# Sargassum -Golden tide or Golden Opportunity

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**University of Greenwich, Faculty of Engineering and Science Research Seminar 9th December 2020**

SLIDE 1


Good afternoon, I am Dr John Milledge and I part of the Algal Biotechnology Research Group at the University of Greenwich. We have been researching methods to exploit members of the genus Sargassum since I joined the team over seven years ago.

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It may appear a little odd to start a presentation with a thank you. Nonetheless, this is the most crucial point of my talk. As many of you may be aware, I was hospitalised by COVID 19 for over four weeks in April. I am incredibly thankful for being a COVID survivor and to all the Darent Valley Hospital staff for saving my life. All the NHS staff were professional, courageous, compassionate. I had the privilege to have met many past and current students of the University of Greenwich during my illness; it made me incredibly proud to be GRE. Thank you to all my colleagues across the university for training such outstanding people.

The support of the University from Jane Harrington down was terrific. I cannot thank my colleagues enough for their support for Pam and me during a tough time.

You may still be asking why a thank you now? Well COVID has changed much and caused many of us to rethink. I too, have reflected and decided to retire at the end of this month. So, this lecture is an opportunity to say thank you and goodbye.

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Before I continue with this seminar, I must make a brief apology.  It is disappointing that the long tail of this disease has left me breathless and lacking concentration; unfortunately, I often stumble and mumble more than I would like, and I'm unable to deliver the 'light and shade' of past live performances. Apologies, I hope the captions increase clarity and that you find the talk informative.

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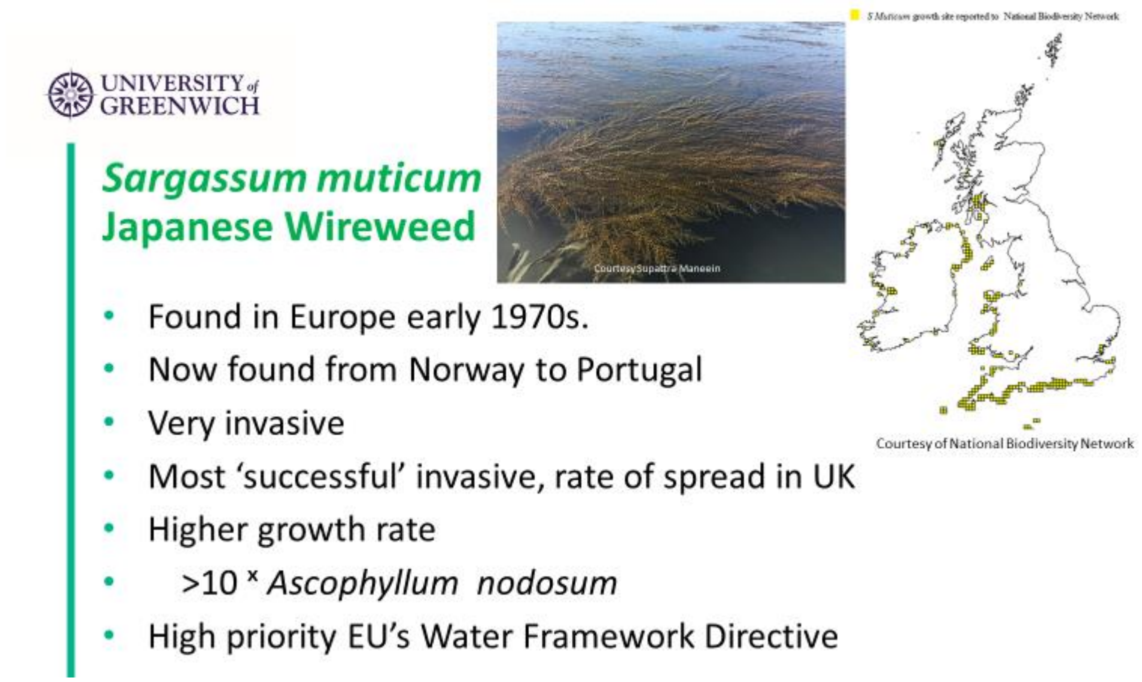

Sargassum is a broad genus or family of brown seaweed consisting of over 300 species. The vast majority of species of Sargassum have a phase of their life cycle than is anchored to the seafloor. Our initial interest was on *S. muticum,* an invasive species to the UK and Europe, which has been causing environmental and economic damage. *Sargassum muticum,* or Japanese wireweed, is native to the northwest Pacific region. It appeared in Europe in the early 1970s and is now found on shorelines from Norway to Portugal. Since its first recorded find in the UK, on the coast of the Isle of Wight, it has spread along the south coast and around the British Isles. The growth rate of *S. muticum* is generally considerably higher than most UK seaweed species, being over ten-times higher than that of *Ascophyllum nodosum*. *S. muticum* has been described as the most 'successful' invasive species in the UK in terms of its rate of spread. The UK has identified *S. muticum* as a species of high priority under the EU's Water Framework Directive, and it causes considerable problems in certain areas of the Kent coast, especially on chalk ledges, and is spreading, possibly displacing native algae.

Globally, invasive species cost US$ 1.4 trillion per year ~5 % of the world economy.

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The clearance of this seaweed is an issue in southern England and has considerable financial and energy costs. The harvesting of *S. muticum* has failed to eradicate it from the coast of the British Isles, but regular harvesting by hand is used as a method to reduce its spread and the problems caused by its growth.  The harvesting of *S. muticum,* in an attempt to control it, results in the need to dispose of large quantities of seaweed biomass. Although *S. muticum* has been exploited for aquaculture in China and as a traditional food in Korea, there is currently no commercial exploitation of this biomass in Europe.

The valorisation of *S. muticum* biomass for fuel and other products could encourage its harvesting and management. We, therefore, examined potential use to encourage harvesting and control. As part of the MacroBioCrude project initially, we examined the potential of *S. muticum* as a fuel.

As we were studying applications for the use of *S. muticum,* we became aware of the increasing problem of holopelagic Sargassum inundations on the beaches of the Gulf of Mexico and the Caribbean together with areas of coastal west Africa.

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Holopelagic or floating Sargassum (*S. natans* and *S. fluitans*) spends all its life cycle floating and never attaches to the seafloor. In the open ocean, it is a tremendous ecological and climate resource. Pelagic Sargassum or Gulfweed has a been described as a floating jungle or golden floating rain forest. It is essential for a diverse range of invertebrates, fishes, sea turtles, birds and mammals, with over 145 species of invertebrates and 100 species of fishes being associated with it. Sargassum of the Sargasso Sea is not only of ecological importance but has a global role in ocean sequestration of carbon, with the Sargassum of the Sargasso Sea being a net sink of $CO_2$ representing ~7 % of the worldwide net ‘carbon pump’.

Although small deposits of *Sargassum* have naturally and regularly occurred on beaches and play a role in stabilising beaches and providing nutrients, the enormous beach inundations in the last ten years are causing economic challenges. Tourism was worth over US$ 29.2 billion in onshore spending in the Caribbean and contributed over 80 % of the regional GDP. Tourists are reported to avoid resorts affected by golden tides.  Sir Hilary Beckles, the Vice-Chancellor of the University of the West Indies, has described the inundation of Caribbean and Mexican Gulf beaches as “an international crisis” and “the greatest single threat” to the Caribbean. It has been estimated that it would take at over US$ 120 million to clean up the *Sargassum* inundations across the Caribbean.

The amount of Pelagic *Sargassum* on the ocean is enormous, and thus the potential for golden. The Sargasso Sea, so-called due to the abundance of *Sargassum,* is the greatest aggregation of seaweed in the world with a total biomass of 10-million tonnes. However, a further new massive accumulation has been observed in satellite imagery since 2011, known as the Great Atlantic Sargassum Belt (GASB), that stretches for over 5000 miles from West Africa to the Gulf of Mexico. The GASB has been estimated to contain over 20 million metric tons of Sargassum biomass.

We received a number inquiries about our work on *Sargassum* muticum and an invitation from the Caribbean Council in 2016 to speak at a conference on Sargassum in the British Islands; however, due other oversea commitments we were unable to attend. Nonetheless, this prompted us to review the current position on the uses and challenges of pelagic Sargassum, “Golden Tides: Problem or Golden Opportunity? The Valorisation of Sargassum from Beach Inundations”. Several conversations were held with a variety of organisation on how the University of Greenwich could help in examining the composition and use of pelagic Sargassum, but finding funding was the major stumbling block. Finally, Dr Debbie Bartlett and I, together with the Turks and Caicos Government, obtained financing from Darwin Plus in 2019 to examine “Sustainable solutions for Sargassum inundations in Turks & Caicos”.

Before we move on to examine our work on pelagic Sargassum inundation lets us first look at some of the key findings of our work on *S. muticum.*

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## *S. Muticum* composition

| | Moisture | Ash | N | C | H | S | O | Salt | HHV |
|---|---|---|---|---|---|---|---|---|---|
| | % total wt. | % dry weight | | | | | | % Ash | kJ $g^{-1}$ dw |
| March 2014 | 79.9 | 29.4 | 4.9 | 30.7 | 4.0 | 1.5 | 29.6 | | 16.4 |
| July 2015 | 85.5 | 33.1 | 3.6 | 30.1 | 4.2 | 0.8 | 28.1 | 46.1 | 12 |
| June 2017 | 85.6 | 32.7 | 3.9 | 27 | 4.7 | 0.6 | 31.1 | 51.5 | 11.5 |

- High Moisture
- High Ash
- Varies seasonally

As with all seaweeds, *S. muticum* has a challenging composition for exploitation as a fuel; it is high in moisture  and rich in inorganic ash . The makeup varies seasonally . The ash content is 15-20 times higher than the wood used in fireplaces, and those of us that have wood burners know how much ash we must remove for our grates burning wood to heat our homes.

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## Biofuels

| Method | Utilises entire organic biomass | Utilises wet biomass | Primary energy product |
|---|---|---|---|
| **Direct combustion** | ✓ | ✗ | Heat |
| **Pyrolysis** | ✓ | ✗ | Primarily solid by slow pyrolysis |
| **Gasification** | ✓ | ✗ [b] (conventional) | Primarily Gas |
| **Biodiesel production** | ✗ | ✗ [c] | Liquid |
| **Hydrothermal treatments** | ✓ | ✓ | Primarily Liquid |
| **Bioethanol production** | ✗ [a] | ✓ | Liquid |
| **Biobutanol production** | ✗ [a] | ✓ | Liquid |
| **Anaerobic digestion** | ✓ | ✓ | Gas |

[a] Polysaccharides require hydrolysis to fermentable sugars. Some of the sugars produced from the breakdown of seaweed polysaccharides are not readily fermented; [b] Supercritical water gasification (SCWG) an alternative gasification technology can convert high moisture biomass; [c] No current commercial process for the wet trans-esterification of wet macroalgal biomass

Methods which require drying may not be energetically viable. The amount of energy to dry Sargassum is nearly as high as the Higher Heating Value of the biomass. Anaerobic digestion (AD) is generally the preferred method for wet biomass and can exploit the entire biomass. It is a relatively simple procedure from an infrastructure/engineering perspective. AD consists of a series of actions by various groups of bacteria that convert organic materials into methane, carbon dioxide, and bacterial biomass in the absence of oxygen. The biogas produced from the AD of seaweed typically contains methane 50–70%, carbon dioxide 30–45% and some hydrogen, hydrogen sulphide and ammonia.

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## Theoretical Methane Potential

| VS Empirical Formula | Methane yield | |
|---|---|---|
| | L $CH_4$ $g^{-1}$ VS | L $CH_4$ $g^{-1}$ TS |
| $C_1H_{1.66}O_{0.7}N_{0.1}S_{0.01}$ | 0.42 | 0.28 |

### Buswell equation stoichiometric calculation

$$C_cH_hO_oN_nS_s + {}^1/_4(4c - h - 2o + 3n + 2s)H_2O = {}^1/_8(4c + h - 2o - 3n - 2s)CH_4 + {}^1/_8(4c - h + 2o + 3n + 2s)CO_2 + nNH_3 + sH_2S$$

molecular formula subscripts, c, h, o, n and s = molar proportion of elements CHONS

Symons, G. E. and A. M. Buswell (1933) The methane fermentation of carbohydrates. Journal of the American Chemical Society 55(5): 2028-2036.
Buswell, A. M. and H. F. Mueller (1952) Mechanism of methane fermentation. Industrial and Engineering Chemistry 44(3): 550-552.

The theoretical methane potential of *S. muticum* can be calculated using a stoichiometric equation, the Buswell Equation. The chemical composition of Sargassum makes it a promising feedstock with a potential methane yield of 0.42 grams per gram of volatile solids (VS). A typical value for sewage sludge is less than 60 % of this.

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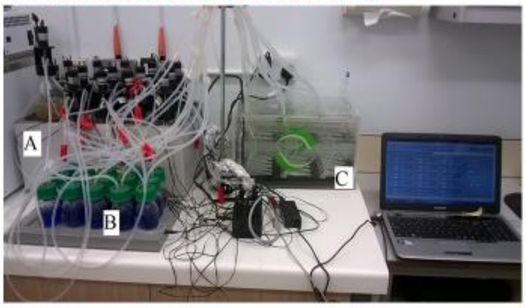
## Methane Potential




**Automatic Methane Potential Test System (AMPTS)**

A) water-bath with controlled temperature and 15 digestion bottles

B) 15 CO2 fixing bottles,

C) A tipping cup volumetric gas measuring device

| Average Methane Yield | % of Theoretical Yield |
|---|---|
| L $CH_4$ $g^{-1}$ VS | |
| 0.10 | 25% |

However, the experimental methane potential was only around 25% of the theoretical.

There are several potential reasons for low practical methane yields from Sargassum.

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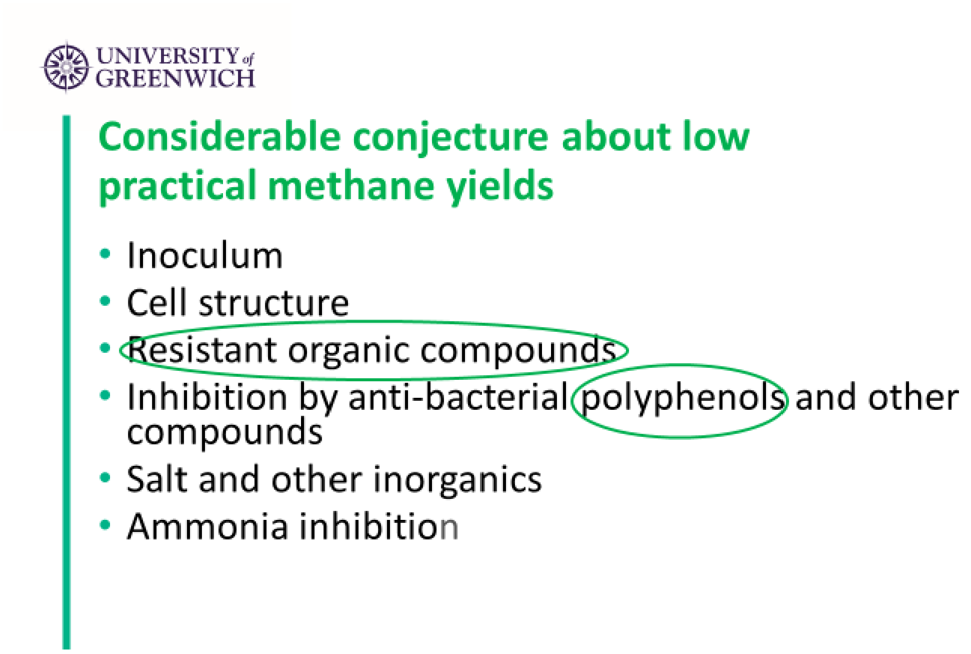


However, two of the most important that we have examined are recalcitrant organic compounds and polyphenolics. It would be great to fully identify the substances that inhibit the anaerobic bacterial breakdown of Sargassum. Supattra is working on phenolic compounds, whilst Mahmud has commenced a new study on iodine. Brominated compounds are known inhibitors of methane production in ruminants. Iodine in seaweed can be valuable in the human diet but can also be problematic in excess. However, little information is available on the effect of iodine on the anaerobic digestion of seaweed.

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## Alginic acid recalcitrant

| Average Gas yield mL $CH_4$ $g^{-1}$ substrate dw | | |
|---|---|---|
| Alginic Acid Sodium Salt | Alginic Acid | Cellulose |
| 76 | 73 | 183 |

Seaweeds can be rich in hydrocolloids such as alginic acid, which can be difficult to breakdown.

The methane yield from both alginic acid and its sodium salt are only around 40% of cellulose.

Sargassum also can be rich in phenolics containing up to 5% of their dry weight.

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## Substrate and phenolic interaction

Highly significant effect (P<0.001)

- Phenolic compounds did **not** inhibit breakdown of the simple compound, glycerol
- High concentrations of epicatechin reduced methane yield from alginic acid
- High concentrations of phloroglucinol reduced methane yield from the sodium salt of alginic acid
- Phenolic compounds may inhibit the breakdown of complex molecules in the initial AD hydrolysis stage

We carried out a study examining the effect on methane yield of three simple, model phenolics, phloroglucinol, gallic acid and epicatechin on four model substrates, glycerol, Cellulose, Alginic acid and the Sodium salt of Alginic acid. A highly significant interaction was found between substrate and phenolics. Although none of the phenolics inhibited the breakdown of the simple compound glycerol, various phenolics appeared to inhibit the breakdown of more complex molecules. Our PhD student Supattra Maneein is trying to identify the phenolics in *S. muticum* and has found the removal of phenolics by 70% methanol improves methane yield.

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## Co-digestion with other wastes

**Co-digestion with crude glycerol a by-product of biodiesel**

| | Ave Methane Yield | % of Theoretical Yield |
|---|---|---|
| | L $CH_4$ $g^{-1}$ VS | |
| Crude Glycerol | 0.26 | 46% |
| *Sargassum muticum* | 0.07 | 17% |
| 50% Crude Glycerol & *S. muticum* | 0.21 | 43% |

**Co-digestion increased biogas yield by 27%**

Co-digestion of Sargassum with other wastes may also be a method of improving methane yield; mixing waste glycerol from biodiesel production with *S. muticum* improved the yield of the combined biomass by 27% relative to the individual materials. ANAEGIA has also found a synergistic enhancement in AD by combining pelagic Sargassum with food-waste.

Seaweed growth, as with terrestrial plants, is seasonal, and the composition of seaweed varies through the growth period; seaweed is not harvested continuously and often only once annually. Therefore, there is a need for effective methods to preserve seaweed to supply biofuel production throughout the year. The seasonal growth of seaweed and the need to preserve and store it are considered as critical barriers to the exploitation of seaweed for biofuel. We already mentioned that drying might not be energetically viable for biofuel production. An alternative low energy preservation method, to drying and dewatering, is ensiling. It is routinely used for the storage of forage for animal feed. In the anaerobic conditions of ensilage, fermentation converts water-soluble carbohydrates into organic acids, mainly to lactic acid, reducing pH and preserving the wet biomass.

Our research has also shown that ensilage may be an energetically viable method of preserving Sargassum for year-round use with low energy losses (<8%).

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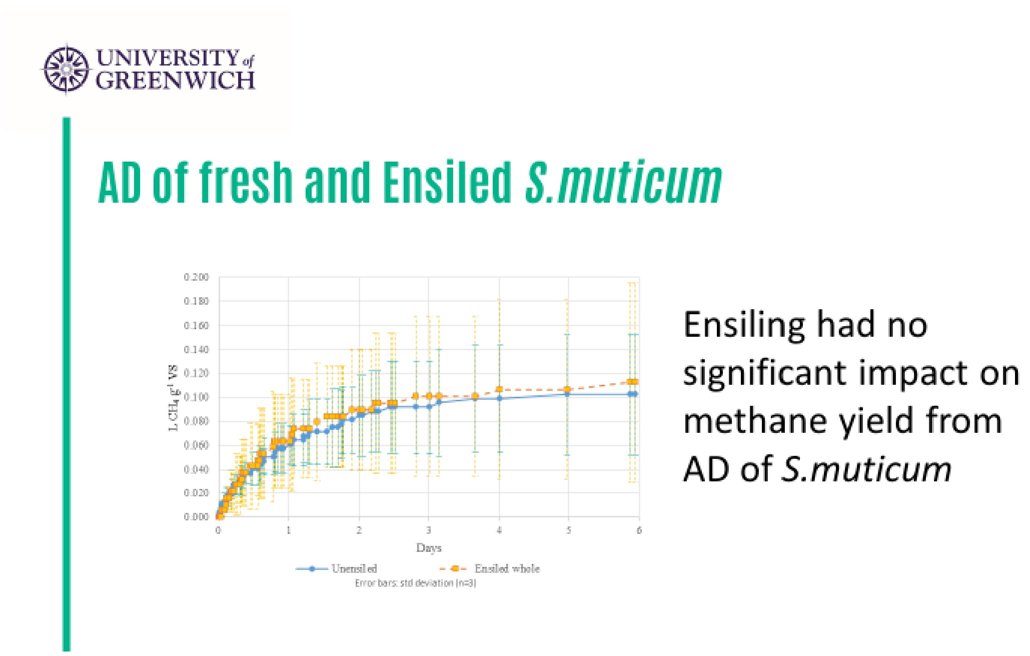


As can be seen in the graph, ensiling had no significant impact on methane yield. As many of you know who deal with biological systems, there is often considerable variability between replicates. All the same ensiling could be a viable alternative to drying for the preservation of Sargassum to overcome the problem of discontinuous supply. We hope that we may examine ensilage as a low-energy, relatively simple technological method of preserving pelagic Sargassum for biofuel and other uses as part of our future work on our Darwin project.

Not only have we look at methods of preservation for long term storage, we also examined some pre-treatments.

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## Salt & Freshwater Washing

- High levels of NaCl known to inhibit AD
- It has been suggested saline algal biomass should be washed in fresh water to reduce the salt content
- Freshwater washing reduced salt by 23% and ash by 6% dw basis.
- Moisture content increased by 4% (85.6 to 89.1%)
- Carbon & Hydrogen content (methane potential) reduced by ~25% on wet weight basis
- Freshwater washing may not be viable

Salt can inhibit methane output. Many researchers have suggested saline algal biomass should be washed in fresh water to reduce the salt content, and washing in fresh water is a pre-treatment step which is often used in a wide a variety of seaweed biofuel research studies. However, fresh water is a precious resource, and although freshwater washing reduced salt, it also reduced organic solids content on a wet weight basis, the feed material for biogas production.  Thus, freshwater washing may not be viable.

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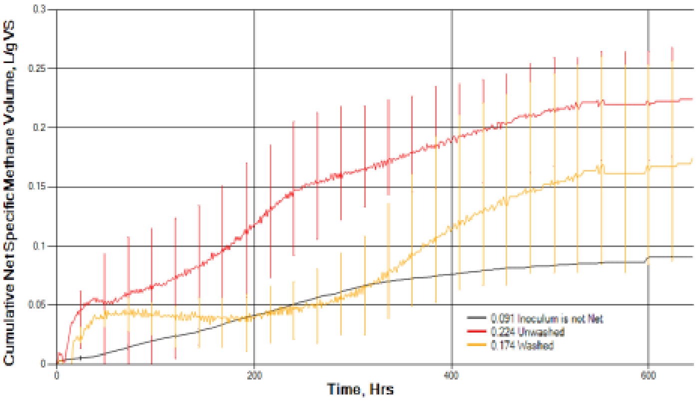
## Net cumulative methane yield unwashed and washed S. muticum




21 % reduction in methane yield, but not statistically significant ($P>0.05$).

Significant ($P<0.01$) delay in peak methane production

Potential causes

- Biota?
- Biochemical?
- Mineral?

Although methane yield was reduced per gram of volatile solids, it was not statistically significant. Nevertheless, peak methane production was delayed significantly. It could be a change in the level of natural microorganisms that aid breakdown, it could be changes in the biochemical makeup or it could be changes in the levels of minerals. Minute traces of Selenium are known to improve methane production from food waste, for example. We hope that the group will be able to study the effect of washing on seaweed further in future projects.

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We also investigated potential pharmaceutical and dental uses of *S. muticum* funded by the High-Value Chemical from Plants Network. Our initial study with IOTA Pharmaceuticals produced a widely cited review, “High-value products from macroalgae: the potential uses of the invasive brown seaweed, *Sargassum muticum”.* We also had a preliminary look at extracting and identifying potential compound of interest.

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## *S. muticum* diethyl ether extract compounds

| UPLC-MS (Acquity) analysis | | Activity indicated in on-line Literature |
|---|---|---|
| Compound | class | |
| Myristamine | Fatty acid amine | broad-spectrum activity against bacteria and viruses |
| Fucosterol acetate | Sterol | anti-osteoporotic |
| Quercetin | Flavonoid | antioxidant |
| Esculin | Flavonoid | vasoprotective |
| Karanjin | Flavonoid | insecticide. |
| Taxifolin | Flavonoid | anti-proliferative effects on cancer cells |
| 3,4,7,8-tetramethoxyflavone | Flavonoid | anti-allergic activity |
| Lupeol | Terpene | anti-inflammatory agent |
| Monoterpene | Terpene | antimicrobial and anti-inflammatory |

Note – the activity indicated by the literature is only a general indication of potential bioactivity. It does **not** mean that there is any proven clinical activity and no claim for such benefits are made by the author

Diethyl ether extracts have also shown that there are several interesting potentially bioactive compounds in *S. muticum.*

It was hoped that we could achieve a win-win situation by removing valuable biological active molecules to suppress bacterial growth whilst improving methane yield.

Periodontal diseases are the most prevalent preventable chronic disease worldwide, and dental cavities are the most common, chronic disease of early childhood. Brushing alone is not always fully effective in eliminating plaque.  In a brief study Business Innovation Voucher funded by the Higher Value Chemical from Plants Network and Mirage Healthcare, we had a quick look at potential dental applications of Sargassum.

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## Zones of inhibition

| | Yield | Conc' of extract |
|---|---|---|
| | | 10% |
| Freeze dried SM Water (40°C), 30-40 min | 6.02-14.7% | S.mutans* |
| | | Control:*** |
| Freeze dried SM Methanol, (Ambient) 45 min | 10.31% | S.mutans* |
| | | Control:*** |
| Control = chlorohexidine mouth rinse | | |
| Zone of inhibition * (<2 mm), **(2-5 mm), ***(>5 mm) | | |

However, trials of *S. muticum* extract as an alternative dental antiseptic to chlorohexidine have been disappointing with the extract not achieving a high degree of bacterial inhibition compared to chlorohexidine. However, our work on *S. muticum* continues, and I hope this brief overview of our work in this area may be of some use to those working on pelagic Sargassum.

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I would now like to move on to our initial results of our work on the composition and methane potential of pelagic Sargassum from inundations on the beaches of Turks and Caicos.

Initial samples were collected by Dr Debbie Bartlett and two University of Greenwich MSc students from Shark Bay, South Caicos in June 2019 under a Turks and Caicos Scientific Research Permit and immediately shipped chilled to our Medway campus. We received four samples collected nearshore:

a) a mixed sample
b) *S. natans VIII*
c) *S. natans I*
d) *S. fluitans*

Fresh samples were analysed for moisture and ash content, and a fresh mixed sample was investigated for methane potential using a CJC methane potential tester. The remaining samples were then freeze-dried for further analysis.

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## High in moisture, ash & salt
## Low in calories

| | % Moisture (ar) | % Ash (dw) | % Ts (ww) | % VS (ww) | % Salt (ww) | HHV KJ $g^{-1}$ |
|---|---|---|---|---|---|---|
| Mixed 'Sargassum' | 82.0% | 46.9% | 18.0% | 9.6% | 2.7% | 9.4 |
| *S. natans VIII* | 86.5% | 34.3% | 13.5% | 8.9% | 2.6% | 10.2 |
| *S natans I* | 87.4% | 35.7% | 12.6% | 8.1% | 2.9% | 10.2 |
| *S fluitans* | 86.3% | 33.6% | 13.7% | 9.1% | 2.6% | 10.3 |

All the fresh samples were high in moisture, ash and salt and low in calories. The unsorted mixed inundation sample was significantly different, being lower in calories and moisture but higher in ash.

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## Difficult to digest – high fibre

| | Ash | Lipid | Total AAs | Total Fibre | Carb' | | Indigestible | Digestible |
|---|---|---|---|---|---|---|---|---|
| Mixed 'Sargassum' | 46.9% | 3.9% | 4.2% | 33.3% | 11.7% | | 80.3% | 19.7% |
| *S. natans VIII* | 34.3% | 3.6% | 3.0% | 37.4% | 21.8% | | 71.7% | 28.3% |
| *S natans I* | 35.7% | 4.5% | 3.8% | 37.0% | 19.0% | | 72.7% | 27.3% |
| *S fluitans* | 33.6% | 4.6% | 3.3% | 31.2% | 27.4% | | 64.8% | 35.2% |

All the samples were rich in fibre. This, together with the high ash means that 65-80% of the solids of pelagic Sargassum is indigestible or difficult to breakdown in the gut or by AD. The protein contents are towards the low end of the protein content (3–16%) reported for brown seaweeds and pelagic Sargassum. However, the amino acid profile compares favourably with the ‘indispensable amino acid’ profile recommended by the World Health Organisation (WHO). It does not appear to be lacking in any amino acid. The lipid contents were relatively comparable across the four samples and similar to those previously reported for brown seaweed, but above those for pelagic Sargassum. The most prevalent fatty acid was palmitic acid, which may play a role in controlling the ‘biofouling’ of the fronds of Sargassum.

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## Arsenic could be problematic

| | | Mixed | *S. natans VIII* | *S. natans I* | *S. fluitans* |
|---|---|---|---|---|---|
| Aluminium | mg kg$^{-1}$ dw | 37.5 | 16.21 | 21.48 | 28.09 |
| Arsenic | mg kg$^{-1}$ dw | 123.69 | 20.94 | 29.76 | 26.25 |
| Cadmium | mg kg$^{-1}$ dw | 0.13 | 0.09 | 0.12 | 0.12 |
| Calcium | mg kg$^{-1}$ dw | 70305.77 | 26019.69 | 28879.26 | 33196.4 |
| Chromium | mg kg$^{-1}$ dw | <0.3 | 0.36 | ND | 0.43 |
| Copper | mg kg$^{-1}$ dw | 2.51 | 1.25 | 2.71 | 2.91 |
| Iron | mg kg$^{-1}$ dw | 3811.37 | 81.58 | 998.56 | 262.02 |
| Lead | mg kg$^{-1}$ dw | 0.26 | 0.48 | 0.28 | 0.37 |
| Magnesium | mg kg$^{-1}$ dw | 12053.19 | 15092.59 | 16546.71 | 16320.64 |
| Manganese | mg kg$^{-1}$ dw | 30.15 | <3 | <3 | <3 |
| Mercury | mg kg$^{-1}$ dw | 0.01 | 0 | 0.01 | 0.01 |
| Phosphorus | mg kg$^{-1}$ dw | 500.65 | 138.3 | 222.15 | 214.28 |
| Potassium | mg kg$^{-1}$ dw | 69359.39 | 7442.57 | 12509.16 | 7771.73 |
| Zinc | mg kg$^{-1}$ dw | 5.81 | 26.49 | 30.88 | 35.64 |

There have been concerns regarding the use of Sargassum, as seaweeds can bioaccumulate metals at concentrations many times above the levels found in the surrounding seawater. in a recent study on the concentrations of fourteen different elements in pelagic Sargassum collected from the Mexican Caribbean coast. Arsenic levels found both in this study and by Rodríguez-Martínez, et al. are above many regulatory limits. Inorganic arsenic is more toxic than organic arsenic, and although many seaweeds accumulate arsenic as less noxious arsenosugars, some species of Sargassum can have up to 80% of their arsenic content as the highly toxic inorganic form. Despite these high levels, there is a lack of information on arsenic speciation in seaweed, and in pelagic Sargassum in particular. More work in this area is required, and care should be taken in the use of pelagic Sargassum as fertilisers and feed supplements until this work is undertaken. Nonetheless, the high ash content of Sargassum could provide minerals and trace elements that are beneficial in both fertilisers and animal feeds. The limited calorific value of Sargassum and limited protein levels, despite an excellent amino acid profile, could limit its potential as a feed.

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## Low Methane yields

Strong negative correlations with both arsenic and phenolics

| | Methane Potential mL CH4 $g^{-1}$ VS | | |
|---|---|---|---|
| | Actual | Theoretical | Actual as % Theoretical |
| Mixed 'Sargassum' | -24 | 496 | -5% |
| *S. natans VIII* | 145.1 | 395 | 37% |
| *S. natans I* | 65.8 | 392 | 17% |
| *S. fluitans* | 112.7 | 392 | 29% |

This appears to be the first study to have attempted to establish the methane potential of 'fresh' pelagic Sargassum. Freeze-drying can not only reduce the mass to be transported but can also preserve biological materials with minimum damage from heat. Our research in this study and other unpublished results at Greenwich have found that there is no statistically significant effect from freeze-drying on methane yields. Thus, freeze-drying appears a suitable technique for preserving pelagic Sargassum for transport for methane potential testing. The reduced weight will also reduce transport costs. A freeze-drier has been installed at the Centre for Fields on Turks and Caicos that will allow us to study seasonal variations and changes in Sargassum stranded on the beach.

The methane potentials from all the substrates were considerably below the theoretical potential. The mixed sample had a methane potential that was not significantly different from the blank. However, the methane potential of a combination of *S. natans VIII, S. natans I* and *S. fluitans* in a ratio typically found in the waters of Turks and Caicos was very similar to that predicted from the methane potentials of the individual species. Thus, there does not appear to be a synergistic or antagonist interaction between the species on methane potential. It seems that inhibitors to methane production must be either present or present at higher levels in the mixed unsorted sample than in individual pelagic species. The composition of the 'mixed Sargassum' mats can be substantially different from the individual species, and methane inhibitors may come from other organisms and materials present in the unsorted samples.

There were strong negative correlations of both arsenic and phenolic content with methane potential. Although a high degree of correlation does not confirm causality, these finds agree with the published literature and our work on *S. muticum*. Arsenic can be highly inhibitory to AD. Phenolics have been implicated as the inhibitor of AD in several seaweed studies. Although the information is still somewhat limited, especially on Sargassum, phenolics appear to be a significant factor in the low methane yield from Sargassum and especially the mixed mats where the phenolic level was highest.

The exploitation of Sargassum, and especially unsorted mixed mats, for biogas, would appear to be very challenging. Sargassum may need to be pre-treated before AD or co-digested with other waste biomass to increase yield.

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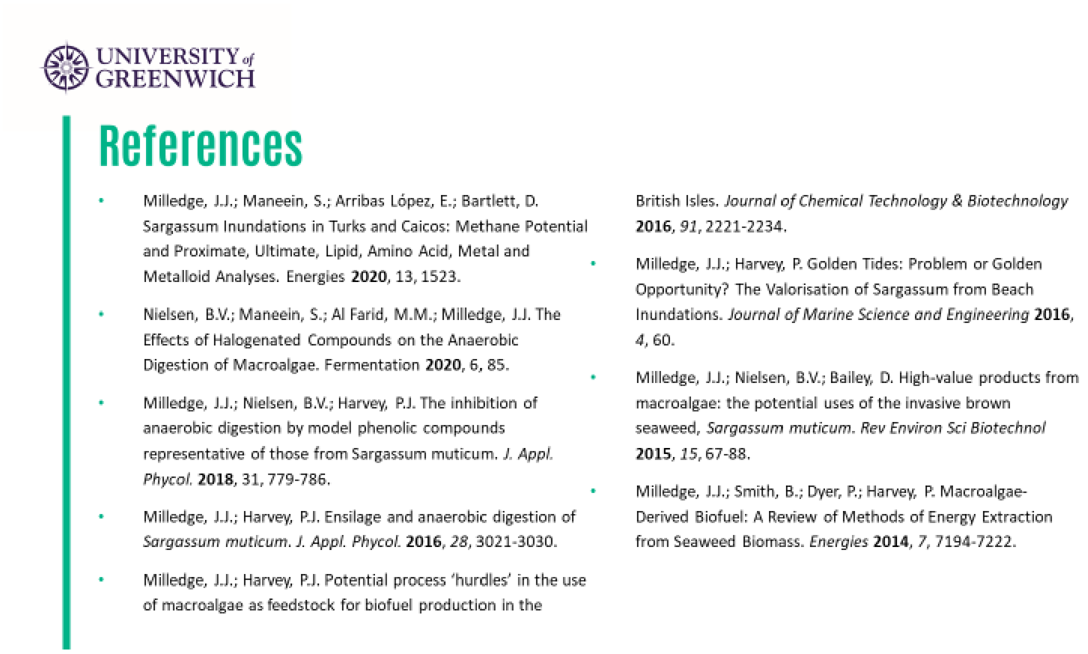
We are expecting to be able to analyse more samples from Turks and Caicos over the coming months to provide further detail on the composition of Sargassum and implications on its use as a source of biogas, fertiliser and feed supplements.

SLIDE 27

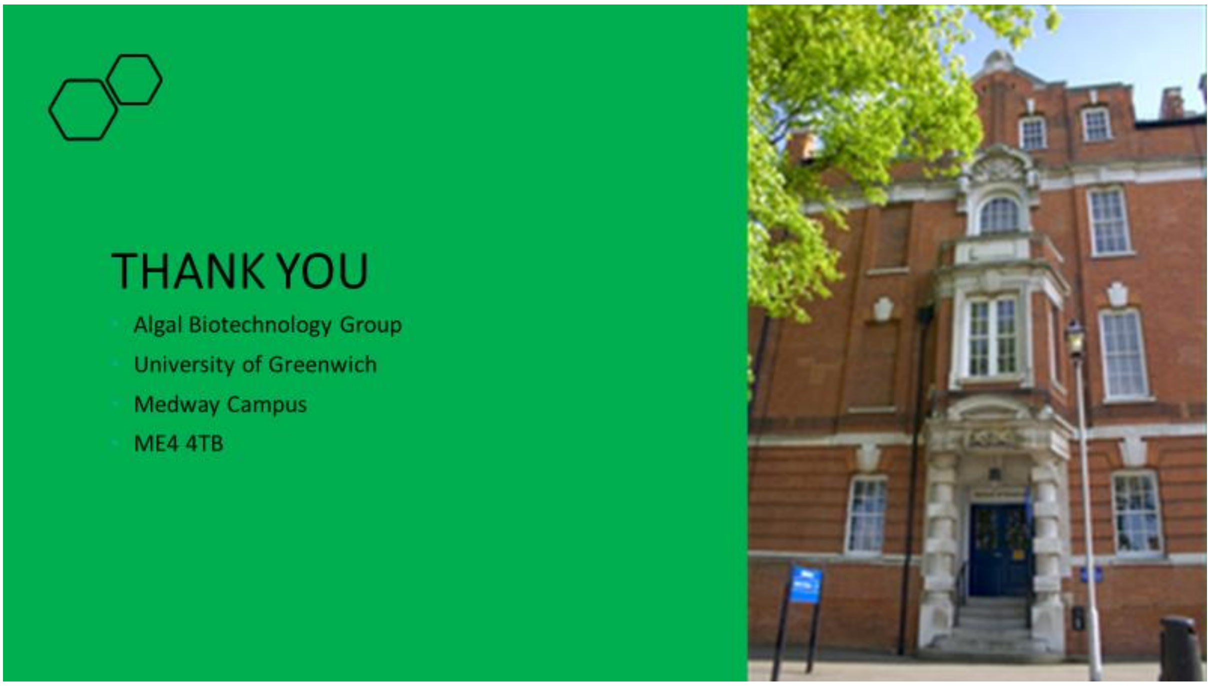



Here are some of the references to our work. I hope that these slides will be available after this seminar. I also have a transcript of my talk, which I also hope to make public.

SLIDE 28


Finally, I would like to thank all my colleagues and collaborators together with the Darwin Fund for funding our work on Turks and Caicos and the other funders of our work on Sargassum. Particular mention must go to Pat Harvey, who brought me to Greenwich and has supported me throughout and encouraged and supported some of my ‘bonkers ideas’. Dr Debbie Bartlett, a chance conversation in the corridor with her led to seven years of research on Sargassum. Many of the best ideas seem to spring from impromptu meetings whilst moving around the campus. Without Debbie’s tenacity, we may never have obtained the Darwin funding.  A special thanks must go to Birthe Nielsen a close collaborator throughout my time in Greenwich who will continue and supervise the work on Sargassum and anaerobic digestion. We have both had the pleasure of supervising our exceptional PhD students. Supattra Maneein’s work has built on our foundations, and I am sure she will produce an outstanding thesis.  Finally, thank you to all the students and staff who have made my time at Greenwich so enjoyable.

SLIDE 29


THANK YOU
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Thank you