Report detailing the results of long term storage

Macro Cascade -Project

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Summary

This report on results of long term storage describes the experimental work with chemical and biological ensiling techniques for storage of algae biomass. The work was done within **Task 2.3** and is based on previous results in **Task 2.1-2.2** in which tests were made on the effect of various silage additives on the conservation of quantity and quality of the algae biomass.

A main objective of ensiling is to reduce pH to below 4.0 which will in general conserve valuable constituents of the biomass. The purpose of the experiments was to demonstrate ensiling methods for brown seaweed (*Saccharina latissima*), developed at DTI and Fermentation Experts in lab-scale and by scaling up to pilot-scale in HDPE barrels and IBC containers in long term conditions. The aim was to evaluate the effect of long term ensiling on pH and other quality parameters as well as to produce algae silage to be used for further experiments with extraction/production of various algae-based products.

Laboratory scale tests at DTI showed that pH of untreated brown seaweed without additives remained above 6, indicating a poor conservation of the seaweed biomass without use of ensiling additives. On the other hand, biological ensiling with molasses+inoculum resulted in a low pH which remained at a low level between 3.8 and 4.6 from week 1 to week 52 of the ensiling experiment. Also, it was possible to maintain a low pH by chemical ensiling with lactic acid, given a sufficient dose of acid is applied for the chemical ensiling. In general, the results indicated that both biological and chemical ensiling can maintain a low pH for an extended period of time. The development in DM content during 52 weeks of ensiling showed no significant main effect of ensiling duration on DM content and no interaction between treatment and ensiling duration. Hence, there is no indication of reduction in DM content over the 52 weeks of ensiling for these 6 treatments, and this also indicates that seaweed biomass can be stored over prolonged periods of time with no or very low loss of DM.

Pilot scale ensiling experiments in barrels by DTI showed that after 373 days of storage, the biomass had separated into a solid fraction floating on top of a liquid fraction, with the liquid fraction constituting 47-53% of the mass. The pH in the silage was significantly reduced compared to the initial level of 6.4 in the fresh biomass; for biomass which was chemically ensiled with lactic acid, pH was 5.04 in the top layer of the solid fraction, 4.48 in the lower layer of the solid fraction and 4.06 in the liquid fraction. In comparison, the biomass treated with sucrose+inoculum had a considerably lower pH of 3.69 in the top solid layer, 3.62 in both the lower solid layer and in the liquid fraction. After ensiling, the content of carbohydrates and particularly glucose (on DM basis) was very low in the liquid fraction for both ensiling methods. For biological ensiling, the liquid and solid fraction accounted for 1.8% and 48.6%, respectively, of the glucose in the original biomass, and for chemical ensiling the corresponding values were 2.5% and 61.7%. This indicates a considerable overall loss of glucose of 36-50% during ensiling due to conversion into fermentation products and with indications of a larger glucose loss for biological ensiling. Hence, in terms of pH level, the biological ensiling with sucrose+inoculum appears superior to chemical ensiling with lactic acid, although the difference will most likely depend on the dose of sucrose as well as the dose of lactic acid. In terms of conservation of glucose during ensiling,

however, chemical ensiling appears to be superior to biological ensiling. The choice of ensiling method may, therefore, depend on the subsequent application of the silage.

In laboratory scale test at FEX, pH was monitored over 11 days and demonstrated that pH was 4.1 after this ensiling duration. The short chain fatty acid pattern included high levels of butyric acid, which suggested that the fermentation had not been dominated only by the lactic acid bacteria, but some other bacteria were involved. This indicates, that the silage process may follow a range of different fermentation pathways.

In the pilot scale test at FEX, the pH dropped from 6.6 to 4.4 during the first week, accompanied by a rise in bacterial numbers from 10^5 cfu/g to 10^7 cfu/g and a temperature rise from 22°C to 34°C. Temperature dropped over the following weeks and stabilized around the temperature of the storage facility. Bacterial numbers were below 10^3 cfu/g after 6 months, and pH remained below approx. 4.3 for 12 months. After 12 months of ensiling, analysis of the headspace gas in the IPC containers indicated, that no CH_4 had been produced, which is highly desirable to avoid during ensiling. During ensiling, the proportion of non-protein nitrogen increased from 11% to 15-21%, indicating a moderate breakdown of protein into non-protein nitrogen. Also, there were some minor changes in the amino acid composition during ensiling. The concentration of carbohydrates was significantly reduced by ensiling for 12 months, by 2.8 percentage points (19%) for glucose, and by 7.6 percentage points (87%) for mannitol, whereas the concentration of fucose was significantly increased by 0.3 percentage points (33%).

Overall, the long term ensiling experiments demonstrate that it is possible to maintain a low pH level in seaweed silage over prolonged periods of time, indicating a good conservation of the biomass. However, changes in the chemical composition does occur during ensiling, e.g. by conversion of carbohydrates to various fermentation products. Therefore, the potential of ensiling as a conservation method for seaweed biomass should be evaluated in relation to the subsequent use of the silage.

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