sp. is feasible and has a high potential.
- Hydrogen yield of 91.3 ± 3.3 L H_2 per kg (VS) of *Sargassum* sp. was attained.
- It was achieved a methane yield of 541 ± 10 L CH_4 per kg (VS) of *Sargassum* sp.
- Potential energy production from *Sargassum* sp. was estimated in 242 GJ ha^{-1} yr^{-1}.
- The value of estimated energy could result in 600 EJ yr^{-1}.

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ABSTRACT

Potential biohythane production from *Sargassum* sp. was evaluated in a two stage process. In the first stage, hydrogen dark fermentation was performed by *Caldicellulosiruptor saccharolyticus*. *Sargassum* sp. concentrations (VS) of 2.5, 4.9 and 7.4 g L^{-1} and initial inoculum concentrations (CDW) of 0.04 and 0.09 g L^{-1} of *C. saccharolyticus* were used in substrate/inoculum ratios ranging from 28 to 123. The end products from hydrogen production process were subsequently used for biogas production.

The highest hydrogen and methane production yields, 91.3 ± 3.3 L kg^{-1} and 541 ± 10 L kg^{-1}, respectively, were achieved with 2.5 g L^{-1} of *Sargassum* sp. (VS) and 0.09 g $L^{-1}$of inoculum (CDW). The biogas produced contained 14–20% of hydrogen. Potential energy production from *Sargassum* sp. in two stage process was estimated in 242 GJ ha^{-1} yr^{-1}. A maximum energy supply of 600 EJ yr^{-1} could be obtained from the ocean potential area for macroalgae production.

1. Introduction

Hythane, a mixture of hydrogen and methane, usually with 10–25% hydrogen in volume, is an important future fuel (Ljunggren and Zacchi, 2010). By adding hydrogen to methane, the H/C ratio is increased, reducing the greenhouse gas emissions. In addition, the fuel efficiency is improved since the narrow range of flammability of methane is extended, the flame speed of methane can be greatly increased, eventually reducing combustion duration and improving heat efficiency, and the quenching distance of methane can be reduced, making the engine easy to ignite with less input energy (Liu et al., 2013). This hydrogen rich source of biofuel can promote an incremental introduction of hydrogen to the fueling infrastructure and accelerate transition of the market towards a hydrogen economy (Bauer and Forest, 2001; Das et al., 2000).

Hydrogen and methane can be biologically produced through a two-stage process coupling dark fermentation and anaerobic digestion (Banks et al., 2010; Lu et al., 2009; Ljunggren and Zacchi, 2010). Hydrogen dark fermentation at hyperthermophilic and extremely thermophilic conditions has been associated to higher productivities (Abreu et al., 2010, 2012). In addition to the high polysaccharide-hydrolysing capacities of many extreme and hyperthermophilic microorganisms, an important advantage is their ability to use most of the reducing equivalents (e.g. NADH, reduced ferredoxin) formed during glycolysis for the production of hydrogen (Verhaart et al., 2010). Extreme thermophilic bacteria, such as *Caldicellulosiruptor saccharolyticus* are reported to approach the theoretical maximum yield of 4 mol H_2 mol^{-1} glucose (van Niel et al., 2002; Willquist et al., 2010). The hydrolysates resulting from dark fermentation, rich in volatile fatty acids (VFA), can be converted to methane through an anaerobic digestion process (Costa et al., 2012). Alternatively a photofermentation process can follow the dark fermentation step, but this alternative has shown limited cost efficiency especially concerning the nutrients requirements

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and the costs of photobioreactors (final report of the Hyvolution EU project – http://www.biohydrogen.nl/hyvolution/32288/9/0/20). In the sequence of the EU project Hyvolution, the project Hytime considers a two step dark fermentation-anaerobic digestion process (http://www.hy-time.eu/hytime/32562/5/0/30).

Several studies have been done on biohythane production from pure sugars and from feedstocks, such as by-products from the agricultural and food industry, municipal waste, or wastewaters (Liu et al., 2013). However, there are no studies describing the production of biohythane in a two-step fermentation process from a marine macroalgae biomass.

Macroalgae cultivation does not involve the use of feed, fertilizer, pesticides or other chemicals. Macroalgae growth is fueled only by natural nutrients in seawater as well as solar energy and carbon dioxide. Moreover, macroalgae contain easily hydrolysable sugars and proteins, low fractions of lignin and high fractions of hemicellulose and a good hydrolysis yield making this biomass suitable for anaerobic fermentation (Briand and Morand, 1997; Nkemka and Murto, 2010). The energy potential of marine biomass is estimated to be more than 100 EJ yr^{-1}, significantly higher than the terrestrial biomass (22 EJ yr^{-1}) or municipal solid waste (7 EJ yr^{-1}) (Chynoweth et al., 2001). *Sargassum* sp. is a genus of brown free floating macroalgae that has a global occurrence.

The main objective of this study is to evaluate the potential of *Sargassum* sp. biomass for the generation of hydrogen enriched biogas (10–25% of H2) and determine the associated potential energy generation. For this, the extreme thermophilic bacterium *C. saccharolyticus* was used for hydrogen production on the first step of the process, followed by biogas production from anaerobic digestion of the resulting fermentation products. *C. saccharolyticus* is used in the present study due to its excellent polysaccharide-hydrolyzing capacity and because it is able to use most of the reducing equivalents formed during glycolysis for the production of hydrogen. *C. saccharolyticus* is referred as relatively insensitive to high pH_2. Moreover, this organism has recently gained increased interest due to its ability to produce thermostable cellulolytic and xylanolytic enzymes, to grow on complex lignocellulosic carbon sources, and to co-metabolize a wide spectrum of monosaccharides including both pentose and hexose sugars.

2. Methods

2.1. Biomass characterization

Sargassum sp. was collected in the spring of 2013 from a location in the north coastline of Portugal (Póvoa de Varzim). The macroalgae was dried at room temperature and then milled into pieces with less than 0.5 cm. *Sargassum* sp. biomass was characterized in terms of total and soluble Chemical Oxygen Demand (COD), total solids (TS), volatile solids (VS), Total Kjeldahl Nitrogen (TKN), fat content, Klason lignin, glucan and xylan content.

2.2. Hydrogen production assays

2.2.1. Culture and medium

C. saccharolyticus DSM 8903 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The culture medium consisted of (per L): KH_2PO_4 0.75 g, K_2HPO_4 1.5 g, $MgCl_2{\cdot}6H_2O$ 0.4 g, NH_4Cl 0.9 g, yeast extract 1.0 g, cysteine-HCl 0.75 g, $FeCl_3{\cdot}6H_2O$ 2.5 mg, NaCl 0.9 g, trypticase 2 g, SL-10 (medium 320 DSMZ) trace elements 1 ml, and resazurin 0.5 mg. The pH was adjusted to 7.2 at room temperature. The culture medium was supplemented with 50 mmol L^{-1} 4-morpholine propanesulfonic acid (MOPS) to increase the buffering capacity of the medium. Medium was reduced with 0.75 g L^{-1} Cysteine-HCl monohydrated. Cellobiose (2 g L^{-1}) was used as the carbon source for growing the culture. The medium was made anoxic by flushing with 100% N_2. The experiments were carried out under sterile conditions. *C. saccharolyticus* was grown at 70 °C.

2.2.2. Experiment set-up

Hydrogen production assays were performed in 160 mL serum bottles containing 50 mL of phosphate-buffered medium (20 mmol L^{-1}) and 50 mmol L^{-1} 4-morpholine propanesulfonic acid (MOPS) flushed with N_2 (100%). The medium was supplemented with trace elements solution SL-10 according to DSMZ 320 medium. Yeast extract and resazurin were added to a final concentration of 0.5 g L^{-1} and 0.5 mg L^{-1}, respectively. Medium was reduced with 0.75 g L^{-1} Cysteine-HCl monohydrated. Three different *Sargassum* sp. concentrations (VS per L) (2.5, 4.9 and 7.4 g L^{-1}) were tested. Before inoculation the bottles containing the different concentrations of *Sargassum* sp. were autoclaved at 121 °C and 1 bar for 15 min. The autoclave functioned as thermal and pressure pretreatment for the *Sargassum* sp.

Two batch series with different initial inoculum concentrations were performed for each *Sargassum* sp. concentration. The inoculum CDW concentrations tested were 0.04 and 0.09 g L^{-1} of precultured *C. saccharolyticus*. Bottles were incubated at 70 °C under shaking (90 rpm). All the experiments were performed in quadruplicate and included controls without *Sargassum* sp. and without *C. saccharolyticus*. Production of hydrogen gas and soluble fermentation products were monitored.

2.3. Methanogenic assays

2.3.1. Inoculum

Anaerobic granular sludge from a brewery industry was used as inoculum in the methanogenic assays. The sludge contained a VS concentration of 0.08 ± 0.01 g g^{-1}. The specific methanogenic activity (SMA) that represents the methane production at standard temperature and pressure (STP) conditions per VS of granular sludge per day, in the presence of acetate (30 mmol L^{-1}) was 156 ± 5 mL g^{-1} d^{-1}, and in the presence of H_2/CO_2 (80/20 v/v, 1 bar) was 375 ± 8 mL g^{-1} d^{-1}. SMA was determined as described in Costa et al. (2012).

2.3.2. Experiment set-up

Methanogenic assays were performed according to the guidelines defined in Angelidaki et al. (2009), with a working volume of 120 mL, at 37 °C. The hydrolysates obtained after H_2 production were added to 600 mL serum bottles containing 20 g of inoculum and basal medium containing $NaHCO_3$ (5 g L^{-1}). pH of the medium was corrected to 7.0–7.2 with NaOH or HCl 2 mol L^{-1}. The vials were sealed and the headspace flushed with N_2/CO_2 (80:20 v/v). Before incubation, the medium was amended with $Na_2S{\cdot}9H_2O$, to a final concentration of 1 mmol L^{-1}.

Blank assays to discount for the residual substrate present in the inoculum were also performed.

The methane accumulated in the headspace of the closed bottles was measured by gas chromatography (GC), with a flame ionization detector (FID), using a gas tight syringe to sample 500 μL. Methane production was corrected for STP conditions (0 °C and 1 bar). Biochemical methane potential (BMP) was defined by the volume of methane produced per unit of COD of substrate added to the assay (Eq. (1)).

$$\mathrm{BMP} = \mathrm{L\ CH_4/kgCOD} = \frac{\mathrm{kg\ COD - CH_4 \times 350(L\ CH_4/kgCOD)}}{\mathrm{kg\ COD_{added(after\ H2\ production)}}} \quad (1)$$

2.3. Analytical methods

Determination of lignin, xylan and glucan was performed according to Sluiter et al. (2008). TKN, ammonium (NH_4^+), TS and VS were measured according to standard methods APHA (1989). Total and soluble COD were determined using standard kits (Hach Lange, Düsseldorf, Germany). Sample filtration was performed prior to soluble COD (CODs) determination. Lipids determination was carried out according to Bligh and Dyer (1959).

Hydrogen concentration in the gas phase was determined by GC using a column molsieve (MS-13 × 80/100 mesh) and thermal conductivity detector Bruker Scion 456 Chromatograph, (Bruker, Massachusetts, USA) with argon (30 mL min^{-1}) as the carrier gas. The injector, detector and column temperatures were 100, 130, and 35 °C respectively. Methane content in the biogas was analyzed by GC (Chrompack 9000) equipped with a FID detector and a 2 m × 1/8″ Chromosorb 101 (80–120 mesh) column, using nitrogen as carrier gas (30 mL min^{-1}); column, injector, and detector temperatures were 35, 110, and 220 °C, respectively.

VFA, ethanol and lactic acid were determined by high performance liquid chromatography using an HPLC (Jasco, Japan) with a *Chrompack column* (6.5 × 30 mm^2); sulfuric acid (0.005 mol L^{-1}) at a flow rate of 0.9 mL min^{-1} was used as mobile phase. Column temperature was set at 80 °C. Detection of VFA, lactic acid and ethanol was made sequentially using a UV detector at 210 nm and a RI detector.

2.4. Data analysis

The modified Gompertz equation was used to describe the progress of cumulative hydrogen production obtained from the batch experiments. Using the cumulative hydrogen production data, corrected to STP conditions (0 °C and 1 bar), the maximum hydrogen production rates were estimated from the fit of the modified Gompertz equation (Eq. (2)).

$$H(t) = P\exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (2)$$

where $H(t)$ is cumulative hydrogen production (mL), P is the hydrogen production potential (mL), Rm is the maximum hydrogen production rate (mL h^{-1}), $e = 2.71828\ldots$, λ represents the lag-phase time (h), and, t is the time (h).

3. Results and discussion

3.1. Substrate characterization

The results of *Sargassum* sp. characterization are shown in Table 1. In general, macroalgae are suitable for biogas production due to the high carbohydrates content that can go up to 60% depending on the species (Costa et al., 2012). Glucan and xylan content of *Sargassum* sp. represents 45% of the VS. These compounds are also suitable for fermentative hydrogen production. The low lipid content makes biodiesel production from *Sargassum* sp. biomass unfeasible. The low values of soluble COD and VS confirm the recalcitrant nature of this substrate. However, the lignin content is low (3.3 ± 0.9% VS). An autoclaving pre-treatment (121 °C and 1 bar for 15 min) was applied to *Sargassum* sp. to increase the soluble COD. After autoclaving the soluble COD increased 10×, corresponding to more than 25% of the total COD.

Table 1
Characterization of *Sargassum* sp.

Sargassum sp.*	Before autoclave	After autoclave
COD_{total} (mg g^{-1})	600 ± 62	562 ± 84
$COD_{soluble}$ (mg g^{-1})	15 ± 0	154 ± 1
TS (mg g^{-1})	896 ± 2	
VS (mg g^{-1})	490 ± 8	
TKN (mg N g^{-1})	20 ± 0	
Fat content (mg g^{-1})	13 ± 0	
Klason Lignin (%VS)	3.3 ± 0.9	
Glucan (%VS)	32.9 ± 2.6	
Xylan (%VS)	11.7 ± 1.3	

* Macroalgae dried at room temperature and milled into pieces with less than 0.5 cm.

3.2. Biohydrogen dark fermentation of Sargassum sp

Cumulative hydrogen production for each ratio substrate/inoculum tested is shown in Fig. 1. The highest hydrogen production was obtained with an initial culture CDW concentration of 0.09 g L^{-1} and concentration of *Sargassum* sp. (VS) of 7.4 g L^{-1}. No hydrogen production was observed in the assay with inoculum concentration (CDW) of 0.04 g L^{-1} and 4.9 g L^{-1} of *Sargassum* sp. (VS).

Higher hydrogen production was achieved with the higher inoculum concentration tested, independently of the ratio substrate/inoculum. Lower hydrogen production (0.30 mmolH_2) was obtained with a substrate/inoculum ratio of 63:1 (2.5 g L^{-1} of *Sargassum* sp. (VS) and 0.04 g L^{-1} of inoculum (CDW) comparing to 1.2 mmolH_2 achieved with a substrate/inoculum ratio of 82:1 (7.4 g L^{-1} of *Sargassum* sp. (VS) and 0.09 g L^{-1} of inoculum (CDW)). These results suggest that inoculum concentration is the main factor affecting hydrogen production from *Sargassum* sp. biomass, independently of the ratio used.

Cumulative hydrogen production data was used to estimate the parameters of the modified Gompertz equation (maximum hydrogen production rate and hydrogen production potential) shown in Table 2. The highest hydrogen production yield was achieved with *Sargassum* sp. concentration (VS) of 2.5 g L^{-1} and 0.09 g L^{-1} of inoculum (CDW) corresponding to hydrogen production of 91.3 mL g^{-1} and a maximum hydrogen production rate of 2.1 mL g^{-1} h^{-1} (Table 2). The lower hydrogen production yield (60.8 mL g^{-1}) and rate (0.54 mL g^{-1} h^{-1}) was observed with 0.04 g L^{-1} of inoculum (CDW) and 2.5 g L^{-1} of *Sargassum* sp. (VS).

For all the conditions tested it was observed that acetate was the sole soluble fermentation product (Fig. 2). The ratio 0.09 g L^{-1} of inoculum (CDW) and 7.4 g L^{-1} of *Sargassum* sp (VS) reached the higher acetate concentration (13 mmol L^{-1}) corresponding also, to the ratio that achieved higher cumulative hydrogen production. Lactate was not produced in any of the conditions tested. *C. saccharolyticus* is capable to directly use NADH for hydrogen production, although, when the hydrogen partial pressure (p (H_2)) is high, the NADH is used by lactate dehydrogenase

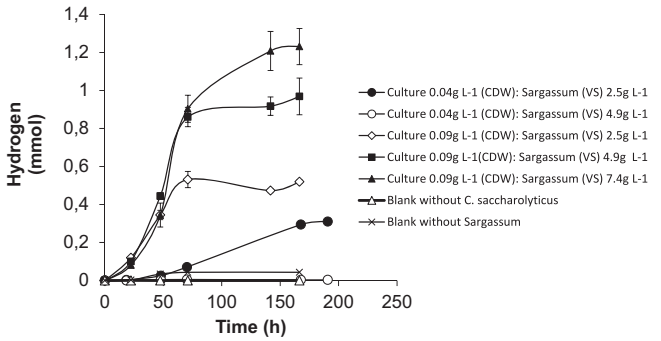

Fig. 1. Cumulative hydrogen production for each ratio inoculum/substrate tested.

Table 2
Hydrogen production results from the first stage process. Modified Gompertz equation parameter values for the different concentrations of *C. saccharolyticus* and *Sargassum* sp. biomass.

Inoculum concentration (CDW) ($g\ L^{-1}$)	Concentration of *Sargassum* sp. (VS) ($g\ L^{-1}$)	Hydrogen production* ($mL\ g^{-1}$)	Hydrogen production rate** ($mL\ g^{-1}\ h^{-1}$)	R^2	Maximum hydrogen partial pressure*** (kPa)
0.04	2.5	60.8 ± 1.4	0.54 ± 0.03	0.999	12.5 ± 0
0.04	4.9	0	0	na	0
0.09	2.5	91.3 ± 3.3	2.1 ± 0.4	0.973	20.9 ± 0.4
0.09	4.9	87.5 ± 2.7	1.83 ± 0.3	0.983	38.9 ± 3.8
0.09	7.4	74.0 ± 2.1	1.56 ± 0.2	0.989	49.5 ± 3.8

* Volume of hydrogen produced per grams of *Sargassum* sp. (VS).
** Volume of hydrogen produced per grams of *Sargassum* sp. (VS) per hour.
*** Above this hydrogen partial pressure $p(H_2)$ no hydrogen was produced.

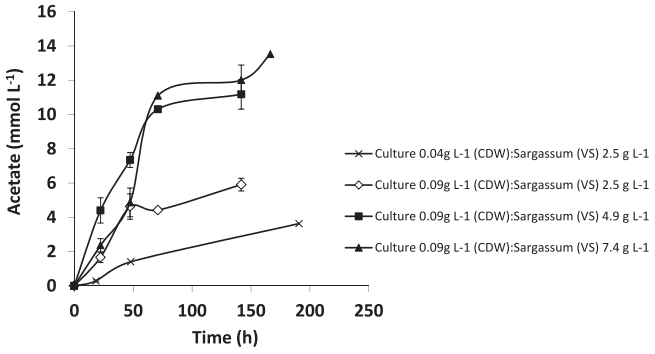

Fig. 2. Acetate formation during hydrogen dark fermentation process from each ratio inoculum/substrate tested.

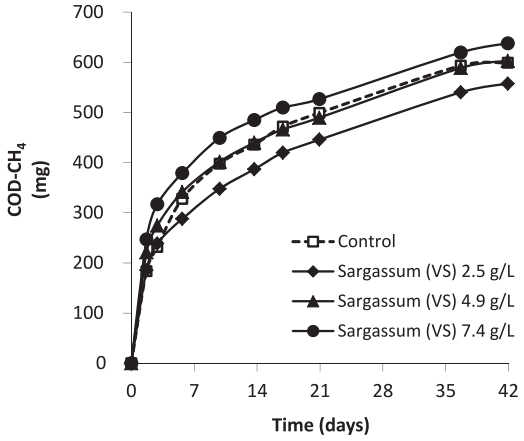

Fig. 3. Specific methane production from the dark fermentation products of each ratio inoculum/substrate. Control assay was performed with substrate (*Sargassum* sp.) and without *Caldicellulosiruptor saccharolyticus*.

to produce lactate instead of acetate and hydrogen (van de Werken et al., 2008). The maximum $p(H_2)$ obtained in this study was 49.5×10^3 Pa, for the ratio 0.09 g L^{-1} of inoculum (CDW) and 7.4 g L^{-1} of *Sargassum* sp. (VS) (Table 2). The $p(H_2)$ achieved was not critical, since lactate formation was not observed as an alternative way for reoxidizing NADH.

3.3. Methane production from Sargassum sp. dark fermentation end products

After H_2 production, the biochemical methane potential of the resulting fermentation end products was assessed in batch tests. The initial total COD varied between 7.1 and 9.6 g L^{-1} (Table 3). The assays lasted 42 days, although 80% of the maximum methane production was achieved around day 20 (Fig. 3). The results from the anaerobic biodegradability assays are shown in Table 3.

The highest methane production per mass (VS) of substrate, 541 ± 10 L kg^{-1}, was attained in the assay performed with the fermentation products of the first stage dark fermentation carried out with concentration of *Sargassum* sp. (VS) of 2.5 g L^{-1}. Higher *Sargassum* sp. concentrations in the dark fermentation step, led to lower methane yields (Table 3), though pH and ammonium concentration at the end of the anaerobic biodegradability assays did not reach inhibitory values. The higher solubilization yield as confirmed by the higher concentration of soluble COD, may suggest the accumulation of potentially inhibitory soluble metabolites.

Substrate solubilisation during the methanogenic step, was higher in the presence of *C. saccharolyticus* suggesting a positive effect of the acidogenic strain in the process of solubilisation even during the methanogenic step at mesophilic conditions (Table 3).

However, the overall efficiency of the methanogenic process was not directly dependent on the presence of *C. saccharolyticus.* For example, in the two assays with concentration of *Sargassum* sp. (VS) of 4.9 g L^{-1} the BMP were 336 ± 14 and 345 ± 10 L kg^{-1}, respectively with and without *C. saccharolyticus*.

3.4. Biohythane production

The present study suggests that biohythane production from *Sargassum* sp. has a great potential. Hydrogen and methane yields

Table 3
Experimental results obtained from the anaerobic biodegradability assays (second stage process).

Dark fermentation conditions		Dark fermentation end products CODt ($g\ L^{-1}$)	BMP* ($L\ kg^{-1}$)	pH	CODs ($g\ L^{-1}$)	NH_4^+ ($mg\ L^{-1}$)
Inoculum concentration (CDW) ($g\ L^{-1}$)	Concentration of *Sargassum* sp (VS) ($g\ L^{-1}$)					
0.09	2.5	7.08 ± 0	541 ± 10	7.3	4.11 ± 0.16	603 ± 1
0.09	4.9	8.06 ± 0.06	345 ± 10	7.1	4.30 ± 0.02	599 ± 13
0.09	7.4	9.58 ± 0.06	281 ± 7	7.1	4.46 ± 0.05	610 ± 14
–	4.9	7.53 ± 0.64	339 ± 14	7.3	3.57 ± 0.24	597 ± 15

* BMP – Methane produced (L) per kg of *Sargassum* sp. (VS) used in the dark fermentation process.

Table 4
Performance of two-stage hydrogen and methane fermentation from different feedstocks (adapted from Liu et al. (2013)).

Substrate	H_2 inoculum	H_2 operation	H_2 yield	CH_4 inoculum	CH_4 operation	CH_4 yield*	% of H_2 in hythane (v/v)	Reference
Wheat straw hydrolysate	Granular sludge	Continuous	89 L kg^{-1} VS	Granular sludge and digested manure	Continuous	307 L kg^{-1} VS	22	Kongjan et al. (2011)
Grass silage	Cow manure sludge	Batch	6.46 L kg^{-1} VS	Cow manure sludge	Batch	467 L kg^{-1} VS	1	Pakarinen et al. (2009)
Cornstalk	Granular sludge and pure culture	Batch	64 L kg^{-1} TS	Granular sludge	Batch	115 L kg^{-1} TS	36	Lu et al. (2009)
Lipid extracted microalgal biomass	Anaerobic sludge	Batch	46 L kg^{-1} VS	Anaerobic sludge	Batch	394 L kg^{-1} VS	10	Yang et al. (2011)
Sweet sorghum biomass	Indigenous microflora	Continuous	10.4 L kg^{-1} VS	Anaerobic sludge	Continuous	107 L kg^{-1} VS	9	Antonopoulou et al. (2008)
Cassava stillage	Anaerobic sludge	Continuous	56.6 L kg^{-1} VS	Continuous	Thermophilic digested cassava stillage	249 L kg^{-1} VS	19	Luo et al. (2010)
Sargassum sp.	*Caldicellulosiruptor saccharolyticus*	Batch	91 L kg^{-1} VS	Granular sludge	Batch	541 L kg^{-1} VS	14	This study
Sargassum sp.	*Caldicellulosiruptor saccharolyticus*	Batch	88 L kg^{-1} VS	Granular sludge	Batch	345 L kg^{-1} VS	20	This study

* kg of VS used in the dark fermentation process.

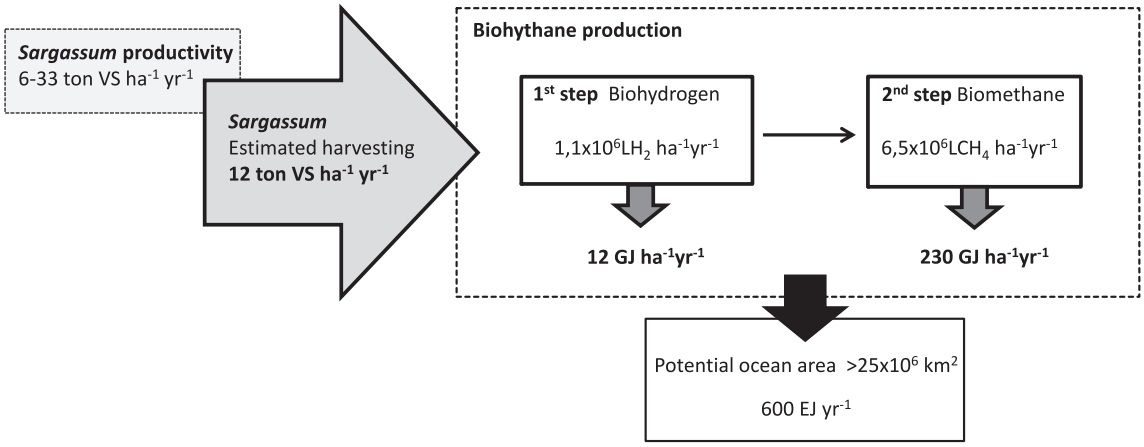

Fig. 4. Potential energy generation from *Sargassum* sp. according to the maximum hydrogen and methane production achieved in the present study.

obtained were higher than others reported in the literature for complex substrates (Table 4).

The maximum H_2 and CH_4 production was achieved with *Sargassum* sp. concentration (VS) of 2.5 g L^{-1} (Tables 2 and 3). This would represent the generation of biohythane with 14% H_2 (Table 4), which is within the optimal range (10–25%). However, it is possible to increase the H_2 percentage in the biohythane up to 20% by using a higher concentration of *Sargassum* sp. (VS) (4.9 g L^{-1}) (Table 4).

3.5. Potential energy generation

Annual productivity of *Sargassum* sp. varies between 6 and 33 ton ha^{-1} yr^{-1} (N'Yeurt et al., 2012) in terms of VS. Considering a feasible sustainable annual harvest amount of 12 ton ha^{-1} yr^{-1}, the energy generation predictable according to the maximum hydrogen and methane production potential achieved in the present study (Fig. 4), is 12 GJ ha^{-1} yr^{-1} from the first step and 230 GJ ha^{-1} yr^{-1} from the second step. According to Reith et al. (2012), the ocean potential area for macroalgae production could exceed 25 × 10^6 km^2. Therefore, the value of energy herein demonstrated of 242 GJ ha^{-1} yr^{-1} could result in a maximum energy supply of 600 EJ yr^{-1}, 6-fold higher the value estimated by Chynoweth et al. (2001), covering theoretically the annual world energy consumption. These figures are theoretical and do not include the energy requirements for the whole process.

4. Conclusion

The highest hydrogen and methane production yields were achieved with *Sargassum* sp. concentration (VS) of 2.5 g L^{-1} and 0.09 g L^{-1} of inoculum concentration (CDW), namely 91.3 ± 3.3 L H_2 and 541 ± 10 L CH_4 per kg (VS) of *Sargassum* sp., resulting in biohythane with 14% hydrogen. Potential energy production from *Sargassum* sp. in two stage process was estimated in 242 GJ ha^{-1} yr^{-1}. Considering the ocean potential area for macroalgae production of 25 × 10^6 km^2, the value of energy herein demonstrated could result in a potential energy supply of 600 EJ yr^{-1}.

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