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3rd Medway Engineering Conference SYSTEMS: from concept to manufacturing 20th June 2018

SARGASSUM – MENACE, METHANE, MOUTH AND MORE

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Algal Biotechnology Group

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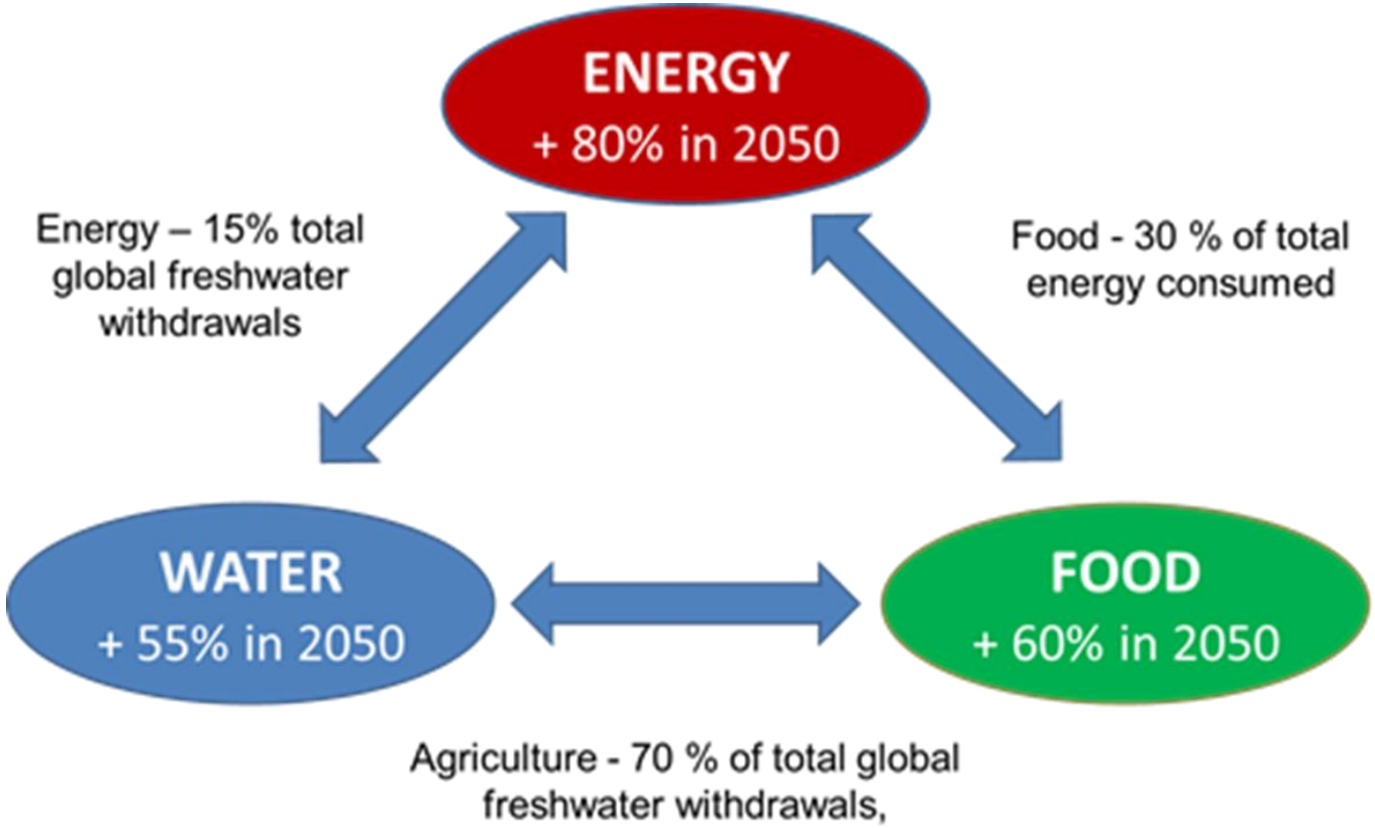
Water – Food - Fuel



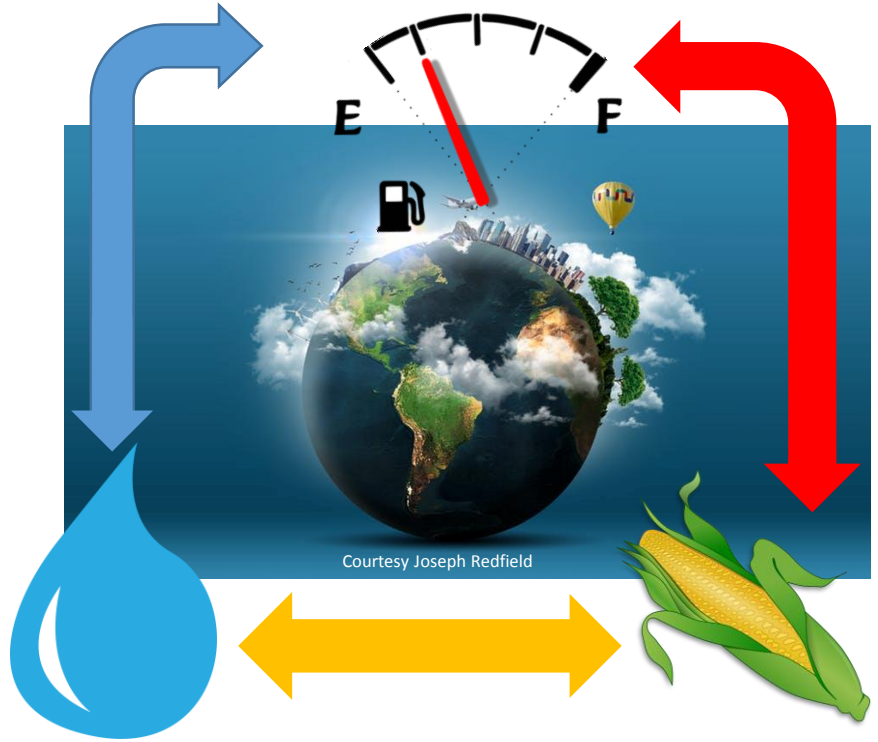


Image courtesy of Prof PJ Harvey

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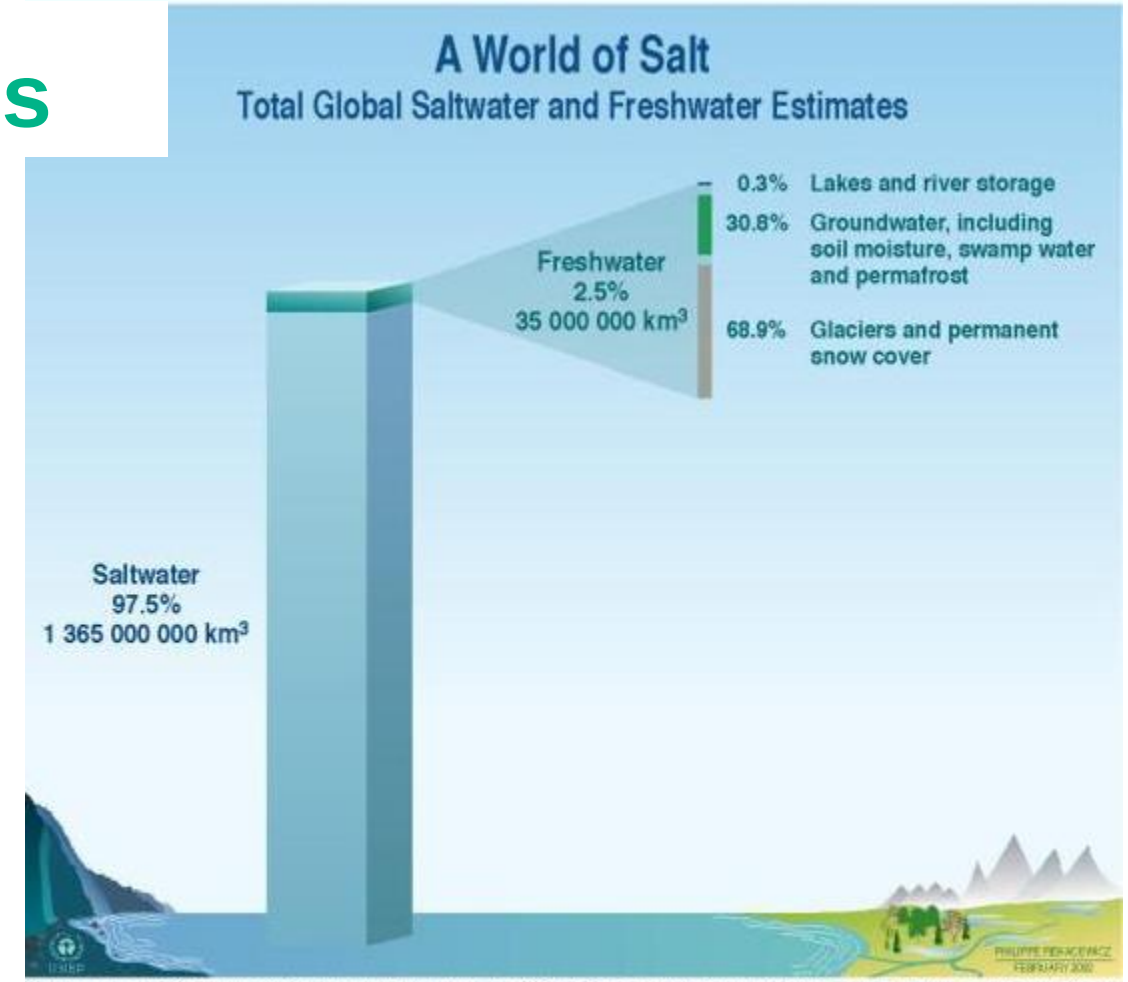
World Water Resources

>97% seawater

<3% fresh water

~0.5% for human freshwater needs.



Source: Igor A. Shiklomanov, State Hydrological Institute (SHI, St. Petersburg) and United Nations Educational, Scientific and Cultural Organisation (UNESCO, Paris), 1999.

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Seaweed Potential

- Oceans cover 70% of the Earth
- Seaweed only ~0.3% of world food tonnage
- Commercial seaweed market 2021- US$ 17.6 billion (Research and Markets, 2016)
- Commercial Macroalgae Production ~100 ˣ microalgae
- China accounts for >70% of the world's total macroalgal production.

TIWARI, B. & TROY, D. (eds.) 2015. Seaweed Sustainability: Food and Non-Food Applications, Amsterdam: Academic Press.

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Menace

Sargassum muticum an invasive species to Northern Europe

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Cost of Invasive Species

- Globally **US$ 1.4 trillion per year** ~5 % of the world economy(1)
- Great Britain **£ 1.7 billion per year**(2)
- UK shipping and aquaculture >**£ 40 million per year**(2)

- 1 Engelen A, Santos R (2009) Which demographic traits determine population growth in the invasive brown seaweed *Sargassum muticum*? Journal of Ecology 97:675-684
- 2 Cook, E. J. *et al* (2013) Impacts of climate change on non-native species. *MCCIP Science Review*, 155-166

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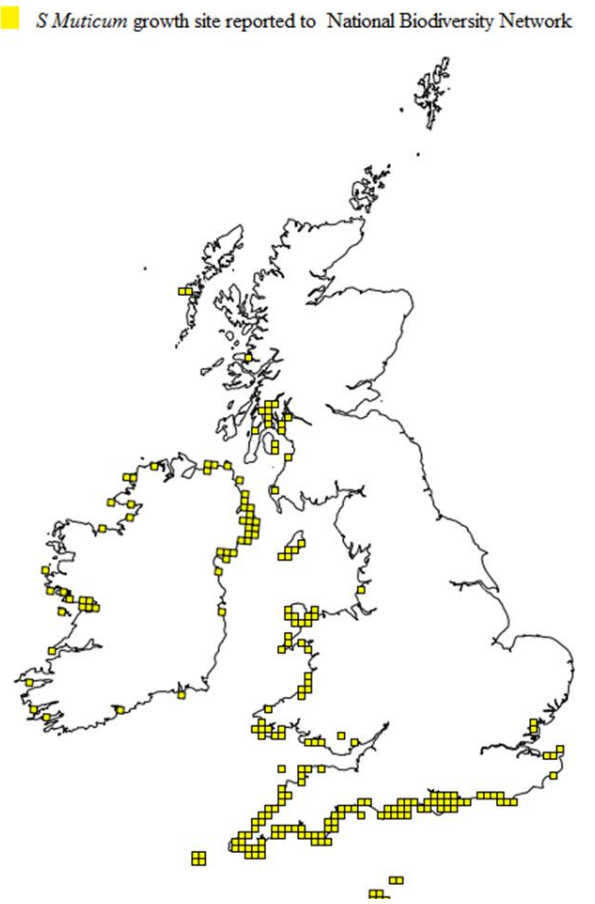
Sargassum muticum Japanese Wireweed



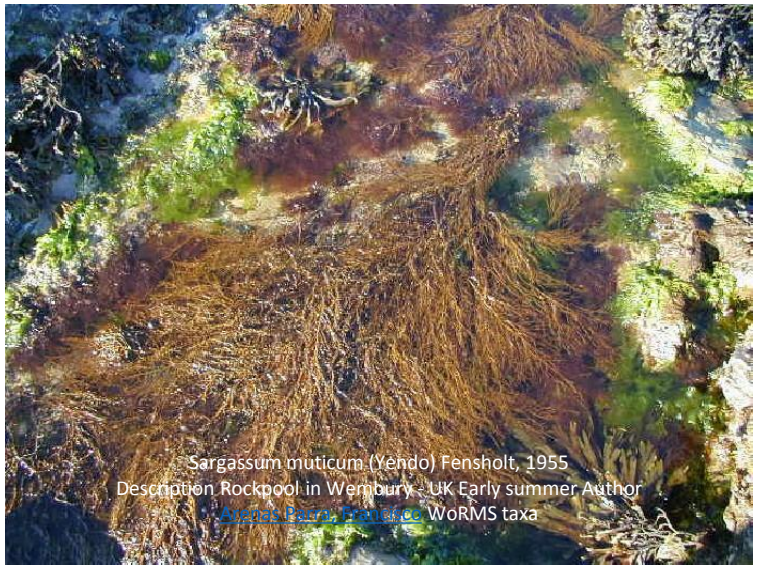
Courtesy of National Biodiversity Network

- Found in Europe early 1970s.
- Now found from Norway to Portugal
- Very invasive
- Most ‘successful’ invasive, rate of spread in UK
- Higher growth rate
- >10 ˣ *Ascophyllum nodosum*
- High priority EU’s Water Framework Directive

Seaweed difficult to control

"Seaweed is like love; even if you push it away, you will not prevent it from coming back."

Adapted from Nigerian Proverb



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Attempts to eradicate *Sargassum muticum* have failed

- Mostly harvested by hand
- Costly ~ £60 tonne^{-1}*
- Large quantities for disposal
- No major commercial exploitation

* Updated from: Critchley AT, Farnham WF, Morrell SL (1986) An account of the attempted control of an introduced marine alga, Sargassum-muticum, in southern England Biological Conservation 35:313-332

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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

- Varies seasonally
- High Moisture
- High Ash

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Methane

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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

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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.

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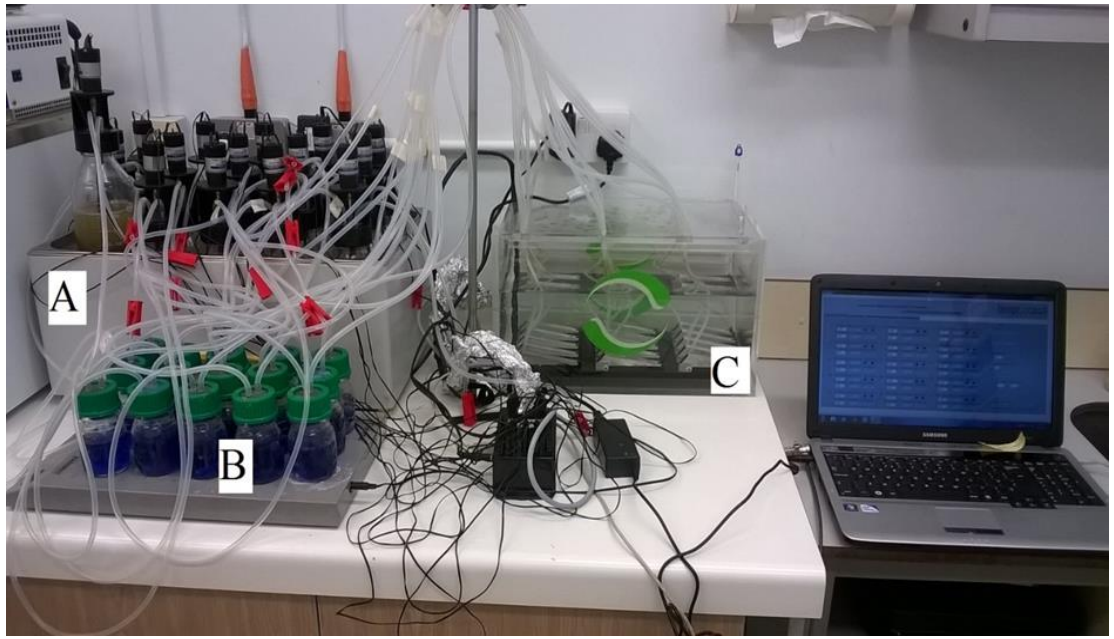
Methane Potential



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%**

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Considerable conjecture about low practical methane yields

- Inoculum
- Cell structure
- Resistant organic compounds
- Inhibition by anti-bacterial polyphenols and other compounds
- Salt and other inorganics
- Ammonia inhibition

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Methane potential factorial design experiment

- 4 substrates

  Readily digested simple organic substance:

  Glycerol

  3 polymers found in seaweed:

  Cellulose, Alginic acid and Sodium salt of Alginic acid

- 3 simple phenolics

  Gallic Acid, Epicatechin and Phloroglucinol

- 4 concentrations - range of phenolic compounds in *S. muticum*

  0, 0.5, 3.5 and 7.5 % of the substrate

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Alginic acid recalcitrant

Substrate			
Glycerol	Alginic Acid Sodium Salt	Alginic Acid	Cellulose
Average Gas yield mL CH_4 g^{-1} substrate dw			
178	76	73	183

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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

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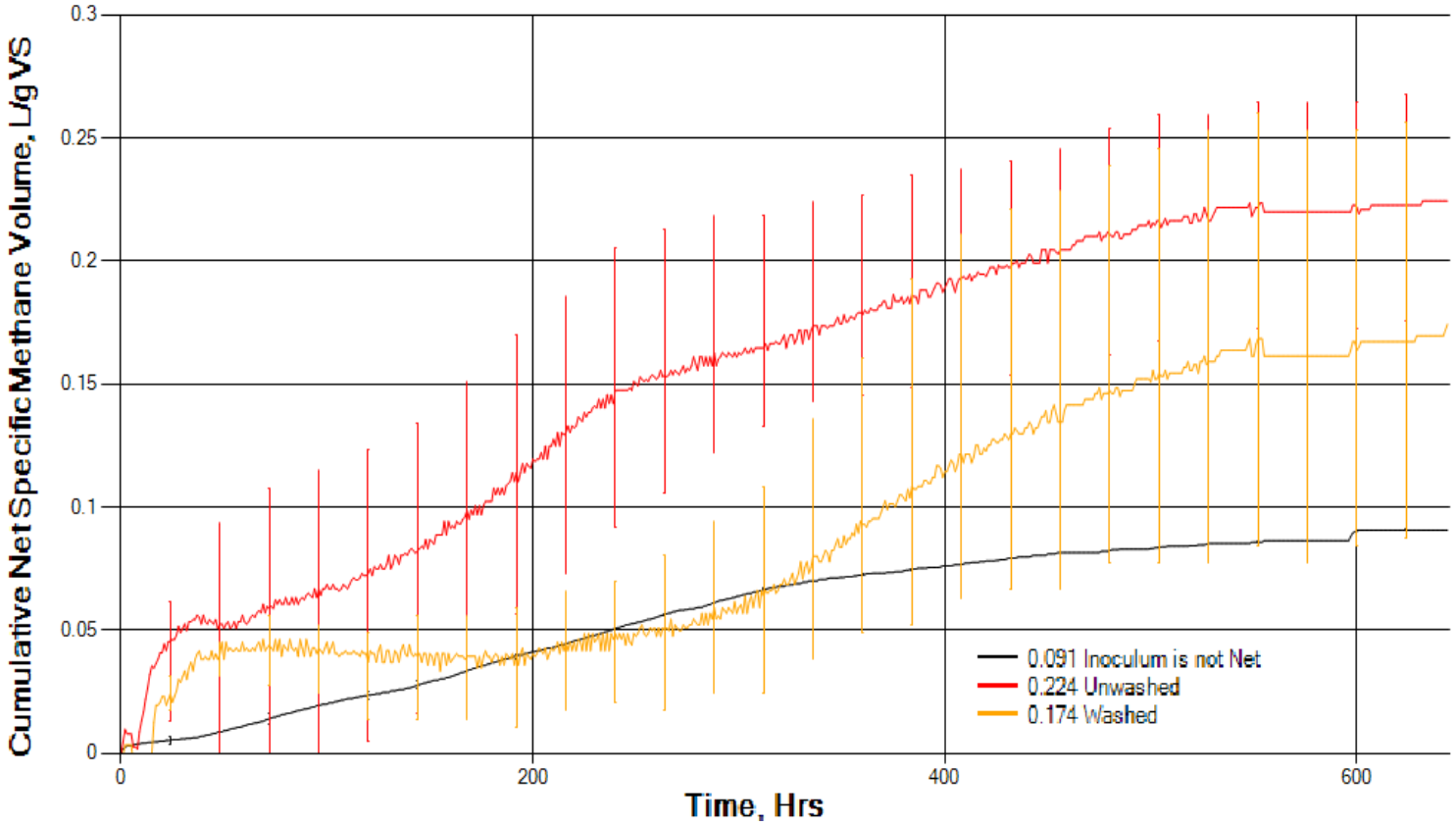
Salt & Freshwater Washing

- 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

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?

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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%

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Mouth

Dental Application of *Sargassum muticum*

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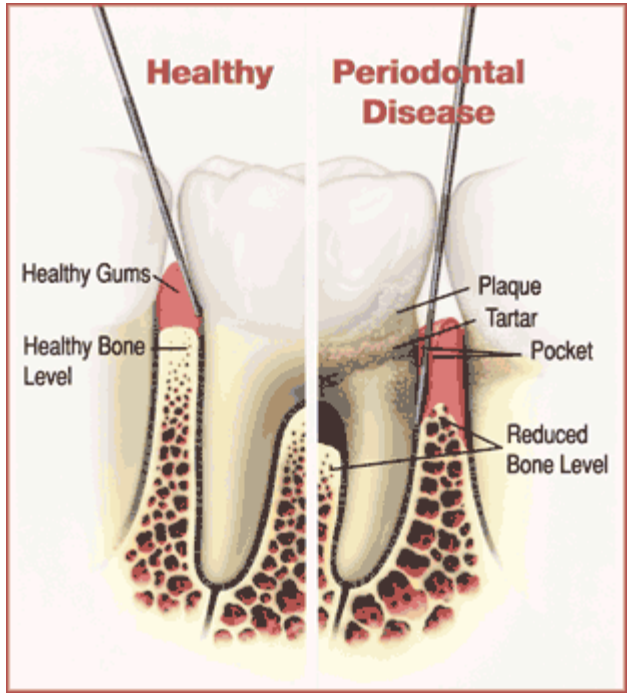
Periodontal Diseases

- Most prevalent preventable chronic disease worldwide[1]
- Dental cavities most common, chronic disease of early childhood[2]
- Brushing alone not always fully effective in eliminating plaque[3]



1. Tamanai-Shacoori, Z et al. Plos One 2014, 9, 8.
2. Karikalan, S; Mohankumar, A. Biotechnol. Res. Commun. 2016, 9, 109-113.
3. Cho, HB et al. J Med Fd 2011, 14, 1670-1676.

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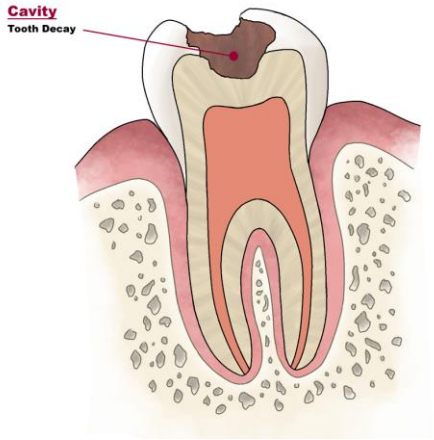

Dental Caries & Periodontal disease

Oral bacterial flora is extremely complex and diverse

Dental plaque, a biofilm consisting of 300 -700 species of bacteria.

Only a few specific species are causative agents of tooth caries

Streptococcus mutans believed to be a major 'player' in dental caries.

Porphyromonas gingivalis gram –ve periodontal pathogen

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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)		

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More

Medical Application of *Sargassum muticum*

Food

Feed

Fertiliser

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S. muticum diethyl ether extract

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

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Phenolics

- Primary role in the structure of seaweed cell walls
- Chemical defence against grazers
- S. muticum > 5% polyphenols
- Antibacterial
- Antioxidant

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Sulphated Carbohydrates

- Chemically very different from land plants
- S. muticum ~8% dw
- Anticoagulant
- Antiviral
- Inhibiting parasite, *Toxoplasma gondi*

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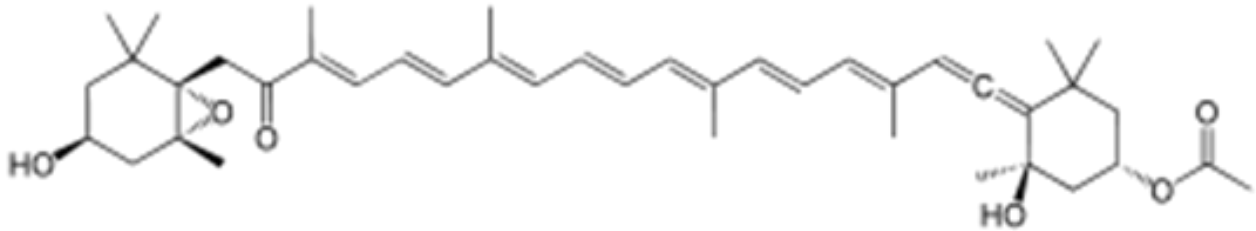
Carotenoids

- Fucoxanthin
- antioxidant
- anti-inflammatory
- anti-obesity
- antitumor



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Biorefinery

Fucoxanthin

€ 9000 g^{-1}

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The seaweed made the world.

John B. Keane (Irish Writer)

Sargassum has potential

But challenges remain

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What else are we doing?

Photographs courtesy of Kelly (Robinson) de Schaun

Massive inundations of pelagic Sargassum known as Golden-tides on the beaches of the Caribbean, Gulf of Mexico and West Africa

Acknowledgements

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CHANGE STARTS HERE

THANK YOU

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