Indigenous algae: Potential factories for biodiesel production

DM MAHARAJH*, R LALLOO
*CSIR Biosciences, Private Bag X2, Modderfontein, 1645
Email: dmaharajh@csir.co.za

1. Abstract

The environmental effects of burning fossil fuels and the increased crude oil prices have triggered increased interest in biofuels. Biodiesel is traditionally produced from oil seed crops, which have lower yields per land area and threaten food security when compared to algae which have high oil yields (~ 90 times more oil per area of land in comparison to the best oil seed crop) and do not require arable land for cultivation. CSIR Biosciences has commenced research in this area with the aim of implementing an algal biodiesel production technology. Lipid producing algal isolates from the United States Department of Energy - Aquatic Species Program (ASP) were obtained to benchmark technology performance. The best strains obtainable were assessed in laboratory studies, the results of which formed the basis of a techno-economic evaluation to identify key variables and parameters influencing the implementation of algal biodiesel production. The model demonstrated a feasible business case (aerial lipid productivity of 26 g/m^2/day) at a basic fuel selling price of ~ R8.98, with an IRR and NPV of ~40% and ~R1.3 bn respectively. The model scaled (529 ha) a facility to supply 10% of the mandatory biodiesel inclusion as stipulated by the Department of Minerals and Energy's biofuels strategy. The model is being applied to drive key research decisions to ensure optimal deployment of resources towards a commercially relevant outcome. The model highlighted the performance of algal strains with high lipid yield and growth rate (aerial productivity) as a key parameter. This led to the commencement of a screening programme to isolate indigenous algal strains capable of high levels of lipid production that would also be suitable to exploit indigenous climatic advantages. Approximately 30% of South African environments favourable for isolating algae have been sampled. Samples were enriched, purified and assessed for lipid content, resulting in a database of indigenous algae. Positive isolates were grown under laboratory conditions to assess growth rates, lipid productivity and yield against *Cyclotella cryptica,* which was the best strain available from the ASP. The sampling to date has yielded 161 algal isolates of which 52 show lipid production. The first positive isolate was presumptively characterised as *Characium spp.* Initial comparisons showed that *Characium spp.* achieved ~70% of the lipid concentration of *C. cryptica*, albeit in a non-production recipe. The current research indicates strong potential for development and implementation of biodiesel production using indigenous algal isolates and has attracted significant interest from government and commercial partners.

2. Introduction

Mankind's continuous use of fossil fuels has caused immense damage to the environment. Fossil fuels are excess carbon that has been removed from the carbon cycle and sequestered within the earth's crust. This was nature's way of protecting itself, however the continuous removal of this sequestered carbon from the earth's crust causes a net increase in carbon in the atmosphere causing various global climatic events such as global warming (Sawayama *et al*. 1995). Fossil fuels have a limited supply and the known resources are reaching rapid exhaustion, sending crude oil prices to record highs. These factors have initiated the global search for renewable, cleaner biofuels (Chisty 1980-81).

Biodiesels are fatty acid methyl esters that are derived from triglycerides by the transesterification process. Biodiesel as an alternative fuel source is increasingly becoming more important because of the diminishing petroleum reserves and environmental problems caused by exhaust gases from fossil fuelled engines. Vegetable oils (sunflower, soya etc.), animal fats and used oils can be processed to yield biodiesel.

Microalgae are responsible for at least 50% of the photosynthetic biomass production on earth (Chisty 2007) and are effective factories for various valuable products such as vitamins, supplements, feeds and biofuels. (Becker 1994; Spolaore *et al.* 2006; Walter *et al*. 2005). Paleobotanical evidence has suggested that microalgae are responsible for major sources of hydrocarbon in a variety of oil-rich deposits dating from the Ordovician period to the present (Moldowan and Seifert 1980; Borrego *et al*.1996). This evidence prompted the onset of research on the use of microalgae as a feedstock for biodiesel production which commenced decades ago. In early stages of microalgal research, focus was on determining the characteristics of microalgae and

their functions in natural waters. The key objective of this work was to use microalgae for waste water treatment. Considerable knowledge on microalgae was gathered during this research phase and can now be applied in promising technologies such as alleviation of pollution problems caused by CO_2 emissions, breakdown of waste products and production of essential products such as proteins, vitamins and biodiesel fuels (Ben-Amotz *et al*. 1983; Becker 1994; Walter *et al*. 2005; Spolaore *et al.* 2006).
Microalgae are categorised in a variety of classes, which are primarily distinguished by their pigmentation, life cycle and basic cellular structure. The four most important classes in terms of abundance are (Borowitzka. 1996):

- Diatoms (Bacillariophyciae), which dominate the phytoplankton of the ocean, but are also found in fresh and blackish water and store carbon in the form of natural oils or as a polymer of carbohydrate.
- Green algae (Chlorophyceae) are also abundant especially in fresh water and commonly found growing in swimming pools. Green algae store their energy in the form of starch, but oils can be formed under certain growth conditions.
- Blue-green algae (Cynophyceae) are very much closer to bacteria and they play an important role in fixing nitrogen from the atmosphere.
- Golden algae (Chrysophyceae) appear yellow, brown or orange in color and they produce natural oils and carbohydrates as storage compounds.

Microalgae require sunlight, water and carbon dioxide to grow. Under optimal conditions, algal cultures can double in population size between two and three times per day. Lipids and fatty acids form a major part of an algal cell, as membrane components, metabolites and storage products (Princen 1982).

Solar energy is renewable compared to energy from fossil and nuclear origin. Solar energy can be beneficially exploited by the application of photosynthetic processes to produce biomass that can be processed to produce fuels and other products through appropriate conversions. The utilisation of solar energy for microalgae growth is attractive for the following reasons:

a) The costs associated with harvesting and transportation of micro algae are relatively low when compared to those associated with biomass from trees and plants (Sheehan *et al*., 1998).
b) Micro algae can be easily processed due to their relatively small sizes (Sheehan *et al*., 1998).
c) Micro algae utilise non arable land and hence do not compete with food crops.
d) Micro algae are capable of utilising CO_2 from harmful flue gasses, thus reducing atmospheric CO_2 levels and global warning (Beneman, 1993).
e) Micro algae are more effective converters of solar energy to organic energy (Borowitzka, 1996).
f) Micro algae have a higher oil yield per unit area than oil crops (Sheehan *et al*., 1998, Chisti 2007).
g) Micro-algae can be grown in effluents resulting in bio-remediation.

Open culture system can be divided into natural waters (lakes, lagoons or ponds) and artificial ponds or containers erected in different ways. Regarding the technical complexity, open systems such as raceway ponds may vary considerably, but they are still much simpler than more recent closed systems for cultivation of microalgae (Borowitzka, 1993). The most common technical design of open pond systems are the raceway cultivators driven by paddle wheels and usually operating at a depth of 15 to 20 cm. Circular ponds that are similar in design are common in Asia and Ukraine (Becker 1994). According to the NREL report, Japanese, French and German governments have invested in R&D of closed bioreactors for micro algae biomass production. This investment was initiated after observing that the algal strains that are promising in the laboratory do not perform well when grown in open pond system.

Materials and methods

Techno-economic assessment

Techno-economic analysis was conducted on literature based values on algal oil production. Sheehan et al 1998 explained that the range of aerial lipid productivity was between 10 and 50 g/m^2/day. We therefore modelled lipid productivities from 10 to 50 g/m^2/day. A conceptual process flow sheet was developed. A detailed analysis of equipment and running cost was conducted and realistic costs obtained through vendor quotations. This information was then used to develop a 10 year cash flow projection model.

University of Hawaii strain evaluations

Three algal strains isolated during the Aquatic Species Program (ASP) were obtained from the University of Hawaii (UH) and tested at CSIR laboratories. These strains were; *Cyclotella criptica, Isochrysis galbana* and *Phaeodactylum tricornutum*. Algae were grown in 50% ASW

media (Sheehan *et al.* 1998) in 250 ml Erlenmeyer flasks with continuous lighting. The first positive isolate (AF6) from the CSIR screening programme was also evaluated. Samples were removed regularly and analysed for optical density at 660 nm (Genesys 20, Spectronic, NY, USA), microscopic cell counts using a haemocytometer and lipid concentration. Lipid analysis was conducted semi- quantitatively as described by Lee *et al.* (1998), using a Perkin Elmer LS55 Florescent spectrophotometer (Perkin Elmer, London, UK).

Screening and isolation protocol

A protocol for the screening and isolation of lipid producing algae was developed from a compilation of literature (Andersen and Kawachi 2005; Day and Brand 2005; Elsey et 2006; Gerwick et al. 1994; Harrison and Berges 2005; MacIntyre and Cullen 2005; Sheehan et al. 1998; Wood and Wingard 2005). Figure 1 illustrates the protocol used.

Laboratory scale raceway evaluation

The first positive isolate (AF6) obtained from the CSIR screening programme was cultured in four 2000 ml Erlenmeyer flasks under constant light until sufficient culture density (>1.0 AU at 660nm) was obtained to form a 10% inoculum for 100l laboratory raceway ponds under continuous light. Artificial fresh water (AF6) (Sheehan *et al.* 1998) was used as growth media supplemented with 0.1% $m.v^{-1}$ $CaCO_3$. The raceway pond operating volume was maintained at ~ 100L by compensation for evaporation. Nitrate and phosphate concentrations were maintained at 10 $mg.l^{-1}$ by adjusting with a concentrated solution of AF6 media (10X). Dry cell weight measurements were conducted according to Hellwig *et al*. 2001. Quantitative fat analysis was conducted on samples at steady operation (day 76 and 134) and analysed quantitatively for total fat content by Soxhlet extraction (Buchi Extraction System B-811, Flawil, Switzerland) and analysis by gas chromotography using methodology as specified by manufacturer. Approxamately four litres of sample were harvested from the raceway pond. Samples were centrifuged at 10 000 RPM for 10 min and washed with de-ionised water prior to drying at 105^{o}C for 24 hours. Dry samples were stored in amber glass samples bottles under nitrogen until analysed. Results were obtained in percentage dry weight.

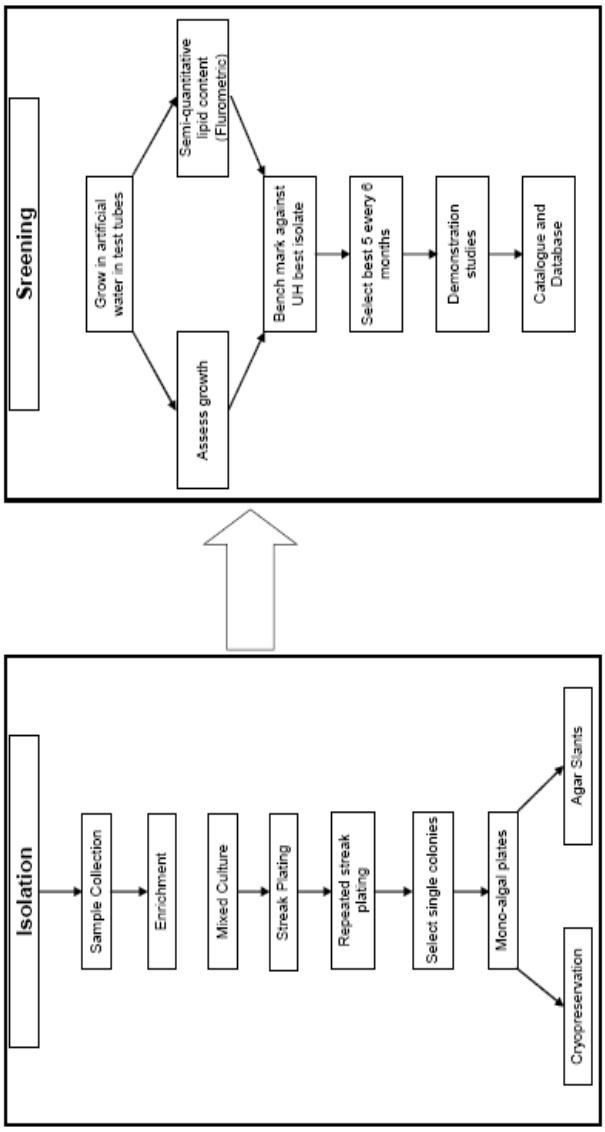

Figure 1 Schematic of screening and isolation protocol

Results and discussion

Techno-economics

Key assumptions (Table 1) were made prior to the development of the techno-economic model. The plant was scaled to produce 10% of the mandatory diesel requirement implemented by DME for 2013. The scale of the plant was varied according to the aerial lipid productivity to meet this requirement; Figure 3 shows graphically the business performance predictions for scenarios of lipid productivities between 10 to 50 g/m^2/day.

Table 1 Techno-economic model input assumptions

Assumption	Amount	Units
Plant scale	Variable	Ha
Inoculum biomass	6.4	Tons
Biomass productivity	30	g./m^2/day
Lipid productivity	Variable	g/m^2/day
Final biomass concentration	1.0	g/L
Production days per year	365	Days
Batches per year	46	#
Total biomass harvested per year	90514	Tons/yr
Lipid content	40	%
Lipid separation efficiency	70	%
Biodiesel produced	40 000	kL/yr
Selling price	8.98	R/L
Carbon dioxide used	90514	Tons/yr
Carbon trading price	202	R/ton

The conceptual flow sheet (Figure 4) designed for the proposed large scale plant was based on experience of algal technologies developed by the team. The process assumes that the plant will utilize CO_2 rich flue gases from power stations and petroleum producers and the nutrients required for algal growth would be obtained from waste water treatment plants that traditionally have nitrate and phosphate concentration of ~10 mg.l^{-1} (Tam and Wong 2000). These compounds are the two most essential for algal growth and concentrations available are ideal for algal culture (Kudela and Dugdale 2000). The price of biodiesel was pitched at the current basic selling price of low sulphur diesel (www.dme.gov.za) in South Africa. The CO_2 utilisation rate of algae was obtained from Chisty *et al*. 2007. Price assumptions for glycerol and spent algal biomass were pitched conservatively at R1 and R0.1.kg^{-1} respectively.

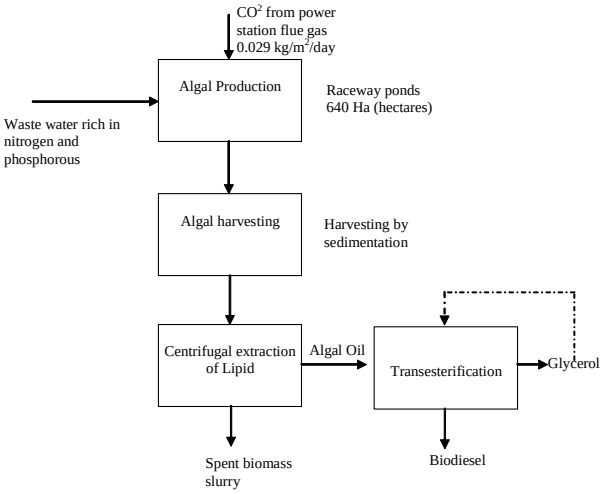

Figure 2 Conceptual algal biodiesel production flow-sheet

Capital cost estimates were sourced from suitable suppliers for the construction of the conceptual plant. Working capital for three years was factored into the cash flow calculations to mitigate operations risks in the startup years of the business. The IRR, NPV, capital investment required and the plant scale for the production of 40 000 KL of biodiesel per annum is depicted in Figure 3. The results presented show the obvious decline in plant size with increased aerial productivity. Furthermore, the model clearly highlights the importance of aerial productivity in the successful development of an outdoor algal biodiesel production facility. The plant is estimated to produce valuable glycerol and biomass as byproducts that result in additional revenue of ~ R5m and R10m per annum respectively. Furthermore the process is envisaged to utilise ~ 90 mega tons of CO_2 per annum resulting in additional revenue from the trading of carbon credits of ~ R18m per annum.

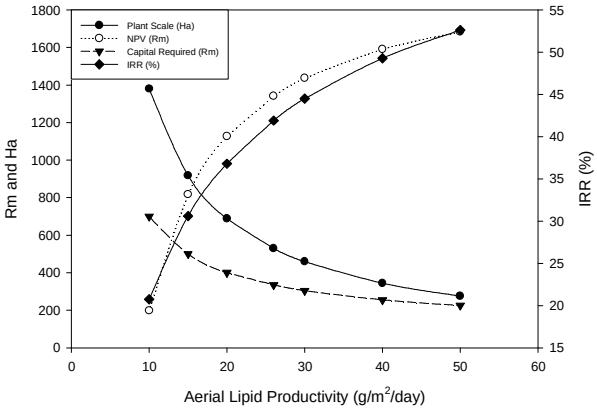

Figure 3 Business predictions at varying aerial lipid productivities

The techno-economic model clearly presents a very promising business case and highlights the key areas that influence the feasibility of the production process such as aerial lipid productivity.

ASP strain evaluations

Figure 2 demonstrates the lipid production profile over a 35 day period of the strains obtained from UH. *C. cryptica* demonstrated the best maximum lipid productivity (13 mg.l^{-1}.day^{-1}). This was ~ 16 times better than the next best strain (*Phaeodactylum tricornutum*).

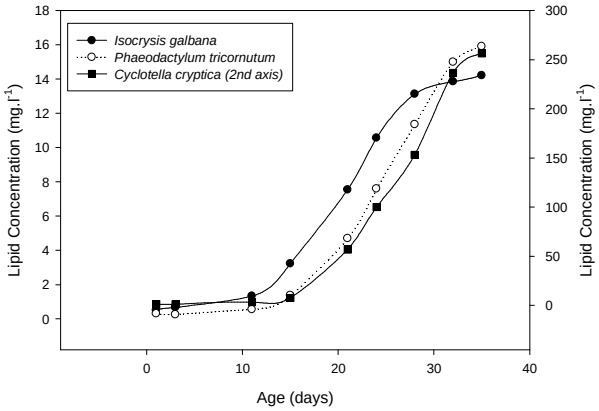

Figure 4 Lipid production profiles of *Isocrysis galbana; Phaeodatylum tricornutum* and *Cyclotella cryptica*

The growth of the cultures were tracked for 25 days and conformed to a typical exponential growth pattern (Figure 4). The best growth rate was observed in *C. cryptica* (0.43), which reached a peak cell concentration of ~ 3.0 X 10^9 cells.ml^{-1}, which translated to ~ 0.7 g.l^{-1} dcw. Under these conditions the lipid content of *C. cryptica* was calculated to ~ 40% total lipid per cell. These results confirmed literature based results using the same organism (Sheehan *et al.* 1998) on defined media.

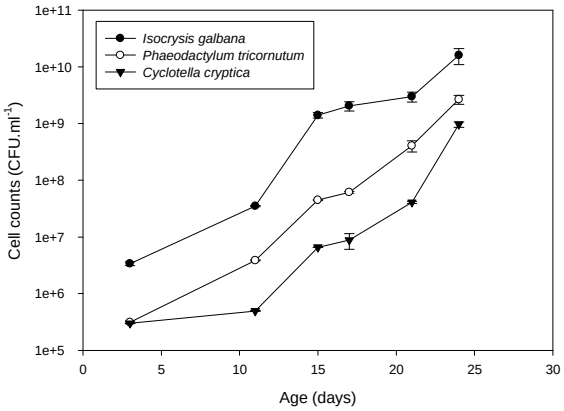

Figure 5 Growth profiles of *Isocrysis galbana; Phaeodatylum tricornutum* and *Cyclotella cryptica*

The lipid productivity of *C. cryptica* was calculated to be 13.3 mg/l/day, which converted to an aerial productivity of 2.66 g/m^2/day in flask growth. Sheehan et al (1998) demonstrated that a typical 10 fold increase in aerial lipid productivity can be observed from indoor flask studies (200 to 1000 ml) to outdoor lab raceway models (3m^2). If we use this result and assume a 10 fold increase in productivity, this will translate to ~ 26 g/m2/day for *C. cryptica* in an outdoor system. This result was inputted into the economic model and resulted in an excellent business case with an IRR of 41%, NPV of R1.3b from a capital investment of R335m. Furthermore, the plant scale could be drastically reduced to only 500 ha.

These experiments were conducted under nutrient sufficient conditions and were hence not optimised to target high lipid production; this is therefore very promising as the process can be significantly improved with additional development.

Screening and isolation

The screening programme has covered ~ 30% of the water bodies available in South Africa, which include coastal and inland waters and has yielded 161 isolates. From these isolates ~ 30% were positive for lipids by fluorescence microscopy. However, a qualitative estimation of lipid content based on image analysis indicates that ~ 90% of the positive isolates demonstrate lipid content of only 20 to 30% that of the UH strains. Six of the positive isolates demonstrated excellent lipid content of ~ 25% more than that observed in the best strains obtained from UH. The screening and isolation programme is still ongoing and quantitative and semi-quantitative lipid production assessments are in progress.

Laboratory scale raceway evaluation

The first positive isolate (*Characium spp*.) was isolated in Gauteng at the Modderfontein Lake (S26^{o}06.534', E028^{o}10.271'). Figure 5 graphically demonstrates the biomass growth of the raceway culture, which conforms to a typical exponential growth pattern with a growth rate of 0.0162 g.l^{-1}.day^{-1}. Samples removed at day 76 (A) and 134 (B) resulted in a lipid content of 12.8 and 14.3% dcw respectively, an increase of only 2 % over a 58 day period. However, this translated to ~ 42 and 130 mg.l^{-1} lipid which is an increase of ~ 3 fold in volumetric lipid concentration, predominantly due to increased biomass growth (Figure 5).

The key nutrients for algal growth (nitrate and phosphate) were never limiting during the raceway trial phase. This finding suggests that although lipid content was conserved the increase in biomass concentration resulted in increased volumetric productivity.

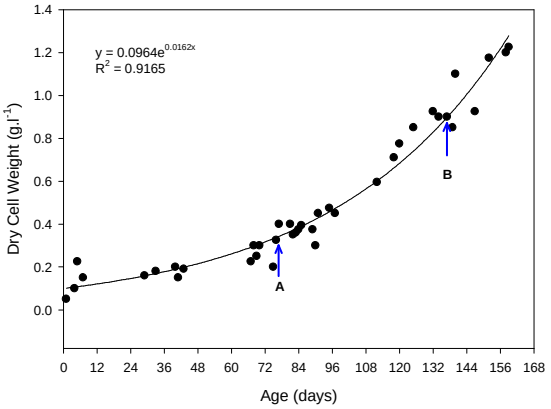

Figure 5 Biomass growth profile of Characium spp. In laboratory raceway pond

Coleman *et al,* 1988b*,* reached similar findings when growing algal strains; *Nannochloropsis salina* and *Nanno Q* on both nitrogen sufficient and nitrogen deficient media. He found that in nitrogen sufficient media, cells continued to grow and there was an increase in volumetric lipid concentration, although the lipid concentration within the cell remained constant. In nitrogen deficient media, the opposite was observed, where there was increase in lipid concentration with the cell while the net volumetric lipid concentration remained constant. It was therefore evident that nitrogen limitation causes an attenuation of cell division but lipid synthesis continues, thus resulting in an increase in lipid concentration within the cell. There is, however, no net increase in volumetric lipid productivity and the nitrogen limitation stress trigger does not increase the activity of the enzymes involved in lipid synthesis. Coleman *et al.,* 1988a also identified a rapid decrease in chlorophyll when cells were grown under nitrogen limitation. This indicated that N limitation caused a decrease in photosynthetic activity and the conversion of carbon. Further research is required to separate the effects of reduced photosynthetic efficiency from the direct effect of N limitation on biosynthetic enzyme activities.

The aerial lipid productivity of this isolate in indoor lab raceway systems was 2.88 g/m^2/day across two incremental points which mimic exponential growth in a continuous system, which can be extrapolated to an outdoor productivity of 28.2 g/m^2/day (Sheeha et al., 1998). This productivity when modelled showed an extremely feasible business case with an IRR and NPV of 43% and R1.4bn respectively at a plant scale of only 480 ha requiring an investment of ~ R300m.

Conclusions

This paper reports progress on algal biofuels research conducted at the CSIR. *Characium spp*. shows promise as a potential lipid producing algal strain. The current database of isolates available at the CSIR show isolates that demonstrate qualitiative lipid production far greater than that of *Characium spp* or the benchmark UH strains tested*.* These isolates are currently being evaluated at laboratory scale and the results obtained from these studies will form the basis of a later stage techno-economic assessment for a proposed production facility. The current research and previous expertise of algal process development and implementation, show great promise for the production of algal bio-fuels to meet South Africa's energy, social and economic priorities.

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