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The effect of mechanical pre-processing and different drying methodologies on bioethanol production using the brown macroalga *Laminaria digitata* (Hudson) JV Lamouroux

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Abstract Macroalgae are capable of generating more organic carbon per hectare than terrestrial plants without requiring land, fertiliser or fresh water to grow. In addition, they avoid the food versus fuel argument as they are not a major food source in Europe. In spite of these benefits, macroalgae are not yet fully exploited as a biomass source for bioenergy or platform chemical production in Europe, with one issue being the high harvesting and processing costs. This paper considers the impact of mechanical pre-processing of *Laminaria digitata* combined with different drying techniques and the effect of these on downstream processing to bioethanol. Results show that mechanically screw pressing macroalgae does enhance conversion to ethanol, but only when the material contains low levels of storage carbohydrates. This occurs in freeze-dried and air-dried samples. The addition of a press aid in the mechanical pre-processing step increases ethanol yields per gramme macroalgae, but due to the presence of the unutilised press aid in the fermentation, ethanol yields were lower overall. The two main findings from this work were (1) simple mechanical processing of *L. digitata* provides homogenisation and pumpability of macroalgae without negatively affecting subsequent microbial conversion to ethanol. (2) At higher carbohydrate concentrations, screw pressing confers no advantage in ethanol yields over strips of unprocessed kelp, making strips the more viable conversion option for low-input, large-scale processing.

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Introduction

Marine biomass includes both macro- and microalgae, both of which are capable of generating more organic carbon per hectare than terrestrial plants (Gao and McKinley 1993; John et al. 2011) and have been identified as potential bioconversion feedstocks (Lardon et al. 2009; Wargacki et al. 2012). This paper focuses on one of the main kelp species growing around the UK coastline (Black 1950), the large, fast-growing macroalgal kelp *Laminaria digitata* as an example biomass feedstock. Previous work by the authors identified substantial changes in *L. digitata* composition due to seasonal variation (Adams et al. 2011a), with July samples containing the maximum proportion of utilisable carbohydrates (Adams et al. 2011b). These compounds include the predominantly β-1,3 glucose polymer laminarin (Nelson and Lewis 1974) and the alcohol sugar mannitol (Horn 2000), both of which can be readily hydrolysed and converted by microbes into a number of products including biofuels and platform chemicals (Suganya et al. 2016). One such a highly researched conversion product, bioethanol (Horn et al. 2000a, b; Adams et al. 2009; Yanagisawa et al. 2011), will be used in this study to assess overall process improvement.

Considerations for processing macroalgal biomass at scale do not appear to be addressed frequently in academic literature. Manns et al. (2016) identifies ultracentrifugal milling which produces reproducible <0.5-mm-diameter particles (Yanagisawa et al. 2011) as being too energy-consuming for large-scale seaweed biorefining and instead focussed on the effect of a less energy-intensive wet milling process for glucose release from *L. digitata* following enzymic

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saccharification. They found that due to the thin structure of the macroalgal blade, the seaweed was cut in a different manner to lignocellulosic material such as straw. Rather than causing a three dimensional defibrillation of the biomass, the macroalgal blade only received a two dimensional disruption when milling at 1- and 2-mm distances as the mill scissioned the blade surface with the depth predominantly untouched. This means that though particle size decreases as milling distance reduces, the expected reduction in a surface area is less than that of three dimensionally reducing lignocellulosic biomass (Manns et al. 2016). One outcome from this work is a reminder that macroalgae is fundamentally different to terrestrial biomass, so processes and expectations applied to terrestrial biomass are not always relevant to this feedstock.

The purpose of this research was to assess simple, scaleable, mechanical processing options for kelps to maximise sugar release and product generation capable of scale up. This builds on published research (Adams et al. 2015) which identified the washing of all types of macroalgae to be a common practice in laboratory scale experiments on macroalgae, with examples including Bruhn et al. (2011), Wang et al. (2011), Meinita et al. (2012) and Park et al. (2012). On an industrial scale, the use of large quantities of fresh water and the logistics regarding handling could benefit some species and downstream processes, but as it also removes glucose and subsequently has a negative effect on ethanol yields (Adams et al. 2015) it is important that more data regarding unwashed macroalgae should be available to inform both industry and academia.

In the reported work below, two studies are presented. The first, using material harvested in July 2013, assessed the use of a screw press to homogenise and macerate the harvested macroalgae and produce an algal preparation which could be pumped either on to shore or to a processing facility, avoiding transport and handling logistics. A subsample of the macroalgae was cut to approximately 15 cm lengths and frozen; the majority of the material was either screw pressed alone or mixed with a press aid of dried, chipped *Miscanthus giganteus* to study the impact of greater maceration. *M. giganteus* is an Asian perennial grass which is classed as an 'energy crop', a high-yielding plant with a low moisture content at harvest (Meehan et al. 2013) capable of growing well on suboptimal land with low fertiliser demands (Robson et al. 2013). A press aid is typically a low-cost, fibrous material added to soft, high-moisture-content biomass such as fruit prior to pressing. In a commercial setting, the inclusion of a press aid allows the separation of juice rather than sauce to be extracted from the fruit (Vincent Corporation 2008). Average particle sizes from both screw pressed samples were analysed; average-sized pieces for each press were then cut from defrosted blades. Samples of all processed materials and blade strips as controls were freeze-dried and used as feedstocks in a fermentation study.

The second study presented compares different drying methods for screw pressed and strips of *L. digitata*. This was conducted on material from a second harvest, made in July 2016. Material from this harvest was either dried at 30 °C in a fan oven, mimicking air-drying, or freeze-dried. Freeze-drying cannot be a large-scale option for processing macroalgae as it is the most expensive process for dehydrating biomass, but it does maintain the quality of the original product which is lost with other drying methods (Ratti 2001). This was therefore used to provide analytical comparisons to the air-dried samples.

Materials and methods

Sample collection and preparation Blades from *Laminaria digitata* were harvested offshore from Aberystwyth beach, Ceredigion, UK (ordnance survey reference SN 581823) in July 2013 and July 2016.

For *L. digitata* harvested in July 2013, the material was cut to 15 cm lengths and separated with one third frozen within 1 h of harvest. The other two thirds were processed with a CP-4 screw press (Vincent Corp., Tampa, FL, USA) with one third processed 'as is' and the last third processed with the addition of a press aid of dried, chipped *Miscanthus giganteus.* This was added at a wet ratio of 10–15 *L. digitata*:1 press aid (54 *L. digitata*:46 press aid by dry weight). Once screw pressed, material was frozen at −20 °C prior to further processing.

For *L. digitata* harvested in July 2016, the material was cut to 15 cm lengths as before and half screw pressed as above without any press aid added. Both strips and screw pressed material were split with proportions of each dried in a drying oven (Unitherm, Russell-Lindsey Engineering Ltd., Birmingham, UK) at 30 °C for 3 days, turning twice a day or lyophilised in a freeze-drier (VirTis Company, USA).

Calculation of screw pressed material area, cut sample preparation and relative press aid addition to the screw pressed *L. digitata* Samples of screw pressed material from July 2013 prepared with and without the press aid were defrosted and measured to determine the length and width (mm) of 50× randomly selected *L. digitata* pieces from each process. These values were then used to calculate the average seaweed particle size for the two screw pressed samples. Previously frozen *L. digitata* strips were then defrosted and cut using a scalpel to the mean sizes for each screw press process. Separately, approximately 100 g sample of the screw pressed material containing press aid was manually separated into each fraction and then dried for 24 h at 75 °C to obtain dry weights for each fraction. The correct proportion of press aid was then added to the smaller *L. digitata* cut pieces prior to its inclusion in the fermentation study.

Fermentation procedure All fermentations were conducted in 250-mL Erlenmeyer flasks capped with foam bungs and stirred using magnetic stirrers on 2× Variomag multipoint 15 stirrers (Fisher Scientific, UK) within large incubators set at 30 °C. The fermenting yeast was *Ambrosiozyma angophorae*, formerly *Pichia angophorae*, (strain 5830, CBS-KNAW, Utrecht, the Netherlands). It was previously cultivated on yeast and mould agar plates (Oxoid) for 72 h before being harvested with small volumes of deionised water into a central collection tube to give an absorbance from a 1:1000 dilution of 0.30 ± 0.05 at 600 nm using a cell density meter (CO8000, WPA Biowave, Biochrom, Cambridge UK). Each fermentation slurry contained 4 g dried *L. digitata* substrate, previously adjusted to pH 4.0 using HCl, 0.4 U laminarinase (*Trichoderma* sp., Megazyme) and 0.5 mL yeast preparation (*A. angophorae* as detailed above) to give a final reaction volume of 200 mL in each flask. All fermentations were run for 72 h with staggered sampling time points taken throughout the period. For each time point, 0.8 mL samples were removed from each flask and heated to 100 °C for 10 min in a hot block. Samples were then frozen prior to analysis.

Post-fermentation analysis by HPLC and ethanol kit Fifty microlitres of each sample for analysis was added to 950 μL 5 mM H_2SO_4 containing 5 mM crotonic acid as an internal standard. Following mixing, this was filtered through a 0.45-μm PVDF Duropore filter (Millex-HV, Millipore, USA) and run through a Rezex ROA organic acid H^+ column at 35 °C with 5 mM H_2SO_4 as the mobile phase at 0.6 mL min^{-1} (Jasco, UK). Concentrations of compounds of interest including polymeric sugars, mannitol and ethanol were determined by refractive index detector and the HPLC software (EZChrome Elite version 3.2, Agilent Technologies, USA) collaborated with a range of standards. Further calculations were subsequently carried out using Excel 2013 (Microsoft). To ensure accurate values were attained though comparative analysis, an ethanol assay kit (Megazyme) was also employed on fermentation samples with methods as per supplier instructions for microplate analysis.

Determination of glucose and laminarin content Soluble laminarin in *L. digitata* was determined with aliquots of macroalgae fermentation slurries pre- and post-fermentation (0 and 72 h) prepared in duplicate with and without 0.1 U laminarinase added (Megazyme). These were prepared at a 0.5 mL final volume of succinic acid buffer (50 mM, pH 4.5) and incubated at 40 °C, 150 rpm, for 2 h to ensure complete conversion of the laminarin to glucose followed by heating to 100 °C for 10 min to ensure assay termination. The released glucose was measured using a GOPOD enzyme assay kit (Megazyme) and any initially present subtracted. Using the assumption of an average 25° of polymerisation in laminarin (Nelson and Lewis 1974), meaning that the average laminarin molecule consists of 25 sugar units (of which an average of 24.5 would be glucose and 0.5 would be mannitol), the molecular weight for laminarin was determined and used to determine the laminarin content from the glucose release value.

Statistical analysis Basic analysis was conducted using Excel 2013 (Microsoft). Univariate and multivariate analyses including post-hoc Tukey honest significant difference (HSD) analysis were conducted using the software programme IBM SPSS statistics (version 22, IBM) following initial data manipulation using Excel 2013 (Microsoft).

Results

Screw pressing study Screw pressing macroalgae is a simple mechanical pre-processing technique to produce a pumpable product with improved transportation properties. Typically, screw pressing is employed as a dewatering process, but for *L. digitata* in this context, screw pressing was unsuccessful in all pressings <10% of water and particulates were separated from 5 kg samples of material processed with or without a press aid (D. Thomas, personal communication). However, the screw press did operate as intended for this study, successfully macerating and homogenising the macroalgae both alone and with a press aid. Material from the first harvest (July 2013) which was screw pressed with and without a press aid was examined, and 50 randomly selected macroalgae fragments from both pressings were removed. Measurements to determine their length and width allowed the average area for both processes to be calculated and used to determine that with the press aid, the fragments generated were >60% smaller by a mean area. These values and related standard errors are seen in Table 1.

Lengths of *L. digitata* blade frozen as control material were defrosted, cut to the average sizes of the two screw pressed macroalgae fragment sizes and refrozen before being freeze-dried with material from both screw pressed processes, strips of blade and the press aid. The ratio of *L. digitata* to press aid was also calculated on a dry weight basis following physical separation of approximately 100 g wet material to give a 1.18

Table 1 Average length, width and area of July 2013 harvested *L. digitata* fragments following screw pressing with and without a press aid present

	L. digitata	*L. digitata* and press aid
Average length (mm)	39.76 ± 3.26	25.12 ± 2.04
Average width (mm)	12.07 ± 1.14	7.85 ± 0.77
Average area (mm^2)	588.59 ± 98.93	220.32 ± 35.53

n = 50, ± = standard error

L. digitata:1 press aid ratio. Together, this data allowed the preparation of fermentation slurries containing both screw pressed products and reproductions of both screw pressed products using cut macroalgae pieces with one also containing the press aid added at the correct ratio. The ethanol yields following the fermentations are shown in Fig. 1 and show significant differences between them ($P < 0.01$). The highest ethanol yields were seen in the slurries which contained the strips of blade, screw pressed material and screw pressed material containing the press aid. Ethanol from *L. digitata* which was cut from strips to the average size of both screw pressed material, with and without additional *M. giganteus* added at the same ratio as when pressed, gave lower ethanol yields. The cut pieces with press aid produced an ethanol yield which was significantly lower than the screw pressed samples, as seen by the lower case letters in Fig. 1 denoting significant differences using Tukey HSD. The press aid *M. giganteus* fermented alone gave no ethanol yields at any of the sampling time points (data not shown).

Drying treatment study Using the second harvest (July 2016) collection of *L. digitata*, a comparison of screw pressed macroalgae and strips was conducted in triplicate following air-drying and freeze-drying treatments to give <10% moisture content in all samples to stabilise them and to reduce variation between samples and treatments. Ethanol yields following the fermentation of these samples are shown in Table 2.

Significant differences were seen in ethanol concentrations between the differently dried and processed treatments ($P < 0.05$), with air-dried strips of *L. digitata* producing the lowest ethanol yields and the significantly different freeze-dried screw pressed fermentations producing the highest. For both air-dried and freeze-dried fermentations, there was a substantial increase in mean ethanol yields due to screw pressing rather than the use of unprocessed macroalgal strips. Despite the *L. digitata* for this study being harvested in the month of July which had previously been shown to have the maximum fermentable carbohydrates within it (Adams et al. 2011a, b), the results in Table 2 from July 2016 gave approximately a one- to two-third decrease in ethanol production compared to that seen in Fig. 1 from the July 2013 harvest. The cause of this decrease following checks on processing parameters was concluded to be due to a low carbohydrate proportion in the 2016 harvested material and was confirmed through compositional analysis. Laminarin quantification assays did not reveal detectable laminarin contents in any of the fermentation slurries pre- or post-fermentation, giving a value of <6 μg laminarin per gramme of dried solids g^{-1}DS for all samples

Table 2 The effect of drying and processing treatments on ethanol yields from *L. digitata* harvested in July 2016

Drying and processing treatment	Mean ethanol yield (μL g^{-1}DS)
Air-dried screw pressed	24.85 ± 7.60^{ab}
Air-dried strips	6.22 ± 2.07^{b}
Freeze-dried screw pressed	42.51 ± 9.14^{a}
Freeze-dried strips	26.13 ± 8.44^{ab}

Mean ± s.e., n = 3, different lower case letters denote significant differences between yields determined by Tukey HSD

DS dry solids

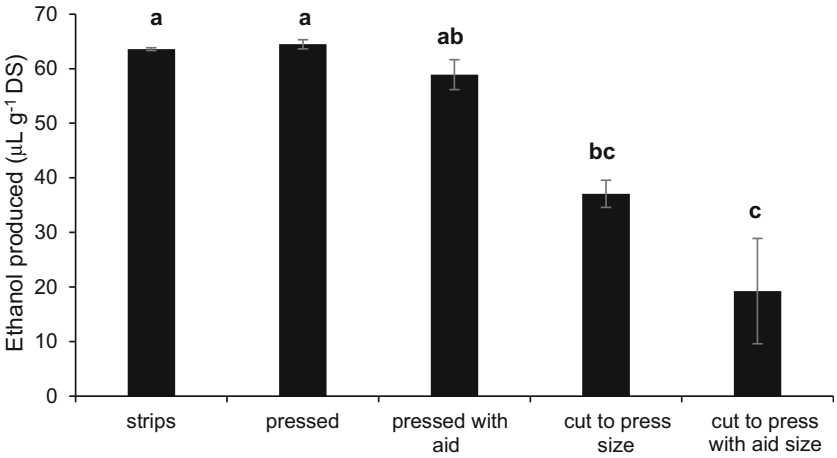

Fig. 1 The effect of differently processed July 2013 *L. digitata* on ethanol yields following 72 h incubation. *Strips*—10–15 cm lengths of blade; *pressed*—following screw pressing; *pressed with aid*—screw pressed with press aid; *cut to press size*—cut to average size of screw pressed material; *cut to press with aid size*—blend of *L. digitata* cut to the average size of material screw pressed with the press aid with the correct ratio of press aid also added. Different *lower case letters* denote significant differences between yields determined by Tukey HSD. *n* = 2. *Error bars* show standard error

(data not shown); neither was the polymeric sugar peaks typically associated with laminarin detected during HPLC analysis. Mannitol was present in all samples and the quantities in the fermentation slurries pre- and post-fermentation shown in Table 3. There were no significant differences ($P > 0.05$) between mannitol concentrations pre- and post-fermentation or between mannitol concentrations from different processing treatments at either time point using Tukey's test. All fermentations had reduced mannitol by the end of the fermentation period, averaging 18.5 mg g^{-1}DS, showing that the yeast could utilise it but not preferentially, as an average of 120.5 mg g^{-1}DS was retained in the slurries after 72 h of incubation.

Discussion

The process of screw pressing *L. digitata* does not generate significant volumes of liquid, with the vast majority (>90%) being retained within the macerated material. This is in direct contrast to other high-moisture-content terrestrial biomass materials such as high-sugar perennial ryegrass which when harvested in June loses >50% of its weight as a liquid extract during the screw pressing procedure (Morris et al. 2008). Following processing for initial trials on the first harvest (July 2013) material, it became apparent that even when working with similarly treated material, the moisture content in 'wet' macroalgae varies considerably. This is particularly noticeable if further processings such as cutting are required, preventing comparative analysis and data interpretation (data not shown). To enable accurate comparisons between differently processed materials, a drying stage is therefore essential. As freeze-dried material is considered the preservation technique most representing the original material (Ratti 2001), this was used to dry the screw pressed material, allowing known dry weights to be used in the initial comparative study presented.

Screw pressing study Three findings can be drawn from the results shown in Fig. 1. The first is that strips of *L. digitata* yielded similar ethanol concentrations per gDS than screw pressed material in this trial, demonstrating no advantage of screw pressing. This is contrary to that in the second study below and will be discussed further there. The second is that significantly higher ethanol yields were seen in the fermentations from screw pressed samples than from the cut macroalgal samples. As the whole strips gave similar ethanol yields to those which were screw pressed, this decrease in yields cannot be due to the maceration effect of screw pressing. Instead, it is hypothesised that the decrease in ethanol yields is due to a loss of soluble laminarin and glucose during the defrosting process prior to cutting the kelp strips to the average particle size. As García-Robledo et al. (2008) notes in their work on the biogeochemical effects of macroalgal decomposition, there is little work on algal decay with most focussing on the degradation of the macroalgal tissue rather than the residues. However, approximately half of the laminarin content of kelps is soluble (Nelson and Lewis 1974), and previous work by the authors (Adams et al. 2015) has demonstrated that a brief washing of kelp with tap water can remove up to 49% of the laminarin present. It is therefore probable that a proportion of laminarin was lost in leachate during the defrost-cut-refreeze steps. This in turn has decreased the available glucose to the yeast and subsequently reduced the concentration of ethanol produced.

The third finding of this study considers the inclusion of a press aid and whether it benefits or hinders processing. A press aid is typically an inert fibre which is available as a cheap, bulk additive to improve the pressing action on the algae (Vincent Corporation 2008), and following its inclusion in this press, the macroalgae particles were >60% smaller by an area. There is a decrease in ethanol yields in samples with the press aid compared to those without in both screw pressed and cut samples, though it is not a significant one. This decrease is unsurprising as fermentations with the press aid alone did not generate any ethanol at detectable limits after 72 h incubation (data not shown). The leaves of *M. giganteus* contain 5% callose (Falter et al. 2015), a β-glucan which is hydrolysable to glucose with the laminarinase used in these fermentations. However, only 17% of the harvested *M. giganteus* is leaf material following senescence (Costa et al. 2014), so the fraction of callose from leaf material in the senesced

Table 3 Mean mannitol contents and utilisation in the fermentation slurries before and after fermentation per gramme dry solids of *L. digitata* from July 2016 harvest

Drying and processing treatment	Pre-fermentation (mg g^{-1}DS)	Post-fermentation (mg g^{-1}DS)	Δ Mannitol concentration (mg g^{-1}DS)
Mean value	139.00 ± 0.27	120.52 ± 0.43	18.48
Air-dried screw pressed	148.41 ± 0.19	128.05 ± 0.34	20.37
Air-dried strips	111.94 ± 0.59	102.83 ± 0.66	9.11
Freeze-dried screw pressed	151.48 ± 0.05	121.63 ± 0.46	29.85
Freeze-dried strips	144.17 ± 0.27	129.58 ± 0.25	14.58

Mean ± s.e., n = 3, no significant differences between yields in each column as determined by Tukey HSD

DS = dry solids

chipped material is therefore <1% of the biomass and thus could not play a significant role in ethanol generation.

The decrease in yield following the addition of the press aid in both screw pressed and cut samples is less than the proportion of press aid added, indicating that combining with the press aid is overall beneficial as regards conversion efficiencies from the macroalgae. Despite the benefits of increased ethanol production per kilogramme of macroalgae, the addition of a press aid has inevitable additional costs for adding and potentially removing the press aid from the residues of pre- or post-fermentation. Because of this, the use of press aids in future macroalgae processing is unlikely to benefit overall.

Drying treatment study Air-drying is a simple, scalable method of drying macroalgae which is employed worldwide (Naylor 1976) but to the authors' knowledge has not previously been combined with screw pressing studies. As the climate in mid-Wales is not always suitable for air-drying, the samples were dried in a fan oven at 30 °C with regular turning, mimicking natural air-drying in warmer and drier climes. Thirty degree Celsius was also the optimal drying temperature for glucose release from kelp identified by Sharma and Horn (2016) in a drying trial across a range of temperatures (21–105 °C).

In the comparative drying study using the second harvested material, all fermentations showed distinctly lower ethanol yields than those seen in the earlier (2013) study. Initial checks on all processing parameters including the use of fresh yeast, pH and temperature checks pre- and post-fermentation were conducted, followed by composition analysis of the macroalgae. Results here showed the macroalgae harvested in July 2016 had below detectable limits of laminarin present, meaning that there was a lower than expected utilisable carbohydrate substrate for the yeast to convert to ethanol. Mannitol was present at relatively high concentrations, providing carbohydrate for utilisation, but the conversion of the alcohol sugar to glucose affects the redox balance and requires expression of enzymes such as mannitol dehydrogenase. *Ambrosiozyma angophorae* has been shown to metabolise mannitol (Lee and Schneider 1987) but will preferentially use glucose directly (Adams et al. 2011b). Mean mannitol concentration values decreased during the fermentation, but a larger proportion of mannitol was converted overall, as the majority of the few laminarin chains present would have had a mannitol terminator molecule present (Read et al. 1996) which would have been released following laminarin degradation.

When comparing between results from the study, freeze-dried fermentations showed higher ethanol yields than their air-dried equivalents, with screw pressed fermentations also yielding ×2–×4 as much ethanol as fermentations containing the larger strips of macroalgae. This contradicts the findings from the earlier study on screw pressing macroalgae and is hypothesised to be due to the low laminarin concentrations. It is widely acknowledged and well documented that conducting experiments with suboptimal conditions can allow the identification of greater genetic or phenotypic variation within the feedstocks, e.g. between genetic variants under stressed conditions (Trontin et al. 2011; Verslues and Juenger 2011), or differences in substrates, e.g. in enzyme additions degrading polymeric structures (Selig et al. 2008). Under more optimal conditions, higher yields may be accrued but with less separation and it is proposed that a similar event has occurred in these two studies. In the first (2013 collection) study, with material containing high concentrations of laminarin, there was excessive glucose from the laminarin available. The yeast utilised the sugars equally in both strips and screw pressed preparations, meaning that there was no difference in yields between the strips and the screw pressed material. In the second (2016 collection) study, with low levels of laminarin present, the glucose availability is hypothesised to have been the rate-limiting factor. In this scenario, any increase in enzyme accessibility to the laminarin through the screw press mechanical pre-processing would have had an impact on the ethanol yields, making them higher for the screw pressed material than for the strips. Support for this hypothesis is that it was seen in both air- and freeze-drying preparations from this harvest collection.

Conclusions The processing of macroalgae through screw pressing does enhance conversion to ethanol, but only when the material contains low levels of storage carbohydrates. Following harvesting at more desirable, higher concentrations of laminarin, an equal concentration of ethanol was produced from processed and strips of *L. digitata*. Another aspect of screw pressing is the addition of a press aid, which was shown to increase ethanol yields per gramme of macroalgae present in the fermentation though the total ethanol yield was below than that produced in fermentations containing screw pressed *L. digitata* alone. Press aid additions would need extra processing, handling and potentially post-maceration separation. The inevitable costs required for these steps, meaning a press aid, are unlikely to be viable or desirable in a future multi-product or biorefinery processing scenario.

Screw pressing or other forms of mechanical pre-processing of macroalgae to create a pumpable product could be beneficial regarding downstream processing, but results from these studies do not indicate that there is any microbial advantage in pressing kelps as regards the conversion of high concentrations of macroalgal carbohydrates to ethanol. This is supported by findings in Manns et al. (2016) who concluded that cutting kelps did not increase sugar release following enzymic hydrolysis but that some processing may be necessary to improve homogeneity.

The macroalgae fermentations produced ethanol even though the proportion of laminarin was low in the material

harvested in July 2016. This shows that despite composition variability between years, macroalgae can still be converted to bioethanol and a range of other fermentable products making it a biomass crop worthy of future research and utilisation.

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References

Adams JM, Gallagher JA, Donnison IS (2009) Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. J Appl Phycol 21:569–574

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. Bioresour Technol 102:226–234

Adams JMM, Schmidt A, Gallagher JA (2015) The impact of sample preparation of the macroalgae *Laminaria digitata* on the production of the biofuels bioethanol and biomethane. J Appl Phycol 27:985–991

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

Black W (1950) The seasonal variation in weight and chemical composition of the common British Laminariaceae. J Mar Biol Ass U K 29:45–72

Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD (2011) Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. Bioresour Technol 102:2595–2604

Costa RMF, Lee SJ, Allison GG, Hazen SP, Winters A, Bosch M (2014) Genotype, development and tissue-derived variation of cell-wall properties in the lignocellulosic energy crop *Miscanthus*. Ann Bot 114:1265–1277

Falter C, Zwikowics C, Eggert D, Bluemke A, Naumann M, Wolff K, Ellinger D, Reimer R, Voigt CA (2015) Glucanocellulosic ethanol: the undiscovered biofuel potential in energy crops and marine biomass. Scientific Reports 5:13722. doi:10.1038/srep13722

Gao K, McKinley K (1993) Use of macroalgae for marine biomass production and CO_2 remediation: a review. J Appl Phycol 6:45–60

García-Robledo E, Corzo A, de Lomas JG, van Bergeijk SA (2008) Biogeochemical effects of macroalgal decomposition on intertidal microbenthos: a microcosm experiment. Mar Ecol Prog Ser 356:139–151

Horn SJ (2000) Bioenergy from brown seaweeds. Doctoral Thesis, Norwegian University of Science and Technology, Trondheim, Norway

Horn SJ, Aasen IM, Ostgaard K (2000a) Production of ethanol from mannitol by *Zymobacter palmae*. J Ind Microbiol Biotechnol 24:51–57

Horn SJ, Aasen IM, Ostgaard K (2000b) Ethanol production from seaweed extract. J Ind Microbiol Biotechnol 25:249–254

John RP, Anisha GS, Nampoothiri KM, Pandey A (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 102:186–193

Lardon L, Helias A, Sialve B, Stayer J-P, Bernard O (2009) Life-cycle assessment of biodiesel production from microalgae. Env Sci Technol 43:6475–6481

Lee H, Schneider H (1987) Ethanol production from xylitol and some other polyols by *Pichia angophorae*. Biotechnol Lett 9:581–584

Manns D, Andersen SK, Saake B, Meyer AS (2016) Brown seaweed processing: enzymatic saccharification of *Laminaria digitata* requires no pre-treatment. J Appl Phycol 28:1287–1294

Meehan PG, Finnan JM, Mc Donnell KP (2013) The effect of harvest date and harvest method on the combustion characteristics of *Miscanthus giganteus*. Glob Change Biol Bioenerg 5:487–496

Meinita MDN, Kang J-Y, Jeong G-T, Koo HM, Park SM, Hong Y-K (2012) Bioethanol production from the acid hydrolysate of the carrageenophyte *Kappaphycus alvarezii* (cottonii). J Appl Phycol 24:857–862

Morris SM, Jackson CJ, Gallagher JA, Kelly S, Donnison IS (2008) High-sugar perennial ryegrass as a bioethanol feedstock. An alternative use for forage grasses. In: Proceedings of 16th European Biomass Conference & Exhibition, Valencia, Spain, 02/06/2008–06/06/2008, 2008. ETA-Florence Renewable Energies pp 60–66

Naylor J (1976) FAO Fisheries Technical Paper No. 159, Production, trade and utilisation of seaweeds and seaweed products. Rome, Italy.

Nelson TE, Lewis BA (1974) Separation and characterization of soluble and insoluble components of insoluble laminaran. Carbohydr Rese 33:63–74

Park J-H, Yoon J-J, Park H-D, Lim DJ, Kim S-H (2012) Anaerobic digestibility of algal bioethanol residue. Bioresour Technol 113:78–82

Ratti C (2001) Hot air and freeze-drying of high-value foods: a review. J Food Eng 49:311–319

Read SM, Currie G, Bacic A (1996) Analysis of the structural heterogeneity of laminarin by electrospray-ionisation-mass spectrometry. Carbohydr Res 281:187–201

Robson P, Jensen E, Hawkins S, White SR, Kenobi K, Clifton-Brown J, Donnison I, Farrar K (2013) Accelerating the domestication of a bioenergy crop: identifying and modelling morphological targets for sustainable yield increase in *Miscanthus*. J Exp Bot 64:4143–4155

Selig MJ, Knoshaug EP, Adney WS, Himmel ME, Decker SR (2008) Synergistic enhancement of cellobiohydrolase performance on pretreated corn stover by addition of xylanase and esterase activities. Bioresource Technol 99:4997–5005

Sharma S, Horn SJ (2016) Enzymatic saccharification of brown seaweed for production of fermentable sugars. Bioresour Technol 213:155–161

Suganya T, Varman M, Masjuki HH, Renganathan S (2016) Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach. Renew Sust Energy Rev 55:909–941

Trontin C, Tisné S, Bach L, Loudet O (2011) What does *Arabidopsis* natural variation teach us (and does not teach us) about adaptation in plants?. Curr Opin Plant Biol 14:225–231

Verslues PE, Juenger TE (2011) Drought, metabolites, and *Arabidopsis* natural variation: a promising combination for understanding adaptation to water-limited environments. Curr Opin Plant Biol 14:240–245

Vincent_Corporation (2008) Press aid. http://www.vincentcorp.com/content/press-aid. Accessed 22 July 2016

Wang X, Liu X, Wang G (2011) Two-stage hydrolysis of invasive algal feedstock for ethanol fermentation. J Integr Plant Biol 53:246–252

Wargacki AJ, Leonard E, Win MN, Regitsky DD, Santos CNS, Kim PB, Cooper SR, Raisner RM, Herman A, Sivitz AB, Lakshmanaswamy A, Kashiyama Y, Baker D, Yoshikuni Y (2012) An engineered microbial platform for direct biofuel production from brown macroalgae. Science 335:308–313

Yanagisawa M, Nakamura K, Ariga O, Nakasaki K (2011) Production of high concentrations of bioethanol from seaweeds that contain easily hydrolyzable polysaccharides. Process Biochem 46:2111–2116