SEAWEED AD Project Final Report

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Project site: http://www.gcu.ac.uk/seaweedforbiofuels/

**Abstract**

• Natural seaweed harvesting for biofuels in Scotland would be restricted to remote island areas. Beachcast seaweed represents an unexploited resource for biofuels and the possibilities for seaweed farming all around the coastline should be investigated

• A mixture of microbiota from ruminants, digester leachate and marine mud makes the most productive anaerobic digestion (AD) inoculum

• Stable AD of *L. hyperborea* requires loading rates of around 1% wet weight/volume final concentration

• Metagenomic analysis was successfully used to ‘fingerprint’ AD microbiota

• Phlorotannins inhibited AD of *Fucaceae* and these were successfully extracted and neutralised. Extracted PT was bioactive with radical scavenging activity.

**Introduction**

There is an urgent need for research into alternative means of producing fuels to replace diminishing and air-polluting fossil fuels. One possibility is to use seaweed as a biomass for biofuel production which does not compete with food production. Since seaweed is a wet biomass, fermentation (and in particular anaerobic digestion for biomethane production) seems the most cost effective means of producing biofuel from seaweed.

The natural productivity of seaweeds is greater than many terrestrial plant systems at approximately 1 ton dry weight per hectare per year in nutrient poor water. In algal cultivation systems however this can be greatly exceeded with, for example, 60 tons dry weight per hectare per year of L. japonicum being produced in China.

The dominant seaweeds in Scotland (and in the North Atlantic in general) have been recorded as the brown kelps *Laminaria* spp., *Ascophyllum nodosum* and *Fucus* spp. In warmer sub-tropical waters *Macrocystis pyrifera* has in particular being investigated extensively for biofuel production.

Substantial amounts of seaweed, *Laminaria* in particular, are anecdotally beach cast every winter with one estimate being for 400,000 tons annually in the Hebridean Islands alone. In other areas, such as the leisure beaches within South Ayrshire council’s jurisdiction, an estimated 10,000 tons of mixed seaweeds are removed as a pollutant annually.

**Overall Goals of the SEAWEED AD**

The overall aims of the present 24 month project were to develop fermentation technologies and apply metagenomic methods to select and study an optimum microbial inoculum and define seaweed processing conditions (including detoxification) for the production of methane biofuel from the anaerobic digestion (AD) of seaweeds. In addition, the presence of bioactive compounds released by bacterial enzymatic hydrolysis was assessed with a consideration as to their add-on value in AD of seaweed.

**Main Results**

The hydrolysis of seaweed polysaccharides is the rate limiting step in anaerobic digestion (AD) of seaweeds. Seven different microbial inocula and a mixture of these (inoculum 8) were therefore compared in triplicate mini-fermenters, each inoculum was grown over four weeks in static culture and assessed for the ability to digest *Laminaria* anaerobically.

All the inocula could degrade *L. hyperborea* and produce methane to some extent. However, an inoculum of slurry from a human sewage anaerobic digester, one of rumen contents from seaweed-eating North Ronaldsay sheep and inoculum 8 used most seaweed volatile solids (VS) (59 and 68% used), suggesting that these each had efficient seaweed polysaccharide digesting bacteria. The human sewage inoculum, an inoculum of anaerobic marine mud mixed with rotting seaweed and inoculum 8 all developed to give higher volumes of methane (41-62.5 mL/g seaweed VS by week 4), compared to other inocula (3.5 and 27.5 mL/g VS). Inoculum 8 also gave the highest acetate production (6.5 mmol/g VS) in a single-stage fermenter AD system and produced most methane (8.4 mL/mmol acetate) in phase II of a two-stage AD system. Overall inoculum 8 was found to be the most efficient inoculum for AD of seaweed. The study therefore showed that selection and inclusion of efficient polysaccharide hydrolysing bacteria and methanogenic archaea in an inoculum offer increased methane productivity in AD of *L. hyperborea*.

DGGE gel analysis of 16SrRNA gene PCR products from AD inocula gave a fingerprint of the diversity of bacteria and archaea in each inoculum. Pyrosequencing generated between 3,000 and 7,000 bacterial and archaeal sequences which so far have been identified to genus level. Preliminary results so far indicate that the main archaeal genus in inoculum 8 was *Methanosarcina* (24-94% of sequences found). This genus was derived from the municipal waste and the anaerobic marine mud in inoculum 8. *Methanobrevibacter* also persisted in low densities in inoculum 8 fermenters and this was a dominant genus in ruminant mother inocula (60-95% of sequences found). This contrasted with the dominance of *Methanosaeta* in human sewage samples, which did not persist in inoculum 8. This supports the contention that mixing microbiota from selected but diverse sources improves seaweed AD.

The AD process on seaweeds was further optimised regarding variables such as diluent composition, temperature, feed rate and continuous or occasional stirred fermentation. It was also concluded that seawater could be used as a diluent for AD in areas where fresh water is prohibitively expensive. Moreover, our results suggest that inoculum 8 is an efficient utiliser of seaweed polysaccharides and pre-treatment with heating or alkali does not improve its use. Continuous seaweed stirring appears not to be necessary for efficient AD for small fermenter volumes. However, on scale-up a 5L static fermenter was found to trap biogas and cause seaweed feed to float, which is undesirable and thus sufficient stirring is advised in order to stop gas entrapment and keep system costs low. Conducting AD of seaweed at 25 and 30°C significantly (*p*<0.05) reduced methane productivity compared to AD at 35 and 37°C but the percentage of methane in the biogas and the percentage of volatile solids (VS) used was similar (52-54% methane; 53-63% VS used). This suggests that seaweed AD with inoculum 8 could proceed at lower temperatures but it would take significantly longer and the yield would be lower.

Feed rate will also determine how quickly AD of seaweed can proceed. From mini-fermenter studies it was recommended that for a good methane yield *L. hyperborea* should not be fed at concentrations above 10% w/v and this would require careful pH adjustment. In later 5L unstirred fermenter scale-up studies FOS/TAC analysis of fermenter leachate showed that product feedback inhibition was likely at feed concentrations of between 10 and 2.5%. Optimum yield (and fermenter stability, requiring no pH adjustment) was achieved at 1.25% w/v feed concentration giving 150 mL biogas/g VS.

A problem with seaweed AD is that phlorotannins (PT), especially in *Fucaceae*, have been found to cause toxicity. Dr Sutherland (the fellow) and his group at GCU found that the brown seaweeds Ascophyllum nodosum and Fucus serratus were, unlike L. hyperborea, toxic to North Ronaldsay sheep microbes and prevented AD. In this report studies at CCMAR have shown that inoculum 8 that was so successful in AD of L. hyperborea was however also susceptible to toxicity by the brown seaweeds *Ascophyllum nodosum* and *Fucus serratus*. This toxicity was shown to be related to high PT concentrations in these seaweeds and it was successfully neutralised by extraction and precipitation or removal of these (results to be published). Extraction of PT whilst markedly improving AD also presented the opportunity to use PT as a bioactive co-product with antimicrobial, antioxidant activities. The PT from *A. nodosum* was extracted (0.4 mg/mL) by using enzymes from individual bacterial strains isolated from North Ronaldsay sheep which was as productive as any physicochemical method tested.

**Conclusions**

The results of this project generated, at lab scale, an optimised inoculum and process parameters with which AD of brown seaweeds can efficiently produce methane biofuel and bioactive co-products. Metagenomics was developed to allow fingerprinting of the active AD fermentation. Scale-up of the process developed and optimised in this project will undergo scale-up and a pilot-scale entrepreneurial venture is expected.