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Efficiency of hydrothermal pretreatment on the anaerobic digestion of pelagic *Sargassum* for biogas and fertiliser recovery

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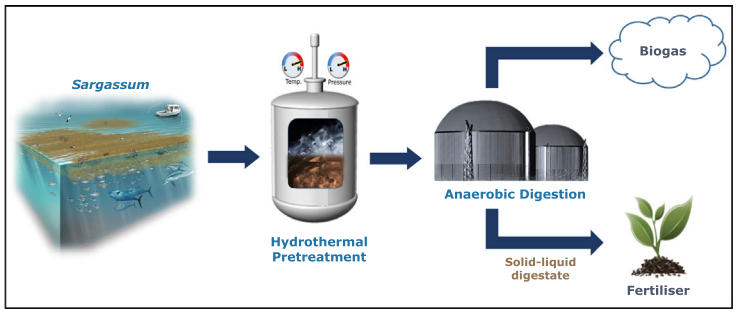
GRAPHICAL ABSTRACT



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ABSTRACT

Pelagic *Sargassum* inundation of coastlines across the North Atlantic Ocean is an ongoing challenge which poses a great threat to economic productivity. This novel study evaluated the valorisation of these invasive seaweeds into biogas and fertiliser using hydrothermal pretreatment and anaerobic digestion technologies. Increasing the severity factor of hydrothermal pretreatment from 1.59 to 3.83 promoted the degradation of organic particulates in *Sargassum*, resulting in a maximum soluble chemical oxygen demand yield of 27,250 ± 75 mg/L or 237% greater than the unpretreated biomass. However, no linear relationship exists between increased solubilisation and biogas productivity. Peak methane recovery of 116.72 ± 2.14 mL/gVS was achieved at severity factor 2.65 with the decrease thereafter attributed to the formation of Maillard reaction products and inhibitory compounds during hydrothermal pretreatment. The hydrogen sulfide content in the biogas generated also diminished from 3% to 1%. Additionally, the digestate of biogas production is pathogen-free, nutrient-rich and exhibits bio-fertiliser potential.

1. Introduction

There is heightened interest in the research and development of non-food source competitive biofuels as a means of counteracting the negative impact of fossil fuel-based economies. According to statistics reported by BP, the years 2007 to 2017 saw growth in global primary energy consumption of an average of 1.5% per annum. However, in 2018, consumption demand doubled that of the previous decade, representing 13,865 million tonnes of oil equivalent, derived from 85% fossil fuels [1]. While there exists a linear relationship between the energy demand and urbanisation, the over utilisation of petroleum-based products for energy generation has increased greenhouse gas emissions and triggered environmental instability. The identification of viable, eco-friendly alternatives to fossil fuels is therefore imperative to

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circumvent these major global challenges.

Biogas is a green, cost-effective bioenergy source with application in electricity generation, cooking, heating and transport. This energy-dense fuel is the main product of anaerobic digestion (AD), the four-step biological process in which organic material is broken down by microorganisms in the absence of oxygen. Methane (CH_4) and carbon dioxide (CO_2) dominate the composition of biogas, representing approximately 60–70% and 30–40%, respectively. Nitrogen, hydrogen sulphide (H_2S) and water vapour are also present in this gaseous fraction (< 1%) [2]. In addition to biogas, a digestate with bio-fertiliser or soil amendment properties is generated from this waste-to-energy process. Utilisation of this anaerobic effluent in horticulture is sustainable since it recycles essential nutrients and organic matter from the feedstock back into soil, thereby eliminating the growing demand for synthetic chemical soil enhancers and their associated negative environmental impact [3,4]. AD technology is well-established technology and has been globally commissioned for the management, treatment and disposal of bio-waste streams including food waste, agricultural residues and sewage sludge [5,6].

Research has also explored the utilisation of macroalgae or seaweeds as opportunity for biogas and fertiliser production. These aquatic plants, taxonomically classified as either green algae (Chlorophyta), brown algae (Phaeophyta) or red algae (Rhodophyta) based on their natural pigmentation and chlorophylls [7], are enriched with carbohydrates (laminarin, alginate, cellulose, fucoidan and mannitol) and possess delignified cell walls with negligible cellulose content [8]. While this unique chemical composition varies with genera and phyla [9], the structure of the algal cell wall matrix supports saccharification and microbial digestion [10]. Ergo, the theoretical biochemical methane potential (TBMP) of macroalgae is superior to that derived from terrestrial biomass. Seaweeds also present vast quantities of nutrients which support their application in agriculture as an organic fertiliser [11,12].

Mass-cultivation of seaweeds for biorefinery utilisation is a sustainable practice given their high photosynthetic activity and CO_2 bioremediation capacity, the avoidance of competition with food crops and their growth, independent of arable land use and a water supply [8]. Hitherto, researchers have shown preference to brown and red macroalgae over green macroalgae, due to the current market demand and available farming strategies [7]. Notwithstanding the knowledge gained, macroalgae remain an under exploited feedstock for large-scale bioenergy and fertiliser production, due to high production costs and harvesting challenges [13]. Several constituents contained within this marine biomass can also restrict these potential applications. Seaweeds possess structurally complex carbohydrates (alginate) and recalcitrant compounds such as insoluble fibre, salt, sulfur, heavy metals and phenolics which may impede microbial degradation and diminish the corresponding methane yield [11,14,15]. The rich heavy metal content (20–40%) of these aquatic plants may also hinder utilisation of the AD-derived digestate in agro-industry. Heavy metals are toxic and accumulate in soils, negatively impacting crop productivity and posing serious risk to public health and the environment [16].

Hydrothermal pretreatment of macroalgae has been identified as the most promising technique to improve the bioavailability of organic matter for microbial hydrolysis, thereby increasing biogas production in the downstream process of AD [17]. This technology, which utilises liquid water heated at moderate temperature (120–200 °C) and pressure (30–150 bar) in the presence of nitrogen, is eco-friendly, net energy positive [18,19,20] and accommodates water-logged feedstock such as seaweeds (70–90 wt% moisture) [7,11,17] without the pre-requisite of a dewatering step prior to treatment [19]. Studies also suggest that incorporating hydrothermal pretreatment prior to AD can enhance the digestate nutrient quality, sterilisation and phytosanitary properties by reducing undesirable odour emissions whilst suppressing the transmission of soil-borne diseases and weed seed germination [3,4].

Despite the ubiquity of brown macroalgae research, little work has been done on pelagic *Sargassum*. To date, studies on these invasive seaweeds have focused on methane productivity through direct AD [15,16,21,22] while a single study examines biological pretreatment [23]. No research has investigated thermal hydrolysis prior to AD for energy optimisation and fertiliser recovery. Thus, this study aims to fill the gap by evaluating biogas and fertiliser co-production from pelagic *Sargassum* seaweeds (*S. fluitans* and *S. natans*) through integrated hydrothermal pretreatment and AD technologies, a topic extensively discussed in previous work [21]. The present research output is of global significance, offering a viable management and disposal solution to the challenge of mass *Sargassum* influx into shorelines of the Caribbean, West Africa, Gulf of Mexico and North America [24,25]. Over the last decade, global anthropogenic changes have effected increased *Sargassum* blooming and deposition, consequently endangering marine ecosystems and rendering vulnerable economies which depend on Tourism and Fisheries [16]. While the clean-up and disposal of this marine biomass is necessary, the current strategies employed are costly and environmentally harmful. The valorisation of these seaweeds into high value-added products such as energy and fertiliser would therefore be a positive and promising development [21].

The key objectives of this study are to: (i) assess the physico-chemical properties of pelagic *Sargassum*; (ii) investigate the effect of hydrothermal pretreatment operating conditions on seaweed solubilisation and microbial methane fermentation; and (iii) explore the viability of utilising the resulting digestate in agriculture as bio-fertiliser by comparing its chemical composition with examples of established international standards.

2. Materials and methods

2.1. Raw substrate and inoculum

Pelagic *Sargassum* seaweed was collected from the neritic waters of Conset Bay, Barbados in June 2018 and the species identified as *S. natans* and *S. fluitans*, based on pod and leaf morphology differences. The fresh biomass was washed with distilled water to remove sea water, sand, dirt and debris. Next, the cleaned seaweeds were sun-dried for two weeks to ensure moisture content reduction, as stipulated by the Import Health Standard for dried and preserved plant material, and fresh plant material for testing, analysis or research outlined by the Ministry of Primary Industries, New Zealand. The sun-dried biomass (2 kg) was then vacuum-packed and exported to New Zealand for chemical analysis and experimentation. Upon receipt in New Zealand, the consignment was heat-treated at 80 °C for 15 h to eliminate contaminants and excess moisture from the feedstock. This treatment process was a secondary requirement for biosecurity clearance. The dried seaweeds were manually shredded and then pulverised in a commercial food grade high-speed multifunction grinder to a particle size of approximately 0.5–1.0 mm. Zipped lock bags were used to store the fine particles which were subsequently preserved at −4 °C until further use. Fig. 1 shows the physical changes made to the substrate during preparation.

The inoculum used for biogas production was sourced from the anaerobic digestate of the Rosedale Watercare wastewater treatment plant located on the North Shore of Auckland, New Zealand, which runs large-scale continuous AD of municipal waste at mesophilic temperature. The inoculum was filtered through a 1 mm sieve to remove large particulates and subsequently stored at −4 °C, until required in biomethane potential (BMP) assay testing. The total solid (TS) and volatile solid (VS) contents of the seed inoculum were 2.16 ± 0.02 wt% and 0.65 ± 0.06 wt%, respectively.

2.2. Hydrothermal pretreatment

Hydrothermal pretreatment was conducted in a 1 L high-pressure batch reactor (Amar Company Ltd, India) equipped with an impeller,

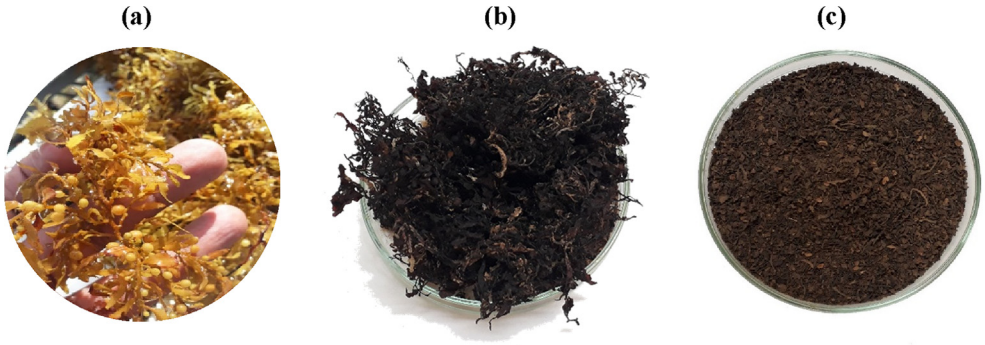

Fig. 1. Pelagic *Sargassum* seaweed (a) fresh from the ocean, (b) sun-dried for 2 weeks and heat treated, and (c) processed and homogenised prior to analysis.

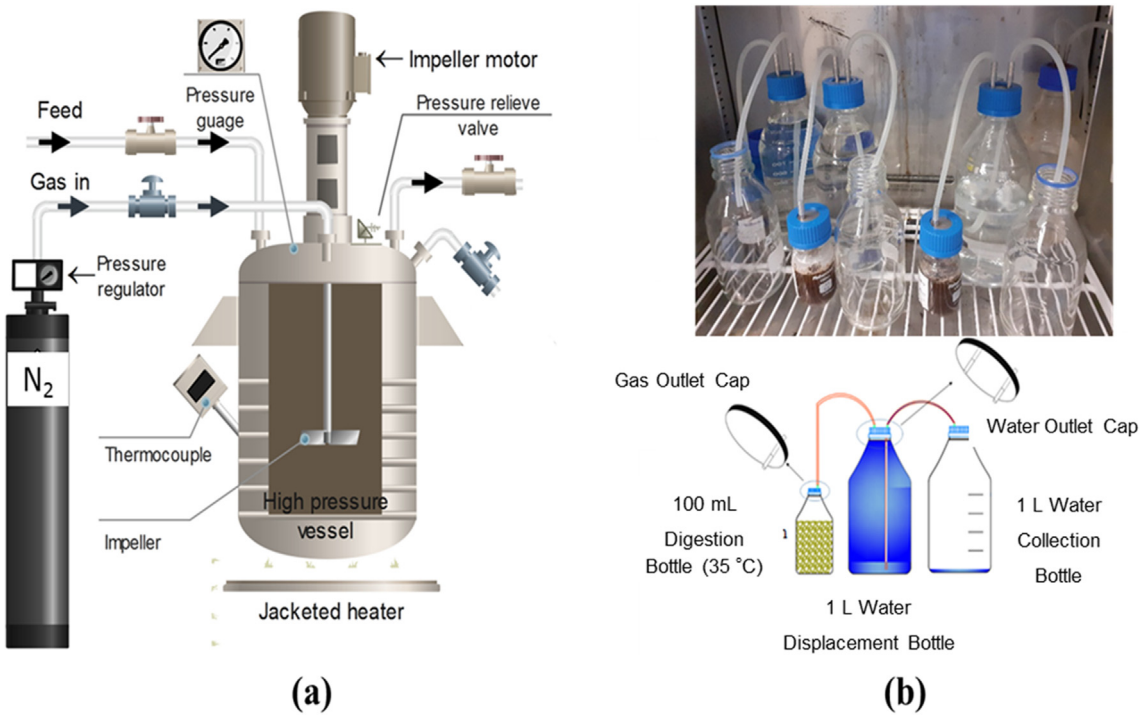

Fig. 2. Experimental setup for (a) hydrothermal pretreatment and (b) anaerobic digestion.

thermocouple and pressure gauge (see Fig. 2a). In each run, a mixture of dried *Sargassum* seaweed and deionised water (solid to liquid ratio of 1:6 w/w) was loaded in the reactor and the device subsequently pressurised with 30 bar N_2 gas and stirred at a speed of 300 rpm to lower energy consumption and minimise the operational costs. Pretreatment at four temperatures (120, 140, 160, 180 °C) was evaluated at retention times ranging from 10 to 30 min. The pretreatment time commenced when the desired internal reactor temperature was reached. At the completion of each run, the reactor was cooled to ambient temperature and the resulting solid–liquid slurry collected for characterisation and microbial bioconversion.

To evaluate and compare the impact of different conditions during hydrothermal pretreatment, reaction time and temperature have been combined into a single parameter called the "reaction ordinate", R_o. This combination is able (i) to unify data on complex reaction systems; (ii) to provide an easy way for comparing results where processing has been conducted under different conditions and using different reactor/equipment scales, and (iii) to facilitate process control by adjusting the operation cycle when operational difficulties dictate changes from standard temperature–time processing profiles [26,27,28,29,30]. The reaction ordinate is defined by Overend and Chornet [28] as

$$R_o = t\exp\left(\frac{T_r - T_b}{14.75}\right) \quad (1)$$

where, t is the time of reaction, T_r is the temperature of reaction and T_b is the base temperature chosen at which there is essentially little or no reaction. In this study as is consistent with prior works [26,28], the base temperature selected was 100 °C. The "severity factor" which represents the severity of the hydrothermal pretreatment process is defined as

$$Severity\ \ Factor\,(SF) = log_{10}R_o \quad (2)$$

The severity factor has been used previously for modelling the hydrothermal pretreatment of biomass and lignocellulosic materials [26,27,28,31], and is closely related to other parameters used for similar approaches in oil and gas or in pulp and paper processes [29,30]. The SF of the different pretreatment conditions employed in this study are shown in Table 1.

The influence of hydrothermal pretreatment on seaweed solubilisation was determined by calculating the chemical oxygen demand (COD) solubilisation yield using Eq. (3) [32]:

$$\text{COD solubilisation } (\%) = \frac{sCOD_{\text{Pretreated}} - sCOD_{\text{Untreated}}}{tCOD_{\text{Untreated}}} \times 100 \quad (3)$$

Table 1
Severity factors (log R_o) of different pretreatment conditions.

Temperature (°C)	Time (min)	SF (log R_o)
120	10	1.59
	20	1.89
	30	2.07
140	10	2.18
	20	2.48
	30	2.65
160	10	2.77
	20	3.07
	30	3.24
180	10	3.36
	20	3.66
	30	3.83

where, sCOD is the soluble chemical oxygen demand (mg/L) and tCOD is the total chemical oxygen demand (mg/L).

2.3. Anaerobic digestion

The batch lab-scale digester set-up employed in this study was adopted from the work of Raspoor et al. [33] and consisted of three sub-units: (1) 100 mL digestion bottle, (2) 1 L water displacement/storage bottle and (3) 1 L water collection bottle (Fig. 2b). Silicon rubber tubing was used to connect the units. Pretreated *Sargassum* was added to the 100 mL digestion bottle and the pH adjusted when necessary to 6.5 ± 0.5 with 5 M NaOH or 5 M HCl solutions to improve the bio-conversion efficiency. Inoculum (5%) was introduced to the feedstock and the slurry flushed with N_2 (99.9% purity) for 1 min to create an environment conducive to anaerobe community proliferation. Thereafter, each digestion bottle was sealed with a metal cap to ensure gas-tight conditions. The total working volume was 80 mL. The samples were incubated for 21 d under mesophilic conditions (35 ± 1 °C). During AD, the biogas generated in the digestion bottle creates pressure which effects water displacement from bottle 2 into bottle 3. Daily biogas production was measured by recording the weight of the water displaced. All experiments were performed in triplicate and the results expressed as mean value ± standard deviation (SD).

At the end of the fermentation process, the net methane yield achieved under each condition was compared to the feedstock's TBMP which was estimated with low error, applying Buswell and Boyle's formula based on the elemental composition (Eq. (4)) [34]. This equation accounts for contributions from C, H, N, O and S, and considers the production of CO_2, CH_4, NH_3 and H_2S during fermentation. Additionally, it assumes complete (100%) material breakdown during AD [34,35]:

$$C_xH_yO_zN_aS_b + \left(x - \frac{y}{4} - \frac{z}{2} + \frac{3a}{4} + \frac{b}{2}\right)H_2O \rightarrow \left(\frac{x}{2} - \frac{y}{8} + \frac{z}{4} + \frac{3a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{x}{2} + \frac{y}{8} - \frac{z}{4} - \frac{3a}{8} - \frac{b}{4}\right)CH_4 + aNH_3 + bH_2 \quad (4)$$

The theoretical methane yield (TMY) can be predicted by Eq. (5) [36]:

$$\text{TMY (mL } CH_4\text{/gVS)} = \frac{22.4 \times 1000 \times \left(\frac{x}{2} + \frac{y}{8} - \frac{z}{4} - \frac{3a}{8} - \frac{b}{4}\right)}{12x + y + 16z + 14a + 32b} \quad (5)$$

where, 22.4 (L) is the volume of 1 mol of gas at standard temperature and pressure, and 1000 is the conversion factor from litres to millilitres.

The efficiency of the AD process at removing sCOD content from the digestate is evaluated using Eq. (6):

$$\text{COD removal efficiency(\%)} = \left(\frac{sCOD_{final} - sCOD_{initial}}{sCOD_{initual}}\right) \times 100 \quad (6)$$

where, $sCOD_{\text{initial}}$ and $sCOD_{\text{final}}$ are respectively the soluble COD (mg/L) before and after digestion.

2.4. Analytical methods

The TS and VS content were determined according to the EPA Standard Methods 2540B and 2540E, respectively. The pH value was measured using a Hanna edge pH meter. An elemental analyser (Thermo Flash 2000) was employed to measure elements (carbon, nitrogen, oxygen, hydrogen and sulfur) in the feedstock and the values used to calculate the carbon to nitrogen (C/N) ratio. The ash content was determined using an Induced Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7700, USA). Chemical oxygen demand (COD) measurements were made using a Hach COD HR test kit 20 to 1500 mg/L and evaluated by a Hach DR3900 spectrophotometer. Ammoniacal nitrogen (NH_3-N) content was assessed with a Hach NH_3-N reagent set, TNT, AmVer (Salicylate), Low Range coupled with Hach DR3900 spectrophotometry. Volatile fatty acid (VFA) content was analysed with Shimadzu QP2010 Plus gas chromatography (GC) configured with a non-polar 5 ms column and equipped with flame ionisation detection (FID). The injection port and FID were set to 250 °C. Initially the column was heated to 100 °C and the temperature held for 1 min. Thereafter, the column temperature was increased to 200 °C at a rate of 10 °C/min and the temperature maintained for 2.5 min at 200 °C. The volatile fatty acids evaluated were acetic acid, propionic acid, *iso*-butyric acid and butyric acid. Biogas composition (CH_4, CO_2 and H_2S) generated in AD was evaluated using an OPTIMA7 portable biogas analyser.

3. Results and discussion

3.1. Characterisation of pelagic Sargassum

3.1.1. Elemental composition

The chemical composition of pelagic *Sargassum* used in this study is presented in Table 2. This marine biomass has a C/N ratio of 21.67 ± 0.21 which lies within the ideal C/N range of 20–30:1 for optimum microbial digestion and fermentation [11]. The pH of the feedstock (7.33 ± 0.16) is also within the acceptable range of 6.5–8.0 for stable anaerobic digester function. Contrariwise, C content of 27.50 ± 0.65 %TS suggests low carbohydrate levels in *Sargassum* and indicates the limited bioavailability of fermentable organic monomers for AD and resulted in the VS/TS ratio of 47.72 ± 0.07. The feedstock moisture level of 20.70 ± 0.93 wt% is also unfavourable for microbial community growth and may reduce substrate methanation [37]. Previous studies have reported optimum conditions for biogas production as VS/TS ratio and water level of 0.70 and 80%, respectively [36].

Table 2
Physico-chemical characteristics of dried *Sargassum*.

Parameters	*Sargassum*	Unit
Proximate analysis:		
Moisture	20.63 ± 0.93	wt.%
TS	79.30 ± 0.93	wt.%
VS	37.84 ± 5.51	wt.%
VS/TS ratio	47.72 ± 0.07	%
Ash	31.82 ± 1.34	wt.%
Fixed C	9.71 ± 0.59	wt.%
Ultimate analysis:		
C	27.50 ± 0.65	%TS
N	1.21 ± 0.06	%TS
H	4.16 ± 0.30	%TS
S	0.82 ± 0.22	%TS
O	34.49 ± 0.18	%TS
C/N ratio	21.67 ± 0.21	–
TMP	142.84	L/kgVS
Gross calorific value	15.66 ± 0.68	MJ/kgVS

Ultimate analysis of pelagic *Sargassum* presents a rich O fraction (34.49 ± 0.18 %TS) which contributes to the energy value of 15.66 ± 0.68 MJ/kgVS, equivalent to the monosaccharide glucose [38]. By contrast, the elemental N and S levels in this marine biomass are low and support microbial bioconversion. Of importance, the N and S content of the feedstock must be monitored during AD as high concentrations can promote NH_3 accumulation and the formation of H_2S, thereby poisoning anaerobic flora and triggering digester failure [39]. The aforementioned challenges can be overcome by feedstock water dilution prior to digestion [11] or extension of the digestion time for inoculum acclimatisation promotion and increased toxicity tolerance [36,40].

Based on the stoichiometric composition of pelagic *Sargassum*, the empirical formula could be expressed as $C_{27.50}H_{4.16}O_{34.49}N_{1.21}S_{0.82}$. The TBMP of this marine biomass was determined to be 142.84 L CH_4/kgVS, suggesting pelagic *Sargassum* as poor feedstock for mono-digestion and biomethane production. In literature, the TBMP of *Sargassum* genus ranges from 119 to 380 L CH_4/kgVS [41,42]. All values shown in Table 2 are expressed as the mean and standard deviation (SD) of three measurements.

3.1.2. Mineral content

Table 3 outlines the metal profile of pelagic *Sargassum*. Ash content of this marine biomass was high, measuring 31.82 ± 1.34 wt% (Table 2). The rich mineral and trace element composition of these seaweeds may be attributed to exogenous factors such as phylum, seasonality, light intensity and anthropogenic changes in the nutritional composition of the growth and sample site (seawater) [43]. Potassium was the major macronutrient in pelagic *Sargassum*, then Ca, Na, S, Al, Mg and P. This result substantiates previous studies on species of the *Sargassum* genus [15,16,44,45]. For the purpose of AD, the macro-nutrients (Na, K, Mg, Ca and P) are essential for anaerobe growth, metabolic activity and biodigester stability. Essentially, the high Na content of this influent (resultant of oceanic growth in conditions predominantly by Na and their salts) may be advantageous for digester stability since it reduces the potential of NH_3-N toxicity through antagonism between these two variables [40]. On the other hand, excessively high Na levels can inhibit methanogen proliferation and function by dehydrating the cells via osmotic pressure [46,47]. In any case, several researchers suggest that the presence of Ca, K and Mg within the feedstock may synergise or compound the antagonistic effect of Na-induced digester toxicity [39,40].

Table 3
Metal profile of pelagic *Sargassum*.

Elements	Measured ± SD (mg/kg DM)
Macro-nutrient:	
Na	14890.69 ± 288.88
Mg	8233.78 ± 170.44
Al	2300.08 ± 57.73
P	855.13 ± 11.89
K	49973.09 ± 1179.36
Ca	48895.20 ± 1232.16
Micro-nutrient:	
V	25.76 ± 0.64
Cr	12.96 ± 0.39
Mn	337.51 ± 7.93
Fe	2398.37 ± 52.52
Co	6.51 ± 0.15
Ni	34.90 ± 0.90
Cu	25.08 ± 0.59
Zn	105.65 ± 2.16
Trace elements:	
As	35.22 ± 0.61
Cd	0.79 ± 0.01
Hg	1.36 ± 0.04
Pb	0.40 ± 0.01

The *Sargassum* genus have high capacity to bioaccumulate metals and metalloids from the environment [15,16,17,45]. In this work, pelagic *Sargassum* contained a large amount of heavy metals in the decreasing sequence Fe > Mn > Zn > As > Ni > V > Cu > Cr > Co > Hg > Cd > Pb (Table 2). During anaerobic fermentation, these non-biodegradable constituents are released from the feedstock, subsequently impacting the biochemical reactions of AD. While some micro-nutrients, namely Fe, Ni, Zn, Cu are required by micro-organisms in trace quantities for methanogen proliferation and methane formation, the accumulation of Pb, Hg, Cd, As and Cr can be toxic and disrupt digester function [48]. The heavy metal concentrations presented in Table 3 are all within the range documented in literature for optimal microbial bioconversion efficiency [49,50].

3.2. Hydrothermal pretreatment of pelagic Sargassum

3.2.1. Solubilisation of Sargassum

Fig. 3 shows the pH, TS, VS and COD solubilisation of *Sargassum* as a function of the SF (Log R_o). In general, increasing the severity of the hydrothermal pretreatment condition applied promoted the hydrolysis of large water-insoluble polymeric components such as carbohydrates, proteins and fibre into low weight water-soluble organic monomers. Consequently, the hydrolysate presented high levels of amino acids and monosaccharides derived from structurally complex sugars, such as alginates. Chemical analysis of the extracts measured greater sCOD (Fig. 3a) and VS (Fig. 3c) content in the liquid phase of the pretreated slurry assayed than the untreated sample. Maximum sCOD recovery of 27,250 ± 75 mg/L and COD solubilisation of 96.12 ± 0.42% were achieved under the harshest pretreatment condition studied of SF 3.83. This yield of solubilised COD is equivalent to an increase of 237.62% compared to the unpretreated *Sargassum*, thus confirming the efficiency of hydrothermal pretreatment at enhancing seaweed degradation and bioavailability for assimilation by microorganisms in AD. Noteworthy, while integrating temperature and exposure time had a positive effect on the solubilisation of organic compounds in *Sargassum* (Fig. 3b), the results suggest that temperature is the most statistically significant variable ($p = 0.05$) at enhancing hydrothermal pretreatment process performance. As the pretreatment temperature increased, this resulted in heightened concentration of fermentable sugars and degradable compounds present in the liquid phase. Similar results have been reported in studies on the hydrothermal pretreatment of food waste [32], municipal solid waste [20,51], sewage sludge [18] and the brown seaweed, *S. latissima* [38].

An inversely proportional relationship also exists between the pretreatment SF and the pH of the liquid phase ($R^2 = 0.8564$). As seen in Fig. 3d, raw *Sargassum* exhibits a pH of 7.33 which subsequently decreased to the range of 6.52–6.98 after pretreatment. The lowest pH (6.52) was measured at the harshest pretreatment condition evaluated of SF 3.83. During hydrothermal pretreatment, water acts as an acid, generating hydrogen ions which reduce the pH value of the resulting solid–liquid slurry to the acidic range [20]. Moreover, reactor heating promotes the formation of NH_3-N, organic acids and release of sulfated compounds from the polysaccharide fucoidan into the pretreated hydrolysate [38].

3.2.2. Spectroscopic analysis of untreated and pretreated Sargassum

Fourier-transform infrared (FT-IR) spectroscopy was performed to show the deconstruction in *Sargassum as* effected by hydrothermal pretreatment. The absorption spectra shown in Fig. 4 is within the range of 450–3500 cm^{-1} and reveals peak similarities between the hydrothermally pretreated seaweeds at SF 3.83 and the raw biomass. The strong broad absorption band at 3406 cm^{-1} and the medium peak at 1408 cm^{-1} correspond to O–H stretching vibrations, characteristic of the presence of hydroxyl groups [42,43] in compounds such as cellulose, hemicellulose and lignin [44].

The peak observed at 2923 cm^{-1} shows the CH_3 and CH_2 stretching

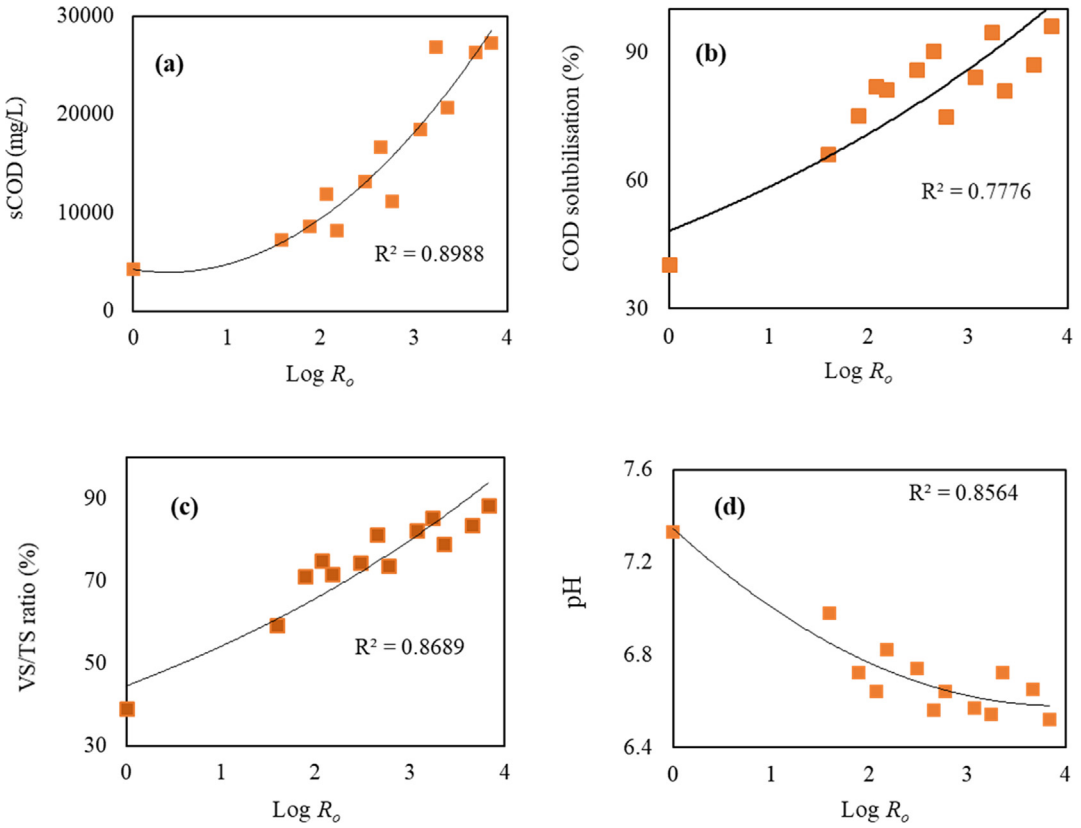

Fig. 3. The influence of pretreatment severity factor (Log R_o) on: (a) sCOD content in the liquid phase, (b) COD solubilisation, (c) VS formation and (d) the pH value of *Sargassum*.

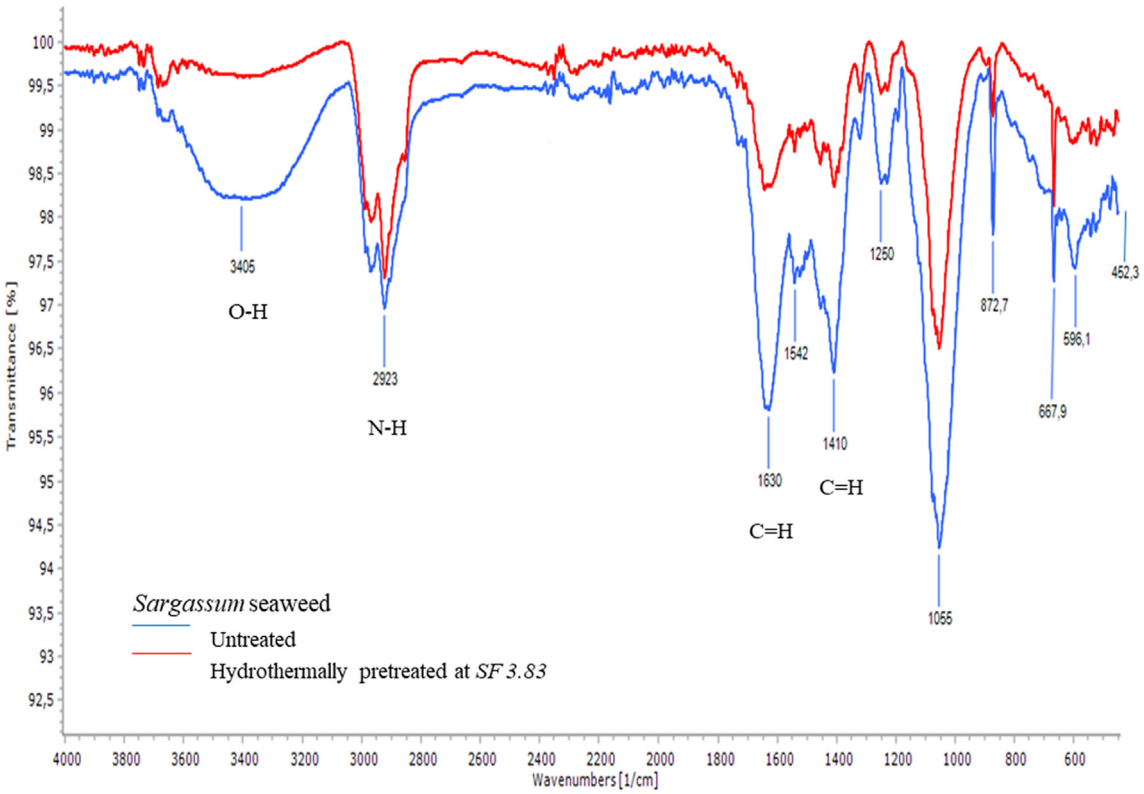

Fig. 4. FT-IR spectra of pelagic *Sargassum* pre- and post- hydrothermal pretreatment.

in polysaccharides, N–H stretching vibrations in aliphatic compounds and chlorophyll groups [43]. The two high band intensities at 1630 cm^{-1} and 1410 cm^{-1} represent C = C stretching and suggests the presence of lignin and aromatic compounds. The weak peak at 1250 cm^{-1} may be attributed to S = O stretching in sulfate esters and C-O stretching in phenols. The sharp band at 1055 cm^{-1} is indicative of the C-O-C stretching of xylans in hemicellulose [44] and the C-N stretching vibration of aliphatic amines in protein [23]. The peak at 873 cm^{-1} shows aromatic C–H bending which is out of the plane and suggests the existence of aromatic ring compounds. The weak peak observed at 668 cm^{-1} represents C-S stretching in sulfates while the band at 452 cm^{-1} corresponds to S-S stretching of disulfide bonds [52]. The FT-IR spectra of pelagic *Sargassum* is similar to *Sargassum wightii* [53], Mexican Caribbean macroalgae consortia [23] and *Saccharina latissima* [23].

In the FTIR spectra presented above, the hydrothermally pretreated seaweed exhibited absorption peaks similar to the unprocessed biomass but with lower intensities. The peaks of interest which correspond to O–H (3406 cm^{-1}), N–H (2923 cm^{-1}), C = C (1630 cm^{-1} and 1410 cm^{-1}) and C–H (873 cm^{-1}) groups, confirm that hydrothermal pretreatment accelerated the hydrolysis of carbohydrates and proteins in *Sargassum* biomass. This finding supports the observation of higher VS and sCOD content in the liquid phase of the pretreated samples than the control as reported in Fig. 3.

3.2.3. Formation of anaerobic digestion inhibitory compounds

The concentrations of the various AD inhibitory compounds generated at each SF is shown in Fig. 5. Typically, protein degradation releases inorganic ammonia nitrogen into the liquid phase as either ammonium ions (NH_4^+) or free ammonia (NH_3). The latter form is the primary cause of AD inhibition since it is freely membrane-permeable [40] and toxic to methanogenic bacteria [54]. In this study, a positive correlation was observed between the SF of pretreatment and NH_3-N accumulation in the supernatant. Higher temperatures (≥160 °C) and longer retention times (≥20 min) were most effective at degrading the proteins in *Sargassum*. The maximum NH_3-N content of 35 ± 2 mg/L was obtained at the SF of 3.83. Notwithstanding, this NH_3-N yield was deemed safe for microorganism activity since it is below 80 mg/L, the minimum value reported in literature for NH_3-N inhibition and stable biodigester function [40]. The authors attribute this result to the low N content (1.21 ± 0.06 %TS) of feedstock and the high dilution factor employed during hydrothermal pretreatment. Montingelli et al. [11] reported the efficacy of high feedstock water dilution at impairing ammonia production while Costa et al. [54] assert that for ammonia inhibition of methane generation to be achieved, the feedstock N content should range from 3.5 to 8.7%.

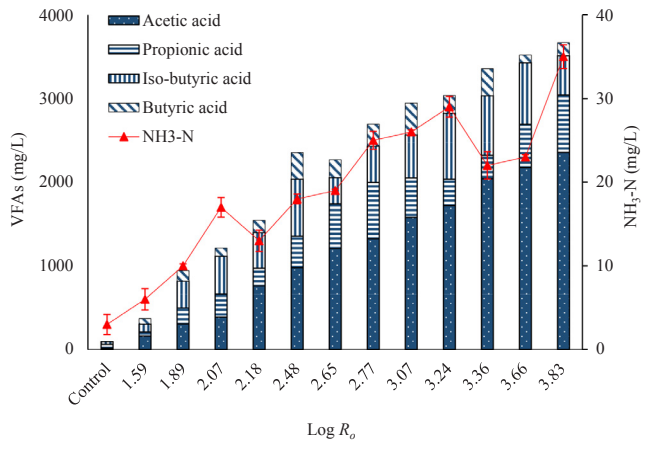

Fig. 5. Recalcitrant compound production profile after hydrothermal solubilisation.

During hydrothermal pretreatment, soluble sugars can be further degraded into short-chain VFAs. These carbon-rich compounds are highly desirable for methanogenesis given their small and easy degradation. However, organic acid accumulation can alter the bio-digester pH to the acidic range, triggering unstable digester performance and mitigating methane productivity. In literature, the VFA inhibitory level for a controlled AD process is > 6000 mg/L [11]. Analysis of the pretreated samples (Fig. 5) reveals a directly proportional increase in VFA production with the SF of the operation condition applied. Acetic acid was the dominant VFA in pretreated samples assayed, increasing in concentration from 15 mg/L in the control to a maximum of 2357 ± 155 mg/L mg/L at SF 3.83. This stable organic acid is an important intermediate compound in methanogen metabolism and its increased formation indicates the favourable potential of hydrothermal pretreatment at improving anaerobic biogas production. Overall, the cumulative VFA yield of the pretreated samples was below the AD inhibitory level. Nkemka and Murto [37] attribute this result to the buffering capacity of water dilution which prevents VFA production and accumulation to toxic concentrations in the bioreactor.

The S content of *Sargassum* may present a challenge to microbial bioconversion as it can promote the synthesis of sulfate-reducing bacteria, thus leading to H_2S production during AD. Hydrogen sulfide is a toxic, corrosive gas which diminishes the quality and limits the economic value of biogas in industry. As such, this parameter must be monitored to optimise the BMP of these seaweeds. In literature, the inhibitory sulfide level is 100 to 800 mg/L for dissolved sulfide and approximately 50–400 mg/L for undissociated H_2S acclimatisation [11]. Digestion studies on *Laminaria digitata* inoculated with bovine slurry report biogas generation with H_2S content of > 200 mg/L [55]. Similar H_2S levels (> 200 mg/L) were quantified in biogas derived from five macroalgal species native to Ireland [56]. Nevertheless, these high H_2S concentrations had no inhibitory effect on methanogenic activity due to swift inoculum acclimatisation [11]. In the present study, the authors anticipate diminished potential for H_2S inhibition of methane production due to the low S content (0.82 ± 0.22 %TS) of the feedstock and the high dilution factor of pretreatment.

3.3. Biogas yield and production rate

After 21 d fermentation time, pretreated *Sargassum* exhibited higher biogas yields than the virgin feedstock, a strong indication that hydrothermal pretreatment improved the microbial biodegradation of solubilised compounds during AD. Under all conditions assayed, maximum biogas recovery was achieved within the first 5 d of digestion due to absence of lag-phase time for methane production. The final methane content in the biogas produced ranged from 45 to 50% in all the digested samples. Fig. 6 presents the methane potential of the raw and pretreated samples at the low (≤2.65) and high (≥2.77) severity factors evaluated. The highest BMP of 116.72 ± 2.14 mL/gVS was achieved at SF 2.65 and represents 81.72% of the TBMP. This energy yield was a significant improvement to the 41.84 ± 3.07 mL/gVS of methane recovered from the raw, untreated biomass. Microbial bioconversion of raw *Sargassum* was low at 29.29% of the TBMP and lies within the range of 27–46% degradability published in literature for this genus [41]. It must be stated that at pretreatment conditions of high intensity (SF ≥ 2.77), the experimental BMP decreased due to Maillard reactions between solubilised sugars and proteins [38]. These chemical reactions create Amadori products or melanoidins which are not easily digested by microbes [57]. Moreover, high pretreatment temperatures (≥160 °C) promote the formation of AD inhibitory compounds (such as NH_3-N, VFAs and phenolics) which substantially reduce the bioavailability of organic matter for methane fermentation [32,38]. Consequently, the authors observed a significant reduction in methane production of 103.90 ± 1.48 mL/gVS to 62.37 ± 2.16 mL/gVS as the severity of the pretreatment condition rose from 2.77 to 3.83.

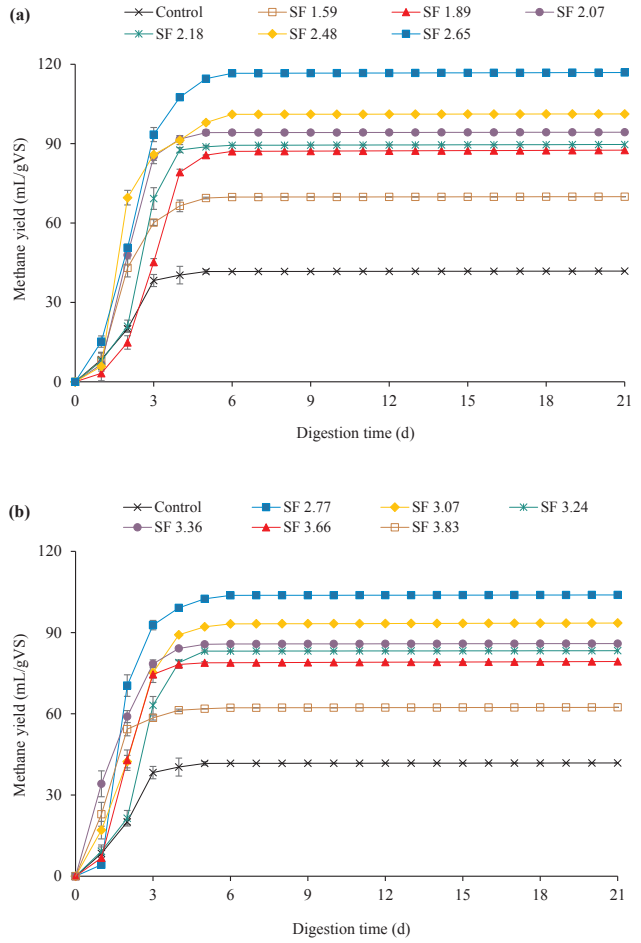

Fig. 6. Cumulative methane yield from *Sargassum* hydrothermally pretreated at (a) low and (b) high severity factors.

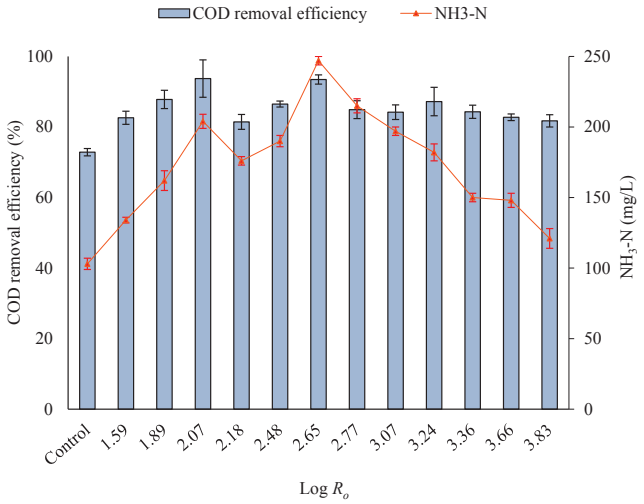

Fig. 7. Effect of anaerobic digestion on COD removal efficiency and NH_3-N formation.

Lin et al. [38] employed the modified Gompertz model to predict bioenergy production from hydrothermally pretreated *S. latissima* biomass. This technique measured the influence of various pretreatment conditions on micro-organism growth and inactivation on bio-methane productivity. The authors reported increased potential for seaweed methanation as the SF of hydrothermal pretreatment increased from 1.48 to 3.24, with peak microbial biodegradation and methane recovery expected at SF 2.65. However, at the highest SF evaluated of 3.83, the formation of recalcitrant compounds during hydrothermal solubilisation inhibited microbial biodegradation and diminished the corresponding methane potential. These results corroborate the experimental data in the present study.

Of importance, hydrothermal pretreatment reduced the H_2S emissions in biogas from 3 to 1%, relative to the unprocessed biomass. This finding is significant as it suggests that during AD, most sulfates and organic sulfur were transferred to the digestate rather than H_2S formation [58]. Proteins are the primary source of sulfur in *Sargassum* and the main contributor to H_2S production, which typically peaks during the initial stages of fermentation due to digester acidification and rapid biogas formation. Hydrothermal pretreatment helps to maintain the digester pH range at 6.8 to 7.8, thereby supporting long-term stable methanogenesis and mitigating the conversion of sulfide to H_2S which occurs at pH values below 6.8 [59].

From the perspective of industrialisation, the energy balance of hydrothermal pretreatment is crucial for full-scale implementation. To support process viability, the energy output from biogas production must outweigh the heat and electricity required to maintain the pretreatment conditions. In this study, the energy balance could not be assessed but based on previous studies, a positive correlation exists between the pretreatment SF and the energy conversion efficiency [32,38]. Despite the absence of energy analysis, it must be emphasized that the optimum pretreatment condition reached in this study is not fixed. As the SF is a function of temperature and time, similar pretreatment conditions may be achieved at lower temperatures with longer exposure times.

In future industrial applications, hydrothermal pretreatment systems equipped with waste heat recovery are recommended to reduce the high investment costs and energy input [32]. The Cambi™ process exemplifies the successful commercialisation of hydrothermal pretreatment for sewage sludge biogas production [14].

3.4. Analysis of bio-fertiliser properties

The market value of digestate intended for land-use depends on compliance with quality assurance standards and guidelines since chemical pollutants and heavy metals can withstand the AD process [3,60]. Analysis of the effluents of *Sargassum* biogas production reveal higher sCOD removal efficiency rates in the hydrothermally pretreated samples than the control sample (Fig. 7). Removal of sCOD from the unpretreated biomass was 72.85 ± 1.04% but subsequently increased when hydrothermal pretreatment was introduced prior to digestion, in a trend similar to that observed for methane production. The maximum sCOD removal efficiency of 93.72 ± 5.30% attained at SF 2.07 was a marginal improvement to the sCOD removal efficiency of 93.46 ± 1.31% achieved at SF 2.65, the optimum condition for biogas generation. Nevertheless, it must be emphasized that even though complete (100%) sCOD removal was not achieved during AD, these results are significant suggesting that most of the organic matter generated during hydrothermal solubilisation can be consumed by micro-organisms in methanogenesis. In literature, high COD removal efficiency rates of 86.7 and 86.1% have also been reported for *S. latissima* pretreated at SF 2.65 and 3.83, respectively, following AD for 11 d [38]. COD removal from the digestate is necessary to prevent soluble organic matter run-off and leaching into ground and surface waters which can cause contamination and trigger major health concerns [3,60].

After 21 d incubation period, all the pretreated samples exhibited higher ammoniacal-N levels in the liquid phase than the unpretreated feedstock. The authors observed a positive correlation between biogas production (Fig. 6) and the digestate NH_3-N content (Fig. 7), suggesting that hydrothermal pretreatment enhanced the microbial biodegradation of volatile solids during AD. Maximum NH_3-N recovery of 248 ± 7 mg/L was achieved at 2.65. Thereafter (SF ≥ 2.77), the NH_3-

Table 4
Macro-nutrient distribution in the digestate (mean ± SD).

Property/ Element	Control (untreated)				Pretreated (*SF 2.65*)			
	Liquid fraction (mg/L)	Recovery (%)	Solid fraction (mg/kg)	Recovery (%)	Liquid fraction (mg/L)	Recovery (%)	Solid fraction (mg/kg)	Recovery (%)
pH	7.67 ± 0.05	–	7.53 ± 0.02	–	7.78 ± 0.02	–	7.84 ± 0.03	–
Total- N	2697.15 ± 62.47	51.83 ± 1.29	2506.69 ± 78.52	48.17 ± 1.03	3282.06 ± 27.44	63.07 ± 1.38	1921.77 ± 62.65	36.93 ± 2.59
P	25.87 ± 0.39	3.03 ± 0.82	829.26 ± 17.99	96.97 ± 0.20	32.89 ± 0.40	3.85 ± 0.17	822.24 ± 9.95	96.15 ± 4.45
K	6039.93 ± 45.90	12.09 ± 1.11	43933.16 ± 755.65	87.91 ± 1.95	6807.22 ± 68.75	13.62 ± 2.12	43165.87 ± 483.46	86.38 ± 3.22
S	517.08 ± 9.51	4.90 ± 1.10	10032.17 ± 214.69	95.10 ± 1.31	921.28 ± 14.28	8.73 ± 1.71	9627.97 ± 152.12	91.27 ± 1.97
Na	1928.67 ± 17.17	12.95 ± 1.34	12962.02 ± 283.87	87.05 ± 2.59	2117.31 ± 24.77	14.22 ± 2.06	12773.38 ± 235.03	85.78 ± 2.30
Mg	486.83 ± 1.61	5.91 ± 0.25	7746.95 ± 127.83	94.09 ± 1.50	507.68 ± 8.43	6.17 ± 1.28	7726.10 ± 63.35	93.83 ± 0.79
Ca	361.21 ± 2.75	0.74 ± 0.11	48533.99 ± 956.10	99.26 ± 3.90	446.73 ± 5.58	0.91 ± 0.18	48448.47 ± 644.36	99.09 ± 2.26

N concentration decreased incrementally due to a high organic loading rate which restricts microbe proliferation and function in batch digester systems [6,13,46,49].

Ammonia is the foundation of fertilisers as it supplies growing crops with readily available N for utilisation. However, high concentrations of NH_3-N (1000 mg/L) prohibit the use of the digestate as bio-fertiliser due to the associated negative environmental impact. In such cases, supernatant treatment methods such as ammonia stripping, struvite precipitation and membrane filtration are recommended to reduce the NH_3 content to industrial emission standards but these technologies are expensive and energy-intensive [4,60]. In this study, the ammoniacal-N in the digested samples was below the industry limit mainly due to high feedstock water dilution, thus eliminating the need for ammonia scrubbing and rendering use of the whole digestate economically viable.

Given that *Sargassum* subjected to hydrothermal pretreatment at SF 2.65 generated the highest methane yield, the nutrient content of the resulting digestate was evaluated to determine bio-fertiliser potential (Table 4). The pH values of the solid-liquid fractions of the digested slurry (7.53–7.84) were higher than that of the raw biomass due to the biochemistry of the AD process [60]. This neutral pH renders the digestate suitable for application to a variety of crops and soil types. Further characterisation of the respective digestate fractions reveal that hydrothermal pretreatment promoted the extraction of N from the substrate into the supernatant. While nitrogen is beneficial to plants for nitrate production during photosynthesis, the low macronutrient content can limit bio-fertilizer potential. Nutrient loading the liquid fraction with P and K would produce high-quality liquid seaweed fertiliser or soil conditioner, thus expanding its marketability for greater opportunities. On the other hand, the solid fraction of the digestate retained most of the essential macronutrients from the original feedstock and can be applied to farmland as bio-fertiliser.

Raw pelagic *Sargassum* exhibits high mineralisation (Table 4) which is also preserved in the digestate after the AD process. Management of the heavy metal levels in organic fertilisers is imperative to prevent arable land pollution and eco-toxicity. World-wide, the afore-mentioned goal has been achieved through the establishment and implementation of environmental limit standards for biosolid heavy metals content in organic fertilisers. Compliance with these standards ensure that fertiliser products marketed for consumption are of a consistent high quality, safe for commercial land-use and pose zero threat to human health and biodiversity [3,4].

Hitherto, no uniform international standard exists for the maximum acceptable levels of Cr, Ni, Cu, Zn, As, Cd and Pb in organic fertilisers [60]. Accordingly, some countries have developed and imposed individualised policies to stringently regulate the concentration of heavy metals in the digestate used in national farming practices [3]. To the best of the authors' knowledge, the Caribbean region lacks soil protection legislation and framework addressing digestate quality, classification and land utilisation. Rather, these developing countries rely heavily on the importation of internationally certified fertiliser products and inevitably adopt the quality control standards imposed in the territories of the supplier. Table 5 presents country-wise examples of the acceptable heavy metal limit values set for biosolids intended as organic fertiliser.

From the data shown in Table 5, hydrothermal pretreatment has a negligible effect on heavy metals extraction from the feedstock into the liquid phase. Analysis of the solid fractions of the digestate recovered from the untreated and treated seaweeds reveal rich heavy metal content, abundant in Zn, Pb, Ni, Cu and As. Notably, the value of these biosolids as organic fertiliser is limited since the concentrations of several of the afore-mentioned elements exceed the maximum acceptable levels stipulated in some countries. Based on the heavy metal content, the effluent from the digestion of *Sargassum* would be acceptable for utilisation solely in the USA. However, the increasingly stringent environmental legislation and policies of Canada, Hong Kong,

Table 5
Comparison of heavy metals in the digestate to various international compost standards.

Digestate	Concentration of heavy metals (mean ± SD)								Units
	Ni	Cr	Cd	Cu	Zn	Pb	Hg	As	
Untreated									
Liquid fraction	0.08 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.06 ± 0.04	0.12 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	4.00 ± 0.28	mg/L
Solid fraction	34.82 ± 0.48	12.95 ± 0.16	0.77 ± 0.05	25.02 ± 0.58	105.53 ± 1.96	40.00 ± 0.57	1.36 ± 0.10	31.22 ± 0.30	mg/kg DM
Pretreated (SF 2.65)									
Liquid fraction	0.14 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.13 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	5.33 ± 0.04	mg/L
Solid fraction	34.76 ± 0.64	12.95 ± 0.15	0.78 ± 0.05	25.05 ± 0.79	105.52 ± 2.48	40.00 ± 0.77	1.35 ± 0.04	29.89 ± 0.46	mg/kg DM
	Maximum permissible concentration in soils								
Canada [1]	62	210	3	100	500	150	0.8	13	mg/kg DM
European Union [2]	25	70	0.7	70	200	45	0.4	–	mg/kg DM
Hong Kong [3]	50	100	1	300	600	100	1	10	mg/kg DM
New Zealand [4]	10	50	0.7	25	75	65	0.2	5	mg/kg DM
United Kingdom [5]	50	100	1.5	200	400	200	1	–	mg/kg DM
USA [6]	420	1200	39	1500	2800	300	17	41	mg/kg DM

[1] Ontario Regulation 394/07 [61].
[2] Saveyn H and Eder P [62].
[3] Hong Kong ORC [63].
[4] New Zealand Standard - NZS 4454 [64].
[5] BSI PAS 110 [65].
[6] USEPA Regulation CFR40/503 [66].

New Zealand and the United Kingdom would prohibit use in these countries. To improve the international appeal and marketability of *Sargassum*-derived digestate as bio-fertiliser, the Hg and As content must be reduced to acceptable levels. Remediation techniques such as immobilisation, phytoremediation and soil washing are inexpensive, eco-friendly and exist in several developed countries [67].

4. Conclusions

This study investigated the influence of hydrothermal pretreatment of pelagic *Sargassum* on biogas recovery and digestate quality. Batch testing confirmed that incorporating hydrothermal pretreatment at SF 1.59 to 3.83 prior to AD increased the degradation and solubilisation of organic components (carbohydrates and proteins) in *Sargassum* for effective and accelerated methane fermentation downstream. Peak methanation of 116.72 ± 2.14 mL/gVS was achieved at SF 2.65. Hydrothermal pretreatment also diminished the concentration of H_2S in biogas from 3% to 1%, thus mitigating challenges associated with biodigester performance and harmful odorous emissions. Nevertheless, desulfurisation of *Sargassum*-derived biogas is recommended for the safe and sustainable utilisation of this energy fuel in industry. The effluent of *Sargassum* biogas production is sterile and nutrient-dense but high Hg and As content would limit its marketability without further treatment. Heavy metal remediation can remove these phytotoxic impurities, thereby improving the digestate quality and satisfying the increasingly stringent environmental and soil protection regulations as enforced by most countries for organic fertilisers. The results of the present work offer great promise for the industrial exploitation of *Sargassum* as feedstock in the seaweed-based biorefinery concept. As such, future research should be devoted to process modelling and simulation to evaluate system design and optimisation for pilot scale study.

CRediT authorship contribution statement

Terrell M. Thompson: Methodology, Investigation, Validation, Writing - original draft. **Brent R. Young:** Supervision, Writing - review & editing. **Saeid Baroutian:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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