

The State of Algal Energy Research

Specific to Bio-Energy Application in South Africa

March 2013



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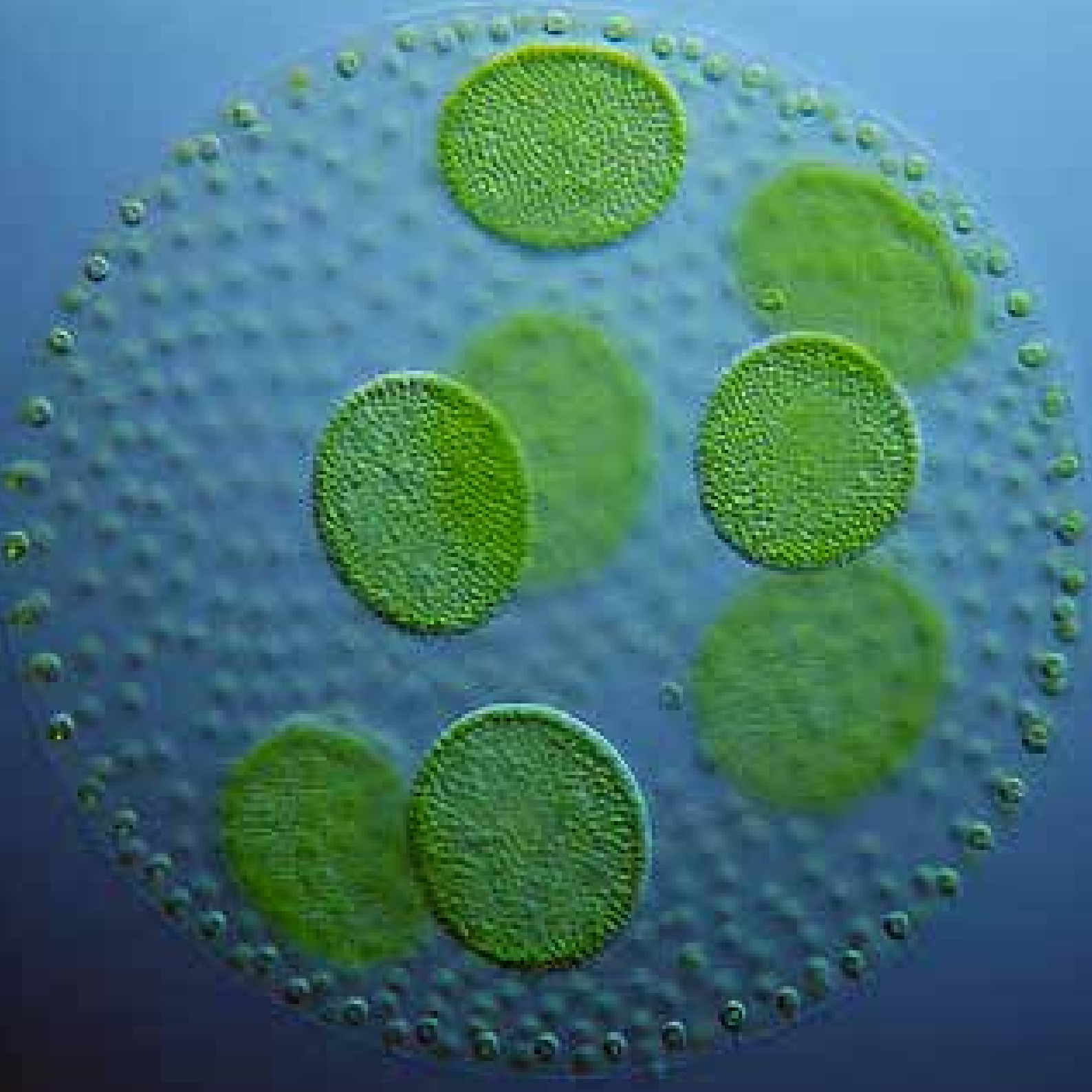
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Introduction

The finite nature of fossil fuel justifies research into alternative fuels from renewable sources. Biofuel refers to liquid, gas and solid fuels primarily produced from biomass. It includes bioethanol, biomethanol, biodiesel and biohydrogen. Among all the biofuels, biodiesel has received the most attention due to its similarity with conventional diesel in terms of chemical and energy content. Therefore, no modification of current diesel engines is required to use biodiesel. An array of biolipids can be used to produce biodiesel. The feedstock of choice for biodiesel production globally depends greatly on the climatic conditions of the country, local soil conditions and feedstock availability. Typical raw materials used globally for biodiesel production are rapeseed, canola, soybean, sunflower and palm oil. Furthermore, various other resources have reportedly been used including jatropha, almond, barley, coconut, copra, fish oil, groundnut, karanja, oat, poppy seed, rice bran, sesame, sorghum and algae.

Algae, like all plants, undergo photosynthesis to grow. As they grow, they accumulate fats and bio-oils that have similar molecular structures to traditional oil. The potential benefits of biodiesel from photosynthetic algae could be significant:

- ▶ Algae can be grown using land and water unsuitable for crop plant or food production;
- ▶ Growing algae consume carbon dioxide; this provides greenhouse gas mitigation benefits;
- ▶ Bio-oil produced by photosynthetic algae and the resultant biofuel will have molecular structures that are similar to the petroleum and refined products we use today;
- ▶ Bio-oils from photosynthetic algae could be used to manufacture a full range of fuels including gasoline, diesel fuel and jet fuel;
- ▶ Algae yield greater volumes of biofuel per acre of production than crop plant-based biofuel sources;
- ▶ Algae used to produce biofuel are highly productive. As a result, large quantities of algae can be grown quickly, and the process of testing different strains of algae for their fuel-making potential can proceed faster than with other crops with longer life cycles.

The objective of this review is provide an overview of current algal energy research being carried out at South African Universities, Universities of Technology and other research institutions in order to:

- ▶ Identify common themes and priorities in energy research;
- ▶ Avoid research duplication
- ▶ Identify possible gaps that are not being covered by current energy research;
- ▶ Compile a profile of energy researchers actively working in the field;
- ▶ Make recommendations on future algal energy research focal areas for South Africa.

This information will be used in the establishment of an algal energy research platform, focused on potential biofuel production.

Prominent Microalgal Energy Researchers in South Africa

Universities of Technology

- ▶ Durban University of Technology
 - Prof. Faizal Bux
- ▶ Mangosuthu University of Technology
 - Prof. Akash Anandraj
- ▶ Tshwane University of Technology
 - Ms. Erika Jordaan
- ▶ Vaal University of Technology
 - Dr. Peter Stegmann

Universities

- ▶ Nelson Mandela Metropolitan University
 - Prof. Ben Zeelie
- ▶ North-West University
 - Prof. Sanette Marx
- ▶ Rhodes University
 - Prof. Keith Cowan
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- ▶ University of Stellenbosch
 - Prof. Emile van Zyl
 - Dr. Raymond Els

- ▶ University of Witwatersrand
 - Prof. Vincent Gray
 - Prof. Stuart Sym

Other Research Institutes

- ▶ Council for Scientific and Industrial Research
 - Mr. Dheepak Maharajh

Prominent Macroalgal Energy Researchers in South Africa

Universities

- ▶ University of Western Cape
 - Dr. Rolene Bauer

Microalgal Energy Researchers





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Overview of Current Algal Research



The main focus area at DUT has been to investigate the biotechnological applications of microalgae. Within this main focus area several components have been researched:

- ▶ Algal bioprospecting;
- ▶ Microalgae for biodiesel production using wastewater as a substrate;
- ▶ Optimisation of growth conditions and maximisation of lipid yields at lab scale as well as utilising a small pilot scale raceway pond;
- ▶ Heterotrophic and autotrophic growth;
- ▶ Evaluating the carbon dioxide sequestration potential of algae from flue gas;
- ▶ Application of algae in wastewater treatment;

- ▶ Work on photobioreactors and downstream processing from harvesting and lipid extraction to the conversion of oil to biodiesel and characterisation thereof.

Algal Biofuel Research



- ▶ **Algal bioprospecting**

Bioprospecting for microalgae was undertaken in freshwater systems of KwaZulu-Natal. A *Chlorella* spp. was isolated from a wastewater treatment plant maturation pond which showed the highest biomass production and lipid content (18% weight per weight (w/w)).

- ▶ **Microalgae for biodiesel production using wastewater as a substrate**

Pre-chlorinated wastewater was shown to be unable to support microalgal growth. However, growth of the selected *Chlorella* spp. in unaltered post-chlorinated wastewater was possible. Biomass and lipid production were much higher when grown in a modified medium as compared to post-chlorinated wastewater. When post-chlorinated wastewater was supplemented with 5 mM Sodium Nitrate (NaNO_3), the growth rate was enhanced with maximal biomass productivity furthermore, a maximum lipid yield was achieved when 25 mM NaNO_3 was added to the wastewater. Low levels of chlorine were shown to enhance microalgal growth. This work revealed that wastewater can be used for the cultivation of microalgae when additional nutrient supplements are added to sustain microalgal growth.

- ▶ **Optimisation of growth conditions and maximisation of lipid yields**

Pulse Amplitude Modulated fluorometry was used to assess microalgal nutrient stress and to monitor its effect on cellular neutral lipids of a *Chlorella* spp. Optimal neutral lipids were produced

when the algal biomass was first maximised followed by complete nutrient stress.

► **Heterotrophic and autotrophic growth**

Cell growth rate and lipid content of a *Chlorella* spp. was determined under photoautotrophic and heterotrophic growth conditions. A higher biomass (3.6 fold) and lipid content (4.4 fold) were observed under heterotrophic growth conditions. The oil produced during heterotrophic growth was superior compared to oil produced during photoautotrophic growth. Under heterotrophic conditions biomass and lipid production costs can also be reduced.

Additional Algal Research

► **Evaluating the carbon dioxide sequestration potential of algae from flue gas**

Current research include bioprospecting for high CO₂ sequestering microalgae.

► **Application of algae in wastewater treatment**

Current research include up-scaling to demonstration plant at a wastewater treatment works.



Recommendations for Future Algal Energy Research

► Life cycle analysis of large scale algal cultivation with regards to energy and economics.





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Overview of Current Algal Research



The main focus areas into algal research at The Centre for Algal Biotechnology (CAB) at MUT has been:

- ▶ Algal bioprospecting;
- ▶ Algal cultivation in photobioreactors;
- ▶ Heterotrophic and autotrophic growth;
- ▶ Optimisation of algal biomass and lipid yield;
- ▶ Algal harvesting and lipid extraction;
- ▶ Conversion of algal oil to biodiesel;
- ▶ Biodiesel characterisation;
- ▶ Bio-hydrogen and bio-aviation fuel production;
- ▶ Cultivation in effluents;

- ▶ Carbon dioxide sequestration;
- ▶ Production of high value algal products.

Algal Biofuel Research



▶ **Algal bioprospecting**

Bioprospecting for indigenous microalgae with high value products are on-going at the CAB. The Centre was part of the first algae bioprospecting project in South Africa. The Consortium (CSIR, DUT and MUT) was funded by TIA and the CSIR. One of the goals of CAB is to house an algae culture bank of high lipid yielding indigenous microalgae. This can be a commercial venture which could produce and sell high value algal species.

▶ **Algal cultivation in photobioreactors**

At the Centre for Algal Biotechnology, cultures are grown in 20L photobioreactors and scaled up to 150L. Instruments are available to characterise the internal conditions of photobioreactors for algal growth. The next phase is to construct a 10 000L tubular photobioreactor. The Centre is also developing a 20L bioreactor with LED lighting, air/CO₂ feed and temperature control. These reactors are ideal for lab culturing and controlled experiments, costing a third of the price of commercial bioreactors.

▶ **Heterotrophic and autotrophic growth**

Both heterotrophic and autotrophic modes of nutrition are investigated. Higher yields of lipids in algae cultivated heterotrophically have been observed. A freely available simple carbon source is incorporated into the growth medium. Radioisotopes were used to trace its pathway into the lipid carb chains. Autotrophic and mixotrophic nutrition is a model under investigation for the improved yield of biodiesel from algae.

► **Optimisation of algal biomass and lipid yield**

The CAB specialises firstly in maximizing algae biomass over the shortest period by optimising the environmental conditions (light, temperature, nutrients) for growth using a PAM fluorometer and photosynthetic rates. Secondly, using the same instrumentation and techniques, the oil content in algal cells is maximised (60% to 80%/cell). These two stages are critical to improving the overall yield of biodiesel. The Centre is equipped with state of the art instruments to assess and monitor the physiology and biochemistry of live cultures of algae. Algae cultures are cultivated in bioreactors ranging from 1000mL to 20L, with an outdoor series of reactors totaling 10 000L.

Algae oil is naturally produced as a mechanism to store chemical energy during nutrient deprivation. Lipid synthesis can be induced by withholding essential nutrients from the growth media or by allowing the culture to grow and deplete the store of nutrients. In order to maximise the neutral lipid cellular content, we expose the culture to extreme nutrient limiting conditions while monitoring the photo-physiology using a specialised fluorometer. A fluorescent microscope indicates changes in the biochemistry, whereby neutral lipids formation in vacuoles are photographed and quantified.

► **Algal harvesting**

Algal harvesting is an on-going process at the CAB, utilizing three common methods (flocculation, centrifugation and settling). The Centre has budgeted for a dewatering portable machine that separates intact biomass while recycling the growth/water medium. The Centre is researching the use of electro-flocculation, which if successful, would be incorporated into the prototype of the new bioreactor. Harvesting at larger scales is a global challenge. Research and development of efficient harvesting systems can only be undertaken at a pilot scale. We are seeking a pilot scale algae plant which would allow for such investigations.

► **Lipid extraction**

Lipid extraction methods used vary from the traditional solvent extraction to more direct methods. The method of drying (solar/oven/air) influences the extraction efficiency. Prior to lipid extraction cultures are stressed. This critical step determines the overall lipid yield and subsequently the biodiesel productivity. The CAB specialises in maximising algal stress while monitoring the physiological stress using state of the art instruments. To date, the Centre has recorded on average between 60% to 80% lipids per algal cell, which is above internationally published yields.

► **Conversion of algal oil to biodiesel**

The extracted oil is converted to biodiesel fatty acid methyl esters (FAMES) using standard transesterification protocols. Research on the characterisation of the biodiesel has shown how the FAMES may vary in carbon chain length and groups of methyl esters. To produce the desired biodiesel, studies have shown how growth conditions, stress factors and extraction methods should be carefully selected. This year (2013) the Centre would acquire instruments that can determine significant parameters (according to EU standards) such as cetane number, flash point and viscosity, which would suggest if the biodiesel is suitable as a fuel source for motor engines.

► **Biodiesel characterisation**

Once the oil is extracted and converted to biodiesel by transesterification, the biodiesel (FAMES) composition is identified and characterised using Gas Chromatography Mass Spectrometry (GC-MS). A carbon chain length of C16 to C18 is preferred. The percentage of C16 and C18 in the total FAME mix is significant to determine the quality of biodiesel. To meet international specifications, the cetane number, viscosity, flash point, density, ash content, carbon residue and the blend and catalysts are

determined. Biodiesel from algae requires further characterization such as oxidation potential, cold filter plugging, and particulate content.

► **Bio-hydrogen and bio-aviation fuel production**

No details at this stage

Additional Algal Research



► **Cultivation in effluents**

Research at the CAB is focusing on using algae to recycle wastewater runoff (leachate) from landfill sites in Durban. We have also been able to recycle acid mine wastewater using a specific algal strain. Studies focus on the physiological responses of algae to these wastewaters. From these experiments the toxicity and lethal concentrations of the hyper-nitrified wastewater effluent could be determined.

► **Carbon dioxide sequestration**

Over the last 10 years the CAB has measured CO₂ sequestration of algae in natural habitats. Pure cultures of these indigenous strains were cultivated in the laboratory and their CO₂ sequestration rates measured using radioisotope 14C. The uptake rates of CO₂ are influenced by environmental parameters. This is an efficient and rapid method to accurately determine the sequestration rate of algae. These rates are useful in calculating the potential carbon credit that can be acquired from the biomass of algae.


► **Production of high value algal products**

Apart from the biofuels produced using algal extracts, high value nutraceuticals such as omega 3 and 6, astaxanthin and beta-carotene are produced. The investigations are at lab scale with the objective of identifying indigenous strains of microalgae that produce high yields of astaxanthin, omega 3 and 6 and beta-carotene.

Recommendations for Future Algal Energy Research

- Harvesting at larger scales is a global challenge. Research and development of efficient harvesting systems can only be undertaken at a pilot scale.





Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



**Tshwane University
of Technology**

We empower people

Tshwane University of Technology



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Overview of Current Algal Research



Algal Biofuel Research




The algal research focus is currently of a pure academic nature and include:

- ▶ Sampling of different water sources in South Africa for algal species;
- ▶ Laboratory optimisation of cultivation and lipid production;
- ▶ Evaluation of the best isolates on different industrial effluents for heterotrophic and autotrophic production of biodiesel;
- ▶ Analysis of the characteristics of algal biodiesel according to international standards.

Recommendations for Future Algal Energy Research

- ▶ Although biodiesel production from algae has great potential, the commercial production thereof is still restricted due to cost of production at a commercial level. Large scale production is mainly for various high value products which is financially more feasible. There are still problems related to isolation, growth etc. of algal species in order to result in optimal lipid production.





Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



Vaal University of Technology

YOUR WORLD IS A BETTER PLACE

Vaal University of Technology



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Overview of Current Algal Research



Additional Algal Research



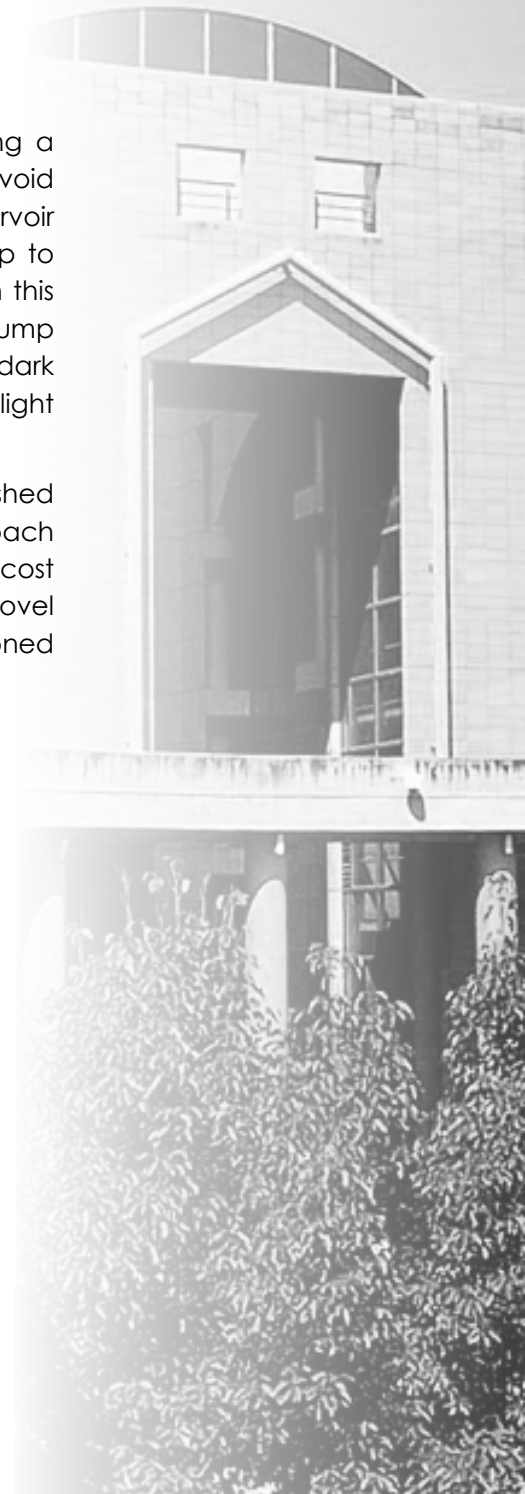
► **Development of a novel photobioreactor to increase the photosynthetic efficiency of algae**


In 2006 a small research group working on algal biotechnology was started. The thrust was the development of a novel photobioreactor to increase the photosynthetic efficiency of algae. An international patent for the reactor was obtained. The reactor was able to increase the photosynthetic efficiency of the algae *Spirulina* (not used in biofuels). The work did however not investigate any bioenergy aspects of algae.

The tubular photobioreactor (TPB) was developed which is capable of stimulating plant growth, especially microalgal

growth. This photobioreactor includes a reservoir for holding a liquid containing the plant material, wherein the reservoir is devoid from light, a pump in fluid communication with the reservoir and a length of transparent tube extending from the pump to the reservoir through which the liquid can be circulated. In this manner, the liquid is capable of being circulated by the pump between a dark phase, wherein the liquid passes through the dark reservoir, and a light phase, wherein the liquid is exposed to light as it passes through the tube in the illuminated environment.

Alternating light and dark cycles in a reactor is usually accomplished by providing pulses of light at a specific wavelength. This approach is limited due to the difficulty of upscaling such a system, its cost and the restricted use of artificial light in such systems. The novel tubular photobioreactor partly alleviates the above mentioned problems.





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**Nelson Mandela
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for tomorrow

Nelson Mandela Metropolitan University



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Overview of Current Algal Research



The NMMU, through its Institute for Chemical Technology, is currently running a large microalgae to energy research and development project funded by the DST (through the TIA) and Eskom. The main components of the project are:

- ▶ Microalgae cultivation;
- ▶ Direct liquefaction of microalgae biomass;
- ▶ Fresh water recovery from the microalgae cultivation platform;
- ▶ Recovery, beneficiation and agglomeration of discard coal using microalgae biomass;
- ▶ Conversion of microalgae biomass and combinations of discard coal and microalgae biomass into energy, chemicals, and liquid fuels (with a specific focus on aviation fuel and heavy fuel oil).



► **Microalgae cultivation**

The focus of research into the cultivation of microalgae at the NMMU is the large-scale cultivation of mixed algal colonies in low-cost photobioreactor (PBR) systems with the view to maximise biomass productivity per unit area. To date a 400 m² microalgae cultivation facility that houses an operational PBR (8000 L capacity) has been established and which is used for microalgae biomass production and R&D.

► **Direct liquefaction of microalgae biomass**

Unlike most research efforts that attempts to produce biodiesel from lipids isolated from microalgae, the direct liquefaction of microalgae biomass into a bio-crude oil, as method of producing chemicals and fuels has been selected. As such, fuels produced are “late generation” biofuels, also referred to as “drop-in” fuels as they are virtually identical to fuels produced from crude oil. This approach has also been selected because it allows for the recovery and recycling of nutrients required for the cultivation of microalgae biomass so as not to compete with food production (for commercial fertilizer).

Direct liquefaction is carried out in a continuous-flow reactor (in-house designed and constructed) capable of processing up to 150 kg of concentrated microalgae slurry per day. Bio-crude produced in this way have been fractionated and characterised (Sasol) and shows that approximately 68% of the total oil is a light or petrol fraction, about 8% a diesel/kerosene fraction and the remainder suitable for use as a heavy fuel oil.

A techno-economic model (Mr.B. Tait of Infinergy) for the production of bio-crude oil from microalgae biomass was developed. For bio-crude oil production the model shows the production costs of oil to be around \$80 per barrel.



► **Fresh water recovery**

Since the microalgae are cultivated in non-potable water (e.g. brack water and municipal grey water), and since 72% of all the solar energy absorbed by the microalgae during photosynthesis is released into the growth medium as heat, significant evaporation of water from the PBR is observed. Recovery of this “biologically distilled” water yields significant amounts of pure water that can be used as either a potable water source, or a de-mineralised water source for industrial application. In so doing, a substantial energy offset is achieved against the energy inputs during cultivation, harvesting, and processing.

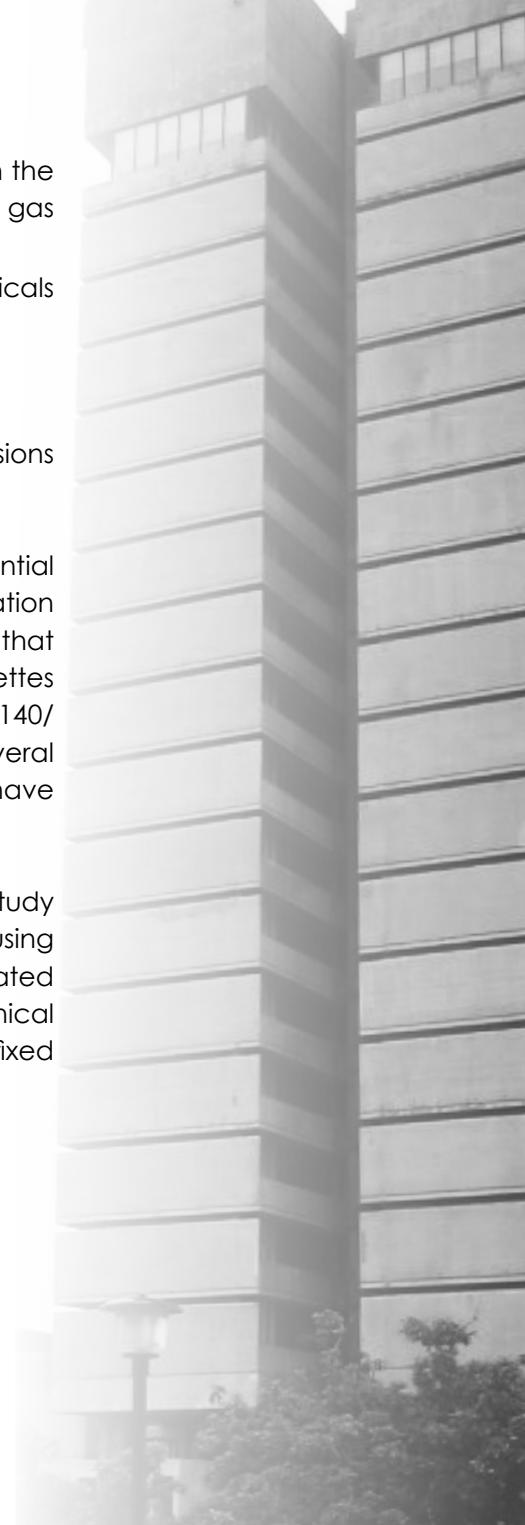
A modular ponding system has been developed for potential rural development that includes a microalgae cultivation system that yields potable water (for human, animal, or irrigation use), biomass for the generation of biogas in a bio-digester (cooking, heating, or electricity), and bio-fertiliser (for gardening or crop cultivation).

► **Recovery, beneficiation, and agglomeration of discard coal using microalgae biomass**

Research has shown that microalgae can be used as a highly effective medium for the recovery and agglomeration of discard coal. Thus, microalgae bind firmly onto fine (waste) coal and acts as a natural binder to give agglomerates (e.g. briquettes) that are both mechanical and weather resistant.

The resultant microalgae-discard coal agglomerates (commonly referred to as “coalgae”) can be used for several applications:

- Direct combustion for energy generation – in comparison to coal alone, coalgae shows slightly increase volatile content, reduced ash, and increased energy content (due to selective removal of free mineral components). Combustion studies




show no significant impact of the microalgae biomass on the combustion of coal in a furnace, but reduces greenhouse gas emissions (GHG emissions), including CO₂ and NO_x;

- Gasification for energy generation or liquid fuels/chemicals production;
- Pyrolysis for liquid fuels production;
- Direct liquefaction for chemicals/liquid fuels production;
- Clean-burning household fuel production – reduced emissions of CO₂ and polyaromatic hydrocarbons(PAH's).

A techno-economic model (Mr.B. Tait of Infinergy) for a potential microalgae cultivation-discard coal recovery and agglomeration business was developed. The results of this model shows that discard coal can be recovered and converted into briquettes containing 10% of microalgae biomass for a total cost of <R140/ton which makes such a business highly attractive. To date several hundred kilograms of briquetted coal using microalgae have been prepared for testing and development purposes.

Funding negotiations are under way for a pre-feasibility study for a 100 ha facility to produce agglomerated waste coal using microalgae (and which should produce sufficient agglomerated coal for a 1000 MW power station) so as to set up a technical demonstration facility somewhere in the RSA adjacent to a fixed point source of CO₂ (e.g. power station).



Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
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Overview of Current Algal Research

- ▶ Hydrothermal liquefaction of microalgae;
- ▶ Algal harvesting using sand filtration and solar drying;
- ▶ Cultivation and harvesting of micro-algae from the Hartbeespoort Dam.



Algal Biofuel Research

▶ Hydrothermal liquefaction of microalgae

The extraction of oil from microalgae by hydrothermal liquefaction was investigated. Studies were carried out on *M. aeruginosa*, *C. meneghinia* and *N. pusilla* at various reaction temperatures and catalyst concentrations. Maximum oil yield was produced at 300°C from *M. aeruginosa*, while a temperature of 340°C was



required for maximum oil yield from the other two strains. Oil obtained from microalgae by hydrothermal liquefaction can be utilised as a substitute for coal in simple gasification processes.

Liquefaction reaction conditions like reaction temperature, biomass loading and reaction atmosphere was studied for producing bio-oil from algae and the effect of these conditions on bio-oil properties and yield was determined. Oil yield was shown to be dependent on the reaction temperature and biomass loading when liquefaction was performed in an inert environment. A substantial increase in oil yield was found at high temperatures and biomass. Moreover, the biomass loading had no significant effect on oil yield at high temperatures when liquefaction was performed in a reducing environment. High C16 fatty acid yields were obtained at 320°C at a 3 wt% biomass loading in a CO₂ atmosphere. When compared to the original feedstock, the bio-oil had a reduced O/C ratio and good combustion properties. Hydrothermal liquefaction was therefore shown to be a feasible method for the production of bio-oil from *Scenedesmus acutus*.

► **Algal harvesting using sand filtration and solar drying**

A promising method of harvesting micro-algal biomass was investigated. Micro-algae from the Hartbeespoort Dam were harvested through a combination of sand filtration and solar drying. This method can be used to increase the energy efficiency and cost effectiveness of an integrated biomass-to-liquids (BTL) process. After bio-oils are extracted, the micro-algal biomass could be sand-filtered, sun-dried and combusted to provide heating for the liquefaction process which is very energy-intensive.

► **Cultivation and harvesting of micro-algae from the Hartbeespoort Dam**

Algal cultivation and harvesting are the most inefficient and costly processes in the production of algal biodiesel. The optimisation of these two processes could result in the successful commercialization



of algal biofuel. Cultivation studies in closed systems revealed that these systems are not feasible due to low biomass concentration achieved. Open-cultivation systems are however only feasible if the infrastructure already exists, for example in the case of the Hartbeespoort Dam.

Three harvesting methods, which makes use of the natural buoyancy of Hartbeespoort Dam algae, gravity settling and a combination of sand filtration and solar drying, were found to be feasible and could be incorporated into a new integrated BTL biodiesel process. Several renewable energy sources from the Hartbeespoort Dam system could be utilized, such as wind, hydro, solar and biomass power to reduce process cost. Wind to concentrate the algal biomass against the dam wall, the hydraulic head of the dam wall to filter and transport biomass to the new integrated BTL facility, and solar power to dry algae which could be combusted to power the extraction unit of the processing facility. Its was calculated that the new process is thermodynamically efficient, exporting 20 times more power than it imports.

Recommendations for Future Algal Energy Research

- ▶ Algal cultivation and harvesting are the most inefficient and costly processes in the production of algal biodiesel. The optimisation of these two processes could result in the successful commercialization of algal biofuel. Cultivation studies in closed systems revealed that these systems are not feasible due to low biomass concentration achieved. Open-cultivation systems are however only feasible if the infrastructure already exists, for example in the case of the Hartbeespoort Dam.





RHODES UNIVERSITY
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Overview of Current Algal Research



The main focus areas into algal research at the Institute for Environmental Biotechnology has been to investigate:

- ▶ An algae-to-energy waste water system;
- ▶ A techno-economic study of algal biofuels;
- ▶ Algal cultivation in a closed photobioreactor;
- ▶ A complete biofuels supply chain project;
- ▶ Bio-methane capture;
- ▶ Microalgae for CO₂ sequestration;
- ▶ Anaerobic digestion;
- ▶ Bio-fertilizers;
- ▶ Natural products from algae.

Algal Biofuel Research



► **Algae-to-energy waste water system**

EBRU was contracted by Peoples Power Africa (PPA) to ensure success of its pilot algae-to-energy waste water based system located at Three Crown Primary School near Queenstown.

► **Techno-economic study of algal biofuels**

A techno-economic study of algae for the use in biofuels for a large multinational company was carried out and several bottlenecks in the algae to biofuels process were identified.

► **Algal cultivation in a closed photobioreactor**

EBRU is in the process of signing an agreement with InnoVenton (NMMU) to explore possibilities in collaboration with Professor Ben Zeelie's group related to algae cultivation in a closed photobioreactor for use in liquefaction and 'green' diesel production.

► **Complete biofuels supply chain project**

Recently, EBRU has partnered with the University of Greenwich in an effort to source funding for a complete biofuels supply chain project. The major thrusts will therefore be to pursue acquisition of funds to demonstrate a complete biofuels supply chain from algae cultivation to final exhaust emission. All the component parts are in place, the IP for each is secure, agreements between the parties are signed and lodged, and a world class scientific steering committee is in place. Once funding is secured this project will move forward.

- ▶ **Bio-methane capture**
No details at this stage

Additional Algal Research



- ▶ **Microalgae for CO₂ sequestration**
An extensive evaluation of the use of microalgae for CO₂ sequestration was concluded and the potential to utilise the resultant biomass as a fuel source.
- ▶ **Anaerobic digestion**
- ▶ **Bio-fertilizers**
- ▶ **Natural products from algae**



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Overview of Current Algal Research

The main focus areas into algal research were to investigate:

- ▶ Algae cultivation ponds linked to a domestic wastewater treatment process;
- ▶ A techno-economic study of algal biofuels;
- ▶ A commercial process to beneficiate organic waste streams and algae.



Algal Biofuel Research

- ▶ **Algae cultivation ponds linked to a domestic wastewater treatment process**

Two large operational algae cultivation ponds (500 m² each) were linked to a domestic wastewater treatment process with the



capacity to generate 3.5 tonnes of algae biomass (dry weight) a year.

► **Techno-economic study of algal biofuels**

A techno-economic study of algae for the use in biofuels for a large multinational company had been carried out and several bottlenecks in the algae to biofuels process were identified.

► **Commercial process to beneficiate organic waste streams and algae**

A current project is being set up to beneficiate sewage in the form of syngas by addressing many of the issues pertaining to the algae to biofuel process. Focus areas include: harvesting, thickening, dewatering and nutrient recycling. Current engagements with the WRC, DEA's Green Fund and TIA is under way for support which will hopefully culminate in a commercial process that can beneficiate dilute organic waste streams (and algae) and recover energy, clean water and nutrients.



University of Cape Town



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Overview of Current Algal Research



Specific research focus at CeBER into microalgal research (in collaboration with Prof. V. Gray from University of Witwatersrand) include:

- ▶ Establishment of target productivity for lipid accumulation;
- ▶ Development of a metabolic assessment framework to guide maximisation of oil production;
- ▶ Isolation, screening and selection of local microalgal species;
- ▶ Effect of microalgal lipid profile on biodiesel quality;
- ▶ Bioreactor design;
- ▶ Effect of nitrogen limitation on *Chlorella vulgaris*;
- ▶ Single or two-stage cultivation processes;
- ▶ Comparison of algal growth and productivity in raceway ponds and airlift reactors;

- ▶ Investigation of methods to determine biomass and lipid analysis in microalgae.

Algal Biofuel Research



- ▶ **Establishment of target productivity for lipid accumulation**

A feasibility study was conducted to identify the necessary targets in terms of productivity for practical algal oil production for biodiesel. Target values are clearly dependent on the crude oil price which has shown substantial variation over the past year. However, the analysis clearly shows that biomass productivities in the range 1.5 to 2.5 g/l/d should be targeted together with a lipid content of 20 to 50%. Further, the benefit of optimising downstream processing for lipid recovery is noted.

- ▶ **Development of a metabolic assessment framework to guide maximisation of oil production**

Key factors to be used as comparators for strain selection were investigated. Lipid productivity is identified as the over-arching factor and is seen to correlate more strongly with biomass productivity than lipid content, based on experimental data. Further factors of importance include ease of cultivation, resistance to contamination, tolerance to a range of environmental conditions and characteristics that improve ease of harvesting and downstream processing. A set of 5 species have been selected to provide the initial grouping for a study based on reported lipid productivity extracted from the literature.

- ▶ **Isolation, screening and selection of local microalgal species**

The lipid productivity of 55 microalgal species was evaluated. Experimental comparison of traits including growth rate, lipid content, lipid productivity and harvesting potential, across 20

days of cultivation in airlift reactors, was carried out for eleven of the species, under both N sufficient (starting $[\text{NO}_3^-]$ 1500 mg.L⁻¹) and deficient (starting $[\text{NO}_3^-]$ 150 mg.L⁻¹) conditions. Considering productivity, ease of harvesting by settling, and fatty acid profile, the most promising species overall were the marine algae *Cylindrotheca* and *Nannochloropsis*, and the freshwater green algae *Scenedesmus* and *Chlorella*.

► **Effect of microalgal lipid profile on biodiesel quality**

A review of the microalgal lipid profile necessary in order to produce biodiesel that met international specifications was carried out. The fatty acid profiles of 11 species of microalgae under nitrogen replete and limited conditions were investigated. It was found that many species did not meet all the specifications, but nitrogen stress generally improved lipid profile.

► **Bioreactor design**

A simplified bioreactor system has been designed and resulted in significant growth of the model organism. This system has much potential for further design improvement that would aid in optimising the system. Upon further optimisation this system may easily be used in the industrial production of microalgae for biodiesel production.

► **Effect of nitrogen limitation on *Chlorella vulgaris***

In order to determine the optimum level of N deprivation, batch cultures of *Chlorella vulgaris* were inoculated at different starting nitrate concentrations. An optimal trade-off between biomass and lipid production was found at a starting nitrate concentration of 170 mg.L⁻¹. A strong correlation was found between the N content of the cells and the pigment and lipid contents. Intermediate levels of N deprivation were shown to enhance overall lipid productivity above that under N sufficient conditions. In addition to enhancing

overall lipid content, low N culture improves triacylglycerol content and fatty acid profile of microalgae, facilitating biodiesel production. The reduced use of N fertilizers could lead to savings in cost and energy, and lower environmental burden.

► **Single or two-stage cultivation processes**

Of the culture regimes tested, N deficient batch culture was found to give the greatest yield of lipid per culture volume. None of the two-stage strategies performed as well, although all were better than the N sufficient batch culture. Fed-batch was the second most promising strategy, but amount and timing of additional nitrate feeding was critical in enhancing biomass concentration without decreasing lipid content.

► **Comparison of algal growth and productivity in raceway ponds and airlift reactors**

The cultivation of *C. vulgaris* in a raceway pond with direct injection of CO₂ enriched air yielded a maximum average productivity of 9.2 mg.L⁻¹.h⁻¹, compared with 12.7 mg.L⁻¹.h⁻¹ in the airlift reactor. When the raceway was not sparged (a more typical operating mode), a significant drop in algal productivity to 1.4 mg.L⁻¹.h⁻¹ was estimated, due to the introduction of gas-liquid mass transfer limitations on carbon provision. In comparison, the air sparged airlift reactor gave an algal productivity of 6.4 mg.L⁻¹.h⁻¹. The increase in productivity of 4.5 fold in the airlift reactor over the raceway pond in the absence of CO₂ enrichment and of 9 fold on comparing the unsparged raceway pond to the CO₂ enriched airlift must be considered in conjunction with the increased energy requirement of the sparged system, strongly influencing both process economics and environmental burden.

► **Investigation of methods to determine biomass and lipid analysis in microalgae**

Methods of biomass and lipid analysis in microalgae were rigorously investigated, compared and evaluated. It was found that, while optical density (OD) was a convenient indirect measure of biomass concentration, in pigmented cells, such as microalgae, variation in pigment content due to culture age or growth conditions could lead to significant error in the estimation of dry weight (DW) from OD. It is recommended that a wavelength outside the range of absorbance by the pigments (e.g. 750 nm) be used, and that the generation of standard curves be carefully considered.


Direct transesterification (DT) was investigated as an alternative to lipid quantification by extraction and transesterification. DT proved a convenient and more accurate method than any of the extraction techniques for quantifying total fatty acid content in microalgae. A combination of acidic and basic transesterification catalysts was found to be more effective than each catalyst individually when the sample contained water. The two-catalyst reaction was insensitive to water up to 10% of total reaction volume. This meant that centrifuged algal samples could be assayed directly without the need for drying of the biomass. Drying of samples was shown to reduce measured lipid content. Storage of samples for up to 7 days gave results within the experimental error of the assay, while longer storage, regardless of temperature, showed slightly lower values for lipid content.

Recommendations for Future Algal Energy Research

- Traits such as ease of harvesting and the cost of oil extraction should be taken into account. However, insufficient published information currently exists to enable comparison across these aspects of a variety of species;



- ▶ Determining the resilience of species by measuring the range of environmental conditions (e.g. pH, temperature, nutrient levels, CO₂ levels, light, etc.) within which the algae remains productive, and quantifying the ease of algal cell harvesting (e.g. by flocculation, filtration or sedimentation) of promising species would be valuable additions to the literature;
- ▶ Unless N can be sourced from a waste stream, the use of N fertilizer comes with associated costs in terms of money and, from a lifecycle perspective, the energy used in its manufacture. In order to reduce both the environmental and cost burden, the use of N in the process should be minimised;
- ▶ In order to further improve yield, novel solutions to the optimal provision of light to the cultures are needed. An alternative to photoautotrophy is mixotrophic or heterotrophic growth. Here carbon and energy are supplied in the form of an organic carbon source. Work in this area has shown great potential for the enhancement of productivity, particularly during the night time hours when the organic C source replaces photosynthesis entirely. However, the benefits need to be carefully considered in terms of the additional cost and energy input associated with the C source. Some of the best sources (e.g. glucose) are expensive and energy intensive to make. Others, such as crude glycerol, could be derived from waste streams (e.g. from biodiesel manufacture);
- ▶ In order to better predict and manipulate the behavior of microalgal cells, a deeper understanding of the signaling and metabolic processes governing lipid accumulation is required;
- ▶ The major energy inputs in a microalgal process are in mixing and harvesting. From other work conducted in the research group, the energy required for constant sparging of compressed gas makes airlift reactors unfeasible from a lifecycle perspective. It is unlikely that airlift bioreactors similar to those used in this study will be the best choice for large-scale production. The energy necessary for pumping to transfer cultures between reactors or processing stages (e.g. into settling tanks) is also likely to be significant, hence this should be minimised.



Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



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Overview of Current Algal Research

The main focus areas into algal energy research at the Department of Plant Sciences have been to investigate and develop:

- ▶ Skills in microalgal cultivation and the up-scale thereof;
- ▶ Microalgae for CO₂ sequestration;
- ▶ High value products from microalgae biomass.



Algal Biofuel Research

- ▶ **Skills in microalgal cultivation and the up-scale thereof**

Developed expertise in growing microalgae in an array of photobioreactors for a number of applications.



Planned, designed, oversaw and commissioned the first commercial scale algal biotechnology production plant at Musina.

Developed a predictive model for microalgal production while doing research on microalgae at the Forschungszentrum Juelich in Germany, Czech Republic and Japan.

Microalgae to biofuels concept is limited by the capacity to produce enough biomass in the shortest possible time and on the smallest possible surface area. The present emphasis is therefore on large scale production techniques.

Additional Algal Research

▶ **Microalgae for CO₂ sequestration**

Helped and oversaw the CO₂ sequestration and bioenergy production project of XSTRATA and Eskom. Intimately involved in the Sentrachem project for using microalgae to sequester CO₂ and to produce O₂.


▶ **High value products from microalgae biomass**

Involved in SASOL project at Upington for the growth of Dunaliella and the production of beta-carotene.

Recommendations for Future Algal Energy Research

- ▶ **The main focus towards biofuels from microalgae should be on developing large scale production techniques.**





Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



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Overview of Current Algal Research



Algal Biofuel Research

Research into algal biofuel has just started and is focused on algal cultivation.





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Overview of Current Algal Research




Research focus areas into algal energy at the Department of Biochemistry include:

- ▶ Algal bioprospecting;
- ▶ Optimization of lipid yield;
- ▶ Algal harvesting and lipid extraction;
- ▶ Heterotrophic and autotrophic growth;
- ▶ Laboratory optimization;
- ▶ Carbon dioxide sequestration by algae.

Algal Biofuel Research

Currently, work is being done into the assembly of oil droplets in algal cells. This includes the effect of nutrient stress on the yield and analyses of the oil associated components. Research also focuses on the use of fluorescent dyes for detection of oil droplets and the use of the confocal microscope to differentiate fluorescent components in the algal cell.





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Overview of Current Algal Research



The main focus areas into algal energy research at the Material and Process Synthesis Research Unit include:

- ▶ Algal bioprospecting;
- ▶ Cultivation in raceway ponds;
- ▶ Conversion of algal oil to biodiesel;
- ▶ Laboratory optimisation;
- ▶ Up-scaling;
- ▶ Potential uses of algae biomass;
- ▶ Heavy metal absorption;
- ▶ Carbon dioxide sequestration.



▶ **Algal bioprospecting**

Identifying South African microalgae that absorbs the highest CO₂ concentration.

▶ **Cultivation in raceway ponds**

One-liter flasks were used to grow algae and test various growth elements that affect growth i.e. nitrate and phosphates. Raceway ponds were built and used to test the growth of algae (*Desmodesmus* sp. from Johannesburg, Zoo Lake).

▶ **Conversion of algal oil to biodiesel**

Looking at optimising and up-scaling of photobioreactors and algae ponds so as to compare the efficiency of both systems. Furthermore, to combine these system with Fisher-Tropsch process in order to produce CO₂ for the algae growth and to set up a biodiesel plant to produce biodiesel from algae oil. Pressed algae biomass may be fed back into the FT process for syngas production.

▶ **Laboratory optimisation**

- Various concentrations of CO₂ were exposed to algae;
- Liquid intensity was investigated;
- Nutrient requirements were investigated and found that nitrates and phosphates played a very important role in the growth of algae.

▶ **Up-scaling**

Photobioreactors and raceway ponds were built and tested outside. Growth in both reactors increased based on lab optimization. However, further enhancement is required on the up-scaled reactors. Open ponds were influenced by factors such as evaporation and rain.

► **Potential uses of algae biomass**

Algae have a high growth rate which makes the daily harvesting of large amounts of biomass possible. This biomass can be used in the transport industry where lipid oil is extracted from algae containing high oil content and processed into biodiesel. Furthermore, biomass can also be used to produce biogas (bio-methane) via an anaerobic digestion process to generate electricity which can be used to sustain the algae ponds. The produced methane can also be used in household stoves. The solid matter from the process may be used as a bio-fertilizer in the agriculture industry. Research intends to use South African fresh water microalgae to produce synthetic gas (syngas) via gasification. This syngas can be used in Fischer-Tropsch process to produce fuel.

Additional Algal Research

► **Heavy metal absorption**

Research is aimed at investigating the reuse of algae biomass for repeated metal biosorption/desorption cycles. Metal desorption will be determined by incubating the algae biomass with an acid under continuous stirring. The re-utilization of the biomass will be carried out by reconditioning it with a strong base followed by reloading with metals. The effect of desorption and re-utilization on alginate beads structure and metal removal efficiency will be investigated. These optimized alginate beads will further be used in a packed bed column. The effect of bed height, flow rate, initial metal ion concentration on biosorption of heavy metals and the availability of light and nutrients on the immobilized biomass will be investigated.




► **Carbon dioxide sequestration**

Carbon dioxide from off-gas can be channeled to algal ponds and absorbed by microalgae. In principle, construction of algal open ponds or photobioreactors at industrial sites such as power plants, has the advantages of CO₂ sequestration which may lead to a reduction in carbon footprints and an increase in carbon credits. For the current crop of mega-factories this is not really a viable option because of the size of the ponds required. However, once one looks at smaller modular factories this suddenly becomes a real possibility. Here a distributed system in the developing world can contribute to job creation and energy/fuel requirements.

Recommendations for Future Algal Energy Research

- **To reduce the cost of algal biofuel production, more research is needed on the optimisation and cost reduction of algal harvesting and drying processes.**





Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



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Overview of Current Algal Research



Algal Biofuel Research



► **Conversion of algal biomass to ethanol or other liquid biofuels**

This group mainly focuses on 2nd generation conversion of total plant biomass to biofuel. However, if one looks at beneficiation from algal biomass, both auxotrophic or heterotrophic, it can be utilized as any other plant biomass as a source of lignocellulose and starch (some algae has both cellulosic and starch content). These components can be enzymatically converted to sugars by engineered yeast and the sugars fermented to ethanol or other liquid biofuels. Another option includes the use of thermochemical processes.



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Overview of Current Algal Research

Algal Biofuel Research


Algal research at the Department of Process Engineering includes:

- ▶ Photobioreactor design;
- ▶ Algal cultivation in photobioreactors;
- ▶ Optimization of lipid yield;
- ▶ Lipid extraction.

Recommendations for Future Algal Energy Research

- ▶ **Finding better ways to cultivate algae at an increasing rate and in a more financial viable way, to create biomass which is the source of energy for our planet.**





Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



University of Witwatersrand



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Overview of Current Algal Research



Specific research focus at the School of Molecular and Cell Biology into microalgal research (in collaboration with Prof. Sue Harrison of the University of Cape Town) includes:

- ▶ Isolation, screening and selection of local microalgal species;
- ▶ Molecular characterization of microalgae;
- ▶ Physiology of lipid and oil production and accumulation;
- ▶ Laboratory protocol development;
- ▶ Optimising airlift photobioreactor design, configuration and operation;
- ▶ Investigating the effects of nitrogen levels on the lipid productivity, algal pigmentation and ultrastructure of *Isochrysis* sp.;
- ▶ Investigating the effect of light intensity on *Isochrysis* sp. growth rate;

- ▶ Investigating the effect of nitrate on *Isochrysis* sp. growth rate;
- ▶ Single or two-stage cultivation processes;
- ▶ Cultivation in wastewater.

Algal Biofuel Research

▶ **Isolation, screening and selection of local microalgal species**

Microalgae were isolated from samples collected from local marine sites. Bubble or aeration flasks have been constructed for the screening and selection of suitable microalgae with respect to oil accumulation capacity. Following the screening study a microalga identified as a *Platychrysis* sp. was selected because it accumulated the largest quantity of lipid.

Over a 100 marine microalgae species have been isolated from sea water samples that have been collected. Mono-cultures have been established from single cells. Four model algal (*Isochrysis* sp., *Platychrysis* sp., *Olithodiscus* sp. and *Pleurochrysis* sp.) species have been selected for the development of a photobioreactor system. Of these the *Platychrysis* species appears to be the most promising.

Successful isolation of a *Chorella* species that can grow and accumulate lipids at low pHs.

▶ **Molecular characterisation of microalgae**

Microalgae collected were identified by molecular techniques.

▶ **Physiology of lipid/oil production and accumulation**

Shake flask experiments were designed to establish the conditions and factors that promote: 1) maximum cell growth, and 2) maximum production and accumulation of lipids and oils. The shake flask experiments confirmed that non-limiting supplies of



nitrogen (N) and phosphate (P) promoted cell growth, but did not promote lipid accumulation. However, it was observed that with the depletion or removal of nitrogen and phosphate, cell division ceased, but lipid accumulation was promoted. Therefore lipid production involves a two phase process: 1) Phase one involves cell production under conditions where N and P supply are non-limiting, and 2) Phase two involves maintaining cell under conditions where N and P supply are limiting, that is, conditions that promote lipid accumulation.

► **Laboratory protocol development**

A procedure to assaying lipid content with respect to Nile Red fluorescence intensity on a flow cytometer has been investigated. A flow cytometer was also used to monitor cell density and to sort cells.

Electron microscopy (EM) and light microscopy (LM) studies have been undertaken to investigate the formation of lipid bodies and the fate of chloroplast in response to nutrient depletion.

► **Optimising airlift photobioreactor design configuration and operation**

A simple two stage tubular airlift bioreactor has been constructed. Photobioreactor experiments involved algal biomass productivities and lipid productivities in response to N and P supply. Studies on the influence of nitrate supply on cell growth rate and lipid accumulation in response to increased light intensity were undertaken under batch conditions.

► **Investigating the effects of nitrogen levels on the lipid productivity, algal pigmentation and ultrastructure of *Isochrysis* sp.**

The lipid production of three marine microalgal species were compared. *Isochrysis* was found to be the most promising. Further experiments were conducted on this species to determine the

effect of varying nitrate concentrations on lipid productivity and the effect of nitrate depletion on algal pigmentation, ultrastructure and lipid production. Upon nitrate depletion an increase in lipid yield was evident however a decrease in biomass productivity was also noticed. This implies that there is an inversely proportional relationship between these two parameters. Lipid productivity is a function of both lipid yield and biomass productivity. At all nitrate concentrations similar lipid productivity's were observed thus indicating that lipid productivity is independent of nitrate concentration. An *Isochrysis* sp. culture was grown over two weeks in f/2 media supplemented with the normal nitrate concentration in this medium. Various parameters were analysed. Upon nitrate depletion in the media it became evident that cell growth continued. This implied that the cells utilise an internal nitrate reservoir upon external nitrate depletion. Thus the internal nitrate content rather than the absolute external nitrate concentration determines the rate of growth hence the initiation of lipid synthesis in *Isochrysis* sp. Furthermore it became evident that the chlorophyll A concentration decreased during the stationary phase implying that the nitrogen in the chlorophyll molecule was utilised when all other nitrogen reserves were depleted. The primary nitrogen reserves are most probably proteins such as Rubisco. The carotenoid to chlorophyll ratio increased upon external nitrate depletion. Carotenoids are lipid in nature and an increase in carotenoid content may be a method of forming a lipid energy reservoir for when optimal conditions return.

The major ultra-structural changes observed during the stationary phase were the accumulation of lipid vesicles, the decrease in the size of the pyrenoid and the dismantling of the chloroplast. A decrease in pyrenoid size may be as a result of the immobilization of Rubisco to be used as a nitrate source. Lipid vesicles form as an energy sink for when optimal conditions return and the dismantling of the chloroplast may be as a result of chloroplast proteins

being used as a nitrate source upon nitrate depletion. Significant morphological changes and changes in pigment content were found with nitrogen stress.

► **Investigating the effect of light intensity on *Isochrysis* sp. growth rate**

A 7L airlift bioreactor was used. According to a comparative analysis of cultures grown in a 1L bubbled vessel and the 7L bioreactor system, the bioreactor was shown to be superior. This was attributed to the increased illumination used in the bioreactor batch run. From this experiment it became evident that light plays a significant role in microalgal growth rate. An experiment was thus conducted where the bioreactor was exposed to varying light intensities to determine at which intensity the maximal growth rate was achieved. It was deduced that sequential changes in the light intensity at varying phases in the life cycle aided in optimising the growth rate.

► **Investigating the effect of nitrate on *Isochrysis* sp. growth rate**

The effect of nitrate on the growth rate of the model organism was also analysed. An increase in the nitrate concentration, in the nutrient supply, led to an increase in the growth rate until the nitrate became saturating. Thereafter the growth rate remained constant. The effluent of the systems, which were fed with media containing greater concentrations of nitrate, was observed to have a longer exponential phase and yielded an increased cell concentration. This is disadvantageous in that an increase in the duration of the second phase of the bioreactor system would lead to an elevated energy cost.

► **Single or two-stage cultivation processes**

Of the culture regimes tested, N deficient batch culture was found to give the greatest yield of lipid per culture volume. None of the

two-stage strategies performed as well, although all were better than N sufficient batch culture. Fed-batch was the second most promising strategy, but amount and timing of additional nitrate feeding was critical in enhancing biomass concentration without decreasing lipid content.

Continuing research involves the design, construction and optimization of lipid production by selected freshwater and marine microalgae species.

► **Cultivation in wastewater**

It is theoretically possible to culture *Pleurochrysis* sp. using wastewater as a nitrogen source and still obtain high (or even higher) specific growth rates. *Pleurochrysis* sp. is a marine microalga and naturally occurs in waters of approximate salinities of 35ppt. Adding wastewater to seawater will reduce the salinity to a significant extent. Results show that this will have no negative effect on the growth of this organism. *Pleurochrysis* sp. cultures double their biomass approximately once per day during exponential growth. *Pleurochrysis* sp. is a lipid producing microalga (20-40% dry weight) and is therefore a suitable organism for a joint wastewater treatment-biodiesel production process.

Recommendations for Future Algal Energy Research

- Traits such as ease of harvesting and the cost of oil extraction should also be taken into account. However, insufficient published information currently exists to enable comparison across these aspects of a variety of species;
- Determining the resilience of species by measuring the range of environmental conditions (e.g. pH, temperature, nutrient levels, CO₂ levels, light, etc.) within which the algae remains



productive, and quantifying the ease of algal cell harvesting (e.g. by flocculation, filtration or sedimentation) of promising species would be valuable additions to the literature;

- ▶ Unless N can be sourced from a waste stream, the use of N fertilizer comes with associated costs in terms of money and, from a lifecycle perspective, the energy used in its manufacture. In order to reduce both the environmental and cost burden, the use of N in the process should be minimised;
- ▶ In order to further improve yield, novel solutions to the optimal provision of light to the cultures are needed. An alternative to photoautotrophy is mixotrophic or heterotrophic growth. Here carbon and energy are supplied in the form of an organic carbon source. Work in this area has shown great potential for the enhancement of productivity, particularly during the night time hours when the organic C source replaces photosynthesis entirely. However, the benefits need to be carefully considered in terms of the additional cost and energy input associated with the C source. Some of the best sources (e.g. glucose) are expensive and energy intensive to make. Others, such as crude glycerol, could be derived from waste streams (e.g. from biodiesel manufacture);
- ▶ In order to better predict and manipulate the behavior of microalgal cells, a deeper understanding of the signaling and metabolic processes governing lipid accumulation is required;
- ▶ The major energy inputs in a microalgal process are in mixing and harvesting. From other work conducted in the research group, the energy required for constant sparging of compressed gas makes airlift reactors unfeasible from a lifecycle perspective. It is unlikely that airlift bioreactors similar to those used in this study will be the best choice for large-scale production. The energy necessary for pumping to transfer cultures between reactors or processing stages (e.g. into settling tanks) is also likely to be significant, hence this should be minimised.



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Overview of Current Algal Research

All work undertaken into algal research at the Department of Animal, Plant and Environmental Science is in collaboration with Prof. V. Gray (School of Molecular and Cell Biology, University of the Witwatersrand) and includes:

- ▶ Optimization of lipid yield;
- ▶ Cultivation in photobioreactors;
- ▶ Algal harvesting;
- ▶ Identification and characterisation of lipids produced by Haptophytes;
- ▶ Biodiesel characterisation;
- ▶ Laboratory optimisation;
- ▶ Bioprospecting and systematics of marine microalgae;
- ▶ Investigating the function of algal cells and their constituents.





► **Optimisation of lipid yield**

Two research projects focus on optimising lipid production in batch culture; one working on a marine organism (*Isochrysis*) and the other on a freshwater organism tolerant to low pH (a novel species of *Scenedesmus*). Both are also grown under bioreactor conditions, using growth criteria determined by batch culture and manipulating various parameters to optimise yield.

► **Algal Harvesting**

One of the model organisms studied shows promise for harvesting (dewatering) because it autoflocculates at certain cell densities. This is compared with flocculation using more routine approaches.

► **Identification and characterisation of lipids produced by Haptophytes**

A third project is not restricted to a single model organism but rather attempts to interrogate lipid production across a promising class of lipid producers, the Haptophyta. A review has shown that the production of lipids alone is only part of the solution to the successful production of biofuel, only certain lipids and in the correct combinations are required. The focus here, then, is to identify the lipids that are made by different representatives across the phylogenetic tree of haptophytes (i.e. representatives of all major lineages) under set conditions and at various stages of the growth curve in batch culture. This work requires clear identification of all representatives used and GC-MS characterisation of the lipids extracted at various stages.

► **Bioprospecting and systematics of marine microalgae**

Inroads have been made in documenting the biodiversity of inshore microalgal flagellates of South African inshore waters, but this is

still in its infancy. Such work requires considerable patience and skill, but remains highly relevant for future biotechnologies, such as identifying potential biofeed organisms (bioprospecting). Work in this laboratory has historically concentrated on (often novel) representatives of a few groups, particularly the haptophytes, dinophytes and prasinophytes (the latter are no longer considered a valid group). Such work requires careful establishment of cultures and exacting light and electron microscopy, complemented by phylogenetic analyses of selected gene sequences.

Additional Algal Research


► Investigating the function of algal cells and their constituents

The functioning of cells, or components thereof, demand more in-depth investigation of organisms of interest. For example, the presence of selected proteins, their locality in the cell, has been followed by immunofluorescence and immune-electron microscopy. In addition, ultrastructural studies complement other investigations (e.g. the fate of potential N-storage bodies during lipid production) and these have formed the focus of this laboratory's contributions to its collaborative work on unraveling programmed death in unicellular algae.

Recommendations for Future Algal Energy Research

- There is too much focus at the coal face too early on, chasing after an instant solution with existing cultures which often prove suboptimal. It would be beneficial if at least some emphasis was placed on fundamental research, such as following cellular responses to stresses known to induce lipid production or understanding how these two processes interface;
- There exists potential for genetically modifying algae.



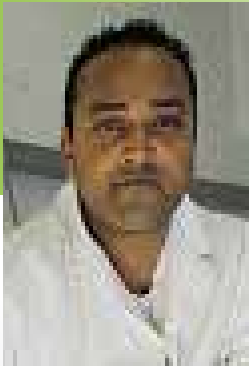


Stakeholders in the SWH industry prepared the South African Solar Thermal Technology Roadmap in 2015 and outlined the goal to have $\frac{1}{2}$ m² of net solar thermal collector area installed for every member of the population by 2030 in South Africa. This relates to an installed capacity of 21 GWh and annual electricity savings of 34 000 GWh, avoiding 23 Million tons of CO₂ every year.



CSIR
our future through science

Council for Scientific and Industrial Research



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Overview of Current Algal Research



The main focus areas into algal energy research at the CSIR has been:

- ▶ Algal bioprospecting;
- ▶ Optimising algal lipid yield;
- ▶ Raceway pond cultivation;
- ▶ Lipid extraction;
- ▶ Conversion of algal oil to biodiesel;
- ▶ Lab optimisation and upscaling;
- ▶ Microalgae for CO₂ sequestration;
- ▶ Obtaining high value products from microalgae biomass.

Algal Biofuel Research



▶ Algal bioprospecting

The CSIR and DUT have completed sampling all provinces of SA and currently hold isolates totaling ~800 between CSIR and DUT.

▶ Optimisation of lipid yield

Work regarding lipid yield optimisation has been focused around nutrient manipulation and carbon feed. Laboratory work has been completed and pilot scale studies are being planned.

▶ Cultivation in raceway ponds

All research to date have been conducted and optimised for raceway pond configuration in laboratory scale model systems.

▶ Lipid extraction

Limited research has been conducted on lipid extraction and further work is currently on-going.

▶ Conversion of algal oil to biodiesel

Work on the conversion of oils to biodiesel has been conducted using the novel micro-reactor approach; this work is currently on-going.

▶ Lab optimisation

Majority of the research focus is around the laboratory optimisation of algal based technologies and are conducted in laboratory raceway systems.

▶ Up scaling

The scale up of technologies are commencing during the early part of 2013.



Additional Algal Research



► Carbon sequestration

Laboratory raceway model studies were conducted using CO₂ gas as a carbon substrate, the research has yielded positive results and plans are in place to scale up this technology.

► High value products

The CSIR has developed a technology for the production of beta-carotene from microalgae that has been commercialised. Their current research focus is around omega three fatty acids as well as other high value products. Isolated microalgae libraries from collaboration with DUT are currently being screened for novel compounds including anti HIV compounds.

Recommendations for Future Algal Energy Research



- Integrated approach to algal products. A method of producing multiple products from a single algal process, that delivers biodiesel and high value products. This approach is expected to ultimately increase the financial feasibility of the process.

Macroalgal Energy Researchers





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Overview of Current Algal Research



Algal research at the Institute of Microbial Biotechnology and Metagenomics includes:

- ▶ Algal bioprospecting;
- ▶ Cultivation in raceway ponds;
- ▶ Cultivation in photobioreactors;
- ▶ Cultivation in effluents;
- ▶ Production of ethanol or other liquid fuels;
- ▶ Bio-hydrogen and methane production;
- ▶ High value algal products such as protein-rich animal feed and pharma compounds;
- ▶ Laboratory optimisation;

- ▶ Algae genomics;
- ▶ Phylogenetic and functional analyses of microbial associations;
- ▶ Bioprospecting for pharma compounds.

Algal Biofuel Research



▶ Cultivation of seaweed (macroalgae)

Exploitation of seaweed as a feedstock for industrial products relies on the ability to cultivate seaweed, as sustainable harvesting is not an ideal option for commercial application. Work is aimed at the establishment of a seaweed cultivation platform for seaweed species of commercial interest. Pilot scale cultivation studies are conducted at the Sea Point Research Aquarium and the Two Oceans Aquarium (Cape Town). Key collaborators include Prof. John Bolton from University of Cape Town (John.Bolton@uct.ac.za) and Dr. Jean-Paul Schwitzguebel (jean-paul.schwitzguebel@epfl.ch) from EPFL, Lausanne, Switzerland. The role of environmental conditions and bacterial populations on spore germination, growth and development are studied. Biochemical interactions between marine algae and associated microbes, and the microbiome's role in pathogen-induced defence, are furthermore investigated. The cultivation programme has made major progress and is planning on extending cultivation practices of macroalgae to bioreactors in 2013 (www.algasolrenewables.com/en/). UWC has had a long association with large scale macroalgal culture in land based seaweed paddle ponds. Commercial, large scale production of macroalgae was initiated in the Southern Cape in 2006, in the Eastern Cape in 2001 and in the Western Cape in 2011. Such studies have provided long term commercial data sets of large scale macroalgal cultivation in a variety of environmental conditions (Nobre, A.M., Robertson-Andersson, D., Neori, A. and Sankar, K. 2010. Ecological-economic assessment of aquaculture

options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. *Aquaculture*, 306: 116-126).

► **Seaweeds for bioremediation, desalination and production of methane**

Since 2009, UWC has been actively involved in research geared towards using seaweeds for bioremediation of aquaculture waste, desalination and production of methane from seaweed biomass. Large scale seaweed cultivation have been shown financially viable when grown as a feed source for abalone, and preliminary results show that methane production from seaweeds may also be commercially viable.

Research programmes initiated in 2013 are focused on:

► **Technologies for methane/hydrogen production from macroalgae biomass**

The technical-economical feasibility and ecological viability of a new process for the sustainable production of synthetic natural gas, via hydrothermal processing of biomass, are under study by Swiss partners (EPFL, Paul Scherer Institute). The hydrothermal process under development is expected to allow an energy efficient conversion of algal biomass to a methane-rich gas. To be economically and ecologically sustainable, the process must be integrated in a biorefinery concept, allowing the recovery of high-value chemicals before the production of biogas or syngas.

Under the umbrella of a community project called "Sustainable Energy for Community Services Initiative" (SECSI), bio digesters will be introduced for the generation of fuel gas for heating and cooking purposes at the University of the Western Cape. Prof. Bruno Pollet (bgpollet@hysasystems.org) and Prof. Bernard Bladergroen

(bbladergroen@uwc.ac.za) will concentrate on CH₄ and H₂ production, respectively, using dark fermentation processes originally. Pollet and Bladergroen are working at the South African Institute for Advanced Materials Chemistry.

► **Conversion of macroalgae biomass into liquid biofuels**

Efficient conversion of unusual sugars, present in marine algae, to biofuels such as ethanol are required. With the use of synthetic substrates and new approaches in synthetic biology, carbohydrate degradation enzymes with novel catalytic activities are mined from marine environments using a high-throughput gene discovery platform incorporating metagenomics and next-generation sequencing.

► **Industrial scale cultivation of algae in bioreactors**

An industry partner Algasol Renewables (www.algasol.com) is a technology company with a unique patented PhotoBioReactor (PBR) for low-cost cultivation of micro-algae for biofuels and fine chemicals. The technology is a flexible (polymer film) PBR floating on water that can be deployed both on land, in ponds, or in the ocean. A worldwide patent covers all key aspects of the PBR technology, and Frost & Sullivan awarded Algasol Renewables the prestigious Global Algae Biofuels Award. Algasol Renewables' modular floating bag technology, a new variation of PBRs, provides a low-cost design coupled with industrial scalability, optimal light exposure, high biomass concentration, low energy consumption, and efficient system control. Algasol Renewables (Miguel Verhein, info@algasolrenewables.com) has committed to modifying the patented floating PBR to accomplish efficient growth and harvesting of macro-algae.



► **A meta-phylogenomic approach to study functional diversity of bacterial communities associated with macro-algae**

Marine macro-algae (seaweeds) are receiving increasing attention as food additives and are attractive renewable feedstocks for producing fuels, chemicals and pharmaceuticals. Both the exploitation of seaweed as cash crops and controlling their prolific growth require a better understanding into the biological processes governing growth and health of algae. Bacterial symbionts are an apparently obligatory requirement that underpins the growth, morphogenesis and lifecycles of algae. At present, this requirement is not understood and the principles underlying the assembly and structure of complex microbial communities are an issue of great concern. Metagenomics has revolutionized biotechnology by paving the way for a cultivation-independent assessment of microbial communities present in complex ecosystems. Bioinformatic analyses further opened the window into the taxonomic and functional diversity of microbial communities. This project uses novel approaches to assess the diversity of microbial symbionts and link these to processes governing algal growth.

► **Bioactive Marine Products**

Metagenomic technologies are exploited to bioprospect for pharmacologically active compounds and antimicrobials from marine algae and associated microbial communities. Mechanisms of action of purified molecules are studied on a molecular level with the aid of nanoparticles, an emerging field that integrates molecular biology and knowledge in chemistry with high resolution imaging. This project is managed in close collaboration with Prof. Kattesh Katti (KattiK@health.missouri.edu) and Prof. Carolyn Henry (henryc@missouri.edu) from University of Missouri.

► **Seaweed as a protein-rich animal feed**

Processed algae biomass is rich in proteins and carbohydrates, making it an ideal feed for livestock. The gut microbiome plays a critical role not only in animal health and productivity but also in meat quality. Gut samples from individual cattle fed on processed algae will be characterized and the gut microbiome's role in beef quality investigated in collaboration with Prof. Louw Hoffman (lch@sun.ac.za).

Recommendations for Future Algal Energy Research

- **Cultivation:** Application of seaweed biofuels relies on expansion of farming area or increased intensity of current farming practices. We need to attain a balance between biofuel production activities and environmental costs. Potential impact of large-scale seaweed cultivation on marine ecosystems and coastal environments need to be assessed.
- **Liquid biofuels:** While depolymerization of seaweed polysaccharides is relatively easy, novel strategies (e.g. based on metabolic engineering in a systems biology approach) for efficient conversion of unusual sugars to biofuels such as ethanol are required.
- **Biogas:** Technical challenges include lowering the cost of the entire supply chain: harvesting, transporting, hydrogen production/conversion, delivery, storage, and end-use applications.



Table 1

Common SWH Research Themes at South African Research Institutions

	CPUT		CUT	MUT	TUT	
	Mr. Kanyarusoke	Mr. Kallis	Mr. Aggenbacht	Prof. Malinga	Prof. Dintchev	
SWH Application						
Domestic	●	●		●	●	
Commercial and Industrial		●		●	●	
Agriculture/Horticulture	●		●			
Swimming pools						
SWH Systems						
Passive direct systems						
• Integrated collector storage	●	●		●	●	
• Convection heat storage unit	●				●	
Passive indirect systems						
• Indirect thermosyphon	●				●	
Active indirect systems						
• Pressurized antifreeze or pressurized glycol					●	
• Drainback				●	●	
Active direct systems						
• Draindown			●		●	
• Flooded open-loop					●	
• Recirculation					●	

	NMMU	NWU	UCT	UJ	UP	US	US	UniVen	WITS		
	Mr. Kleyn	Mr. van Niekerk	Ms. De Groot	Mr. Bhamjee	Prof. Meyer	Prof. Xia	Mr. Gauché	Mr. Joubert	Prof. Sankaran	Dr. Ferrer	Prof. Cronje
	•	•	•		•	•	•	•	•	•	•
		•			•	•	•	•		•	•
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	•										
	•										

Table 1

Continued...

	CPUT	CUT	MUT	TUT
Mr. Kanyarusoke				
Mr. Kallis				
Mr. Aggenbacht				
Prof. Malinga				
Prof. Dintchev				

SWH System Components

Integrated collector storage systems

• Collector Type						
↳ Tank type					•	
↳ Tube type	•			•		
↳ Compound parabolic		•				
• Thermal performance improvements						
↳ Vessel design		•		•	•	
↳ Glazing systems	•				•	
↳ Selective coatings		•			•	
↳ Methods of insulation				•	•	
↳ Reflector configurations		•				
↳ Phase change materials				•		
↳ Optimising design parameters	•	•			•	
↳ Extending heat transfer area				•		

Convection heat storage unit systems

• Collector Type						
↳ Flat plate (Harp)	•				•	
↳ Flat plate (Serpentine)	•					
↳ Evacuated tube (Direct-flow)	•					
↳ Fully flooded						
• Thermal performance improvements						
↳ Storage tank design	•				•	

Table 1

Continued...

	CPUT	CUT	MUT	TUT	
	Mr. Kanyarusoke	Mr. Kallis	Mr. Aggenbacht	Prof. Malinga	Prof. Dintchev
SWH System Components					
↳ Collectors					
- Cover material					●
- Glazing systems					●
- Absorber plates	●				
- Selective coatings					●
↳ Optimising design parameters					●
↳ Methods of insulation					●
↳ Extending heat transfer area					●
Indirect thermosyphon systems					
• Collector Type					
↳ Flat plate (Harp)	●				●
↳ Flat plate (Serpentine)	●				
↳ Flat plate with heat pipe	●				●
↳ Evacuated tube (Direct-flow)					●
↳ Tube-in-sheet	●				
↳ V-trough	●				
• Heat Exchanger Configurations and Types					
↳ Internal					●
↳ External					
↳ Wraparound					
↳ Plate-type	●				
↳ Coiled tube type					
↳ Tube-in-shell type					

Summary

Prominent algal biofuel research in South Africa can be broadly summarised as follow:

- ▶ Bioprospecting for indigenous algae from a wide range of marine and freshwater systems across the country;
- ▶ Microalgal selection based on high biomass and lipid productivity;
- ▶ Algal selection based on its ability to proliferate in municipal and industrial effluents;
- ▶ Cultivation of algae in raceway ponds and several photobioreactors;
- ▶ Optimisation of photobioreactor design in order to improve algal growth and reduce reactor cost;
- ▶ Investigation of optimal cultivation and harvesting methods for algae;
- ▶ Up-scaling of algal cultivation;
- ▶ Cultivation of algae in different municipal and industrial effluents;
- ▶ Comparison of single- and two-stage cultivation systems;
- ▶ Optimisation of microalgal growth and lipid production by investigating the effect of nutrient supply, optimal illumination and different algal growth modes (heterotrophic and photoautotrophic);
- ▶ Investigation of optimal microalgal lipid extraction methods;
- ▶ Conversion of microalgal oil to biodiesel and the characterization thereof;
- ▶ Determining the effect of microalgal lipid profile on biodiesel quality;
- ▶ Investigation of microalgal ultra-structural properties in order to better understand lipid production;
- ▶ Investigation of the function of algal cells and their constituents;
- ▶ Direct liquefaction of algal biomass to produce bio-crude oil;
- ▶ Preparation of techno-economic studies to determine the feasibility of microalgal biodiesel production;
- ▶ Preparation of a techno-economic feasibility and ecological viability study for the conversion of macroalgal biomass to methane-rich gas;
- ▶ Investigation of pre-existing infrastructures for biodiesel production, e.g. Hartbeespoort Dam;

- ▶ Demonstration of a complete biofuel supply chain from microalgal cultivation to final exhaust emissions;
- ▶ Utilisation of algal biomass for the production of ethanol or other fuels;
- ▶ Utilisation of CO₂ from FT process for microalgal growth and in turn the use of algal biomass to fuel the FT process;
- ▶ Up-scaling of algal biofuel production systems.

Recommendations for Future Algal Energy Research

Algae present multiple possibilities for fuel end-products such as biodiesel, ethanol, methane, jet fuel, biocrude and more via a wide range of process routes. Each of these process routes presents its own set of opportunities, parameters, dynamics and challenges. Producing biodiesel from algae provides the highest net energy as converting oil into biodiesel is a much less energy-intensive method than its conversion to other fuels. Large scale production from algae is presently only feasible for high value products.

The high cost of producing fuel from algae is mainly due to costly and inefficient cultivation and harvesting methods.

Currently, there are three primary methods (with variations and combinations of these) used for algae cultivation: open and closed pond systems and photobioreactors. Cultivation studies in closed systems revealed that these systems are not feasible due to low biomass concentrations achieved. Furthermore, the energy required for constant sparging of compressed gas makes airlift reactors unfeasible from a lifecycle perspective. It is unlikely that airlift bioreactors will be the best choice for large-scale production. The energy necessary for pumping to transfer cultures between reactors or processing stages (e.g. into settling tanks) is also likely to be significant, hence this should be minimised.

Open-cultivation systems are proposed to only be feasible if the infrastructure already exists, for example in the case of the Hartbeespoort Dam. Furthermore cultivation costs (in any type of reactor or pond) are increased due to nutritional requirements of algae. Unless nitrogen can be sourced from a waste stream, the use of nitrogen fertilizer comes with associated costs in terms of money and, from a lifecycle perspective, the energy used in its manufacture. In order to reduce both the environmental and cost burden, the use of nitrogen in the process should be minimised. Also, due to the scarcity of freshwater in South Africa, it would be beneficial if algae could be cultivated in alternative water sources for e.g. municipal and industrial effluents. Here it is possible to achieve twin benefits, which could result in cost-effective treatment of wastewater and secondly, owing to the readily available nutrients in the medium, it also reduces costs of algal biomass production. Another approach that can ultimately increase the financial

feasibility of algal biofuel would be to look at an integrated approach to algal products. A method of producing multiple products from a single algal process, that delivers biofuel and high value products.

Biofuel production costs can be reduced by improving algal biomass and lipid yield. In order to improve yield, novel solutions to optimal provision of light to the cultures are needed. Moreover, alternative algal growth modes like mixotrophic or heterotrophic growth instead of photoautotrophy have shown promise. Here carbon and energy are supplied in the form of an organic carbon source. Work in this area has shown great potential for the enhancement of productivity, particularly during the night time hours when the organic carbon source replaces photosynthesis entirely. However, the benefits need to be carefully considered in terms of the additional cost and energy input associated with the carbon source. Some of the best sources (e.g. glucose) are expensive and energy intensive to make. Alternatively, crude glycerol could be derived from waste streams (e.g. from biodiesel manufacture).

Selection of the optimal algal strain is a key component of a successful algal biofuel venture. Traits such as ease of harvesting and the cost of oil extraction should also be taken into account when selecting algal species for biofuel production. Insufficient published information currently exists to enable comparison across these aspects of a variety of species. Determining the resilience of species by measuring the range of environmental conditions (e.g. pH, temperature, nutrient levels, CO₂ levels, light, etc.) within which the algae remains productive, and quantifying the ease of algal cell harvesting (e.g. by flocculation, filtration or sedimentation) of promising species would be valuable additions to the literature.

There is too much focus at the coal face too early on, chasing after an instant solution with existing cultures which often prove suboptimal. It would be beneficial if at least some emphasis was placed on fundamental research, such as following cellular responses to stresses known to induce lipid production or understanding how these two processes interface. This will enable researchers to better predict and manipulate the behavior of

microalgal cells, understanding the signaling and metabolic processes governing lipid accumulation. Possibilities into genetically modifying algae also need to be explored.

In order to advance towards commercial production of algal biofuel, some focus should be on developing large scale production techniques. Moreover, life cycle analysis of large scale algae cultivation with regards to energy and economics is also required.

There also exists potential for biofuel and biogas production from macroalgae (seaweed). However, the application of seaweed biofuels relies on expansion of farming area or increased intensity of current farming practices. A balance between biofuel production activities and environmental costs needs to be attained. Potential impact of large-scale seaweed cultivation on marine ecosystems and coastal environments need to be assessed.

While depolymerization of seaweed polysaccharides is relatively easy, novel strategies (e.g. based on metabolic engineering in a systems biology approach) for efficient conversion of unusual sugars to biofuels such as ethanol are required.

A technical challenge into biogas production from macroalgae include lowering the cost of the entire supply chain: harvesting, transporting, hydrogen production/conversion, delivery, storage, and end-use applications.

