Changes in higher heating value and ash content of seaweed during ensiling.

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Abstract

A problem in the use of macroalgae for biofuel is that harvesting of seaweed is generally seasonal, and there is a need to preserve and store seaweed to supply year-round production processes. Ensiling is a widely used preservation method in agriculture, but there is little research on ensiling seaweed.

The changes in ash content, higher heating value (HHV) and dry matter (DM%) of algal biomass together with mass loss (ML) during ensilage for a year was studied for two species of seaweed, *Laminaria digitata* (LD),and *Palmaria palmata* (PP) with and without the addition of *Lactobacillus plantarum*. The mean ash content of the two species was significantly different (LD 24.3% and PP 18.0%) and remained constant after 90 days ensiling. The mean HHV before ensiling for PP was higher, 14.2 kJ g^{-1}, compared to LD, 11.9 kJ g^{-1}. Both the species ($P < 0.05$) and ensilage period ($P < 0.05$) had a significant effect on HHV. The overall DM% of the ensiled LD (22.4%), and PP (22.0%) were similar with a gradual increase in the DM% after 90 days ensiled. There was no effect of the ensiling with or without *L plantarum* on DM%. There was a continuous wet matter loss during ensilage, and although the HHV of the ensiled wet biomass increased as the macroalgae became drier over time the energy available from each kilogram of wet macroalgae ensiled declined over the year to 78% in LD and 59% in PP.

Keywords: Seaweed; Macroalgae; Ensilage; Higher Heating Value; *Phaeophyceae; Rhodophyceae; Laminaria digitata; Palmaria palmata;*

Introduction

There is a drive to find alternative sustainable feedstocks for chemicals and energy production. In this context marine macroalgae, or seaweed, are receiving attention (Milledge et al. 2014; Chen et al. 2015; Kerrison et al. 2015). Marine macroalgae, unlike terrestrial crops, do not require agricultural land for cultivation with many species growing in brackish conditions or seawater, avoiding competition for the fresh water required for direct food production (Chen et al. 2015; Tiwari and Troy 2015). The potential biomass yield of macroalgae per unit area is also often higher than that of terrestrial plants with, for example, farmed brown seaweeds yields of ~13.1 kg dry weight (dw) m^{-2} yr^{-1} compared to ~10 kg dw m^{-2} yr^{-1} from sugarcane (Kraan 2013; Rajkumar et al. 2014). Despite their obvious potential, there are yet no economically-viable commercial-scale quantities of fuel from macroalgae, although there has in the past been large scale macroalgae harvesting for the production of potash and acetone (Neushul 1989; Kelly and Dworjanyn 2008).

Any use of macroalgae as a biomass source for commercial scale biofuel production will need a reliable and continuous supply of biomass. A key problem is that the harvesting of most crops is seasonal and is undertaken when the crop is at an optimal point in its growth cycle e.g. high soluble sugars and high dry matter content for rye grass (McDonald 1981). This applies to macroalgae also, and species have shown seasonal variation in their suitability for conversion to biofuels (Adams et al. 2011b; Tabassum et al. 2016b). Macroalgae also decompose on removal from the marine environment. Thus there is a need to preserve and store macroalgae to supply a continuous biofuel production process. However, the preservation of seaweed by oven drying is not energetically viable for biofuel production and solar drying in the UK is impractical due to the large areas required and unfavourable climatic conditions (Milledge et al. 2015; Tiwari and Troy 2015). An alternative preservation method is ensiling, which is routinely used at large scale for the storage of forage for animal feed. During crop ensilage, acid fermentation under anaerobic conditions converts water-soluble carbohydrates into organic acids, mainly lactic acid. As a result the pH decreases, bacterial growth is inhibited and the moist crop is preserved (Ashbell and Weinberg 2005). Ensiling conditions can be achieved from either spontaneous anaerobic lactic acid fermentation initiated by naturally-present bacteria on the crop or by the addition of a starter culture (McDonald 1981; Oude Elferink et al. 1999; Shinya and Yukihiko 2008).

Despite its widespread use in terrestrial agriculture there has been relatively little research on the ensiling of seaweed biomass in order to satisfy year round continuous process demand (Herrmann et al. 2015; Milledge and Harvey 2016a). However, understanding of ensiling of seaweed is absolutely crucial for a substantial and sustainable seaweed biofuel industry (Herrmann et al. 2015). Research on the ensiling of seaweed has been studied sporadically since the 1950s (Black 1955; Lee 1977), with more recent work focusing on lactic acid fermentation of seaweed for novel-food production (Uchida and Miyoshi 2013), and on the effect of ensiling upon methane production from anaerobic digestion of seaweed (Herrmann et al. 2015; Milledge and Harvey 2016a). Despite this renewed interest, the changes occurring in the macroalgae during its ensilage, and in particular the effects on energy content of the ensiled macroalgae remain poorly understood.

The aim of the present work was to investigate energy content changes in the biomass of macroalgae during ensiling with the objectives of examining the changes in the higher heating values, sample mass after ensiling, dry matter and the proportion of ash remaining after ignition in two macroalgae species, commercially harvested in Europe (Edwards and Watson 2011; Milledge and Harvey 2016b), over a one year ensilage period, with and without the addition to the ensiling treatment of a *Lactobacillus plantarum* starter culture.

Methods

Macroalgae samples and ensiling

Samples of two macroalgae species; a brown Phaeophyceae, *Laminaria digitata* (LD) and a red Rhodophyceae, *Palmaria palmata* (PP) were collected from beaches on the Gower Peninsular, Wales, UK (Ordnance Survey SS 4130 8877) at the spring low tide in November 2013. The samples were rinsed in sea water and drained overnight at 4 °C. A baseline 3 × 50 g was grab-sampled from each species on the day of collection. The remainder biomass from each individual species was then chopped with a garden shredder (Bosch AXT 25 TC) and halved. One half of the biomass from each species was treated (labelled "T") by spraying it with a fresh culture of *Lactobacillus plantarum* NCIMB 41028 (Genus ABS) made up according to the manufacturer's specifications and applied at a rate of 1×10^6 colony forming units (CFU) g^{-1} fresh weight of seaweed before mixing, giving sample groups LD T and PP T, the other half was not treated with *L. plantarum* starter culture and left to naturally ensile

due to the effect of compression and an anaerobic environment. These untreated samples were labelled “U” giving the sample groups LD U and PP U. Due to the quantity of biomass available, the treated and untreated portions were divided into 100 g (*L. digitata*) and 50 g (*P. palmata* sub-samples and placed in food grade polythene bags (Vogue, UK) and sealed at a 99.9% vacuum (Minipack-torre, Dalmino, IT). The evacuated and sealed samples of each species were stored at ambient temperature 20 - 25 °C with no additional compression of the seaweed other than that caused by evacuation of the bags. After ensiling for 0, 6, 10, 16, 31, 63, 181, 270 and 365 days, 3 randomly selected bags were removed from both the treated and untreated silage bags available and stored at -18 °C to arrest any further biological activity before the contents were tested.

Bags from both the treated and untreated groups were defrosted and suspended before the seal was broken, the leachate drained for 10 minutes, and the wet mass lost per kilogram ensiled due to the ensiling process calculated for each sample.

pH determination

For three analysis dates (ensiling 0, 31and 365 days) the pH of the resulting liquid leachate was measured (Jenway 3510) and the mean overall pH of the material calculated.

Dry matter determination

The percentage dry mass (DM%) of the samples selected for each analysis date (0, 6, 10, 16, 31, 63, 181, 270 and 365 days after ensiling) was assessed using lyophilisation (Christ Alpha 1-4; 97 hr cycle, 1.65 mBar, ice condenser -53 °C, shelves + 20 °C),. The lyophilised material was ground and passed through a 100 mesh sieve (0.150 mm).

Ash content determination

The ash content of the lyophilised samples was determined using the British Standards dry oxidation method (550 °C) for determination of ash content in solid biofuels (BSI 2009).

Higher heating values (HHV) determination

For each analysis date, samples of ~0.5g lyophilised material were pelletised using a Specac hydraulic press, fitted with a 13 mm diameter die, and applying a gauge-pressure of 1000 kg. Pellets were used in order to prevent small particles being swept out of the combustion

capsule during calorimetry. Each pellet was visually examined prior to calorimetry to assess friability. Higher heating values HHV, or calorific values (CV), were measured using a Parr Model 1341 Bomb Calorimeter, with the included sulphate and nitrate contribution to HHV calculated from titration with standard sodium carbonate solution, using the UKAS method for determination of calorific value (BSI 2010). Two determinations of HHV were carried out for each sample.

Energy losses

The average of the initial ensiled biomass energy remaining during ensilage was calculated using the experimental data obtained for: HHV; wet matter losses; and dry matter and ash content.

The destruction of organic matter by anaerobic bacteria over time has been described by first order integrated rate equation (Rittmann and McCarty 2001; Uzir and Mat Don 2007; Murphy and Baxter 2013):

Equation 1

$$A = 100\ e^{-kt}$$

Where A is the percentage of the compound remaining, t is the time (d) and k is the reaction rate constant (d^{-1}). If the HHV remains constant then Equation 1 could be used to describe the reduction in biomass energy during anaerobic digestion or ensilage. A first rate order equation has been used to describe the hydrolysis of maize silage during ensilage (Pabón Pereira et al. 2009) and the destruction of ascorbic acid during lactic acid fermentation (Di Cagno et al. 2011). However, first order rate equations for anaerobic systems may give only a "moderate agreement" for destruction of biomass as the substrate can be heterogeneous (Murphy and Baxter 2013). A better fit that reflects different destruction rates of the biomass components can be obtained by using two first rate expressions, one for the rapidly degrading material and another for slower degrading fraction (Murphy and Baxter 2013). The percentage of energy remaining in a biomass during ensilage could thus be described;

Equation 2

$$\mathbf{B = (100 - P)e^{K_1 t} + Pe^{K_2 t}}$$

Where B is percentage of energy remaining, t is time ensiled (d^{-1}), k_1 and k_2 are rate constants, P is the percentage of slow degrading biomass energy. Equations 1and 2 were fitted to the data using Microsoft Excel 2013 solver to optimise P, k_1 and k_2 by minimising the sum of the square of the differences between the results derived from the experimental data and those calculated from the equations.

Statistical Analyses

Excel 2013 (Microsoft), IBM SPSS 23 and MINITAB 16 (Minitab Inc.) software were used for Analysis of Variance (ANOVA) and all other statistical analyses. ANOVA was conducted to compare the effects macroalgae species, ensilage period, ensilage treatment and their interactions on both HHV and ash. To remove the strong effect of the species on the analysis further ANOVA models of time ensiled, ensilage treatment and their interactions on HHV and ash were performed for each species. Polynomial regression equations were calculated using MINITAB for the rate of mass loss per kilogram ensiled for the combined LD T and LD U results and for the combined PP T and PP U results.

Results

Changes in pH during ensiling

The pH of *L. digitata* silage leachate fell from 6.32 (standard deviation S.D. 0.07) on day zero to pH 3.21 (S.D. 0.02) for the treated samples by day 31 after ensiling and pH 3.43 (S.D. 0.02) for the untreated silage samples. For *P. palmata,* by day 31 post ensiling, the initial pH of 7.12 (S.D. 0.07) dropped to 3.94 (S.D 0.09) and 4.00 (S.D. 0.07) for the leachate of the treated and untreated silage samples respectively. After 365 day ensiling period the overall mean pH of ensiled macroalgae leachate of *L. digitata* was 3.46 (S.D. 0.02) for the material treated with *L. plantarum,* and significantly lower ($P<0.05$) than the pH 3.98 (S.D. 0.13) for the untreated and naturally ensiled material. For *P. palmata,* after 365 day storage period, the overall pH of the *L. plantarum* treated material was 4.10 (S.D. 0.07), statistically significantly lower ($P<0.05$) than for the untreated material pH 4.49 (S.D. 0.17). The pH for the ensiled

sample of *P. palmata* was statically significantly higher ($P < 0.05$) than that for *L. digitata* at both 31 and 365 days of ensiled storage.

Effects of ensiling on pellet formation

Ensiled lyophilised macroalgae samples readily formed pellets. However, the pellets from *L. digitata* ensiled for period of >180 days were visually more friable than the samples ensiled ≤31 days in contrast to the situation with samples of *P. palmata*, which showed no visual differences in friability over time.

Changes in the observed dry mass of ensiled macroalgae

The overall mean DM% of the ensiled *L. digitata* and *P. palmata* were similar (22.4%, 22.0% respectively, Table 1) and there was no effect of the ensiling treatment on overall mean DM%. The profile for DM% change with time of ensiling for each species was also similar: after an initial period of ~90 days ensiling during which time DM% remained constant, DM% increased at a linear rate over the next ~100 days ensiling then ceased to increase further (Figure 1).

By contrast, from mass measurement of the ensiled macroalgae samples mass loss (ML) occurred from the outset of ensiling, (Figure 2). By the end of the 365 day storage period, the maximum mass loss was 48% and 45% for the treated and untreated *L. digitata* and 65% for both the treated and untreated *P. palmata*. The rate of mass loss per kilogram ensiled during ensiling can be described by similar polynomial equations for both for *L. digitata* (Equation 3) and for *P. palmata* (Equation 4) with a coefficient of determination (R^2) >0.9.

Equation 3 (mass loss during ensiling *L. digitata*)

$$\mathrm{ML} = 52.0 + 2.42\mathrm{t} + 0.00369\mathrm{t}^2$$

Equation 4 (mass loss during ensiling *P. palmata*)

$$\mathrm{ML} = 108 + 3.81\mathrm{t} + 0.00676t^2$$

Where ML is, mass lost (g kg^{-1}) and t is time ensiled (d),

Ash Determination

The results for ash content analysis for *L. digitata* and *P. palmata* during ensiling are given in Figure 3 and show the effect of the number of days ensiled on ash content for both treated and untreated samples of the two seaweed species. The difference in percentage ash content between the two species is statistically significant ($P < 0.05$). The effect of number of days ensiled is not significant for *L. digitata* or *P. palmata*. There is no statistical difference in the ash content of macroalgae treated with *L. plantarum* versus the untreated samples.

Higher Heating Values and energy content

The effect of the number of days the macroalgae has been ensiled on the HHV is shown in Figure 4. The mean initial HHV for *P. palmata* was higher than for *L. digitata* (14.2 kJ g^{-1} and 11.9 kJ g^{-1} respectively). Overall, the ANOVA revealed that both the species ($P < 0.05$) and ensilage period ($P < 0.05$) had a statistically significant effect on HHV, but the effect of pre-ensiling treatment (spraying with a fresh culture of *L. plantarum*) was not significant. There was also, a statistically significant interaction between species and treatment with *L. plantarum* ($P < 0.05$), indicating that the effect of treatment on HHV is species dependent: the mean HHV was higher for treated *L. digitata,* 12.6 kJ g^{-1} compared to the untreated, 12.1 kJ g^{-1}. There is lower variability in the HHV data for material ensiled without the addition of *L. plantarum* (untreated) with the standard deviation being consistently lower (0.3) than that for treated material (0.9). The overall average HHV for *P. palmata* was higher for the untreated material (15.4 kJ g^{-1}) compared to the treated material (15.1 kJ g^{-1}), i.e. the reverse of that found for *L. digitata*.

Using the data in Figures 3 Figure and 4 the average HHV of the volatile solids (VS) or organic matter of the ensiled material was calculated (Figure 5). The average of the initial ensiled biomass energy remaining during ensilage was calculated using the data from Figure 1, Figure 2 and Figure 5 and the results are displayed as markers in Figure 6. Equation 1 did not produce well-fitted trend-lines. However, there was good agreement between the trend-lines (*) produced by Equation 2 and the data calculated from the experimental results for HHV, DW and mass losses (Figure 6)). The coefficient of determination (R^2), rate constants (k_1 and k_2) and proportion of slowly degraded biomass energy (P) are given in Table 2.

Discussion

The initial average ash content of *L. digitata* (24.3%) is similar to that previously reported for *L. digitata* (25.8%) (Ross et al. 2008). The ash content of *P. palmata* (18.0%) is towards the lower end of the typical ash content reported for *P. palmata* (12-35%) (Tiwari and Troy 2015). The ash content of seaweeds varies throughout the year (Tabassum et al. 2016a) and differences in ash content may be due to the time of year that the samples were collected and where they were collected from. The seaweeds in this study were collected from the seashore rather than cultivated offshore.

Dewatering and demineralisation are considered inherent features of ensiling terrestrial crops (Jones and Jones 1995). Herrmann et al. (2015) found that the ash content of biomass of five macroalgae species reduced after 90 days ensiling with the average ash of the macroalgae effluents exceeding that of the ensiled biomass by 74 g kg^{-1} total solids (TS). However, the results of the current study found no statistical different change in the ash content for *L. digitata* or *P. palmata* during ensiling. Milledge and Harvey (2016a) also found no significant change in the ash content of *Sargassum muticum* during ensilage, although there was a statistically significant loss of sodium chloride (salt). Salt loss was not measured during the current study. Low salt concentrations can stimulate microbial growth, but high salt concentrations ($\geq$10 g l^{-1}) are known to inhibit anaerobic systems through an increase of osmotic pressure or dehydration of methanogenic microorganisms (Lefebvre and Moletta 2006; Hierholtzer and Akunna 2012; Roberts et al. 2016). The composition and content of inorganic salts can also influence the product yields and bio-oil properties from thermal treatments (Ross et al. 2008; Rowbotham et al. 2013; Yanik et al. 2013). Low salt and sulphur feedstocks are favoured for both gasification and AD, and thus ensilage may yield downstream process benefits in biofuel production if salt and sulphur contents are reduced.

The macroalgae samples in this study were washed with seawater. In the study by Herrmann et al. (2015) the macroalgae samples were washed with cold tap water to remove adherent sand and impurities, but in the work by (Milledge and Harvey 2016a) the seaweed was not washed. These differences in pre-treatment could be a potential factor in the difference between the studies in the loss of inorganic material during ensiling. However, the species and environmental growth conditions may also have large effects. Further research is needed to study the effect of pre-treatment on ensiling of seaweed.

The initial average HHV of volatile solids for the baseline non-ensiled *L. digitata* is 15.7 kJ g^{-1} is lower than that reported by Ross et al. (2008), 17.6 kJ g^{-1}. This difference in initial HHV may be due to differences in the time of year when the macroalgae were harvested as the composition of macroalgae is known to change throughout the growing season (Black 1948; Adams et al. 2011a; Milledge and Harvey 2016a). The variation in relative chemical composition of macroalgae during the growing season will have implications for not only ensilage, but methods of energy production from macroalgae such as gasification and anaerobic digestion. More research is needed to establish the effect of seasonal composition changes in macroalgae on ensilage and subsequent processing.

The initial HHV of the organic matter of *P. palmata* is higher than *L. digitata*. This difference in HHV is likely to be due to differences in composition. The HHV of proteins and lipids are typically higher than those of carbohydrates (Merrill and Watts 1955; Heaven et al. 2011), and *P. palmata* has protein and lipid contents that are higher than those reported for *L. digitata* (Tiwari and Troy 2015).

The data for the change in HHV of the total solids of the biomass during ensiling (Figure 4) for treated and untreated *L. digitata* and *P. palmata* indicate that there is an initial increase in HHV followed by a decrease. The initial increase in HHV was at first thought to be due to a loss of inorganic matter, but there was no statistical different change in the ash content for *L. digitata* or *P. palmata* during ensiling. The change in HHV of the organic matter during ensiling for *L. digitata* and *P. palmata* (Figure 5) shows a similar early pattern to HHV for the total solids. Simple sugars (mono and disaccharides) have a lower HHV and are generally more rapidly broken down by microorganisms than complex carbohydrate, protein or lipid (Merrill and Watts 1955; Heaven et al. 2011; Kawai and Murata 2016), thus the initial increase in HHV of both the VS and TS could be due to the consumption of the readily available sugars by bacterial and residual seaweed respiration. Declining respiration rates in land plant silages have been shown to occur with cessation of respiration when the pH drops below 3.0 (McDonald 1981).

Ensiling of seaweed was found to have a statistical significant effect on HHV for *L. digitata* and *P. palmata.* Herrmann et al. (2015) found that concentration of C, N and H based on the TS content of the 5 seaweeds slightly increased after ensiling for 90 days, indicating a rise in

HHV with ensiling, but Milledge and Harvey (2016a) found no statistically significant difference in HHV of *S. muticum* non-ensiled and ensiled for 60 days. However, the data in the current study for *P. palmata* non-ensiled and ensiled for 63 days (Figure 4) (similar to period of ensilage used in the study by Milledge and Harvey (2016a)) shows a statistically significant difference with the average HHV increasing from 14.2 kJ g^{-1} to 15.9 kJ g^{-1} over the 63 day ensiling period. The data in the current study also shows a statistically significant effect for the interaction between species and ensilage on HHV, and therefore differences in the seaweed species and the ensiling period may be the reason for difference in the findings of Herrmann et al. (2015); Milledge and Harvey (2016a) and this study.

Although the percentage of dry matter increased for the two macroalgae species with time during ensiling, showing that they had become dryer due to the observed loss of leached liquid, the actual physical mass of the macroalgae left was also declining due to bacterial anaerobic respiration and volatilisation of low molecular weight fatty acids. Loss of mass (ML) from the seaweeds during ensilage was initially rapid with 24-46% of the overall total loss occurring in the first 31 days of 365 day ensiling period. This is a similar pattern to that found in ensiling high moisture content terrestrial crops (~85% moisture) where the major loss occurs in the first 26 days with peak flow of leachate typically occurring around 10 days post ensiling (Gebrehanna et al. 2014).

The percentage of original biomass energy remaining after ensilage for *L. digitata* and *P. palmata***,** calculated from percentage dry matter, dry matter loss and HHV (Figure), shows that there is a rapid energy loss during the initial stage of ensilage for both species followed by a more gradual loss reflecting the pattern for dry matter losses found in this study and the study by Herrmann et al. (2015). *P. palmata*, which although having a higher HHV than *L. digitata*, has a more rapid rate of mass lost over the one year storage period. There appears to be considerable variation between species in terms of overall energy loss. The energy losses from the Rhodophyceae *P. palmata* (38-44%) are considerably higher than those from the Phaeophyceae *L. digitata* (21-22%). The genetic class of the seaweed may influence the changes occurring after ensiling. Herrmann et al. (2015) studied the ensiling of 5 species of seaweed, and although the HHV was not measured, considerable difference were found in both TS and VS losses between algal species ensiled for 90 days. The energy loss for the Phaeophyceae, *S. muticum*, was less at ≤ 8% for an ensiling period of 60 days (Milledge and Harvey 2016).

The HHV of the ensiled wet biomass will increase as the macroalgae become drier, but as the actual mass of the macroalgae reduces, the energy available from each kilogram of wet macroalgae originally ensiled will decline (21-44% depending on the species ensiled) to such an extent that, subject to the production costs entailed, it will be uneconomic to store the material further. There will be an economic cut-off of storage time compared to energy loss during ensilage. Data from commercial seaweed farms are only available on a very limited scale (Dijk and Schoot 2015), and although here the rate of mass lost for both *L. digitata* and *P. palmata* was calculated the lost monetary value of declining mass cannot currently be calculated. However, this work lays the foundation of a storage/energy loss model. There is a need for more quantitative data on all parts of the seaweed biofuel process especially at scale. However, the losses of energy content during a year in ensiled storage are still considerably below the energy required to dry seaweed which is equivalent to ~80% of the energy content of the seaweed biomass (Milledge et al. 2015).

Although the total carbohydrate content of *Laminaria* (31-61%) and *Palmaria* (38-74%) are similar (Tiwari and Troy 2015). There are considerable differences in the primary and storage carbohydrates (Percival 1979; Kraan 2012; Tiwari and Troy 2015). The main polysaccharides of brown seaweeds are alginate, laminarin, fucans and cellulose with the primary storage reserve carbohydrate being laminarin. In red algae the predominant polysaccharides are agars and carrageenans with the primary reserve carbohydrate being floridean starch (Tiwari and Troy 2015). There are also considerable differences in the resistance of these polysaccharides to bacterial breakdown and the monosaccharide produced (Lobban and Wynne 1981; Roesijadi et al. 2010; Kawai and Murata 2016). These variations in carbohydrates and differences in their binding ability and breakdown during ensiling may be the potential reasons for the differences observed in the friability of pellets formed from the ensiled biomass of the two species of seaweed studied.

First order rate equations do not describe the energy loss from seaweed biomass during ensilage due to the heterogeneous nature of seaweed and differences in the resistance of the chemical components of seaweed to bacterial breakdown. A better expression of energy loss during ensiling was obtained by using two first rate expressions, one for the rapidly ‘degrading’ material and another for the slower ‘degrading’ fraction. The difference in the

saccharide composition may be part of the reason for the differences in energy losses and rate constants in Equation 2 for *P. palmata* compared to *L. digitata.* However, energy loss from seaweed during ensiling is not only the result of the destruction of organic matter by anaerobic bacteria, but also effluent losses (Herrmann et al. 2015; Milledge and Harvey 2016a). Moreover, changes in the activity of the microbiota during ensiling will cause variations not only in the organic compounds broken down, but also those produced. Nevertheless, the energy losses from ensiling seaweed can be described by a relatively simple equation formed from two first rate expressions. Further research is required to interpret the equation and the various components of it.

Both *L. digitata and P. palmata.* achieved a pH <4.3, recommended for grass silage (Genever 2011), by day 31 of ensiled storage. However, due to the high water content of seaweed silage, relative to typical terrestrial forage crops, the pH required in seaweed ensilage to completely inhibit *Clostridial* fermentation and the production of butyric acid may be lower than that recommended for grass. Final pH values in this study were considerably lower, pH 3.2-3.4 for *L. digitata* and pH 3.5-4.0 for *P. palmata,* than those found in other studies of seaweed ensiling, 4.7 (Black 1955), 4-5.7 (Herrmann et al. 2015) and 4.9-5.1 (Milledge and Harvey 2016a). This study found a statistically significant effect of species on pH, and the differences in final pH found between this study and others may be due to the species of seaweed studied, but further work is required to ascertain the exact biochemical changes and resultant pH changes occurring in ensiling for various species of seaweed.

The addition of *Lactobacillus, such as L. plantarum*, enhances the silage making process in terrestrial crops with a more rapid pH reduction and a more stable product (Davies et al. 1998; Wang et al. 2014). This process is used commercially, and proprietary strains and mixture of *Lactobacillus* are routinely applied to land based forage crops in silage making. In this present work the pH, one of the main indicators of the quality of the ensiling process, after 30 and 365 days, for both species of seaweed studied is less for the treated samples, and therefore the use of *L. plantarum* results in a lower pH throughout the storage period of the silage, resulting in a preserved macroalgae biomass with potentially greater overall stability. Specific *Lactobacillus* strains have been examined with the purpose of improving the fermentation of land grown silage crop and the inhibition of the growth of spoilage microorganisms (Santos et al. 2013), and further work on the use of other silage starter cultures is required to find the most suitable for seaweed ensilage.

In conclusion; this study found that there were significant changes in HHV of the biomass during ensiling of seaweed, despite no statistical different changes in the ash content for *L. digitata* or *P. palmata* during ensiling. The ensiling process and leachate production brings about changes in the relative organic composition of some macroalgae species during ensilage. Thus the mass and energy loss during ensilage of seaweed varies with species, and can be considerable. However, the HHV of the material remained relatively constant after day 31 post ensiling, and importantly it was the loss of mass over time from the ensiled seaweed which reduced the energy available per kg of seaweed originally ensiled. This will have an impact on species selection, waste management and the economic and energetic viability of a continuous macroalgal biofuel process. However, it should be noted that the energy losses during ensilage are less than energy required for drying seaweed, and ensilage may be a viable technique for the preservation of seaweed in temperate climates for the production of bioenergy by wet processes such as anaerobic digestion and fermentation.

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References

Adams JMM, Ross AB, Anastasakis K, Hodgson EM, Gallagher JA, Jones JM, Donnison IS (2011a) Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion. Bioresource Technol 102:226-234

Adams JMM, Toop TA, Donnison IS, Gallagher JA (2011b) Seasonal variation in *Laminaria digitata* and its impact on biochemical conversion routes to biofuels. Bioresource Technol 102:9976-9984

Ashbell G, Weinberg ZG (2005) Silage production and utilization. FAO, Bet Dagan, Israel

Black WAP (1948) The seasonal variation in chemical constitution of some of the sub-littoral seaweeds common to Scotland. Part III. *Laminaria saccharina* and *Saccorhiza bulbosa.* J Soc Chem Ind 67:172-176

Black WAP (1955) The preservation of seaweed by ensiling and bactericides. J Sci Food Agric 6:14-23

BSI (2009) Solid biofuels -determination of ash content.

BSI (2010) Determination of the gross heat of combustion (calorific value)

Chen H, Zhou D, Luo G, Zhang S, Chen J (2015) Macroalgae for biofuels production: progress and perspectives. Renewable and Sustainable Energy Rev 47:427-437

Davies DR, Merry RJ, Williams AP, Bakewell EL, Leemans DK, Tweed JK (1998) Proteolysis during ensilage of forages varying in soluble sugar content. J Dairy Sci 81:444-453
Di Cagno R, Minervini G, Rizzello CG, De Angelis M, Gobbetti M (2011) Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. Fd Microbiol 28:1062-1071
Dijk Wv, Schoot JRvd (2015) An economic model for offshore cultivation of macroalgae. EnAlgae project,, Swansea
Edwards M, Watson L (2011) Aquaculture explained cultivating *Laminaria digitata*. Irish Sea Fisheries Board, Galway
Gebrehanna MM, Gordon RJ, Madani A, VanderZaag AC, Wood JD (2014) Silage effluent management: A review. J Environ Manage 143:113-122
Genever L (2011) Making grass silage for better returns. Agriculture and Horticulture Development Board (AHDB), Huntingdon
Heaven S, Milledge J, Zhang Y (2011) Comments on 'Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable'. Biotechnol Adv 29: 164-167
Herrmann C, FitzGerald J, O'Shea R, Xia A, O'Kiely P, Murphy JD (2015) Ensiling of seaweed for a seaweed biofuel industry. Bioresour Technol 196:301-313
Hierholtzer A, Akunna JC (2012) Modelling sodium inhibition on the anaerobic digestion process. Water Sci Technol 66:1565-1573
Jones DIH, Jones R (1995) The effect of crop characteristics and ensiling methodology on grass silage effluent production. J Agr Eng Res 60:73-81
Kawai S, Murata K (2016) Biofuel production based on carbohydrates from both brown and red macroalgae: recent developments in key biotechnologies. Int J Mol Sci17:145
Kelly MS, Dworjanyn S (2008) The potential of marine biomass for anaerobic biogas production a feasibility study with recommendations for further research. The Crown Estate, Scotland
Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: Site and species selection. Biomass and Bioenergy 80:229-242
Kraan S (2012) Algal polysaccharides, novel applications and outlook. In: Chang C-F (ed) Carbohydrates - comprehensive studies on glycobiology and glycotechnology. vol 22. InTech, Rijeka, Croatia.
Kraan S (2013) Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. Mitig Adapt Strategies Glob Chang 18:27-46
Lee MH (1977) Studies on the feed value of seaweed silage for the exploitation of feedstuff resources. Korean J Animal Sci 19:91-94
Lefebvre O, Moletta R (2006) Treatment of organic pollution in industrial saline wastewater: a literature review. Water Res 40:3671-3682
Lobban CS, Wynne MJ (eds) (1981) The biology of seaweeds vol 17. Botanical monographs Blackwell Scientific, Oxford
McDonald P (1981) The biochemistry of silage. Wiley, Chichester
Merrill AL, Watts BK (1955) Energy values of foods: basis & duration, slight revised February 1973. Agricultural Handbook vol 74. US Department of Agriculture, Washington, DC
Milledge JJ, Harvey PJ (2016a) Ensilage and anaerobic digestion of *Sargassum muticum* J Appl Phycol:1-10 doi:10.1007/s10811-016-0804-9
Milledge JJ, Harvey PJ (2016b) Potential process ‘hurdles’ in the use of macroalgae as feedstock for biofuel production in the British Isles. J Chem Technol Biotechnol 91:2221-2234

Milledge JJ, Smith B, Dyer P, Harvey P (2014) Macroalgae-derived biofuel: A review of methods of energy extraction from seaweed biomass. Energies 7:7194-7222
Milledge JJ, Staple A, Harvey P (2015) Slow pyrolysis as a method for the destruction of Japanese Wireweed, *Sargassum muticum*. Environ Nat Resources Res 5:28-36
Murphy J, Baxter D (eds) (2013) The biogas handbook science, production and applications. Woodhead Publishing, Oxford.
Neushul P (1989) Seaweed for war: California's World War I kelp industry. Technol Culture 30:561-583
Oude Elferink SJWH, Driehuis F, Gottschal JC, Spoelstra SF (1999) Silage fermentation processes and their manipulation. Paper presented at the FAO Electronic Conference on Tropical Silage, http://www.fao.org/waicent/faoinfo/agricult/agp/agpc/gp/silage/pdf/paper2.pdf
Pabón Pereira CP, Zeeman G, Zhao J, Ekmekci B, van Lier JB (2009) Implications of reactor type and conditions on first-order hydrolysis rate assessment of maize silage. Water Sci Technol 60:1829-1836
Percival E (1979) The polysaccharides of green, red and brown seaweeds: their basic structure, biosynthesis and function. Brit Phycol J. 14:103-117
Rajkumar R, Yaakob Z, Takriff MS (2014) Potential of the micro and macro algae for biofuel production: a brief review. BioResources 9:1606-1633
Rittmann BE, McCarty PL (2001) Environmental biotechnology: principles and applications. McGraw-Hill, Singapore
Roberts KP, Heaven S, Banks CJ (2016) Quantification of methane losses from the acclimatisation of anaerobic digestion to marine salt concentrations. Renew Energy 86:497-506
Roesijadi G, Jones SB, Snowden-Swan LJ, Zhu Y (2010) Macroalgae as a biomass feedstock: a preliminary analysis. U.S. Department of Energy, Washington
Ross AB, Jones JM, Kubacki ML, Bridgeman T (2008) Classification of macroalgae as fuel and its thermochemical behaviour. Bioresource Technol 99:6494-6504
Rowbotham JS, Dyer PW, Greenwell HC, Selby D, Theodorou MK (2013) Copper(II)-mediated thermolysis of alginates: a model kinetic study on the influence of metal ions in the thermochemical processing of macroalgae. Interface Focus 3:20120046
Santos AO, Avila CLS, Schwan RF (2013) Selection of tropical lactic acid bacteria for enhancing the quality of maize silage. J. Dairy Sci 96:7777-7789
Shinya Y, Yukihiko M (eds) (2008) The Asian biomass handbook -a guide for biomass production and utilization. The Japan Insitute of Energy, Tokyo
Tabassum MR, Xia A, Murphy JD (2016a) The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel. Bioresource Technol 209:213-219
Tabassum MR, Xia A, Murphy JD (2016b) Seasonal variation of chemical composition and biomethane production from the brown seaweed *Ascophyllum nodosum* Bioresource Technol 216:219-226
Tiwari B, Troy D (eds) (2015) Seaweed sustainability: food and non-food applications. 1 edn. Academic Press, Amsterdam
Uchida M, Miyoshi T (2013) Algal fermentation-the seed for a new fermentation industry of foods and related products. Jarq-Japan Agric Res Quart 47:53-63
Uzir MH, Mat Don M (2007) Biochemical engineering - A concise introduction. University Sains Malaysia, Penang
Wang MS, Yang CH, Jia LJ, Yu KF (2014) Effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation characteristics and aerobic stability of whipgrass silage in laboratory silos. Grassl Sci 60:233-239

Yanik J, Stahl R, Troeger N, Sinag A (2013) Pyrolysis of algal biomass. J Anal Appl Pyrolysis 103:134-141

Tables and figures

Table 1 Overall mean and standard deviation (S.D.) and species means for percentage dry mass (DM%) and mass lost from the samples over the ensiling time (ML, g kg^{-1} ensiled) for *Laminaria digitata* (LD) and *Palmaria palmata* (PP) (Numbers with different superscripts within columns are significantly different (P<0.01).

	Overall by Species			Overall by Treatment			Overall species by Treatment				
								LD		PP	
		Mean	S.D.	Treat	Mean	S.D.	Treat	Mean	S.D.	Mean	S.D.
DM%	LD	22.4 $^{\gamma}$	3.88	T	22.6 $^{\alpha}$	5.35	T	23.2 $^{\alpha}$	0.870	22.2 $^{\alpha}$	1.11
	PP	22.0 $^{\gamma}$	4.85	U	21.4 $^{\alpha}$	3.90	U	21.9 $^{\alpha}$	0.600	21.9 $^{\beta}$	0.89
ML (g kg^{-1})	LD	219 $^{\gamma}$	153	T	298 $^{\alpha}$	184	T	241 $^{\alpha}$	155	357 $^{\alpha}$	196
	PP	376 $^{\delta}$	194	U	292 $^{\alpha}$	198	U	200 $^{\alpha}$	139	398 $^{\alpha}$	194

Table 2 The coefficient of determination (R^2), rate constants (k_1 and k_2) and proportion of slowly degraded biomass energy (P) for equation 2 to fit the data in Figure 6

Sample	K_1	P	K_2	R^2
	d^{-1}		d^{-1}	
LD T	0.8	92%	0.0004	0.9
LT U	0.3	88%	0.0004	0.7
PP T	0.1	67%	0.0004	0.9
PP U	0.1	66%	0.0005	0.9

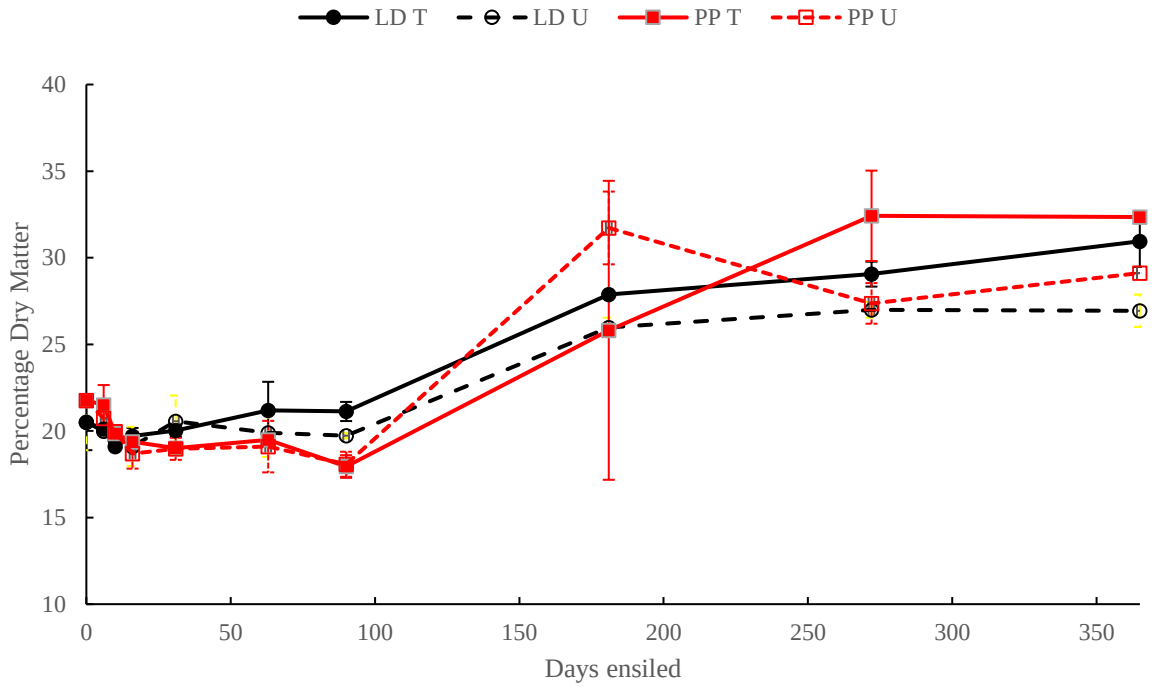

Figure 1 Percentage dry mass of ensiled macroalgae samples of *Laminaria digitata* (LD) and *Palmaria palmata* (PP) over a 365 day storage period, where T indicates samples sprayed with a fresh culture of *L. plantarum* and U indicates untreated macroalgae. Error bars: S.D (n=3)

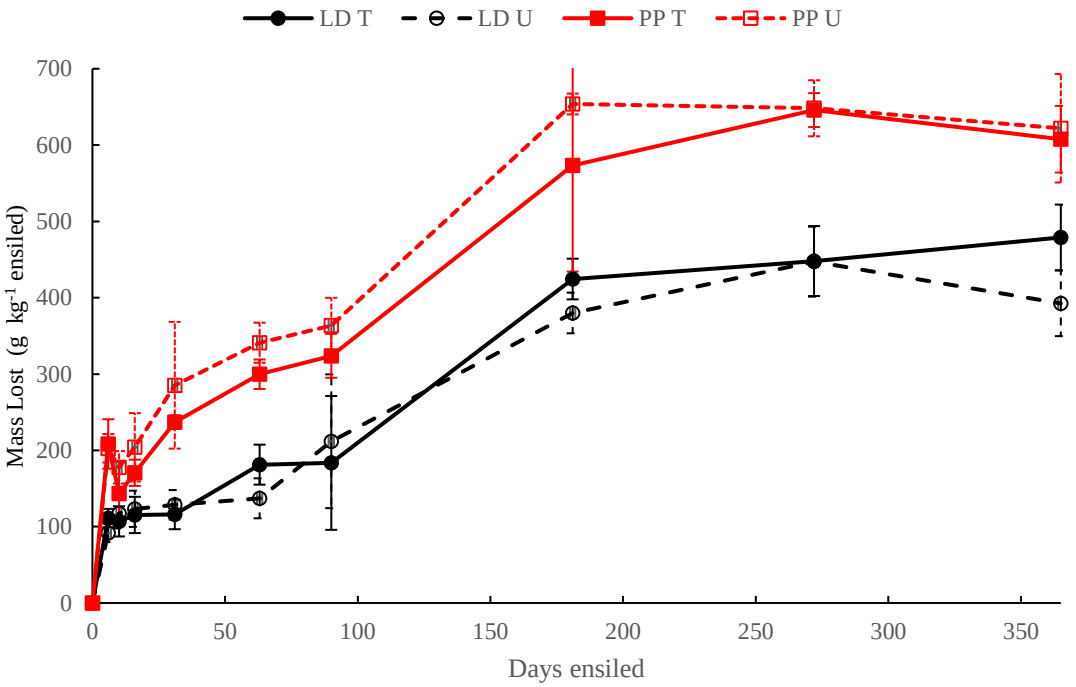

Figure 2 Mass lost (g kg^{-1} ensiled) from ensiled macroalgae samples of *Laminaria digitata* (LD) and *Palmaria palmata* (PP) over a 365 day storage period, where T indicates samples sprayed with a fresh culture of *L. plantarum* and U indicates untreated macroalgae. Error bars: S.D. (n=3).

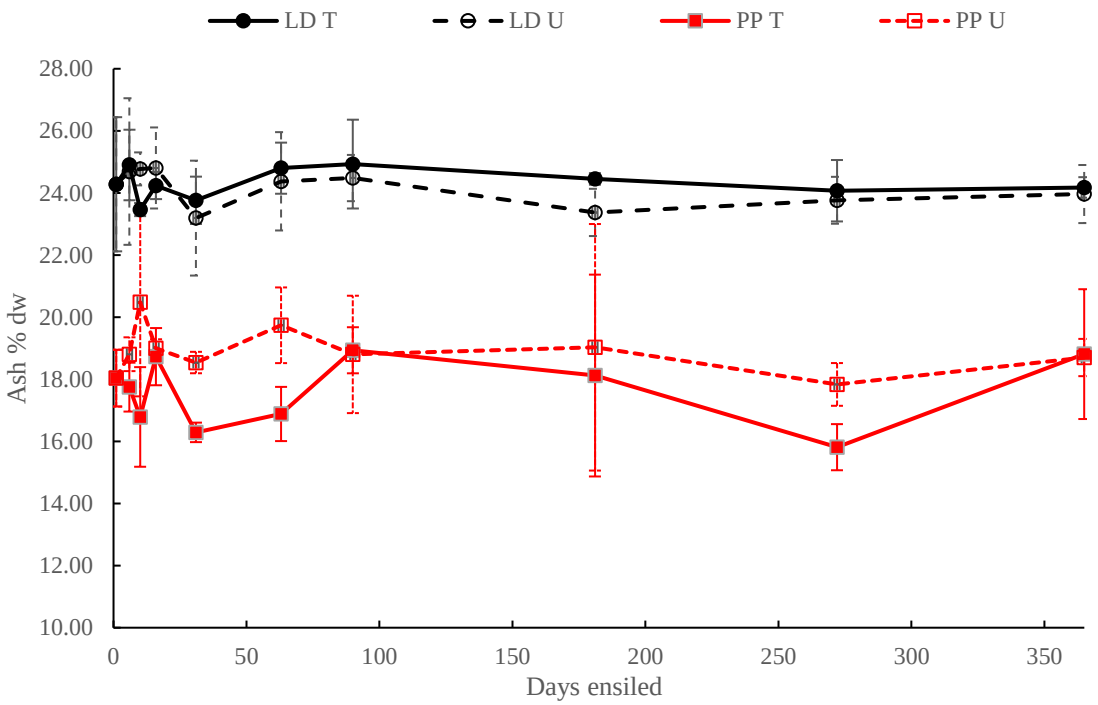

Figure 3 Changes in ash content during ensiling Changes in ash content of ensiled macroalgae samples of *Laminaria digitata* (LD) and *Palmaria palmata* (PP) over a 365 day storage period, where T indicates samples sprayed with a fresh culture of *L. plantarum* and U indicates untreated macroalgae. Error bars: S.D. (n=3*2)

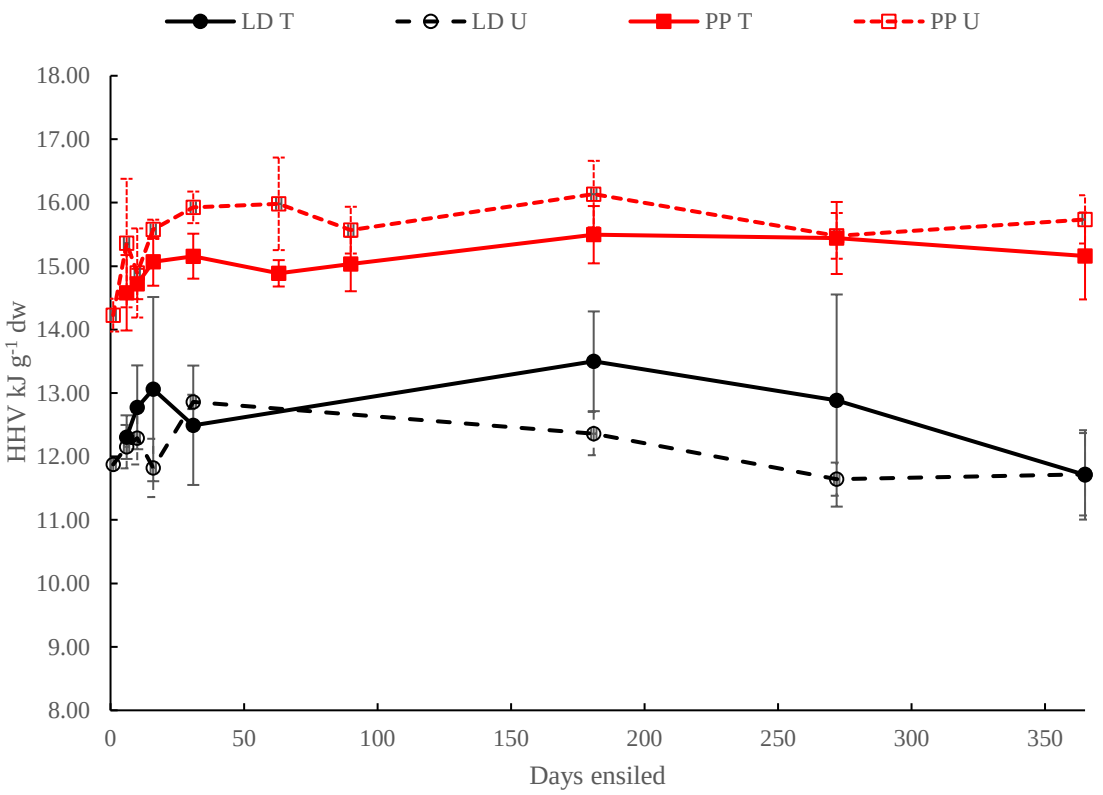

Figure 4 HHV of biomass of ensiled macroalgae samples of *Laminaria digitata* (LD) and *Palmaria palmata* (PP) over a 365 day storage period, where T indicates samples sprayed with a fresh culture of *L. plantarum* and U indicates untreated macroalgae. Error bars: S.D. (n=3*2)

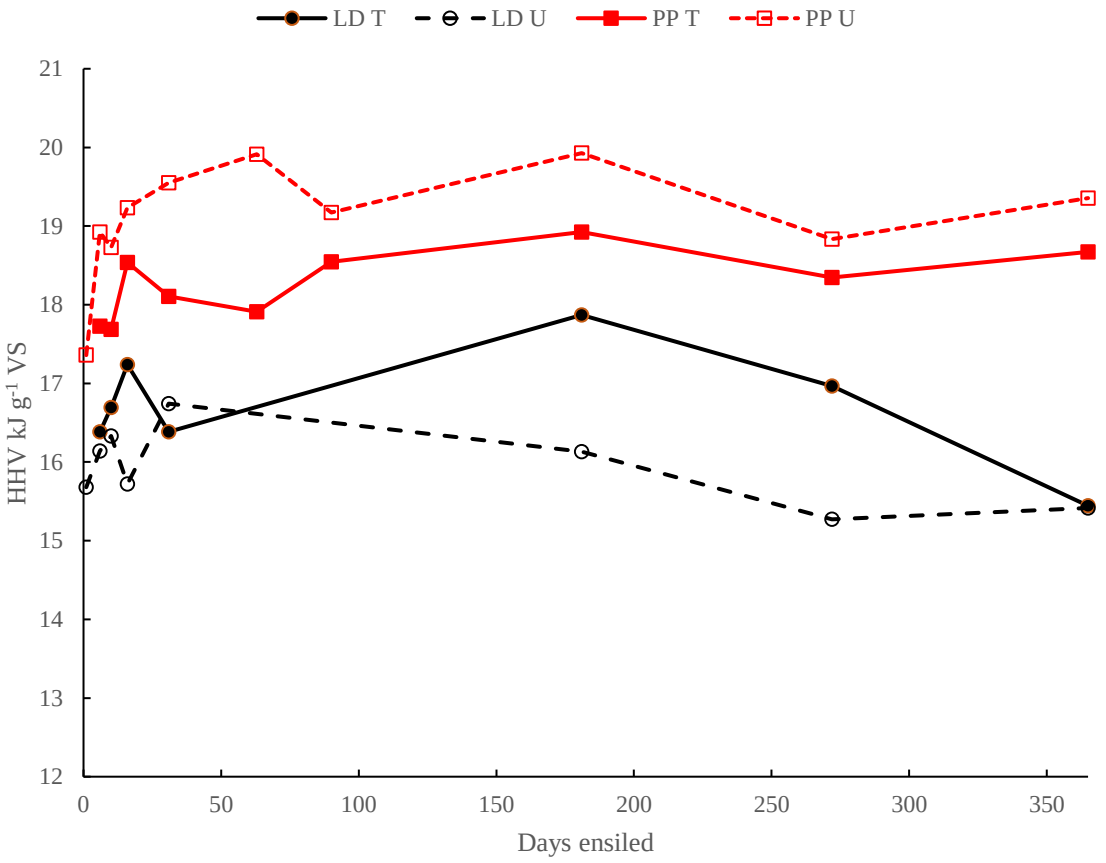

Figure 5 HHV of organic matter in biomass (VS)) of ensiled macroalgae samples of *Laminaria digitata* (LD) and *Palmaria palmata* (PP) over a 365 day storage period, where T indicates samples sprayed with a fresh culture of *L. plantarum* and U indicates untreated macroalgae.

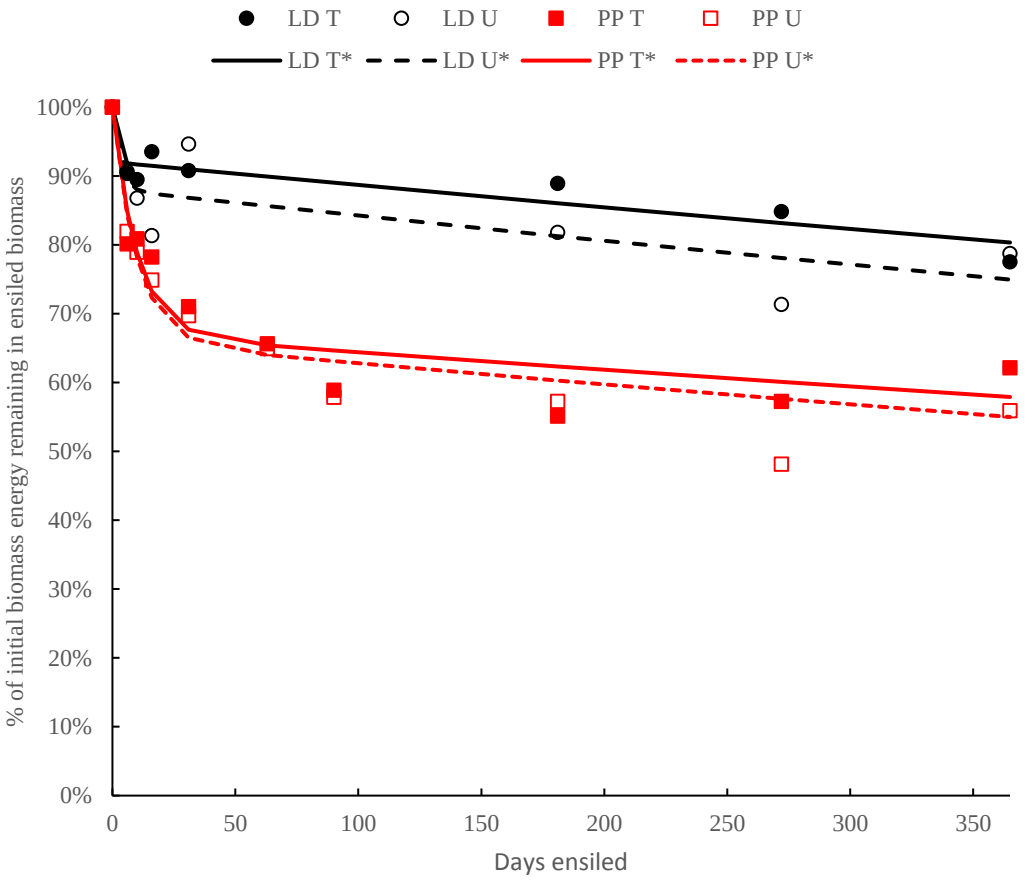

Figure 6 Percentage of initial biomass energy remaining in ensiled macroalgae samples of *Laminaria digitata* (LD) and *Palmaria palmata* (PP) over a 365 day storage period, where T indicates samples sprayed with a fresh culture of *L. plantarum* and U indicates untreated macroalgae. The trend-lines derived from Equation 2 are indicated by *.