ENSILING OF SUGAR KELP BIOMASS FOR BIOREFINING

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ABSTRACT: Seaweeds are generally harvested at certain seasons to obtain optimal yield and quality for specific purposes. Year-round biorefining of seaweed, therefore, requires storage of the biomass from harvest to processing. We investigated ensiling as a method for preserving seaweed biomass for subsequent biorefinery. In lab-scale and pilot-scale experiments of up to one-year duration, sugar kelp (*Saccharina latissima*) biomass was preserved by either biological ensiling by means of lactic acid bacteria (LAB) fermentation or by chemical ensiling by addition of lactic acid to reduce pH. The results clearly demonstrated that the ensilability of sugar kelp was positively correlated with the glucose content of the biomass. The ensiling process could be optimized by application of molasses and further improved by addition of LAB inoculum. The doses of molasses and lactic acid were important for pH after biological and chemical ensiling, respectively. Biological ensiling reduced the content of glucose and increased the content of fermentation products. In conclusion, ensiling appears to be an interesting preservation method for seaweed biomass, which may be optimized by application of ensiling additives. However, ensiling also changes the chemical composition of the biomass, and the applicability of ensiling for preserving seaweed biomass may depend on the subsequent use of the silage in biorefinery processes.
Keywords: Macroalgae, storage, fermentation, biorefinery, chemical composition, quality.

1 INTRODUCTION

Seaweeds are generally harvested at specific seasons to obtain optimal yield and/or quality. However, it is often desirable with year-round production of seaweed-based products to optimize the use of facilities and labor. This emphasizes the need for storage of the biomass from harvest until processing, with optimal preservation of valuable constituents. Drying constitutes one way of preserving seaweed biomass, but a major drawback is the large energy requirement due to low dry matter (DM) content in seaweed biomass, typically below 20% and even as low as 10% (Herrmann et al., 2015). Ensiling offers an alternative storage method without the need for drying.

Ensiling is a well-established method for preservation of wet biomass, which is widely used for storage of forage for animal production. Recent research has focused on ensiling as a possible preservation method for seaweed biomass (Herrmann et al., 2015; Milledge & Harvey, 2016; Sandbakken et al., 2018). The basic principle of ensiling is to reduce the pH of the biomass to around 4.0 to ensure stable conditions with minimal degradation and storage loss.

The pH may be reduced either by 'biological ensiling', 'chemical ensiling' or by a combination of these two principles. In biological ensiling, pH is decreased by lactic acid bacteria (LAB), which use water soluble carbohydrates (WSC) as the primary substrate for production of organic acids which acidify the biomass (Borreani et al., 2018). This has been demonstrated in different seaweed species including sugar kelp (*Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders 2006) by Herrmann et al. (2015). In chemical ensiling, pH is reduced by addition of organic or mineral acids, which has been demonstrated for *S. latissima* by Sandbakken et al. (2018). In combinations of biological and chemical ensiling, organic or mineral acids may be added to the biomass at a lower dose compared to chemical ensiling, and this acid dose may reduce pH enough to facilitate further activity of LAB and, hence, lead to a desirable fermentation pathway during ensiling.

For biological ensiling of seaweed, it can be difficult to achieve sufficient acidification due to high buffering capacity (BC) of the biomass as well as low concentrations of WSC and LAB, although the 'ensilability' differs between seaweed species (Herrmann et al., 2015). Whereas it is difficult to change the BC of a given seaweed biomass, it is possible to add both WSC and LAB as ensiling additives to improve the ensiling process. Addition of WSC and LAB has been shown to have positive effects on the fermentation quality of soybean silage when applied individually and with a larger effect when applied in combination (Ni et al., 2017). Sugar beet molasses may be added as a source of WSC, and a range of commercial LAB products may serve as inoculum. However, very little research has been conducted on application of WSC and LAB as ensiling additives for seaweed biomass.

A wide range of acids may be applied for chemical ensiling of biomass. Sulfuric acid and formic acid have been evaluated for preservation of *S. latissima* (Sandbakken et al., 2018). Other acids may also be relevant for ensiling of seaweed.

Previous studies of ensiling of seaweed have focused on application of the biomass for e.g. biogas production (Herrmann et al., 2015; Milledge & Harvey, 2016), but also on the preservation of the seaweed carbohydrates laminarin and mannitol for biofuels and chemicals (Sandbakken et al., 2018). In general, however, only little research has focused on the quality of seaweed silage and the preservation of valuable constituents of the biomass to be used for value-added products.

An important prerequisite for ensiling is to obtain anaerobic conditions, and vacuum packing has been shown to offer a model system for lab-scale ensiling (Johnson et al., 2005). The vacuum bag ensiling system has also been applied for ensiling of seaweed (Milledge & Harvey, 2016; Redden et al., 2017), and the system is useful for studying the effect of various parameters during ensiling, with the possibility of producing a large

number of small silage samples for destructive sampling over time. Since seaweed is harvested seasonally, frozen seaweed is sometimes used for ensiling experiments (Milledge & Harvey, 2016; Sandbakken et al., 2018). There are indications, however, that freezing may reduce the microbial activity of the seaweed biomass (Sandbakken et al., 2018), and it is relevant to verify the potential effect of freezing on the ensilability of seaweed biomass.

The aim of this study was to investigate ensiling as a preservation method for *S. latissima* biomass for subsequent biorefining purposes. A number of factors affecting ensiling was studied in lab-scale ensiling experiments of up to one year duration as well as in pilot-scale ensiling experiments of one year duration. The effect of ensiling was evaluated by analysis of pH and content of valuable constituents such as carbohydrates and protein.

2 MATERIALS AND METHODS

2.1 Biomass resources

Cultivated *S. latissima* was harvested from three different sites: 1) Ocean Rainforest (ORF) cultivation site in Funningsfjordur, Faroe Islands, 2) Scottish Marine Institute (SAMS) cultivation site near Oban, Scotland, 3) Aarhus University (AU) cultivation site near Grenaa, Denmark. Seaweed from ORF was harvested on 13th July 2016 and 14th May 2018, seaweed from SAMS and AU was harvested 12th June and 26th June 2018, respectively.

In general, seaweed biomass was frozen at -18°C from harvest until the start of the ensiling experiment. However, pilot-scale ensiling was done with fresh seaweed from ORF and SAMS.

For an experiment with ensiling of frozen versus fresh seaweed, biomass harvested by ORF 14th May 2018 was either ensiled directly or frozen at -18°C for 14 days before ensiling.

2.2 Ensiling additives

SiloSolve MC (Chr. Hansen A/S) containing *Enterococcus faecium* DSM 22502, *Lactococcus lactis* SR3.54 and *Lactobacillus plantarum* CH6072 was used as LAB inoculum. The dry inoculum was suspended in water to 11 g/kg.

Sugar beet molasses (Nordic Sugar, Denmark) was used as a source of WSC. The molasses contained minimum 43% sucrose and maximum 26% water, and a dose of 24 g of molasses per kg FM corresponded to minimum 10.3 g sucrose per kg FM. For application, a solution was prepared by dissolving 300 g molasses in 150 g of water.

Lactic acid was applied as a 80% solution (Lactol Vinoferm, Brouwland).

2.3 Lab-scale ensiling

In the general procedure for lab-scale ensiling, the seaweed biomass was ensiled in vacuum bags (model SR 120 x 550 PA/PE 0.090 mm, by LogiCon Nordic, Kolding, Denmark), which were vacuum packed by a Webomatic vacuum packing machine (M. Type C 15-HL, M. No 0310TB1000. Webomatic, Bochum, Germany). Each vacuum bag corresponded to an experimental unit.

Frozen seaweed biomass was thawed overnight, and effluent was collected and weighed. The solid fraction of the biomass was chopped manually into pieces of approx. 2-4 cm^2 size. A total of 50 g of biomass (fresh matter, FM) was loaded into each vacuum bag, i.e. the solid fraction and liquid fraction was used in the same proportion as in the original biomass before draining.

The doses of additive solutions of a specific treatment were added directly into the bag with the biomass, and biomass and additive solution were mixed thoroughly in the bag. The bag was then folded and put into a second bag which was vacuum packed and put into a third bag which was also vacuum packed. Bags for ensiling durations of 4 months or longer were vacuum packed in two additional layers of 90 µm and 150 µm PA/PE, respectively, to minimize the risk of oxygen diffusion.

After sealing, the vacuum bags were stored at 20°C for the pre-planned ensiling duration after which either two or three replicate bags per treatment and ensiling duration were frozen at -18°C until analysis.

2.4 Pilot-scale ensiling

For pilot-scale ensiling, freshly harvested seaweed was chopped to 5-20 cm (ORF) or 5-10 cm (SAMS) particle size and mixed with the given ensiling additive. In the ORF experiment, seaweed was loaded into 68 L neck drum barrels in high density polyethylene (HDPE) with screw lid (ORF), either with an aqueous solution with 10.3 g sucrose and 0.022 g LAB inoculum (SiloSolve by Chr. Hansen, Denmark) per kg FM for biological ensiling or 7 g lactic acid per kg FM for chemical ensiling. In the SAMS experiment, seaweed was loaded into 60 L open top drum barrels in HDPE, either with no additive or with an aqueous solution of sucrose and LAB inoculum as used in the ORF experiment.

The biomass was compacted as firmly as possible, and the barrels were closed tightly and stored at 17°C for 7 days (ORF) or at 9-15°C for 7 days (SAMS) and subsequently in an unheated barn for 12 months (ORF) or 11½ months (SAMS).

After ensiling, the barrels were opened and the silage from each barrel was separated into a solid fraction and a liquid fraction by use of a sieve, and the fractions were weighed, and samples were frozen at -18°C until analysis.

2.5 Analyses

The pH was measured before ensiling and after ensiling by use of a pH probe.

For carbohydrate composition analysis, biomass samples were oven-dried at 65°C and stored in air-tight bags at room temperature until analysis. 0.32 g dried samples were hydrolyzed with 1.5 mL 72% w/w H_2SO_4. Carbohydrates were tested using a modified version of ASTM E 1758 – 01 (2015), using an Aminex HPX-87P column at 80°C with a 0.5 mL/min flow-rate with inclusion of mannitol as reference sample.

The contents of lactic acid and acetic acid in ensiled biomass were analysed in centrifuged supernatant from homogenized ensiled samples (slush). A measured quantity of 1.5M H_2SO_4 was added to adjust pH of samples to 1-3. Samples were analysed in a similar way as the method described by Andersson and Hedlun (1983) in an Agilent 1100 series HPLC using Aminex HPX-87H HPLC column at 50°C using 5 mM H_2SO_4 mobile phase and a flow of 0.4 ml/min.

Data analyses were done by use of the proc mixed procedure in the SAS software, release 9.3.

3 RESULTS AND DISCUSSION

3.1 Lab-scale ensiling

A 28-day experiment with different ensiling additives showed significant effects on pH of both additive treatment and ensiling duration as well as interaction between these factors (all $P<0.001$) (Figure 1). This means that the time required to reach a low pH differed between ensiling methods: Chemical ensiling by addition of lactic acid (12 g/kg FM) immediately reduced pH to around 4.5 and below. When adding both LAB inoculum and molasses (24 g/kg FM), pH was reduced rapidly (within one week) to around 4.5 and below.

Without any additive treatment, pH remained above 6.0 for 28 days, whereas LAB inoculum (SiloSolve) reduced pH slightly and slowly, and addition of molasses reduced pH more and slightly faster. This indicates that biological ensiling of this *S. latissima* batch may be limited especially by lack of water-soluble carbohydrates but also by lack of naturally occurring LAB on the biomass.

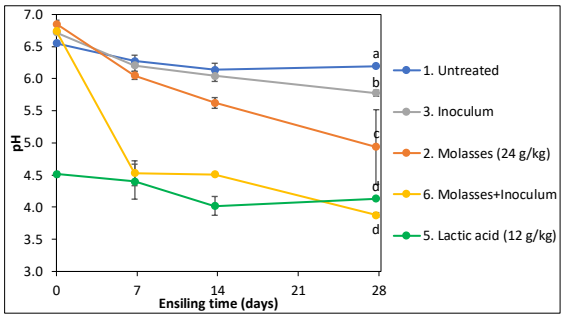

Figure 1: pH development during ensiling of *S. latissima* (ORF July 2016) with various additives, with focus on biological and chemical ensiling. Each point represents the mean of two replicates, and error bars indicate standard deviation of the two replicates.

Dose-response experiments were performed in order to study the effects of the doses of molasses and lactic acid on pH in the silage. Increasing the dose of molasses up to 36 g/kg FM showed a non-linear relationship with pH reduction up to approx. 24 g/kg FM and no further decrease beyond this dose, with a minimum pH level between 4.0 and 4.5 (Figure 2). This indicates a saturation point for biological ensiling; LAB may not produce more acid when pH has dropped to a certain level, even when there is more available sugar.

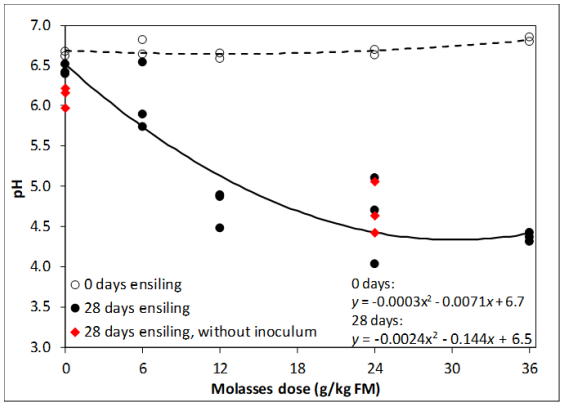

Figure 2: pH before ensiling and after 28 days of ensiling of *S. latissima* biomass (ORF July 2016) depending on the applied dose of molasses. All samples were treated with inoculum (SiloSolve MC), except where otherwise stated. Each point represents a single silage sample.

For chemical ensiling with lactic acid, pH decreased linearly with increasing doses of lactic acid up to 7.5 mL/kg FM, with a pH of approx. 4.0 at the highest dose (Figure 3). There was an increase in pH during the first week of ensiling, but the dose-response relationship was consistent for ensiling durations from 7 to 28 days. The initial increase in pH indicates that it may take some time to reach an equilibrium between biomass and acid after adding the acid, and this may cause a challenge for rapid determination of the buffering capacity of a certain batch of seaweed biomass. It should also be noted that the relationship between the dose of acid and the pH in the silage may differ between different batches of seaweed biomass, particularly due to variation in biofouling as heavy fouling (in particular with calcifying organisms) may increase the buffering capacity.

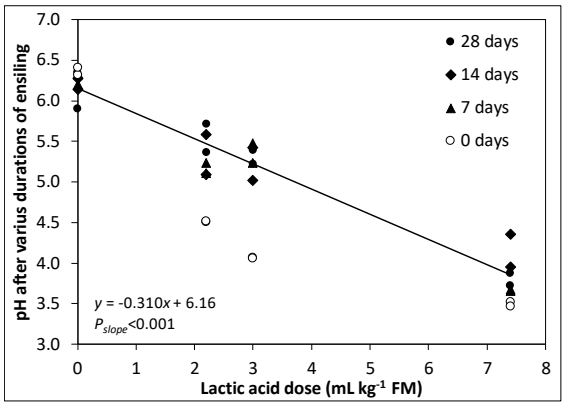

Figure 3: pH after different durations of chemical ensiling of *S. latissima* (ORF July 2016) in vacuum bags with various doses of lactic acid. Each dot represents the observed pH for one silage bag, and the line represents the predicted relationship across the ensiling durations of 1, 2 and 4 weeks, i.e. 0 weeks of ensiling was not included in the regression analysis.

To study the differences in ensilability between different sources of *S. latissima*, an experiment included biomass harvested on three sites, i.e. Faroe Islands (ORF), Scotland (SAMS) and Denmark (AU). The initial

pH in the biomass varied between 5.9-6.7. After biological ensiling for 28 days without any additives (untreated), pH differed significantly between the three batches, with high pH in biomass from ORF, intermediate pH in biomass from SAMS and very low pH in biomass from AU (Figure 4). In treatments with addition of molasses+inoculum (24 g/kg FM molasses corresponding to approx. 85 g/kg DM), pH was relatively low in all three biomasses, but still with a slightly higher pH in biomass from ORF. The results clearly demonstrate differences in the ability to ensile between different sources of seaweed even within the same species. These differences emphasize that the need for ensiling additives may differ between batches of seaweed. It would be very useful with a rapid analysis to get an indication of the need for ensiling additives and the required dose for a given batch of seaweed.

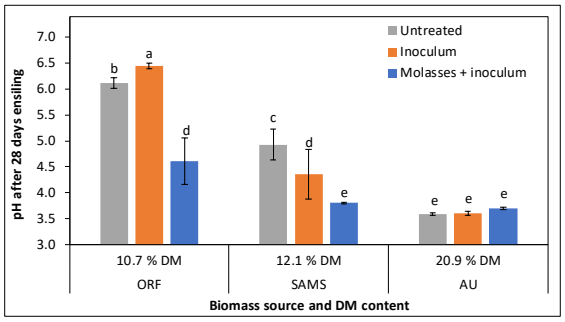

Figure 4: pH after 28 days of ensiling of three sources of *S. latissima* biomass and with various additive treatments. Each column represents the mean of three replicates, error bars indicate the standard deviation, and letters indicate LSD groups (P<0.05).

The differences between batches of *S. latissima* in ability to ensile biologically may be related to the buffering capacity as well as the initial content of fermentable sugars. For the biomasses from SAMS and AU, the total concentration of glucose in the fresh biomass (DM basis) was analysed and differed significantly with 14.7% glucose in biomass from SAMS and 47.8% in biomass from AU (Figure 5). This very large difference in initial glucose content is most likely involved in the differences in ensilability between the batches as seen in Figure 4. The results also demonstrate a considerable reduction in glucose during ensiling, particularly for the batch from AU where the glucose concentration was reduced to 24.4% after 28 days of ensiling, i.e. approx. half of the glucose was consumed during the ensiling process (Figure 5).

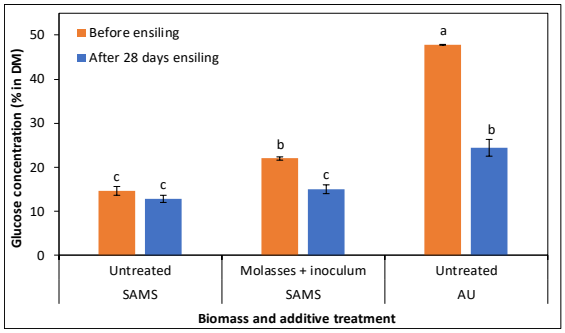

Figure 5: Total glucose concentration before ensiling and after 28 days of ensiling of *S. latissima* biomass from SAMS in Scotland (without and with addition of molasses+inoculum) and Aarhus University (AU) in Denmark. Each column represents the mean of two replicates, error bars indicate standard deviation, and letters indicate LSD groups for all treatments (P<0.05).

Seaweed biomass may have to be stored over long periods, and long-term effects of ensiling are import. The pH was followed over a 12-month period for *S. latissima* without any additives, with molasses+inoculum for biological ensiling and with lactic acid for chemical ensiling. In consistency with Figure 1, pH remained high in biomass without any additives (Figure 6). For both biological ensiling and chemical ensiling, on the other hand, pH was retained at a low level (≤4.3) over the whole period. There was no significant change in DM content in the biomass over 12 months of ensiling. This illustrates the potential of ensiling to stabilize the biomass over longer periods of time.

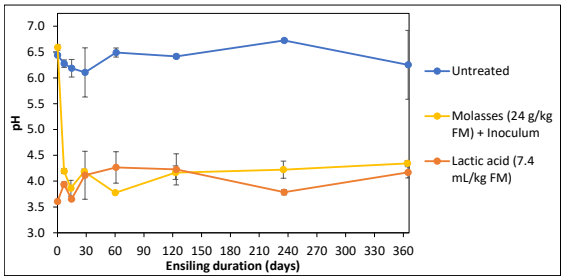

Figure 6: pH development during 12 months of biological ensiling and chemical ensiling of *S. latissima* (ORF July 2016). Each point represents the mean of two replicates, and error bars indicate standard deviation of two replicates.

Although the biomass was stabilized at a low pH level during ensiling, changes in the chemical composition did occur during ensiling. Figure 7 (upper figure) shows an initial decrease in glucose during the first 4 months of biological ensiling but no significant change in glucose content from months 4 to 12 of ensiling. The fermentation process resulted in a large initial increase in lactic acid concentration which remained relatively stable from months 4 to 12 of ensiling. In general, only little acetic acid was produced. For chemical ensiling with lactic acid, the content of glucose did not change significantly over the 12-month period, but there was a slight reduction in lactic acid content from months 4 to 12 of ensiling (Figure 7, lower figure). These results clearly demonstrate that the method

of ensiling may affect the chemical composition of the silage. This may have implications for the subsequent applicability of the seaweed silage. If the silage is to be used for e.g. ethanol fermentation, chemical ensiling may be preferable, since this seems to preserve the glucose in the silage. On the other hand, if the silage is to be used for e.g. butanol fermentation, the conversion from glucose to lactic acid during ensiling may not pose a problem, since the lactic acid can serve as a substrate for butanol producing bacteria. Hence, the applicability of ensiling of seaweed and the choice of ensiling method may depend on the subsequent utilization and biorefining of the seaweed silage.

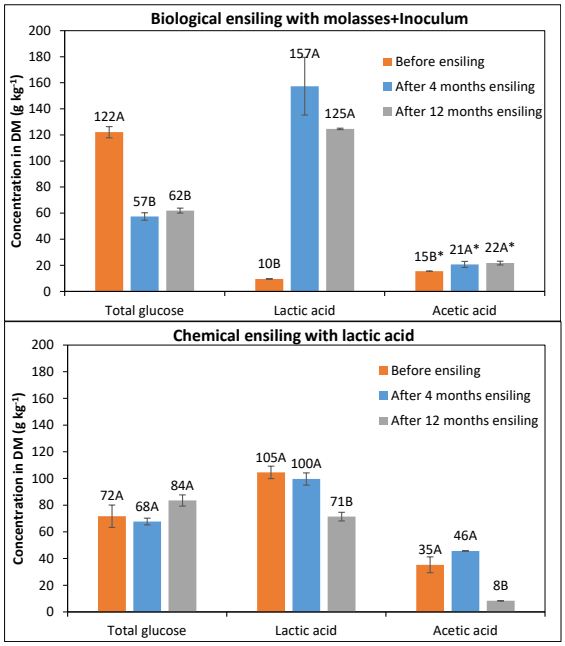

Figure 7: Concentration of chemical components in *S. latissima* biomass (ORF July 2016) before ensiling and after ensiling in vacuum bags for 4 or 12 months with either biological ensiling with molasses+inoculum (upper figure) or chemical ensiling with lactic acid (lower figure), analysed in dried, hydrolyzed samples. Each column represents the mean of two replicates, error bars indicate standard deviation of the two replicates, figures represent the value and letters indicate LSD groups within each additive treatment and chemical component. *Overall, the effect of ensiling duration was only nearly significant with P=0.052.

Most of the experiments were carried out using frozen seaweed biomass. However, there are indications that freezing of seaweed biomass may reduce microbial activity during subsequent ensiling (Sandbakken et al. 2018). The effect of freezing was studied in an ensiling experiment with unfrozen versus frozen *S. latissima* biomass. The initial pH value in fresh biomass was 6.1-6.4 and was not affected by freezing. After 27 days of biological ensiling without any additives, pH was significantly lower in silage from unfrozen biomass than in silage from frozen biomass, and pH also differed depending on if molasses had been added (Figure 8). However, there was no difference in pH when LAB inoculum or molasses+inoculum had been added. This confirms, that freezing of the seaweed biomass can have a negative effect on the subsequent ensiling of the biomass, and the effect seems to be related to the microbial activity in the biomass. When interpreting the results of ensiling experiments done with frozen seaweed biomass, this should be taken into account, and fresh seaweed biomass may be expected to ensile better than indicated by experiments with frozen biomass. Moreover, the experiment shows that the effect of freezing seaweed biomass before ensiling may be counteracted by adding LAB inoculum. Hence, the presented results from ensiling experiments with frozen seaweed biomass are still considered as valid for the factors studied such as effects of doses of molasses and lactic acid and the conversion of glucose, as also indicated by the pilot-scale ensiling experiments which were done by use of fresh, unfrozen biomass (see below).

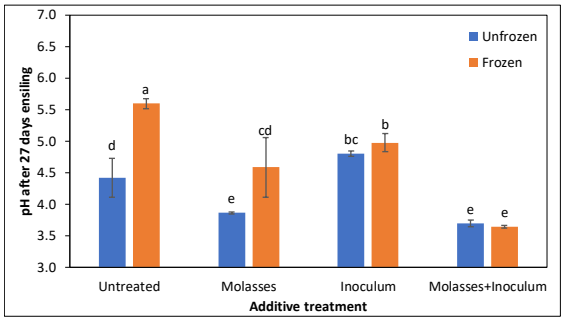

Figure 8: pH after 27 days of ensiling of *S. latissima* biomass (ORF May 2018) with various additive treatments and with or without freezing before ensiling. Each column represents the mean of two replicates, error bars indicate the standard deviation, and letters indicate LSD groups.

3.2 Pilot-scale ensiling

Pilot-scale ensiling of *S. latissima* biomass was done by use of fresh, unfrozen biomass from ORF on the Faroe Islands and SAMS in Scotland. When opening the barrels after 12 and 11½ months of ensiling, the biomass had separated into a solid fraction floating on top of a liquid fraction. There was some fungal contamination on the surface of silage from SAMS, indicating ingression of oxygen during the ensiling period. In contrast, there was no contamination of silage in the barrels from ORF, most likely because the lids on this type of barrels closed more tightly.

The pH was generally low in all barrels, both with biological ensiling with sucrose+inoculum and with chemical ensiling with lactic acid, and even in the surface layer of SAMS barrels which were contaminated (Table I). For the SAMS experiment, one barrel was ensiled without any additives (Barrel 3) and, interestingly, this barrel also ensiled well and obtained a very low pH between 3.4 and 3.7. There was no control barrel without additives in the ORF experiment.

The carbohydrate composition was analysed both for fresh biomass and for the solid and liquid fractions of the ensiled biomass (Table II). The glucose concentration was considerably higher in the fresh biomass from SAMS (17.6%) than in the fresh biomass from ORF (9.8%), and the higher glucose content may explain the lower pH in silage from the SAMS biomass. The concentration of mannitol was much higher in biomass from SAMS than in biomass from ORF. During ensiling, the concentrations of glucose and galactose were reduced considerably in the liquid fraction with more moderate

changes in the solid fraction (Table II).

Table I: pH in solid and liquid fractions after 12 months (ORF) and 11½ months (SAMS) of ensiling of *S. latissima* in barrels. Values represent the mean of four measurements for solid fractions and two measurements for liquid fractions. For barrels from ORF, there was no fungal growth, and the fraction 'Upper part of solid fraction' represents approx. two thirds of the total solid fraction. For barrels from SAMS, pH was measured directly in the top-layer (2-3 cm depth) and in the mixture of the remaining part of the solid fraction.

Experiment / seaweed source	Barrel no. and additive treatment	Fraction	pH
ORF	Barrel 1: Sucrose (10.3 g/kg FM) + SiloSolve	Upper part of solid fraction	3.69 ± 0.02
		lower part of solid fraction	3.62 ± 0.01
		Liquid fraction	3.62 ± 0.01
	Barrel 2: Lactic acid (7 g/kg FM)	Upper part of solid fraction	5.04 ± 0.17
		lower part of solid fraction	4.48 ± 0.29
		Liquid fraction	4.06 ± 0.02
SAMS	Barrel 1: Sucrose (10.3 g/kg FM) + SiloSolve	Top-layer (moldy)	3.74 ± 0.10
		Bottom+middle layer	3.34 ± 0.01
		Liquid fraction	3.34 ± 0.00
	Barrel 2: Sucrose (10.3 g/kg FM) + SiloSolve	Top-layer (moldy)	4.15 ± 0.34
		Bottom+middle layer	3.39 ± 0.05
		Liquid fraction	3.35 ± 0.00
	Barrel 3: Control, no additive	Top-layer (moldy)	3.65 ± 0.02
		Bottom+middle layer	3.43 ± 0.05
		Liquid fraction	3.38 ± 0.00

Table II: Carbohydrate composition before and after approx. one year of ensiling of *S. latissima* from ORF (12 months) and SAMS (11½ months) in barrels. Total carbohydrate content was analysed after acid hydrolysis. Values represent the mean and standard deviation of two replicate analyses per batch and fraction.

Experiment / seaweed source	Barrel no. and additive treatment	Fraction	Glucose	Galactose	Fucose	Mannitol
			(% in DM)			
ORF	Fresh unensiled	Total	9.8% ± 3.4%	2.4% ± 0.6%	3.4% ± 1.6%	9.5% ± 0.2%
	Barrel 1: Sucrose (10.3 g/kg FM) + SiloSolve	Solid	10.1% ± 0.1%	2.1% ± 0.2%	1.1% ± 0.1%	5.1% ± 0.6%
		Liquid	0.8% ± 0.3%	1.0% ± 0.0%	1.4% ± 0.2%	3.2% ± 0.7%
	Barrel 1: Lactic acid (7 g/kg FM)	Solid	12.3% ± 0.8%	2.9% ± 0.2%	2.0% ± 0.0%	2.4% ± 0.4%
		Liquid	0.7% ± 0.5%	0.9% ± 0.0%	1.1% ± 0.1%	3.3% ± 0.5%
	P-value		*<0.001*	*<0.001*	*0.028*	*<0.001*
SAMS	Fresh unensiled	Total	17.6% ± 0.2%	2.5% ± 0.0%	1.7% ± 0.1%	24.8% ± 0.1%
	Barrel 1: Sucrose (10.3 g/kg FM) + SiloSolve	Solid	11.5% ± 0.1%	2.5% ± 0.1%	1.8% ± 0.1%	28.3% ± 0.1%
		Liquid	2.6% ± 0.4%	0.4% ± 0.4%	1.0% ± 0.0%	40.8% ± 2.3%
	Barrel 2: Sucrose (10.3 g/kg FM) + SiloSolve	Solid	8.5% ± 0.7%	2.1% ± 0.1%	1.7% ± 0.1%	23.9% ± 2.2%
		Liquid	2.0% ± 0.2%	0.9% ± 0.2%	1.0% ± 0.1%	40.3% ± 2.1%
	Barrel 3: Control	Solid	8.0% ± 0.1%	2.0% ± 0.1%	1.2% ± 0.0%	31.4% ± 0.2%
		Liquid	1.5% ± 0.2%	0.0% ± 0.0%	0.6% ± 0.2%	45.3% ± 2.5%
	P-value		*<0.001*	*<0.001*	*<0.001*	*<0.001*

A mass balance for FM, DM and glucose was calculated for the barrels from ORF (Table III). The solid and liquid fractions both constituted about half of the FM mass in these two barrels, whereas the solid fraction constituted the main proportion of the DM mass. The liquid fraction was nearly depleted for glucose. Biological ensiling with addition of sucrose+inoculum gave a recovery of approx. 100% of the DM (with a recovery slightly above 100% indicating some uncertainty) and 51% of the glucose. Chemical ensiling with lactic acid gave a recovery of 84% of the DM and 64% of the glucose. As with the lab-scale ensiling experiments, the pilot-scale experiments indicated a considerable consumption of glucose during ensiling, particularly for biological ensiling. However, the relatively high DM recovery indicated a relatively low overall loss during ensiling, although some of the constituents of the biomass may be converted to fermentation products.

Table III: Mass balance after 12 months of ensiling of *S. latissima* from ORF in barrels.

Barrel no. and additive treatment	Fraction	Mass balance (% of initial total weight per barrel)		
		Fresh matter	Dry matter	Glucose
Barrel 1: Sucrose (10.3 g/kg FM) + SiloSolve	Solid	54.3	68.1	48.9
	Liquid	47.7	32.7	1.8
	Total	102.0	100.8	50.7
Barrel 2: Lactic acid (7 g/kg FM)	Solid	45.5	48.2	61.7
	Liquid	52.1	35.3	2.5
	Total	97.6	83.5	64.2

4 CONCLUSIONS

Lab-scale and pilot-scale ensiling experiments with *S. latissima* indicate that both biological and chemical ensiling can be used for efficient preservation of wet seaweed biomass for at least one year. However, considerable changes in chemical composition may occur during ensiling, particularly with conversion of glucose to lactic acid during biological ensiling. These changes may affect the subsequent applicability of the seaweed silage. The study also emphasizes the differences in ensilability between different batches of seaweed even within the same species. This is a challenge when giving recommendations for ensiling practice. Future research should focus on methods for rapid prediction of the ensilability and the requirement for ensiling additives for a given batch of seaweed biomass.

5 REFERENCES

[1] Andersson, R. & Hedlund, B. (1983). HPLC Analysis of Organic Acids in Lactic Acid Fermented Vegetables. Zeitschrift für Lebensmittel Untersuchung und-Forschung, 176:440-443.

[2] Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E. 2018. Silage review: Factors affecting dry matter and quality losses in silages. Journal of Dairy Science, 101(5), 3952-3979.

[3] Herrmann, C., FitzGerald, J., O'Shea, R., Xia, A., O'Kiely, P., Murphy, J.D. 2015. Ensiling of seaweed for a seaweed biofuel industry. Bioresource Technology, 196(Supplement C), 301-313.

[4] Johnson, H.E., Merry, R.J., Davies, D.R., Kell, D.B., Theodorou, M.K., Griffith, G.W. 2005. Vacuum packing: a model system for laboratory-scale silage fermentations. Journal of Applied Microbiology, 98(1), 106-13.

[5] Milledge, J.J., Harvey, P.J. 2016. Ensilage and anaerobic digestion of *Sargassum muticum*. Journal of Applied Phycology, 28(5), 3021-3030.

[6] Ni, K., Wang, F., Zhu, B., Yang, J., Zhou, G., Pan, Y., Tao, Y., Zhong, J. 2017. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. Bioresource Technology, 238, 706-715.

[7] Redden, H., Milledge, J.J., Greenwell, H.C., Dyer, P.W., Harvey, P.J. 2017. Changes in higher heating value and ash content of seaweed during ensiling. Journal of Applied Phycology, 29(2), 1037-1046.
[8] Sandbakken, I.S., Sæther, M., Funderud, J., Aasen, I.M. 2018. Acid preservation of *Saccharina latissima* for application as a carbon source for fermentation to biofuels and chemicals. Journal of Applied Phycology, 30, 3581-3588.
[9] SAS, I. 2010. SAS/STAT. Release 9.3, Vol. 9.3, SAS Institute. Cary, NC, USA.

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