

GLYCEROL PRODUCTION BY HALOPHYTIC MICROALGAE: STRATEGY FOR PRODUCING INDUSTRIAL QUANTITIES IN SALINE WATER

P.J.Harvey⁽¹⁾, M.Psycha⁽²⁾, A.Kokossis⁽²⁾, A.L.Abubakar⁽¹⁾, V.Trivedi⁽¹⁾, R.Swamy⁽¹⁾, A.K.Cowan⁽³⁾, D.Schroeder⁽⁴⁾, A.Highfield⁽⁴⁾, G.Reinhardt⁽⁵⁾, S.Gartner⁽⁵⁾, J.McNeil⁽⁶⁾, P. Day⁽⁶⁾, M.Brocken⁽⁷⁾, J.Varrie⁽⁸⁾, A Ben-Amotz⁽⁹⁾,

⁽¹⁾School of Science, University of Greenwich Central Avenue, Chatham Maritime, Kent ME4 4TB, and Institute of Advanced Study, University of Durham, Cosin's Hall, Palace Green, Durham, DH1 3RL

+44-20-8331-9972 p.j.harvey@gre.ac.uk

⁽²⁾National Technical University of Athens,

⁽³⁾ Institute for Environmental Biotechnology, Rhodes University, PO Box 94, Grahamstown 6140 South Africa

⁽⁴⁾ Marine Biological Association of the UK

⁽⁵⁾ IFEU – Institute for Energy and Environmental Research, Wilckensstr. 3, 69120 Heidelberg, Germany

⁽⁶⁾ Aquafuel Research Unit 1020 Helly Close Kent Science Park, Sittingbourne, Kent ME9 8HL

⁽⁷⁾ Evodos Takkebijsters 17A, 4817 BL, Breda, The Netherlands

⁽⁸⁾ Flamingo Moon (Pty) Ltd, Block B Milestone House, Sovereign Drive, Irene, South Africa

⁽⁹⁾ Seambiotic Ltd., Azrieli Center, Tel-Aviv, Israel

ABSTRACT: Our aim is to increase the range of commercially viable biofuels by developing a low-cost system for producing industrial quantities of glycerol from the halophytic microalga, *Dunaliella*, cultivated on saline water. Glycerol can be combusted at very high efficiencies and with very low emissions without chemical alteration or the addition of combustion enhancers using a new commercial CHP technology based on the McNeil cycle, and holds the key to developing an entirely new, environmentally-sustainable biofuel industry sector. Glycerol will also serve as an intermediate to replace various fossil oil-based bulk chemicals in the biorefineries of the future. Glycerol is currently available from biodiesel manufacture, but is also synthesised de novo by natural halophytic microalgae that grow in highly saline environments (salt pans and desalination non-potable waters) across the globe. In this paper new strains of halophytic microalgae from Namibia were characterised for glycerol productivity and the data used in engineering models to optimise production and process designs. Our preliminary data indicate that in water-based biorefineries the economic potential of the venture will improve dramatically as one shifts from single-product into multiproduct processes. Based on current prices of glycerol, both glycerol and β -carotene need to be extracted from *Dunaliella* for profitability.

Keywords microalgae, biorefinery, cultivation, biofuel, supply chain

1 INTRODUCTION

The history of biofuel technology adoption reveals a repeating pattern of government subsidies and government mandates since the 1860s to promote biofuel use. The aim in general has been to create jobs in the agricultural sector and to decrease reliance on fossil fuels, and government interventions have taken place depending on the price and availability of fossil fuels. In recent times the IPCC Climate Change report (2007) has impressed the need for CO₂ mitigation measures, and biofuel technologies are able to play a role in reducing CO₂ emissions. In this paper we analyze the promise of glycerol as a new biofuel.

1.1 Properties of glycerol as a biofuel

Glycerol is a 1,2,3 propane triol - a simple alcohol. It has a high viscosity (1030cP at 22°C) and high activation energy (auto-ignition temperature of 370°C), properties which seem unlikely to commend it as a transport fuel. It also has been tainted with a poor emissions profile on combustion [1], and at first sight its energy density per kg appears moderate (~16 MJ/kg). It has, nonetheless a high volumetric energy density (see Fig.1). However, its promise as a biofuel of the future is as a result of the novel McNeil combustion cycle [2], which allows standard production compression ignition engines to combust glycerol at high efficiencies and with very low emissions without chemical alteration or the addition of combustion enhancers. When combusted within diesel engines glycerol produces no combustion particulate, no SO_x, reduced primary NO_x, extremely low VOC and aldehyde emissions and no catalyst poisons, and these parameters have been independently verified [3].

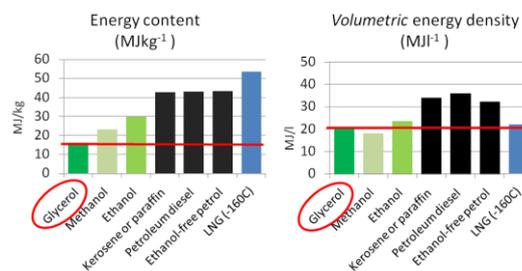


Figure 1: Energy content of glycerol compared to other fuels

Aquafuel have established the specification of glycerol from biodiesel manufacture (for CHP combustion to electricity), from trials of several thousand hours using adapted 2, 4 and 12 cylinder engine sets (20-400 kW). When emission abatement equipment is employed NO_x emissions 1/10 of the proposed Marpol Tier III can be achieved. Such low emissions would allow ship owners and operators to comply with the strictest future legislation at European and international levels and national authorities to meet their air quality engagements e.g. SECA, which requires that from January 2010 vessels entering certain ports for more than 2 hours must now only burn a fuel of max sulphur content of 0.1%. As the requirement for the reduction of emissions from internal combustion engines grows; low emissions fuels and systems will be required to meet very strict limits within the transport sector.

Combustion is also more energy efficient than any known fossil, bio or synthetic fuel (34 – 37 % (10 – 30kW), 40 – 42% (up to 1 MW) and 42 – 48% (over 1

MW) electrical efficiency), and engine performance with glycerol has been proven. Glycerol also has an extremely low volatility (1mm Hg at 125.1°C) and would therefore meet SOLAS regulations. In fact glycerol fuel stores could be safely used as thermal batteries, storing heat for CHP applications because glycerol also has a high boiling point (290°C), relatively and high heat capacity (221.9 J/(mol K) at 25°C). The correct combustion of glycerol within compression ignition engines results in glycerol being a highly desirable future biofuel from most perspectives.

1.2 Production of Glycerol

Glycerol can be sourced from plant oil as a by-product of transesterification in fatty acid methyl ester (biodiesel) manufacture with methanol, approx. 1 mole for every 3 mole of biodiesel. Volumetrically, it represents ca. 10% of the initial reactants. However, depending on the feedstock and the process adopted, glycerol by-product may contain significant and variable amounts of excess alcohol, water, catalyst, soaps and residual fats and oils, which necessitates some degree of post-reaction clean-up before combustion. In light of the current prices of glycerol (see Fig 2), this makes the margins for its clean-up extremely tight.

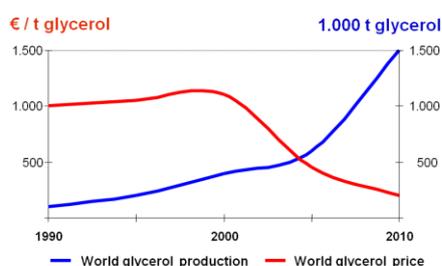


Figure 2: Current global production from biodiesel and its relation with price

More significantly, there is mounting concern over the negative impacts of growing plant oil for biodiesel manufacture, represented by so-called ILUC factors, which casts a cloud over the continued expansion of this source of glycerol. It is therefore of great interest that glycerol can also be produced by microbes directly [4-15].

During World War 1 glycerol was produced commercially from yeast fermentation of sugar as a feedstock in explosive manufacture, when demand exceeded supply from soap manufacture [5]. However since 1964 it has been known that halophytic microalgae will also produce glycerol, as much as 85% of their dry matter when placed in high salinity environments [6-9, 12-13]. On exposure to hyperosmotic stress and in response to decreased water activity, the rate of glycerol formation greatly increases, accumulating under the control of a channel protein which closes to conserve the glycerol within the cell, so that an osmotic equilibrium with the external environment can be maintained and osmotic pressures across the outer cell membrane countered. The most extreme example of this osmoregulation is found in various *Dunaliella* species that grow in salinities that vary from <1 to ~4M NaCl equivalent. In salt ponds across the globe, algae that produce glycerol in this way have been discovered and classified.

In this paper we have screened saline waters in

Namibia for halophytic microalgae and conducted a simple analysis of costs derived from study of process engineering designs for purifying glycerol from the halophyte *Dunaliella*. We show that the price of glycerol is currently too low for economic sustainability of the microalgal glycerol supply chain. Our data strengthen the need for approaches based on algal biorefineries if the goal of providing “Sustainable Energy for all by 2030” is to be met using biofuel.

2 METHODS

Algal strains were collected from saline water at the Sam Nujoma Algal Research Centre of Namibia and isolated by the Marine Biological Association Plymouth Culture Collection using conventional cell picking techniques. Isolated cultures were maintained and cultivated in modified Johnson’s Medium (Johnson et al., 1968) at 12:12 photoperiod, pH 7.5, average of 4395Wm² photosynthetically active irradiation (PAR) and 23 ± 0.2°C temperature.

PCR and sequencing of several genes was undertaken on the strains and phylogenetic analyses were performed using Neighbour-Joining and Bayesian methods.

For glycerol induction cultures were maintained for 14 d in medium with 1M NaCl then transferred to fresh media with varying NaCl concentration. Glycerol extraction was according to Ben-Amotz, Avron (1973). Glycerol contents were measured at 410 nm according to Chen et al., (2011).

For SCF, algae cells were centrifuged at 3000g then subject to SCF extraction. A glass beaker containing algal cells was placed in the high pressure reaction vessel. The pressurised CO₂ was pumped into the reaction vessel and heated to the desired temperature. A series of experiments were conducted in temperatures ranging from 25-60°C and pressures from 100-200 bar to determine optimum conditions for glycerol extraction. Only one parameter was changed at a time for example temperature was kept constant and pressure was gradually increased by 10 bar at a time until a desirable value was achieved. Another important aim of these experiments was to establish best parameters for maximal cell rupture. The experiments performed at 50°C and 200 bar provided best results hence these conditions were chosen for further extraction studies.

Process flowsheeting combined commercial software (Aspen Plus) with data available from the literature and selected industrial partners. Process units included scrubbers, cultivation ponds, pumps and a wide range of separators (distillation, evaporators, flash drums, homogenizers, spray dryers, screen separators, settling tanks, drying beds and osmotic tanks). Property models included NRTL database. The bench-mark was a single-product flowsheet producing glycerol and processing the alga *Dunaliella*, nutrients, seawater and CO₂ (in 25°C and 1 atm) in a cultivation pond (33°C, pH7.2, 15 cm depth) as available from harvesting 0.5 Ha. The conversion of *Dunaliella* was fixed at 3.5 tonnes biomass per year. Starting with glycerol, the study addressed the parallel production of β-carotene considering different solvents and different operating conditions (mainly pressures). Solvents studied included ethanol, cyclohexane, n-hexane, and trichloroethane. Comparisons were drawn on the basis of the economic potential still neglecting capital costs as annualisation (10 years payback time) proved

only a small fraction of total material and operating costs.

3 RESULTS

3.1 Bioprospecting for glycerol producing microalgae

Sequencing and phylogenetic analyses of the 28S rRNA gene of three strains T35, T36 and T37 of *Dunaliella* isolated from saline waters of Namibia showed that the strains were genetically distinct from other *Dunaliella* strains (see Fig. 3).

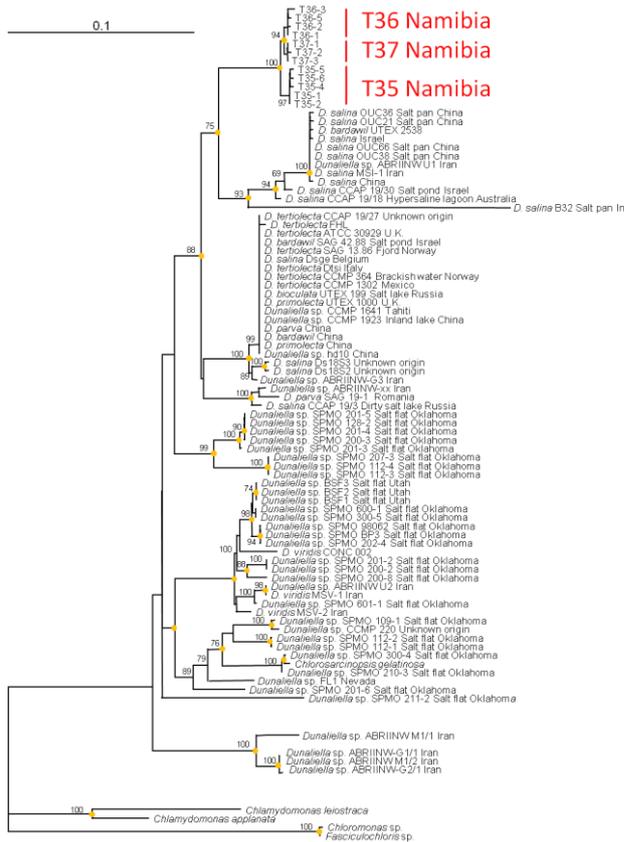


Figure 3: Microalgal phylogenetic tree constructed for *Dunaliella* showing the distance in relatedness of strains T35, T36 and T37 isolated from Namibia.

3.2 Cultivation, harvesting and extraction using supercritical CO₂.

Growth curves for strains T35, T36 and T37 were similar. Table 1 lists the doubling times which decreased ~5-fold to ~0.7d at 30°C compared to 23°C and decreased with increasing salinity.

Table I: Specific growth rate and doubling time of T35 T36 and T37.

Strain	Specific growth rate μ (1M NaCl) (d ⁻¹)	Doubling time (d) (23°C)
T35	0.144	4.8
T36	0.182	3.8
T37	0.164	4.2

When T35 was transferred to fresh medium after 14 days growth, glycerol productivity increased with increasing salinity (see Fig. 4).

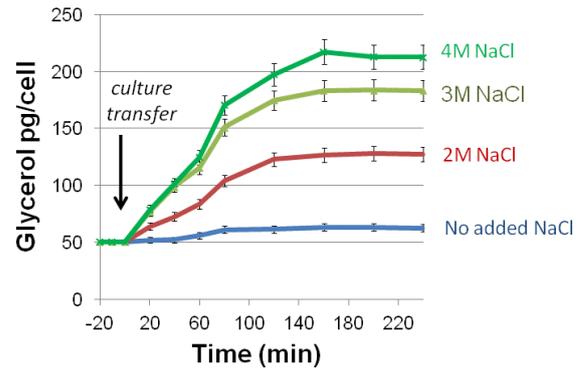


Figure 4: Effect of salinity on the growth of *Dunaliella* T35, T36 and T37. Cells were cultivated for 14 days in media containing 1M NaCl then transferred to fresh media containing increasing levels of NaCl as shown.

Using these data and those drawn from the literature a series of alternative process engineering flowsheets were constructed.

The first process flowsheet (Fig. 5) used the following assumptions: Cells are cultivated in sea water and nutrients for optimized growth rate and supplied with CO₂-enriched flue gas. They are transferred to osmotic stress ponds/tanks for 2 days during which time glycerol accumulates. Here the cells are neutrally buoyant in a high specific gravity, high viscosity brine and are therefore harvested by centrifugation but since they have no rigid cell wall and can be easily ruptured when excessive shear force is applied, a mild centrifugation approach is adopted such as that described by the Evodos system. This provides a separation sharpness equivalent to 11,000 – 15,000 g at only 3,000 g applied force. Glycerol is now enriched and separated from non-polar compounds including β -carotene by solvent extraction.

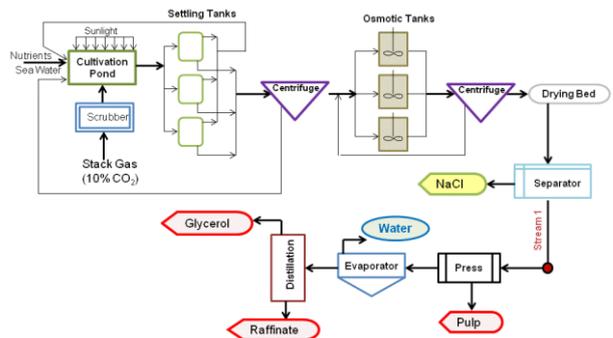


Figure 5: Process engineering flow sheet for the production of glycerol only.

In the next series of process engineering flowsheets, design improvements were aimed at optimising for the production of both glycerol and β -carotene. The first attempt used ethanol, trichloroethane, cyclohexane, and alum/ferric chloride as solvents and a wide range of devices. In the final iteration, cyclohexane was replaced by n-hexane and trichloroethane and alum/ferric chloride were not used, bearing in mind the requirement for protein meal as a feed supplement for e.g. fish. By systematic assessment of costs and performance requirements, the design flow sheet depicted in Fig. 6

was derived. After centrifugation, ethanol is mixed with the cells to extract glycerol and β -carotene, then the latter is separated from the mixture using n-hexane. Glycerol is recovered last after ethanol and water have been removed. The residue is now available as a fish feed supplement.

From analyses of the flow sheets, costs of the designs were now considered using the prices of glycerol (€250 / tonne) and β -carotene (€400/kg) and without considering a possible fall in price of the latter as scaling for glycerol production escalates. The results of the analysis are presented in Table II. They were calculated assuming a 0.5 Ha cultivation pond producing 3.5 tonnes biomass. The cost of algae cultivation (CF) includes the cost of electricity (€0.100/KW), fertilizers (N, P, K, Fe) and other chemicals, Domestic Land City Taxes, Manpower, and fresh water. The cost of CO₂ is assumed to be supplied free of charge from flue gas, although this could be considerable (€400/tonne). Sea water is estimated at €0.25/m³.

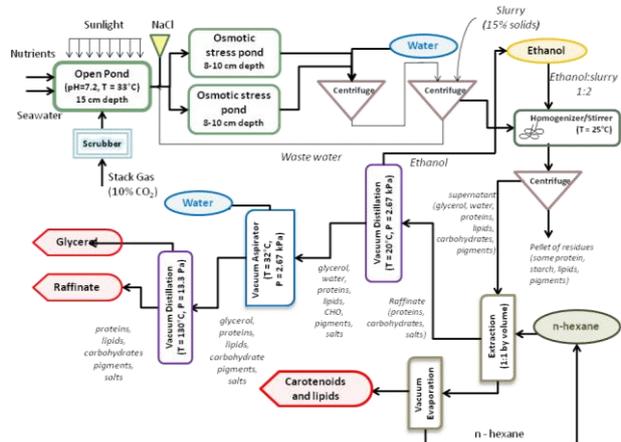


Figure 6: Process engineering flow sheet for the production of glycerol, β -carotene and a feed supplement (raffinate) for fish.

iterations of process design aimed at improving performance to extract both β -carotene and glycerol.

Table II shows that separation costs accounted for a significant proportion of the total cost and that the use of different solvents was able to increase savings by 50%. Considering the apparent impact of separation, significant improvements are expected with the use of process-to-process integration and thermally-integrated units i.e. there is considerable scope in the analysis for process integration, which has not yet been undertaken. For example, in a FP7 BIOCORE project, process integration was able to decrease the use of energy and water by 50% and 65% respectively (*personal communication*). Process integration is the subject of future analyses. Nevertheless, within the boundaries described the analysis shows that at current prices of glycerol the production of glycerol alone from microalgae is unlikely to be profitable. On the other hand there are significant opportunities for establishing a profitable business based on both glycerol and β -carotene products i.e. the inclusion of a single additional product transformed the economics from infeasible to highly profitable. This is likely to be even more profitable if lipid-protein by-products to meet current huge global demand for fish feed supplements are included. Experiments are now underway using supercritical fluid extraction with CO₂ to differentially fractionate *Dunaliella* (see Fig. 7) and analyses of the extracts are underway to identify further products of potential value.

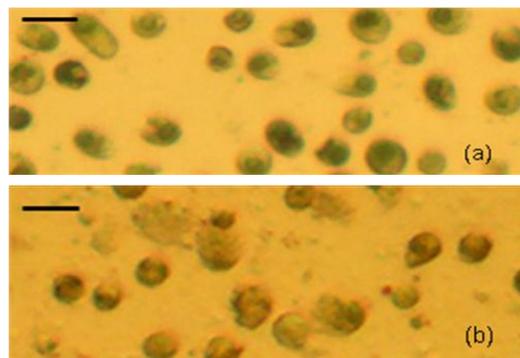


Figure 7: Images of *Dunaliella* cells after harvesting by centrifugation at 3,000g (a) before, and (b) after supercritical fluid extraction for 2h at 50°C 200 bar with CO₂. Bar=6µm

	Glycerol only	I: Glycerol & β -carotene	II: Glycerol & β -carotene	III: Glycerol & β -carotene
Cost (€/yr)				
Solvents (CS)	—	1,865,720	3,437,333	2,067,206
Ethanol	—	739,544	739,544	739,544
Cyclohexane	—	741,217	741,217	—
n-hexane	—	—	—	1,327,663
Alum/Ferric Chloride	—	384,959	384,959	—
Trichloroethane	—	—	1,571,613	—
Energy (CE)	880,147	2,639,711	2,639,711	1,305,193
Cultivation CF)	539,928	539,928	539,928	539,928
Products (WP)	48,305	14,329,313	14,329,313	14,329,313
Glycerol	48,305	48,305	48,305	48,305
β -carotene	—	14,281,008	14,281,008	14,281,008
Profit/loss	-1,371,770	9,283,954	7,712,341	10,416,986

CS: Cost of Solvents

CE: Cost of Energy

CF: Cost of Cultivation Input (Feed)

WP: Worth of Products

Table II: Cost analysis of producing glycerol from *Dunaliella*, together with analysis based on 3 successive

4 DISCUSSION AND CONCLUSIONS

Climate change mitigation by reducing CO₂ emissions in the atmosphere has a global context, and is evidenced by the 191 states that have signed and ratified the Kyoto Protocol. In turn, biofuel adoption strategies – subsidies and mandates - now come under a similar global spotlight. However there is now a widespread debate as to where society's agricultural outputs should be best directed i.e. food, feed or fuel? This debate is highly relevant in the context of global population growth. Moreover, the availability and discovery of new seams of fossil fuel continues to place fossil fuel as a cheaper alternative to non-fossil fuel and this suggests that the use of fossil fuels might continue unabated, as in the past, unless Society as a whole acts to pay for the environmental benefits of implementing non-fossil technologies such as those described in this paper based on glycerol. This adds further impetus to understanding

what the costs are to Society, and how the implementation of non-fossil bioenergy technologies might affect local communities.

This paper shines a spotlight on glycerol, which, when sourced from halophytic microalgae, could serve as a remarkable new biofuel with environmentally sustainable benefits and capable of contributing to meeting Society's energy requirements. As demonstrated by the simple exercise of screening saline waters in Namibia for microalgae, the opportunities for bioprospecting for new strains of glycerol-producing microalgae tailored for a wide range of environments are immense. Their cultivation in highly saline non-potable waste or marine water offers the opportunity for glycerol production around the globe. As such glycerol technologies will not compete with agricultural land for their production, nor will they encroach upon displacement / land use rights compared to other biofuel / bioenergy options. Glycerol cultivation ponds would bring "High-Tech" agriculture to rural communities as well as improve the education of the local communities and eliminate poverty, and create new jobs. They would also integrate well with communities: they offer enhanced work place safety and aesthetics and are compatible with parks and urban Greenbelts. Furthermore, unlike biofuels derived from either plant or microalgal lipids, glycerol is a "finished" fuel: it does not require chemical modification or molecular reformation. This aspect adds benefit to any microalgal production facility requiring electricity and heat, since glycerol sourced from the microalgae could be used directly as a fuel to meet the combined heat and power requirement.

Glycerol is classified as carbon neutral under the EU Renewables Directive 2010. Its use in power generation as an alternative to hydrocarbon fuels would therefore contribute to CO₂ reduction emissions aligned with new policy targets set out in the Climate and Renewable Energy Package of 2009. By way of example, producing 145 MW electricity from glycerol in a CHP engine would equate to displacement of 263,529 tonnes fossil diesel @ 3.135 tonnes CO₂ per tonne diesel, thus eliminating 826,163 tonnes CO₂ per annum. Similarly, heat capture from glycerol CHP at ~190 MW could eliminate ~ 313,120 tonnes of CO₂ from natural gas combustion. By burning 0.7 million tonnes of glycerol in CHP, the total possible CO₂ elimination is > 1 million tonnes per annum. The use of glycerol could therefore contribute substantially to meeting the world's first legally binding targets set for a nation to cut its greenhouse gas emissions, outlined in the UK Climate Change Bill 27 November 2008.

The future of all transportation sectors, excepting air, is increasingly focused upon hybrid and electric technologies, which will result in future internal combustion engine modes for hybrid, such as series hybrid where the vehicle has a battery driven electric motor for propulsion, and carries a small generator to charge the battery to act as a range extender. These are more related to electrical generators than the current propulsion requirement. Glycerol would be an ideal fuel for such future systems.

However the commercial viability of algae-based biofuels production ultimately depends on economics. Regardless of technological and biological breakthroughs, the commercial marketplace will not have an appetite for funding capital intensive energy projects unless the risk-return ratio is acceptable to debt and

equity financiers. A number of companies and government organizations have previously assessed different production designs and offered estimates of costs for algae systems. The most popular of designs previously analysed include open ponds, open raceways, and closed photobioreactors. Generally these assessments have taken a first-order look at capital and operations and maintenance (O&M) costs. The capital costs are usually broken down into costs associated with algal biomass growth, harvesting (removal of the biomass from the culture), dewatering (getting the algae to an acceptable concentration for further processing), and algal oil extraction systems. In addition, there are more traditional project costs such as engineering, permitting, infrastructure preparation, balance of plant, installation and integration, and contractor fees. O&M costs generally include expenses for nutrients (generally N-P-K), CO₂ distribution, water replenishment due to evaporative losses, utilities, components replacement, and labour costs.

The simple analysis presented here suggests that without subsidy from government or public-private partnership to meet CO₂-mitigation mandates, the costs of glycerol production will be too great to compete with fossil-derived fuels.

On the other hand, the alternative scenarios based on production of β -carotene as well as glycerol open up new opportunities for production of high value nutraceuticals and feed alongside glycerol. They also open up new challenges in approaching the concept of the "algal biorefinery", namely, how will such a refinery develop a balanced market-led portfolio of products driven by production of glycerol as a biofuel from *Dunaliella* without over supply to one or more market sectors taking by-products of the process? Clearly supply chain management will be critical in the modern algal biofuel enterprise development.

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