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Effect of temperature on kinetics of biogas production from macroalgae.¹ Edward Membere *, ² Paul Sallis^{1,2} School of Civil and Geosciences, Newcastle University Upon Tyne, NE1 7RU, United Kingdom¹ University of Port Harcourt, Choba, Rivers State, Nigeria*Corresponding author: E-mail addresses: edward.membere@yahoo.com (E.Membere), paul.sallis@ncl.ac.uk (P. Sallis)**Abstract**

An assessment was carried out on the effect of temperature on the anaerobic digestion of *Laminaria digitata* biomass, in batch reactors (25, 35, 45 and 55 °C) with a hydraulic retention time of 40 days. The first order, modified Gompertz and logistics models were used to obtain the kinetic parameters of the biogas production process. Results indicate the chemical composition of the algae substrate could be written as $C_{316}H_{613}O_{289}N_{13}S_1$, with a theoretical methane yield of $336 \pm 0.86 \text{ L CH}_4 \text{ kg VS}^{-1}$. Experimental methane yield obtained from the reactors for 25, 35, 45, and 55 °C were 318 ± 1.58 , 293 ± 1.11 , 271 ± 0.98 and $352 \pm 0.63 \text{ mL CH}_4 / \text{gVS}$ respectively. Their $R^2 > 0.90$ indicate both models fits well for predicating kinetics of methane production. The lowest k_h (0.31), high biodegradability index (0.96) and lag time (9.3 - 11.7 days) were obtained for 55 °C.

Keywords: Kinetics, Methane, Biodegradability, Macroalgae, Anaerobic digestion.

1. Introduction

Biofuel production from algae is known as third-generation biofuel [1], to differentiate first and second generation biofuels produced from terrestrial biomass which is less sustainable for their production [2]. Macroalgae or microalgae are photosynthetic organisms growing in aquatic environments [3]. Their biomass can be degraded biologically [4]. Whereas microalgae, which are unicellular and have been the focus of intensive research to various products; bioethanol and biodiesel [5], production of methane gas [6] and hydrogen [7], seaweed (marine macroalgae), sometimes known as marine plant crop [8] has received little attention as a prospective feedstock [9]. Hence, their utilization globally is low [4]. Algal biomass are known energy crops because they can trap and store solar energy as expressed by Demirbas [3]; that algae are photosynthetic aquatic organisms that convert water, sunlight and carbon-dioxide into algae biomass.

Many researchers have pointed out the inherent benefit seaweed has over other feedstocks, namely; use of large land mass for cultivation is avoided [4], no competition with conventional agricultural resources [10], large scale mariculture [11], high coastal biomass [9], contains sulfated fucans and proteins [12] and high carbohydrate content (the polysaccharides of alginate, laminarin and mannitol), with zero lignin and low cellulose content making them biodegradable as biofuels during anaerobic processes [9], and they undergo a more complete hydrolysis than terrestrial biomass [5]. For brown algae, alginates form the dominant cell wall / intercellular structural matrix making them a good potential source of methane and hydrogen production as a result of the high carbohydrate content [4].

The process and application of anaerobic digestion is a robust low-tech/low-cost process that is well understood in generating bioenergy as biogas [9]. It has been identified as a viable means of producing carbon neutral energy [13], while also reducing uncontrolled greenhouse emissions [14]. Other advantages are energy recovery, pollution control [15], destruction of pathogens [16], and the production of nutrient rich sludge that can be used as an agricultural fertilizer.

Kinetic analysis is an effective way in determining the key steps in anaerobic digestion process [17], which helps in pilot plant studies to provide better data for reactor designs and operation, leading to more efficient process performance and reduced reliance on skilled operators [18]. Mathematical models (kinetics) are used to demonstrate the effects of changing certain design parameters [19]. They help to describe the kinetic behaviour of biologically mediated processes with digesters. To operate an anaerobic system effectively, and predict how the system will respond to changes in feed and other operating conditions, appropriate models need to be developed [20]. Mathematical modeling of anaerobic digestion process was motivated by the need for efficient operation of AD systems in the early 1970's [21]. Models using the kinetics of microorganisms growth and chemical reactions to predict the behavior of systems have long been reported [22]. Various models have been used to estimate the kinetic parameters; first order hydrolysis constant k_h [23], maximum specific growth rate μ_{max} , lag time λ , methane production time and rate [24]. Fang [17] stated that the modified Gompertz model has been used to describe the kinetics of methane production in anaerobic digestion process. One common feature among the models is that they predict and calculate biogas and methane production rate, which are both very important parameters

for design of an efficient biogas plant [22]. Kinetic models are divided into two classes; structural and un-structural models [17], whereas the former considers metabolic pathways making it generally complicated, the latter is simpler [25]. The application of un-structural models such as Gompertz and Monod equation has been previously used to describe anaerobic digestion of lignocellulose waste with rumen microorganisms [17].

The aim of this research was to demonstrate the application of un-structural models such as the first order, Gompertz equation and logistics model on anaerobic digestion of macroalgae feedstock to estimate and predict the kinetic parameters at different temperatures. Although, reported studies on the influence of digestion temperature on *L. digitata* is scarce and or limited, similar laboratory-scale study has been carried out at 20, 35 and 45 °C to demonstrate the feasibility of biogas production at different temperatures and how it influence the cumulative and methane concentration but did not take into account the kinetics of the digestion processs [26]. Hence, this study intends to add and broaden the knowledge of this substrate to already existing literature.

1.1 Materials and methods

1.1.1 *Algae collection, pretreatment, and storage*

Algal biomass *Laminaria digitata* (LD) used in the batch reactor experiments were collected from shallow water during low tide at Seaton Sluice, 55.0836° N, 1.4744° W, Northumberland UK (NZ 3350) on 5th July, 2015. The seaweed was transported in 30 litre bags and was immediately washed to remove marine salts and sediments. The reactors feedstock were prepared using only the frond; the stipe and holdfast were discarded as reported elsewhere [27]. The fronds were roughly chopped by hand to a particle size of about 10 cm using a

knife, and to obtain the dry algal substrate, the roughly chopped frond were oven dried at 70 °C for between 24 - 48 hrs. This was then pulverized with a Kenwood 100 coffee blender to particle size generally < 1mm. All samples were stored at 4 °C in air tight gas bag until required.

1.1.2 *Inoculum*

The batch reactors were inoculated with a mixed methanogenic sludge from a full-scale running anaerobic digester (Cockle Park Farm, Newcastle), operating on grass silage, pig and cow manure. It had following characteristics; pH 7.5, 21.2 %TS, 60 %VS (%TS), 0.019 sulphur and C: N of 0.061.

1.1.3 *Substrate characterization and analysis*

Characterization of the macroalgae feedstock used in this study are summarized in Table 1.

Table 1: Physiochemical characteristics of *Laminaria digitata* macroalgal feedstock

The dried macroalgae prepared had a TS content of approximately 94%, and a VS content of about 65%, giving a fairly high VS/TS ratio of 0.69, indicating mostly organic digestible matter in the feed. The C: N ratio was 21.6: 1 which is close to the optimal range (25 - 30:1) for stable anaerobic digestion [28]. C: N values as high as 27.5:1 [29], and 22.3:1 [30] have been reported for *L. digitata*. It has a very low lignin content (0.67%), indicating the storage carbohydrates should be accessible to fermentation since a high lignin content results in reduced biodegradability of the biomass by microbial processes, hence limiting digestibility and gas production [31].

The pH was measured using a Jenway 3010 pH meter. The total solids (TS) and volatile solids (VS) were determined gravimetrically using methods described in [32]. %TS was obtained by placing the sample in triplicate into an oven for 24 hrs at 104 °C and subsequently placed in a furnace at 550 °C between 1 - 2 hrs to obtain the volatile solids content. [32]. Samples were analysed for carbon, nitrogen and sulphur content using an Elemental VarioMAX CNS analyser. Fibre analysis was carried out using standard methods in Elemental microanalysis laboratory, UK.

1.2 BMP studies at different temperatures

1.2.1 *Batch studies*

The batch test was divided into four different temperatures range and carried out according to Membere et.al., [27], briefly described below; The incubation was carried out in a water bath at temperatures of 25 °C, 35 °C, 45 °C, 55 °C. The batch reactors consisted of 500 mL Duran bottles (actual internal volume 580 mL) fitted with rubber stoppers inserted to serve as an outlet port for biogas collection in gas bags and as a purging port for nitrogen flushing of the headspace. Before starting the BMP test, all reactor bottles were pressure tested for air leakage, and once the experiment has commenced, for nitrogen or methane leakage using a Thermo-Scientific GLD ProLeak detector used to check any CO₂, NO₂, and CH₄ leaks. The required amount of inoculum and substrate was evaluated for each reactor on a VS basis using a ratio of 3: 1 (3g VS_i / L: 1g VS_f / L). This was to ensure adequate destruction of the volatile solids and overcome possible VFA inhibition [23]. The inoculum and substrate were then placed inside the reactor and the solution was made up to 500 mL with deionized water. The rubber stoppers were then used to close the bottles, and the headspace (approx. 80 mL) was flushed for 5 min with pure (99.99%)

N₂ gas to establish anaerobic conditions. The tube clamp was used to close the PVC tube ensuring all the bottles were gas-tight without the gas bags.

Triplicates reactors were used to overcome inoculum variability, sample heterogeneity and allow statistical significance [23]. Each digester was mixed manually by shaking for 15 - 30s once a day.

Biogas collection and methane measurement was done as described in [27].

The methane potential and production rate from biogas production were studied in this experiment. Assays with inoculum alone were used as controls and the methane produced from this inoculum were subtracted from the sample assays [33].

1.2.2 Kinetic study on batch experiment

From the experimental elemental analysis determination, the empirical formulae (C_aH_bO_cN_dS_e) of the macroalgae composition was calculated [34]. This was used to develop a stoichiometric equation using the Buswell Equation, Eqn 1-1 [35], to obtain the theoretical methane potential (BMP_{theo}), ammonium yields and carbon dioxide (CO₂) volumes that can be produced when the macroalgae feedstock is broken down by a consortium of microorganisms present in a batch reactor [36].

Using the calculated (BMP_{theo}), the biodegradability index was determined. The biodegradability index is defined as the ratio of the observed BMP to the Buswell theoretical methane yield (BMP_{theo}) [29, 35].

Although the BMP_{theo} gives a rough idea of the strength of a substrate's biogas potential, experimental assays must be used to ascertain the actual potential [27]. The degradation kinetics (derived from ultimate methane yield at infinite digestion time) was used in this study.

The degradation kinetics were assumed to follow a first-order degradation rate, Eqn 1-2 [23, 34];

$$B = B_0 \cdot [1 - \exp(-k \cdot t)] \quad \text{Eqn 1-2}$$

Where B ($\text{mL CH}_4 \text{ gVS}^{-1}$) is the cumulative methane yield, B_0 ($\text{mL CH}_4 \text{ gVS}^{-1}$) is the ultimate methane yield, k (day^{-1}) is the first-order rate constant and t (d) is the time.

The first order kinetics for hydrolysis of particulate organic matter used a linear regression model based on the empirical relationship (Eqn 1-2), and is used to determine the rate and extent of degradation, where the value of k (slope of the linear plot) represents the characteristics of a given substrate, and gives the time required to generate a ratio of the ultimate methane potential [23].

But the linear form of the first-order model which is in an exponential form cannot be used to adequately account and predict the cumulative methane production through the entire process particularly after the exponential phase [37]. A nonlinear regression model, the modified Gompertz equation (Eqn 1-3), is mostly used to account for the lag phase (λ) duration, biomethane potential (B_0) and the μ_{max} biogas production rate [1] ;

$$B = B_0 \cdot \exp \left\{ -\exp \left[\frac{\mu_{max} e}{B_0} (\lambda - t) + 1 \right] \right\} \quad \text{Eqn 1-3}$$

Several authors have further modified the Gompertz equation to estimate the cumulative biogas production [38], and also applied in this study is the modified logistics model (Eqn 1-4).

$$B = \frac{B_0}{\left\{ 1 + \exp \left[4 \mu_{max} \frac{\lambda - t}{B_0} + 2 \right] \right\}} \quad \text{Eqn 1-4}$$

Using both models, the kinetic parameters (λ , μ_{max} , B_0) of each reactor were estimated using nonlinear least-square regression analysis in MATLAB[®](R2016a) software. The statistical indicators R^2 (correlation coefficient) and root mean square error (RMSE) were calculated [28, 39]. The RMSE is a standard statistical metric used to measure model performance [40]. Both the R^2 and RMSE (lowest value) were used to access best-fitted model [41].

$$RMSE = \left[\frac{1}{m} \sum_{j=1}^m (d_j)^2 \right]^{1/2} \quad \text{Eqn 1-5}$$

Where ' m ' is the number of data pairs; j is the j th values; Y is measured methane yield; ' d ' is the difference between experimental and predicted methane yield.

1.3 Results and Discussion

1.3.1 Experimental batch study

The characteristics of the substrate and ultimate analysis are given in Table 1. From the atomic weight of the elements, the stoichiometric description of the algae is derived as $C_{316}H_{613}O_{289}N_{13}S_1$. Theoretical biomethane and ammonium yield, calculated using the Buswell equation for the algae (*L. digitata*) with VS

(65%) per kg of the algae weight contribution is shown in Table 2. The BMP_{theo} obtained was $366 \pm 0.1 \text{ L CH}_4 \text{ kg VS}^{-1}$ which is similar to $368 \text{ L CH}_4 \text{ kg VS}^{-1}$ [29], and $335 \text{ L CH}_4 \text{ kg VS}^{-1}$ [27], but lower than $479 \text{ L CH}_4 \text{ kg VS}^{-1}$ [30] reported for *L. digitata*. *Laminaria species* are known to exhibit variation in biomass composition across the year (seasonal variation) which can alter the carbohydrate concentrations composition dramatically [42], and probably explains the difference in reported gas production yields above. The biodegradability index obtained for the reactors was 0.96 at 55 °C and 0.80 at 35 °C, Table 3, compares very well to 0.78 reported for *L. digitata* [29] and (0.81) for *S. latissimi* [30]. This gives an indication of how well the substrate was degraded and how the BMP yield compared to the theoretical biomethane yield [35]. Higher biodegradability indices correspond to higher digestion efficiencies [29].

Table 2 Generation of the stoichiometric equation and theoretical assessment of biogas production from macroalgae (collected in July 2015).

Table 3 Bio-methane production for macroalgae using results of BMP and theoretical analysis.

The cumulative biogas and methane production, daily methane production, and % methane content, with respect to the retention time of 40 days for all the reactors is shown in Figure 1 and Figure 2. The cumulative biogas production obtained in the reactors for 25 °C, 35 °C, 45 °C, and 55 °C are 559 ± 1.2 , 639 ± 1.0 , 558 ± 1.1 and $501 \pm 0.2 \text{ ml}$ respectively. These results show a trend of 35 °C > 25 °C > 45 °C > 55 °C, Figure 1 A. The cumulative biogas produced by the reactor with a temperature of 35 °C is 14.5%, 14.5%, and 27.5% higher than the yield of the reactors which are at 25 °C, 45 °C and 55 °C.

Figure 1 A) Cumulative biogas production B), % Methane

Figure 2 A) Cumulative methane production B) Daily methane production (mL CH₄ / gVS).

A similar trend has been observed by Vanegas and Bartlett [26], on the effect of temperature on anaerobic digestion of *L. digitata*, using three different temperatures (20 °C, 35 °C, and 45 °C), with reactors incubated at 35 °C producing the highest biogas. This trend was also reported by Varel et.al., [43], with *Spirulina maxima*, biogas production was higher at the mesophilic (35 °C) temperature than thermophilic temperature (55 °C). The methane yield obtained shows a different trend of 55 °C > 25 °C > 35 °C > 45 °C, Figure 2 A. This trend indicates that acclimatisation plays a critical role for the thermophilic temperature (55 °C) which began to work best after day 20 - 30 (see steep slope in Figure 2 A). The results suggest the activity of the methanogenic bacteria [39], process of adaptation of the inoculum to the various temperatures, the inoculum ability to produce a number of specific enzymes capable of hydrolysing the main polysaccharides of *L. digitata* (cellulose, laminarin, fucoidan and mannitol) to biogas [26], and the degradation rate (k_h) [27], depend on the reactors operation temperatures. This in turn influences the rate of biogas production, as the solubility of both of CH₄ and CO₂ decreases with increase in temperature [44]. Therefore, in batch reactors operating under different temperature conditions, using unacclimatised inoculum, the mesophilic temperature 35 °C seems more effective for biogas production than thermophilic 55 °C for macroalgae but as the experimental run progresses, acclimatisation of the inoculum takes place and thermophilic fermentation may be the preferred temperature for methane production for *L. species*.

The kinetic parameter (k_h) was calculated for each set of temperature conditions by the procedure described elsewhere [23] and shown in Figure 3 and Table 3.

The k_h values varied from 0.31, 0.45, 0.54 to 0.69 (d^{-1}) in increasing order for 55, 35, 45 and 25 °C. k_h values of 0.33 - 0.36 [27] and 0.19 – 0.22 [30], and 0.08 - 0.21 [45] for the mesophilic temperature of 35 °C has been previously reported for *Laminaria species*. The higher k_h values mean a shorter degradation time, and k_h can display a huge variability which can be specific to particular process system [46]. From Figure 1 A, the thermophilic reactor at 55 °C with the lowest k_h of 0.31 was more inhibited than the other reactors. As previously reported, k_h is a kinetic parameter that increases as the degradation rate increases [47]. The R^2 values, (Figure 3) indicate a good fit of the first order rate model.

Figure 3 First order plot of cumulative methane production of *Laminaria digitata* at various temperature range.

The methane composition in the biogas was determined using a GC-FID instrument as described by Membere et.al., [27]. The % methane evaluated was multiplied by the daily measured biogas volume from the gas bags giving the volume of methane produced at room temperature. The total volume of methane produced daily was calculated by using an equation as previously described in [27], and the volume normalised to dry gas at STP [48]. The measurement was carried out daily for the first 10 days, thereafter between once and twice a week, as between 80 and 90% of methane production is normally achieved within 8 - 10 days [27].

Biomethane potential and daily methane volume measured is shown in Figure 2 A and B. The cumulative methane (CH_4) produced was highest for 55 °C with a value of 352 ± 0.63 ml CH_4 g VS^{-1} with methane content increasing from 3% on day 1 to about 68% by day 21, Figure 1 B. The cumulative methane production for 25, 35 and 45 °C are 318 ± 1.58 , 293 ± 1.11 and 271 ± 0.98 ml CH_4 g VS^{-1} .

These are similar to results obtained ($267 - 288 \text{ L CH}_4 \text{ kg VS}^{-1}$) for mono-digestion of natural *L. digitata* and ($258 - 296 \text{ L CH}_4 \text{ kg VS}^{-1}$) for cultivated *S. latissimi*, at 37°C [29], and documented values of up to $280 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}^{-1}$ for the brown seaweed *Laminaria* [49]. Allen et al., [30], reported the highest BMP yield $342 \text{ L CH}_4 \text{ kg VS}^{-1}$ for *Saccharina latissima* among 10 species of seaweed, with $218 \pm 4.1 \text{ L CH}_4 \text{ kg VS}^{-1}$ for *L. digitata* at 37°C with a C: N ratio of 22.5.

The % methane for 25°C reactor increased from 5% on day 1 to about 78% on day 21 while the 35°C reactor increased from 13% methane content on day 1 to 75% on day 10 and the 45°C reactor increased from 15% on day 1 to 68% by day 13. The percentage of CH_4 in the biogas was higher for the 25, 35 and 45°C digesters (81, 69, 68% at $t = 13$ days) than the 55°C digesters (34% at $t = 13$ days). This suggests that some acclimatization of the inoculum was occurring in the 55°C reactor between day 1 and day 13.

1.3.2 Kinetic study using modified Gompertz and logistics model

The modified Gompertz equation was used to fit the cumulative methane data obtained from the batch reactors. Table 4 shows the results of the estimated kinetic parameters based on the Gompertz model, which indicates that it can be used to predict the methane yield potential, maximum methane production rate and duration of the lag phase [24].

Table 4 Results of kinetics study (Modified Gompertz and Logistics model)

The soundness of the model results was evaluated by plotting the predicted cumulative methane values against experimental values as shown in Figure 4. The maximum predictable biomethane potential of the algae is shown in Table 4, with batch reactors operating temperatures of 25, 35, 45 and 55°C , found to be 415, 291, 316 and $518 \text{ mL CH}_4/\text{gVS}$ respectively. This shows the reactor

with operating temperature of 55 °C should have a maximum methane yield followed by 25 °C. This trend seemed to follow the cumulative experimental values, as shown in Figure 2 A. The lag phase was found to be in between 0.11 – 9.3 d. Reactor 45 °C (0.11 d) and 35 °C (1.5 d) shows faster degradation, this could be as a result of the acclimatization of the inoculum at this temperatures whereas reactor 55 °C (9.3 d), showed there was initial inhibition of the anaerobic biomass as depicted in Figure 2 A. Inhibition can be attributed to several factors, apart from non-acclimatization of the inoculum at 55 °C to the substrate as shown in this study, drop in pH to about 5.5 -5.9 after 1 - 2 days is also a factor that can cause a reduction in biogas production [26]. The R^2 values which are the coefficient of determination, for reactors 25, 35, 45 and 55 °C were 0.99, 0.99, 0.98 and 0.99. This shows the predicted values give a good fit to experimental values. The RMSE values were between 5.5 -13.3 L CH₄ kg VS⁻¹. Figure 4 A shows the comparison of experimental and predicted cumulative methane production, the R^2 values agree with kinetic results in Table 4. This shows that the modified Gompertz equation fitted the data from the kinetics study of methane production from *Laminaria* feedstock predicting reliably both the lag time and maximum methane potential.

Using the logistics model, the estimated kinetic parameters are also shown in Table 4. To evaluate the robustness of model results from the logistic model, the predicted cumulative methane production was plotted against the measured values, as shown in Figure 4 B. The maximum predictable biomethane potential of the algal substrate from the logistic model with operating temperatures of 25, 35, 45, and 55 °C are 343, 289, 301 and 390 mL CH₄ g VS⁻¹, respectively. The lag time was between 1.2 – 11.7 days with R^2 values of 0.99, 0.99, 0.97, and 0.99 in order of increasing temperature. The RMSE values (Table 4) were

between 6.5 - 17.2 L CH₄ kg VS⁻¹. Similar results of between 9.7 - 13.9 L CH₄ kg VS⁻¹ has been reported by researchers studying kinetics using the logistics model [39]. Comparison of the predicted logistics models with experimental cumulative methane production for all the reactors is shown in Figure 4 B. The R² obtained from Figure 4 A (0.99, 0.99, 0.98, 0.99) and B (0.99, 0.99, 0.97, 0.91) for 25 °C, 35 °C, 45 °C and 55 °C are similar to the predicted values in Table 4, indicating the Gompertz and logistics model fits well for the kinetics of methane production, lag time determination and maximum methane potential. From the RMSE values (Table 4), which represents a statistical indicator to measure the model error [50], the Gompertz model appears to be better suited than the logistics model for modelling the data set with a good fit and indicating, for instance at 55 °C the error point was 13.3 ml compared to 17.2 ml using the logistics model which is a deviation of about 22% compared to the Gompertz model.

Figure 4 Comparison of predicted A), modified Gompertz; and B), logistics models with experimental cumulative methane production.

1.4 Conclusion

The effect of temperature on biogas and methane yield from macroalgae, *L. digitata* was investigated in 500 mL batch reactors running at 25, 35, 45 and 55 °C. The results demonstrated the feasibility of producing biogas at all the digestion temperatures, and this parameter had an influence on cumulative biogas production. The theoretical methane yield, biodegradability index, modelled biogas and methane production rates were all assessed for the reactors. These results were compared with the experimentally obtained values. From the results the cumulative biogas production was best at 35 °C, while overall methane yield potential was best at 55 °C.

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Figure 5: A) Cumulative biogas production, and B) % Methane

Figure 6: A) Cumulative methane production, and B) Daily methane production

Figure 7 First order plot of cumulative methane production of *Laminaria digitata* at various temperature range.

Figure 8 Comparison of predicted A) modified Gompertz and B) logistics models with experimental cumulative methane production.

Table 5: Physiochemical characteristics of *Laminaria digitata* macroalgal feedstock

Physical analysis		Fibre analysis		Elemental composition	
% Moisture	6.3%	Neutral Detergent Fibre	7.0%	Carbon ©	30.8%
%TS	93.7%	Acid Detergent Lignin	0.7%	Hydrogen (H)	5.0%
%VS	65.0%	Acid Detergent Fibre	20.3%	Nitrogen (N)	1.4%
TOC	36.1%	Oil A (Ether Extract)	0.5%	Oxygen (O)	37.6%
C/N RATIO	21.6	Total Oil (Oil B)	1.4%	Sulphur (S)	0.26%

Table 6 Generation of the stoichiometric equation and theoretical assessment of biogas production from macroalgae (collected in July 2015).

Component	Number of atoms per mole of algal biomass	Atomic weight	Weight contribution (Kg/t)	%
Carbon	25.69 (316.21)	12	369.96	37.0
Hydrogen	49.8 (612.92)	1	4.98	0.50
Nitrogen	1.02 (12.57)	14	20.02	2.0
Oxygen	23.48 (288.92)	16	600.96	60.1

Sulphur	0.08 (1)	32	8.32	0.83
$\text{C}_{316.21}\text{H}_{612.92}\text{O}_{288.92}\text{N}_{12.57}\text{S}_1 + 28.44 \text{ H}_2\text{O} \rightarrow 158.68 \text{ CO}_2 + 157.52 \text{ CH}_4 + 12.57 \text{ NH}_3 + \text{H}_2\text{S}$				
9238.14	+ 511.95	6982.02	+ 2520.3664	+ 213.7138 +34
	9750.08502		9750.097	
0.65 kg + 308.65 H ₂ O → 171.30 kg CO ₂ + 170.05 kg CH ₄ (algae is 65% VS dry wt)				
Density of CH ₄ = 0.714 kg m ⁻³ , Density of CO ₂ = 1.96 kg m ⁻³				
Gas by volume → 238.17 m ³ CH ₄ + 87.40 m ³ CO ₂ = 325.565 m ³ biogas @ 47.77% CH ₄				
Theoretical maximum methane production: 238.17 m ³ CH ₄ / 650 kg VS: 366.42 L CH ₄ /kg VS				
%CH ₄ = 47.77 %, CO ₂ = 48.11 %, NH ₃ = 3.81%, H ₂ S = 0.303 %				

Table 7 Biomethane production for macroalgal using results of BMP and theoretical analysis.

Reactors	Theoretical yield (L CH ₄ kg VS ⁻¹)	BMP yield (L CH ₄ kg VS ⁻¹)	Biodegradability index	K (d ⁻¹)
Algae (<i>L. digitata</i>)	366.42 ± 0.86			
25 °C		318 ± 1.58	0.87	0.69
35 °C		293 ± 1.11	0.80	0.45
45 °C		271 ± 0.98	0.73	0.54
55 °C		352 ± 0.63	0.96	0.31

Table 8 Results of kinetics study (Modified Gompertz and Logistics model)

Parameter	Modified Gompertz				Logistic Model			
	25 °C	35 °C	45 °C	55 °C	25 °C	35 °C	45 °C	55 °C
Cumulative methane produced - Experimental (ml CH ₄ /reactor/gVS)	318	293	271	352	318	293	271	352
Cumulative methane produced - predicted (ml CH ₄ /reactor/gVS)	334	285	272	366	323	279	268	356
Biomethane potential - predicted (ml CH ₄ /gVS)	372	285	284	442	329	279	271	369
Max biomethane potential - predicted (ml CH ₄ /gVS)	415	291	316	518	343	289	301	390
μ _{max} (ml/day)	12.9	26.3	10.9	14.1	15.5	28.4	11.2	17.1
Lag phase (λ)	5.9	1.5	0.11	9.3	8.3	2.1	1.2	11.7
R ²	0.99	0.99	0.98	0.99	0.99	0.99	0.97	0.99
RMSE	11.25	5.45	13.37	12.78	6.47	10.23	17.19	7.91

RMSE – Root mean square error

Highlights

- *Macroalgae, Laminaria digitata used as a feedstock for anaerobic digestion.*
- *Influence of temperature on L. digitata fermentation.*
- *Kinetics of biogas production from L. digitata.*
- *Evaluated chemical composition of L. digitata feedstocks.*
- *Evaluated theoretical biomethane and ammonium yield.*
- *Biodegradability index of L. digitata at different temperature.*
- *Modelled biogas and methane production rates of L. digitata.*