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Improving gaseous biofuel production from seaweed *Saccharina latissima*: the effect of hydrothermal pretreatment on energy efficiency

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Abstract

Marine macroalgae (seaweed) is a promising feedstock for producing biohydrogen and biomethane via dark fermentation and anaerobic digestion, respectively. However, one of the limiting steps in the biological process is the conversion of polymeric carbohydrates into monomeric sugars. Here hydrothermal pretreatments were assessed for hydrolysis and subsequent production of biohydrogen and biomethane from the brown seaweed *Saccharina latissima*. The solubilization of *S. latissima* improved with increasing temperatures from 100 to 180 °C, resulting in a maximum yield of 0.70 g soluble chemical oxygen demand/volatile solid (sCOD/g VS); equivalent to an increase of 207.5% compared with untreated seaweed. However, the yield of the derived monomeric sugar mannitol peaked at 140 °C and decreased with increasing temperatures, likely due to production of fermentative inhibitors. Microstructural characterization revealed that the algal structure was significantly damaged, and the major chemical groups of carbohydrates and proteins were weakened after pretreatment. Regardless of hydrothermal temperatures, biohydrogen yield only slightly increased in dark fermentation, while biomethane yield significantly increased from 281.4 (untreated *S. latissima*) to 345.1 mL/g VS (treated at 140 °C), leading to the sCOD removal efficiency of 86.1%.

The maximum energy conversion efficiency of 72.8% was achieved after two-stage biohydrogen and biomethane co-production. In comparison, considering the energy input for pretreatment/fermentation/digestion, the highest process energy efficiency dropped to 37.8%. Further calculations suggest that a significant improvement of efficiency up to 56.9% can be achieved if the heat from pretreatment can be recovered.

Keywords: Macroalgae; hydrothermal pretreatment; energy efficiency; biohydrogen; biomethane.

1. Introduction

In a world concerned with climate change and poor air quality associated with excessive use of non-renewable fossil fuel, biofuels will play an increasingly more significant role in future energy systems [1]. The European Union (EU) encourages the optimization of renewable energy systems to meet more and more stringent sustainability criteria. The EU has introduced a cap on the contribution of food-based biofuels, starting at 7% of energy in the transport sector in 2021 and reducing progressively to 3.8% by 2030 [2]. Third-generation biofuel feedstocks are free from arable land use. These include advanced gaseous biofuels (biomethane and biohydrogen) derived from microalgae and macroalgae (seaweeds), which show significant potential to satisfy advanced biofuel targets [3-8]. The EU target for advanced biofuels has been set at a minimum share of 3.6% of the total fuel consumption by 2030 as compared to the target of just 0.5% for 2021 [2]. The advantages of using algae include for higher growth rates and production yields, higher rate of carbon dioxide fixation, negligible quantities of hemicellulose and lignin, versatile biofuel production and freedom from competition for agricultural land [9].

Biohydrogen and biomethane can be produced through biological technologies such as fermentation and anaerobic digestion, respectively. Seaweeds (including brown, green, and red) grow naturally and are cultivated worldwide. The harvest is greatest in Asian countries (China, Philippines, Japan, Korea). China annually harvests 3.2 million tonnes of seaweeds [4]. In the European context, Ireland is one of the largest seaweed producers, producing 29,500 tonnes per annum [4]. Typical brown seaweeds (such as *Laminaria digitata* and *Saccharina latissima*) and green seaweeds (such as *Ulva lactuca*) are rich in organic matter, including carbohydrates and proteins. Brown seaweeds are relatively high in carbohydrates in the form of polysaccharides (mannitol, laminarin and alginate) which are easily degradable [10]. Some species of green seaweeds may grow faster than brown seaweeds; however green seaweeds contain high levels of proteins, leading to unfavorable low carbon to nitrogen ratios [11]. Seaweeds have the capacity to produce a vast array of high-value liquid (such as bioethanol and biodiesel) and gaseous (such as biohydrogen and biomethane) biofuels [12-14]. It has been reported that gaseous biofuels may have certain advantages over liquid biofuels [9]: (1) the greenhouse gas (GHG) savings in producing and using gaseous biofuels is greater than that for liquid biofuels; (2) the pollutant emissions using gaseous biofuels as transport fuels are much less than that using liquid biofuels; and (3) gaseous biofuels can take advantage of the existing natural gas grid system for low energy input distribution.

Polymeric carbohydrates are the most important components in seaweeds for gaseous biofuel production employing biological processes. The main groups of carbohydrates found in seaweeds are laminarin, alginate, cellulose, fucoidan, and sugar alcohol mannitol [15]. Biohydrogen and biomethane can be produced through biological technologies via fermentation and anaerobic digestion, respectively. However, the rigid cell wall structure and high molecular weight organics in seaweeds may adversely affect cell disintegration and prolong fermentation time. Allen et al. assessed ten species of seaweeds for biomethane potential, and found that some species (such as *Ascophyllum nodosum and Fucus serratus*) exhibited biodegradability indices as low as 0.19−0.34 [16]; as such 66 to 81% of volatile material did not degrade during digestion over 30 days. Similar to lignocellulosic feedstock, seaweeds in fermentation and anaerobic digestion require firstly the conversion of polymeric carbohydrates into readily available monomeric sugars (such as glucose and mannitol). The carbohydrate monomers derived from land-based biomass are dominating by glucose and xylose; biohydrogen and biomethane produced from these carbohydrates have been extensively investigated [17-20]. However, the use of seaweeds as feedstock and their fermentation performance have received less attention, especially in two-stage fermentation systems for co-production of biohydrogen and biomethane.

In order to convert polymeric carbohydrates to monosugars efficiently, an effective pretreatment process prior to fermentation and anaerobic digestion is necessary. To date, different pretreatment methods have been developed, including mechanical, chemical, thermal, and biological methods [21-27]. However, factors that influence pretreatment efficiency and, consequently, biofuel yields, are highly affected by seaweed composition (such as salts, polyphenols, polysaccharides type) [28]. Hu et al. found the different acids (such as H_2SO_4, HCl, H_3PO_4) can effectively damage the dense surface of algae and remove a small portion of protein and sulfate polysaccharide in seaweed *Enteromorpha* [29]. The thermal pretreatment of brown seaweed *Nizimuddinia zanardini* released more than 80% of components such as mannitol and led to a 22% higher methane yield compared to untreated seaweed [30]. Among those methods, mild acid treatment using sulfuric or hydrochloric acid was found to be effective in hydrolysis of polysaccharides in seaweeds at relatively low temperatures. Acid is able to cleave the β-1-4-glycosidic bond of complex polysaccharides to associated monomers. Monosugars derived from hydrolysis can be readily used by acidogenic bacteria and methanogenic archaea for sequential biohydrogen and biomethane production. Yin et al. reported that the solubilization of *Laminaria japonica* increased 1.9 fold after microwave pretreatment in the presence of 1% sulfuric acid, thereby improving the sequential biohydrogen production from 15 to 28 mL/g dry weight [31]. However, the use of acid in pretreatment may inevitably lead to formation of fermentative inhibitors (such as furfural, and levulinic acid) as a consequence of sugar decomposition. Furthermore, alkaline addition is typically necessary in order to balance the pH value of the pretreated solution before biological processes. Jung et al. reported a negative correlation between hydrogen yield and the produced 5-(hydroxymethyl)furfural in hydrolysate [32]. The presence of furfural (15 mM) reduced the biohydrogen production rate by 28.9%, likely due to the competitive reduction reaction converting furfural aldehyde to alcohol, inhibiting the reaction of protons to hydrogen [33].

As an eco-friendly alternative, hydrothermal pretreatment may be applied to seaweeds to improve hydrolysis and increase the biodegradability index in fermentation and digestion. Hydrothermal pretreatment has been used for the fractionation of macroalgae biomass. The excellent solvent properties of water as a reaction medium and the high moisture content of macroalgal biomass make this technology promising for the direct use of algae in the production of biofuels and high added-value compounds [34]. For hydrothermal pretreatment, liquid hot water is used at temperatures from 100 to 374 °C under high pressure, corresponding to conditions below the water critical point [35]. The high hydrothermal temperatures can weaken H-bonding in water, allowing for autoionization of water into acidic hydronium ions (H_3O^+) and basic hydroxide ions (OH^-) [7]. Acidic hydronium ions can act as catalysts that may facilitate the hydrolysis of biomass. Despite the advantages of hydrothermal pretreatments, some drawbacks may still present, particularly when employing high temperatures. For instance, Jard et al. investigated the effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalgae *Palmaria palmata*, and found that higher temperatures resulted in a substantial decrease in methane potential (−13% at 160 °C and 180 °C, up to −31% at 200 °C) [36].

A previous study suggested that *S. latissima* has the highest specific methane yield of seaweeds available in Ireland [16]. The potential energy yield from *S. latissima* may be up to 365 GJ ha^{-1} yr^{-1} [16]; this further leads to the calculation that at least 6124 ha of coastal area would be required to grow seaweed to satisfy Ireland's 2020 target in advanced biofuels [4]. Minimisation of coastal area used is dependent on innovations in cultivation and harvesting of seaweed, but in terms of this paper more significantly in terms of increasing biofuel yield per unit of seaweed and optimisation of energy efficiency in the overarching bioenergy systems. Therefore, *S. latissima* was selected as the feedstock for further assessment in this study. To the best of our knowledge, there is a clear research gap on the use of hydrothermal pretreatment of brown seaweed (in this case *S. latissima*) for two-stage biohydrogen and biomethane production. Studies of such systems are very rare in the scientific literature. The research output can provide an answer as to how energy-efficient hydrothermal pretreatment is in terms of improving gaseous biofuel production from *S. latissima*. The objectives of this study are to: assess the effect of hydrothermal pretreatment on the physicochemical properties of *S. latissima*; evaluate the biohydrogen and biomethane potential from pretreated *S. latissima*; and assess the energy feasibility of the cascading two-stage fermentation system by calculating energy conversion efficiency and energy process efficiency.

2. Materials and Methods

2.1. Feedstock and inocula

Feedstock: *S. latissima* was harvested in June in West Cork, Ireland. The collected algal samples were washed with tap water to remove sands and other impurities, and then cut to a particle size of approximately 5 mm by a mincer (Buffalo Heavy Duty Mincer CD400). The samples were then dried at 105 °C in an oven and pulverized to 0.02 mm mesh size. The pulverized samples were stored at 4 °C before use. The physicochemical characteristics of dried biomass are presented in Table 1.

Inoculum for hydrogen fermentation: Mixed fermentative bacteria used in biohydrogen potential (BHP) assays were originally sourced from a biogas plant treating pig slurry, dairy slurry and food waste at mesophilic temperatures. The digestate from the biogas plant was prepared by passing through a 1 mm sieve and was degassed for two weeks under anaerobic conditions to reduce any residual gas production. The digestate was then stored in a laboratory digester, and was fed cellulose as a substrate to maintain the microbial activity. The obtained digestate was heat-treated at 100 °C for 30 min in an autoclave (Sanyo MLS-3780, Japan) to deactivate methanogenic microorganisms. Subsequently, the pre-heated sludge was cultured in an anaerobic environment to enrich hydrogen-producing bacteria. The composition of the culture medium used was as follows (per liter): glucose, 20.0 g; tryptone, 3.0 g; yeast extract, 1.0 g; NaCl, 3.0 g; K_2HPO4, 2.5 g; $MgCl_2$, 0.1 g; $FeCl_2$, 0.1 g; L-cysteine, 0.5 g; vitamin solution, 10.0 mL; and trace element solution, 10.0 mL. The acclimatization procedure was repeated three times to ensure hydrogen-producing bacteria were fully activated.

Inoculum for anaerobic digestion**:** The inoculum for the biomethane potential (BMP) assays was sourced from the laboratory digester, which was run at mesophilic temperature using cellulose as a substrate. Large particles in the original inoculum were removed through a 1 mm sieve. Prior to the BMP experiments, the sieved inoculum was degassed for two weeks.

2.2. Hydrothermal pretreatment

Fig. 1 presents the experimental schematic including hydrothermal pretreatment, dark hydrogen fermentation and anaerobic digestion. The hydrothermal pretreatment of *S. latissima* biomass was performed in a 500 mL batch reactor (Parr Instrument Company 4500, USA). The pretreatments were carried out under a stirring speed of 500 rpm for 30 min at 100, 120, 140, 160, and 180 °C. Our previous studies have demonstrated that the optimal pretreatment conditions for a wide range of feedstock (such as grass silage, cassava residue and food waste) were in the processing temperature of 135–140 °C and time of 15–20 min [1, 2]. Deng et al. found that the optimal acid pretreatment was achieved at 135 °C for 15 min with a total reducing sugar yield of 0.33 g/g VS of grass silage [37]. When using food waste as feedstock, the optimal hydrothermal pretreatment condition was found to be 140 °C for 20 min in terms of the solubilization efficiency of organic components (namely carbohydrates and proteins) [38]. As compared to pretreatment time, hydrothermal temperature plays a more significant role in affecting the effectiveness of feedstock hydrolysis. Based on the results from the above studies, this study chose the temperature range from 100 to 180 °C and process time of 30 min.

In each run, 7.5 g volatile solid (VS) of *S. latissima* biomass was brought to the reactor along with a volume of 250 mL deionized water. The pretreatment time was precisely measured, starting from when the algal sample reached the set temperature. After each run, the reactor was allowed to cool down to room temperature and the treated samples were taken out for further processing.

2.3. Two-stage dark fermentation and anaerobic digestion

Dark hydrogen fermentation**:** The cascading fermentation system included two stages, namely, dark hydrogen fermentation followed by anaerobic digestion. The first stage dark fermentation for BHP assays was carried out in duplicate in the AMPTS II system (Bioprocess Control, Sweden). To analyse the effects of hydrothermal temperature on fermentative hydrogen production, five experimental groups were performed based on the hydrothermal conditions (100, 120, 140, 160, and 180 °C). Untreated *S. latissima* biomass was used as a comparison group. In BHP assays, 3 g VS of algal hydrolysates were added into each glass fermenter. Subsequently, 30 mL of activated inoculum for dark fermentation was added to each fermenter. The total liquor volume was then adjusted to 300 mL with deionized water. The initial pH was adjusted to 6.5 ± 0.1 with 6 M NaOH and 6 M HCl solutions. The bottles were sealed and purged with nitrogen gas for 5 min to ensure anaerobic conditions. The bottles were then placed in a water bath at 35.0 °C and the dark hydrogen fermentation lasted for 48 h.

Anaerobic digestion**:** After dark fermentation for 48 h, the effluents from each bottle were inoculated with seed inoculum at an inoculum to substrate VS ratio of 2:1 (based on original VS before dark fermentation) for second-stage anaerobic digestion. The bottles were also sealed, purged with nitrogen gas for 5 min, and placed in a water bath at 35.0 °C. The anaerobic digestion trials ran for 11 days to allow for maximum degradation of substrates. During dark fermentation and anaerobic digestion, carbon dioxide in the produced gas was removed by passing the biogas through a 3 M NaOH solution. The gas flow was measured, and the volume was automatically normalized to standard conditions (0 °C, 1 atm) and zero moisture content by the AMPST II system.

2.4. Analytic methods

The differences of algal biomass surface microstructures before and after pretreatment were observed by a field emission Scanning Electron Microscope (SEM; Hitachi S3700, Japan). The biomass samples were processed by coating with a thin layer of gold under vacuum before the SEM observation. The changes in chemical composition and functional groups were assessed by a Fourier Transform Infrared Spectroscopy analyser (FTIR; Nicolet 5700, USA) equipped with a universal attenuated total reflection accessory. The total solid (TS), VS and ash content of the inoculum were analyzed by using the standard method of drying of the sample for 24 h at 105 °C and subsequent heating for 2 h at 550 °C [39]. A spectrophotometer (Hach DR890, USA) coupled with a Hach DRB200 reactor was employed to determine the chemical oxygen demand (COD) of algal samples. The concentration of soluble metabolic products (SMPs; such as acetic acid and butyric acid) was analyzed on a gas chromatography system (GC; Agilent 7820A, USA) equipped with a flame ionization detector and a DB-FFAP column. The temperatures of the injection port and the flame ionization detector were both set at 250 °C. The initial column temperature was set at 100 °C, increased to 200 °C at a heating rate of 10 °C/min, and then held for 2.5 min. Before injecting to the GC, the liquid samples were first centrifuged at 5000 rpm for 5 min and then adjusted with hydrochloric acid to ensure an acidic pH. The quantification of each component was determined by comparing with a standard SMP solution. The concentrations of mannitol, glucose, xylose, galactose, and arabinose were determined on a high-performance liquid chromatography (HPLC) system (Agilent 1200, USA) using refractive index detector and Aminex HPX-87P column at 85 °C with H_2O as mobile phase at 1 mm/min.

The severity factor of the hydrothermal pretreatment under different conditions was calculated based on Eq. 1 [40]. Severity factor is an expression of the combined effect of temperature and time of the hydrothermal pretreatment.

$$Severity\ factor = log\ (t \times e^{\frac{T-100}{14.75}}) \qquad \text{(Eq. 1)}$$

where t is the time of the hydrothermal pretreatment in min and T is the temperature of the pretreatment in °C. For each pretreatment condition, the severity factor was determined as 1.48 (100 °C, 30 min), 2.07 (120 °C, 30 min), 2.65 (140 °C, 30 min), 3.24 (160 °C, 30 min), and 3.83 (180 °C, 30 min).

The biodegradability index (BI) was defined as the ratio of actual methane yield in batch trial to the calculated theoretical methane yield. The theoretical methane yield was calculated using the Buswell equation on the basis of elemental C, H, O and N content. Biomethane yield was simulated by the modified Gompertz equation as described in Eq. 2. The derived kinetic parameters (H_m, maximum methane yield potential, mL/g VS; R_m, peak methane production rate, mL/g VS/d; and λ, lag-phase time of methane production, d) were calculated using Origin 8.5 software.

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

2.5. Energy balance

Process energy input: The process energy input involved in the cascading system comprises includes for hydrothermal pretreatment, dark fermentation and anaerobic digestion. The total energy needed (Q_{in}; kJ/g fresh weight) can be estimated as per Eq. 3 [41]:

$$Q_{in} = Q_{Htpt} + Q_{Fer} + Q_{AD} \quad \text{(Eq. 3)}$$

Where Q_{Htpt} is the energy input for hydrothermal pretreatment, Q_{Fer} is the energy input for fermentation, and Q_{AD} is the energy input for anaerobic digestion.

The energy input in each step can be determined using Eq. 4:

$$Q_{Htpt}/Q_{Fer}/Q_{AD} = (V_{reac}\rho_w Cp_w + m_{s,fresh}Cp_{s,fresh}) \times (T_{reac} - T_{amb})/m_{s,fresh} \quad \text{(Eq. 4)}$$

Where the liquid fraction in the reactor (V_{reac}) is assumed to be water with a specific heat (Cp_w) of 4.2 kJ/kg·K. The hydrothermal pretreatment temperature (T_{reac}) varies based on each experimental run, while the ambient temperature (T_{amb}) is assumed as 25 °C. The temperature of dark fermentation and anaerobic digestion is 35 °C as per the experimental conditions. The $m_{s,fresh}$ is the fresh weight of *S. latissma* fed into the reactor, and $Cp_{s,fresh}$ is the corresponding specific heat based on the fresh mass. The specific heat of fresh *S. latissima* is averaged from the data presented for three seaweed species by Wang et al. over different temperatures [42]. No change in the heat capacity with pretreatment or fermentation is assumed. Minimum loss in mass between the different stages is neglected [41].

Process energy efficiency and energy conversion efficiency: The process energy out is associated with the energy yield of hydrogen and methane produced in dark fermentation and anaerobic digestion; and can be estimated specific to the input substrate as per Eq. 5 & 6 [43]:

$$E_{H_2} = V_{H_2,VS} \times LHV_{H_2} \quad \text{(Eq. 5)}$$

$$E_{CH_4} = V_{CH_4,VS} \times LHV_{CH_4} \quad \text{(Eq. 6)}$$

$V_{H_2,VS}$ and $V_{CH_4,VS}$ is the volumetric yields of hydrogen and methane per unit VS substrate used, respectively.

The energy conversion efficiency (η_{ece}) is defined as the ratio of the heating value in hydrogen and methane to the total heating value of the added substrate. Calculations are conducted based on the lower heating values (LHV) of hydrogen (242 kJ/mol) and methane (801 kJ/mol) [44].

$$\eta_{ece} = (E_{H_2} + E_{CH_4})/E_{algae} \times 100 \quad \text{(Eq. 7)}$$

The energy value of algal biomass is calculated based on the modified Dulong Formula as shown in Eq. 8 [45]:

$$E_{algae} = 337C + 1419(H - 0.125O) + 23.26N \quad \text{(Eq. 8)}$$

where C, H, O, and N represent the weight percentages of each element in total VS.

In contrast, taking into account the energy input in relation to hydrothermal pretreatment, dark fermentation and anaerobic digestion, the overall process efficiency (η_{pe}) can hence be represented based on Eq. 9:

$$\eta_{pe} = (E_{H_2} + E_{CH_4} + Q_{recov})/(Q_{Htpt} + Q_{Fer} + Q_{AD} + E_{algae}) \times 100 \quad \text{(Eq. 9)}$$

In which Q_{recov} is the potential heat recovered from hydrothermal pretreatment (this becomes 0 as per the experimental set-up). The potential heat recovery was evaluated considering cool-down of the hydrolysates from process temperatures to 50 °C with an efficiency of heat transfer of 90% [46, 47]. As per the batch experimental set-up, no heat was recovered. However, due to the operation at elevated temperatures, cooling down of the pretreated algal hydrolysates can provide an interesting opportunity for heat recovery with subsequent use in providing heat for fermentation and anaerobic digestion.

3. Results and discussion

3.1. *Saccharina latissima* characterization before and after hydrothermal pretreatment

S. latissima primarily contains a high amount of carbohydrates (such as mannitol, alginate, laminarin, low concentration of cellulose and negligible lignin), the content which varies significantly with seasons [48]. As a structural component of the cell wall, alginate is the most abundant carbohydrate and may account for 40% of dry weight in some species; structural cellulose has been reported at concentrations of approximately 10% of dry weight; the storage carbohydrates (including laminarin and mannitol) vary considerably between 5–32% and 2–25% of dry weight, respectively [48, 49].

Table 1 presents the compositions of dried seaweed *S. latissima* used in this study. The ultimate analysis showed that the energy value of *S. latissima* was 17.1 kJ/g VS, which is comparable to that of monosaccharide (for example glucose with an energy value of 15.6 kJ/g VS). *S. latissima* used in this study presents a carbon to nitrogen ratio of 14.3. However, large variations were observed in carbon to nitrogen ratios depending on the harvest seasons and locations [16, 50]. As a brown seaweed *S. latissima* is rich in carbohydrate mannitol, which is a sugar alcohol with a variety of applications [51]. Mannitol can be readily used by microorganisms to produce biofuels. Typical amino acids derived from proteins in *S. latissima* include glutamic acid, aspartic acid, and glycine [15]. The fermentable sugars and amino acids are originally in the form of slowly biodegradable high-weight molecular polysaccharides. To improve biofuel production, it is beneficial to pretreat to effectively release monomers from *S. latissima*.

SEM images of untreated and pretreated *S. latissima* biomass with different magnification are shown in the supplementary material (Fig. S1). Hydrothermal pretreatment had a critical impact on the surface morphology of seaweed. The untreated *S. latissima* biomass showed a flat surface with sharp edges and some cracks (Fig. S1 a and b). The surface of hydrothermally treated seaweed was observed to be more uneven and eroded (Fig. S1 c and d). The algal structure was loosened and formed a rugged surface; more internal structure of the samples was exposed. With more ridges and grooves formed, the roughness of the algal surface increased greatly. The damaged surface structure could facilitate diffusion of the hydrolytic enzymes and the degradation of algae by methanogenic archaea.

Fig. 2 shows the FTIR spectra of *S. latissima* biomass before and after hydrothermal pretreatment, which exhibits similar peaks at different wave numbers. This is because the process of hydrothermal pretreatment does not introduce new chemical groups to the solid fraction of seaweed after pretreatment, due to the fact that only water is used during pretreatment. It indicates that there are two absorption peaks existing in the hydrogen bonding area ranging from 2500 to 4000 cm^{-1}. The major peak located at 3454 cm^{-1} is assigned to the stretching vibrations of hydrogen bonded O−H groups and N−H groups, indicating the presence of carbohydrates and proteins in *S. latissima* biomass [52]. The weak peak at 2923 cm^{-1} is derived from the vibration of C−H group in the polysaccharides in the *S. latissima* biomass. The peak at 1640 cm^{-1} is assigned to the C=O group of amides [53], which arises due to the presence of proteins. The peak around 1415 cm^{-1} may attribute to the C−C stretching vibration of aromatic structure [54]. The peak at 1058 cm^{-1} is characteristic of the C−N stretching vibration of aliphatic amines [55], which confirms the presence of proteins.

As shown in the FTIR spectra, raw seaweed had strong stretching vibration peaks related to the O−H and N−H groups (3454 cm^{-1}), while these absorption intensities decreased in the pretreated seaweed, suggesting decomposition of carbohydrates and proteins. In a similar trend, the vibration peaks corresponding to the C−H and C−C groups (2923 and 1415 cm^{-1}) also decreased, which was due to hydrolysis of polysaccharide substances after hydrothermal pretreatment. The decrease of C=O and C−N vibration peaks (1640 and 1058 cm^{-1}) further confirmed the degradation of proteins in treated seaweed.

The complex structural components of *S. latissima* can be partially transformed from water-insoluble fraction (macromolecular polymers) to water-soluble fraction (low molecular weight organics), thereby increasing the soluble COD production during hydrothermal pretreatment. Fig. 3 a presents the soluble COD yield from *S. latissima* after pretreatment at different temperatures. The untreated seaweed contained a certain amount of soluble organics, which resulted in a yield of 0.23 g sCOD/g VS. By increasing the hydrothermal temperature to 180 °C, the COD yield significantly increased with the highest value of 0.70 g sCOD/g VS (equivalent to an increase of 207.5%). This significant increase in soluble COD production indicates that hydrothermal pretreatment could significantly promote the solubilization of *S. latissima*.

However, the monomer sugars (such as mannitol and glucose) may be further degraded to inhibitory by-products (such as furfural and levulinic acid), especially at high hydrothermal temperatures. Fig. 3 b shows the mannitol yield from *S. latissima* at varying temperatures. The mannitol yield from raw *S. latissima* was 17.8 mg/g VS. With increasing temperature from 100 °C to

140 °C, the mannitol yield increased from 23.0 to 32.2 mg/g VS. Further increasing temperature led to decreased mannitol yield with the lowest yield of 21.8 mg/g VS obtained at 180 °C. The decrease in mannitol yield was ascribed to the fact that higher hydrothermal temperatures favoured the degradation of mannitol to inhibitory by-products. This phenomenon has been previously observed when pretreating other substrates (such as trehalose) [56].

3.2. First-stage dark fermentation for hydrogen production

The solubilized *S. latissima* biomass under different hydrothermal conditions were subject to first-stage dark hydrogen fermentation, as shown in Fig. 4. The biohydrogen yield from untreated seaweed was obtained as 11.7 mL/g VS after fermentation of 48 h. The pretreatment at 100 °C slightly increased the biohydrogen yield to 13.7 mL/g VS. Further increasing the temperature resulted in the decrease in biohydrogen yield; this decrease in yield is postulated to be due to the decreased concentration of monosaccharides (such as mannitol) and the presence of potential inhibitors. This result suggests that the seaweed *S. latissima* may not be a good candidate to produce hydrogen through dark fermentation. As a major monosaccharide derived from *S. latissima*, mannitol has been previously investigated for biohydrogen production [57-59]. The theoretical hydrogen yield from mannitol was calculated as 615.4 mL/g mannitol following the acetate pathway (see Eq. 10). The practical hydrogen production from mannitol achieved ranged from 209.2 to 224.2 mL/g [57-59]. This suggests the efficiency of hydrogen production is less than 40%. Comparatively, the theoretical hydrogen yield from glucose was determined as 498.0 mL/g glucose (see Eq. 11). Previous studies have achieved hydrogen production efficiencies of up to 90% [57].

$$\text{Mannitol: } C_6H_{14}O_6 + 4H_2O = 2CH_3COO^- + 4H^+ + 2HCO_3^- + 5H_2,\ \Delta G'_0 = -180.6\ \text{kJ/mol} \qquad \text{(Eq. 10)}$$

$$\text{Glucose: } C_6H_{12}O_6 + 2H_2O = 2CH_3COO^- + 2HCO_3^- + 4H_2,\ \Delta G'_0 = -215.7\ \text{kJ/mol} \qquad \text{(Eq. 11)}$$

Where $\Delta G'_0$ as indicated in Eq. 10 and 11 is the free energy change under the standard condition. The free energy change of glucose reaction (−215.7 kJ/mol) is more negative than that of mannitol reaction (−180.6 kJ/mol), suggesting that the fermentative metabolism of glucose is thermodynamically more favourable than that of mannitol. This result implies that fermentative bacteria thermodynamically prefer to consume glucose as compare to mannitol, which thereby provides a clue as to why mannitol resulted in a less efficient hydrogen production.

Table 2 presents the SMPs profile after dark hydrogen fermentation of *S. latissima* biomass. SMPs primarily consisted of acetate, butyrate, and small amounts of propionate, iso-butyrate, valerate, iso-valerate and caproate. The untreated *S. latissima* yielded SMPs of 5.73 g/L, of which the sum of acetate and butyrate comprised 86.0% of total SMPs. This indicates that the degradation of mannitol mainly followed the acetate and butyrate pathways during hydrogen fermentation, as shown in Fig. 5. The pretreated *S. latissima* exhibited similar levels of SMP production, which is in accordance with the minor change of hydrogen production after pretreatment. No significant change on the metabolic pathways was observed using pretreated seaweed as the substrate, as acetate and butyrate were still the dominant SMPs, comprising 82–90% of total SMPs.

3.3. Second-stage anaerobic digestion for methane production

The hydrogenogenic effluents from the first-stage fermentation contain a significant amount of SMPs (such as acetate and butyrate), which are favourable substrates for methanogens in anaerobic digestion. The effects of hydrothermal pretreatment on subsequent biomethane production are shown in Fig. 6 a. The biomethane yield from the effluent of untreated *S. latissima* was 281.4 mL/g VS after digestion of 11 d with a biodegradability of 63.4% (see Table 3). When increasing the hydrothermal temperature to 140 °C, the highest biomethane yield of 345.1 mL/g VS was achieved, corresponding to an increase of 22.6% as compared to that without pretreatment. The biodegradability of *S. latissima* significantly increased to 77.7%. It was noted that the pretreatment at 180 °C reduced the biomethane yield to 278.7 mL/g VS. This was ascribed to the toxic effect caused by inhibitory by-products. A previous study demonstrated that thermal treatment of algal substrate, which contained abundant sugars and amino acids, could cause binary interactions between the carbonyl group (–C=O) and amino group ($–NH_2$), leading to the generation of various fermentative inhibitors (such as methylfurfural, pyrazine compounds, and nitrogen-containing Maillard compounds) [60]. The presence of these inhibitors decreased biomethane production by 43.8% as compared to that in the absence of inhibitors [60]. Therefore, this finding suggests that the optimization of hydrothermal pretreatment is necessary considering the competitive reactions between hydrolysis of polysaccharides and decomposition of mono-sugars.

The soluble COD removal efficiency after second-stage anaerobic digestion is shown in Fig. 6 b. The trends of COD removal were similar to that of biomethane production. The COD removal efficiency of the untreated seaweed was 79.5%; this increased to 86.7% and 86.1% when the seaweed was pretreated at 120 and 140 °C, respectively. Further increasing temperature to 180 °C resulted in the lowest COD removal efficiency of 67.4%. This indicates that anaerobic digestion was not capable of removing all the produced COD during hydrothermal solubilization. A higher hydrothermal temperature contributed to a higher generation of COD, but inevitably generated more inhibitory by-products. This, in return, resulted in a lower COD removal efficiency after fermentation and anaerobic digestion.

Table 3 shows the kinetic parameters of biomethane production from *S. latissima* by employing the modified Gompertz model. The parameters of biomethane production were evaluated in terms of methane yield potential (H_m), peak methane production rate (R_m), lag-phase time (λ), and peak time (T_m). The modelling results confirmed that hydrothermal pretreatment at 100–160 °C could improve methane yield potential from *S. latissima* by 14.2%–23.7%. Similarly, the peak methane production rate was also enhanced by 8.9%–22.6%. By applying hydrothermal temperature at 180 °C, both methane yield potential and methane production rate decreased to some extent. These results are in agreement with the experimental data.

3.4. Energy balance of *Saccharina latissima* biomass in dark fermentation and anaerobic digestion

The results of the energy conversion efficiency are compared to the different process energy efficiencies under three different scenarios, which include: (1) process energy efficiency as per experimental conditions; (2) process energy efficiency with potential heat recovery; and (3) process energy efficiency with reduced water use for hydrothermal pretreatment by 30%, as shown in Fig. 7. The energy conversion efficiency at all conditions shows the higher value compared to the energy process efficiency. The reason for that is obviously due to the fact that the calculation of energy conversion efficiency excludes the external energy supply for pretreatment, dark fermentation and anaerobic digestion. The total energy conversion efficiency of untreated seaweed was calculated as 59.6%, of which the efficiency of hydrogen was only 0.7% and the efficiency of methane was 58.8%. The hydrothermal pretreatment at 140 °C led to the highest energy conversion efficiency of 72.8%, due to the fact that the highest methane yield was obtained under this condition. It is noted that not all the energy of *S. latissima* was transformed into hydrogen and methane; around 27.2%–41.3% of total energy was still unexploited. This result could be attributed to the following reasons: (1) the degradation efficiency of seaweed substrate was not 100%; (2) a minority of energy was consumed for supporting microbial growth and reproduction; and (3) the formation of unfermentable byproducts during hydrothermal pretreatments.

Taking into account the external energy to supply heat for pretreatment, fermentation and anaerobic digestion, the highest process energy efficiency was achieved as only 37.8% when no pretreatment was applied. Unlike the energy conversion efficiency which peaked at 140 °C, the process efficiency continued to drop with increased temperatures, due to a higher need for external heat. This suggests that the additional methane generated was not sufficient to overcome the added heat required to maintain the temperature of pretreatment. Even with reducing the water use by as much as 30%, the process energy balance showed minimal improvement. However, a significant gain in process energy efficiency was obtained if the heat from the hydrolysates was recovered. A new peak of process energy efficiency was obtained at 160 °C of 56.9%. Thus, the use of waste heat is of importance, whereby the easiest use is to maintain the heat for fermentation and anaerobic digestion. Upon further system optimisation and integration, the reported cascading system may open a valuable approach for seaweed processing and valorisation.

3.5. Comparison of gaseous biofuels production from macroalgae with literature

Table 4 provides relevant studies on the pretreatment of various macroalgae and the resulting biohydrogen and biomethane production. Macroalgae including brown (such as *Laminaria japonica* and *Sargassum sp.*) and red (such as *Gelidium amansii*) algae have been investigated for one-stage biohydrogen production and two-stage biohydrogen and biomethane co-production. There are many pretreatment methods that have been studied in the literature, including thermal, thermal acid and microwave acid pretreatments [61]. These pretreatment methods could lead to an increase in biofuel production to different extents. For example, biohydrogen yield from *L. japonica* was significantly enhanced (by 143%) after combining thermal and hydrochloric acid pretreatment [62]. Yin et al. investigated the effect of combined microwave-acid pretreatment on fermentative hydrogen production from *L. japonica* [31]. The results showed that pretreatment at 140 °C with 1% H_2SO_4 for 15 min increased biohydrogen yield from 15 to 28 mL/ g dry weight [31]. In comparision, the research on two-stage fermentation using pretreated macroalgae has seldom been investigated. Costa et al. demonstrated the feasibility of biohydrogen and biomethane co-production from thermally pretreated *Sargassum* sp., which could greatly improve the energy conversion efficiency from macroalgae [63]. In the present paper, hydrothermal pretreatment has been successfully applied to improve biofuels production from brown seaweed *S. latissima*. The biomethane yield was enhanced by 22.6% under optimal condition. The biodegradability of untreated *S. latissima* was 63.4%, and raised to 77.7% with hydrothermal pretreatment at 140 ^{o}C for 30 minute in a two stage system. Coupled with methane fermentation, the two-stage fermentation process achieved the highest overall energy efficiency from seaweed, demonstrating the feasibility for future commercial applications.

4. Conclusions

Hydrothermal pretreatment of seaweed *S. latissima* could significantly break down the recalcitrant macro- and micro-structures of seaweed and was proven to be effective to produce gaseous biofuels. The major findings are as follows:

1) The first-stage biohydrogen yield only slightly increased via dark fermentation after pretreatment, while the second-stage biomethane yield significantly increased by 22.6% under the optimal pretreatment temperature of 140 °C. The maximum energy conversion efficiency of 72.8% was achieved after two-stage biohydrogen and biomethane co-production.
2) When considering the extra energy input for pretreatment, fermentation and digestion, the highest process energy efficiency obtained was 37.8% using untreated seaweed. This efficiency continued to drop with increasing pretreatment temperature, suggesting the additional methane generated was not sufficient to overcome the energy required to maintain the pretreatment

temperature.

3) However, a significant increase in efficiency (to 56.9%) can be achieved through heat recovery from hydrothermal pretreatment at 160 °C. Upon further system optimisation, this enables the possibility of combining thermochemical and biological treatments for seaweed exploitation and can promote circular bioeconomy concepts.

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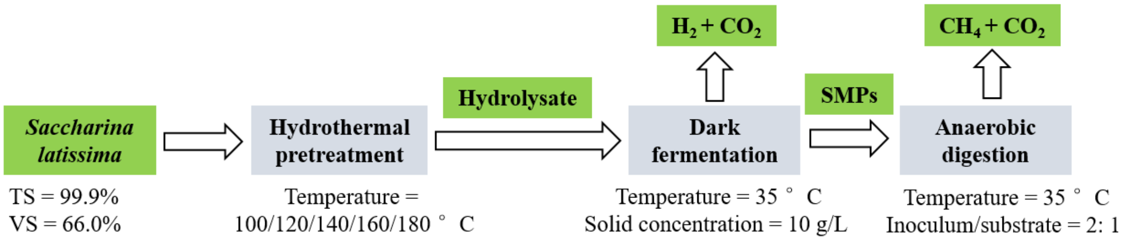
Table 1 Compositional analysis of dried seaweed *Saccharina latissima.*

Table 2 Soluble metabolite products (SMPs) profile after dark hydrogen fermentation of *Saccharina latissima* biomass.

Table 3 Kinetic parameters of biomethane production from *Saccharina latissima* biomass.

Table 4 Biohydrogen and biomethane production from macroalgal biomass with various pretreatments.



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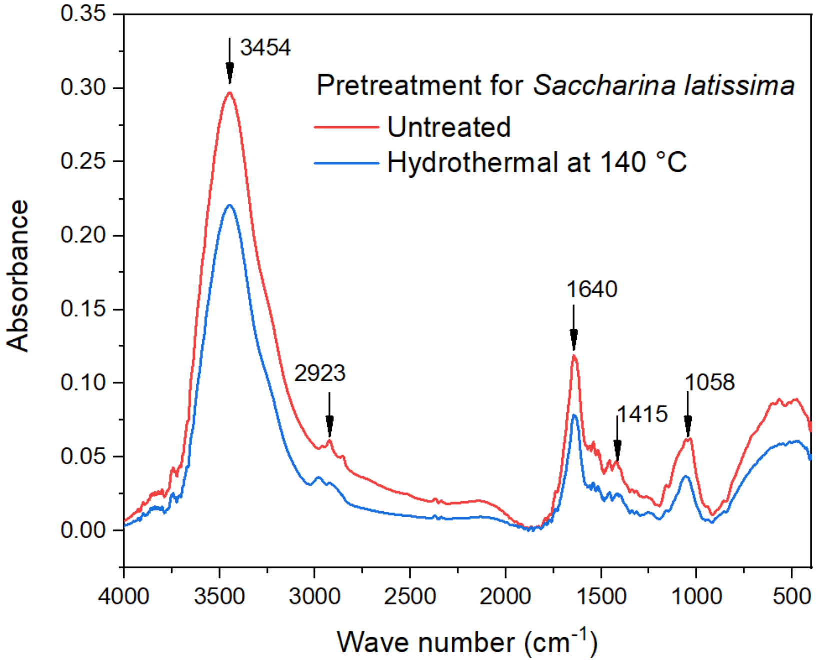

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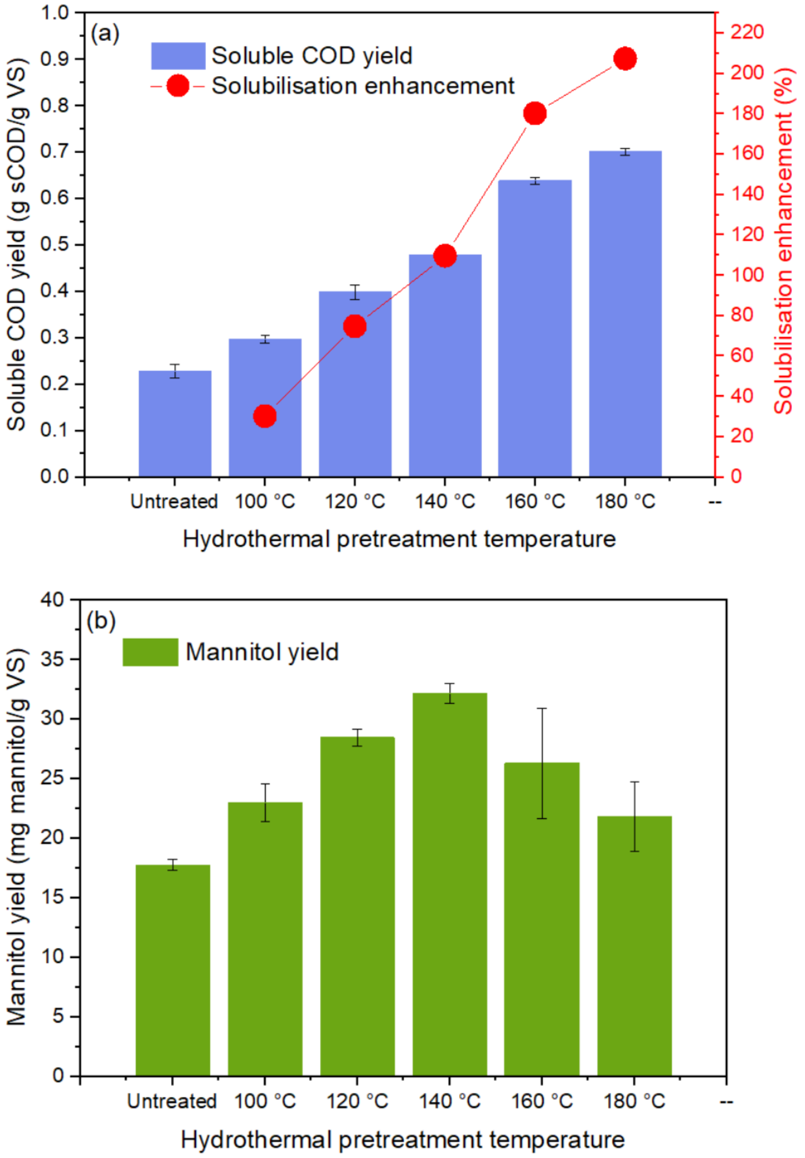

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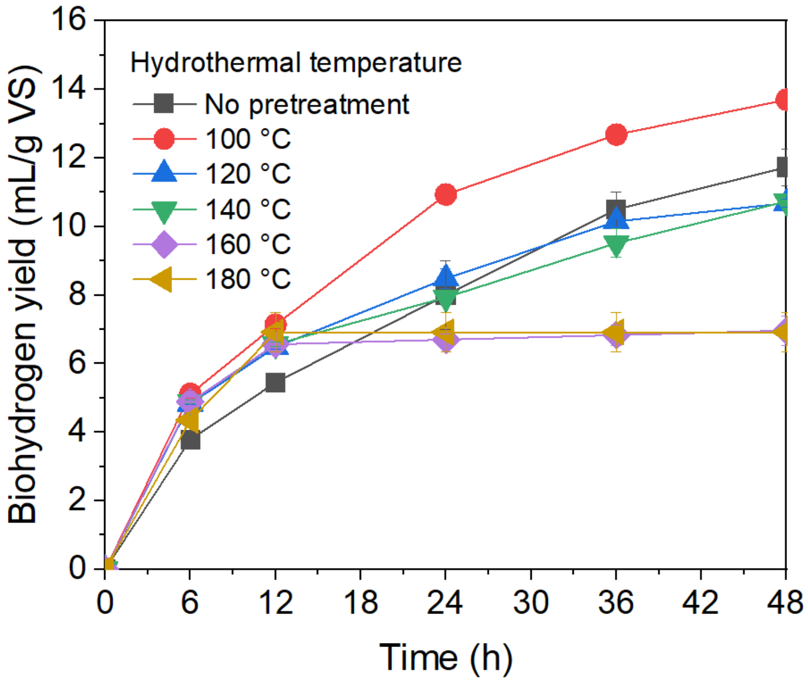
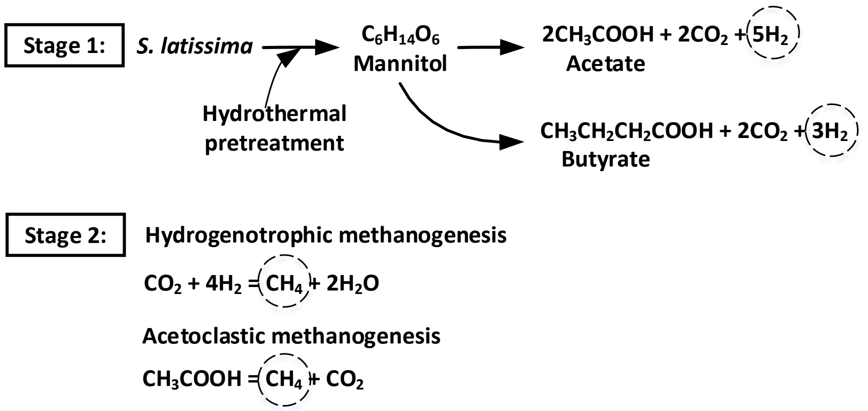


Fig. 4. Biohydrogen yield from *Saccharina latissima* after hydrothermal pretreatment. Data are presented as mean ± standard deviation.



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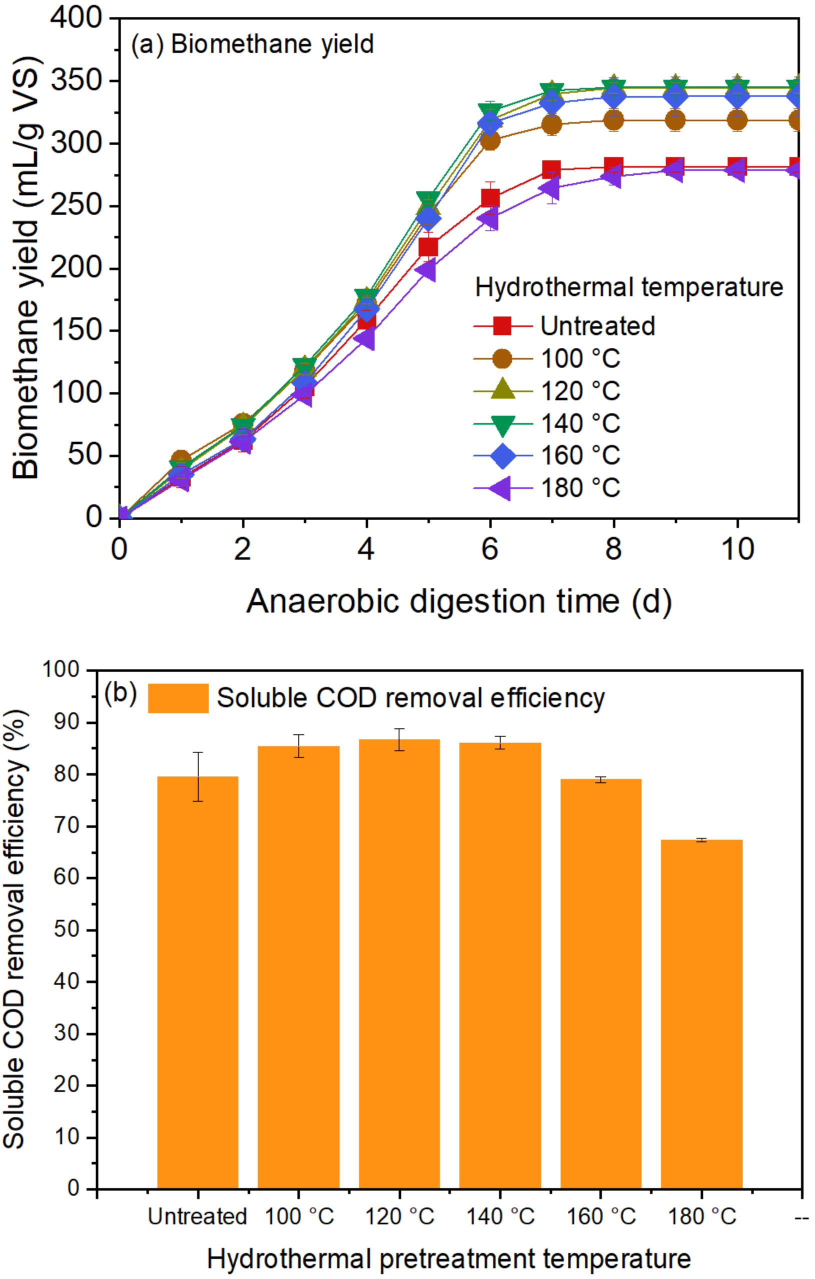

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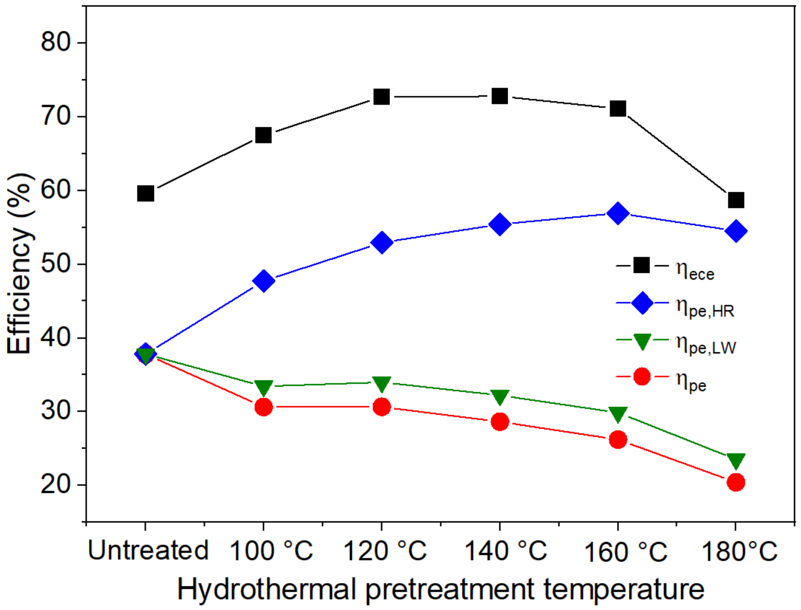

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Table 1 Compositional analysis of dried seaweed *Saccharina latissima.*

Parameter	*S. latissima*
Proximate analysis (wwt%)	
TS	99.9
VS	66.0
VS/TS (%)	66.1
Ultimate analysis (%TS)	
Carbon	31.5
Hydrogen	4.0
Oxygen	28.4
Nitrogen	2.2
C/N mass ratio	14.3
Energy value (kJ/g VS)	17.1
Theoretical biomethane yield (mL/g VS)	443.9

Table 2 Soluble metabolite products (SMPs) profile after dark hydrogen fermentation of *Saccharina latissima* biomass.

Compositions (g/L)	Untreated	100 °C	120 °C	140 °C	160 °C	180 °C
Acetic acid	3.58	3.30	3.67	3.82	3.76	3.70
Propionic acid	0.30	0.14	0.12	0.21	0.19	0.43
Iso-butyric acid	0.21	0.20	0.14	0.20	0.17	0.18
Butyric acid	1.33	1.13	0.78	1.25	0.43	0.81
Iso-valeric acid	0.14	0.15	0.09	0.15	0.10	0.13
Valeric acid	0.14	0.18	0.14	0.20	0.20	0.20
Caproic acid	0.02	0.02	0.02	0.02	0.01	0.06
Total SMPs	5.73	5.12	4.96	5.84	4.87	5.51

Table 3 Kinetic parameters of biomethane production from *Saccharina latissima* biomass.

Pretreatment	Experimental methane yield (mL/gVS)	Peak production rate (mL/gVS/d)	Kinetic model parameters					Biodegradability (%)
			H_m[a] (mL/gVS)	R_m[b] (mL/gVS/d)	λ[c] (d)	T_m[d] (d)	R^2	
Untreated	281.4±4.2	58.5±4.1	294.1	59.4	1.1	2.9	0.991	63.4
100 °C	318.8±9.1	71.9±1.9	336.1	64.7	0.9	2.8	0.982	71.8
120 °C	344.6±3.9	74.4±3.8	363.7	70.5	1.2	3.1	0.985	77.6
140 °C	345.1±6.3	78.1±3.1	363.5	72.4	1.2	3.0	0.983	77.7
160 °C	337.8±15.5	76.3±3.6	355.4	72.8	1.4	3.2	0.984	76.1
180 °C	278.7±2.4	54.9±1.7	293.7	52.6	1.0	3.1	0.993	62.8

Note: [a] H_m is maximum methane yield potential; [b] R_m is peak methane production rate; [c] λ is lag-phase time of methane production; [d] T_m is peak time of methane production (calculated as: $T_m = H_m/R_m/e + \lambda$).

Table 4 Biohydrogen and biomethane production from macroalgal biomass with various pretreatments.

Macroalgae species	Pretreatment	Hydrogen yield	Methane yield	Increases in hydrogen and methane production	Ref.
Laminaria japonica	Thermal; T=150–180 °C; t=5–40 min	69.1–109.6 mL H_2/g COD_{added}	/	From –10.7% to +63.9% (H_2)	[62]
Laminaria japonica	Combined acid + thermal; T=60, 110, 160 °C; t=5, 22.5,40 min; HCl dosage=0, 6, 12% (w/w)	9.5–163.1mL H_2 g/ dry algae	/	From –86% to +143% (H_2)	[32]
Laminaria japonica	Combined microwave-acid + thermal, T=140 °C; t= 15 min, H_2SO_4 dosage=0, 0.5, 1 and 2% (v/v)	15.0–26.8mL H_2/g dry algae	/	From –0.6% to +77.5% (H_2)	[31]
Laminaria digitata and *Arthrospira platensis*	Combined acid + thermal, T= 95, 115 and 135 °C; t=15 min; H_2SO_4 dosage=2.5 and 5% (v/v)	60.5–70.6 mL H_2/g VS	/	From –7.2% to +16.7% (H_2)	[64]
Gelidium amansii	Combined acid + thermal, T=120, 150, 180 °C; t=15 min; H_2SO_4 dosage=0.5, 1, 1.5% (w/w)	0–37.0 mL H_2/g dry algae	/	N.A.	[65]
Sargassum sp.	Thermal, T=121 °C; t=15 min	60.8–91.3 mL H_2/g VS	345–541 mL CH_4/g VS	N.A.	[63]
Saccharina latissima	Hydrothermal, T=100, 120, 140, 160, 180 °C; t=10 min	6.9–13.7 mL H_2/g VS	278.7–345.1 mL CH_4/g VS	From –41.0% to +17.0% (H_2) From –9.6% to +22.6% (CH_4)	This study