JAMES COOK UNIVERSITY
AUSTRALIA

High Energy Algal Fuels

A partnership between JCU, USyd, AMCRC, MBD Energy & ARENA

macro
Centre for Macroalgal Resources & Biotechnology

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Executive Summary

The fundamental driver for the production of high energy fuels is the productivity of macroalgal biomass. This factor had a more significant effect on the production of biocrude due to much larger variance in biomass productivity between species than biocrude yield. Notably, all biocrude produced from macroalgae was similar in biochemical composition and therefore quality. Therefore, the key selection criteria for high energy fuels from macroalgae are the upstream selection of species and these are largely independent of the HTL conversion process and subsequent refining to renewable fuels. Both the conversion process of biomass to biocrude by HTL and the refining of biocrude to renewable high energy fuels are feasible without significant technical hurdles at the pilot scale.

A key driver for the success of the development of high energy fuels from renewable biomass is the ability to deliver an economic product. An important component of this equation is the derivation of the maximum value of the biomass through the delivery of high value co-products. These will provide the major economic return on the production of biomass with the delivery of biocrude being a lesser contributor to the overall value-chain. In addition to co-products there is a tangible economic value to the bioremediation services provided by macroalgae when cultured in targeted water sources rich in nutrients. This integrated model then provides for economic value of macroalgae as both a service and a product. As with co-products, the provision of a bioremediation service will be a key driver for economic feasibility of high energy algal fuels.

The most direct and accessible source of nutrient rich waste water in Australia is municipal waste water. Australia discharges 75% of municipal waste water into the environment. This provides a resource of 4.3×10^6 m^3 of water day^{-1} for possible use for the cultivation of freshwater macroalgae with the beneficial reduction of nutrients and contaminants. The freshwater macroalgal genus *Oedogonium* is well adapted to cultivation in municipal waste water, has a high biomass productivity, biocrude yield and consequently biocrude productivity. It also has a biochemical profile that delivers higher value co-products and through the production process provides a quantifiable bioremediation service. Modeling this integrated production system delivers a financially viable outcome when factoring both product and service revenue, including the production of high energy algal fuel as biocrude.

Project Outline

High Energy Algal Fuels is a Research and Development Project defined by two parallel programs

Research and Development (Program 1) and **Demonstration** (Program 2)

Program 1 provides the scientific blueprint for the development and production of high-energy fuels (Jet A1 and renewable diesel) and valuable co-products from macroalgal biomass.

Program 2 demonstrates the commercial scale production of biomass for biocrude. This program will provide the blueprint to support and implement cost-effective, large-scale macroalgal production, and provides for the development of macroalgae as a flexible and economic feedstock.

Project Collaborators

Program 1 Research and Development

The aim of Program 1 was to provide the scientific blueprint for the development and production of high-energy fuels (Jet A1 and renewable diesel) and valuable co-products from macroalgal biomass.

This was achieved with the delivery of a clear blueprint that identifies the key steps for the production of high energy fuels and co-products from macroalgae. The production of high energy fuels is a function of biomass productivity (g dry biomass. m^{-2}. day^{-1}) and biocrude yield (g biocrude. 100 g $biomass^{-1}$) with biocrude productivity (g biocrude. m^{-2}. day^{-1}) being the defining metric for the delivery of high energy fuels. The fundamental driver for the production of high energy fuels is the productivity of macroalgal biomass. This factor had a more significant effect on the production of biocrude due to much larger variance in biomass productivity between species than the yield of biocrude yield. Notably, all biocrude produced from macroalgae was similar in biochemical composition and therefore quality. Therefore, the key selection criteria for high energy fuels from macroalgae are the upstream selection of species and are largely independent of the HTL conversion process and subsequent refining to renewable fuels. Both the conversion process of biomass to biocrude by HTL and the refining of biocrude to renewable high energy fuels are feasible without significant technical hurdles at the pilot scale.

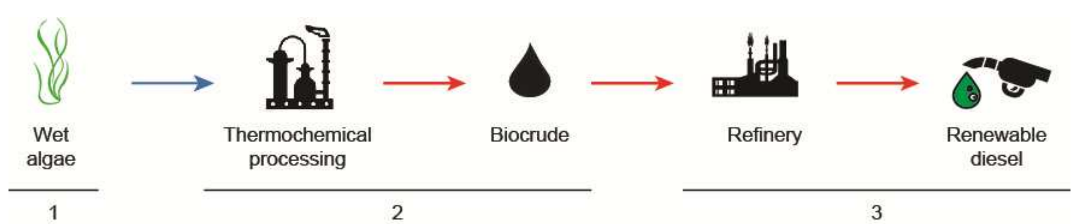
A scientific blueprint for the development and production of high-energy fuels and valuable co-products from macroalgal biomass

The blueprint for the development and production of high energy fuels and valuable co-products relies on three key processes

1 Selection of species with high biocrude productivity

2 Optimisation of HTL for conversion of biomass to biocrude

3 Refining of biocrude to high energy fuels



This successful blueprint is predicated on the identification of species that have **high biomass productivity** and a biochemical profile that provides for a **high yield of biocrude**. We also identify that the conversion of all macroalgal biomass to biocrude, through the targeted platform of hydrothermal liquefaction (HTL), results in a product of similar quality. Consequently, the upgrading of the biocrude to high energy fuels as Jet A1 and renewable diesel is an equivalent process regardless of feedstock species with equivalent resultant products.

1 Selection of species with high biocrude productivity

The key metric for the successful production of high energy algal fuels is the productivity of biocrude per unit area [g biocrude.m^{-2}.day^{-1}] which is an outcome of biomass productivity per unit area [g dry weight.m^{-2}.day^{-1}] and yield of biocrude per unit biomass [g biocrude.100 g dry weight biomass^{-1}] (1).

To develop and proof this blueprint we initially assessed **40 species** of freshwater and marine macroalgae for their resilience and biomass productivity under intensive cultivation resulting in the selection of **six target species** for detailed research and development for conversion to biocrude through HTL. These six species, four marine (*Derbesia tenuissima*, *Ulva ohnoi*, *Chaetomorpha linum*, *Cladophora coelothrix*) and two freshwater (*Oedogonium* sp., *Cladophora vagabunda*), were then cultivated to quantify biomass productivities and converted to biocrude to quantify the yield and quality of the biocrude produced. This resulted in the ranking of the six species in terms of biomass productivity (Figure 1) and composition (Table 1), biocrude yield (Table 2) and consequently biocrude productivity (Figure 2). Notably the quality of biomass varied significantly between species (Table 1), however, the quality of biocrude was very similar (Table 3). The three most productive species based on the key metric of biocrude productivity were *Ulva ohnoi*, *Derbesia tenuissima* and *Oedogonium* sp. (Figure 2) and on this basis were selected for scale-up, conversion and refining of biomass to biocrude, and subsequently to high energy fuels (Program 1). These species (and species within these genera) were also then targeted for the research and development of valuable co-products (Program 1) and the commercial scale production of biomass for biocrude and co-products (Program 2).

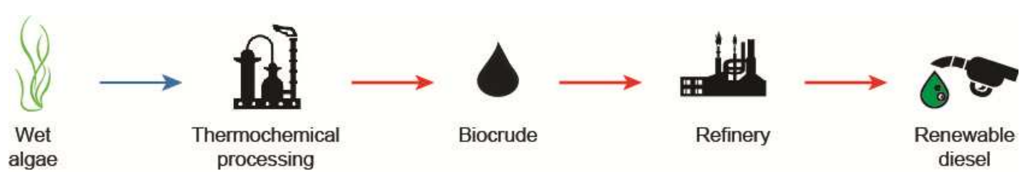


Figure 1. Biomass productivity of macroalgae. Macroalgae were cultivated in an outdoor tank system for comparison of feedstock for the production of biocrude and the calculation of biocrude productivity. Full experimental details are provided in (1). The data show means (n = 3 ± SE) of productivity as dry weight of marine and freshwater macroalgae.

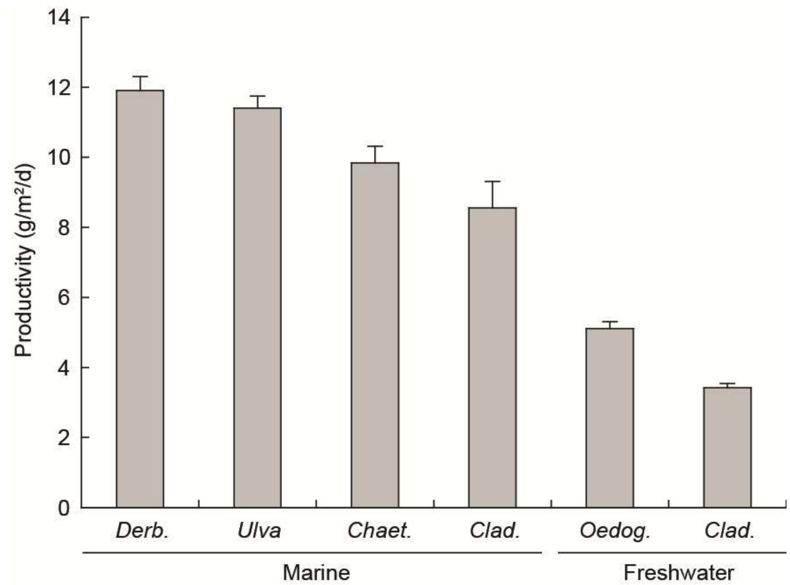


Figure 2. Biocrude productivity of macroalgae. Productivity was calculated on experimental data for biomass productivity and biocrude yield to select the best marine and freshwater species for scale-up production of biomass and conversion to biocrude and co-products. Full experimental details are provided in (1) and (2). The data show means (n = 3 ± SE) of productivity (dry weight of biocrude following conversion of marine and freshwater macroalgae).

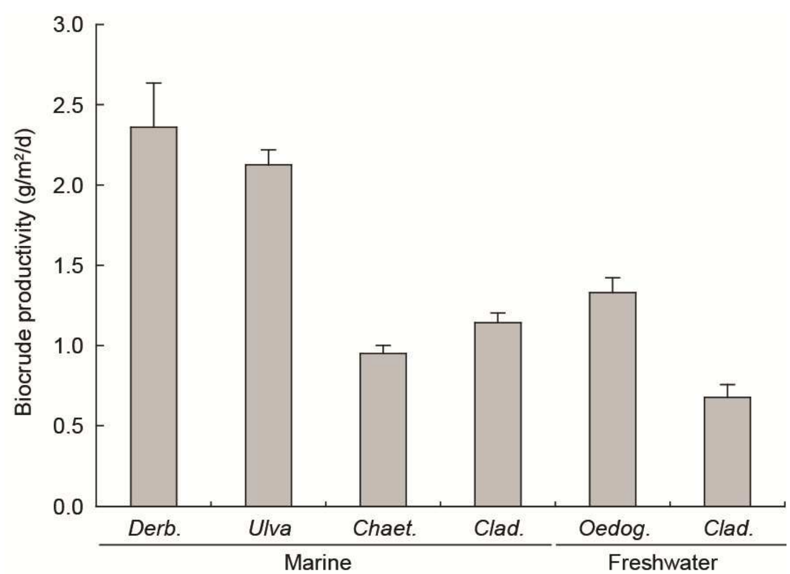


Table 1. Proximate, biochemical and ultimate analysis of macroalgae. Macroalgae were cultivated outdoors in 50L cylindrical tanks. Full experimental details of the cultivation and analysis of biomass are provided in (1) and (2). The data show means (n = 3 ± SE) of content dry weight of marine (M) and freshwater (FW) macroalgae.

Species	*Derb.*	*Ulva*	*Chaet.*	*Clad.*	*Oedog.*	*Clad.*
Source	M	M	M	M	FW	FW
Proximate (wt%)						
Ash	34.7	30.7	36.6	25.5	20.6	17.8
Moisture	6.4	7.2	5.1	6.7	6.5	5.7
Biochemical (wt%)						
Lipid	10.4	1.9	3.3	4.6	9.4	5.3
Protein	21.6	16.3	11.1	17.8	22.5	26.8
Carbohydrate	26.9	43.9	43.9	45.4	41.0	44.4
Ultimate (wt%)						
C	29.2	27.7	26.5	30.9	36.6	37.5
H	4.8	5.5	4.1	5.0	5.7	5.9
O	27.4	41.1	31.0	34.9	30.9	32.9
N	4.5	3.5	3.4	5.2	4.8	6.5
S	2.8	5.0	2.1	2.3	0.4	1.8
HHV (MJ/kg)	12.4	11.7	10.3	12.7	15.8	16.4

Table 2. The yield of products and biocrude productivity from the hydrothermal liquefaction of macroalgae. The experimental yields of biocrude, biochar and aqueous and gas products were quantified from hydrothermal liquefaction of cultured macroalgal biomass. Yields are presented with ash (*wt%, dw*) and without ash (*afdw*) for comparison of the yield from organic matter only. Full experimental details are provided in (1). The data show means (n = 3 ± SE) of yield of products following conversion of marine (M) and freshwater (FW) macroalgae.

Species	*Derb.*	*Ulva*	*Chaet.*	*Clad.*	*Oedog.*	*Clad.*
Source	M	M	M	M	FW	FW
Yield (wt%, dw)						
Biocrude	19.7 ± 1.6	18.7 ± 0.8	9.7 ± 0.4	13.5 ± 1.0	26.2 ± 2.6	19.7 ± 1.8
Biochar	8.1 ± 1.4	12.1 ± 1.1	8.4 ± 1.6	10.4 ± 0.8	10.2 ± 2.5	18.7 ± 2.9
Aqueous + Gas	72.2 ± 2.2	69.2 ± 0.3	82.0 ± 1.6	76.1 ± 0.4	63.6 ± 2.6	61.7 ± 3.3
Biocrude *(afdw)*	33.4 ± 2.7	30.1 ± 1.3	16.6 ± 0.7	20.0 ± 1.5	35.9 ± 3.6	25.7 ± 2.3
Productivity (g/m^2/d, dw)						
Biocrude	2.4 ± 0.3	2.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	0.7 ± 0.1

dw = dry weight basis; afdw = ash-free dry weight basis

Table 3. Ultimate analysis and energy recovery of biocrude produced by the hydrothermal liquefaction of macroalgae. The biocrude was produced using an experimental batch reactor and analysed to provide data on the elemental composition of the biocrude which provides for the calculation of the energy content of the biocrude. Notably, the HTL process upgrades the energy content of the biomass from a range of 10-16 MJ.kg-1 to 33-34 MJ.kg-1 by increasing the proportion of carbon more than two-fold and reducing the proportion of oxygen 3-4 fold from biomass to biocrude. The data show an energy recovery of more than 50% for the three best species. Full experimental details are provided in (1). The data show means (n = 3 ± SE) of content dry weight of marine (M) and freshwater (FW) macroalgae.

Species	*Derb.*	*Ulva*	*Chaet.*	*Clad.*	*Oedog.*	*Clad.*
Source	M	M	M	M	FW	FW
Biocrude (wt%)						
C	73.0	72.6	70.9	71.6	72.1	71.1
H	7.5	8.2	7.7	8.0	8.1	8.3
O	10.6	11.0	11.4	10.6	10.4	10.6
N	6.5	5.8	6.8	7.1	6.3	6.8
S	0.7	0.4	0.1	0.9	0.8	1.3
HHV (MJ/kg)	33.2	33.8	32.5	33.3	33.7	33.5
ER (%)	52.5	54.0	30.6	35.3	55.7	40.3

HHV = higher heating value; ER = energy recovery

2 Optimisation of HTL

A comparison of the quality of biocrude produced from both marine and freshwater macroalgae with fossil crude shows that the biocrude produced from macroalgae has a lower content of carbon and a higher content of nitrogen. The content of sulphur, a key element in determining the quality of biocrude, is similar to that of conventional fossil crude (Table 4).

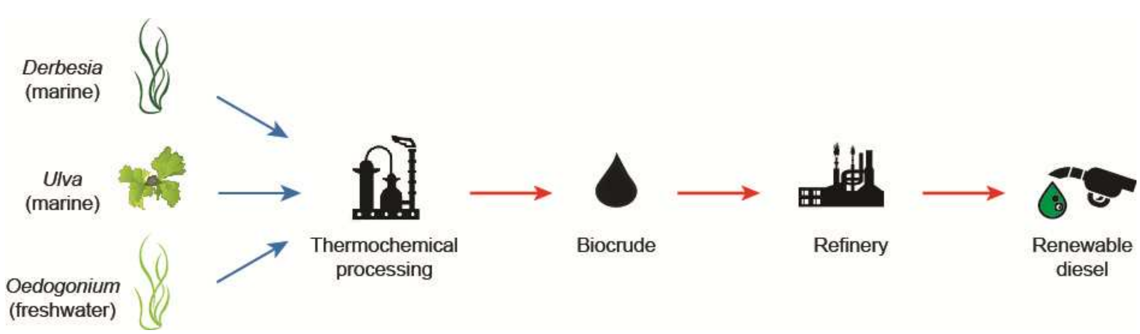
Table 4. Comparison of the ultimate composition of biocrude as the average composition of six species of macroalgae and conventional fossil crude

	Biocrude	Fossil crude
C	72	83
H	8	14
O	11	1
N	7	1
S	1	1
HHV (MJ/kg)	33	42

HHV = higher heating value

The resultant outcome is a higher degree of refining to deliver renewable high energy fuels.

Consequently, cultivation methods were optimized for the production of macroalgal biomass specifically for the production of biocrude using pre- and post-harvest treatment to improve the quality of the feedstock. This used the three target species of *Ulva*, *Derbesia* and *Oedogonium* with a pre-harvest treatment of the removal of nitrogen to minimize its concentration in the biomass, and a post-harvest treatment of washing to minimize the content of ash (3).



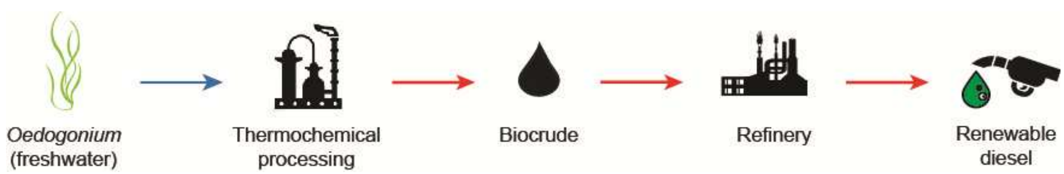
The pre-harvest treatment of nitrogen removal (N-) reduced the content of heteroatoms (nitrogen and sulphur) in the biocrude compared to biomass where the supply of nitrogen was uninterrupted (N+) (Table 5). However, in this process there is a clear trade-off between biomass productivity and the quality of biocrude, with the decrease in nitrogen in culture resulting in a decrease in biomass productivity but an increase in biocrude quality through a decrease in the content of nitrogen and sulphur (Table 5). An analysis of the trade-off between biomass productivity, and consequently biocrude productivity and quality shows that *Oedogonium* sp. has the smallest loss of productivity while retaining the highest quality of biocrude and on this basis it was selected for scale-up production of optimized biomass for biocrude to refine to high-energy fuels. The post-harvest treatment of washing reduced the ash (salt) content of the biomass thereby increasing yield as a proportion of dry weight but not of ash free dry weight (3). This process will provide benefits in the HTL process through minimizing corrosion and interference with catalysts but did not affect yield or quality.

Table 5. Yield and ultimate composition of biocrude produced by the hydrothermal liquefaction of macroalgae. Biomass was converted to biocrude using hydrothermal liquefaction using a batch reactor and the effects of the biochemical composition, as a result of pre-and post-harvest treatment of biomass, on biocrude yield and composition determined. Full experimental details are provided in (3). The data show means (n = 4 ± SE) of yield and content dry weight of biocrude following conversion of macroalgae as not starved (N+), starved (N-), not washed (A+) and washed (A-)

Species	*Derbesia*				*Ulva*				*Oedogonium*			
Treatment	N+	N+	N−	N−	N+	N+	N−	N−	N+	N+	N−	N−
	A+	A−	A+	A−	A+	A−	A+	A−	A+	A−	A+	A−
Biocrude yield (wt%)												
	24.6	36.3	21.5	29.5	19.6	24.2	20.9	22.8	30.7	33.4	27.9	28.1
Biocrude composition (wt%)												
C	71.9	72.2	73.5	73.5	71.9	73.2	73.1	72.0	71.7	71.7	71.7	72.3
H	7.7	7.9	7.6	7.5	7.2	8.1	6.9	6.9	7.4	7.3	6.6	7.3
O	11.7	11.3	14.8	14.8	12.0	11.9	16.2	15.9	13.8	13.8	17.2	17.0
N	6.1	6.7	3.2	3.0	6.4	5.7	2.7	3.0	5.3	5.3	2.2	2.1
S	0.8	0.7	0.3	0.2	0.7	0.5	0.2	0.2	0.4	0.3	0.0	0.0
HHV	33.0	33.3	33.0	32.9	32.3	33.8	32.0	31.6	32.2	32.2	31.1	32.1

3 Refining to High Energy Fuels

The refining of biocrude to high energy fuels used biocrude produced from optimized biomass of *Oedogonium* sp. that was deplete of nitrogen. *Oedogonium* is a resilient, robust and highly productive genus of macroalgae that has, as part of this project, been successfully integrated into a range of industries producing waste water (see Program 2). As such *Oedogonium* is the key freshwater target species to implement cost-effective, large-scale production of macroalgae, and provides for the development of renewable biomass as a flexible feedstock (see Program 2). *Oedogonium* was produced at scale to deliver 40 kilograms (dry weight) of nitrogen deplete dry biomass which was subsequently converted to biocrude using the parameters derived from laboratory and pilot-scale research at the University of Sydney, School of Chemistry and School of Chemical Engineering.



The biocrude was produced by industry partner Licella Pty Ltd using its proprietary Cat-HR process at its pilot-plant facilities at Somersby, NSW. This biocrude was produced under commercial-in-confidence arrangements and subsequently successfully upgraded to high energy fuels at the Instituto de Technologia Quimica, Universitat Politēcnica de Valēncia (ITQ-UPV), Spain. The biocrude was distilled and the distilled liquids hydro-processed using a conventional hydrotreatment catalyst typically used in hydrodesulphurization, and a hydrocracking catalyst under higher hydrogen pressure. The biocrude was successfully hydro-treated and hydrocracked to yield a diesel range of product within, or close, to commercial diesel specifications. A full technical report has been provided to ARENA by Licella Pty Ltd (Dr Bill Rowlands) under commercial-in-confidence arrangements. The report also identifies improvements in the process to maximize on-specification diesel yields and quality.

In summary, there is a clear blueprint for the successful production of high energy fuels from macroalgal biomass. The selection of species with high biomass productivity and a high yield of biocrude through HTL provides for the delivery of high biocrude productivity. Notably, the yield of biocrude from biomass is directly correlated to the carbon content of the biomass with a significant linear correlation (R^2=0.96) between the yield of biocrude and the content of carbon (within a range of 30%-50% carbon) of $Y_{biocrude} = 0.885 \times C_{biomass} - 7.455$. This provides a rapid screen for prospective species which can then be trialed for biomass productivity as the key criteria for success.

In delivering this blueprint the program (Program 1) has more specifically identified *Ulva ohnoi* and *Derbesia tenuissima,* and the genera *Derbesia* and *Ulva* more broadly, as being amenable to intensive cultivation in brackish and marine (salt water) environments with high biomass productivities. These species can be converted with high yield to biocrude using HTL providing a high recovery of energy. In addition, the program has specifically identified *Oedogonium* as the key genus for biomass production in freshwater where its biochemical profile can be manipulated to improve the quality of biocrude while minimizing impact on yield. The remaining objective of Program 1 is to provide the scientific blueprint for the development and production of valuable co-products from macroalgal biomass as these will play a key role in defining the economics of delivering a high energy algal fuel.

4 Co-products

A key driver of the success of the development of high energy fuels from renewable biomass is the ability to deliver an economic product. An important component of this equation is the derivation of the maximum value of the biomass through the delivery of high value co-products. In many cases these may provide the major economic return on the production of biomass with the delivery of biocrude being a lesser contributor to the overall value-chain. It is important to acknowledge the market context for the development of macroalgae as a resource for the production of biocrude as there are strong markets for macroalgal biomass with annual global production in 2012 exceeding 23 million tonnes (wet weight) with an estimated value of more than $US 8 billion (SOFIA 2014). This resource is predominantly for traditional SE Asian markets for direct human consumption (9 million tonnes) and the production of phyco-colloids (14 million tonnes). World production of farmed macroalgae (seaweeds) more than doubled between 2000 and 2012 and there is a growing demand in traditional markets, and also in new markets as animal feeds and supplements, and fertilizers and plant stimulant products for agriculture (SOFIA 2012).

We assessed the suitability of the three target species (*Ulva*, *Derbesia*, *Oedogonium*) for their use as an alternative product (if used as whole biomass) or as a co-product (if used as a resource to extract high-value components) (Table 6). This assessment was primarily based on the biochemical composition of the biomass (Table 1) and on an understanding of current and potential markets for each species and extracts thereof, as follows. Detailed studies of the use of biomass of each species are described in Program 2 where the biochemical profile of the biomass from Demonstration Projects was carefully assessed, as produced at scale, and the quality of products determined.

Table 6. Suitability of macroalgae for the production of commodities

Application	Commodity	*Derbesia*	*Ulva*	*Oedogonium*
		Marine	Marine	Freshwater
Human food	Whole biomass	✓	✓✓	✓
	Dietary supplement	✓	✓✓	✓
Nutraceuticals	Polyunsaturated fatty acids (PUFA)	✓✓		✓✓
	PUFA Omega-3	✓✓		✓✓
	PUFA Omega-6	✓		✓
	Sulfated polysaccharides		✓✓	
Animal feed	Whole biomass	✓	✓	✓✓
	Protein extract	✓✓	✓✓	✓✓
Fertiliser	Whole biomass	✓	✓	✓
	Biochar	✓	✓✓	✓✓
Bioenergy	Biomass - direct combustion	✓		✓✓
Liquid fuels	Biocrude	✓	✓	✓✓

✓ = macroalgae potential as a feedstock; ✓✓ = macroalgae attractive as a feedstock

Ulva

The marine macroalga (seaweed) *Ulva ohnoi* has both high productivity under large scale culture (see Program 2) and a high yield of biocrude (1). It is also a desirable human food product that is used in Japanese (Aosa), Chinese and SE Asian cuisine and is cultivated (*Ulva ohnoi* and related foliose morphologies) across Japan and China. As such it is a valuable food resource with competing markets for whole biomass as a human food and as a food ingredient. The value of the dried biomass in the food market ranges from $1500 – $8000 per tonne depending on quality. It is also used as a dietary supplement including in Western diets based on its mineral profile and polysaccharide profile. Many species of *Ulva*, including *Ulva ohnoi*, are rich in insoluble and soluble fibres (ulvans) which are effective in treating metabolic syndrome with anti-inflammatory properties (4). The rich mineral and carbohydrate profile of *Ulva* also makes it suitable feedstock for composts (5), biochar (6) and liquid plant stimulants. *Ulva* also contains extractable proteins (7, 8).

Oedogonium

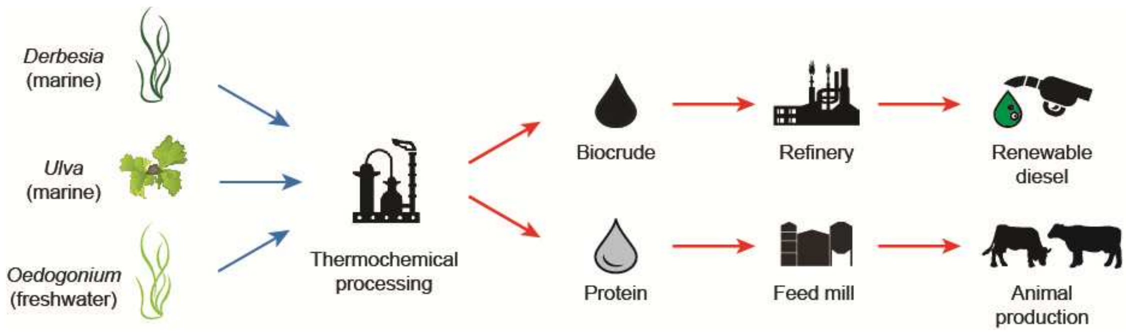
The freshwater macroalga, *Oedogonium*, is unique in that it there is no production or market for this genus, or for freshwater macroalgae more broadly. However, in addition to have high biomass productivity in culture (9, 10) and being suitable for effective conversion to biocrude, it has a biochemical profile that makes it suitable to a diversity of applications (11-13). The high lipid and protein content of the biomass make it a strong candidate for the development of human and animal feed products (11, 12) and for bioenergy (11). The high protein content makes it suitable for use as a human food as whole biomass. The high protein and low ash content makes it suitable as an animal feed or supplement, and for the extraction of proteins. The high lipid content also makes it a suitable feedstock for the extraction of lipids and results in an HHV that makes it attractive as a bioenergy resource including the production of biocrude (1-3). Overall, *Oedogonium* ranks as the feedstock with the most diverse applications.

Derbesia

In a similar manner to *Oedogonium*, *Derbesia* is unique in that it there is no production or market for this genus. However, *Derbesia* has high biomass productivity (8) and a high yield of biocrude (1-3), and is simple to cultivate at scale with consistent and predictable growth (see Program 2). *Derbesia* also has a very attractive biochemical profile due to its high lipid content which is rich in omega-3 fatty acids (13, 14) and high protein content for a marine macroalga (8). This biochemical profile makes it attractive as a human food or supplement (15) and as a bioresource for the extraction of polyunsaturated fatty acids, in particular omega-3 fatty acids (8). It is also attractive as a bioresource for the extraction of proteins (8).

It is within this content that we have considered the importance of co-products using a case study of the potential production and value of high energy algal fuels and protein from the three target species of macroalgae.

Quantitative assessment of the contribution of high energy fuels and co-products to value



To assess the potential contribution of co-products to the value of high-energy fuels we quantified the contribution of extracted protein prior to the conversion of the remainder of the biomass to biocrude (2). This has the dual benefit of providing a valuable co-product while also reducing the proportion of nitrogen in the biomass thereby improving the overall quality of the biocrude. The projected value of the three species of macroalgae was increased by 45-77% through the extraction of protein prior to the conversion of residual biomass to biocrude (Table 7) (2). This was done using a biocrude value equivalent to 85.50% of fossil crude based on the specific gravity of biocrude and fossil crude. Given the rapid decrease in the value of fossil crude to approximately half of this value the contribution of co-products has become even more important (up to 70%) and highlights the importance of targeting co-products to increase value.

The extraction of protein has the added benefit of reducing the content of nitrogen in the biomass and consequently the biocrude produced after the extraction of co-products is of a higher quality (3). The co-product process also then allows for the optimisation of biomass productivity through the provision of nitrogen such that it is not a limiting factor for growth. The nitrogen is assimilated into protein and extracted as a valuable co-product (3). Consequently, the development of co-products is advantageous from both a production and product perspective, including the improvement of the quality of biocrude.

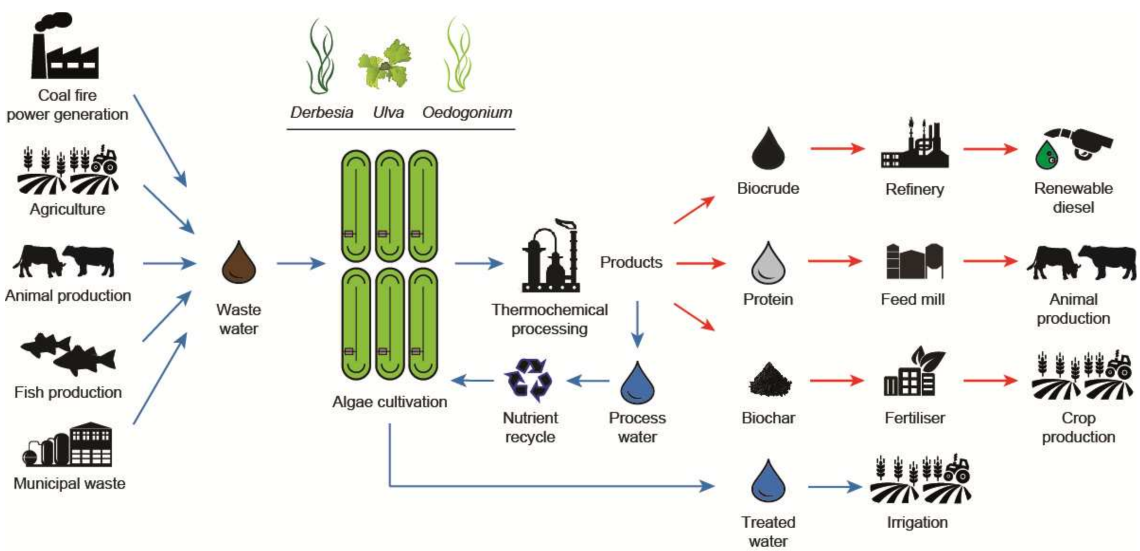
Table 7. Projected productivity and value of commodities produced by macroalgae.

Data show macroalgae projected productivities (P, in metric t/ha/yr) and values (V, in US$/ha/yr) of commodities produced by marine (M) and freshwater (FW) macroalgae through different scenarios including conversion to biodiesel (1), biocrude (2), extraction of protein (3), and HTL conversion of residual biomass to biocrude after protein extraction (4). Theoretical values of protein extract plus biocrude from residual biomass (5) is also presented. Note that theoretical values (V) are rounded to the nearest $100 for each scenario.

Scenario			1	2	3	4	5
Commodity			Biodiesel	Biocrude	Protein	Biocrude - Protein	3 + 4
Price (US$/t)			941	682	432	682	
Species	Source						
Derbesia	M	P	1.8	7.1	9.4	5.4	
		V	$1,700	$4,800	$4,100	$3,700	$7,700
Ulva	M	P	0.6	4.6	6.8	3.4	
		V	$600	$3,100	$2,900	$2,300	$5,200
Oedogonium	FW	P	0.8	3.3	4.2	2.5	
		V	$800	$2,300	$1,800	$1,700	$3,500

5. Services

In addition to co-products there is a tangible economic value to the services provided by macroalgae when cultured in targeted water sources. Macroalgae have high productivity and during growth assimilate CO_2, nitrogen and phosphorous into biomass. There is a similar bioaccumulation of metals and trace elements into biomass. The rapid growth of macroalgae therefore also provides the opportunity to provide a service in the remediation of waste waters rich in nitrogen, phosphorous, metals and trace elements. This provides the capacity to deliver an integrated system for the production of macroalgae for high energy algal fuels and co-products with the concomitant bioremediation of waste waters as a service (16, 17). In addition, the integration model removes or reduces the requirements for the supply of water, CO_2, nutrients and trace elements required for intensive cultivation. This integrated model then provides for economic value of macroalgae as both a service and a product. Notably, the value of both the service and the product will vary with each production model as the biochemical profile of the biomass will reflect the properties of the water in which it is cultivated. For example, the cultivation of macroalgae in the waste water from intensive land-based agriculture or aquaculture results in biomass higher in proteins due to the higher nitrogen content in the water. This biomass then has higher value as a human food and/or animal feed or supplement. In contrast, the cultivation of macroalgae in the waste water from energy generation, mining or mineral-processing results in biomass in metals and trace elements due to the presence of these elements in the water. This biomass then has lower value as it is excluded from the human or animal food chain but can be used for the production of energy, biochar and/or fertilizer. In addition, the integration model will vary for marine and terrestrial applications with each species being restricted by its capacity to tolerate environmental parameters.



The potential for the integrated model of production is described for each of the selected species of *Ulva*, *Derbesia* and *Oedogonium*. Notably, the importance of the integrated model in deriving economic value resulted in this process being the focus of Program 2 (demonstrate the commercial scale production of biomass for biocrude) as the bioremediation service component of the model is essential to support and implement cost-effective, large-scale macroalgal production and provide for the development of macroalgae as a flexible and economic feedstock.

Ulva

Ulva ohnoi has a broad environmental tolerance for a marine macroalga (seaweed) and can be success cultivated from brackish to hyper-saline salinities. Similarly, it can tolerate high temperatures and is therefore a very robust species for integration into marine and brackish water bioremediation systems (18). It has particular application in the bioremediation of waste waters from land-based marine aquaculture where temperature and salinity vary markedly depending on the tropical wet-dry season cycle (19).

Derbesia

Derbesia has a limited range of salinity tolerance (30-38 ppt) and is therefore restricted to cultivation in marine aquaculture. However, it is robust to environmental factors others than salinity and has a high growth rate, high affinity for nutrient-rich environments, and attractive biochemical composition (lipids, proteins, antioxidants) (20). It has restricted application to the bioremediation of waste-waters from land-based marine aquaculture where salinity is has low variability.

Oedogonium

The freshwater macroalga *Oedogonium* integrates into the broadest range of industries to provide a service in the bioremediation of wastewaters. As such it has been a focus of Program 2 and is a unique outcome providing Australian industries with an option to remediate wastewaters from municipal, agriculture (including freshwater aquaculture) (11), mining, mineral processing and energy generation processes (21). In this regard, the isolation, identification, scalable production and processing of *Oedogonium* is an innovative outcome of the project and has no parallel in Australia or internationally.

Program 2 Demonstration

The aim of Program 2 was to demonstrate the commercial scale production of biomass for biocrude. This program aims to provide the blueprint to support and implement cost-effective, large-scale macroalgal production, and provide for the development of macroalgae as a flexible and economic feedstock.

This was achieved with the production of biomass for biocrude with a focus on the delivery of the marine macroalga *Ulva ohnoi* and the freshwater macroalga *Oedogonium*. The program delivered a blueprint and pilot-scale facilities, and supported commercial facilities to implement cost-effective, large-scale macroalgal production. Integrated pilot-scale facilities were implemented at four sites (one marine and three freshwater) demonstrating the successful integration of biomass production for bioremediation with concomitant bio-products, including biocrude. The integration of macroalgal production with the treatment of waste waters is a key outcome and is identified as a critical factor for the successful development of macroalgae as a flexible and economic feedstock in Australia.

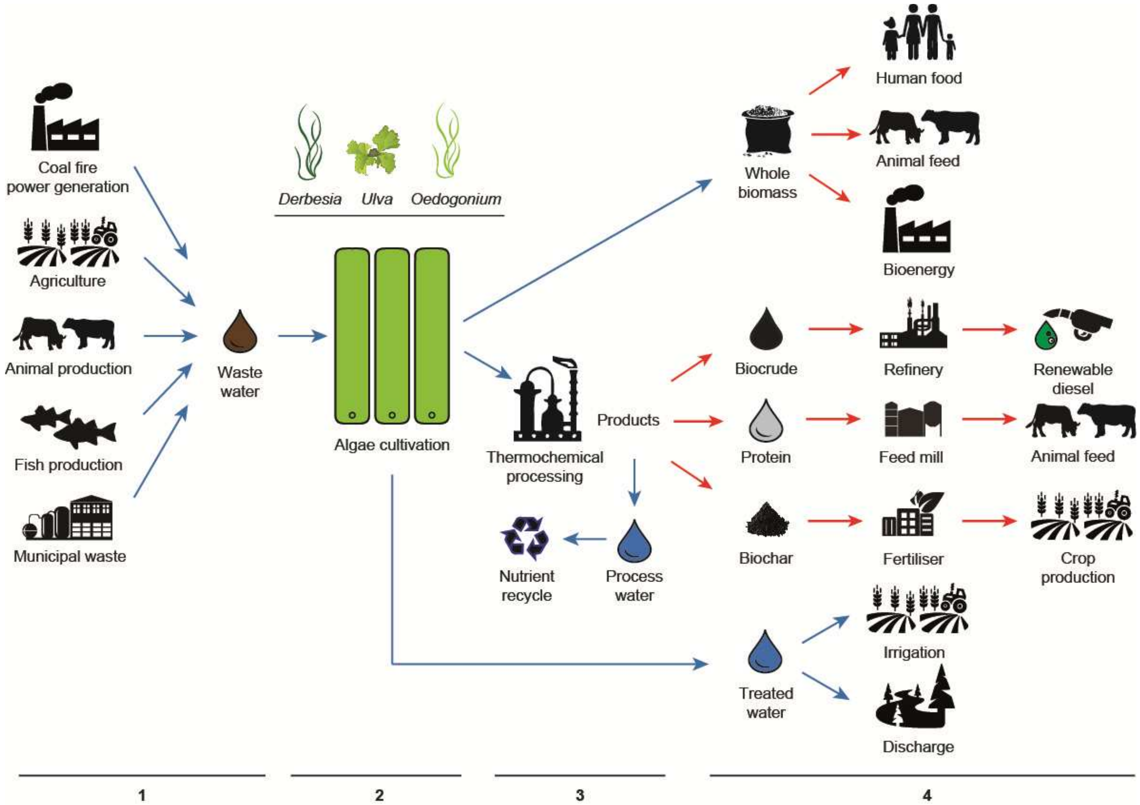
A blueprint to support and implement cost-effective, large-scale macroalgal production, and provides for the development of macroalgae as a flexible and economic feedstock

The blueprint to support and implement cost-effective, large-scale macroalgal production, and provide for the development of macroalgae as a flexible and economic feedstock relies on three key processes.

1 Selection of robust species across multiple waste streams

2 Development of integrated scale-up culture for bioremediation

3 Development and assessment of products including biocrude



The blueprint relies primarily on the cost-effective, large-scale production of macroalgae identified in Program 1. These species are the marine species *Ulva ohnoi* and *Derbesia tenuissima* (and species within these genera) and the freshwater macroalga *Oedogonium* sp. (and species within the genus). The blueprint clearly identifies that the cost-effective large-scale production of macroalgal biomass in Australia will initial rely on integration with the treatment of waste waters. This provides two benefits, a reduction in the cost of production through the provision of water and nutrients (nitrogen and phosphorous) and an increase in value through the provision of bioremediation services. The resultant biomass has a value depending on quality as determined by its biochemical composition. Biomass derived from the bioremediation of waste water from agriculture, aquaculture (freshwater and marine) has a higher value than that derived from the bioremediation, which in turn has a higher value than that derived from the treatment of waste water from industrial production and energy generation. This is due to its value in human and animal feed as compared to bioenergy applications.

To develop and proof this blueprint we delivered eight demonstration projects, at varying scales, to assess the services and products from macroalgae across a range of industries. All demonstration projects began with an initial assessment of the capacity to cultivate macroalgae in the waste water from an industrial source by bringing that water to JCU and assessing its quality. Macroalgae were then cultivated at increasing scales in this water and once developed beyond the capacity on-campus at JCU projects were implemented at scale at industry sites with close collaboration, or in partnership, with industry. These larger-scale demonstration projects were critical for the delivery of biomass to assess products as the quality of the biomass is a function of the quality of the water resource in which the biomass is cultured. As such the same species cultured in a different water source will result in a differentiated product stream(s). Products are described for each demonstration project with a focus on the role of biochemical composition in dictating products.

The Demonstration projects are presented based on species in order of development of scale.

1 *Ulva ohnoi* in parabolics at JCU (biocrude, ulvans, nutraceuticals, human food)

2 *Ulva ohnoi* at Pacific Reef (ulvans, nutraceuticals, human food, biochar, fertiliser)

3 *Derbesia tenuissima* in parabolics at JCU (biocrude, food, lipids, anti-oxidants, nutraceuticals)

4 *Oedogonium* in parabolics at JCU (biocrude, bioenergy, protein, animal feed)

5 *Oedogonium* at GFB (biocrude, bioenergy, protein, animal feed)

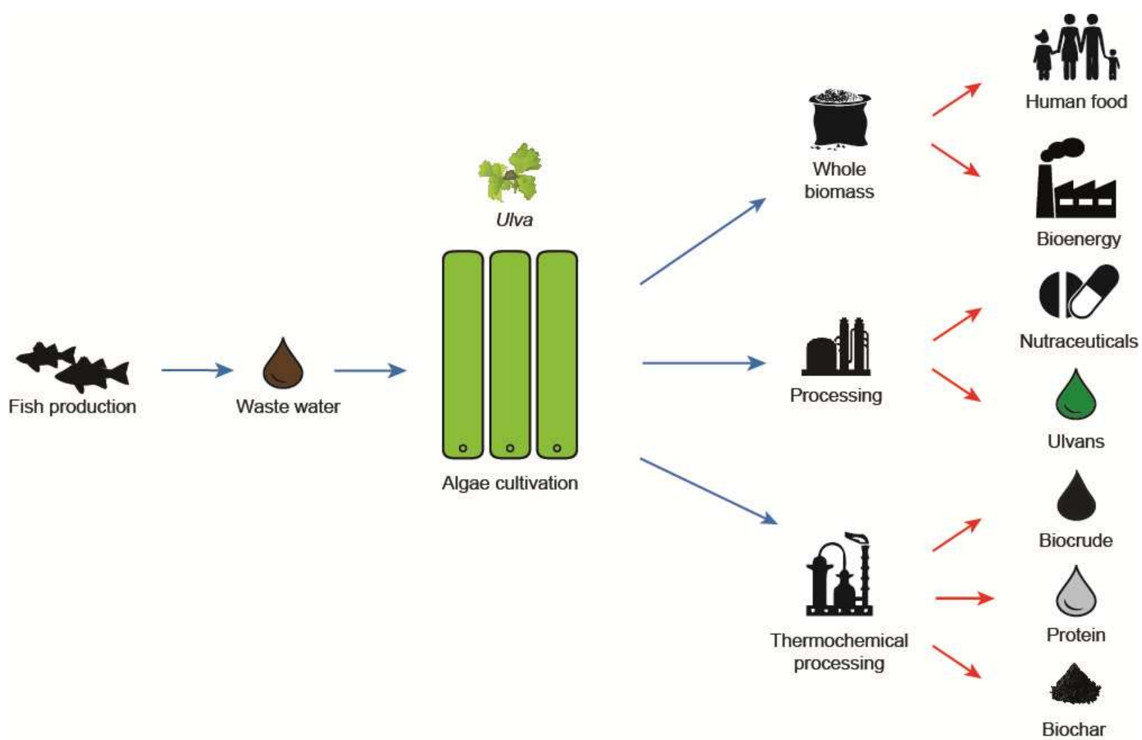
6 *Oedogonium* at Tarong (biochar, bioenergy)

7 *Oedogonium* at Townsville City Council MWWT plant (biochar, protein, animal feed)

8 *Oedogonium* in HRAPs at MARFU (biochar, fertilizer, protein, animal feed)

Demonstration Project 1 – *Ulva ohnoi* at Marine and Aquaculture Research Facilities Unit @ JCU

This first long-term demonstration project tested the pilot-scale production of *Ulva ohnoi* over a six month period using an array of parabolic tanks with a total volume of 32,500 L (3 x 10,833 L). The array was integrated in to the waste stream from a Barramundi breeding and hatchery facility (Mainstream Aquaculture) located in the Marine and Aquaculture Research Facilities Unit (MARFU) (8). This facility provided the required nutrients to maintain the growth of the macroalgae. Concentrations of nitrate and phosphate were analysed three times per week and N ($NaNO_3$) and P (NaH_2PO_4) were added to maintain concentrations at approximately 10 and 2 mg.L^{-1} respectively. Productivity was measured weekly and the key biochemical parameters of the biomass (C, N, Ash) and of the key co-product of *Ulva ohnoi* (soluble fibres = ulvans) were quantified every four weeks. The total biomass produced was also milled, homogenized and analysed for total lipids, fatty acids, fibres, amino acids, ash and moisture content. The protein content was determined from the sum of all amino acids. Total carbohydrates were determined by difference. These values were used to calculate projected annual productivities of bio-products by multiplying the content of individual bioproducts in the bulk sample by the annual biomass productivity estimation [$P_{bioproduct} = (P_{biomass} * C)/100$ where $P_{bioproduct}$ is the productivity of a bio-product (t ha^{-1} $year^{-1}$), $P_{biomass}$ is the productivity of the biomass (t ha^{-1} $year^{-1}$) and C is the concentration of the bio-product as a percentage of dry weight].



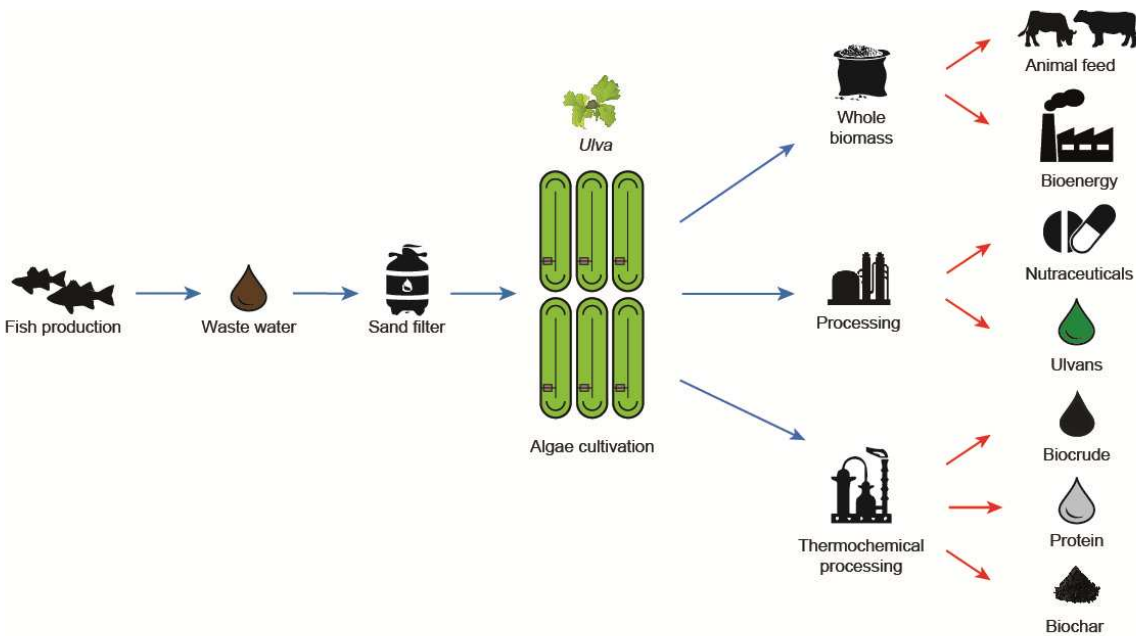
Ulva ohnoi was highly productive across the six month period which included the dry (June – October) and wet (November – January) seasons of the tropical Austral year with an average biomass productivity of 38 g dw m^{-2} day^{-1} (138 t dw ha^{-1} yr^{-1}) (8). This far exceeds the recognized commercial benchmark of 20 g dw m^{-2} day^{-1}. However, productivity was variable and stochastic ranging between 16 and 77 g dw m^{-2} day^{-1}. The lipid content of *Ulva ohnoi* was 2.3%, the soluble fibre (ulvan) content 12%, and the protein content 13% dw. The land-based cultivation of *Ulva ohnoi* produced 2.8 t ha^{-1} $yr^{-1}$of lipids, 18.4 t ha^{-1} $yr^{-1}$of protein and 16.4 t ha^{-1} $yr^{-1}$of soluble fibre (8).

As a comparison, the average annual productivity of soybeans is ~3 t ha^{-1} yr^{-1} with an average lipid content of 18% and crude protein content of 40% resulting in productivities of 0.5 t ha^{-1} $yr^{-1}$of lipids and 1.2 t ha^{-1} $yr^{-1}$of proteins.

Ulva ohnoi offers a niche opportunity to deliver multiple products through a biorefinery process where the salts and ulvans can first be extracted at low temperature. The proteins can then be extracted at a higher temperature and the remaining biomass used for transformation to biocrude or bioenergy. *Ulva ohnoi* also has value as whole unprocessed biomass a human food and nutraceutical with biomass being used in food taste trials in Japan (Aosa) and in nutraceutical trials for metabolic syndrome (4).

Demonstration Project 2 *Ulva ohnoi* at Pacific Reef Fisheries, Ayr, Queensland

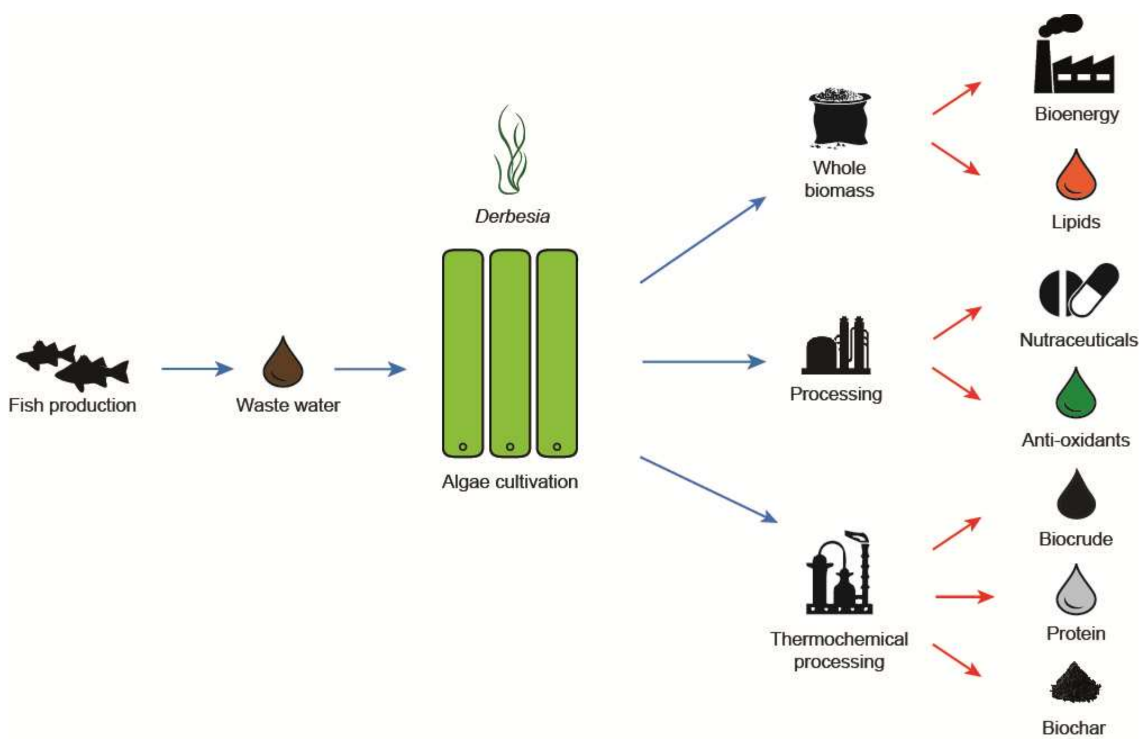
Pacific Reef Fisheries is one of Australia's largest producers of prawn (900 tonnes yr^{-1}) and an industry leader in best practice and sustainable aquaculture. PRF facilitated an initial six month trial using eight long raceways (20 m x 2.2 m x 0.8 m) and tank infrastructure in an area previously used to hold brood stock and raise juvenile prawns. The first six months of the PRF project determined proof-of-concept utilizing waste water from brackish water culture of prawns on-site. This initial phase was 16 times the scale of the MARFU project and demonstrated the feasibility of continuous large-scale production of *Ulva ohnoi* over the dry and wet season of the Austral tropics. Key management parameters for the production of *Ulva ohnoi* were determined in particular the flux requirements of nitrogen and phosphorous and filtration for optimised light transmission in water. In addition harvesting, drying and processing methods were developed for biomass produced at scale. Biomass was used in scale trials for human food (Aosa) and animal feed supplements.



The success of this initial trial led to the commercial development of Australia's first macroalgal production facility by MBD Energy in partnership with Pacific Reef Fisheries. The MBD Facility at PRF is the first production facility for macroalgae in Australia and is an integrated model that provides a commercially valuable bioremediation service for the discharge water from the production of prawns (black tiger, *Penaeus monodon*) and fish (cobia, *Rachycentron canadum*) while producing *Ulva ohnoi* for use as a human food and organic fertilizer. The facility is comprised of a sand filter (50 m x 10 m x 3m) where incoming water is filtered and dissolved organic nitrogen (DON), which is not available for uptake by algae, is converted to dissolved inorganic nitrogen (DIN as NO_3^- and NH_4^+) which is rapidly assimilated by algae into biomass. The sand filter also provides for conversion of dissolved nitrogen to dinitrogen gas through denitrification and provides for clear water with high light penetration. This filtered nutrient rich water is then delivered to two large high rate algal ponds (HRAP) as the preferred cost-effective production system for macroalgal biomass. Each HRAP (50 m x 8 m x 0.4 m = 85,000 L) is used to cultivate *Ulva ohnoi*. The production of *Ulva ohnoi* has run successfully for 18 months through two wet seasons when environmental fluctuations, and therefore production risk, are highest. Production from the HRAP system is approximately 60 tonnes ha^{-1} yr^{-1}. The HRAP system produces biomass for large scale product trials as human food and as an ingredient of organic fertilizers with the concomitant remediation of water such that the levels of nitrogen (0.5-1.0 $mg.L^{-1}$) and phosphorous (0.1-0.4 $mg.L^{-1}$) are similar to those of the coastal intake water for the aquaculture of prawns and fish. The resultant reduction in the discharge of nutrients to environmental receiving waters provides PRF with the capacity to increase production within existing infrastructure or expand the farm production through new infrastructure while remaining within discharge compliance limits. The integrated of production of *Ulva ohnoi* has facilitated the addition of 30 Ha of new ponds at PRF, an increase in production area of 33%.

Demonstration Project 3 – *Derbesia tenuissima* at Marine and Aquaculture Research Facilities Unit @ JCU

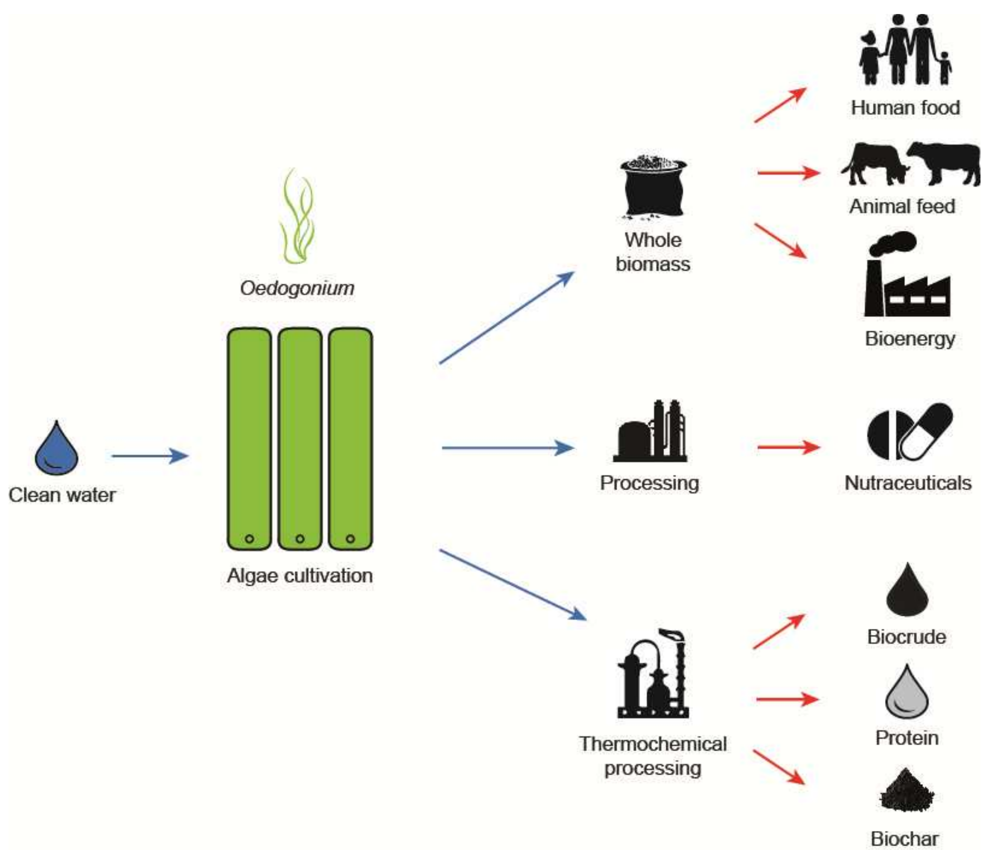
The long-term demonstration project tested the pilot-scale production of *Derbesia tenuissima* over a six month period using an array of parabolic tanks with a total volume of 32,500 L (3 x 10,833 L). The array was integrated in to the waste stream from a Barramundi breeding and hatchery facility at the Marine and Aquaculture Research Facilities Unit (MARFU) which provided the required nutrients to maintain the growth of the macroalgae (8). This project was run in conjunction with Demonstration Project 1 (*Ulva ohnoi* @ MARFU) providing for a direct comparison of productivity and products. Details of the system and operation are presented in Demonstration Project 1. Productivity was measured weekly and the key biochemical parameters of the biomass (C, N, Ash) and of the key co-product of *Derbesia tenuissima* (total lipids, total fatty acids, n-3 polyunsaturated fatty acids = PUFAn-3) were quantified every four weeks. The total biomass produced was also milled, homogenized and analysed for total lipids, fatty acids, fibres, amino acids, ash and moisture content. The protein content was determined from the sum of all amino acids. Total carbohydrates were determined by difference. These values were used to calculate projected annual productivities of bioproducts by multiplying the content of individual bioproducts in the bulk sample by the annual biomass productivity estimation [$P_{bioproduct} = (P_{biomass}*C)/100$ where $P_{bioproduct}$ is the productivity of a bioproduct (t ha^{-1} $year^{-1}$), $P_{biomass}$ is the productivity of the biomass (t ha^{-1} $year^{-1}$) and C is the concentration of the bioproduct as a percentage of dry weight].



Derbesia tenuissima had consistent in biomass productivity across the six month period which included the dry (June – October) and wet (November – January) seasons of the tropical Austral year with an average biomass productivity of 15.3 g dw m^{-2} day^{-1} (56 t dw ha^{-1} yr^{-1}). This is 2.5 times lower than that of *Ulva ohnoi* (138 t dw ha^{-1} yr^{-1}) (8). Biomass productivity was consistent ranging between 8 and 20 g dw m^{-2} day^{-1}. The lipid content of *Derbesia tenuissima* was seven times higher than in *Ulva ohnoi* and the protein twice as high. *Derbesia tenuissima* has one of the highest concentrations of lipids of all seaweeds and has use as a functional human whole food and as a nutraceutical supplement with strong anti-oxidant activity. However, regardless of the higher content of bio-products, the amount per unit area (bio-product productivity) was similar or higher in *Ulva ohnoi* due to its higher biomass productivity. Consequently, *Derbesia tenuissima* has a high value as a whole food or nutraceutical product but delivers significantly less productivity and bio-product through a biorefinery process where the key parameter associated with bio-product productivity (t dw ha^{-1} yr^{-1}) is consistently higher for *Ulva ohnoi* based on a higher biomass productivity per unit area (8).

Demonstration Project 4 – *Oedogonium* at Marine and Aquaculture Research Facilities Unit @ JCU

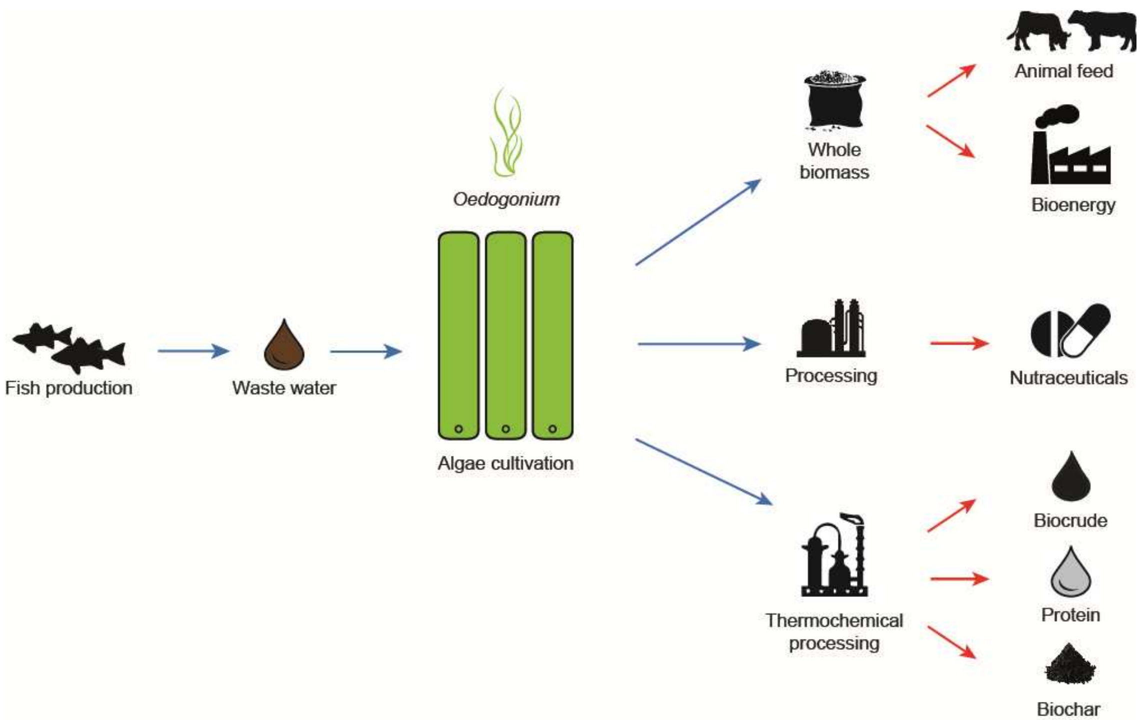
The demonstration projects with the broadest application to the delivery of high energy algal fuels and co-products are based on the integrated production of *Oedogonium* across a diversity of waste water streams from multiple industries. This is because there are larger and more diverse freshwater waste streams than marine given that the vast majority of industries and human activities are based on a reliable and clean supply of freshwater. Consequently, the interest in the integrated production of macroalgae in freshwater with the concomitant bioremediation of the water for re-use or discharge to the environment exceeded all expectations. The longer-term scaled production of *Oedogonium* in Program 2 has been trialed in waste water from freshwater aquaculture (11), energy generation (combustion of coal) (21, 22) and municipal waste water (sewage) (23). These demonstration projects were developed from the initial research in Program 1 which identified *Oedogonium* as the genus for cultivation and the methods for cultivation. They were also facilitated by this initial demonstration project at MARFU that developed and delivered the prototype scale technology for production (24) and implementation at multiple sites (freshwater aquaculture and municipal waste water). This long-term Demonstration project (Demonstration Project-4) tested the pilot-scale production of *Oedogonium* sp. (Genbank Accession number KF606977) over multiple periods of six months using an array of six parabolic tanks with a total volume of 65,000 L (6 x 10,833 L). The array was integrated in to a control system at the Marine and Aquaculture Research Facilities Unit (MARFU) with the capacity to provide CO_2 and pH.



The system was designed to allow for the delivery of water from industry partners water which could then be trailed at scale for its effects on the growth of *Oedogonium* and consequently on the degree of remediation of the water. It also provided for biomass cultured in each water source at scale for the testing of quality and the development and quality of bio-products. This is because the quality of biomass is a direct outcome of the quality of the water source. The provision of all biomass for the pilot-scale production of biocrude (University of Sydney and Licella) was supplied from this demonstration project where the quality of water was modified to provide biomass low in nitrogen (see Program 1). This biomass was also used to assess the suitability for slow pyrolysis (25), direct combustion (26, 27) and co-combustion with coal (28). This Demonstration Project also delivered the designs and operational parameters for the integration of the production of *Oedogonium* with waste water from freshwater aquaculture (DP-5), energy generation (DP-6) and municipal waste water (DP-7). These Demonstration Projects were delivered after long term trials where water from the aquaculture farm, power station and waste water treatment plant was trucked on-site, used for culture, and remediation quantified. Importantly, this Demonstration Project (DP-4) mitigated the risk of establishing facilities on remote sites by ensuring that *Oedogonium* could be produced successfully with concomitant bioremediation prior to investment in on-site demonstrations.

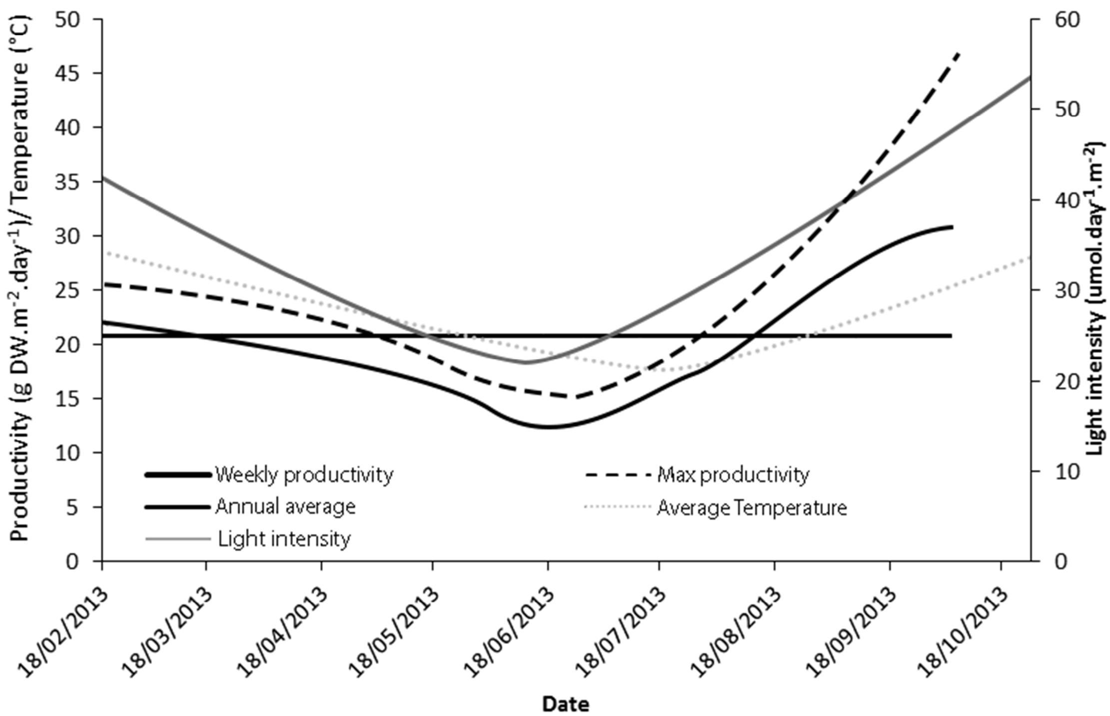
Demonstration Project 5 – *Oedogonium* at Good Fortune Bay Fisheries, Kelso, Queensland

Good Fortune Bay (GFB) is one of Australia's largest producers of barramundi producing 800 tonnes yr^{-1} at two facilities, one marine and one freshwater, in tropical North Queensland. GFB facilitated a long term trial at their freshwater facility at Kelso (Townsville) that ran continuously for more than 18 months. The demonstration project used three custom built 27,000 L parabolic troughs (25 x 2 m, surface area 50m2, water depth 0.75 m) using continuous flow-through water from the final capture pond for discharge water from production, prior to release. The water flowed directly from the pond through a 400 L sand filter to remove particulates and through the troughs and into a discharge area.



This demonstration project provided important baseline data for the production of *Oedogonium* over an eighteen month period allowing for predictive models to be established for the supply of nutrients and carbon for optimized growth, the manipulation of nutrient regimes for optimized protein yields and the provision of biomass for multiple product trials. The production of biomass ranged from 15 -35 g dw m^{-2} day^{-1} with an average of 19.5 g dw m^{-2} day^{-1} (70 tonnes ha^{-1} yr^{-1}) with noticeable fluctuation across seasons even within the tropics. There was a strong correlation between productivity and light with productivity all affecting productivity (Figure 4).

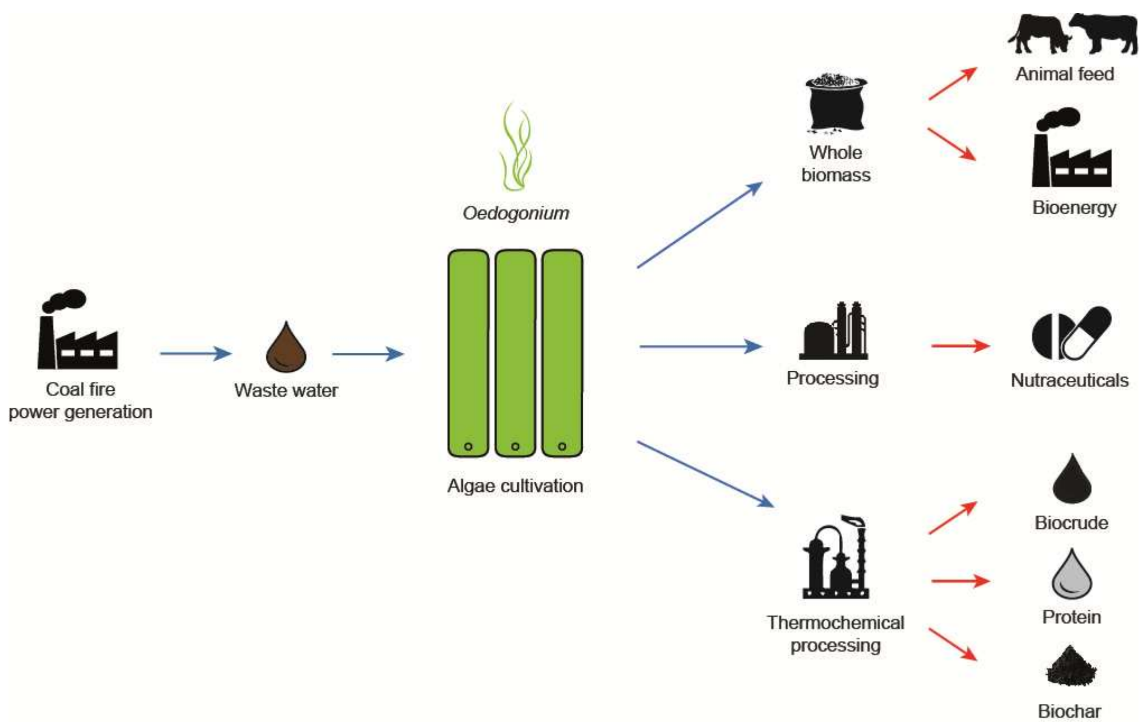
Figure 4.



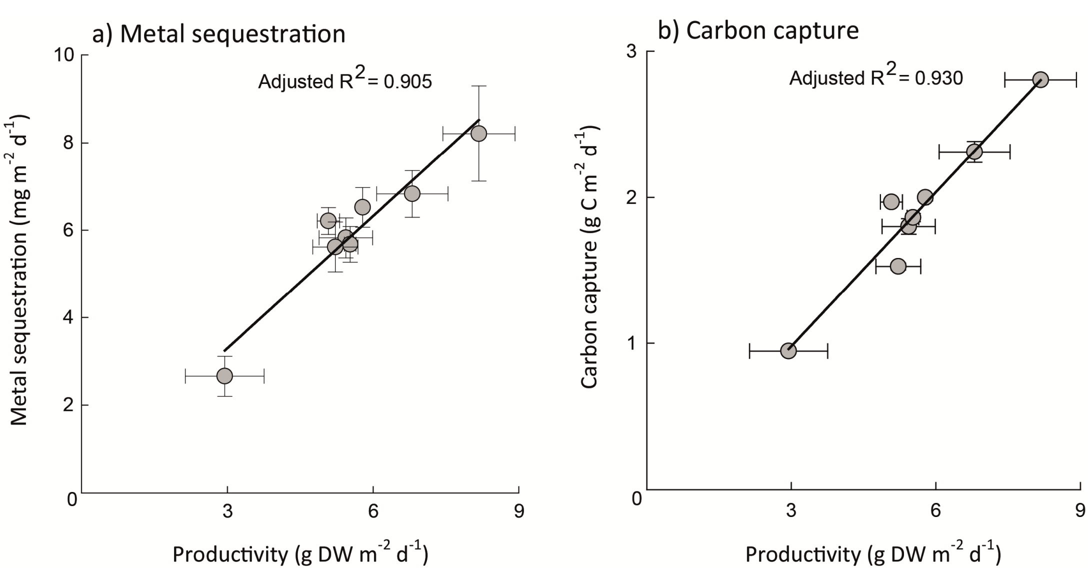
This mean productivity matches the commercial benchmark of 20 g m^{-2} day^{-1} (73 tonnes ha^{-1} yr^{-1}). The relationship between productivity and the flux of nitrogen and phosphorous was determined to model water supply, nutrient concentration, nutrient flux and productivity. *Oedogonium* requires 0.09 g m^{-2} day^{-1} of nitrogen and 0.04 g m^{-2} day^{-1} of phosphorous to maintain growth without nutrient limitation at scale, while simultaneously maintaining a high rate of nutrient uptake and efficiency (29). These data strongly support the integrated culture of freshwater macroalgae with freshwater aquaculture for nutrient recovery and bioremediation. These trials were further extended to produce *Oedogonium* under optimized conditions with productivities of 24 -36 g dw m^{-2} day^{-1} which are the highest reported for freshwater macroalgae. Notably, the protein content of biomass increased with increasing nitrogen content in the biomass (up to 18%) reflecting increase nitrogen supply in the water. Furthermore, the quality of protein is high with 43% of the protein being essential amino acids with high levels of methionine and lysine which cannot be synthesized by animals and must be provided in their diets (11). The quantity and quality of protein in *Oedogonium* is equivalent to, or higher than, many terrestrial crops used as a protein source in animal feeds. It can therefore provide a novel source of protein for the agricultural sector and contribute to the sustainability of intensive animal production through bioremediation (11).

Demonstration Project 6 – *Oedogonium* at Tarong Power Station, Tarong, Queensland

Tarong Power Station in SE Queensland is a large coal-fired power station producing 1400 megawatts (MW) of electricity. It produces ash from the combustion of coal which is washed from into a 46,000 megalitre (ML) ash dam. This water is stored and evaporation concentrates the metals and metalloid contaminants in the ash water (AW). The ash water in Tarong contains elements in excess of the Australian and New Zealand Environment and Conservation Council (ANZECC) water quality guidelines (Al, As, Cd, Cr, Cu, Ni, Se and Zn) and as such the water must either be stored indefinitely or cleaned to ANZECC standards prior to discharge or re-use. There are few treatment options for ash water and as such it is also often a legacy contaminant that poses a persistent environmental risk after the decommissioning of power stations. The cultivation of *Oedogonium* was trialed in AW from Tarong, with the initial trials using water transported from Tarong Ash Dam to JCU (DP-4) (22, 30). Following the successful isolation of an endemic species of *Oedogonium* from Tarong (Genbank accession KF606974) and cultivation in AW a demonstration project was established at the MBD Energy Algal Pilot Plant adjacent to Tarong Power Station. *Oedogonium* was cultivated in 15,000 L parabolic tanks on site using AW that was first passed through a 10 µm filter to remove fine suspended solids. Flue gas from the power station was used to provide CO_2 and this was controlled by pH which was between 8.4 and 8.6 (21).



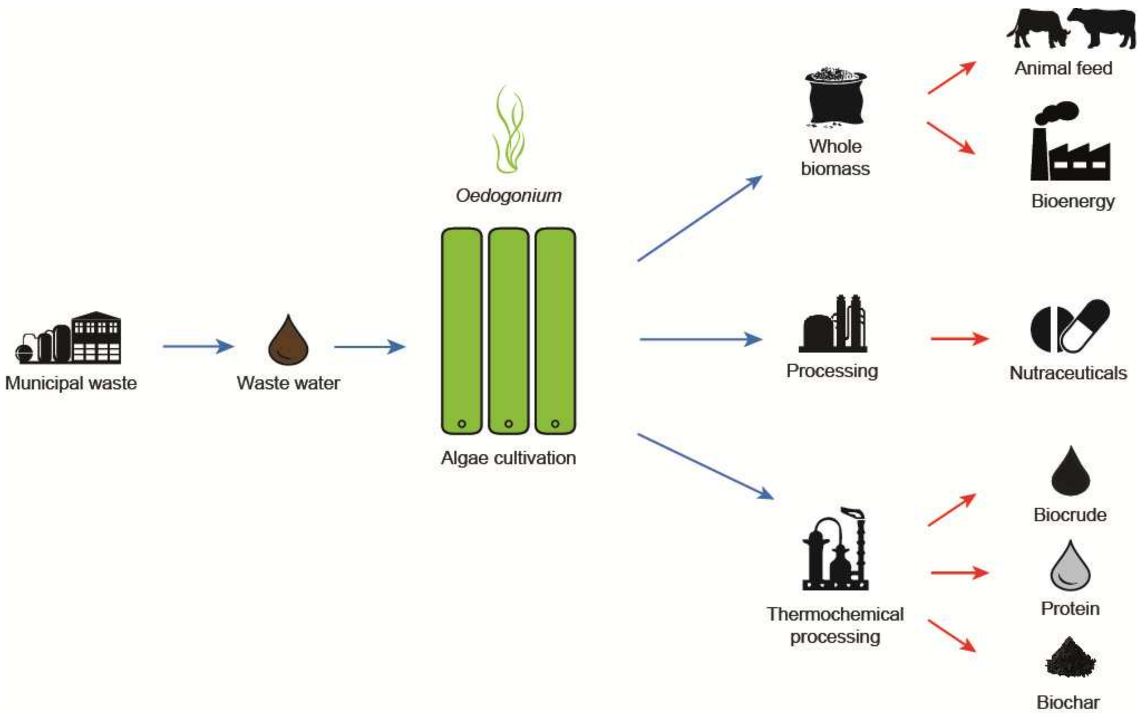
This demonstration project provided important baseline data for the production of *Oedogonium* in metal contaminated water for the remediation of metals and carbon. Nutrients were added to the culture system to maintain growth as the AW was low in nitrogen and phosphorous. *Oedogonium* was successfully cultivated in the July-August period when ambient air temperature ranging from 1°C-16°C with a 50% increase in biomass over 3-4 day culture cycles. This resulted in an average biomass productivity of 5.6 g dw m^{-2} day^{-1} (21). While below productivities for tropical Australia the study demonstrates the ability to continuously cultivate macroalgae in temperate regions with expectations of productivities exceeding 20 g dw m^{-2} day^{-1} from spring through to autumn. Importantly, there was a strong linear relationship between the sequestration of total metals (mg m^{-2} day^{-1}) and productivity. Similarly there was a strong linear correlation between the sequestration of carbon (g C m^{-2} day^{-1}) and productivity (21). There was differential sequestration of metals with aluminium and zinc being remediated most rapidly followed by arsenic, copper and nickel, and then cadmium, chromium and selenium. The bioremediation model from this quantitative data predicts that if the area of the ash dam (200 Ha) was dedicated to the production of *Oedogonium* rather than the storage of waste water the power station would remediate the predicted rate of metal inputs each year, and over five years return the ash dam to zero discharge of contaminants with the exception of selenium (21). Overall, this project demonstrated for the first time that macroalgae can be cultivated in simple low-input open culture systems at a coal-fired power station to remediate waste water and deliver biomass. Notably, the growth rate in the lowest part of the annual production cycle was comparable to terrestrial perennial grasses targeted for carbon capture and energy production (*Miscanthus* spp.). However, the rate of carbon capture relative to emissions is negligible while rate of metal sequestration is significant and material at realistic scaled scenarios. Finally, in this demonstration project we were able to show that the biomass, even with levels of sequestered metals, is a suitable feedstock for the production of biochar as a soil ameliorant (21, 31). Slow pyrolysis of the biomass of *Oedogonium* produced at Tarong could be modified to immobilize metals in macro-molecular structure of the biochar. Biochar produced at 750°C balanced yield, carbon recalcitrance and metal immobilization to provide a material for the improvement of low fertility soils, in particular those associated with adjacent coal mining sites (21, 31).



Demonstration Project 7 – Oedogonium at TCC Cleveland Bay WTP

The Townsville City Council Waste water Treatment Plant (TCC WWTP) at Cleveland Bay is a major facility treating 29 ML of sewage per day, with a maximum secondary treatment capacity of 75 ML day^{-1} and a peak wet weather flow of 145 ML day^{-1}. The plant is designed to treat the wastewater from a population of 126,000 EP (equivalent people). The waste water is treated to a secondary level prior to discharge to the receiving waters of Great Barrier Reef. The cultivation of *Oedogonium* (Genbank accession KF606977) was trialed in primary and secondary treated waste water from the Cleveland Bay, with the initial trials using water transported from Cleveland Bay WWTP to the macroalgal cultivation facility at JCU. *Oedogonium* was successfully cultivated at small scale in both primary and secondary treated sewage with further larger scale trials using primary treated sewage based to the high concentration of available nutrients using a low exchange rate (23). The primary effluent has nitrogen and phosphorous concentrations of 27 mg L^{-1} and 5 mg L^{-1} respectively. *Oedogonium* had biomass productivities of 7-10 g dw m^{-2} day^{-1} in this system, similar to other small scale trials, with nutrient removal rates of 0.50 g N m^{-2} day^{-1} and 0.11 g P m^{-2} day^{-1} (23). Chemical oxygen demand, microbial counts and metals were also significantly reduced in the treated water. The biomass produced had a consistent biochemical profile and based on the verified model derived from this Program (see Program 1) this would yield 27% of the dry weight of the biomass as biocrude for conversion to drop-in fuels (23). Overall, this pilot-scale work demonstrated that freshwater macroalgae can be used to treat multiple components of municipal wastewater and simultaneously deliver biomass that can be converted to biocrude for the production of drop-in fuels.

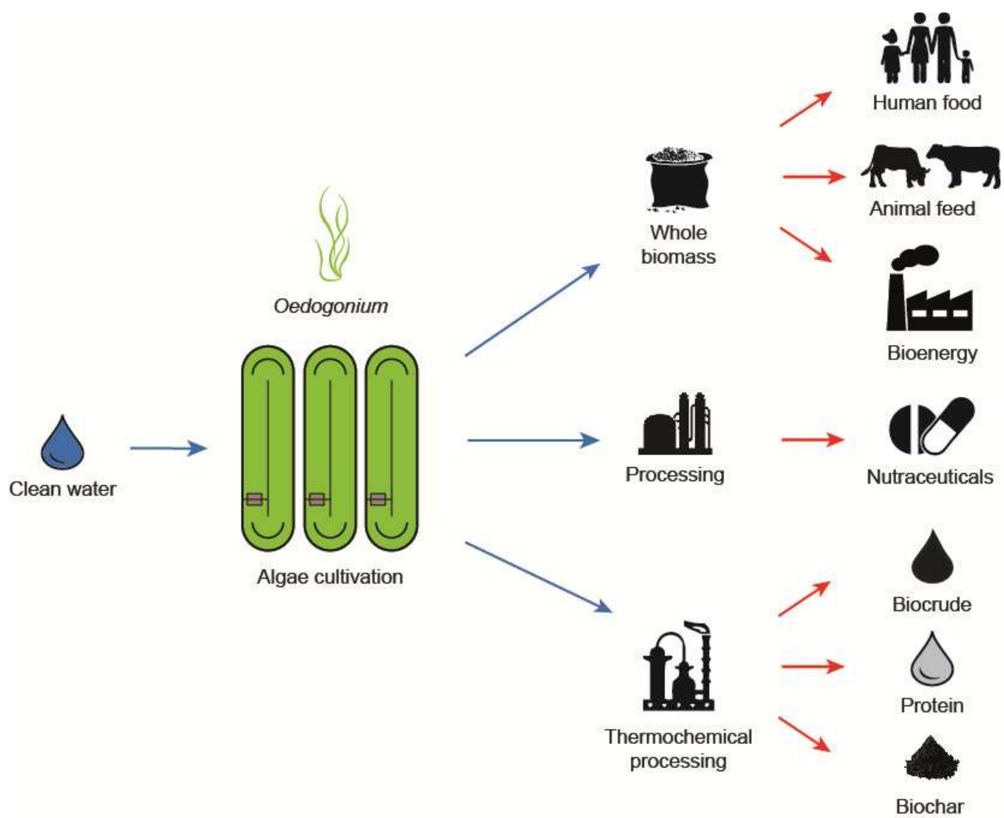
Following the successful cultivation in sewage from the WWTP a demonstration project was established in collaboration with the TCC at the Cleveland Bay WWTP to trial the large-scale cultivation of *Oedogonium* in secondary treated sewage to quantify improvements in water quality and biomass productivities. The primary effluent has a nitrogen and phosphorous concentration of 27 mg L^{-1} and 5 mg L^{-1} respectively, with this concentration reduced during the treatment process to less than 3.5 mg L^{-1} of nitrogen and 0.5mg.L^{-1} of phosphorous. This secondary effluent is then passed through a 1 micron membrane filter before being used as the source of water and nutrients in the demonstration project. *Oedogonium* was cultivated in three custom designed 27,000 L parabolic troughs (see Demonstration Project 5) (25 x 2 m, surface area 50m^2, water depth 0.75 m) on site at the TCC WWTP using secondary effluent. *Oedogonium* was maintained in suspended culture through aeration provided by a central aeration line. The tanks were maintained under continuous flow with an exchange rate of one volume per day (27,000 L day^{-1}) and have been operational for three months with continued experimentation supported by MBD Energy and the Townsville City Council.



This ongoing demonstration project has provided important baseline data for the production of *Oedogonium* in municipal waste water which is a very available resource that is currently treated to high levels at significant cost, particularly in environmental sensitive regions such as those adjacent and discharging to the World Heritage Listed Great Barrier Reef. This Demonstration Project is re-defining wastewater from a risk factor for the environment to a valuable resource for beneficial use prior to discharge. It allows for the efficient recovery of nutrients and metals from the treated waste water thereby improving quality prior to discharge with the concomitant delivery of biomass. The production of biomass has ranged from 10 -21 g dw m^{-2} day^{-1} with an average of 15 g dw m^{-2} day^{-1} (55 tonnes ha^{-1} yr^{-1}) with continual improvements in productivity as we optimize operational parameters for water flow and stocking density. The cultivation of *Oedogonium* has resulted in a 25% decrease in nitrogen and a 61.5% decrease in phosphorous from the secondary treated water. With the development of the overall program there are parallels between large-scale demonstration projects, with improved biomass productivities as each project is developed through managing nutrient and carbon flux. For example, the production of *Oedogonium* at GFB Kelso (Demonstration Project 5) was optimized over a year prior to delivering steady state productivity based on stocking density and flux (Figure 4). The continual improvement to reach an optimized steady state is the primary objective of the ongoing work at the Cleveland Bay WWTP with ongoing product development of the biomass.

Demonstration Project 8 – *Oedogonium* at JCU – High Rate Algal Ponds

The High Rate Algal Pond (HRAP) is the most cost-effective infrastructure for the large-scale, land-based production of algal biomass based on extensive engineering and production research. This has also been demonstrated in this program with a comparative assessment of the cost of infrastructure for the cultivation of *Ulva ohnoi* resulting in the implementation of HRAPs as the commercial platform. However, there has been no testing of the production of freshwater macroalgae, the fundamental basis for the delivery of bioremediation and biomass, in HRAPs. This is the critical 'next-step' in scale production and understanding the operation and management of *Oedogonium* in HRAP is essential for the uptake and dissemination of integrated bioremediation and biomass production in freshwater waste streams from agriculture, municipal waste, mining, mineral processing and energy generation. These scale systems also provide biomass for larger trials of the use of biomass, in particular animal feed trials which require a consistent supply of larger quantities of biomass over time. This is important as *Oedogonium* has a nutritional profile making it suitable as a feed ingredient and supplement for ruminants under intensive and extensive production.



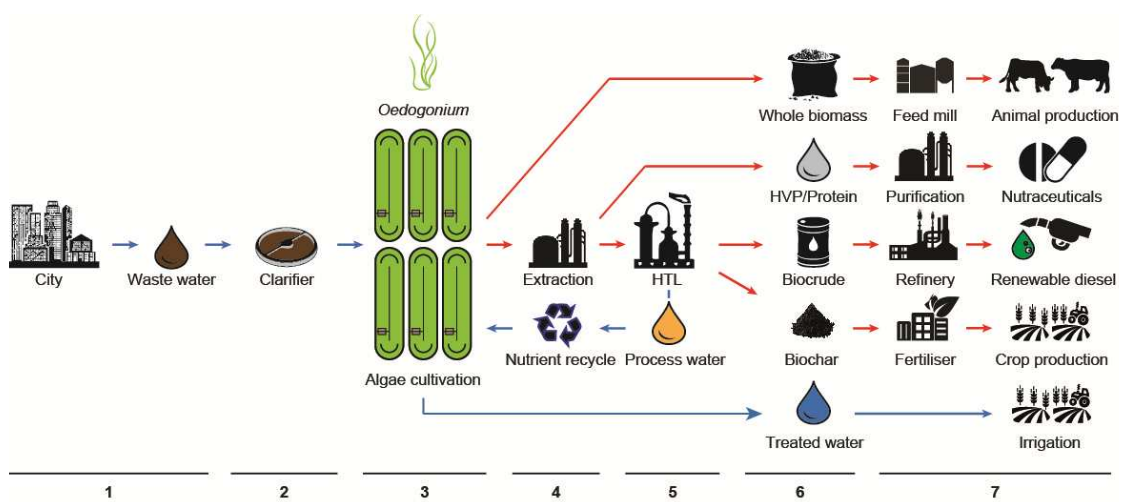
Three high rate algal ponds (22 m l x 3.4 m w, depth 0.8 m, water depth 0.4m) have been designed and implemented at JCU to complement the smaller pilot-scale facilities that have been the key infrastructure for all projects to date. The HRAP system includes customized harvesting equipment based on Demonstration Projects 5 and 7 and trials have been implemented using *Oedogonium* (Genbank accession KF606977) to optimize paddlewheel speed and current velocity (mixing), nutrient and carbon flux, and stocking density (light quantum) to maximize productivity based on the interaction between these parameters. We have reduced energy usage to a quarter of that of research scale infrastructure (circular tanks, parabolic tanks and raceways) (per m^2 of production area) with further improvements pending. These trials will continue beyond the ARENA program as the basis for expansion and implementation of the integration of freshwater macroalgae in the treatment of agricultural and municipal waste water.

Importantly, the most direct and identifiable source of nutrient rich waste water in Australia is municipal waste water. Australia produced 2.095×10^9 m^3 of municipal wastewater in 2013 (AQUASTAT database) of which 1.580×10^9 m^3 (75%) was discharged into environmental receiving waters. The remaining 25% of secondary water was reused, mainly for irrigation. This provides a resource of 4.3×10^6 m^3 of treated water day^{-1} that is discharged for possible use for the cultivation of freshwater macroalgae with the beneficial reduction of nutrients and contaminants. The re-use of municipal waste water and its discharge are a priority area for the Department of the Environment with improved outcomes for marine water quality. Given the understanding of the productivity of the biomass, the cost and productivity of infrastructure and the value and application of bio-products, including multiple products under a biorefinery concept with biocrude as a target component, we have used this model for a preliminary techno-economic evaluation.

Preliminary economic assessment of the production and macroalgal biomass and biocrude

We undertook a preliminary economic assessment of the production of macroalgal biomass and biocrude in Australia. This assessment reports that the production of renewable fuels from algae cultivated in land-based systems in Australia is not economically viable, either at present or in the near future, as a standalone industry in which algae is produced with the sole purpose to generate biofuel. Consequently, we describe and assess an integrated biorefinery process for freshwater macroalgae, based on the research in the HEAF project, to create an economically sustainable investment opportunity.

The economic assessment is of an integrated biorefinery model to produce 100,000 tonnes of dried biomass per annum yielding ~200,000 barrels of high-energy biocrude per annum (equivalent to 25,000 tonnes). The process flow diagram below describes the integrated biorefinery approach. The enterprise must be sited adjacent to a city generating sufficient wastewater and nutrients [**1**]. The enterprise is effectively a hybrid treatment facility using a primary clarifier to remove particulates [**2**] and treating dissolved components with the algae cultivation production area [**3**] with clean (treated) water leaving the facility. The wet algal biomass is then processed in two stages, firstly, [**4**] the extraction of high-value products (HVP) and feed ingredients (including protein) and, secondly, [**5**] the hydrothermal liquefaction of the residual biomass to biocrude and biochar. A range of products will be generated [**6**] that can then be distributed to third-parties for either direct use or further product development [**7**]. The model is based on the value of products at [**6**].



The base model assumes no cost for the supply of nutrient-rich water as water is delivered as municipal waste [**1**]. For production of 100,000 tonnes of dried biomass per year, a production footprint of 1370 ha is required for freshwater macroalgae growing at a demonstrated rate of 22 g m^{-2} day^{-1}. The fixed costs to build this production facility [**2 + 3**] would be US$122.7 million, with a variable cost of US$33.7 million per annum. The feasibility of high-energy fuels from algal biocrude hinges on the generation of multiple co-products including high-value animal feed ingredients and agricultural crop fertilizer (biochar). Thermochemical processing – hydrothermal liquefaction – is the preferred pathway to generate high-energy biofuels because of its capability to process wet biomass, its use of the entire organic component of the biomass and the generation of biocrude that can be refined directly into high-energy fuel. The fixed costs to build a two-stage processing facility to deliver high-value products and biocrude [**4 + 5**] from 100,000 tonnes of dried algae per annum (equivalent to ~500,000 tonnes of wet biomass) would be US$40.8 million, with a variable cost of US$3.1 million per annum. The facility would create a diversified product stream [**6**] to ensure that there was resilience within the enterprise to fluctuating product prices.

Over a 10-year period the production of 831,436 tonnes of dry algae [**2 – 6**] has a cost of $466 per tonne of algae, based on a total production investment of $387 million. Over this same time period, the cost of processing per tonne is $78 per tonne of algae, with a total processing investment of $65 million. The combined value of all processed products is $378 per tonne of algae, generating $307 million in revenue over 10 years, but falling well short of the combined production and processing costs of $544 per tonne. We found that, at the base model value of $324/tonne of biocrude (equivalent to $50 a barrel of WTI crude oil), the majority of the revenue in the enterprise would be derived from HVP and protein for animal feed (>70% of revenue at present day values) yet still with a negative Net Present Value (NPV) over a 10 year period for the enterprise. We then show in a sensitivity analysis that this would only change if the price of crude oil increased to $1263/tonne of biocrude (or $200/barrel) at which point there would be a positive NPV over a 10 year period.

However, we then added an additional revenue stream at the start of the process [**2 + 3**] for the treatment of municipal water for a population of 1,200,000 (which provides sufficient nutrients for the production of ~100,000 tonnes of dry biomass per annum), as the enterprise also provides a water treatment service for this population. By factoring in water treatment into the model at $360/ML (almost half the cost of existing municipal wastewater treatment facilities) we achieve a payback period of less than 5 years (i.e. a positive NPV) while retaining a 5% discount rate.

Using this preliminary analysis we demonstrate the importance of integrating biomass production with wastewater to deliver a new technology for Australia that generates a range of new products using non-arable land and that can service the treatment requirements of an expanding urban population with point-source waste streams. This integrated approach would effectively operate as a hybrid wastewater treatment facility in which the nutrient and water in the system are considered as resources for re-use and value-adding opportunity. The externalities of this kind of enterprise would represent a significant contribution for regional development in Northern Australia where the space and potential are both compatible with this economic assessment of high-energy algal biofuels.

Technical Appendix

The summarized outcomes of the HEAF Project as described above have been delivered through a foundation of peer-reviewed outputs to provide methods of best practice to deliver macroalgal biomass through integrated production systems and conversion of biomass to bioenergy (with a focus on biocrude) and bioproducts. The HEAF Project was deliberately executed to maximize the accessibility of information for industry, Government and the public. The strategy was, where possible, to deliver outputs in the form of peer-reviewed publications to ensure quality in science and technology. This provides the broadest accessibility to reliable data from a reviewed source. The accompanying technical appendix is a comprehensive listing of the outputs of the project under the themes of ‘energy’, ‘co-products’ and ‘services’. These publications are accessible electronically through scientific journals where each carries a digital object identifier (doi) as the international standard for document identification.

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