Deliverable 2.3: Report on biological and chemical ensiling techniques for storage of algae biomass for valorisation medium value-added products

Authors, Institutions

Søren Ugilt Larsen, DTI

Nicolaj Ma, DTI

PaulinaTamez, FEX

Søren Kjærulff, FEX

Lars Nikolaisen, DTI

Anne-Belinda Bjerre, DTI

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Summary

This report describes experimental work with biological and chemical ensiling techniques for storage of seaweed biomass, allowing subsequent use of the biomass for medium value-added products. The work was done within **Task 2.2** and the two sub-tasks, **Task 2.2.1** on biological ensiling and **Task 2.2.2** on combined ensiling and acid addition. Lab-scale ensiling experiments with the brown seaweed species *Saccharina latissima* have been performed by use of a vacuum bag system and focusing on either biological or chemical ensiling or combinations of these approaches. pH development during ensiling was used as a general indicator of the ensiling process for the primary screening of the most promising process. For the selected processes, further analyses were done to elucidate quality aspects of seaweed ensiling.

For biological ensiling process, the results clearly demonstrate that the ensilability varies significantly between different sources/batches of *S. latissima*. Batch with high glucose content (47.8 % in dry matter, DM) ensiled better than batch with low glucose content (14.7 %in DM). For seaweed with low ensilability, addition of a source of fermentable sugar such as molasses or glucose was found to improve the ensiling process significantly, and a non-linear relationship between molasses dose and final pH in the silage was found. This relationship may, however, differ between batches depending on e.g. the buffering capacity (BC) of the biomass.

Although lack of fermentable sugar may be the primary limiting factor for biological ensiling of seaweed, the results also demonstrate that addition of lactic acid bacteria (LAB) inoculum may improve the ensiling process, particularly in combination with a sugar source and with a more pronounced effect for frozen seaweed than fresh seaweed. Hence, the microbial population in the seaweed biomass may also be a limiting factor for ensiling. There were relatively small differences in the effect of three different types of LAB inoculum tested. Altogether, combined addition of molasses and LAB inoculum appears to favour biological ensiling and ensure low pH in silage of seaweed biomass with low ensilability.

Moreover, other parameters such as the degree of biomass grinding, temperature, addition of enzymes have also been investigated. The degree of grinding of the biomass before ensiling had relatively small effect on final pH in the silage, and biomass with and without dewatering (14.5 vs. 10.5 % DM) responded similarly to various ensiling additives, although dewatered biomass appeared to require a higher dose of lactic acid. pH in the silage after ensiling at 20°C was significantly lower than at 5°C and 37°C. Addition of cellulase sometimes reduced pH but not sufficiently.

A range of organic and mineral acids were also tested as ensiling additives for chemical ensiling, either alone or in combination with inoculum or cellulase. The results demonstrated the importance of acid dose and practical implications of determining the required dose of a given acid to obtain a target pH of e.g. 4.0. pH often increased from the target pH, particularly for strong acids, possibly due to lack of equilibrium between the biomass and acid at the initial measurement of pH. A common linear relationship was found between lactic acid dose and pH after 1 to 4 weeks of ensiling, and a BC of 70

mL kg^{-1} DM (83 g kg^{-1} DM) was found for this seaweed batch. Application of half doses of lactic acid or acetic acid did not enhance subsequent activity of LAB to reduce pH sufficiently.

During long-term ensiling over 32 weeks for 6 different treatments, the DM content did not decrease significantly, but there was a non-linear fresh matter (FM) loss during ensiling with FM losses ranging between 5.5 and 8.0 % after 32 weeks.

In terms of quality changes during ensiling, biological ensiling for 4 or 16 weeks reduced the content of glucose significantly, whereas the content of mannitol was unaffected. The fermentation of glucose (from the seaweed and from the added molasses) resulted in significant increase in the content of especially lactic acid but also some increases in acetic acid, ethanol and glycerol. For chemical ensiling with lactic acid (without molasses), the content of lactic acid increased significantly during 16 weeks ensiling. For both biological and chemical ensiling, the lactic acid concentrations indicated hydrolysis of polymeric carbohydrates from seaweed during ensiling which served as a substrate for LAB.

Analysis of amino acids (AAs) showed that the proportion of total AAs and protein-bound AAs as percentage of total crude protein was reduced during 16 weeks of ensiling, indicating degradation of true protein and AAs during ensiling. However, the results also indicated that application of ensiling additives can reduce the degradation. The proportion of true protein as percentage of total crude protein was reduced by 26 percentage points without additives but only 13 and 10 percentage points with molasses+inoculum and lactic acid, respectively. Essential AAs constituted 41.8 % of the total AA content before ensiling, and the AA profile appeared to change little during ensiling. There was an indication of increased antioxidant activity of seaweed during ensiling with molasses + inoculum.

Product lacto-fermented rapeseed-seaweed was developed by Partner FEX. A solid state fermentation was carried out with a combination of two types of seaweed and rapeseed meal. A combination of three lactic-acid fermentative bacteria was used. After several trial at a small scale, a final blend and process was scaled up. The final fermentation blend was carried out in a 2-step fermentation process where ground seaweed and rapeseed meal were blend and fermented for nearly 20 days at room temperature. The mix was fermented anaerobically using ensiling as a method for this purpose. The product is gently dried to ensure bacteria and functional metabolites remain viable. Total moisture content is of about 11%. The products were analysed in regards of their metabolite fingerprint that included identification and quantification of compounds. Among these, several phenolic compounds with recognized antioxidant and anti-inflammatory effects were found.

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