Semi-continuous anaerobic digestion of the marine micro-algal species *I. galbana* and *D. salina* grown under low and high sulphate conditions

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Highlights:

- Marine micro-algae successfully digested at marine salt concentrations (~33 g L^{-1})

- Stable digestion of new feedstocks at up to 4.7 g SO_4 L^{-1}, well above marine levels

- CH_4 yield at high SO_4 affected more by substrate competition than H_2S inhibition

- Batch and semi-continuous CH_4 yields similar but well below theoretical potential

- Apparent struvite and sulphur precipitation may present challenges at full scale

Keywords

Marine micro-algae, semi-continuous anaerobic digestion, inhibition, adaption, hydrogen sulphide, biogas.

Abstract

Anaerobic digestion of marine micro-algae is a necessary step for their incorporation into the future portfolio of biofuels. Digestion of marine feedstocks can pose operational issues associated with competition and toxicity to the microbial consortium. This research examined

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the marine species *Isochrysis galbana* and *Dunaliella salina* continuously cultivated in a tubular photobioreactor using a low sulphate medium; *D. salina* was also cultivated with a high sulphate medium (4.7 g SO_4 L^{-1}). Harvested micro-algal biomass was used as feedstock in semi-continuous digestion with a salt-adapted inoculum. Stable operation was achieved with reasonable specific methane production (SMP) despite a short (15-day) retention time. SMP for *I. galbana* and *D. salina* was 0.244 and 0.233 L CH_4 g^{-1} volatile solids (VS), with VS destruction 32% and 48% respectively. SMP ranged from 62-94% of the biochemical methane potential, but was only 32-49% of theoretical methane yields, indicating pre-treatments may be beneficial. Changing from low to high sulphate *D. salina* reduced the SMP to 0.193 L CH_4 g^{-1} VS with a rise in H_2S production. Under semi-continuous digestion, evidence for sulphide precipitation and oxidation was observed, which were not seen in batch analyses. This highlights the importance of conducting continuous rather than batch studies, to avoid overlooking these effects.

1   Introduction

Anaerobic digestion (AD) is a proven energetically efficient way to convert organic materials into biofuel in the form of biogas. This technology is commonly used to remediate and stabilise organic wastes, such as wastewater biosolids and agricultural residues; while recent research is focussed on utilising non-traditional biomass sources. Macro and micro-algae have frequently been suggested as substrates for the development of biofuels and of high value products that can avoid the 'food vs fuel' argument [1-4]. Currently, research into the anaerobic digestion of micro-algae, and particularly of marine species, is primarily limited to laboratory analysis using batch biochemical methane potential assays, with only a very few long-term continuous experiments undertaken [5-8]. Reported methane yields vary depending

on the algal species, operating conditions, pre-treatment methods and source of inoculum [9]. Methanogenesis from marine micro-algae and other feedstocks at marine salinities has been successfully demonstrated under batch conditions using halotolerant inoculum, but research into the effects of continuous operation on methanogenesis is also very limited [10].

If the production of algal biomass is to become widespread it is likely that cultivation will have to be undertaken within saline water bodies, as this is the only realistic option for large-scale production that limits competition with terrestrial crops for fresh water [11]. High salinities may present difficulties in the AD process, however, with inhibition and toxicity resulting from the high concentration of cations Na^+, Ca^{2+}, K^+ and Mg^{2+}. Studies on various types of feedstocks have reported a range of concentrations that result in moderate to strong inhibition and toxicity [12]. Particular attention has been given to the most abundant cation Na^+ which is often reported to be inhibitory at concentrations between 3.0 and 12.0 g Na^+ L^{-1} using non-acclimatised mesophilic consortia [12, 13]. Inhibition and toxicity reduces dramatically with the use of marine sources of inoculum, and Na^+ concentrations of 35 g L^{-1} can be tolerated in batch culture [12, 14] through gradual adaptation to high salinities. Inoculum from a non-acclimated digester, such as one used in the treatment of municipal wastewater biosolids, may have difficulty acclimatising to shock conditions under batch testing. It can, however, be adapted to continuous digestion of feedstocks at salinities in excess of marine values, with little impact on methane yields [15].

Probably the greatest concern for digestion of marine micro-algae for biogas production is the potential competition for organic electron acceptors from sulphate-reducing bacteria (SRB). The sulphate concentration in seawater is typically around 2.7 g SO_4 L^{-1} [16]. Although sulphur is required at concentrations between 1 - 25 mg S L^{-1} for healthy operation of an AD

system, at higher concentrations SRB can outcompete methanogens for acid intermediate products, thus reducing the biogas yield and energy potential [17]. SRB activity also leads to the formation of inhibitory/toxic reduced sulphur compounds, with HS^- in the aqueous phase and H_2S in both the aqueous and gaseous phases, in proportions dependent on the equilibrium conditions. Marine micro-algae may also have a relatively high biomass sulphur content compared to that of freshwater species [18], further increasing the potential for sulphide inhibition of the digestion process.

To alleviate the combined impacts of excess anions and cations (particularly SO_4^- and Na^+) and of sulphate in an AD system, washing of marine macro-algae to remove excess salts has become normal practice [19]. This is not generally an appropriate approach for micro-algae, however, as they are harvested at much lower concentrations. Centrifugation and re-suspension in fresh water is possible, as demonstrated by Santos et al. [5] who reported a 71% increase in methane production after a washing process was applied. This does, however, require high energy and fresh water inputs, which may limit its potential uptake at an industrial scale [20, 21]. An emerging area of focus in AD is thus the potential for digestion of marine species of micro-algae suspended within high salinity water media utilising salt-adapted inoculum [10, 14].

This paper reports on cultivation of two strains of marine micro-algae selected from a previous study [18], and on the assessment of their methane potential under batch conditions and in semi-continuous digestion. Gas production kinetics in batch tests were modelled using a pseudo-parallel first order equation [22], allowing estimation of the readily degradable proportion of the biomass and comparison with data from the previous study. The semi-continuous study used an inoculum adapted to marine concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+}

cations [15]. One of the species was grown under both low and high sulphate conditions to allow the impact of the sulphate to be assessed independently from that of the other ionic species. The work is thus novel with regard to the feedstocks and conditions used, and adds to the limited literature on semi-continuous digestion of marine micro-algal species.

2 Materials and methods

2.1 Feedstock

Marine micro-algal strains *Isochrysis galbana* and *Dunaliella salina* were obtained from the culture collection of the National Oceanographic Centre in Southampton, UK. Low sulphate cultures of *I. galbana* and *D. salina* were grown on Jaworski's Medium (JM) made up with tap water and additional chloride salts at (g L^{-1}) 27.4 NaCl, 3.2 MgCl$_2$·6H$_2$O, 1.51 CaCl$_2$ and 0.7 KCl to give molar ratios of 1:0.87:0.03:0.03:0.02 for Cl, Na, Mg, Ca and K, similar to mean ocean ratios [16]. For the high sulphate *D. salina* feedstock, 4.73 g L^{-1} SO$_4$ as MgSO$_4$ was added and MgCl$_2$·6H$_2$O reduced accordingly to maintain the Mg concentration.

The micro-algal feedstocks were continuously cultivated in a tubular photobioreactor (PBR) with a working volume of ~320 L located in a greenhouse at the University of Southampton, UK (50.934 N, 1.398 W) and provided with additional artificial illumination consisting of 480 W of 3500 K cool white fluorescent tubing (Figure 1). Culture temperature was regulated at approximately 25 $^{\circ}$C by a thermocirculator with a heat exchanger in the riser. Low sulphate *I. galbana* was cultivated over a 21-day period in October and November, while *D. salina* was cultivated at low sulphate concentrations in March and April (32 days) and at high sulphate concentrations in April and May (14 days). Average horizontal irradiance monitored

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at a University site in these periods using a Sensol mono-crystalline silicon irradiance sensor (IKS Photovoltaik, Germany) was 66.8, 124.3 and 174.4 W m^{-2}, respectively. These values were between 6-10% lower than the estimated long-term averages for these periods obtained from the Joint Research Council's PVGIS site [23].

Each cultivation run was inoculated with 160 L of a laboratory-grown pure culture of the relevant micro-algae, which was pumped into the PBR and topped up with fresh culture medium. The reactor was operated in batch mode for two days before continuous feeding with the relevant culture medium began at 70 L day^{-1}. Samples of the culture were monitored for total suspended solids (TSS) content and observed under the microscope to determine whether contamination had occurred. The collected effluent was centrifuged and the resulting algal paste and supernatant were frozen at -17 ˚C until required. *I. galbana* was harvested using a Powerfuge Pilot continuous centrifuge (CARR Centritech) and the *D. salina* cultures were harvested using a milk creamer disk stack continuous centrifuge.
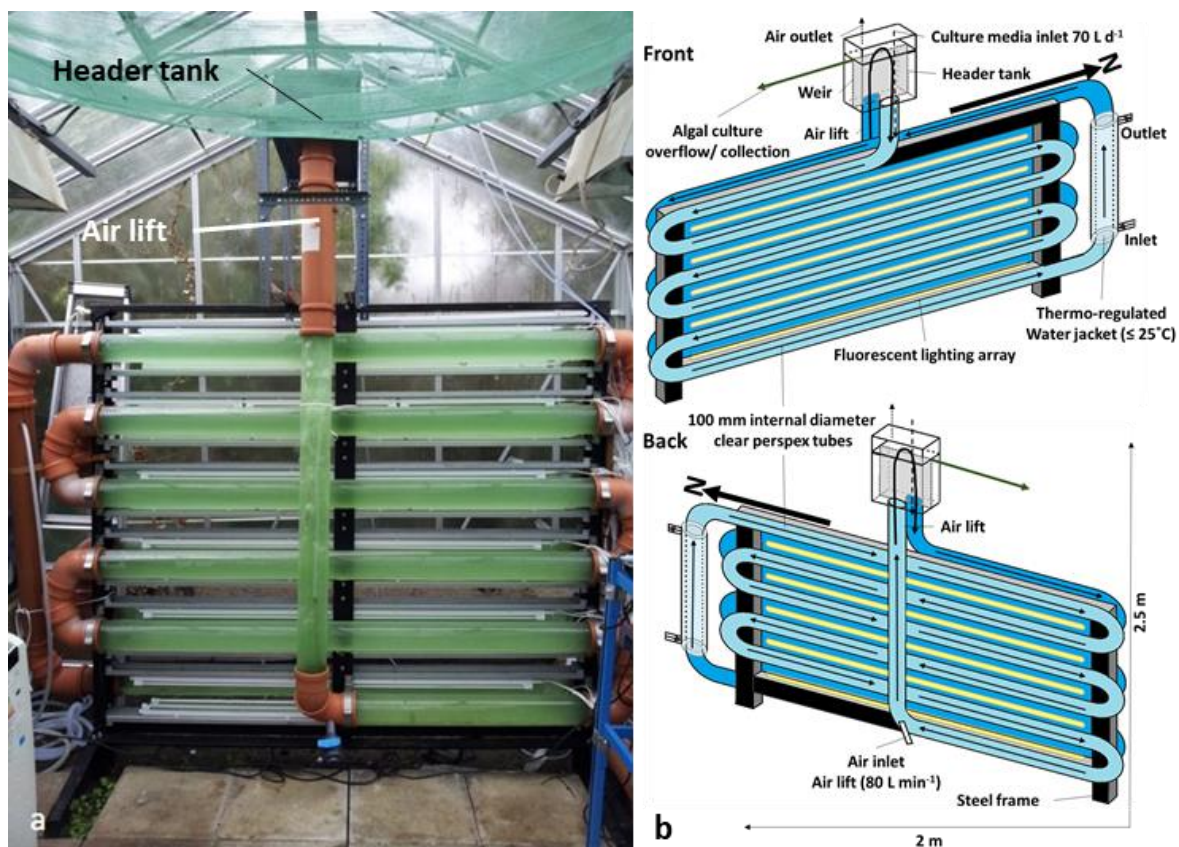
Figure 1 Photobioreactor: (a) start-up of algal cultivation, (b) schematic showing airlift, cooling jacket, lighting arrays, culture media addition and collection point and header tank.

2.2 Semi-continuous digestion

The work was carried out in 6 continuously-stirred digesters constructed from PVC tube, each with a working volume of 0.5 L. The top plate had a gas outlet, an access port sealed with a rubber bung, and a draught tube liquid seal providing access for an asymmetric bar stirrer driven by a 40 rpm motor. Digester temperature was maintained at $37 \pm 0.5\ ^{\circ}C$ in a thermostatically-controlled water bath. Feedstock addition and digestate removal was carried out daily via the access port, using a 50 mL syringe. Biogas was collected in a gas-impermeable bag and the daily volume was measured using a weight-type water displacement gasometer [24]. All gas volumes reported are for dry biogas (i.e. without water vapour) at standard temperature and pressure (STP) of 0 °C and 101.325 kPa.

The inoculum used was obtained from digesters that had previously been fed on a synthetic substrate and acclimatised to marine concentrations of chloride salts, as described in Roberts et al. [15]. The digesters were fed on the original synthetic substrate for the first five days of operation, then switched to the algal feedstocks. These were prepared by re-suspending the harvested algal paste in the medium to a known solids content (Table 1); this differed between the species depending on the mass harvested, to ensure that the system could be run for at least three hydraulic retention times (HRTs). 33 mL of feedstock was added daily to each digester, after removal of an equivalent amount to maintain the working volume, giving a HRT of 15 days. Two digesters were initially fed on *I. galbana*, and the other four on low sulphate *D. salina*. On day 51, the feed to one pair of digesters was switched from low sulphate *D. salina* to *D. salina* grown in high sulphate conditions (*D. salina* SO_4). On day 62, feeding of one *I. galbana* digester was stopped to conserve the available feedstock and extend the running period for the remaining digester. Feeding of this digester was stopped on day 71. All continuous digestion experiments were thus carried out in duplicate apart from the last 10 days of operation on *I. galbana*.

2.3   Biochemical methane potential (BMP)

This assay was carried out in 0.5-L digesters which were mixed manually once per day. Inoculum was taken from a mesophilic digester treating municipal wastewater biosolids (Millbrook, Southampton, UK). The inoculum-to-substrate ratio used was approximately 4.5:1 on a volatile solids (VS) basis. Tests were carried out at 37 ± 1 ˚C in duplicate against blank controls with no substrate added and against a positive cellulose control (C6288, Sigma-Aldrich Ltd, UK). Biogas was collected in 1-L cylinders using a 75% sodium chloride

barrier solution adjusted to pH 2 with sulphuric acid to minimise losses of CH_4 through dissolution. Biogas composition was analysed each time the collection cylinder was emptied. The volume of methane was calculated by multiplying the dry biogas volume by the measured methane percentage, corrected so that $\%CH_4$ plus $\%CO_2 = 100\%$ to take account of the initial headspace contents. The BMP for a given test substrate was determined by calculating the cumulative volume of methane produced from each test digester; subtracting the average cumulative STP methane production from the inoculum-only controls; and dividing the result by the weight of substrate VS added to each test digester. The average value in L CH_4 g^{-1} VS for all test digesters fed on a given substrate was taken as the final BMP value.

2.4   Analytical methods

Total suspended solids (TSS) and total and volatile solids (TS and VS) were measured using Standard Methods 2540 D and G, respectively [25]. pH was measured using a FE20/EL20 pH meter (Mettler Toledo, UK) with a combination glass electrode calibrated in buffers at pH 7.0 and 9.2 (Fisher Scientific, UK). Alkalinity was determined by titration with a 0.25 N H_2SO_4 solution to endpoints of 5.7 and 4.3 to allow determination of total (TA), partial (PA) and intermediate alkalinity (IA) [26]. Total Kjeldahl Nitrogen (TKN) and Total Ammonia Nitrogen (TAN) were determined using a Kjeltech digestion block and a Büchi steam distillation unit, according to the manufacturers' instructions. Volatile fatty acid (VFA) concentrations were measured using a Shimadzu 2010 GC. Elemental composition was determined using a FlashEA 1112 Elemental Analyzer (Thermo Finnigan, Italy), with methionine, l-cystine, pasta, basil leaf and sulphanilamide as standards and vanadium pentoxide added as a catalyst for sulphur determination. Biogas composition was determined

using a Varian CP-3400 gas chromatograph (GC) with a mixed gas standard of 65% CH_4 and

35% CO_2 (v/v) for calibration (BOC, UK). H_2S gas was analysed using a H_2S-AE sensor

(Alphasense Ltd, UK) at a flow of 500 mL min^{-1}. The sensor was calibrated with a gas

mixture containing 404 ppm H_2S, 35.19% CO_2, and the balance CH_4 (SIP Analytical Ltd,

UK).

Analytical determinations for feedstock properties were carried out in triplicate unless noted.

Analysis of digestate and biogas was carried out approximately weekly on single samples

from duplicate digesters without measurement replicates.

2.5 Calculation of theoretical methane potential and calorific value, soluble H_2S, kinetic biodegradability coefficients and sulphur removal rates

Theoretical methane potential (TMP) was calculated using the Buswell equation [27], with

elemental composition data from direct measurement of C, H, N and S, and O obtained by

difference, on a %VS basis. The theoretical calorific value (TCV) of the feedstocks was

calculated according to the Boie equation [28]. The higher heat value (HHV) of CH_4 was

taken as 39.84 MJ m^{-3} at STP.

The kinetics of the BMP test were modelled using the pseudo-parallel first order model

shown in equation 1, where Y (L CH_4 g^{-1} VS) is the specific methane production at time t

(day), Y_{max} is the measured or estimated ultimate methane yield (L CH_4 g^{-1} VS), k_1 is the first

order rate constant (day^{-1}) for readily biodegradable material, k_2 is the first order rate constant

(day^{-1}) for less readily biodegradable material, and P is the proportion of readily

biodegradable material [22].

$$Y = Y_{max}(1 - Pe^{-k1} - (1 - P)e^{-k2})$$ (1)

Soluble H_2S was calculated using Henry's Law based on the measured headspace H_2S concentration. The un-ionised fraction of H_2S was determined according to Standard Method 4500-S^{2-} H [25], with the total soluble sulphide fraction presumed to consist of H_2S and HS^- due to the operational pH range within the digesters.

Sulphur removal rates as sulphide were calculated using equation 2, where %S_{rem} is the percentage removal rate of sulphur as sulphide, $S_{(in)}$ is the mass (g) of organic and inorganic sulphur entering the reactor and $S_{(out)}$ is the calculated mass of sulphur exiting the reactor as sulphide in both the aqueous ($H_2S_{(aq)}$ and HS^-) and gas (H_2S) phases.

$$\%S_{rem} = \frac{100 \; x \; S_{(out)}}{S_{(in)}}$$ (2)

3 Results and discussion

3.1 Algal cultivation

Figure 2a shows the TSS content in the PBR during the cultivation periods. The greatest overall yield came from *D. salina* SO_4 grown in the spring and early summer when irradiances were highest. TSS concentration in this period reached 0.62 g L^{-1}, almost double that for *I. galbana* at the same number of days after inoculation. The run with *D. salina* produced similar TSS concentrations to those for *I. galbana,* possibly due to similar irradiance levels for the periods in which they were cultured (data not shown). After the sharp

initial increase in TSS there was a continuing trend of TSS accumulation indicating that slightly higher dilution rates could have been achieved for all species.
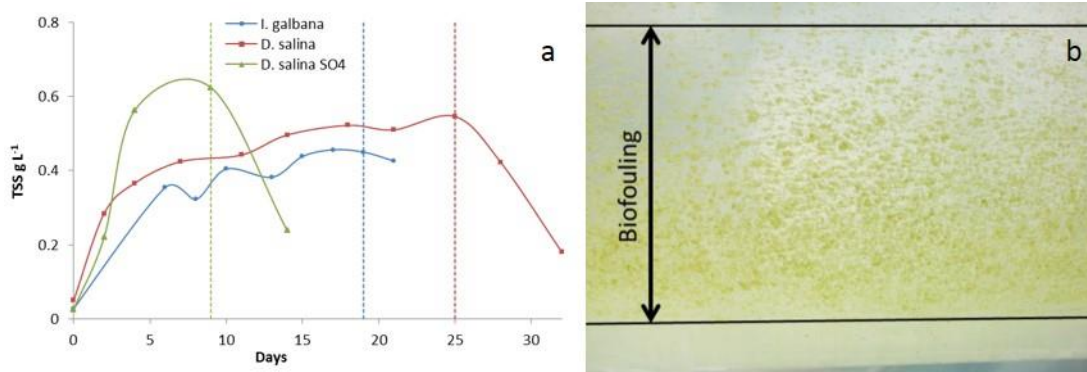

Figure 2 (a) Culture total suspended solids (n=1) content during cultivation of *I. galbana*, *D. salina* and *D. salina* SO_4. Vertical dotted lines indicate the occurrence of significant biofouling. (b) Biofouling on lower part of horizontal photobioreactor tube during cultivation of *I. galbana*.

During each cultivation run, there was some contamination and biofouling within the PBR, with the latter leading to reductions in TSS content (Figure 2a) and thus in growth yield. This biofouling occurred particularly in the lower third of the Perspex tube (Figure 2b), where scouring by the plastic 'followers' (small particles with a density close to that of water, used to clean the inner surface of the tube) was less effective. *I. galbana* exhibited minor biofouling within 15 days, with invasive pennate micro-algae observed from day 19-21. *D. salina* remained a pure culture for longer, exhibiting biofouling within 10 days, which appeared to become growth limiting after 25 days, and with an invasive pennate micro-algae observed after 31 days. Biofouling of *D. salina* SO_4 began within five days. On day 12 of the run with *D. salina* SO_4 the tubing that supplied fresh culture medium failed, resulting in a reduction in volume due to evaporative loss, leading to reduced mixing and aeration and

allowing the temperature and dissolved oxygen concentrations within the PBR to become

potentially toxic to micro-algae.

3.2   Feedstock characteristics

The characteristics of the three algal feedstocks as used in the BMP test, i.e. in the form of an

algal paste after centrifugation, are shown in Table 1.

Table 1 Characteristics of algal paste

Parameter	Unit	*I. galbana*			*D. salina.*			*D. salina* SO_4			*I. galbana* [a]	*D. salina* [a]
TS	% WW	33.3	±	0.3	32.7	±	0.3	28.5	±	0.3	-	-
VS	% TS	56.3	±	0.1	77.8	±	0.4	80.5	±	0.2	67.2	50.4
TKN	% VS	6.1	±	0.1	6.4	±	0.0	9.6	±	0.0	6.61	6.10
CV [b]	MJ kg^{-1} VS	23.0	±	0.6	23.6	±	1.0	24.0	±	0.2	23.4	20.0
Elemental composition												
C	% VS	51.2	±	0.4	52.5	±	0.4	55.1	±	1.2	52.9	43.7
H	% VS	6.5	±	0.2	7.0	±	0.2	7.0	±	0.20	7.9	8.0
N	% VS	5.9	±	0.1	6.0	±	0.1	8.8	±	0.1	6.7	6.9
S	% VS	0.3	±	0.0	0.9	±	0.0	2.4	±	0.0	4.0	5.5
C/N [d]	-	8.5			8.2			5.7			8.0	7.2

TS = total solids, VS = volatile solids, TKN = total Kjeldahl Nitrogen, CV = calorific value, WW = wet weight

Data are shown as average ± SD, n = 3

[a] Values for batch cultivation of the same algal cultures reported in [18].

[b] Measured Higher Heat Value (HHV)

[d] C/N ratio calculated using TKN on a VS basis

VS as a percentage of TS varied considerably, in part due to the different moisture contents of the samples. If the VS content is corrected to allow for the estimated quantity of salts present in the liquid fraction, the revised average VS contents are 60.2 ± 0.0, 83.4 ± 0.4 and 87.8 ± 0.4 %TS for *I. galbana*, *D. salina* and *D.salina* SO_4 respectively. The two *D. salina* samples had similar carbon and hydrogen contents, but the high sulphate *D. salina* SO_4 contained more nitrogen, possibly indicating a higher protein content. The C/N ratio for all samples was outside the favourable range for anaerobic digestion of between 20 – 40:1 [12], and was similar to those reported in the literature for other algal cultures [3]. Theoretical calorific values (HHV) were 21.9, 23.4 and 25.5 MJ kg^{-1} VS for *I. galbana*, *D. salina* and *D. salina* SO_4, respectively, showing reasonable agreement with measured CV and thus providing support for the elemental composition data.

The low sulphate species had sulphur contents around 0.4 - 0.9 %VS, while the sulphur content of the *D. salina* SO_4 was 2.4 %VS. These values were markedly lower than those previously found for *I. galbana* and *D. salina* cultivated under batch conditions using the same medium [18], although the sulphur content of the *D. salina* SO_4 was within the range of 1.8 – 5.5 %VS found for marine species. This difference may have been due to culture conditions. Harvesting of the algae during the exponential phase rather than the stationary phase of growth could affect the composition, with the micro-algae reducing reproduction and protein synthesis in favour of carbohydrate and lipid storage [29-31].

After dilution of the algal paste the average feedstock VS concentrations for the semi-continuous digestion trial were 15.5 ± 0.0, 23.5 ± 0.0 and 28.1 ± 0.2 g VS kg^{-1} WW, corresponding to organic loading rates (OLR) of 1.03, 1.57 and 1.87 g VS L^{-1} day^{-1} for *I. galbana*, *D. salina* and *D. salina* SO_4 respectively at the chosen HRT of 15 days.

3.2.1 BMP test results

The BMP test ran for 87 days. The cellulose control gave a methane yield of 0.428 ± 0.003 L CH_4 g^{-1} VS added, close to the expected value and indicating a healthy inoculum. Results for the test samples are shown in Table 2 and Figure 3. The final value for *I. galbana* was taken as 0.315 L CH_4 g^{-1} VS: this appeared to be still rising slightly, but the increase over a 5-day period was less than 0.6% of the total methane production at the end of the test. The values for *D. salina* and *D. salina* SO_4 were 0.248 and 0.290 L CH_4 g^{-1} VS respectively and these appeared to have reached a final plateau. The kinetic coefficients obtained by modelling were similar for the two *D. salina* samples, indicating similar degradation kinetics, although the higher sulphate sample had slightly higher values for *P*, the proportion of readily degradable material and for k_2, the decay coefficient for the less degradable fraction. In contrast the *I. galbana* sample had a lower proportion of readily degradable material and a higher value of k_1, the coefficient of the more degradable fraction. *D. salina* and *D. salina* SO_4 had produced 92% and 97% of their maximum CH_4 yield respectively by day 15 of the BMP test, whereas *I. galbana* had produced only 84% suggesting that this substrate might perform less well in semi-continuous digestion at shorter HRT.

Table 2 Experimental BMP values and kinetic constants obtained from modelling

Species	Measured BMP [a]			Y_{max}	P	k_1	k_2	R^2 [b]
	L CH_4 g^{-1} VS			L CH_4 g^{-1} VS		day^{-1}	day^{-1}	
I. galbana	0.315	$\pm$	0.005	0.310	0.61	1.42	0.07	0.998
D. salina	0.248	$\pm$	0.000	0.250	0.80	0.92	0.06	0.997
D. salina SO_4	0.290	$\pm$	0.001	0.290	0.87	0.92	0.08	0.994

BMP = Biochemical Methane Potential, Y_{max} = estimated ultimate methane yield, k_1 = 1st-order rate

constant for readily biodegradable material, k_2 = 1st-order rate constant for less readily biodegradable

material, P = Proportion of readily biodegradable material

[a] Measured BMP values are shown as average ± range, n = 2

[b] R^2 value indicates correlation between experimental and modelled data
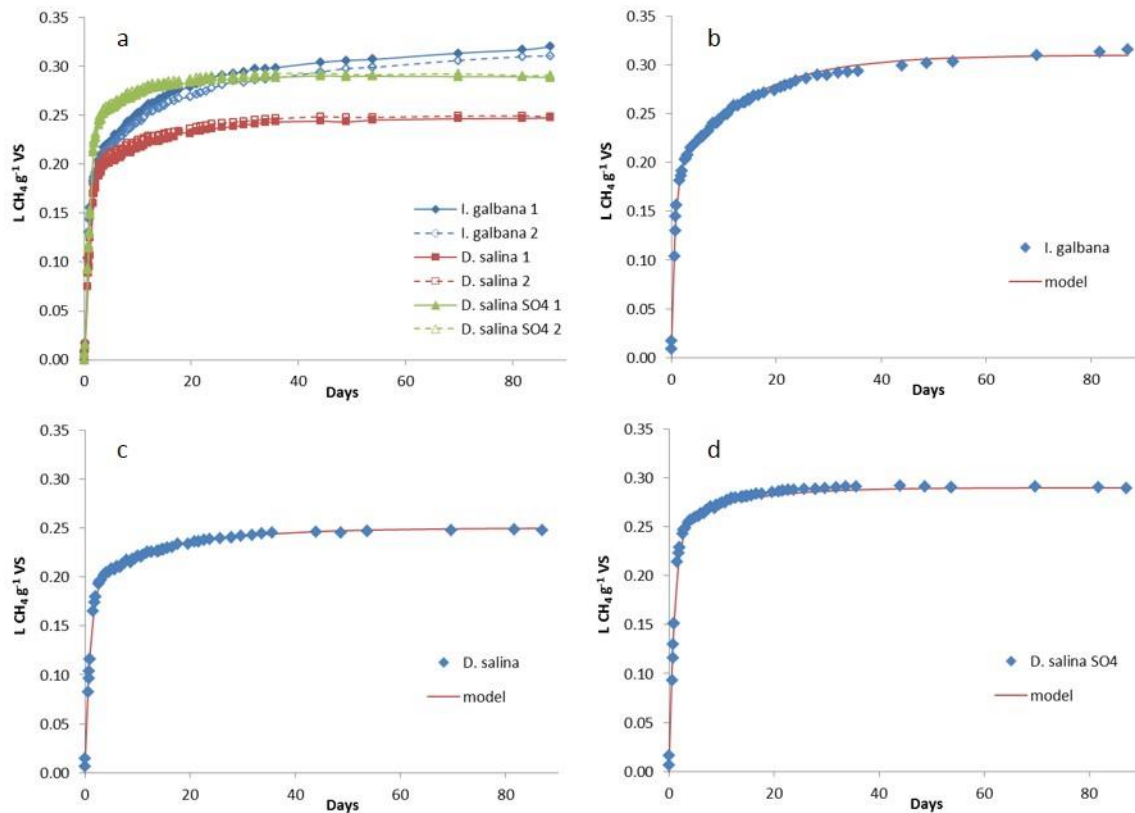

Figure 3 Cumulative net specific methane yield of marine micro-algae showing (a)

experimental data for duplicates (n=2) for each species; and kinetic models with average

values of duplicates for (b) *I. galbana*, (c) *D. salina* and (d) *D. salina* SO4.

Values from kinetic modelling showed broadly similar patterns to those found in previous

laboratory growth trials with the same species, but also some differences. Roberts et al. [18]

reported specific methane yields of 0.349 and 0.276 L CH_4 g^{-1} VS for *I. galbana* and *D.

salina* cultivated in batch mode in 20-L containers and harvested with a continuous

centrifuge, which are around 10% higher than in the current study. The kinetic coefficients were P 0.71 and 0.89; k_1 1.93 and 1.36; k_1 0.15 and 0.13 for *I. galbana* and *D. salina*, respectively. BMP values and kinetic coefficients in the current trial (Table 2) were consistently lower, by similar ratios for each species, suggesting that these values reflect genuine differences with respect to the relative degradability of different fractions. One factor contributing to these differences may be the cultivation methods used in each case: Roberts et al. [18] noted that samples from larger-scale algal cultivation systems showed more recalcitrance to anaerobic degradation, and suggested this could be due to factors such as exposure to turbulence-induced shear stresses. The relatively low BMP value for the current sample of *I. galbana* in particular may also reflect the less favourable time of year for cultivation in comparison with the two *D. salina* samples. Elsewhere in the literature, BMP values of 0.204 and 0.323 L CH_4 g^{-1} VS have been reported for *D. salina* [14, 32] and between 0.009 and 0.408 L CH_4 g^{-1} VS for *Isochrysis* sp. [5, 33].

The samples in this study, and also those in Roberts et al. [18], were frozen for storage before use in the BMP assay. Freezing itself is a form of pre-treatment that can affect biogas productivity [34, 35]. For material grown in large-scale systems, however, this change may be small, especially in relation to the effects of other types of pre-treatment aimed at enhancing biogas production [36]. Although not ideal, freezing has been practiced in many studies of this type [10, 37-40] to allow storage and homogenisation of material.

Taken together these results tend to support the growing body of evidence that, while a component of the BMP value may depend on the algal species, a significant proportion of the variability between reported results is due to factors such as growth and storage conditions. While BMP tests provide a useful indication of the potential of a particular sample, the result

is not an absolute value for that species, or even a particularly robust one unless information on all growth and assay conditions is available. Analysis of kinetic coefficients may, however, provide additional insight into the relative biodegradability of different species.

3.3   Semi-continuous digestion trial

3.3.1 Biogas production

Volumetric biogas production (VBP) in all digesters stabilised quite rapidly after the change to algal feedstocks on day 6 (Figure 4a), reaching average values after 3 HRT of around 0.62 and 0.39 L L^{-1} day^{-1} for *D. salina* and *I. galbana* respectively, with the lower value for *I. galbana* partially reflecting the lower OLR. Specific methane production (SMP) also stabilised rapidly (Figure 4b), and reached average values of 0.237 and 0.247 L CH_4 g^{-1} VS, respectively. Biogas methane content for *I. galbana* was significantly higher at ~65% compared to ~60% for *D. salina* (Figure 4c), possibly indicating a slightly higher lipid content in the former [41].

The change of feedstock to *D. salina* SO_4 in one pair of *D. salina* digesters on day 51 produced a slight reduction in VBP (Figure 4a), despite the small increase in OLR from 1.57 to 1.87 g VS L^{-1} day^{-1}. The SMP fell immediately, and continued to decline until around day 76 after which it stabilised at an average of 0.180 L CH_4 g^{-1} VS (Figure 4b). Meanwhile the VBP in the *D. salina* digesters had also fallen slightly to 0.58 L L^{-1} day^{-1} but the SMP remained close to its previous values, averaging 0.230 L CH_4 g^{-1} VS. Biogas methane content in the *D. salina* SO_4 digesters stabilised at around 62%, around 1% higher than the final value for *D. salina* (Figure 4c).
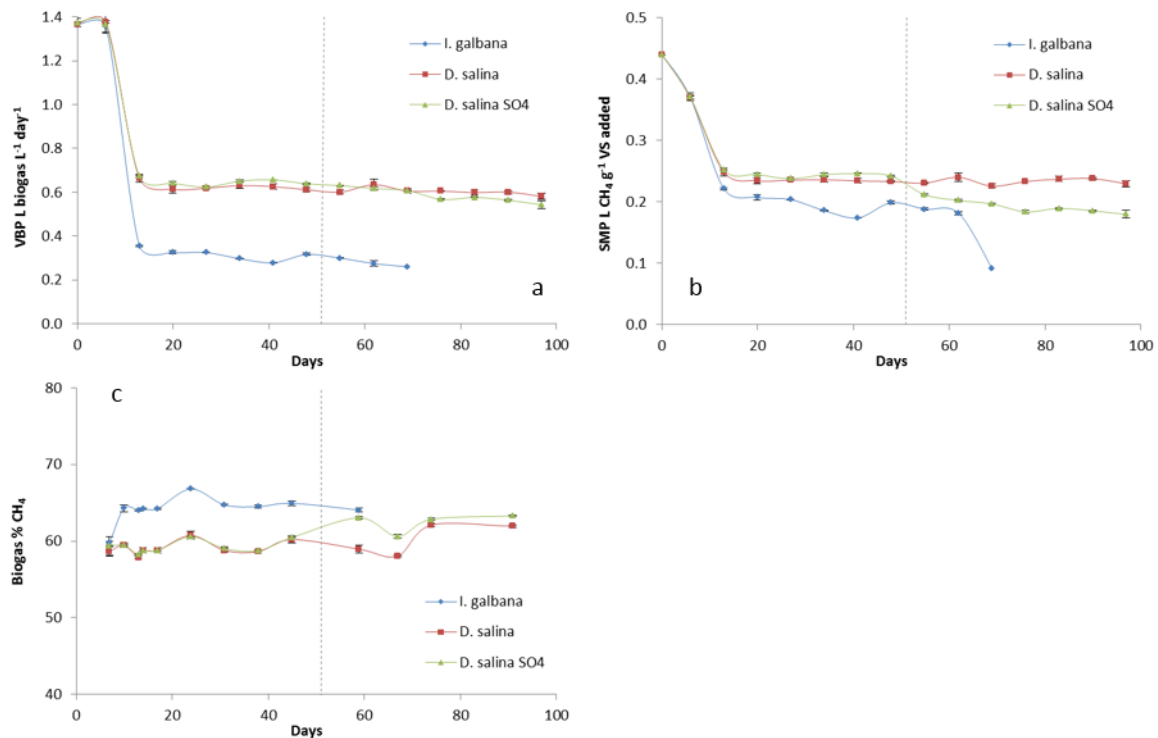
Figure 4 Weekly average values of (a) volumetric biogas production (VBP), (b) specific methane production (SMP), (c) biogas methane content (%CH$_4$) during the experimental period. Vertical line indicates switch to *D. salina* SO$_4$ feedstock in one pair of digesters on day 51. Points show average for duplicate digesters (n=2), error bars show range.

3.3.2 Sulphate and sulphide

Figure 5a shows the biogas H$_2$S content during the experimental run. For the low sulphate algal substrates *I. galbana* and *D. salina*, H$_2$S remained below 500 ppmv, equivalent to a specific production of <0.2 mL H$_2$S g^{-1} VS added (Figure 5b). The switch to *D. salina* SO$_4$ caused a rapid increase in H$_2$S production, which stabilised within 3 HRT at around 22000 ppmv, equivalent to ~7 mL H$_2$S g^{-1} VS.

Calculated values for dissolved H$_2$S follow the same trend as biogas H$_2$S content, but in the first 2-3 weeks of operation the proportion of HS$^-$ also rose due to changes in digester pH. Total soluble sulphide calculated from headspace composition for the low sulphate substrates

thus initially showed a small increase (Figure 5c), before stabilising at around 9-10 mg S L^{-1}.

For the *D. salina* SO$_4$ the calculated value for H$_2$S$_{(aq)}$ increased to around 70 mg L^{-1}, and total

sulphide increased to 1.1 g S L^{-1} after 3 HRT (Figure 5c). The increase in sulphide is likely to

be due to the growth of sulphate-reducing bacteria (SRB) stimulated by the gradual washing-
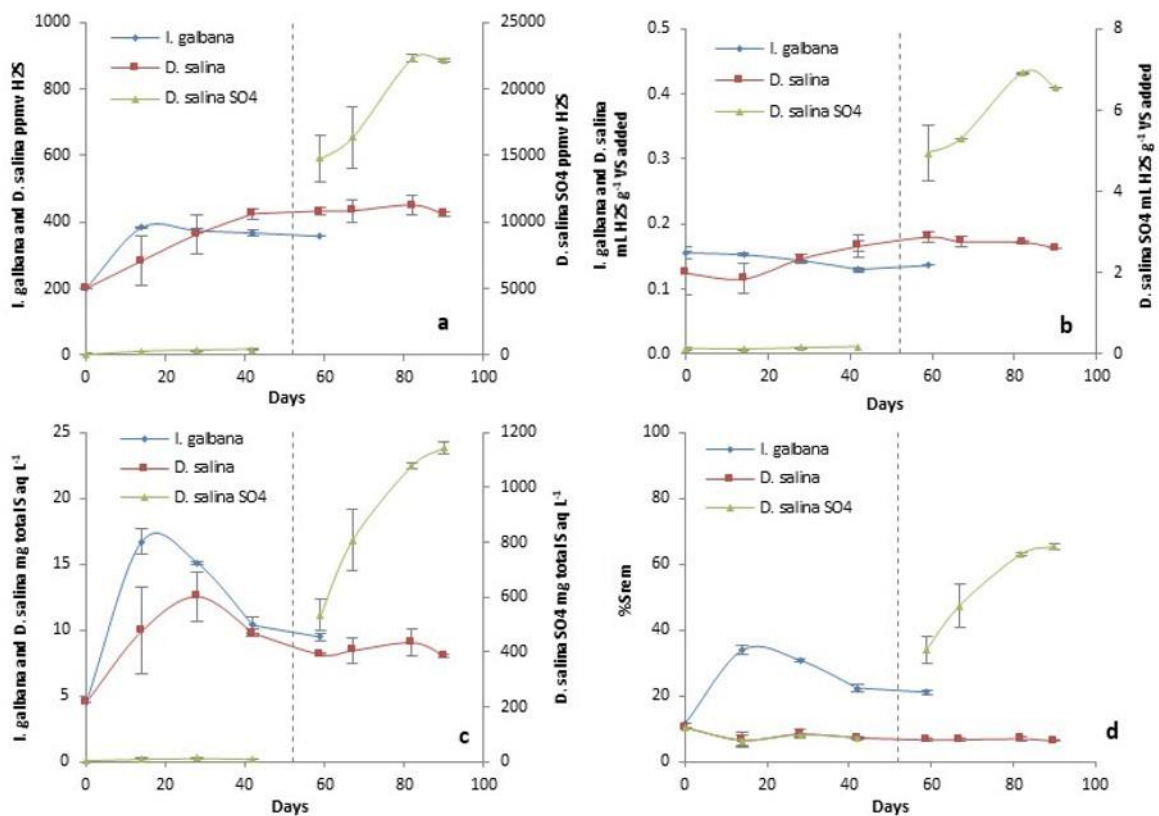
in of sulphate in the feedstock, which stabilised when it reached equilibrium.

Figure 5 (a) H$_2$S as parts per million by volume (ppmv), (b) mL H$_2$S g^{-1} VS, (b) mg L^{-1}

soluble sulphide, (d) sulphur removal rate %S$_{rem}$. Points show average values for duplicate

digesters (n=2), error bars show range. Vertical dashed line shows introduction of *D. salina*

SO$_4$.

Figure 5d shows %S$_{rem}$, i.e. the proportion of sulphur leaving the reactor in the gas and liquid

phase, calculated from the headspace H$_2$S content, as a percentage of that entering in the

feedstock as biomass or sulphate. The %S$_{rem}$ value for *I. galbana* after three HRT was 21 %.

The low sulphur *D. salina* reactors had relatively stable %S_{rem} values of 6-8% suggesting the reactors were in steady state with regard to sulphur breakdown, while for *D. salina* SO$_4$ %S_{rem} appeared to stabilise at around 65%.

3.3.3 Operational parameters

TAN concentrations in all digesters declined from day 6 onwards (Figure 6a), reflecting the new feedstock properties. After 3 HRT values appeared to have stabilised at around 0.7 and 1.0 g N L^{-1} for *I. galbana* and *D. salina*, respectively. Following the switch to *D. salina* SO$_4$ on day 51 the TAN concentration in this pair of digesters rose to 1.4 g N L^{-1}; while in the other two pairs the TAN concentrations at the end of the run had fallen by around 0.1 g N L^{-1} from their previous values. pH values also fell, stabilising at around 7.4 and 7.2 for *I. galbana* and *D. salina*, and rising to 7.7 after the change to *D. salina* SO$_4$ (Figure 6b). The observed increase in pH from 7.5 to 7.7 in the high sulphate *D. salina* SO$_4$ reactors (Figure 6b) would alter the speciation of sulphide, enabling one order of magnitude more HS$^-$ than H$_2$S to remain dissolved in the digestate.

Trends in TA and PA reflected the changes in TAN concentration, with PA values around 70% of TA, although there was some variation between duplicate digesters during the first 3 HRT (Figure 6c and d). Average TA values at the end of the run were 4.2, 6.6 and 9.4 g CaCO$_3$ L^{-1} for *I. galbana*, *D. salina* and *D. salina* SO$_4$ respectively. Average IA values for *I. galbana* remained fairly stable at around 2 g CaCO$_3$ L^{-1}, while for *D. salina* IA fell to around 1 g CaCO$_3$ L^{-1} and for *D. salina* SO$_4$ it rose to over 2.5 CaCO$_3$ L^{-1} by the end of the run (Figure 6e). These variations led to some fluctuations in the IA/PA ratio, but no strong trends. By the end of the run IA/PA values had settled at around 0.44 for *I. galbana* and *D. salina* and 0.38 for *D. salina* SO$_4$ (Figure 6f), indicating stable operation in all cases. Total VFA

concentrations in all reactors fell rapidly at the start of the run (Figure 6g), stabilising below 40 mg COD L^{-1}. The change in feedstock to *D. salina* SO$_4$ led to an increase in total VFA from day 60, but only to around 200 mg COD L^{-1}, and this subsequently declined to around 60 mg COD L^{-1} by the end of the run. The main component in this transient VFA peak was propionic acid, with a maximum concentration of 130 mg L^{-1}.

Digestate TS and VS content stabilised rapidly after the introduction of the algal feedstocks (Figure 6h). VS content was similar in all digesters at between 1.0-1.3% of wet weight (WW) from day 14 onwards. Differences in digestate TS content and in VS/TS ratio (Figure 6i) reflect the degree of dilution of the algal paste in the saline medium used to re-suspend it, as well as the original composition of the paste and the degree of solids destruction. By the end of the run, VS destruction calculated from comparison of the VS contents of the feed and digestate was around 32.0% for *I. galbana*, 47.8% for *D. salina* and 53.4% for *D. salina* SO$_4$ (Figure 6j). Estimation of VS destruction based on the dry weight of biogas produced gave values of around 36.9, 43.8 and 34.6%, respectively. The discrepancy between these values in the case of *D. salina* SO$_4$ is discussed below.
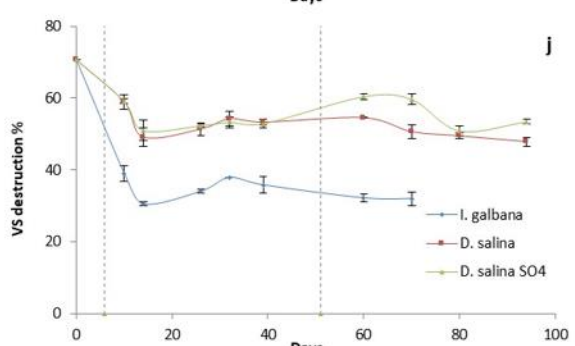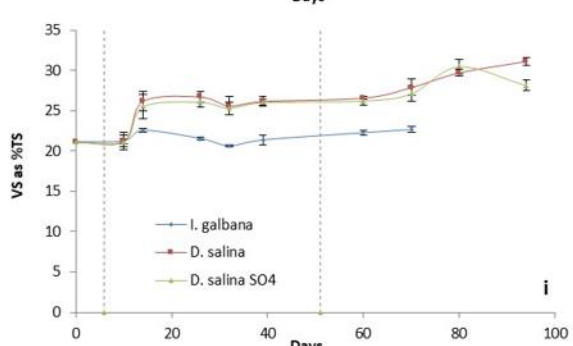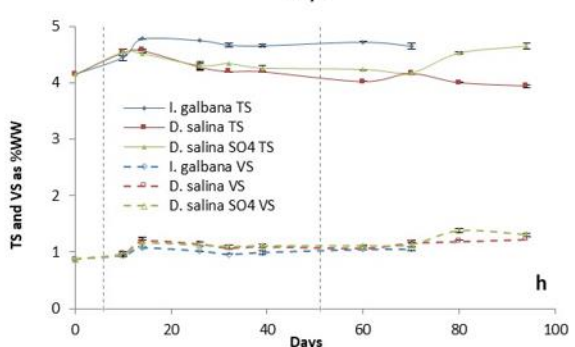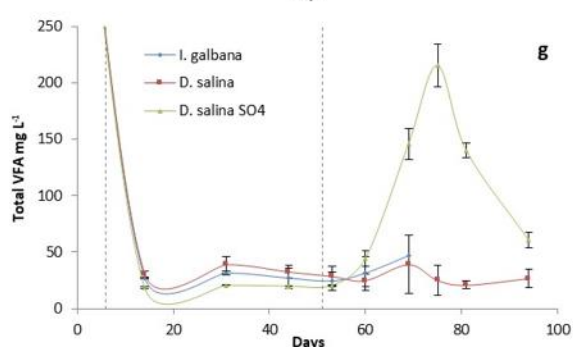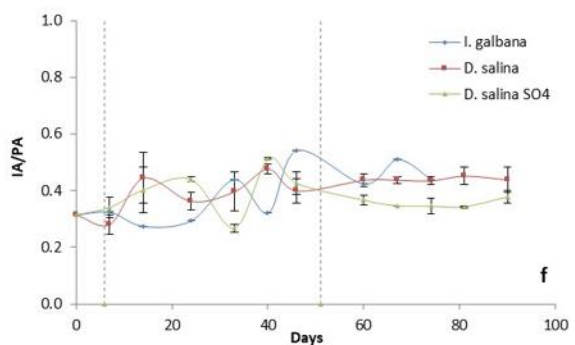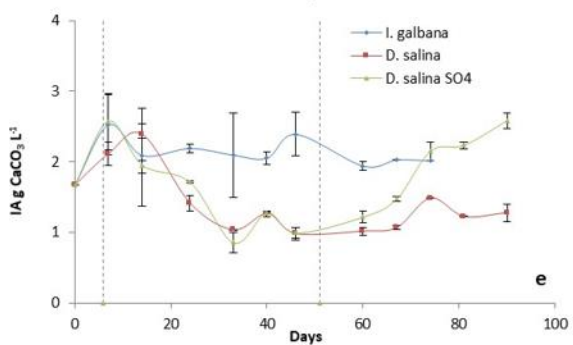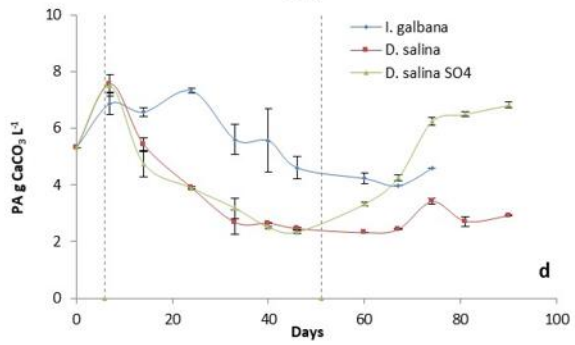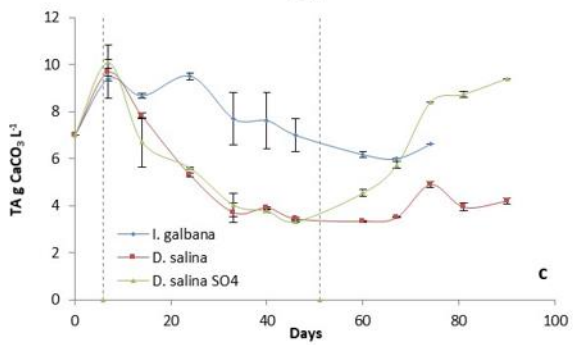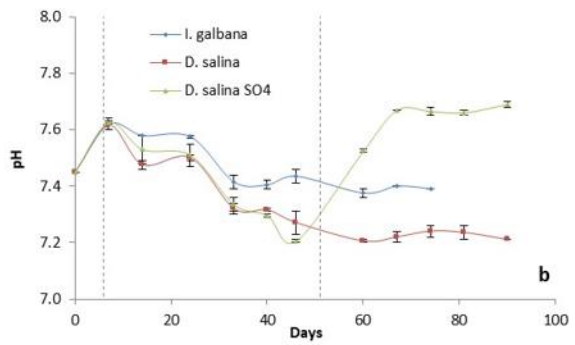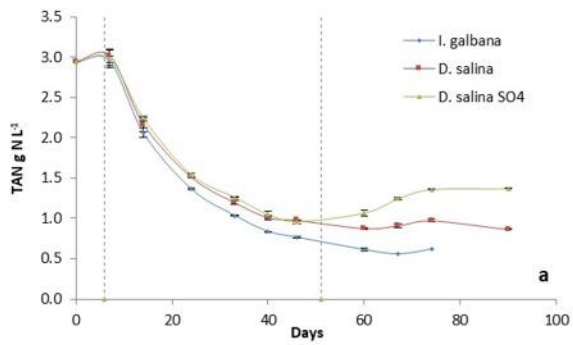
Figure 6 (a) Total ammonia nitrogen (TAN), (b) pH, (c) total alkalinity (TA), (d) partial alkalinity (PA), (e) intermediate alkalinity (IA), (f) IA/PA, (g) total volatile fatty acids (VFA), (h) total solids (TS) and volatile solids (VS) content, (i) VS/TS and (j) VS destruction. Points show average values for duplicate digesters (n=2), error bars show range. Vertical dashed lines indicate introduction of *I. galbana* and *D. salina* on day 5 and *D. salina* SO$_4$ on day 51.

3.3.4 Residual biogas potential

Residual biogas potential (RBP) was measured in-situ after continuous operation had ended. The duplicate reactors for *D. salina* and *D. salina* SO$_4$ showed reasonably good agreement, with average values respectively of 0.009 and 0.016 L biogas g^{-1} of total VS added during the run, or 0.41 and 0.64 L biogas g^{-1} VS L day based on the final applied OLR. These values appear to reflect the lower specific gas production and VS destruction of the *D. salina* SO$_4$ observed during continuous operation. Cumulative residual gas production for these reactors plateaued around 60 days after the end of feeding. The agreement between values for each of the two *I. galbana* reactors was less good, probably due to the different end dates and the shorter overall running time (3.7 and 4.5 HRT respectively). Gas production from these reactors took more than 100 days to plateau after the feeding ended, however, confirming the initial view based on the BMP results that this material is slow to degrade.

3.3.5 Energy considerations

TMP values predicted from the Buswell equation were 0.494, 0.527 and 0.557 L CH$_4$ g^{-1} VS for *I. galbana*, *D. salina* and *D. salina* SO$_4$ respectively (Table 3). As expected, TMP, BMP and SMP followed a declining trend, with TMP the highest value and SMP the lowest. The TMP is calculated assuming that all organic carbon and hydrogen is converted to biogas, thus

giving a maximum upper threshold. The BMP value shows the maximum methane production that occurs in batch conditions over a prolonged period: in this instance, 87 days. The SMP shows the actual methane production under continuous conditions at a particular OLR and HRT. The decrease in methane potential from TMP to BMP is due to the presence of recalcitrant material that is not consumed and converted to biogas within the BMP, but is included within the TMP [42]. Differences between the BMP and SMP may be due to the following: continuous operation, which removes a proportion of biomass and undigested substrate daily; potential inhibition from the salt content in the feedstock; and possible competition from SRB and other microorganisms for fermentative products.

If the fraction of material that is anaerobically biodegradable in semi-continuous digestion is known, as here, the TMP value can be adjusted to reflect this. When the TMP is adjusted based on the %VS destruction in CSTR operation, the agreement with the measured SMP is good (Table 3). *I. galbana* has a lower adjusted TMP than its SMP, which may indicate the degradable fraction contains a higher proportion of lipids than the undegraded material; whereas the adjusted TMP for both *D. salina* samples is close to the SMP suggesting that the composition of the degraded and undegraded fractions is similar.

Measured BMP values for *D. salina* and *D. salina* SO_4 were 47.0% and 52.0% of their respective TMPs, similar to the degree of VS breakdown achieved in the semi-continuous trial. For *I. galbana*, however, the BMP was 63.8% of the TMP, well above the value predicted from the semi-continuous VS destruction: this indicates that greater degradation can be achieved with sufficient time and again supports the view that this substrate would benefit from a longer HRT in semi-continuous digestion, or some form of biomass retention reactor where the liquid and solids retention times can be uncoupled to allow a longer period

for solids degradation. *I. galbana* and *D. salina* SO_4 showed higher conversion of measured CV into methane in the BMP test, but *D. salina* showed relatively high conversion in the semi-continuous digestion, probably as it was least affected by either the short HRT or the sulphur content.

Table 3 Theoretical and actual methane production and calorific value conversion

Parameter	Unit	*I. galbana*	*D. salina*	*D. salina SO_4*
TMP	L CH$_4$ g^{-1} VS	0.494	0.527	0.557
Measured BMP	L CH$_4$ g^{-1} VS	0.315	0.248	0.290
SMP [a]	L CH$_4$ g^{-1} VS	0.244 ± 0.013	0.233 ± 0.007	0.180 ± 0.008
Adjusted TMP [b]	L CH$_4$ g^{-1} VS	0.182	0.231	0.193
BMP as % of TMP	%	63.8%	47.0%	52.0%
SMP as % of BMP	%	77.2%	94.0%	62.2%
Energy value of CH$_4$ from BMP	kJ g^{-1} VS	12.6	9.9	11.5
% of measured CV converted to CH$_4$	%	54.8%	41.9%	48.1%
Energy value of CH$_4$ from SMP	kJ g^{-1} VS	9.7	9.3	7.2
% of measured CV converted to CH$_4$	%	42.3%	39.3%	29.9%

TMP = Theoretical methane potential (Buswell), BMP = biochemical methane potential (batch assay), SMP = specific methane potential (semi-continuous digestion) CV = calorific value

[a] Average of daily values in duplicate digesters between day 45-51 for *I. galbana* and between day 91-97 for *D. salina* and *D. salina* SO4 (last 7 days of each run),

[b] Calculated from TMP multiplied by volatile solids destruction based on gas production in semi-continuous digestion

4   Discussion

The only marine micro-algal species for which the SMP in semi-continuous digestion has been reported is *Nannochloropsis salina*, with a value of 0.13 L CH$_4$ g^{-1} VS when digested

mesophilically at an OLR of around 2 kg VS m^{-3} day^{-1} and an average HRT of 120 days [43]. This increased to 0.27 L CH_4 g^{-1} VS after a thermal pre-treatment was applied. The SMP for *I. galbana* and *D. salina* in the current work was greater than the value for *N. salina* without pre-treatment, and only slightly lower than the value for pre-treated *N. salina*, despite the relatively short HRT in this study. When compared to SMP values for freshwater species, the results from this study are towards the higher end of the reported range. One of the highest is from Ehimen et al. [37] who obtained an SMP for *Chlorella* of 0.302 L CH_4 g^{-1} VS digested mesophilically at OLR 5 kg VS m^{-3} day^{-1} and HRT 15 days after cell wall rupture to release lipids, followed by co-digestion of both fractions. At the lower end of the range *Scenedesmus* spp. grown in an open raceway had a SMP of 0.139 L CH_4 g^{-1} VS when digested mesophilically at OLR from 2.0-3.5 kg VS m^{-3} day^{-1} and HRT from 12-20 days: this low value was tentatively attributed to the recalcitrant cell wall and the high inorganic solids present [38]. An intermediate SMP value of 0.240 L CH_4 g^{-1} VS, similar to those found in this study, was obtained for *Chlorella vulgaris* cultivated on wastewater and digested mesophilically at OLR between 1.0-2.6 kg VS m^{-3} day^{-1} and HRT of 16-28 days [44]. The highest reported SMP from continuous digestion was for *Spirulina maxima* at 0.35 L CH_4 g^{-1} VS at an OLR of 0.67 kg VS m^{-3} day^{-1} and a HRT 30 days; higher loadings could not be sustained, however, as ammonia inhibition caused digester failure [45]. Several other studies have also reported inhibition or failure due to low C:N ratios when digesting micro-algae at OLR of 3-5 g VS L^{-1} day^{-1} and HRT from 10-20 days [34, 45, 46]. No signs of ammonia inhibition were observed in the present study, due to the low feedstock N concentration at the applied OLR as well as the relatively low VS destruction. For example, of the three test samples *D. salina* SO_4 had the highest TKN content of 9.61 %VS and was fed to the digesters at the highest feedstock VS content of 28.1 g VS kg^{-1} WW. The maximum possible digestate TAN concentration for *D. salina* SO_4, assuming complete degradation of TKN into TAN

with no allowance for uptake into the digester's microbial biomass, would therefore be around 2.7 g N kg^{-1} WW, which is below the typical inhibitory range in mesophilic conditions [12, 47].

Comparison of the results for the different species and growth conditions used in this study showed the SMP of the *D. salina* SO_4 was lower than that for *I. galbana* and also lower than that for *D. salina*, despite its higher TMP and BMP. This could be caused by two factors: inhibition of methanogens and fermentative bacteria by the presence of H_2S in aqueous solution; and/or substrate competition between SRB and methanogenic archaea. On switching from *D. salina* to *D. salina* SO_4 the headspace H_2S content rose rapidly, driving up the $H_2S_{(aq)}$ and HS^- in solution. By the end of the run with *D. salina* SO_4 the calculated value for $H_2S_{(aq)}$ was in the range 70-80 mg L^{-1}, which has been reported as inhibitory in some conditions [12]. In response to this rise in sulphides there was a small increase in VFA concentrations, consisting mainly of propionic acid. During the same period, however, the pH in the *D. salina* SO_4 digesters rose to between 7.65-7.70, in comparison with a pH value of 7.21 for the *D. salina* feedstock. This is explained by the fact that sulphide compounds exhibit buffering capacity within anaerobic reactors via the following reversible reaction: $H_2O + CO_2 + HS^- \leftrightarrow H_2S + HCO_3$ [48]. Increasing concentrations of soluble sulphide due to SRB activity will drive off H_2S into the headspace, favouring the forward reaction and increasing the reactor alkalinity and pH, which is also influenced by TAN. The conversion of sulphate also increases the pH by the consumption of H^+, leading to higher partial alkalinity and a lower IA/PA ratio. The small increase in VFA in the *D. salina* SO_4 digesters thus had little effect on pH, and after day 67 the VFA concentration began to fall, reaching less than 50 mg L^{-1} by the end of the run. In contrast the low sulphate digesters, in which the pH was lowerpH, experienced no decline in SMP and the VFA concentration remained consistently low, yet the

IA/PA ratio was slightly higher indicating a reduced buffering capacity. Any inhibition in the *D. salina* SO_4 digesters was therefore not as a result of increased acidity.

It has been reported that propionate degradation can be inhibited at concentrations of undissociated $H_2S_{(aq)}$ above 100 mg L^{-1}, due to inhibition of either the obligate hydrogen producing propionate-degraders or the hydrogenotrophic methanogens [49]. O'Flaherty and Colleran [50] found inhibition of syntrophic propionate-degrading activity and severe inhibition of the acetate-degrading methanogens under a sulphate loading of 4 g SO_4 L^{-1} similar to the concentration used in this study. In this study the increase in $H_2S_{(aq)}$ was accompanied by an increase in propionate concentration, but this was only temporary, again indicating that sulphide inhibition was not a major issue for the *D. salina* SO_4 digesters.

The above results thus favour the alternative explanation, that the lower SMP in the *D. salina* SO_4 digesters is likely to be due at least in part to the consumption of fermentative products by SRB and the subsequent production of H_2S. Evidence to support this comes from the two methods of calculating VS destruction, based respectively on measurements of digestate VS and on biogas production. These showed reasonably good agreement for the two low-sulphate feedstocks; but for *D. salina* SO_4 there was a quite a wide discrepancy, with VS destruction of 53.4% based on digestate solids content and of 34.6% based on gas production. This difference supports the view that a proportion of the energy potential from degradation of VS was not going into biogas production. To obtain an estimate of the SMP without substrate competition from SRB, the SMP for *D. salina* SO_4 can be multiplied by the ratio of VS destruction based on digestate solids to the apparent VS destruction based on biogas. The resulting scaled value of 0.278 L CH_4 g^{-1} VS is closer to the BMP value of 0.290 L CH_4 g^{-1} VS and to the TMP of 0.297 L CH_4 g^{-1} VS, while preserving the expected sequence of these

parameters. Comparison of this scaled value with the actual SMP of 0.180 L CH_4 g^{-1} VS suggests the 'missing' methane yield is 0.098 L CH_4 g^{-1} VS. At a conversion rate of 0.35 L CH_4 g^{-1} COD this has a COD value of 0.28 g COD g^{-1} VS, and at the applied OLR of 1.87 g VS L^{-1} day^{-1} this corresponds to 0.52 g COD L^{-1} day^{-1}. Stoichiometrically the conversion of 1 g of sulphate requires 0.67 g of COD, although in practice higher ratios of COD to SO_4 are typically required for complete sulphate reduction [51]. As the daily input of sulphate from the saline medium was around 0.16 g SO_4, the 'missing' methane COD equivalent of 0.26 g COD day^{-1} is more than sufficient to reduce the applied sulphate load, and corresponds to a conversion rate of 1.66 g COD g^{-1} SO_4 added. If this ratio is divided by 65%, the final value for %S_{rem} in the *D. salina* SO_4 digesters, the conversion rate becomes 2.54 g COD g^{-1} SO_4 removed. These calculations of the energy represented by the reduction in SMP support the view that this is more due to competition than inhibition, even under the relatively high sulphide conditions seen in the digesters with a *D. salina* SO_4 feed.

The method used to estimate the sulphur removal rate %S_{rem} is vulnerable to errors from several sources, and can only really be applied when the digesters have reached a steady state. Calculation of soluble H_2S is reliant on an accurate value for pH, temperature and H_2S concentration within the headspace. These in turn are dependent on good sampling and analysis techniques: for instance, the time between sample removal and pH measurement is critical as degassing of CO_2 can increase the sample pH and shift the equilibrium, with slight differences at lower concentrations resulting in a greater variation in % removal than that at higher concentrations. At the operational pH range within the digesters, the speciation of H_2S could represent a change of two or three orders of magnitude in ionised sulphide. Using the methods described, the estimated %S_{rem} values for *I. galbana*, *D. salina* and *D. salina* SO_4 were respectively 21 %, 6 % and 65 %. These values are lower than those obtained in other

studies on sulphate removal: for example, Harada et al. [52] reported an 85 – 88% removal of sulphate from upflow anaerobic sludge blanket (UASB) reactors treating wastewaters at COD/SO_4 ratios of 0.8-16.7, without reactor failure. Rizvi et al. [53] reported a lower sulphate removal rate of 76% possibly due to operating at lower temperatures between 25 and 30 ˚C. In this case, however, the %S_{rem} calculation includes sulphur input in the biomass in the form of proteins etc as well as in the medium. In this study operation remained relatively unaffected by the addition of excess sulphate, indicating that digesters fed on marine algal feedstocks can become stable and maintain biogas production under high sulphate loadings.

Solids precipitation. As the digestate sulphate concentration was not monitored during the trial a full sulphur balance could not be derived; but in addition to conversion into H_2S, sulphur may be deposited as a precipitate either in elemental form or as a mineral composite, and may thus be unreactive. At the end of the run when the digesters were opened for cleaning a thin crystalline deposit could be seen in the low sulphate digester treating *D. salina* (Figure 7a and c), which could potentially have been struvite [15, 54]. In the *D. salina* SO_4 digesters a thin (1-2 mm) white/yellow biofilm with occasional crystals can be seen in the headspace region. (Figure 7b and d). This film may be a result of H_2S oxidation by sulphide oxidising bacteria (SOB) [55, 56], since atmospheric oxygen was admitted into the digester as a result of opening the feeding port at each feed addition. Kobayashi et al. [56] observed a thick white biofilm growth in the headspace of an agricultural AD plant treating cattle slurry: by addition of 1% v/v oxygen to the biogas produced, H_2S within the headspace was reduced by more than 50%. In the current work it is likely that the method of feeding allowed sufficient intrusion of air to enable some H_2S oxidation to occur. Evidence for this comes from the GC analysis of the headspace gas, which showed that 10-15% was not in the form of CH_4, CO_2 or H_2S. Of this 2-3% is estimated to be water vapour, and the remainder is likely to

be air. At an initial 21% oxygen content, this would represent a greater addition than the 1%

concentration which was introduced to promote SOB activity by Kobayashi et al. [56]. The

deliberate addition of low concentrations of oxygen has been widely used to scrub biogas of

H_2S [57]. This in turn increases the partial pressure between the headspace and digestate,

removing H_2S from solution and reducing the concentration and potential inhibitory effects

[56]. In addition to conversion to H_2S and HS^-, it is thus likely that a proportion of sulphur

was removed via these routes.

Figure 7 Images of deposits within the digesters at the end of the run

for low sulphate *D. salina* ((a) and (c)), and high sulphate *D. salina*

SO_4 reactors ((b) and (d)).

5   Conclusions

Marine micro-algae were successfully digested without washing to reduce salt content : due to growth media carry-over, the digesters thus operated at marine salt concentrations (~33 g L^{-1}). The results confirmed the feasibility of this approach and indicate how anaerobic digestion of marine micro-algae can be incorporated into the future portfolio of biofuels. Using a salt-adapted inoculum, stable operation was achieved at a short (15-day) retention time. Specific methane production for *I. galbana* and *D. salina* was 0.244 and 0.233 L CH_4 g^{-1} volatile solids (VS), with VS destructions of 32% and 48% respectively. These SMP values respectively represented 77 % and 94 % of the biochemical methane potentials, but only 49% and 44% of theoretical methane yields, indicating that pre-treatments may be beneficial. Stable digestion was achieved for the high sulphate *D. salina* SO_4 grown in a medium containing 4.7 g SO_4 L^{-1}, which is well above typical marine sulphate concentrations. This did, however, represent a limitation on the process as the SMP from *D. salina* under these conditions was reduced to 0.193 L CH_4 g^{-1} VS, with a rise in biogas H_2S concentration to above 22000 ppmv. Despite the high sulphide concentrations there was no evidence of long-term inhibition of the methanogenic population, and the loss in methane yield could be attributed to competition between methanogens and SRB for available electron acceptors. To improve methane yield under high sulphate input conditions it would be necessary to prevent this type of loss, possibly through inhibition of the SRB themselves or by other measures to control sulphide toxicity. The work highlighted the difficulties associated with determining partitioning of H_2S under anaerobic conditions, and the impact that this may have on digester alkalinity and pH. Under continuous digestion there were also signs of sulphide oxidation and precipitation with the potential for the formation of complex insoluble salts (such as struvite), which was not seen in batch analyses: this phenomenon may also present challenges in full scale operation.

Acknowledgements

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable

Authors Contributions

Keiron P. Roberts, Sonia Heaven and Charles J. Banks contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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