# Chapter 15
# Enhancing Methane Production from Spring-Harvested *Sargassum muticum*


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**Abstract** *Sargassum muticum* is a brown seaweed which is invasive to Europe and currently treated as waste. The use of *S. muticum* for biofuel production by anaerobic digestion (AD) is limited by low methane ($CH_4$) yields. This study compares the biochemical methane potential (BMP) of *S. muticum* treated in three different approaches: aqueous methanol (70% MeOH) treated, washed, and untreated. Aqueous MeOH treatment of spring-harvested *S. muticum* was found to increase $CH_4$ production potential by almost 50% relative to the untreated biomass. The MeOH treatment possibly extracts AD inhibitors which could be high-value compounds for use in the pharmaceutical industry, showing potential for the development of a biorefinery approach; ultimately exploiting this invasive seaweed species.

**Keywords** Biofuel · Biogas · Seaweed · Post-harvest treatment

## 15.1 Introduction

*Sargassum muticum* is considered a menace as it is an invasive seaweed species to Europe [1]. Although this seaweed species has the potential to be exploited for its pharmacological and biomedical value, it is treated as 'waste' as there are no current utilisation methods. Methods to utilise this seaweed in a biorefinery approach could have positive economic and environmental implications.

Seaweed shows potential as a feedstock for biofuel production via methods such as anaerobic digestion (AD) that can handle wet biomass [1]. During AD, organic material is converted in an oxygen-free environment into $CH_4$ and carbon dioxide in a series of degradation steps carried out by different groups of bacteria and Archaea. However, the use of *S. muticum* as a feedstock for AD is currently limited by its low $CH_4$ yields [1]. This could partly be due to potential inhibitors of AD in seaweed; secondary metabolites extracted from *Asparagopsis taxiformis* showed inhibitory

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effects against the ruminal microbial population and $CH_4$ production in the first 96 h [2]. The removal of other components of seaweed, such as polyphenols and salts, have also been associated with $CH_4$ yield enhancements [3].

This study aims to remove potential inhibitors of AD from *S. muticum* using aqueous methanol (MeOH). To the authors' knowledge, this is the first study to determine the biochemical methane potential (BMP) of aqueous MeOH treated *S. muticum* and subsequently, its prospective use as a feedstock for AD. The BMP of MeOH treated residues is also compared to washed seaweed, a pre-treatment method that enhances $CH_4$ production rates from summer-harvested *S. muticum* [4].

## 15.2 Experimental Methods

### *15.2.1 Sample Preparation*

*S. muticum* samples were collected in May (spring) from Broadstairs, UK (TR399675). Samples of *S. muticum* were rinsed with deionised water ($dH_2O$) to remove sand and any residues from the seawater, stored at −18 °C, and freeze-dried (FD) (−55 °C, 48 h).

Three types of samples were prepared from spring-harvested *S. muticum* samples (Table 15.1). The MeOH treated residues (MTR) from three replicates were pooled and air-dried under the fume hood (24 h). For each replicate, the extracts from the sequential extraction were pooled and dried (Genevac™ Concentrator EZ-2). The wash solutions from the sequential washing were pooled and freeze-dried (−55 °C, 48 h), herein referred to as washed extract. Extraction yields were measured gravimetrically.

**Table 15.1** Treatment methods of *Sargassum muticum* for BMP determination

| Sample type | Treatment | Conditions |
|---|---|---|
| FD samples | Freeze-dried seaweed ground (Lloytron®, Kitchen Perfected coffee grinder) to a fine powder | – |
| Washed residues | 5 sequential washes of ground FD spring samples (1:10 solid: solvent ratio) (n = 6) | FD samples mixed in deionised water, centrifuged (3,900 rpm, 20 min; Eppendorf, Centrifuge 5810R), repeated ×5 |
| 70% MeOH treated residues | 3 sequential 70% aqueous MeOH (v/v) extractions of ground FD spring samples (1: 30 solid: solvent ratio) (n = 3) | FD samples mixed in 70% MeOH, incubated (room temperature (21.5 °C), 90 min), centrifuged (3,900 rpm, 20 min), repeated ×3 |

### 15.2.2 Dry Weight and Ash Content

Residues and extracts were dried in a vacuum oven at 105 °C overnight to determine their dry weight (DW) [4]. Ash and volatile solids (VS) contents were determined according to [5].

### 15.2.3 Biochemical Methane Potential (BMP) Determination and Specific $CH_4$ Yield Calculation

The inoculum was collected from an anaerobic digester treating paper-making waste at Smurfit Kappa Townsend Hook Paper Makers, Kent, United Kingdom. The inoculum was 'degassed' in a water bath (37 °C, 7 days) to minimise its contribution to the $CH_4$ yields during the BMP test [6], and then homogenised using a handheld blender (Philips™) before use.

The Automatic Methane Potential Test System II (AMPTS II) was used to measure $CH_4$ production. Replicates were made containing 2 g VS content of each sample type in Table 15.1. The inoculum was added to make an inoculum-to-substrate ratio of 5 and made up with water to 400 g. Blanks with only inoculum and water were used to calculate the net $CH_4$ production from *S. muticum*, removing the $CH_4$ contribution by the inoculum. Reactors were mixed continuously (150 rpm) and incubated at 37 °C. $CH_4$ volumes were recorded daily over 28 days and corrected to water vapour content at 0 °C, 101.325 kPa.

Specific $CH_4$ yields were calculated by multiplying the $CH_4$ potentials and the amount of residue remaining after the washing and extraction processes if 1 kg WW of *S. muticum* was treated according to Table 15.1.

### 15.2.4 Statistical Analysis

Excel (2016) was used for t-tests, and IBM SPSS version 25 was used for one-way and two-way ANOVA (with post-hoc analysis). Dependent variable: net cumulative $CH_4$ yield. Independent variables: treatment (FD samples, washed residues, MTR), day (time during BMP test).

**Table 15.2** Volatile solids (VS) and ash content of residues and extracts as % of the dry weight (DW)

| Samples | VS (% DW) | Ash (% DW) |
|---|---|---|
| FD *S. muticum* | 74.7 | 25.3 |
| Washed residues | 85.5 | 14.5 |
| Washed extract | 60.1 | 39.9 |
| MeOH treated residues | 82.7 | 17.3 |
| MeOH extract | 64.9 | 35.1 |

## 15.3 Results

### *15.3.1 Dry Weight and Ash Content of Extracts and Residues*

The material removed by washing FD *S. muticum* (42.8% DW) was significantly higher than those extracted by 70% aqueous MeOH (35.3% DW) (t-test, $p < 0.05$). The washed residues had a higher VS content and a lower ash content compared to MTR, corresponding to the higher ash content in the washed extract compared to the MeOH extract (Table 15.2). MTR and washed residues have significantly lower ash content and higher VS content relative to the FD samples (one-way ANOVA, $p < 0.05$).

### *15.3.2 $CH_4$ Production Profile and Specific $CH_4$ Yield*

Figure 15.1 shows the $CH_4$ production profile of FD samples, washed residues and MTR during the BMP test. A significant interaction between days of incubation and

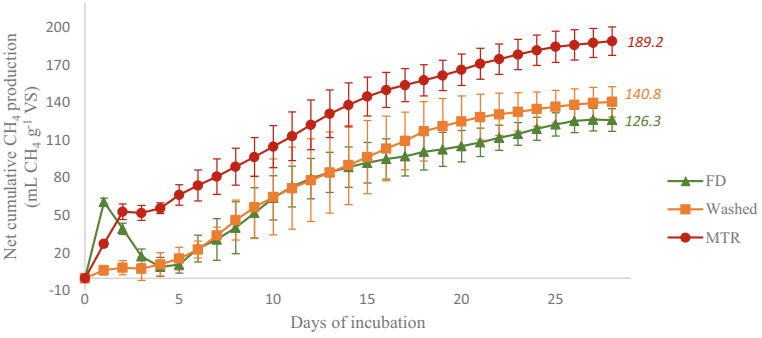


**Fig. 15.1** Net cumulative $CH_4$ production of FD samples (FD [n = 3]; green triangles), washed residues (Washed [n = 6]; orange rectangles) and MeOH treated residues (MTR [n = 4]; red circles) over the duration of the BMP test (28 days). Final $CH_4$ potentials (after 28 days) are shown in italics in their respective colours. Error bars represent standard deviations

**Table 15.3** Specific $CH_4$ yield of FD samples, washed residues and MeOH treated residues

| Samples | Specific $CH_4$ yield (L $CH_4$ $kg^{-1}$ WW *S. muticum*) |
|---|---|
| FD *S. muticum* (n = 3) | 12.7 ± 0.9 |
| Washed residues (n = 6) | 9.1 ± 0.8 |
| MeOH treated residues (n = 4) | 14.3 ± 0.8 |

the treatment on the net cumulative $CH_4$ production was found (two-way ANOVA, $p < 0.05$). Post-hoc comparisons revealed that, except for day 2, the $CH_4$ production profile of MTR was significantly different from those of FD samples and washed residues for the duration of the BMP test ($p < 0.05$). In contrast, the $CH_4$ production profiles do not differ significantly between washed residues and FD samples ($p > 0.05$), except for days 1 and 2 when the mean net cumulative $CH_4$ production by washed residues was significantly lower (up to 54.5 mL $CH_4$ $g^{-1}$ VS) than FD samples ($p < 0.05$). Ultimately, while the final $CH_4$ potentials (after 28 days) of FD and washed residues were not significantly different ($p > 0.05$), those of the MTR's were statistically higher than both the FD samples and washed residues (one-way ANOVA, $p < 0.05$).

The specific $CH_4$ yield produced by MTR is statistically higher than the FD samples by 12.6% (t-test, $p < 0.05$) (Table 15.3). Comparatively, washing reduces the specific $CH_4$ yield by 28.3% relative to the FD samples; mean yields were statistically different (t-test; $p < 0.05$).

## 15.4 Discussion

Washing FD *S. muticum* removed 7.5% more compounds than 70% aqueous MeOH. This is partly attributed to the higher ash content removed by water, which may be due to its higher solubility in water compared to MeOH. Higher extraction yields by water compared to alcoholic solvents are also found in the literature [7, 8]. The compounds removed by water had negative implications for AD of spring *S. muticum.* Despite enhanced VS content of 10.8% relative to the FD samples, washed residues still have lower specific $CH_4$ yields (3.6 L $CH_4$ $kg^{-1}$ WW) and statistically similar $CH_4$ potentials to FD samples.

MTR showed higher VS content relative to FD samples, but lower VS content relative to washed residues. MTR produced 49.8% and 34.4% higher $CH_4$ potential relative to the FD and washed residues, respectively. The highest $CH_4$ production potential achieved by MTR could, therefore, be related to the removal of potential inhibitors of AD from *S. muticum.* MeOH extracts of *Sargassum spp.* showed antibacterial activity against *Bacillus subtilis*, with up to half the inhibitory potential of tetracycline, a potent antibiotic with anti-methanogenic activity [9, 10].

Other potential inhibitory compounds of AD include polyphenolic compounds and fatty acids [11]. However, polyphenolic and lipid content of the three sample

types showed no correlation to the $CH_4$ potential (data not shown). Repeats of this experiment showed lower $CH_4$ potentials from MTR, but are nevertheless higher than FD samples. Higher $CH_4$ potentials in this experiment could be due to incomplete removal of aqueous MeOH. Further analysis of the antimicrobial potential and identification of compounds present in the aqueous MeOH extracts are needed to clarify their inhibitory potentials. The identification of compounds with a pharmacological or biomedical value may also allow for the development of a biorefinery approach.

## 15.5 Conclusion

Treating spring *S. muticum* with aqueous MeOH prior to AD can be an effective method to enhance $CH_4$ production, making it a more suitable feedstock compared to untreated *S. muticum*. This pre-treatment method shows potential in a biorefinery approach to ultimately exploit this invasive seaweed species when potential uses of compounds extracted by MeOH are identified.

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