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Bio-methane Potential Test (BMP) using inert gas sampling bags with macroalgae feedstock.

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Abstract

An approach to Bio-methane potential test (BMP) was carried out at mesophilic temperature of 35ºC with Supel™ inert gas sampling bags as biogas collection and storage bags, using selected seaweed (macroalgae) as substrate. Samples were given a range of pre-treatments from washing, drying and macerating. Dried laminaria digitata (DD) with 68.14 % VS (%TS) produced the highest BMP of 141 ± 5.77 L CH4 / kg VS, with methane content increasing to about 70%, while the lowest BMP of 93.35 ± 5.03 L CH_4 / kg VS with methane content of about 65 % was obtained for fresh laminaria digitata (FD) with 72.03 % VS (%TS). Methane yields of 97.66 and 67.24 m^3 CH_4 / t wet weight based on BMP results were obtained for DD and FD. Both DD and FD achieved within 28% and 38% of the theoretical BMP value based on the Buswell equation, respectively. The total methane (V) produced was computed based on ;

$$V = X_1 + X_2 - X_3 \text{ corrected to Standard temperature and pressure (STP)}$$

where X_1 = daily calculated headspace methane volume, X_2 = daily measured volume of methane in gas bags, X_3 = previous day headspace methane volume. An advantage of this approach is the volumetric measurement of gas produced directly from the gas bags, hence it does not require liquid displacement or pressure transducers. Results from a second set of freshly collected sample seaweed sample showed it was in agreement with published BMP values. All analysis were carried out without mineral supplementation.

Keywords: Biogas, Biodegradability, BMP, Seaweed, Methane, Algae

1. Introduction

Anaerobic biodegradability (AB) is a terminology now used to describe Bio-chemical methane potential (BMP) [1-3]. It is defined as the fraction of compound(s) converted to biogas (methane and carbon dioxide) under oxygen-free conditions mediated by a diverse mixture of microorganisms for an indefinite degradation time. But in practice the degradation time is definite and methane potential estimated from extrapolation of the experimented degradation curve [4]. AB can be determined by the volume of biogas produced, or the amount of substrate depleted or the formation of intermediates and end products [3]. The biochemical methane potential (BMP) test is the procedure developed to measure the volume of methane produced [1,5]. The assay was developed as a standardized method to determine the ultimate biodegradability [6] and associated methane yield during the anaerobic methanogenic fermentation of organic substrates [7]. It is a proven and reliable method to obtain the extent and rate of organic matter conversion to methane [8]. The parameter, ultimate methane potential (λ_{max}) from the BMP assay is regarded to a great extent as the determining factor for both design and economic details of a biogas plant [5]. The experimental BMP approach is simple; a characterized [9] and quantified organic substrate is mixed with a known anaerobic inoculum in a suitable medium (minerals and water) under defined operating conditions where the gas evolved is quantified by a specified measurement system until gas production virtually ceases [10]. Mixtures of nitrogen (N_2) 70-80% and carbon dioxide (CO_2) 20 -30% are used as headspace gas to create anaerobic conditions, these prevent pH - change in the water phase due to CO_2 from the headspace of the reactors [11], pure N_2 alone has been also used [10]. Blank controls are included to account for the biogas produced from the inoculum alone, these are termed endogenous tests [1].

The blank control gives an idea of the volume of biogas produced by the substrate alone [5]. Glass bottles with rubber septums as closed vessels are normally used (Figure 1-1). The volume of the bottles range between 0.1 L - 2 L [5] to 0.1 -120 L [1], all depending on the homogeneity of the substrate used. It is recommended that samples and blank assay should be carried out in triplicate for statistical significance [5] because the BMP assay uses inoculum from different sources with varying quality and these can be relatively heterogeneous [10, 11]. Furthermore, the biological approach in determining methane potential leads to substantial uncertainty hence triplicate samples should be used as a minimum [11].

Figure 1-1: Bio-methane potential reactor and sampling illustration [11].

Generally, the anaerobic biodegradability assay is used in triplicate [4] ; to establish biodegradability of substrate for products (biogas /intermediates) formation, determination of the ultimate biogas potential and rate of biodegradation. In the first category, most methods are based on monitoring biogas using gasometric techniques [1, 3-5, 11] while different chemical analysis techniques are used to quantify formation of intermediates or substrate depletion [3]. In the gasometric methods, biogas is quantified either manometrically, by measuring pressure increase in constant volume or volumetrically as volume increase under constant pressure [1, 3, 5], and also by gas chromatography [1, 2].

Volumetric methods comprise three approaches; displacement of a piston of a glass syringe inserted into the reactor, liquid displacement method using an alkaline solution for washing the biogas, or absorbing CO_2 and collection of the biogas in a gas sampling bag with low permeability [1], e.g. aluminium foil bags [12]. During the manometric method, biogas produced in the reactors creates a proportional

overpressure which are measured by pressure transducers of various kinds [3]. Both methods require a complementary gas analyser to obtain percentage composition of methane in the biogas.

Seaweeds are marine macro-algae which can be biologically degraded to methane [13].They can be utilized as a new promising biomass for the low-carbon economy,and recently have attracted attention as possible feedstocks for biorefinery ventures ([14]. Biorefineries are regarded as a sustainable technology that converts biomass into various marketable products, and energy [15]. Macroalgae have the potential of becoming a viable aquatic energy crop [16-18], but energy production from macroalgae is still limited due to economic viability [19].Figure 1-2 illustrates the current biofuel products from algae [19].

Figure 1-2: Current renewable fuel sources from algae [19]

2. Materials and Methods

2.1 Collection, pretreatment and storage

Algal biomass *Laminaria digitata (LD)* and *Laminaria Hyperborea (LHY)* used in the batch experiments were freshly collected from shallow water during low tide at Culler coats Bay, Tyne and Wear (NZ3572) on 19th December, 2013. The seaweed were transported in 1 m bags and were immediately washed to remove marine salts and sediments which can cause mechanical problems in digesters. Sand is known to be abrasive to moving parts such as mixers and pumps while salt removal leads to more stable digestion [20]**.**

In preparation of the feedstocks, only the frond was used for *LD,* while stipe (stem) were used for the *LHY*. Two categories of pre-treatment were carried out on both samples to obtain fresh slurry and a dried algal powder. For the slurry the

fronds were roughly chopped by hand to particle size of about 10 mm, while the stipe was broken to smaller pieces < 5 mm using knives and hammer mill. Approximately 250 g of each were then macerated with 250 ml of distilled water using a kitchen blender to give consistent thick slurry (particles generally < 2 mm) suitable for direct addition to the reactors. The algal powder was obtained by oven dying the sample at 104 ^{O}C for 24 hrs and then pulverized with a Kenwood 100 coffee blender to particle size generally < 1 mm. Both types of pre-treated sample were labelled in 1 litre containers and stored at 4 ^{O}C until required.

2.2 **Inoculum**

The specific methanogenic activity test (SMA) is normally used to check the quality of inoculum in anaerobic digesters. It is an indication of the efficiency of anaerobic treatment process because it measures the rate of the methanogenic activity under defined substrate conditions [21]. The SMA test is a quick and simple way to get information about the percentage of active methanogenic microorganism in a sludge, and also estimate the rate of maximum methane production of a reactor at a particular sludge density [22], or capability [23] to convert volatile fatty acids into methane under ideal conditions [23]. The test is performed with acetate, or acetic acid, or mixture of acetic, propionic and butyric acids [24], because in non-gastrointestinal environments like anaerobic digesters, acetate is one of the major intermediates of fermentation [22] and is regarded as the principle precursor of about 70% of methane produced under typical operating conditions [25].The inoculum used was collected from laboratory scale mesophilic anaerobic digesters running in the environmental engineering laboratory, Newcastle University. It had been stored at 4^{o}C for between 1- 4 weeks before use, and had the following characteristics; pH 7.33, 13.95% TS and 58.77% VS (%TS). The inoculum was pre-incubated using 2L

reactor bottles at 35^{o}C for 3 days with waste beer COD concentration 117 g /L to restore / reactivate the methanogenic activity. Active biomass was confirmed by good biogas production (1L biogas / L reactor / d) with 50 – 70 % methane content in the biogas (Figure 2-1).

Figure 2-1: % Methane composition in biogas using waste beer as substrate

Before using the pre-incubated inoculum for both SMA and BMP tests it was de-gassed between 3-5 days until biogas production was negligible. The SMA test was carried out by adding different amounts of sodium acetate (NaAc) (1g HAc = 1.37 g NaAc) to 98 ml of inoculum (2 g VS/L) in 0.5 L reactor bottles and the volume made up to 400 ml with de-ionised water. Then the procedure described for the BMP assay [22] was used to carry out the SMA test. Acetate (0.5 - 2.0 g/L) was used as substrate since approximately 72% of methane formed during anaerobic digestion is from acetic acid [26].

2.3 **Characterization of the sample**

pH was measured on the prepared substrate prior to digestion using a Jenway 3010 pH meter. The total solids (TS) and volatile solid [27] as % TS, were determined gravimetrically using methods described in [28]. VS was obtained by placing the sample in triplicate into an oven for 24hrs at 104^{O}C, and these solids subsequently placed in a furnace at 550^{O}C for 1 - 2 hrs to obtain the volatile solids content.as a fraction of the total solid (%TS) [28]. Total Kjeldahl Nitrogen (TKN) was determined using Turbotherm acid digestion and Vapodest 30S steam distillation apparatus (C Gerhardt Lab Supplies,UK).10 ml of the samples were digested by the Turbotherm in Kjeldahl tubes with H_2SO_4 and a $K_2SO_4/CuSO_4$ Kjeltab tablet. The digestate was then neutralised and steam distilled as described for ammonical

nitrogen analysis [28]. The Total protein content was estimated by multiplying the TKN value by 6.25 [7, 29]. To obtain the percentages of carbon, hydrogen and nitrogen for the generation of stoichiometric description of biomass, the fresh slurry samples were firstly oven dried at 70^{o}C for multiple 30 minutes periods until constant weight obtained to remove moisture content, and passed through 1 mm sieve before CHN analysis. Each pre-treated substrate stock was sampled and tested in triplicate for total carbon (C), hydrogen (H) and nitrogen (N) on a total solid basis. The ultimate analysis for the fresh samples was carried out by Micro elemental Ltd,UK using a CE Instruments (now Thermo) elemental analyser model EA1110 for CHN and a Fisons instrument (now Thermo) elemental analyser model NA2000 for oxygen and sulphur. The Instruments were calibrated and verified using certified reference chemical, acetanilide 141 d traceable to NIST primary standards (ASTM 2005). A confirmation analysis was done for CHN using (Carlo Erba 1108 Elemental Analyser,confidence limit <0.3%) by the Chemistry Department in Newcastle University Upon Tyne.

2.4 **Assessment of Bio-methane potential energy from the Buswell equation.**

When the atomic or organic fraction composition of a compound is known, it is possible to calculate the theoretical bio-methane potential (BMP_{theo}) [4]. From the experimental elemental analysis determination, the empirical formulae ($C_aH_bO_cN_dS_e$) can be calculated [10]. A stoichiometric equation can be developed using the Buswell equation (Equation 1) [20] to obtain the BMP_{theo} and Carbon Dioxide (CO_2) volumes produced when a substrate is broken down by a consortium of micro-organisms present in a digester.

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad [1]$$

Assuming a total stoichiometric conversion of the organic compounds to methane and carbon dioxide the methane yield (*BMP_{theo}*) from the Buswell equation can be calculated from Equation 2 ; [10].

$$BMP_{theo}\ yield = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) 22.4}{12n + a + 16b} \left(STP\ \frac{lCh_4}{g - VS}\right) \quad [2]$$

2.5 **Modified Bio-methane potential assessment of pre-treated Substrate.**

The modifield assessment was carried out in a water bath at mesophilic temperature of 35^{O}C. The batch reactors consisted of 500 ml Duran bottles (actual internal volume 580 ml) fitted with rubber stoppers (Fisher brand Height 30 mm, bottom 29 mm) with a 4 mm diameter stainless steel tube (45 mm long) inserted to serve as an outlet port for biogas collection in gas bags and as a purging port for Nitrogen flushing of the headspace. The plastic bottle caps were used to hold the stoppers in place (Figure 2-2) preventing any frictional movement of the stoppers as a result of biogas pressure build-up in the reactors and preventing loss and oxygen penetration into the reactors. A flexible PVC (non- oxygen / methane permeable) tubing connector 0.5 cm long was attached to the stainless, and a tube clip was used to close the tube (Figure 2-2). Before starting the BMP test all reactor bottles were pressure tested for air leakage, and once the experiment has commenced, nitrogen or methane leakage using a thermo-scientific GLD ProLeak detector used to check any CO_2, NO_2 and CH_4 leaks. The required amount of inoculum and substrate was evaluated for each reactor on a VS basis using a ratio of 3:1 (6 g VS / L : 2 g VS / L). This was to ensure adequate destruction of the volatile solids and overcome possible

VFA inhibition [5, 24]. The inoculum and substrate was then placed inside the reactor and the solution was made up to 400 ml with of de-ionised water. The rubber stoppers were then used to closed the bottles, and the headspace (approx. 160 ml) was flushed for 5 minutes with pure (99.99 %) N_2 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 samples were used to overcome inoculum variability, sample heterogeneity and allow statistical significance [5, 11]

Figure 2-2: Modifield BMP reactor and gas collection bag

Biogas collection

Biogas collection started after 24 hrs of digestion. Any biogas production was initially contained within the headspace of the closed reactor and caused a causes a proportional pressure increase within the reactors. Supel^{TM} inert gas sampling bags were attached to the PVC tubing connectors daily for collection of biogas. This was achieved by releasing the clamps allowing the biogas to flow into the bags after which they are reclamped before removal, ensuring no air penetration into the reactor bottles. The collected biogas was allowed to equilibrate at room temperature 22 ± 3 ^{O}C before compositional analysis and volume determination. The gas bags contained septa from which the gas was collected by gas syringe for analysis. It is assumed that composition of the gas bag is proportional to the headspace of the reactors.

Biogas and methane measurement

The methane composition in the biogas was determined using a GC-FID instrument (Carlo-Erba 5160 GC) in split mode with the injector at 150°C and FID at 300°C.

Hydrogen was used as carrier gas at a flow rate through the column of 1 ml / min. Using a 100 µl sample Lock syringe (Hamilton,USA), duplicate headspace samples (100 ul) were taken from the sample bags and injected manually into the GC with the inlet in a split mode (flow rate 100 mls / min giving a split ratio of 100 :1). After the initial injection the GC temperature programme and data acquisition commenced. Separation was performed on a HP-PLOT-Q capillary column (30 m x 0.32 mm i.d) packed with 20 um Q phase. The GC was held isothermally at 35°C for 90min and heated to 250°C at 10°C / min and held at final temperature for 10 minutes. Methane standards were prepared prior to each analysis from 100 % analytical grade CH_4 (BOC Gases, UK) by injecting duplicate samples to make a five –point standard curve in the range 20-100 % CH_4. The volume of biogas produced was measured at room temperature 22 ± 3 ^{O}C using a 100 ml BD Plastipak syringe to remove all biogas from the gas bags. The methane composition (%) calculated was multiplied by the measured biogas volume giving the volume of methane produced at room temperature. Measurement was carried out daily for the first 10 days, as between 80-90% of methane production is normally achieved within 8-10 days [11], thereafter it was sufficient to measure twice week. Total volume of methane (V) produced daily was calculated by using Equation 3 and corrected to STP with Equation [4] [30];

$$V = X_1 + X_2 \ - X_3 \qquad [3]$$

where; X_1 = daily calculated headspace methane volume ,X_2 = daily measured volume of methane in gas bags, X_3 = previous day headspace methane volume.

$$V_d = V \cdot \frac{(p - p_w) \cdot T_O}{p_o - T} \qquad [4]$$

Where V_d = volume of dry gas in normal state, in mL_N ; V = volume of gas as read off, in ml ; p = pressure of gas at time of reading, in hPa ; p_w = vapour pressure of water as a function of temperature of the ambient space,in hPa ; T_o = normal temperature, 273 K ; p_o = normal pressure, 1013 hPa ; T = temperature of the gas or ambient ,K

2.6 **Determination of the kinetic decay constant and lag phase.**

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 Raposo, Fernández-Cegrí [10] stated that two experimental methods can be used; the $B_{o\text{-}experimental}$ (calculated by dividing the net methane production by weight of sample on (VS or COD basis) at STP conditions and $B_{o\text{-}kinetic}$ (derived from ultimate methane yield at infinite digestion time). The latter method is mainly used.

The $B_{o\text{-}kinetic}$ is assumed to follow a first order degradation rate [5, 10, 31];

$$B = B_O.[1 - exp(-k.t)] \qquad [5]$$

where *B (mL CH_4 gVS^{-1})* is the cumulative methane yield, B_o *(mL CH_4 gVS^{-1})* is the ultimate methane yield, *k* (day^{-1}) is the first order rate constant and t (d) is the time.

The equation is a linear regression model based on the empirical relationship, and is used to determine the rate and extent of degradation, where the value of *k (slope of the linear plot)* shows the characteristics for a given substrate, and gives the time required to generate a ratio of the ultimate methane potential [5]. It should be noted that, if $B_{o\text{-}kinetic}$ differs from $B_{o\text{-}experimental}$ by more than 10%, then *k* is not valid because the kinetic model cannot be used to explain data obtained as the experimental data does not fit the proposed model Equation 4 [10].

2.7 **Second Set of Seaweed Samples**

In order to check and validate the proposed batch method, a second set of seaweed samples were collected during low tide at Seaton Sluice, Whitley Bay (NE26) on 29th August 2014. Samples were subjected to the same pre-treatment described in Section 2.1. The prepared feedstocks were : *Fresh Laminaria Hyperborea Frond (FHL*), *Fresh Laminaria Hyperborea Stipe (FHS), Dried Laminaria Hyperborea Frond (DHL), Dried Laminaria Hyperborea Stipe (DHS) and Fresh Laminaria Digitata Frond (FDL*), *Fresh Laminaria Digitata Stipe (FDS), Dried Laminaria Digitata Fond (DDL), Dried Laminaria Digitata Stipe (DDS).* Table 2-1 shows the characteristics of the samples.

Table 2-1: Charateristics of macroalgal samples

3. **Results and discussion**

3.1 **Inoculum**

The SMA was carried out at four different acetate concentrations (0.5 g ,1.0g, 1.5 g and 2.0 g / L) each combined with 2 g VS / L of inoculum to ensure substrate limitation did not occur [32]. Figure 3-1 show that the higher acetate concentrations (1.0, 1.5, 2.0) gave higher cumulative methane production rates. The daily methane production ranged between 13.73 mL CH_4 g HAc^{-1} d^{-1} on day 2 to 81.11 mL CH_4 g $HAc^{-1}d^{-1}$ on day 8 (data not shown), while the lowest acetate concentration of 0.5 g produced between 5.36 mL CH_4 g HAc^{-1} d^{-1} – 27.05 mL CH_4 g $HAc^{-1}d^{-1}$ on day 8. These values show a low methanogenic yield of the inoculum compared to typical values of 350 mL CH_4 gVS^{-1} d^{-1} obtained for granular sludge with acetate as substrate [24] and 1000 mL CH_4 gVS^{-1} d^{-1} for acetoclastic methanogens [32]. The final methane composition was around 70% for all acetate concentration obtained, except 0.5 g (50 % methane).

Figure 3-1 : a) plot of cumulative methane at different HAC concentration b) methane

composition obtained at different concentrations HAC concentration.

3.2 **Characterisation of macroalgal substrates**

The physiochemical properties of the samples and inoculum were measured in terms of pH, TS, VS, TKN and elemental analysis as shown in Table 3-1.

Table 3-1: Elemental and physical analysis of macroalgal samples

Results showed that VS constitute a major part of the macroalgal biomass, ranging from 63.19 % in *DHY* to 72.03 % of TS in *FD.* pH was in the range of 7.0 - 7.18 in all the reactor bottles before commencing digestion, which is ideal for methanogenic bacteria [4]. Table 3-2 outlines the stoichiometric equation of the pre-treated algal samples while the analysis in Table 3-3 shows that fresh laminaria digitata (FD) with 5.6 % VS should give the maximum theoretical yield of 335.36 L CH_4 / kg VS. Using this methodology, the theoretical maximum methane composition (% methane in biogas) and the maximum biogas attainable from each sample is shown in Table 3-4.

Table 3-2: Elemental components for generation of the stoichiometric equation for macrogal samples.

Table 3-3: Theoretical prediction of biogas production from macroalgal samples using the Buswell Equation [20]

Table 3-4: Theoretical methane yields for pre-treated macroalgal samples

3.3 **CH_4 production**

Bio-methane production potential was measured under controlled conditions (35^{O}C) for 32 days. The cumulative and daily methane production profile are shown in Figure 3-2 and Figure 3-3, respectively. Contribution from background CH_4 produced by the inoculum was deducted from the cumulative yield in evaluating the data. The appearance of the graph (Figure 3-1) conforms with the typical assay [5].

Samples of *Laminaria Digitata* and *Hyperborea* were subjected to a range of pre-treatments from washing, drying and macerating.

Figure 3-2: Cumulative BMP for macroalgal samples ; FD, FHY, DD, DHY

Figure 3-3: Daily BMP evolution for macroalgal samples FD, FHY, DD and DHY

Cumulative CH_4 yield obtained was the highest for washed and dried laminaria digitata, with a value of 150 ± 5.77 L CH_4 / kg VS, with methane content increasing to about 70% (Figure 3-3), while fresh laminaria digitata gave the lowest cumulative yield of 100 ± 5.03 LCH_4 / kg VS, attaining 65% methane content. Chynoweth, Turick [8] have documented values up 280 LCH_4 / kg VS for the brown seaweed laminaria, and between 126 -174 LCH_4 / kg VS for the fresh green seaweed Ulva [20]. Analysis of Figure 3-1 indicates that there is no linearity of methane production rate over the time period of maximum biogas production. Figure 3-2 shows that methane production increased within the first 72 hrs, followed by a decline in production to a basal level, then a transient recovery on day 26 based on this BMP method (after 10 days of biogas accumulation in the reactor headspace before measurement).

Biogas production started with an almost negligible lag time in all experimental bottles, which confirms good microbial activity of the inoculum (as a result of pre-incubation), and rapid digestibility of some macroalgal components as a result cell wall disruption from the pre-treatment. Macroalgal cells have a tough and protective cell wall which makes them highly resistant to bacterial attack [33], producing low methane yields during fermentation process. Pre-treatment process can aid the decomposition the cells and improve methane production [34]

Figure 3-3: Macroalgal methane composition for pre-treated samples FD,FHY,DD and DHY.

From Figure 3-1, the steep initial curve for all macroalgal substrates is indicative of fast degradation rates (k), with values ranging from 0.33–0.36 /day

(Table 3-5). This suggests that basic pre-treatments can improve hydrolysis rates [18] and enhance biogas production and yield [16, 20]. The values are comparable to (0.23 / d) obtained for dried Ulva, (0.433 / d) food waste and (0.239 / d) for grass silage [20]. The R^2 values (Figure 3-4) indicate a good fit of the first order rate model, log (ln) ((B_O–B) / B_O) against time.

Figure 3-4: First order plot of the cumulative methane production of macroalgal samples FD,FHY,DD and DHY.

Of all the substrates, FD had the lowest C:N ratio at 8.61:1 (Table 3-1) while the other substrates were in the range 15:1 - 30:1 which has been proposed as being optimum for anaerobic digestion [35]. Although there was no apparent inhibition of methane production when the C:N ratio was less than 20:1, it is assumed that the imbalance between carbon and nitrogen requirements of the anaerobic microflora [36] could eventually lead to elevated ammonia levels in the bioreactors, leading to failure [37, 38]. Ammonia toxicity is due to the accumulation of total ammonia nitrogen (TAN), but specifically it is mainly from free ammonia (NH_3) which inhibits methanogens, leading to the accumulation of volatile fatty acids (VFAs) [39]. The selection of an appropriate inoculum to substrate ratio is one way of overcoming VFA toxicity, allowing continued biogas production as the sludge (inoculum) acclimatises to the substrate content or any inhibitory substances present [40]. The inoculum to substrate ratio of 3:1 used in this research has been recommended by various authors [5, 24], and as a result, inhibition from known inhibitory compounds such as sulphide and phlorotannins, which are well described components of brown algae (phaeophytes) [41], did not occur in these batch tests.

Table 3-5 compares the BMP results with the theoretical methane potential estimated from Equation 1. The fresh (FD) and dried (DD) samples achieved 28% and 36% of their theoretical values respectively. Allen [20] reported between 36% to 42% of the theoretical value achieved for pre-treated *Ulva* samples. The estimated methane yield was 103.56 m^3 CH_4 / t wet for DD and 72 m^3 CH_4 / t for FD, confirming that dried samples generated a higher volumes of methane than the fresh samples.

Table 3-5: BMP results compared to theoretical yield.

3.4 **Methane Production (Second Seaweed Samples)**

The BMP results (cumulative methane production) is shown in Figure 3-5. The appearance of the graph for all samples agrees with typical example proposed by Angelidaki [5]. Interestingly, both FDL (160.55 ± 1.44 L CH_4 / kg VS) and FDS (160.94 ± 2.68 L CH_4 / kg VS) achieved the highest BMP followed by DDL (150.03 ± 0.78 L CH_4 / kg VS), while FHL (107.49 ± 3.16 L CH_4 / kg VS) showed the lowest BMP after 38 days incubation at 35^{O}C. The values obtained for FDL differ significantly from the first BMP results (Figure 3-1) supporting the fact that both seasonal and compositional variation of macroalgae can affect BMP values [42]

Figure 3-5: Cumulative BMP for macroalgae samples (Second sample of seaweed)

Comparing the steeper curve between Figure 3-1 and Figure 3-5, the degradation rate (k) was slightly lower for second seaweed samples with values ranging from 0.22 - 0.34 (Figure 3-7). The maximum percentage of methane obtained in all reactors was above 60% (Figure 3-6).

Figure 3-6 : Percentage of *Methane in biogas from BMP test as figure 3-5.*

Figure 3-7: First order plot of cumulative methane production as figure 3-5.

4. Conclusion

Marine seaweed are detrimental to the amenity of coastal bay often causing eutrophication in water bodies. It has been reported that the approximate quantity of energy in algae is about 6 calorie / g of which only about 40 % is released from mesophilic methane fermentation studies while the remaining 60 % in the algae biomass is resistant to release through decomposition partly because many cells and walls remain intact through the fermentation process [34].Macroalgae pretreatment before fermentation is used to overcome this limitation

A proposed modified new BMP method using Supel^{TM} inert gas sampling bags as biogas collection and storage system on all reactors were studied with macroalgea as substrate. Pretreatment processes of washing, macerating and drying were under taken to assess the algae strain with higher bio-methane potential. Washed and dried laminaria digitata produced the highest BMP of 141.45 ± 5.77 L CH_4 / kg VS with k (0.36 d^{-1}) and methane content of about 70% during the period of experimentation. It can be concluded that both pre-treatment of the algae and pre-incubation of the inoculum aided in the faster degradation rate observed in all the substrate. The results shows that macro-algae has the potential to be a viable source of generation of gaseous biofuel which are now known as third generation biofuel [19] to differentiate first and second generation from terrestrial biomass which have significant negative opinion to limit their production [43, 44]. Results as shown from the experiment two (Figure 3-5) proved the method is in agreement with a typical BMP test appearance.[5]

The proposed modified BMP approach has certain inherent advantage over current methods in use;

I. Gas measurement converted to STP is carried out directly from the gas bags at ambient conditions, so do not require liquid displacement or pressure transducers.

II. Volume of methane produced is also directly measured from the gas bags.

III. Larger volume of reactor and substrate of heterogeneous nature can be added / used.

IV. Room for easy modification and adaptability to suite specific BMP process.

V. Easy application.

A disadvantage to this method could be the cost of the gas bags. It is highly recommended that in applying this approach the duration of experimentation should exceed the typical 30 day period for batch assay depending on substrate used as evident in observed gas production after day 30 in this work. Hassan et.al. [11] has proposed a 50 day period in their approach. Care should be taken not to have too large a headspace in the reactor bottle leading to erroneous biogas and methane estimation.

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